HUNGARIAN MOLECULAR
LIFE SCIENCES
2015

PROGRAMME
&
BOOK OF ABSTRACTS

HOTEL EGER-PARK
EGER, HUNGARY
27-29 MARCH 2015

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Dear Colleagues,

The Hungarian Biochemical Society (MBKE), the Hungarian Genetical Society (MAGE) and the Hungarian Association for Cell and Developmental Biology (SFBT) organize the second joint conference, entitled „Hungarian Molecular Life Sciences 2015”, in Eger, Hungary, March 27-29. Our scientific societies are intent on maintaining the traditions of the first, highly successful “Hungarian Molecular Life Sciences 2013” meeting. Scientists are anticipated to attend from a broad range of institutions of higher education as well as from research institutes under the egis of the Hungarian Academy of Sciences or other governmental bodies. This large-scale meeting, adhering to the traditions of each association, concurrently will also constitute the XI. Hungarian Genetics Conference, the XVIII. Symposium of Cell and Developmental Biologists, and the forthcoming Convention 2015 of the Hungarian Biochemical Society. The goal of the conference is to establish a common forum for colleagues working in the fields of classic and molecular biochemistry, cell and structural biology, developmental biology, classic and molecular genetics, the molecular biology of human diseases, systems biology, synthetic biology, genomics and bioinformatics. We look forward to a conference with a creative and friendly atmosphere, where former acquaintances are renewed and new collaborative partnerships are formed; a symposium of class and uniqueness.

On behalf of the organisers:

Beáta Vértessy	**Miklós Erdélyi**	**Gábor Szabó**
Lajos Haracska	**Péter Kaló**	**Miklós Sass**
Hungarian Biochemical Society	**Hungarian Genetical Society**	**Hungarian Association for Cell and Developmental Biology**

GENERAL INFORMATION

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CONGRESS ORGANISERS

- *Magyar Biokémiai Egyesület (Hungarian Association for Biochemistry)*
- *Magyar Genetikusok Egyesülete (Hungarian Genetical Society)*
- *Sejt- és Fejlődésbiológiai Szakosztály (Hungarian Association for Cell and Developmental Biology)*

ORGANISING COMMITTEEE

Miklós Erdélyi[1], Lajos Haracska[2], Péter Kaló[3], Miklós Sass[4], Gábor Szabó[5], Beáta Vértessy G.[6]

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Műegyetem rakpart 3, Budapest H-1111
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http://www.biostruct.org

TECHNICAL ORGANISERS

Diamond Congress Ltd.
1255 Budapest, Pf. 48.
Tel: 1-225-0209
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WEBSITE OF THE CONGRESS

http://www.hunlifesci.hu

WEBSITE OF THE HUNGARIAN BIOCHEMICAL SOCIETY

http://www.mbkegy.hu

WEBSITE OF THE HUNGARIAN GENETICAL SOCIETY

http://www.magenegy.hu/hu/

VENUE

Hotel Eger Park
3300 Eger
Szálloda utca 1-3.
Tel.: +36-36/522-200

OPENING HOURS OF THE REGISTRATION

Friday, 27 March, 2015.	-	**10.00 – 20.00**
Saturday, 28 March, 2015.	-	**08.00 – 19.00**
Sunday, 29 March, 2015.	-	**08.00 – 13.00**

ONSITE CONTACT NUMBERS

Éles-Etele Nóra / Varga Attila
06/70-9438-543, 06/20-936-2969
Diamond Congress Ltd.

OFFICIAL LANGUAGE

Official language of the Congress is English (no translation is available).

Registration fee (incl. VAT)	Payment till 15 February	Payment after 15 February
Registration fee for industrial participants	55.000 Ft	60.000 Ft
Registration fee for senior researchers*	50.000 Ft	55.000 Ft
Registration fee for junior researchers**	35.000 Ft	40.000 Ft
Registration fee for exhibitors	30.000 Ft	30.000 Ft
Registration fee for Accompanying persons	27.000 Ft	27.000 Ft

**Only for participants with academic background*
***Junior researcher: Ph.D. and university student, or researcher under 30*

Registration fees include admission to the scientific sessions, admission to the exhibition, welcome reception, banquet dinner, coffee breaks, Lunches.

Accompanying persons' registration fee and exhibitor's registration fee is not valid for admission to the scientific part of the Conference, and these fees do not include congress materials, only meals and social events.

ORAL PRESENTATIONS (PL-01 – PL-07, CL-01 – CL-03, O-001 – O-085)

The schedule of the oral presentations can be seen in the detailed programme of this booklet. Speakers and session chairs are kindly requested to keep the time of the presentations. Make sure to bring your presentation file written on a properly closed CD ROM or USB flash drive. Presenters are kindly requested to give their presentation file to the technicians in the lecture rooms preferably half day before beginning of the corresponding session.

Oral presentations: Room I, Room II, Room III.

POSTER PRESENTATIONS (P-001 – P-165)

Poster presenters can mount their posters from 10.00 on Friday, 27 March, 2015. Posters will be identified by poster numbers, which are indicated in the author index and on the floor plan (inner, back cover page of this programme booklet).

Poster sessions: Room Liget
Pins are to be provided to fix the posters by the technical organisers.

Presenting authors having **odd numbers** should be at their posters between **20.30 – 22:30 on Friday, 27 March.** Presenting authors having **even numbers** should be at their posters between **16.30 – 18.30 on Saturday, 28 March.**

AWARDS OF BEST POSTER PRESENTATIONS

The organisers intend to appoint a professional jury who will evaluate the best 3 presented posters.

EXHIBITON

In accordance with the conventions of the conference, parallel to the scientific sessions a professional exhibition is to be organised. Please have a look at the exhibition floor plan of the booklet.

ACCOMMODATION

Hotel rooms are booked under the name of the participants. Congress participants may occupy the rooms from 14:00 on the day of arrival and should arrange the check out until 10:00. The hotel ensures a luggage room. The guarded parking lot of the hotel is available for our participants free of charge. Guests are kindly requested to settle their extra room bills (such as phone calls, drinks and minibar) prior to departure.
The room prices include buffet breakfast, VAT and city tax. In the Hotel Eger-Park the price also contains the usage of wellness facilities (pools, jacuzzi, sauna park and steam bath).

SOCIAL PROGRAMMES *(incl. in the registration fees)*

Friday, 27 March, 2015	Welcome dinner (Restaurant of Hotel Park)
Saturday, 28 March, 2015	Concert (Basilica) and Banquet (Conference hall of Hotel Eger)
Saturday & Sunday, 28-29 March, 2015	Lunches (Restaurant of Hotel Park)
Friday – Sunday, 27-29 March, 2015	Coffee breaks (indicated in the programme)

All participants and accompanying persons will receive a personal badge upon registration. You are kindly requested to wear your name badge when attending the meetings or social events.

Extra consumption, which is not included in the menus are kindly requested to settle prior to departure.

CANCELLATION POLICY

Cancellations on registration and hotel reservation can be made only in writing. The refund for cancellations made on and **prior to 10 March, 2015** is 100%. After this date the conference secretariat has to pay the advanced payments to the hotel, and there is no way to refund in case of latter cancellation.

PAYMENT, INVOICES

The price of the ordered services will be indicated on the final invoice according to the Hungarian official financial rules. Official final invoices and receipts for fees paid by the participants will be handed over on site at the registration desk. Please forward them to the financial department of the Institute.

LIABILITY AND INSURANCE

The organisers cannot accept liability for any personal accidents, loss of belongings or damage to private property of participants and accompanying persons that may occur during the Congress.

SCIENTIFIC PROGRAMME

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FRIDAY, 27 March 2015		
14:00 – 14:10	**Opening**	
Plenary lectures I *Chairperson: Éva Kondorosi*		
14:10 – 14:40	**PL-01**	**THE ROUTES OF REPROGRAMMING TO ALTERNATIVE STATES OF PLURIPOTENCY** Andras Nagy *Mount Sinai Hospital, Lunenfeld-Tanenbaum Research Institute, Toronto, Canada*
14:40 – 15:10	**PL-02**	**UNDERSTANDING ESTROGEN RECEPTOR TRANSCRIPTION IN BREAST CANCEr** Jason S. Carroll *Cancer Research UK, University of Cambridge, Cambridge, United Kingdom*
15:10 – 15:40	**PL-03**	**DEF1 PROMOTES THE DEGRADATION OF POL3 FOR POLYMERASE EXCHANGE TO OCCUR DURING DNA-DAMAGE–INDUCED MUTAGENESIS IN SACCHAROMYCES CEREVISIAE** Andreea Daraba, Vamsi Krishna Gali, Miklós Halmai, Lajos Haracska, Ildikó Unk *The Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged*
15:40 – 16:10	**Coffee break**	
Plenary lectures II *Chairperson: Mikós Sass*		
16:10 – 16:40	**PL-04**	**REVEALING NOVEL MOLECULAR ELEMENTS OF CELL DEATH PARADIGMS. THE NEUTROPHIL EXTRACELLULAR TRAP** László Fésüs *Department of Biochemistry and Molecular Biology, DE-MTA Stem Cells, Apoptosis and Genomics Research Group, Faculty of Medicine, University of Debrecen*
16:40 – 17:10	**PL-05**	**ROLE AND MECHANISMS OF AUTOPHAGY IN DROSOPHILA** Gábor Juhász *Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary, and Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged*
17:10 – 17:40	**PL-06**	**MEMBRANES AND LIPIDS IN THE CELLULAR HEAT STRESS MANAGEMENT** László Vígh, Gábor Balogh, Zsolt Török, Imre Gombos, Mária Péter,Tim Crul, Begum Peksel, Bálint Csoboz, Attila Glatz, Ibolya Horváth *Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary*

FRIDAY, 27 March 2015		
Plenary lectures II - *continued*		
Chairperson: Mikós Sass		
17:40 – 18:00	**PL-07**	**INDEPENDENT PARALLEL FUNCTIONS OF P19 PLANT VIRAL SUPPRESSOR OF RNA SILENCING REQUIRED FOR EFFECTIVE SUPPRESSOR ACTIVITY** Éva Várallyay[1], Enikő Oláh[1], Zoltán Havelda[2] *[1]Diagnostic Group, Agricultural Biotechnology Research Institute, NARIC, Gödöllő, Hungary; [2]Plant Developmental Biology Group, Agricultural Biotechnology Research Institute, NARIC, Gödöllő*
18:00 – 18:10	**Technical break**	
Company lectures		
18:10 – 18:20	**CL-01**	**NEXT-GENERATION PROMEGA CELL-BASED ASSAYS TO STUDY CELLULAR HEALTH AND RESPONSES** Zsolt Somlai *Bio-Science Ltd., Budapest*
18:20 – 18:30	**CL-02**	**HIGH CONTENT SCREENING: WIDEFIELD AND CONFOCAL IMAGING BASED AUTOMATED RESEARCH SOLUTIONS FROM MOLECULAR DEVICES** Nándor Nagy *Bio-Science Ltd., Budapest*
18:30 – 18:50	**CL-03**	**IMAGE-BASED HIGH CONTENT SCREENING: STATE OF THE ART CONTRACT RESEARCH SERVICES AT SOFT FLOW** György Lustyik, Árpád Czéh, Erika Lantos, György Nagyéri, Viktória Németh, Marianna Merki *Soft Flow Hungary Kft., Pécs*
19:00 – 20:30	**Welcome dinner (Restaurant of Hotel Park)**	
20:30 – 22:30	**Poster discussion with drinks (odd poster numbers)**	

SATURDAY, 28 March 2015		
Parallel session #1 – Molecular Mechanism of Deseases I – Room I. *Chairperson: Márta Széll*		
9:00 – 9:20	**O-001**	**THE CHANGING LANDSCAPE OF GENETIC TESTING** István Balogh *University of Debrecen, Faculty of Medicine, Division of Clinical Genetics, Debrecen*
9:20 – 9:40	**O-002**	**ASSOCIATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ POLYMORPHISMS WITH INFLAMMATORY BOWEL DISEASE IN A HUNGARIAN COHORT** András Penyige[1], Szilárd Poliska[2], Péter László Lakatos[3], Mária Papp[4], Károly Palatka[4], László Lakatos[5], Tamás Molnár[6], László Nagy[2] *[1]Department of Human Genetics, University of Debrecen, Debrecen; [2]Department of Biochemistry and Molecular Biology, University of Debrecen; [3] 1st Department of Medicine, Semmelweis University, Budapest; [4] 2nd Department of Medicine, University of Debrecen, Debrecen; [5] 1st Department of Medicine, Csolnoky F. County Hospital, Veszprem; [6] 1st Department of Medicine, University of Szeged*
9:40 – 10:00	**O-003**	**PROTEIN PHOSPHATASE-1 IS INVOLVED IN THE MAINTENANCE OF NORMAL HOMEOSTASIS AND IN UVA IRRADIATION-INDUCED PATHOLOGICAL ALTERATIONS IN HACAT CELLS AND IN MOUSE SKIN** Dóra Dedinszki[1], Adrienn Sipos[1], Andrea Kiss[1], Róbert Bátori[1], Zoltán Kónya[1], László Virág[1,2], Ferenc Erdődi[1,2], Beáta Lontay[1] *[1]Department of Medical Chemistry, University of Debrecen, Debrecen; [2]MTA-DE Cell Biology and Signaling Research Group, Faculty of Medicine, University of Debrecen, Debrecen*
10:00 – 10:20	**O-004**	**LIVER METABOLIC ALTERATIONS INFLUENCE PXE-LIKE PHENOTYPE** Borbála Vető[1], Caroline Bacquet[1], Hugues de Boussac[1], Attila Horváth[2], Endre Barta[2], Dávid Jónás[2], László Buday[1], Bálint L. Bálint[2], László Nagy[2], András Váradi[1], Tamás Arányi[1] *[1]Institute of Enzymology, Research Center for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2]Department of Biochemistry and Molecular Biology, Medical and Health Science Center, University of Debrecen, Debrecen*
10:20 – 10:40	**O-005**	**INTRODUCING A NEW MEMBER IN DNA DAMAGE TOLERANCE PATHWAY** Lili Döme *Biological Research Centre of the Hungarian Academy of Sciences Mutagenesis and Carcinogenesis Research Group, Szeged*
10:40 – 11:00	**O-006**	**ANALYSIS OF THE FIRST 1500 PREIMPLANTATION GENETIC SCREENING DONE IN HUNGARY USING ARRAY COMPARATIVE GENOMIC HYBRIDIZATION** Attila Vereczkey, Éva Margittai, Marianna Csenki, Gyöngyvér Téglás, László Nánássy *Versys Clinics Humán Reproduction Institute, Budapest*
11:00 – 11:20	**Coffee break**	

SATURDAY, 28 March 2015		
Parallel session #2 – Protein Structure, Function and Modelling – Room II. *Chairpersons: Tamás Hegedús, László Nyitray*		
9:00 – 9:20	**O-007**	**MOLECULAR MECHANISM OF NUCLEOTIDE-DEPENDENT ACTIVATION OF THE KTRAB K^+ TRANSPORTER** András Szöllősi[1,2], Ricardo S. Vieira-Pires[1], Celso M. Teixeira-Duarte[1], João H. Morais-Cabral[1] *[1]IBMC, Molecular and Cell Biology Institute, University of Porto, Porto, Portugal; [2]Semmelweis University, Department of Medical Biochemistry, Budapest*
9:20 – 9:40	**O-008**	**CHARGED SINGLE ALPHA HELICES: PREDICTION, ANALYSIS AND MODELING** László Dobson[1], László Nyitray[2], Zoltán Gáspári[1] *[1]Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest; [2]Eötvös Loránd University, Department of Biochemistry, Budapest*
9:40 – 10:00	**O-009**	**MULTIPLE FUZZY INTERACTIONS IN THE MOONLIGHTING FUNCTION OF THYMOSIN-B4** Ágnes Tantos[1], Beáta Szabó[1], András Láng[2], Zoltán Varga[3], Mónika Bokor[4], Kálmán Tompa[4], András Perczel[2], Péter Tompa[1,5] *[1]Institute of Enzymology, RCNS, Hungarian Academy of Sciences, Budapest; [2]Institute of Chemistry, Eötvös Loránd University Budapest; [3]Institute of Materials and Environmental Chemistry, RCNS, Hungarian Academy of Sciences, Budapest;[4]Institute for Solid State Physics and Optics, Wigner Research Centre for Physics, Hungarian Academy of Sciences, Budapest; [5]VIB Department of Structural Biology, Vrije Universiteit Brussel, Brussels, Belgium*
10:00 – 10:20	**O-010**	**DISCRETE MOLECULAR DYNAMICS CAN PREDICT HELICAL PRESTRUCTURED MOTIFS IN DISORDERED PROTEINS** Lajos Kalmár[1], Dániel Szöllősi[2], Tamás Horváth[1], Tamás Hegedűs[2], Péter Tompa[1] *[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2]MTA-SE Molecular Biophysics Research Group, Hungarian Academy of Sciences, Budapest; Department of Biophysics and Radiation Biology, Semmelweis University, Budapest*
10:20 – 10:40	**O-011**	**COMPLEX DNA-PROCESSING ACTIVITIES ARE CONSERVED BETWEEN BACTERIAL AND HUMAN GENOME-GUARDING HELICASES** Gábor M. Harami[1], Martina Máté[1], Nikolett T. Nagy[1], Junghoon In[2], Veronika Ferencziová[1], Yeonee Seol[2], Kata Sarlós[1], Yuze Sun[2], Keir C. Neuman[2], Mihály Kovács[1] *[1]Department of Biochemistry, ELTE-MTA “Momentum” Motor Enzymology Research Group, Eötvös Loránd University, Budapest; [2]Laboratory of Molecular Biophysics, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, United States*

SATURDAY, 28 March 2015		
Parallel session #2 – Protein Structure, Function and Modelling – Room II. – *continued*		
Chairpersons: Tamás Hegedús, László Nyitray		
10:40 – 11:00	**O-012**	**COMPUTATIONAL TOOLS INDICATE THE IMPORTANCE OF FLEXIBILITY AND INTERMOLECULAR INTERACTIONS IN MULTIDRUG RECOGNITION OF ARYL HYDROCARBON RECEPTOR** Dániel Szöllősi[1,2], Áron Erdei[1,2,3], Gergely Gyimesi[4], Tamás Hegedűs[1,2] *[1]MTA-SE Molecular Biophysics Research Group, Hungarian Academy of Sciences, Budapest; [2]Department of Biophysics and Radiation Biology, Semmelweis University, Budapest; [3]Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest; [4]Institute of Biochemistry and Molecular Medicine, University of Bern, Bern, Switzerland*
11:00 – 11:20	**Coffee break**	
SATURDAY, 28 March 2015		
Parallel session #3 – Regulatory RNAs – Room III.		
Chairpersons: Tamás Orbán, Dániel Silhavy		
9:00 – 9:20	**O-013**	**R-LOOPS AS POTENT TARGETS OF THE APOBEC CYTIDINE DEAMINASE-MEDIATED INNATE IMMUNITY** Lóránt Székvölgyi[1], András Szántó[1], László Halász[1], Zsolt Karányi[2], Péter Nánási, György Fenyőfalvi[1], Eszter Csoma[3], Gábor Szabó[1] *[1]Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen; [2]Department of Internal Medicine, Faculty of Medicine, University of Debrecen, Debrecen; [3]Department of Microbiology, Faculty of Medicine, University of Debrecen, Debrecen;*
9:20 – 9:40	**O-014**	**FUNCTIONAL DISSECTION OF A PLANT ARGONAUTE** Károly Fátyol, Márta Ludman, József Burgyán *Agricultural Biotechnology Institute, National Agricultural Research and Innovation Centre, Gödöllő*
9:40 – 10:00	**O-015**	**TISSUE AND CIRCULATING MICRORNAS IN THE DIAGNOSIS OF ADRENAL TUMORS** Zoltán Nagy, Péter Igaz *2nd Department of Medicine, Semmelweis University, Budapest*
10:00 – 10:20	**O-016**	**ARTIFICIAL MIRTRON-DERIVED SMALL REGULATORY RNAS: A POTENTIAL TOOL FOR EFFECTIVE GENE EXPRESSION REGULATION** Anita Schamberger, Tamás I. Orbán *Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest*
10:20 – 10:40	**O-017**	**SMALL REGULATORY RNAS IN RESPONSE TO AMBIENT TEMPERATURE SENSING IN *ARABIDOPSIS*** Ivett Baksa[1], Irina Mohorianu[2], Tamás Dalmay[2], György Szittya[1] *[1]NARIC, Agricultural Biotechnology Institute, Epigenetics Group, Gödöllő; [2]UEA, School of Biological Sciences, Norwich, United Kingdom*
10:40 – 11:00	**O-018**	**LINKING RNA SILENCING TO NON-STOP DECAY** Tibor Csorba[1], István Szádeczky-Kardoss[1], Tünde Nyikó[1], János Taller[2], József Burgyán[1], Dániel Silhavy[1] *[1]NAIK-MBK, Gödöllő; [2]Georgikon Kar, Pannon Egyetem, Keszthely*
11:00 – 11:20	**Coffee break**	

SATURDAY, 28 March 2015

Parallel session #1 – Molecular Mechanism of Diseases II. – Room I.

Chairperson: István Balogh

11:20 – 11:40 **O-019** **SYSTEMATIC ANALYSIS OF SOMATIC MUTATIONS IN CANCER: UNCOVERING FUNCTIONAL PROTEIN REGIONS IN DISEASE DEVELOPMENT**

Bálint Mészáros[1], András Zeke[1], Attila Reményi[1], István Simon[1], Zsuzsanna Dosztányi[2]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2]Department of Biochemistry, Eötvös Loránd University, Budapest

11:40 – 12:00 **O-020** **EPIGENETIC MODIFICATIONS IN HUNTINGTON'S DISEASE**

László Bodai[1], Judit Pallos[2], Wan Song[2], Nóra Zsindely[1], J. Lawrence Marsh[2]

[1]Department Biochemistry and Molecular Biology, University of Szeged, Szeged;[2]Department Developmental and Cell Biology, University of California at Irvine, Irvine, CA, United States

12:00 – 12:20 **O-021** **THE UNIVERSE OF MONOGENIC HUMAN DISEASES: IS IT REALLY EXPANDING?**

Nikoletta Nagy[1,2,3], Márta Széll[1,3]

[1]Department of Medical Genetics, University of Szeged, Szeged; [2]Department of Dermatology and Allergology, University of Szeged, Szeged; [3]MTA SZTE Dermatological Research Group, University of Szeged, Szeged

12:20 – 12:40 **O-022** **MAGNESIUM HOMEOSTASIS IN PLATELETS**

Sanjeev Kiran Gotru[1,2], Simon Stritt[1,2], Wenchun Chen[1,2], Karen Wolf[1,2], Peter Kraft[3], Guido Stoll[3], Vladimir Chubanov[4], Thomas Gudermann[4], Bernhard Nieswandt[1,2], Attila Braun[1,2]

[1]Department of Vascular Medicine, University Hospital Würzburg, Germany; [2]Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg, Germany; [3]Department of Neurology, University Hospital Würzburg, Germany; [4]Walther-Straub-Institute for Pharmacology and Toxicology, LMU, Munich, Germany

12:40 – 13:00 **O-023** **GENOTYPE-PHENOTYPE CORRELATIONS IN *DROSOPHILA COL4A1* MUTANTS: HUMORAL IMMUNE RESPONSE, MUSCULAR DYSTROPHY WITH VARIOUS PROGRESSION, CENTRONUCLEAR/MYOFIBRILLAR MYOPATHY, AND OPTIONS OF TREATMENT**

Ildikó Kelemen-Valkony[1], Márton Kiss[1], András Attila Kiss[1], Mónika Radics[1], Nikoletta Popovics[1], Katalin Csiszár[2], Mátyás Mink[2]

[1]Department of Genetics, University of Szeged, Szeged; [2]John A. Burns School of Medicine, University of Hawaii, Honolulu, United States

13:00 – 13:20 **O-024** **CONSEQUENCES OF LOSS OF RETINOL SATURASE ENZYME IN MICE**

Zsolt Sarang[1], Gábor Nagy[2], Tibor Sághy[1], Zsuzsa Szondy[1]

[1]Apoptosis Signal Research Group, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen; [2]Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen

SATURDAY, 28 March 2015		
Parallel session #1 – Molecular Mechanism of Diseases II. – Room I. - *continued* *Chairperson: István Balogh*		
13:20 – 13:40	**O-025**	**REGULATION OF CELL CYTOTOXICITY BY CYSTEINE CATHEPSINS** Janko Kos[1,2], Milica Perišić Nanut[2], Jerica Sabotič[2], Špela Magister[2], Anahid Jewett[3] *[1]University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia; [2]Jožef Stefan Institute, Department of Biotechnology, Ljubljana, Slovenia; [3]The Jane and Jerry Weintraub Center for Reconstructive Biotechnology, and Division of Oral Biology and Medicine, UCLA School of Dentistry, University of California, Los Angeles, United States*
13:40 – 14:30	**Lunch (Restaurant of Hotel Park)**	

SATURDAY, 28 March 2015		
Parallel session #2 – Developmental Genetics I. – Room II. *Chairperson: József Mihály*		
11:20 – 11:40	**O-026**	**PROTEIN PHOSPHATASE 4 INTERACTS WITH CENP-C AND REGULATES KINETOCHORE INTEGRITY** Zoltan Lipinszki[1], Stephane Lefevre[2], Matthew S. Savoian[1,3], Martin R. Singleton[2], David M. Glover[1], Marcin R. Przewloka[1] *[1]Department of Genetics, University of Cambridge, Downing Street, Cambridge, United Kingdom; [2]Macromolecular Structure and Function Laboratory, Cancer Research UK, London Research Institute, London, United Kingdom; [3]Present address: Institute of Fundamental Sciences, Massey University, Palmerston North, New Zealand*
11:40 – 12:00	**O-027**	***IN SITU* FUNCTIONAL ANALYSIS OF DIFFERENT *CIS*-ACTING ELEMENTS IN THE *BXD* REGULATORY REGION** Viktória Kiss, László Sipos *Institute of Genetics, Biological Research Centre of Hungarian Academy of Sciences, Szeged*
12:00 – 12:20	**O-028**	**DEVELOPMENTAL AUTOPHAGY IS REQUIRED FOR EYE PATTERNING IN *DROSOPHILA*** Viktor A. Billes[1], Tibor Kovács[1], Péter Lőrincz[2], Ágnes Regős[1], Anna Manzéger[1], Miklós Erdélyi[3], József Mihály[3], Miklós Sass[2], Tibor Vellai[1] *[1]Department of Genetics, Eötvös Loránd University, Budapest; [2]Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest; [3]Institute of Genetics, Biological Research Centre, Szeged*
12:20 – 12:40	**O-029**	**ELABORATION AND APPLICATION OF AN *IN VIVO* ASSAY TO DETECT AND QUANTIFY CHROMOSOME LOSS** János Szabad[1], István Soós[2] *[1]University of Szeged, Faculty of Medicine, Department of Biology, Szeged; [2]Szent István University, Faculty of Applied Arts and Pedagogy, Szeged*

SATURDAY, 28 March 2015		
Parallel session #2 – Developmental Genetics I. – Room II. - *continued*		
Chairperson: József Mihály		
12:40 – 13:00	**O-030**	**CHARACTERIZING THE ROLE OF THE NM23 HOMOLOG NDK-1 IN CELL MIGRATION AND APOPTOTIC ENGULFMENT** Zsolt Farkas[1], Luca Fancsalszky[1], Sára Sándor[2], Tamás Orbán[2], Maja Herak Bosnar[3], Tibor Vellai[1], Anil Mehta[4], Krisztina Takács-Vellai[5] *[1]Department of Genetics, Eötvös Loránd University, Budapest; [2]Institute of Enzymology, Research Centre for Natural Sciences, Budapest; [3]Laboratory for Molecular Oncology, Division of Molecular Medicine, Rudjer Bošković Institute, Zagreb, Croatia; [4]Medical Research Institute, Ninewells Hospital Medical School, University of Dundee, Dundee, United Kingdom; [5]Department of Biological Anthropology, Eötvös Loránd University, Budapest*
13:00 – 13:20	**O-031**	**DEVELOPMENTAL PLASTICITY AND REGULATION OF BLOOD CELL DIFFERENTIATION IN *DROSOPHILA MELANOGASTER*** Viktor Honti[1], Gábor Csordás[1], Gergely István Varga[1], Róbert Márkus[1], Ferenc Jankovics[2], Éva Kurucz[1], István Andó[1] *[1]Biological Research Centre of the Hungarian Academy of Sciences, Institute of Genetics, Immunology Unit, Szeged; [2]Biological Research Centre of the Hungarian Academy of Sciences, Institute of Genetics, Developmental Genetics Unit, Szeged*
13:20 – 14:30	**Lunch (Restaurant of Hotel Park)**	

SATURDAY, 28 March 2015		
Parallel session #3 – Membrane, Transport, Trafficking – Room III.		
Chairpersons: László Homolya, László Vígh		
11:20 – 11:40	**O-032**	**MEMBRANE LIPID-THERAPY IN OPERATION: THE MODE OF ACTION OF THE MEMBRANE INTERACTING STRESS PROTEIN CO-MODULATORS** Imre Gombos[1], Tim Crul[1], Gábor Balogh[1], Mária Péter[1], Ákos Hunya[1], Begüm Peksel[1], Bálint Csoboz[1], László Vígh Jr.[1], Stefano Piotto[2], Ibolya Horváth[1], Zsolt Török[1], László Vígh[1] *[1]Laboratory of Molecular Stress Biology, Institute for Biochemistry, Biological Research Centre, Szeged; [2]Department of Pharmaceutical and Biomedical Sciences,University of Salerno, Salerno, Italy*
11:40 – 12:00	**O-033**	**DEPLETION OF ENDOPLASMIC RETICULUM CA2+ PROVOKES IMPORT OF REDUCTANTS FROM THE CYTOSOL** Gergely Kosztyi[1], Beáta Lizák[1], Petra Ágoston[1], Julia Birk[2], Davide Montero[2], Richard Zimmermann[3], Christian Appenzeller-Herzog[2], Gábor Bánhegyi[1] *[1]Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest; [2]Division of Molecular & Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland; [3]Medical Biochemistry & Molecular Biology, Saarland University, Homburg, Germany*

SATURDAY, 28 March 2015		
Parallel session #3 – Membrane, Transport, Trafficking – Room III. - *continued*		
Chairpersons: László Homolya, László Vígh		
12:00 – 12:20	**O-034**	***IN SILICO* AND *IN VITRO* VALIDATION OF ISOFORMS OF ORGANIC ANION TRANSPORTING POLYPEPTIDES** Daniella Kovacsics, Izabel Patik, Csilla Özvegy-Laczka *Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest*
12:20 – 12:40	**O-035**	**DISSECTION OF THE CATALYTIC CYCLE OF PGP USING WALKER A MUTANTS** Gábor Szalóki[1], Orsolya Bársony[1], Dóra Türk[2], Szabolcs Tarapcsák[1], László Csanády[3], Gábor Szabó[1], Gergely Szakács[2], Katalin Goda[1] *[1]Department of Biophysics and Cell Biology, University of Debrecen, Debrecen; [2]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [3]Department of Medical Biochemistry, Semmelweis University, Budapest*
12:40 – 13:00	**O-036**	**ROLE OF THE N-TERMINAL TRANSMEMBRANE DOMAIN IN THE ENDO-LYSOSOMAL TARGETING AND FUNCTION OF THE HUMAN ABCB6 PROTEIN** Gergely Szakács, Katalin Kiss, Nóra Kucsma, Anna Brozik *Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest*
13:00 – 13:20	**O-037**	**THE EFFECT OF LIGAND STIMULATION AND ERBB2 COEXPRESSION ON THE HOMODIMERIZATION OF ERBB3** Peter Nagy[1], Tímea Váradi[1], Mario Brameshuber[2], Gerhard J. Schütz[2], János Szöllősi[1] *[1]Department of Biophysics and Cell Biology, University of Debrecen, Debrecen; [2]Institute of Applied Physics, Vienna University of Technology, Vienna, Austria*
13:20 – 14:30		**Lunch (Restaurant of Hotel Park)**
SATURDAY, 28 March 2015		
Parallel session #1 – Genome Organization and Mobile Genetic Elements – Room I.		
Chairperson: Zoltán Ivics		
14:30 – 14:50	**O-038**	**HOW DO GENES JUMP: INSIGHTS FROM CRYSTAL STRUCTURES AND MORE…** Franka Voigt[1], Lisa Wiedemann[2], Irma Querques[1], Cecilia Zuliani[1], Eike C. Schulz[1], Anna Rubio Cosials[1], Lotte M. Lambertsen[1], Aleksandra Bebel[1], Zoltán Ivics[2], Orsolya Barabás[1] *[1]European Molecular Biology Laboratory, Heidelberg, Germany; [2]Paul Ehrlich Institute, Langen, Germany*
14:50 – 15:10	**O-039**	**A NOVEL TRANSGENIC ORGAN MODEL TO SCREEN FOR THE GENETIC DRIVERS OF TUMORIGENESIS** Andrea Nagy[1], Liza Hudoba[1], Gergely Imre[1], Katalin Hegedűs[1], Ildikó Fekete[1], Thomas Rülicke[2], Lajos Mátés[1] *[1]Institute of Genetics, Biological Research Centre, Szeged; [2]Institute of Laboratory Animal Science, University of Veterinary Medicine, Vienna, Austria*

SATURDAY, 28 March 2015		
Parallel session #1–Genome Organization and Mobile Genetic Elements–Room I.-*continued*		
Chairperson: Zoltán Ivics		
15:10 – 15:30	**O-040**	***HELRAISER*: A RESURRECTED *HELITRON* TRANSPOSON FROM THE BAT GENOME** Ivana Grabundzija[1,2], Simon A. Messing[2], Csaba Miskey[3], Andreas Gogol-Döring[4], Ilija Bilic[1], Vladimir Kapitonov[5], Tanja Diem[3], Anna Dalda[1], Jerzy Jurka[5], Fred Dyda[2], Zsuzsanna Izsvák[1], Zoltan Ivics[3] *[1]Max Delbrück Center for Molecular Medicine, Berlin, Germany; [2]NIH, Bethesda, United States; [3]Division of Medical Biotechnology, Paul Ehrlich Institute, Langen, Germany; [4]German Center of Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Germany; [5]Genetic Information Research Institute, Mountain View, California, United States*
15:30 – 15:50	**O-041**	**THE MECHANISM OF AGEING: PRIMARY ROLE OF TRANSPOSABLE ELEMENTS IN GENOME DISINTEGRATION** Ádám Sturm[1], Bernadette Hotzi[1], Évi Saskői[1], Mónika Kosztelnik[1], Bianka Gordos[1], Anna Tarnóci[1], Zoltán Ivics[2], Tibor Vellai[1] *[1]Department of Genetics, Eötvös Loránd University, Budapest; [2]Paul Ehrlich Institute, Langen, Germany*
15:50 – 16:10	**O-042**	**SEARCH FOR THE PRIMATE-SPECIFIC FUNCTIONS OF SETMAR, A DOMESTICATED TRANSPOSASE-DERIVED PROTEIN IN APES AND HUMANS** Csaba Miskey[1], Andreas Gogol-Döring[2], Marta Swierczek[1], Zsuzsanna Izsvák[1], Donald V. Hucks[3], Cedric Feschotte[3], Zoltán Ivics[1] *[1]Paul Ehrlich Institute, Langen, Germany; [2]Deutsches Zentrum für Integrative Biodiversitätsforschung, Leipzig, Germany; [3]Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT, United States; [4]Max Delbrück Center for Molecular Medicine, Berlin, Germany*
16:10 – 16:30	**O-043**	**EXCISION EFFICIENCY VERSUS TRANSGENIC RATE: *SLEEPING BEAUTY* AND *PIGGYBAC* TRANSPOSITION IN MAMMALIAN STEM CELLS AND EMBRYOS** Orsolya Kolacsek, Zsuzsa Erdei, Ágota Apáti, Sára Sándor, Kornélia Szebényi, András Füredi, Balázs Sarkadi, Tamás I. Orbán *Institute of Enzymology, Research Centre of Natural Sciences, Hungarian Academy of Sciences, Budapest*
16:30 – 18:30	**Poster discussion with coffee break (even poster numbers)**	
19:30 – 20:00	**Concert (Basilica)**	
20:30	**Banquet (Conference hall of Hotel Eger)**	

SATURDAY, 28 March 2015		
Parallel session #2 – Developmental Genetics II.–Room II.		
Chairperson: Tibor Vellai		
14:30 – 14:50	**O-044**	**POLY(ADP-RIBOSYL)ATION AS A CENTRAL REGULATOR OF CELLULAR LIFE AND DEATH DECISION MAKING** László Virág *Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen*
14:50 – 15:10	**O-045**	**DMUSP5 COUPLES UBIQUITIN HOMEOSTASIS TO DEVELOPMENT AND APOPTOSIS IN DROSOPHILA** Levente Kovács[1,2], Olga Nagy[2], Margit Pál[1,2], Octavian Popescu[3], Andor Udvardy[2], Péter Deák[1,2] *[1]Department of Genetics, Faculty of Sciences, University of Szeged, Szeged; [2]Institute of Biochemistry, Biological Research Center of the Hungarian Academy of Sciences, Szeged; [3]Interdisciplinary Institute of Bio-Nano-Sciences, Molecular Biology Center, Cluj-Napoca, Romania*
15:10 – 15:30	**O-046**	**LOSS OF THE AUTOPHAGY GENE ATG16 PROMOTES ALCOHOL TOLERANCE IN DROSOPHILA** Kata Varga, Péter Nagy, Katarina Arsikin, Attila L. Kovács, Gábor Juhász *Eötvös Loránd University, Department of Anatomy, Cell and Developmental Biology, Budapest*
15:30 – 15:50	**O-047**	**THE ANALYSIS OF PLANT GENES CONTROLLING BACTEROID DIFFERENTIATION IN NITROGEN-FIXING NODULES OF *MEDICAGO TRUNCATULA*** Péter Kaló[1], Beatrix Horváth[1], Ágota Domonkos[1], Anikó Gombár[1], Gyöngyi Kováts[1], Attila Kereszt[2], Attila Szűcs[2], Edit Ábrahám[2], Ferhan Ayaydin[3], Károly Bóka[4], Éva Kondorosi[2] *[1]NARIC, Agricultural Biotechnology Institute, Gödöllő; [2]Institute of Biochemistry, Biological Research Center, Szeged; [3]Cellular Imaging Laboratory, Biological Research Center, Szeged; [4]Department of Plant Anatomy, Eötvös Loránd University, Budapest*
15:50 – 16:10	**O-048**	**UNRAVELING THE FUNCTIONS OF CYTOSKELETAL PROTEINS IN THE NUCLEUS** Péter Vilmos, Ildikó Kristó, Umesh Kumar Gautam, Csaba Bajusz *Biological Research Center of the HAS, Szeged*
16:10 – 16:30	**O-049**	**INTEGRATION OF STRESS, ABA AND LIGHT SIGNALING PATHWAYS IN PLANTS** László Szabados, Mary Prathiba Joseph, Bogáta Boros, Ákos Hornung, Csaba Papdi, László Kozma-Bognár *Institute of Plant Biology, Biological Research Centre, Szeged*
16:30 – 18:30	**Poster discussion with coffee break (even poster numbers)**	
19:30 – 20:00	**Concert (Basilica)**	
20:30	**Banquet (Conference hall of Hotel Eger)**	

SATURDAY, 28 March 2015		
Parallel session #3 – Signaling, Cell-cell Communication – Room III. *Chairperson: Zsuzsa Szondy*		
14:30 – 14:50	**O-050**	**PROTEIN KINASES: ENZYMES WORKING IN SIGNALING BRIGADES** Attila Reményi *MTA Lendület Protein Interaction Group, Institute of Enzymology, Research Center for Natural Sciences, Hungarian Academy of Sciences, Budapest*
14:50 – 15:10	**O-051**	**NOVEL INTERACTIONS AND REGULATORY ROLES OF PROTEIN PHOSPHATASES IN ENDOTHELIAL CELLS** Róbert Bátori, Bálint Bécsi, Beáta Lontay, Ferenc Erdődi *Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen*
15:10 – 15:30	**O-052**	**TNFα REVERSE SIGNALING INHIBITS THE LPS-INDUCED PRO-INFLAMMATORY CYTOKINE FORMATION BY UPREGULATING TGF-β IN MACROPHAGES** Zsuzsa Szondy, Anna Pallai *Department of Biochemistry and Molecular Biology, Research Center of Molecular Medicine, University of Debrecen, Debrecen*
15:30 – 15:50	**O-053**	**THE ROLE OF HEAT SHOCK TRANSCRIPTION FACTOR HSF-1 IN THE COORDINATION OF CELLULAR STRESS RESPONSES IN *CAENORHABDITIS ELEGANS*** János Barna[1], Mónika Kosztelnik[1], Ákos Ipcsics[1], Péter Srajner[2], Beatrix Kovács[1], Petra Körmendi[1], Dániel Kovács[1], Ibolya Stiller[2], Katalin Lengyel[1], Bernadette Hotzi[1], Tímea Sigmond[1], Gábor Bánhegyi[2], Tibor Vellai[1] *[1]Department of Genetics, Eötvös Lorand University, Budapest; [2]Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest*
15:50 – 16:10	**O-054**	**OVEREXPRESSION OF A HETEROLOGOUS POLYAMINE OXIDASE, INVOLVED IN POLYAMINE CATABOLISM, ACTIVATES BOTH NECROTIC AND VACUOLAR PROGRAMMED CELL DEATH IN TOBACCO** Szabolcs Tóth, László Kovács, Ákos Mendel, Anna Szentgyörgyi, Erzsébet Kiss, Ottó Toldi *Szent István University, Institute of Genetics and Biotechnology, Gödöllő*
16:10 – 16:30	**O-055**	**POTENTIAL ROLE OF SINGLET OXYGEN IN INTRA- AND INTER-CELLULAR SIGNALING EVENTS IN MICROALGAE** Imre Vass[1], Ateeq ur Rehman[1], Péter B. Kós[1], Zsuzsanna Deák[1], Ferhan Ayaydin[2], Csaba Tömböly[3], István Z. Vass[1] *[1]Institute of Plant Biology, Biological Research Centre, HAS, Szeged; [2]Cellular Imaging Laboratory, Biological Research Centre, HAS, Szeged [3]Institute of Biochemistry, Biological Research Centre, HAS, Szeged*
16:30 – 18:30	**Poster discussion with coffee break (even poster numbers)**	
19:30 – 20:00	**Concert (Basilica)**	
20:30	**Banquet (Conference hall of Hotel Eger)**	

SUNDAY, 29 March 2015		
Parallel session #1 – Regulation of Gene Expression, Epigenetics – Room I. *Chairpersons: Imre Boros, Lóránt Székvölgyi*		
9:00 – 9:20	**O-056**	**INFECTION OF HUMAN CELLS WITH LENTIVIRAL VECTOR LEADS TO HIGHLY REPRODUCIBLE GENOME-WIDE DNA METHYLATION CHANGES** Tamás Arányi[1,2,4], Daniel Stockholm[1,2,3], Roseline Yao[1,2], Thibaut Wiart[2], Yoshiaki Yamagata[1,2], Anne Galy[1,2], András Paldi[1,2,3] *[1]UMR951, Evry, France; [2]Genethon, Evry, France; [3]EPHE, Evry, France; [4]UEVE, Evry, France*
9:20 – 9:40	**O-057**	**SOME POLYCOMB-GROUP PROTEINS CAN MODULATE THE REGULATORY CAPACITY OF ACTIVE CIS-REGULATORS** Izabella Bajusz[1], Henrik Gyurkovics[1], József Mihály[1], Fabiene Cléard-Karch[2], Francois Karch[2], László Sipos[1] *[1]Institute of Genetics, Biological Research Center, Szeged; [2]Department of Genetics and Evolution, University of Geneva, Switzerland*
9:40 – 10:00	**O-058**	**COMPLEX AUTOREGULATION OF THE KEY TRANSLATION TERMINATION FACTOR: EUKARYOTIC RELEASE FACTOR 1 IN PLANTS** Andor Auber, Tünde Nyikó, Levente Szabadkai, Dániel Silhavy Plant RNA Biology Group, Agricultural Biotechnology Institue, NARIC, Gödöllő
10:00 – 10:20	**O-059**	**THE ROLE OF P53 DURING TRANSCRIPTIONAL BLOCKAGE** Barbara Nikolett Borsos[1], Ildikó Huliák[1], Zsuzsanna Újfaludi[1], Tibor Pankotai[1], Imre Miklós Boros[1,2] *[1]Department of Biochemistry and Molecular Biology, University of Szeged, Szeged; [2]Institute of Biochemistry, Biological Research Center, Szeged*
10:20 – 10:40	**O-060**	**THE CCR4-NOT COMPLEX CONNECTS THE DIFFERENT LEVELS OF GENE EXPRESSION** Zoltán Villányi[1], Ishaan Gupta[2], Christopher Hughes[2], Olesya O. Panasenko[1], Lars M. Steinmetz[2,3,4], Martine A. Collart[1] *[1]Department of Microbiology and Molecular Medicine, University of Geneva, Faculty of Medicine, Geneva, Switzerland; [2]European Molecular Biology Laboratory (EMBL), Genome Biology Unit, Heidelberg, Germany; [3]Stanford Genome Technology Center, Stanford University, Palo Alto, CA, United States; [4]Department of Genetics, Stanford University School of Medicine, Stanford, CA, United States*
10:40 – 11:00	**O-061**	**THE EFFECT OF HISTONE MODIFICATIONS ON NUCLEOSOME STABILITY AND THE BENEFIT OF PAN-NICKING** László Imre[1], Zoltán Simándi[2], Attila Horváth[2], László Halász[1], György Fenyőfalvi[1], Péter Nánási[1], András Szántó[1], Hiroshi Kimura[3], László Nagy[2], Lóránt Székvölgyi[1], Gábor Szabó[1] *[1]Department of Biophysics and Cell Biology, University of Debrecen, Debrecen; [2]Department of Biochemistry and Molecular Biology, University of Debrecen; [3]Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan*
11:00 – 11:20	**Coffee break**	

SUNDAY, 29 March 2015

Parallel session #2 – Systems Biology – Room II.

Chairperson: Péter Csermely

9:00 – 9:20 **O-062** **GENERAL PRINCIPLES OF TRANSCRIPTOMIC CHANGES FOLLOWING SINGLE-GENE DELETION IN *SACCHAROMYCES CEREVISIAE***

Károly Kovács[1], Zoltán Farkas[1], Dorottya Kalapis[1], Patrick Kemmeren[2], Frank C. P. Holstege[2], Richard A. Notebaart[3], Csaba Pál[1], Balázs Papp[1]

[1]Biological Research Centre, Hungarian Academy of Sciences, Szeged; [2]Molecular Cancer Research, University Medical Center Utrecht, Utrecht, The Netherlands; [3]Radboud University, Nijmegen, The Netherlands

9:20 – 9:40 **O-063** **THE EVOLUTIONARY POTENTIAL OF UNDERGROUND METABOLISM**

Richard A. Notebaart[1], Balázs Szappanos[2], Bálint Kintses[2], Ferenc Pál[2], Ádám Györkei[2], Balázs Bogos[2], Viktória Lázár[2], Réka Spohn[2], Bálint Csörgő[2], Csaba Pál[2], Balázs Papp[2]

[1]Radboud Institute for Molecular Life Sciences, Centre for Bioinformatics (CMBI) and Systems Biology (CSBB), Radboud University Medical Centre, Nijmegen, The Netherlands; [2]Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged

9:40 – 10:00 **O-064** **A COMPREHENSIVE SYSTEMS BIOLOGICAL STUDY OF AUTOPHAGY-APOPTOSIS CROSSTALK DURING ENDOPLASMIC RETICULUM STRESS**

Orsolya Kapuy[1], Marianna Holczer[1], Margita Márton[1], Anita Kurucz[1], P.K. Vinod[2], Gábor Bánhegyi[1]

[1]Semmelweis University, Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Budapest; [2]University of Oxford, Department of Biochemistry, Oxford, United Kingdom

10:00 – 10:20 **O-065** **INTRATUMOR HETEROGENEITY IN RENAL CELL CARCINOMA PATIENTS**

Lőrinc Sándor Pongor[1], Zsófia Sztupinszki[2], Balázs Győrffy[3]

[1]2nd Department of Paediatrics, Semmelweis University, Budapest; [2]1st Department of Paediatrics, Semmelweis University, Budapest; [3]MTA TTK Lendület Cancer Biomarker Research Group, Budapest

10:20 – 10:40 **O-066** **DISEASES IN THE HUMAN INTERACTOME**

Jörg Menche[1,2,3], Amitabh Sharma[1,2], Maksim Kitsak[1,2], Susan Dina Ghiassian[1,2], Marc Vidal[2,4], Joseph Loscalzo[5], Albert-László Barabási[1,2,3,5]

[1]Center for Complex Networks Research and Department of Physics, Northeastern University, Boston, United States; [2]Center for Cancer Systems Biology (CCSB) and Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, United States; [3]Center for Network Science, Central European University, Budapest; [4]Department of Genetics, Harvard Medical School, Boston, United States; [5]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, United States

10:40 – 11:00 **O-067** **COMPPI: A CELLULAR COMPARTMENT-SPECIFIC DATABASE FOR PROTEIN–PROTEIN INTERACTION NETWORK ANALYSIS**

Daniel V. Veres, Dávid M. Gyurkó, Peter Csermely

Department of Medical Chemistry, Semmelweis University, Budapest

11:00 – 11:20 **Coffee break**

SUNDAY, 29 March 2015		
Parallel session #1 – Stem Cells – Room I.		
Chairpersons: Éva Rajnavölgyi, István Szatmári		
11:20 – 11:40	**O-068**	**EPIGENETIC REGULATION OF RETINOIC ACID DEPENDENT EMBRYONIC STEM CELL DIFFERENTIATION** Zoltán Simándi[1], Attila Horváth[1], Ixchelt Cuaranta-Monroy[1], Lászlo Nagy[1,2,3] *[1]Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen; [2]MTA-DE "Lendulet" Immunogenomics Research Group, University of Debrecen, Debrecen; [3]Sanford-Burnham Medical Research Institute at Lake Nona, Orlando, FL, USA*
11:40 – 12:00	**O-069**	**RYBP AS A NEW PLAYER IN DIFFERENTIATION OF STEM CELLS** Melinda K. Pirity *Biological Research Centre, Hungarian Academy of Sciences, Szeged*
12:00 – 12:20	**O-070**	**DIFFERENTIATION AND ONTOGENY OF CD45+ STELLATE CELLS IN CHICKEN EMBRYO** Dávid Dóra, Nándor Nagy *Semmelweis University, Faculty of Medicine, Department of Human Morphology and Developmental Biology, Budapest*
12:20 – 12:40	**O-071**	**IDENTIFICATION OF A NOVEL STEM CELL NICHE FACTOR IN DROSOPHILA** Ferenc Jankovics, Brigitta Kiss, Melinda Bence, Miklós Erdélyi *Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged*
12:40 – 13:00	**O-072**	**LICENSING BY INFLAMMATORY CYTOKINES ABOLISHES HETEROGENEITY OF IMMUNOSUPPRESSIVE FUNCTION OF MESENCHYMAL STEM CELL POPULATION** Enikő Szabó[1], Roberta Fajka-Boja[1], Éva Kriston-Pál[1], Ákos Hornung[1], Ildikó Makra[1], Gyöngyi Kudlik[2], Ferenc Uher[3], Róbert László Katona[1], Éva Monostori[1], Ágnes Czibula[1] *[1]Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged; [2]Institute of Enzimology, Budapest; [3]Stem Cell Biology Unit, National Blood Service, Budapest*
13:00 – 13:20	**O-073**	**PATIENT SPECIFIC INDUCED PLURIPOTENT STEM CELLS (IPSCS) AND THEIR NEURONAL DIFFERENTIATION IN ALZHEIMER'S DISEASE** Julianna Kobolák[1], Abinaya Chandrasekaran[1,2], Anna Ochalek[1,2], Tamás Bellák[1], Viktor Szegedi[1], Eszter Varga[1], Hasan Avci[1], Shuling Zhou[1,3], Karolina Szczesna[1], Béla Smidth[1], András Dinnyés[1,2,4] *[1]BioTalentum Ltd., Gödöllő; [2]Molecular Animal Biotechnology Laboratory, Szent István University, Gödöllő; [3]Institute for Veterinary Science, University of Copenhagen, Denmark; [4]Departments of Equine Sciences and Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, The Netherlands*
13:20– 13:40	**Closing and award ceremony**	
13:40	**Lunch (Restaurant of Hotel Park)**	

SUNDAY, 29 March 2015		
Parallel session #2 – Microbial Genetics – Room II.		
Chairpersons: Ferenc Olasz, György Pósfai		
11:20 – 11:40	**O-074**	**THE RESULTS OF WHOLE-GENOME ANALYSES (WGA) FORCE CHANGES IN PROKARYOTE SYSTEMATICS AND RESULT NEW CONCEPTS IN ECOLOGY** Károly Márialigeti[1], Tamás Felföldi[1], András Táncsics[2] *[1]Department of Microbiology, Faculty of Science, Eötvös Loránd University, Budapest; [2]Regional University Center of Excellence in Environmental Industry, Szent István University, Gödöllő*
11:40 – 12:00	**O-075**	**GENOME INTEGRITY AND PATHOGENICITY ISLAND TRANSFER IN STAPHYLOCOCCUS** Judit E. Szabó[1,2], Dóra Szabó, Orsolya Dobay, Beáta G. Vértessy[1,2] *[1]Department of Applied Biotechnology, BME, Budapest; [2]Institute for Enzymology, RCNS, Hungarian Academy of Sciences, Budapest; [3]Institute for Medical Microbiology, Semmelweis University, Budapest*
12:00 – 12:20	**O-076**	**DEVELOPMENT OF A HIGHLY PRECISE, PORTABLE GENOME ENGINEERING METHOD** Bálint Csörgő, Ákos Nyerges, István Nagy, György Pósfai, Csaba Pál *Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged*
12:20 – 12:40	**O-077**	**RETICULATE EVOLUTION IN A GROUP OF YEAST SPECIES** Mátyás Sipiczki, P.W. Pfliegler, Enikő Horváth *Department of Genetics and Applied Microbiology, University of Debrecen, Debrecen*
12:40 – 13:00	**O-078**	**ENHANCING RECOMBINANT PROTEIN SOLUBILITY WITH A SAMP FUSION PARTNER** Sándor Varga[1], Ganesh Pathare Ramnath[2], Erzsébet Baka[1], Marius Boicu[2], Balázs Kriszt[3], András Székács[1], József Kukolya[1], István Nagy[2] *[1]National Agricultural Research and Innovation Centre Research, Institute of Agro-Environmental Sciences, Department of Applied and Environmental Microbiology, Budapest; [2]Max Planck Institute of Biochemistry, Department of Molecular Structural Biology, Martinsried (Planegg), Germany; [3]Szent István University, Institute of Aquaculture and Environmental Safety, Department of Environmental Safety and Ecotoxicology, Gödöllő*
13:00 – 13:20	**O-079**	**ENGINEERED RIBOSOMAL RNA OPERON COPY-NUMBER VARIANTS OF *E. COLI* REVEAL THE EVOLUTIONARY TRADE-OFFS SHAPING RRNA OPERON NUMBER** Zsuzsanna Győrfy[1], Gábor Draskovits[1], Viktor Vernyik[1], Frederick F. Blattner[2], Tamás Gaál[3], György Pósfai[1] *[1]Institute of Biochemistry, Synthetic and Systems Biology Unit, Biological Research Centre of the Hungarian Academy of Sciences, Szeged; [2]Scarab Genomics LLC, Madison, WI, United States [3]Departmen of Bacteriology, University of Wisconsin-Madison, Madison, WI, United States*
13:20– 13:40	**Closing and award ceremony**	
13:40	**Lunch (Restaurant of Hotel Park)**	

SUNDAY, 29 March 2015

Parallel session #3 – DNA Repair and Carcinogenezis – Room III.

Chairpersons: Lajos Haracska, Dávid Szüts

11:20 – 11:40 **O-080** **BRCA1 PROMOTES ERROR-FREE BYPASS OF REPLICATION STALLING DNA LESIONS**

Bernadett Szikriszt[1], Orsolya Pipek[2], János Molnár[1], Judit Zsuzsanna Gervai[1], Gábor E. Tusnády[1], Charles Swanton[3], Zoltán Szállási[4], István Csabai[2], Dávid Szüts[1]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2]Department of Complex Physics, Eötvös Loránd University, Budapest; [3]Cancer Research UK London Research Institute, London, United Kingdom; [4]Department of Systems Biology, The Technical University of Denmark, Lyngby, Denmark

11:40 – 12:00 **O-081** **POLYMERASE EXCHANGE MEDIATED TRANSLESION RNA SYNTHESIS**

Vamsi K. Gali, Éva Bálint, Ildikó Unk

DNA Repair Group, Institute of Genetics, Biological Research Centre of the Hungarian Academy of Sciences, Szeged

12:00 – 12:20 **O-082** **A GENOME-WIDE APPROACH TO LINK GENOTYPE TO CLINICAL OUTCOME BY UTILIZING NEXT GENERATION SEQUENCING AND GENE CHIP DATA OF BREAST CANCER PATIENTS**

Balázs Győrffy[1], Lőrinc Pongor[1], Máté Kormos[1], Christos Hatzis[2], Lajos Pusztai[2]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2]Yale Comprehensive Cancer Center, Yale School of Medicine, New Haven, CT, United States

12:20 – 12:40 **O-083** **DETECTION OF URACIL WITHIN DNA USING A SENSITIVE ANTIBODY-LIKE SENSOR FOR *IN VITRO* AND *IN VIVO* APPLICATIONS**

Gergely Róna[1,2], Ildikó Scheer[1,2], Kinga Nagy[1,2], Hajnalka L. Pálinkás[1,3], Gergely Tihanyi[1,2], Máté Borsos[1], Soma Zsótér[1], Angéla Békési[1], Judit Eszter Szabó[1,2], Dobay Orsolya[4], Szabó Dóra[4], Beáta G. Vértessy[1,2]

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12:40 – 13:00 **O-084** **RECOMBINATION ASSOCIATED DNA SYNTHESIS IN HUMAN CELLS**

Péter Burkovics[1,2], Lili Döme[1], Szilvia Juhász[1], Marek Sebesta[2], Lumir Krejci[2,3,4], Lajos Haracska[1]

[1]Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged; [2]Department of Biology, Masaryk University, Brno, Czech Republic; [3]National Centre for Biomolecular Research, Masaryk University, Brno, Czech Republic; [4]International Clinical Research Center, Center for Biomolecular and Cellular Engineering, St. Anne's University Hospital Brno, Brno, Czech Republic

SUNDAY, 29 March 2015		
Parallel session #3 – DNA Repair and Carcinogenezis – Room III. - *continued* *Chairpersons: Lajos Haracska, Dávid Szüts*		
13:00 – 13:20	**O-085**	**QUANTIFYING DNA REPAIR PATHWAY ABERRATIONS IN TUMOR BIOPSIES IN ORDER TO DETERMINE RESPONSE TO CHEMOTHERAPY** Zoltán Szállási[1,2] *[1]Department of Systems Biology, The Technical University of Denmark, Lyngby, Denmark; [2]Children's Hospital Informatics Program at the Harvard-Massachusetts Institutes of Technology Division of Health Sciences and Technology (CHIP@HST), Harvard Medical School, Boston*
13:20– 13:40	**Closing and award ceremony**	
13:40	**Lunch (Restaurant of Hotel Park)**	

ABSTRACTS - PLENARY LECTURES

PL-01
THE ROUTES OF REPROGRAMMING TO ALTERNATIVE STATES OF PLURIPOTENCY

Andras Nagy

Mount Sinai Hospital, Lunenfeld-Tanenbaum Research Institute, Toronto, Canada

The ability to reprogram somatic cells to a pluripotent state is paradigm shifting for both biology and medical research. Reprogramming continues to challenge many of our assumptions about the specification of cellular phenotypes and yet, despite major efforts, we still lack a complete molecular characterization of the process. To address this gap we generated a comprehensive molecular description of the reprogramming cascade toward two distinct pluripotent states. We explored alternative outcomes of somatic reprogramming by fully characterizing reprogrammed cells independent of preconceived definitions of reprogrammed iPSC states. We demonstrate that manipulating the expression level of the reprogramming factor influences cells arrival to a non-ES cell-like or ES cell like pluripotent state. This bifurcated process has been characterized with multiple "omic" platforms, consisting of the transcriptome (microRNA, lncRNA and mRNA), CpG methylation, ChIP-sequencing (for chromatin marks: H3K4me3, H3K27me3 and H3K36me3), in addition to quantitative mass spectrometry profiling of the global and cell surface proteome. This dataset enables cross-referencing between "omic" platforms, which facilitates deeper understanding of the cascade of molecular events driving the generation of pluripotent cells.

PL-02
UNDERSTANDING ESTROGEN RECEPTOR TRANSCRIPTION IN BREAST CANCER

Jason S. Carroll

Cancer Research UK, University of Cambridge, Cambridge, United Kingdom

Estrogen Receptor (ER) is the defining feature of luminal breast cancers, where is functions as a transcription factor. The discovery of ER-DNA interaction regions from ER+ breast cancer cell lines has revealed that ER rarely associates with promoter regions of target genes and instead associates with enhancer elements significant distances from the target genes. The genomic mapping of ER binding events also revealed the enrichment of DNA motifs for Forkhead factors. The Forkhead protein FOXA1 (HNF3a) was subsequently shown to bind to ~half of the ER binding events in the genome and was required for ER to maintain interaction with DNA. We have extended on these findings to explore ER and FoxA1 functional interactions in breast cancer, with a specific focus on changes in binding dynamics that occur during endocrine resistance. We have utilized ChIP-seq in primary tumor material, coupled with functional analysis, to identify mechanisms that govern FoxA1-ER chromatin interactions and the variables that alter binding capacity. In addition, we have recently established a method for rapid unbiased discovery of protein interacting complexes, which we have applied to discover ER and FoxA1 associated proteins. We find an unexpected interaction between ER and progesterone receptor in ER+ breast cancer. We show that PR is a negative regulator

of the ER complex, where it is important for modulating cellular growth. These findings help delineate the complexes that influence ER transcriptional activity and ultimately impinge on tumor progression and drug sensitivity.

PL-03
DEF1 PROMOTES THE DEGRADATION OF POL3 FOR POLYMERASE EXCHANGE TO OCCUR DURING DNA-DAMAGE–INDUCED MUTAGENESIS IN SACCHAROMYCES CEREVISIAE

Andreea Daraba, Vamsi Krishna Gali, Miklós Halmai, Lajos Haracska, Ildikó Unk

The Institute of Genetics, Biological Research Centre,
Hungarian Academy of Sciences, Szeged, Hungary

The genome is constantly under attack from endogenous and exogenous genotoxic agents, and also possesses an inherent level of instability. DNA damage can promote genome instability and directly lead to various human diseases, particularly cancer, neurological abnormalities, immunodeficiency and premature aging. To avoid such deleterious outcomes, cells have evolved an array of DNA repair pathways, which carry out what is typically a multiple-step process to resolve specific DNA lesions and maintain genome integrity.

However if DNA damage is unrepaired prior to the onset of the S phase of the cell cycle the consequence is stalling of the replication machinery, which poses a challenging problem for the cells. Stalled replication forks can undergo DNA breakage and recombination that can lead to chromosomal rearrangements and cell death. To ensure survival, cells have evolved different mechanisms that can sustain DNA replication on damaged templates. These so-called DNA damage tolerance or DNA damage bypass processes allow replication to continue on damaged DNA without actually removing the damage. DNA damage tolerance is achieved through two main mechanisms: template switching and translesion synthesis (TLS). Template switching is inherently error-free, as replication continues by using the undamaged nascent sister chromatid as a template for the bypass of the lesion, whereas during TLS, specialized polymerases take over the nascent primer end from the replicative polymerase and carry out synthesis opposite the DNA lesion in an error-free or error-prone way. By generating mutations in the genome, these polymerases are the drivers of carcinogenesis in humans. TLS polymerases synthesize DNA with a high error rate and are responsible for damage-induced mutagenesis, so their activity must be strictly regulated.

Polymerase exchange is a key, although poorly understood step in translesion synthesis (TLS). During this process, the stalled replicative polymerase has to hand over the primer end to a TLS polymerase that can continue synthesis by inserting a nucleotide opposite the lesion and/or extending from it.

Using *Saccharomyces cerevisiae* as a model system, we have identified Def1as a new factor essential for DNA damage induced mutagenesis. We show that Def1 promotes the ubiquitylation and subsequent proteasomal degradation of of the replicative polymerase after DNA damage treatment. We demonstrate that the noncatalytic subunits of the replicative polymerase are not affected by UV-induced degradation and that they can form a complex with the TLS polymerase Rev1. Based on our results we propose a new model for polymerase exchange at stalled replication forks. During

replication, when the replicative polymerase stalls at a DNA lesion, the catalytic subunit is ubiquitylated by a Def1-dependent manner and removed from the stalled replicative polymerase complex through proteasomal degradation. We hypothesize that a TLS polymerase takes over the place of catalytic domain and teams up with the remaining noncatalytic subunits of the replicative polymerase, at the stalled fork to form a new complex capable of executing DNA lesion bypass.

PL-04
REVEALING NOVEL MOLECULAR ELEMENTS OF CELL DEATH PARADIGMS. THE NEUTROPHIL EXTRACELLULAR TRAP

László Fésüs

Department of Biochemistry and Molecular Biology, DE-MTA Stem Cells, Apoptosis and Genomics Research Group, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

In parallel to describing molecular details of apoptosis the cell death research community has revealed novel forms of regulated cell death, such as necroptosis (mediated by rip1 and -rip3 kinase), autophagic death (initiated by autophagy regulators), parthanatos (over-activation of PARP) and pyroptosis (induced by inflammasome activated capspase-1). The release of chromatin and the formation of a neutrophil extracellular trap (NET) is the result of a unique form of regulated neutrophil cell death, called "NETosis". The NET is a highly ordered structure of DNA and selected proteins capable to eliminate pathogenic microorganisms. Biochemical determinants of the non-randomly formed stable NETs have not been revealed so far. Studying the formation of human NETs it was observed by immunohistochemistry and biochemical techniques that polyamines were incorporated into the NET. Inhibition of myeloperoxidase, which is essential for NET formation and can generate reactive chlorinated polyamines through generating hypochloric acid, decreased polyamine incorporation. Proteomic analysis of the highly reproducible pattern of NET components revealed cross-linking of net proteins through chlorinated polyamines. Presence of transglutaminase-formed ε(γ-glutamyl)lysine and bis-γ-glutamyl polyamine bonds could be also observed among NET components presumably catalysed by the detectable transglutaminase 1 enzyme. Competitive inhibition of intracellular protein crosslinking by externally added monoamines led to less incorporated polyamines into the NET and disturbance of the crosslinking pattern of NET proteins. This resulted in the loss of the ordered structure of the NET and significantly reduced capacity to trap bacteria. These findings provide explanation how the organized structures of DNA-protein complexes in NETs are formed in a reproducible and ordered manner to efficiently neutralize microorganisms at the first defence line of the innate immune system.

PL-05
ROLE AND MECHANISMS OF AUTOPHAGY IN DROSOPHILA

Gábor Juhász

Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest, Hungary, and Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

During the catabolic process of autophagy, cytoplasmic material is transported to the lysosome for degradation and recycling. This way, autophagy contributes to the homeodynamic turnover of proteins, lipids, nucleic acids, glycogen, and even whole organelles. Autophagic activity is increased by adverse conditions such as nutrient limitation, growth factor withdrawal and oxidative stress, and it generally protects cells and organisms to promote their survival. Misregulation of autophagy is likely involved in numerous human diseases including aging, cancer, infections and neurodegeneration, so its biomedical relevance explains the still growing interest in this field. Studies on model organisms, including the fruit fly Drosophila, have contributed significantly to our understanding of the physiological and pathological roles of this process, and the molecular mechanisms of autophagosome formation and fusion with lysosomes. At the meeting, I will discuss established models and some of our recent advances in this field.

PL-06
MEMBRANES AND LIPIDS IN THE CELLULAR HEAT STRESS MANAGEMENT

László Vígh, Gábor Balogh, Zsolt Török, Imre Gombos, Mária Péter,Tim Crul, Begum Peksel, Bálint Csoboz, Attila Glatz, Ibolya Horváth

Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Important diseases are known to be associated with abnormal stress protein (HSP) levels and characteristic membrane defects. We investigate the interconnection between changes of lipidome, fluidity- and microdomain organization of plasma membranes and the simultaneously altered expression and cellular distribution of HSP molecular chaperones, in mammalian cells. A comparative lipidomics study explored key lipid molecular species with the potential to activate specific HSP signaling pathways, to initiate the membrane association of specific HSP subclasses or, strongly affecting lipid droplet (LD) biogenesis. Fever-like heat stress or heat-analogous mild membrane stress promoted TG synthesis both in mammalian cells and in the "fission yeast" (*S.pombe*), a model organism in molecular and cell biology. Our data first emphasize the pivotal role of nutrient supply (in this case for PUFAs) in modifying responses to stress and highlight the need for the careful control of culture conditions when assessing cellular responses *in vitro*. A subpopulation of HSPs is membrane associated: via their specific lipid interactions HSPs can control major attributes of the membranes like fluidity or curvature and hence, generation of stress signals in a feed-back loop.

The mode of action of various drug candidates, capable to refine HSP profile (inducers, silencers) by targeting specific membrane microdomains, - with considerable therapeutic benefit -, will also be discussed. Since a dysregulated stress protein response is found in a large number of important diseases our observations offer new opportunities for clinical interventions.

References:

- *Brameshuber et al, J.Biol. Chem. 2010; Balogh et al, BBA, 2010;*
- *Horvath and Vigh, Nature, 2010; Haldimann et al, J.Biol.Chem, 2011;*
- *Balogh et al, PLoS ONE, 2011; Gombos et al, PloS ONE, 2011;*
- *Peter et al, Mol. Membr. Biol,, 2012; Crul et al, Curr. Pharm. Des., 2012;*
- *Horvath et al., Progr. Lipid. Res., 2012; Balogh et al, FEBS L 2013;*
- *Gungor et al, PLoS ONE, 2014; Törok et al, BBA, 2014*

PL-07

INDEPENDENT PARALLEL FUNCTIONS OF P19 PLANT VIRAL SUPPRESSOR OF RNA SILENCING REQUIRED FOR EFFECTIVE SUPPRESSOR ACTIVITY

Éva Várallyay[1], Enikő Oláh[1], Zoltán Havelda[2]

[1] *Diagnostic Group, Agricultural Biotechnology Research Institute, NARIC, Gödöllő, Hungary*
[2] *Plant Developmental Biology Group, Agricultural Biotechnology Research Institute, NARIC, Gödöllő, Hungary*

Virus infection in plants induces host defence reactions such as activation of RNA interference, a sequence specific RNA degradation mechanism, mediated by small interfering RNAs (siRNAs). RNAi is induced by the double stranded viral RNA. SiRNAs are produced by DICER enzymes and loaded into the RNA induced silencing complex (RISC). Loaded RISC will recognize all RNA species which have sequence complementary to the loaded siRNA and block its activity by cleavage or by translational inhibition. To counteract RNA silencing viruses express viral suppressor proteins of RNA silencing (VSR) which are able to disarm the virus infection induced host defence. VSRs interfere also with the microRNA (miRNA) pathway, which is an important gene regulatory mechanism involved in developmental processes. Argonaute 1 (AGO1) is the key regulator of the miRNA pathway. AGO1 homeostasis is controlled by the action of miR168 as a complex refined feedback regulatory loop. In our previous work we have shown that in virus infected plants the induction of AGO1 mRNA expression is a potential host defence mechanism, while the virus mediated induction of miR168 is a viral counter defence strategy **[1]**. Using a transient expression system and virus infection studies it was revealed that the p19 VSR of Tombusviruses is mainly responsible for the induction of miR168 **[1]**. We showed that several viruses mediate the control of AGO1 accumulation in different test plants and induce the transcriptional activity of the MIR168a gene. Using a transient protein expression assay, we proved that VSRs of different viruses are responsible for the induced accumulation of miR168, which is associated with the control of AGO1 level **[2]**. In this paper **[3]** we used a mutant p19 VSR (p19-3M) disabled in its main suppressor function, siRNA binding, to investigate the biological role of VSR mediated miR168 induction. Infection with the mutant virus carrying p19-3M VSR resulted in suppressed recovery phenotype in spite of the presence of free virus specific siRNAs. Analysis of the infected plants revealed that the mutant p19-3M VSR is able to induce miR168 level controlling the accumulation of the antiviral

AGO1 and this activity is associated with the enhanced accumulation of viral RNAs. Our data indicate that p19 VSR possesses two independent silencing suppressor functions, viral siRNA binding and the miR168 mediated AGO1 control, both of which are required to efficiently cope with the RNA silencing based host defence. This finding suggests that p19 VSR protein evolved independent parallel capacities to block the host defence at multiple levels.

Our work was supported by OTKA K78351 and PD78049.

Keywords: *plant virus, RNAi, AGO1 regulation*

References:

[1] *Varallyay E, Valoczi A, Agyi A, Burgyan J, Havelda Z (2010) Embo J 29:3507-3519;*
[2] *Varallyay E, Havelda Z (2013) Mol Plant Pathol 14:567-575;*
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ABSTRACTS - COMPANY LECTURES

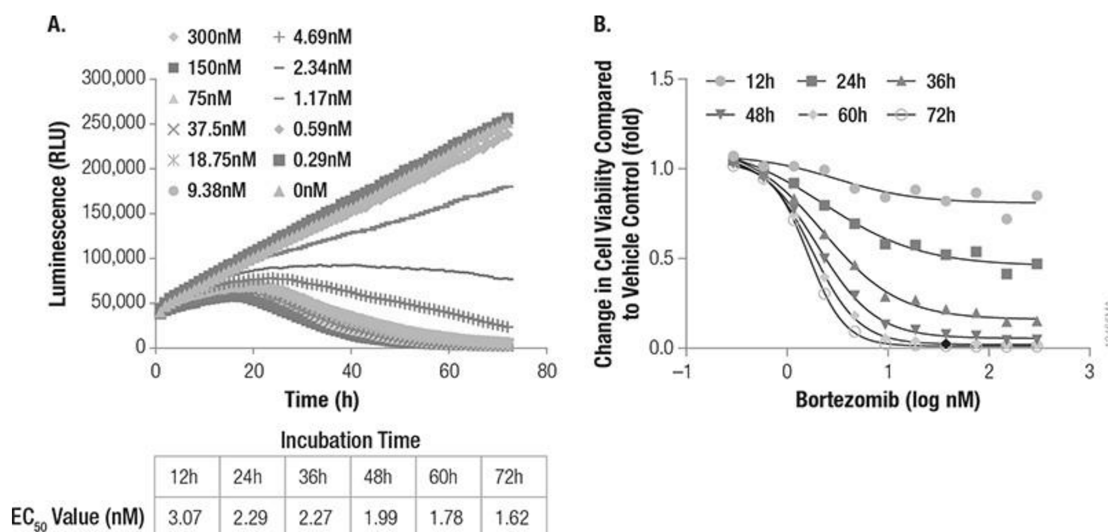
CL-01
NEXT-GENERATION PROMEGA CELL-BASED ASSAYS TO STUDY CELLULAR HEALTH AND RESPONSES

Zsolt Somlai

Bio-Science Ltd., Budapest, Hungary

The assessment of cell health and cellular responses after experimental manipulation continues to be a very important aspect of experimental biology.

In this context, robust and simple to perform **cell-based assays** are highly valuable tools to study different cellular responses. This talk will provide an overview of the most sensitive and easy to-use cell-based assay formats **to study cell health and cellular pathway activation** with focus on our **new real time and 3D cultures assays**. In addition, we will address the combination of these methods (**multiplexing**) to determine several parameters simultaneously facilitating the interpretation of results and improve data quality.



	12h	24h	36h	48h	60h	72h
EC_{50} Value (nM)	3.07	2.29	2.27	1.99	1.78	1.62

Figure 1: *ReaTime-GloTM: Assay plate yields the same data as many end-point assay plates.*

<u>Keywords:</u> *cell-based microplate assays, multiplexing, 3D cultures*

CL-02
HIGH CONTENT SCREENING: WIDEFIELD AND CONFOCAL IMAGING BASED AUTOMATED RESEARCH SOLUTIONS FROM MOLECULAR DEVICES

Nándor Nagy

Bio-Science Ltd., Budapest, Hungary

High Content Screening is a robust and constant emerging possibility for cell/tissue/organism analysis.

Among others, as an image based fully automated solution it has the benefits of having **morphometry analysis**, publicable pictures and **multi-parametric measurements**.

Built-in application software modules ensure easy and robust analysis – with setting only a few parameters!

Two main instruments are availible for different purposes.

ImageXpress Micro XLS is a **widefield fluorescent** and optional **brightfield/phase** microscopy based system with **environmental control** and **liquid handling** options.

ImageXpress Ultra is a **true point-scanning confocal** microscopy instrument with up to **four lasers** for robust and fully automated confocal imaging and analysis.

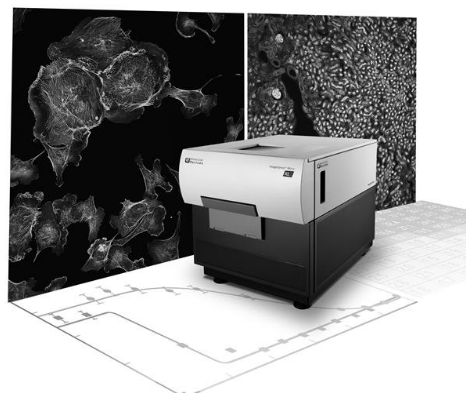


Figure 1: *ImageXpress Micro XLS High Content Screening System*

Examples of suitable applications:

- 3D Imaging Of Cancer Spheroids
- Stem Cell Research
- Whole Organism Imaging
- Live Cells tracking
- Label-free unmodified cell analysis
- Fast kinetic measurements
- Colocalization
- Receptor internalization
- Kinaze activation
- Cell signaling by translocation

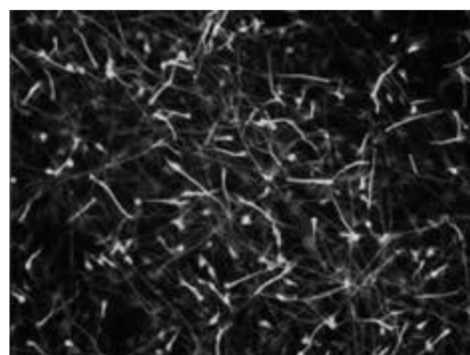


Figure 2: *Differentiated neurons imaged in a 96 well plate at 10x magnification with three different fluorescent markers*

<u>Keywords:</u> *High Content Screening, imaging, microscopy*

CL-03
IMAGE-BASED HIGH CONTENT SCREENING: STATE OF THE ART CONTRACT RESEARCH SERVICES AT SOFT FLOW

György Lustyik, Árpád Czéh, Erika Lantos, György Nagyéri, Viktória Németh, Marianna Merki

Soft Flow Hungary Kft., Pécs, Hungary

Soft Flow Hungary Kft. (SFH) is a contract research organization with capabilities to provide the discovery and preclinical studies such as the testing of pharmaceutical targets related mainly to immunology, angiogenesis and apoptosis.

We prefer high throughput analyses (HTA) such as automated flow cytometry using mainly multiplex bead based assay systems, and we can utilize both commercially available and own bead sets to evaluate the interested mediator(s) or even (the effects of) the relevant environmental impacts such as the mycotoxins. We are interested in hybridome techniques, assay development and validation in addition to the total project management, regulatory compliance and/or consultancy services.

The HTA automated, microscope based imaging systems such as our new Olympus Scan^R platform can be also useful to examine the biological activity in single cells, cultures or (even) whole organisms following treatments with thousands of (even expositor) agents. Those instruments/methods are increasingly applied as well to identify substances such as small molecules, peptides, or RNAi that alter the phenotype of a cell/population in a desired manner. Working with the standardized multi-well plates, simultaneously multiple features of cells/compartments or organisms can be measured/characterized, and a large number of both fluorometric and morphologic data points can be collected per cell (or any events).

As described elsewhere, as contract services, our Scan^R can be applied for a wide range of measurements related to e.g. gene/protein expression, intracellular transport, translocation, cell proliferation and cell cycle analysis, bacterial infection, protein (co-)localisation, automated FISH analysis, micronuclei and comet assays, apoptosis, calcium flux, cell differentiation, cell migration, cytotoxicity, signaling pathway analysis, etc.

Assay development occured in SFH with our Scan^R can be also performed/offered depending on *inter alia* the target (protein) marker(s), cell modell and culture conditions, surface/plate type, reagents, probes, compounds, assay kinetics and the optimization processes.

We may conclude that HTA systems especially the microscope based platforms can be used for the large-scale, objective and cost-effective screening of cellular biological systems.

Keywords: *high-throghput assays, assay development*

ABSTRACTS - ORAL LECTURES

O-01
THE CHANGING LANDSCAPE OF GENETIC TESTING

István Balogh

University of Debrecen, Faculty of Medicine, Division of Clinical Genetics, Debrecen, Hungary

The molecular genetic testing of an individual's genetic status is currently changing. The technical improvement of DNA sequencing resulted in the development of next-generation sequencing (NGS) methods, which are able to sequence very high amount of genetic material. NGS is quickly reaching the clinical use. There are several different strategies of the development of NGS assay systems depending on the clinical goal. Targeted sequencing is performed where a single gene is to be tested (BRCA1, CFTR) or when a group of genes are analyzed in case genetic heterogeneity. If a Mendelian disease is suspected with unknown cause, the entire coding region, the exome of the individual will be tested preferably together with the parents or affected siblings. A limited exome sequencing is also possible where clinically established genes are tested (less than 5000). By exome sequencing, depending on the settings, app. 20,000 single nucleotide variations are detected. It is also possible to sequence the entire genome of an individual resulting in millions of variations. This amount of data, the complexity of the analysis, the ethical considerations, and result interpretation would clearly require a multidisciplinary team consisting of clinical geneticists, clinical laboratory geneticists, technology experts and bioinformaticians. Though there are still limitations of the techniques (method validation, standardization, quality assurance, reference materials, mutation databases) and serious concerns of the use of the genetic data, the great potential of the NGS (genetic screening, non-invasive prenatal testing, pharmacogenomics) will clearly change the way of genetic testing and will likely lead to the paradigm shift in the medicine as in the future everyone's genome will be sequenced.

O-02
ASSOCIATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR-γ POLYMORPHISMS WITH INFLAMMATORY BOWEL DISEASE IN A HUNGARIAN COHORT

András Penyige[1], Szilárd Poliska[2], Péter László Lakatos[3], Mária Papp[4], Károly Palatka[4], László Lakatos[5], Tamás Molnár[6], László Nagy[2]

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[2] *Department of Biochemistry and Molecular Biology, University of Debrecen, Hungary*
[3] *1st Department of Medicine, Semmelweis University, Budapest, Hungary*
[4] *2nd Department of Medicine, University of Debrecen, Debrecen, Hungary*
[5] *1st Department of Medicine, Csolnoky F. County Hospital, Veszprem, Hungary*
[6] *1st Department of Medicine, University of Szeged, Szeged, Hungary,*

Inflammatory bowel diseases (IBDs) are chronic inflammatory disorders with an autoimmune origin affecting the gastrointestinal tract. It is clinically classified into two major subphenotypes: Crohn's disease (CD) and ulcerative colitis (UC). IBD shows increasing incidence in the last few years in

Hungary. Since genetic susceptibility of patients plays an important role in the development and pathogenesis of IBD, it is important to identify new susceptibility genes. Peroxisome proliferator-activated receptor gamma (PPARγ) is expressed in the colon and has protective effects against inflammatory processes. Polymorphisms of PPARγ have been examined in disorders with autoimmune origins such as psoriasis, psoriatic arthritis, and asthma. However, only a few studies have examined the association of PPARγ polymorphisms in IBD so far. Our aim was to examine the association of four polymorphisms of PPARγ in a well-characterized Hungarian IBD cohort. In all, 575 CD, 103 UC patients, and 486 sex- and age-matched controls were examined. Four polymorphisms of PPARγ (rs10865710 [C-681G], rs2067819, rs3892175, and rs1801282 [Pro12Ala]) were genotyped by TaqMan genotyping assays.

Tests of HWE were carried out among CD, UC, and control groups separately for all loci, all polymorphisms were found to be in equilibrium in all three groups and the allele and genotype frequencies did not differ significantly among the cohorts. When the strength of association of Pro12Ala polymorphism with CD and UC phenotypes was examined, the homozygous variant of the minor allele (Ala/Ala) of Pro12Ala was found to be significantly less common in CD patients compared to controls, confirming a significant protective effect compared to homozygous common genotype (odds ratio [OR] = 0.33; 95% confidence interval [CI] = 0.12–0.94; P = 0.03). In contrast, no significant statistical association was detected between the Pro12Ala and UC susceptibility. Since genetic association analysis based on haplotypes is more powerful than single SNP analysis, we assessed the association between haplotypes and CD or UC. Despite of similar allele frequencies, the overall frequencies of haplotypes were significantly different between the control and either CD or UC patients (CD/control $P = 3.58 \times 10^{-6}$; UC/control $P = 2.24 \times 10^{-19}$) and between the two diseased groups, too ($P < 10^{-4}$). The GAGG haplotype of PPARγ confers a protective effect in CD; however, it is not significant, but in UC it has a protective effect with a significant level (OR = 0.14; 95% CI: 0.05–0.42; $P = 3.78 \times 10^{-5}$), while GAGC increases the risk of UC (OR = 6.70; 95% CI: 3.41–13.17; $P = 3.85 \times 10^{-10}$).

Conclusions: In the present study we demonstrated a significant association between PPARγ polymorphisms and the development of CD and UC at single loci level and also in haplotype combinations.

Keywords: *IBD, peroxisome proliferator-activated receptor gamma, SNP, haplotype lysis*

O-03

PROTEIN PHOSPHATASE-1 IS INVOLVED IN THE MAINTENANCE OF NORMAL HOMEOSTASIS AND IN UVA IRRADIATION-INDUCED PATHOLOGICAL ALTERATIONS IN HACAT CELLS AND IN MOUSE SKIN

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The number of UV radiation-induced skin diseases such as melanomas is on the rise. **The altered behavior** of keratinocytes **is often coupled with signaling** events **in which Ser/Thr specific** protein kinases and phosphatases regulate various cellular functions. In the present study the role of protein phosphatase-1 (PP1) was investigated in the response of HaCaT cells and mouse skin to UV radiation. PP1 catalytic subunit (PP1c) isoforms, PP1cα/γ and PP1cδ, are all localized to the cytoskeleton and cytosol of keratinocytes, but PP1cδ was found to be dominant over PP1α/γ in the nucleus. PP1c-silencing in HaCaT cells decreased the phosphatase activity and suppressed the viability of the cells. Exposure to 10 J/cm^2 UVA dose induced HaCaT cell death and resulted in 30 % decrease of phosphatase activity. PP1c-silencing and UVA irradiation altered the gene expression profile of HaCaT cells and suggested that the expression of 19 genes was regulated by the combined treatments with many of these genes being involved in malignant transformation. Microarray analysis detected altered expression levels of genes coding for melanoma-associated proteins such as keratin1/10, calcium binding protein S100A8 and histone 1b. Treatment of Balb/c mice with the PP1-specific inhibitor tautomycin (TM) exhibited increased levels of keratin1/10 and S100A8, and decreased level of histone 1b proteins following UVA irradiation. Moreover, TM treatment increased pigmentation of the skin which was even more apparent when TM was followed by UVA irradiation**. Our data identify PP1 as a regulator of the normal homeostasis of keratinocytes and the UV-response.**

Keywords: *protein phosphorylation, UV radiation, keratinocyte*

O-04
LIVER METABOLIC ALTERATIONS INFLUENCE PXE-LIKE PHENOTYPE

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ABCC6-related diseases, such as *Pseudoxanthoma elasticum* (PXE) and *Generalized Arterial Calcification in Infancy* (GACI) can be attributed to the loss-of-function of the *ABCC6* gene. Although *ABCC6* is mainly expressed in the liver the disease has dermatologic, ocular and cardiovascular symptoms. The severity of the symptoms is highly variable between patients. We have investigated the transcriptional regulation of the gene. We have mapped the binding of CCAAT/Enhancer binding protein ß (C/EBPß) and the conserved hepatocyte nuclear factor 4α (HNF4α). Short-term activation of the ERK1/2 cascade leads to decreased HNF4α binding on *ABCC6*. We also observed similar decrease of HNF4α binding genome-wide in ChIP-Seq experiments. Furthermore, in accordance with transcription factor binding alterations, we have observed changes in the pattern of histone modifications. We have proved that HNF4α is directly phosphorylated by ERK1/2 *in vitro* at several sites. The effect of phosphomimetic HNF4α mutants was also investigated by luciferase reporter gene assays. Our results suggest that ERK1/2 activity contributes to HNF4α metabolic balance in hepatocytes. We consider that metabolic status of the patients contributes to the PXE-associated phenotypes.

O-05
INTRODUCING A NEW MEMBER IN DNA DAMAGE TOLERANCE PATHWAY

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Appropriate replicaton is essential for the cells to preserve their genomic inegrity. Due to endogenous and exogenous effects mutations can accumulate in the DNA, and the remaining of the alterations can lead to the blockage of the replication fork leading to double strand breaks and genomic rearrangements. Several mechanisms are evolved to protect the maintenance of the genom and ensure the continuation of the replication on the damaged site. These are so called damage tolerance pathways which refer to the fact that many cases the lesions are not removed perfectly, but tolerated as the replicative apparatus steps over the problematic region. In the damage bypass pathway the replicative DNA polymerase is removed from the damaged site and a translesion polymerase takes its place, which can pass the lesion and the replication can be continued. On the other hand, breakage of the replication fork generates double strand breaks, which can be repaired

by homologous recombination or non-homologous end joining. Deficiency of several genes involved in these pathways can lead to serious genetically inherited disorders and cancer predisposition. Recent years our laboratory has identified several new members of these damage tolerance pathways and in my talk I will introduce an exciting new player.

O-06
ANALYSIS OF THE FIRST 1500 PREIMPLANTATION GENETIC SCREENING DONE IN HUNGARY USING ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

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Preimplantation Genetic Diagnosis for Aneuploidy with array Comparative Genomic Hybridization is a widely used technique to improve the outcome of assisted reproduction techniques. It refers to the screening of the chromosomes of embryos and oocytes for numerical chromosome errors. Historically it is developed to increase the success of *in vitro* fertilization cycles in women with advanced maternal age, or those who are suffering from recurrent miscarriage or repeated implantation failure. Our aim was to retrospectively analyze the data obtained at our clinic between May 2011 till present. Array Comparative Genomic Hybridization was performed on single blastomeres. Among many others, we have analyzed the rate of the euploid, single and complex aneuploid (including double aneuploidies) embryos, the prevalence of viable aneuploidies and the average number of transferred embryos per cycle. Array Comparative Genomic Hybridization is a reliable and robust technique utilizing DNA chip technology. The whole process can be performed within 12-24 hours enabling fresh embryo transfer following cleavage stage embryo biopsy. Hopefully, further clinical trials will support the acceptance of the method in reproductive medicine and this will make a significant positive difference in clinical outcomes for couples who require *in vitro* fertilization to establish a successful pregnancy and ultimately a healthy live birth.

Keywords: *in vitro fertilization, preimplantation genetic diagnosis for aneuploidy, array Comparative Genomic Hybridization*

O-07
MOLECULAR MECHANISM OF NUCLEOTIDE-DEPENDENT ACTIVATION OF THE KTRAB K^+ TRANSPORTER

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KtrAB is a member of the large Ktr/Trk/HKT superfamily that includes ion transporters with crucial roles in environmental adaptation of non-animal cells: plants, fungi, archaea and bacteria. Strikingly, these transporters closely resemble K^+ channels both at the level of their membrane embedded

subunits and of the cytosolic RCK (Regulating the Conductance of K^+) regulatory domains. K^+ uptake via KtrB is the primary step during adaptation to osmotic upshock in some bacteria and is thought to be regulated by the cytosolic nucleotide-binding protein, KtrA. It is yet a puzzle how KtrA regulates ion flux in KtrB transporters. We solved the crystal structure of the KtrAB complex from *B. subtilis* at 3.5 Å resolution in the ATP-bound conformation (*Nature* 496(7445):323-9). The structure of isolated KtrA was also solved both in ADP and ATP and showed an octameric ring that undergoes ligand-dependent asymmetrical changes. Conformational differences in the structure of the KtrA ring in ADP *vs.* ATP prompted the idea of nucleotide-dependent functional regulation of KtrB by ring expansion and constriction, respectively. Monitoring accessibility changes of engineered cysteines in KtrB upon ligand binding to KtrA revealed conformational rearrangements at one of the KtrB-KtrA contact sites. Such movements were supported by a low-resolution structure of KtrAB in an inactive conformation. Further biochemical and functional data suggests a novel activation mechanism in the KtrAB complex through a nucleotide-dependent allosteric interplay between the contact and activation sites. Therefore, we report for the first time the structural basis for propagation of gating ring movements towards the activation site of a K^+ transporter. While the activation machinery of KtrB is markedly different compared to K^+ channels, it might be ubiquitous among homologues of KtrB, the landmark uptake system in the Ktr/Trk/HKT family.

O-08
CHARGED SINGLE ALPHA HELICES: PREDICTION, ANALYSIS AND MODELING

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The Charged Single Alpha Helix (CSAH) is a structural motif only recognized in the past decade. It is characterized by a high density of charged residues (primarily glutamate, lysine and arginine) that are arranged in a characteristic alternating pattern. The exact role of CSAH segments is elusive in general except for some specific cases like myosin IV. The first regular surveys for CSAH regions revealed that they are often overlapping with regions predicted to form coiled coils and/or to be disordered. CSAH-containing proteins are relatively rare in proteomes and are preferentially associated with RNA-binding function, although direct contact between CSAH regions and RNA is unlikely. To assess this elusive connection, we have chosen to perform detailed analysis on proteins of the paraspeckle, a recently identified subnuclear component in eukaryotic cells with a supposed role in gene expression regulation. We have found that the three proteins investigated (denoted NONO, PSPC1 and SFPQ) exhibit a highly conserved domain organization and a largely conserved CSAH segment at the C-terminal part of their predicted coiled coil regions. The structure of the NONO-PSPC1 'core' dimer has been recently solved by X-ray crystallography, but this structure does not contain the predicted CSAH segment. According to the proposed higher-order organization of the core dimers, we have generated atomic-level models of hexameric NONO-PSPC1 fragments that contain all possible subunit-subunit interactions expected to be present in larger multimers. The models contain the extended coiled coil and CSAH regions which are presumably involved in multimerization. Our results support a steric role of the CSAH segments, namely, the

positioning of the genuinely disordered C-terminal segment of one monomer to the specific 'cavity' of a neighboring core dimer. We stress that our models are still incomplete but still might yield valuable insight into the role of CSAH segments in the protein family.

Keywords: *coiled coil, structural modeling, proteome, protein function*

O-09
MULTIPLE FUZZY INTERACTIONS IN THE MOONLIGHTING FUNCTION OF THYMOSIN-Β4

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Thymosine β4 (Tβ4) is a 43 amino acid long intrinsically disordered protein (IDP), which was initially identified as an actin-binding and sequestering molecule. Later it was described to have multiple other functions, such as regulation of endothelial cell differentiation, blood vessel formation, wound repair, cardiac cell migration, and survival. The various functions of Tβ4 are mediated by interactions with distinct and structurally unrelated partners, such as PINCH, ILK, and stabilin-2, besides the originally identified G-actin. Although the cellular readout of these interactions and the formation of these complexes have been thoroughly described, no attempt was made to study these interactions in detail, and to elucidate the thermodynamic, kinetic, and structural underpinning of this range of moonlighting functions. Because Tβ4 is mostly disordered, and its 4 described partners are structurally unrelated (the CTD of stabilin-2 is actually fully disordered), it occurred to us that this system might be ideal to characterize the structural adaptability and ensuing moonlighting functions of IDPs. Unexpectedly, we found that Tβ4 engages in multiple weak, transient, and fuzzy interactions, i.e., it is capable of mediating distinct yet specific interactions without adapting stable folded structures.

Keywords: *Thymosin β4, PINCH, ILK, stabilin, intrinsically disordered protein, fuzzy complex, uncoupled binding and folding, weak interaction, moonlighting*

O-10
DISCRETE MOLECULAR DYNAMICS CAN PREDICT HELICAL PRESTRUCTURED MOTIFS IN DISORDERED PROTEINS

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Intrinsically disordered proteins (IDPs) lack a stable tertiary structure, but their short binding regions termed Pre-Structured Motifs (PreSMo) can form transient secondary structure elements in solution. Although disordered proteins are crucial in many biological processes and designing strategies to modulate their function is highly important, both experimental and computational tools to describe their conformational ensembles and the initial steps of folding are sparse. Here we report that discrete molecular dynamics (DMD) simulations combined with replica exchange (RX) method efficiently samples the conformational space and detects regions populating α-helical conformational states in disordered protein regions. While the available computational methods predict secondary structural propensities in IDPs based on the observation of protein-protein interactions, our ab initio method rests on physical principles of protein folding and dynamics. We show that RX-DMD predicts α-PreSMos with high confidence confirmed by comparison to experimental NMR data. Moreover, the method also can dissect α-PreSMos in close vicinity to each other and indicate helix stability. Importantly, simulations with disordered regions forming helices in X-ray structures of complexes indicate that a preformed helix is frequently the binding element itself, while in other cases it may have a role in initiating the binding process. Our results indicate that RX-DMD provides a breakthrough in the structural and dynamical characterization of disordered proteins by generating the structural ensembles of IDPs even when experimental data are not available.

Keywords: *disordered proteins, structural ensemble, molecular dynamics*

O-11
COMPLEX DNA-PROCESSING ACTIVITIES ARE CONSERVED BETWEEN BACTERIAL AND HUMAN GENOME-GUARDING HELICASES

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Homologous recombination (HR) is the most efficient mechanism that can lead to error-free repair of DNA double-strand breaks, the most dangerous form of DNA damage. However, HR must be strictly controlled because excessive HR can lead to fatal genome rearrangements. Bacterial and eukaryotic RecQ-family helicases exert a broad range of pro- and antirecombinogenic activities, acting on a variety of HR-intermediate DNA structures. Our single-molecule and ensemble biophysical experiments and in vivo HR precision assays have recently revealed unexpected complex DNA-restructuring activities of E. coli RecQ helicase, raising the concept that the enzyme is capable of multiple means of processing that are dependent of the geometry of the DNA substrate encountered. We found these mechanisms to be conserved in Bloom's syndrome (RecQ-family) helicase, the key enzyme responsible for HR quality control in humans. We assign central roles to conserved DNA-binding accessory domains of the bacterial and human enzymes in directing the activities of the catalytic core towards efficient and controlled DNA processing. We provide a mechanistic model of helicase architecture supporting a versatile, DNA structure-dependent enzymatic behavior that appears functionally optimized for multiple processes preserving genome integrity.

Keywords: *DNA, protein, structure, DNA repair, helicase, enzyme, mechanism*

O-12
COMPUTATIONAL TOOLS INDICATE THE IMPORTANCE OF FLEXIBILITY AND INTERMOLECULAR INTERACTIONS IN MULTIDRUG RECOGNITION OF ARYL HYDROCARBON RECEPTOR

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The nonspecific binding of multidrug ABC transporters plays an important factor in limiting the bioavailability of drugs. The mechanism of multidrug binding (MDB) is largely unknown because of the hydrophobic nature of the interacting chemicals and transmembrane proteins. Moreover, the large size of the system including the bilayer and a transporter also limits computational studies. Since knowledge on MDB can facilitate designing drugs and strategies to modulate drug metabolism (transport, oxidation, conjugation), we aimed to understand MDB of a small promiscuous domain, the PAS-B domain of aryl hydrocarbon receptor (AhR) that might be extrapolated to decode MDB of large transporters. As flexibility has been indicated to play a role in recognition of multiple drugs, we performed different types of molecular dynamics simulations to characterize the dynamics and stability of various PAS domains exhibiting physiological functions associated with either specific or nonspecific interactions. Moreover, *in silico* docking to conformational ensembles was employed to characterize the differences between the interactions of strong, poor, and non-binders. Our results indicate that PAS domains with no ligand binding function or with a prosthetic group are more stable compared to the multidrug binding AHR PAS domain. Simulations with different force fields resulted in conformations with significantly different binding pockets that warn the field for careful selection of tools and interpretation of computational results. Finally, the docking results suggest that ligand specificity is determined not only by the ligand binding PAS-B domain, but other parts of AhR or its interacting partners.

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Keywords: *AhR, xenobiotics binding, molecular dynamics, ensemble docking*

O-13
R-LOOPS AS POTENT TARGETS OF THE APOBEC CYTIDINE DEAMINASE-MEDIATED INNATE IMMUNITY

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It is well known that R-loops have an adverse effect on genome integrity but a growing body of evidence points toward their role in normal physiological processes. Using DNA-RNA immunoprecipitation-coupled deep sequencing (DRIP-seq) and biophysical analyses we show that these structures are prevalent on retrotransposons (LINEs, SINEs, LTRs) and they lie in molecular proximity (i.e. within 2-10 nm) with single-stranded DNA. Transcription inhibition completely prevents their formation, but remarkably, they are also highly sensitive to reverse transcriptase inhibitors suggesting that a significant portion of these structures form by reverse transcription. By differential DNA denaturation PCR (3D-PCR) we reveal that R-loops are potent targets of APOBEC cytidine deaminases, a family of proteins that introduce C-to-T mutational showers into attacking viral genomes. These features tie R-loop formation and APOBEC-mediated innate immunity to the molecular same pathway.

Keywords: *R-loop, APOBEC, innate immunity*

O-14
FUNCTIONAL DISSECTION OF A PLANT ARGONAUTE

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RNA silencing (or RNA interference) is an evolutionarily conserved gene regulatory mechanism with fundamental implications in many biological processes. It is triggered by double-stranded RNA (dsRNA) and causes shutdown of the expression of genes containing sequences identical or highly similar to the initiating dsRNA. In plants, RNA silencing acts at both the RNA and DNA levels. Mechanisms of silencing at the RNA level include mRNA cleavage or translational repression (post-transcriptional gene silencing, PTGS) whereas at the DNA level they involve DNA and/or histone methylation and subsequent transcriptional gene silencing (TGS). All these manifestations of RNA silencing rely on the action of small RNA (sRNA) molecules of 21–24 nucleotide (nt) that derive from the processing of the dsRNA trigger by RNaseIII-like enzymes. One strand of the sRNA duplexes is

incorporated into an Argonaute (AGO) containing RNA-induced silencing complex (RISC) to guide sequence-specific inactivation of targeted RNA or DNA.

Plant Argonautes fall into three major phylogenetic clades. Members of the first clade (AGO1/AGO5/AGO10 in *Arabidopsis*) primarily bind 21 nt small RNAs (most miRNAs and 21-nt siRNAs) and are the main executors of PTGS. The 24-nt siRNAs that derive mostly from transposons and repetitive sequences are incorporated into AGOs that belong to clade 2 (AGO4/AGO6/AGO9 in *Arabidopsis*,). They silence the transcription of the genomic loci, from which the 24 nt siRNAs originate. Clade 3 includes *Arabidopsis* AGO2, AGO3 and AGO7. AGO7 is a highly specialized Argonaute. It regulates the auxin signaling pathway via production of TAS3 *trans*-acting siRNAs (tasiRNAs). The *Arabidopsis* AGO2 and AGO3 genes are very similar to each other and they are likely the result of an evolutionarily recent duplication event. Until recently, relatively little has been known about the function of AGO2 compared to other Argonautes. AGO2 displays both additive and overlapping activities with AGO1, however newer data also highlight its essential and independent function in various anti-pathogenic defense pathways (both anti-viral and anti-bacterial) and genotoxic stress responses (via its involvement in DNA double-strand break repair). Considering the significance of the above processes it is important to functionally characterize a plant AGO2 protein in greater detail. We chose to study the AGO2 protein of *Nicotiana benthamiana* because, this plant is amenable to a number of powerful molecular biological and biochemical techniques and is also susceptible to a wide range of plant pathogens (viruses, bacteria, oomycetes, fungi). We have cloned the *N. benthamiana* AGO2 gene and expressed it in various *in vitro* and *in vivo* systems. A sensitive, AGO2-specific *in vivo* reporter system has been established to monitor the activity of AGO2. We have demonstrated that AGO2 is capable of exerting translational repression on target genes through different mechanisms depending on the location of miRNA binding site. We have also developed an *in vitro* cleavage assay and showed that AGO2 can cleave target RNAs in an ATP independent and context sensitive fashion. Based on structural and bioinformatics considerations several functionally important amino acid residues have been identified that affect miRNA loading, cleavage activity and translational repressive potential of AGO2. In summary, we have initiated a detailed molecular dissection of a plant AGO2 protein, which already provided significant novel information on this previously poorly understood RNA silencing executor.

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O-15
TISSUE AND CIRCULATING MICRORNAS IN THE DIAGNOSIS OF ADRENAL TUMORS

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Differential expression of microRNAs has been described in a several tumors, and microRNAs are considered to be major players in tumorigenesis. MicroRNAs can be exploited as markers of malignancy and prognosis. Our research group was among the first to describe the tissue microRNA expression profiles of adrenocortical and adrenomedullary tumors and identified microRNA markers of malignancy. Overexpressed oncogenic and underexpressed tumor suppressor

microRNA have been described in both adrenocortical and adrenomedullary tumors. Since the histological diagnosis of both adrenocortical and adrenomedullary tumors is difficult, microRNA markers of malignancy could be very useful in clinical practice. The expressional difference of *hsa-miR-503* and *hsa-miR-511* appeared to be the best marker of malignancy reaching 100 % sensitivity and 97 % specificity for the diagnosis of adrenocortical cancer. Overexpressed *hsa-miR-1225-3p* turned out to be useful for the diagnosis of adrenomedullary pheochromocytoma recurrence. By *in silico* microRNA target prediction and pathway analysis, microRNA-mediated damage of the G2-M cell cycle checkpoint was identified as the primary event in microRNA deregulation in adrenocortical cancer, whereas Notch-signaling is affected by overexpressed *hsa-miR-1225-3p* in recurrent pheochromocytomas. Beside tissue microRNAs, we have recently examined the circulating microRNA profiles of adrenocortical tumours, and five overexpressed microRNAs (*hsa-miR-100, hsa-miR-181b, hsa-miR-184, hsa-miR-210* and *hsa-miR-483-5p*) in plasma samples adrenocortical cancer were identified that could be used for the differentiation of benign and malignant tumors. Circulating microRNAs might enable the preoperative diagnosis of malignancy and thereby would be of major clinical significance. The biological significance of circulating microRNAs is unclear, and we have raised a hypothesis that the relative overrepresentation of circulating microRNAs with predominant tumor suppressor activity in the blood might be related to a tumor surveillance mechanism.

Keywords: *microRNA, adrenal, tumor, diagnosis, malignancy, tissue, circulating*

O-16
ARTIFICIAL MIRTRON-DERIVED SMALL REGULATORY RNAS: A POTENTIAL TOOL FOR EFFECTIVE GENE EXPRESSION REGULATION

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Small regulatory RNAs are the mediators of RNA interference. The endogenously encoded microRNAs (miRNAs) represent one of the most significant groups of these molecules. The single-stranded mature miRNAs are about 20–24 nucleotides in length, and play very important roles in many cellular processes. They form complex gene regulatory networks comparable to that of transcription factors by targeting different mRNA molecules via sequence complementarity. Most of the miRNA molecules are generated via the canonical miRNA processing pathway, but there are also emerging data on various alternative miRNA maturation routes. Mirtrons represent the most prominent group of the alternatively processed miRNAs. They reside in short introns, which are essentially equivalent to the precursor form of the given miRNA. Thus, the first step of the mirtronic miRNA processing is different from the canonical one: the pre-miRNA is cleaved out from the primary transcript by the splicing machinery instead of the Drosha/DGCR8 complex.

Mirtrons, owing to their special features, are promising genetic tools for the regulation of gene expression: (i) the mirtron-derived miRNA expression can be easily followed, since the expression of the host (marker) gene and the intronic mirtron is strongly coupled, (ii) this ‘co-expression’ is driven by a Pol II promoter, which allows the usage of inducible and tissue specific promoters, (iii)

more than one mirtron can be inserted into one marker gene as distinct introns (e.g. designed against different targets), (iv) the first step of their processing is independent from the main miRNA processing machinery. Here, we report the design and development of EGFP marker gene-embedded artificial mirtron sequences to silence our model gene, the ABCG2 membrane transporter protein. We tested the processing of small RNAs from various artificial mirtrons, and investigated their silencing capacity at mRNA and protein levels in both reporter assays and on the ABCG2 target gene expression. Our results suggest that artificial mirtrons show good potential for gene expression regulation, and they are promising candidates as genetic tools for both basic research and gene therapy applications.

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O-17
SMALL REGULATORY RNAS IN RESPONSE TO AMBIENT TEMPERATURE SENSING IN *ARABIDOPSIS*

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Temperature is a key environmental limiting factor for plants, and as sessile organisms it is absolutely essential for them that they grow and develop accordingly. Within the about 12-27^{0}C ambient temperature range plants have large differences in growth and developmental changes, such as flowering time, disease resistant pathways, and temperature also has multiple effects on the clock. Although temperature influences a lot of crucial mechanisms in a large extent, the process, how temperature is sensed and integrated into development is largely unknown. From an earlier study we know that chromatin structure has a key role in ambient temperature sensing in *Arabidopsis*. It was shown that alternative H2A.Z histone containing nucleosomes acts, like regulators of ambient temperature transcriptome. In plants short 21-24 nucleotides long non coding RNAs (sRNA) have been recognised as important gene expression regulators both transcriptionally and post-transcriptionally. Furthermore sRNAs also have a major function during epigenetic regulation of chromatin structure. To test our hypothesis we use high-throughput sequencing and bioinformatical methods to identify, profile and describe conserved and non-conserved sRNAs in *Arabidopsis thaliana* during different ambient temperature conditions. Furthermore we also describe sRNA cleaved mRNAs through genomic-scale deep-sequencing. We found that the 24 nt long sRNAs, which plays important role to regulate genes transcriptionally are more diverse at 27 ^{0}C in mRNA coding regions. Our preliminary results may suggest that 24 nt long sRNAs have an important role to regulate gene expression and help plants to adapt to higher temperature. In addition our observation correlate with other studies saying that there is a relationship between DNA methylation and H2A.Z within genes. Understanding the molecular mechanisms of ambient temperature response of sRNAs will give us important knowledge that can be adapted to crop species and used in future breeding programs. This is particularly important, because the projected

increases in mean global temperature as well as predicted extremes of temperature in the next 100 years suggesting significant threats to wild plants and agricultural production.

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Keywords: *Small RNAs, Chromatin remodelling, epigenetics, temperature, Arabidopsis*

O-18
LINKING RNA SILENCING TO NON-STOP DECAY

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RNA silencing is a conserved pathway that leads to sequence-specific degradation of target RNAs. The effector of RNA silencing is the RNA Induced Silencing Complex (RISC), comprising minimally of one Argonaute protein (the so-called slicer) and a 21-24 nt short RNA (small interfering RNA or microRNA) that confers sequence-specificity to the cleavage. Following the RISC-mediated endonucleolytic cleavage, the 3′ part of the cleaved target RNA is degraded by XRN 5′-to-3′ exonuclease (in plants XRN4). The 5′ RISC-cleavage product may be harmful for the cellular machinery since it may encode for truncated proteins, therefore needs to be eliminated fast. How the 5′ end cleavage product is degraded is not completely understood. It was proposed that 5′-to-3′ exonucleases are involved in this process. Here we show that Non-stop decay (NSD), which is an RNA quality control system that eliminates aberrant mRNAs lacking the stop codon, is responsible for the degradation of 5′ cleavage product of RNA silencing and that on-going translation is required for this to happen. Our finding points toward an intimate connection between RNA silencing and NSD in plants. We propose that channeling RISC cleavage products into NSD pathway is conserved among eukaryotes.

Keywords: *RNA silencing, non-stop decay, cleavage product, RNA quality control*

O-19
SYSTEMATIC ANALYSIS OF SOMATIC MUTATIONS IN CANCER: UNCOVERING FUNCTIONAL PROTEIN REGIONS IN DISEASE DEVELOPMENT

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Recent cancer genome projects aim to provide a deeper understanding of the molecular basis of the most common genetic disease. The results of these projects have already served us with valuable insights. On one hand, the identified somatic mutations verified known cancer genes that – in their mutated form – drive tumorigenesis and metastasis. On the other hand, various genes that were previously not studied in the context of cancer formation showed a high accumulation of somatic mutations. In both cases the position and nature of these mutations can often be translated to the disruption of protein function and structure, or in a wider sense to the disruption of biological processes and pathways serving as a tool to better understand the molecular principles of tumor formation.

Although some attempts have recently been made at the systematic discovery of significantly mutated protein regions, all current methods have serious limitations. Here we present the first method that is able to pinpoint proteins and protein regions that harbor a significant amount of cancer-related missense mutations or in-frame insertions and deletions using a unified statistical model for all three mutation types. Furthermore, using the annotations of mutations not only is it possible to obtain position-specific information about protein regions involved in cancer, but also to tie them to specific cancer types.

Our testing showed that even this restricted amount of input information (only somatic mutations that have a local effect of the synthesized protein) is enough to re-capture the majority of known cancer genes. Furthermore, our analysis can reveal novel protein regions previously not known to be involved in cancer and link known cancer proteins to a broader range of cancer types. This can provide a platform for the more complete mapping of cancer pathways. Our results show that albeit a much broader range of genetic alterations occur in cancer (nonsense/frame-shift mutations, loss of heterozygosity or disruption of expression levels), the significant accumulation of local somatic mutations appear near-ubiquitously in the affected genes offering a relatively straight-forward way for their identification.

Apart from somatic genetic alterations, another substantial contribution to cancer development comes from germline mutations. Although germline mutations affect a markedly different spectrum of genes than their somatic counterparts, the majority of them fall into the same protein regions that show enrichment of somatic mutations. This indicates that despite the differences between somatic and germline development of cancer, the two mechanisms share a central core-set of genes that represent common essential elements of the protein network.

Our approach can not only identify genes that are essential to cancer formation, but can also pinpoint specific protein regions that – via incorporating a wide range of biological annotations – show

cancer-related mutations preferentially targeting structured protein regions, especially kinases and sites of post-translational modifications. This further marks the essential role of kinase mediated signaling in cancer.

O-20
EPIGENETIC MODIFICATIONS IN HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a fatal, adult-onset inherited neurodegenerative disorder. Although several treatment options are currently under development, a cure for HD is not available. Huntington's disease is caused by the expansion of a polymorphic polyglutamine domain in the huntingtin protein encoded by the *IT15* gene. Due to the dominant gain of function nature of the mutation genetic disease models of HD were readily developed in various model organisms. These models provided much needed insight into the molecular pathomechanism of the disease and might also give clues to general pathomechanisms in proteopathies.

Transcriptional dysregulation is one of the earliest changes in Huntington's disease pathogenesis. We used a *Drosophila* model of HD to investigate epigenetic factors that may be in the background of these changes. Over the years we found that altering the levels of histone post-translational modification activities, including acetylation and methylation, are affecting HD pathogenesis. We identified the specific histone acetyltransferase and histone deacetylase enzymes involved in this process and found that reduced histone deacetylase levels suppress phenotypes induced by the expression of the toxic mutant huntingtin fragment. The involvement of histone methylation in HD pathogenesis seems to be more complicated. By analyzing the effects of mutant forms of methyltransferase and demethylase enzymes modifying different amino acid residues on histone tails we found that in some cases enzymes having opposite general effects on transcriptional activity may influence HD phenotypes similarly, suggesting gene specific transcriptional effects.

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O-21
THE UNIVERSE OF MONOGENIC HUMAN DISEASES: IS IT REALLY EXPANDING?

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During the past few decades, the investigative technologies of molecular biology – especially sequencing – underwent huge advances, leading to the sequencing of the entire human genome, as well as to the identification of several candidate genes and the causative genetic variations that are responsible for monogenic diseases. According to the publicly available databases there are approximately 10000 monogenic human disorders with already uncovered genetic background. At the same time we also realized that in many cases the mutations of the very same gene are responsible for different monogenic diseases that were previously defined by different names based on their first describers. Careful genotype-phenotype analysis in these diseases often lead to the assumption in fact that these monogenic diseases are not different entities, but rather clinical variants of a disease spectrum caused by mutations in the same gene. In the view of the above train of thought we suppose that in fact the universe of monogenic diseases is not expanding: in many cases we rather experience tendency of unification. In our lecture we will bring clinical cases recent examples from our own: the careful examination of the Brooke-Spiegler syndrome, the familial cylindromatosis and the multiple familial trichoepitheliomatosis type 1 group of genodermatosis caused by the mutations of the *CYLD* gene and the Papillon-Lefevre syndrome, the Haim-Munk syndrome and the aggressive periodontitis type 1 group of human diseases caused by the mutations of the *CTSC* gene.

Keywords: *monogenic skin diseases, cylindromatosis gene, Brooke-Spiegler syndrome, familial cylindromatosis, multiple familial trichoepitheliomatosis type 1*

O-22
MAGNESIUM HOMEOSTASIS IN PLATELETS

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Magnesium is *the second* most abundant intracellular cation in platelets and contributes to the maintenance of low intracellular calcium $[Ca^{2+}]_i$ levels. Mg^{2+} forms a complex with ATP, regulates the activity of more than 300 enzymes, and in addition competes with Ca^{2+} for binding sites on several EF-hand domain proteins. Interestingly, Mg^{2+} is a natural antagonist of platelet reactivity since an elevated Mg^{2+} concentration inhibits platelet aggregation and thrombus formation *in vivo*. In line with this, altered Mg^{2+} homeostasis is described in many cardiovascular diseases, especially in patients with coronary heart disease, arrhythmias and stroke. Although Mg^{2+} influx has been described in platelets, the molecular composition of the Mg^{2+} transport system and its signalling function are unknown. So far, more than 20 Mg^{2+} transporters and channels have been identified in eukaryotic cells. In platelets, we found that only MagT1 and TRPM7 are predominantly expressed.

Magnesium transporter 1 (MagT1) has been described as an important component for Mg^{2+} uptake in lymphocytes. The MagT1 gene is located on the X chromosome and genetic ablation of MagT1 function causes abnormal T cell function (X-MEN syndrome) in humans. To elucidate the role of Mg^{2+} transport in platelets, we analysed a knock-out mouse line of MagT1 ($Magt1^{-/-}$). In the tMCAO model of ischemic stroke, $Magt1^{-/-}$ mice develop an increased brain infarct volume. Furthermore, a faster occlusion time of mesenteric arteries and shorter bleeding time were observed in $Magt1^{-/-}$ mice. In line with this findings, $Magt1^{-/-}$ platelets were hyperresponsive to platelet agonists *in vitro* and a strongly enhanced Ca^{2+} influx and ATP secretion were observed after platelet activation. Since $[Mg^{2+}]_i$ was nearly normal in resting $Magt1^{-/-}$ platelets, we assume that Mg^{2+} transport or downstream signalling of MagT1 antagonizes Ca^{2+} responses during platelet activation. Altogether, our *in vivo* results indicate that altered MagT1 function in males may be a risk factor for thrombo-inflamatory diseases.

Transient receptor potential melastatin-like 7 channel (TRPM7) is a ubiquitously expressed bi-functional protein comprising a TRP channel segment linked to a cytosolic α-type serine/threonine protein kinase domain. To elucidate the role of TRPM7 in platelets, we analysed a megakaryocyte- and platelet-specific knock-out of TRPM7 ($Trpm7^{PF4Cre}$) mouse line. Here we report that $Trpm7^{PF4Cre}$ mice develop macrothrombocytopenia due to accelerated turnover of platelets and impaired platelet production by megakaryocytes. $Trpm7^{PF4Cre}$ platelets displayed altered Mg^{2+} homeostasis, increased content and aberrant organization of microtubules, which was accompanied by an abnormal distribution of cell organelles. These results suggest TRPM7 as a key regulator of Mg^{2+} homeostasis and cytoskeletal rearrangements, and point to a possible involvement of TRPM7 in platelet disorders.

O-23
GENOTYPE-PHENOTYPE CORRELATIONS IN *DROSOPHILA COL4A1* MUTANTS: HUMORAL IMMUNE RESPONSE, MUSCULAR DYSTROPHY WITH VARIOUS PROGRESSION, CENTRONUCLEAR/MYOFIBRILLAR MYOPATHY, AND OPTIONS OF TREATMENT

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Type IV collagenopathy is a multisystemic disease associated with mutation of the *COL4A* gene. Patients present with severe vascular, ocular, renal, cerebral, and muscular complications. Mouse models recapitulate the symptoms, though the limitations in mouse genetics hamper large-scale experiments that would decipher the molecular mechanism involved in the disorder. Therefore, *Drosophila* mutants carrying mutations in the homologous *col4a1* gene offer a tractable genetic model of type IV collagenopathy.

In our *Drosophila* model with type IV collagen mutations, we have observed *en gross* cell degeneration following cell detachment from the basement membrane and hypothesized that *col4a1* mutants may suffer from chronic inflammation and manifest an immune response. We have observed overexpression of immune reaction-associated genes *Metchnikowin, Diptericin, Diptericin B, Attacin A, Attacin C* and *edin,* the enhanced functions of which collectively result in intestinal dysfunction and a shortened life-span of the mutants.

The onset of muscular dystrophy, as the dominant phenotype in these mutants, is either rapidly or slowly progressing, depending on the position of the mutation within the COL4A1 protein. Dimorphism in muscular dystrophy progression is supported by biochemical evidence. In mutants with rapidly progressing muscular dystrophy, proteolytic cleavage of the myofibrillar proteins myosin heavy chain, actin, and tropomyosin was observed. A more severe manifestation of the recessive phenotype was observed in double mutant trans-heterozygotes with myofibrillar/centronuclear myopathy, SR-stress, and lack of sarcomeres. These results confirm role of type IV collagen in muscle differentiation and sarcomere formation.

In a translational study aimed at conformational correction of the mutant COL4A1 protein we used osmolytic treatment resulting in extra- and intercellular hyperhydration. The treatment proved successful as it prolonged the otherwise 20% shorter life span of the mutants to levels comparable with wild-type animals and exerted beneficial effect on cellular and molecular markers.

The markers discovered by the above studies generated a well-established basis for large-scale, high-throughput drug screening tests and are anticipated to significantly contribute to the development of novel treatment options for this disorder.

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O-24
CONSEQUENCES OF LOSS OF RETINOL SATURASE ENZYME IN MICE

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Retinoids acting via retinoid (RAR) and retinoid X (RXR) receptors have been reported to modulate several functions of mononuclear phagocytes, such as proliferation and phagocytosis of Fc-opsonized particles or yeast cells. Previously we have found that retinoids also enhance clearance of apoptotic cells by upregulating several phagocytosis-related receptors co-receptors and bridging molecules such as Mertk, CD14, Tim 4, TG2 and C1qb.

We have also reported that macrophages express retinoic acid producing RALDH enzymes and are capable of producing retinoids. Indeed, with HPLC-MS technique and using RARE lacZ transgenic mice, in which the lacZ expression is dependent on endogenous retinoid production, we detected the presence of RA in apoptosing thymus and peritoneal macrophages of LXR agonist injected mice, respectively. Based on the MS-settings specific for this peak, it seems to be a dihydro-RA derivative. Production of dihydro-RAs requires the retinol saturase (retsat) enzyme. In the apoptosing thymus and in bone marrow macrophages, following LXR agonist or ATRA treatment, we detected the expression of retsat enzyme. Using knock out mouse strain we found that retsat deficient mice develop splenomegaly at old age and are positive for anti-nuclear antibodies indicating development of autoimmune disease. Autoimmune disease might be a consequence of impaired apoptotic cell clearance therefore we tested the in vitro phagocytic capacity of knock out macrophages but there was no difference between wild type and KO cells. We also compared the gene expression of phagocytosis-related genes and found decreased expression of the PS receptor Tim4 in KO macrophages following LXR ligation and in apoptosing thymus. In the spleen of the KO mice we detected increased number of active caspase 3 positive cells indicating that clearance of dead cells might be delayed in these mice.

Our result show that and dihydro-retinoids might be involved in the regulation of phagocytosis and loss of retsat enzyme leads to development of autoimmune disease which might be the consequence of decreased in vivo phagocytic capacity of macrophages.

O-25
REGULATION OF CELL CYTOTOXICITY BY CYSTEINE CATHEPSINS

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Cysteine cathepsins are lysosomal peptidases involved at different levels in the processes of the innate and adaptive immune responses. Some, such as cathepsins B and L are expressed constitutively in most immune cells. In cells of innate immunity they play a role in cell adhesion and phagocytosis. Other cysteine cathepsins are expressed more specifically. Cathepsin X promotes dendritic cell maturation, adhesion of macrophages and migration of T cells. Cathepsins S and F are implicated in major histocompatibility complex class II antigen presentation, whereas aminopeptidases cathepsins C and H, expressed in cytotoxic lymphocytes (CTLs) and natural killer (NK) cells, are involved in processing progranzymes into proteolytically active forms, which trigger cell death in their target cells. Cathepsin C has been proposed as the main protease to generate active granzymes from their precursor forms by proteolytic cleavage of the N-terminal dipeptide. In cathepsin C-null mice and patients with Papillon-Lefèvre syndrome lacking expression of cathepsin C, cathepsin H has been suggested as an alternative pro-granzyme B convertase. Cathepsin W is expressed predominantly in NK cells, however, it is not localized in secretory granules, like cathepsins C and H, but mainly in the endoplasmic reticulum, suggesting a role in ER-resident proteolytic machinery.

Like in other cells, the activity of cysteine cathepsins in immune cells is controlled by endogenous cystatins, cysteine protease inhibitors. Of these, cystatin F is the only cystatin that is localized in endosomal/lysosomal vesicles and thus able to bind progranzyme convertases cathepsins C and H. It is produced and translocated to vesicles as an inactive disulphide-linked dimer. After proteolytic removal of its N terminal peptide by cathepsin V, cystatin F becomes a monomer and a potent inhibitor of cathepsins C and H with the potential to regulate progranzyme processing and cell cytotoxicity. We demonstrated that the level of active cystatin F in NK cells is related to lower expression and activity of cathepsins C and H and impaired NK cytotoxic function. Target cells may secrete inactive dimeric cystatin F which after internalization enter endosomal/lysosomal vesicles and after activation inhibits cathepsins C and H and down-regulates cell cytotoxicity. Due to the specific roles of cathepsins C, H and V and cystatin F in regulating cell cytotoxicity, they are potential therapeutic targets for improving anticancer cell therapy or impairing cytotoxicity in immune disorders.

Keywords: *proteases, cell cytotoxicity, cathepsins*

O-26
PROTEIN PHOSPHATASE 4 INTERACTS WITH CENP-C AND REGULATES KINETOCHORE INTEGRITY

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The cyclical nature of pathways regulating Eukaryotic cell division requires tight coupling between protein phosphorylation and dephosphorylation driven by the antagonistic actions of protein kinases and phosphatases. Although roles of the multiple kinases are largely understood, the functions and substrates of protein phosphatases (PPase) are for the most part mysterious. To fully understand mitotic progression it is necessary to comprehend PPase functions, one of the major remaining challenges in the field of mitosis. Protein Phosphatase 4 (PP4), a ubiquitous Ser/Thr phosphoprotein phosphatases that belong to the PP2A-type of PPases was recently identified as an important regulator of cell cycle. PP4 often functions as a heterotrimeric complex consisting of one evolutionarily conserved catalytic subunit (PP4c) that associates with a structural regulatory subunit 2 and a variable regulatory subunit 3. We found that the conserved EVH1 domain of the regulatory subunit 3 of *Drosophila* PP4, Falafel, directly interacts with the key centromeric protein CENP-C (Lipinszki et. al, 2015, Nat. Commun. 6:5894). Unlike other known EVH1 domains that interact with Pro-rich ligands, the crystal structure of the Falafel amino-terminal EVH1 domain bound to a CENP-C peptide, called FIM (Falafel-interacting motif), revealed a new target-recognition mode for PP4. This mode of targeting PP4 to its substrate(s) appears very different to other Ser/Thr protein phosphatases such as PP1 and PP2A. We also showed that binding of Falafel to CENP-C is required to bring PP4 activity to centromeres in order to maintain CENP-C and attached core kinetochore proteins at chromosomes during mitosis.

Keywords: *Mitosis, Protein Phosphatase 4, CENP-C, crystal structure, Drosophila*

O-27
IN SITU FUNCTIONAL ANALYSIS OF DIFFERENT *CIS*-ACTING ELEMENTS IN THE *BXD* REGULATORY REGION

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During embryonic development, proliferating cells are getting committed to different cell fates to create different tissues. This process is regulated by epigenetic factors generating tissue- specific gene expression profiles maintained during the life of a cell and transmitted to its descendants by modifications of higher order chromatin structure.

To study the process of epigenetic gene regulation, the homeotic bithorax-complex (BX-C) of Drosophila is an excellent model-system. Subtle alterations of the chromatin structure of BX-C results in easily detectable segmental transformation. The three genes of BX-C are regulated by nine large, segment-specific *cis*-regulatory regions. The appropriate active or inactive conformation of these regulatory regions is maintained by the TRITHORAX or POLYCOMB group of proteins, binding to specific elements in the regulatory regions, called Trithorax- or Polycomb-Response-Elements (TRE or PRE), respectively.

We studied *in situ* the role and the functioning of *bithoraxoid* (*bxd*) *cis*-regulatory region of BX-C. This regulatory region is responsible for the correct expression level of the *Ultrabithorax* homeotic gene (*Ubx*) in parasegment 6 of *Drosophila*. Five DNA fragments in *bxd* were identified previously bearing embryonic enhancer activity using transgenic assays. In our experiments, two of these fragments, S1 and S2, straddling the neighboring *bxd* PRE, were deleted using an enhanced form of gene conversion developed by our group. We also generated several other deletions, which remove additional regulatory elements in the *bxd* region. Changes in gene expression patterns in embryos and larvae were followed using immuno-histochemistry and native GFP fluorescence combined with high resolution confocal microscopy. The adult mutant phenotypes were scored and a sensitive functional analysis for flying capability was also performed. We found that S1 and S2 have significant roles in the regulation of the expression of *Ubx*. Our results suggest that S1 is a part of the *bxd* PRE and S2 cooperates with the PRE and neighboring late enhancers. We try to understand the mechanism of this cooperation, hereby to answer how maintenance elements and enhancers can affect chromatin structure and functioning of regulatory regions. We hope our experiments will contribute to the understanding of the general and the specific roles of different *cis*-acting elements.

O-28
DEVELOPMENTAL AUTOPHAGY IS REQUIRED FOR EYE PATTERNING IN *DROSOPHILA*

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Autophagy (cellular "self-eating") is a highly conserved, lysosome-mediated, self-degradation process of eukaryotic cells. As a main route of eliminating superfluous (damaged, non-functioning or harmful) cytoplasmic constituents, autophagy is required for maintaining cellular homeostasis. It also provides energy for the survival of cells under starvation and it is also implicated in other stress responses. The core mechanism of autophagy involves more than 30 ATG (autophagy-related) proteins, which are evolutionarily conserved from yeast to mammals, forming four distinct protein complexes to execute autophagy. Exploring the function of autophagy in animal development is still in the initial phase. Autophagy has a role in cellular differentiation and, via or independently of apoptosis, in cell death, thereby removing, for example, larval tissues during metamorphosis in the fruit fly *Drosophila melanogaster*.

The compound eye of *Drosophila* is one of the most intensively studied and best understood model organs in the field of developmental biology. This organ, together with other external parts of the head, develops from a larval primordium called eye-antennal imaginal disc. Imaginal discs are epithelial bilayers; in the case of eye-antennal imaginal disc one layer is the disc proper, which is built up from columnar cells and gives rise to the retina. Cells of the disc proper divide, grow, and then undergo differentiation into photoreceptors and accessory cells. The border between the dividing and differentiating cells is marked by the morphogenetic furrow (MF) that progresses from the posterior to anterior direction within the disc. The putative role of autophagy in *Drosophila* eye development is hardly unknown and contradictory.

We examined the accumulation of autophagic structures in the eye disc, and found a characteristic pattern, especially within and behind (i.e. in the differentiation zone) the area of the MF. We further demonstrate that downregulation of *Atg* genes in the developing eye tissue by a driver being also active in the MF can severely compromise eye patterning in adult animals through enhancing apoptotic cell death. Moreover, we identified two binding sites of the HOX protein Labial in the genomic region of the key autophagic gene *Atg8a*. We detected the expression of *labial* in columnar cells in front of and within the MF in the eye disc, and found alterations in *Atg8a* expression in these cells in genetic backgrounds defective for *labial*. These data may reveal a novel developmental function of autophagy: its death-suppressing role is essential for most differentiating cells in the *Drosophila* eye disc to survive, thereby acting as a prerequisite for eye morphogenesis. Since this live-or-die cell fate decision is likely to occur in several cell types during development, autophagy may play a more fundamental role in tissue patterning than previously thought.

Keywords: *autophagy, eye development, HOX/Labial, transcriptional control, Drosophila, cell death, pattern formation, differentiation*

O-29
ELABORATION AND APPLICATION OF AN *IN VIVO* ASSAY TO DETECT AND QUANTIFY CHROMOSOME LOSS

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An assay - to detect the *in vivo* loss of $mwh^{+}Y$, a genetically engineered *Y* chromosome - in cells of the *Drosophila* wing primordia was published recently (G3: Genes, Genomes, Genetics **2**, 1095, 2012.) Loss of the $mwh^{+}Y$ chromosome in any of the wing disc cells - in a *multiple wing hairs* homozygous background - leads to the formation of an *mwh* mosaic spot (clone) in the emerging wing. The frequency and the size of the *mwh* clones enable the detection and quantitative evaluation of genetic and/or agents to induce chromosome loss. Using this novel technique, we analyzed the effects of loss- and gain-of-function *lodestar* mutant alleles, known mutagens as well as a few environmental agents (Mutation Research **763**, 18, 2014.). The relationship between aneuploidiy and cancer formation, principles of the technique and its application in screening potential chromosome-loss-inducing environmental agents will be discussed.

O-30
CHARACTERIZING THE ROLE OF THE NM23 HOMOLOG NDK-1 IN CELL MIGRATION AND APOPTOTIC ENGULFMENT

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Abnormal regulation of cell migration and altered rearrangement of the cytoskeleton are fundamental properties of metastatic cells. In this project we investigated the role of NDK-1, the *Caenorhabditis elegans* homolog of the first described metastasis inhibitor NM23-H1 in these processes. We overexpressed worm NDK-1 and human NM23-H1 and H2 in MDA-MB231T, an invasive breast carcinoma cell line. Overexpression of NDK-1, similar to its human counterparts, significantly suppressed the migratory potential of MDA-MB231T cells, showing that the nematode serves as a tractable model system to monitor the effects of the *nm23* gene family.

Next we examined the function of NDK-1 in distal tip cell migration in *C. elegans*. During larval development the gonadal leader cells, called distal tip cells (DTCs), guide the migrating gonad arms

which eventually form two symmetric U-shaped tubes. We found that *ndk-1* loss of function mutants display DTC migration defects. Epistasis analysis using mutants of the α-integrin *ina-1* and its downstream functioning motility-promoting signaling module (referred to as CED-10 pathway) suggests that NDK-1 acts downstream of CED-10/Rac.

As DTC migration and engulfment of apoptotic corpses are analogous processes, both partially regulated by the CED-10 pathway, we investigated defects of apoptosis in *ndk-1* mutants. Embryos and germ cells defective for NDK-1 showed an accumulation of apoptotic cell corpses. NDK-1::GFP is expressed in gonadal sheath cells, specialized cells for engulfment and clearence of apoptotic corpses in germ line, which indicates a role for NDK-1 in apoptotic corpse removal. Epistasis analysis based on apoptotic phenotypes confirmed that *ndk-1* acts downstream of *ced-10*. In addition to the CED-10 pathway, engulfment in the worm is also mediated by the CED -1 pathway. Furthermore, NDK-1 shows a genetic interaction with DYN-1/dynamin, a downstream component of the CED-1 pathway.

In summary, we propose that NDK-1/NDPK represents a converging point of CED-10 and CED-1 pathways in the process of cytoskeleton rearrangement We first linked an NDPK, NDK-1 to apoptotic engulfment and corps removal in *C. elegans*. In ongoing experiments we monitor NDPKs' function in a human monocyte cell line to see whether the apoptotic function ascribed to NDK-1 is conserved in human.

Keywords: *NDK-1, apoptotic engulfment, cell migration*

O-31
DEVELOPMENTAL PLASTICITY AND REGULATION OF BLOOD CELL DIFFERENTIATION IN *DROSOPHILA MELANOGASTER*

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As a result of conservation and convergent evolution, the hematopoiesis of the fruit fly *Drosophila melanogaster* and that of vertebrates show striking similarities, thereby making *Drosophila* an excellent model organism to study the development of the immune system.

The blood cells (hemocytes) of the *Drosophila* larva are localized in separate hematopoietic compartments: the lymph gland, the sessile tissue, and the circulation. In these compartments, besides prohemocytes, three effector hemocyte types can be found: the phagocytic plasmatocytes, the melanizing crystal cells and the capsule forming lamellocytes.

Using a transgenic *in* vivo lineage tracing system, we found that although the lymph gland differentiates in separation from the other two compartments, the effective immune response requires the concerted action of all three hematopoietic compartments. Our experiments also revealed that plasmatocytes, which were previously believed to be terminally differentiated blood

cells, are capable of transforming into non-phagocytic, but encapsulating hemocytes, the lamellocytes. Our transgenic system also enabled us to reprogram crystal cells that do not convert into lamellocytes upon immune induction, which implies that crystal cells may lack certain elements of the signal transduction pathways that are necessary for lamellocyte differentiation.

To analyse the development and the function of hematopoietic compartments, we developed an *in vivo* immunostaining and confocal video microscopy method, by which we observed that the hemocytes of the sessile tissue and those of the circulation are in dynamic steady state, and the mobilization of hemocytes does not initiate lamellocyte differentiation by itself. We discovered that upon immune induction, hemocytes contact each other with special structures, which may be the key to the communication during non-cell-autonomous differentiation events. Our latest results imply that hematopoietic compartments interact with each other during their development, and also in the course of immune response. Our next goal is to shed light on this crosstalk, which may be the basis of the concerted action of the hematopoietic compartments.

This research was supported by grants from the Hungarian Science Foundation, OTKA grant NK 101730 (IA), TÁMOP 4.2.2.A-11/1KONV-2012-0035 (IA). This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4.A/2-11-1-2012-0001 'National Excellence Program' (for VH and GIV).

Keywords: *Drosophila, hematopoiesis, differentiation, plasticity*

O-32
MEMBRANE LIPID-THERAPY IN OPERATION: THE MODE OF ACTION OF THE MEMBRANE INTERACTING STRESS PROTEIN CO-MODULATORS

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Aging and pathophysiological conditions are linked to distinct membrane changes which modulate membrane-controlled molecular switches, causing dysregulated heat shock protein (HSP) expression. Modifying the plasma membrane ultrastructure by chemical compounds a selective remodeling of the HSP expression could be achieved. HSP co-modulators are small molecules which do not induce the classical heat shock protein response *per se*, but rather amplify or silence the expression of heat shock proteins, induced by mild physical or pathophysiological stresses. These co-modulator molecules are unique drug candidates because they may regulate HSP expression in diseased cells, without significantly affecting their healthy counterparts. Using *in vitro* molecular dynamic simulation, experiments with lipid monolayers, ultrasensitive fluorescence microscopy on giant unilamellar vesicles and live cells we demonstrate examples how these co-modulator compounds interact with lipids and how they alter the organization of cholesterol-rich membrane domains (rafts) and related signaling pathways. According to our state of the art lipidomics data the effect of a co-modulator molecule depends on the initial membrane lipid composition.Since a deregulated heat shock response (HSR) is found in a large number of important

diseases, our observations offer new opportunities for clinical interventions and innovative therapies. The lipid-specific refinement of HSR could help us to understand why a small subpopulation of heterogeneous cells could determine the outcome of important disease states.

O-33
DEPLETION OF ENDOPLASMIC RETICULUM CA2+ PROVOKES IMPORT OF REDUCTANTS FROM THE CYTOSOL

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The endoplasmic reticulum (ER) lumen is separated from the cytosol by a continuous membrane network that isolates a microenvironment resembling the extracellular milieu. The most notable differences between the ER luminal and the cytosolic microenvironments are the higher Ca^{2+} concentration and the more oxidized redox poise, which are both indispensable for the proper functioning of the organelle. Luminal redox poise of the ER is known to affect Ca^{2+} fluxes across the ER membrane by both Ca^{2+} pumps and channels; however, an opposite relationship has not been explored in detail. Recent observations revealed that depletion of Ca^{2+} from the ER resulted in an immediate reductive shift in the ER, but the underlying molecular mechanism remained unclear.

We hypothesized that the reductive shift could be due to a change in ER membrane permeability, for example through the opening of a pore. One possible candidate is the Sec61 translocon peptide channel, which was proven to act as a non-specific pore besides cotranslational peptide translocation.

By using an ER luminal fluorescent redox sensor we investigated if a transport process underlies the rapid reduction of the ER lumen, which could be linked to Ca^{2+}-dependent opening of the Sec61 channel. We found that translocon opener puromycin provoked the same reductive shift in the ER lumen as ER Ca^{2+} depletion, which can be prevented by anisomycin. On the other hand, plugging the Sec61 channel with anisomycin could not limit the effect of Ca^{2+} depletion by the Ca^{2+} pump inhibitor thapsigargin. Silencing of Sec61alpha also did not prevent the reductive shift in response to Ca^{2+} release, indicating that opening of the translocon does not directly participate in this process.

To assess if ER luminal Ca^{2+} depletion increases the import of reductants we pharmacologically decreased cellular glutathione which potently hindered the reducing transition seen after ER Ca^{2+} depletion.

Based on these results, we postulate that Ca^{2+} depletion opens a so far unknown pore in the ER membrane, which allows the permeation of reducing compounds from the cytosol. The resulting luminal redox shift can serve as a feedback mechanism, which promotes the refilling of Ca^{2+} stores; moreover, it can promote the elimination of misfolded proteins.

Keywords: *endoplasmic reticulum, calcium, glutathione, translocon*

O-34
IN SILICO AND *IN VITRO* VALIDATION OF ISOFORMS OF ORGANIC ANION TRANSPORTING POLYPEPTIDES

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Organic Anion Transporting Polypeptides (OATPs) are transmembrane proteins that mediate the uptake of large, negatively charged or amphipathic molecules. Among their important physiological functions, OATPs transport various endogenous compounds across the plasma membrane, such as hormones and bile acids, and they are also involved in the uptake of several xenobiotics, including anticancer, antiviral and cholesterol-lowering drugs. In addition, the altered expression of some OATPs in tumors suggests that these proteins may also play an important role in pathological processes. The 11 known human OATP proteins are encoded by a family of genes termed SLCO (for Solute Carrier family of the OATPs). Based on *in silico* data, most of these genes code for multiple transcript variants resulting in various potential OATP isoforms. In the case of SLCO3A1, alternative splicing has been shown to result in at least two functional proteins that localize differently in normal tissues. In addition, the presence of two truncated isoforms of OATP1B3 has also been demonstrated in cancer tissues. Putative isoforms of other OATPs are not yet characterized.

One of the least characterized organic anion transporters is OATP6A1. Of the 9 splice variants of SLCO6A1 present in the AceView database, three correspond to isoforms that are likely to be functional based on size. To validate these OATP6A1 isoforms, we analyzed the presence of SLCO6A1 transcript variants in cDNA obtained from healthy tissues.

Our analysis revealed the presence of at least two isoforms of OATP6A1 in the testis and only one version in the liver. When we determined the topology of these two isoforms by prediction methods, we found that the shorter isoform, lacking 47 amino acids, is predicted to form a protein with only 10 transmembrane helices. It is generally accepted, though not proved experimentally, that OATPs need 12 transmembrane helices to become functional. To test the activity of the shorter isoform, we have expressed both the shorter version and the full length OATP6A1 protein in insect and human cell lines and analyzed their expression and function. Our results help elucidate the structure-function relationships of OATPs and may allow the prediction of other functional OATP isoforms..

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O-35
DISSECTION OF THE CATALYTIC CYCLE OF PGP USING WALKER A MUTANTS

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P-glycoprotein (Pgp) belongs to the family of ATP-binding cassette proteins (ABC transporters), which bind and hydrolyze ATP to catalyze the translocation of their substrates through membranes. The basic archiecture of ABC transporters is highly conserved, consisting of two homologous halves each built from a transmembrane domain (TMD) and a cytosolic nucleotide binding domain (NBD). The exact molecular mechanism linking ATP binding and/or hydrolysis to conformational changes resulting in substrate transport is not known. Based on crystal structures of the mouse Pgp and bacterial transporters a universally accepted model suggests that the association and dissociation of the NBD dimer interfaces propagates to the TMDs switching them from the high substrate affinity inward facing conformation to a low substrate affinity outward facing conformation, however, the exact sequence of the conformational snapshots is unknown, yet. To analyze the relation of ATP binding and hydrolysis to the conformational switch of the TMDs we exploited the unique property of a conformation sensitive anti-Pgp antibody (UIC2) recognizing a complex extracellular epitope. Various pre- and post-hydrolytic catalytic intermediates of Pgp were stabilized and characterized in terms of their UIC2-reactivity, nucleotide and substrate affinity and the rates of the formation and dissociation of the above intermediates were also determined in case of wild-type and Walker A mutant Pgps.

Our results suggest that single Walker A mutations allow substrate stimulated ATP hydrolysis and residual substrate transport, while mutations of the key Walker A lysines at both NBDs stabilize Pgp in the inward open, substrate binding conformation. We have found that nucleotide binding is sufficient to switch Pgp from the high substrate affinity inward facing to the low substrate affinity outward facing conformation in both wild-type and single Walker A mutant Pgp variants. Pgps trapped in the post-hydrolysis state by phosphate analogues are still in the low drug affinity conformation, while release of nucleotides resets them to the inward open conformation.

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O-36
ROLE OF THE N-TERMINAL TRANSMEMBRANE DOMAIN IN THE ENDO-LYSOSOMAL TARGETING AND FUNCTION OF THE HUMAN ABCB6 PROTEIN

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ABCB6, a member of the ATP-binding cassette (ABC) transporter family, was first identified as a mitochondrial regulator of cellular porphyrin biosynthesis. Recently, we and others have shown that ABCB6 is a glycoprotein present in the plasma membrane and the endo/lysosomal compartment, and also demonstrated that ABCB6 function is not required for de novo heme biosynthesis. Using a variety of imaging modalities including confocal and electron microscopy we confirm the endo-lysosomal localization of ABCB6 and show that the protein is internalized from the plasma membrane through endocytosis, to be distributed to multivesicular bodies and lysosomes. In addition to the canonical nucleotide binding (NBD) and transmembrane domains (TMD), ABCB6 contains a unique N-terminal transmembrane domain (TMD_0), which does not show sequence homology to known proteins. We investigated the functional role of these domains through the molecular dissection of ABCB6. We find that the folding, dimerization, membrane insertion and ATP binding/hydrolysis of the core ABCB6 complex devoid of TMD_0 is preserved. However, in contrast to the full-length transporter, the core ABCB6 construct is retained at the plasma membrane, and does not appear in Rab5-positive endosomes. TMD_0 is directly targeted to the lysosomes, without a passage to the plasma membrane. Collectively, our results reveal that TMD_0 represents an independently folding unit, which is dispensable for catalysis, but has a crucial role in the lysosomal targeting of ABCB6.

O-37
THE EFFECT OF LIGAND STIMULATION AND ERBB2 COEXPRESSION ON THE HOMODIMERIZATION OF ERBB3

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Dimerization or the formation of higher-order oligomers is required for the activation of ErbB receptor tyrosine kinases. The heregulin receptor ErbB3, a kinase-deficient receptor, must heterodimerize with other members of the family, preferentially ErbB2, to form a functional signal transducing complex. Previously we have characterized the homoclustering of ErbB1 and ErbB2 using quantitative biophysical techniques and we have shown that ErbB1 is largely monomeric in the absence of activation and at low expression levels, and it undergoes ligand-induced homoassociation and heteroclustering with ErbB2. To the contrary, ErbB2 forms large homo-

oligomers in quiescent state from which it is removed to form heteroclusters with EGF-activated ErbB1. Here, we applied single molecule imaging capable of detecting long-lived and mobile associations for determining the size of ErbB3 homoclusters in order to extend the quantitative model outlined above. ErbB3 was found to be almost exclusively monomeric in cells expressing it as the only member of the ErbB family, whereas ~10% of the protein formed homodimers if ErbB2 was coexpressed. The process of heregulin-induced formation of ErbB3 homodimers in the absence of ErbB2 coexpression was inhibited if ErbB2 was also present in the membrane due to the formation of ErbB2-3 heterodimers. In conclusion, ErbB3 resembles ErbB1 in that it is largely monomeric in quiescent cells and ligand binding initiates two competitive processes, the formation of homodimers and heteroassociations with ErbB2.

Keywords: *receptor tyrosine kinase, receptor dimerization, single molecule fluorescence detection*

O-38
HOW DO GENES JUMP: INSIGHTS FROM CRYSTAL STRUCTURES AND MORE…

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Transposons constitute a large fraction of modern genomes and play important roles in evolution, adaptation, and disease development. They can autonomously move around in and between genomes and provide attractive genetic engineering tools. The movement of transposons is generally catalyzed by transposase enzymes that are encoded on the elements themselves. However, their molecular mechanisms are generally poorly understood.

In this talk, I will present novel insights into the mechanisms of two transposons: the popular vertebrate genetic engineering tool, *Sleeping Beauty* (SB), and a vancomycin resistance conferring conjugative transposon (CTn).

For *Sleeping Beauty*, we reconstitute transposon end cleavage *in vitro* and show that the initial assembly steps of transposition follow a distinct pathway, different from better-studied homologues like *Human Mariner 1* or *Mos1*. We also determine the first crystal structure of the SB transposase catalytic domain and use it to design mutants to probe the mechanism of transposition. Our structure also explains previously identified hyperactive mutations in the SB transposase and allows us to engineer further improved variants.

Conjugative transposons (CTns) provide an efficient mechanism to transmit antibiotic resistance genes across bacterial populations, greatly contributing to the spread of antibiotic resistance, one of the greatest health challenges of the 21st century. They often sequester antibiotic resistance or virulence genes and can autonomously move these together with their own DNA sequence from the genome of one bacterium to another. Here, we reconstitute the initial steps of a prototypical CTn and determine the first structure of a CTn integrase – DNA complex. These results provide insights

into long debated questions regarding the mechanism of conjugative transposition, and might, on the long term, help develop novel approaches to fight the spread of antibiotic resistance.

O-39

A NOVEL TRANSGENIC ORGAN MODEL TO SCREEN FOR THE GENETIC DRIVERS OF TUMORIGENESIS

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Cancer is the leading cause of death in the developed world. According to estimates from the International Agency for Research on Cancer, there were 8.2 million cancer deaths in 2012 worldwide and by 2030 the global burden is expected to grow to 13 million deaths simply due to the growth and aging of the population.

Cancer development can be considered as an evolutionary process within our body, driven by genetic instability generating genetic variations and natural selection analogous to Darwinian evolution. Significant emphasis has recently been placed on the characterization of the human cancer genome. However, the genetic complexity of cancer has complicated the identification of the so called "driver" mutations facilitating disease progression, among the more abundant "passenger" mutations found in tumours. To date ~140 "driver" genes with mutations conferring a selective growth advantage to the tumour cells have been reliably identified. Importantly, methods based on mutation frequency alone can only prioritize but cannot unambiguously identify "driver" genes. Accordingly, besides the continuation of tumour sequencing efforts, the scientific world is currently shifting the focus on functional studies to identify "driver" mutations and explore that how they influence the development and progression of cancer.

The harmful "driver" mutations typically occur somatically during cancer development. Consequently, classical transgenic animals, produced by the modification of the germline, are frequently inadequate for the creation/testing of such mutations. Lately we have developed an alternative method for somatic transgenesis in mice, based on an *ex vivo* gene therapy protocol, avoiding the modification of the germline and allowing the rapid *in vivo* analysis of artificially created "driver" mutations. For the efficient chromosomal delivery of transgenes we harness the hyperactive *Sleeping Beauty* transposon system. We rely on large sequence databases of human cancer samples, like the COSMIC collection, to identify recurrent somatic mutations that may affect putative „driver" genes. For modeling loss-of-function mutations affecting mostly tumorsuppressor genes we design artificial microRNAs silencing our candidate genes, based on the structure of the endogenous miR30a and miR155. For modeling gain-of-function mutations typically occurring in oncogenes we overexpress mutant cDNAs. In addition to the candidate gene approach, our mouse model is perfectly suited for carrying out genetic screening procedures.

Keywords: *cancer, cancer „driver" gene, transposon*

O-40
HELRAISER: A RESURRECTED *HELITRON* TRANSPOSON FROM THE BAT GENOME

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Helitron transposons belong to a superfamily of DNA transposons recently discovered by computational analysis of eukaryotic genomes. Unlike the majority of the DNA transposons, which utilize a conservative "cut and paste" mode of transposition, *Helitron* transposons represent a structurally distinct group of mobile elements presumed to employ replicative rolling-circle transposition for their amplification in the host genome. These elements were found in the genomes of organisms belonging to all eukaryotic kingdoms including mammals, where their propensity to capture and shuffle genes might have played an important evolutionary role. However, to date, there is no evidence for an active autonomous *Helitron* transposon in any organism, and all available knowledge about these interesting mobile elements stems from *in silico* analyses. We have resurrected an active *Helitron* element belonging to the *HeliBat1* transposon family found in the little brown bat (*Myotis lucifugus*) genome. We have demonstrated the activity of the reactivated bat *Helitron* element in human cells and, for the first time, we provide an insight into the transposition and gene capture mechanisms, target site properties, transposition efficiency and life cycle of these elements *in vivo*.

O-41
THE MECHANISM OF AGEING: PRIMARY ROLE OF TRANSPOSABLE ELEMENTS IN GENOME DISINTEGRATION

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Understanding the molecular basis of ageing remains a fundamental problem in biology. In multicellular organisms, while the soma undergoes a progressive deterioration over the lifespan, the germline—the cell lineage from which reproductive cells are derived—is essentially immortal. Genomic instability in somatic cells increases with age, and accumulating evidence indicates that the disintegration of somatic genomes is accompanied by mobilisation of mutagenic elements called transposable elements (TEs); when mobilised, TEs can disrupt coding or regulatory sequences. In

contrast, TEs are effectively silenced in the germline by the Piwi-piRNA system. Here we propose that TE repression transmits the persistent proliferation capacity and thereby the non-ageing phenotype of the germline. The Piwi-piRNA pathway also operates in tumorous cell lines and in somatic cells of certain organisms, including hydras, which likewise exhibit immortality. However, in somatic cells lacking the Piwi-piRNA pathway, gradual chromatin decondensation increasingly allows the mobilisation of TEs—mainly self-replicating elements—as the organism ages. This can explain why the mortality rate rises exponentially throughout adult life in most animal species including humans.

Keywords: *Lifespan determination, Age-related diseases, Retroelements, Repetitive sequences, Chromatin relaxation, non-coding small RNAs, Methylation, Cancer*

O-42
SEARCH FOR THE PRIMATE-SPECIFIC FUNCTIONS OF SETMAR, A DOMESTICATED TRANSPOSASE-DERIVED PROTEIN IN APES AND HUMANS

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The *Hsmar1* family of *mariner* transposons invaded an ancestral primate genome about 50 million years ago. Transposition of these elements gave rise to thousands of *Hsmar1*-derived inverted repeats and the SETMAR gene, which originated from the fusion of a pre-existing SET gene to a *Hsmar1* transposase open reading frame. The function of the resulting novel SETMAR is yet enigmatic. The SETMAR protein methylates histone H3 K36 through its SET domain. In addition, using *in vitro* assays we and others showed that SETMAR binds a 19-bp DNA sequence motif located in *Hsmar1* transposon IRs. Many of these sequence motifs are conserved enough in primate genomes to provide hundreds of potential binding sites for the SETMAR protein. Furthermore, a subset of *Hsmar1*-derived MITEs comprises the palindrome regions of the *hsa-mir-548* family of miRNA genes in primates. These miRNA genes may also serve as specific sites for SETMAR binding *in vivo.*

In this study we scrutinized a model, which assumes that SETMAR and its numerous IR-derived binding sites scattered across the genome constitute an anthropoid primate-specific gene-regulatory network.

We created transgenic human cell lines overexpressing SETMAR, or its mutant derivatives deficient in K36 methylation or IR-binding. SETMAR expression was knocked down with siRNAs. We applied ChIP-sequencing to determine the chromosomal binding sites of SETMAR. In parallel, whole transcriptome sequencing was performed to relate the positions of SETMAR binding sites with the genome-wide expression changes in the presence *vs.* absence of SETMAR.

Our results, which derive from an overlay of the genome-wide binding and gene expression data, support the hypothesis that SETMAR has primate-specific functions.

Keywords: *primate evolution, transposon domestication*

O-43
EXCISION EFFICIENCY VERSUS TRANSGENIC RATE: *SLEEPING BEAUTY* AND *PIGGYBAC* TRANSPOSITION IN MAMMALIAN STEM CELLS AND EMBRYOS

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The *Sleeping Beauty* (SB) and *piggyBac* (PB) DNA transposons represent an emerging new gene delivery technology, potentially suitable for human gene therapy applications. Previous studies revealed important differences between these transposon systems, depending on the cell types examined and the methodologies applied. However, efficiencies cannot always be compared due to differences in applications. In addition, "overproduction inhibition", a phenomenon believed to be a characteristic of DNA transposons, can remarkably reduce the overall transgenic rate, emphasizing the importance of transposase dose applied. Therefore, due to lack of comprehensive analysis especially in certain stem cell types, researchers are often forced to optimize the technology for their own "in house" platforms.

We investigated the transposition of several SB (SB11, SB32, SB100X) and PB (mPB and hyPB) variants in different cell types, including human embryonic stem cells (hESCs), at three levels: comparing the *excision efficiency* of the reaction by real-time PCR, testing the overall *transgenic rate* by detecting cells with stable integrations, and determining the average *copy numbers* when using different transposon systems and conditions. We concluded that a high excision activity is not always followed by a high transgenic rate, as exemplified by the hyperactive transposases, indicating that the excision and the integration steps of transposition are not as strongly coupled as previously thought. In general, all levels of transposition show remarkable differences depending on the transposase used and cell lines examined, being the least efficient in hESCs. In spite of the comparably low activity in those special cell types, the hyperactive SB100X and hyPB systems could be used in hESCs with similar transgenic efficiency and with reasonably low (2-3) transgene copy numbers in the generated cell lines.

To optimize the transposon based transgenesis in the laboratory rat, a preferred model for most physiology and toxicology studies, we applied the SB100X system to establish a homozygous rat strain constitutively expressing a calcium indicator protein GCaMP2. We developed an effective and considerably fast methodology for creating a low copy number transgenic rat line by using the hyperactive SB system in a combined genotype/phenotype screening approach. Characterizing the expression of the calcium sensor protein and monitoring calcium homeostasis in several tissues, we provided evidence that this transgenic rat model expressing a calcium-sensitive protein, especially

when combined with other genetically engineered rat disease models, will provide a valuable system for further developmental, pharmacological and toxicological studies.

We believe that our detailed characterizations of the DNA transposons in various mammalian systems will contribute to their efficient use in mammalian transgenesis applications, as well as to their potential applicability for gene therapy purposes in the future.

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O-44
POLY(ADP-RIBOSYL)ATION AS A CENTRAL REGULATOR OF CELLULAR LIFE AND DEATH DECISION MAKING

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Poly(ADP-ribosyl)ation (PARylation) is a reversible protein modification with wide ranging biological functions. Concerted action of PAR polymerase (PARP) and PAR glycohydrolase (PARG) enzymes is responsible for the synthesis and rapid degradation of the polymer, respectively. PARylation has been implicated in the regulation of chromatin structure, replication and transcription, but its classical signaling function comes to light after DNA damage when, depending on the intensity and reparability of the genotoxic injury, it contributes either to cell survival (by enhancing DNA repair) or cell elimination mainly by regulated necrosis. As a dual regulator of cell fate, and a mediator of autophagy, certain forms of apoptosis and necrosis, the role of PARP-1 must be kept under tight control and it must be deeply integrated into complex regulatory circuitries, to ensure precise decision making at critical control points of a cell's life. Here we provide an overview of recent paradigm shifts in our understanding of PAR as a signal, metabolic regulator and structural element formed in physiological and pathological cell death scenarios.

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O-45
DMUSP5 COUPLES UBIQUITIN HOMEOSTASIS TO DEVELOPMENT AND APOPTOSIS IN DROSOPHILA

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Ubiquitylation is a reversible posttranslational modification which uses a small protein, called ubiquitin. An enzyme cascade catalyzes the attachment of ubiquitins to proteins. This covalent modification changes enzymatic activities, facilitates protein-protein interactions or targets proteins to proteasomal degradation. This dynamic process is implicated in many basic cellular functions including cell cycle and cell death. For all these functions, the ubiquitylation requires a pool of free mono-ubiquitins which is maintained most prominently by ubiquitin recycling. Members of the proteases family called deubiquitylating enzymes or DUBs are implicated in ubiquitin recycling since they can remove ubiquitins from target proteins or process polyubiquitins. Although DUBs seem to be indispensable for the maintenance of ubiquitin homeostasis, their precise physiological importance is still poorly understood.

In the genetically well tractable Drosophila model, 46 DUBs have been identified, but the function of most of them is still unknown. One of them is Usp5, an evolutionarily conserved DUB enzyme involved in the disassembly of unanchored ubiquitin chains. Our genetic analysis shows that the Drosophila orthologue of the human Usp5 (DmUsp5) is essential for normal development. A heterologous complementation experiment confirmed functional homology between the *DmUsp5* gene and its yeast homologue, Ubp14. Loss of *DmUsp5* function results in late lethality that is accompanied by the accumulation of unanchored polyubiquitin chains. It also stabilizes p53 and induces a high incidence of apoptosis in larval brains and imaginal discs. In addition to this, the expression of *reaper* and *hid*, but not the *grim,* pro-apoptotic genes becomes elevated in *DmUsp5* mutants. Most importantly, the expression of another, proteasome-associated DUB, *DmUsp14* increased highly in *DmUsp5* mutants. It was shown in the unicellular budding yeast that Ubp6 is expressed and progressively deubiquitylate proteasome-bound substrates at times of ubiquitin depletion. Elevated *DmUsp14* expression together with dominant cycloheximide sensitivity indicates that loss of DmUsp5 cause ubiquitin stress in these animals. These observations suggest that the DmUsp5 DUB enzyme plays a critical role in regulating apoptosis and – together with DmUsp14 - moderating ubiquitin homeostasis in Drosophila.

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Keywords: *ubiquitin homeostasis, deubiquitylation, apoptosis, Usp5, Drosophila*

O-46
LOSS OF THE AUTOPHAGY GENE ATG16 PROMOTES ALCOHOL TOLERANCE IN DROSOPHILA

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The catabolic process of autophagy ensures cellular and organismal homeodynamics via lysosomal degradation and recycling of intracellular material. Autophagy defects lead to the buildup of damaged proteins and organelles, impaired survival of stress and infections, and progressive neurodegeneration. Here we show that Drosophila null mutants of Atg16, an essential autophagy gene, exhibit increased tolerance during alcohol exposure. Corazonin-producing neurosecretory cells have been recently implicated in alcohol-induced sedation in flies. As the selective knockdown of Atg16 or other Atg genes in these cells also increases alcohol tolerance, our data altogether suggest that autophagy is required in corazonin-producing cells for the organismal sedation response to alcohol, likely preventing intoxication this way.

O-47
THE ANALYSIS OF PLANT GENES CONTROLLING BACTEROID DIFFERENTIATION IN NITROGEN-FIXING NODULES OF *MEDICAGO TRUNCATULA*

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Legumes form root nodules to accommodate nitrogen-fixing soil bacteria termed rhizobia. Rhizobia invade nodule cells wherein they are hosted intracellularly in plant derived membrane compartments called symbiosomes. In *Medicago truncatula* nodules, the bacterial partner undergo genome endoreduplication cycles and enlargement process resulting in irreversibly differentiated nitrogen fixing bacteroids that convert atmospheric dinitrogen into ammonia. This terminal differentiation process is directed by the host plant. In order to identify plant genes that control the differentiation of rhizobia, *M. truncatula* symbiotic mutants were identified, characterized and analyzed for bacterial differentiation in their nodules. In one of the ineffective (Fix-) symbiotic mutants, bacteria were released from infection threads and colonized the nodule cells but non-elongated rod shaped of rhizobia could be observed indicating the failure of bacteroid differentiation. In other mutants bacterial differentiation is induced but not completed. The detailed symbiotic phenotype of the deficient nodules and the transcriptome of both partners were analyzed.

One of the most remarkable mutants had a deletion in a single member of the family of the large nodule-specific cysteine-rich (*NCR*) genes indicating the specific role of this peptide in bacterial differentiation. The possible role of the identified plant genes in bacteroid differentiation will be discussed.

Keywords: *symbiotic nitrogen fixation, Sinorhizobium, senescence, bacteroid differentiation, plant mutants*

O-48
UNRAVELING THE FUNCTIONS OF CYTOSKELETAL PROTEINS IN THE NUCLEUS

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The investigation of the cell nucleus in the past decade provided increasing and surprising evidence that homologues, variants or fragments of numerous cytoskeletal proteins are present in the nucleus. These nuclear components include actin, actin-binding and actin-related proteins, crosslinking proteins and more than 10 different myosin and kinesin motor proteins. Our laboratory is primarily interested in the nuclear function of the evolutionarily conserved actin regulating protein, Moesin. Our experiments revealed that in the nucleus Moesin localizes to the nucleoplasm, the transcriptionally active chromosome regions and occasionally to the nucleolus. We also found that the transport of Moesin into and out from the nucleus are regulated processes.

However, nuclear localization does not prove nuclear function, therefore the central question about the existence of cytoskeletal elements in the nucleus today is whether this localization has any biological significance? In other words, is the nuclear localization simply the result of passive engulfment of cytoskeletal proteins during the course of opened or semi-closed mitosis or these components have essential functions in the nucleus during interphase? One possible explanation to this problem is provided by the recent re-discovery of the so-called spindle matrix. The spindle matrix is constituted mainly by nuclear-derived proteins that reorganize during the cell cycle to form an elastic gel-like matrix which serves as a base for the assembly of spindle microtubules and makes a significant contribution to the movement of the chromosomes during mitosis. In accordance with this idea, we found evidence that Moesin has a direct spindle function and is a new member of the spindle matrix.

Based on the spindle matrix model one can speculate that the interphase nucleus simply serves as a storage place for these mitotic skeletal elements. But recent results claim that this is not the case, nuclear cytoskeletal proteins are involved in essential nuclear functions. In the case of Moesin, we found for instance that it participates in the process of transcription and/or in mRNA export. But evidence are mainly indirect today due to a serious technical problem. While most spindle matrix proteins are exclusively nuclear throughout interphase, thereby enabling the investigation of their nuclear function, the cytoskeletal components of the nucleus, for example actin and Moesin, are mostly cytoplasmic with pivotal cytoplasmic roles. Thus, there is no good mean to selectively disrupt nuclear function without disrupting cytoplasmic functions.

We decided to resolve this issue and designed two approaches, and in the case of Moesin and actin we think we are now close to answer the question what is the nuclear function of these cytoskeletal elements in the interphase nucleus.

Keywords: *nucleus, transcription, nuclear transport, Moesin, actin*

O-49
INTEGRATION OF STRESS, ABA AND LIGHT SIGNALING PATHWAYS IN PLANTS

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Plant growth and development are regulated by external environmental factors which are coordinated into internal hormonal signals. One of the most important environmental stimuli is light which controls multiple developmental processes in the life cycle of the plant. Light modulates the balance between hormones such as ABA and GA which are implicated in seed development, transition from dormancy to germination, vegetative to reproductive stage, flowering, seed yield and fertility. Light signals can modulate responses to extreme environmental conditions such as drought or high salinity also. We report data of two C2H2-type zinc finger factor families, which influence ABA signaling and are implicated in light regulation. ZFP3, a nuclear C2H2 zinc finger protein and the closely related ZFP subfamily acts as a negative regulators of ABA- suppressed germination. Analysis of ZFP3ox plants revealed multiple phenotypic alterations, such as semidwarf growth habit, defects in fertility and enhanced sensitivity of hypocotyl elongation to red but not to far-red or blue light. Analysis of genetic interactions with phytochrome *phyA*, *phyB* and *abi* mutants indicates that ZFP3 enhances red light signaling by photoreceptors other than phyA, and additively increases ABA insensitivity conferred by the *abi2*, *abi4* and *abi5* mutations. PhyB-mediated red light signaling and ABI5 was found to be epistatic to ZFP3 in the control of red light-dependent photomorphogenesis. ZFP3 and the related ZFP subfamily of zinc finger factors therefore regulate light and ABA responses during germination and early seedling development. Another group of C2H2 zinc finger factors ZLC1 (At3g46080) and ZLC2 (AT3G46070) are closely related to ZAT7, and were shown to enhance the expression of stress and ABA-induced ADH1-LUC reporter gene. Overexpression of these genes can reduce salt tolerance of transgenic Arabidopsis plants. Recent genome-wide transcript profiling revealed that a number of stress-induced genes are upregulated in phytochrome interacting factor 1 (*pif1)* mutant, including the ZAT7, ZLC1 and ZLC2 zinc finger factors. These results suggest, that the newly identified zinc finger factors are components of important signaling system, which integrate light, stress and hormonal regulation.

O-50
PROTEIN KINASES: ENZYMES WORKING IN SIGNALING BRIGADES

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Protein kinases control diverse cellular processes by playing a major role in cell signaling as intracellular enzymes. In addition to their upstream activators and downstream substrates, they bind to other signaling proteins. Thus, protein kinases rarely work alone, they are normally part of multi-protein signaling complexes where their impact on a specific biological outcome (e.g. cell divison, apoptosis or differentiation) depends on the protein-protein interactions that they form with other dedicated signaling proteins. Using mitogen activated protein kinases (MAPK) as a model system we studied the interactions that govern the assembly of specific MAPK dependent signaling complexes. Our latest results reveal how ERK5, ERK2 and JNK MAPK signaling modules assemble into functional complexes. This set the stage for the discovery of novel compounds that may selectively interfere with specific functions of ubiquitous MAPKs through targeting the interactions rather than the cata lytic capacity of the members of a specific "signaling brigade".

Keywords: *cell signaling, protein kinase, structural biology*

O-51
NOVEL INTERACTIONS AND REGULATORY ROLES OF PROTEIN PHOSPHATASES IN ENDOTHELIAL CELLS

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Myosin phosphatase (MP) consists of protein phosphatase-1 catalytic subunit (PP1cδ) and myosin phosphatase target subunit-1 (MYPT1) and is involved in controlling the barrier function of endothelial cells by regulating the phosphorylation level of the 20 kDa light chain of non-muscle myosin-II. In our recent studies we identified protein phosphatase 2B, termed also calcineurin, as an interacting partner of MP, and the phosphatase that were able to dephosphorylate the phosphatase inhibitory sites (Thr696 and Thr853) in MYPT1 thereby influencing the regulatory function of MP. The goal of our present study was to identify further binding partners of MP in order to gain a better insight of the physiological role of MP in endothelial cells.

Endothelial nitric oxide synthase (eNOS) is responsible for the production of nitric oxide (NO), an important regulator of smooth muscle relaxation and oxidative cellular processes. The activity of eNOS is enhanced by phosphorylation at Ser1177, while increased phosphorylation at Thr495 has an inhibitory influence. It has been shown that PP1c is involved in the regulation of eNOS via dephosphorylation at Thr495, however, the role of the regulatory subunit(s) is unknown yet. Our present results demonstrate the interaction of MYPT1 and eNOS in endothelial cells (EC) by several methods as well as in tsA201 cells following co-expression of both Flag-MYPT1 and myc-eNOS.

Purified myc-eNOS phosphorylated by Rho-kinase at Thr495 was dephosphorylated by PP1c alone, but the extent of dephosphorylation was increased when PP1c was complexed with MYPT1. Upon PKC activation by PMA, and/or inhibition of PP1 and PP2A by calyculin-A (CLA) or tautomycin (TM, phosphorylation of Thr495 was stimulated in both bovine pulmonary endothelial cells (BPAEC) as well as in tsA201 cells transfected with eNOS plasmid accompanying with decreased NO production. Measurements of transendothelial resistance of BPAECs exhibited differential effects of PMA, CLA and TM on the EC barrier function.

In summary, our present data implicate MP in the regulation of NO production via its interaction with and dephosphoryaltion of eNOS as well as suggest the combined roles of PKC, PP1 and PP2A in the control of EC barrier function.

This work was supported by grants from the Hungarian Scientific Research Fund OTKA K109249 and PD104878.

O-52

TNFα REVERSE SIGNALING INHIBITS THE LPS-INDUCED PRO-INFLAMMATORY CYTOKINE FORMATION BY UPREGULATING TGF-β IN MACROPHAGES

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More and more antibodies targeting TNF-α are used in the treatment of certain human chronic inflammatory diseases. TNF-α is a proinflammatory cytokine which is produced in both soluble and membrane bound (mTNF-α) forms in macrophages. Part of the clinically used antibodies only neutralize soluble TNF-α, while others simultaneously trigger mTNF-α as well. In our experiments the effect of mTNF-α crosslinking was studied in macrophages on the proinflammatory cytokine production. We found that in resting macrophages TNF-α is found in secretory vesicles, and a small amount also appears on the cell surface as mTNF-α. Triggering of mTNF-α results in CREB phosphorylation and production of the anti-inflammatory cytokine TGF-β via activation of jun and p38 kinases. Upon LPS stimulation the levels of mTNF-α were transitionally induced: first from the secretory granuli, than as a result of de novo synthesis. Then they were decreased due to metalloprotease activation. Activation of mTNF-α inhibited the induction of many LPS-induced cytokines. This effect was mediated by TGF-β. Interestingly, we have noticed that LPS-induced soluble TNF-α also reduces LPS-induced pro-inflammatory cytokine formation indicating that soluble TNF-α also have a negative feed back effect. Based on these observation we propose that those antibodies are really anti-inflammatory, which can trigger mTNF-α. mTNF-α can also be triggered by TNF receptor bearing cells. Our data indicate that mTNF-α also contributes to the apoptotic cell-induced TGF-β production in engulfing macrophages, thus it mediates partly the anti-inflammatory effect of apoptotic cells.

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through the New Hungary Development Plan cofinanced by the European Social Fund and the European Regional Development Fund.

Keywords: *mTNFα, TGF beta, LPS, macrophage*

O-53
THE ROLE OF HEAT SHOCK TRANSCRIPTION FACTOR HSF-1 IN THE COORDINATION OF CELLULAR STRESS RESPONSES IN *CAENORHABDITIS ELEGANS*

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Protein damage caused by various environmental stresses induces distinct cell protective mechanisms, such as the heat shock response. During heat shock response HSF1 (*heat shock transcription factor 1*) helps to refold or degrade damaged proteins via activation of chaperone gene expression.

The majority of HSF-1 target genes identified so far encode for heat shock proteins (HSPs). However during the last decade HSF-1 has been implicated in fundamental physiological and pathological processes that expand beyond heat shock response. HSF-1 has been shown to influence cell growth and metabolism, development, aging, and immunity as well as carcinogenesis and tumor progression.

Using *in silico* approaches we have identified novel HSF-1 target genes in the nematode *Caenorhabditis elegans* encoding key components of cellular stress pathways. These include necrosis, autophagy, oxidative stress response and the unfolded protein response (UPR). Here we show that HSF-1 is required for effective ER stress response upon protetoxic stress by regulating UPR genes. Our results suggest that HSF-1 is at the hub of cellular stress signaling in *C. elegans*. We aim to confirm these findings using human cell lines.

Keywords: *stress response, heat shock factor, unfolded protein response*

O-54
OVEREXPRESSION OF A HETEROLOGOUS POLYAMINE OXIDASE, INVOLVED IN POLYAMINE CATABOLISM, ACTIVATES BOTH NECROTIC AND VACUOLAR PROGRAMMED CELL DEATH IN TOBACCO

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Plant polyamine (PA) metabolism is highly modulated by endogenous cues and environmental factors, especially those imposing stress. Salt stress in many plant species has been linked to readjustment of PA titers, mostly by changing the levels of putrescine, spermidine and spermine, through activation of PA biosynthetic genes. Transgenic plants overexpressing genes encoding PA-synthesizing enzymes exhibited increased stress tolerance; while supression of their expression exerted opposite effects, in the majority of cases. However, most of the previous studies did not give any conclusive evidence for an underlying mechanism by which PAs exert their tolerance effects.

To gather information about the mechanism by which PAs provide stress tolerance, we intended to deplete PA-pool in transgenic tobacco plants by introducing strawberry polyamine oxidase (*FvPAO*1) under a constitutive CAMV35S promoter, which catabolises (and partly recycles) Spd and Spm. The reaction yields Δ^1-pyrroline and 1,5-diazabicyclononane, respectively, along with 1,3-diaminopropane (1,3-Dap) and hydrogen peroxide (H_2O_2), a nodal point in the reaction of plant cells to stress.

The highest transgene expressor tobacco plants showed a 10x increase in the relative expression of PAO genes (chimaeric and endogenous) and a 150% increase in the activity of the encoded PA-oxidase both in leaves and roots (line T13). Over a 20% loss in chlorophyll level, and a 32% decrease of protein content were detected in T13 plants compared to controls, that was associated with a higher ion leakage and a marked appearance of H_2O_2 in the apoplast (**Figure 1**).

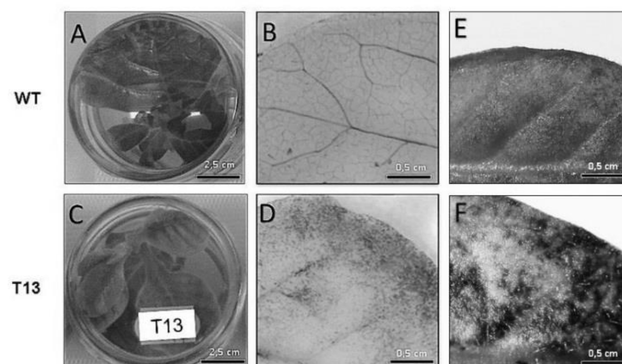

***Figure 1** – Leaf phenotypes (A,C), H_2O_2 distributions (B,D – reddish-brown coloration) and appearance of oxidative stress (E,F – blue coloration) in PAO (C,E,F) and WT control plants (A,B,E).*

The apoplastic H_2O_2 burst coincided with leaf-areas showing oxidative stress identified by methylene blue, a specific redox indicator. Monitoring relative expression levels of the necrotic PCD-specific *TGase* gene, and a vacuolar PCD-specific *SAG*12 gene revealed that increasing catabolic flux of PAs , activates PCD through an elevated ethylene/total-PA ratio and oxidative stress. The symptomes of PCD showed a mixture of normally separated necrotic and vacuolar cell death as was proven by monitoring the expression of specific marker genes (*TGase*, *SAG*12) throughout the progression of PCD.

Keywords: *polyamines, oxidative stress, PCD*

O-55

POTENTIAL ROLE OF SINGLET OXYGEN IN INTRA- AND INTER-CELLULAR SIGNALING EVENTS IN MICROALGAE

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Singlet oxygen ($^1O_2$) is an important reactive oxygen species in plant systems, which is formed mainly during photosynthetic light energy conversion. Due to its high reactivity $^1O_2$ can induce irreversible modifications of cellular components leading to loss of biological functions and damage of living cells. However, $^1O_2$ influences also the expression of a large number of genes in bacteria, plants, and animal systems, which subsequently activate or modify transcription factors and thereby participate in signaling pathways. While the damaging aspects of $^1O_2$ are reasonably well, knowledge has just started to accumulate regarding the role of $^1O_2$ in signaling. Such knowledge is especially scarce in case of cyanobacteria, such as *Synechocystis* PCC 6803, which represents a very useful model system of photosynthetic organisms due its small genome size and natural transformability. $^1O_2$ related studies in microalgal cells were hampered for a long time by the lack of methods allowing detection of intracellular $^1O_2$. We have successfully removed this bottleneck by developing a chemical trapping method based on the measurement of O_2 consumption due to $^1O_2$ induced oxidation of added histidine, and utilized this method in the characterization of $^1O_2$ production and scavenging in various *Synechocystis* 6803 mutants. We have also applied genome wide transcript profiling to identify $^1O_2$ inducible genes in *Synechocystis* 6803 and found a couple of promising candidates, which could be involved in $^1O_2$-mediated intracellular signaling. An important finding of our work is the demonstration that previously unknown metabolites are excreted from cyanobacterial cells, which are capable of extracellular $^1O_2$ production and represent very interesting candidates for intercellular communication. We have also studied $^1O_2$ production in the dinoflagellate *Symbidinium*, which represents the photosynthetic partner in coral symbiosis. Our data show that intracellular $^1O_2$ concentration is regulated by environmental factors, which are known inducers of the expulsion of *Symbidinium* cells from coral tissues, leading to the phenomenon of coral bleaching that threatens important ecosystems worldwide. We have also found extracellular $^1O_2$ sensitizer(s) which are excreted from *Symbidinium* cells. These compounds could play a role in the maintenance or breakup of the symbiotic interaction between the photosynthetic *Symbiodinium* cells and their animal host. In conclusion, our data demonstrate the formation of $^1O_2$ both inside and outside of microalgal cells and indicates the potential of this important reactive oxygen species to participate in both intracellular and intercellular signaling events.

Keywords: *Singlet oxygen, signaling*

O-56
INFECTION OF HUMAN CELLS WITH LENTIVIRAL VECTOR LEADS TO HIGHLY REPRODUCIBLE GENOME-WIDE DNA METHYLATION CHANGES

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Lentiviral vectors (LV) are efficient tools for gene transfer.They are frequently used as vectors *in vivo* and *in vitro* in laboratory experiments and clinical trials. After infection LVs integrate the genome and interact with the chromatin. It is therefore of major importance to investigate theireffect on the DNA methylation of human cells. In the present study we tested in primary human haematopoietic and Jurkat cells the effect of infectionof both wild-type and integration deficient LVs. We used the Infinium Illumina450K BeadChip assay and developed the double average technique (DAT) to detect DNA methylation changes. We demonstrated that independently of the capacity of vectors to integrate soonafterinfection a great number of CpGs undergo DNA methylation increase. These methylation changes revealed to be highly reproducible in preferentially targeted genomic regions.Interestingly, these regions are found in CpG islands of non-expressed genes. The consequences of these epimutations will be discussed.

O-57
SOME POLYCOMB-GROUP PROTEINS CAN MODULATE THE REGULATORY CAPACITY OF ACTIVE CIS-REGULATORS

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Developmental biologists established the Bithorax complex (BX-C) in Drosophila melanogaster as a powerful model system of epigenetic regulation. The protein-coding genes of the BX-C (*Ubx, abd-A, Abd-B*) are sufficient to ensure the normal segmental identity of nine posterior segments of the fruit fly. Each homeotic gene is responsible for the identity of more than one morphologically different segment. It is possible through the action of nine segment specific cis-regulatory regions, responsible for setting the appropriate segment specific level of transcription of the homeotic genes. The advantage of the system is that, even slight alterations of the regulation of BX-C change the appearance of the affected segments.

The activity state of the cis-regulatory regions is set up early in embryogenesis by transiently present morphogens, and maintained throughout development by epigenetic mechanisms. The repressed

state of cis-regulators is mediated by specific DNA regions called PREs (Polycomb Response Elements) where the epigenetic repressor POLYCOMB GROUP proteins act. If the PREs deleted, or one or more POLYCOMB GROUP proteins are mutated, gain of function type homeotic transformations occur: the concerned segments become similar to a segment posteriorly to it.

According to the canonical view, these transformations are the consequence of the ectopic activation of one or more cis-regulatory regions, leading to elevated level of transcription of the homeotic genes in more anterior segments. We have proposed an alternative hypothesis: if PREs retain some silencing function even in the active domains, and at least a subset of POLYCOMB type repressors acts as silencers, the gain of function transformations can be solely the consequence of an increased regulatory capacity of the cis-regulators in their cognate segment. We term this phenomenon "hyper-activation".

Because the phenotypic outcome of the two processes is the same, the presence of ectopic activation of cis-regulators has to be monitored different Polycomb mutant backgrounds to decide between the above two hypothesizes. We used different reporter constructs, integrated into the *iab7* cis-regulator in order to directly monitor the activity state of this cis regulator.

In situ replacement of the *iab7* PRE with the *bithraxoid* PRE resulted in a decrease of the regulatory output of the *iab-7* cis-regulators also suggested modulatory role of different PREs. Our findings explain the apparent paradox that different cis-regulatory domains of the Abd-B transcription unit have greatly different effects on the expression level of Abd-B, despite the fact that each regulatory region harbors enhancers of comparable strength.

We propose that, in addition to being key elements in maintaining the "off" state of cis-regulatory regions, PREs are responsible for the segment-specific regulation level of expression of BX-C genes by modulating the regulatory capacity of active cis-regulatory regions quantitatively.

Keywords: *PRE, Polycomb, chromatin domain, repression, silencer,*

O-58
COMPLEX AUTOREGULATION OF THE KEY TRANSLATION TERMINATION FACTOR: EUKARYOTIC RELEASE FACTOR 1 IN PLANTS

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The expression of the components of the basic gene expression machineries should be strictly controlled. Autoregulatory circuits play critical role in the stabilization of the expression of these genes. Overexpression of the translation termination factors could lead to translation termination at coding codons, while reduced expression of termination factors might result in frequent translational readthrough.

It has been known for decades that the expression of the key prokaryotic translation termination factor is stabilized by an autoregulatory circuit. Although several lines of evidence support that expression of the key eukaryotic translation termination factor (eRF1) is also precisely regulated, the mechanism of eRF1 control has remained unsolved.

Here we show that in plants, a highly complex autoregulatory system evolved to stabilize the expression of eRF1. We found that two additional translation termination coupled events, translational readthrough and Nonsense-mediated decay (NMD), which is a conserved eukaryotic quality control system, play critical role in the eRF1 autoregulatory circuit. Our data suggest that this complex eRF1 autoregulatory mechanism helps the plants to keep the balance between translation termination, readthrough and NMD. The mechanism and the evolution of eRF1 autoregulatory system will be shown.

Keywords: *translation termination, autoregulation, Nonsense-mediated decay, readthrough,*

O-59
THE ROLE OF P53 DURING TRANSCRIPTIONAL BLOCKAGE

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P53 is a well-known tumor suppressor gene, which is affected by inactivating mutations in the majority of the human cancer related diseases. P53 is a sequence-specific transcription factor that preferentially binds to specific sequences of target genes. Under regular conditions, the P53 is present in a low level while following DNA damage, the P53 protein level increases and it blocks the cell cycle prior to S phase allowing repair of the broken DNA.

In addition to its several well-characterised functions, P53 has been recently described to affect transcriptional elongation by a so far less well-understood mechanism. In accord with this function, P53 can be found at the coding regions of actively transcribed genes and physically associated with the RNA polymerase II (RNAPII) elongation complex. It has been shown that the human P53 interacts with RNAPII in *Saccharomyces cerecisiae*. We have also reported recently that P53 localized to actively transcribed gene regions of the Drosophila polytene chromosomes. This observation led us to investigate whether P53 is present at specific gene regions which are not direct targets of it. Specifically we wanted to examine the co-localization of P53 and RNAPII at specific gene regions and the interaction between P53 and RNAPII upon transcriptional elongation blockage.

We found that P53 binds to the regulatory regions of genes, which have not been reported earlier as P53 direct targets. We found that P53 and RNAPII co-localized to the examined regions and this localization was ceased upon transcriptional blockage. P53 interacts, presumably with the serine 5-phosporylated (S5P) form of RNAPII which might implicate that P53 associates with the transcriptionally active form of RNAPII. The block of transcription by actinomycin D strenghtens the interaction between the two proteins. Furthermore we found that the chromatin associated P53 and RNAPII were localised to the same nuclear positions upon transcriptional blockage. High concentration of actinomycin D treatment resulted in an increase in P53 protein level, and in a decrease RNAPII level with a concomittant reduction in the S5 and S2 phoshorylation forms of RNAPII. Finally, our data indicate that the transcriptional blockage-induced RNAPII degradation is regulated by the ubiquitin proteasome system.

These observations highlight a mechanism by which the transcriptional blockage could be resolved upon different kinds of DNA damages. The mechanisms we propose here helps to understand how

the RNAPII is degraded on a transcribed unit in order to allow access for repair proteins. This is most likely a cell defense mechanism to avoid production of truncated or mutated transcripts of essential genes that would endanger the cell viability.

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Keywords: *P53, RNAPII, transcriptional elongation blockage*

O-60
THE CCR4-NOT COMPLEX CONNECTS THE DIFFERENT LEVELS OF GENE EXPRESSION

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Recent studies suggested that a sub-complex of RNA polymerase II composed of Rpb4 and Rpb7 couples the nuclear and cytoplasmic gene expression stages by associating with newly made mRNAs in the nucleus and contributing to their translation and degradation in the cytoplasm. We showed that a subunit of the Ccr4-Not complex, Not5, is essential in the nucleus for the cytoplasmic functions of Rpb4. Not5 interacts with Rpb4; it is required for the presence of Rpb4 in polysomes, for interaction of Rpb4 with the translation initiation factor eiF3. We additionally determined that Not5 is needed in the cytoplasm for the co-translational assembly of RNA polymerase II. This stems from the importance of Not5 for the association of the R2TP Hsp90 co-chaperone with polysomes translating *RPB1* mRNA to protect newly synthesized Rpb1 from aggregation.

Subunits of many multi-protein complexes were identified in aggregates isolated from *not5Δ* cells suggesting that Not5 influences co-translational assembly of many molecular machines. Hence to explore the role of Ccr4-Not, and Not5 in particular, in regulating the post-transcriptional fate of mRNAs at a global level, we identified all mRNAs associated with the scaffold of the complex, Not1, by native RNA immunoprecipitation, in wild type and *not5Δ* conditions.

In my presentation I will summarize our results and discuss the role of the Ccr4-Not complex in regulating the gene expression circuitry of eukaryotic cells.

O-61
THE EFFECT OF HISTONE MODIFICATIONS ON NUCLEOSOME STABILITY AND THE BENEFIT OF PAN-NICKING

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The effect of various posttranslational histone tail modifications (PTMs) on nucleosome stability was compared by exposing agarose embedded nuclei to treatments with salt or intercalator dyes, determining the remaining fraction of histones using PTM specific antibodies and laser scanning cytometry. Steep elution profiles could be measured in nuclei of all phases of the cell cycle by both salt and intercalator treatment in the case of H3K4me3 and H3K27ac marks, while the nucleosomes carrying a number of different other marks were relatively resistant, similarly to bulk histone-GFP. Destabilization of the H3K4me3 marked TSS proximal nucleosomes was uniform along the genome, as revealed by chip sequencing, when doxorubicin was used as the intercalator. Nicking treatments of the nuclei did not affect the stability of nucleosomes carrying H3K4me3 or H3K27ac, while those of the second group were all destabilized. To interpret these results we suggest that the H3K4me3 and H3K27ac active marks specify dynamic nucleosomes accomodating already relaxed DNA sequences, while most other nucleosomes hold the DNA in constrained superhelices. In accordance with this hypothesis, endogeneous nicks were mapped by chip sequencing in the vicinity of active promoters of human peripheral blood lymphocytes, and in several other cell types. In nuclear halos, two topologically isolated chromatin domains were demonstrated in all phases of the cell-cycle; superhelical loops and the nuclear lamina enclosed compartment harboring nicks. Importantly, the latter domain accomodates the sites of in vivo nucleoside analogue incorporation upon transcription as well as replication. These observations lend support for a model where the role of transient nicks in transcriptional regulation and in higher-order chromatin organization are integrated.

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O-62
GENERAL PRINCIPLES OF TRANSCRIPTOMIC CHANGES FOLLOWING SINGLE-GENE DELETION IN *SACCHAROMYCES CEREVISIAE*

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What are the general patterns and principles behind transcriptomic responses following genetic perturbations? Our work is the first to analyse this question in a systematic manner. What gene features determine the extent of transcriptomic changes following a single-gene deletion? How specific are these changes to the deleted gene? Are they mostly adaptive or rather deleterious? A recent systematic dataset, provided by the Holstege lab, allowed us to address these questions on an unprecedented scale. This dataset contains information on genome-wide mRNA expression changes for 1484 viable knock-out strains of *Saccharomyces cerevisiae*. Our results indicate that the number of up- or downregulated genes show strong positive correlation with both the fitness contribution and the degree of pleiotropy of the deleted gene. While we revealed a significant overrepresentation of functional relatedness between the deleted and the responsive genes, this effect is small and most changes do not occur within functional modules. By overlaying the gene expression data on a genetic interaction map of yeast, we demonstrate that most expression changes are unlikely to provide compensation following gene deletion. Our results do not support that gene specific adaptive expression changes are widespread. Next we started to investigate how widespread harmful expression changes are. Our experimental results so far suggest that harmful expression changes can happen following single-gene deletion, meaning that part of the fitness loss can be indirectly caused by expression misregulation.

O-63
THE EVOLUTIONARY POTENTIAL OF UNDERGROUND METABOLISM

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A central unresolved issue in evolutionary biology is how to predict the emergence of innovations for the forecast of evolutionary adaptation. The current hypothesis is that innovations emerge from low-level side-reactions that are enhanced to physiologically relevant level by mutations. Many of these side-activities are known for enzymes, however, their role in adaptation towards novel environments has remained largely unknown, not least because these issues demand analyses at the

level of the entire metabolic network. Here, for the first time we provide a comprehensive computational model of the underground metabolism of an organism (*Escherichia coli*), which allowed us to conduct an integrated genome-wide *in silico* and experimental survey to characterize the evolutionary potential of *E. coli* to adapt to hundreds of nutrient conditions. We estimate that at least ~20% of the underground reactions that can be connected to the existing network confer a fitness advantage under specific environments when their activity is increased. Moreover, the good agreement between the *in silico* and experimental results demonstrate that the genetic basis of evolutionary adaptation is computationally predictable via underground metabolism.

O-64
A COMPREHENSIVE SYSTEMS BIOLOGICAL STUDY OF AUTOPHAGY-APOPTOSIS CROSSTALK DURING ENDOPLASMIC RETICULUM STRESS

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One of the most important tasks of a cellular organism is to maintain its genetic integrity with respect to various stress events. Endoplasmic reticulum (ER) has a crucial role in sensing cellular homeostasis by controlling the synthesis and packaging of secreted and membrane proteins, metabolism and several signalling processes. ER stress results in cytoprotective autophagy-dependent self-eating followed by apoptotic cell death. Our primer goal was to explore the dynamical characteristic of the control network of decision making between life and death with respect to ER stress.

In our comprehensive study both molecular and theoretical biological techniques were incorporated. Using different ER stressors (such as tunicamycin, thapsigargin, DTT) we confirmed that autophagy-dependent survival always precedes apoptotic cell death. When apoptosis turned on autophagy quickly got inactivated suggesting a mutual antagonism between the two mechanisms. We claim that an activation threshold for apoptosis inducer is crucial to guarantee the one-way-directionality of cell death under ER stress. We explored the features of this threshold by introducing both autophagy activators and inhibitors, and transient treatment with excessive level of ER stressor was also performed. Using various methods of mathematical modelling (such as computational simulations, bifurcation analysis) we suggest that the activation profile of autophagy is sigmoid, while the apoptosis inducer has an irreversible bistable characteristic with a particular threshold for its activation with respect to ER stress. We claim that a double negative feedback loop between autophagy and apoptosis inducers has key role in the response mechanism of ER stress.

Since ER stress is tightly connected to diabetes, various liver and degenerative diseases, the long-term significance of our work shows medical purposes.

Keywords: *endoplasmic reticulum stress, autophagy, apoptosis, crosstalk*

O-65
INTRATUMOR HETEROGENEITY IN RENAL CELL CARCINOMA PATIENTS

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Introduction: Next-generation sequencing technologies have provided us the possibility to sequence entire human genomes, which may help diagnostics, research and drug discovery. Tumor sample sequencing is one of the most difficult tasks, since tumors are usually genetically heterogeneous. This heterogeneity may foster the survival of tumor cells, which may be interpreted as an evolutionary process. In the results presented by Swanton et al [1], a heterogeneous tumor had a higher probability to acquire some kind of drug resistance mechanism, which may become a selection marker after a treatment.

Methods and results: Using publicly available datasets deposited in the EGA (European Genome/Phenome Archive) repository, we were able to establish a link between mutation frequency distributions in sequencing datasets and the degree of tumor heterogeneity.

The analysed data contained whole exome sequencing (WES) datasets of eight renal cell carcinoma (RCC) patients. Each patient had a sequenced normal sample, paired with 8 intratumor samples. Somatic mutations where identified using the MuTect algorithm [2], using it's default settings. Since many mutations had either low coverages or had low occurrences, we filtered mutations that had less than 15x coverage either in the normal or tumor samples, and/or the mutations that occurred less than 5x in the tumor sample.

Our analysis was able to split the patients into two groups: 1) homogeneous subgroup, which contained fewer mutations, that had higher frequencies in the sequencing datasets, and 2) heterogeneous subgroup, which contained many mutations, with very low frequencies in the sequencing datasets.

Discussion: The distribution of the somatic mutation frequencies in next-generation sequencing data may be a good measure for the degree of tumor heterogeneity, which may help estimate a more exact prognosis.

Keywords: *Tumor, heterogeneity, somatic, next-generation sequencing*

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O-66
DISEASES IN THE HUMAN INTERACTOME

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Recent advances in disease gene identification and high-throughput mapping of physical interactions between gene products offer new opportunities to explore the role of molecular networks in human disease. Here we show that proteins associated with the same disease display a statistically significant tendency to agglomerate in the same neighborhood of the interactome, offering quantitative evidence for the existence of well-localized and potentially identifiable disease modules. Most important, we find that the network-based location of each disease module determines its pathobiological relationship to other diseases. For example, disease pairs with overlapping modules show significant co-expression patterns, symptom similarity, and comorbidity; those that reside in separated network neighborhoods are pathobiologically and clinically distinct. The proposed interactome-based framework offers systematic avenues to discover common molecular roots between clinically unrelated disease phenotypes even if they do not share disease genes, and helps identify the biological role of GWAS genes of small effect size and low genome-wide significance.

Keywords: *Network medicine, protein-protein interaction networks, disease relationships*

O-67
COMPPI: A CELLULAR COMPARTMENT-SPECIFIC DATABASE FOR PROTEIN–PROTEIN INTERACTION NETWORK ANALYSIS

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The analysis of protein-protein interaction networks (interactomes) help the systems level understanding of the organization and functions of intact and diseased cells. Currently used interactomes often contain a large number of biologically unlikely interactions because the interacting proteins are not localized at the same subcellular compartment.

ComPPI is a compartmentalized protein-protein interaction database containing cellular compartment-specific interactions of proteins enabling an extensive, compartmentalized

interactome analysis (URL: http://ComPPI.LinkGroup.hu). ComPPI helps the user to filter biologically unlikely interactions, where the two interacting proteins have no common subcellular localizations. Also to predict novel properties, such as compartment-specific biological functions.

The integrated ComPPI database covers four species (*S. cerevisiae*, *C. elegans*, *D. melanogaster* and *H. sapiens*). The compilation of nine protein–protein interaction and eight subcellular localization data sets had four curation steps including a manually built, comprehensive hierarchical structure of >1600 subcellular localizations.

ComPPI provides confidence scores for the subcellular localizations of the proteins based on the type (experimental, predicted or unknown) and number of sources and for the protein–protein interactions calculated from the localization scores of the interacting partners.

In order to facilitate the browsing of the database we designed a user-friendly web application. The Search page displays the subcellular localization for individual proteins, their interactions and the likelihood of their interactions considering the subcellular localization of their interacting partners. Download options of search results, whole-proteomes, organelle-specific interactomes and subcellular localization data are available on the Download page. The website also contains an extensive Help with a detailed step-by-step Tutorial.

Our analysis of the compartmentalized protein interactome showed that protein localization data induce profound changes in the properties of currently used interactomes, and helps to predict novel compartment-specific biological functions especially for translocating proteins.

Due to its novel features, the ComPPI database offers a great help to find protein interactions having a high confidence regarding their subcellular localization and/or change their subcellular localization in disease or during the aging process. ComPPI is useful for the analysis of experimental results in biochemistry and molecular biology, as well as for proteome-wide studies in bioinformatics and network science helping cellular biology, medicine and drug design.

Keywords: *protein-protein interaction, subcellular localization, network, systems biology*

O-68
EPIGENETIC REGULATION OF RETINOIC ACID DEPENDENT EMBRYONIC STEM CELL DIFFERENTIATION

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Retinoids are morphogens and have been implicated in cell fate commitment of embryonic stem cells (ESCs) to neurons. Their effects are mediated by RAR and RXR nuclear receptors. However, early transcriptional and epigenetic events resulting in cell-type specific gene activation or repression is less understood.

Comprehensive genome-wide studies were carried out to determine how RAR:RXR targets are defined in early stem cell differentiation. We provide data how stem cell specific transcription factor Oct3/4 regulate cell type-specific response to retinoic acid. We also demonstarte how co-activator P300 and co-repressor SMRT and HDAC3 are involved in chromatin remodeling. Moreover we show that a novel co-factors, Protein aRginine Methyl Transferase (PRMT) 1 also play key roles in determining retinoid regulated gene expression and cellular specification in a multistage neuronal differentiation of murine ESCs. PRMT1 acts as a selective modulator, providing the cells with a mechanism to reduce the potency of retinoid signals on regulatory "hot spots". Lack of PRMT1 leads to reduced nuclear arginine methylation, dysregulated neuronal gene expression and altered neuronal activity.

Overall, we show how well established and novel co-regulators control key transcriptional events of stem cell differentiation.

Keywords: *embryonic stem cell, retinoic acid, epigenetics*

O-69
RYBP AS A NEW PLAYER IN DIFFERENTIATION OF STEM CELLS

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It has become increasingly clear in recent years that Polycomb Group (PcG) complexes (namely Polycomb repressive complexes [PRCs] 1 and 2 (PRC1 and PRC2) govern the cell fate decisions and differentiation. Findings from last year turned the widely held notion of the PcG recruitment upside-down by showing that the initial step inverts classical model that PRC2 is the initiator of the recruitment process. Recent findings also described the presence of alternate canonical and non-canonical PRCs, which opened up a number of interesting new questions in this field.

The *mouse* PcG member Ring1 and Yy1 binding protein (Rybp; or also known as Dedaf (Death Effector Domain Associated Factor) is a member of the non-canonical PRC1 multimeric complex with unspecified biologic function. We have shown that the mouse Rybp is critical for embryonic development and that haploinsufficiency of Rybp causes failure of neural tube closure in a subset of embryos and may also play a role in terminal differentiation. We have also derived Embryonic Stem (ES) cells bearing homozygously *($rybp^{-/-}$)* and heterozygously *($rybp^{+/-}$)* disrupted *rybp* genes, thereby providing a molecular genetic approach to assess the role of *rybp* in lineage commitment and differentiation. By utilizing these cell lines we have shown that Rybp is a critical regulator of cardiac differentiation. We have also shown that in the absence of Rybp neural differentiation is impaired. These suggest that the proper dosage of Rybp is important for differentiation. Results and possible mechanisms responsible for this phenomenon will be discussed. The possible functions of Rybp as the member of multimeric protein complexes containing PcG proteins will be also addressed.

Keywords: *Differentiation, Embryo, Mouse, Polycomb, Rybp, Stem Cell*

O-70
DIFFERENTIATION AND ONTOGENY OF CD45+ STELLATE CELLS IN CHICKEN EMBRYO

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Apart from the classical antigen presenting cells of the lymphoid organs, almost every organ contains highly ramified stellate-shaped cells expressing hematopoietic markers and MHC II antigen. The embryonic origin, phenotype of these cells and how they colonize a given organ primordium are less known. The avian embryo is well suited for studying the origin, differentiation and tissue specific colonization of hematopoietic cells. The accessibility of the chick embryo allows manipulations not feasible in mammalian systems.

The aim of our study is to determine the embryonic appearance and differentiation of the avian hematopoietic cells (HSCs) cells and investigate their ontogeny using embryonic microsurgery methods. CD45 (common leukocyte antigen) is the earliest surface marker for the chicken HSCs. During our immunocytochemical characterization three types of CD45+ cells were found in the early chicken embryo. The one type of cells had small, round morphology in the lumen of yolk sac blood islands and associated to the aorta. A subpopulation of the round CD45+ cells coexpresses the CD41/CD61 antigen, which are cells of thrombocytic lineage. The other types of the CD45+ cells were either amoeboid-shaped Lep100+/Grl1+/AcPhosphatase+ macrophages or stellate-shaped with long, ramified and slender processes and scattered uniformly in the embryonic mesenchyme. These stellate-shaped CD45+ cells colonize not only the lymphoid, but most of the non-lymphoid organs and tissues. During their differentiation the CD45+ stellate-shaped cells co-express CD44, Csfr1 molecules and acquire MHC class II antigen.

To study whether the CD45+ stellate-shaped cells of the avian embryo can be considered a different subpopulation of the circulating CD45+ cells or instead they have a common stem cell originating from the extraembryonic blood islands, yolk-sac without embryo were cultured in the egg for additional 72 hours and the dissected embryo was cultured in vitro in three-dimensional collagen gel matrix. In the case of cultured yolk sac blood islands large number of CD45+/MHC II+/Csfr1+ stellate cells (exclusively of extraembryonic origin) differentiated, while in the explanted embryo only CD45+ cells with round-morphology developed. Using GFP-transgenic chicken yolk-sac chimeras, where normal chick embryo was transplanted onto GFP-yolk sac, we show that the majority of the intraembryonic stellate-shaped CD45+ cells are generated in the yolk-sac.

Taken together, our result suggest that yolk-sac derived CD45+ HSC has the capacity to actively migrates through the embryonic mesenchyme and colonizes each organ primordia and are able to differentiate into stellate and dendritic cells.

O-71
IDENTIFICATION OF A NOVEL STEM CELL NICHE FACTOR IN DROSOPHILA

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In adult metazoans, tissue homeostasis is maintained by stem cells, which are characterized by their ability to self-renew and generate differentiating progeny. In vivo, stem cells reside in anatomically and physiologically limited microenvironments called niches. Balance between self-renewal of stem cells and production of differentiating cells is maintained by complex signalling interactions between niche cells. Although discoveries of the last decades improved our understanding about the biology of stem cells enormously, the mechanisms that govern the formation, size and signalling of *in vivo* niches remain less well understood.

Owing to their complicated anatomic structures, identification and analysis of mammalian stem cell niches has been extremely difficult. However, studies on stem cell niches of model organisms, such as *Drosophila* provided valuable information about their general organisation and function. Most of the mechanistic insights into how niches operate have been obtained using the *Drosophila* ovarian germ-line stem cell niche. In the Drosophila ovarian niche, germ-line stem cells (GSCs) are surrounded by two somatic cell types: cap cells and escort cells. Intrinsic epigenetic mechanisms in the GSCs and local signals emanating from the somatic cells maintain GSC fate and initiate the differentiation of GSC daughter cells.

In a large scale RNAi screen, we have identified *small ovary* (*sov*), a putative transcription factor to be essential for *Drosophila* germ line development. By FLP/FRT based mitotic recombination and subsequent complementation analysis, loss of function *sov* alleles were isolated and tested for germ cell defects. Mutants displayed rudimentary female gonads suggesting a role for *sov* in GSC niche function. In mutant niches, differentiation of GSC daughter cells is not initiated and permanent proliferation of the GSCs generates small-sized stem cell tumours. Genetic analysis revealed that *sov* functions in somatic cells of the germ-line niche and it is required non-autonomously in the GSCs for germ cell differentiation. We showed that *sov* restricts niche size by mediating communication events between stem cells and somatic niche cells. Investigation of various signalling pathways indicates that *sov* negatively regulates TGF-β signalling to restrict niche size and GSC proliferation.

In addition to its role in the maintenance of the adult niche, *sov* is essential for niche formation during development. Throughout niche formation, mutant somatic niche cells adopt inappropriate fates indicating a regulatory function of *sov* in specification of escort cell and cap cell precursors.

Keywords: *Drosophila, stem cell, niche, ovary, germ line*

O-72

LICENSING BY INFLAMMATORY CYTOKINES ABOLISHES HETEROGENEITY OF IMMUNOSUPPRESSIVE FUNCTION OF MESENCHYMAL STEM CELL POPULATION

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Mesenchymal stem cells (MSCs) have been a subject of intense research in recent years due to their beneficent properties in therapy of numerous pathological conditions. When MSCs are used for therapy of immunological diseases, they get into an inflammatory environment altering the effectiveness of the treatment. Moreover, MSC population was found to be heterogeneic in immunosuppressive capacity. To elucidate how inflammatory environmental factors impact the intrinsic immunosuppressive heterogeneity of mouse MSC population individual MSC clones were generated and characterized. Adipogenic but not osteogenic differentiation and pro-angiogenic activity of five independent MSC cell lines were similar. Regarding osteogenic differentiation, MSC3 and MSC6 clones exhibited poorer capacity than MSC2, MSC4 and MSC5. To analyze the immunosuppressive heterogeneity, *in vitro* and *in vivo* experiments have been carried out using T-cell proliferation assay and delayed-type hypersensitivity (DTH) response, respectively. MSC clones inhibited T-cell proliferation with remarkable differences setting up the following order among clones: MSC2≥MSC5>MSC4>MSC3>>MSC6. Nonetheless, the differences between the immunosuppressive activities of the individual clones disappeared upon pre-treatment of the cells with pro-inflammatory cytokines, a procedure called licensing. Stimulation of the clones with IFN-γ and TNF-α was followed by increased inhibition of T-cell proliferation at a similar high level. Nitric oxide (NO) and prostaglandin E2 (PGE2) were determined to play key role in the immunofunction of the MSC clones. The above findings were also supported by *in vivo* results. Without licensing, MSC2 inhibited, while MSC6 did not influenced DTH response. In contrast, pre-stimulation of MSC6 with inflammatory cytokines evoked strong suppression by this clone as well. In sum, our findings suggest that MSC population is functionally heterogeneous in term of immunosuppressive function, however, this variability is largely reduced under pro-inflammatory conditions.

Keywords: *mesenchymal stem cells, heterogeneity, immunosuppression*

O-73
PATIENT SPECIFIC INDUCED PLURIPOTENT STEM CELLS (IPSCS) AND THEIR NEURONAL DIFFERENTIATION IN ALZHEIMER'S DISEASE

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Patient-derived induced pluripotent stem cells (iPSCs) are providing a useful tool to model the pathology of certain diseases, especially when the affected tissue is hard to study, like in the CNS. Pluripotent cells, carrying the genotype of a given patient, can be differentiated in vitro to neural precursor cells (NPCs) which are then used to obtain neuronal and glial cells in culture. These cells offer a valuable platform which allows the investigation of the patient-specific pathomechanism of a disease. Furthermore, NPCs and their derivates can be used for *in vitro* drug testing assays. Finally, these cells serve as an unlimited source of immune compatible cells for cell replacement therapies to treat neurodegenerative diseases such as Parkinson's or Alzheimer's disease.

Our aim is to develop a highly efficient and reproducible assay to generate neurons and glial cells from healthy and Alzheimer's disease patients' iPSCs. Here we compared the use of various small molecules and recombinant proteins in a step-wise manner, which selectively induces neural differentiation of iPSCs into NPCs and, later, into different neural linages. The treatment induced neural rosette formation followed by the occurrence of neuroepithelial cells (NEPs) resulting in a homogenous population of NPCs. At the NPC-stage all small molecules were withdrawn allowing for the terminal differentiation of mature post-mitotic neurons. Neuronal lineage was confirmed by immunocytochemistry (ICC) and RT-qPCR for neural and neuronal specific genes. Furthermore, 2D and 3D neuronal cultures were investigated in electrophysiological and calcium imaging measurements. Abeta 40/42 ratio was also determined as one of the major pathological sign of the AD. We have established a highly reproducible and efficient procedure to generate patient-specific neuronal cells form iPSCs.

This work was supported by grants from EU FP7 projects (STEMMAD, PIAPP-GA-2012-324451; Anistem, PIAPP-GA-2011-286264; EpiHealth, HEALTH-2012-F2-278418; EpiHealthNet, PITN-GA-2012-317146; D-BOARD, FP7-HEALTH-2012-INNOVATION-1-305815).

O-74
THE RESULTS OF WHOLE-GENOME ANALYSES (WGA) FORCE CHANGES IN PROKARYOTE SYSTEMATICS AND RESULT NEW CONCEPTS IN ECOLOGY

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Carl Woeses' revolutionary SSU RNA cataloguing based phylogenetics set new conception to microbial ecology and systematics. Researchers of both scientific fields switched from the investigation of the phenotype to the analysis of the genotype. Based on the existing molecular genetic and biochemical techniques a consensus methodology rapidly emerged (PCR, clone library preparation, SSU RNA coding gene sequencing) and a consensus genetic species definition developed in the investigation of Prokaryotes and their communities. In the numerical data analyses similarity gave way to homology. This methodology soaked the whole of microbiology and slowly infiltrated similar research concerning higher Eukaryotes, resulting in a biodiversity blast.

The consensus genetic species definition of bacteria is still based on 16S rRNA gene sequence, and whole genome DNA-DNA hybridization similarity values, though results of Next Generation Sequencing (NGS) based Whole-Genome Analyses (WGA) want new standards. Methodologies developed to delimit the core genome or the recognition of fuzzy species help the development of a new consensus. The polyphasic species conception however, still emphasizes the importance of the phenotype even in the genomic era.

We performed multilocus sequence analyses embracing catechol dioxygenase, and alkane 1-monooxygenase genes with species of the genus *Rhodococcus*. Results clearly indicate the phylogenetic power of the *AlkB* gene sequences in resolving species boundaries in different sub-clades of the genus, thus bring us closer to the species delimitation in the genus. Moreover such „functional" genes with phylogenetic power help to understand the ecology of the species in the genus *Rhodococcus*. *Rhodococcus* species dominated communities have eminent role in the remediation of petroleum contaminated sites.

Based on similar examples we argue on the need of parallel use of the „traditional" consensus molecular methodology and the NGS approach to help the achievement of sound taxonomic and ecology results.

O-75
GENOME INTEGRITY AND PATHOGENICITY ISLAND TRANSFER IN STAPHYLOCOCCUS

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Despite its general role as the depository of genetic information, the DNA molecule possesses inherent chemical reactivity that results in frequent and various modifications even under normal physiological conditions. The major pathways of DNA repair are eminently conserved among free-living species from bacteria to multicellular eukaryotes. In prokaryotes, the bacterial genome sometimes includes genetic elements that can be mobilized to form a more or less independent unit of replication (pathogenicity islands (PIs)) that can spread virulence factors and toxins among different bacterial strains. There is an intimate relationship between pathogenicity islands and helper phages in Staphylococcus aureus. In many instances, helper phages are essential for excision of Staphylococcus aureus pathogenicity islands [1]. We have proposed a novel mechanism for control of SaPI de-repression in Staphylococcus aureus where upon helper phage infection, phage-encoded dUTPase first sanitizes the nucleotide pool by hydrolyzing cellular dUTP and then binds to the Stl repressor, leading to de-repression and SaPI activation [2]. The Stl repressor is a strong inhibitor of dUTPase,

We became aware of the interesting and – to our knowledge - previously not addressed fact that none of the sequenced Staphylococcus aureus genome (a total of 49 strains) contains endogenous dUTPase gene. However, in most, but not all of these strains, prophage-encoded dUTPase genes are present. S. aureus strains (eg RN450 and RN4220) that are cured of prophages and do not encode any dUTPase. This is a very unusual situation, as dUTPase is generally considered to be ubiquitous and essential for viability in all biological organisms (expect viruses), since lack of dUTPase leads to heavily elevated levels of uracil (dUMP) moieties in DNA that induce futile cycles of excision repair and finally chromosome fragmentation. With these considerations, it was of immediate interest to check the uracil level in S. aureus genomic DNA. We found that in the two strains checked so far (MSSA and MRSA), uracil-DNA content is around (40 +/- 4) U/10^6 bases, ie order of magnitude higher than in any other wild type organism.We also discovered that whereas in most organisms just two enzymatic activities (dUTPase and uracil-DNA glycosylase) are involved in regulating uracil-DNA metabolism, in the Staphylococcal cell four relevant proteins (dUTPase, uracil-DNA glycosylase, and their respective inhibitors, Stl and SaUGI) are present. When in preliminary sequence analysis studies we looked at the genomic location of the SaUGI gene on the S. aureus chromosome, it was also intriguing to find that SaUGI is in almost all cases encoded within the SCCmec chromosomal cassette that carries the antibiotic resistance genes, as well. This may involve co-regulated expression of genes for antibiotic resistance and genome metabolism.

Keywords: *DNA damage and repair, uracil-DNA, Staphylococcus*

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O-76
DEVELOPMENT OF A HIGHLY PRECISE, PORTABLE GENOME ENGINEERING METHOD

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Multiplex Automated Genome Engineering (MAGE) has expanded the repertoire of genome engineering towards unprecedented throughput and efficiency. MAGE uniquely allows complex editing of multiple genomic loci, and thereby enables the generation of a desired genotype as well as combinatorial cell libraries within a practical timescale and at a reasonable cost. However, previous applications aiming to upscale the level of genome restructuring shed light on major limitations that impede the success of future genome engineering endeavors.

Increasing the number of targets in multiplex genome engineering is prone to lower overall fidelity and boosts off-target mutagenesis. This is due to the increased number of iterative modifications in cells with perturbed native mismatch-repair functions. These off-target effects place a practical limit on reprogramming of the target organism and could render the design-build-test-learn cycle of synthetic biology ineffective.

In a previous attempt we significantly improved fidelity of MAGE, however notable off-target activity still remained after extensive multiplex editing (Nyerges, Á. et al. Nucl. Acids Res. 42, e62–e62). As a solution we designed a novel strategy for overcoming off-target noise related limitations and in turn simplified and increased the portability of MAGE. We characterized a dominant mutation in a key protein of the methyl-directed mismatch repair (MMR) system and integrated its power to precisely disrupt mismatch-repair in target cells. With the integration of this advance, we developed a new workflow for genome engineering and demonstrated its applicability for high-throughput genome editing by efficient modification of multiple loci without any observable off-target mutagenesis.

Another main limitation of synthetic biology is that protocols for efficient genome editing are not portable to a wide range of living hosts and are often restricted to laboratory model organisms. Due to the highly conserved nature of the bacterial MMR system, the application of dominant mutations provides a unique solution for this limitation. By placing the entire synthetic operon that enables efficient genome engineering into a broad-host vector, we successfully adapted MAGE to a wide range of hosts and applied the strategy for genome editing in biotechnologically and clinically relevant enterobacteria.

These advances allow the maturation of multiplex genome engineering into a more versatile genome editing tool and paves the way towards the efficient production of highly valuable bio-products, more precise therapeutic applications and the investigation of central biological issues outside model organisms.

Keywords: *genome engineering, synthetic biology, off-target effects*

O-77
RETICULATE EVOLUTION IN A GROUP OF YEAST SPECIES

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Reproduction by cell division of organisms produces lineages that are independent of one another. The evolutionary histories of these lineages can be accurately represented by bifurcating trees. If these lineages exchange genetic material or recombine in any way, the trees become networks. Networking (melding) of evolutionary histories (also referred to as reticulation) occurs at three principle levels: chromosomal (meiotic recombination), population (neocombination) and species (interspecies hybridization and horizontal gene transfer) level. Whereas chromosomal-level and population-level reticulation are common in most sexual species, the evolutionary role of interspecies reticulation is doubted in certain larger eukarytic clades, either due to an actual dearth of genetic exchange among the lineages belonging to these clades or because of a lack of appropriate data. Very little is known about reticulation at species level or above in fungi. Here we present data which indicate that six *Metschnikowia* (Ascomycota) species share a pool of diverse (non-homogenised) chromosomal rDNA repeats. The Bayesian phylogenetic, network and neighbour-net splits analyses revealed that the repeats differing from each other in regions corresponding to hairpin loops of the D1/D2 domains of the large subunit rRNA and in the ITS1 and ITS2 segments had not evolved in a vertical tree-like way but by reticulation involving interspecies exchange of sequences. We also show that this exchange may take place by rare mating of cells of the species.

Keywords: *recombination, rDNA, interspecies hybridisation*

O-78
ENHANCING RECOMBINANT PROTEIN SOLUBILITY WITH A SAMP FUSION PARTNER

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With the advantages of recombinant gene technology a plethora of heterologous gene expression systems have been developed, aiming to obtain high amount of pure and properly folded proteins suitable for crystallography, enzyme reactions and many other purposes. However, the success rate of expressing a protein at high quantity in soluble form is low, while the frequency of insoluble

protein aggregation/precipitation or misfolded protein production is much higher, if the protein is expressed at all. In order to enhance the expression level and to prevent precipitation one possible solution is to fuse solubility protein tag(s) to the target protein either N- or C-terminally. Solubility proteins can stabilize the protein structure at various chemical conditions and in addition they may enhance the expression level as well. Up to now there is no one single affinity and solubility tag combination that could be used for every proteins to eliminate protein solubility problems, therefore existing fusion tags that could solve problems should be tested or new solubility protein tag candidates can be searched thus further increasing the chance to find optimal purification conditions.

In this presentation a novel fusion protein for recombinant protein expression in *E. coli* (potentially usable in any kind of cells and in vitro expression systems) is described. The fusion protein is the 90 AA long sequence of Ta0895 a putative Small Archaeal Modifier Protein (SAMP) from *Thermoplasma acidophilum*. The AA sequence of the protein of interest was fused to the carboxyl terminus of SAMP. This fusion partner imparts favorable solubility, proper folding, reduced interaction with host proteins and prevented the target protein from precipitation during purification. A purification tag was joined to the fusion protein for affinity chromatography purposes. This recombinant expression system is particularly advantageous for rapid expression and high yield purification of proteins that have low solubility or difficult to express in heterologous systems.

O-79
ENGINEERED RIBOSOMAL RNA OPERON COPY-NUMBER VARIANTS OF *E. COLI* REVEAL THE EVOLUTIONARY TRADE-OFFS SHAPING RRNA OPERON NUMBER

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Ribosomal RNA (rrn) operons, characteristically present in several copies in bacterial genomes (7 in *E. coli*), play a central role in cellular physiology. We investigated the factors determining the optimal number of rrn operons in *E. coli* by constructing isogenic variants with 5 to 10 operons. We found that the total RNA and protein content, as well as the size of the cells reflected the number of rrn operons. While growth parameters showed only minor differences, competition experiments revealed a clear pattern: 7-8 copies were optimal under conditions of fluctuating, occasionally rich nutrient influx, and lower numbers were favored in stable, nutrient-limited environments. We found that the advantages of quick adjustment to nutrient availability, rapid growth, and economic regulation of ribosome number all contribute to the selection of the optimal rrn operon number. Our results suggest that the wt rrn operon number of *E. coli* reflects the natural, „feast and famine" life-style of the bacterium, however, different copy numbers might be beneficial under different environmental conditions. Understanding the impact of the copy number of rrn operons on the

fitness of the cell is an important step towards the creation of functional and robust genomes, the ultimate goal of synthetic biology.

O-80
BRCA1 PROMOTES ERROR-FREE BYPASS OF REPLICATION STALLING DNA LESIONS

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BRCA1 plays an important role in homologous recombination, regulating the recruitment of RAD51 to single stranded DNA. At broken replication forks the failure of HR-mediated fork restart may allow the inaccurate joining of broken chromosome ends, giving rise to the chromosome instability phenotype characteristic of BRCA1 mutant tumours. BRCA defective tumours generally respond better to treatment with alkylating agents. To investigate whether this is due to their chromosome instability or to a more general role of BRCA1 in replicative lesion bypass, we performed genome-wide analyses on wild type and mutant DT40 chicken lymphoblastoma cells treated with alkylating agents. Using whole-genome sequencing we determined the mutagenic spectrum of the methylating agent methyl methanesulfonate and the crosslinking agent cisplatin. In case of cisplatin, we found that the mutations occur at neighbouring purine bases, indicating that cisplatin exerts its mutagenic effect primarily through intrastrand crosslinks. Importantly, in BRCA1 knockout cells there was a massive increase in the rate of base substitutions induced by either agent, while the mutagenic spectra were mostly unchanged. At the same time we only observed a limited level of chromosome instability. The base substitutions are likely caused by incorrect nucleotide incorporation during translesion DNA replication. Thus in normal cells BRCA1 promotes an error-free alternative to translesion DNA synthesis, and the increased rate of mutagenesis in BRCA1 mutants may contribute to the oncogenic effect of the loss of BRCA1 function.

Keywords: *DNA damage bypass, BRCA1, mutagenesis, whole genome sequencing*

O-81
POLYMERASE EXCHANGE MEDIATED TRANSLESION RNA SYNTHESIS

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When DNA replication is blocked by DNA lesions, DNA damage tolerance (DDT) mechanisms are activated that can sustain replication on damaged templates without removing the damage. One mechanism of DDT is translesion synthesis (TLS), where specialized DNA polymerases with lowered selectivity and fidelity replace the replicative polymerase and synthesize across the damage, and/or extend from it. Error-free bypass contributes to genomic stability, whereas error-prone damage bypass increases instability. Previously we identified Def1 as a protein that mediates the switch between the replicative and TLS DNA polymerases during DNA damage bypass in the yeast *Saccharomyces cerevisiae* (Daraba et al. 2014).
Transcription can also be blocked by DNA damages in the transcribed strand. Transcription blocking lesions can be removed by transcription-coupled repair, during which the lesion is excised from the re-annealed double-stranded DNA, and repair synthesis restores the original sequence. Cyclobutane pyrimidine dimers (CPDs) represent a strong block to RNAPII. However, transcription of an active gene in an UV-irradiated Chinese hamster cell line recovers much earlier than the removal of CPDs or other photoproducts could take place, suggesting the existence of a damage bypass mechanism operating during transcription. So far translesion RNA synthesis has been thought to be performed by RNAPII itself with the aid of elongation factors. Purified TFIIF, TFIIS, Elongin and CSB were shown to help RNAPII to bypass certain DNA lesions. However, it is still poorly understood how other transcription blocking lesions, like CPDs can be bypassed, and what other factors influence transcriptional fidelity.
In this study we present evidence that similar to DNA replication, transcription stalled at DNA damage sites can be rescued by polymerase exchange mediated translesion RNA synthesis.

Keywords: *translesion synthesis, RNA polymerase II, transcription elongation*

Reference:

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O-82

A GENOME-WIDE APPROACH TO LINK GENOTYPE TO CLINICAL OUTCOME BY UTILIZING NEXT GENERATION SEQUENCING AND GENE CHIP DATA OF BREAST CANCER PATIENTS

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To date, the identification and targeting of driver genes delivered suboptimal results in breast cancer. Nevertheless, we need to identify clinically relevant mutations to spot candidate genes for targeted therapy or for new personalized clinical trials. It is a general view in cancer therapy that the genetic background of the tumor determines the patient's chances for survival. However, the linkage between somatic mutations and clinical outcome is complex and to a large extent unknown.

It is known on the other hand that individual somatic mutations can affect a diverse set of other genes and thereby the effects of the mutation can be "leveraged" via a network of altered protein products. Gene expression can be directly estimated from RNAseq measurements using next generation sequencing. At the same time, large-scale datasets with extensive clinical characteristics are accessible in the growing databases of microarray data. Harvesting the combined prognostic value of these studies is a challenge (e.g. one has to link different annotation databases; microarray data on breast cancer are collected in multiple studies, etc.).

We have set up a pipeline enabling the functional validation of a discovered mutation for any gene in a large breast cancer cohort by connecting genotype to gene expression signature and employing this signature for survival analysis. The pipeline was validated to reassure the correlation between RNA-seq and microarray data ($r2=0.73$, $p<1E-16$). In addition, a set of established genes that are known to influence patient survival were used as test set (e.g. TP53, $p<1E-16$ and PIK3CA, $p=8.1E-07$).

In summary, our aim was to combine available genotype data generated by next generation sequencing (NGS) with gene expression data generated by gene chips in order to establish a framework for assessing the leveraged effect of genotype on clinical outcome.

Keywords: *breast cancer, microarrays, biomarker, SNP, survival, Cox regression, ROC analysis, TCGA*

O-83
DETECTION OF URACIL WITHIN DNA USING A SENSITIVE ANTIBODY-LIKE SENSOR FOR *IN VITRO* AND *IN VIVO* APPLICATIONS

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Uracil is usually considered to be a mistake when appearing in DNA. Most polymerases cannot distinguish between deoxyuridine and deoxythymidine, leading to uracil incorporation during DNA synthesis. Oxidative deamination of cytidine resulting also results in uracil, which leads to genomic instability. Genomic uracil is specifically recognised by the uracil-DNA glycosylase (UDG) superfamily, initiating the first step in base excision repair by, cleaving the N-glycosidic bond between the pyrimidine ring and deoxyribose, leaving apyrimidinic (AP) sites.

However, genomic uracil also appears under normal physiological conditions, such as in *Bacillus subtilis* PBS1 and PBS2 phages, *Yersinia enterocolitica* ΦR1-37 phage. It also has a major role in HIV infection, in B lymphocyte function during somatic hypermutation and class-switch recombination. Inhibitors targeting pathways involved in proper dUTP/dTTP pool maintenance, like the *de novo* thymidylate biosynthesis pathways, are in focus of cancer treatment. Thus several different fields in biology from phage genetics, lentiviral infection mechanisms, antibody maturation to chemotherapeutic approaches in cancer treatment all rely on genomic uracil occurrence. Thus a truly reliable, fast and easy method in gaining quantitative and qualitative information on uracil levels in DNA is of high importance for *in vitro* and *in vivo* studies.

Currently available genomic uracil quantification methods vary in specificity, sensitivity and price. These methods excise uracil, or scarf the DNA, and do not rely on *in situ* detection. There are several examples for quantification of non-orthodox DNA bases in the literature, such as 8-hydroxy-2'-deoxyguanosine, thymine glycol and 7-methylguanosine. Such routinely used uracil sensor was not yet available to date. Our new method uses a catalytically inactive UNG, capable of binding but not excising uracil. The uracil bound UNG sensor was designed in a way that it could be detected with conventional antibodies in dot-blot or ELISA applications along with possible *in situ* detection using an immunocytochemical approach. Our method delivers the sensitivity of MS based methods, with as low as 0.1 uracil / million bases, while also giving the possibility to gain position and sequence specific information on genomic uracil content. We validated our uracil sensor in several applications, using samples derived from CJ236 dut-, ung- *E.coli* strain, BL21 (DE3) ung-151 *E.coli* strain and also mouse embryonic fibroblast cells with altered base excision repair background in combination with the chemotherapeutic drug, 5FdUR. Our measurements are in good agreement with current results from the literature but omitting most of the limitations of the currently available methods, while delivering a powerful method at low cost, and great potentials.

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O-84
RECOMBINATION ASSOCIATED DNA SYNTHESIS IN HUMAN CELLS

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Replicating cell encountering DNA lesion has to quickly and efficiently response to ensure integrity of its genome. Several mechanisms have been suggested to deal with the damage at the replication fork. The damage tolerance (DT) and homologous recombination (HR) dependent DNA repair pathways constitute two alternative pathways, which can rescue stalled replication forks. DT pathway uses special DNA polymerases called translesion synthesis (TLS) polymerases, which can bypass the damaged DNA template. HR mechanism also need DNA extension, but the contributing DNA polymerases and the regulation of the polymerase usage on D-loop substrate is not clear yet.

The strict regulation of the usage of DT and HR pathways guarantees optimal replication and living condition to the dividing cell. PCNA is one of the key replication factors which has a crucial role in regulating the replication and repair processes via its posttranslational modifications as ubiquitilation and SUMOylation. Ubiquitilation of PCNA by Rad6/Rad18 complex is essential for the proper function of the TLS polymerases. Recently, the human PCNA SUMOylation was described, which has an anti–recombinogenic and a double strand break prevention function in vivo, but its biochemical function remained ascertain.

In our current work we analyzed the recombination mediated DNA extension and the regulation of this process.

Keywords: *Damage tolerance, PCNA SUMOylation, Homologous recombination*

O-85
QUANTIFYING DNA REPAIR PATHWAY ABERRATIONS IN TUMOR BIOPSIES IN ORDER TO DETERMINE RESPONSE TO CHEMOTHERAPY

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Genomic instability, the ability to accumulate and tolerate genomic aberrations, is one of the central driving mechanisms of cancer. DNA repair pathway aberrations are a major contributor to this process. In fact, the actual DNA repair pathway aberration in a given cancer has a profound impact on the biology of the tumor and in cases it also determines the efficacy of a particular genotoxic therapy. However, identifying and quantifying the actual DNA repair pathway aberration in a tumor biopsy is not a trivial task. We will present here several next generation sequencing based strategies to achieve this goal that also yielded clinically applicable response predictors to specific genotoxic agents.

Keywords: *DNA repair pathway, cancer chemotherapy, next generation sequencing*

ABSTRACTS - POSTER PRESENTATIONS

P-001
AUXIN-INDUCED PROTEIN DEGRADATION IN *DROSOPHILA MELANOGASTER*

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Recently a variety of in vivo systems have been developed for inducible degradation of proteins. They allow the direct analysis of protein functions overcoming the limitations of experiments influencing the protein expression at gene or mRNA level. However, protein degradation techniques are widely used in yeast and in mammalian cell lines only a few methods are available in *Drosophila.*

In plants, the auxin-inducible degron (AID) pathway induces the auxin dependent proteasomal degradation of target proteins with the contribution of SKP1/CUL1/F-box (SCF) ubiquitin ligase complex. In the SCF complex the F-box protein TIR1 is responsible for the auxin hormone dependent binding of proteins containing the AID motif. Due to the high conservation of Skp1 protein the ectopically expressed TIR1 is able to incorporate into endogenous SCF complexes and degrade the AID tagged target proteins in non-plant cells. Our aim was to establish a novel *in vivo* protein degradation technique in *Drosophila* using the plant AID system.

To examine whether the plant derived AID system can be functional in *Drosophila*, we co-expressed TIR1 and the AID tagged dominant negative form of Nanos protein in female germ line. The double transgenic females laid eggs in which bicaudal embryos developed. Upon auxin administration, however, the dominant negative bicaudal phenotype was suppressed in a concentration dependent and a reversible manner, indicating that the AID protein degradation system was active in *Drosophila*. To make the AID system versatile and easy to use, we designed a CRISPR based homologous recombination vector system by which AID tagging of either Drosophila protein can be performed. To prove that the vector system was functional we *in situ* tagged the *vasa* gene with AID-GFP double tag. We induced two double strand breaks in *vasa* gene by CRISPR and provided a donor vector containing the AID-GFP tag. Precise homologous recombination took place. We showed that DNA sequence of the AID-GFP tag successfully incorporated into the *vasa* gene from which AID-GFP tagged Vasa protein was expressed. We showed proper expression and subcellular localization of the AID-GFP tagged Vasa protein. We also showed that the tagged *vasa* gene was fully functional since in homozygous condition it resulted in wild type viable and fertile phenotype.

In this study, we demonstrated that the AID system is able to induce reversible protein degradation in *Drosophila.* Furthermore our results extend the *in vivo* applicability of this protein degradation technique. We developed a method by which we successfully tagged the endogenous *vasa* gene with AID-GFP. This fly line allows us the further examination of the efficiency of AID system at various developmental stages.

Keywords: *AID system, Drosophila, homologous recombination*

P-002
ANALYSIS OF INEFFECTIVE NODULATION MUTANTS IN *MEDICAGO TRUNCATULA*

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In order to discover plant components involved in rhizobial invasion, bacteroid differentiation and effective functioning of the symbiotic nodule *M. truncatula* mutants with ineffective (Fix-) symbiotic phenotypes were identified and characterized. Mutants developed white non-fixing nodules and showed symptoms of nitrogen starvation and retarded growth were selected. Eight mutants which showed complete reduction in nitrogenase activity were further characterized for their symbiotic phenotype. One of the ineffective mutants termed as 7Y showed extreme pigmentation in the nodules 2 weeks after infection with rhizobia indicating a strong pathogen response in the non-functional nodule. The 7Y mutant shows the pigment accumulation irrespective of the rhizobial strains (*S. meliloti* 1021, 2011 and ABS7, *S. medicae* WSM419) used for inoculation. Expression analysis of symbiosis specific genes suggests that 7Y is an early Fix- mutant compared to our other ineffective plant mutants. Microscopic analysis of 7Y indicates that the gene impaired in 7Y is required for persistence of rhizobia in the symbiotic cells.

Keywords: *symbiotic nitrogen fixation, Medicago, rhizobia*

P-003
THE ROLE OF U-BOX UBIQUITIN LIGASES IN PLANT-MICROBE INTERACTIONS

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Ubiquitination is a very powerful post-translational modification that plays major regulatory roles in many different biological processes. More and more details are discovered about the diversity of building the ubiquitin chains or the various ways of targeting substrates. All these characteristics allow ubiquitination not only to mediate proteolysis, but also to contribute in many other controlling events, so to become an integral part of diverse cellular functions. The E3 ubiquitin ligases -one of the three enzymes involved in linking ubiquitin to protein targets- are of particular interest as they confer substrate specificity during this process. In plants, the U-box domain containing E3 family has undergone a large gene expansion that may be attributable to biological processes unique to the plant life cycle. Research on these genes from several different plants has started to elucidate a range of functions for this family, from self-incompatibility and hormone responses to defence and abiotic stress responses.

We work on the characterization of two different kinds of U-box containing proteins that have role in the process of nitrogen-fixing symbiosis in *Medicago truncatula*. Beside the functional U-box they contain ARM-repeats, but the rest of the modules are different. Their mutant phenotypes show striking dissimilarities, just as their overexpression. We aim to discover the possible functions and interacting partners of these E3 ubiquitin ligases. On one hand this can reveal their role during the investigated symbiotic process, and, on the other hand, it can suggest their participation in other biological processes in plant, like other plant-microbe interactions or development.

This work was supported by the Hungarian OTKA Funds K76843 and NK105852.

P-004
THE ANALYSIS OF THE ROLE OF A CYSTEINE-RICH RECEPTOR-LIKE PROTEIN KINASE REQUIRED FOR SYMBIOTIC NITROGEN FIXATION BETWEEN *MEDICAGO TRUNCATULA* AND *SINORHIZOBIUM MELILOTI*

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The symbiotic association between *Medicago truncatula* and *Sinorhizobium meliloti* results in the formation of nitrogen-fixing nodules. In symbiotic nodules rhizobia differentiate into bacteroids which reduce atmospheric nitrogen. In order to identify plant genes required for rhizobial invasion, bacteroid differentiation and effective functioning of the symbiotic nodule, *Medicago truncatula mutants* with ineffective (Fix-) symbiotic phenotype were identified and characterized. The genetic analysis revealed a new allele of the formerly identified *dnf5* mutant. The ineffective *dnf5-2* mutant developed small white nodules without the characteristic zonation of indeterminate nodules. Light and electron microscopic analysis of the invasion of the symbiotic cells host non-elongated rod shaped bacteria and the failure of bacteroid differentiation in the *dnf5-2* mutant. We generated double mutants of *dnf5* and other ineffective symbiotic mutants to analyze their symbiotic phenotype and hierarchy in the symbtioc interaction. To identify the gene impaired in the *dnf5-2* mutant, positional cloning experiments has been initiated that identified a gene encoding a receptor-like protein kinase with a cysteine rich domain (*NCK* –nodule specific cysteine rich receptor like kinase). Complementation of the *dnf5-2* mutant with the wild-type *NCK* gene restored the symbiotic phenotype. The expression analysis of some marker genes associated with pathogen and senescence responses showed induction in the *dnf5-2* mutant.

Our data suggest that the *NCK* gene is involved in the differentiation of the bacterial partner and controlling the plant defense revealed the induction of defense responses

Keywords: *mutant, symbiotic nitrogen fixation, Medicago truncatula, gene cloning*

P-005
THE *DROSOPHILA* FORMIN DDAAM FUNCTIONS AS AN EFFECTOR OF THE PLANAR CELL POLARITY PATHWAY DURING AXON GROWTH IN THE DEVELOPING BRAIN

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In the developing nervous system growth cones have an essential role in guiding axons to their correct target sites. Directed growth cone motility in response to extracellular cues is produced by the coordinated regulation of peripheral F-actin and central microtubule networks. Key regulators of actin dynamics are the so called nucleation factors, such as formins, which promote actin assembly by associating with the fast-growing end of actin filaments, and facilitate the formation of unbranched filaments.

We have previously examined the function of the *Drosophila* formin dDAAM in the embryonic CNS, where this protein shows a strong accumulation in the developing neurites. Genetic analysis suggested that dDAAM plays a major role in the regulation of axonal growth by promoting filopodia formation in the growth cone. We noticed that *dDAAM* exhibits a strong expression in certain regions of the larval and adult brain as well. Specifically, in the developing mushroom body dDAAM is highly enriched in the newly born axons suggesting that dDAAM might be a general regulator of *Drosophila* axonal development. Consistently, by loss of function analysis we detected axonal projection defects in the mushroom body. To identify proteins that may act together with dDAAM in the regulation of axonal growth, we carried out a genetic interaction analysis. We demonstrated that *dDAAM* interacts with the core PCP (Planar Cell Polarity) proteins during axon guidance. Moreover, we identified Rac as the most likely activator of dDAAM in the developing nervous system.

Keywords: *formin, actin, axon growth, Drosophila, PCP*

P-006
ANALYSIS OF THE TRANSMISSION OF TRANSPOSON-TRANSGENIC FLUOROPHORE PROTEIN IN RABBIT

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The DNA Class II transposons have been excessively used for the generation of transgenic animals, such as mouse, rabbit, pig, etc. Some of these transgenic lines are established with the same transgene construct thus capable for identify the differences in some terms of reproduction between

species. These transgenic boars and rabbits contains Venus fluorescent reporter driven by a CAGGS promoter allowing the presence of transgene ubiquitously.

Kues at al examined transposon-transgenic boars with CAGGS-Venus transgene construct, each boar were hemizygous and carrying 3 copies from the transgene. It has been shown that all of the boar spermatozoa derived from a hemizygous boar were uniformly fluorescent independently their genotype. Fluorescence signal was detected in the postacrosomal sheath, in the midpiece and in the tail. In addition the embryos fertilized with the semen of the same boars shown Venus fluorescence before the transcriptional activation of the genome (Kues, PLoS ONE, 2012).

With the previously generated transposon-transgenic laboratory rabbit line we investigated on the appearance of the transgene in the germ cells. These rabbit line also carrying Venus fluorophore driven by CAGGS promoter. We follow the fate of the Venus protein in rabbit spermatozoa and early stage rabbit embryos with molecular and microscopic methods. With confocal microscopy we analyzed the population of spermatozoa derived from heterozygous and homozygous animals. All the spermatozoa derived from a homozygous animal were Venus fluorescent in the postacrosomal sheath, in the midpiece and in the tail. With FACS analysis we could distinguish between the homozygous and the heterozygous sperm population based on the Venus fluorescence. Moreover we analyzed the Venus fluorescence of the embryos fertilized with transgenic sperm in different stages of embryogenesis.

The comparative analysis of rabbit and boar sperm will determine if the spermatozoa of transgenic rabbits shown a similar genotype-independent, uniform Venus fluorescence like the boar spermatozoa or display some other phenotype.

The project supported by OTKA-108921.

Keywords: *transposon, rabbit, Venus, spermatozoa*

P-007

A SINGLE GENE OF THE LARGE NODULE SPECIFIC CYSTEINE RICH GENE FAMILY HAS A UNIQUE ROLE IN BACTEROID DIFFERENTIATION IN THE *MEDICAGO TRUNCATULA*-RHIZOBIUM SYMBIOTIC ASSOCIATION

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The most effective forms of biological nitrogen fixation is the endosymbiosis, wherein nitrogen-fixing bacteria are hosted in cells of the symbiotic nodule. In the symbiotic cells rhizobia are encompassed by a plant derived membrane in the nodules cells and differentiate into bacteroids to

be able to reduce atmospheric nitrogen. In the *Medicago truncatula – Sinorhizobium meliloti* symbiotic interaction rhizobia undergo genome endoreduplication cycles and enlargement.

Our aim was to identify plant genes involved in the nodule colonization by bacteria, differentiation of the rhizobia, and effective function of the root nodule in *Medicago truncatula – Sinorhizobium meliloti* nitrogen-fixing symbiosis. We have isolated fast neutron mutagenized mutant plant developing ineffective (Fix-) nodules, *dnf7-2*. This mutant shows defect in bacterial elongation and persistence of bacteria in the nitrogen-fixation zone of the symbiotic nodules. The combination of classical positional cloning and microarray-based gene identification was applied to clone the *DNF7* gene encoding a member of the nodule-specific cysteine-rich (*NCR*) gene family, *NCR169*. The complementation experiments with the fluorescent-tagged version of NCR169 restored the mutant phenotype and revealed that localization into the symbiosome space.

Our result indicate that the deletion of a single member of the NCR gene family leads to defect in bacteroid differentiation and ineffective symbiotic nitrogen fixation.

Keywords: *symbiotic nitrogen fixation, NCR peptides, bacteroid differentiation*

P-008
APPLICATION OF THE CRISPR-CAS9 SYSTEM FOR THE *IN VIVO* TARGET SPECIFIC MODIFICATION OF A DROSOPHILA GENE CODING FOR AN ACTIN BINDING PROTEIN

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Members of the Ezrin-Radixin-Moesin (ERM) family of evolutionarily conserved, actin binding proteins act as crosslinker between membrane proteins and the actin cytoskeleton therefore they play important role in cytoskeletal rearrangements. Their function in cell migration and metastasis is currently in the focus of intensive research. ERM proteins localize in the cytoplasm and at the cell cortex, however our laboratory observed previously that they are detectable in the cell nucleus as well.

Our laboratory is studying the nuclear function of Drosophila Moesin (Moe), the only representative of the ERM family in the fly. Because the nuclear transport mechanism of Moe is unknown, therefore studying its nuclear function alone is not possible today. We decided to overcome this difficulty with the application of a novel genom editing technology, the CRISPR-Cas9. Our aim was to tag the genome-encoded Moe protein with a nuclear export signal (Moe-NES). In this case, Moe-NES will be exported permanently from the nucleus, so it can not carry out its nuclear activity while the cytoplasmic functions of Moe remain intact.

For the generation of Moe-NES, we used several laboratory methods (various PCR techniques, DNA cloning, *Drosophila* embryo injection), designed multiple target sites for Cas9 and numerous mutagenic, adapter and sequencing primers. With the help of these tools we created the guide RNA coding vectors, injected them into early fly embryos and tested the Cas9 cleavage efficiency by PCR. Next we built up the donor construct which contains two segments of the *moesin* gene one of which

is equipped with the NES sequence. After the injection of guide RNAs together with the donor construct, the mutant flies can be detected in the F1 generation by red fluorescent eye marker.

In conclusion, we succesfully applied the CRISPR-Cas9 system in the case of *Drosophila melanogaster*. The phenotype analysis of Moe-NES flies, hence the investigation of the biological significance of the nuclear localization of the fly ERM protein, is in progress.

Keywords: *actin, nucleus, nuclear export, Moesin*

P-009
SEX-SPECIFIC REGULATION OF DEVELOPMENT, AGEING AND BEHAVIOUR IN *CAENORHABDITIS ELEGANS*

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In several animal species – including humans – the two sexes significantly differ in anatomy, physiology, behaviour, learning and lifespan. Although sex determination is well characterized in numerous species, the molecular mechanisms by which sex-specific differences are established remain largely unknown.

In the nematode *Caenorhabditis elegans,* the two sexes – the self-fertilizing hermaphrodites (which are essentially females capable of sperm production) and males – are different in many features. The terminal regulator of the sex determination pathway in *C. elegans* is the transcription factor TRA-1 (sexual TRAnsformer). TRA-1 is orthologous to mammalian Gli (Glioma-associated) proteins. TRA-1 activity is essential in sex-determination, but until now only a few direct TRA-1 targets have been identified. Our group determined a list of potential TRA-1 target genes in the *C. elegans* genome, using *in silico* methods, and started to characterize these factors. Two of the most interesting targets are *daf-16/FOXO* and *goa-1*/$G\alpha_{oi.}$

DAF-16 is the sole *C. elegans* homologue of the human FOXO (forkhead box O) protein. DAF-16 is the terminal transcription factor of the insulin/IGF-1 signalling pathway, which has a central role in the regulation of development, metabolism, stress response and aging. We observed that hermaphrodites live longer than males, and this difference depends on the activity of DAF-16. Furthermore, the expression of *daf-16* is directly regulated by TRA-1. Our results suggest that DAF-16 activity is adjusted in a sex-specific manner, leading to differences in development and aging between the two genders.

goa-1 encodes the α subunit of the trimeric G protein (belonging to the Go/Gi class). In *C. elegans, goa-1* has important role in several processes and behaviours, including development, neuronal migration, synaptic transmission, locomotion, male mating, and olfactory-mediated behaviours. We show that TRA-1 directly activates *goa-1* to regulate sex-specific behavioural patterns in *C. elegans*.

Together, our results provide a mechanistic insight into how development, longevity and behaviour in *C. elegans* are specified unequally in the two genders.

Keywords: *sex determination, ageing, development, behaviour, TRA-1/Gli, DAF-16/FOXO, GOA-1/* $G\alpha_{oi.}$

P-010
PRODUCTION OF HIGHLY INBRED ZEBRAFISH (*DANIO RERIO*) LINE WITH INTERSPECIFIC GYNOGENESIS

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Contrary to other model animals, highly homogeneous/inbred strains of zebrafish (*Danio rerio*) are not available to the scientific community. Only two gynogenetic lines (C32 strain and another line) and some partially inbred strains (SJD, SJC, SJA derived from DAR, C32, and AB, respectively and the IM from India strain) were established but genetic analysis of them, revealed that 7% (C32), 11% (SJD) 15% (SJA) and 5% (IM) of tested loci were polymorphic in them, respectively. However, it is not clear whether these types of strains have such a "high" genetic diversity in zebrafish or will become extinct.

Gynogenesis is one of the fastest ways to produce highly inbred strains. However, the production of gynogenetic zebrafish lines has some disadvantages (i.e., logistical difficulties related to sperm, high mortality levels of embryos and larvae, biased sex ratio, low quantity and variable quality sperm, etc.). In this study, we have initiated the production of a highly inbred line with gynogenesis using interspecific cryopreserved sperm to avoid disadvantages of the process. However the interspecific ginogenesys were successful, only 184 larvae hatched from more than 18,000 eggs. Most of them died of different developmental disturbances at different developmental stages. Finally, 14 individuals reached adulthood. Five of them (4 males and one putative female) have not produced offspring, while 7 males and 2 females were fertile. These individuals were used to produce the F1 generation. Until now in the F1 generation consists of 46 individuals produced by 1 couple and only one individual displays the male phenotype.

Our results show that irradiated and cryopreserved sperm of carp and goldfish can be used for gynogenesis in zebrafish. However, adequate control groups were not available to check the sperm and egg quality before the treatments because the unfertilized, haploid and interspecific hybrid individuals (from carp/goldfish X zebrafish crosses) have a similar phenotype and are not viable. Nevertheless, the survival rate was lower than 1% as it was expected. We observed a sex-biased nature of the highly homogenous lines both in the gynogenetic (male biased) and the F1 generation (female biased; ~98%). Presumably this is a result of the multi chromosomal sex determination of *Danio rerio*

To the best of our knowledge, this is the first experiment that produced gynogenetic zebrafish individuals with interspecific, cryopreserved sperm.

This work was supported by OTKA (105393) and KTIA-AIK-12-1-2012-0010 projects

Keywords: *gynogenesys, zebrafish, Danio rerio, interspecific, cryopreserved sperm*

P-011
DE NOVO GENOME SEQUENCING FOR IDENTIFICATION OF SEX DETERMINING GENE IN AFRICAN CATFISH (*CLARIAS GARIEPINUS*)

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In the last decades our groups have been investigating the molecular genetics of sex determination in fish. During this work one of our targets was the African catfish (*Clarias gariepinus*). Following the carp, this species is produced in the second largest volume in Hungary. In addition, the African catfish is also an important object for biological research. (genome manipulation, hybridization, sex reversion, reproductive fish endocrinology, etc.). Despite all these investigations, the available DNA-RNA sequence information and data on genetic background of the species are very limited.

During our previous work two sex specific molecular genetic markers and strong genetic sex determination were found in this species (Kovacs et. al 2001).

Our aim now is identification of a master sex-determining gene of African catfish. Among catfish species only *Ictalurus punctatus* genome is available. However this species is not a close relative of *Cl. gariepinus* and not a good basis for a positional cloning approach. Moreover in the family of air-breathing or labyrinth catfishes (*Clariidae*) both female heterogamety and male heterogamety have been described like in many other families or orders among fishes. This is why we have started a De Novo genome sequencing project to get closer to the master sex-determining gene. Paired-end and Mate-pair libraries were designed for Illumina HiSeq 2000. The alignment of about 711 million unique reeds (71 billion bases) just finished. It is mean ~60x coverage of the African catfish genome, but the prediction and annotation of the genes in the first draft genome are just started.

The rapid development of genome sequencing technologies allows a new and faster strategy to get sequential information from the sex chromosomes. Sex-specific markers are good starting points for identification of genetic regions and genes involved in sex determination. Moreover the *De Novo* sequencing process produces a large amount of additional sequence data. The analyses of this data represent a major challenge and good opportunity to get the whole genome sequence of *Cl. gariepinus,* however, additional genome and transcriptome sequencing must be carried out to reach these future aims.

This work was supported by OTKA (105393) project.

Keywords: *Clarias gariepinus, sex determination, De Novo genome sequencing*

P-012
THE SHORT STOP (SHOT) GENE IS REQUIRED FOR EFFICIENT EPITHELIAL CLOSURE IN DROSOPHILA MELANOGASTER

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Several developmental processes, such as dorsal closure of the fruit fly *Drosophila melanogaster* embryo, involve a coordinated series of cellular activities that are very similar to those required for wound healing. Therefore, dorsal closure of the fruit fly embryo provides an excellent model system for *in vivo* analysis of the molecular and cellular events occurring during the closure of a small epithelial opening. Dorsal closure represents the last major morphogenetic movement during embryogenesis, when two opposed epithelial sheets converge toward the midline where they meet, sealing a hole at the dorsal surface of the embryo. The fusion of the epithelial sheets takes place by a zipper-like mechanism, as the opposing surfaces are zipped together at the most anterior and posterior ends of the dorsal hole. Genetic screening, biochemical and cell biological approaches have uncovered some of the structural and signaling molecules required for these closure events. Several studies have highlighted the importance of reorganization of actin-based structures, such as filopodia and lamellipodia, but the function of the microtubule network is very poorly understood.

To uncover novel components required for microtubule reorganization and function, we have applied an RNAi-based screening method combined with automated *in vivo* video microscopy and we identified the *short stop (shot)* to be essential for the zippering of the dorsal hole. Shot belongs to the conserved family of the spectraplakins, gigantic structural proteins with functional domains binding to actin filaments, microtubules and cell adhesion complexes. Detailed cell biological analysis revealed that *shot* controls the morphology of the MT network in the epithelial cells by regulating dynamic properties of microtubule growth.

We propose that in epithelial cells *shot* coordinates the interactions of distinct cytoskeletal components, which enables the cells to rapidly restructure their cytoskeleton and adopt an organization appropriate to the physiological requirements of the closure. Our work provides insights how mechanisms integrating individual cytoskeletal elements into complex, highly shaped functional patterns contribute to a developmental process at the organism level.

Keywords: *dorsal closure, short stop (shot), microtubule network, drosophila, cytoskeleton*

P-013
THE ROLE OF LIPID METABOLISM DURING *DROSOPHILA* SPERMATOGENESIS

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Defects occurring during postmeiotic stages of *Drosophila* spermatogenesis are the most common phenotype of male sterile mutants. Most of the gene products necessary in later steps are synthesized before meiosis and stored until needed, so even a weak hypomorphic mutant could show late phenotype. Spermatid elongation and individualization are known to require extensive membrane biosynthesis and remodelling. Increasing number of lipid metabolic enzymes shows important role during all stages of sperm development, for example in the biosynthesis of phosphatidylinositol (PI) and their phosphorylated forms. We identified a hypomorphic male sterile allele of an important gene of lipid biosynthesis, the *Drosophila CDP-DAG synthase* (*CdsA*). The CdsA enzyme catalyzes the synthesis of cytidin diphospate diacylglycerol (CDP-DAG) from phosphatidic acid (PA). We characterized the $CdsA^{ms}$ mutant with classical and molecular genetic methods and analysed its lipid composition using mass spectrometry. We found that male sterility in $CdsA^{ms}$ mutant is caused by defects in spermatid individualization and by enlargement of axial membranes and mitochondria inside the developing cyst. Lipidomic analysis of the $CdsA^{ms}$ mutant testes revealed important changes in lipid composition, such as the elevation of PA, and the reduction of PI, but no change was detected in the level of the mitochondrial specific lipid, cardiolipin. We could partially rescue the male sterile phenotype by overexpressing of PI-synthase in a $CdsA^{ms}$ mutant background. Measurement of the lipid class levels revealed that PI-synthase overexpression did not lead to the elevation of PI level, but rather to the repression of the strongly elevated PA. Lipidomic analysis of the $CdsA^{ms}$ mutant with or without PI-synthase overexpression highlights the importance of correct lipid composition and the relevance of PA as a central hub in lipid synthesis during postmeiotic spermatid development.

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P-014
THE ROLE OF *HOX* GENES IN *NOTCH* RECEPTOR EXPRESSION IN *C. ELEGANS*

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The *Hox (Homeobox)* genes are responsible for the establishment of the proper tissue identity along the anterior-posterior axis of the bilaterian animals. They encode transcription factors with conserved DNA-binding preferences that specify cell fates by regulating the expression of their target genes. *ceh-13 (Caenorhabditis elegans Hox-13)*, a member of the anterior *Hox* homology group, has various functions in regulating embryogenesis. *ceh-13(sw1)* homozygous mutant animals exhibit variable abnormal morphology, reduced fertility, severe embryonic and larval lethality, and have a slow growth rate. *Notch* signaling is crucial for several steps of embryonic development, and has an important role in the function of the adult nervous system as well. In *Caenorhabditis elegans*, there are two *Notch* homologous receptors encoded by the *glp-1* and *lin-12* genes. The *Hox* genes and *Notch* receptors have human relevance, as they are implicated in organ development, so their mutations can result in tumorigenesis, and diseases associated with disruptions in neural cell fate determination. Previous research at our department identified CEH-13 binding sites in the intronic regions of *glp-1* and *lin-12 Notch* homologous receptors. Based on these findings, gfp-fused reporter constructs were generated to examine the neural expression of *ceh-13*, *glp-1* and *lin-12*, which was proved to be similar in the case of the genes in question.

Taken these data into consideration, we studied the expression of the two *Notch* homologous genes, raising the question whether it is affected by *ceh-13*. To determine the *glp-1* and *lin-12* mRNA levels in control versus *ceh-13* deficient backgrounds, we applied a real-time qPCR experiment. Based on these studies, we can conclude that *ceh-13* influences the expression of *glp-1* and *lin-12*.

As a part of this project, we aimed to characterize *ceh-13* expression. Applying a translational fusion CEH-13::GFP reporter-construct, it would be possible to get more information about the function of *ceh-13* in organizing neural circuits. To address this issue, the *ceh-13::gfp* strain was crossed with the *ceh-13(sw1)* deletion mutant strain. If the construct was functional, it would be able to rescue the *ceh-13(sw1)* phenotype. We compared the morphology of *ceh-13::gfp* strain with that of *ceh-13(sw1)* mutant strain, moreover, we examined the neural expression pattern of different *ceh-13::gfp* lineages.

Accumulating evidence indicates that both *Hox* genes and *Notch* homologous receptors function in the regulation of the nervous system, therefore have an impact on behavior. We currently carry out behavioral assays to investigate this question.

Keywords: *Hox, Notch, C. elegans*

P-015
IDENTIFICATION OF THE INTERACTION PARTNERS OF THE FORMIN DDAAM REQUIRED FOR AXON GROWTH

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In the developing nervous system growth cones have an essential role in guiding axons to their correct target sites. Directed growth cone motility in response to extracellular cues is produced by the coordinated regulation of peripheral F-actin and central microtubule networks. Key regulators of actin dynamics are the so called nucleation factors, such as the Arp2/3 complex and formins, that promote the formation of new actin filaments. We have previously shown that the *Drosophila* formin *dDAAM* plays a major role in the regulation of axonal growth by promoting filopodia formation in the growth cone. In order to better understand how dDAAM functions during neuronal development, an LC-MS/MS analysis was carried out to identify potential partners that work together with dDAAM during axonal growth regulation. Interestingly, the list of the potential binding partners includes several microtubule binding proteins, and consistently, we found that, besides its role in actin assembly, dDAAM is also able to interact with the microtubules directly. Currently, we are investigating the mechanism as to how dDAAM induced actin assembly might be coupled to the regulation of microtubule dynamics in axonal growth cones.

P-016
CHARACTERIZING THE MAJOR PROTEIN COMPONENTS OF *DROSOPHILA* SPERM PROTEOME

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Sperm leucyl-aminopeptidases (S-Laps) are metalloproteases from the M17 Merops family. There are 8 known S-Laps in *Drosophila*, all of which are testis specific. It is suggested that most of the genes encoding sperm leucyl-aminopeptidases were created by either gene duplication or retroposition. In adults they are exclusively expressed during spermatogenesis and are mainly upregulated in the meiotic and post-meiotic phases. S-Laps represent the major part of proteins within the mature sperm, twice as much as tubulin that is the main component of the tail. There are amino acid exchanges among the 7 conserved amino acid residues in the active site of S-Laps compared to the M17 aminopeptidases, suggesting they gained novel function.

Our goal was to investigate the molecular function of this abundant protein family during spermatogenesis. We identified S-Lap mutants and found male sterile phenotypes that we characterized using classical genetic and molecular biological methods. Our results suggest that

their function is not entirely redundant during sperm development. We described the subcellular localization of S-Lap proteins using GFP-tagged transgenes and found specific enrichment in mitochondria, the biggest organelle of mature *Drosophila* sperm. We identified specific mitochondrial localization signal sequence in S-Laps.

During spermiogenesis the elongation of spermatids highly depends on the right localization and proper function of mitochondria. In meiotic cells every mitochondria localized in the cytoplasm migrates near the nucleus, aggregates, fuses and creates two mitochondrial derivatives. The fused mitochondria are elongated with microtubules forming the tail, probably providing the energy needed for the process and later on for the motility of the sperm and also giving the rigidity of the almost 2mm long tail by paracrystalline accumulation. We investigated the structure and function of mitochondria in S-Lap mutants and found abnormal post-meiotic mitochondria development. Our results may contribute to the understanding of the organization and function of mitochondria during spermatogenesis and in mature sperm.

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Keywords: *spermatogenesis, Drosophila, leucyl-aminopeptidases*

P-017
ROLE OF VESICULAR TRANSPORT IN ACROSOME FORMATION AND NUCLEAR ELONGATION DURING SPERMATOGENESIS

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Spermatocytes undergo a massive, 150-fold growth in length during spermatogenesis in *Drosophila*. The whole membrane surface will be 5 times larger, which needs intensive membrane synthesis and rearrangement as well as increased vesicular transport. One example for the intensive membrane rearrangement is the Golgi-, lysosome- and endosome-derived acroblast, the site of the acrosome formation. Acrosome has an important role generally in the fertilization and early embryogenesis.

We investigated the role of vesicular transport using various mutations of vesicular transport proteins. Vps54 (Scat) protein, a subunit of GARP complex is required in retrograde transport of vesicles towards *trans*-Golgi. A P-element insertion in *scat* gene causes male sterile phenotype with no matured, motile sperms. We confirmed that the P-element insertion results in a null mutant and we rescued the male sterile phenotype with a wild type transgene and with the remobilization of the P element. We found that early steps of sperm development are normal however individualization does not take place in *scat* mutant testes. Another typical phenotype is the abnormal nuclear structure in elongated cysts of the mutant. Analyzing different organelle specific markers showed that other aspects of spermatogenesis including chromatin remodeling (histone-protamine substitution) and basal body formation are normal. We examined the structure and the function of the Golgi and the vesicular transport using several Golgi-localized and other vesicular transport proteins and we found that acrosome is missing and the vesicular transport is damaged in

scat mutant testes. We tested one other transport protein, Cog5 (Fws), which is part of the intra-Golgi transport specific COG complex. We found individualization and nuclear elongation abnormalities in *fws* mutant similarly to *scat*. Our results suggest that not only the GARP complex but the whole intact Golgi is necessary for the proper acrosome formation. Moreover our results highlight the importance of a functional Golgi (including retrograde and intra-Golgi vesicular transport) in the formation and function of acroblast, which connects nuclear elongation and acrosome formation with each other.

Supported by EMBO Installation Grant No.1825, OTKA NF 101001.

Keywords: *spermatogenesis, Drosophila*

P-018
THE FUNCTION OF HEADCASE IN THE HEMATOPOIESIS OF *DROSOPHILA MELANOGASTER*

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Against microbial and eukaryotic parasites, the fruitfly (*Drosophila melanogaster*) possesses an effective immune response, which consists of humoral and cellular elements. The immune cells of *Drosophila*, the hemocytes, are located in distinct compartments and differentiate in multiple waves under the control of phylogenetically conserved epigenetic and transcription factors. Although the three larval hematopoietic compartments - the sessile tissue, the circulation and the lymph gland - have different mesodermal origin, they all contribute to the formation of the effector cells: the phagocytic plasmatocytes, the melanotic crystal cells and the capsule forming lamellocytes. In our screens, which were aimed to identify genes that play an important role in the control of the differentiation of hemocytes, we isolated *headcase* (*hdc*), the ortholog of the human tumor suppressor HECA, which is a repressor of differentiation in numerous developmental processes of *Drosophila*. We found that *hdc* is expressed in the lymph gland of naive larvae, however, upon immune induction the hemocyte leaving the organ differentiate into lamellocytes and lose *hdc* activity. This suggests that *hdc* may be a regulator of hemocyte differentiation.

Our newly generated *hdc-Gal4* driver and a number of hemocyte-specific transgenic drivers were applied to silence *hdc* by RNA interference, and immunological markers and *in vivo* transgenic reporters were used to follow hemocyte differentiation in all hematopoietic compartments.

In the lymph gland, *hdc* expression was not restricted to hemocytes, but was also observed in the Posterior Signaling Center (PSC), the hematopoietic niche that blocks the differentiation of hematopoietic progenitors in this compartment. Silencing of *hdc* with a general hemocyte specific driver resulted in the loss of plasmatocyte specific marker expression in all hematopoietic compartments. Most surprisingly however, lymph gland specific *hdc* silencing resulted in lamellocyte differentiation in each hematopoietic compartment. Moreover, this regulatory function was confined to the PSC.

Our results show that Hdc - expressed in the PSC cells - plays a regulatory role in the differentiation of lamellocytes. The nonautonomous effect of the factor suggests a potential crosstalk of the hematopoietic compartments with unknown molecular background.

Our work is supported by OTKA NK-101730 and TÁMOP 4.2.2.A-11/1KONV-2012-0035 grants. The research of G. I. Varga and V. Honti was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program'.

Keywords: *Drosophila melanogaster, lamellocytes, differentiation, head*

P-019
CHARACTERIZATION OF ZEBRAFISH MUTANTS WITH IMPAIRED SURVIVAL OF FAST-PROLIFERATING PROGENITOR CELLS IN THE VISUAL SYSTEM

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In zebrafish larvae the most extensive cell proliferation during post-embryonic stages can be observed in the ciliary marginal zone (CMZ) of the retina and in the proliferative zones of the optic tectum (OT).

In a recent genetic screen, we isolated several mutants that show elevated levels of apoptosis specifically in these two highly proliferative tissues at 2 days post fertilization (dpf). Interestingly, after 2 dpf the apoptotic phenotype recedes and some of these mutant larvae can survive up to a week, but at 5 dpf they all show a severe disorganization of the OT. Mapping and characterization of one the mutants revealed that the mutation affects *gins2*, a gene encoding an essential member of the eukaryotic replication complex.

As the replication complex is widely believed to be universally required for cell proliferation, using the *gins2* mutant we want to examine why only certain fast-dividing cell populations are affected by its absence, whereas other, slower dividing cells are seemingly not affected.

P-020
THE *ARCHIPELAGO* TUMOR SUPPRESSOR GENE IS REQUIRED FOR SPERM INDIVIDUALIZATION IN *DROSOPHILA*

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The F-box protein FBXW7 *Drosophila* orthologous Archipelago (*ago*) is widely known for its role in cell cycle as a regulator of CycE, dMyc, Mei-p26 levels. *Ago* also plays role in trachea formation, and it regulates programmed cell death by promoting or hindering apoptotic pathways. Here we present a*rchipelago* function in spermatogenesis. One of the latest steps of *Drosophila* spermatogenesis is individualization, where the elongated 64-spermatids of a cyst gain their own membrane, and become individual sperm. The process starts with actin cone formation around each of the elongated nuclei. The cones start a simultaneous migration, shrivelling unnecessary cell components to the basal end of the cyst. Meanwhile a non-apoptotic caspase cascade activates to facilitate the process by degrading the unnecessary cytoplasmic material. The migrating actin cones first emerge a cystic bulge, and at the end of the cyst, they help the forming of the waste bag. Two E3 ligase complexes (Cul3-Roc1b-Klhl10, Cul1-SkpA-Ntc) are known to regulate the process of individualisation by the regulation of caspase cascade activation, and proteasome function. The *ago* gene encodes a protein that is an F-box subunit of an SCF E3 ligase complex. We identified a male and female sterile allele of *ago*. *Ago*^{ms} allele is semilethal with a few sterile adult escapers. *Ago*^{ms} allele is caused by a transposon insertion in the 5′ end of the gene. We showed that *ago* expression level is significantly decreased in the *ago*^{ms} mutant. Testing the expression of the three annotated *ago* transcripts (ago-RA ago-RB and ago-RC) we find that the ago-RB is specifically absent in the mutant testis. Characterizing the male sterile phenotype, we observed elongated cysts with spermatid individualization defects in *ago*^{ms} mutant testis. We showed that caspase cascade is active in a*go*^{ms} mutants, however the actin cones are scattered, and unable to form cystic bulges, and waste bags. We showed the cysts without individualization die in an apoptotic process. We rescued the phenotype by overexpressing the mCherry-Ago protein in *ago*^{ms} mutant background and observed the accumulation of the transgene in the nuclei of the spermatocytes in early stages. In later stages mCherry-Ago localise to the base of actin cones in the cystic bulge, showing a similar pattern to the Cul1-SkpA-Ntc E3 ligase subunits. Our results suggest that Ago has an independent or additional role in the known individualization specific SCF complex.

Supported by EMBO Installation Grant No.1825, OTKA NF 101001

Keywords: *Drosophila, spermatogenesis, E3 ligase*

P-021
DEGRADATION OF SECRETORY GRANULES IN *DROSOPHILA* LATE LARVAL AND EARLY PUPAL SALIVARY GLAND CELLS

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At the beginning of metamorphosis of the common fruit fly *(Drosophila melanogaster)*, the glue granules of salivary gland cells are exocytosed in order to fix the pupa to a solid surface. However, many granules are retained in these cells, and are subsequently broken down by fusing with lysosomes. This specific type of autophagy is called crinophagy. Thus, this tightly regulated catabolic process in salivary glands is part of the normal fly development.

Crinophagy was discovered using electron microscopy by Robert E. Smith and Marylin G. Farquar in 1966. The molecular mechanism of this process is still unknown. We developed a new fluorescent reporter system for the monitoring of crinophagy that we named GlueFlux. This biosensor is based on the simultaneous use of two fluorophores, DsRed and GFP, both linked to a secretory granule-specific glue protein. This allows the visualization of nascent secretory granules as yellow (red and green) spots in the fluorescent microscope. After fusion with lysosomes, GFP is quenched in the acidic lumen of the resulting vesicles, so degrading secretory granules appear as red spots.

We are currently carrying out a targeted loss-of-function screen to test whether known regulators of macroautophagy, the best-characterized pathway of this intracellular self-degradation process, are involved in crinophagy.

Keywords: *Drosophila, Glue granule degradation, Lysosome, Salivary gland,*

P-022
THE ROLE OF EPG4 IN AUTOPHAGY IN DROSOPHILA

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Autophagy is an evolutionarily conserved catabolic system, which is characteristic to all eukaryotic cells. This mechanism plays a critical role in removal of unnecessary or damaged cellular components by the lysosomal system to recycle them. The major form of autophagy is macroautophagy. During this process the cell forms double-membrane vesicles (called autophagosome) containing portions of cytosol and delivers them to the lysosome. Macroautophagy is involved in neurodegenerative diseases, myopathies, cancer, aging and it is necessary for maintaining cellular homeostasis.

The fruit fly, *Drosophila melanogaster* is a well-functioning model organism to study autophagy. During metamorphosis the larval organs (including the fat body) are eliminated by intensive

autophagy. Stress induced autophagy occurs in starved feeding larvae, so the effect of a gene of interest on the autophagic process can be observed. In our work, we investigated the role of *Drosophila* epg4 gene (the homolog of the mammalian tumour suppressor gene EI24/PIG8, in fruit fly called tank) in autophagy. In mouse EI24 causes accumulation of insufficient autolysosomes and p62. In contrast in *C. elegans*, the formation of the isolation membrane is not sufficient in epg4 mutants.

Our experiments were performed on mutant and RNAi stocks. We used FLP/FRT recombinase system to generate mosaic animals. Our data suggest, that the lack of epg4 gene influence in the late stages of autophagic process.

P-023
A SYSTEMS BIOLOGICAL VIEW OF LIFE-AND-DEATH DECISION AFTER ENDOPLASMIC RETICULUM STRESS – THE ROLE OF PERK PATHWAY

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One of the most important tasks of a cellular system is to maintain its intrinsic homeostasis against external stimuli. To keep the proper balance of both secreted and membrane proteins is controlled in the endoplasmatic reticulum (ER). Accumulation of misfolded proteins due to different ER stressors (such as thapsigargin, DTT) leads to the activation of unfolded protein response (UPR). The primer role of UPR is to reduce the bulk of damages and try to drive back the system to the former or a new homeostatic state by self-eating dependent autophagy, while excessive level of ER stress results in apoptotic cell death.

Our study focuses on the PERK-controlled branch of UPR, where the PERK-induced ATF4 is the transcription factor of both Gadd34 and CHOP. We confim that autophagy is regulated by Gadd34, while CHOP activates apoptosis during ER stress. The main question of this project immediately arises: how the two qualitatively different stress responses can be regulated similarly by the same ATF4 molecule. Our experiments have revealed a strict order of autophagy and apoptosis. The survival mechanism is always followed by the suicide loop, but how these processes can be controlled by the same ATF4 and their targets is still unknown. We claim that the activation of surviving and self-killing mechanisms in proper order is controlled by a negative and a positive feedback loops. This regulatory network makes possible smooth, continuous activation of autophagy during ER stress, while the induction of apoptosis is irreversible and switch-like. Using our knowledge in molecular biological techniques and systems biological tools our goal is to give a qualitative description about the dynamical behaviour of the system by exploring the key regulatory motifs.

Experimental data have been shown that ER stress is tightly connected to generic or degenerative disorders (such as Alzheimer's disease and Parkinson's disease). Studying the signalling pathways of UPR and their role in maintaining ER proteostasis thus have medical importance.

Keywords: *autophagy, apoptosis, endoplasmic reticulum stress*

P-024
NOVEL ROLE FOR GALECTIN-1 IN T-CELLS UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

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Secreted, extracellular galectin-1 (exGal-1) but not intracellular Gal-1 (inGal-1) has been described as a strong immunosuppressive protein due to its major activity of inducing apoptosis of activated T-cells. It has been previously reported, that T-cells express Gal-1 upon activation, however its participation in T-cell functions remains largely elusive. To determine Gal-1 function expressed by activated T-cells we have carried out a series of experiments. We have shown that Gal-1, expressed in Gal-1-transgenic Jurkat cells or in activated T-cells, remained intracellularly, indicating that Gal-1-induced T-cell death was not a result of an autocrine effect of the *de novo* expressed Gal-1. Rather, a particular consequence of the intracellular Gal-1 (inGal-1) expression was that T-cells became more sensitive to extracellular Gal-1 (exGal-1) added either as a soluble protein or bound to the surface of a Gal-1-secreting effector cell. This was also verified when susceptibility of activated T-cells from wild type or Gal-1 knockout mice to Gal-1 induced apoptosis were compared. Murine T-cells expressing Gal-1 were more sensitive to the cytotoxicity of the exGal-1 than their Gal-1 knockout counterparts. We also conducted a study with activated T-cells from patients with systemic lupus erythematosus (SLE), a disease in which dysregulated T-cell apoptosis has been well described. SLE T-cells expressed lower amounts of Gal-1 than healthy T-cells and were less sensitive to exGal-1. These results suggested a novel role of inGal-1 in T-cells as a regulator of T-cell response to exGal-1 and likely contributing to the deficiency of T-cell apoptosis in lupus.

Keywords: *intracellular galectin-1; avtivated T-cells; Systemic lupus erythematosus; Apoptosis*

P-025
CAS9-MEDIATED MUTAGENESIS OF CORE AUTOPHAGY GENES IN DROSOPHILA

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Autophagy, an evolutionarily conserved intracellular degradation process, is involved in numerous disorders including cancer, neurodegeneration, myopathy and aging. Autophagy is thus becoming a widely investigated topic thanks to its biomedical relevance, because of which null mutants are needed for clear-cut genetic analyses. Commonly used mutagenesis techniques have either

extremely low efficiency (homologous recombination) or they are limited by the requirement of existing transposon insertion lines. The most recent solution for this problem is the CRISPR/Cas9 in vivo mutagenesis system. CRISPR/Cas9 is a sequence-specific nuclease from a prokaryotic immune response pathway. The system has two critical components: 1. the Cas9 nuclease, and 2. the target gene specific guide-RNA. Cas9 endonuclease makes a double strand cleavage in DNA at a site specified by its guide-RNA. In the last 2-3 years, this system has been successfully applied for mutagenesis in Drosophila as well as several other eukaryotic model organisms. In this work, we crossed two transgenic lines to each other for mutagenesis: one expressing Cas9 (obtained from a public stick center) and a second one expressing a gene-specific guide-RNA (generated by us). In this strategy, we identify mutant lines using PCR and/or western blots for the selective autophagy cargo p62, which accumulates to high levels if autophagy is disrupted. We have successfully generated mutants for the first try for all five core autophagy genes that we targeted (Atg5, Atg8b, Atg9, Atg14, and Atg101), which would have been impossible with conventional mutagenesis schemes.

P-026
RETINOIDS INDUCE NUR77-DEPENDENT APOPTOSIS IN MOUSE THYMOCYTES

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Nur77 is a transcription factor, which plays a determinant role in mediating T cell receptor-induced cell death of thymocytes. In addition to regulation of transcription, Nur77 contributes to apoptosis induction by targeting mitochondria, where it can convert Bcl-2, an anti-apoptotic protein into a proapoptotic molecule. Previous studies have demonstrated that retinoids are actively produced in the mouse thymus and can induce a transcription-dependent apoptosis in mouse thymocytes. Here we show that retinoic acids induce the expression of Nur77, and retinoid-induced apoptosis is completely dependent on Nur77, as retinoids were unable to induce apoptosis in Nur77 null thymocytes. In wild-type thymocytes retinoids induced enhanced expression of the apoptosis-related genes FasL, TRAIL, NDG-1, Gpr65 and Bid, all of them in a Nur77-dependent manner. The combined action of these proteins led to Caspase 8-dependent Bid cleavage in the mitochondria. In addition, we could demonstrate the Nur77-dependent induction of STAT1 leading to enhanced Bim expression, and the mitochondrial translocation of Nur77 leading to the exposure of the Bcl-2/BH3 domain. The retinoid-induced apoptosis was dependent on both Caspase 8 and STAT1. Our data together indicate that retinoids induce a Nur77-dependent cell death program in thymocytes activating the mitochondrial pathway of apoptosis.

Keywords: *apoptosis, Nur77, retinoids, thymocytes*

P-027
AUTOPHAGY-DEPENDENT REGENERATION OF STEM CELLS IN THE *DROSOPHILA* MALE GERMLINE

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Autophagy is a highly conserved lysosome-dependent catabolic process of eukaryotic cells which can protect cell from several stress factors. It has an essential role in the enzymatic degradation of damaged proteins and organelles. Autophagy has also important roles in cellular differentiation and development, by mediating remodelling of cytoplasmic constituents. In early spermatogenesis of *Drosophila*, there are two different populations of stem cells and the somatic and the germline stem cells (GSCs), GSCs are connected to somatic stem cells (CSCs) and Hub cells (non-dividing somatic cells). CSCs and Hub cells are keeping a special microenvironment (niche) for maintaining the GSCs on undifferentiated form. GSCs are dividing by asymmetric division to give rise to two distinct daughter cells. One of them functions as an ordinary stem cell and the other one will left the stem cells' niche and start it's differentiation into spermatogonial cell. Both the aging process and the accumulation of cellular damage can cause the loss of GSCs. The lost stem cells can be replenished (new GSC will occupy the empty niches) by dedifferentiation of spermatogonial cells or symmetric division of GSCs. The ectopic expression of the differentiation factor *bam* can induce rapid GSCs loss, thus without GSCs we can observe only dedifferentiation. In our study we investigated the potential role of autophagy during this dedifferentiation process. During GSC regeneration, we detected elevated levels of the autophagic process. Dedifferentiation of germline GSCs could be suppressed by deficiency of autophagy. Furthermore, the accumulation of multilamellar bodies could also be observed by electron microscopy and we examined intense colocalization between mitochondrial and autophagic structures by fluorescence microscopy. Interestingly, in the *Atg7* loss-of-function mutant background, *bam* ectopic expression was unable to mediate GSC's loss. From these results we suggest that autophagy has an essential role in dedifferentiation of the *Drosophila* male germline stem cells, and mitophagy can be a possible key mechanism for underlying the role of autophagy function in GSCs' regeneration. Moreover, *bam*-triggered elimination of GSCs is an *Atg7*-dependent mechanism.

Keywords: *autophagy, regeneration, stem cell, germline, Drosophila*

P-028
TGF-ß SIGNALING PATHWAY AND AUTOPHAGY

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Autophagy is a highly conserved process of eucaryotic cells that is responsible for degradation of damaged, dysfunctional or harmfull organelles and macromolecules. It is one of the basic mechanisms of defense against stress. Beyond these crucial functions of autophagy it has also a proven role during morphogenesis as it elevates to an extraordinary high level before the cell death of larval organs in *Drosophila melanogaster*. Considering the current knowledge of the process and molecular regulation of autophagy, it has strong biomedical, social and economic significance.

During insect metamorphosis the increasing autophagic activity is involved in the elimination of larval organs. Many studies show that cells can avoid apoptosis by autophagy in certain cases of development. Besides the increased levels of autophagic processes in these surviving cells an intensification of transforming growth factor (TGF)-ß signaling pathway has been observed. TGF-ß pathway is a highly conserved pathway that has an important role in aging. Since *autophagy related genes* (Atg) also have a deep influence in the regulation of lifespan, certain relationship can be hypothesized between autophagy and TGF-ß signaling. Our goal was to examine the possible relationship between TGF-ß signaling and autophagy in *Drosophila melanogaster.*

We showed, that silencing the TGF-ß receptor caused the appearance of atg8 positive structures,suggesting that the receptor could be a negative regulator of autophagy.

SARA is a downstream member of TGF-ß signaling pathway, and is required for TGF-ß inernalization. We used a dominant negative form of *sara* ($sara^{F678A}$), carrying an amino acid change in the protein phosphatase binding domain. Due to the amino acid change, protein phosphatases were unable to inactivate the TGF-ß receptor, hence the receptor accumulates in a punctate pattern in the citoplasm of $sara^{F678A}$ overexpressing cells. In cells where $sara^{F678A}$ was overexpressed, autophagy could not be induced by starvation and these cells were unable to perform developmental autophagy as well. In those cells many p62 could be also observed, indicating a serious malfunction in the autophagic processes. These results were confirmed by using the constitutively active form of TGF-ß receptor, as we got similar results.

By co-expressing the dominant negative form of *sara* ($sara^{F678A}$) and Akt/PKB RNAi (insulin signaling pathway member), the later could rescue the effect of $sara^{F678A}$ as the appearance of mcherry-atg8 positive structures could be observed. This epistasis analysis result suggest, that TGF-ß receptor signaling cascade inhibits autophagy via insulin signaling pathway.

Taking the above into account we suggest that in *Drosophila melanogaster* TGF-ß pathway could act as a negative regulator of autophagy, possibly by inhibiting premature developmental autophagy.

P-029
INVESTIGATING THE ROLE OF HPS1 AND HPS4 PROTEINS IN AUTOPHAGY

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The normal biogenesis of the lysosomal compartment and the proper function of the associated degradation pathways, endocytosis and autophagy, is essential for maintenance of cellular homeodynamics.

Hermansky-Pudlak syndrome is a genetic disorder caused by mutations of the *Hps* genes. Hps1 and the Hps4 proteins form the BLOC-3 complex, which is required for the biogenesis of lysosomal-related organelles such as melanosomes and pigment granules. The aim of my work was to analyze whether loss of BLOC-3 complex has any effect on autophagic function.

I used *Hps1* and *Hps4* mutant *Drosophila melanogaster* stocks for my experiments. In fluorescence microscopy I found that the autolysosomes were formed but the autophagic cargo was not completely degraded. We confirmed this with ultrastructural analysis of the fat body of the mutant larvae, as a significant proportion of the autolysosomes showed impaired degradation of cytoplasmic structures. Furthermore, both *Hps1* and *Hps4* mutants showed reduced lifespan.

Mon1 protein has a structure similar to *Hps1,* and is an important regulator of endo-lysosomal maturation. Because the BLOC-3 genes showed a relatively weak disturbation of autophagy, we wondered whether there is any redundancy between *Hps1* and *Mon1*. Therefore I recombined the mutant alleles of *Hps1* and *Mon1* to the same chromosome. In contrast to *Hps1* and *Mon1* single mutants which were viable and semi-lethal respectively, the double mutants showed synthetic larval lethality and a much stronger defect in endocytosis and autophagy. Additionally, I observed melanotic tissue mass in the double mutant larvae, which is similar to certain endocytosis defective mutant fly stocks.

In summary, my results show that loss of BLOC-3 leads to decreased level of autophagic degradation as a likely result of abnormal biosynthetic transport to lysosomes. We also discovered a genetic interaction between *Hps1* and *Mon1*, and propose a functional redundancy between these two genes.

P-030
AUTOPHAGY (MITOPHAGY) PLAYS CRUCIAL ROLE IN TISSUE REMODELLING DURING MESENCHYMAL-TO-EPITHELIAL TRANSITION (MET) IN RAT MESENTERIC MESOTHELIAL CELLS

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We previously described that Freund's adjuvant treatment induces an acute inflammation (epithelial-to-mesenchymal transition –EMT) and parallel with these events the morphology of mesothelial cells has remarkable changed: they became cuboidal shaped, lost their polarity, the basement membrane desintegrated, an increasing number of cytoplasmic organelles (specificly the number of mitochondria) was observed. However, the cellular richness of the meosthelial cells disappeared during the recovery period, following inflammation when original mesothelial phenotype was re-established.

Autophagy is known to have the pivotal role in tissue remodelling and allows cells to control the number and turnover of intracytoplasmic organelles while maintaining viability upon stress stimuli. The characteristic components of this degradative pathway are the autophagosomes/autolysosomes containing cytoplasmic components targeted into lysosomes for degradation. Among the numerous extracellular stimuli that might induce autophagy, the role of TNF-α is well established. Sex steroids have also been described as potential players in the process under inflammatory conditions. Our previous data showed a significant *in loco* synthesis of extragonadal estradiol and a synergistic elevation with TNF-α that is known not only to induce autophagy, but upregulating aromatase enzyme it can contribute to local synthesis of estradiol as well.

Since autophagy promots tissue remodelling, we investigated whether autophagy may play role in the recovery process of mesothelial cells. Using conventional electron microscopy, we found some autophage vacuoles (AVs) even under control conditions but the number of AVs markedly increased five days after treatment. As the regeneration progressed, many autophagolysosomes, lysosomes and degenerating mitochondria were observed in the cytoplasm of mesothelial cells. There were no detectable elements of the degradative pathway by the eleventh day after treatment in the cytoplasm of the cells and by this time the morphological re-establishment of the mesothelium was accomplished. Our detailed morphometrical analysis (15-15 electron micrographs/3 independent experiments) confirmed our morphological observations. Microtubule-associated protein light chain 3 (LC3) is a widely used and well-validated biomarker of autophagy. Detecting the conversion of LC3-I to its active form, LC3-II is a commonly used method to monitor the formatin of AVs. Our Western blot results showed that LC3-II expression gradually increased between D5 and D8 correlating with the excessive number of autophage vacuoles observed during this period.

We consider that in our *in vivo* system autophagy presumably induced by both extragonadal estradiol and TNF-α has a pivotal role in the elimination of intracytoplasmic organelles and helps in the recovery of mesothelium following inflammation.

Keywords: *autophagy, mitophagy, mesenchymal-to-epithelial transition (MET), mesothelial cells*

P-031
GENETIC EVIDENCE OF THE ROLE OF PCNA POST-TRANSLATIONAL MODIFICATIONS IN DNA DAMAGE TOLERANCE

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DNA damage bypass mechanisms are required to avoid replication fork collapse. Post-translational modification of Proliferating cell nuclear antigen (PCNA) plays a key role in these processes by recruiting essential proteins implicated in DNA damage bypass. PCNA can be monoubiquitylated at K164 by the Rad6-Rad18 ubiquitin ligase complex. Through this modification, PCNA can interact with low-fidelity Y-family polymerases to promote translesion synthesis. Monoubiquitylated PCNA can be polyubiquitylated by a further ubiquitin-conjugating complex to promote template switching, an error free process.

In our study we used a $PCNA^{K164R}$ mutant DT40 chicken B lymphoblastoma cell line, which is hypersensitive to DNA damaging agents such as methyl methanesulfonate (MMS) or cisplatin due to the fact that PCNA cannot be ubiquitylated. Indeed, by expressing a PCNA rescue construct we were able to restore the sensitivity similar to the wild type. PCNA-ubiquitin fusion proteins have been reported to mimic the monoubiquitylated PCNA, therefore we created and stably expressed (in the $PCNA^{K164R}$ cell line) two further constructs, $PCNA^{K164R}$-ubiquitin and $PCNA^{K164R}$-$ubiquitin^{K63R}$ fusions. Because of the ubiquitin K63R mutation, ubiquitin could not be polyubiquitylated, which facilitates the investigation of the effects of this post-translation modification. We investigated MMS and cisplatin sensitivity and also determined the influence of PCNA ubiquitylation on polymerase recruitment by measuring T-T cyclobutane pyrimidine dimer (CPD) bypass. Cell lines expressing the fusion displayed resulted similar sensitivity and rate of translesion synthesis to the wild type, suggesting that the polyubiquitylation of PCNA is not necessary to protect cells from replication-stalling DNA damage.

Keywords: *DNA damage bypass, PCNA, DT40*

P-032
THE EFFECT OF RADIATION ON MURINE BRAIN PERICYTES

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Introduction: Pericytes are main components of the brain microvascular region, having pleiotropic functions. Apart of regulating the integrity and normal homeostasis of the blood-brain barrier they play important role in brain inflammation also. The objective of our study was to investigate pericyte radiosensitivity by quantifying radiation induced DNA damage and repair kinetics by the γ-H2Ax assay. The assay is based on the detection of the phosphorylated form of the H2AX histone protein, which is highly sensitive and dose-dependent, but due to the quick repair process changes are only

informative for short time period after irradiation. Long-time persisting foci indicate a repair-deficiency and an on-going genetic instability.

Methods: Primary pericyte cultures were established from the brain of young C57Bl/6 mice. Cells were grown and irradiated on coverslips with different doses (0.01, 0.05, 0.1, 0.5, 2 and 4 Gy). After irradiation, cells were incubated for different time periods and γ-H2Ax assay was used to detect DNA double strand breaks (DSBs) and repair kinetics. For immunofluorescence staining, fixed and permeabilized cells were incubated with anti-γ-H2AX antibody. For quantitative analysis, foci were manually counted by a fluorescent microscope by using a 100x objective. For statistical analysis Pearson's t-test was used. Differences were accepted as statistically significant if $p<0.05$.

Results: The level of nuclear foci showed a strict dose-dependency within the tested radiation dose range (0.01 – 4 Gy) 1 hour after irradiation. The number of foci decreased after longer incubation times due to DNA repair mechanism. Pericytes showed fast repair kinetics after high doses, but a certain level of residual damage could be identified even 3 days after irradiation. Interestingly, the level of residual damage after low doses was very close to the level of residual damage after high doses.

Conclusions: These finding indicates an incomplete repair mechanism after both low and high doses of ionizing radiation. This shows that even very low doses can induce an ongoing genetic instability. Thus it is important to consider that the effect of applied low doses in human diagnostics incorporates the risk for IR-induced carcinogenesis.

P-033
THE GENOME OF THE CHICKEN DT40 BURSAL LYMPHOMA CELL LINE

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The DT40 chicken lymphoma cell line has been an important tool in the study of several biological processes due to the relative ease of conventional genome editing caused by its high ratio of targeted over non-targeted homologous recombination. As exact sequence information highly increases the success of gene targeting, a study has been conducted to determine the genome of DT40 cells by whole genome shotgun sequencing and to identify any genomic alterations that contributed to the unique features of this cell line.

On comparison with the *Gallus gallus* reference genome and genomic data from two further domestic strains, a relatively normal karyotype was found, except for the polyploidy of chromosomes 2 and

24. Both the number and the spectrum of single nucleotide polymorphisms compared to the reference genome were similar to that of other sequenced chicken breeds, and only a minor increase in the relative number of short indels was found.

We catalogized all coding mutations that are unique to DT40 and tried to identify possible driver mutations during oncogenic transformation. Although no classical tumor genes were found affected, we suspect a deletion of two residues in PIK3R1 and a frameshift deletion in ATRX to be relevant.

The DT40 cell line was immortalized by infection with avian leukosis virus (ALV), so we analyzed the number and positions of possible viral insertion sites. In addition to the already known insertion into the Myc gene and two endogenous retroviral sites, we also found two novel ALV insertion sites in the genes FAM208B and SLC13A5 with a yet unknown relevance.

These findings are consistent with the view that DT40 is a cell line with a relatively normal genome, meaning that it is a suitable model system to obtain valid experimental results. In order to further promote research in the DT40 field, all genomic data obtained and analyses performed, including a list of all mutated genes and a searchable *de novo* genome assembly are also available online (http://dt40.enzim.ttk.mta.hu).

Keywords: *DT40, genome sequencing*

P-034
ESTABLISHMENT OF AN IN VITRO REPLICATION SYSTEM FOR STUDYING REPLICATION BYPASS MECHANISMS

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UV light frequently generates photodimers in DNA between two pyrimidine bases, and these lesions will block the movement of replication forks later, during DNA replication. Such replications blocks can cause serious mutations if not bypassed, or even if bypassed with error prone processes. Previous investigations in our laboratory mapped the potential DNA damage bypass pathways in DT40 chicken cells, using a plasmid based test system, which enabled us to differentiate between the potentially error-prone translesion synthesis and the error-free template switching. Those studies initiated the development of a simpler and effective *in vitro* test system, where considerable replicated material is obtained allowing further tests on the outcome of replication and studies on the replication structures and protein factors.

Our system involves SV40 large T antigen that was expressed in a baculoviral expression system, cytosolic extracts obtained from HeLa cells, and a buffered replication mix containing NTPs, dNTPs, and an ATP regeneration system.

First, the system was calibrated using DNA vectors with or without functional replication origin sequences. The nature of the obtained material was tested with restriction digestion using methylation sensitive restriction endonucleases (Dpn I and Mbo I) in order to discriminate between truly replicated (Dpn I resistant) and non-replicated (Dpn I sensitive) DNA.

We managed to replicate either lesion-free plasmid DNAs, or a plasmid containing the UV photoproduct, cyclobutane pyrimidine dimer (CPD). Transforming the purified and Dpn I digested material obtained from this CPD containing template into E. coli resulted in colonies that contained plasmid molecules derived from *in vitro* replicated DNA. Sequencing the replicated plasmids revealed error-free damage bypass in most cases, suggesting a template switch to the sister chromatid. In addition, signs of translesion synthesis were also observed in some cases. Further characterisation of this *in vitro* system will allow molecular studies of DNA damage bypass at fully assembled replication forks.

Keywords: *DNA damage, DNA replication, DNA bypass-mechanisms, in vitro replication, SV40 T antigen*

P-035
THE FUNCTION OF BRCA1 IN CHICKEN DT40 CELLS IN THE RESPONSE TO ALKYLATING DNA DAMAGE

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BRCA1 (Breast cancer associated gene 1) is a tumor suppressor gene, normally expressed in a range of tissues including cells of the breast and ovary. It promotes genome integrity and it is a vital participant in cellular responses to DNA damage. This protein has been implicated in multiple biochemical and biological functions. The BRCA1 protein interacts with other proteins set up multiple, distinct protein complexes with functions in DNA repair and DNA damage signalling. BRCA1 protein plays an important role in the process of the error-free homologous recombination (HR), a double strand break repair mechanism that can also help rescue collapsed replication forks. Mutations of BRCA1 increase the risk of the development of breast and ovarian cancer.

In this study we compared a BRCA1 knock-out chicken DT40 bursal lymphoma cell line to wild type DT40 cells. In our experiments we examined the sensitivity of these cell lines to alkylating(-like) agents, namely MMS (methyl methanesulfonate) and cisplatin, by methylcellulose colony survival assays and color-coded cytometry-based cell growth assays. These agents directly damage DNA, causing long-term damage without cell cycle phase specificity. The rates of treatment-derived mutations were determined by next-generation sequencing of the whole genomes of the examined cell lines. Following either MMS- or cisplatin-treatment, the BRCA1$^{-/-}$ mutant cell lines displayed a characteristic increase in short deletions relative to the control cells. The mutational spectra were not changed in the BRCA1 mutant cell line compared to the wild-type. Including heterozygous mutants in our analysis, we observed that the BRCA1 knock-out cell line was more sensitive to alkylating agents than the wild-type or BRCA1$^{+/-}$ cells. We analyzed DNA damage markers in these cells by immunfluorescence and immunoblotting assays as well. The results supported the sensitivity and mutagenesis data.

Keywords: *BRCA1, tumor suppressor gene, DNA repair*

P-036
UVB INDUCES A MAJOR GENOME-WIDE REARRANGEMENT OF RNA POLYMERASE II AT TRANSCRIBED HUMAN GENES

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Transcription of DNA is continuously disturbed by damaged DNA triggered by various genotoxic effects from endogenous and also environmental sources. Transcription coupled repair (TCR) has been described to take part in the rapid restoration of blocked transcription by elimination of DNA lesions from the transcribed strand of active genes. However, the mechanism of TCR in individual target genes has been well studied, the precise global mechanism by which the action of RNA polymerase II (Pol II) transcription is regulated following UVB irradiation during the DNA repair processes is still not well understood.

In order to study the effect of UV on Pol II transcription we treated MCF7 human cells with the non-lethal dose of UVB and we accessed the DNA-bound Pol II distribution. We found that about 90% of the promoters of expressed genes showed reduced Pol II occupancy 2-4 hours following UVB irradiation, which was restored to “normal” or higher levels 5-6 hours after the treatment. Interestingly, we found a smaller set of the active genes, where the enrichment of Pol II was not decreased after UVB irradiation at the promoter regions, but increased throughout the entire transcription unit. We also observed that promoters, where Pol II clearance occurred, the behaviour of TFIIH but not of TBP highly resembled that of Pol II suggesting that at these genes TFIIH might be sequestered for DNA repair upon UVB treatment.

In conclusion, our study uncovers a global negative regulation of Pol II transcription initiation on the large majority of transcribed genes following non-lethal UVB irradiation, with the exception of a small subset of genes (including regulators of repair, cell growth and survival), where Pol II escapes this negative regulation.

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P-037
A NOVEL SOMATIC GENE TRANSFER SYSTEM IN MICE

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Today's science uses an enormous amount of transgenic animal models. Therefore, transgenic technologies are central to the field of molecular biology and biotechnology. In spite of this, the creation of animal models fulfilling all aspects of scientific requirements is often difficult. Fundamental problems are the low efficiency of genomic transgene integrations and the poor or sometimes completely missing transgene expression. The classical pronuclear microinjection method is suitable for germline transgenesis generally yielding transgene concatemers made of 20-300 copies in the genome of the host organism. Such transgene clusters often undergo DNA methylation and gene silencing thereafter, resulting in weaker or completely missing expression. These drawbacks can be avoided by the so called active transgenesis, where a pronuclearly introduced helper enzyme actively integrates transgenes. Still germline transgenesis remains being expensive, laborious and time consuming. Our laboratory successfully developed an alternative somatic transgenesis method in the mouse liver that avoids the modification of the germline and allows the rapid and even simultaneous analysis of numerous transgenes. By that, in accordance with the principle of the 3 Rs (Replacement, Reduction and Refinement), it also allows significant reduction in the number of experimental animals.

The liver is a highly regenerative organ, in rodents 10% intact liver tissue is sufficient for total liver regeneration. Consequently, in a mutant genetic background where genetically normal hepatocytes have a selective advantage, extensive liver repopulation can be achieved. If those normal hepatocytes are also genetically modified, an efficient transgenic cell replacement system may be created, in order to build organs containing a very high percentage of transgenic cells. We harness the mouse model of human tyrosinemia type I. for the creation of such a transgenic cell replacement system and the hyperactive *Sleeping Beauty* transposon for the efficient chromosomal delivery of our transgenes.

This novel somatic gene transfer system is particularly suitable for the investigation of carcinogenesis and the underlying mechanisms of distinct liver diseases. Sporadic cancer is a disease dependent on the accumulation of somatic genetic changes therefore transgenic animal models created by conventional germline transgenesis are often inadequate for the investigation of mechanisms driving its development. Germline transgenic strains are more adequate models of germline hereditary cancer predisposition syndromes.

Keywords: *Tyrosinemia type I, somatic transgenesis, transposon*

P-038
DEVELOPMENT OF AN *IN VITRO* RNAI EFFICIENCY MONITORING SYSTEM

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RNA interference (RNAi) is a biological process in which small non-coding RNA molecules silence gene expression transcriptionally or post-transcriptionally. In the past 15 years, this cell physiological phenomenon has become a powerful tool to analyze loss-of-function phenotypes, allowing analysis of gene function when null-mutant alleles are not available. It needs to be considered that RNA-based silencing is a method to reduce (knock down) but not eliminate gene function. Therefore, it is important to design and use as efficient target gene silencer RNAs as possible in functional genetic studies. Consequently, there is a growing need for rapid, quantitative screening to confirm small non-coding RNAs efficiency on target gene knockdown.

Our aim is to develop an *in vitro* cell culture system screening for the most effective target gene silencer RNAs. In this approach, a marker gene can be used as an indicator of the effectiveness of a small non-coding RNA on gene silencing. More specifically, we use a fluorescent reporter gene (eGFP) fused to a short, 22 nt sequence from any given target gene located in the marker gene's 3' UTR. This short sequence serves as the target site of the tested silencer RNA loaded into the RNA-induced silencing complex (RISC), leading to the degradation of the eGFP mRNA due to full complementarity between these two RNA species. Subsequently, the target gene knockdown efficiency of a silencer RNA can be measured based on the decrease in the level of eGFP fluorescence intensity or eGFP gene expression.

In our experimental design, the marker gene is expressed from a single genomic integration site created by the *Sleeping Beauty* transposon system in a HeLa cell line that leads to equal level of fluoresence intensity in the cell culture. It means much more accurate measurement of the RNAi efficiency compared to other screening systems based on a transiently expressed marker gene in which the copy number of the delivery vector can vary from cell to cell. On the field of RNAi three types of RNA molecules are applied: small interfering RNA (siRNA), short hairpin RNA (shRNA) and the most recent artificial microRNA (amiRNA). Our system uses the novel improved, amiRNA-based strategy because it has numerous advantages over the use of other types of silencer RNAs. AmiRNAs can be expressed from any RNA-polymerase-II-dependent promoter and embedded in UTRs or introns of coding transgenes which broadens their usage for tissue-specific and/or inducible expression studies and allows the co-expression of a reporter gene. Considering these advantages, we hope that our RNAi efficiency monitoring system will become a fast, reliable and cost effective method for functional genetic approaches.

Keywords: *RNAi, RNAi effectiveness, Transposon*

P-039
THE ROLE OF Β-ADRENERGIC RECEPTOR IN THE BILE SALT SIGNALING

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Taurocholate, one of the major mammalian bile acid is known to regulate hepatocyte polarization, canalicular network formation (1), and trafficking of certain apical (canalicular) membrane proteins, such as the bile salt export transporter, ABCB11 (2). Taurocholate acts through a cAMP-Epac-MEK-mediated activation of LKB1 and AMPK, however, the mode of action is unknown. It has been demonstrated that activation of the β-adrenergic receptors in hepatocytes elevates canalicular expression of ABCB11 in an Epac-MEK-dependent manner (3). An alternative, PKA-dependent regulatory mechanism of ABCB11 trafficking has also been proposed (2, 3), which seems to be independent of the β-adrenergic receptor. Based on these observations, we hypothesized that β-adrenergic receptors are involved in the taurocholate-mediated canalicular trafficking of ABCB11.

To test our hypothesis we used MDCK cells, since they express β-adrenergic receptors, but have no detectable NTCP, a taurocholate uptake transporter expression. Using this model system we measured the changes in the intracellular cAMP levels in response to taurocholate in the presence and absence of various receptor agonists and antagonists. The phosphorylation levels of downstream targets of the cAMP-Epac-MEK signaling pathway, such as LKB1 and AMPK, as well as of the cAMP-PKA signaling pathway, such as CREB, were also determined. Our results revealing the role of β-adrenergic receptor in the taurocholate-mediated cellular responses may contribute to the understanding the mechanism how taurocholate regulates ABCB11 trafficking and hepatocyte cell polarization.

This work has been supported by the Momentum Program of the Hungarian Academy of Sciences (LP2012-025), and a research grant from the Research and Technological Innovation Fund (KTIA_AIK_12-1-2012-0025).

Keywords: *taurocholate, β-adrenergic receptor, cAMP, LKB1, AMPK*

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P-040
INVESTIGATION OF POTENTIAL STEROL-BINDING SITES IN THE ABCG1 PROTEIN

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The ABCG1 protein belongs to the ATP binding cassette (ABC) transporter G subfamily. As regards its function, ABCG1 have been proposed to play a role in HDL-dependent cellular lipid/sterol regulation and can induce apoptosis by an unknown mechanism. Studies investigating the HDL-dependent efflux of different sterol derivatives identified cholesterol, desmosterol, 7-ketocholesterol, etc., as potential substrates of ABCG1. Moreover, accumulation of desmosterol was observed in brain of Abcg1 knock-out mice. However, direct interactions between ABCG1 and these sterols have not been demonstrated yet; neither the regions of ABCG1 interacting with sterol compounds are revealed. Several previous studies identified specific amino acid sequences in proteins involved in cellular sterol homeostasis. The Cholesterol Recognition Amino acid Consensus (CRAC, L/V-$X_{(1-5)}$-Y-$X_{(1-5)}$-R/K) and Sterol Binding Element (SBE, L/M-XX-L-XX-L) motives were examined in several proteins and identified as an important sequence in the interaction with sterol molecules.

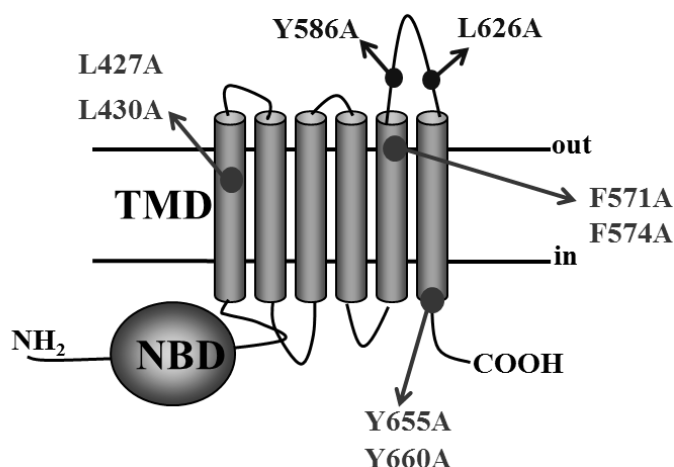

Fig. 1. *Mutations of the putative CRAC and SBE motives in ABCG1*

In the present work, we mutated the putative CRAC and SBE motives in the ABCG1 protein by replacing the central tyrosine or leucines to alanine by using site-directed mutagenesis. The different ABCG1 variants were expressed in both mammalian cells and Spodoptera frugiperda 9 (Sf9) insect cells. The expression and localization of these CRAC and SBE mutants were investigated in Hela and HEK cells by Western blot analysis and confocal microscopy, respectively. The apoptotic effects of the ABCG1 mutants were compared to that of wild type protein using a High Content Screening instrument.

In addition, the function of the ABCG1 variants was assessed by measuring their ATPase in Sf9 cell membrane preparations. Using this approach the interaction of ABCG1 mutants with different sterol compounds can also be measured. Combining the results obtained with mammalian and Sf9 cells

allowed us to map the sterol-sensing regions in the ABCG1 protein, and establishes a link between the different functions of the transporter.

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P-041
INVESTIGATION OF NON-SYNONYMOUS SNPS IN THE GENE ENCODING HUMAN ORGANIC ANION TRANSPORTING POLYPEPTIDE 4A1

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Organic Anion Transporting Polypeptides (OATP) encoded by *SLCO* (Solute Carrier of the OATPs) genes are plasma membrane proteins which mediate sodium-independent cellular uptake of various amphipathic endo- and xenobiotics (bile acids, hormones, clinically important drugs) with secondary active transport. A total of 11 human OATPs are known, which are expressed in several important barrier organs of the human body and their proper functions are essential in many physiological processes (e.g. hepatic circulation, detoxification, the coordination of metabolism). Therefore, genetic variations present in OATP-encoding genes may lead to the development of diseases (e.g. Rotor syndrome is caused by mutations in the genes of OATP1B1 and 1B3) and altered pharmacokinetics of drugs. OATP4A1 is a ubiquitously expressed protein, involved in the uptake of steroid and thyroid hormones, prostaglandin E2 and taurocholate. Overexpression of OATP4A1 has also been documented in colon and ovarian cancers, though its role in tumor progression and survival has not yet been defined.

Our aim was to investigate genetic variations in the gene encoding OATP4A1. We set up a PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method for the detection of two non-synonymous single nucleotide polymorphisms (SNP) in the *SLCO4A1* gene (rs34419428 and rs1047099). Using this method, we determined the frequencies of these SNPs in a Hungarian population consisting of 65 healthy individuals. We found that the allele frequencies within this population are in agreement with data found in international databases for the European population. We also examined the OATP4A1 polymorphisms in a population of colon cancer patients where we compared individual genotypes with clinical data and OATP4A1 expression levels. We found that the investigated two polymorphisms of OATP4A1 do not increase the risk of colon cancer development but the minor allele of the c.209G>A SNP (rs34419428, causing R70Q change) decreases OATP4A1 protein expression in tumor cells. We also demonstrated that this allele (c.209A) exists in linkage disequilibrium with the minor allele (c.232A) of the c.232G>A SNP (rs1047099, resulting in V78I).

To better understand the importance of these two SNPs, the polymorphic variants of OATP4A1 were also characterized by *in vitro* functional assays to evaluate their effect on the expression, localization and function of OATP4A1.

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P-042
FUNCTIONAL EXPRESSION OF HUMAN ORGANIC ANION TRANSPORTING POLYPEPTIDES (OATPS) IN SF9 INSECT CELLS REVEALS SODIUM-FLUORESCEIN AS A GENERAL OATP SUBSTRATE

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Organic Anion Transporting Polypeptides (OATPs) are encoded by the SLCO (Solute Carrier family of the OATPs) gene superfamily. The 11 human OATPs are transmembrane proteins involved in the uptake of numerous amphipathic and anionic compounds of endogenous (e.g. steroids, prostaglandins, bile acid etc.) or exogenous (e.g. statins, antibiotics, anticancer drugs) origin. In addition to their important physiological roles, OATPs have also been shown to influence the pharmacokinetics of several clinically relevant drugs, underlining the importance of these proteins in transporter-drug interaction testing. However, to examine drug-OATP interactions, precise and reliable model systems are required. Such models and functional assays are indispensable in elucidating the mechanism of OATP-mediated transport, a process that is not yet fully understood. Moreover, new experimental systems are critical in the case of two poorly characterized members of the family, OATP5A1 and 6A1, for which there are no functional assays available at all.

The aim of the present study was to test the applicability of a baculovirus-Sf9 (*Spodoptera frugiperda*) insect cell expression system for the analysis of human OATP function. To verify the functionality of the expressed human OATPs, we investigated the accumulation of two previously described fluorescent substrates of OATP1B1 and 1B3, sodium-fluorescein and fluorescein-methotrexate. In Sf9 cells transiently overexpressing human OATP1B1 or 1B3, we found a temperature- and specific inhibitor-sensitive transport of these two substrates. Moreover, transport kinetics of these substrates were comparable to that measured in mammalian cells, demonstrating that these transporters are fully functional in insect cells. We also tested the accumulation of the two aforementioned fluorescent compounds in Sf9 cells overexpressing the other members of the human OATP family. Based on these results we describe sodium-fluorescein as a general substrate of human OATPs. Furthermore, we also show that most human OATPs are triggered by an inwardly directed H^+ gradient.

In conclusion, we demonstrated that human OATPs are functional when expressed in insect cells, and that this *in vitro* model system is a suitable tool for functional characterization and further substrate screening of human OATPs.

Funding: this research has been supported by the Hungarian Research Fund (OTKA, K-109423) and by MEDinPROT.

P-043
A POTENTIAL ROLE OF THE PUTATIVE MITOCHONDRIAL TRANSPORTER ABCB6 IN VACUOLAR DETOXIFICATION

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ABCB6, a member of the ATP-binding cassette (ABC) transporter family, was first identified as the human ortholog of the yeast mitochondrial ABC exporter Atm1p. Subsequent studies suggested that ABCB6 catalyzes the mitochondrial import of a heme synthesis intermediate, thereby serving as an important regulator of cellular porphyrin biosynthesis. Based on these conflicting findings ABCB6 is usually discussed in the context of mitochondrial ABC transporters, despite mounting evidence supporting its extramitochondrial localization. Recently, we and others have shown that ABCB6 is a glycoprotein present in the plasma membrane and the endo/lysosomal compartment, and also demonstrated that ABCB6 function is not required for de novo heme biosynthesis. The identification of the endolysosomal localization of ABCB6 paved the way for studies aimed at the elucidation of its physiological function. ABCB6 is homologous to the heavy metal tolerance factor 1 (HMT1) of *Caenorhabditis elegans* and *Schizosaccharomyces pombe*. HMT1 confers tolerance to heavy metal toxicity in *S. pombe* and tolerance to cadmium in *C. elegans*. Since HMT1 is expressed in the lysosome-like vacuoles, our aim was to test the role of the human ABCB6 protein in metal tolerance. Subcellular localization of ABCB6 was analyzed by confocal microscopy; the ability of ABCB6 to rescue the cadmium-sensitive phenotype of HMT1-deficient S. pombe strains was assayed in cytotoxicity experiments. Our results will be presented in the context of the prediction that ABCB6 is a functional orthologue of HMT1, pumping transition metal complexes into the interior of acidic vesicles.

P-044
SUBSTRATE BINDING AND TRANSPORT BY WALKER-A MUTANT PGPS

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P-glycoprotein (Pgp) is an ABC transporter that is able to extrude a large variety of chemotherapeutic drugs from cells, causing multidrug resistance of cancer cells. The protein consists of twelve transmembrane alpha-helices forming the substrate binding site, and two nucleotide binding domains (NBD) involved in ATP binding and hydrolysis. Based on crystal structures of several ABC transporters, Pgp is believed to alternate between an inward and an outward facing conformation, characterized by high and low substrate binding affinities, respectively.

Despite accumulating structural and functional data, it is still unknown how ATP binding and hydrolysis are connected to the conformational changes that allow transmembrane transport. To elucidate partial catalytic reactions, we studied Pgp variants carrying mutations in the conserved Walker A region (K433M and K1076M) of either the N-terminal or C-terminal ABC domains or both.

Although mutation of these key residues have been shown to abolish ATPase and transport activity, we found that single mutants possessed a residual drug efflux activity (the double mutant variant was indeed inactive). Confocal microscopic image analysis showed that both the single and double mutant Pgp variants sequester vinblastine-bodipy in the plasma membrane, whereas wild-type Pgp can efficiently catalyze transmembrane transport. Fluorescence cross-correlation analysis proved that the sequestered vinblastine-bodipy strongly co-localize with the mutant Pgp molecules. Since the vinblastine-bodipy staining of the plasma membrane could be competed with Pgp substrates, these results suggest that mutations of the key Walker A lysines stabilize Pgp in the inward open, substrate binding conformation. Using transition state analogs, single mutants could be trapped in the outward open (low substrate affinity) conformation, suggesting that single mutations allow the transition between the two conformations.

Taken together, we show that mutation of a single Walker A lysine is compatible with a residual transport activity. Analysis of the partial catalytic reactions suggests that ATP binding brings about the conformational change needed to switch Pgp from the inward facing to the outward facing conformation.

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P-045
COMPLETE GENOME CHARACTERIZATION OF NOVEL AVIAN PICORNAVIRUSES (ORIVIRUS A1 AND A2): IDENTIFICATION OF POTENTIAL RECOMBINATION EVENTS AND VARIABLE GENOME REGIONS

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Members of the family *Picornaviridae* are small viruses with positive sense, single-stranded RNA genome which are capable of infecting different vertebrate species including birds. The monophyletic avihepatovirus phylogenetic cluster of picornaviruses contains the members of genera *Avihepatovirus*, *Avisivirus* and "Aalivirus" which were originally identified from domestic ducks, and turkey. The characteristic genome feature of these avian picornaviruses is the presence of three different types of 2A proteins in tandem. Our research group was identified and characterized the complete genome of a novel avian picornavirus provisionally called as Orivirus A1 (OrV-A1) from cloacal samples of diarrhoeic domestic chicken (*Gallus gallus domesticus*) using viral metagenomic and RT-PCR methods [1]. The OrV-A1 was detectable in 6 of the 12 cloacal samples (50%) of apparently healthy chickens. Based on the results of sequence and phylogenetic analyses the OrV-A1 could be the founding member of a novel picornavirus genus ("Orivirus") which belongs to the avihepatovirus phylogenetic cluster. The genome of OrV-A1 contains (i) a type II internal ribosomal entry site (IRES), (ii) a single 2A protein with no identifiable function, and (iii) multiple repeated sequence motifs followed by an AUG-rich region at the 3′ UnTranslated Region

(UTR). The repeated motifs show significant sequence identity to the multiple "Unit A" sequences of the phylogenetically distant megriviruses (genus *Megrivirus*). The presence of a novel single 2A and the megrivirus-like "Unit A" motifs suggest multiple recombination events in the evolution of Orivirus A1 [1]. Sequence analysis of the viral metagenomic contigs revealed the presence of a second orivirus genotype (Orivirus A2), which was presented in the same sample as Orv-A1. The results of sequence comparison analysis between the complete genome of OrV-A2 and OrV-A1 suggest that the most variable genome region is not the immunodominant VP1 (86.6% amino acid /aa/ identity) but the 2A (65.6% aa identity). This result suggest an accelerated pace of evolutional changes in the 2A non-structural genome region in contrast to the other parts of the genome.

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Keywords: *virus, avian picornavirus, chicken picornavirus, genome, recombination,*

References:

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P-046
CONSTRUCTION OF A PROPHAGE-FREE, HYBRID-GENOME E. COLI BL21 HOST STRAIN BY DIRECTED GENOME SHUFFLING

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E. coli BL21(DE3) is a widely used host strain for recombinant protein production. While being a superior protein producer, the strain is poorly accessible to genetic engineering (poor transformability by plasmids, unpredictable recombination events, propensity for lysis). On the other hand, K-12 strains are easy to manipulate genetically. Moreover, genome streamlining and elimination of mobile genetic elements, as well as genes involved in generation of mutations were shown to yield further improvements in the applicability of the K-12 host cell. We set out to combine the favorable traits of BL21 and K-12. Taking advantage of the "clean"-genome (mobile genetic element free, reduced-genome) K-12 derivative MDS42, a BL21 strain-based hybrid-genome E. coli was constructed.

K-12 and BL21 have similar core genomes (99% core sequence identity with notable variations), but possess significantly diverged, laterally transferred genomic islands, including prophages and other mobile genetic elements. Marked segments of "clean"-genome K-12 MDS42 were sequentially transferred to BL21 by P1 transduction to replace regions occupied by prophages. To further improve engineering, the genomic region responsible for host restriction and modification was removed. To ensure that the resulting cell lines possess the desired characteristics, after each genome re-arranging step a recombinant strain pool was screened for efficient protein-expression and for preserving good growth profiles. The T7 polymerase-expressing construct (important for

recombinant protein production) of the original BL21(DE3) strain was replaced by a modified, tightly controlled lac-T7 polymerase system. Altogether, 9 regions of the BL genome were replaced by the corresponding K-12 MDS42 sequence, and all prophages were eliminated from the genome. The resulting, BL21-based strain harbors an estimated 270 kb K-12 sequence, retains the excellent protein producing features, displaying unaltered stress tolerance and increased genomic stability.

Keywords: *genome reduction, prophage, protein overexpression*

P-047
GENETIC EXAMINATION OF INTERSPECIES *SACCHAROMYCES* HYBRIDS

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Hybridization among *Saccharomyces* species takes place frequently in winery and natural environment and it is easy to form ’artificial’ interspecies *Saccharomyces* hybrids in the laboratory. Most of these hybrids are sterile, cannot produce viable ascospores (equivalents of gametes) that causes their death under extreme conditions (vegetative cells are more sensitive to harsh conditions than ascospores). However, the hybrid sterility is not absolute. We found in a previous study that certain artificial (lab-made) *Saccharomyces cerevisiae* x *S. uvarum* hybrids (mainly allopolyploids) could regain the ability of sexual reproduction by losing one of the parental chromosomes containing the mating type regulatory locus. Genetic characterization of these dihybrids (two-species hybrids) and their offspring allowed us to gain an insight into the complexity of the mechanism of the evolution of interspecies hybrids (usually only genomic chimeras) occurring in nature. Here we report on the production and analysis of *S. uvarum* x *S. kudriavzevii* hybrids. The hybrids were created by the mass-mating technique of spores and/or cells and then investigated by physiological (e.g. sporulation, fermentation of sugars, growth morphology in liquid medium) tests and molecular (e.g. Polymerase Chain Reaction – Fragment Length Polymorphism of chromosomal markers, mtDNS polymorphism, electrophoretic karyotyping) genetic methods. The hybrid strains showed high degree of phenotypic similarity but turned out to be heterogeneous in genome structure, stability and segregation. Our results are partly consistent with our earlier data obtained with a different species combination.

Keywords: *interspecies hybridization,*

P-048
THREE-SPECIES *SACCHAROMYCES* HYBRIDS: FORMATION AND ANALYSIS

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In last decades several Saccharomyces strains have been isolated from oenological environments, which possess mosaic (chimera) genomes. Those chimeric genomes contain genes from two or three Saccharomyces species. For example CID1 is a so-called trihybrid which received genes from three species, Saccharomyces cerevisiae, S. uvarum and S. kudriavzevii. A possible way of formation of such chimeric yeasts involves two successive hybridization events among three species and elimination of unnecessary genes, chromosome fragments or even entire chromosomes (genome stabilization). To study the process, we created artificial three-species hybrids (trihybrids) in the laboratory. The hybrids were generated in two steps: first, two species were hybridized and then segregants of their hybrids were hybridized with the third species. The combinations were: (S. cerevisiae x S. uvarum) x S. kudriavzevii and S. cerevisiae x (S. uvarum x S. kudriavzevii). The obtained trihybrid strains were characterized and analyzed by various genetic and molecular methods. All hybrids had unstable chimerical genomes consisting of incomplete genomes of the hybridized species. Each hybrid had a unique combination of the chromosomal markers of the parental strains, indicating that the hybridization of multiple Saccharomyces species does not follow a common pattern.

Keywords: *trihybrid, interspecies hybridization*

P-049
MECHANISM OF ANTIBIOTIC SELF-RESISTANCE OF PRIMYCIN PRODUCING *SACCHAROMONOSPORA AZUREA* STRAINS

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Many species of Actinomycetes produce bioactive natural products and possess resistance systems for these metabolites, which are frequently clustered and co-regulated with antibiotic biosynthesis genes. Microorganisms require one or more self-resistance determinants to produce antibiotics; these encode proteins that inactivate the antibiotic, facilitate its export, or modify the host to render it insensitive to the antibiotic. Multiple resistance mechanisms are often found; in such cases, it is not known whether any one resistance mode is sufficient to ensure survival or antibiotic production.

Several recent findings support the *hypothesis* that antimicrobial resistance is not a new phenomenon and antibiotic producer bacteria represent a reservoir of drug resistance genes also called intrinsic *resistome*. The concept of antibiotic *resistome* is closely related to the spreading of resistance genes *via* horizontal gene *transfer; on the other hand resistome* composition of antibiotic-producing organisms are also correlated with improved yields and increased *production* of useful bioactive compounds.

We have previously shown that Saccharomonospora azurea a member of rare Gram-positive Actinomycete genera; belonging to the family Pseudonocardiaceae; produces a *non-polyene macrolide* lactone antibiotic complex, called primycin. Macrolides constitute a group of antibiotics mainly active against Gram-positive bacteria. *Topically administered* primycin is a highly potent agent against *broad range* of *infections* caused by Gram-positive b*acteria* including *MRSA strains; however the self-protection* mechanism(s) employed in the *antibiotic*-producing *Saccharomonospora* strains are completely uncharacterized. To provide new insights into the molecular basis of antibiotic self-resistance in primycin producing *S. azurea* strains, two interrelated *approaches such as whole* genome sequencing *and* transcriptome profiling *were applied.*

Even though the *S. azurea* strains analyzed in this study differ markedly in their *ability* to produce primycin (SZMC-14600 and NA-128 with high- and low-antibiotic production respectively); *comparative structural genomics studies revealed* a highly similar overall coding capacity between these two intraspecific strains. However *quantitative RT-PCR confirmed* whole transcriptome analyses *revealed considerable differences* in *expression of genes* confer resistance to macrolide antibiotics.

***Keywords**: resistome, macrolide antibiotic, self-resistance mechanism*

P-050
THE IDENTIFICATION OF TWO NOVEL ALLELES OF THE *RSD* (REGULATOR OF SYMBIOSOME DIFFERENTIATION) GENE IN *MEDICAGO TRUNCATULA*

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Legumes and rhizobia are able to form symbiotic nitrogen-fixing interaction. This assosiation induces the formation of a new organ called root nodule wherein nitrogen fixation takes place. Bacteria enter to the nodule cells via endocytosis-like process and form endosome-like structures termed symbiosomes. In symbiosomes bacterium undergo differentiation and develop into bacteroids to be able to reduce atmospheric nitrogen.

In order to dissect the symbiotic interaction between *Medicago truncatula* and its compatible symbiotic partner, *Sinorhizobium meliloti* we analyzed two ineffective symbiotic (Fix-) mutants, *dnf8* (does not fix nitrogen) and the NF-FN9290 (Nobel Foundation-Fast Neutron). Both mutants showed the symptoms of nitrogen deficiency under symbiotic conditions and the microscopic investigation showed less number of bacteria in the cells of the invasion zone and only few invaded cell in the

fixation zone. The genetic mapping experiments indicated that the *dnf8* and NF-FN9290 loci mapped to the same region in chromosome 7 and F1 allelism test proved that they are allelic. Microarray-based cloning identified a deletion corresponding to the mapped loci in *dnf8*. Using PCR-based markers we delimited the size of the deletion about 340 kbp, containing about 60 genes. The analysis of the gene content of the genomic region identified a gene, encoding a C_2H_2-type transcription factor recently cloned from a *M. truncatula* insertion symbiotic mutant. The sequence of the *RSD* (Regulator of Symbiosome Differentiation) gene was determined from NF-FN 9290 background which identified a two bp deletion in the coding sequence of the *RSD* gene.

We carried out complementation tests to verify that beside the *RSD* gene no other gene in the deleted region is required for the effective nitrogen-fixing symbiosis. To identify target genes of the C_2H_2-type transcription factor, transcriptome analysis of the *dnf8* mutant has been initiated and down-regulated genes will be verified by quantitative RT-PCR.

P-051
DELETION ANALYSIS OF *SALMONELLA* GENOMIC ISLAND 1

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The *Salmonella* genomic island 1 (SGI1) conferring multidrug resistance (MDR) appeared in the 80's in *Salmonella enterica serovar Typhimurium*. This multiresistant pandemic clone with seemingly enhanced virulence has rapidly spread all over the world in the 90's. SGI1 proved to be an integrative mobilizable genetic element that contains a complex In104 integron encoding the resistance for ampicillin, chloramphenicol, florphenicol, streptomycin, spectinomycin, sulphonamides and tetracycline. The prototype and many variants with altered resistance spectra have appeared in numerous endemic *Salmonella* serovars and some *Proteus mirabilis* strains, which means significant health risk for humans and livestocks. Some ORFs of the conserved SGI1 backbone are shown to be involved in site-specific excision/integration of the island, but majority of ORFs have unknown functions. The large conjugative plasmids of IncA/C family proved to be the specific helpers of SGI1 transfer, however genetic basis of this connection is mostly unexplored.

Aims of our group are to investigate the molecular mechanisms of the horizontal transfer of SGI1 to identify the relevant ORFs and non coding sequences involved and to explore the genetic background of connection between SGI1 and IncA/C helper plasmids in the conjugative transfer. In this work we have generated directed deletions by the one-step gene-inactivation method using λ Red recombinase mediated homologue recombination in the backbone of streptomycin/spectinomycin resistance. SGI1-C variant integrated chromosomally in laboratory *E. coli* strain. This set of deletion mutants makes possible to study the role of SGI1 regions in the conjugal transfer promoted by the IncA/C plasmid R55 and in specific functions such as excision which is affected by flhDC-like regulator ParB homologue KorB encoded by R55.

By comparing the conjugation frequency of deletion mutant and wild type islands and PCRs indicative of excision activities we aim to map all SGI1 ORFs involved in excision and transfer. Based

on these results we would like to deduce and construct the fully transfer competent minimal SGI1 model system for further investigations.

Keywords: *Salmonella genomic island 1, Integron, IncA/C family plasmids, Multidrog resistance, Horizontal gene transfer*

P-052
CONSERVED SYNTENY AND GENE ORDER DETERMINATION IN *SCHIZOSACCHAROMYCES*

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In the near past it was assumed that genes within eukaryotic genomes are randomly distributed. For now, analyzes of the increasing amount of accessible annotated genome sequences provides evidences that challenge the aforementioned view. For example, genes of similar expression or genes co-regulated by common transcription mechanisms tend to cluster more often than expected by chance. Several factors have been examined by other laboratories that contribute to the formation of gene clusters in the Saccharomyces lineage. It was found that intergenic distance seems to be the strongest predictor of synteny conservation beside co-expression and the local density of essential genes. Our aim was to investigate the determinants of gene order conservation in the ancient clade of fission yeasts which has a distinct evolutionary history from budding yeast using *Sch. pombe* as reference genome, since the genomes of *Schizosaccharomyces octosporus*, *Sch. cryophilus* and *Sch. japonicus* were sequenced and assembled.

First we revealed the synteny relationships among the four *Schizosaccharomyces* species. Strikingly, we observed that 2117 genes of more than 4200 1:1:1:1 orthologs remained in conserved synteny blocks that consist of at least five genes. Next, the evolutionary rates of genes from some randomly chosen conserved and non-conserved blocks were compared. Interestingly, there were no significant differences. Thereafter the biological role of the genes in those blocks were examined. Finally, the seemingly strongest factors of gene order conservation: intergenic distance (from cds to cds), co-expression (in response to environmental stress) and local essential gene density were investigated. We found that the mean intergenic distance on the edges of the conserved blocks were almost twice longer than the mean intergenic distance within the blocks. As for the number of essential genes, we also noticed that conserved blocks show much higher density of them than non-conserved blocks. Co-expression in this case seems to be not as important as thought before.

Keywords: *synteny, gene order, Schizosaccharomyces, bioinformatics.*

P-053
NEXT GENERATION SEQUENCING: POWERFUL TOOL FOR TARGETING NOVEL CELLULOLYTIC MODEL ORGANISMS

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Lignocellulose is the most abundant and renewable source of organic carbon in the biosphere. In nature, it is found almost exclusively in plant cell wall. For utilization of this raw material as biofuel or oligosaccharides and sugars, a number of biomass conversion methods have been employed ranging from direct chemical methods like acid hydrolysis to biological methods such as application of cellulase enzymes. So any process that could efficiently and economically convert cellulosic material to glucose would be of high industrial significance.

Bacteria adapted to lignocellulose as sole source of energy and carbon express a series of different hydrolases (cellulases, xylanases, mannanases) in order to degrade this recalcitrant substrate. Model organisms and their enzyme systems like the aerobic *Thermobifida fusca, Cellulomonas flavigena* or the anaerobic *Clostridium thermocellum* are well-characterized and applied for industrial processes. On the other hand, nowadays new model organisms could also be found via NGS. The recently described *Saccharophagus degradans* has the most complex hydrolytic enzyme system as it harbors up to 110 different polysaccharide degrading enzyme encoding genes. Here we report the polysaccharide degrading system of two recently isolated new species new genus candidate bacterial strain (K07 and K13) based on their finished de-novo genome projects. Genome sequencing of the strains was performed by combining sequencing-by-synthesis on MiSeq system (Illumina) with 454 FLX pyrosequencing (Roche). Assembly was accomplished using in house developed workflow. Automatic annotation of the genomes was achieved by Prokka.

The outstanding cellulose degrader, compost-inhabiting, mesophilic, bacterium K07 belongs to the Gram-negative saccharophagus-clan. The 4,23 Mbp genome (G+C contents 51,26%) is well-equipped with genes for plant cell wall degradation. The sequence analysis of the annotated genome reveals 81 predicted glycoside-hydrolase (GH) enzymes belonging to 28 different GH-families. Many of these enzymes exhibit unusual domain architecture, including the presence of long polyserine linker adaptors (PSL; 25-61aa) between the polysaccharide binding- and hydrolytic-domains. K07 encodes a large set of GH43 putative xylanase enzymes - 12 isoenzymes with different domain structures. The use of malectin for polysaccharide binding in several GH enzymes seems to be a peculiar feature of strain K07.

K13 (4,28 Mbp; GC content 52%) is a thermotolerant bacterium (Topt~50 °C), its taxonomic position is near the Gram-positive *Paenibacillus* group. The analysis of the annotated genome revealed the existence of 40 putative glycoside-hydrolases belonging to 24 different GH-families. From this

enzyme pool 17 enzymes belongs to endoxylanases, 10 to cellulases, 5 to galactosidases, 3 to mannanases, 2 to amylases and only one pectate lyase was found. GH5 is the largest glycosyl hydrolase family in K13 (6 title). Interestingly at least one of the different cellulase, xylanase and mannanase enzymes harbor 3 consecutive S-layer domains at the C- or the N-terminus. It looks like that strain K13 uses a full set of cell surface bonded polysaccharide-degrading enzymes. More interestingly, dozen long proteins decorated with cadhesin, IG, fibronectin and different cellulose-binding domains contain also S-layer triplet domain motive. These structures may act as arms for anchoring the polysaccharide substrates to the cell surface.

P-054
qPCR ASSAY FOR MEASURING *TRICHODERMA* PEPTAIBOL SYNTHETASE GENE EXPRESSION

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Species of the imperfect filamentous fungal genus *Trichoderma/Hypocrea* (Ascomycota, Pyrenomycetes, Hypocreales, Hypocreaceae) are well known for the production of peptaibols or peptaibiotics. Trichoderma species have been studied widely due to their production of industrial enzymes, antibiotics and their action as biocontrol agents. The peptaibols and the related peptabiotics are 7-20 amino acid length nonribosomal peptides, which have antibiotic and antifungal activity. A database of peptaibol structures currently lists 316 structures grouped into nine distinct subfamilies. Peptaibols are synthesized by non-ribosomal peptide synthetases (NRPSs). NRPS enzimes are modular and act in stewise assembly from amino acid monomers. The moduls are subdivided into domains. Each module are responsible for incorporation of one amino acid and contain minimum three domains. Every domain catalyse different reactions, from which the substrate activation, the substrate covalent bindig and the peptide binding are essential. In addition, other domains can catalyse some further non-essential modifications.

Identification of peptaibols can be utilized by analytical and molecular biological methods, however so far universal qPCR assay for detect and measure gene expression based on peptaibol synthetase encoding genes is absent in the literature. We designed three TaqMan Assays for analysing the *Trichoderma* peptaibol NRPS expression with degenerated primers. Due to the redundance of the domains must be to select unique region for the assay design, the target was the acetyltransferase domain, which every peptaibol NRPS contains only one copy. Alignment of different *Trichoderma* peptaibols NRPS acetyltransferase domains showed three conservative region, which we targeted with three degenerated assays.

Keywords: *peptaibol, Trichoderma, NRPS, quantitative PCR*

P-055
FROM GENOMICS TO PROTEOMICS IN THE FIELD OF ANTIBIOTIC RESEARCH

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As the problem of antimicrobial resistance becomes more widespread, the need for new anti-infective agents is more urgent than ever. Actinomycetes are known as one of the most significant pharmacologically important metabolites producers with over 10.000 bioactive compounds. Currently, the revolution of "omic" technologies has changed the scenario of natural product research and soon leads *to the conclusion that,* capacity of microbes to produce bioactive secondary *metabolites* was underestimated. These high-throughput experimental techniques supported by bioinformatics tools could provide a wide range of omics data, allowing *depth*-analysis for understanding complex biological processes. As a result of these new perspectives significant amount of research *has focused* on detection, function, and biosynthesis of polyketide synthase (PKS) and non-ribosomal peptide synthase (NRPS) enzyme complexes.

The *recently announcement genome project focused on a rare* Actinomycetes, clearly demonstrated that *Saccharomonopora azurea* is a *prolific source* of structurally diverse *secondary metabolites, however* these *in silico* information are not to tie to products in the laboratory till now. Our previous structural and functional genomics efforts revealed *transcriptional active* gene clusters corresponding to PKS, NRPS and PKS/NRPS metabolisms spanned a genomic region of over 300 kb.
To get additional and deeper insights into the molecular basis of secondary metabolism, proteomes of two intraspecific *S. azurea* strains were analyzed (SZMC-14600 and NA-128 with high- and low-antibiotic production respectively) *under different growth conditions.* Total proteins were separated on two-dimensional (2D) gels. Isoelectric focusing was performed on 7-cm IPG strips (pH 3 to 10; Bio-Rad). For the second dimension, the IPG strips were applied on the top of 12.5 % SDS-PAGE and the proteins were resolved. Comparative peptide profiles of the two *S. azurea* strains revealed considerable quantitative and qualitative differences. Some of the differentially expressed and clearly detectable protein spots that could have potential effects on the secondary metabolite production were identified by ingel trypsin digestion via peptide mass fingerprinting (PMF) using MALDI TOF/TOF MS.

Keywords: *proteome, antibiotics, 2D peptide gel-electrophoresis*

P-056
COMPARATIVE CHARACTERIZATION OF BACTERIAL AND HUMAN PYRUVATE AND ALPHA-KETOGLUTARATE DEHYDROGENASE MULTIENZYME COMPLEXES RECONSTITUTED FROM RECOMBINANT COMPONENTS: BINDING STOICHIOMETRY, PH-DEPENDENCE AND REACTIVE OXYGEN SPECIES GENERATION

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Individual recombinant components of pyruvate and alpha-ketoglutarate dehydrogenase multienzyme complexes (PDHc, α-KGDHc) of human and bacterial (*E. coli*) origin were expressed and purified from *E. coli* with optimized protocols. The four multienzyme complexes were each reconstituted under optimal conditions in different stoichiometric ratios in view of the still debated stoichiometry. Activity measurements detecting NADH production were used to determine binding stoichiometry with the highest catalytic efficiency at physiological pH. Since some of these complexes were shown to possess 'moonlighting' activities under such pathological conditions that are often accompanied by acidosis, maximal activity was also determined at a lower pH of 6.3. Reactive oxygen species (ROS) generation is a pathologically relevant feature of these complexes hence the efficacy of superoxide generation by the complexes with optimal stoichiometry was determined by the acetylated cytochrome c reduction method in both the forward and the reverse catalytic directions. Various known respective affectors of physiological activity and ROS production, including Ca^{2+}, ADP, lipoylation status or pH, were investigated. The human complexes were also reconstituted with the most prevalent human pathological mutant of the E3 component, G194C and characterized; the G194C mutation was previously reported by us to stimulate ROS generation by isolated hE3. Beyond the optimal complex stoichiometries, the major conclusions of this study are that: *i.* PDHc is equally strong to α-KGDHc as a producer of ROS; *ii.* Both bacterial complexes also generate ROS, *iii.* The only complex among those investigated here of which its E1 component or E1-E2 subcomplex generate significant amount of ROS relative to the intact complex is the human α-KGDHc; *iv.* incorporation of the G194C mutant of hE3 into reconstituted α-KGDHc and PDHc results in decreased activity by both and higher ROS generation by α-KGDHc in the reverse reaction.

P-057
THE BENEFITS AND HAZARDS OF NEW GENERATION SEQUENCING IN GENETIC DISEASES

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With the heavily expanding field of genomics and large datasets of functional information linked to genes, there is a growing demand for the early identification of genetic diseases or factors that are playing roles in the susceptibility to diseases. Also the recognition of genetic information plays a key role in personalized medical treatment especially in the case of cancerous diseases. The most advanced, state of the art method for sequencing is based on new generation sequencing techniques. The large amount of sequence information and low cost makes them the ideal way for genetic disease diagnostics. During routine diagnostics we have identified several platform dependent and independent drawbacks of these unmatched machines. Presenting the benefits and hazards of the New Generation Sequencing methods could enlighten the importance of deep knowledge of the techniques, from the wetlab and sample preparation, through the sequencing platform, to the end of bioinformatical data processing.

Keywords: *NGS, diagnostics, genetic diseases*

P-058
MOLECULAR BIOLOGICAL CHARACTERIZATION OF H3.3 K27M WITH BIOPHYSICAL TECHNIQUES

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The H3.3 K27M histone mutation defines clinically and biologically distinct subgroups of high-grade gliomas (pHGGs). Exon sequencing has identified this mutation in about 30% of pediatric glioblastoma multiformes and 70% in brain stem tumor diffuse intrinsic pontine gliomas. The molecular biological consequences of this driver mutation as well as how the substitution affects pathways contributing tumor initation and progression are not well understood. In order to clarify the molecular background of H3.3 K27M we raised the question whether the mutation is able to modify the nucleosome structure or kinetics properties of the histone protein. By performing *in vitro* nucleosome reconstitution followed by energy transfer based structural analyses we observed a mildly opened conformation of nucleosomes containing the mutant H3.3 histone. In fluorescence recovery after photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS)

measurements the H3.3 K27M nuceosomes showed a faster repopulation rate and higher mobile fraction compared to their wild-type counterpart. The immobile fraction was analyzed by a laser scanning cytometer (LSC)-based *in situ* salt elution assay, revealing an increased salt-dependent stability of the chromatin incorporated mutant histone. Our results suggest that the disease caused by the H3.3 K27M histone is related to the altered mobility and diffusion kinetics of mutant nucleosome core particles.

Keywords: *chromatin, histone, epigenetics, glioblastoma*

P-059
MUTATION SCREENING IN RARE MYOPATHIES

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The myopathies are neuromuscular disorders in which the primary symptom is muscle weakness due to dysfunction of muscle fiber. Other symptoms of myopathy can include include muscle cramps, stiffness, and spasm. The aim of our study was to identify the underlying genetic abnormalities in case of two patients with rare myopathies. A 4-year-old Hungarian boy presenting with the clinical and histological findings of nemaline myopathy type 2, and a 5-month-old infant with the histological diagnosis of centronuclear myopathy were investigated. Peripheral blood samples were taken from the patients and genomic DNA was isolated. Direct sequencing of the *NEB* and *MTM1* genes was performed. The mutation screening of the *NEB* gene revealed two previously unreported heterozygous mutations: a deletion (c.24527_24528delCT p.P8176fsX8179) in exon 174 and a duplication (c.24250_24253dupGTCA p.T8085fsX8100) in exon 171 in the DNA sample of the patient with nemalin myopathy. In case of the patient suffering from centronuclear myopathy, the genetic analysis detected a hemizygous nonsense mutation (c.1456C/T p.Arg486X CM990881) in the *MTM1* gene. These mutations result in the formation of premature termination codons, thus tey presumably leading to truncated nebulin and myotubularin proteins. Our investigations have great importance for the affected families since they help family planning. Hopefully, these findings might also provide the basis of future studies for the development of novel therapeutic modalities in neurogenetic disorders.

Keywords: *neuromuscular disorders, nemaline myopathy, centronuclear myopathy, NEB gene, MTM1 gene*

P-060
COMPARISON OF TUMOR-ASSOCIATED AND NORMAL MYOFIBROBLASTS DERIVED FROM PATIENTS BEARING GASTROINTESTINAL TUMORS

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The predominant cells in the microenvironment of various epithelial tumors are myofbroblasts (MFs), which have an important contribution to cancer development. The basic roles of these spindle-shaped stromal cells are promotion of wound healing and involvement in tissue remodeling and deposition of the extracellular matrix. Their functions depend largely on the gene expression program, which influence their contractile, secretory and migratory capacities. In the tumor microenvironment MFs might support tumor cell invasion and formation of metastasis by acquiring novel functions resulting from an altered gene expression profile as compared to cells in the healthy tissue unaffected by the tumor. To better understand the contribution of MFs in tumor invasion and matrix remodeling, we studied resection samples derived from gastrointestinal tumor patients. Immunohistochemical analysis showed α-smooth muscle actin- and vimentin-positive MFs in increased number and with altered morphology in the tumor affected tissue. In order to analyze the possible functional differences we compared the migratory capacity of primary MFs of healthy tissues and that of those isolated from surgical samples obtained from colorectal and oesophagus tumors. By scratch wound assays we demonstrated that tumor-derived MFs exhibited higher migration rate than their normal counterparts. A comparison of the expression of selected genes on low density gene expression array revealed that numerous genes involved in tumorigenesis, metastasis, matrix remodeling and cell migration showed altered expression level in tumor-associated MFs compared to MFs localized in the healthy tissue. These observations further support the morphological and functional differences between these cells. Finally, we tested whether genetic or epigenetic alterations such as differences in DNA methylation and in histone acetylation and/or methylation status detectable between tumor-associated and normal MFs could explain the shift in gene expression profile. Immunocytochemistry, immunoblot and promoter DNA methylation analysis revealed slight epigenetic differences between tumor-associated and normal myofibroblasts, however, no significant genetic deviations within the most frequently mutated cancer genes were detectable. Thus, the demonstrated alterations and the observed gene expression changes suggest the involvement of epigenetic mechanisms in determining the morphological and functional characteristics of these cells. Our results on tumor-associated MFs contribute to the better understanding of the impact of MFs in supporting tumor progression.

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P-061
ANTI-PROLIFERATIVE ACTIVITY OF CITRATE-COATED SILVER NANOPARTICLES IN CERVICAL CANCER CELLS

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Given the exceptional toxicity of silver nanoparticles (AgNPs) against a broad spectrum of Gram-positive and negative bacteria and several pathogenic fungi AgNPs have been used in the last decade as potent antibacterial materials in household products and in medicine. In addition, AgNPs induce cellular stress and apoptosis in several mammalian cell types as well therefore their cytotoxic propensity could be exploited to kill cancerous cells. The revolutionary application of AgNPs as chemotherapeutical drugs seems very promising nevertheless the exact molecular mechanisms underlying AgNP-induced cell death have to be elucidated in details before clinical administrations. It has been demonstrated that after cellular uptake AgNPs release highly toxic silver ions within the cell, which eventually results in the activation of apoptotic pathways. Although several studies revealed the cytotoxic effect of AgNPs, it is still unclear how some physical and chemical parameters like particle size, shape, the utilized reducing agent, the coating and the functionalizing groups might influence the anti-cancer activity of silver nanoparticles.

In the present study we applied biocompatible citrate-coated, quasi-spherical silver nanoparticles of approximately 5 nm diameter on HeLa cervical cancer cells. By transmission electron microscopy the uptake of nanoparticles into HeLa cells has been verified as we observed nanoparticles both on the surface and inside the cells where the dispersed and aggregated AgNPs were largely localized in membrane coated vesicles. The amount of cell-associated and intracellular silver was quantified by atomic absorption spectroscopy. We observed significantly reduced viability of cancer cells upon AgNPs administration, and the toxicity of the particles proved to be time and concentration dependent. Staining AgNP treated HeLa cells with cleaved caspase 3 specific antibody verified the induction of apoptosis during AgNP mediated cell death. This finding was reinforced by significantly elevated mRNA levels of apoptosis related genes like Bax, caspase 3 and superoxide dismutase 1, and by decreased expression of the anti-apoptotic survivin measured by real-time quantitative PCR. Relative 5-bromo-2-deoxyuridine incorporation indicated significantly reduced proliferation capacity of HeLa cells already 6 hours upon AgNP treatment.

Our results show that cancer cells are able to take up the spherically shaped silver-citrate nanoparticles which attenuate cell proliferation and induce cancer cell death by apoptosis. On a broad scale, silver nanoparticles represent a feasible platform for the rational design of therapeutically useful anticancer agents so long as their actions are fine-tuned with bioactive functionalizing molecules.

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P-062
RESISTANCE MECHANISMS IN SILVER-CITRATE NANOPARTICLE TREATED CANCER CELLS

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Multidrug resistance (MDR) continues to be the major challenge in tumor therapy despite the significant advancements in the treatment of cancer over the last decades. MDR cancer cells evolved a marvelous adaptation capacity to toxic insults as they are able to activate a combination of resistance mechanisms when chemotherapeutic agents are applied. One of the most relevant resistance actions involves the induction of drug efflux transporters, like P-glycoprotein, which could actively export cytotoxic drugs, preventing their cellular accumulation. Lately, new nanotechnology-based strategies gathered grounds as these might provide the potential to overcome MDR. Silver nanoparticles (AgNPs) possessing unique chemical and antibacterial features are the most commonly used nanomaterials. Previous studies indicated that AgNPs induce cellular stress and apoptosis in several mammalian cell types thus we could take advantage of their cytotoxic properties to kill cancer cells. AgNPs seem to be very promising novel chemotherapeutic agents however the exact molecular background of their cytotoxicity has to be elucidated.

In this study we treated drug-sensitive (Colo205) and P-glycoprotein overexpressing drug-resistant (Colo320) colon adenocarcinoma cells with citrate-coated AgNPs of approximately 35 nm size and measured cell viability. AgNPs killed both cells in a concentration dependent manner, and drug-resistant cells showed higher viability compared to drug-sensitive Colo205. This result confirmed the higher survival and the resistance of Colo320 cells to anticancer agents. The intake of AgNPs by these cancer cells was verified by electron microscopy. The increased number of caspase positive Colo205 cells and the elevated mRNA levels of apoptotic and stress marker genes (bax, p21, sod-1, caspase-3) proved the activation of programmed cell death in Colo205 cells, however elevated expression of the anti-apoptotic survivin was detected in Colo320 cells. The amount of reactive oxygen species in AgNP treated Colo205 and Colo320 cells was significantly increased compared to control suggesting the induction of the mitochondrial apoptotic pathway. Immunodetection of p53 showed abundant amounts and nuclear export of the protein in Ag-citrate nanoparticle treated drug sensitive colon cancer cells.

Our results show that both drug resistant and sensitive cells take up AgNPs, which induce apoptosis via activation of p53 pathway in the sensitive cell line. However Colo320 cells manifest various resistance mechanisms to combat the stressor and survive. AgNPs represent a feasible platform for the rational design of therapeutically useful anticancer compounds, acting as single agents or in combination with conventional drugs, so long as their actions are fine-tuned with bioactive functionalizing molecules.

This project was founded by TÁMOP-4.1.1.C-12/1/KONV-2012-0014.

Keywords: *Silver nanoparticles, multidrug resistance, cancer, apoptosis*

P-063
ISOLATION AND CHARACTERISATION OF A NOVEL NEPOVIRUS

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In our group, we study the role of small interfering RNAs (siRNA) and post-transcriptional gene silencing (PTGS) in the antiviral defense of plants. Virus infected plants display various symptoms with different type and severity and these symptoms can reduce the crop yields enormously. Although the molecular mechanisms behind the virus induced disease symptoms are in the focus of many research groups, however the process still poorly understood. By studying symptoms during naturally occurring virus infections, we observed interesting viral symptoms on begonia plants (*Begonia elatior*) infected with an unknown virus. The occurring symptoms are ringspots, which change diurnally. To identify the virus we infected some differentiating host plants with the unknown virus and we concluded this virus may belong to the genus of Nepoviruses or Ilarviruses. We tested some previously described group-specific degenerate primers recognizing both genuses. Our experiments confirmed that the examined virus is a Nepovirus. The Nepoviruses (nematode-transmitted, polyhedral particles) are a wide group and infect many plant families. Probably, Nepoviruses cause the most serious viral diseases of crops and are of serious dismay to quarantine authorities worldwide. A Nepovirus genome consists of two RNA species, both of which are transcribed as single polyproteins.

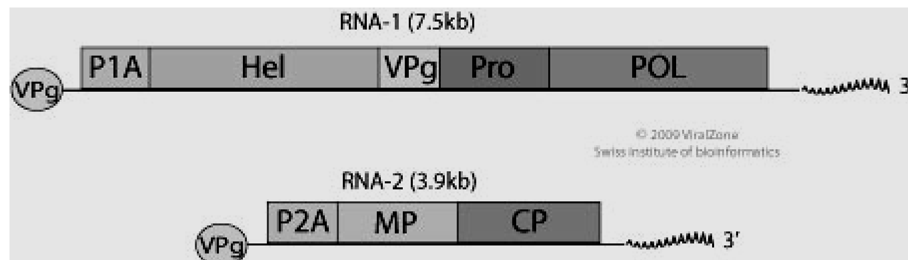

***Figure 1**: Nepovirus segmented, bipartite linear ssRNA(+). Segment has a VPg linked to its 5' end and a 3' poly(A) tract. RNA-1 encodes the helicase, polymerase and proteinase, and RNA-2 encodes for the capsid and movement prote*

The genus is separated into three subgroups (A, B, C), which based on the size of RNA-2, sequence similarity and serological relationship. Using the group-specific primers we determined that our virus belongs to the subgroup B. Complete nucleotide sequences of subgroup B viruses were retrieved from NCBI GenBank, and were aligned using ClustalW2 to find conserved regions. Overlapping fragments of the viral genome were amplified by primers located in the conserved regions and cloned into pGEM-T Easy for sequencing. Sequences of the fragments were stitched together using CAP3 sequence assembler. The assembled viral genome was compared to other subgroup B viral genomes by the blastn algorhythm.

The isolate has been named as Hungarian begonia ringspot virus (HBRSV), which is a member of the subgroup B within the Nepovirus group.

This study was funded by the Young Researcher Career Development Program of the Ministry of Agriculture.

Keywords: *Begonia, Ringspot, Nepoviruses, Subgroup B*

P-064
TYPE IV COLLAGEN DOMAINS IDENTIFIED VIA INTERALLELIC COMPLEMENTATION REGULATE MUSCLE DIFFERENTIATION AND SARCOMER FORMATION

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Recent clinical studies and mouse genetic analyses indicate that basement membrane defects resulting from mutations of the *COL4A1* gene cause muscle weakness, contractures, cramps, muscular dystrophy, and atrophy. In-depth study of these phenotypes is still missing and important details of the mutant COL4A1-associated muscle-specific pathomechanism remain unexplored. In this study, *Drosophila* models with *col4a1* trans-heterozygote double mutants were generated and used for analyses of mutation-associated cellular mechanisms and resulting muscle phenotypes.

Comprehensive studies of eight *Drosophila col4a1* mutants were carried out using genetic analyses, protein expression data, and immunohistochemistry that focused on the myopathic phenotype of the the striated single-layer muscle fibers of the common oviduct. The recessive phenotype of the mutants manifested as embryonic lethality of the homozygotes. Trans-heterozygotes, however, proved viable by interallelic/intragenic complementation. Subsequently, trans-heterozygotes, identified by balancer loss, were prepared in all forward and reciprocal allelic combinations (56) in experiments generating 22,969 individual flies.

The phenotypic features of the double mutants included accumulation of the COL4A1 protein within the sarcoplasmic reticule (SR-stress), central nuclei, formation of actin cables without interdigitation and sarcomere formation consistent with myofibrillar/centronuclear myopathy, and lack of costamers. COL4A1 domains involved in these pathologic processes proved to be direct or indirect integrin binding sites or motifs regulating triple helical assembly. Punctate integrin staining that co-localized with the costamers, was replaced in mutants by diffuse, uneven distribution of the protein.

Results collectively demonstrate that type IV collagen domains play critical roles in determing the achore function of integrins during sarcomer formation. Thus the genetic insults exerted by *col4a1* mutations clearly identify type IV collagen as a novel etiologic factor during muscle fiber differentiation and sarcomere formation.

This research was supported by the Hungarian Scientific Research Fund OTKA, contract nr. NN 108283 to M.M.

P-065
OVEREXPRESSION OF ANTIMICROBIAL PEPTIDE GENES AND INTESTINAL DYSFUNCTION IN *DROSOPHILA* TYPE IV COLLAGEN MUTANT

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Multicellular eukaryotes are constantly exposed to microbial challenges; to combat against infection they are armed by sophisticated repertoire of host defense mechanisms. In the fruit fly *Drosophila melanogaster* reactions of the systemic and local innate immunity defend the animals exclusively. The host immune response against pathogens includes the physical barriers of epithelia, located beneath the cuticle, in tracheae, and in the alimentary tract. These cell sheets produce antimicrobial peptides and reactive oxygen species upon pathogenic challenge, which in turn may damage the epithelia. We have recently reported massive cell degradation following detachment from the basement membrane in temperature-sensitive, type IV collagen *col4a1 Drosophila* conditional mutant, affecting the striated muscle fibers of the common oviduct, the circular smooth muscle and the epithelial cells of the intestine. We therefore hypothesized that the mutant may suffer from chronic inflammation and exert a robust immune response. We demonstrate overexpression of antimicrobial peptide genes in the *col4a1* mutant. We identified *Acetobacter cerevisiae* and *Lactobacillus plantarum* as cultivable members of the gut flora. Alteration of the gut microbiome included marked decrease of the bacterial counts in the mutant. Mutant and wild-type flies performed similar sensitivity to the challenge by the intestinal bacteria at elevated temperature. The *col4a1* mutation caused intestinal dysfunction in young flies otherwise observed during aging and reduced their longevity remarkably. Antibiotic treatment increased the lifespan of the animals. Our results demonstrate that detachment and degeneration of the gut cells from the basement membrane, a yet unexplored issue, disrupts the physical barrier and provides a primary cause for antimicrobial peptide overexpression and intestinal dysfunction in *col4a1* mutant.

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Keywords: *extracellular matrix, intestinal dysfunction, collagen type IV, col4a1, Drosophila, microbiome, antimicrobial peptides*

P-066
SILVER NANOPARTICLES INDUCE CANCER CELL APOPTOSIS IN A P53 INDEPENDENT MANNER

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Loss of function of the tumor suppressor p53 is observed frequently in human cancers thereby challenging the drug induced apoptotic elimination of tumor cells from the body. This phenomenon is a major concern and provides much of the impetus for current attempts to develop a new generation of anti-cancer drugs capable of inducing apoptosis in a p53 independent manner. Because of their unique cytotoxic propensities silver nanoparticles (AgNPs) seem promising nano-inspired chemotherapeutic agents in future cancer therapy. However, the exact mechanism behind the activity of AgNPs in tumor suppressor deficient cancer cells is not fully understood, therefore we investigated their effects on osteosarcoma cells with different p53 genetic backgrounds.

In this study 5 nm and 35 nm sized, citrate coated AgNPs were used on wild type p53 expressing U2Os and on p53 deficient Saos-2 osteosarcoma cells and the main apoptotic responses (i.e. viability, proliferation, caspase 3 activation) were investigated upon AgNP expositions.

We found that although differently sized AgNPs stimulate p53 signaling, AgNPs are able to kill p53 deficient Saos-2 cells as well suggesting that AgNPs can induce apoptosis in a p53 independent manner. We observed significant mitochondrial dysfunction by detecting mitochondrial protein release into the cytoplasm, loss of mitochondrial membrane potential and increased level of reactive oxygen species upon AgNP administrations in both U2Os and Saos-2 cells. According to these data we concluded that the primary action of AgNPs is to induce mitochondrial stress which drives cancer cell apoptosis in a p53 independent manner.

Our results indicate that osteosarcomic cancer cells are able to uptake both 5 nm and 35 nm spherical shaped silver-citrate nanoparticles which induce cancer cell apoptosis by targeting mitochondria. Since elimination of p53 function is one of the most commonly observed impairments in human cancers, the fact that AgNPs are inducers of p53 independent apoptotic mechanisms, enhances their potential and renders them attractive novel candidates for the rational design of therapeutically useful anticancer agents.

This project was founded by TÁMOP-4.1.1.C-12/1/KONV-2012-0014.

Keywords: *Silver nanoparticles, p53, cancer, apoptosis*

P-067
IONIZING RADIATION INDUCED MITOCHONDRIAL DYSFUNCTION IN MOUSE BRAIN

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Introduction: Increasing number of epidemiological and animal experiments point to the brain toxicity of low-dose irradiation, especially if delivered at a young age. Mitochondrial damage is a potential non-targeted effect of low dose radiation. Since brain mitochondrial damage is involved in several neurological diseases, the aim of this study was to investigate the ionizing radiation induced mitochondrial dysfunctions in mouse brain.

Methods: Ten-day and ten-week old C57/Bl6 mice were locally irradiated on their head with 0.1 Gy and 2 Gy. The cortex, cerebellum and hippocampus were separated. The activity of the four main enzyme complexes of the mitochondrial electron transport chain (Complex I-IV) and superoxide dismutase (SOD), as well as protein carbonylation levels were measured from mitochondrial enriched fraction. Mitochondrial ROS levels were quantified in single cell suspension of brain tissue by Mitosox Red staining.

Results: At early time points after irradiation, mitochondrial enzyme activity changes of mice irradiated at 10 days or 10 weeks were mild and mainly the activity of Complex I and III were altered. Interestingly, at one week after irradiation with 0.1 Gy, the activity of all four complexes increased in the hippocampus of adult mice. SOD activity changes were more prominent in young mice, at early time points after irradiation. At late time points after irradiation, the activity of Complex I, III and IV decreased in both young and adult mice, the changes were more characteristic 6 months after irradiation. There was a massive dose-dependent decrease in protein carbonylation in young mice, while ROS levels did not increase, and SOD activity was inconsistent.

In conclusion, mitochondrial damages after ionizing radiation were mostly late effects, the severity of changes increased with the dose and time. Young mice were more sensitive to irradiation, Complex I and III were the most affected. At early time points protein carbonylation and SOD activity showed opposite changes, but at late time points, SOD activity did not change. These results suggest that SOD might be responsible for the early compensatory antioxidant mechanisms while at late time points other systems might be active.

This work was supported by the FP7 – CEREBRAD project.

P-068
OSMOLYTIC TREATMENT MITIGATES SEVERE MYOPATHY ASSOCIATED WITH IV COLLAGEN MUTATIONS IN *DROSOPHILA*

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In mammals the A1 and A2 chains of type IV collagen are components of the ubiquitous basal lamina as in the case of *Drosophila*. Mutations of the *COL4A1 gene,* both in mice and humans, are phenotypically pleiotropic and systemic in their clinical manifestation. Missense or intron splicing site mutant alleles of the human *COL4A1* gene cause vascular instability, porencephaly, and serious damage to the kidneys, eyes, and muscle. Treatment of these complex conditions is yet unresolved and a versatile animal model system is urgently needed for targeted translational, pharmacological studies for effective medical care for this devastating condition.

We have recently identified an allelic series of dominant temperature-sensitive (DTS) mutations within the *col4a1* gene in *Drosophila*, generated by independent ethyl-methane-sulfonate mutageneses, whereas recessive alleles were found exclusively in the head-to-head organized *col4a2* gene. DNA sequence analysis revealed that, consistent with the mutagen, all mutations were G to A transitions resulting in Gly to Asp, Glu or Ser substitutions. The DTS *col4a1* mutants demonstrated severe, condition-dependent muscular dystrophy or myofibrillar/centronuclear myopathy.

We surmised that in the mutants, amino acid substitutions within COL4A1 perturb the protein’s native conformation and osmolytic treatment induced hyperhydration could be beneficial in conformational correction and mitigation of the phenotype.

In translational experiments, designed to determine whether a pharmacological approach can be used to mitigate the effects of the mutant phenotype in the line carrying the *col4a1*^{G552D2} mutant allele, osmolytic treatments significantly ($p < 0{,}05$) increased the mutants’ life span, restoring it to the level of the wild-type animals, and simultaneously improved signs of muscular dystrophy. The results indicate that osmolytic treatment may be a beneficial treatment option in improving type IV collagenopathy.

This research was supported by the Hungarian Scientific Research Fund OTKA, contract nr. NN 108283 to M.M.

P-069
DAF-21 CONTROLS DAF-16 TRANSCRIPTION FACTOR ACTIVATION IN AN ISOFORM-SPECIFIC MANNER

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DAF-16, a conserved forkhead box O (FOXO) transcription factor, is a master regulator of a systemic stress response, innate immunity and contributes to longevity in *Caenorhabditis elegans*. DAF-16 is a major target of the insulin-like signaling (ILS) pathway. DAF-16 activity is mainly regulated by its localization. The nematode heat-shock protein 90 ortholog DAF-21 is involved in stress tolerance, development and muscle function. In this study, we investigated the interaction between DAF-21 and DAF-16.

We found that silencing *daf-21* has an isoform specific effect on DAF-16: inhibiting nuclear translocation of the isoform DAF-16a while changing expression pattern of the DAF-16d/f isoform. Besides, we were able to show that the DAF-16 inhibition results in a decrease in DAF-16-dependent expression of certain target genes - induced either by heat-shock or by *daf-2(RNAi)*. We also observed that *daf-21* is required for the lifespan extension induced by *daf-2(RNAi)* or by a mutation in the *daf-2* gene. Currently, we further investigate the nature of interaction between DAF-21 and DAF-16 in *C. elegans*.

Our findings shed light on a novel, DAF-21-dependent modulation of the insulin-like signaling pathway through compartmentalization and function of the different DAF-16 isoforms. Our novel data on the potential role of Hsp90/DAF-21 in DAF-16 expression is consistent with recent reports on the transcriptional regulation of DAF-16d/f. This regulation connects various stress responsive mechanisms which might have implications in immunity and longevity.

P-070
ONE MUTATION, TWO PHENOTYPES: A SINGLE NONSENSE MUTATION OF THE *CTSC* GENE CAUSES TWO CLINICALLY DISTINCT PHENOTYPES

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Papillon-Lefévre (PLS; OMIM 245000) and Haim-Munk syndromes (HMS; OMIM 245010) are phenotypic variants of the same rare disease caused by mutations of the *cathepsin C* (*CTSC*) gene and exhibit autosomal recessive inheritance.

Our aim was to identify the diseases causes mutations of the *CTSC* gene in two clinically distinct Hungarian patients. In order to elucidate any familiar relationship between the two investigated patients haplotype analysis was performed.

Mutation screening and polymorphism analysis were performed by direct sequencing of the *CTSC* gene.

Mutation screening of the *CTSC* gene from the two patients revealed the presence of the same heterozygous nonsense mutation (c.748C/T; p.Arg250X). However, one of the patients exhibited the PLS phenotype and the other exhibited the HMS phenotype. Although these patients were not aware that they were related, haplotype analysis - especially the genotypes of the rs217116 and the rs217115 polymorphisms - clearly indicates that the patients are carrying the same haplotype, while the unrelated controls carrying several different haplotypes.

Our results demonstrate that PLS and HMS are the phenotypic variants of the same disease and, additionally, exclude the presence of a putative genetic modifier factor within the *CTSC* gene that is responsible for the development of the two phenotypes. We suggest that this putative genetic modifier factor is located outside the *CTSC* gene or, alternatively, the development of the different phenotypes is the consequence of different environmental and/or life style factors.

Keywords: *Papillon-Lefévre syndrome, Haim-Munk syndrome, allelic variants, cathepsin C gene, nonsense mutation*

P-071
GENE EXPRESSION-BASED PREDICTION OF LYMPH NODE STATUS IN BREAST CANCER

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Introduction: Infiltration of the axillary lymph node affects prognosis, determines the stage and thus the treatment for breast cancer patients. Besides their advantages axillary block dissection and even sentinel lymph node biopsy have considerable comorbidities: lymphedema, sensory and motor neuropathy. Although 20-40 % of sentinel positive patient have no additional lymph node metastasis, the guidelines still recommend extensive surgery. In the last decade, numerous multigene prognostic tests have been developed for breast cancer (e.g. Mammaprint, OncotypeDX), but none of these predict the axillary lymph node status. The most accurate models for predicting nodal status are currently based on clinical parameters (e.g. MSKCC nomogram, Tenon score, Mayo score). Although these models are validated widely, they show significant variance in accuracy between geographical regions. These models are mostly based on subjective variables, whereas multigene tests can be more objective.

Aim: Our goal was to develop a new, personalized multigene classifier to identify patients with low risk of metastasis in the axillary lymph nodes.

Methods: Using the publicly available GEO database repository we established a database containing clinical and microarray data for 2341 breast cancer patients. The gene expression measured on Affymetrix HG-U133A and HG-U133 Plus2.0 arrays were normalized with MAS5 algorithm in R using the simpleaffy package.

The patients were divided into the St Gallen molecular subtypes. In addition, we divided the luminal B group into HER2 positive and negative subcohorts. We identified genes differently expressed in lymph node positive and negative patients by employing empirical Bayes moderated t-statistics test for each group separately. These top genes were combined into a 30-gene signature. Using the expression values of these 30 genes, Euclidean distances were computed between patients to identify the molecularly most similar, "nearest" cluster of patients. For each patient this gives a different cluster of nearest patient. To predict the lymph node status we examined the proportional lymph node positivity of patients in the nearest cluster.

Results: To optimise our algorithm we used leave one out cross-validation for all of the 2341 patients. We also tested varying the number of selected nearest patients. The best setting reached a negative predictive value of 93% and accuracy of 73% across these patients. In case of a validation set of 506 patients the negative predictive value and the accuracy of our test was 82% and 62%.

Summary: Here, we demonstrate the feasibility to develop a gene-chip based classifier to identify nodal positivity with a high negative predictive value.

Keywords: *breast cancer, lymph node, gene expression, biomarker*

P-072
DEVELOPMENT OF PROTEIN AND CELL BASED SYSTEMS TO STUDY ISOPEPTIDASE ACTIVITY OF TRANSGLUTAMINASE 2

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Transglutaminase 2 (TG2) is a ubiquitously expressed multifunctional protein. It has various enzymatic activities, the best studied is Ca2+-dependent transamidase activity leading to protein crosslinking or amine incorporation. TG2 has a poorly studied isopeptidase activity when the previously formed γ-glutamyl-ε-lysine or amide bonds are cleaved. There is no protein based method for characterisation of this isopeptidase activity.

S100A4 turned out to be a good lysine donor substrate of TG2 (see poster of Beáta Biri et al.). Its crosslinking by TG2 to a fluorescein labelled glutamine donor dodecapeptide (fl-pepT26) and consequent separation from the free peptide resulted in a product containing single γ-glutamyl-ε-lysine isopeptide bond. Its enzymatic cleavage was demonstrated by the visualization of the released fl-pepT26 by gel electrophoresis and the detection of glutamate instead of glutamine in the peptide by mass spectrometry. Cleavage of the isopeptide bond could be followed in real time using fluorescent polarization measurements and the effect of Ca2+ concentration, substrate and inhibitor

were characterised. Mutant TG2 variants with separated transamidase and isopeptidase activities could be also tested by this newly developed assay.

To explore the in situ effect of TG2, the huntingtin exon 1 fragment expressing MEF and rat PC12 cells were transduced by viral vectors containing TG2 mutants (in collaboration with Mauro Piacentini, University of Rome Tor Vergata, Rome, Italy and Martin Griffin, Aston University, Birmingham, UK). Although the presence of TG2 variants led to some differences between cells, they have not led to huntingtin fragment crosslinking. Further experiments are needed to confirm or challenge previous claims that crosslinking of huntingtin proteins by TG2 is an important factor for Huntington disease development.

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Keywords: *transglutaminase 2, isopeptidase activity, S100A4, Huntington's disease*

P-073
NEWLY IDENTIFIED SOMATIC MUTATIONS IN THE PIK3CA GENE AND THEIR IMPORTANCE IN THERAPY DEVELOPMENT FOR THE KLIPPEL-TRENAUNAY SYNDROME

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Klippel-Trenaunay syndrome (KTS) is characterized by unilateral angio-osteohypertrophy and caused by the somatic mutations of the *PIK3CA* gene. The aim of our work was the genetic analysis of two unrelated patients suffering from KTS, a 56-year old woman and a 4-year old girl, in order to decide whether these patients are suitable for the newly developed rapamycin therapy. This therapy is currently available for patients with overgrowth syndromes and with verified PIK3CA somatic mutation as part of a clinical trial. DNA isolated from peripheral blood and from the affected tissue was used for the analysis. The direct sequencing of the coding regions of the *PIK3CA* gene was performed. In the case of the 56-year old patient, the analysis revealed several differences between the sequencing results of the peripheral blood and the affected tissue, but these differences were all the consequence of the *PIK3CA* pseudogene and they were not causative somatic mutations underlying in the background of the observed symptoms. In the case of the 4-year-old girl a missense somatic mutation was identified (c.1624G/A p.Glu542Lys) in the affected tissue, but not in the

peripheral blood. The mutation burden of the affected tissue sample was only 4%. This mutation is in the helical region of PIK3CA, and has been found in other patients with PIK3CA-related overgrowth. The detection of the underlying causative somatic mutation has great importance, since the opportunity to take part in the recently initiated rapamycin clinical trial is now available for this patient.

Keywords: *Klippel-Trenaunay syndrome, PIK3CA gene, overgrowth syndromes, rare diseases*

P-074
CONGENITAL MUSCULAR DYSTROPHY IN *DROSOPHILA* TYPE IV COLLAGEN MUTANTS: RAPID PROGRESSION ASSOCIATED WITH ALTERED INTEGRIN BIDING SITES WITHIN COL4A1

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Congenital muscular dystrophy (CMD) is a clinically and genetically heterogeneous group of inherited muscle disorders. Patients present with progressive muscle weakness and elevated creatine kinase levels. Muscle biopsy further reveals fiber size variability, fibrosis, necrotic fibers and, additionally, central myonuclei that normally appears in regenerating muscle. In CMD cases, mutations were identified in multiple genes, including myofibrillar basement membrane components laminin alpha-2, integrin alpha-7, and collagen type VI. Type IV collagen *COL4A1* mutations have recently been identified in patients presenting with central nervous system and ophthalmic pathology and CMD, consistent with diagnoses of Walker–Warburg Syndrome or Muscle–Eye–Brain disease.

In this study, we have identified the mutation sites in an allelic series of *Drosophila col4a1* conditional, temperature-sensitive mutants and determined mutation-specific cellular and biochemical features of the manifestations of symptoms. We have observed, at restrictive condition, severely compromised groups of muscle fibers in all mutants within the single-layer striated muscle of the common oviduct, fulfilling morphologic criteria of CMD.

The CMD phenotype progressed rapidly in *col4a1* mutants that directly or indirectly affect integrin-binding sites within the COL4A1 protein. In these mutants, beta-PS integrin expression was uneven with intracellular accumulation of the integrin protein. Slowly progressing CMD was noted in the rest of the mutants, with mutation sites in the C-terminal portion of the COL4A2 polypeptide. Both dystrophic groups presented central nuclei.

The results demonstrate that the dominant phenotype of the *col4a1* mutants is CMD, with rapid progression in mutants with compromised integring-binding sites within COL4A1.

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P-075
RESULTS OF GENETIC INVESTIGATION AND HAPLOTYPE ANALYSIS IN EUROPEAN PEDIGREES WITH BROOKE-SPIEGLER SYNDROME

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Brooke-Spiegler syndrome (BSS; OMIM 605041) is an autosomal dominant rare monogenic skin disease characterized by skin appendage tumors including cylindromas, trichoepitheliomas, and/or spiradenomas. The gene responsible for BSS, the cylindromatosis gene (*CYLD*), is localized at 16q12-q13 and codes for an enzyme with deubiquitinase activity. Our aim was to identify the causative mutation on the *CYLD* gene in a Spanish family with BSS and to perform haplotype analysis in international cooperation with Dutch and Austrian research groups.

Direct sequencing of the coding regions and the flanking introns of the *CYLD* gene revealed a heterozygous nonsense mutation (c.2272C/T, p.R758X) in exon 17 in affected family members of the Spanish pedigree. This mutation has been previously identified in several families thus this part of the gene could be a mutational hotspot. Based on these information, haplotype analysis was performed to elucidate whether the mutation - carried by the Spanish, Dutch and Austrian pedigrees - is the results of the same founder effect or independent mutation events occurred in the past.

Based on our results, the haplotype analysis demonstrated that the same mutation was the result of three independent mutational events.

Keywords: *Brooke-Spiegler syndrome, cylindromatosis gene, nonsense mutation, haplotype analysis*

P-076
GENERATION OF GENETICALLY MODIFIED MICE USING TALEN TECHNOLOGY

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Mice with specific gene modification are valuable tools for studying developmental processes and various diseases. To understand gene function, knock-out animal models are generated. In conventional gene targeting methods, genetic modifications are introduced into mouse embryonic stem (ES) cells through homologous recombination, which is a complex and time-consuming process, with low efficiency. Recently, the discovery of gene editing nucleases (like ZFNs: zinc-finger nucleases, TALENs: transcription activator-like (TAL) effector nucleases and the CRISPR/Cas9-system: clustered regularly interspaced short palindromic repeat/CRISPR-associated protein 9)

revolutionized the field of genome manipulation. The production of genetically modified mice has been greatly accelerated by novel approaches using direct injection of DNA or mRNA of site-specific nucleases into one-cell-stage embryos, to generate DNA double-strand breaks at specified sequences leading to the generation of targeted mutations (Thomas et al., 2013). Co-injection of a single-stranded or double-stranded DNA template homologous to the target site can introduce precise point mutations or additional sequences into the genome (Yang et al., 2013).

In our study we have generated genetically modified mice by knocking out the Tmem86a gene via specific, TALEN-mediated gene editing. The process has involved four major steps: 1) design and validation of TALEN constructs; 2) *in vitro* transcription and purification of TALEN mRNA; 3) microinjection of TALEN mRNA into one-cell-stage mouse embryos and transfer of the injected embryos into pseudopregnant mice; finally, 4) genotyping of the offsprings to identify mutations. A total of 491 zygotes were injected and transferred into 31 pseudopregnant mothers, which then delivered 54 live pups. Mismatch sensitive nuclease (T7E1) assay has been used for the genetic screen, resulting in the identification of 6 pups that carry mutations in the targeted gene. Detailed sequence analysis revealed, that majority of the determined mutations cause frameshifts and disrupt the gene function; therefore these mutants are suitable for the generation of stable knock-out (KO) model. The process of generating the KO founders took only one month, which is very favourable compared to the approximately 8-10 months for similar results with the traditional ES cell targeting method.

The major advantages of using the novel nuclease mediated gene targeting technologies, compared to the conventional and more standard stem-cell based strategy, are the higher efficiency and the faster workflow without time-consuming cell culturing and extensive clone characterisation before embryo-injections. TALENs are highly effective in inducing mutations at specific genomic loci, consequently TALEN-mediated mutagenesis in zygotes is a robust, economically viable alternative of the conventional gene targeting in mice.

This study was supported by the RESOLVE /FP7-HEALTH-F4-2008-202047/ and NKTH/KPI (Bonus Resolve, OMFB-01660/2009) grants.

Keywords: *gene targeting, genome engineering, TALEN*

P-077

COMBINING STANDARD CLINICAL METHODS WITH PCR SHOWED IMPROVED DIAGNOSIS OF INVASIVE PULMONARY ASPERGILLOSIS

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Poor life expectations of patients with different hematological malignancies and prolonged neutropenia are significantly influenced by life-threatening invasive pulmonary aspergillosis. The major causative agent of the infection is the Aspergillus fumigatus, an opportunistic pathogen. Recently the galactomannan-ELISA immunoassay (Aspergillus GM-EIA) is the gold standard diagnostic method which is a rapid, non-invasive tool. The major drawback is however that it is not able to identify the pathogen on species level. We have found a unique target sequence in the

Aspergillus fumigatus genome, allowing specific nucleic acid based detection of the pathogen by using sensitive quantitative PCR system (facC-PCR).

In a one year period 27 patients with different hematological malignancies and prolonged neutropenia were involved in our prospective case-control study. Sera were successively screened for galactomannan (GM) antigen and for *Aspergillus fumigatus* specific fungal nucleic acid targets. Furthermore thoracic computed tomography (CT) was invariably performed and bronchoscopy with lavage (BAL) when clinically indicated.

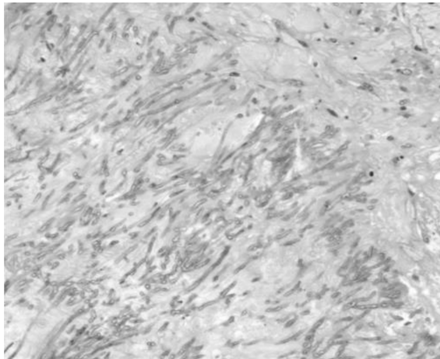

Figure 1: Postmortem histological findings showing congestion and focal atelectasis of the lung (x40 magnification).

PMH proved the presence of Aspergillus hyphae in the lung in the case of one episode with consecutive positive PCR results that was misdiagnosed with both CT and consistent negative serology. Detailed evaluation highlighted invasive Aspergillus hyphae which could be further confirmed by PAS staining.

We managed to assess the discriminatory value of combined biomarker screening by considering *facC*-PCR results together with GM-serology. Assessing the discriminatory power of the biomarker methods diagnostic odds ratios (DORs) were calculated. For GM-EIA it was 15.33 and for *facC*-PCR 28.67. According to Cohen's kappa our in-house PCR method showed a fair agreement with *Aspergillus* GM-EIA.

In one hand our primary goal was to successfully integrate our in-house PCR method in the routine clinical practice. On the other hand we want to highlight the importance of combining standard clinical methods (*Aspergillus* GM-EIA, CT, BAL) with rapid, cost efficient qPCR (e.g. *facC*-PCR) to establish an accurate and prompt diagnosis. Moreover we started to optimize a new post PCR method, high resolution melting point analysis, allowing species specific identification of the pathogens.

Keywords: *invasive pulmonary aspergillosis, combination testing, neutropenia*

P-078
MICROSATELLITE MARKERS DEVELOPED FOR RED DEER USING GENOME SEQUENCE DATA

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Red deer, *Cervus elaphus*, the Wonder Deer is surrounded by amazement and historical legends in the Hungarian culture and the most desirable and highly prized "royal" game in Europe. Today it is the prime member of the megafauna in Hungary and due to record trophies stands in the center of wide attention. This motivated our ambition of whole genome sequencing and developing the toolkits for DNA based genotype profile analyses. The size of the Hungarian wild population is over 100000, and thousands were bred in farms.

Microsatellite loci are widely applied DNA markers in forensic genetics and population genetics studies as well as for wildlife conservation and parentage testing in animal breeding. Detecting and controlling the valuable paternal and maternal lineages is a very important need for animal breeders to be able to select the eligible phenotypes. In some mammal species, usually in domesticated stocks like cattle, swine, dog, etc., molecular methods have already been developed to perform parentage tests. Such genetic tools can also aid game management.

The accessibility of next-generation sequencing (NGS) technology has transformed the research in life sciences, and in many cases, this revolution calls for the redesign of previous experimental methods. We launched the first Hungarian de novo genome program of a mammal, red deer, and used genome sequences to develop novel microsatellite markers. A repeat motif search on de novo assembled red deer genome sequences was done with the help of QDD software kit, and primers have been designed for microsatellite loci using Primer3. Scaffolds of red deer genome have been aligned to cattle as reference using BLAST, to select X and Y chromosomal regions, and so sex chromosome linked microsatellites.

More than 978,000 microsatellites were identified in the red deer genome, and primers were successfully designed for 73,870 genomic loci, which provided a rich source of marker development. Eighteen sex chromosome linked microsatellites were selected for PCR amplification, and in a population study 13 of these proved to be polymorphic. By the help of these markers 57 X and 15 Y haplotypes have been identified so far, as well as the Y chromosome evolution and an X chromosome-based hind-stag-calf match have been demonstrated.

Keywords: *Cervus elaphus, red deer, microsatellites, NGS, bioinformatics*

P-079
SURFACTANT PROTEIN EXPRESSION IN THE AVIAN THYMIC MEDULLA

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The cortical and medullary thymic epithelial cells are responsible for different functions. Cortical cells express attractive signal(s) for T cell progenitors, while the medullary epithelial cells participate in the negative selection of autoreactive T lymphocytes. Two unique structures are formed from the medullary epithelium: cyst and multicellular and unicellular Hassall's bodies. The thymic epithelium develops from the foregut entoderm like the type I and II pneumocytes and Clara cells. During the last two decades turned out, that the role of surfactant is more complex, than only to prevent the collapse of alveoli during deep expiration. The surfactant SP-A and SP-D proteins are collectins, which protect the lung from bacterial, viral and other infections.

To determine the avian thymic dendritic cells we used a monoclonal antibody (mAb) 74.3 (Jeurissen et al, 1992) which recognized chicken specific follicular dendritic cells. In a chimeric experiment,-accidentally-turned out that this mAb recognized type II pneumocytes. Using polyclonal antibody against SP-B provided evidence that 74.3 mAb recognized type II pneumocytes. At the same time, avian thymus stained with anti SP-B indicated its presence in the medulla which confirm the mammalian data. Transmission electron micrographs showed that the same structural granules can be found in the epithelial cells of the thymic cysts as are present in the type II pneumocytes. Double staining (MHC class II and SP-B) proved that the epithelial cells of the cysts express MHC class II antigen and the lumen of the cysts is full of SP-B.

Conclusion: Thymic cyst epithelium expresses MHC class II antigen like thymic reticular cells, additionally produces SP-B like type II pneumocytes, which are MHC class II negative.

Keywords: *chicken thymus, thymic cyst, surfactant protein-B*

P-080
METAPEAK: PEAK-BASED ENRICHMENT PROFILING OF CHROMATIN IMMUNOPRECIPITATION-SEQUENCING (CHIP-SEQ) DATA

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In the post-genomic era it is important to investigate multiple biological events (e.g. transcription factor binding, histone modifications) in parallel over hundreds of thousands of genomic regions as gene bodies, transcription start sites or termination sites. Typically, signal intensities obtained from chromatin Immunoprecipitation sequencing (ChIP-seq) are plotted as a single curve or heatmap

over a meta-gene that represents all gene bodies in the genome. A number of meta-profiler algorithms exist that use various strategies to plot the average ChIP signal intensity over a meta-gene. These approaches perform well if the signal is symmetric about the mean, but a general problem immediately emerges if the data follow an asymmetrical distribution. When the signal is positively or negatively skewed, the arithmetic mean is no longer a good estimator for the expected value of the ChIP profile. We have developed Metapeak, an open-source python software that builds meta-gene profiles from noise filtered ChIP-seq peaks and make it possible to draw unbiased conclusions when the data are asymmetrically distributed.

This work was supported by the European Union (FP7/MCA-CIG) and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 'National Excellence Program', and also by the Hungarian Scientific Research Fund (OTKA-PD).

Keywords: *metagene analysis, bioinformatics, algorithm*

P-081
MECHANOPHARMACOLOGICAL SCREENING PLATFORM

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In addition to chemical signals most of the cells are able to sense the mechanical aspects of their microenvironment. Different mechanical cues, sheer stress or change in the substrate stiffness can influence wide scale of cellular responses (e.g. proliferation, adhesion or migration) therefore they play essential role in physiological and pathological condition e.g. wound-healing. In the present work we focus on complex relation of cell viability, adhesion and mechanosensation especially durotaxis triggered by pharmaceutical compounds. We demonstrate the use of a well-defined elasticity tunable polyacrylamide gel system as a novel durotaxis platform coupled to impedance measuring system (ECIS) which is a highly sensitive and real-time technique to follow cellular adhesive and migratory capacity.

Our aim is to detect the effect of 11 drugs on durotactic behavior of two major cell types, fibroblasts (3T3) and keratinocytes (HaCaT) involved in wound repair in vivo .

Specific cytoskeletal inhibitors (blebbistatin, calyculin-A, colchicine, cytochalasin-B, cytochalasin-D, nocodazole, phalloidin) common drugs, anti-inflammatory compounds (diclofenac, ibuprofen) and beta-blockers (metoprolol, propranolol) were tested. At first cytotoxicity assays were performed by MTT and AlamarBlue test to determine the minimal toxic concentrations. Then durotaxis measurements were applied in cells pretreated with drugs in lower – non toxic - concentrations. Finally, those concentrations which abolished durotaxis were studied by impedimetry on adhesion and wound healing in ECIS system.

Our results showed that specific inhibitors of the cytoskeletal elements were all able to inhibit durotaxis at the 0.25-10 uM range; however, at these levels they (e.g. cytochalasin-D, colchicine,

nocodazole) also interfered with cell adhesion and delayed wound-healing in both cell types. Interestingly, beta-blocker propranolol and metoprolol could inhibit durotaxis of both cell types at low, micromolar concentrations without affecting their viability, adhesion or migratory capacity considerably. Nevertheless, anti-inflammatory drugs had no effect on the studied cell biological responses. Comparison of the two cell lines revealed higher sensitivity of 3T3 fibroblasts towards drug effects in both viability and durotaxis assay as well as in the ECIS wound-healing assay.

The present workflow might be suitable to manage mechanosensitivity studies and used in the future for mechanophramacological screening purposes.

Keywords: *durotaxis, mechanophamacology*

P-082
MOLECULAR CHARACTERIZATION OF TRANSGENIC MOUSE MODEL OF LONG QT SYNDROME

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In cardiac myocytes the Iks channel is composed of a pore-forming α-(KCNQ1) and the modulatory β-subunits (KCNE1), also KCNQ1 alone assembles to form voltage-gated potassium channel, the presence of KCNE1 is required to reproduce the kinetic properties of the native IKs channel. The mutation of IKs channel causes prolonged QT interval of ECG wave. IKs the slowly activating cardiac potassium current is an important determinant of myocardial repolarization. The aim of our experiment was to create a transgenic mouse model carrying a transgene construct with a missense mutation in G52R-KCNEl gene, first identified in a long QT syndrome family. The missense mutation G to A at position 154 in the KCNE1 gene leads to an amino acid substitution of arginine for glycine at position 52. The mutant G52R-KCNE1 has a dominant negative effect on IKs current targeting the β-IKs channel subunit.

To generate these Long QT mouse models, 763 embryos were microinjected with a lentiviral G52R-KCNE1 vector and 268 embryos were pronucleus injected, 896 were transferred into recipients. Two transgenic founder mice were identified out of 80 offspring.

The F1 mice belong to both transgenic lines expressed tissue specificaIly the mutant human KCNE1 mRNA in the heart.

However we could not detect the mutant human KCNE1 protein expression in the heart of the pronucleus injected line. And our examination shows human G52R-KCNE1 protein in heart of the lentivector mouse line by Isoelectric focusing (IEF). Suprisingly, the ratio of homozygous offspring were significantly lower than expected in this transgenic line, when two heteroygote mouse were mated.

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P-084
EXAMINING GENOME INTEGRITY MAINTENANCE IN HUMAN CELL LINES BY SITE-SPECIFIC, BIALLELIC GENE KNOCK-OUT TECHNOLOGY

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Numerous physiological pathways with multiple protein factors play diverse role in the faithful maintenance of genomic integrity which is indispensable for life. The object of our studies, dUTPase, catalyzes the hydrolysis of dUTP into pyrophosphate and dUMP, supporting low cellular dUTP/dTTP ratio thus immunity against genomic uracil accumulation [1]. It has been shown that deficiency in dUTPase function leads to cell death in several organisms e.g. in *Mycobacterium smegmatis* [2] and in *Drosophila melanogaster* [3].

The molecular mechanism of thymine-less cell death, induced by the lack of dUTPase, is poorly understood although several routinely applied chemotherapeutic drugs in the clinic (fluoropyrimidines, methotrexate and its derivatives); interfere with the *de novo* thymidylate biosynthetic pathway. Overexpression of dUTPase causes partial resistance against fluoropyrimidines, while its deficiency sensitizes the cells. Therefore a better knowledge of dUTPase role and function is particularly important in medicine. Until now, dUTPase function was only investigated with gene silencing [4] but gene knock-out strategy has not been applied.

Our aim is to investigate the potential mechanism of cell death caused by dUTPase knock-out in human cancer cell lines, thus gaining a better understanding how cancer cells get resistant to chemotherapeutic agents. We applied a state-of-the-art method, the zinc finger nuclease (ZFN) technology, which allows site-specific, poliallelic genomic manipulations. Considering the fact that a knocking-out a putative essential gene, we first had to provide the cells with conditional exogenous source of the protein, otherwise a viable knock-out cell line could not be established. Therefore an exogenous floxed dUTPase source was also integrated to another well-established point of the genome, which expresses both the nuclear and the mitochondrial isoforms. Expression could be terminated at any desired time point by the help of a ligand inducible Cre recombinase.

With the inducible dUTPase knock-out cell line we will be able to clarify the role of dUTPase in genome integrity maintenance and their involvement in clinical therapy. The applied combined knock-out/integration strategy could also be used for other potentially essential genes which could be of wide interest.

Acknowledgements: This work was supported by grants from the Hungarian Scientific Research Fund (OTKA NK84008, OTKA K109486).

Keywords: *genomic integrity, dUTPase, gene knock-out, zinc finger nuclease*

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P-085
RESTRICTION ENZYME BODY DOUBLES AND PCR CLONING: ON THE GENERAL USE OF TYPE IIS RESTRICTION ENZYMES FOR CLONING

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The procedure described here allows the cloning of PCR fragments containing a recognition site of the restriction endonuclease (Type IIP) used for cloning in the sequence of the insert. A Type IIS endonuclease - a Body Double of the Type IIP enzyme - is used to generate the same protruding palindrome. Thus, the insert can be cloned to the Type IIP site of the vector without digesting the PCR product with the same Type IIP enzyme. We achieve this by incorporating the recognition site of a Type IIS restriction enzyme that cleaves the DNA outside of its recognition site in the PCR primer in such a way that the cutting positions straddle the desired overhang sequence. Digestion of the PCR product by the Body Double generates the required overhang. Hitherto the use of Type IIS restriction enzymes in cloning reactions has only been used for special applications, the approach presented here makes Type IIS enzymes as useful as Type IIP enzymes for general cloning purposes. To assist in finding Body Double enzymes, we summarised the available Type IIS enzymes which are potentially useful for Body Double cloning and created an online program (http://group.szbk.u-szeged.hu/welkergr/body_double/index.html) for the selection of suitable Body Double enzymes and the design of the appropriate primers. We routinely use this procedure to bypass this frequently arising problem.

Keywords: *type IIS restriction enzyme, body double enzyme, molecular cloning*

P-086
HETEROLOGOUS EXPRESSION AND *IN VITRO* ANALYSIS OF ACCELERATELY EVOLVING DROSOPHILA PROTEINS INVOLVED IN TELOMERE MAINTENANCE

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Our laboratory is interested in the maintenance of genome integrity and telomere structure in *Drosophila melanogaster*. In eukaryotic cells telomeres prevent the chromosome ends from being detected as DNA double strand breaks and protect the coding regions from degradation. Telomere “capping” by the multi-subunit complex *shelterin*, expressed in higher eukaryotes, averts triggering of the DNA damage signaling pathways. In Drosophila this capping process is performed by a putative complex called *terminin*. Terminin is believed to consists of HOAP, HipHop, Ver and

DTL/Moi protein subunits and is able to bind to DNA in a sequence-independent manner. HP1 is generally regarded as the fifth subunit of the putative complex, thought it is not strictly terminin-specific, as terminin proteins localize only at chromosome ends while HP1 plays a role at non-telomeric regions as well. Deletion of the gene of HP1 or any terminin protein results in telomere fusions. Consequently, while Drosophila and higher eukaryotes use different mechanism for telomere formation the shelterin and terminin protein complexes fullfill similar functions.

Curiously, HP1 is evolutionary highly conserved, while terminin proteins manifest an accelerated rate of evolution. However, the existence of terminin complex so far is not proven experimentally. Physical interactions among terminin proteins have been demonstrated *in vitro*, but no biochemical studies have been performed to investigate the formation of the complex in details.

A further interesting aspect is given to studying telomere protection by the hypothesis that the accelerated evolution of terminin components may have implications in driving speciation. Speciation is the process by which new species arise. We hypotised that a rapid rate of telomere protective terminin evolution might play a role in enhancing the separation and preventing the mixing of differently evolving gene sets present in unique chromosomes. Motivated by these facts we used biochemical approaches to study the terminin complex. We reconstituted terminin by coexpressing its subunits in bacteria and studied the role of the accelerately evolved parts of terminin components in complex assembly.

Our results suggest the existence of stable subcomplexes of terminin and question the organisation of the complex as was suggested by others previously. We found that the accelerately evolved parts of Ver and DTL are unlikely responsible for their stable interaction.

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Keywords: *terminin, telomere, accelerated evolution, protein complex*

P-087
MLO GENE POLYMORPHISM IN *TRITICUM MONOCOCCUM* L.

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Mlo (*Mildew resistant locus o*) genes have a key role in powdery mildew infection; presence of the wild-type protein is necessary for successful plant invasion by the fungus. The wild type gene encodes a plant-specific 60 kDa heptahelical protein, which is anchored to the cell membrane by seven transmembrane helices. A loss of function mutation was found in powdery mildew resistant plants in wild population of barley (*Hordeum vulgare* L.). Homozygotes for this recessive *mlo* allele showed stable broad-spectrum resistance against the fungus (*Blumeria graminis* f.sp. *hordei*). Till today many *Mlo* orthologs have been identified in various plant species. Despite of finding orthologs in all three wheat genomes (*TaMlo-A*, *TaMlo-B*, *TaMlo-D*), no similar mutant has been found in hexaploid wheat (*Triticum aestivum* L.). Different cultivars of the diploid *T. monococcum,* whose

genome is closely related to the "A" genome of the hexaploid wheat, show resistance to powdery mildew.

Therefore, we use these resistant cultivars as starting material to clone and sequence the entire *Mlo* gene in order to find null mutations. The MLO protein has three regions, which are required for fungus infection. First we studied one of these regions in *T. monococcum* cultivars (MVGB1150-1159). We extracted and pooled DNA from at least ten individual plants of each cultivar. PCR amplifications of the *Mlo* gene were carried out with primers designed for the *TaMlo2* gene and the obtained fragments were cloned. Inserts from six clones of each cultivar were sequenced and the obtained sequences were aligned, which resulted in two groups. Blast search of the GenBank was carried out using the consensus sequences. One group showed 98 % homology to the *TaMlo2* gene while the other showed 80 % homology to the barley *Mlo-h1*gene. Although a number of SNPs were found in both groups, the variability within the first group was larger than in the second one.

Our future plan is to sequence the entire *Mlo* gene of *T. monococcum* and identify loss of function mutations, which than might be bred into hexaploid bread wheat to develop powdery mildew resistant elite varieties.

Keywords: *mlo, Triticum, polymorphism*

P-088

ROLE OF THE C-TERMINAL LONG DISORDERED REGION OF HUMAN UPF2 IN FLY-CASTING AND THE MECHANISM OF NONSENSE-MEDIATED MRNA DECAY

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Nonsense-mediated mRNA decay (NMD) is a cytoplasmic surveillance system that identifies and degrades mRNAs containing premature termination codons (PTC), thus prevents the production of truncated and inappropriate proteins. The mechanism of the NMD system largely depends on the binding and communication of proteins and protein complexes far distant from each other. During our earlier work we analyzed the sequence, structure and function of proteins and their potential homologues participating in NMD using bioinformatics methods. We have observed that many disordered/unstructured proteins and protein regions are found in NMD. While this distance-tolerance is one of the basic characteristics of disordered protein regions, they may significantly participate in the long-range interactions through their enhanced hydrodynamic radius, speeding up the molecular recognition (fly-casting) of proteins located in distant complexes.

In this work we aimed to examine the role of the C-terminal long disordered region of human UPF2 and its interaction with UPF1, which are the two key proteins in the mechanism of NMD. UPF2 binds to its partner protein UPF1 through a natively unfolded region which gains an 'alpha-helix – linker region – beta-hairpin' structure during the interaction with the UPF1 CH domain. While both the UPF1 and UPF2 proteins are parts of different complexes associated to the mRNA, and may take place far distant from each other, it seems that the long and evolutionary conserved unstructured

region on UPF2 is able to facilitate the interaction of the two proteins through the fly-casting mechanism. To prove this, first we analysed the C-terminal part of UPF2 using discrete molecular dynamics (DMD) simulation. Meanwhile, we also aimed to examine the UPF1-UPF2 interaction *in vitro* by altering the length of the long unstructured region and monitoring its effect on the binding

During the DMD simulations show that the long disordered region of UPF2 remains totally elongated, while the secondary structural elements on the UPF1-binding site can be unambigously recognized. This observation is consistent with our conception of the importance of fly-casting in the UPF1-UPF2 interaction. We will verify this conception with the ongoing *in vitro* experiments, and provide evidence to the role of disorder in the NMD process.

Keywords: *DMD, fly-casting, in vitro experiments, NMD, protein disorder, UPF1, UPF2*

P-089
INHIBITION OF THE EUKARYOTIC *DROSOPHILA MELANOGASTER* DUTPASE BY STL, A *STAPHYLOCOCCAL* REPRESSOR PROTEIN

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The enzyme dUTPase plays a key role in maintaining sufficiently low cellular dUTP/dTTP ratio via effective hydrolysis of dUTP into dUMP and pyrophosphate. This enzymatic reaction is thought to be evolved in order to prevent hyperactivation of uracil excision repair process, which carries the risk of DNA double strand brakes or even cell death [1].

As most pro- and eukaryotes and also some viruses encode their own dUTPase enzyme, an essential role of dUTPase activity is expected in case of an active base excision repair system. This expectation has already been verified for several species (recently for example *Mycobacterium smegmatis* [2] and the fruitfly [3]. dUTPase is therefore a potential therapeutic target in certain infectious diseases [2] but also in cancer treatment [4]. Though small molecule dUTPase inhibitors do exist [5], an effective proteinaceous inhibitor of the enzyme may promote identification of a potentially alternative synergistic inhibition site at the dUTPase surface.

It has been shown recently that the *Staphylococcal Φ11* phage dUTPase inhibits DNA-binding ability of Stl, a pathogenicity island repressor protein of *Staphylococcus aureus* [6, 7]. Subsequent quantitative characterization of this interaction revealed that Stl acts as a highly potent inhibitor of the *Φ11* phage dUTPase, vice versa [7]. In addition, it also shows cross-species effects by inhibiting mycobacterial dUTPases both *in vitro* and *in vivo* [8].

In hope of identifying Stl as a broad-spectrum dUTPase inhibitor, we tested its in vitro effect on the eukaryotic dUTPase of *Drosophila melanogaster*. Using steady-state activity assay we observed approximately 40% decrease in enzymatic activity in case of pre-incubation of dUTPase and Stl. Notably, for dUTPase pre-incubation with dUTP, no decrease in activity upon Stl addition was observed. We further confirmed a strong interaction between *D. melanogaster* dUTPase and Stl by native gel electrophoresis. Additionally, we carried out electrophoretic mobility shift assays to investigate the effect of protein complex formation on DNA binding affinity of Stl. In order to further

investigate this intriguing cross-specific interaction, mass spectrometry and protein crystallization experiments are planned.

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P-090
METASTASIS-ASSOCIATED S100A4 SERVES AS A SPECIFIC AMINE SUBSTRATE FOR TISSUE TRANSGLUTAMINASE-2

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S100A4 belongs to the calcium-binding EF-hand S100 protein family and acts both intra- and extracellularly through binding to various interaction partners. It is a regulator of cell migration, its overexpression is strongly associated with certain inflammatory diseases and tumor metastasis, and it is considered as a prognostic marker for poor patient survival in a number of cancers. Tissue transglutaminase-2 (TG2) is a multifunctional protein that mainly acts as a calcium-dependent cross-linking enzyme; however, some of its functions are based on specific protein-protein interactions. It is involved in a number of processes including extracellular matrix remodeling, adhesion, migration, as well as tumor growth and metastasis. TG2 has been recently suggested to have a role in S100A4-mediated tumor cell migration.

We provide evidence that S100A4 is an interacting partner (with submicromolar K_d) and a specific amine-donor of TG2. Fluorescence polarization assay show that TG2 forms an isopeptide bond to Lys100 of S100A4 as determined by mass spectrometry. The amine-donor lysine is localized in the C-terminal random coil region of S100A4. ELISA binding assays reveal that the enzyme activity is not necessary for the interaction: S100A4 also binds to TG2 in the presence of a specific transamidase inhibitor (ZDON) or to an enzymatically inactive mutant. Measurements in the presence of GTP (inducing the closed conformation of the enzyme) indicate that the interaction is only formed in the open conformation of TG2. We also find that other S100 proteins (S100B, S100P, S100A2, S100A6 and S100A10) are not substrates of TG2. Cell adhesion assay using impedimetrics (xCELLigence)

demonstrates that on fibronectin-coated surface, TG2 and S100A4 (in the presence of $CaCl_2$) considerably increase cell adhesion of A431 epithelial carcinoma cell line. In conclusion, S100A4 is the first protein to be described as a highly specific amine substrate of TG2. Both proteins have important roles in tumor progression, thus examining their interaction and function could lead to better understanding of the development of metastasis.

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Keywords: *S100A4, transglutaminase-2, metastasis, protein-protein interaction*

P-091
INTRAMOLECULAR INTERACTIONS IN THE SCAFFOLD PROTEIN TKS4

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The scaffold protein Tks4 (Tyrosine kinase substrate with four Src homology 3 domains) is a member of the “p47phox-related organizer superfamily”. Tks4 contains a phosphoinositol lipid-binding *phox* (PX) domain, four Src Homology-3 (SH3) domains and several proline-rich regions (PRR), which are putative SH3 interacting sites. Mutations of the Tks4 gene are associated with Frank-Ter Haar syndrome, an autosomal-recessive disorder that is characterized by skeletal, cardiovascular, and eye abnormalities. Tks4 was shown to be required for embryonic development and adipocyte differentiation. It is essential for the formation of actin-rich membrane protrusions like podosomes and invadopodia, that are required for the motility of normal and transformed cells. Therefore, beyond the physiological functions of Tks4 (e.g motility of macrophages) it plays a crucial role in pathological situations, such as cancer cell invasion and metastasis. Tks4 is regulated by the epidermal growth factor (EGF) signaling pathway and required for EGF induced lamellipodia formation. EGF induces the translocation of Tks4 from the cytoplasm to the plasma membrane, where it associates with the EGF receptor. Src kinase is required both for this association and for the phosphorylation of Tks4 on several tyrosine residues. These phosphorylation events may act as a “switch” between the inactive, cytoplasmic state and the active, membrane-bound state of the protein. Besides the association with the EGF receptor, the function of lipid binding PX domain is also required for membrane translocation. It has been demonstrated that p47phox, which is another member of the same protein family, is capable of autoinhibition. The N-terminal region of Tks4 and p47phox share many similarities. Both proteins possess a PX domain at the N-terminus, followed by two SH3 domains (so called “tandem SH3”) and a PRR. Intramolecular interactions between the tandem SH3 domain and the PRR and between the second SH3 domain and PX domain of p47phox results in an autoinhibitory conformation. Based on the conserved structural features of the p47phox and the Tks4 proteins and the fact that an intramolecular interaction between the third SH3 and the PX domains of Tks4 has also been reported we have hypothesized that Tks4 is also capable of autoinhibition in a similar way. In this study we aim to identify all intramolecular interactions within

the N-terminal part of the Tks4 protein (residues 1-476) that are necessary for the autoinhibited conformation. The conserved structural and functional elements, like the PX domain, three SH3 domains and the PRR of this region, or combinations of these are expressed as individual recombinant proteins in *E. coli*. Systematic screening for interactions between the obtained protein fragments is in progress. We have already shown that the tandem SH3 domain (SH3-1 and SH3-2) of TKS4 binds the PRR with a Kd of ~30 μM. This interaction might be important for positioning the third SH3 domain to the close proximity of the PX domain to achieve an effective inhibition of its membrane binding activity.

This work was supported by OTKA K 83867 and LENDÜLET grants.

Keywords: *Tks4, scaffold protein, intramolecular interaction*

P-092
CCTOP: CONSENSUS CONSTRAINTED TOPOLOGY PREDICTION METHOD

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The Constrained Consensus TOPology (CCTOP) is a novel consensus topology predictor based on 10 state-of-the-art topology prediction methods. In addition to utilizing 10 different prediction algorithm, CCTOP automatically incorporates previously determined structural, experimental and bioinformatic topology information from the PDBTM, TOPDB and TOPDOM databases by using the probabilistic framework of hidden Markov model. The accuracy of CCTOP algorithm has been tested on a newly compiled benchmark set, containing 170 transmembrane protein sequences with known 3D structures, which were never seen earlier by any of the used topology prediction algorithms nor CCTOP, and on which CCTOP outperforms the state-of-the-art methods by 10-30%. Topology predictions can be preceded by signal peptide prediction and transmembrane-globular protein discrimination. The reliability of each prediction is calculated as well, which was shown to correlate with the topology prediction accuracy.

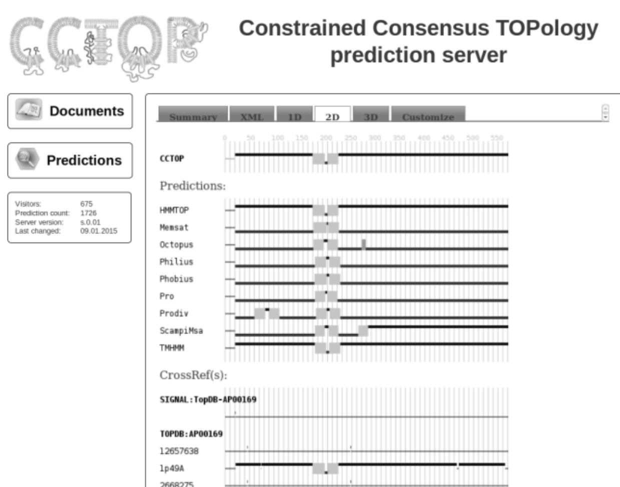

Figure 1: *Visual representaion of the prediction results.*

A server-based application providing visual and graphical presentation of the topology prediction also available at http://cctop.enzim.ttk.mta.hu (Figure 1).

Keywords: *transmembrane protein, topology prediction, Hidden Markov Model*

P-093
ASSESSING THE COMPLIANCE OF DYNAMIC PROTEIN STRUCTURAL ENSEMBLES WITH EXPERIMENTAL NMR DATA

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Generation of dynamic structural ensembles that are compatible with experimentally determined mobility parameters is an increasingly important approach for the understanding subtle details of protein function. There are multiple methods to obtain such ensembles, the two most important ones being ensemble-restrained molecular dynamics and sub-ensemble selection from a large pool of conformers. Nevertheless, for the evaluation of such ensembles in terms of their correspondence to experimental parameters – both those used for the generation of the ensemble and those that were not – there are very few standardized tools available. Here we report a complete recoding of our previously published approach, CoNSEnsX (compliance of NMR-derived structural data to experimental parameters) providing both more functionality and enhanced user interface. In particular, the new tool is capable of handling multiple independent residual dipolar coupling data sets and can perform the superposition of the conformers submitted. The functionality of the new program is demonstrated on structural ensembles of ubiquitin and the PDZ domains of PSD-95.

Keywords: *restrained molecular dynamics, general order parameter, protein internal dynamics, residual dipolar couplings*

P-094
DOES VIRAL FUZZINESS CONTRIBUTE TO MOLECULAR MIMICRY OF EUKARIOTIC MOTIFS?

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Viral proteins evade host systems by hijacking the cellular regulatory machinery of the host via short linear motifs (LMs). LMs are low-complexity protein segments, which mediate specific protein-protein interactions. Although both eukaryotic and viral motifs are preferably located in disordered

protein environments, we hypothesize that protein regions embedding the viral motifs enable them to outperform the motifs of the host system.

To probe this tenet, we analyzed the coarse-grained structural properties of the protein regions, which flank the viral motfs and compared them to the corresponding segments of the eukaryotic system. Using the viral motif database (http://elm.eu.org/infos/viruses) we characterized the length and degree of disorder of the flanking regions and found the increase in these properties as compared to that of eukaryotic motifs. These results support that viral motifs act via an 'excluded-volume' mechanism, when longer disordered arms sterically inhibit the access of the host proteins to the same target.

We also identified intrinsically disordered binding (IDB) sites in the enviroment of LMs, which could contribute to binding by serving as non-specific anchor site and increasing the local concentration of the viral protein. We found that protein regions neighboring the viral are enriched in IDBs, and likely to establish buttressing interactions with the target. Taken together these parameters could account for the efficiency of viral motifs as compared to their human counterparts.

This Work was supported by LP2012-41 Momentum Program of the Hungarian Academy of Sciences, University of Debrecen, Department of Biochemistry and Molecular Biology

Keywords: *fuzziness, disorder, linear motifs, virus-host interaction*

P-095

FUNCTIONAL RELEVANCE OF DNAJA1 AS A NOVEL INTERACTING PARTNER OF HUMAN TRANSGLUTAMINASE 2

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Human transglutaminase 2 (TGM2) is a multifunctional crosslinking enzyme which has a large number of interacting partners contributing to its diverse biological and pathological functions such as cell growth, differentiation, adhesion, migration, apoptosis, neurodegenerative disorders, liver diseases, metastasis and cancer. However, the molecular interactions between TGM2 and its partners, which govern these processes, are largely unknown because of the lack of adequate information regarding these interacting proteins. Thus the present study aims to identify novel interacting partners of TGM2 and explore its functional significance. To achieve this we use NB4 cell line as a model because TGM2 expression is undetectable in wild type NB4 cell line but increases upon ATRA (all *trans* retinoic acid) treatment by several folds. To identify the proteins interacting with TGM2 we employed GST pull down assays and subsequent mass spectrometry analysis. We obtained several novel TGM2 binding proteins, namely Tubulin α, Histone H2A and heat shock protein 40 (HSP 40)/DNAJA1 in addition to some known interacting partners such as human Glutathione S Transferase (hGST-P1) validating the experimental approach. Since DNAJA1 and human TGM2 have been reported to be involved in various pathological processes (cancer,

Huntington and Alzheimer disease) we chose DNAJA1 as one of the candidate protein for further analysis. We performed GST pull down experiment, ELISA, Biacore and co-immunostaining studies with TGM2 overexpressing HEK cells and confirmed that TGM2 and DNAJA1 interact with each other and they colocalize in the cytoplasm.

TGM2 has four domain structure and we used TGM2 domain mutants to determine the exact binding domain/site of DNAJA1. ELISA experiments show that core domain of TGM2 is the most important domain in this interaction. These results were also confirmed via Biacore experiments. Furthermore, we determined via in vitro BPA incorporation experiment that DNAJA1 is a glutamine donor substrate of TGM2. The effect of DNAJA1 on TGM2 activity was also explored via ELISA and the results suggest that DNAJA1 increases the crosslinking activity of TGM2. We are performing in situ BPA incorporation experiments to check this effect of DNAJA1 on TGM2 activity using DNAJA1 overexpressing and down-regulated HEK-TGM2 cells.

P-096
DYNAMIC STRUCTURAL ENSEMBLES OF THE 3RD PDZ DOMAIN OF PSD-95

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PSD-95 (postsynaptic density-95) is one of the most thoroughly studied postsynaptic proteins. It contains 3 PDZ domains, of which PDZ3 contains an unusual C-terminal extension. It has been shown that the presence of this additional alpha-helical segment influences the peptide binding activity of PDZ3. NMR spectroscopy revealed that this intramolecular allosteric effect is of largely entropic nature: side chain dynamics of the PDZ3 domain is influenced by the presence or absence of the extra segment. Our aim was to generate dynamic structural ensembles using the backbone and side-chain order parameters available for the PDZ3 domain. We have performed calculations using our own implementation of order parameter-based ensemble restraining in GROMACS 4.5.5. The first question to be answered is how the incorporation of both backbone and side-chain parameters influences the conformational space represented by the ensembles. Detailed analysis of the interactions involved in peptide binding and the identification of a possible allosteric communication route will be presented.

Keywords: *restrained molecular dynamics, general order parameter, protein internal dynamics*

P-097
UBIQUITIN-DEPENDENT PHOSPHORYLATION OF ERK5 IN A THREE COMPONENT SIGNALING COMPLEX

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Mitogen-activated protein kinases (MAPKs) are well-conserved elements of human signal transduction. Mammals possess four classical MAPK pathways that play critical roles in many biological processes, e.g. proliferation, differentiation, stress-induced signaling and apoptosis. ERK5 and c-jun N-terminal kinase (JNK) pathways have paralogues protein elements and share a common upstream avtivator (MEKK3). Structural and biochemical studies of MEKK3-MKK5-ERK5 interactions can give a mechanistic insight on how signaling cascades using common components and can achieve functionally distinct and specific outcomes.

Pull-down and fluorescence polarization (FP) based assays showed that minimally two MKK5 interacting regions are required to bind ERK5. A Phox and Bem1 (PB1) domain and a linear motif (D-motif) from MKK5 cooperate to mediate high affinity binding to ERK5. MAPK activation depends on a linear binding motif found in all MAPK kinases (MKK). I present the crystal structure of ERK5 in complex with an MKK5 construct comprised of the PB1 domain and the linear binding motif. Structural and biochemical characterization revealed that the MKK5 PB1 domain cooperates with the MAPK binding linear motif to achieve substrate specific binding. In addition this domain also enables co-recruitment of the upstream activating enzyme and the down-stream substrate into one signaling competent complex. The upstream activator kinase for MKK5 is MEKK2/3, which also activates MKK7 that in turn activates JNK signaling. ERK5 and JNK signaling is functionally distinct and I demonstrate that ERK5 pathway activity is diminished upon MEKK2/3 ubiquitination by XIAP (X-linked Inhibitor of Apoptosis Protein). Interestingly, JNK activation is not inhibited by ubiquitination of this shared upstream activator kinase.

Keywords: *MAPK, paralogous enzymes, ubiquitiation*

P-098
TRANSCRIPTIONAL REGULATORY INTERACTIONS IN THE CHEMOIMMUNE SYSTEM INCLUDING TRANSPORTERS, METABOLIC ENZYMES, AND TRANSCRIPTION FACTORS

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Multidrug ABC transporters have been indicated as major factors in cancer multidrug resistance (MDR). While several efficient MDR transporter inhibitors have been developed, failure of clinical trials indicated that modulating the function of the transporters is insufficient to eliminate cancer drug resistance. Transporters are part of the chemoimmune/chemodefense system, which neutralizes toxic and therapeutic compounds entering our body. This system also includes metabolic enzymes chemically transforming xenobiotics and nuclear receptors sensing these molecules thus activating the transcription of enzymes and transporters. The modulation of the chemoimmune system is hindered by the lack of knowledge on the function of multidrug binding transporters, enzymes and receptors as a coherent network. To overcome this limitation we study the cooperation and co-regulation of the members of this system by analyzing mRNA expression changes upon drug treatment in various cell lines. We constructed a pipeline to analyze the results of mRNA chip experiments of human cell lines treated with over 1,300 different chemical compounds, publicly available in the gene expression database of Broad Institute. Raw microarray data were background corrected, normalized and expression changes at different thresholds were calculated. mRNA expression changes of drug transporters, metabolizing enzymes, and nuclear receptor genes were extracted and experiments were clustered based on the expression patterns of these chemoimmune proteins. The grouped expression patterns were correlated with experimentally determined data on chemical structure, metabolism and drug transport. Our results were supplemented with information from public databases (e.g. CTD, DrugBank) to infer metabolic and regulatory pathways in the chemoimmune system.

Acknowledgements: *The support of KTIA-AIK-12-2012-0025, OTKA K 111678, and Bolyai Fellowship to TH is gratefully acknowledged.*

Keywords: *systems biology, chemoimmune system, drug metabolism*

P-099
USING A MOLECULAR SWITCH TO DECIPHER PROTEIN-PROTEIN INTERACTIONS WITHIN THE LIVING CELL: WHAT CAN WE LEARN ABOUT HORIZONTAL GENETRANSFER?

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Bacterial genomes may include phage-related mobile genetic elements that encode virulence enhancing factors e.g. toxins termed as pathogenicity islands. There is an intimate relationship between pathogenicity islands and helper phages in *Staphylococcus aureus,* as *S. aureus* pathogenicity islands (SAPIs) are under endogenous repressor control by SAPI specific Stl proteins that are encoded by the pathogenicity island. Helper phages, on the other hand, encode proteins with derepressor functions. After infection, the derepressor protein relieves Stl-exerted repression. A phage-encoded dUTPase was shown to be responsible for derepression of the pathogenicity island SAPIBov1. The mechanism of this derepression[1] is of direct interest for understanding transmission of virulence and pathogenic factors.

Our aim was to construct a switchable model to characterize the Stl driven repression and the dUTPase induced derepression within cellular environments. We have chosen *Mycobacterium smegmatis* as a model to build this system, as we already have various dUTPase mutant strains to test. Therefore, we designed and created a strain, where the following construct was integrated into the genome. Stl binding site was cloned in a promoter element driving expression of a β-galactosidase reporter protein to test functional repression by Stl protein in the cell. Binding of Stl to its recognition site represses/activates gene expression from the reporter gene. The coding region of Stl was randomly mutagenized to characterize interaction with its binding site. Interaction of Stl with various (mutant) dUTPase proteins can also be tested.

This system is aimed at providing an assay background to check different mutants of dUTPases and Stl proteins for their preserved or diminished functionality in cellular environment.

Reference:

1. *Szabó, J. E. et al. Highly potent dUTPase inhibition by a bacterial repressor protein reveals a novel mechanism for gene expression control. Nucleic Acids Res.* ***42,*** *11912–20 (2014).*

P-100
ROLE OF THE NUDT9H DOMAIN OF TRPM2

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TRPM2 is a Ca^{2+}-permeable cation channel that belongs to the M subfamily of Transient Receptor Potential (TRP) channel proteins. TRPM2 is a homotetramer, each monomer contains a cytosolic N-terminal TRPM-homology region, six transmembrane helices with an architecture typical to that of

voltage-gated cation channels, and a cytosolic coiled-coil region followed by a NUDT9-Homology (NUDT9H) domain at the C-terminus.

TRPM2 activates in the presence of Ca^2, ADP-Ribose (ADPR), and PIP_2 (*Toth and Csanady, 2012*). ADPR binds to the NUDT9H-domain which displays ~35% identity with NUDT9, a monomeric mitochondrial ADPR-pyrophosphatase with available crystal structure (*Shen et al., 2003*). At present, it is unclear whether NUDT9H is an active enzyme itself, or just an ADPR-binding domain. In contrast to the extremely high solubility of NUDT9, NUDT9H solubility is very low: when overexpressed in bacterial systems it forms inclusion bodies, and reprecipitates upon removal of chaotropic agents. This might be a consequence of the tetrameric nature of TRPM2, in the context of which it is unclear how the four N-terminal domains and the four NUDT9H domains interact with each other and/or with the transmembrane segments. The fact that ADPR binding to NUDT9H is necessary for channel activation suggests an extended interface between NUDT9H and some partnering segment within the complex. Surface exposure of this interface region may account for the reduced solubility of NUDT9H when expressed in isolation.

Here we explore this hypothesis by expressing soluble NUDT9, insoluble NUDT9H, as well as different NUDT9/NUDT9H chimeras. By monitoring the solubility of the chimeras, we attempt to narrow down the search for the functional interface of NUDT9H. In addition, we try to identify the minimal substitutions required for increasing NUDT9H solubility. Finally, we use soluble NUDT9 to test hydrolysis of various substrates/ligands that activate the TRPM2 channel.

P-101
INTERACTION BETWEEN ATF2 TRANSCRIPTION FACTOR AND JNK MITOGEN ACTIVATED PROTEIN KINASE

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Activating transcription factor-2 [ATF2, CREB-2] is a member of leucine-zipper family of DNA-binding protein and contributes to various stress response by binding to CRE (cAMP response element) or AP-1 consensus sequences. ATF2 is activated through the MAPK (mitogen activated protein kinase) pathway by phosphorylation of the preferred Ser/Thr residues, but several studies conflict about the specificity or phosphorylation sites of the activating MAPK kinases (p38, ERK, JNK).

Our aims were to investigate the binding specificity of ATF2 to MAPKs and explore the structural background of the binding.

Our results clearly demonstrated that the transactivation region of ATF2 specifically bind JNK1 with high affinity (Kd ≈0.2 μM), but the binding to p38α and ERK2 is insignificant. This contradicts many published results. The minimalized binding element contains a C2H2 type zing finger domain which partially overlaps with a MAPK docking motif. This block enables a relatively strong interaction to JNK compared to classical D-motif containing peptides. Incubation with 2mM EDTA strongly reduced the binding affinity, which proves that the zing-finger is essential for the strong binding. According to our model [Figure 1.], Zn^{2+} staples the two histidines of the docking motif, thus it can

form an α-helix. Such a rigid structure may enhance binding by reducing entropic costs. We also demonstrated that the K48 residue strongly contribute for the interaction.

We plan to solve the structure of the peptide-protein complex to validate our model. To explore the specific role of the ATF2-JNK1 interaction in mammalian cell, we would like to switch off the interaction selectively with light using photo-caged-lysine unnatural amino acid, caging K48.

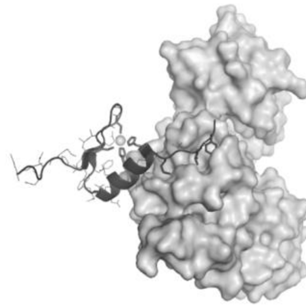

Figure 1: *pep-ATF2 in complex with JNK1*

Keywords: *docking interaction, zing-finger, MAP kinase*

P-102
COMPUTATIONAL ANALYSIS OF HOMINOID-SPECIFIC *DE NOVO* GENES EXPRESSED IN THE BRAIN

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Although the majority of evolutionarily new genes arise from previously existing ones through duplication, some originate from noncoding DNA sequences. These genes are called *de novo* genes, and the proteins encoded by them are the *de novo* proteins. The past years have seen an increase in the number of *de novo* genes identified in various species but primarily in humans. Interestingly, a considerable amount of these human- or hominoid-specific *de novo* genes are expressed in the brain but their exact role is elusive.

The aim of this study is to analyze hominoid-specific de novo protein-coding genes previously identified in the literature. An exhaustive computational analysis is performed to obtain clues about the molecular function of at least some of these *de novo* proteins. We start by careful re-checking of the *de novo* status of the described protein-coding genes and then apply various tools for sequence analysis, structure- and function prediction to obtain clues about their potential function. Our preliminary results show that many of these proteins possess a long intrinsically disordered segment and that in many cases even for the folded parts the predicted three-dimensional structures cannot be regarded as reliable. Therefore, a subset of proteins has been selected for more in-depth analysis whose results will be detailed.

Keywords: *de novo protein, protein evolution, structure prediction*

P-103
DYNAMIC STRUCTURAL ENSEMBLES OF THE TANDEM PDZ1-PDZ2 DOMAINS OF PSD-95

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The tandem PDZ1-PDZ2 domains form a structurally independent unit near the N-terminus of the postsynaptic density protein-95 (PSD-95). The two PDZ domains seem to have a rigid structure in their apo state, however, NMR measurements indicate that peptide binding to both domains considerably increases interdomain mobility. Molecular modeling studies also indicated that the complex has a larger flexibility than the unliganded state form of the protein due to the presence of a 5-residue long linker that allows the two PDZ domains to reorient.

The aim of this study was to generate structural ensembles of the tandem PDZ1-PDZ2 domains of PSD-95 by ensemble-restrained molecular dynamics. NMR-based general order parameters, reporting dynamics on the ps-ns time scale, were incorporated using our own extension of GROMACS 4.5.5. An important additional feature of the calculations is that the order parameters are used separately for the two domains modeling their independent reorientation on the ligand-bound state.

Our results show that we are able to model the independent tumbling of the PDZ domains and can generate structural ensembles that reflect the individual fast motions of the domains while also being able to account for interdomain mobility. Detailed analysis of the role of dynamics in peptide binding will also be presented.

Keywords: *restrained molecular dynamics, general order parameter, protein internal dynamics, interdomain mobility*

P-104
A SERINE PROTEASE INHIBITOR BOMAPIN REQUIRES FOR UVB STRESS RESPONSE

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Bomapin (SPB10) is a serine protease inhibitor that promotes cell proliferation under normal circumstances in hematopoietic and myeloid leukaemia cells, while in the absence of growth factors it can induce apoptosis. We identified *Bomapin* as one of the most dramatically upregulated gene in keratinocytes following UVB radiation. To learn about the possible biological function of *Bomapin* induction upon UVB treatment we used immortalised keratinocyte (Hker E6SFM, HaCat) and

melanoma (A375) cell lines. As a first step we analysed the kinetics of induction by determining the mRNA level in different time intervals following irradiation (2h, 8h, and 24h). We found that *Bomapin* mRNA level increased 2 and especially 8 hours after UVB irradiation (80 J/m^2), while 24h after the treatment the mRNA decreased in all examined cell lines. The mRNA level of *Bomapin* was also increased by a low intensity (20 J/m^2) UVB irradiation in primer keratinocyte cells. Accordingly, the transient change of mRNA level suggests that Bomapin might be involved in the UVB induced stress response. The elevated mRNA level resulted in higher Bomapin protein level as well in a time dependent manner (2h, 8h, and 24h) in Hker cells. Moreover UVB treatment resulted in a time dependent Bomapin transport from cytoplasm to the nucleus in U2OS cells. Based on our result we concluded that the Bomapin protein could be an essential player in the UVB induced stress response.

This work was supported by grants from TÁMOP-4.2.2-08/1-2008-0001, TÁMOP-4.2.2.A-11/1/KONV-2012-0035 and TÁMOP-4.1.1.C-12/1/KONV-2012-0014.

Keywords: *Bomapin, UVB stress, stress response*

P-105
BIOLOGICAL EFFECTS OF B-SHEET ANALOGS DESIGNED ON THE BASIS OF ANGIOGEN INHIBITOR ANGINEX

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The growth of solid tumors stimulates the formation of new blood vessels, a process called angiogenesis, therefore inhibition of this pathway is a promising trend in cancer therapy. In such methods the bioactive proteins involved in angiogenesis are suitable targets. A new synthetic oligopeptide, anginex (invented by Kevin H. Mayo) preventing the formation of new blood vessels, thus inhibits tumor growth. However our experiments showed that anginex is highly aggregated in solution probably due to its ß-sheet structure. Furthermore according to the literature its half-life *in vivo* is likely short. Therefore we started to synthetize anginex analogs in which specific amino acids were substituted in β-sheet by β-amino acids or β-amino acids with cyclic side-chains. These constructs did not pocess the above mentioned disadvantages of anginex. Our experiments aimed to reveal whether anginex analogs retained the functional properties of anginex, and characterize the anti-angiogenic mechanism of anginex.

Effects of the molecules were tested on cell proliferation and cell viability of a murine brain microvascular endothelial cell line, bEnd.3. A strong inhibition was observed in case of the viability of endothelial cells after treatment with anginex, and few anginex analogs as well. Determining the mechanism of inhibition further studies were carried out with anginex examining its effect on cell

proliferation. Our results showed that anginex was toxic to the cells instead ofinhibiting the cell proliferation of bEnd.3.

Our data suggest that effectiveness and secondary structure of anginex can be retained by certain substitutions, and the decrease of cell viability after anginex treatment is the consequence of its toxicity.

P-106
PUZZLING OUT: THE BEGINNING OF A LONG WAY TO CHART THE ROLE OF *PF*CCT STRUCTURAL ELEMENTS IN FUNCTION

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Malaria is one of the most significant causes of morbidity and mortality worldwide. In *P. falciparum* phospholipid biosynthesis has an essential role in synthesis of membranes. The most prevalent way of *de novo* biosynthesis is Kennedy-pathway, where the reaction catalysed by CTP:phosphocholine cytidylyltransferase[1,2] (CCT) (CTP+ChoP→CDPCho) is rate-limiting. Because of the persistent need of Plasmodium for membrane synthesis during its life cycle, *de novo* phospholipid biosynthesis emerges as a target for new generation antimalarial drugs[3].

CHO-MT58, a mutant cell line was proved to be an appropriate tool for investigating intracellular function of CCT. In this cell line, the endogenous CCT activity decreases dramatically at 40 °C, blocking membrane synthesis and ultimately leading to apoptosis. We have demonstrated for the first time that heterologously expressed *Pf*CCT is able to complement endogenous CCT activity in mammalian cells. Thus, a suitable system has been established for functional investigation of structural elements of *Pf*CCT.

In order to investigate the role of different structural elements in enzymatic function we redesigned the structural gene of *Pf*CCT obtaining a modular system where different domains are easy to remove or exchange. Here we designed a series of different truncation and deletion constructs (cf Fig. 1) to reveal the role of Plasmodium specific sequences. In parallel, heterologous expression experiments of different constructs in the mutant CHO-MT58 and its isogenic wild type control cell lines are performed to validate the reported model system.

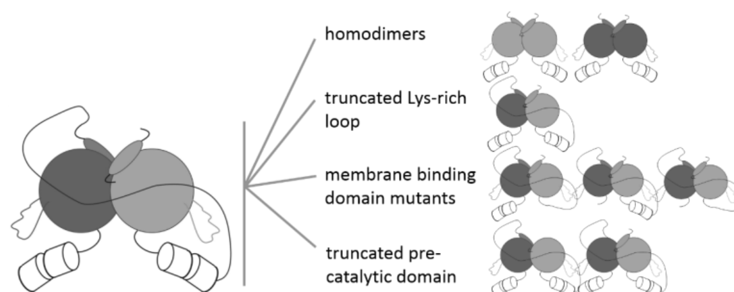

Figure 1.*Design of various PfCCT mutants to reveal segment-specific roles*

This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'.

References:
1 *Nagy et al. FEBS J 2013*
2 *Nagy et al. Angew. Chem. 2014*
3 *Vial et al. PNAS 2004*

Keywords: *malaria, lipid biosynthesis, CHO cells*

P-107
N-TERMINAL TAIL OF DR0550 NUDIX HYDROLASE ACTS AS AN INTRINSICALLY DISORDERED INTRAMOLECULAR CHAPERON

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Proteins of the Nudix hydrolase superfamily are widely distributed among all classes of organisms and they have a typical pyrophosphatase activity. One of the Nudix hydrolases, called DR0550 in *Deinococcus radiodurans* contains significant intrinsically disordered regions on the N- and C-terminus that are not present in non-extremophile homologues. IDPs play a crucial role in many processes of living organisms due to their specific structure and ensuing specific features, including chaperone function.

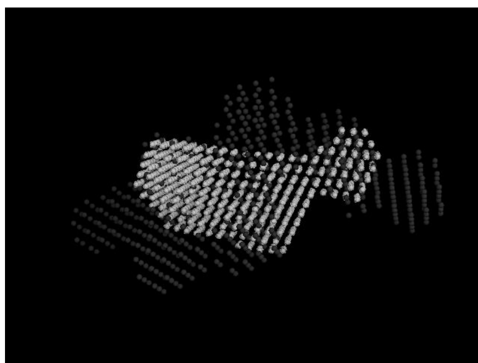

Figure 1: *Aligned structures of the Nudix wild type and the scarmbled mutant according to SAXS measurements*

We chose to examine DR0550 Nudix hydrolase with the aim of shedding light on the potential chaperone function of its disordered segments. Our hypothesis is that we can measure significant differences between the stress-tolerance of the WT and mutant enzymes due to the presumed intramolecular chaperon activity of the disordered tails. We created different mutants (delta-C, delta-N, N-term, scramled) and examined the stress-tolerance of the wild type (WT) and the mutant Nudix forms. It became evident that the C terminal region is indispensible for the proper folding of

the protein, but the N-terminal region can be removed without significant activity loss. To understand the molecular mechanism of the intramolecular chaperon activity the sturctures of the different protein constructs were investigated by many spectorfotometric techniques (CD, DSF, SAXS, FT-IR) as well as by bioinformatic analysis (DMD).

Keywords: *intramolecular chaperon, IDP, small angle X-ray scattering,*

P-108
GENOME-WIDE ANALYSIS OF FUZZY COMPLEXES

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Activities of intrinsically disordered (ID) proteins are often related to adopting a well-defined structure upon molecular interactions. Conformational heterogeneity however, could also be retained in bound forms of ID proteins. In these systems, the disordered state is not only maintained in one or more interacting partners, but is also a pre-requisite for the biological function. This phenomenon is termed as fuzziness. In fuzzy complexes the structural ensemble is shifted according to the incoming signals and enable a variety of functional outputs. Classification of fuzzy complexes and the underlying mechanisms have been published (TiBS 2008, TiBS 2011).
In the present work we investigate the abundance of proteins, which are predicted to form fuzzy complexes. In *Homo sapiens*, *Saccharomyces cerevisiae* and *Escherichia coli* about 80% of the ID proteins contain >30 AA long regions, which are devoid of a well-defined structure in the context of other proteins. In eukaryotes, the majority of fuzzy regions flank short peptide motifs or folded molecular interaction sites. In accord, 87% of proteins, which contain eukaryotic linear motifs (ELM) encompass fuzzy protein segments. Thereby the fuzzy regions facilitate positioning of short motifs and/or enable combinatorial usage of multiple motifs. Based on analysis of GO categories, proteins with fuzzy regions are distinguished in molecular transducer activities and within the transcription machinery. 81% of the proteins, which mediate crosstalk in signaling pathways are also equipped with fuzzy regions. All these results suggest that fuzziness enables context-dependent regulation of protein complexes.

This Work was supported by LP2012-41 Momentum Program of the Hungarian Academy of Sciences, University of Debrecen, Department of Biochemistry and Molecular Biology

Keywords: *fuzziness, linear motif, signaling*

P-109
CHARACTERISTIC MUTATIONS IN TRANSMEMBRANE PROTEINS ASSOCIATED WITH HUMAN DISEASES

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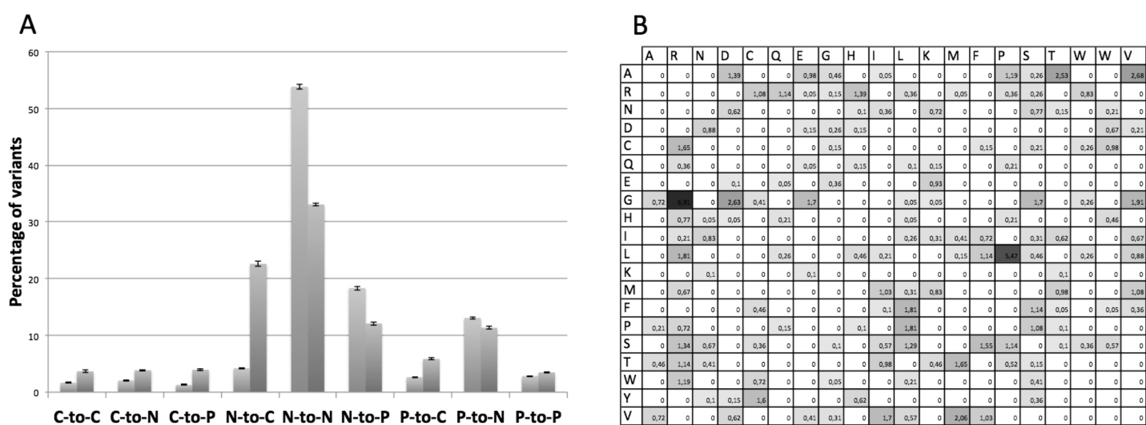
Transmembrane protein coding genes are commonly associated with human diseases. To understand the differences between disease causing mutations and natural polymorphisms in these proteins, we analyzed missense genetic variations from the UniProt database, and mapped these positions to the transmembrane protein topology from the Human Transmembrane Proteome database (http://htp.enzim.hu). We found characteristic differences in the spectrum of amino acid changes within the transmembrane region. In the case of disease associated mutations the non-polar to non-polar and non-polar to charged amino acid changes are frequent (Figure 1A). Comparing these mutations to the natural polymorphisms the non-polar to charged type of changes are rare while non-polar to non-polar changes are the most frequent mutations. Investigating disease associated mutations more deeply revealed that glycine to arginine and leucine to proline changes are the most responsible for these findings (Figure 1B). Furthermore, the mutations to positively charged amino acids are more common in the middle region of the lipid bilayer, where they can cause more severe anomalies. Interestingly, genes harboring glycine to arginine mutations are strongly connected to the chromosome X. This knowledge could help to understand the effect of disease associated mutations in transmembrane proteins and help to prioritize genetic variations in personal genomic investigations.



	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	W	V
A	0	0	0	1,39	0	0	0,98	0,46	0	0,05	0	0	0	0	1,19	0,26	2,53	0	0	2,68
R	0	0	0	0	1,08	1,14	0,05	0,15	1,39	0	0,36	0	0,05	0	0,36	0,26	0	0,83	0	0
N	0	0	0	0,62	0	0	0	0	0,1	0,36	0	0,72	0	0	0	0,77	0,15	0	0,21	0
D	0	0	0,88	0	0	0	0,15	0,26	0,15	0	0	0	0	0	0	0	0	0	0,67	0,21
C	0	1,65	0	0	0	0	0	0,15	0	0	0	0	0	0,15	0	0,21	0	0,26	0,98	0
Q	0	0,36	0	0	0	0	0,05	0	0,15	0	0,1	0,15	0	0	0,21	0	0	0	0	0
E	0	0	0	0,1	0	0,05	0	0,36	0	0	0	0,93	0	0	0	0	0	0	0	0
G	0,72	6,91	0	2,63	0,41	0	1,7	0	0	0	0,05	0,05	0	0	0	1,7	0	0,26	0	1,91
H	0	0,77	0,05	0,05	0	0,21	0	0	0	0	0,05	0	0	0	0,21	0	0	0	0,46	0
I	0	0,21	0,83	0	0	0	0	0	0	0	0,26	0,31	0,41	0,72	0	0,31	0,62	0	0	0,67
L	0	1,81	0	0	0	0,26	0	0	0,46	0,21	0	0	0,15	1,14	5,47	0,46	0	0,26	0	0,88
K	0	0	0,1	0	0	0	0,1	0	0	0	0	0	0	0	0	0	0,1	0	0	0
M	0	0,67	0	0	0	0	0	0	0	1,03	0,31	0,83	0	0	0	0	0,98	0	0	1,08
F	0	0	0	0	0,46	0	0	0	0	0,1	1,81	0	0	0	0	1,14	0,05	0	0,05	0,36
P	0,21	0,72	0	0	0	0,15	0	0	0,1	0	1,81	0	0	0	0	1,08	0,1	0	0	0
S	0	1,34	0,67	0	0,36	0	0	0,1	0	0,57	1,29	0	0	1,55	1,14	0	0,1	0,36	0,57	0
T	0,46	1,14	0,41	0	0	0	0	0	0	0,98	0	0,46	1,65	0	0,52	0,15	0	0	0	0
W	0	1,19	0	0	0,72	0	0	0,05	0	0	0,21	0	0	0	0	0,41	0	0	0	0
Y	0	0	0,1	0,15	1,6	0	0	0	0,62	0	0	0	0	0	0	0,36	0	0	0	0
V	0,72	0	0	0,62	0	0	0,41	0,31	0	1,7	0,57	0	2,06	1,03	0	0	0	0	0	0

Figure 1:* *A, *Changes of amino acid types (C=charged, N=non-polar, P=polar), blue coloumns representing polymorphisms, red coloumns representing disease-associated genetic variants.* ***B,*** *The amino acid change matrix of disease-associated genetic variants, the one-letter code representing the amino acids, the more deeper the color the more frequent the mutation*

Keywords: *transmembrane, genetic disease, mutation*

P-110
ANAPHASE PROMOTING COMPLEX SUBUNITS WITH UNUSUAL CHARACTERISTICS

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The eukaryotic cell cycle is unidirectional and irreversible process. This is ensured by the ubiquitin mediated proteolysis of cell cycle regulators. A polyubiquitin chain is attached to the substrate by an enzyme cascade, then the target protein is degraded by the proteasome. The specificity of this process relies on the activity of the ubiquitin-protein ligase, or E3 enzyme. The Anaphase Promoting Complex/Cyclosome (APC/C) is an E3 enzyme, which plays a key role in cell cycle regulation during mitosis and the G1 phase. The process of the cell cycle is well conserved in eukaryotic organisms, so the cell cycle related enzymes.

The APC/C is a large protein complex containing 13-15 well conserved subunits. One of the smallest of them, the cdc26 subunit was successfully detected in yeast and in human cell cultures and lately our group identified two putative cdc26 proteins in *Drosophila melanogaster*. These two proteins are different in size and amino acid sequence, but both contain a highly conserved, short N-terminal region. Our aim is to characterize these two subunits through genetic analysis.

Genetic characterization of P element insertion alleles of one of the genes revealed pupal lethality. Larval brain preparations of these mutants show mitotic arrest characteristic to the loss of function phenotypes of other essential APC/C subunits. We denominate this gene as *DmCdc26*. The other gene proved to be nonessential, but it could complement the loss of function phenotype of *DmCdc26*, therefore it was dubbed by us as *DmCdc26-like*.

To determinate whether they are integral subunits of APC/C complex, we use affinity purification, native polyacrylamide gel electrophoresis and Western blotting. For these experiments transgenic *Drosophila melanogaster* strains were established overexpressing the haemagglutinin tagged cdc26 and cdc26-like. We have used these lines to create double mutants with transgenic lines carrying either a FLAG tagged Apc3 or Apc10, both are subunits of the *Drosophila's* APC/C. The results of the native-PAGE combined with Western blotting are promising, suggesting that both Cdc26 and Cdc26-like belong to the APC/C complex.

Keywords: *cell cycle, ubiquitylation, APC/C, Cdc26, Cdc26-like*

P-111
SPECIFIC MODIFICATIONS OF MEMBRANE-ASSOCIATED PROTEINS FOR LIVE CELL APPLICATIONS

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Membrane associated proteins have important functions in living cells including signal transduction, cell adhesion and antigen presentation. A special class of lipidated membrane proteins contains a glycosylphosphatidylinositol (GPI) glycolipid moiety at the C-terminus, and the lipid chains of the GPI anchor are responsible for the membrane association. GPI-anchored proteins are accumulated in cholesterol-rich membrane microdomains of the outer leaflet of the membrane bilayer, that is lipid rafts. A unique feature of GPI-anchored proteins is that isolated compounds are reinserted into the cell membrane with the retention of the function. Accordingly, the exogenous introduction of fluorescent GPI-anchored protein analogues into cell membranes is a useful method for visualizing the cellular traffic of membrane associated proteins and for engineering cell surfaces. We have recently shown that cholesterol can be applied for anchoring proteins to the plasma membrane of live cells without perturbing the membrane.[1] In this construct a small molecule fluorophore is applied in the lipid headgroup as a reporter, and it was evidenced that the headgroup fluorescence can unambiguously be assigned to the attached protein without using protein-specific fluorescent antibodies. In order to widen the scope of the method, the anchorable proteins are investigated by conjugating semisynthetic proteins to the fluorescent cholesterol anchors (Figure 1). In order to prepare illustrative proteins, an enzymatic method is investigated for the preparation of semisynthetic proteins from N^{α}-protected protein thioester building blocks and the details of the method are presented.

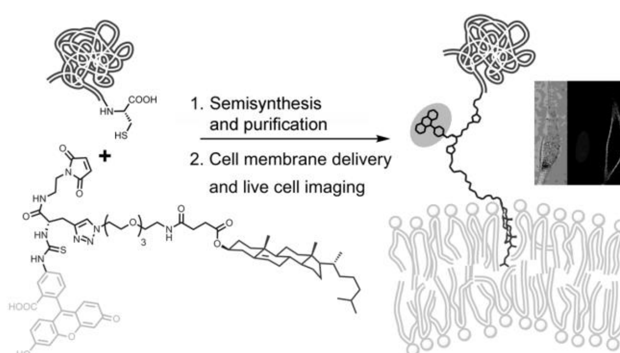

Figure 1 *Cell membrane delivery of semisynthetic proteins with fluorescent reporters*

References:

[1] Schäfer et al. "Preparation of Semisynthetic Lipoproteins with Fluorescent Cholesterol Anchor and their Introduction to the Cell Membrane with Minimal Disruption of the Membrane." *Bioconj. Chem.* **24**, 1684-97 (2013)

Keywords: *membrane protein, GPI anchor, native chemical ligation*

P-112
IN VITRO TYROSINE PHOSPHORYLATION OF SRC HOMOLOGY 3 DOMAINS FOR STRUCTURAL STUDIES

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Src homology 3 (SH3) domains are protein-protein interaction domains in eukaryotes involved in various intracellular signalization pathways. More than 200 human proteins possessing one or more SH3 domains are known. These play essential roles in many physiological or pathological cellular processes (e.g. the regulation of cellular proliferation or the development of cancer). SH3 domains bind short, proline rich sequences within intrinsically unstructured regions of partner proteins. More and more evidences have been accumulated in the recent years showing that phosphorylation on different conserved tyrosine residues of SH3 domains is a common regulatory strategy. Most of these tyrosines are part of the ligand binding groove, and the result of this post-translational modification was the inhibition of partner binding *in vivo* in the majority of reported cases. However, *in vitro* studies demonstrating and comparing the effects of tyrosine phosphorylation on individual tyrosine residues (e.g. partner binding assays) has not been published yet. Atomic resolution structures would also be essential to fully understand the role and function of the introduced phosphate groups. These studies would, however, require phosphorylated proteins on the milligram scale. Here we successfully overcome this technical limitation, and aim to investigate the structural and functional consequences of phosphorylation *in vitro*. Several SH3 domains of adaptor proteins (e.g. Grb2) and tyrosine kinases (members of Abl and Tec tyrosine kinase families) have been chosen as model systems for our experiments. All of these were shown to be phosphorylated *in vivo* both by site specific and high throughput methods according to the PhosphoSitePlus database. In all cases, several inter- or intramolecular interaction partners (proline rich regions) are known. We have successfully expressed and purified the selected SH3 domains. Several tyrosine kinases that were suspected to be able to phosphorylate our proteins were tested in *in vitro* kinase assays. We found that most of the SH3 domains could be phosphorylated on one or more physiologically relevant tyrosine residues by the recombinant kinase domain of Ephrin B1 receptor. This kinase domain proved to be perfect for *in vitro* phosphorylation experiments because: 1) it can be expressed in high quantities in *E. coli* bacteria, 2) activates itself by autophosphorylation and 3) shows remarkable stability under the required conditions. Our approach allowed us to upscale the *in vitro* kinase reactions and obtain highly purified, phosphorylated SH3 domains by ion exchange chromatography. Phosphorylated tyrosines were identified by mass spectrometry. According to our preliminary results, the SH3 domain of Abl1 kinase phosphorylated on two *in vivo* relevant tyrosine residues (Y70 and Y115) show reduced affinity to a peptide ligand corresponding to the binding motif of the 3BP-1 protein (residues: 616-625). Experiments with other phosphorylated SH3 domains and screening for optimal crystallization conditions for X-ray diffraction studies are in progress.

This work was supported by OTKA K 83867 and LENDÜLET grants and by the MTA Postdoctoral Fellowship Programme.

Keywords: *SH3 domain, tyrosine phosphorylation, ligand binding*

P-113
RELATIONSHIP BETWEEN INTERFACES AND BINDING STRENGTH OF INTRINSICALLY DISORDERED PROTEIN COMPLEXES

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The concept of structural disorder is now widely accepted, bioinformatic predictions suggest that about 50% of human proteins have at least one long disordered region. Most of IDP functions are carried out by molecular recognition, i.e. via transient or permanent binding to a structured partner. To understand the role and functioning of disordered proteins, it is very important to map the mechanism(s) of recognition and binding. The common view is that disorder separates binding strength from specificity. This feature would enable IDPs to engage in highly specific, yet rather weak and reversible interactions. Scattered observations, however, show that this may not be true without exceptions. Our hypothesis is that the binding strength of IDP complexes depends on the mode of recognition and the type of interaction, which enables weak and strong, specific and non-specific interactions alike.

The aim of the project is to find out what influences the binding strength (and specificity) of protein complexes of which at least one of the partners is disordered. We have been assembling complexes of disordered proteins for which both the structure and the dissociation constant (Kd) are known, and we will build a publicly available database. We analyze the collected data from several aspects: distribution of Kd values, physical and chemical properties of the interface, eg. size, proportion of hydrophobic interface, amino acid composition, distribution of contact types etc. We examine and compare these properties in the case of strong (low Kd values) and weak (high Kd values) complexes. We use a reference database built from complexes of completely ordered proteins.

Keywords: *disordered proteins, protein complexes, specificity*

P-114
A NANNY MODEL FOR REGULATION OF HALF-LIFE OF INTRINSICALLY DISORDERED PROTEINS

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Intrinsically disordered proteins (IDPs) and ID regions commonly occur in eukaryotic cells and are strongly implicated in protein function. Precise regulation of protein turnover is essential for a variety of cellular functions, such as the cell cycle, genome maintenance and DNA repair, apoptosis

or cell survival (Millar AJ et al 2011). It was found that IDPs are inherently unstable and undergo proteasomal degradation by default. It has been proposed that they can escape from degradation by interacting with a protective protein partner, called a "nanny". Here we test the hypothesis that nannies control the levels of their ID clients thereby regulating their function using the AP-1 transcription complex as a model system in which ID containing protein, c-Fos interacts with c-Jun.

c-Fos, c-Jun and their mutants are fused to EGFP and mRFP and expressed in bacteria system. Directed evolution system is being established for AP-1 complex. Two random libraries with higher and lower mutation rates have been generated from full length cFos and cJun DNA sequence. A new, robust and high-throughput in-vitro Eukaryotic transcriptional assay has been establishing in our laboratory. The method will allow for sensitive detection and precise quantification of the newly transcribed, unlabelled RNA and is particularly useful for quantification of strong transcriptional factors and used for functional mutant's selection on the basis of increased transcriptional fitness. Moreover, the method is further optimized/normalized on the amount of Fos put into the reaction, which is given by the GFP fluorescence intensity. Isolated clones possessing desired transcriptional activity will be sequenced and structural characteristics are determined with the softwares, IUPred and PONDR VSL1.

We also managed to develop conditions to purify c-Jun and to solubilize and purify c-Fos, which has a tendency to aggregate. The c-fos mutants will be sequenced, the mutations mapped on the complex, the corresponding mutant proteins expressed, their effect on protein structure, on protein-protein and protein-DNA interactions of the complex will be characterized. In conclusion, we will probe whether c-Jun serves as a nanny for c- Fos. We will establish relationships between transcriptional activity, protein disorder and half-life.

Keywords: *Intrinsically disordered proteins, nanny model, in-vitro transcriptional assay, directed evolution*

P-115
THE CHARACTERISATION OF THE MAPKAPK2 AND THE ERK5 INTERACTION

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The MAPK kinases and their substrates like the MAPKAPK kinases have an important role in the cell's signaling apparatus. In my project I'm examinig the interaction between the ERK5 and the MK2 kinases. Our measurements between the linear docking motifs of the MK2 protein and the ERK5 kinase indicated an interaction between the two proteins.

Aims: The interaction between the two proteins was examined in vitro with GST pulldown and with in vitro kinase assays. The interaction's strenght was quantified with fluorescence polarisation assay. The interaction was also examined in cell lines. The current aim is to investigate what function could the interaction have. It's also our goal to solve the erk5-mk2 complex structure by x-ray crystallography.

Results: A new interaction was discovered between ERK5 and MK2. Measurements suggests that ERK5 is capable of phosphorylating MK2. Kinase assays measurements between other MAPKAPK

kinases suggests that ERK5 specifically activates the MK2 MAPKAPK kinase. The phosphorylation occurs mostly on the 222. threonin on MK2. The fluorescence polarisation assay results suggests that the linear docking motif of the MK2 attaches to the ERK5's docking groove. The interaction's strenght is around 0,5 μM. The interaction was also shown in cell lines in fragment complementation assays.

Keywords: *MAPK signaling, ERK5, MK2*

P-116
STRUCTURE AND LIFETIME OF THE INTRINSICALLY UNSTRUCTURED PLANT DEHYDRIN ERD14 *IN VIVO*

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ERD14 belongs to the family of late embryogenesis abundant (LEA) proteins, which mostly consists of IDPs noted for stress-related functions. LEA proteins appear to have broad, rather non-specific and redundant chaperone activity as demonstrated by a range of non-physiological substrates. In terms of their molecular mechanism of action, they have been suggested to act by a combination of partner binding, entropic exclusion, space filling and membrane stabilization, however, have never been tested in vivo. ERD14 was previously shown by NMR to be largely disordered in vitro, with its few conserved regions (K-segments) sampling transiently ordered secondary structures. As disordered proteins are very sensitive to proteolysis in vitro and their cellular level is strictly regulated (mainly by rapid elimination), aimed to determine the in vivo half-life of a functional disordered protein in prokaryotic and eukaryotic cells. To this end we overexpressed recombinant ERD14 in E. coli cells for in-cell NMR structural studies and lifetime experiments and introduced the purified protein into A2780 human cells. Albeit structurally disordered, the stability of ERD14 was comparable to that of a globular protein both in E. coli and human cells.

Keywords*: intrinsically disordered protein, in vivo lifetime, chaperon, in cell NMR*

P-117
CHARACTERIZATION OF SPECIES-SPECIFIC INHIBITORY EFFECT ON DUTPASES BY A TRANSCRIPTION FACTOR ENCODED BY *STAPHYLOCOCCUS AUREUS*

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Proteins responsible for genome integrity are preferred targets in drug therapies against various diseases. Accordingly, dUTPase, an enzyme, that is essential for cell viability in many organisms, is subjected as a target of several drug research projects.

It was recently shown that Φ11 phage dUTPase is capable of interacting with a *Staphylococcus aureus* repressor protein called Stl during phage infection[1]. Our group has started to investigate the interaction between Stl and dUTPases and showed that Stl is able to inhibit enzymatic activity of Φ11 dUTPase. On the other hand, it was found that Stl can be a potent inhibitor of not only Φ11 dUTPase [2], but other homotrimeric dUTPases, such as the *Mycobacterium tuberculosis* (MTB) dUTPase.

The exploration of species-specific elements of this proteinaceous inhibition may serve significant information for designing further species-specific inhibitors. The characterization of the interaction between dUTPases and Stl would also establish the grounds of the *in vivo* inhibition of dUTPases by Stl. A proteinaceous dUTPase inhibitior would ease the investigation of the effects of dUTPase inhibition on DNA metabolism. Therefore we set out to explore the inhibition mechanism of Stl with the help of kinetic methods.

Our results show that Stl inhibition has a slow and tight binding character in case of all investigated dUTPases, however, the extent of the inhibition is differs between the dUTPases from different species. For in-depth transient kinetic characterization of the dUTPase – Stl interaction we used Φ11 and MTB quasi wild type dUTPase variants containing a tryptophan substitution at the active site. By fluorometer and stopped-flow, we found that the Stl is a competitive inhibitor in case of both Φ11 and MTB dUTPases. This indicates also that the difference between the interaction of these dUTPases and Stl is not the result of active site differences. Further investigation has shown that in case of Φ11 dUTPase three Stl molecules are able to bind to the homotrimeric enzyme with similar affinity, while in case of MTB dUTPase the affinity of the third Stl molecule is somewhat lower, which leads to the observed differences in the extent of inhibition under our assay conditions.

To summarize our results we have characterized the species independent and species specific aspects of Φ11 phage and MTB dUTPase inhibition by Stl. We have found that the interaction of these dUTPases and Stl is very similar for the individual active sites and the species specific differences in the extent of inhibition are the results of the differences within the quaternary structure of these dUTPases. We propose that the interaction with Stl is similar also for the other dUTPases.

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P-118
DETERMINATION OF ERA REGULATED GENES BY THE META-ANALYSIS OF TRANSCRIPTION FACTOR BINDING SITES IN MCF-7 CELL LINE DERIVED FROM VARIOUS EXPERIMENTAL METHODS

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Estrogen receptor alpha (ERα) is a member of the nuclear receptor superfamily and is known as a key hormone-regulated transcription factor in three-quarters of the breast cancer cases. The activation of ERα by 17β-estradiol results binding of the receptor to transcription regulatory regions of the DNA and influences the expression of specific genes.

One of the greatest opportunities in nowadays' research is the ability to manage large datasets and draw biologically relevant conclusions from them. Currently, there are many ways to investigate functional genomic features of sequencing data; so, to elucidate the effect of ERα transcription factor binding in MCF-7 cell line, we re-analyzed all available ChIP-seq, RNA-seq, GRO-seq and DNase-seq as well as H3K4me2 experiments with bioinformatic methods.

We matched the consensus 189,964 ERα ChIP peaks to GRO-seq, DNaseI hypersensitivity and H3K4me2 signals and to level of RNA-seq expression – that were influenced by estradiol treatment –, and each binding site was given a score (from -5 to +5) based on the cumulative change of the different features. Scores of the peaks were clustered and showed clearly distinct sets.

Next, we decided to investigate further those genes that were previously annotated to the binding sites with the highest score. After defining the possible genes that are influenced by estrogen treatment we identified their upstream regulators and canonical pathways.

In the literature, a part of our identified genes were published in association with breast cancer, but some genes were not published; so, later on we plan to determine the relationship between genes and breast cancer and we have to perform a deeper analysis of clusters one by one.

The project is funded by the Internal Research University Grant entitled „Dissecting the genetic and epigenetic components of gene expression regulation in the context of the 1000 genomes project". Balint L. Balint is Szodoray fellow of the Medical Faculty of the University of Debrecen.

P-119
MAPPING THE ESTROGEN RECEPTOR ALPHA BINDING SITES DERIVED FROM ER POSITIVE CELL LINES IN THE LIGHT OF THE 1000 GENOMES PROJECT

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Estrogen Receptor alpha (ERα) is a hormone-regulated transcription factor expressed in most of endocrine-related cancers (such as breast and endometrial cancer) in women. ERα is an important target for endocrine therapy of breast cancer, although resistance to treatment occurs in a significant number of the patients. Differences in response to the treatment can be derived from genetic variations between individuals. The aim of our study was to investigate the effects of SNP-s in the ERα binding sites (ERBSs).

ERα ChIP-Seq data were collected from public database. We selected four ER positive cell lines derived from breast (MCF-7 and T47D) and endometrial cancers (ECC-1 and Ishikawa). Raw data were re-analyzed by our bioinformatic pipeline to determine the common and individual ERBSs of each cell line. The resulting peak sets were compared with the dbSNP database, then the common ERBSs were annotated, giving 29 intronic gene-associated ones. These genes encode proteins including DNA-binding ones, regulatory enzymes and some which have role in cell adhesion. Some of these were selected to be validated by experimental methods. We investigated the presence of ERBS by chromatin immunoprecipitation followed by qPCR (ChIP-qPCR) in the control and estradiol (E2) treated MCF-7 cells and in parallel we examined the gene expression changes upon the treatment.

Results of the ChIP experiments showed ER enrichment at the selected ERBSs in E2 treated MCF-7 cells. Genes hosting the ERBSs showed significant changes in expression upon ligand treatment. Characterization of the newly characterized ER target genes may help us to understand the effects of SNPs on ER binding events.

The project is funded by the Internal Research University Grant entitled „Dissecting the genetic and epigenetic components of gene expression regulation in the context of the 1000 genomes project". Balint L. Balint is Szodoray fellow of the Medical Faculty of the University of Debrecen.

P-120
IDENTIFICATION OF HISTONE ACETYLTRANSFERASES AFFECTING AGING IN *DROSOPHILA*

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The continuous changes that occur during the process of aging can be observed not only in the anatomy and physiology of an organism, but also at cellular and molecular levels by extensive changes in gene expression patterns. The best known epigenetic marks connected to aging are DNA methylation and post-tanslational modification of histones that cooperate in chromatin remodeling, leading to a dynamic regulation of gene expression. The acetylation status of core histones plays a major role in the regulation of chromatin structure. Acetylation is modulated by the antagonist Histone AcetylTransferase (HAT) and Histone DeACetylase (HDAC) proteins. HAT proteins transfer the acetyl group from acetyl coenzyme A to the ε-NH_3^+ groups of internal lysine residues of core histone N-terminal domains. Introduction of the acetyl group to lysine neutralizes its positive charge, thus it loses its affinity to the negatively charged DNA. This might lead to the loosening of chromatin structure and increased transcription.

To date two HDAC enzymes, Rpd3 (homolog of human HDAC1/2) and Sir2 (homolog of human SirT1) have been linked to aging in *Drosophila* and increased longevity could be achieved by modulating the levels of these factors. We hypothesized that if HDACs influence aging than the opposing enzyme group, the HATs might also affect it. Therefore we designed experiments to identify HAT genes participating in the regulation of longevity in *Drosophila*.

First, we aimed to determine the expression level of a selected group of HAT genes in aging flies. For this we collected w^{1118} male flies with the ages of 1 day, 3 days, 1 week, 2 weeks, 4 weeks and 6 weeks old and measured the expression level of HAT genes in head tissues by qRT-PCR. We found that the expression levels of *enok* and *nejire* decreased during aging, thus these genes seem to be potential candidates to affect the acetylation status of histones during the process of aging. Next we set up crosses to test the effects of altering the level of the *nejire* gene (encoding the Drosophila CREB-Binding Protein) on longevity, but found that both overexpression and suppression of *nejire* lead to lethality during development.

Keywords: *aging, histone acetyltransferase, Drosophila*

P-121
REVEALING THE *IN SITU* FUNCTION OF THE *BXD* TRE IN MAINTAINING THE ACTIVE STATE OF THE *BXD CIS*-REGULATORY REGION OF *DROSOPHILA*

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In the post-genomic era, one of the main challenges facing biology is answering the question of how different cell types and cell lineages, deriving from the zygote, utilize the very same genome differently during development. Part of the answer must be sought at the level of higher order chromatin structure.

It is well known that the alteration of the chromatin structure is a substantial process of epigenetic regulation of gene expression. In *Drosophila*, the bithorax complex (BX-C) is an exquisitely convenient model system to study epigenetic regulation. In *Drosophila*, two classes of genes, the Polycomb group and the trithorax group, are required to act in concert to maintain the expression of numerous key regulator genes by regulating the chromatin structure. The POLYCOMB group proteins are epigenetic silencers required for the repression of target genes by condensing chromatin structure, whereas the TRITHORAX group proteins are known to be epigenetic activators with the function to keep target genes in open chromatin conformation. The regulation of chromatin conformation depends on special DNA-sequences, called Polycomb Response Elements (PREs), containing binding sites for the POLYCOMB group proteins and the Trithorax Response Elements (TREs), containing binding sites for the TRITHORAX group proteins, respectively.

In the *bxd*/*pbx cis*-regulatory region the TRE (*bxd* TRE) located in the vicinity of the PRE (*bxd* PRE), establishing a so called Maintenance Element (ME). The ME is responsible for maintaining the active or the inactive conformation state of the chromatin from the late embryonic stages to the adult stage. While the *in situ* function of the *bxd* PRE is well known (*Sipos et al*., 2007), the *in situ* function of the *bxd* TRE element is not revealed yet.

Our main goal is to define the *in situ* function of the described *bxd* TRE in its original genomic context. We would like to find out its real contribution to the maintenance of the active state and its possible role in the initiation step. Our results help to reveal the role of the TRE in the regulation of the distant target promoter (*Ubx*) and in the remodeling of the local chromatin state.

Keywords: *Drosophila, epigenetics, maintenance, Trithorax Response Element (TRE)*

P-122
THE EFFECT OF HISTONE MODIFICATIONS AND DNA SUPERHELICITY ON NUCLEOSOME STABILITY

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The effect of various posttranslational histone tail modifications (PTMs) on nucleosome stability was compared by exposing agarose embedded nuclei to treatments with salt or intercalator dyes, determining the remaining fraction of histones using PTM specific antibodies and laser scanning cytometry. Steep elution profiles could be measured in nuclei of all phases of the cell cycle by both salt and intercalator treatment in the case of H3K4me3 and H3K27ac marks, while the nucleosomes carrying a number of different other marks were relatively resistant, similarly to bulk histone-GFP. The difference is not due to the nucleosome-free neighboring regions in the case of the promoter/enhancer-proximal H3K4me3 and H3K27ac nucleosomes, since eluting by salt ~50% of the nucleosomes prior to intercalator elution did not change the apparent H3-GFP stability. Destabilization of the H3K4me3 marked TSS proximal nucleosomes was uniform along the genome, as revealed by chip sequencing, when doxorubicin was used as the intercalator. Nicking treatments of the nuclei did not affect the stability of nucleosomes carrying H3K4me3 or H3K27ac, while those of the second group were all destabilized. To interpret these results we suggest that the H3K4me3 and H3K27ac active marks specify dynamic nucleosomes accomodating already relaxed DNA sequences, while most other nucleosomes hold the DNA in constrained superhelices.

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P-123
NUCLEAR FUNCTION FOR THE ACTIN BINDING CYTOSKELETAL PROTEIN, MOESIN

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Moesin, the well-known cytoplasmic actin binding protein is the only member of the evolutionary conserved mammalian ERM (Ezrin-Radixin-Moesin) protein family in *Drosophila melanogaster*. ERM proteins are responsible for the organization of the cortical actin network and anchor membrane proteins to it. Our laboratory has demonstrated previously that Moesin is present in the interphase nucleus but the biological significance of this localisation remained unknown.

We are studying the exact localization and function of Moesin in the interphase nucleus both in *Drosophila* larval salivary gland cells and cultured cells. The localization experiments showed that Moesin accumulates as a ring at the nuclear envelope; it is present in the nucleoplasm, in some chromosome regions and occasionally in the nucleolus. We found that the quantity of Moesin in the nucleus increases upon heat stress, which suggests that the transport of Moesin to the nucleus is a regulated, active process.

To further analyze the chromosomal localization of Moesin, we performed immunostaining experiments on larval polytene chromosomes. These experiments revealed that Moesin binds to the euchromatic regions where it colocalizes with the active form of RNA Polimerase II. Moesin staining was especially strong in the chromosome puffs which are special euchromatic regions with extremely high transcriptional activity. We could also show that in the puffs Moe is involved in transcription rather than in the formation of transcriptionally active chromatin. Treatment of the chromosomes with a drug specifically inhibiting and disassembling the RNA Polymerase complex resulted in the detachment of both PolII and Moesin from the chromosomes, further supporting the idea that Moesin is part of the transcription complex.

Next, we performed a screen in cultured Drosophila S2R+ cells to identify the proteins that are responsible for the nuclear transport of Moesin. Our results show that the Nucleoporin98 (Nup98) and Ribonucleic acid export 1 (Rae1) proteins are involved in the nuclear export of Moesin. We confirmed these results in live animals as well. It has been shown that both proteins in interaction with each other are involved in the nuclear export of mRNA to the cytoplasm.

In summary, our results strongly argue that Moesin participates in the process of transcription and/or in mRNA export, therefore we are currently working on to distinguish between these two possible functions of nuclear Moe.

Keywords: *actin, nucleus, transcription, mRNA export, Drosophila*

P-124
THE TAF10-CONTAINING TFIID IS NECESSARY FOR THE ECDYSONE INDUCING LARVAL-PUPAL TRANSITION IN *DROSOPHILA MELANOGASTER*

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The binding of TFIID - which plays a role in the assembly of the preinitiation complex- to the promoter regions of target genes is a key step of the eukaryotic transcription initiation. TFIID consists of TBP associated proteins (TAFs) and the TATA-binding protein (TBP), which have a role in the recognition of the TATA box and help to bind components of further TFII complexes to promoter regions. In addition to TFIID complexes, TAF proteins are also indispensable in the assembly of GCN5 containing histone acetyltransferase complexes. In higher order eukaryotes, two

GCN5 containing histone acetyltransferase complexes have been identified; the SAGA (Spt-Ada-Gcn5 acetyltransferase) and the ATAC (Ada2a containing) complexes. In *Drosophila melanogaster*, TAF10 is part of the dSAGA complex, while the dATAC complex does not contain any TAF proteins.

We generated Taf10 null mutant *Drosophila melanogaster* to investigate the role of TAF10 protein containing complexes in the regulation of specific biochemical processes. The transcriptome analysis of dTAF10 and dATAC mutant animals revealed expressional changes of a few thousands of genes. Since TAF10 is a member of the TFIID complex, we hypothesized that the gene expressional changes resulting from the absence of TAF10, were caused mainly by the deficiency of TFIID function. To support this assumption, we investigated if additional TFIID subunits can bind to their target genes in TAF10 null mutants. We found that the lack of TAF10 does not influence the binding of TAF1 and TAF4, while TAF5 can bind to its target regions with a decreased affinity. In addition, in the absence of TAF10, the binding of RNA polymerase II was not changed at the polytene chromosomes.

TAF10 mutant animals died at late L3 or pupal stage, which phenotype can be partially rescued. We propose that the main reason of the lethality is that the *Halloween* genes (*spookier, phantom, disembodied, shadow, shade)*, which are involved in the molting hormone ecdysone synthesis, show reduced expression levels in the absence of TAF10. In TAF10 mutants, the transcriptional changes can be also explained by the absence of ecdysone. In summary, our results suggest that for the assembly of all TFIID the presence of TAF10 protein is not a general requirement, however, TAF10 containing TFIID complexes have important roles at specific stages of the Drosophila development.

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Keywords: *TAF10, SAGA, Halloween genes*

P-125
PROTEIN KINASE A SIGNALING IN THE DIMORPHIC FISSION YEAST *SCHIZOSACCHAROMYCES JAPONICUS*

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The dimorphic pathogenic fungi, such as the human pathogen *C.albicans* or the corn smut *U.maydis*, which cause serious agricultural damage, are big problems in our civilization. Fungal dimorphism is an ability which allows the cells to switch between unicellular (yeast) and filamentous (hyphae) forms. Predominantly, the mitogen activated protein kinase (MAPK) and protein kinase A (PKA) molecular pathways are responsible for dimorphic switch, besides sensing and integrating the extracellular signals. Both pathways are essential to infect their hosts. These pathways are also important in higher eukaryotes and mutations or alterations in these networks can result malignant tumours (cancer) in humans.

The high similarity of these pathways among eukaryotes enables us to use model organisms which can be handled easier. Such organism is the less known dimorphic yeast *Schizosaccharomyces japonicus* which is haploid, non pathogenic and its genome sequence has been already annotated.

In our department we have constructed a PKA deletion mutant of *Sch. japonicus* to determine the molecular background of dimorphic switch. We performed the RNA-Seq technique, a relatively new method, to identify the target genes of PKA in the mutant. The mutation affected the transcription level of 1896 genes which were predominantly important in carbohydrate metabolism, signaling and transmembrane transport. Finally, we compared our results with those available for other fungi.

Keywords: *RNA-Seq, protein kinase A, Schizosaccharomyces japonicus*

P-126
GENE EXPRESSION CHANGES BEHIND SYMPTOM DEVELOPMENT IN VIRUS INFECTED PLANTS

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In compatible plant-virus interaction, the virus alters the host plant metabolism in order to replicate its genome and spread cell to cell in the plant. As a result virus specific symptoms, typical for each plant-virus interactions appears. Severe *symptoms result reduction in crop yield and deteriorate quantity and quality of the harvested plant products.*

In our previous study we observed that some plant-virus interactions are able to decrease the expression of the important housekeeping genes (shut-off) which persist for several weeks.*

*Our g*enome wide analysis of gene expression changes with microarray technology testing different plant-virus interactions revealed that the intensity of symptoms correlated with the presence of shut-off. In shut-off showing plant-virus interactions (CrTMV and CymRSV infected *N.benthaminana*) serious changes in the expression level of thousands of genes, including induction of stress genes was revealed while these changes were absent in interactions not showing shut-off (TCV infected *N.benthaminana*). This was confirmed by Northern blot analysis testing the expression of central stress genes: GST, PRQ, PRP and SAR. To exclude that serious changes, appeared in shut-off presenting interaction, are consequences of necrotic processes, infection studies with a mutant virus inducing no necrosis was carried out. Northern blot analysis showed changes in the expression of important housekeeping genes (Gapdh, Rubisco) similar to that of the wild type virus proving that shut-off is not a consequence of necrosis. According to our previous *in vitro* run on transcription experiments, shut-off manifests in the nucleus*. We assumed that either changes in the chromatin methylation, or drastic changes in the expression of some master transcription factors can be responsible for widespread transcriptional shut-off of the affected genes. Methylation changes are carried out by methyltransferases and are further regulated by AGO4. We have found that the level of AGO4 and some methyltransferases were down regulated in crTMV, CymRSV infected plants while were not changed during TCV infections. As we could not see changes in the methylation status of Rubisco, search for key transcription factors or key regulator master genes has been started. We confirmed the microarray predicted changes in the expression level of a BZIP, a WRKY and a NAC like transcriptional factors and also of a DEAD box helicase. Changes in the expression level

of pheophorbideA oxigenase (PAO) as a potent master regulator enzyme, having an important role in the chlorophyll degradation and senescence were also investigated.

As a result of our experiments we have found some key regulation steps which can help in the identification of molecular processes lying behind symptom development in virus infected plants.

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*Havelda, Z. et al. (2008). Plant J **55**(2): 278-288.

Keywords: *plant virus, symptom development, shut-off*

P-127

THE CHANGES IN GENETIC REGULATION OF THE SPLEEN IN LACK OF TRANSCRIPTION FACTOR NKX2.3

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The spleen has major immunological and haematological roles like filtration of certain types of bacteria and the old and damaged blood cells. In this regard, the examination of the developmental events of the spleen is highly important. The normal ontogenesis of the spleen requires the adequate function of certain transcription factors. The major factors are Nkx3.2, Nkx2.5, Sox11, Pbx1, Tlx1 and Wt1. These transcription factors regulate in a hierarchical genetic network and the dysfunction of these factors causes the lack of the entire spleen. However, the lack of the factor Nkx2.3 the spleen is developed but it is smaller and the distribution of its cells are altered. Our aim is the determination of the role of transcription factor Nkx2.3 in the regulatory genetic network. In our experimental system we used RNA samples derived from spleens of Nkx2.3 -/- and wild-type of new-born mice. The quantitative changes in the expression of the different genes was examined with real-time PCR technique.

According to our results the loss of Nkx2.3 influences the expression of the other ontogenetic transcriptional factors. The defect in spleen development caused by the lack of Nkx2.3 can be explained by the changes in the expression pattern. In the course of our investigations we determined the reduction of the gene expression of Nkx3.2, Nkx2.5, Sox11, Pbx1, Tlx1 and Wt1 as consequence of lack of transcription factor Nkx2.3. Therefore, this transcription factor has an important regulatory role in the genetic and transcriptional network of the early spleen development.

Keywords: *spleen, development, transcription factors*

P-128
R-LOOPS AS POTENT TARGETS OF INNATE IMMUNITY

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Though considered rare transcriptional byproducts, the three-stranded nucleic acid structures consisting of an RNA:DNA hybrid and a displaced DNA strand termed R-loops have been proven to possess a Janus faced nature. They can be essential players in cellular processes (e.g. class switch recombination, mitochondrial DNA replication, bacterial plasmid replication, etc.) and at the same time they pose threats to genomic stability.

Using DNA:RNA immunoprecipitation coupled with deep sequencing (DRIP-seq), differential DNA denaturation PCR (3D-PCR), qPCR and bioinformatical analyses we reveal that these structures are potent targets of APOBEC cytidine deaminases, a family of proteins that introduce C-to-A mutational showers into attacking viral genomes. We suggest that R-loops play a central role in APOBEC-mediated innate immunity.

P-129
THE ROLE OF CHEMOSENSORY STIMULI IN THE CELLULAR STRESS RESPONSE OF THE *CAENORHABDITIS ELEGANS*

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The survival of living organisms depends on the ability to properly respond to environmental stimuli, i.e. stress. This response involves mechanisms from molecular changes to behaviours governed by the nervous system. Each cell has its own stress response, which is regulated by various master regulators, such as FOXO, HSF, or NF-κB transcription factors. FOXO translocates to the nucleus upon oxidative and metabolic stress, and induces the co-ordinated transcription of hundreds of genes. The activity of FOXO regulates the immune response and determines longevity in invertebrates. However, it is largely unknown whether neuronal signals would evoke the cellular stress response and/or integrate the individual cells' responses at the organismal level.

The nematode *Caenorhabditis elegans* is a widely used model organism in the research of aging and neuroscience. Its nervous system (comprising 302 neurons) acquires information mainly from dissolved or volatile substances of the environment. The chemosensory system of the *C. elegans* has direct effect on the development and survival of the animal. Here, we investigate the relationship between chemosensory stimuli and the activation of the FOXO orthologue DAF-16.

A DAF-16:GFP expressing strain (TJ356) was applied during the experiments. Animals have been exposed to different stressors (heat shock, oxidative stress, starvation) and chemosensory stimuli (diacetyl, benzaldehyde). The localisation of the DAF-16::GFP was assayed by fluorescent microscopy, enabling us to follow the activation of the stress response.

We showed that stressing the animals resulted in the translocation of the DAF-16 to the nucleus in a dose dependent manner. Treatment with cc. diacetyl did not affect the localisation of DAF-16 neither influenced the kinetics of stress dependent translocation. In contrast, cc. benzaldehyde treatment caused DAF-16 translocation to the nucleus. This reaction was more visible in young adult worms compared to gravid animals. Currently, we investigate the underlying mechanism of this phenomenon.

To summarise, the benzaldehyde activates the DAF-16/FOXO transcription factor through a yet unidentified mechanism. Our results raise the possibility of regulating the lifespan of nematodes through chemosensory neurons. The understanding of this phenomenon can widen our knowledge about influencing the signalling pathways determining lifespan.

P-130
GENOME WIDE INVESTIGATION OF DIFFERENTIAL EXPRESSION OF SWEET PEPPER (CAPSICUM ANNUUM) MICRO RNAS DURING FRUIT FORMATION

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Plant developmental processes are regulated by the complex interactions of mRNAs and small regulatory RNAs. The discovery of the small RNA mediated RNA interference revealed a new functions of the RNAs molecules and became one of the most intensively researched scientific fields. MicroRNAs (miRNAs) are small (21–22 nucleotide long), non-coding RNAs produced by sequential processing of genome-coded long single-stranded RNA molecules exhibiting highly specific stem-loop structures. The stem-loop structure (miRNA precursor) is recognized and cl

eaved by RNase III enzyme (DCL1)producing a short RNA duplex, from which one of the strands (mature miRNA) is incorporated into the RNA-induced silencing complex (RISC) while the complementary strand (miRNA*) strand is usually degraded. It has been demonstrated that miRNA-loaded RISC complexes are responsible for mRNA cleavage or in some cases for translational inhibition. Recent studies showed that miRNAs have extremely critical functions in almost all biological and metabolic processes includingdevelopment, hormone responses, biotic and abiotic stresses. The recent development next-generation sequencing technology allows the genome wide expression analyses of small regulatory RNAs and mRNAs in parallel. This approach has been successfully applied for model plants, but its application on important agricultural crops is still limited.

The aim of our research is to gain genome wide information about the expression of pepper miRNAs in order to get comprehensive and detailed information about the biological role of miRNAs during

fruit formation. We have defined small RNA and mRNA profiles of the pepper fruit in four phenological conditions.In the later time points the fruit was dissected for placenta, seed and pulp to analyse the tissue specific expression of miRNA. The Illumina sequencing provided more than 200 million reads. Comparative bioinformatics analyses identified 182 miRNAs,which are already known from the literature. Using different bioinformatics tools we identified 90 potentially new, pepper specific miRNAs. Comparative expression analyses of known and new miRNAs indicatethat miRNAs are central regulatory component of pepper fruit formation. The small RNA northern blot analyses for selected known and new miRNAs confirmed our sequencing results.

We have also started the analyses of potentially new pepper specific miRNAs by transient expression of their precursors. Our long term goal is to identify miRNAs, and theirs target mRNAs, which are associated with control of economically important traits.

Supported by; OTKA K109438

Keywords:*microRNA, high-throughput sequencing, RNA interference, pepper, development*

P-131
NGS AIDED DIAGNOSTICS REVEALED THE PRESENCE OF RECENTLY DESCRIBED VIRUSES IN HUNGARIAN GRAPEVINE PLANTATIONS

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Lifespan of grapevine plantations is based on their health and fitness which decreases considerably if pathogens – viruses and viroids – are present. Maintenance of economically important traits needs vegetative propagation of grape varieties. Latent infection of the propagation material – below the detection threshold of traditional testing methods – can serve as a starting point for epidemics in young plantations. Fast evolution in sequencing techniques established the possibility to obtain viral metagenomics information on the investigated plants. During virus infection small interfering RNAs having the same sequence of the infecting viruses are formed. Deep sequencing of the small RNA population extracted directly from field plants offers a unique opportunity to reveal any virus or viroid present in the sample, either expected or not*. The aim of our study was to develop a new, high throughput, molecular biology-based sensitive diagnostic method for reliable detection of virus infection in grapevine.

Small RNA libraries representing different grape plantations across Hungary were produced and sequenced using the Illumina platform. Bioinformatics methods were used to trim adapters from the resulted sequences and filter out grapevine-specific small RNAs. The remaining sequences were

analysed for the presence of known grapevine-infecting viruses by BLAST against reference genomes of known viruses or by *de novo* assembly of the sequenced small RNA reads.

NGS by Roche 454** or by smallRNA sequencing in Illumina platform*** of grapevine plantations showing decline or virus specific symptoms identified two new viruses not described in Hungary before: Grapevine Syrah virus-1 and Grapevine Pinot gris virus, respectively. We identified these two viruses which showed widespread distribution according to our small RNA data. Their presence was further proved by RT-PCR in most of our samples. Sequence analysis of the PCR-amplified fragments revealed relationships between lineages and can serve as a base for the development of new diagnostic methods in order to detect these new viruses during the production of virus-free propagation material.

Our work was supported by the Hungarian Ministry of Agriculture and the KTIA_AIK_12 -1-2013-0001 project. N. Czotter participates in the Program for Reinforcement of Scientists of the Hungarian Ministry of Agriculture and is a PhD student of the Doctoral Scool of Plant and Horticultural Sciences at the University of Pannonia.

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Keywords: *grapevine, virus, diagnostics, smallRNA-NGS*

P-132
IDENTIFICATION OF HOST GENES FOR VIRAL SYMPTOM DEVELOPMENT BY COMPARING *ARABIDOPSIS* ECOTYPES

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The main antiviral system of plants is based on the small interfering RNA (siRNA) mediated post-transcriptional gene silencing (PTGS). The genomic RNA of positive single stranded RNA (+ssRNA) viruses functions as a genuine mRNA in the host cyt oplasm. The double stranded RNA molecules produced by viral replication trigger PTGS. During this process, siRNAs are generated that results in the degradation of the viral RNA. As a counter-measure, viruses utilise silencing supressor molecules. Besides their primary viral target sequences, siRNAs can hybridise to complementary host mRNAs causing “off target” effects. This interference with the host gene expression highly depends on the degree of sequence complementarity, therefore natural selection for less complementary host sequences can attenuate the severity of symptoms. Furthermore, the recently discovered family of viral induced, host derived vasiRNAs may also contribute to the viral symptoms developed in plants.

Our goal is to investigate the molecular background of symptom development and to identify the host factors for the attenuation of symptoms in a model plant (*Arabidopsis thaliana*) using advanced high throughput sequencing technologies. There are genetically distinct geographic variants of

Arabidopsis called ecotypes in which genetic heterogenity is greatly reduced as a result of inbreeding. This makes them particularly useful for comparative genetic analysis and genetic mapping. There are hundreds of Arabidopsis ecotypes available from different public sources and many of them also have their genomes resequenced. According to previous studies and our preliminary results, the severity of symptoms developed by different ecotypes upon infection by the same virus can vary greatly.

To find host factors causing this phenomenon we started to infect 92 ecotypes with four +ssRNA viruses (*Ribgrass Mosaic Virus*, *Turnip Crinkle Virus*, *Turnip Mosaic Virus*, *Turnip Yellow Mosaic Virus*) belonging to different families. Five, four-weeks-old individual plants of every ecotypes were treated either with virus or mock. The symptoms were scored after four weeks. We have already found promising ecotypes showing marked differences in their responses to viral infection. Having a suitable ecotype pair selected we will purify total RNA from plants of each ecotypes. Following fractionation of the purified RNA specimens by size and type, we will prepare libraries for whole transcriptome, sRNA-ome and degradome (sRNA-mediated degradation products of mRNAs) sequencing. By comparing the differential gene expression profiles of virus- and mock-treated ecotypes we will be able to identify the host genes "off targeted" by virus derived siRNA. We also intend to clarify the role of vasiRNAs in the development of viral symptoms.

If we manage to understand the processes during symptom development in a model plant, we would also be able to attenuate the symptoms in economically important plants to reduce crop loss.

Keywords: *viruses, siRNA, silencing, Arabidopsis thaliana, high throughput sequencing*

P-133
POLYCISTRONIC ARTIFICIAL MIRNAS MEDIATED RESISTANCE TO WHEAT DWARF VIRUS IN BARLEY IS HIGHLY EFFICIENT AT LOW TEMPERATURE

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The infection of *Wheat dwarf virus* (WDV) strains, on barley results in dwarf disease imposing severe economic losses on crop production. Since the natural resistance resources are limited it is imperious to elaborate a biotechnological approach providing effective and safe immunity to wide range of WDV strains. Since vector insect (*Psammotettix alienus)* mediated WDV infection occurs during cool periods in the nature it is inevitable to elaborate a technology which effective at lower temperature.

The micro-RNA (miRNA) pathway is an important post transcriptional gene regulatory mechanism involved in developmental processes. MiRNAs are ~21 nucleotide long non-coding RNA molecules which are generated by sequential processing of longer precursor molecules. The plant miRNA loci encode capped and polyadenylated transcripts (pri-miRNAs), which are processed to pre-miRNAs in the nucleus by the Dicer-like enzyme DCL1. The genetically defined pre-miRNAs possess a self-complementary fold- back structure that is processed to a double stranded intermediate comprising the miRNA and the complementary miRNA* strands, respectively. The miRNA-miRNA* duplex is unwound and the miRNA is subsequently loaded in the RISC (RNA-induced Silencing Complex)

and guide the RISC to degrade any RNA possessing sequence complementary to the miRNA. It can be specify RNA targets by remodeling the miRNA precursors rendering them to produce artificial miRNA (amiRNA).

We utilized a barley miRNA precursor backbone (hvu-miR171) to design artificial miRNAs targeting different conservative sequence elements of the WDV strains. The potential amiRNAs sequences were selected to minimize the off-target effects in barley and tested in a transient sensor system to select the most effective constructs at low temperature. Based on the gained data a polycistronic amiRNA precursor construct was built (VirusBuster171) expressing three amiRNAs simultaneously. The construct was transformed into barley under the control of a constitutive promoter (maize *Ubi1*). The transgenic lines were kept at 12-15 ^{0}C to mimic the autumn and spring conditions when major WDV infection takes place. We were able to establish stable transgenic lines developing no symptoms and possessing phenotype similar to the mock inoculated plants.

Our study demonstrate that amiRNA technology can be an efficient tool to introduce highly efficient resistance in barley against a DNA virus belonging to the Geminiviridae family and this resistance is effective at low temperature when the natural insect vector mediates the infection process.

Our work supported by OTKA (81937K)

Keywords: *artificial miRNA, WDV, barley, low temperature*

P-134
THE ROLE OF DCL2 AND DCL4 IN PLANT-VIRUS INTERACTION

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RNA silencing, is a well conserved pathway in eukaryotes. In plants it is the primary antiviral mechanism. RNA silencing is initiated by the cleavage of a DICER-LIKE (DCL) protein, on a heavily structured RNAs or double stranded RNAs (dsRNA) processesing dsRNAs into small interfering RNAs (siRNA). These siRNAs guide the RNA Induced Silencing Complex (RISC) to target RNA molecules having complementary sequences of the loaded siRNAs, resulting in a drastic down regulation of the target RNAs. DCL4 and DCL2 have been characterized to be the most important anti-viral DCLs acting hierarchically in infected Arabidopsis plants, by processing the viral RNA exclusively to 21 and 22nt siRNA respectively. Moreover DCL4 has been described to be the primary anti viral DCL. Cymbidium ringspot virus (CymRSV) is a single, positive stranded RNA virus, belonging to the Tombusvirus genus. The open reading frame 5 (ORF5) of the virus encodes a suppressor of RNA silencing called P19. In this study we used a version of CymRSV in which the p19 was inactivated resulting in a suppressor deficient virus, referred to as Cym19S. To assess the importance of DCL2 and DCL4 in antiviral response transgenic *Nicotiana benthamiana* plants in which DCL2, DCL4 or both have been knocked down by hairpin constructs were infected by CymRSV or Cym19S. Cym19S viral RNA accumulated significantly higher in DCL2&4 but not in DCL2 or DCL4 single knockdowns. DCL2 and DCL4 knockdown plants showed recovery from viral symptoms similarly to wt plants, however if both DCL2 and DCL4 knocked down simultaneously the recovery of infected plants is abolished. These findings indicate that both DCL2 and DCL4 are

similarly important in the antiviral response of *N. benthamina* suggesting that the role of these two DCLs may vary in different plant-virus combinations.

P-135
USE OF THE CRISPR/CAS9 SYSTEM FOR GENOME EDITING IN TOBACCO

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Targeted modification of plant genomes can facilitate basic research as well as plant biotechnology. Using sequence-specific nucleases genes can be modified in a precise and predictable manner, helping the understanding of their *in planta* function, as well as has the potential to accelerate plant breeding. Until recently, site-specific cleavage of genomes has relied on engineered meganucleases and artificial bipartite enzymes composed of a DNA-binding domain linked to an endonuclease domain (zinc finger nucleases (ZFNs) and transcription activator like effector nucleases (TALENs)). A major drawback of all of these systems is that, they require the design and construction of large modular proteins which is both laborious and expensive.

The latest development in genome editing is the emergence of RNA guided nucleases. The most widely used of these systems is based on the type II clustered regularly interspaced short palindromic repeat (CRISPR) loci and the CRIPR associated 9 (Cas9) endonuclease from *Streptococcus pyogenes*. CRISPR/Cas9 is a representative of the recently described RNA guided prokaryotic adaptive immune systems, which can cleave invading nucleic acids (plasmids and phages) in a sequence specific manner. In early 2013, the CRISPR/Cas9 system has been re-purposed for the use of an engineered single guide RNA (sgRNA) to cleave any 20 nt DNA sequence that lie 5′ to a NGG motif (PAM, protospacer adjacent motif). Due to its simplicity, the modified CRISPR/Cas9 system has since been successfully used for genome editing in many organisms, including a number of plant species.

Here we report the use of the CRISPR/Cas9 system in the virological model plant *Nicotiana benthamiana* to study the function of the Argonaute 2 (AGO2) gene. AGO2 has recently been shown to play major roles in anti-pathogenic defense pathways and genotoxic stress responses. To target the AGO2 gene, we decided to generate a single component system to express the Cas9 protein and the appropriate sgRNAs from the same plasmid. With the use of such system, we hope to achieve higher gene modification efficiency compared to multi plasmid systems, where efficient co-delivery of the various components could be problematic. The pK7WG2D binary plasmid vector was used to co-express the plant codon optimized spCas9 gene and sgRNAs specific for the coding region of AGO2. Following agroinfiltration into *N. benthamiana* leaves, the targeted regions of the AGO2 gene were PCR amplified and sequenced. With two of the tested sgRNAs, we detected Cas9 introduced mutations at the expected positions in the AGO2 gene, demonstrating that the CRISPR/Cas9 system is suitable to specifically target genes in *N. benthamiana*. We also created "nickase" versions of the Cas9 protein (one of the two nuclease domains was mutagenized: D10A and H840A), which could be used to increase the efficiency of homology directed gene replacement. To circumvent the potential toxicity issues associated with constitutive expression of the Cas9 protein, β-estradiol

inducible expression plasmids were also constructed. In summary, the CRISPR/Cas9 tool-box created by us will be valuable for future work to perform targeted gene modifications in *N. benthamiana*.

This work was supported by the Hungarian Scientific Research Fund (NK105850).

P-136
IDENTIFICATION AND EXAMINATION OF RABBIT MICRORNA-S IN DIFFERENT DEVELOPMENTAL STAGES OF EMBRYOS AND STEM CELLS

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MicroRNAs (miRNA) are ~22nt long, non-coding RNAs, which have important regulatory role in several fundamental biological processes by posttranscriptional targeting the 3′ UTR of mRNAs causing translational repression or the degradation of the mRNA. They are very important in maintenance of the pluripotency and promoting cell differentiation.

Our aim is to establish a real embryonic stem cell line from rabbit, to obtain germline chimaeras. With this goal we started to investigate which miRNAs can be found in different developmental stages of rabbit embryos and in stem-like cells. It would be important to understand their role in embryo development and to know which kind of modifications would be necessary to maintain the pluripotency of the stem-like cells.

Because of no published rabbit miRNA sequences was available at the beginning of our study, samples derived from different stages of rabbit embryos and stem cells were sequenced by using SOLID platform. The results were checked for new rabbit miRNA sequences according to known human, mouse and bovine miRNAs.

1693 new rabbit sequences were identified. From these sequences three clusters were chosen to further analysis, known to be stem-cell specific in mouse and human, and some extra miRNA known to have important role in early differentiation. We compared two different methods to RNA isolation based on RNA purity and integrity. We tested the hypothesis whether the read numbers getting by SOLiD sequencing are suitable for predicting the gene expression level.

We could identify where these miRNAs are localised in the rabbit genome, and clarified the structures of these clusters. First we measured the expression of selected miRNAs in 6 rabbit tissues or embryo derived samples by real-time PCR, then in 6 day embryo, epiblast, hypoblast and trophoblast cells. We found that the ocu-miR-290 and ocu-miR-302 clusters are stem cell specific, but no specificity was found regarding the ocu-miR-17-92 cluster. Not surprisingly, PGCs are really similar to ESCs, while iPSCs express the pluripotency markers at a very high level, what clearly shows the success of the reprogramming.

At last, we examined the expression of the miR-191 and miR-302 miRNAs in 6-day embryo by in situ hybridization. We found that the miR-302 show higher expression in embryoblast than in trophoblast, which is consistent with our real-time PCR data.

Keywords: *microRNA, regulatory RNAs, rabbit, stem cell*

P-137
SMALL RNA REGULATION OF PLANT NB-LRR DEFENSE GENE FAMILY

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During their whole life plants have to cope with the attack of broad range of pathogens with different infection strategies. The first line of defense against non-viral pathogens is provided by the pattern recognition receptors (PRRs) that recognize conserved pathogen-associated molecular patterns (PAMPs) and activate PAMP-triggered innate immunity (PTI). However, PTI can be suppressed by the pathogen, usually through secreted proteins called effectors. Since this is an evolutionary arms race - as a second line of defense - plants evolved resistance genes (R), which code intracellular receptor proteins. These proteins recognise the given pathogen effector protein and this recognition initiate effector triggered immunity (ETI). Most resistance proteins has a specific structure, they contain nucleotide-binding and leucin-rich repeat domain (NB-LRR). Most plant genomes contain a large number of NB-LRR genes so continuous expression of these genes in absence of infection could mean a very large fitness cost. Unlike animals, in plants viral RNA or DNA is recognised not by immune receptor but RNA silencing (RNAi) system and after the recognition this system can target and degrade the invading nucleic acids. During this process double stranded RNA is diced by DCL enzymes and viral small RNA molecules (21-24 nt vsiRNAs) are generated. These vsiRNAs can incorporate into AGO complexes and can target complementer viral sequences for degradation. In plants small non-coding RNA molecules can also derive from different endogenous RNA silencing pathways. They have two groups depending on their biogenesis: siRNAs and miRNAs. These endogenous small RNAs have an important role in regulation of plant development, physiological processes and stress tolerance.

Based on the recent studies innate immunity and RNA silencing are closely related. It is turned out that NB-LRR genes are silenced by mir482 family in different plant species. In case of virus infection the level of mir482 miRNAs is decreased in inoculated leaves. Since mir482 miRNAs cannot silence NB-LRR resistance genes, their mRNAs are translated and can protect plant from the invaders. One of the possible explanation of this phenomenon that silencing suppressor proteins of the virus can bind mir482 miRNAs but this theory was not proved experimentally.

To test the hypothesis we infected tomato plants with wild type tomato bushy stunt virus (TBSV) and with silencing suppressor deficient TBSV mutant (TBSV-P19stop). Since infection with TBSV-P19stop also decreased the level of mir482 miRNAs in inoculated leaf, we concluded silencing suppressor protein of the virus was not responsible for the mir482 level reduction. We also analysed one tomato mir482 miRNA - mir482a - expression pattern in different tomato organs. The highest expression was detected in shoot tip and likewise the leaf miRNA level also reduced in case of virus infection. Therefore regulating function of mir482a appears not only in inoculated leaf right after infection but in other organs, a few weeks later. Salicylic acid, jasmonic acid and ethylene mediated pathways play central role in plant immune system. For further analysis of mir482a role in plant pathogen interaction tomato plants were treated with these hormones and mir482a level was analysed with northern blot. Our results contribute the understanding of the mir482 family role in plant immune response.

Acknowledgement: *This work was supported by OTKA (grant number:K-106170)*

Keywords: *NB-LRR resistance gene, miRNA, RNA silencing,*

P-138
ENDODERM DERIVED SONIC HEDGEHOG REGULATE THE EXTRACELLULAR MATRIX PATTERNING DURING ENTERIC NERVOUS SYSTEM DEVELOPMENT

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The enteric nervous system (ENS) is a large neural network in the wall of the intestine which is colonized by a small number of enteric neural crest cells (ENCCs). These multipotent stem cells originate from vagal level of neural tube and migrate rostrocaudally along the entire length of the gastrointestinal tract to differentiate as neurons and glial cells that form the ganglionated ENS. Incomplete migration of ENCCs leads to Hirschsprung disease, a congenital disorder characterized by the absence of enteric ganglia along variable lengths of the distal intestine. Inductive interactions between gut epithelium and mesenchyme have been suggested to regulate the migration and differentiation of ENCCs. However, little is known about the function of epithelial derived factors, such as Sonic hedgehog (Shh), how they influence the intestinal extracellular matrix expression during ENS development.

Hindgut from 6 day old chicken embryo was cultured in the presence of Shh protein or after injection of Shh overexpressing replication competent retrovirus (RCAS)-virus. In presence of Shh the hindgut is aganglionic, while in the presence of Shh inhibitor (cyclopamine) large and ectopic ganglia developed. Shh treatment strongly induced the expression of chondroitin sulphate proteoglycans (CSPGs) such as versican and collagen type IX, whereas cyclopamine reduced the expression pattern of these inhibitory matrix molecules. These results indicate that versican and collagen IX is a candidate for mediating the effects of Shh on ENCC migration. Shh also inhibited the proliferation and promoted the differentiation of ENCCs. Abnormalities of NCC migration and extracellular pattern formation are characteristic of two human intestinal disorders, Hirschsprung disease and intestinal neuronal dysplasia. Our results support an essential role for epithelial-mesenchymal interactions in these aspects of ENS development.

P-139
ARE ADENOSINE A3 RECEPTORS REQUIRED FOR PROPER PHAGOCYTOSIS OF APOPTOTIC CELLS?

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Clearance of apoptotic cells by phagocytosis (efferocytosis) is a fundamental biological process that is carried out by macrophages and other professional phagocytes. The recognition and subsequent engulfment can be broken down into three steps (find me, eat me, digest me). These steps can be characterized by the appearance and interaction of specific molecules that orchestrate the course of efferocytosis.

Previous *in vitro* studies have revealed that purinergic adenosine A3 receptors are involved in the efficient chemotactic movement of phagocytes. We set up an *in vivo* experimental system to test the effect of the loss of the receptor in the *in vivo* clearance of apoptotic thymocytes. To induce apoptosis in the thymus we administered intraperitoneally dexamethasone to wild type and adenosine A3 knock out mice. Clearance of apoptotic cells in the thymus has been tested with the help of flow cytometry and immunohistological techniques.

Our results demonstrate that the loss of the A3 receptor doesn't lead to significant changes of the examined parameters related to the efferocytosis. Our data indicate that A3 receptors don't have critical role in the apoptotic cell clearance.

P-140
GMCSF INDUCED EPITHELIAL-TO-MACROPHAGE TRANSITION OF MESENTERIC MESOTHELIAL CELLS: AN *IN VITRO* STUDY

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Intraperitoneal injection of Freund's adjuvant induces acute peritonitis. This inflammation process caused remarkable changes in the morphology of the mesenteric mesothelial cell: flat squamous epithelial cells become cuboidal shaped, they lose their apical-basolateral polarity, many of them tend to detach from the basement membrane, and the cell-cell interactions are lost indicating that mesothelial cells undergo a biological process known epithelial-to-mesenchymal transition (EMT). Our previous results showed that concomitant with these morphological changes macrophage markers' (ED1, OX43 and CD68) started to express in these cells. We also found that in the peritoneal wash the number of macrophages is significantly increased. The question arose whether mesothelial

cells detaching from the mesentery can contribute to the significantly increased pool of peritoneal macrophages.

At the first step we used different cell culture media to provide optimal microenviroment for primary mesenteric culture. Our main goal was to induce epithelial-to-macrophage differentiation *in vitro* circumstances. The transforming growth factor beta (TGFβ) is known to induce EMT. Our previous data showed during peritonitis TGFβ is secreted into the peritoneal cavity indicating that TGFβ plays important regulatory role in our system as well. We treated our primary mesenteric culture with granulocyte-macrophage-colony-stimulating factor (GMCSF), TGFβ and a mixture of GMCSF and TGFβ.

TGFβ contained culture medium induced fibroblastic transformation of the mesothelial cells. GMCSF alone and in combination with TGFβ caused morphological changes that were similar to Freund's adjuvant treatment. By the time of the treatment we could detect increased expression of ED1 as well. Our immunocytochemical results proved that under these *in vitro* conditions mesothelial cells can undergo EMT and can differentiate into macrophages.

By these experiments we were able to provide a good *in vito* model to study the steps and regulation of epithelial-to macrophage transition.

Keywords: *EMT, macrophages, mesothelial cells, primary mesenteric culture*

P-141
INCREASED SAM POOL AND PA/ETHYLENE RATIOS IN TRANSGENIC *NICOTIANA BENTHAMIANA* PLANTS RESULTED IN MARKED CHANGES IN SALT TOLERANCE, PROTEIN CONTENT AND BIOMASS PRODUCTION

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The plant hormone ethylene is an essential signaling molecule involved in many plant processes including: germination, flower development, fruit ripening and responses to many environmental stimuli. Convincing evidence suggests that besides ethylene, polyamines (PAs) are also involved in the regulation of these processes. Although their biosynthetic pathways are interrelated, the physiological functions of PAs and ethylene are distinct and at times antagonistic, particularly during leaf and flower senescence. This provides an effective means to understand the mechanism by which the balance between the two can be established for manipulating the above processes.

The main hub of this interrelation is S-adenosylmethionine (SAM) being the common precursor of both PA and ethylene synthesis. Our present work focuses on the understanding how the differently altered PA-to-ethylene ratios affect plant development, abiotic stress tolerance and senescence. Two types of transgenic *Nicotiana benthamiana* plants were produced. One set of transgenic plants carried a heterologous *SAM-synthase* gene, while the other plant set were transformed with a *SAM-decarboxylase* gene, both were isolated from *Fragaria vesca* and were driven by a constitutive CaMV35S promoter. By overexpression of *SAM-synthase*, we were intending to increase the SAM-pool to look at the relative hierarchy among the most abundant pathways competing for SAM

(**Figure 1**). Furthermore, we were also intending to alter PA/ethylene ratios by overexpressing *SAM-decarboxylase* that holds the potential to re-allocate larger proportion of SAM through the polyamine pathway at the expense of ethylene biosynthesis. This may allow us to see how plant development, abiotic stress tolerance and senescence are influenced in transgenic plants.

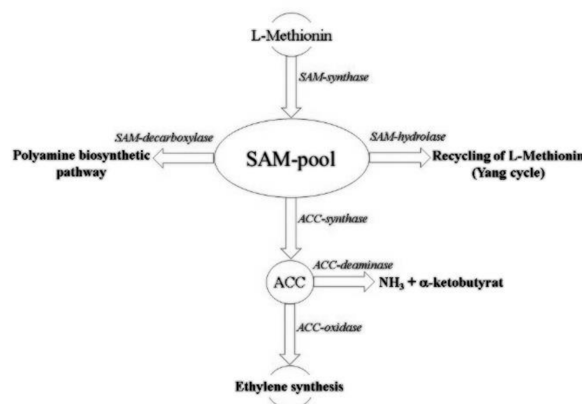

***Figure 1**. Metabolic pathways of SAM-flux*

To draw proper inferences, changes in the relative expression of transgenes and genes encoding the most important enzymes involved in PA and ethylene metabolism were detected, complemented with determinations of PA, ethylene, chlorophyll and protein contents. Dry matter accumulation, chlorophyll contents and the integrity of biological membranes were also examined in transgenic and control plants that were subjected to salt, osmotic and oxidative stress. Results of the above experiments together with the involved conclusions will be given in the present paper.

Keywords: *ethylene, polyamines, SAM*

P-142
BIASED OR PARTIAL AGONISM? QUANTIFYING FUNCTIONAL SELECTIVITY AT THE ADRENERGIC A_{2C} RECEPTOR

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Although G protein-coupled receptors (GPCRs) are traditionally categorized as Gs-, Gq-, or Gi/o-coupled, their signaling is regulated by multiple mechanisms. GPCRs can couple to several effector pathways, having the capacity to interact not only with more than one G protein subtype but also with alternative signaling or effector proteins such as arrestins. Moreover, GPCR ligands can have different efficacies for activating these signaling pathways, a characteristic referred to as biased agonism or functional selectivity.

Guided by drug discovery efforts made in the field of biased agonism, in this work we evaluated the differences in the ability of agonists acting at the α_{2C}-AR to modulate cAMP accumulation, cytoplasmic Ca^{2+}-release, β-arrestin recruitment and receptor internalization. A detailed comparative pharmacological characterization of G protein-dependent and -independent signaling pathways was carried out using structurally different agonists (norepinephrine, phenylephrine, brimonidine, BHT-920, oxymetazoline, clonidine, moxonidine, guanabenz).

Our results showed that a series of agonists displayed pathway selective activities, causing different activation of G-protein-dependent and G-protein-independent signaling pathways compared with the endogenous agonist norepinephrine. Applying the relative activity method (RA) we were able to derive numerical values for the degree of signaling bias. Determination of bias factors allowed us to perform a comprehensive analysis of ligand bias and to identify several weakly biased agonists towards β-arrestin recruitment or receptor internalization. Our results also revealed that agonists with only subtle differences in structure can still display functional selectivity, as seen for norepinephrine and phenylephrine. Phenylephrine was 11.3 and 16.2 times more active than norepinephrine to induce β-arrestin recruitment than to inhibit cAMP accumulation or to stimulate Ca^{2+}-release.

In summary, beside the fact that the observed differences in signal transduction broaden our understanding on α_{2C}-AR signaling, studying all this stages of receptor activation may help link specific signaling properties to desired therapeutic activities and/or unwanted effects, ultimately paving the way for better/safer drugs.

P-143
THE REGULATORY CONNECTION BETWEEN NRF2 TRANSCRIPTION FACTOR AND AMPK ENERGY SENSOR

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NRF2 (NF-E2-related factor 2, NFE2L2) transcription factor is a master regulator of oxidative and xenobiotic stress response and it is also important in cell homeostasis maintenance. Besides regulating phase I and phase II drug metabolism genes, it can also influence cellular responses to DNA damage, intermediary metabolism and mitochondrial function.

Previous studies have shown that there is a link between autophagy dependent self-eating and NRF2. Under normal conditions NRF2 is bound to its repressor Keap1 (Kelch-like ECH associating protein 1), in the cytosol to facilitate its degradation. When the level of ROS and electrophiles increases Keap1 gets inactivated by oxidation and NRF2 becomes free. The active NRF2 translocates into the nucleus and promotes transcriptions of its target genes.

Our study is based on previous experiments by ELTE, Department of Genetics that confirm a negative regulatory loop between NRF2 and AMPK (*Caenorhabditis elegans* ortholog skn-1 and aak-2, respectively). AMPK (AMP-activated protein kinase) is a key cellular energy sensor and highly conserved in eukaryotic organisms. AMPK is activated by nutrient deprivation caused increased intracellular AMP levels. This kinase can inhibit cell proliferation and biosynthetic processes meanwhile autophagy gets activated. Collaborating with ELTE, Department of Genetics we investigate the same regulatory loop in *Homo sapiens.* First we silenced Nrf2 in HEK293 cells (human embryonic kidney). Then oxidative stress was induced by TBHP (*tert*-Butyl hydroperoxide) or starvation was mimicked with 2DG (2-Deoxy-D-glucose). The expression of AMPK (AMPKα1,

ULK1 Ser 555) autophagy (LC3) and apoptotic markers (pro-Casp3, PARP, CHOP) were followed by Western blot. We verified the efficiency of silencing with quantitative PCR.

According to our results we can conclude that NRF2 negatively regulates autophagy through AMPK inhibition with respect to either oxidative stress or starvation in human cell line as well as their orthologs in *C. elegans*.

Keywords: *NRF2, AMPK, autophagy, oxidative stress*

P-144

THE PLASMA MEMBRANE CA^{2+}ATPASE CAN REGULATE CA^{2+} SIGNALING BY CONTROLLING PHOSPHATIDYLINOSITOL 4,5-BISPHOSPHATE LEVEL

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Plasma membrane Ca^{2+} ATPases (PMCAs) eject Ca^{2+} ions from the cytosol to the extracellular space thus controlling Ca^{2+} signaling of cells. The function of PMCA is regulated by several factors such as Ca^{2+}-calmodulin, phosphorylation by protein kinases and by the signaling phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP_2). Molecular dynamic simulations revealed four PIP_2 binding pockets at the interface between the cytoplasmic part of the PMCA and the plasma membrane. Using a fluorescently labeled PIP_2 sensor we found that by binding PIP_2, PMCAs can create a PMCA-PIP_2 pool that protect PIP_2 molecules in the plasma membrane from depletion by phospholipase C and decrease receptor-mediated inositol 1,4,5-triphosphate (IP_3) formation. By forming a PIP_2 pool, PMCA can control the level of PIP_2 in the plasma membrane that might affect various cellular functions regulated by PIP_2.

The work was supported by grants from OTKA (CK 80283, K 101064) and TRANSRAT KMR_12-1-2012-0112.

P-145
REVEALING THE FUNCTIONS OF PROTEIN PHOSPHATASE Z1 IN *CANDIDA ALBICANS* WITH PROTEOMIC METHODS

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Protein phosphatase Z is a novel type of fungus specific Ser/Thr protein phosphatases. Recently we have identified its ortholog in *Candida albicans* and disrupted the CaPPZ1 gene in order to reveal its functions. The *cappz1* deletion mutant was tolerant to toxic cations, it was sensitive to salts and cell wall damage agents as well as to oxidative stress conditions. We suggested that the gene product was implicated the cation homeostasis, cell wall integrity, stress response and morphology of the opportunistic pathogen, but the molecular mechanisms underlying the phenotypes of the mutant remained enigmatic. To identify potential CaPPZ1 targets we adopted proteomic methods for the comparison of the *cappz1* deletion mutant and the genetically matching QMY23 control strain. Here we report on the preliminary results of this approach. Proteins extracted from the two strains were separated by two-dimensional gel electrophoresis. Gels were stained either for total protein with RuBPS or for phosphoproteins with Pro-Q Diamond. The changes in the protein and/or phosphorylation levels were analyzed with the Delta2D software. Selected protein spots were excised from the gels, were digested with trypsin and the resulting peptides were identified by LC-MS/MS mass spectrometry. We found 14 proteins that exhibited a large (more than 2-fold) and significant change in their expression/degradation upon the deletion of the phosphatase. We also identified 25 phosphoproteins in which the level of phosphorylation was altered significantly in the mutant. In 5 cases both the protein content and the level of phosphorylation was affected by the mutation. According to their predicted physiological functions the proteins pinpointed in our study are related to protein synthesis, oxidative stress response, regulation of morphology and metabolism. These findings are in broad agreement with the mutant phenotypes and suggest some novel functions for the CaPPZ1 phosphatase in the regulation of gene expression and/or proteolysis.

This work was supported by the OTKA grant K108989.

Keywords: *protein phosphorylation, protein phosphatase Z, C. albicans, phosphoproteomics*

P-146
SEMI-RATIONAL INHIBITOR DESIGN BASED ON THE STRUCTURE OF THE MITOGEN ACTIVATED PROTEIN KINASE

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Mitogen activated protein kinases (MAPKs) are part of the multi-tiered kinase cascade that plays a key role in signaling transduction networks. These kinases regulate many processes in cells such as division, apoptosis, and differentiation. MAPKs are activated by dedicated Mitogen activated protein kinase kinases (MAP2Ks) that interact with substrate proteins through linear binding motifs. These docking motives bind to the MAPKs docking grooves, which determine the specificity of the interaction between MAPKs and their partner molecules.

Our research focused on designing small molecule inhibitors for the docking grooves of the ERK2, JNK1 and p38 MAP kinases based on well-known 3D crystal structures. With the help of the Organocatalysis Research Group at the MTA TTK research center, we were able to produce novel decalin derivatives which can be specific inhibitors of ERK2, JNK1 and p38. These novel molecules may bind to docking grooves of MAPKs based on in-silico docking.

To enhance the binding of inhibitor candidates, we would like to target this docking groove with different Michael-acceptor groups. We designed several peptides (based on former binding peptides [Figure 1.]) which contain a Michael-acceptor unnatural amino acid. The Michael-acceptor residue may react in Michael-addition reaction with cysteine in docking grooves if it's in a sterically correct position. The peptide inhibitors that had a successful Michael-addition bind irreversibly to the MAPKs' docking grooves, decreasing their activity.

Our plans include semi-high-throughput screening of inhibitors which target the docking groove with Michael-addition strategy. Our goal is to develop inhibitors that are specific to the three MAPKs, by binding irreversibly to their targets' docking grooves.

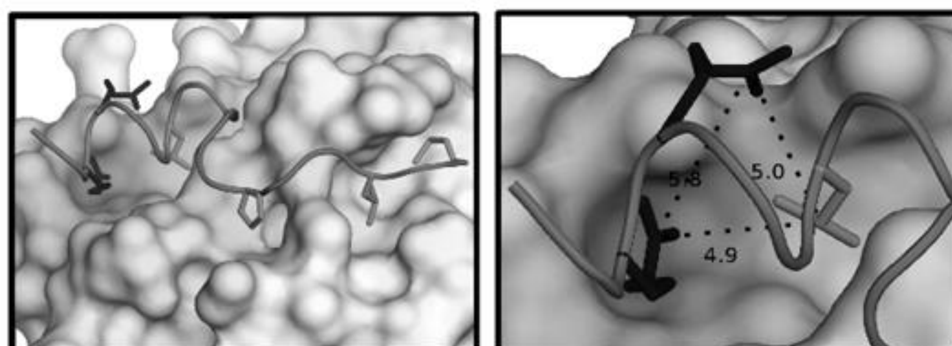

Figure 1: *ERK2-pepMNK1complex*

Keywords: *docking groove, decalin derivatives, Michael-acceptor, MAP kinase*

P-147
THE ROLE OF HSF-1 TRANSCRIPTION FACTOR IN THE FORMATION OF HEAT SHOCK ACTIVATED ER STRESS

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The proteostasis of a cell is influenced by a number of environmental and physiological effects. The heat shock response (HSR) is a fundamental mechanism, which responses to temperatures higher than optimal; heat shock proteins (HSP) are synthesized for repairing the misfolded/unfolded protein accumulation. HSF-1 (heat shock transcription factor 1) is a transcription factor that plays major role in the regulation of HSR and thus in the protection of cells and organisms from heat, inflammation, oxidative stress etc. Under normal circumstances the HSF-1 is in its inactive, monomer form maintained by several chaperons and intramolecular interactions. After heat shock HSF-1 is transformed into its active, homotrimer form. Entering the nucleus it binds to promoters containing specific heat shock elements (HSE), enhancing the transcription of various genes.

According to *in silico* researches by Department of Genetics, ELTE on *Caenorhabditis elegans* and *Homo sapiens* there are numerous genes coding ER stress response proteins with HSF-1 binding sites in their promoter regions. They successfully identified the direct regulatory relationship between the HSF-1 and the ER localized BiP (Grp78) ortholog *C. elegans* protein, HSP-4.

Our goal was the experimental confirmation of the *in silico* results in human embryonic kidney, HEK 293 cells.

First we optimized the temperature for heat shock treatment. According to some previous publications mild increase in temperature (39-40°C) induces ER stress, while higher temperatures (43°C) inhibit it. We introduced three different temperatures: 40, 42 and 43°C, the cells were heat stressed for 40 minutes, then we examined the appearance of ER stress markers after 0, 3, 6, 12 and 24 hours recovery.

We could detect ER stress under all three temperatures: elevated BiP, p-PERK expression and XBP1 mRNA splicing were demonstrated. For the confirmation of HSF-1's direct function we repeated the experiments with HSF-1 specific siRNA silenced cells. After gene silencing the expression of BiP was not reduced and XBP1 slicing also could be detected.

Our current results imply that in human cells the HSF-1 transcription factor does not mean sole and/or direct connection between the activation of HSR and ER stress response. However to widen our knowledge about the subject further experiments are planned.

Keywords: *unfolded protein response, heat shock, heat shock factor*

P-148
THE ROLE OF MYOSIN PHOSPHATASE IN REGULATING THE SNAP25 PROTEIN

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Synaptic proteins are regulated by reversible phosphorylation and dephosphorylation. The phosphorylation state of these proteins are determined by protein kinases, and phosphatases. The SNARE (Soluble NSF Attachment Protein Receptor) complex consists of three proteins: syntaxin, synaptobrevin, and the SNAP25 proteins and plays a crucial role in the docking of synaptic vesicles to the presynaptic membrane in neurons. SNAP25 is responsible for the connection between the synaptobrevin (vesicle bound), and syntaxin (plasma membrane bound) proteins. Although several regulatory Ser/Thr phosphorylation sites of SNAP25 are known to be regulated by protein kinases such as protein kinase A and C have been characterized but we have little information about protein phosphatases responsible for dephosphorylation. Our previous studies showed that a Ser/Thr specific protein phosphatase (PP1) called myosin phosphatase (PP1M) is the major PP1 enzyme at the presynaptic densities. This holoenzyme consists of a myosin binding regulatory (MYPT1) and the PP1c catalytic subunits. The detailed study of the PP1M interacting proteins in synaptosomes revealed that MYPT1 interacts with the SNARE complex through syntaxin as well as SNAP25. Our goal was to investigate the regulation of SNAP25 by PP1M and Rho activated kinase (ROK) holoenzyme, which is known to act on the same phosphorylation sites as PP1M. We successfully identified the subunits of PP1M and SNAP25 in B50 *neuroblastoma* and differentiated PC12 rat *pheochromocytoma* cell lines. The protein-protein interaction of MYPT, PP1c and SNAP25 was determined by immunoprecipitation, immunofluorescence and surface plasmon resonance techniques. The silencing of MYPT decreased the total protein phosphatase activity and the cell viability by 30% and 25%, respectively. Using *in vitro* kinase assay, mass spectrometry analysis and siRNA knockdown of MYPT in B50 cells, we detected two potential PP1M/ROK regulation sites of SNAP25, namely Thr138 and Ser187. By site-directed mutagenesis we created T138A and S187A mutant SNAP25 plasmids, and these proteins were expressed and purified and were used in *in vitro* kinase assay. Only the wild type SNAP25 but not the alanine mutant forms was phosphorylated by ROK. The ROK-phosphorylated SNAP25 was applied together with PP1M and the phosphorylation level of both SNAP25 Ser187 and Thr138 was found to be decreased. Using freeze-thaw permeabilization we introduced Flag-KEPI PP1M inhibitor proteins into murine cortical synaptosomes. The SNAP25 phosphorylation on both residues as well as the rate of the neurotransmitter release were decreased. Our data suggest that ROK and PP1M may play a crucial role in the regulation of neurotransmitter release by mediating the phosphorylation level of SNAP25 at Thr138 and Ser187 residues.

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Keywords: *SNAP25, Myosin Phosphatase*

P-149
DIRECT ASSESSMENT OF CALCIUM SIGNALS IN HEPATOCYTES ISOLATED FROM RATS TRANSGENIC FOR GCAMP2 CALCIUM SENSOR

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Calcium ions play important roles in several physiological processes as a seconder messenger molecule. Numerous methods are available to measure the changes of the intracellular calcium level; however, the utilization of genetically encoded calcium indicators has recently been spreading due to the fact that these sensors can selectively be expressed in different cell types and readily be used without further manipulation. Especially beneficial is the application of genetically engineered Ca2+indicators in certain cell types, such as hepatocytes, which from the traditional calcium dyes are effectively extruded by multispecific transporters. In the present work, hepatocytes from constitutive (CAG) promoter-driven GCaMP2 homozygous transgenic rats were isolated and cultured in monolayer and collagen sandwich configurations. A comparative analysis was performed with cells obtained from animals of three different age groups (3, 7-8, and 23+ week old rats). Since hepatocytes undergo substantial changes following isolation, the cultures were studied at different time points ranging from 1 to 7 days after seeding. The mRNA expression levels of the Ca2+sensor, as well as two characteristic hepatic transporters (Ntcp and Bsep)were assessed by qPCR. The protein expression and the cellular localization of the transgene were determined by Western blotting, and immunofluorescence staining, respectively. Intracellular Ca2+levels were measured by confocal microscopy at each time point in all three age groups. Our results indicated that CAG-promoter driven GCaMP2 expressed in the liver at high levels regardless of the age of the animal. The expression of the Ca2+sensor was rapidly restored after isolation and remained stable in cultured hepatocyte up to 7 days; however the cellular localization of GCaMP2 was uneven, and underwent redistribution during culturing. The applicability of the Ca2+sensor to assess intracellular calcium changes in cultured hepatocytes was demonstrated by Ca2+signals elicited by ATP and ionomycin. The Ca2+levels and responses were also determined in various subcellular compartments, such as submembrane and cytosolic regions as well as in the Golgi compartment and the nucleus.

Our results clearly demonstrate that the rat transgenic for GCaMP2 can provide a proper in vitro test system for studying calcium homeostasis in hepatocytes.

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Keywords: *GCaMP2, transgenic rat, calcium signaling, hepatocytes, collagen sandwich culture*

P-150
SYSTEMATIC IDENTIFICATION OF MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) DOCKING MOTIFS IN THE HUMAN PROTEOME REVEAL A WIDESPREAD AND DYNAMICALLY EVOLVING SYSTEM

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Although mitogen-activated protein kinases (MAPKSs) are one of the best characterized members of intracellular signaling pathways, our knowledge on their substrates is still very limited. However, we do know that the MAPK activating kinases, pathway regulators and most substrates associate with these kinases through auxiliary linear motifs called D-motifs. Here we present a novel attempt to chart out the human MAPK interactome by directly detecting MAPK-docking motif containing proteins. Using a combination of structural homology modelling and rigorous sequence-based methods, we were able to identify more than a hundred potentially novel MAPK partners. Experimental validation using recombinant protein arrays, fluorescence anisotropy titrations and cell-based methods confirm the validity of this approach. Our studies suggest that specific MAPK-interacting D-motifs are remarkably widespread in the human proteome. These novel partner proteins are involved in a number of intriguing cellular functions. Although the MAPKs themselves are an ancient heritage, their substrates and other partnerships evolve much faster than previously anticipated.

P-151
3D ENGINEERED NEURAL TISSUE FROM HUMAN INDUCED PLURIPOTENT STEM CELLS

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Three dimensional cultures have shown more physiological relevance (improved cell survival, enhanced neuronal differentiation, better cell–cell and cell–matrix interactions) compared to traditional two dimensional cell culture systems. Differentiation of patient-derived induced

pluripotent stem cells (iPSCs) into a three dimensional (3D) engineered neural tissue (ENT) provides advantages to study the pathophysiology of neurodegenerative disorders such as Alzheimer's disease, frontotemporal dementia (FTD) or spinocerebellar ataxia (SCA). 3D ENTs also allow preclinical analyses of selected neural drug candidates and could be a promising tool for neuro-toxicology.

In this study fibroblasts and mononuclear blood cells were isolated from genetically and clinically well-characterized patients, reprogrammed into iPSCs and differentiated into neurons using an air-liquid interface based, scaffold-free system which allowed generation of a compact 3D neural tissue without added growth factors (Krause et al., 2009).

After 6 weeks of differentiation the 3D ENTs were characterized with electrophysiology (multi-electrode array, MEA), calcium imaging technique and immunocytochemical methods.

MEA recordings and calcium imaging revealed that ENT neurons exhibited spontaneous firing activity and the evidence of functional synapses. Immunocytochemistry analyses confirmed these results with the presence of various neuronal and glial markers such as beta III Tubulin, MAP2, NF200kD, GFAP, OSP and different synaptic proteins. The ENT structures were fairly homogenous and the majority of cells differentiated into neurons, but we have found areas with strong nestin positivity, which indicates less differentiated cell types, therefore further optimization towards simultaneous and complete differentiation would be beneficial.
In conclusion, these findings demonstrate that 3D ENTs neurons might be suitable for drug development and studying the pathophysiology of different neurological and psychiatric disorders, including major pathologies such as Alzheimer's disease.

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P-152
HUMAN PLURIPOTENT STEM CELL DERIVED DENTATE GYRUS PROX1 NEURONS AND MEDIAL GANGLIONIC EMINENCE GABA INTERNEURONS AS SYSTEMS FOR IN VITRO DISEASE MODELLING

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Human embryonic stem (ES) cells and induced pluripotent stem (IPS) cells represent unique model systems for investigating the molecular machinery of neurodevelopment and neuronal disorders. These self-renewable cell lines can be used to generate different neuronal types in vitro, and to study the differentiation process into various neuronal types. Along this line, individual cell lines

generated from different healthy or diseased individuals can also be compared by investigating molecular and cellular phenotypes.

We used established and published protocols to generate hippocampal dentate gyrus granule cells and forebrain GABA interneurons. The dentate gyrus protocol is based on dual SMAD inhibition, Wnt- and SHH inhibition after generating embryoid bodies from pluripotent stem cells, which procedure results in forebrain specific Sox2 and Nestin positive neuronal progenitor cells (NPCs). This intermediate cell type can be further differentiated into Prox1-expressing mature neurons. Using this protocol we compared the Wnt-antagonist effects of recombinant Dkk1 and XAV939, a small molecule used in other protocols. The forebrain GABA protocol exploits SMAD inhibition combined with SHH signaling to generate Nkx2.1-expressing medial ganglionic eminence precursors, which give rise to GABAergic interneurons. We characterized both progenitors and differentiated neurons by immunocytochemistry, Ca-imaging, single cell electrophysiology and quantitative PCR.

The prospect of investigating neuropsychiatric disorders by reprogramming somatic cells of patients into IPS cells and using these differentiation protocols is tantalizing. Several such studies on disease models have been able to recapitulate certain aspects of these disorders in vitro. The introduced protocols will be discussed in the context of IPS cell based in vitro disease modeling.

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P-153

THE ROLE OF SHORT-CHAIN FATTY ACIDS IN ADIPOGENIC DIFFERENTIATION OF CHORION-DERIVED MESENCHYMAL STEM CELLS

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GPR41 and GPR43 are G-protein-coupled receptors activated by short-chain fatty acids that are produced by gut microbiota through fermentation of non-digestible carbohydrates. These receptors are expressed in the gut epithelium where they function as metabolic sensors but they are also expressed in several other tissues considered to be metabolically important, such as adipose tissue.

Recent studies proved the connection between GPR43 and adipocyte differentiation in mice but only limited data are available on human cells. The aim of this study was to investigate the implication of GPR41 and GPR43 receptors in human adipogenesis in chorion-derived mesenchymal stem cells (cMSCs).

cMSCs isolated from four donors were differentiated into adipocytes in the presence of short-chain fatty acids (acetate, propionate and butyrate), the ligands of these receptors, and a selective GPR43 agonist. We found a marked inhibition of adipogenic differentiation (characterized by staining and quantification of lipid droplets) as a result of propionate and GPR43 agonist treatments. The adipogenesis was also influenced by the timing of treatments: cMSCs were more sensitive in the first and the last periods of the 14-day long differentiation as shown by less number of differentiated adipocytes compared to the counterparts treated in the middle of the period.

The expression of GPR41, GPR43, and the propionate receptor OR51E2 as well as several adipocyte marker genes was determined by RT-PCR in undifferentiated and adipo-differentiated cMSCs. Surprisingly, GPR43 transcripts were not detected either in the undifferentiated or in the differentiated samples. GPR41 was expressed only in some samples at low levels while OR51E2 expression was pronounced. PanPPARg, FASN, and leptin adipocyte marker genes were upregulated, while the transporter FABP4 gene was downregulated during adipogenesis. Based on our RT-PCR results we hypothesize that the inhibition of adipogenesis takes places via GPR41 or OR51E2 receptors but we cannot exclude the operation of a receptor-independent pathway either.

Keywords: *free fatty acids, cMSC, GPR41, GPR43, OR51E2*

P-154
THE ROLE OF SYNDECAN-4 IN MYOBLAST PROLIFERATION

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The muscle progenitor satellite cells mitotically and physiologically quiescent in healthy muscle, they are stimulated by local damage to proliferate extensively and form myoblasts that will subsequently differentiate and fuse to form muscle fibers. The molecular mechanisms controlling this process are poorly understood, particularly the signaling pathways operating early in regeneration that lead to the initial activation and proliferation of the satellite cells. The type-I transmembrane heparan sulfate proteoglycan syndecan-4 is expressed ubiquitously regulating essential cellular processes (e.g. cell migration); furthermore, it is a marker of the satellite cells. Here we show a transient upregulation of syndecan-4 expression; and simultaneous low level of myostatin, a negative regulator of muscle growth (growth and differentiation factor 8; GDF8) during the early stages of skeletal muscle regeneration. Furthermore, co-immunoprecipitation experiments revealed the interaction of syndecan-4 and precursor promyostatin in a heparan sulfate-dependent manner. Silencing of syndecan-4 in C2C12 myoblasts decreased the proliferation rate of the cells. Flow cytometric analysis revealed that syndecan-4 silencing decreased the progression of myoblasts from G1- to S-phase of the cell cycle, which was accompanied by the elevated level of p21(Waf1/Cip1) and the decreased amount of cyclin E. Interestingly, the level of myostatin was increased in syndecan-4 silenced cells, and enhanced activity of PI3K/Akt signaling was observed.

These data can explain the essential role of syndecan-4 in both skeletal muscle development and regeneration.

P-155
LACK OF RYBP IMPAIRS NEURAL DIFFERENTIATION OF EMBRYONIC STEM CELLS

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Rybp (Ring1A and Yy1 Binding Protein) is a member of the non-canonical Polycomb Repressive Complex 1 with essential role in early embryonic development. We have previously reported that alterations of Rybp dosage in mouse models induced striking neural tube defects (NTDs), exencephaly, disorganized neurocortex and coloboma of the eye. However, the exact molecular mechanism responsible for the *in vivo* phenotypes remained unknown.
We aimed to uncover underlying molecular events that are responsible for the observed phenotypic changes. We used mouse embryonic stem (ES) cells as *in vitro* models of development. Since *in vitro* neural differentiation processes follow the *in vivo* neuroectodermal development, we utilized wild type *(rybp$^{+/+}$)* and *rybp* null mutant *(rybp$^{-/-}$)* ES cells and differentiated them towards neural lineages *in vitro.* We used retinoic acid (RA) to facilitate neural development. Differentiation revealed clear morphological differences between the wild type and the *rybp* null mutants the latter consistently producing poor neuron-like outgrowths in cultures. Further molecular and immunostaining studies confirmed altered expression of key neural, glial markers (e.g. Pax6, Nestin, Tuj1, Olig2) and members of the RA signal transduction pathway (e.g. Stra6, Crabp2) suggesting that *rybp* has important effect on neural lineage entry by ES cells. In the *rybp* null mutants, we have also found a complete defect in the induction of tumor suppressor Plagl1/Lot1/Zac1, which is an important regulator of neural differentiation processes. Furthermore, Plagl1 was the first gene unrelated to tumor suppressor p53 that was found to regulate cell cycle progression and apoptosis, these two fundamental genetic programs. In the *rybp$^{-/-}$* cells the expression of several cell-cycle regulators (e.g. Ccnd2, p21) are also altered. Thus, our findings showed that normal dosage of Rybp is indispensable for the proper neural differentiation and lack of functional Rybp severely impairs the formation of neural cell types.

Keywords: *embryonic stem cells, neural differentiation, Rybp, Plagl1*

P-156
MESENCHYMAL STEM CELL-DERIVED GALECTIN-1 PLAYS ROLE IN PHYSIOLOGICAL AND PATHOLOGICAL VESSEL FORMATION

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Tumor cells are supported by surrounding tumor-associated stroma (TAS) cells and soluble or cell-bound factors produced by these cells. In spite of the importance of the stroma cells especially vessels in tumor development and survival, their origin has not yet properly revealed. The present assumption is, one part of the stroma cells comes from the recruited healthy cells which are transformed to act as TAS. The other source of TAS can originate from bone marrow mesenchymal stem cells (MSCs) which can migrate and accumulate into the tumor mass. Among the factors which contribute to the tumor growth galectin-1 (Gal-1) with proangiogenic activity produced by solid tumor cells and/or stromal cells has been identified. However, MSC-derived Gal-1 has not yet been characterized, therefore our research has focused on the analysis whether Gal-1 secreted by MSCs support angiogenesis in *in vitro* and *in vivo* model systems.

Pre-vascular co-culture system is used to study the role of MSC-derived Gal-1 in angiogenesis *in vitro*. This system is presumed to model a late step of angiogenesis when periendothelial cells, which can originate from MSCs and endothelial cells get into an intimate contact and generate pre-vascular network. Wild type MSC^{wt} and Gal-1 deficient $MSC^{Gal\text{-}1\text{-}/\text{-}}$ isolated from bone marrow of wild type and Gal-1 knockout mice, respectively, were co-cultured with H5V mouse heart capillary endothelial cells and the evolved pre-vascular network was evaluated. The extent of the pre-vascular structures greatly depended on the expression of Gal-1 by MSCs since Gal-1 deficient MSCs supported significantly less formation of the pre-vascular structures than their wild type counterparts. Confocal microscopy analysis showed that the structures consisted of both cell types, and direct contact between endothelial and mesenchymal stem cells was essential to the development of network. The role of secreted Gal-1 was also verified by cultivation of MSC^{wt}/H5V co-culture in the presence of thiodigalactoside (TDG), a minimal ligand of Gal-1. The length of structures were notably decreased during TDG treatment compared to that of an indifferent disaccharide, saccharose.

To prove these findings *in vivo*, MSCs were either subcutaneuosly co-injected with mammary tumor cells or transplanted intravenously into tumor bearing mice. MSC^{wt} supported tumor growth in both tumor model, while $MSC^{Gal\text{-}1\text{-}/\text{-}}$ failed to enhance the enlargement of mammary carcinoma. Histologic examination of the tumor tissues revealed that the microvessel density of control and the $MSC^{Gal\text{-}1\text{-}/\text{-}}$ treated tumors were similar but it increased significantly in the presence of MSC^{vt}.

Our results prove that MSC derived Gal-1 plays important role in angiogenesis as it was shown in an *in vitro* system modelling the physiological angiogenesis and in an *in vivo* animal tumor model representing pathological vascularization.

Keywords: *mesenchymal stem cells, angiogenesis, tumor*

P-157
BIOLOGICAL EFFECT OF MOUTHWASH COMPOUNDS ON DENTAL STEM CELLS AND GINGIVAL EPITHELIUM

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In the last decades several types of dental stem cells (DSCs) have been identified from the dental pulp (DPSC), the periodontal ligament (PDLSC), the apical papilla (SCAP) etc. Recent studies, *in vitro and in vivo* have revealed a wide range of plasticity of these cells and demonstrated that DSCs are promising applicants of clinical and regenerative dentistry. Among these cells the mesenchymal origin PDLSCs have an underlined significance as their high proliferative capacity and multipotent (e.g. osteogenic or chondrogenic) differentiation provides an important therapeutical target; however, the cell biological integrity of these cells is frequently disturbed by the routinely used irrigative compounds applied as components of root canal irrigant mixtures (e.g. hydrogen peroxide - H_2O_2 and chlorhexidine - CHX).

In the present study our objectives were to monitor cell physiological effects (cell adhesion, spreading, viability, CD markers) of two traditional (H_2O_2, CHX) and a novel (ClO_2) dental irrigative compound in PDLSC and in gingival epithelial cells (HGEp) as referent ones.

Chemicals were applied in the following concentrations: 3% H_2O_2, 0.2% CHX, 0.025% ClO_2. PDLSCs were isolated from periodontal ligament of the root of impacted 3rd molar tooth and cultured in alpha-MEM/10% FBS, HGEps in CnT-24 media. The cell adhesion/spreading were evaluated by impedimetry in a real-time system (xCELLigence SP). Cell viability was measured by WST-1 assay. Morphology of the cells was evaluated by phase contrast microscopy (Nikon TMS). Immunocytochemistry was used to detect mesenchymal stem cell markers: STRO-1, CD-90 and CD-105.

Chlorine dioxide proved to express mild effects on spreading of PLDSCs and in HGEp and elicited less cytotoxic effects compared to the traditionally used irrigative compounds. Studies of cell viability (morphology, WST-1 and Cell Index) demonstrated that ClO_2 has no significant effect on the viability of PLDSCs. The active ingredients of mouthwash (e.g. H_2O_2 and CHX) actually applied in the clinical routine for the endo- or periodontitis management had toxic effect on PLDSCs; while the relatively novel and hyper pure compound, ClO_2 proved to be less toxic and the DSCs were able to regenerate after the treatment.

Keywords: *PDLSC, DPSC, chlorine dioxide, hydrogen peroxide, chlorhexidine*

P-158
EXAMINATION THE EXPRESSION OF PLURIPOTENCY RELATED MARKERS IN THE GONADS AND PRIMORDIAL GERM CELLS OF DIFFERENT TYPES OF CHICKEN BREEDS

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The chicken embryo is an important model system for developmental and stem cell biology, primarily because of easy access to the embryos. Primordial germ cells (PGCs) are the precursors of the germ cell lineage, which will differentiate into mature spermatozoa and oocytes. Researchers used the avian model to reveal the cellular origins and migratory pathways of PGCs. Unlike in mammals, avian PGCs use the bloodstream for transportation to the future gonadal ridge. This unique feature gives us the opportunity to collect PGCs from the dorsal aorta of embryos. These cells can be maintained *in vitro* for long periods of time. The most recent works focus on the molecular mechanisms regulating the early germ cell development.

Avian PGCs have significant potential as a cell-based system for preservation of avian germ cells. Long-term maintenance of avian PGCs *in vitro* has tremendous potential because it can be used to deepen our understanding of the biology of PGCs and also a promising option for gene banks and genome preservation.

In our study along with the standard White Leghorn breed we also examined two native Hungarian breeds, the Hungarian Partridge colour and the Transylvanian Naked neck.

First of all we adapted the method of feeder-free *in vitro* cultivation of PGCs. We isolated the PG cells from the blood of HH16 chicken embryos. Using SSEA-1 and EMA1 immunostaining we annotated that our cultivated cells were truly PG cells. After that we designed primers which are specific to stem cell- and PGC-associated genes (such as CVH, cPouV, cNanog, cSox2, cGATA4, cCDX2, cDAZL), and then tested their expression pattern in chicken embryonic gonads and cultivated PGCs.

We examined the expression of the marker genes in the right and left gonads of the embryos separately. The expression was asymmetric in both sexes and there was a significant difference between females and males regarding the difference of the expression of OCT4 and VASA in the left and right gonads.

In the future we are planning to investigate the role of miRNAs during the differentiation of chicken germ cells and to profile the expression of stem cell specific miRNAs in bilateral gonads.

The expression pattern of the PGCs enables us to determine whether the long term culture or the freezing procedure affects the cell developmental potential. The obtained knowledge can be used to maintain the PGCs of our native Hungarian chicken breeds in an undifferentiated state and therefore preserve their genetic material.

Keywords: *primordial germ cell, chicken embryo, gene expression*

P-159
AUTOPHAGY IS REQUIRED FOR INTESTINAL STEM CELL MAINTENANCE AND HYPERPLASIA CAUSED BY UVRAG DEFICIENCY IN DROSOPHILA

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Stem cells are critical for the maintenance of adult tissues and organs, including the intestinal tract of mammals and Drosophila. Here we show that intestinal stem cells in Drosophila depend on high levels of baseline autophagic degradation. Stem cells lacking core autophagy genes fail to maintain proliferative capacity and are progressively lost, leading to increased leakage of the gut and premature death of affected animals. In contrast, loss of the putative autophagy regulator UVRAG, a tumor suppressor potentially involved in colorectal cancer, causes abnormal proliferation of stem cells and accumulation of undifferentiated progeny. We find that hyperplasia induced by lack of UVRAG requires a functional autophagy pathway, which raises the possibility of applying autophagy inhibitors for treating UVRAG-deficient cancers. Our results establish the fundamental role of autophagy for gut functioning through long-term maintenance of intestinal stem cells, which is also important for longevity.

P-160
ONTOGENIC STUDY OF THE BURSAL STEM CELLS USING A NOVEL MONOCLONAL ANTIBODY

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The bursa of Fabricius plays a central role for the development of B lymphocytes in birds. During embryonic development the bursal epithelial anlage is colonized by B cell precursors (prebursal stem cells) to form the follicle buds which ultimately develop into lymphoid follicles with central medullary and outer cortical region. Previous cell transplantation experiments suggested that posthatched bursa of Fabricius may contain an undifferentiated B cell population (bursal stem cells) which regenerates the bursal follicles after B cell depletion. As an effort to characterize the different B cell populations we have produced a large panel of monoclonal antibodies (mAbs) by immunizing mice with cell suspension of spleen and bursa of Fabricius of guinea fowl (*Numida meleagris*).

One of these antibodies (clone: 7H3) was found to recognize a cell surface antigen (molecular weight: ~ 70 kDa) expressed by CD45+/chB6+ pre B cells in the developing bursa of Fabricius. Double immunofluorescence labeling proved that nearly all B cells of embryonic bursa co-expressed the 7H3 antigen. However, after hatching the 7H3 expression of the bursal follicles gradually diminished, and it was lost, except a subpopulation of cortical B cells.

Based on this expression pattern we hypothesized that 7H3 mAb could be a candidate marker for the prebursal and bursal stem cells. To study the ontogeny of 7H3+ cells during bursal regeneration we experimentally induced B cell depletion of the bursa. B cell depletion was induced by Cy treatment and infectious bursa diseases virus (IBDV) in inoculation. A subpopulation of cortical cells survived, which expressed 7H3 antigen. Recovery of the bursal follicles started by proliferation of 7H3+/chB6+ cells in the cortex and 15 days later followed the medulla.

These data suggest that 7H3 mAb is a suitable marker for the bursal stem cells and provide a unique tool to better understand the dynamics of bursal B cell population during normal development and regeneration.

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ROLE OF BFGF AND EGF IN NEURAL ROSETTE FORMATION

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Neurons derived from human induced pluripotent stem cells (hiPSCs) are providing a new model system to study neurodegenerative diseases and drug development. To generate neurons, hiPSC are first induced to become neural progenitor cells (NPCs) through inhibition of SMAD signalling and then further differentiated to neurons. Two mitogens at varying concentrations are usually applied to stimulate neural stem cell proliferation: basic Fibroblast Growth Factor (bFGF) and Epidermal Growth Factor (EGF). We compared whether low (10 ng/ml) or high (25 ng/ml) concentrations of bFGF and EGF could affect neural rosettes formation or the neural progenitor cell population. ICC studies revealed that all examined lines were positive for NPC markers (N-cadherin, Pax6, Nestin) independent of the applied mitogen concentration. However, high concentrations of bFGF and EGF significantly reduced the area of the lumen of neural rosettes ($P < 0.01$). Similarly, at high concentrations of bFGF and EGF the proportion of large neural rosettes was significantly reduced in all studied cell lines ($P < 0.05$). The expression of anterior and posterior NSC, neural crest, and neural rosette markers were compared. Additionally, NPCs were differentiated terminally and neurons were analysed after 14 days. Qualitative and quantitative studies were performed on the expression of beta-III Tubulin and Map2 to compare the neurons from all four cell lines. Further studies on neurons generated from these NPCs are needed to analyse the impact of bFGF and EGF on electrophysiological activity of the differentiated neurons.

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Keywords: *bFGF, EGF, neural rosettes, NPC*

P-162
TRANSPORTER EXPRESSION PROFILING DURING HEPATIC DIFFERENTIATION OF HUMAN PLURIPOTENT STEM CELLS

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Numerous ABC transporters are expressed in the liver and play essential role in various hepatic functions including detoxification, bile secretion, and cholesterol metabolism. Most cell-based hepatic test systems used in basic research and pharmaceutical studies are based on primary cell cultures or cancerous cell lines, therefore, their availability or applicability is greatly limited, which especially applies for human liver model cells. Pluripotent stem cells can be utilized as a cell source for pharmacological models. Although several protocols are available for hepatic differentiation of stem cells, the attention devoted to the transporter composition of the progeny cells in insufficient, despite the fact that the applicability of these cells in pharmacological tests is greatly determined by their transporter assortment.

In the present work, we differentiated human embryonic stem celllines toward to hepatic lineage using previously published differentiation protocols (Si-Tayeb et al. 2010, Cai et al. 2012), and performed a comparative study on differentiation, hepaticfunctions, and comprehensive expression profiling. The primary objective of our study was to reveal the changes in expression levels of liver transporter genes, especialy that of ABC transporter genes at various stages of differentiation. The progress of differentiation was followed by a custom-designed qPCR array assessing the mRNA expression levels of different markers, including pluripotency, germ line, and hepatocyte marker genes. Protein expressions of several liver markers were also verified, and the hepatic functions of the progeny cells were determined by measuring the albumin and urea secretion. In parallel, the transporter assortment in the differentiated cells was assessed using an additional qPCR array specially designed for human ABC proteins and hepatic uptake transporters.

Our results indicated that most liver specific genes and transporters crucial for hepatic functions are markedly induced during hepatic differentiation of stem cells. The progeny cells possessed an ABC transporter assortment, which is typical for hepatocytes; however, the expressions levels did not reach that found in adult liver samples. Noteworthy that the generated human stem cell-derived hepatocyte-like cells greatly suppress the most commonly used human liver model cell line, HepG2, in terms of expression profile and mRNA levels of the studied transporters.

Our results call for further improvement of differentiation protocols and provide a tool for testing the quality of stem-cell derived hepatic model cells.

Si-Tayeb K, et al. „Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells.”Hepatology (2010) 51(1):297-305.

Cai J, et al. „Protocol for directed differentiation of human pluripotent stem cells toward a hepatocyte fate.”(June 10, 2012), StemBook, ed. The Stem Cell Research Community

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Keywords:*pluripotent stem cell, hepatic differentiation, ABC transporter*

P-163
AN EXPERIMENTAL TEST OF THE ADAPTIVE GENOME STREAMLINING HYPOTHESIS

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Genome reduction is a prominent evolutionary process that pervades nearly all major bacterial lineages. Two main hypotheses has been suggested why some prokaryotic genomes are especially compact and contain reduced gene sets. The non-adaptive loss scenario suggests that relaxed purifying selection together with a strong mutational bias towards deletions in bacterial lineages account for genome reduction. In sharp contrast, the streamlining hypothesis posits that selection acts to reduce genome size to improve cellular economics via two mechanisms. It minimizes the metabolic burden of replication, and it allows concomitant reduction in cell volume. In this work, we explicitly tested assumptions of the genome streamlining hypothesis. By extending a previous series of deletions of genomic segments, we reduced the *Escherichia coli* genome by up to 22 %. The resulting 75 multiple-deletion series (MDS) strains provided an unprecedented opportunity to study the phenotypic consequences of genome reduction, not least because the deleted segments harbor genes that have been repeatedly lost in E.coli relatives. The following major conclusions were drawn. Eradication of large genomic segments i) had no beneficial effects on growth rates, ii) reduced metabolic yield under nutrient starvation, and iii) caused a major perturbation of genome-wide gene expression. iv) A systematic experimental survey revealed that multiple-deletion strains exhibit severe defects in nutrient utilization. Last, v) there was no significant association between genome size and cell size. In sum, we failed to find systematic evidence for beneficial effects of genome reduction in most cellular traits investigated.

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COMPLEMENT COMPONENT 1Q-BINDING PROTEIN IS PRESENT IN SOME NERVE TERMINALS AND INVOLVED IN MATERNAL ADAPTATIONS OF THE HYPOTHALAMUS

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Maternal adaptations represent a homeostatic challenge whose counterbalance is expected to include alterations in the functioning of specific brain regions. Our hypothesis was that alterations associated with the postpartum behavioral and hormonal state are present at the proteome level. The hypothalamus has a pivotal role in the coordination of maternal behaviors and neuroendocrine responses. Since neuronal connections play particularly important roles in complex brain processes, we isolated synaptosome fractions from the hypothalamus to investigate proteomic changes in mother rats. Non-maternal dams deprived from their pups immediately after the parturition were used as controls. The preparation of synaptosomes was performed using Ficoll gradient centrifugation, and the validation of the method was carried out with electron microscopy. 2D-DIGE full stain technique combined with LC MS/MS was used to separate proteins and determine protein level differences. We have identified 21 significant protein changes, among which we found 7 increases and 14 decreases in dams with their litter compared to control animals. One of the proteins with reduced level in rat dams was Complement component 1q-binding protein(C1qbp), which is a multifunctional and multicompartmental protein involved in inflammation and infection processes, ribosome biogenesis, regulation of apoptosis, transcriptional regulation and pre-mRNA splicing. The reduction of C1qbp in maternal synaptosomes was validated with Western blot technique and immunohistochemistry. C1qbp immunoreactivity was present in some hypothalamic regions, mostly in fibers and terminals, and also in some cell bodies. The C1qbp-immunoreactive terminals showed highest density in the preoptic area and the mediobasal region of hypothalamus while cell bodies were most abundant in the magnocellular preoptic and paraventricular nuclei. The density of C1qbp-immunoreactive terminals and the number of C1qbp-immuoreactive cell bodies were decreased in dams as compared to control animals.

The results suggest that pup nursing is associated with reduced C1qbp protein levels, which may play a role in the maternal adaptation of the hypothalamus.

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PROTEOMIC ANALYSIS OF THE MEDIAL PREFRONTAL CORTEX AND THE HYPOTHALAMIC PREOPTIC AREA IN MOTHER RATS

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The highest center of maternal responsiveness lies within the medial prefrontal cortex while the preoptic area of the hypothalamus regulates critically important components of maternal behaviors, e.g. pup retrieval in rodents. Therefore, we dissected these two brain regions to investigate proteomic changes in rat dams. The control group consisted of mothers, which were deprived of their pups immediately after the parturition.

2-DIGE minimal dye technique combined with LC-MS/MS was used to separate proteins and determine protein level differences. We have identified 32 different significant protein changes in the medial prefrontal cortex, among which we found 25 increases and 7 decreases in dams compared to control animals. In the preoptic area, we found 18 different significant protein changes, 13 increased and 5 decreased in dams.

Alpha-crystallin B chain showed the greatest protein level changes in both of the analyzed brain regions: it increased in the cortex, but decreased in preoptic area in dams. Astrocytic phosphoprotein PEA-15 and alpha-internexin also showed considerably increased protein levels in the cortex, and alpha-internexin also increased in the preoptic area. These protein level changes were validated with Western blot technique while their distributions were described with immunohistochemistry.

The functional classification of the altered proteins was made according to Uniprot and Gene Ontology protein databases. The altered proteins participate in neurotransmission, synaptic plasticity, neuron development, protein and glucose metabolism, cytoskeleton organization, oxidative stress and apoptosis according to their classification. The interactions of these proteins were determined and visualized with Elsevier Pathway Studio Platform. On the bases of our results, pup nursing is associated with cortical and preoptic protein level changes, the majority of the proteins showed increased level in dams, and the greatest protein level changes are associated with neuronal development and apoptosis.

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