BIOKÉMIA

A Magyar Biokémiai Egyesület internetes folyóirata

XXXVIII. évfolyam 3. szám

2014. augusztus

Annual Meeting of the Hungarian Biochemical Society

University of Debrecen
Life Science Building
Debrecen, Hungary

24-27 August, 2014

Final Programme and Abstract Book

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A Magyar Biokémiai Egyesület internetes folyóirata

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XXXVIII. ÉVFOLYAM 3. SZÁM **2014. augusztus**

Final Programme & Abstract Book of the Annual Meeting of the Hungarian Biochemical Society (HBS), 2014

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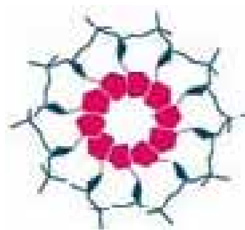
ANNUAL MEETING OF THE HUNGARIAN BIOCHEMICAL SOCIETY



University of Debrecen
Life Science Building
Debrecen, Hungary

24 – 27 August, 2014

Final Programme and Abstract Book

INVITATION

Dear Colleagues,
It's our pleasure to announce that the **Annual Conference of the Hungarian Biochemical Society** http://www.mbkegy.hu/ will take place in **Debrecen between August 24-27.** This follows the very successful „Hungarian Molecular Life Sciences 2013" joint meeting with the geneticists and cell biologists.

The topics include the followings: Biocrystallography and structural biology; Signaling and post translational modifications; Genomics and Epigenetics; Genome structure, function and maintenance; Biochemical pharmacology; Cell death and differentiation; Stem cells; Lipidomics and Proteomics; Membrane Biochemistry; Pathobiochemistry.

The official language of the Conference is English.

The section chairs and the organizing committee finalized the program based on the submitted abstracts. The announcement and all relevant information can be found on the homepage of the Annual Meeting (http://www.mbkegy.hu/annualmeeting.php). The final program as well as the abstracts will be published in the 3rd volume of the "Biokémia", the official journal of the Society.

The conference will be held in the Life Science Building of the University of Debrecen. The lecture halls, the poster exhibition area and that of the company exhibitors will be situated in one building, while the restaurants and many of the accommodations are located in close vicinity.

We hope that you will join us in this meeting and will share your latest results with the scientific community. We also would like to ask you to call the attention of fellow colleagues to this conference.

On behalf of the organizers:

József Tőzsér
Chair of the Conference
Department of Biochemistry and Molecular Biology
Faculty of Medicine.
University of Debrecen

László Fésüs
President of Hungarian Biochemical Society

Beáta G. Vértessy
Secretary General of Hungarian Biochemical Society

GENERAL INFORMATION

Congress organizers

Hungarian Biochemical Society;
Department of Biochemistry and Molecular Biology,
University of Debrecen

Organizing Committee

József Tőzsér[1], László Fésüs[1], Beáta Vértessy G.[2], Nóra Elek[1]

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Phone: 06 1 3826707

Department of Applied Biotechnology, Budapest University of Technology and Economics (BME), Szt. Gellért tér 4, Budapest H-1111

Venue

University of Debrecen, Life Science Building
4032 Debrecen, Egyetem tér 1, Hungary

Parking possibility

You will get a ticket at the entrance gate. Please, handle it to the staff at the Registration desk in order to having it stamped and signed. At the end of each day the driver has to give it to the guard at the gate before leaving the campus. This way the reduced price of a daily ticket is 1.000,- HUF.

Opening hours of the registration

Sunday, 24 August, 2014:	14.00 – 19.00 h
Monday, 25 August, 2014:	08.00 – 18.30 h
Tuesday, 26 August, 2014:	08.00 – 18.00 h
Wednesday, 27 August, 2014:	08.00 – 12.00 h

Internet connection at the venue

Wireless Internet access is available on the ground floor during the conference days.

Official language

Official language of the annual meeting is English.

Registration fees (without VAT)		Early fee before 31st May	Late fee after 31st May
For non-members	Registration fee for junior researchers[*]	40 500 Ft	49 500 Ft
	Registration fee for senior researchers[**]	52 500 Ft	60 000 Ft
	Registration fee for industrial partners	60 000 Ft	67 500 Ft
	Registration fee for accompanying persons[***]	22 400 Ft	22 400 Ft
For HBS members	Registration fee for junior researchers[*]	27 000 Ft	33 000 Ft
	Registration fee for senior researchers[**]	35 000 Ft	40 000 Ft
	Registration fee for industrial partners	40 000 Ft	45 000 Ft

[*] Junior researcher: Ph.D. and university student, or researcher under 30.
[**] Senior researcher: only for participants with academic background.

Registration fee includes coffee breaks, lunches, dinners during the conference lecture days, cultural programme, conference materials, conference attendance, and admission to the exhibition.

Accompanying persons' registration fee is not valid for admission to scientific programmes and it does not include conference materials, only meals and social events.

Oral presentations
We would like to draw your attention to the following organizational information:

You must bring your presentation on a memory stick or your own laptop if you feel more comfortable. The memory stick or the laptop must be brought to the projection desk half an hour before the beginning of the morning or afternoon sessions to allow for laptop setup, equipment checks, and a brief run through the presentation.

Speakers and session chairs are kindly requested to keep the time of presentations. *Oral presentations*: *Lecture Halls F.014 – 015 and F 008 – 009 on the ground floor.*

Poster presentation
Poster boards will be placed on the ground floor next to Lecture Hall F 015 -016. We are planning to have one long poster session between 15.00 – 18.00 h on Tuesday, 26th of August. To locate your assigned poster board please, look for the board marked with the number of your poster. You can check your number in this booklet. Poster presenters can mount their posters from 10.00 h on the day of Poster session.

Posters will be available for collection from 8.00 h on Wednesday, 27 August at the venue of poster session.

Awards of best young lecturers and poster presentations

The organizers will set up a professional committee to evaluate the best three oral presentations and host a poster competition at the annual meeting.
Sigma-Aldrich LTD. has offered the awards for the best young lecturers and UD-GenoMed Ltd. has offered the awards for the best poster presentations. The winners will get vouchers that can be expended. The prizes are:

	Best Young Lecturers	The best poster presentations
1st prize	100 000 HUF	50 000 HUF
2nd prize	80 000 HUF	40 000 HUF
3rd prize	60 000 HUF	30 000 HUF

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Exhibition

A professional exhibition is to be organized parallel to the scientific sessions accordingly to the traditions of the annual meetings of the Hungarian Biochemical Society. The exhibition will be held at the coffee break area.

Social programmes

Sunday, 24 August, 2014 — Wine and cheese party with Bio-Science Ltd.
Monday, 25 August, 2014 — Dinner and relaxation at “Fráter Tanya”
Monday & Tuesday, 25-26 August, 2014 — Lunches
Monday & Tuesday, 25-26 August, 2014 — Coffee breaks (indicated in the programme)
Tuesday, 26 August, 2014 — Poster session with discussions amongst the posters
Tuesday, 26 August, 2014 — Grill party in front of the Main Building of the University of Debrecen

Cancellation policy

Cancellations on registration can be made only in writing. The refund for cancellations made on and prior to 30 June is 100 %. After this date the conference secretariat has to pay the advanced payments to the venue, restaurant etc. 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 invoice according to the Hungarian official financial rules. It means that food and drink costs will be listed separately on the invoice. Accommodation is not included in the registration fee, but a special conference reservation code will be provided for the hotel registration.

All bank charges and commissions are paid by the participants. Please inform your bank about this when ordering the transfer. Payment on site is not allowed.

Liability, 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

24th August, 2014

Session I. Opening Plenary Session

14.00- ***Arrival and registration***

Chairs: József Tőzsér, Beáta Vértessy, László Fésüs

17:00-17:10 ***Opening remarks***
László Fésüs, Zoltán Szilvássy, József Tőzsér

17:10-17:15 ***Tankó Award Ceremony***

17:15-17:40 E1-1
Butterflies, bacteria, books. My way…
Pál Venetianer
Institute of Biochemistry, Biological Research Centre, HAS, Szeged

17:40-18:05 E1-2
From genes to genomes and back again
László Nagy
Department of Biochemistry and Molecular Biology, Research Center of Molecular Medicine, University of Debrecen, Debrecen; Sanford-Burnham Medical Research Institute, Orlando, FL, USA

18:05-18:45 E1-3
Understanding estrogen receptor transcription in breast cancer
Jason Carroll (FEBS National Lecturer)
Cancer Research UK, University of Cambridge, Cambridge, UK

18:45-18:45 E1-4
Bio-Science Award (presented by Ágnes Tátrai, CEO, Bio-Science Ltd.)
Molecular mechanisms of autophagosome fusion with lysosomes in Drosophila
Takáts Szabolcs
Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest

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19:00- ***Wine and cheese party with Bio-Science Ltd*****.**

25th August, 2014

Session II. Membrane Biochemistry

Chairs: Csilla Laczka, László Homolya

9:00-9:25 E2-1
Péter Nagy
Beyond classical dimerization: subtle regulation of the clustering and function of ErbB proteins
Department of Biophysics and Cell Biology, University of Debrecen, Debrecen

9:25- 9:50 E2-2
Viola Pomozi
Chemical chaperon therapy reduces calcification in mice expressing misfolded human ABCC6 mutants
Institute of Enzymology, Research Centre for Natural Sciences, HAS, Budapest

9:50- 10:15 E2-3
Enikő Kállay
Calcium acts as a first messenger and suppresses colon cancer development by binding to the calcium sensing receptor (a G protein-coupled receptor)
Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria

10:15- 10:20 E2-4
Róbert Király
An attempt to modify reciprocally the opposed transamidase and γ-glutamyl hydrolase activities of human transglutaminase 2
Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen

10:30- 10:45 E2-5
Lívia Marton
CHO-MT58: A model system for functionality studies of Plasmodium falciparum CTP:phosphocholine cytidylyltransferase
Institute of Enzymology, Research Centre for Natural Sciences, HAS, Budapest

Session III. Genomics and Epigenetics

Chairs: Imre Boros, László Nagy

9:00-9:25 E3-1
Tibor Pankotai
Mechanistic insights into the transcriptional arrest in the presence of Double Strand Breaks
Department of Biochemistry and Molecular Biology, University of Szeged, Szeged; Department of Cancer Biology, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), France

9:25-9:50 E3-2
Zsolt Boldogkői
Transcriptional Interference Networks
Department of Medical Biology, Faculty of Medicine, University of Szeged, Szeged

9:50- 10:15 E3-3
Bálint L. Bálint
Functional annotation of Estrogen Receptor binding sites in the light of the 1000 Genomes Project
Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen; Center for Clinical Genomics and Personalized Medicine, Debrecen

10:15- 10:30 E3-4
András Viczián
Tissue-specific aspects of UVR8 photoreceptor controlled signalling in Arabidopsis thaliana
Institute of Plant Biology, Biological Research Centre, HAS, Szeged

10:30- 10:45 E3-5
Levente Kovács
Deubiquitylases involved in maintenance of ubiquitin homeostasis
Department of Genetics, Faculty of Sciences, University of Szeged, Szeged

Session II. Membrane Biochemistry

10:45-11:00 E2-6
Roland Takács
The role of septins through exerting influence on store operated Ca2+ entry (SOCE) in chondrogenesis
Department of Anatomy, Histology and Embryology, Faculty of Medicine, University of Debrecen, Debrecen

----
11:00-11:20 ***Coffee break***

Session III. Genomics and Epigenetics

10:45- 11:00 E3-6
Zsolt Czimmerer
Identification of STAT6 as a transcriptional repressor during alternative macrophage activation
Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen

----
11:00-11:20 ***Coffee break***

Session IV. Cell Death and Differentiation

Chairs: Zsuzsa Szondy, József Mihály

11:20-11:45 E4-1
Tibor Vellai
to be announced

11:45- 12:10 E4-2
Viktor Honti
Plasticity of blood cell lineages and hematopoietic compartments in *Drosophila melanogaster*
Institute of Genetics, Immunology Unit, Biological Research Centre, HAS, Szeged

12:10- 12:35 E4-3
Zsuzsa Szondy
Macrophages engulphing apoptotic cells produce a novel retinoid to enhance phagocytosis as well as apoptosis of neglected thymocytes
Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen

Session V. Omics: Lipidomics, Proteomics

Chairs: Éva Hunyadi-Gulyás, Balogh Gábor

11:20- 11:45 E5-1
Éva Klement
Protein phosphorylation – enrichment and mass spectrometry analysis
Biological Research Centre, Laboratory of Proteomics Research, HAS, Szeged

11:45- 12:10 E5-2
Evelyn Orsó
Platelet storage lesion and release of extracellular vesicles from senescent platelets: a lipidomic and proteomic approach
Institute for Laboratory Medicine and Transfusion Medicine, University of Regensburg, Germany

12:10- 12:35 E5-3
Gábor Balogh
Lipids, as key players in cellular stress management and in pathogenesis of metabolic syndrome
Institute of Biochemistry, Biological Research Centre, HAS, Szeged

Session IV. Cell Death and Differentiation

12:35- 12:50 E4-4
Katalin Pászty
Consequences of death receptor mediated apoptotic cleavage of the plasma membrane Ca2+ATPase
Molecular Biophysics Research Group, HAS, Budapest

12:50- 13:05 E4-5
Endre Kristóf
Irisin modifies the differentiation program of subcutaneous human adipocytes and induces "browning" *ex vivo*
Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen

13:05- 13:20 E4-6
Zoltán Simándi
PRMT1 and PRMT8 regulate retinoic acid dependent neuronal differentiation with implications to neuropathology
Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen

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13:20-15:00 ***Lunch***

Session V. Omics: Lipidomics, Proteomics

12:35- 12:50 E5-4
Rita Sinka
The role of lipid metabolism during the *Drosophila* spermatogenesis
Department of Genetics, University of Szeged, Szeged

12:50-13:05 E5-5
Róbert Dóczi
A flexible experimental system to study *in vivo* protein phosphorylation in plants
Department of Plant Cell Biology, Agricultural Institute, Centre for Agricultural Research, HAS, Martonvásár

13:05- 13:20 E5-6
Péter Lábiscsák
Targeted proteomics method and multiplexed immunobead-based assay for identification of salivary biomarkers in oral squamous cell carcinoma
Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen

----
13:20-15:00 ***Lunch***

Session VI. Stem Cells

Chairs: Balázs Sarkadi, István Szatmári

15:00-15:25 E6-1
András Nagy
Alternative Sources of Stem Cells for Tissue Repair
Department of Obstetrics and Gyneacology, Mount Sinai Hospital, Lunenfeld-Tanenbaum Research Institute; Institute of Medical Sciences, University of Toronto, Toronto, Canada

Session VII. Genome Structure, Function and Maintenance

Chairs: Mihály Kovács, Lajos Haracska

15:00-15:20 E7-1
Beáta Vértessy
Interplay between gene expression regulation and sanitization of the cellular nucleotide pool to preserve integrity of mobile genetic elements
Institute of Enzymology, Research Centre for Natural Sciences, HAS Budapest; Department Applied Biotechnology, BME, Budapest

Session VI. Stem Cells

15:25- 15:50 E6-2
András Dinnyés
Patient specific induced pluripotent stem cells and their neuronal differentiation
Biotalentum Ltd; Gödöllő; Molecular Animal Biotechnology Lab, Szent István University, Gödölő; Departments of Equine Sciences and Farm Animal Health, Faculty of Veterinary Medicine Utrecht University, The Netherlands

15:50- 16:15 E6-3
Ágota Apáti
ABC transporters in human pluripotent stem cells
Institute of Enzymology, Research Centre for Natural Sciences, HAS, Budapest

----
16:15- 16:35 ***Coffee break***

16:35-17:00 E6-4
István Szatmári
Enhanced embryonic stem cell-derived dendritic cell development by transcription factor mediated programming
Stem cell Research Laboratory, Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen

17:00-17:15 E6-5
Zsuzsanna Valkó
Chondrogenesis is regulated by poly(ADP-ribose) polymerase
Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen

Session VII. Genome Structure, Function and Maintenance

15:20- 15:40 E7-2
Mihály Kovács
RecQ helicases support precise recombination via complex activities that are enhanced by macromolecular crowding
Department of Biochemistry, ELTE-MTA “Momentum” Motor Enzymology Research Group, Eötvös Loránd University, Budapest

15:40- 16:00 E7-3
Lóránt Székvölgyi
Histone point mutations driving human disease: a biophysical perspective
Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen

16:00-16:15 E7-4
Zsuzsanna Ujfaludi
UVB induces a major genome-wide rearrangement of RNA polymerase II at transcribed human
Department of Biochemistry and Molecular Biology, Faculty of Sciences and Informatics, University of Szeged, Szeged

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16:15- 16:35 ***Coffee break***

16:35-16:55 E7-5
Gábor Szabó
Discontinuities present in the genome continuously
Department of Biophysics and Cell Biology, University of Debrecen, Debrecen

16:55- 17:15 E7-6
Károly Fátyol
Cloning and characterization of argonaute proteins of Nicotiana Bentahamiana
Agricultural Biotechnology Institute, National Agricultural Research and Innovation Centre

Session VI. Stem Cells

17:15- 17:30 E6-6
Ixchelt Quaranta Monroy
Highly efficient differentiation of embryonic stem cells into adipocytes by ascorbic acid
Department of Biochemistry and Molecular Biology, Research Center for Molecular Medicine University of Debrecen, Medical and Health Science Center, Debrecen

17:30- 17:45 E6-7
Csaba Matta
Ser/Thr-specific phosphoprotein phosphatases are key regulators of chondrogenesis
Department of Anatomy, Histology and Embryology, Faculty of Medicine, University of Debrecen, Hungary

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18:30- ***Social program: Fráter tanya (bus transport)***

Session VII. Genome Structure, Function and Maintenance

17:15- 17:30 E7-7
Szilvia Juhász
A new interaction partner for ubiquitylated PCNA
Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged

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18:30- ***Social program: Fráter tanya (bus transport)***

26th August, 2014

Session VIII. Signaling and Posttranslational Modifications

Chairs: László Buday, Ferenc Erdődi

9:00-9:25 E8-1
Attila Mocsai
to be announced

9:25- 9:50 E8-2
László Virág
Poly(ADP-ribosyl)ation as a redox regulatory mechanism
Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen

Session IX. Biocrystallography and Structural Biology

Chairs: Beáta Vértessy, László Nyitray

9:00- 9:25 E9-1
Attila Reményi
Linear binding motif surfaces on protein kinases: a hot-spot for generating signaling diversity
"Lendület" Protein Interaction Group, Institute of Enzymology, Research Center for Natural Sciences, HAS, Budapest

9:25-9:50 E9-2
Veronika Harmat
Size selection mechanisms of acylaminoacyl peptidases
MTA-ELTE Protein Modelling Research Group, Budapest; Laboratory of Structural Chemistry and Biology, Institute of Chemistry, Eötvös Loránd University, Budapest

Session VIII. Signaling and Posttranslational Modifications

9:50- 10:15 E8-3
László Buday
Tks scaffold proteins in tyrosine kinase signalling
Institute of Enzymology, Research Centre for Natural Sciences, HAS, Budapest

----
10:15-10:35 ***Coffee break***

10:35- 10:50 E8-4
Beáta Lontay
Myosin phosphatase regulates gene expression via mediating arginine methylation in human hepatocarcinoma cells
Department of Medical Chemistry, Medical and Health Science Center, University of Debrecen, Debrecen

10:50-11:05 E8-5
Beáta Biri
The significance of the metastasis-associated S100A4 protein in cell adhesion and migration of A431 epithelial carcinoma cell line
Department of Biochemistry, Eötvös Loránd University, Budapest

11:05-11:20 E8-6
Anna Pallai
mTNF-alpha signaling inhibits LPS-induced proinflammatory cytokine formation by upregulating TGF-β in macrophages
Department of Dental Biochemistry, Research Center of Molecular Medicine, Debrecen

Session IX. Biocrystallography and Structural Biology

9:50-10:15 E9-3
Gergely Nagy
Composite aromatic box: structural motif for binding and enzyme-catalyzed conversion of quaternary ammonium substrates
Institute of Enzymology, Research Centre for Natural Sciences, HAS; Department of Biotechnology and Food sciences, Faculty of Chemical and BioEngineering, Budapest University of Technology and Economics, Budapest

----
10:15- 10:35 ***Coffee break***

10:35- 10:50 E9-4
Péter Ecsédi
Symmetric or asymmetric: interaction of S100 proteins with annexin-A2
Department of Biochemistry, Eötvös Loránd University, Budapest

10:50-11:05 E9-5
Gyula Batta
„Invisible" Conformations of a Disulfide Constrained Antifungal Protein Disclosed by Cold and Heat Unfolding, Nuclear Magnetic Resonance Saturation Transfer and Molecular Dynamics Calculations
Department of Chemistry and Biology, University of Debrecen, Debrecen

11:05-11:20 E9-6
Éva Gráczer
Essential role of E270 in K+-activation and domain closure of Thermus thermophilus isopropylmalate dehydrogenase
Institute of Enzymology, Research Centre for Natural Sciences, HAS, Budapest

Session IX. Biocrystallography and Structural Biology

11:20-11:35 E9-7
Katalin Nagy
Structural and kinetic studies on the skin-specific retroviral-like aspartic protease 1 (ASPRV1)
Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen

11:35-11:50 E9-8
Andrea Kiss
Role of phosphorylation in the regulation of nuclear transport of myosin phosphatase targeting subunit
Department of Medical Chemistry and Faculty of Medicine, University of Debrecen, Debrecen

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12:00- 13:00 ***General Assembly of the Hungarian Biochemical Society (HBS)***

13:00-14:15 ***Lunch***

Session X. Plenary Session: HBS Awardees

Chair: László Fésüs, HBS president

14:15-14:20 **Presentation of HBS Awards**

14:20-14:40 E10-1
Kata Sarlós (HBS Awardee 2013)
Translocation of RecQ helicase along single-stranded DNA
Department of Biochemistry,"Momentum" Motor Enzymology Research Group, Eötvös Loránd University, Budapest; Department of Cellular and Molecular Medicine, Faculty of Health Sciences, Panum Institute, University of Copenhagen, Copenhagen, Denmark

14:40-15:00 E10-2
Gergely Róna (HBS Awardee 2014)
Phosphorylation adjacent to the nuclear localization signal of human dUTPase abolishes nuclear import: structural and mechanistic insights
Institute of Enzymology, Research Centre for Natural Sciences, HAS, Budapest

Poster Session

15:00-18:00 Poster session P-1-65

18:00- ***Grill party***

27th August, 2014

Session XI. Biochemical Pharmacology

Chairs: Péter Arányi, György Kéri

9:00-9:25 E11-1
György Kéri
Signal transduction therapy of cancer targeting cancer driver genes related pathways
MTA-SE Pathobiochemistry Research Group, Department of Medical Chemistry, Semmelweis University; Vichem Chemie Research Ltd., Budapest

9:25-9:50 E11-2
Péter Arányi
Protein kinases – the most fashionable therapeutic targets
Ethics Committee for Clinical Pharmacology, Medical Research Council of Hungary, Budapest

9:50-10:15 E11-3
Sándor Bátori
Preclinical development of a kinolol derivative for Alzheimer's disease
Ubichem Research Ltd., Budapest

Session XII. Pathobiochemistry and Organelle Biochemistry

Chairs: Ferenc Gallyas, József Mandl

9:00-9:25 E12-1
Miklós Csala
Diabetes mellitus and the endoplasmic reticulum
Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest

9:25-9:50 E12-2
Balázs Sümegi
Prevention of poly(ADP-ribosyl)ation and inactivation of ATF4/Creb2 by PARP inhibitor protects cells via ATF4-MKP-1-MAPK pathway
Departments of Biochemistry and Medical Chemistry, University of Pécs, Medical School, Pécs; Nuclear-Mitochondrial Interactions Research Group, HAS, Budapest; [3]Szentágothai Research Center, Pécs

9:50-10:15 E12-3
Péter Bai
The role of PARP-2 in the regulation of SREBP1
Department of Medical Chemistry, Faculty of Medicine, University of Debrecen; Research Center for Molecular Medicine, University of Debrecen; MTA-DE Lendület Laboratory of Cellular Metabolism Research Group, Debrecen

Session XI. Biochemical Pharmacology

10:15-10:30 E12-4
Éva Csősz
Selected reaction monitoring as a versatile tool for biomarker discovery
Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen

10:30-10:45 E11-5
Tibor Docsa
Effect of carbohydrate-based glycogen phosphorylase(gp) inhibitors on the hepatic glycogen metabolism
Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen

10:45-11:00 E11-6
Endre Kókai
Characterization of the interaction between the adenosine receptor 2A and cathepsin D protease in macrophages
Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen

Session XII. Pathobiochemistry and Organelle Biochemistry

10:15-10:30 E13-4
Lilla Nagy
Glycogen phosphorylase inhibitors enhance pancreatic β –cell function and hepatic mitochondrial metabolism
Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen

10:30-10:45 E12-5
Tamás Kardon
Role of ADP dependent glucokinase in respiratory burst of differentiated HL-60 cells
Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University Budapest

10:45-11:00 E12-6
Szilvia Benkő
Molecular mechanisms of IL-1β production and NLRP3 inflammasome function in LPS-activated human macrophage subtypes
Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen

11:00-11:20
Poster Award Ceremony

Young Lecturer Award

Closing ceremony

Abstracts
E1 - Plenary lectures

E1-1

Butterflies, bacteria, books. My way...

Pál Venetianer,
Institute of Biochemistry, Biological Research Centre, HAS, Szeged

In this Béla Tankó Memorial lecture the awardee tries to summarize his scientific carreer, spanning more than fifty years, from classical enzymology to molecular genetics, acknowledgeing the roles of his masters and disciples, concentrating on selected papers that for some reason proved to be memorable for him, and commenting on some of his books.

E1-2

From Genes to Genomes and Back Again

László Nagy
Department of Biochemistry and Molecular Biology, Research Center of Molecular Medicine, University of Debrecen, Debrecen; *Sanford-Burnham Medical Research Institute, Orlando, FL, USA*

One of the most fascinating problems in biology is to understand how the different cells of the human body develop and function relying on the same genetic information or blueprint under normal physiological conditions and also in diseases. The principal and fundamental difference between the various cell types is the expression of their genomes. The distinct gene expression patterns are generated and maintained by molecular switches or transcription factors. As the result of the completion of mammalian genome projects and the technological developments of the past decade now it is possible to get a complete and comprehensive picture on the role and activity of such factors. It is also in our reach to create maps of the transcription factors' genomic binding activities, interactions with other factors and the identification of the network of regulated genes. We used a special group of molecular switches, so called nuclear hormone receptors, as the objects of our studies and characterized their activities in key cell types of the immune system such as macrophages and dendritic cells as our model systems during the last two decades. The lecture will illustrate how our understanding of mammalian gene regulation has changed by the comprehensive and integrated usage of genomics and bioinformatics analyses. We will also show that the gene regulatory networks uncovered provide insights into the integration of immune and metabolic processes.

E1-3

Understanding estrogen receptor transcription in breast cancer

<u>Jason S. Carroll</u>
Cancer Research UK, University of Cambridge, Cambridge, UK

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.

E1-4

Molecular mechanisms of autophagosome fusion with lysosomes in Drosophila

Szabolcs Takáts, Karolina Pircs, Péter Nagy, Ágnes Varga, Manuéla Kárpáti, Krisztina Hegedűs, Attila L. Kovács, Miklós Sass, Gábor Juhász
Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest

Autophagy is a catabolic process responsible for the degradation and recycling of all kinds of intracellular material, including organelles such as mitochondria. About 20 years ago, yeast genetic screens identified a set of evolutionarily conserved Atg genes required for autophagosome formation in eukaryotic cells. Functional analysis of the corresponding genes in different model organisms revealed that autophagy defects contribute to various human diseases. In contrast, how autophagosomes gain competence for fusion with lysosomes in animal cells has remained unknown for a long time. We carried out an RNAi screen for autophagy in *Drosophila*, and identified three genes encoding SNARE proteins essential for the fusion of autophagosomes with lysosomes. This SNARE complex assembles from Syntaxin 17 recruited to autophagosomes, ubisnap (SNAP-29), and the lysosomal membrane protein Vamp7. Moreover, we showed that the loss of *Syntaxin 17* results in neuromuscular dysfunction and early death of adult flies (Takats 2013 J Cell Biol). In addition, we identified all 6 subunits of the HOPS tethering complex in our screen as required for autophagosome-lysosome fusion. We found that HOPS physically binds to Syntaxin 17, and their interaction is specific for autophagy, as Syntaxin 17 appears to be dispensable for the fusion of endosomes with lysosomes and lysosome biogenesis, unlike HOPS (Takats 2014 Mol Biol Cell). Much of the work done on autophagy is based on the study of *Atg* genes, and aims to dissect the order in which these gene products orchestrate autophagosome formation. We think that identifying the molecular machinery involved in the clearance of autophagosomes, and elucidating how these factors function together to promote autophagosome-lysosome fusion will fill an important gap in our knowledge, and may open up new research avenues to explore in physiology and pathology. We thank the Hungarian Scientific Research Fund (OTKA K83509) and the Wellcome Trust (087518/Z/08/Z) for funding.

Abstracts
E2 - Membrane Biochemistry

E2-1
Beyond classical dimerization: subtle regulation of the clustering and function of ErbB proteins

Tamás Kovács, Ágnes Szabó, János Szöllősi, Péter Nagy
Department of Biophysics and Cell Biology, University of Debrecen, Debrecen

ErbB proteins (ErbB1-4, ErbB1=epidermal growth factor receptor, EGFR) constitute one of the most important growth factor receptor families. They are receptor tyrosine kinases whose activation is thought to involve ligand-induced dimerization. Although the basic phenomenon of dimerization-activation coupling has been convincingly shown to be essential, questions about the existence of larger receptor clusters in quiescent and stimulated cells and about the role of the plasma membrane in the regulation of receptor association have been debated for years. We have shown using flow cytometric homo-FRET measurements and N&B (number and brightness) analysis that ErbB1 is mainly monomeric in the absence of stimulation and it undergoes EGF-induced dimerization. However, at high expression levels even this receptor forms preformed receptor clusters. As opposed to this kind of behavior, ErbB2 forms large receptor associations involving tens of receptors in quiescent cells and these clusters disassemble upon activation. Large-scale clusters of ErbB2 colocalize with caveolin membrane domains. Although the ligand-induced changes in the association of receptors are thought to be regulated by the extracellular domain, we assumed that the transmembrane domain also plays a permissive role in these processes. We modified the membrane dipole potential, a potential due to the presence of oriented dipoles close to the surface of the membrane. Both constitutive and ligand-induced homoassociation of ErbB1 and ErbB2, and their heteroassociations increased upon elevating the dipole potential. This phenomenon was followed by enhanced ligand-induced tyrosine phosphorylation. In addition to changing the clustering of receptors the dipole potential also influenced the binding of ligands to the receptors. Taken together, these results show that the environment of ErbB proteins generated by the plasma membrane fine-tunes the clustering and functioning of these growth factor receptors. Grant support: OTKA 103906, 101337; TÁMOP-4.2.2.A-11/1/KONV-2012-0025.

E2-2
Chemical chaperon therapy reduces calcification in mice expressing misfolded human ABCC6 mutants

Viola Pomozi[1], Christopher Brampton[2], Krisztina Fülöp[1], Ailea Apana[2], Natália Tőkési[1], Olivier Le Saux[2], András Váradi[1]
[1]Institute of Enzymology, RCNS, HAS, Budapest; [2]Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawaii, Honolulu, HI, USA

The ABCC6 transporter is expressed mainly in the liver, in the basolateral plasma membrane compartment of the cells. Mutations in the *ABCC6* gene may lead to the development of two genetic diseases: Pseudoxanthoma elasticum (PXE, OMIM 26480) and Generalized Arterial Calcification of Infancy (GACI, OMIM 208000). Furthermore, a missing allele of *ABCC6* is a genetic risk factor in coronary arterial disease. In order to better understand how mutations in the *ABCC6* gene lead to development of abnormal calcification symptoms, and to establish potential therapeutic treatment for patients carrying *ABCC6* mutations, we have set up a complex experimental strategy to determine the structural and functional consequences of disease-causing mutations in the human ABCC6 transporter. The transport activity of the protein was determined *in vitro*, and the subcellular localization of wt and mutant proteins was investigated both *in vitro* and *in vivo*. The major aim of our study was to identify mutants with preserved transport activity but failure in intracellular targeting, as these mutants are candidates for functional rescue. Sodium 4-phenylbutyrate (4PBA), an FDA-approved drug, has been shown to act as a chemical chaperon and to restore the reduced cell surface expression of certain mutated plasma membrane proteins. An *in vivo* functional assay was set up to analyze the effect of 4-PBA to the function of ABCC6 variants. The expression of the human WT ABCC6 in mouse liver reduced significantly the calcification phenotype, while disease-associated ABCC6 mutants expressed in the liver of *Abcc6-/-* mice do not reduce calcification. However, administration of 4-PBA attenuates *in vivo* calcification by directing the mutants to the plasma membrane. These results indicate that 4-PBA restored both the localization and the physiological function of certain ABCC6 mutants, suggesting that 4-PBA treatment would be a feasible and viable therapeutic solution for selected patients with PXE and GACI. Funds: NIH RO1HL108249 USA, PXE International USA, OTKA NK 81204 and OTKA K 104227 Hungary.

E2-3

Calcium acts as a first messenger and suppresses colon cancer development by binding to the calcium sensing receptor (a G protein-coupled receptor)

Enikö Kállay
Department of Pathophysiology and Allergy Research, Medical University of Vienna,Vienna, Austria

In normal colonic epithelial cells high extracellular calcium inhibits proliferation and induces differentiation and this sensitivity is lost during colon tumourigenesis. Whitfield suggested that the molecular switch that turns on differentiation and off proliferation is the calcium-sensing receptor. Multiple signalling cascades, critical for cell survival, differentiation or cell death are mediated by calcium. Within the cell, calcium controls a number of signalling pathways, including those that regulate cell growth and cell death. Key to altering the function of the cell is changing the intracellular concentration of calcium, achieved by an interaction among membrane components, intracellular organelles, calcium pumps, and ion channels. Calcium is one of the best characterized second messengers in the cell. However, with the cloning of the extracellular calcium-sensing receptor it became evident, that calcium can act even as a first messenger by binding to this G protein-coupled receptor. The calcium-sensing receptor binds calcium and mediates its effects without facilitating immediate calcium entry. Even though downstream effects involving activation of phospholipase C and inositol trisphosphate could lead to opening of endoplasmic reticulum calcium stores and ultimately to store operated calcium entry. The calcium-sensing receptor interacts with various heterotrimeric G proteins to control selectively downstream signalling pathways. This cell-surface receptor functions as a key transducer of signals from the extracellular milieu to the intracellular environment. The calcium-sensing receptor integrates a variety of extracellular metabolic stimuli which preferentially activate distinct intracellular signalling cascades in a process known as ligand-directed targeting of receptor stimulus. CaSR signalling is specific for type of the cell, expression of G protein isoforms, enzymes, and adapter proteins that control the assembly of signalling scaffolds. Funded by the European Union Grant "Multifaceted CaSR", the Austrian Research Foundation, the Herzfelder'sche Familienstiftung.

E2-4

An attempt to modify reciprocally the opposed transamidase and γ-glutamyl hydrolase activities of human transglutaminase 2

Róbert Király[1], Kiruphagaran Thangaraju[1], Zsófia Nagy[1], Russell Collighan[2], Zoltán Nemes[1], Martin Griffin[2], László Fésüs[1, 3]
[1]Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen; [2]School of Life and Health Sciences, Aston University, Birmingham, UK; [3]Stem Cell, Apoptosis and Genomics Research Group of Hungarian Academy of Science, Debrecen

Transglutaminase 2 (TG2) is a multifunctional and conserved protein in humans taking part in various intra- and extra-cellular processes. TG2 has different enzymatic activities. The best studied one is the Ca^{2+}-dependent transglutaminase activity which covers several kinds of protein modifications. TG2 also has a poorly studied isopeptidase activity where the previously formed isopeptide bonds are cleaved. Our aim was to analyse the effect of site directed mutations on these opposed activities and determine whether it is possible to separate the transamidase and isopeptidase activities of TG2. TG2 mutants have been found which are deficient in but still have some remnant isopeptidase or transamidase activity (0-30% based on the applied assays) and have similar or significantly higher other type of activity compared to the wild type TG2. Their activities, kinetic parameters, GTP binding have been analysed. As another approach to separate the different type of transglutaminase activities inhibitors were tested on isopeptidase and transamidase activities of TG2. We have found inhibitors which have slightly different effect on the different type of transglutaminase activities. The developed models may be applicable to reveal the unknown roles of different types of transglutaminase activities in biological processes. Support: Magyary Zoltán Postdoctoral Scholarship*, TRANSCOM – Marie Curie IAPP, TRANSPATH – Marie Curie ITN, Research University grant from the University of Debrecen, Hungary. *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 – Elaborating and operating an inland student and researcher personal support system convergence program'.

E2-5

CHO-MT58: A model system for functionality studies of *Plasmodium falciparum* CTP:phosphocholine cytidylyltransferase

Lívia Marton, Gergely N. Nagy, Nóra Kucsma, Gergely Szakács, Beáta G. Vértessy
Institute of Enzymology, Research Center for Natural Sciences, Hungarian Academy of Sciences

Plasmodium falciparum malaria is still one of the most threatening infectious diseases. In *P. falciparum* phospholipid biosynthesis has a key role in synthesis of membranes and membrane elements. The most prevalent way of *de novo* biosynthesis is Kennedy-pathway, where the reaction catalyzed by CTP:phosphocholine cytidylyltransferase (CCT) (CTP+ChoPCDPCho) is rate-limiting (Nagy et al. *FEBSJ* 2013). 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. CHO-MT58 cell line was proved to be an appropriate tool for investigating intracellular function of CCT harboring a point mutation in its endogenous CCT, causing thermo-sensitivity.[3] At 40 °C the endogenous CCT activity decreases dramatically, which blocks membrane synthesis and ultimately leads to apoptosis. Whereas supply of external phosphatidylcholin or heterologous expression of rat CCT can successfully complement the mutant strain. our aim was to develop a model system using this inducible CCT "knock out" cell line to study the functionality of heterologously expressed *Pf*CCT constructs in cellular environment, excluding the effect of endogenous CCT. In this study we transfected CHO-K1 and CHO-MT58 cells with CCT constructs and incubated them at permissive and non-permissive temperatures. To verify the rescue we performed FACS with these and with cells transfected with catalytically inactive *Pf*CCT as control. Having obtained a higher ratio of active *Pf*CCT expressing cell population at 40°C than at 37°C, 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. Besides, the possibility to assess the functionality of mammal or *Pf*CCT enables selectivity test of novel antimalarials acting on this pathway. 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'.

E2-6

The role of septins through exerting influence on store operated Ca^{2+} entry (SOCE) in chondrogenesis

Roland Takács[1], Csaba Matta[1], Tamás Juhász[1], János Fodor[2], László Csernoch[2], Nicolai Miosge[3], Róza Zákány[1]
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Septins belong to a family of proteins that is highly conserved in eukaryotes and is increasingly recognized as a novel component of the cytoskeleton. All septins are GTP-binding proteins that form hetero-oligomeric complexes and higher-order structures, including filaments and rings. Septins form plasmamembrane domains juxtaposed to the endoplasmic reticulum membrane punctae which are important in the STIM1 – ORAI1 signalling. The knock down of certain septin types (2, 4 and 5) or the inhibition of their remodeling inhibits SOCE and the related downstream signalling processes. Septins have no previously described role in chondrogenesis and their role in other differentiational processes is also sparsely described. According to previous results of our laboratory, SOCE is necessary for chondrogenesis, therefore we aimed to provide a link between septin function and chondrogenesis. Our studies were carried out in migratory chondrogenic progenitor cells (CPC) differentiated in alginate pearls. We verified the expression of the septin types associated with SOCE and the components of SOCE at the mRNA level. The pearls, containing 20.000 cells each, were treated with the inhibitor of septin remodeling, forchlorfenuron (FCF). We performed transient gene-silencing of the discussed septin types with an shRNA vector designed based on a published study, examined SOCE functions using cells loaded with the calcium-sensitive dye Fura-2 with an appropriate set-up to measure single cell calcium levels. Eventually, we compared chondrogenesis in control pearls to FCF-treated and shRNA-treated pearls by chondrogenic marker expression-analysis and histological stainings. Our results demonstrate the presence of a link between SOCE and chondrogenic processes in CPC cells. Further elucidating this link and our present findings provide a novel function of the septin protein family. This work was sponsored by the grant TÁMOP-4.2.2/B-10/1-2010-0024.

Abstracts
E3 - Genomics and Epigenetics

E3-1
Mechanistic insights into the transcriptional arrest in the presence of Double Strand Breaks

Tibor Pankotai[1,2], Celine Bonhomme[2], Audrey Furst[2], David Chen[3], Dirk Eick[4], Isabella Sumara[2], Evi Soutoglou[2]

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Double-strand breaks (DSBs) occur frequently in the genome during genome replication or by DNA damaging agents. DNA lesions affect fundamental DNA-dependent nuclear processes, such as replication and transcription. We have developed an experimental system where DSBs are induced at coding regions of RNA polymerase II transcribing genes. We have started to study the kinetics of RNA polymerase II transcription inhibition in the presence of DNA breaks. We observed that induction of the break led to transcription inhibition and the restoration of transcription closely followed the dynamics of the repair of breaks. We confirmed by chromatin-immunoprecipitation that the break induction led to displacement of RNA polymerase II affecting both the elongation and the initiation of transcription. Our results show that this is dependent on one of the major kinase in DNA damage repair called DNAPKcs. We also investigated the downstream steps of RNA polymerase II removal and we claimed that it was a multistep process involving additional kinases and ubiquitin ligases NEDD4 and CUL3. At the last step of break dependent transcriptional silencing the RNA polymerase II is targeted for proteasome dependent degradation. These data demonstrate that the DNA damage repair complexes and proteasomal system have a synergistic and active role in transcriptional silencing during the DSB repair by removing the RNA pol II from the transcribing region. We show here that DNA lesions occurring at transcribed regions cause a transient repression until the lesion is repaired. This is probably a cell defense mechanism to avoid production of truncated or mutated transcripts in essential genes whose alterations in their gene expression would endanger cell viability. Understudying the role of DNAPKcs, in preventing RNA pol II bypassing a DSB might be a key in avoiding the production of mutated transcripts that could lead to cancerous phenotypes.

E3-2
Transcriptional Interference Networks

Zsolt Boldogkői[1], Péter Oláh[1], Csanád Imreh[2], Dóra Tombácz[1]

[1]Department of Medical Biology, Faculty of Medicine, University of Szeged, Szeged; [2]Department of Computer Algorithms and Artificial Intelligence, Faculty of Science and Informatics, University of Szeged, Szeged

Earlier we proposed the existence of a previously unrecognized layer of genetic regulation termed transcriptional interference networks (TINs), which is based on the interplay between the adjacent genes through their transcriptional machineries. TINs are essential genetic units, which are assumed to be especially important in processes exhibiting a definite time course of gene expressions, such as embryonic development, tissue regeneration, response to external stimuli, metabolism, viral life cycle etc. The function of TINs is supposed to coordinate the switch between the ON and OFF states of the interacting genes, as well as to reduce the transcriptional noise. Furthermore, TINs simplify the regulation of genes, because they diminish the need for the change in the composition and/or the chemical modifications of the transcription factors. The operation of TINs is demonstrated by the analysis of gene expression and genomic organization of pseudorabies virus, a neurotropic herpesvirus. The transcriptome of the virus has been analyzed using real-time RT-PCR, as well as Illumina HiScan and PacBio RSII platforms. Additionally, we have developed a mathematical approach based on graph-based modeling of the TINs.

E3-3

Functional annotation of Estrogen Receptor binding sites in the light of the 1000 Genomes Project

Dóra Bojcsuk[1], Attila Horváth[1], Lilla Ozgyin[1], Edina Erdős[1], Endre Barta[1], László Nagy[1,2] and Bálint László Bálint[1]
University of Debrecen, Faculty of Medicine, Department of Biochemistry and Molecular Biology, Center for Clinical Genomics and Personalized Medicine; [2]MTA-DE "Lendulet" Immunogenomics Research Group, Debrecen

Estrogen Receptor is a key determinant of breast cancer development and progression, both the presence and the absence of the Estrogen Receptor (ESR1) is a critical component of the signaling pathways in the disease. The most widely used cell line to investigate breast cancer is the MCF-7 cell line, isolated in 1970 from the pleural effusion of a 69 years old breast cancer patient. MCF-7 cells are growing only in the presence of estrogen, the phenol red used in the media is sufficient activator for its growth. Although MCF-7 cells contributed to major discoveries in the field of estrogen receptor signaling, its wide usage in many laboratories conducted to results that had significant disconcordances. We decided to perform a meta-analysis of the published MCF-7 ESR1 ChIP-Seq datasets available in the public databases and to identify the common set of the Estrogen Receptor binding sites in the human genome based on all the published datasets. Using the results of the meta-analysis, we investigated the impact of the individual SNP-s available in the dbSNP and 1000Genomes Project databases on the signaling patways in these cells. We have identified the genes that have intronic binding sites for Estrogen Receptors and the Upstream Regulators of these genes.

E3-4

Tissue-specific aspects of UVR8 photoreceptor controlled signalling in Arabidopsis thaliana

András Viczián[1], Kata Terecskei[1], Roman Ulm,[2], Ferenc Nagy[1]
[1]Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Szeged; [2]Department of Botany and Plant Biology, University of Geneva, Geneva, CH

UV-B RESISTANCE 8 (UVR8) photoreceptor initiates UVB-specific signalling cascades orchestrating expression of a range of genes resulting in different developmental responses. Despite this broad range of effects, UV-B can not penetrate deep into the plant tissues. UVR8 is expressed uniformly in the model plant Arabidopsis thaliana but the spatial aspects of UVR8 action are not yet elaborated. The aim of our study is to unravel to what extent the cell/tissue specific signalling contributes to UVR8-controlled responses. To this end we created transgenic uvr8 mutant plant lines expressing UVR8 fused to the YELLOW FLUORESCENT PROTEIN (YFP) under the control of different tissue-specific promoters. The detailed characterization of these lines including YFP-UVR8-dependent photomorphogenesis and observation of UVR8-dependent changes in gene expression has also been performed. We used these transgenic lines to monitor how tissue-specific expression of UVR8 can regulate the nuclear accumulation of the ELONGATED HYPOCOTYL 5 (HY5) transcription factor. Furthermore, we could analyse the tissue-specific aspects of induction of several UV-B responsive promoters, including HY5. Our results suggest that UVR8-controlled signalling is at least partly mediated in a tissue-autonomous fashion. The work was supported by grants OTKA-81399, K-108559 and TAMOP-4.2.2.A-11/1/KONV-2012-0035.

E3-5

Deubiquitylases involved in maintenance of ubiquitin homeostasis

Levente Kovács[1], Olga Nagy[2], Margit Pál[1,2], Octavian Popescu[3], Péter Deák[1,2]
[1]*University of Szeged, Faculty of Sciences, Department of Genetics, Szeged;* [2]*Institute of Biochemistry, Biological Research Centre, HAS, Szeged;* [3]*Interdisciplinary Institute of Bio-Nano-Sciences, Molecular Biology Center, Cluj-Napoca, Romania*

Ubiquitylation is a reversible posttranslational modification in which the removal of the ubiquitin moieties is mediated by a family of proteins known as deubiquitylating enzymes or DUBs. In Drosophila, 46 DUBs have been identified, but the function of most of them is still unknown. Genetic analysis of mutant phenotypes in the Drosophila model organism can provide important information in elucidating the function of DUBs. Usp5 is 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 an other, proteasome-associated DUB, DmUbp6 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 DmUbp6 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 DmUbp6 - moderating ubiquitin homeostasis in Drosophila. This work is supported by HURO/1101/173/2.2., TAMOP-4.2.2.A-11/1/KONV-2012-0035 and TAMOP-4.1.1.C-13-1-KONV grants.

E3-6

Identification of STAT6 as a transcriptional repressor during alternative macrophage activation

Zsolt Czimmerer[1*], Bence Dániel[1*], Attila Horváth[1*], Gergely Nagy[1], Laszlo Nagy[1,2]
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Macrophages are participating in the maintenance of tissue homeostasis, protection against pathogen infections and tissue repair. Macrophage pehenotypes and functions are influenced by their molecular microenvironment including pathogen-derived molecules, cytokines and lipid mediators. The two end points of macrophage polarization are INFγ/LPS-mediated classical (M1) and IL-4/IL-13-induced alternative (M2) macrophage activation. M1-type macrophage activation results in enhanced bactericidal capacity and pro-inflammatory properties. In contrast, M2-type macrophages have anti-inflammatory properties, and therefore participate in tissue regeneration, protection against parasitic infections and also in the regulation of tumor progression. IL-4 activates both IL4Rα-JAK-STAT6 and phosphoinositide 3-kinase (PI3K) pathways resulting in alternative macrophage activation-specific gene expression changes. Although, the mechanism of IL-4/STAT6-dependent transcriptional activation is well understood, the IL-4-regulated transcriptional repression is not characterized. We combined genome-wide ChIP-seq and RNA-seq analysis for better understanding of the IL-4-mediated negative regulation of gene expression in murine macrophages. We characterized the STAT6 cistrome and H4 acetylaton in IL-4 stimulated mouse macrophages and we found extensive overlap between STAT6-bound and H4 acetylated genomic regions. We observed that one cluster of IL-4-induced STAT6 peaks was associated with decreased H4 acetylation. Next, we compared the STAT6-bound genomic regions to IL-4-regultated genes and we could show extensive association between decreased H4 acetylation at STAT6-bound region and IL-4-mediated repression. In addition, we identified several immunologically relevant IL-4-repressed genes including Fos, CD14, Tlr2 and Nlrp3 in association with decreased H4 acetylation at STAT6-bound region. Taken together, these results suggest that IL-4 may modulate immunological properties of macrophages via STAT6-mediated direct transcriptional repression. Currently we are in the process of identification of the mechanism of IL-4/STAT6-mediated direct transcriptional repression in macrophages using genomic and biochemical approaches.

Abstracts
E4 - Cell Death and Differentiation

E4-1

Tibor Vellai

E4-2

Plasticity of blood cell lineages and hematopoietic compartments in *Drosophila melanogaster*

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During the last century, the fruit fly, *Drosophila melanogaster* proved to be an excellent model organism to study innate immunity. Similarly to vertebrates, the blood cells (hemocytes) of the fly differentiate in multiple waves and are localised in separate hematopoietic compartments. In our studies, we used an *in* vivo transgenic lineage tracing system to follow the fate of hemocyte lineages throughout metamorphosis. We concluded that, although the hematopoietic compartments of the larva differentiate from independent hemocyte lineages, the compartments act in concert to defend the larva from invaders. We also showed that phagocytic hemocytes of the larva (the plasmatocytes), which were previously believed to be terminally differentiated blood cells, are capable of transforming into non-phagocytic, capsule forming cells, the lamellocytes. The plasmatocyte-lamellocyte conversion involves dramatic changes in cell morphology, marker expression and cell function. Our next goal is to understand the mechanisms that regulate the integrity and concerted action of the hematopoietic compartments and to identify the factors that are responsible for plasmatocyte plasticity. 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 GIBV).

E4-3

Macrophages engulphing apoptotic cells produce a novel retinoid to enhance phagocytosis as well as apoptosis of neglected thymocytes

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The thymus provides the microenvironment, in which thymocytes develop and reach the mature T cell stage. Due to the random nature of T cell receptor (TCR) production, 90% of CD4+CD8+ thymocytes produced express a TCR that does not recognize peptide loaded self MHC molecules found in the thymus and will undergo a default death pathway named "death by neglect". The thymocyte apoptosis program *in vivo* is completed by the clearance of apoptotic cells by professional phagocytes. Our previous studies indicated that the in vivo apoptosis program of thymocytes involves retinoid production by engulfing macrophages. Here we show that lipid content of the engulfed apoptotic cells will trigger the synthesis of retinoids in macrophages. The retinoids produced enhance the phagocytosis capacity of macrophages by activating retinoid receptor dependent signaling pathways, which upregulate the expression of various phagocytosis receptors. In addition, the retinoids are released and enhance the apoptosis of neglected thymocytes. Retinoids induce apoptosis via activating retinoid receptor (RAR)γ, which initiates an apoptosis program dependent on the synthesis of Nur77. Glucocorticoids are thought to be produced locally by thymic epithelial cells to initiate apoptosis in the neglected thymocytes. Retinoids also enhance the glucocorticoid-dependent apoptosis of thymocytes acting via RARα/RXR. We show that the ligated receptor directly interacts with the glucocorticoid receptor to stimulate its transcriptional activity. Our data indicate that a complex crosstalk between apoptotic cells and macrophages ensures the efficient death and clearance of neglected thymocytes, and one of the mediators of this crosstalk is a new not fully characterised retinoid. Supported by OTKA K104228, K83865, NK105046) and the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 „VÉD-ELEM" project. The project is implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.

E4-4

Consequences of death receptor mediated apoptotic cleavage of the plasma membrane Ca^{2+}ATPase

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Calcium plays an important role in almost all cellular processes including cell proliferation, differentiation and cell death. Plasma membrane Ca^{2+}ATPases (PMCA) are essential elements of the calcium handling toolkit: their main task is to remove excess calcium from the cytosol after many kinds of stimuli. Previously we showed that PMCA4b is cleaved and irreversibly activated during apoptosis (1-3). Here we studied death receptor mediated apoptotic fragmentation of PMCA4b and its effect on cellular Ca^{2+} handling. We developed an sh-PMCA4 HeLa cell line in which the endogenous PMCA4b expression was diminished. To achieve high PMCA4b expression, HeLa cells were transfected with PMCA4b constructs. Both PMCA4b expressing and sh-PMCA4 HeLa cells showed typical apoptotic morphology – nuclear fragmentation and apoptotic blebbing – upon anti-Fas antibody treatment. Moreover, PMCA4b retained its plasma membrane localization all along the treatments. However, the propidium iodide uptake of the cells, which indicates decreased plasma membrane integrity, was much more pronounced in sh-PMCA4 than in PMCA4b expressing cells. To test the integrity of calcium homeostasis of the apoptotic cells, we co-transfected HeLa cells with mCherry-PMCA constructs and the genetically encoded fluorescent calcium indicator, GCaMP2 and induced cytosolic Ca^{2+} increase by the calcium ionophore, A23187. In anti-Fas treated PMCA4b expressing HeLa cells, the A23187 induced calcium signal was significantly reduced compared to cells with diminished PMCA4b expression. Previously we showed that the PMCA4b-ct125 mutant corresponding to the caspase-3 cleavage fragment was fully and constitutively active. Here we found that expression of mCherry-PMCA4b-ct125 in Hela cells eliminated excess Ca^{2+} more quickly than the full length mCherry-PMCA4b. These data suggest that the apoptotic fragmentation of PMCA4b results in effective Ca^{2+} clearance from the apoptotic cells preventing excessive calcium overload. Supported by OTKA CK 80283 and K 101064 and TRANSRAT KMR_12-1-2012-0112.

References: 1) Pászty et al. (2007) Ann N Y Acad Sci, 1099: 440-50; 2) Pászty et al. (2005) Biochem J, 391: 687-92; 3) Pászty et al. (2002) J Biol Chem, 277: 6822-9.

E4-5

Irisin modifies the differentiation program of subcutaneous human adipocytes and induces "browning" *ex vivo*

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Recent studies showed high incidence of metabolically active brown adipose tissue (BAT) in healthy adult humans and highlighted the strong negative correlation between obesity and BAT amount. There are at least two different thermogenic fat depot types because humans have "classical brown" and "beige" adipocytes. Irisin is a recently identified peptide hormone which induces a browning program in subcutaneous white adipose tissue in mouse models. In humans, however, an escalating debate revolves around the secretion and metabolic effects of irisin. Our aim was to clarify whether human recombinant irsin was able to induce a browning program on differentiating human adipocytes. Human primary preadipocytes obtained from herniotomy were differentiated into white or brown adipocytes with or without long or short-term irisin treatment. Expression of white, brown and general adipocyte markers were determined by RT Q-PCR or immunoblotting and changes in morphology (size of lipid droplets, Ucp1 and Cidea content of single adipocytes) were visualized and quantified by Laser Scanning Cytometry. Functional analysis was carried out using a Seahorse Bioscience XF-96 Analyzer.

Irisin administration during white adipogenic differentiation resulted in a significant overexpression of brown adipocyte marker genes (UCP1, ELOVL3, CIDEA, CYC1, PGC1A). Irisin treated cells had smaller lipid droplets, more mitochondrial DNA, higher functional mitochondrial respiration and contained more Ucp1 and Cidea protein than the *in vitro* differentiated white adipocytes. We conclude that irisin is able to induce a browning program on differentiating human subcutaneous white adipocytes *ex vivo*. 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' which provided personal support to E. K., TÁMOP-4.2.2.A-11/1/KONV-2012-0023 grant and the Hungarian Academy of Sciences.

E4-6

PRMT1 and PRMT8 regulate retinoic acid dependent neuronal differentiation with implications to neuropathology

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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, transcriptional co-factors required for cell and gene-specific retinoid signaling are not known. We found that Protein aRginine Methyl Transferase (PRMT) 1 and 8 have 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 "hotspots". PRMT8 is a retinoid receptor target gene itself and acts as a cell type specific transcriptional co-activator of retinoid signaling at later stages of differentiation. Lack of either of them leads to dysregulated neuronal gene expression and altered neuronal activity. Importantly, PRMT8 regulates a distinct set of genes, including markers of gliomagenesis. PRMT8 is almost entirely absent in human glioblastoma tissues. We propose that PRMT1 and PRMT8 serve as a rheostat of retinoid signaling to determine neuronal cell specification in a context-dependent manner, and might also be relevant in the development of human brain malignancy.

Abstracts E5 - Omics: Lipidomics, Proteomics

E5-1
Protein phosphorylation – enrichment and mass spectrometry analysis

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Phosphorylation is one of the most important post-translational modifications (PTM). The majority of intracellular proteins bear this PTM. It has regulatory functions; consequently, it is of transient nature and generally occurs at low abundance. Extracellular proteins also can be phosphorylated. Some Golgi-residing kinases have been identified recently. An indispensable analytical tool in the characterization of protein phosphorylation is mass spectrometry. High accuracy mass measurements and characteristic fragmentation upon collisional activation facilitate peptide identification, as well as modification site assignments. However, successful phosphorylation analysis requires enrichment of the modified peptides. The most straightforward approach is metal ion affinity chromatography utilizing preferential phosphate binding to certain metal ions (Fe^{3+}, Ga^{3+}, Ti^{4+}). Binding conditions greatly influence the efficiency and selectivity of the enrichment. This is especially crucial in complex mixtures where different modifications of acidic nature (e.g. glycopeptides with terminating sialic acid) interfere with phosphopeptide enrichment. Potentials and limitations of mass spectrometry paired with the enrichment methods for the analysis of protein phosphorylation will be illustrated with two different biological examples: i) PTM characterization of a single protein: *in vivo* phosphorylation of phytochrome B participating in light adaptation of plants and ii) phosphorylation analysis of a protein mixture of wide complexity and dynamic range, with a variety of other PTMs: the identification of phosphorylated proteins in human serum.
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E5-2
Platelet storage lesion and release of extracellular vesicles from senescent platelets: a lipidomic and proteomic approach

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Aging platelets develop storage lesion, characterized by decreased activation in response to agonists and enhanced release of platelet extracellular vesicles (PL-EVs). We have established an *in vitro* model for human platelet senescence by 5 days blood banking of platelet concentrates. Platelets and subfractions of PL-EVs were characterized in the presence/absence of high density lipoprotein subfraction-3 (HDL_3)/ apolipoproteinA-I (apoA-I) by nanoparticle tracking analysis, flow cytometry, lipidomic and proteomic profiling. Subsets of PL-EVs were separated into subfractions by density gradient centrifugation. Different PL-EVs show overlapping size of 60-260 nm, but differ in their lipid and protein composition. Low-, intermediate- and high-density PL-EVs are enriched in lipid markers for plasma membrane, intracellular membrane/granule and mitochondria, respectively. In addition they also carry several protein markers, implicated for neurodegenerative diseases. The exosomes are enriched in markers of lipid-rafts and multivesicular bodies. During platelet senescence, HDL_3/apoA-I reduce PL-EVs by 62%, and the decrease correlates with the concentration of added apoA-I. In parallel, the agonist-induced platelet activation increases, indicating improved platelet function. In addition, HDL_3/apoA-I promotes cholesteryl ester exchange and increases the ratio of bis(monoacylglycero)-phosphate/ phosphatidylglycerol as well as phosphatidic acid/ lysophosphatidic acid. Different lipid composition of PL-EVs suggests their unique origins, overlapping with platelet granule secretion. Dense PL-EVs likely represent autophagic vesicles released during platelet activation, and exosomes resemble multivesicular bodies and lipid-rafts. HDL_3/apoA-I influences membrane homeostasis of platelets by improving intracellular lipid processing and vesicular transport. Supported by the European Community FP7/2007-2013, grant No. 202272 IP-LipidomicNet.

E5-3

Lipids, as key players in cellular stress management and in pathogenesis of metabolic syndrome

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Earlier we reported how membrane composition could modulate the stress response. The numerous sensors at the top of the pathways are interconnected by the parameters of the chemical and physical state of membrane. Membrane alterations, signal pathways from membranes to heat shock protein (Hsp) genes and Hsps themselves play fundamental roles in the aetiology of several human diseases, such as type 2 diabetes and cancer. Improving the cellular stress response via Hsp induction provided protection against insulin resistance after consuming a high fat diet, and was coupled with reduced fat stores in mice. Interestingly, acute heat stress is able to activate lipid droplet (LD) formation in different cell lines. Moreover, in the HSF1 -/- MEF cells, where the heat-inducibility of Hsps is almost completely abrogated, the LD formation was largely enhanced upon thermal stress. To gain insight into interconnection between stress response, membrane-lipid signalling and LD biogenesis, the yeast model *Schizosaccharomyces pombe* was studied. Mass spectrometric analysis revealed that *S. pombe* responded to heat stress with profound reorganisation of its whole lipidome. In addition to the bilayer stabilizing compositional shift of membrane lipids, enhanced production of signal lipids, and the storage lipid triglyceride (TG) were observed. In the trehalose synthase yeast mutant, where trehalose – that has been shown to stabilize proteins and membranes during heat shock – cannot be formed, the stress-induced TG synthesis and the signal lipids generation were more pronounced than in wild-type cells. Furthermore, the almost totally TG-synthesis deficient (dga1/plh1) mutant *S. pombe* cells displayed cell cycle arrest and striking elevation of diglyceride level upon heat stress. Consequently, LDs may have a key role in the stress-regulation of lipid mediator and membrane lipid metabolism. Moreover, we suggest that LDs may have some unexpected "holdase"-like function as protective reservoirs for unfolded proteins formed due to stress. S*upported by grants from the Hungarian Basic Research Fund (OTKA NK100857, K82097, PD109539).*

E5-4

The role of lipid metabolism during the *Drosophila* spermatogenesis

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The existence of similar male sterile phenotype in flies, mice and human strongly suggests that many of the genes required during spermatogenesis have been evolutionary conserved. Male sterile mutations of *Drosophila* exhibit a broad range of phenotypes and affect all stages of spermatogenesis. Spermatid individualization is an especially interesting step of the spermatogenesis because it requires an unusual amount of membrane remodelling using a well-defined actin structure. *Drosophila* spermatids increase 150-fold in length and fivefold greater total surface area following individualization. Increasing number of lipid metabolic enzymes show important role during all stages of spermatogenesis, for example in the biosynthesis of phosphatidylinositol and their phosphorylated forms. We have started the genetic characterization of mutant lines in which membrane transport related genes are affected. One of them is the *CdsA* gene which encodes for phosphatidate cytidyltransferase, CdsA enzyme, which catalyzes the synthesis of CDP-DAG from phosphatidic acid, which is an important player of phosphatidylinositol and cardiolipin biosynthesis. Phosphatidic acid itself and phosphoinositides play important roles in lipid signalling and membrane trafficking, while cardiolipin is an important component of the inner mitochondrial membrane. We characterized the *CdsA^{ms}* mutant with classical and molecular genetic methods and analysed its lipid composition using mass spectrometry. Lipidomic analysis of the mutant revealed the importance of phosphatidic acid in mitochondrial membrane formation during spermatid development. Supoorted by EMBO Installation Grant No.1825, OTKA NF 101001 and TÁMOP 4.2.4.A/2-11-1-2012-0001.

E5-5

A flexible experimental system to study *in vivo* protein phosphorylation in plants

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Our knowledge about protein kinase substrates in plants is scarce. Protein kinases are important components of signalling pathways, and due to sessile lifestyle, there are about twice as many kinases in plants than in mammals. Plant growth is highly dependent on changing environmental conditions, thus understanding the mechanisms by which plants regulate adaptation to environmental factors is of paramount importance. Mitogen-activated protein kinase (MAPK) cascades are well-conserved signalling modules in all eukaryotes. In plants, MAPK pathways have been mainly shown to play important roles in stress signalling, but they are also involved in regulating developmental processes. Plant MAPKs are most similar to human ERK-type MAPKs. ERK1/2 have over 100 substrates, whereas there are only about ten known *in vivo* substrates in the model plant *Arabidopsis*. Because knowledge on substrates is essential to functional understanding of MAPK pathways, the aim of our research is to identify novel MAPK substrates in plants. To this end, we have developed an efficient experimental system, in which substrate candidates are transiently expressed in protoplasts with or without co-expression of activated MAPKs. Changes in phosphorylation status of the expressed proteins are detected by separation of differently migrating phosphorylated and non-phosphorylated isoforms. Samples are analysed by capillary isoelectric focusing coupled with nanofluidic immunoassay. This system is extremely sensitive, capable of quantitatively detecting subtle changes in isoform distribution. To the best of our knowledge this novel, state-of-the-art method has not been yet used in plant research. Furthermore, expression of epitope-tagged proteins in transfected protoplasts enables highly flexible experimental arrangements. We believe that this efficient experimental approach to identify kinase substrates in plant cells has a great potential to contribute to our understanding of signalling pathways in plants. Supported by OTKA grant K101250. R.D. is a Bolyai Fellow of the Hungarian Academy of Sciences.

E5-6

Targeted proteomics method and multiplexed immunobead-based assay for identification of salivary biomarkers in oral squamous cell carcinoma

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Oral squamous cell carcinoma (OSCC) constitutes 90% of oral cancer, which is the 6th most common malignancy with increasing incidence and mortality rate. Hungarian population occupies the top places of statistics regarding OSCC incidence. Current diagnostic and screening tools for the OSCC are scalpel biopsy and histopathological evaluation. Delayed detection is likely to be a primary reason for the discovery of the high mortality rate and this supports the need for biomarkers to improve early detection. The human saliva as a biological fluid for the detection of diagnostic and prognostic biomarkers could be collected easily and non-invasively. The analysis of tumor-specific proteins may provide an opportunity for early diagnosis and detection of OSCC.

The aim of our research was to develop and validate a targeted proteomic SRM (Selected Reaction Monitoring) based method for examination of potential oral cancer protein biomarkers previously reported in the literature. Furthermore our aim was to determine the level of previously reported OSCC-related salivary cytokines using multiplexed immunobead-based technology (Luminex). Using these two methods we would like to establish an SRM-based OSCC biomarker panel valid for the Hungarian population. SRM method was developed for 14 human proteins and in a pilot experiment samples from 5 control and 12 OSCC patients were analyzed in duplicates.

In case of multiplex measurements a custom 6plex Milliplex kit was used to analyze 9 control and 26 OSCC patient samples in duplicates. The levels of IL-1 alpha, IL-1 beta, IL-6, IL-8, TNF-alpha and VEGF were examined. Our results show that we could set up a targeted proteomic approach for detection and relative quantification of 14 previously reported OSCC related proteins from human saliva. Based on the preliminary results protein S100-A9, catalase, thioredoxin, IL-6, IL-8 and TNF-alpha seem to be useful biomarkers for OSCC detection in the Hungarian population. Funded by OTKA 105034 grant.

Abstracts
E6 - Stem Cells

E6-1

Alternative Sources of Stem Cells for Tissue Repair

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The discovery of a defined set of transcription factors that can induce reprogramming of somatic cells to pluripotent stem cells (iPSCs) has had an unprecedented impact on our view on future cell transplantation-based tissue repair and restoration of faulty physiological functions. Somatic cell reprogramming is a several weeks long process through which cells reach pluripotency, the developmental state similar to embryonic stem cells. This cascade of events and the driving forces behind the phenomenon are very poorly understood. It is, however, crucial to uncover the fine details of this process in order to comprehend the true properties of iPSCs and so better tailor their future therapeutic use. We have recently developed a reprogramming method utilizing a transposon-mediated delivery of the reprogramming transgenes. This system has several advantages over the viral delivery-based alternative. Most notably, it allows for a seamless removal of the transgenes once pluripotent stem cells have been generated and they are no longer needed for stem cell self renewal. We also combined the doxycycline inducible transgene expression system with the transposon delivery-based reprogramming and found that these transgenes are very efficiently regulatable by adding or withdrawing doxycycline. *In vivo* differentiated somatic cells derived from iPSCs can be reprogrammed to "secondary" iPSCs (2ºiPSc) by simply adding doxycycline to the culture medium. By analyzing the effect of reprogramming factor expression dynamics on the reprogramming process, we have discovered additional classes of pluripotent cells that might have a different spectrum of phenotypes than that of ES cell-like iPS cells. Here, we explore alternative outcomes of somatic reprogramming by fully characterizing reprogrammed cells independently of preconceived definitions of partial or fully reprogrammed iPSC states. These alternative pluripotent states may have different therapeutic values depending on the target disease.

E6-2

Patient specific induced pluripotent stem cells and their neuronal differentiation

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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 disease 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 diseased iPSCs. Here we compared the usage 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 measurements. We have established a highly reproducible and efficient procedure to generate patient-specific neuronal cells form iPSCs. Supported by grants from EU FP7 projects (STEMMAD, PIAPP-GA-2012-324451; STEMCAM, PIAP-GA-2009-25118; Anistem, PIAPP-GA-2011-286264; EpiHealth, HEALTH-2012-F2-278418; EpiHealthNet, PITN-GA-2012-317146; D-BOARD, FP7-HEALTH-2012-INNOVATION-1-305815) and the SZIE Research Project of Excellence (17586-4/2013/TUDPOL).

E6-3

ABC transporters in human pluripotent stem cells

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ATP-binding Cassette (ABC) transporters have key roles in various physiological functions as well as providing chemical defense and stress tolerance in human tissues. Since the human embryonic stem cells (hESC) require special protection during development, and some of the differentiated tissues are also well protected against toxic agents, an important task is to follow the changes in the expression and localization of the ABC multidrug transporters during these early developmental processes. Therefore the mRNA expression levels of the 48 human ABC proteins were examined in pluripotent hESCs and differentiated cell types (cardiomyocytes, neuronal cells and mesenchymal stem cells). We found that the mRNA expression pattern of the 48 human ABC proteins clearly distinguished the pluripotent and the respective differentiated cell types. Our further results regarding the expression and localization of the most important ABC transporter proteins strengthened the mRNA expression data. We found that the most prevalent multidrug transporter in the undifferentiated hES cells was the ABCG2/BCRP protein. This protein was expressed on the cell surface and, although this expression was heterogeneous, our results showed that ABCG2 may significantly contribute to the defense mechanisms in pluripotent stem cells during various stress conditions. These results may also help to obtain further information concerning the specialized cellular functions of other selected ABC transporters. The authors appreciate the gift of HUES9 cell line by Dr. Douglas Melton, HHMI. Research in our laboratory has been supported by Hungarian Scientific Research Fund [NK83533], Hungarian Brain Research Program [KTIA_13_NAP-A-I/6] and by the National Development Agency [KTIA_AIK_12-1-2012-0025 and KMR_12-1-2012-0112].

E6-4

Enhanced embryonic stem cell-derived dendritic cell development by transcription factor mediated programming

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The application of dendritic cells (DCs) to elicit responses to various antigens provides a promising approach to immunotherapy. However, only a limited number of DCs can be obtained from adult progenitors (bone-marrow cells or monocytes). Pluripotent embryonic stem (ES) cells can provide an unlimited source for DC generation, however it remains a big challenge to steer directional differentiation because ES-DCs are typically immature with poor immunogenicity. To characterize the developmental program of ES-DCs, we determined and compared the transcript levels of several DC affiliated transcription factors in ES-DCs and bone marrow (BM)-derived DCs. Our gene expression analysis revealed that the well-established myeloid transcription factor-triad (Irf8, Pu.1 and Id2) is highly expressed in both ES-DCs and BM-DCs. However three additional DC affiliated transcription factors (Spi-B, Runx3 and Irf4) had an impaired expression in ES-DCs compared to BM-DCs. Consistent with this finding our gain of function analysis revealed that Spi-B instructed, ES cell-derived mesoderm progenitors gave rise more DC precursors, suggesting that this factor positively regulates the myeloid ES-DC differentiation. This work was funded by the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 "VÉD-ELEM". This project is co-financed by the European Union and the European Social Fund.

E6-5

Chondrogenesis is regulated by poly(ADP-ribose) polymerase 1

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Given their relative abundance and ease of isolation, chorion-derived mesenchymal stem cells represent an ideal cellular model for studying chondrogenic differentiation. Previous studies have demonstrated the potential of extracellular matrix (ECM) molecules as three-dimensional (3D) scaffolds for promoting chondrogenesis. TGF-beta and BMP2 were significantly effective in promoting the expression of chondrogenic markers including COl2A1, aggrecan, COMP and Sox9. We identified the regulatory role of PARP1, a chromatin associated enzyme, in chondrogenic differentiation of mesenchymal stem cells. We found that expression COl2A1, ACAN, COMP is PARP1 dependent but the expression of Sox9 is independent of PARP1. Taken together these data suggest that PARP1 regulates chondrogenesis in a Sox9 independent way. 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'. Direct costs of this study were supported by the Hungarian Science Research Fund (OTKA K82009 and K112336).

E6-6

Highly efficient differentiation of embryonic stem cells into adipocytes by ascorbic acid

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In the past 10 years the prevalence of obesity related diseases has increased worldwide. Due to metabolic complications related to obesity, adipose tissue has been recognized as an endocrine organ. Therefore, several models have been set up for dissection of fat cell differentiation. A major limitation of essentially all of these models is that they are unable to provide information about the entire differentiation program. Hence, the early stages of this process remain largely unexplored. In principle, embryonic stem cells (ESCs) could allow us to get insight into the early phases of any differentiation including adipogenesis on different genetic backgrounds. However, heterogeneity of ESC cultures combined with the low efficiency of the differentiation are major limitations of such studies of adipogenesis. Here we present that ascorbic acid (AsA) in addition to the adipogenic differentiation cocktail enables the robust and efficient differentiation of ESCs to mature adipocytes within 27 days. Such ESCs derived adipocytes mimic the gene-expression profile of *in vivo* isolated adipocytes remarkably well. Moreover, the differentiated cells are in a monolayer, allowing a broad range of genome-wide studies of adipocyte differentiation to be performed. This model system provides a novel cellular model for the field that will promote our understanding on the upstream molecular events during early (ESCs to preadipocyte) and late (preadipocyte to adipocyte) differentiation. L.N. is supported by a grant from the Hungarian Scientific Research Fund (OTKA K100196). L.N. and I.C.-M. are recipients of TÁMOP-4.2.2.A-11/1/KONV-2012-0023VÉD-ELEM grant implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund. Z.S. and G.N. are recipients of TÁMOP 4.2.4.A/2-11-1-2012-0001/A2-JÁDJ-13. Microarray studies and Next Generation Sequencing were carried out at the Center for Clinical Genomics and Personalized Medicine at the University of Debrecen, Medical and Health Science Center.

E6-7

Ser/Thr-specific phosphoprotein phosphatases are key regulators of chondrogenesis

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Reversible phosphorylation of specific Ser/Thr residues of key signalling components plays a determining role in the regulation of chondrogenesis *in vitro*. While signalling pathways controlled by protein kinases including PKA, PKC, and mitogen-activated protein kinase (MAPK) have been mapped in great details, available data relating to the specific role of phosphoprotein phosphatases (PPs) in differentiating chondroprogenitor cells or in mature chondrocytes is relatively sparse. PPs are clearly equally important as protein kinases to counterbalance the effect of reversible protein phosphorylation on specific substrates. Over the last ten years, our laboratory has been investigating the involvement of PP1, PP2A and PP2B (calcineurin) in the context of chondrogenesis in an embryonic chicken limb bud-derived micromass model system. While PP1 and PP2A appear to negatively regulate chondrogenic differentiation and maintenance of the chondrocyte phenotype as the PP1 and PP2A-specific inhibitor okadaic acid significantly enhances cartilage matrix production, calcineurin is an important stimulatory mediator during chondrogenesis. Blockage of calcineurin activity by cyclosporine A exerts a chondrogenesis-suppressing effect. We also described a reciprocal regulation between calcineurin activity and cytosolic calcium concentration in chicken chondroprogenitor cells during differentiation. We recently documented the involvement of PP2A in mediating mechanotransduction pathways in chondrifying micromass cultures; mechanical stimulation resulted in a decline of PP2A activity but a steep increase in PKA activity. The mRNA expression profile analysis of novel PPs (PP4 and PP5) have been also been carried out on various chondrogenic model systems. Since PPs are implicated to be important mediators during the pathogenesis of osteoarthritis, this makes them potential therapeutic targets to be exploited in the close future.

Abstracts E7 - Genome Structure, Function and Maintenance

E7-1

Interplay between gene expression regulation and sanitization of the cellular nucleotide pool to preserve integrity of mobile genetic elements

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Transfer of phage related pathogenicity islands of *Staphylococcus aureus* (SaPI-s) was recently reported to be activated by helper phage dUTPases (1). This is a novel function for dUTPases otherwise involved in preservation of genomic integrity by sanitizing the dNTP pool (2,3). We investigated the molecular mechanism of the dUTPase-induced gene expression control using direct techniques. The expression of SaPI transfer initiating proteins is repressed by proteins called Stl. We found that Φ11 helper phage dUTPase eliminates SaPIbov1 Stl binding to its cognate DNA by binding tightly to Stl protein. We also show that dUTPase enzymatic activity is strongly inhibited in the dUTPase:Stl complex and that the dUTPase:dUTP complex is inaccessible to the Stl repressor. Our results disprove the previously proposed G-protein like mechanism of SaPI transfer activation. We propose that the transfer only occurs if dUTP is cleared from the nucleotide pool, a condition promoting genomic stability of the virulence elements. We also report that the *Staphylococcal* Stl is capable of jumping the species barrier for dUTPase inhibition. To our knowledge, this is the first indication that dUTPase activity can be controlled by a protein inhibitor. We demonstrate that Stl strongly inhibits mycobacterial, Drosophila and human dUTPases. We also found that expression of the inhibitor protein Stl in *Mycobacterium smegmatis* led to high cellular dUTP levels and moderate colony forming. Therefore, we propose that Stl may considered as a general dUTPase inhibitor and may used as a reagent in dUTPase inhibition experiments either *in vitro* or *in situ* and *in vivo*. Funding: OTKA NK84008, K109486, MedinProt.

1) Tormo-Más et al (2010) *Nature*, 465, 779–82
2) Vértessy and Tóth (2009) *Acc Chem Res*, 42, 97–106
3) Leveles et al (2013) *Acta Crystallogr D Biol Crystallogr*, 69, 2298–308.

E7-2

RecQ helicases support precise recombination via complex activities that are enhanced by macromolecular crowding

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RecQ helicases are ubiquitous enzymes that play central roles in genome maintenance via their unique capability to process non-canonical DNA metabolic intermediates. Based on a comprehensive set of single-molecule and ensemble biophysical experiments, we developed a mechanistic model to explain how the prototypical and widely conserved domain architecture of E. coli RecQ helicase promotes complex DNA processing behaviors. This feature results from dynamic interactions of conserved DNA-binding domains attached to the RecQ helicase motor core. The preferential binding of the winged-helix domain to duplex DNA ahead of the progressing helicase, together with that of the helicase-and-RnaseD-C-terminal (HRDC) domain to the displaced single-strand DNA, trigger a switch from processive unwinding of DNA duplexes to shuttling and repeated unwinding upon reaching the end of the duplex segment. We find that the complex activities of RecQ helicase are selectively enhanced under conditions mimicking the physiological crowded environment. These findings provide insight into the mechanism of efficient processing of DNA recombination and repair intermediates by RecQ helicases. This work was supported by the Human Frontier Science Program (RGY0072/2010) and the "Momentum" Program of the Hungarian Academy of Sciences (LP2011-006/2011).

E7-3

Histone point mutations driving human disease: a biophysical perspective

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Substitution mutation of lysine 27 to methionine in the human *H3F3A* gene (coding for the histone variant H3.3) has been recently identified as a driver mutation for the brain tumor Pediatric Glioblastome Multiforme. The dominant negative disease phenotype caused the mutant histone is not understood at the molecular level. Here we apply various *in vitro* and *in vivo* model systems (E.coli, *S. cerevisiae*, human cell lines) and state of the art genetic and biophysical approaches to reveal how the histone H3K27M point mutation affect the nanostructure of nucleosome core particles (NCPs) and influence the integrity of chromosomes. We tested the viability and stress tolerance of H3K27M-expressing *S. cerevisiae* strains under different metabolic (YPD, YPGal, YPG) and stress conditions (H2O2, campthotecin, ethoposide, hidroxyurea, MMS, UV), and we find that the K27M mutation does not cause any growth defect in this model organism. We performed bulk- and single-molecule FRET measurements on *in vitro* reconstituted nucleosomes and observed a slightly opened conformation of the H3.3K27M nucleosomes, however the overall stability of the mutant NCPs has been similar to wild-type. Importantly, by FRAP (fluorescence recovery after photobleaching) and FCS (fluorescence correlation spectroscopy) we reveal a significantly lower mobile fraction and slower repopulation rate of the H3.3K27M histones. The latter suggests that the disease caused by the H3.3K27M histone is related to the altered mobility and diffusion kinetics of mutant nucleosome core particles. 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', also by the Hungarian Scientific Research Fund (OTKA-PD) and CRP-ICGEB international research grant.

E7-4

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.

E7-5

Discontinuities present in the genome continuously

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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 by 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 any of the following marks were relative resistant, similarly to bulk histone-GFP: H3K27me1, H3K27me2, H3K27me3, H3K9me1, H3K9me2, H3K9me3, H3K36me3, H3K4me0, H3K4me1, H3K4me2, H3K9ac, H3K14ac. The H2A-H2B dimers already dissociated in the conditions when the differences between the H3-H4 tetrasomes carrying different PTMs were recorded. Destabilization of the H3K4me3 marked TSS proximal nucleosomes was rather uniform along the genome, as revealed by chip sequencing, when doxorubicin was used as the intercalator. Nickase treatment of the nuclei did not affect the stability of nucleosomes carrying H3K4me3 or H3K27ac, while those of the second group were all destabilized. A possible interpretation of these results is 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 the mouse embryonic stem cells studied. Nicked DNA is highly sensitive to mechanical stimuli, as shown by our molecular combing studies, suggesting that the nicks revealed serve as predilection points for the various ds chromatin fragmentation phenomena observed in normal cells under protein denaturing condition. In accordance with the dichotomous conduct of the nucleosomes in the elution studies, two topologically isolated chromatin domains were demonstrated in nuclear halos, in all phases of the cell-cycle: superhelical loops and the nuclear lamina enclosed compartment harboring nicks, sharing this localization with the sites of in vivo nucleozide analogue incorporation upon transcription as well as replication. Supported by OTKA 72762, 101337 and TÁMOP 4.2.2.A-11/1/KONV-2012-0023 "VÉD-ELEM" (implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund). L.I. is supported by TÁMOP 4.2.4. A/2-11-1-2012-0001 National Excellence Program.

E7-6

Cloning and characterization of argonaute proteins of *Nicotiana benthamiana*

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Plants respond to viral infection by a defensive mechanism which relies on recognition, and subsequent inactivation of the invading foreign nucleic acids. Years of research has uncovered striking similarities between this process and RNA silencing, an important cellular phenomenon responsible for regulating gene expression at the transcriptional and post-transcriptional levels. Beside the mechanistic resemblance, many of the molecular players employed by the two processes are also shared. For example, the protein complexes (RNA induced silencing complexes, RISCs) that are executing the final steps of both RNA silencing and viral restriction, contain members of the highly conserved Argonaute (AGO) protein family. One of the most important biochemical properties of AGO proteins is their ability to bind small RNAs, which ensures specific recognition of the substrate RNA molecules (viral RNAs, mRNAs) by RISCs. The tobacco species, *Nicotiana benthamiana* is a widely used experimental host in plant biology, due mainly to the large number of viruses that can successfully infect it. Additionally, it is also susceptible to a wide variety of other plant pathogens (such as bacteria, oomycetes, fungi), making this species a cornerstone of host-pathogen research. Recently, the complete genome sequence of *N. benthamiana* has become available from several sources. Using this information, we have cloned cDNAs of the nine *N. benthamiana* Argonaute proteins and the predicted full-length proteins were produced in various expression systems. Additionally, we have developed in vivo reporter assays to monitor the activity of the cloned Argonaute proteins. These tools will be invaluable for future efforts to better understand the molecular details of plant-virus interactions. This work was supported by the Hungarian Scientific Research Fund (NK105850).

E7-7

A new interaction partner for ubiquitylated PCNA

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Accurate replication of the whole genome with every cell division is a prerequisite for maintaining genomic stability. Processing of stalled replication forks during the process of genomic duplication is critical for completion of DNA replication. Since stalled forks are inherently unstable and prone to collapse leading to double-strand breaks and chromosomal rearrangements thus they pose a serious threat to genomic integrity. Cells possess different mechanisms to ensure the continuity of replication on damaged templates. These are often collectively called damage tolerance pathways, since the lesions are not repaired, but "tolerated" as the cells find a way to overcome the defect of replication stalling. These mechanisms include DNA damage bypass, homologous recombination (HR)-dependent repair and non-homologous end-joining (NHEJ)-dependent repair to deal with fork collapse. Although replication stalls frequently a delicate balance of damage bypass, homologous recombination and non-homologous end-joining could ensure survival and at the same time effectively prevent increased mutagenesis, gross chromosomal rearrangement, and carcinogenesis. Genomic instability has been documented as a preceding step for multiple inactivation of tumor suppressor genes and activations of proto-oncogenes that can lead to cancer. In our study we are focusing on the regulation of PCNA ubiquitylation to give more insight into the mechanism of DNA damage tolerance pathways. Here we identify a new player which interacts with ubiquitin- PCNA and participates in DNA damage bypass. This work was supported by TÁMOP 4.2.4.A/2-11-1-2012-0001 Nemzeti Kiválósági Program, A2-EPFK-13-0069.

Abstracts E8 - Signaling and Posttranslational Modifications

E8-1

Attila Mocsai

E8-2

Poly(ADP-ribosyl)ation as a redox regulatory mechanism

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Classical signal transduction pathways rely on the interplay of kinase cascades and protein phosphatases. However, these signaling routes can be modulated by a diverse set of stimuli interfering with one or more steps of these cascades. Poly(ADP-ribosyl)ation (PARylation) is a protein modification implicated in the regulation of various cellular pathways ranging from DNA repair, transcriptional control to autophagy and cell death. Poly(ADP-ribose) polymerase-1 (PARP-1) is the most characterized enzyme in the 17 member PARP family. Our recent data suggest that PARylation signaling is closely intertwined with MAP kinase cascades in the control of osteogenic differentiation-coupled cell death. In connection with this we review the plethora of evidence suggesting multilevel connections between PARylation and conventional signaling routes and propose that PARylation by PARP-1 represents a novel signaling (or signal modifying) factor that should be considered especially in redox-active environments. 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'. Direct costs of this study were supported by the Hungarian Science Research Fund (OTKA K82009 and K112336).

E8-3

Tks scaffold proteins in tyrosine kinase signalling

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Signalling pathways utilising tyrosine kinases play crucial roles in the regulation of cell proliferation and movement. Impairment of these pathways may lead to important public health diseases such as malignant cancer or diabetes mellitus. Our laboratory focuses on scaffold proteins which are implicated in tyrosine kinase signalling. We have showed that the scaffold protein Tks4 plays an important role in the EGF signalling as, in response to EGF, Tks4 is tyrosine phosphorylated and associated with the activated EGF receptor. This association is not direct but requires the presence of Src tyrosine kinase. Treatment of cells with LY294002, an inhibitor of PI 3-kinase, or mutations of the PX domain reduces tyrosine phosphorylation and membrane translocation of Tks4. Furthermore, a PX domain mutant (R43W) Tks4 carrying a reported point mutation in a Frank-ter Haar syndrome patient shows aberrant intracellular expression and reduced phosphoinositide binding. Silencing of Tks4 is shown to markedly inhibit HeLa cell migration in a Boyden chamber assay in response to EGF or serum. In addition, we have generated a Tks4 knock-out mouse which shows very dramatic morphological changes compared to wild type mice. Our preliminary experiments suggest that Tks4 may have a crucial role in the differentiation processes as mesenchymal stem cells isolated from the KO mice have significantly reduced potential to differentiate to adipose and bone tissues than that of stem cells isolated from wild type mice.

E8-4

Protein phosphatase-1 is involved in the maintenance of normal homeostasis and 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 only PP1cδ was found 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 20 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 histon 1b. Treatment of Balb/c mice with the PP1-specific inhibitor tautomycin (TM) exhibited increased levels lead to increased expression 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.

E8-5

The significance of the metastasis-associated S100A4 protein in cell adhesion and migration of A431 epithelial carcinoma cell line

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S100A4 belongs to the Ca^{2+}-binding vertebrate-specific EF-hand S100 protein family. The biological function of S100A4 is due to its interactions with binding partners such as non-muscle myosin IIA, annexin A2, p53 and several other proteins. By binding to myosin IIA S100A4 disrupts myosin filaments, thus increases cell motility by changing the balance between the forward and reverse forces at the leading edge of polarized cells. S100A4 could also act extracellularly as an angiogenic and motility-inducing factor. Emerging data from S100A4 studies suggest that it is a regulator of tumor cell motility, a prognostic marker for poor patient survival in a number of cancers and a potential therapeutic target protein. Our aim was to study the extracellular and intracellular effect of S100A4 on the adhesion and migration of A431 human epithelial carcinoma cell line. We stably transfected A431 cells with bicistronic pIRES2-EGFP construct expressing both S100A4 and GFP. Adhesion of cells was investigated with real-time label-free impedance-based assays: xCELLigence system and Electric Cell-substrate Impedance Sensing (ECIS). Cell migration was studied using NeuroProbe® chemotaxis chamber and ECIS automated wound healing assay. According to our results, extracellular S100A4 reduces cell adhesion on fibronectin-coated surface in a concentration-dependent manner. S100A4 induces chemotaxis of non-transfected A431 cells at a 1 μM concentration optimum. Intracellular S100A4 results in an increase in early cell adhesion (spreading). Furthermore, cells that stably express S100A4 show an enhanced motility in both wound healing and EGF- or soluble S100A4-induced chemotactic motility tests. These migration assays give further evidence about the significance of S100A4 in enhancing cell motility acting both intra- and extracellularly and could be used for testing S100A4 inhibitors in the future. Supported by the Hungarian Scientific Research Fund (OTKA NK81950 and K108437).

E8-6

mTNF-alpha signaling inhibits LPS-induced proinflammatory cytokine formation by upregulating TGF-β in macrophages

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Tumor necrosis alpha (TNFα) exists in two isoforms: the soluble sTNFα which is a trimer built up of 17 kDa sized subunits and the membrane-bound 26 kDa sized mTNFα. Increasing evidence suggests that following engagement with TNF receptor mTNFα initiates a reverse signaling pathway, but the details have not been described yet. For characterization of TNFα-production we examined the mRNA levels both in resting and in bacterial lipopolysaccharide (LPS) activated bone marrow derived macrophages by Q-PCR, the amounts of secreted sTNFα by ELISA technique, while the amount of mTNFα by flow cytometry. To study mTNFα signaling, mTNFα was crosslinked by coated antibodies and the signaling was studied by the Proteome Profiler Human Phospho-MAPK Array as well as the secreted cytokines were studied by Cytokine arrays. Our results indicate that following LPS stimulation mTNFα is originated first from the stored cytosolic pool and the *de novo* synthesis contributes to the late expression of mTNFα. TNFα appears first in the membrane, but later it is cleaved by metalloproteases to form sTNFα resulting in a very little steady state mTNFα concentrations on the cell surface. mTNFα reverse signaling induces the production of TGF-beta in mouse bone marrow derived macrophages. In turn, TGF-beta acts back on macrophages and triggers the upregulation of the dual specific phosphatase 1 (DUSP1) and IL-10 via activating the MKK3/MKK6 signaling pathway. As a result triggering mTNFα leads to the downregulation of LPS-induced signaling and the consequent proinflammatory responses (IL-6 production) in macrophages. Our data indicate that some of the neutralizing anti-TNFα antibodies used in human therapy which trigger mTNFα signaling might exert their anti-inflammatory effects via the mTNFα signaling pathway as well. This study was supported by TÉT_10-1-2011-0028.

Abstracts E9 - Biocrystallography and Structural Biology

E9-1

Linear binding motif surfaces on protein kinases: a hot-spot for generating signaling diversity

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Protein kinases regulate many aspects of cellular life by phosphorylating hundreds or thousands of proteins according to a given biological logic. By studying members of two protein kinase systems (MAP kinases and NDR/LATS kinases) structurally we established how short linear motifs contribute to efficient and specific phosphorylation events in partner proteins. My talk will demonstrate how linear motifs bind to novel surfaces on protein kinases and I will discuss how these protein-peptide type interactions has been likely propelling protein kinase based signaling network evolution and thus contributed to the signaling logic that we currently see in living organisms. This work was supported by the Wellcome Trust and by the "Lendület" Grant from the Hungarian Academy of Sciences.

E9-2

Size selection mechanisms of acylaminoacyl peptidases

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Acylaminoacyl peptidase (AAP), a member of the prolyl oligopeptidase family cleaves the acylated N-terminal residue of oligopeptides. AAP was shown to exert regulation over protein maturation and degradation processes and cell survival, possibly through being an upstream modulator of the proteasome. AAP is active in tetrameric form, however structural information is only available for its two archeal orthologs with low homology, a dimeric and a hexameric enzyme. Enzymes of the family impose a size limitation on their substrates by forcing them to bind in a cavity between their two domains. There are different strategies within the family for making the buried active site accessible for only oligopeptides. i) Prolyl oligopeptidase and *A. pernix* AAP show conformational gating by fluctuating between a catalytically active conformational state with the active site buried and an opened conformation with distorted active site. ii) In case of dipeptidyl peptidase IV and *P. horikoshii* AAP a homomultimer enzyme is formed and side entrances between the two domains and/or a channel system are responsible for selecting oligopeptides for cleaving. We solved the crystal structure of *A. pernix* AAP in complex with a covalent substrate-like inhibitor to clarify the roles of enzyme-substrate interactions in the active/inactive conformational transition of the type i) mechanism. We collected a low resolution data set from the mammalian enzyme sufficient for recognizing the organization of monomers in the tetramer and the topology of interdomain and intermolecular interactions that helps deepen our understanding of how catalytic efficiency and substrate size-selectivity are maintained simultaneously within the prolyl oligopeptidase family. We acknowledge the European Synchrotron Radiation Facility for providing synchrotron radiation facilities and Hungarian Scientific Research Fund (OTKA) for financial support (grants PD101095, NK101072).

E9-3

Composite aromatic box: structural motif for binding and enzyme-catalyzed conversion of quaternary ammonium substrates

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Trimethyl ammonium moieties with diffuse positive charge are often recognized within sterically restricted protein environment *via* cation-π interaction from aromatic residue(s). Numerous enzymes convert substrates possessing such moieties, playing key roles in metabolism as well as in pathogenesis. Here we aim to characterize the architecture of ligand recognition by these enzymes focusing on the choline binding site of P. falciparum choline kinase and CTP:phosphocholine cytidylyltransferase combined with analysis of available cognate enzyme structures. We designed a series of point mutants for choline interacting residues of these enzymes and tested their catalytic performance and ligand binding capability. Exchange of charged residues to non-charged analogues yielded profound perturbation of enzyme efficiency and ligand binding in several instances, while retaining the aromatic character of residues recognizing choline moiety by cation-π interactions was still somewhat compatible with catalysis. In contrast, the elimination of the aromatic ring of the latter residues resulted in practically inactive enzyme forms. These results prove the valid distinction of charged versus cation-π interactors and demonstrate their key functional role. By further performing a full range interrogation of the Protein DataBank for quaternary ammonium ligand moiety, we found that an overwhelming majority of enzyme:ligand complex structures (86 independent enzyme hits, i.e. 93%) possess a similar ligand binding site, constituted by one or two aromatic as well as charged or polar residues. This emerging universal enzyme binding motif termed "composite aromatic box" is clearly distinct from the well-known aromatic box or aromatic cage architecture found abundantly at receptor proteins without enzymatic function (78 % of relevant hits). Recognition of this common enzyme structural solution, present in a wide range of protein family clans, provides a spectacular example of parallel evolution.

E9-4

Symmetric or asymmetric: interaction of S100 proteins with annexin-A2

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S100A4 is a small, dimeric EF-hand Ca^{2+}-binding protein. Increased concentration of the S100A4 promotes metastasis formation through interactions with partner proteins like non-muscle myosin-IIA (NMIIA), p53 and annexin A2 (ANXA2). ANXA2 is a non-EF-hand-type Ca^{2+}-binding protein that exhibits Ca^{2+}-dependent phospholipid binding. A fully symmetric interaction of ANXA2 with S100A10 has been previously shown to control activation of matrix metalloproteases (MMPs) via regulating the level of active plasmin. MMPs then can induce matrix degradation and invasion. Through phosholipid binding ANXA2 anchors S100A10 to the membrane, while plasminogen and its activators bind to the C-terminal lysine of S100A10. Here we provide evidence that S100A4 also facilitates plasminogen activation in a similar manner. Besides we present the 2.4 Å crystal structure of the S100A4–ANXA2 complex. The structure reveals an interesting asymmetric binding: the N-terminal residues of one ANXA2 embraces the dimer S100A4 (as in the case of the S100A4–NMIIA complex), while another ANXA2 makes a weaker interaction with one of the hydrophobic pocked formed upon Ca^{2+}-binding. In the apo form of ANXA2, part of the N-terminal domain snuggles to the globular part of the protein. In the complex this part opens up to make interaction with the S100 proteins. The intrinsically disordered N-terminal tail of ANXA2 folds into two helices with a linker region upon complex formation. While the N-terminal helix is formed in both bound ANXA2s (Val^5, Ile^8, Leu^9 and Leu^{12} make hydrophobic contacts), the C-terminal helix is observed only in the ANXA2 bound to the "waist" of the S100A4 dimer. In the latter hydrophobic, ionic and sulfur-aromatic interactions can be identified. Our results suggest that the extracellular S100A10 and S100A4 could promote tumor cell invasion as plasminogen receptors by forming symmetric and asymmetric complexes with ANXA2, respectively. Supported by OTKA NK81950, K108437, K109486 and MedinProt.

E9-5

„Invisible" conformations of a disulfide constrained antifungal protein disclosed by cold and heat unfolding, nuclear magnetic resonance saturation transfer and molecular dynamics calculations

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Interconversion between conformational states in proteins is being recognized as a key factor contributing to their function. There is growing evidence that structures invisible by most of the conventional techniques may exist and even could be functionally relevant. Our cold and heat unfolding experiments with the Penicillium AntiFungal protein revealed that the maximum population (~70%) of visible conformations is reached at room temperature in between the -15°C…+80°C range, as we found by ^{15}N Nuclear Magnetic Resonance spectroscopy. This observation can be explained by the different dynamics states of the protein "dark matter" if compared to the observable well folded state. We detected a fraction of the invisible states in Chemical Exchange Saturation Transfer experiments. Our experimental data and *in-silico* molecular dynamics calculations indicate the presence of dynamic structures differing in the conformation at certain loop residues and around both termini. We conclude that the unfolding kinetics of this disulfide constrained protein is rather complex, that underlines the importance of hidden conformations for explaining function. 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' and also by the Hungarian National Fund with grant No. OTKA - ANN 110821.

E9-6

Essential role of E270 in K⁺-activation and domain closure of *Thermus thermophilus* isopropylmalate dehydrogenase

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The role of an active site residue, E270 of *Thermus thermophilus* 3-isopropylmalate dehydrogenase has been tested by site-directed mutagenesis. The E270A mutant exhibits largely reduced (~1 %) catalytic activity and negligible activation by K^+ compared to the wild-type enzyme. The X-ray structure of E270A indicates a bound water molecule in the place of K^+. Small Angle X-Ray Scattering and Förster Resonance Energy Transfer experiments have revealed an essential role of E270 in stabilisation of the active domain-closed conformation of the enzyme, but K^+ is not required for domain closure. E270 additionally positions K^+ into close proximity of the nicotinamide ring of NAD^+. QM/MM calculations have shown that activation energies in the mutant increased with 4.0 kcal/mol compared to the wild-type, K^+ bound structure. Based on chemical thermodynamics it can be expected that the reaction rate decreases 150 to 1000 fold, which result is in very good agreement with the experimentally observed 242-fold decrease in the k_{cat} value of the mutant enzyme. In fact, the electron-withdrawing effect of K^+ may assure polarisation of the ring for occurrence of the hydride-transfer. The financial support by the grants OTKA (NK 108642) of the Hungarian National Research Fund is gratefully acknowledged. We would also like to thank the synchrotron facilities EMBL (Hamburg Outstation, Germany) and Helmholtz Zentrum Berlin (Germany) for the allocation and provision of synchrotron beam time (FP7/2007-2013 No. 226716). D.I.S. and P.V.K. acknowledge support from the German Ministry of Education and Science (BMBF) project BIOSCAT, Grant 05K20912.

E9-7

Structural and kinetic studies on the skin-specific retroviral-like aspartic protease 1 (ASPRV1)

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ASPRV1 (also referred as SASPase) is a skin-specific retroviral-like aspartic protease which is expressed in the transitional layer of skin by keratinocytes during their differentiation. Through this process keratinocytes become corneocytes and part of the cornificated barrier. The role of the stratified, cornified squamous epithelium is to prevent water loss and block the entry of foreign substances. SASPase is expressed in stratum granulosum as a complete protein (SASP37, 37 kDa) and further processed to a shorter autoprocessing product (SASP28, 28 kDa) and to its active form (SASP14, 14 kDa). The natural substrate of SASP14 is the profilaggrin protein which contains filaggrin monomer repeats. During profilaggrin processing the linker sequences are cleaved by SASPase, leading to the release of filaggrin monomers. These monomers are cross-linked to keratin-cytoskeleton by transglutaminases and further processed to short peptides. Degradation products constitute a part of natural moisturizing factor having key role in the moisturizing of the skin. Our goal was to characterize SASP14 and explore its expressional, enzymatic and structural properties. The structure of homodimeric SASP14 was predicted by homology modeling and we analyzed the dimer interface. Mutations were designed based on the proposed model to study the role of dimer interface residues. After the mutagenesis we have expressed the dimer interface mutant GST-SASP14 fusion proteins in *E. coli* cells and purified them with affinity chromatography. Values of dimerization constant (K_{dapp}) and urea dissociation (UC50) were determined after HPLC analysis using synthetic decapeptides and compared to the values of wild type SASP14. This work was supported by the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM project (to J.T.) and by the Hungarian Science and Research Fund (K-101591 to J.T.).

E9-8

Role of phosphorylation in the regulation of nuclear transport of myosin phosphatase targeting subunit

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In myosin phosphatase (MP) holoenzyme PP1 catalytic subunit (PP1c) is complexed with myosin phosphatase targeting subunit (MYPT1). MYPT1 is generally distributed between the cytoplasm and the nucleus of cells, and its nuclear import is dependent on NLS sequence located close to the N-terminus. In THP-1 leukemic cells MYPT1 is localized almost exclusively in the nucleus. Treatment of the cells with the phosphatase inhibitor calyculin-A (CLA) induces translocation of MYPT1 from the nucleus to the cytoplasm followed by increased phosphorylation at Thr696 and Thr853 residues, which are implicated in the inhibition of MP. Inhibition of Rho kinase, the major kinase for the inhibitory sites, did not influence the localization pattern suggesting, that the inhibitory sites might not be involved in mediation nuclear transport of MYPT1. While MYPT1 translocates from the nucleus to the cytoplasm, the distribution of PP1c between the subcellular fractions remains approximately the same, suggesting that nucleocytoplasmic shuttling of MYPT1 requires its dissociation from PP1. These data suggest that the phosphorylation site may be located close to both the N-terminal NLS and the PP1c-binding motif. A recent proteomic study identified Ser20 and Thr22 as novel casein kinase 2 (CK2) phosphorylation sites in MYPT1, but the ability of CK2 to phosphorylate MYPT1 and the physiological relevance of the phosphorylation was not investigated. Therefore, we initiated *in vitro* phosphorylation experiments using recombinant CK2 and MYPT1 N-terminal peptides as substrates, in which Ser20 and/or Thr22 residues are mutated to Ala. We identified Thr22 as a CK2 phosphorylation site in MYPT1. Western blot analysis of longer N-terminal fragments also proved the incorporation of phosphate group to Thr residue in MYPT1 upon phosphorylation by CK2. This raises the possibility that Thr22 is responsible for the regulation of MYPT1 localization, however, phosphorylation of this residue in cells and its implication in the nuclear import/export processes of MYPT1 requires further evidences. 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' (A.K.). Direct costs of this study were supported by TÁMOP-4.2.2.A-11/1/KONV-2012-0025 (F.E.) and the Hungarian Scientific Research Fund OTKA K109249 (F.E.).

Abstracts
E10 - HBS Awardees

E10-1

Translocation of RecQ helicase along single-stranded DNA

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The information of life is encoded by DNA, which is exposed to several external (e.g. UV) and internal (e.g. oxidative stress) damaging impacts. DNA is also constantly manipulated during everyday cell functioning. It is replicated, organized and reorganized in the chromatin structure, transcribed, disentangled to allow segregation during cell division, etc. These processes set a challenge for the cell in preserving the integrity of the genome.
RecQ-family DNA helicases are evolved to fulfill essential roles in genome maintenance. The family is named after the emblematic *E. coli* RecQ, helicase, and members are present in all living organisms from bacteria to humans. Deficiency of three of the five human forms causes severe hereditary diseases, associated with cancer and premature ageing (e.g.: Bloom, Werner and Rothmund-Thomson Syndromes). They play key roles in homologous recombination based DNA repair, and in handling the consequences of conflicts of replication. These functions involve convoluted enzyme activities modulated by the DNA structure and the interacting partners present. DNA helicases are motor enzymes, utilizing the energy of ATP hydrolysis to perform directional movement along single-stranded DNA (translocation), which results in the separation of the two strands of the double helix from each other (unwinding). Translocation is an elemental activity to investigate on the way of understanding the mechanism of a helicase enzyme. In the work presented here, we created a comprehensive, mechanistic model about the translocation of *E. coli* RecQ helicase along single-stranded DNA, based on evidences acquired by complex kinetic analysis, including pre-steady-state and steady-state methods. This model allows the deeper understanding of the underlying molecular mechanism of the complex physiological functions of these ubiquitous motors.

E10-2

Phosphorylation adjacent to the nuclear localization signal of human dUTPase abolishes nuclear import: structural and mechanistic insights

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Phosphorylation adjacent to nuclear localization signals is involved in the regulation of nucleocytoplasmic transport. The nuclear isoform of human dUTPase, an enzyme essential for genomic integrity, has been shown to be phosphorylated on a serine (S11) residue in the vicinity of its nuclear localization signal, however, the effect of this phosphorylation is not yet known. To investigate this issue, here we employ an integrated set of structural, molecular, and cell biological methods. We show that NLS-adjacent phosphorylation of dUTPase occurs at the M phase of the cell cycle. Comparison of the cellular distribution of wild-type dUTPase to hyper-phosphorylation and hypo-phosphorylation mimicking mutants suggests that phosphorylation at S11 leads to exclusion of dUTPase from the nucleus. Isothermal titration microcalorimetry and additional independent biophysical techniques show that the interaction between dUTPase and importin-α, the karyopherin molecule responsible for "classical" NLS binding, is weakened significantly in the case of the hyper-phosphorylation mimicking mutant. The structures of the importin-α-wild-type and the importin-α- hyper-phosphorylation mimicking dUTPase nuclear localization signal complexes provide structural insights into the molecular details of this regulation. The data indicate that post-translational modification of dUTPase during the cell cycle may modulate the nuclear availability of this enzyme.
Supported by the Hungarian Scientific Research Fund (OTKA NK 84008, PD72008), Baross program of the New Hungary Development Plan (3DSTRUCT, OMFB-00266/2010 REG-KM-09-1-2009-0050), the Hungarian Academy of Sciences (TTK IF-28/2012), and the European Commission FP7 Biostruct-X project (contract number 283570). G.R. is recipient of Young Researcher Fellowships from the Hungarian Academy of Sciences. J.T. is the recipient of the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. B.K. is a National Health and Medical Research Council Research Fellow.

Abstracts
E11 - Biochemical Pharmacology

E11-1

Signal transduction therapy of cancer targeting cancer driver genes related pathways

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Signal transduction therapy has become a leading area of modern drug research aiming to inhibit the pathomechanism based validated target molecules in cellular signaling. The goal in cancer treatment would be to permanently eradicate all cancer cells from a particular patient. Our goal is to develop drugs that can eliminate certain types of cancer, via killing differentiated cancer cells and even cancer stem cells with the efficient inhibition of a certain set of driver genes and survival factors, thus inducing apoptosis. This therapeutic approach has to be accompanied by proper and sophisticated molecular diagnostics, utilizing genomics for identifying the driver genes in a particular tumor. Our Nested Chemical Library™ (NCL) technology is based on a knowledge based approach where focused libraries of kinase inhibitors and signaling inhibitors around more than 600 scaffolds are used for hit finding and to generate pharmacophore models. We have developed nM lead molecules against a series of kinases and signaling molecules. Recently we have developed a DriverHitLibrary which has lead compounds against 107 Driver gene related targets out of the identified 138 driver genes. Using a proper combination therapy against the identifeid driver genes in a particular cancer model all the cancer cells can be eliminated. We have also developed a phenotypical screening based cancer stem cell killing program which is accomplished with the following steps: Using our NCL™ kinase inhibitor library we have developed and will further develop kinase inhibitors which kill more than 95% of certain cancer cells (including cancer stem cells). Those compounds which kill certain cancer cell lines have been tested in clonogenic assay for a direct proof of CSC killing. Using diagnostics tools and "Target Fishing" technology we identify markers of the cancer stem cells. We provide a personalized therapy and molecular diagnostic protocol to select who will respond to a particular cancer stem cell killing drug.

E11-2

Protein kinases – the most fashionable therapeutic targets

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About 25% of all registered drugs exert their effects via G-protein coupled receptors. That family of molecular targets has quite a few members whose roles in health and disease have not been fully understood yet. Still, the interest of big pharma is now focussed towards other targets. Signal transduction pathways involved in cell proliferation or death and cell specific differentiated functions are regulated by protein kinase cascades. Thus their importance in cancer, inflammatory and metabolic diseases is unquestionable. Selecting protein kinases as molecular targets in drug research was attempted already many years ago but without much success. Selectivity was rather difficult to achieve with low molecular weight inhibitors due to the close similarity of the active centers of those enzymes. That resulted in unacceptable side effect profiles. Better understanding of the pathomechanism of diseases representing high unmet medical need at the molecular level gave a new impetus to this kind of research and a number of protein kinase inhibitor drugs and antibodies to receptor tyrosine kinases were registered and are now used in the clinics. Even more impressive, as it is seen from the analysis of clinical trials initiated last year in Hungary, is the increasing frequency of use of these molecular targets in drug research and development.

E11-3
Preclinical development of a kinolol derivative for Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognition and memory impairment. At present, there are no proper therapies for AD. Curative or disease-modifying therapies are urgently needed to offset the upcoming AD epidemic. Unfortunately, many therapeutic agents have failed at several stages of development. In the course of the research work aiming at finding new lead molecules for CNS diseases we found interesting small molecular weight compounds that showed promising properties to develop them in the field of AD. The lead optimization of the hit structure led to the recognition of a 8-hydroxy derivative with one chiral C-atom. We elaborated chiral synthesis for both enantiomers. In the meantime, in the frame of a grant from the Hungarian Government, we solved the large scale synthesis of the selected compound and performed its physico-chemical characterization. Our drug candidate (Q134) shows remarkable activity in protection of oxidative stress on primary rat neuronal cells or in neuroprotection against Aβ on primary rat cortical neurons. In disease models on mice the quinolol compound showed beneficial anxiolytic effects, exhibited complete rescue of the memory impairment induced by *i.c.* injection of oligomeric Aβ, exhibited complete rescue of decrease in pre- and post-synaptic proteins induced by *i.c.* injection of oligomeric Aβ and rescues the memory impairment induced by scopolamine in an amnesia model. Q134 showed excellent selectivity on the CEREP Diversity profile. Q134 has high plasma protein binding and acceptable pharmacokinetic parameters. Metabolically it is very stable. On MNT test it proved to be non-mutagenic. The presentation will give the results of the full ADME-T characterization. Our drug-candidate compound has good chances to start Phase I investigations in 2015. The preclinical development and the planed Phase I study are supported by the grant: KMR_12-1-2012-0072.

E11-4
Selected reaction monitoring as a versatile tool for biomarker discovery

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Selected reaction monitoring (SRM) is a targeted mass spectrometry method being able to specifically detect and quantify analytes of interest. The SRM-based targeted proteomic methods permit multiplexing and in most cases are more cost-effective than the classical antibody-based techniques. Another advantage of the targeted methods is their high flexibility, the possibility to design and validate SRM transitions and the simultaneous measurement of multiple proteins. These features together make the SRM-based methods a good candidate for biomarker studies, where the simultaneous detection of specific proteins from low amounts of sample is critical. We have developed and validated several SRM-based methods for the detection and quantification of proteins of interest in different pathological conditions. Proteins identified in previous studies as possible biomarkers for oral squamous cell carcinoma (OSCC), Alzheimer disease and diabetic retinopathy have been studied in saliva and tear samples of patients and controls. A statistical model for the reliable analysis of SRM-data was developed and the utility of the SRM method development, method validation and statistical analysis workflow in analyzing biomarkers characteristic for different diseases is highlighted. This research was funded by TÁMOP 4.2.4. A/2-11-1-2012-0001, TÁMOP-4.2.2.A-11/1/KONV-2012-0045 and OTKA 105034.

E11-5
Effect of carbohydrate-based glycogen phosphorylase (gp) inhibitors on the hepatic glycogen metabolism

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Glycogen phosphorylase (GP) together with glycogen synthase regulate glycogen metabolism, where GP is responsible for glycogen breakdown and hence has major role in hepatic glucose production (HGP). Therefore GP inhibition has been suggested to provide a way to reduce HGP and thereby could be used in the treatment of type II diabetes. Hereby, we evaluated the metabolic properties of a novel potent GP inhibitor, KB228 (N-(3,5-dimethyl-benzoyl)-N′-(β-D-glucopyranosyl)urea K_i = 937 nM) synthesized in our laboratory. KB228 enhanced catabolism as evidenced by measuring the rate of glycolysis and mitochondrial oxidation. Interestingly, mitochondrial membrane potential did not increase despite the increased mitochondrial activity suggesting enhanced mitochondrial uncoupling. For the better understanding of cellular responses to the action of GP inhibitors we are tested their effects on insulin levels and their interactions on the energy expenditure (mitochondrial functions, glycogen metabolism). The mouse-derived MIN6 cells retain glucose-stimulated insulin secretion as isolated islets. It was demonstrated that MIN6 produce pancreatic endocrine cells and secrete insulin in response to glucose and other secretagogues. It is possible to use this cell line as a model to research the development, cell differentiation and function of pancreatic islets. Using MIN6 cells we have determined the effects of some best inhibitor molecules to the glucose-stimulated insulin secretion. Supported by TÁMOP-4.2.2.A-11/1/KONV-2012-0025, Bolyai János Research Fellowships from the Hungarian Academy of Sciences and the University of Debrecen (5N5X 1IJ0 KUDT 320).

E11-6
Characterization of the interaction between the adenosine receptor 2A and cathepsin D protease in macrophages

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Here we are focusing on the specific role of the C-terminal intracellular domain in regulating adenosine receptor 2A (A2A) mediated signal transduction pathways in macrophages. Although the precise function of the C-terminal tail remains elusive, it has been demonstrated that the C-terminus juxtamembrane segment is required for proper folding of the receptor. The X-ray crystal structure of the human A2A suggests that the C-terminal domain of A2A can serve as a surface for protein binding. This assumption is supported by the fact that eight accessory proteins have already been indentified to bind to the A2A receptor in neuronal cells. However, similar interaction of the A2A has not yet been referred in macrophages. In order to identify proteins interacting with the C-terminal domain of the A2A we created and screened a mice macrophage cDNA library by yeast two hybrid methods. When we used the C-terminal 120 amino acids of the A2A as "bait" we identified cathepsin D aspartyl protease as a putative interacting protein. This interaction was confirmed by immunoprecipitation and pull down methods in macrophages. To examine the functional role of A2A – cathepsin D interaction we analyzed the effect of the A2A agonist and antagonist treatment on the maturation of cathepsin D. We found that the specific activation of A2A receptor increased while the antagonist treatment decreased the amount and the enzyme activity of the cathepsin D matured form in macrophage cell line and intraperitoneal macrophage cells. We observed that the pharmacological treatment of the A2A receptor modified the cellular localization of the cathepsin D in macrophages, too. We also checked the effect of cathepsin D inhibition on the A2A mediated cytokine production of macrophages. We detected lower level of the proinflammatory cytokine TNF-alpha and higher level of the anti-inflammatory cytokine IL10 production of the activated macrophage cells in the presence of cathepsin D inhibitor. Our results demonstrate that A2A mediated signalization regulates the maturation of cathepsin D and inhibition of the protease has effect on the A2A regulated pro- and anti-inflammatory cytokine production of macrophages. Supported by OTKA 84685 MB08A.

Abstracts E12 - Pathobiochemistry and Organelle Biochemistry

E12-1
Diabetes mellitus and the endoplasmic reticulum

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Type 2 diabetes mellitus is often associated to obesity and sedentary lifestyle, and it is characterized by pancreatic β-cell dysfunction. The disease evolves on the ground of insulin resistance and often remains unnoticed as long as the damaged peripheral responsiveness can be compensated for by an enhanced insulin secretion. The combination of central obesity, dyslipidemia, hypertension and insulin resistance is referred to as the metabolic syndrome, which typically turns into a real diabetes mellitus upon the failure of strained β-cells. The striking similarities between these features and the symptoms of the Cushing's disease strongly suggest the involvement of an excessive glucocorticoid activity in spite of normal plasma cortisol levels in the metabolic syndrome. Investigations of the last decade shed light on the prominent role of the endoplasmic reticulum (ER) in the physiology and pathology of insulin-mediated metabolic control. Normal β-cell function and viability are dependent on appropriately controlled insulin processing in the ER. Insulin resistance can at least partly develop due to ER stress and the consequent unfolded protein response (UPR) in various tissues. Enhanced local (so called pre-receptor) cortisol production in the lumen of the ER might be responsible for an intracellular Cushing's disease upon overfeeding. In addition, the aggravating insulin resistance, which causes hyperglycemia, hyperfattyacidemia and a growing insulin demand, leads to β-cell dysfunction and β-cell apoptosis through an incurable ER stress. Our studies focus on the nutrient sensor function of the ER in health and disease as well as on potential drug targets in the organelle offering new means of prevention and/or treatment of the obesity-related metabolic diseases. Supported by the Hungarian Scientific Research Fund (OTKA 104113 and 106060) and by the Hungarian Research and Technological Innovation Fund (KMR_12-1-2012-0074). É.K. is a grantee of the Bolyai Research Scholarship of the HAS.

E12-2
Prevention of poly(ADP-ribosyl)ation and inactivation of ATF4/Creb2 by PARP inhibitor protects cells via ATF4-MKP-1-MAPK pathway

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Oxidative stress-induced JNK and p38 MAP kinase activation occur in several diseases and contribute to diseases progression. Several studies showed that PARP inhibitors reduce JNK and p38 MAP kinase activation which is important in inflammatory and cell death pathways, but its molecular mechanism is not yet understood. Analyzing oxidative stress induced MAP kinase (ERK, JNK and p38 MAPK) activation we show the prominent role of PARP inhibitor (PJ_{34}) induced MKP-1/Dusp1 expression in JNK and p38 MAPK inactivation, and reduction of mitochondrial damage and cell death. Transcription factors involved in MKP-1 expression (Hsf1, 2 and 4, and Creb1 and ATF4/Creb2) were analyzed, and showed that suppression of ATF4/Creb2 prevents PARP inhibitor induced MKP-1 expression, mitochondria protection and cytoprotection. PARP-1 catalyzed the poly-ADP-ribosylation of ATF4 inhibited ATF4-binding to CRE-elements while no binding was observed to mutated CRE elements giving clear mechanism for the ATF4 inactivation by PARP-1. PARP-1 binding to CRE-element was facilitated by self poly-ADP-ribosylation, and was inhibited by PARP inhibitor. These data show that PARP-1 inhibition by preventing ATF4/Creb2 poly-ADP-ribosylation and inactivation initiates MKP-1 expression which inactivates JNK and p38 MAP kinases preventing mitochondrial damage and cell death. PARP inhibitor induced ATF4-MKP-1-MAPK retrograde pathway can be important in oxidative stress related diseases, and because of the critical role of JNK activation in the tumor-initiating capacity of cancer stem cells the PARP inhibitor induced JNK inactivation emphasize the importance of PARP inhibitors in cancer therapy. 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'.

E12-3

The role of PARP-2 in the regulation of SREBP1

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The metabolic role of poly(ADP-ribose) polymerase-2 (PARP-2) was recently identified, it's deletion impairs the function of the adipose tissue and the endocrine pancreas. Furthermore, in the absence of PARP-2 the expression of SIRT1 was induced leading to enhanced mitochondrial biogenesis in skeletal muscle and liver. To date PARP-2 was the only PARP enzyme shown to regulating hepatic metabolism. In the current study we wanted to gain insight into the impact of PARP-2 on hepatic lipid metabolism. Besides the known decrease in triglyceride content, cholesterol levels were induced in the livers of PARP-2-/- mice. To uncover the molecular background, we analyzed changes in steady-state mRNA levels upon the knockdown of PARP-2 in HepG2 cells and revealed higher expression of sterol-regulatory element binding protein (SREBP)-1 dependent genes. We demonstrated that PARP-2 is a suppressor of the SREBP1 promoter, and the suppression of the SREBP1 gene depends on the enzymatic activation of PARP-2. Consequently, the knockdown of PARP-2 enhances SREBP1 expression that in turn induce the genes driven by SREBP1 culminating in higher hepatic cholesterol content. We did not detect hypercholesterolemia, higher fecal cholesterol, higher hepatic or fecal bile acid content or increase in serum LDL suggesting that the excess cholesterol is not exported from the liver. To our surprise serum HDL levels decreased in the PARP-2$^{-/-}$ mice. In cells and mice where PARP-2 was deleted we observed decreased ABCA1 mRNA and protein expression that is probably the explanation for lower HDL levels. Apparently, the deletion of PARP-2 rearranges lipid metabolism. In the absence of PARP-2 hepatic triglyceride levels are reduced, while cholesterol levels are upregulated and cholesterol accumulates in the liver. In the circulation HDL levels decrease suggesting impaired cholesterol transport between the liver and the periphery suggesting a risk factor for peripheral cholesterol deposition. Supported by grants from OTKA (K108308, K105872), TÁMOP-4.2.2. A-11/1/KONV-2012-0025. P.B. and M.S. are recipients of Bolyai fellowship, the laboratory is supported by the Lendület program of the Hungarian Academy of Sciences.

E12-3

Glycogen phosphorylase inhibitors enhance pancreatic β–cell function and hepatic mitochondrial metabolism

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Glycogen phosphorylase (GP) cooperating with glycogen synthase regulates glycogen metabolism, hence has major role in hepatic glucose production (HGP). Therefore, GP inhibition is a potential target to modulate glucose levels in type II diabetes. We evaluated the metabolic effects of glucose-based GP inhibitors KB228 (N-(3,5-dimethyl-benzoyl)-N'-(β-D-glucopyranosyl)urea K_i = 937nM) and BeVa335 (3-β-D-glucopyranosyl-5-(2-naphtyl)-1,2,4-triazole K_i= 0.411 nM) synthetized by laboratory of Somsák László. In vivo, single *i.p*. injection of KB228 increased glucose sensitivity in lean (chow-fed) and diabetic, obese (C57/Bl6J mice on 60% fat hypercaloric diet for 3 months) mice linked with glucose excursion to the liver. KB228 enhanced oxygen consumption and RQ suggesting higher mitochondrial activity, which was probably linked to the overexpression of uncoupling protein-2 (UCP2) in animal and cellular models (HepG2) under both normoglycemic and hyperglycemic conditions. Besides, KB228 induced mammalian target of rapamycin complex 2 (mTORC2), which may take an active part in increased glycogen deposition in the cells. Further-more, we have observed elevated glucose-induced insulin release upon KB228 administration hence we investigated this effects on cellular model of pancreatic β-cells, MIN6 insulinoma cell line. We have discerned further unexpected metabolic rearrangements: increased mitochondrial function and insulin production as well as facilitated insulin secretion in MIN6 β-cells after two-day treatment. Moreover, these inhibitors intensified mTORC2 activation manifested by increased AKT phosphorylation. KB228 and BeVa335 treatment induced MIN6 proliferation together with the islets of Langerhans from mouse (C57/Bl6J, chow-and HFD-fed treated with KB228 (90mg/kg)) pancreas. Our results suggest that GP inhibitors do not only reduce HGP but induce beneficial metabolic rearrangements as well. Supported by OTKA K108308, TÁMOP-4.2.2. A-11/1/KONV-2012-0025. P.B., A.T. and T.D. were supported by the Bolyai fellowship. The laboratory is supported by the Lendület program of the Hungarian Academy of Sciences.

E12-5

Role of ADP dependent glucokinase in respiratory burst of differentiated HL-60 cells

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ADP dependent glucokinase (ADPGK) is a specific kinase using ADP to phosphorylate glucose instead of ATP. ADPGK is highly expressed in human and rodent leukocytes, but its role is not well known. Glucose-6-phosphate produced by ADPGK can be further metabolised through various pathways e.g. the pentose-phosphate pathway thus contributing to NADPH production. During phagocytosis glucose-6-phosphate breakdown is shifted towards pentose-phosphate pathway providing high amounts of reduced NADPH for the respiratory burst. Differentiation in DMSO induced HL-60 cells cause an approx. 10 fold increase in the expression of ADPGK according to qPCR. This difference was also present on the protein level and enzyme activities supported the induction also. Differentiated HL-60 cells silenced for ADPGK showed significant decreased glucokinase activity compared to non-silenced differentiated cells. Transfection of both cell types with ADPGK siRNA decreased superoxide production in differentiated cells compared to non-differentiated cells as well. Our results suggest that ADPGK has a potential role in bactericidal properties of neutrophil granulocytes influencing respiratory burst via the pentose-phosphate shunt at the NADPH level. This work was supported by OTKA 101226

E12-6

Molecular mechanisms of IL-1β production and NLRP3 inflammasome function in LPS-activated human macrophage subtypes

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IL-1β is a pro-inflammatory cytokine that has indispensable role in orchestrating innate and adaptive immune responses via regulating cell death, tissue repair, inflammation or T cell polarization. One of the main sources of IL-1β production is the activated macrophages (MFs). Depending on the tissue environment MFs differentiate to morphologically and functionally different populations. We aimed to investigate the molecular mechanisms of Nlrp3 inflammasome activation and subsequent IL-1β secretion of different human macrophages in response to LPS and ATP stimulation. Macrophages were generated from human peripheral blood in the presence of granulocyte-macrophage colony stimulating factor or macrophage colony stimulating factor which mimic immuno-stimulatory (GM-MF) or tissue repair (M-MF) functions, respectively. M-MFs and GM-MFs were stimulated with ultrapure LPS in the presence or absence of ATP. Our results show that though both types of LPS-activated MFs secrete IL-1β in the presence of ATP, in the case of M-MFs IL-1β is released rapidly and only for a short time period, while IL-1β secretion by GM-MFs is sustained. Consistently, pro-IL-1β and NLRP3 proteins were expressed with the same time kinetics leading to the activation of caspase-1 enzyme. While the virtual enzyme activity of caspase-1 was comparable in the two macrophage types, we measured substantial differences in the activation of signaling pathways, as well as in the effect of IL-10 neutralizing antibody, and in the expression of IL-1Ra and that of the ecto-ATPases. Due to intensive studies, the general mechanism of Nlrp3 inflammasome activation is well characterized, nevertheless our results demonstrate that the actual inflammasome activation and IL-1β secretion is substantially determined by the molecular characteristics of a given cell. Financial support: OTKA K-109429, TÁMOP-4.2.2.A-1/1/KONV-2012-0023. S.B. is receiver of Lajos Szodoray Post.Doc. Fellowship and János Bolyai Post.Doc. Fellowship.

Abstracts
P - Poster Session

P-1
Selection and characterization of Noggin specific aptamers

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Bone morphogenic proteins (BMPs) are members of TGF-β (transforming growth factor-β) superfamily. These extracellular signalling proteins regulate embryonic development through binding to their cognate receptors. BMPs are also expressed in the dental mesenchymal and epithelial cells and are involved in tooth development. Noggin is a BMP-specific antagonist protein that prevents induction of BMP activated signalling pathways. It has been shown that the expression of Noggin in osteoblasts was elevated when the cells were treated with BMPs suggesting a negative regulatory loop of BMP signalling. We set out to isolate Noggin selective aptamers for enhancing bone morphogenesis of stem cells by disrupting BMP-Noggin interaction.
Aptamers are single stranded RNA or DNA molecules that are selected from a random single stranded DNA or RNA library to bind specifically to various targets (e.g. proteins) after folding into well-defined spatial structures. Aptamers are isolated by an *in vitro* selection process, referred to as Systematic Evolution of Ligands by EXponential enrichment (SELEX). We applied a modified two phase selection procedure to isolate Noggin selective aptamers. In the first phase, the BMP interacting peptide motif of Noggin was used as selection target. For the second phase of SELEX, we produced GST-tagged Noggin by in vitro translation and applied the purified protein as ligand of selection. Following completion of selection procedure, several aptamer candidates were characterized by fluorescence anisotropy. The BMP-Noggin disrupting capacity of most promising aptamers was also evaluated by Amplified Luminescent Proximity Homogenous Assay (ALPHA). The luminescence measurements demonstrated that one of the selected aptamers effectively blocks the interaction between 6XHis-tagged BMP-2 and GST-tagged Noggin implying competition of aptamer with BMP-2. Currently, we set up a bone cell differentiation system to study the effect of aptamers on bone morphogenesis.

P-2
Identification Of the transcriptome, small rna pattern and their targets in a model plant by high-throughput sequencing

Ivett Baksa[1], Tamás Nagy[2], Eszter Barta [2], Zoltán Havelda[4], Dávid Silhavy[5], József Burgyán[3], György Szittya[1]
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Nicotiana benthamiana has an important role in many plant molecular biological researches. It is a widely used model species for plant-pathogen interactions and also an excellent target plant for agroinfiltration and a platform for industrial production of recombinant proteins. Since it is also susceptible to the large number of diverse plant viruses, it is a common experimental host in plant virology. Virus infected plants display various symptoms with different type and severity and these phenotypic alterations are the consequence of altered gene expression of the host plant. Gene expression is regulated at several levels to ensure normal development and appropriate responses to biotic and abiotic stresses. One of the post-transcriptional regulatory mechanisms relies on endogeneous short RNAs that are 21-24 nt in length. Here, we use deep-sequencing and computational methods to identify profile and describe conserved and non-conserved miRNAs in five *N.benthamiana* tissues. A total of 22 conserved miRNA families were identified in all five tissues and 19 known but non-conserved miRNAs were also found. In addition to known miRNAs, we also found 25 new *N.benthamiana* specific miRNAs together with their star strands. Genomic-scale high-throughput sequencing of miRNA cleaved mRNAs identified 41 target mRNAs of known and 36 target mRNAs of new *N.benthamiana* – specific miRNAs. It could be a useful tool for any other scientist who works with *N. benthamiana*.

P-3

Characterization of the DNA remodeling activity of HLTF protein

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The DNA in our cells is continuously damaged by different agents, such as UV irradiation, reactive oxygen species, metabolites and chemicals. These agents are changing the structure of the DNA molecule. To avoid these mutations many DNA repair mechanisms have evolved. These mechanisms are able to set back the original structure of the DNA double helix but some damages get to the S phase of the cell cycle where they can cause the stalling of the replication fork, chromosomal breaks and cell death. To avoid these possibilities the DNA damage bypass pathway has evolved which can protect the stalled replication fork by different ways. The main step of the pathway is the monoubiquitylation of the PCNA protein, which is the processivity factor of the polymerases by Rad6/Rad18 complex at the lysine 164 position. After this modification the replicative polymerase can be changed by an alternative polymerase, which is able to synthesize through the lesion. In an other error free mechanism the monoubiquitylated PCNA becomes polyubiquitylated by the Mms2/Ubc13/HLTF complex through lysine 63 residues, therefore HLTF can reverse the replication fork. On this newly emergent so-called chicken foot structure the stalled strand can be finished using the newly synthesized sister strand as a template. The third possibility is an alternative template switching mechanism. Our study is focusing on HLTF protein and the better understanding of the function and regulation of the DNA damage bypass pathway. We are analyzing the mechanism and function of HLTF DNA remodeling activity. Our ultimate goal is to shed light on the whole molecular mechanism of the damage bypass. 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'.

P-4

The role of myosin phosphatase-eNOS interaction in nitrite production

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Endothelial nitric oxide synthase (eNOS) produces nitric oxide (NO) which is involved in the regulation of smooth muscle relaxation and many other physiological processes. eNOS activity is regulated by phosphorylation of different Ser an Thr residues. It has been demonstrated that the protein phosphatase-1 (PP1) catalytic subunit (PP1c) is involved in the regulation of eNOS via dephosphorylation at Thr495, however, the role of the regulatory subunit(s) is unknown yet.

We investigated whether myosin phosphatase (MP) consisting of PP1cδ and myosin phosphatase target subunit-1 (MYPT1) is involved in the dephosphorylation of eNOS. The interaction of MYPT1 and eNOS in endothelial cells (EC) was demonstrated by several methods as well as in tsA201 cells co-expressing 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. tsA201 cells, , overexpressing myc-eNOS exhibited higher NO level as revealed by nitrite measurements. Upon PMA, calyculin-A (CLA) and tautomycin (TM) treatment phosphorylation of Thr495 was stimulated in both BPAEC cells and tsA201 cells transfected with eNOS plasmid and it was accompanied with decreased NO production. We demonstrated that MP and eNOS interacts in EC's and MP is the phosphatase holoenzyme which dephosphorylates phospho-eNOS-Thr495, thus it may be involved in the activation of this enzyme coupled with increased NO production. This work was supported by grants from the Hungarian Scientific Research Fund OTKA K109249 and TÁMOP-4.2.2.A-11/1/KONV-2012-0025.

P-5

The role of protein phosphatase inhibitors on the nitrit production

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Endothelial nitric oxide synthase (eNOS) produces nitric oxide (NO) which is involved in the regulation of smooth muscle relaxation and many other physiological processes. eNOS activity is regulated by phosphorylation of different Ser an Thr residues. It has been demonstrated that the protein phosphatase-1 (PP1) catalytic subunit (PP1c) is involved in the regulation of eNOS via dephosphorylation at Thr495, however, the role of the regulatory subunit(s) is unknown yet. We investigated whether myosin phosphatase (MP) consisting of PP1cδ and myosin phosphatase target subunit-1 (MYPT1) is involved in the dephosphorylation of eNOS. The interaction of MYPT1 and eNOS in endothelial cells (EC) was demonstrated by several methods as well as in tsA201 cells co-expressing 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. tsA201 cells, , overexpressing myc-eNOS exhibited higher NO level as revealed by nitrite measurements. Upon PMA, calyculin-A (CLA) and tautomycin (TM) treatment phosphorylation of Thr495 was stimulated in both BPAEC cells and tsA201 cells transfected with eNOS plasmid and it was accompanied with decreased NO production. We demonstrated that MP and eNOS interacts in EC's and MP is the phosphatase holoenzyme which dephosphorylates phospho-eNOS-Thr495, thus it may be involved in the activation of this enzyme coupled with increased NO production. This work was supported by grants from the Hungarian Scientific Research Fund OTKA K109249 and TÁMOP-4.2.2.A-11/1/KONV-2012-0025.

P-6

Cytokine production and NLRP3 inflammasome activation in GM- and M-MFs

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IL–1β is synthesized as an inactive pro-IL-1β form and cleaved by a multi-protein complex called NLRP3 inflammasome. Activation of inflammasome requires two signals: the first signal (TLR4 activation by lipopolisacharide - LPS) leads to the synthesis of pro-IL–1β and the inflammasome components, the second signal (P2X7R activation by ATP) results in the assembly of the inflammasome and the cleavage of pro-IL–1β by caspase-1 enzyme. Macrophages are one of the main sources of IL–1β. Depending on the tissue environment monocytes differentiate into alternative macophage subpopulations We aimed to investigate the molecular mechanisms of IL-1β secretion from different human macrophages in response to LPS and ATP. Macrophages were generated from human peripheral blood in the presence of granulocyte-macrophage colony stimulating factor (GM–CSF) or macrophage colony stimulating factor (M-CSF) which indicate the immuno-stimulatory (GM-MF) or the tissue repair (M-MF) functions of the cells. On day 5, M-MFs and GM-MFs were stimulated with ultrapure LPS in the presence of ATP. We show that the rapid release of IL–1β by M-MFs is associated with increased expression of NLRP3 and pro-IL–1β at an early timepoint which is followed by a rapid down-regulation. In contrast, GM-MFs produce IL–1β at a later timepoint but in comparable amount. The experiments with caspase-1 inhibitor indicate the critical involvement of the enzyme in IL–1β secretion by both macrophage types. Further regulatory differences were observed between M- and GM-MFs during analysis of the activation of key signal transduction pathways. We can conclude that the actual IL-1β production by a particular cell strongly depends on the cell type and its typical features influenced strongly by environmental mediators. Financial support: OTKA K-109429, TAMOP-4.2.2.A-1/1/KONV-2012-0023.

P-7

Identification of protein phosphatase interacting proteins from normal and UVA-irradiated HaCaT cell lysates by surface plasmon resonance based binding technique

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Identification of the interacting proteins of phospho-serine/threonine (P-Ser/Thr) specific protein phosphatases is an important issue in determining the cellular roles of these enzymes. We designed a method based on surface plasmon resonance (SPR) binding technique to isolate the protein phosphatases and their interacting partners from cell lysates. Microcystin-LR (MC-LR), a potent inhibitor of protein phosphatase-1 (PP1), -2A (PP2A), PP4, PP5 and PP6, was biotinylated and immobilized to streptavidin-coupled sensor chip surface. Biotin-MC-LR captured PP1 catalytic subunit (PP1c) stably and the biotin-MC-LR-PP1c complex was able to further interact with the regulatory subunit (MYPT1) of myosin phosphatase. Biotin-MC-LR coated sensor chip surface in the Surface Prep unit of Biacore 3000 captured PP1c, PP2Ac and their regulatory proteins including MYPT1, MYPT family TIMAP, inhibitor-2 as well as PP2A-A and –B subunits from normal and UVA-irradiated HaCaT cell lysates as revealed by dot blot analysis of the recovered proteins. Our results imply that biotin-MC-LR is a suitable capture molecule in SPR for isolation of protein phosphatase interacting proteins from cell lysates in sufficient amounts for immunological detection. This work was supported by OTKA K109249 from the Hungarian Scientific Research Fund; TÁMOP-4.2.2/B-10/-1-2010-0024, TÁMOP-4.2.2.A-11/1/KONV-2012-0025 and TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program' supported by the European Union and the State of Hungary, co-financed by the European Social Fund.

P-8

Effect of the glycogen phosphorylase inhibitor molecules on the cell cycle of tumour cell lines

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Metabolic reprogramming in cancer cells provides energy and important metabolites required to sustain tumour proliferation. Enhanced glycogen metabolism is a common feature of cancer cell metabolism, and therefore may represent novel anticancer therapeutic targets. Glycogen primarily acts as an intracellular storage of glucose and fulfils important roles in both nonmalignants and cancer cells under conditions of oxygen and nutrient deprivation. GP is the main enzyme that catalyses the release of glucose from glycogen. The major role of liver glycogen is to supply glucose to the circulation maintaining the normal blood glucose level. In muscle and liver, the accumulation and breakdown of glycogen are regulated by the reciprocal activities of glycogen synthase (GS) and glycogen phosphorylase (GP). The control of these enzymes can occur by reversible phosphorylation by hormonal and neuronal stimulation and by allosteric effectors. GP which catalyses the first step of glycogen degradation can be inhibited by glucose and its analogues. Metabolic reprogramming in cancer cells provides energy and important metabolites required to sustain tumour proliferation. We extend our research studying the connection of the energy derived from glycogenolysis and the cell cycle of human tumour cell lines. Using cell permeable GP inhibitors in synchronized cells we was resolved the energy requirement of the restriction point in G1 through S phase and its connection to the inhibition of glycogenolysis. By the assay of several glucose derivatives as possible inhibitors of GP structure-activity relations will be also analyzed. Supported by TÁMOP-4.2.2.A-11/1/KONV-2012-0025, Bolyai János Research Fellowships from the Hungarian Academy of Sciences and the University of Debrecen (5N5X 1IJ0 KUDT 320).

P-9

Lipopolisacharide induces IL-1β production via P2X7 receptor - independent mechanisms in human GM-MFs

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IL-1β is synthesized as a proprotein, which is proteolytically processed to its active form by a multi-protein complex called NLRP3 inflammasome. Activation of NLRP3 inflammasome requires an initial priming step for the expression of the inflammasome components and of pro-IL-1β and a second signal, like the extracellular ATP, for the assembly and activation of the inflammasome. Under metabolically stressful conditions like inflammation and cell damage ATP accumulates in the extracellular space. The stimulation of ATP-sensing purinergic receptor P2X7 leads to K^+ efflux and mediates inflammasome activation. We aimed to study the molecular mechanisms of IL-1β production and secretion by LPS-activated human macrophages in the presence or absence of exogenous ATP. Monocytes were separated from human peripheral blood and cultured for five days in presence of granulocyte-macrophage colony stimulating factor (GM–CSF) to become inflammatory macrophages (GM-MFs). The macrophages were activated by ultrapure LPS in the presence or absence of ATP. Our results show that GM-MFs secrete substantial amounts of cleaved IL-1β upon LPS treatment in the presence of ATP. This cytokine secretion is associated with increased expression of NLRP3 and pro-IL-1β and depends on P2X7 receptor activation. It was described that human monocytes release mature IL-1β upon LPS stimulation due to autocrine production of ATP. Contrary to these results the IL-1β release by GM-MFs in the absence of ATP is independent of P2X7 receptor. Notably ATP release was detected upon LPS activation; however neither apyrase, an enzyme that hydrolyzes extracellular ATP nor the inhibition of ATP releasing channel pannexin-1 nor the P2X7 receptor inhibitor did not affect IL-1β secretion. These data suggest that P2X7 signaling was not required for IL-1β release in response to LPS in human GM-MFs. Financial support: OTKA K-109429, TÁMOP-4.2.2.A-1/1/KONV-2012-0023.

P-10

Alternatively activated macrophages possess enhanced angiogoenic capacity in response to RXR activation through different enhancer usage

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Retinoid X Receptor (RXR) is a ligand inducible transcription factor implicated in various metabolic and immune processes. RXR is the obligate heterodimerization partner of several nuclear receptors for instance PPARs or LXRs. There is a great debate on the field about the individual action of RXR. Our previous results in mouse macrophages (MQs) show that RXR activation leads to the establishment of a pro-angiogenic phenotype through the regulation of the vascular endothelial growth factor alpha (Vegfa) which can not be recapitulated through the activation of the two main heterodimeric partner (PPARg, LXR) in MQs. Activating RXR does not lead to the redistribution of the receptor assayed by ChIP-Seq. These results suggest that RXR signaling has a unique readout in mouse MQs and the RXR cistrome remains static in the presence of the activator ligand. Based on these we decided to map the genomic binding sites of the receptor in IL-4 differentiated, alternatively activated MQs. Surprisingly, we could show more than 7000 significantly upregulated RXR binding sites in IL-4 differentiated MQs compared to those grown in the absence of IL-4. According to these results it seems that IL-4 reprogrammes the nuclear receptor binding landscape. Due to the fact that alternatively activated MQs participate in wound healing and tumor progression it is more interesting whether these cells are even more pro-angiogenic upon RXR activation. Using 3C sequencing we revealed the potential enhancers participating in the regulation of Vegfa. We showed that IL-4 differentiation leads to enhanced RXR binding on a set of very long range enhancers and interestingly on a novel one located -41kb far from Vegfa. Finally, we could show at the protein level that activation of RXR in IL-4 differentiated MQs resulting in a more pronounced VEGFA secretion which may indicate that these cells are even more pro-angiogenic upon RXR activation than their counterparts differentiated in the absence of IL4.

P-11

WFS1 promoter polymorphisms as putative genetic components of type 2 diabetes mellitus

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Diabetes mellitus has become one of the most significant health problems worldwide. It is known that the disease is a complex phenotype, being determined by both environmental and genetic factors as well as by their interactions. However the complete list of genetic components of the disease has not been identified yet. The purpose of this research project was to investigate, if certain genetic factors can be considered as risk factors of diabetes mellitus. The *WFS1* gene seemed to be a promising candidate, thus three polymorphisms (two SNPs: rs4273545, rs4689388 and one VNTR: rs148797429) were selected in the promoter region of the gene for analysis. Buccal DNA samples were collected and purified from 368 type 2 diabetic and 588 control people. Restriction endonuclease based method, allele-specific polymerase chain reaction and melting curve analysis were employed for genotyping, and the obtained results were evaluated in a case-control setup. Since the chosen polymorphisms are in close proximity to each other, we applied linkage analysis as well. As no complete linkage disequilibrium was observed, we also carried out a haplotype analysis. Putative biological function of the polymorphisms was examined with luciferase reporter assay. We successfully applied melting curve analysis for genotyping the rs148797429 VNTR. In the control population all genotype frequency values were in Hardy-Weinberg equilibrium ($p>0.1$). The SNPs showed significant difference between the control and diabetic population. After the Bonferroni correction, the A allele of the rs4689388 ($p = 0.000225$), and the T allele of the rs4273545 ($p = 0.000341$) was significantly more common in the diabetic population. One haplotype (A-2x-T) seems to be a risk factor (O.R. = 3.18, $p = 0.0000184$) for type 2 diabetes. *In vitro* analysis of the polymorphisms is currently in progress. This work was supported by the Hungarian grant OTKA K83766.

P-12

The role of the B” subunit of PP2A phosphatase complex in the dephosphorylation of *Oryza sativa* retinoblastoma-related protein-1

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The phosphorylation of plant retinoblastoma-related (RBR) proteins by cyclin dependent kinases (CDKs) is well documented, but the counteracting plant phosphatases have not been identified yet. We found that rice retinoblastoma-related protein-1 (OsRBR1) underwent reversible phosphorylation during the cell division cycle, and the OsRBR1 protein interacted with the B” subunit of rice protein phosphatase 2A (OsPP2A B”). This association required intact pocket domains in OsRBR1 and the second calcium binding EF-hand motif in OsPP2A B”. Immunoprecipitation experiments revealed that OsPP2A B”, OsPP2A catalytic subunit subtype II, PSTAIRE-type CDK and OsRBR1 were in the same protein complex, indicating a physical association between the phosphatase, the kinase and their common substrate. OsPP2A B” contains three predicted CDK phosphorylation sites: Ser95, Ser102 and Ser119. The *in vitro* phosphorylation of Ser95 and Ser119 with PSTAIRE-kinases was demonstrated by mass spectrometry. We generated a series of phosphorylation site mutants to mimic the dephosphorylated or phosphorylated states of OsPP2A B”, and confirmed that all of the three predicted sites can be phosphorylated. Yeast two-hybrid experiments indicated that the full phosphorylation of OsPP2A B” may promote the OsPP2A holoenzyme formation. A mutant mimicking the triple phosphorylation of OsPP2A B” showed higher activity in phosphatase assays. According to our data, the phosphatase activity of OsPP2A associated with its substrate OsRBR1 is regulated by phosphorylation and Ca^{2+} binding of its B” subunit. Supported by OTKA grant NK-69227 and the Hungarian Social Renewal Operational Program (TAMOP-4.2.2-A-11/1/KONV-2012-0025 grant). E.Á. was supported by the Bolyai Research Fellowship of HAS.

P-13

Studies on the NOD-like receptor family member human NLRC5 protein

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NOD-like receptor (NLR) family member proteins have important role in the regulation of innate immune responses, as these pattern recognition receptors are involved in the recognition of microbial- and danger-associated molecular patterns in vertebrates. The NOD-like receptor family CARD domain containing 5 (NLRC5) protein is unique within the NLR protein family: it is the largest member due to the unusually high number of leucine-rich repeats (LRRs) and the presence of an untypical caspase activation and recruitment domain (CARD). NLRC5 plays a major role in the regulation of MHC I transcription, it is involved in the regulation of various signal transduction pathways, but its direct ligand is unidentified and its involvement in the inflammasome activation is still controversial. To correlate biochemical findings with the proposed homology models of full-length human NLRC5 protein published recently by our laboratory, we performed expression studies on NLRC5. To test the reliability of commercially available anti-Flag and anti-NLRC5 antibodies for Western blot, we expressed NLRC5-Flag fusion protein in 293T cells. For co-immunoprecipitation examinations we utilized myc-NLRC5 fusion protein construct. The overexpressed fusion proteins were successfully identified by anti-Flag or anti-myc antibodies, while the tested anti-NLRC5 antibodies were found to be less effective. We performed alkaline phosphatase treatment in order to clarify possible phosphorylation. We successfully confirmed the potential oligomerization of the NLRC5-Flag fusion protein by gel filtration of total cell lysates. An MRM-based method was also designed to detect NLRC5 without antibodies. We optimized the quantitative measurement of the NLRC5 protein using isotope-labelled oligopeptide, and measured the level of endogenous protein after treatment of HaCaT cells. This work was supported by the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 „VÉD-ELEM" project.

P-14

Calcium binding of a defensin-like antifungal protein does not contribute to the inhibition of antimicrobial activity – a structural biology approach

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Recent biological studies suggest that high cation concentration interferes with the antifungal activity of defensin-like antifungal proteins. Furthermore, it was shown in the case of *Penicillium* antifungal protein that cytosolic free calcium ion concentration increases in sensitive organisms in response to the impact of this antifungal agent, though without permeabilisation of the plasma membrane. In order to disclose the role of calcium in the antimicrobial mechanism, we investigated the calcium-binding properties of *Penicillium* antifungal protein by nuclear magnetic resonance spectroscopy, isothermal titration calorimetry, molecular dynamics simulations, and generated mutants of this protein and compared its antifungal activity to the wild type. We proved that *Penicillium* Antifungal Protein binds only calcium cation, however the binding affinity is in the sub-milimolar region and binding of calcium does not influence appreciably the three dimensional structure of the protein. We eliminated the calcium binding site of *Penicillium* antifungal protein by asparagine-serine mutation, that decreased the calcium-binding affinity by an order of magnitude. Our results suggest that calcium binding does not contribute to the putative interference between cation binding and the antifungal activity of this protein. Perhaps, the observed inhibitory effect of calcium is connected to a yet unknown signaling mechanism. This research was supported by the Grant OTKA ANN-110821, and 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'.

P-15

Study of anti-Warburg effect of AMPK activators and antimetabolite drugs on MCF7 cell model

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AMPK is an energy sensor that regulates cellular metabolism. When activated by a deficit in nutrients, AMPK stimulates mitochondrial energy production, while turning off energy-consuming processes to restore energy balance. We analyzed AMPK activation in MCF7 ductal breast cancer cells. AMPK can work as an anti-Warburg agent via enhancing mitochondrial biogenesis.

In MCF7 cells we studied the combined action of the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) and the antimetabolite folate analog, Methotrexate (MTX). AICAR is an AMP mimetic that is able to activate AMP-activated protein kinase. On the other hand, MTX is a folate analog inhibiting the synthesis of purine bases and hence DNA duplication. AICAR and MTX exerted an additive effect on MCF7 cells and caused inhibition of cell proliferation. We observed increased mitochondrial activity as evidenced by DioC6 and TMRE measurements, while glycolytic flux decreased. The combination of AICAR and MTX also disrupted the phases of the cell cycle, and the cells were accumulated in the G1 and G2 phase. These data suggest that the combined application of AICAR and MTX slows down cell cycle through an anti-Warburg effect.

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P-16

Visualizing and understanding experimentally determined protein dynamics at the atomic level: methods and applications

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The internal dynamics of proteins is recognized as one of the key factors determining their molecular function and efficiency. Recently developed methods allowing the combination of experimental data (primarily obtained by NMR spectroscopy) and molecular dynamics simulations are capable of yielding ensemble models or proteins that reflect the observed dynamics of proteins at several time scales [1]. Besides, due to the ensemble-based nature of the method, individual conformers are expected and often indeed found to be closer to ideal geometry than those obtained by conventional structure determination methods [2]. After cross-validation with experimental data not used in the course of the calculations [3], the ensembles can be analyzed to assess the role of correlated motions and changes in residue-residue interactions, as well as the similarities and differences between ensembles in different functional states. Based on the free GROMACS molecular dynamics package, we have implemented several methods for the generation of protein structural ensembles that reflect experimentally determined dynamics at different time scales [4,5]. In the presentation, the methodology and its applications to various proteins like different parvulins, the 3rd PDZ domain of PSD95 and the C-terminal domain of the Rev1 polymerase will be discussed.

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P-17

The charged single alpha-helix in paraspeckle-forming proteins

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The Charged Single Alpha Helix (CSAH) is a recently identified structural motif often overlapping with regions predicted to form coiled coils and/or to be disordered [1]. The exact structural and functional role of CSAH segments has been characterized only in some myosins [2]. Our systematic survey revealed that although CSAH-containing proteins are relatively rare (in humans, they are found in ~0.2% of the proteome), they are preferentially associated with some molecular functions, one of the most prevalent being RNA-binding [3]. In order to investigate the role of CSAH segments in RNA-binding proteins in more detail, we have chosen the proteins of the paraspeckle, a novel subnuclear RNA-binding component of eukaryotic cells. We have shown that all of the proteins investigated (NONO, PSPC1 and SFPQ) contain conserved domain organization and CSAH segments located at the C-terminal parts of their predicted coiled coil regions. Using the experimentally determined structure of the human NONO-PSPC1 dimer and suggestions about its higher-order organization [4], we have built atomic-level models of hexameric NONO-PSPC1 fragments. These models contain extended coiled coil and CSAH regions missing from the crystal structure. Our results suggest that the CSAH segments might have a role in the exact spatial positioning of the dimers relative to each other during paraspeckle formation. Funding by the Hungarian Scientific Research Fund (OTKA 104198) and the European Union (TÁMOP-4.2.1.B-11/2/KMR-2011-0002) is acknowledged.

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P-18

NMDA receptor expression in neoplastic cells

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The NMDA type glutamate receptors (NMDAR) are non-selective cationic channels, which are mostly permeable for Ca^{2+}. Subunits of the receptor (NR1, NR2, NR3) function as diheterotetrameric assemblies. NR1 may contain a nuclear localization signal, which binds (or releases) importin-α depending on the phosphorylation status of NR1. This may result in nuclear shuttle of various transcription factors or other proteins and nuclear transport of NR1 subunit is also conceivable. Pharmacological modification of NMDA receptors resulted in intracellular Ca^{2+} concentration changes in melanoma cells, dominantly detectable in the area of the nucleus. Therefore we aimed to describe the expression pattern and subcellular localization of NMDA receptor subunits in different neoplastic cells. A2058 and WM35 human melanoma cells and MCF7 invasive ductal breast carcinoma cells were used for our experiments. mRNAs were detected with RT-PCRs, Western blots on samples of cell lysate fractions to detect NMDA receptor subunit proteins in different cellular compartments were performed and subcellular localization was also investigated with fluorescent immunocytochemistry. RT-PCR showed that mRNAs of all types of subunits (NR1, NR2A, NR2B, NR2C, NR2D, NR3A, NR3B) are present in all cell lines. Fractionated Western blots showed that NR1 and NR3 subunits are present throughout in the cells. NR2B protein was not detectable in any cell lines, while NR2A seemed to be expressed in the cytoplasmic fraction of all cell lines but was present in the plasma membrane fraction only of melanoma cell lines. NR1-NR3B immunocytochemisty revealed that the two subunits colocalize inside the nuclei rather than the cytoplasm. To elucidate functional aspects of our observations further experiments are required. 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'.

P-19

The effect of different treatments on the characteristic of crosslinked proteins in Neutrophil Extracellular Trap

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Mature neutrophils are suitable for binding and sensing different types of pathogens via their receptors. Activated neutrophils release granule peptides and a chromatin resulting in neutrophil extracellular trap (NET) formation which contributes significantly to the control of pathogens and minimizes damage to the host cells. NET is an early process of the cell death and is not a result of the leak out of disintegrated cells. Further function of NETs is to provide a high local concentration of antimicrobial peptides therefore serving not only as a physical but a chemical barrier to prevent the spread of pathogens. A number of proteins participating in NET formation were identified by different research groups. Our aim is to examine how the characteristic of these crosslinked proteins changes during NET generation after various treatments. NET proteins were analyzed with mass spectrometry on a 4000QTRAP mass spectrometer and data were evaluated using StavroX protein cross-link examination software. Acknowledgement of the funding source: TÁMOP-4.2.2.A-11/1/KONV-2012-0023.

P-20

Role of tissue transglutaminase 2 (TGM2) in the differentiation. death and cytokine production of all-trans retinoic acid (ATRA) and arsenic-trioxide (ATO) treated NB4 leukaemic cells

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Acute promyelocytic leukemia (APL) NB4 cells can be differentiated to neutrophil granulocytes by all-trans-retinoic-acid (ATRA) treatment used as a differentiation therapy in patient with APL to result in terminal differentiation of promyelocytic cells. This differentiation method results in up and down regulation of several thousand genes to generate functional neutrophil granulocytes with abilities for quick immune responses. One of the most up-regulated genes in ATRA induced maturation of NB4 cells is transglutaminase 2 (TGM2). Silencing of TGM2 expression in NB4 cells revealed functional contribution of TGM2 to adhesion, migratory, phagocytic capacity of neutrophils and superoxide (ROS) production. (Balajthy Z, et al. Blood. 2006; Csomós K, et al. Blood. 2010). Arsenic trioxide (ATO) has recently been identified as an effective drug for treatment of APL. ATO combined with ATRA shows a synergistic effect, which further prolongs survival of APL patients in a dose dependent manner. ATO also affects transactivation of transcription factors resulting in activation of the cellular signaling pathways leading to ROS generation by the NADPH oxidase system during arsenic exposure. These events may explain the ability of ATO to induce partial differentiation and apoptosis leading the complete remission in relapsed APL patients. Retionic acid syndrome or differentiation syndrome (DS) is a complication associated with the treatment of APL with ATRA and ATO, in which induction and secretion of CC chemokines (CCL2/MCP-1, CCL22/MDC, CCL23/MIP-3, CCL24/MPIEF-2) and cytokines (IL1B and IL8) result in both tissues infiltration by neutrophils and then organ damage through ROS production of differentiating APL cells. Knocking down TGM2 expression in ATRA differentiated NB4 cells suppressed both chemokines/cytokines and ROS production suggesting a role of TGM2 in triggering DS (Csomós K, et al. Blood. 2010). Moreover TGM2 knockdown cells showing higher apoptosis rate upon a combined treatment, which indicates the role of TGM2 in the aspect of cell death. ATRA/ATO combined treatment could further enhance the already increased induction of TGM2 by ATRA. Surprisingly, this combined treatment did not increase but suppressed ROS production. When we knocked down the TGM2 expression, in ATRA/ATO treated cells ROS production was inhibited further. Future experiments can reveal the various regulatory pathways, dependent or not on TGM2, which influence ROS production of differentiating promyelocytes upon ATRA or ATRA/APO treatment. Nevertheless, it is worth considering to modify TGM2 expression together with these therapeutic protocols for the attenuation the inflammatory phenotype of differentiation syndrome.

P-21

Pituitary Adenylate Cyclase Activating Peptide enhance the differentiation of UMR106 osteoblast cell line

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Pituitary Adenylate Cyclase Activating Peptide (PACAP) has important regulatory roles in the differentiation of central nervous system and several peripheral tissues. However, little is known about the connection of PACAP signalling pathways to osteogenesis or bone regeneration. We aimed to clarify whether exogenous PACAP has any effect on the osteogenic differentiation of UMR106 osteoblastic cell line. PACAP 1-38 as an agonist and PACAP 6-38 as an antagonist of PACAP receptors were applied. UMR106 cells express preproPACAP and PAC1 receptor, indicating a functional PACAP signalling. Administration of the neuropeptides did not alter the morphology and viability of UMR106 cells but increased their proliferation. PACAPs enhanced bone matrix secretion: production of collagen type I was elevated and increased expression of alkaline phosphatase was detected. PKA activation influences osteogenic differentiation and it is believed as one of the most important downstream targets of PACAP signalling pathways. Expression either of PKA mRNA or of protein increased, while those of CREB and Runx2 transcription factors were not altered. Elevated nuclear signal of Runx2 was detected, along with increased osterix and/or BMP 2, 4, 6, and 7 expressions. The application of PACAPs increased the mRNA and protein expression of SHH and PTHrP but did not alter the expression of IHH. Our results demonstrate that PACAP enhances bone formation in the UMR106 osteoblastic cell line and PKA and Hedgehog signalling both are mediators of this effect. Interestingly, PACAP 6-38 did not behave as an antagonist, although slight differences in the effects of the two peptides were observed.
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P-22

Relative quantification of human defensins in colonic epithelial cells using targeted mass spectrometry-based analysis

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Defensins represent an important group of antimicrobial peptides associated with multiple functions in order to act as a first line of defense mechanism. The two human subfamilies of defensins (α and β) differ in their peptide length, location of disulphide bonds, their precursor structures and in the site of expression. β-defensin 1 was described as a non-inducible peptide of the natural immune system. Elevated level of inducible β-defensin 2 expression determined in stool samples was described in the inflamed digestive tract whereas increased β-defensin 3 levels were described in colonic mucosa of patients with ulcerative colitis suggesting their role in the inflammatory response. β-defensin 4 was demonstrated as a synergistic partner of Lysozyme-C and it has a strong additive effect with β-defensin 3 suggesting its role in the immune response against different types of pathogens. We have developed a Selected Reaction Monitoring-based mass spectrometry method for the analysis of human beta defensins which may be an alternative way for defensin quantification. The multiplex feature and the flexibility of the developed method is advantageous compared to classical ELISAs because the level of multiple proteins can be determined simultaneously from one sample. In this study Caco-2 cell lines challenged with IL-1β treatment as pro-inflammatory stimulus were used. As a result of IL-1β activation the levels of β-defensin 2 and β-defensin 3 were increased significantly compared to controls as measured in both the cell lysates and cell culture supernatants demonstrating the inducible feature of these defensins. In contrast, the levels of β-defensin 1 and β-defensin 4 remained unchanged upon IL-1β stimulation indicating their constitutive expression and unresponsiveness to IL-1β. These results demonstrate that the targeted proteomics method developed here offers an alternative semi-quantitative approach for the analysis of human β-defensins of immunological significance. This research was funded by TÁMOP 4.2.4. A/2-11-1-2012-0001, TÁMOP-4.2.2.A-11/1/KONV-2012-0045, TÁMOP 4.2.2.A-11/1/KONV-2012-0023 and Marie-Curie Cross-Talk program FP7-215553 (2007-2013).

P-23

Interaction between the PPM1L protein phosphatase and the Ig kappa protein

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PPM1L is a metal ion dependent serine/threonine phosphatase that regulates ceramide transport, as well as stress and apoptotic signaling pathways. In addition, this phosphatase may be implicated in DNA damage response and can be considered as a potential tumor suppressor. To elucidate its functions further we searched for its interacting partners with the main emphasis on the role of protein interactions in the recovery from DNA damage. First we cloned PPM1L cDNA into the pGBKT7 plasmid for yeast two-hybrid screening of a human bone marrow cDNA library. In the first round of screening found five potential PPM1L interacting partners: MHC, immunoglobulin kappa, beta-globin, actin related protein and beta-2-microglobulin. After eliminating false positives and weak interactions we focused our interest on immunoglobulin kappa. We confirmed the interaction with a pairwise yeast two hybrid experiment and two-way immunoprecipitation. In immunohistochemistry experiments we found that PPM1L accumulated mostly in the nucleolus while the immunoglobulin kappa had an even distribution in the U2OS osteosarcoma cells. The Pearson's correlation index for colocalization was > 0.5 in all experiments. Using FLIM-based microscopic FRET, we found 4.5% FRET efficiency between PPM1L and IgG kappa in the nucleolus, where the phosphatase was the most abundant. Our data indicate that PPM1L and immunoglobulin kappa can interact with each other and exhibit a weak colocalization in the nucleolus of the U2OS cancer cells. Since the overexpression of immunoglobulin kappa is considered as a tumor marker, and the protein contains a potential phosphorylation site for ATM or DNA-dependent protein kinase the interaction may have physiological relevance. Supported by the Hungarian Social Renewal Operational Program TAMOP-4.2.2-A-11/1/KONV-2012-0025 grant.

P-24

Identification of host genes involved in symptom development during virus infection in *Arabidopsis thaliana* ecotypes

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One of the most pressing problems of the 21st century is to meet the nutritional requirements of the growing population, which requires significant increase in the level of current food production. Since the size of the available land for cultivation can no longer be increased, it is important to increase crop yields and reduce crop losses. Plant viruses often cause serious damage to various crop plants and are important agents behind crop losses. However, we know very little about the molecular mechanisms of symptom development during viral infection. Therefore, our research aims to understand these processes and to reduce viral symptoms and yield losses. In our experimental system we use various ecotypes of *Arabidopsis thaliana,* collected from different habitats, to study the development of symptoms during viral infection. We are examining the symptoms caused by Cucumber Mosaic Virus, Ribgrass Mosaic Virus, Turnip Crinkle Virus and Turnip Mosaic Virus on these different ecotypes. Standardization of the infections had been done by purified virions propagated on their host plants. Sixty *Arabidopsis* ecotypes were infected with the different viruses and viral symptoms were evaluated three weeks after inoculation. In our experimental setup, we are looking for virus and ecotype combinations where the symptoms differ from each other significantly. The selected ecotypes with different viral symptoms will be used to identify the genes involved in symptom formation using both genetic mapping and high throughput molecular biology methods. Acknowledgements for the Ministry of Rural Development for supporting Sz.K. in the Young Researcher Career Development Program.

P-25

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. This study was supported by Hungarian grants from the TÁMOP-4.2.2.A-11/1/KONV-2012-0023.

P-26

The selective binding of S100A4 to non-muscle myosin II isoforms is determined by a single amino acid substitution

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Non-muscle myosins (NMII) play an important role in various cellular functions such as cell polarization, cell migration and cytokinesis. Despite having a high degree of sequence similarity the three NMII paralogs show different enzymatic properties and partially different subcellular localization, consequently they have isoform specific functions. Via ionic interactions the NMII rods assemble into „minifilaments" which is the functional forms of these non-processive motor proteins to generate force. S100A4 is an EF-hand calcium–ion binding protein that interacts selectively with the C-terminal coiled-coil and the non-helical tailpiece region (46 residues) of the NMIIA and NMIIC isoforms, thereby causing filament disassembly and leading to enhanced migration of tumor cells. Our aim was to determine the structural requirements of the selective binding of S100A4 to NMII isoforms. On the one hand, we carried out combinatorial paralog scanning experiment by phage display to determine the contribution of the α-helical S100A4 binding region of NMII to the isoform selectivity. This experiment pointed out a single amino acid substitution in NMIIB compared to NMIIA and NMIIC that caused a 60-fold reduction in the binding affinity. On the other hand, we studied the role of the sequentially most diverse non-helical tailpiece region of the S100A4 binding site. According to our results, the NMIIA tail is the most favorable among the three isoforms regarding the binding affinity. By observing the binding kinetics of dimeric NMII constructs to S100A4 it was found that the non-helical tailpiece affects significantly the on rate constant of the interaction. Finally, we suggest that the higher stability of NMIIB coiled-coil region of the S100A4 binding site could also contribute to its reduced binding capability. The results will be discussed in light of a mechanistic model of S100A4 induced filament disassembly. Supported by OTKA grants (NK81950 and K108437).

P-27

PARP-1 ablation alters eicosanoid and docosanoid signaling and metabolism in a murine model of contact hypersensitivity

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Poly(ADP-ribose) polymerase (PARP)-1 is a pro-inflammatory protein. The inhibition of PARP-1 reduces the activity of several pro-inflammatory transcription factors that results in reduced production of pro-inflammatory cytokines, chemokines, matrix metalloproteinases, inducible nitric oxide synthase culminating in reduced inflammatory in skin and other organs. We aimed to investigate the effect of the deletion of PARP-1 on PUFA and PUFA metabolite composition in mice under control conditions and in oxazolone (OXA)-induced contact hypersensitivity reaction (CHS). We elicited CHS using OXA in PARP-1$^{+/+}$ and PARP-1$^{-/-}$ mice, then the concentration of PUFAs and PUFA metabolites were assessed in lipidomics experiments in the diseased skin. When comparing control, unsensitized PARP-1$^{+/+}$ and PARP-1$^{-/-}$ mice we observed that the level of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) increased in PARP-1$^{-/-}$ mice. In parallel we observed higher expression of fatty acid binding protein 7 (FABP7) in PARP-1$^{-/-}$ mice that is considered as to be a specific carrier of DHA and EPA. Furthermore, the metabolites of DHA and EPA (considered mainly anti-inflammatory or pro-resolving factors) were higher as compared to the metabolites of arachidonic acid (considered mainly pro-inflammatory) both in control (unsensitized) and OXA-sensitized PARP-1$^{-/-}$ mice. Our data suggest that the genetic deletion of PARP-1 affect the PUFA-homeostasis of the skin bringing about an anti-inflammatory milieu (increased DHA and EPA levels, higher DHA and EPA metabolite levels) that is probably an important component of the anti-inflammatory action of PARP-1 inhibition.

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P-28

Oxytocin receptor gene polymorphisms are associated with human directed social behavior in dogs

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The oxytocin system has a crucial role in human sociality; several results prove that polymorphisms of the oxytocin receptor (OXTR) gene are related to complex social behaviors in humans. Dogs' parallel evolution with humans and their adaptation to the human environment has made them a useful species to model human social interactions. Previous research indicates that dogs are suitable models for behavioral genetic research. The aim of our study was to identify SNPs in the OXTR gene in different dog breeds, which could have associations with social behaviour. Buccal DNA samples were collected in a non-invasive way with cotton swabs. Genomic DNA was isolated using standard protocol. The sequence of protein coding and regulatory regions was amplified by polymerase chain reaction (PCR) and determined by direct sequencing on five different dog breeds. From the 8 identified SNPs 4 (rs8679682, -212AG, -73CG and -49CG) were subsequently genotyped by PCR-RFLP method. The PCR products were analyzed by agarose gelelectrophoresis. Two polymorphisms (19131AG, rs8679684) were genotyped by real time PCR using sequence specific probes. The two other SNPs (-93TC, rs22927829) were investigated by allelespecific amplification (ASA). Eight SNPs were found in the OXTR gene, 5 of them have not yet been described previously. 4 SNPs (-212AG, -93TC, -73CG and -49CG) is located in the 5' UTR (untranslated) region, one (rs22927829) were found in the first exon, one (rs8679682) in the second exon, and two (rs8679684, 19131AG) in the 3' UTR region. The identified polymorphisms were genotyped in approximately 800 dogs (14 breeds), including Border Collies, German Shepherds and Siberian Huskies. The -212AG polymorphism was associated with proximity seeking, the rs8679684 SNP with Friendliness toward an unfamiliar human in German shepherds and Border Collies. The -93TC SNP was associated with activity and movement during handling in Siberian Huskies. Our results suggest that the single nucleotide polymorphisms in the OXTR gene might contribute to the genetic background of dog activity and social behaviour.

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P-29

Activation of RAR pathway induces migration of cells derived from human glioblastoma multiforme

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Glioblastoma multiforme (GBM) is a highly malignant astrocyte-derived brain tumor. Available therapies based on cytotoxic compounds are not efficient and even can lead to worse prognosis. All-trans retinoic acid (ATRA) has been shown to promote the cellular differentiation, has an anti-proliferative effect and also known as apoptotic agent, thus testing ATRA in GBM therapy is reasonable. ATRA acts through the activation of Retinoic Acid Receptor (RAR), which forms an obligate heterodimer with Retinoid X Receptor (RXR). RAR:RXR binds to the retinoic acid response element and regulates cell type specific gene expression. However, retinoid signaling has been extensively studied in gliomagenesis, its biological function and direct targets are not clearly understood. In our study, human glioma cells treated with ATRA showed decreased proliferation. Unexpectedly, ATRA treatment resulted in increased migration of these cells, which *in vivo* might result in increased tumor invasiveness. In order to determine ATRA-induced transcriptional events in glioma cells, we carried out RNA-seq and chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-seq) experiments. We found that over one thousand genes were regulated upon ligand activation, including the well-established astrocyte marker Gfap, several citokines (e.g. Tgfb1, Vegfa, Il6) and genes related to cellular migration (e.g. Mmp2). Integrated analysis of these genome-wide results allowed us to identify novel direct RAR:RXR targets in glioma. These observations suggest a more complex and controversial effect of RAR:RXR pathway in glioma, which caution us to apply ATRA as a therapeutic compound in GBM treatment.
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P-30

Inositol phosphates induce DAPI fluorescence shift

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The polymer inorganic polyphosphate (polyP) and inositol phosphates, such as IP_6 (inositol hexakisphosphate; also known as phytic acid), share many biophysical features. These similarities must be attributed to the phosphate groups present in these molecules. Given the ability of polyP to modify the excitation-emission spectra of DAPI (4′,6-diamidino-2-phenylindole) we decided to investigate if inositol phosphates possess the same property. We discovered that DAPI-IP_6 complexes emit at around 550 nm when excited with light of wavelength 410-420 nm. Inositol pentakisphosphate (IP_5) is also able to induce a similar shift in DAPI fluorescence. Conversely, inositol trisphosphates (IP_3) and inositol tetrakisphosphates (IP_4) are unable to shift DAPI fluorescence. We have employed this newly discovered feature of DAPI to study the enzymatic activity of the inositol polyphosphate multikinase (IPMK) and to monitor phytase phosphatase reactions. Finally, we used DAPI-IP_6 fluorescence to determine the amount of phytic acid in plant seeds, facilitating the biotechnology industry in their efforts to obtain low phytic acid grain. Using an IP_6 standard curve this straight-forward analysis revealed that among the samples tested, borlotti beans possess the highest level of IP6 (9.4 mg/gr) while the Indian urad bean the lowest (3.2 mg/gr). The newly identified fluorescence properties of DAPI-IP_5 and DAPI-IP_6 complexes allow the levels and enzymatic conversion of these two important messengers to be rapidly and reliably monitored. We believe that this reliable method can be easily adapted to IP_6 measurement from any biological samples.

P-31

The effect of phenyl glycosides on the activity of protein phosphatases and on the survival of HeLa cells

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Reversible phosphorylation of proteins regulates several important cellular processes (metabolism, apoptosis). The phosphoserine/threonine specific protein phosphatase-1 (PP1) and -2A (PP2A) are two major types of phosphatase, which are responsible for the dephosphorylation of more than 90 % of cellular phosphoproteins. Our aim was to study the inhibitory potency and the cell death inducing effects of gallic acid coupled glucose derivative hexa-hydroxy-diphenoyl-tannin compounds (tellimagrandin 1, praecoxin B, mahtabin A, pedunculagin, 1,2-Di-O-Galloyl-4,6-HHDP-β-D-glucose) and selenoglucosides also. Our results show that tellimagrandin 1 (IC_{50}=0.1-0.22 μM) has more potent inhibitory effect on PP1 than does PGG, while inhibitory potency of other derivatives (praecoxin B, mahtabin A, pedunculagin or 1,2-Di-O-Galloyl-4,6-HHDP-β-D-glucoside varies slightly (0.13-4 μM). Inhibition of PP2Ac occurred at ~100-fold higher IC_{50} values for each derivatives compared to that of determined for PP1c. In contrast, selenoglycosides were without effect or increased the activity of PP1c and PP2Ac in a concentration range of 150-400 μM. One selenoglycoside exerted a weak inhibitory effect on PP1c. In cell survival studies tellimagrandin 1 induced cell death in the range of 5-25 μM concentrations, 1,2-Di-O-Galloyl-4,6-HHDP-β-D-glycoside and pedunculagin were effective in higher concentrations (10-100 μM), while up to 100 μM praecoxin B and mahtabin A were without effect. Acetylation of selenoglycosides increased their viability reducing effect. We conclude that the cell death inducing effect of the PGG derivatives and selenoglycosides are due to a broader biological influence, not just phosphatase inhibition. In seleneoglycosides acetylation of glucose hydroxyls and coupling with hydrophobic aromatic ring appear to be required for phosphatase activation. Supported by OTKA K109249, TÁMOP-4.2.2/B-10/1-2010-0024, TÁMOP-4.2.2/A-11/1/KONV-2012-0025, TÁMOP-4.2.4.A/2-11-1-2012-0001

P-32

Studies on the human NOD-like receptor family CARD domain containing 5 (NLRC5) protein

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The NOD-like receptor family CARD domain containing 5 (NLRC5) protein is a unique member of NLR protein family due to its high molecular mass, the presence of an untypical caspase activation and recruitment domain and an unusually high number of leucine-rich repeats. We have published homology models for both the monomeric and a homo-heptameric full-length human NLRC5 protein. The aim is to correlate biochemical findings with the proposed models. We have expressed Flag-tagged NLRC5 in 293T cells. Anti-Flag and anti-NLRC5 antibodies were tested for the identification of the fusion protein. The overexpressed protein was identified successfully by anti-Flag antibodies, while the tested anti-NLRC5 antibodies appeared to be less effective. Flag-tagged NLRC5 was detected in both nuclear and cytosolic cellular fractions by Western blot. Gel filtration of total cell lysates was performed to determine the molecular weight of NLRC5 and results suggest potential oligomerization of the overexpressed NLRC5. An MRM-based method was also designed which was found to be specific for the NLRC5, and using this method we proved that the transfected cells could express the NLRC5 protein contrary to the non-transfected cells. The work was supported in part by the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 VÉD-ELEM project (to S.B. and J.T.) and by the Hungarian Science and Research Fund (K-101591 to J.T.). S.B. is receiver of Lajos Szodoray Postdoctoral Fellowship and Janos Bolyai Postdoctoral Fellowship. A.V. is receiver of Ányos Jedlik Predoctoral Fellowship (TÁMOP 4.2.4.A/2-11-1-2012-0001; A2-JÁDJ-12-0186).

P-33

Molecular characterization of GTD endophytic and pathogenic fungi

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Grapevine trunk diseases are amongst the most important diseases in vineyards worldwide and in Hungary. GTD are caused by several pathogenic fungi (e.g. *Phaeoacremonium aleophilum, Phaeomoniella chlamydospora, Eutypa lata, Fomitiporia mediterranea, Diplodia seriata*). There is very limited information about the pathogens in Tokaj wine region nowadays. Pathogenic fungi were isolated from 207 symptomatic grape trunks from the Tokaj wine region in 2013. The samples with foliar symptoms originated from four areas: Szemere (Degenfeld); Dorgó (Disznókő); Szarvas (Kereskedőház), and Várhegy (Patrícius) vineyards. The woody tissue samples were cut in a small part, and were disinfected before putting on malt extract agar medium and incubated at room temperature for 3-8 days. MagNaLyser (Roche) was used for disruption, and isolation was carried with NucleoSpin Plant II. The ITS4 and ITS5 primers were used for PCR amplification of the internal transcribed spacer regions. Purified amplicons were sequenced by Mycrosinth, Austria. The sequences were aligned with deponated reference sequences with Clustal X program, and manually corrected with GeneDoc. Phylogenetic analyses were performed with MEGA 5.05 366 pure fungal cultures were isolated and identified till now. The majority of the isolates (56 %) were determined as *Diplodia seriata* (*Botryosphaeriaceae*). Other fungi, like *Fusarium* sp. (13.9 %), *Alternaria* sp. (9.3 %), *Aspergillus* sp. (7.9 %), *Mucor* sp. (7.1 %), *Diaporthe* sp. (3.3 %), *Trichoderma* sp. (1.4 %), *Epicoccum* sp. (0.5 %), *Penicillium* (0.3 %) and *Xanthomendoza* sp. (0.3 %) were also identified from grapevine trunk samples. This work was supported by the TÁMOP 4.2.4.A/2-11-1-2012-0001 National Excellence Program . The project is co-financed by the European Union and the European Social Fund. E. S. was supported by the Research Grant of the University of Debrecen. The research was supported by COST Action FA1303.

P-34

The PARP inhibitor PJ34 has a photosensitizing effect on A431 (human squamous skin carcinoma) cell line

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UVA radiation influences cellular function, generates radical oxygen species (ROS), alters signalling events and causes DNA damage. Poly(ADP-ribosyl)ation is a posttranslational protein modification catalyzed by poly(ADP-ribose) polymerase enzymes (PARPs). The most prominent member of the family is PARP-1, which is activated by DNA damage induced for example by oxidative stress or UV irradiation. To investigate the role of PARP-1 in the UVA induced cell death, A431 human squamous skin carcinoma) cells were irradiated in the presence of specific PARP inhibitors (PJ34 and Veliparib). Surprisingly, PJ34 sensitized cells to UVA irradiation at a low and non toxic dose (2.5 J/cm^2) of UVA, but Veliparib and PARP-1 silencing didn't have any effect. In the presence of PJ34, single and double DNA breaks were detected soon after UVA irradiation. 4 hours after UVA irradiation high caspase-3 and caspase-8 activity was measured only in the case of PJ34 + UVA treatment. The pan caspase inhibitor Z-VAD-fmk, Z-DEVD–fmk and Z-IETD–fmk had protective effect against PJ34+UVA induced cell death as assessed by MTT cell viability assay. The highest ROS production could be detected immediately after UVA irradiation in the case of PJ34+UVA treatment. Although antioxidants (ascorbic acid, trolox) decreased ROS production, they did not have any effect on the phototoxicity of PJ34. Based on our results we suggest that the photosensitizing effect of PJ34 is independent from its PARP inhibitory activity and from ROS production. Supported by TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program'.

P-35

Structural biology of preventive DNA repair mechanism

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The occurrence of modified bases in DNA is attributed to two major factors: incorporation of altered nucleotide building blocks and chemical reactions on bases within the DNA structure. Several enzyme families are involved in preventing incorporation of non-canonical bases playing a "sanitizing" role. The catalytic mechanism of action of these enzymes has been revealed for a number of representatives in clear structural and kinetic details. This time the focus is on those examples where straightforward evidence has been produced using high resolution structural studies. Comparing the protein fold and the architecture of the enzyme active sites, two main classes of sanitizing (d)NTP phosphorylases can be assessed that are distinguished by the site of the nucleophilic attack. In enzymes associated with attack at the α-phosphorus, it is shown that coordination of the γ-phosphate group is also ensured by multiple interactions. In contrast, enzymes catalyzing attack at the β-phosphorus atom mainly coordinate the- and the β-phosphate only. Characteristic differences are also observed in the role of the metal ion cofactor (Mg^{2+}) and in coordination of the nucleophilic water. Using different catalytic mechanism embedded in different protein folds, these enzymes present an example for convergent evolution. This work was supported by the Hungarian Scientific Research Fund OTKA NK 84008, and K109486, the Baross Program of the New Hungary Development Plan (3DSTRUCT, OMFB-00266/2010 REG-KM-09-1-2009-0050), the Hungarian Academy of Sciences (TTK IF-28/2012) and the European Commission FP7 Biostruct-X project (contract No. 283570).

P-36

Investigation on the role of tissue transglutaminase in AdipoGENESIS of mice

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Adipose tissue is a specialized connective tissue that functions as the major storage site for fat in the form of triglycerides. Adipose tissue is present in mammals in two different forms: white and brown adipose tissue, with white adipose tissue being abundant. It has a well-organized vasculature and is highly innervated. As our knowledge on adipose tissue is continuously growing, new insights into normal physiological regulation as well as new strategies for treatment of obesity and its related disorders may be expected. Tissue transglutaminase (TGM2) can be a notable target for investigation on adipose tissue development as the expression pattern for TGM2 suggests that it promotes differentiation to clinically important cell types. To substantiate a potential role of TGM2 in adipogenesis, we study its effects on *in vivo* adipose tissue formation in mouse models. We have detected the presence of TGM2 in both brown and white adipose tissues. Although wild type and TGM2 KO mice produce the same amount of adipose tissue, we have found that the expression of ADIPOQ and UCP1 adipocyte differentiation markers is lower in KO animals. Glucose tolerance of the strains is highly similar at the age of week 16; however, prolonged differential expression of ADIPOQ may contribute to development of glucose intolerance that has been detected by others at age of week 24. We also compare tissue characteristics of animals such as size and density of both adipocytes and blood vessels.
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P-37

A modular system to study the effect of protease inhibitors on the action of Human Immunodeficiency Virus type 2 (HIV-2)

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The Human Immunodeficiency Virus type-2 (HIV-2) is a causative agent of acquired immune deficiency syndrome (AIDS). Statistical data on the prevalence of HIV-2 are widely lacking, but it is estimated that about 1-2 million infections in the West African region are caused by this virus, excluding the recent surge of HIV-2 infection rates in Europe and America. The viral protease is a homodimeric aspartyl protease that is crucial for the viral life-cycle, leading to the development of mature, infection competent virions. Protease inhibitors are potent antiretroviral agents, yet their effect on HIV-2 is still to a large extent, understudied. Utilizing a wild-type HIV-2 vector backbone, we have developed a modular cassette system where the efficacy of clinically used protease inhibitors can be studied for various serotypes of HIV-2 protease both in enzymatic and cell culture assays. The protease was expressed in *E. coli* and its activity was characterized using high-performance liquid chromatography, the stability of the enzyme was then determined by studying its autodegradation/autoinhibition, and inhibition profiling assays were carried out for clinically used protease inhibitors. Moreover, we are studying the common treatment-associated resistance mutations of the enzyme; to determine their effect on the kinetics of the viral protease and its susceptibility to clinical inhibitors. Given the absence of a standardized protocol and the antigenic variability of clinical isolates, we believe that the inhibition profiling assays performed with our modular system is expected to provide an accurate measure of the efficacy of currently used protease inhibitors on HIV-2. This work was supported by the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 "VÉD-ELEM" and by the Hungarian Science and by the Hungarian Science and Research Fund (OTKA 101591).

P-38

Autophagy in tissue regerenration

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Regeneration is the ability of multicellular organisms to replace damaged tissues and regrow lost body parts. This process relies on cell fate transformation that involves changes in gene expression as well as in the composition of the cytoplasmic compartment, and exhibits a characteristic age-related decline. Here, we present evidence that genetic and pharmacological inhibition of autophagy—a lysosome-mediated self-degradation process of eukaryotic cells, which has been implicated in extensive cellular remodelling and aging—impairs the regeneration of amputated caudal fins in the zebrafish (*Danio rerio*). Thus, autophagy is required for injury-induced tissue renewal. We further show that upregulation of autophagy in the regeneration zone occurs downstream of MAPK/ERK signalling, to protect cells from undergoing apoptosis and enable cytosolic restructuring underlying terminal cell fate determination. In addition, autophagy is also required for germ cell regeneration in the *Drosophila* testis. These novel cellular functions of the autophagic process in tissue regeneration imply that the role of cellular self-digestion in differentiation and tissue patterning is more fundamental than previously thought.

P-39

The role of poly(ADP-ribose) polymerase-2 in the cholesterol homeostasis

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Poly(ADP-ribose) polymerase (PARP)-2 enzyme, known as a DNA damage repair enzyme is a member of the PARP enzyme family. Our research group showed, that PARP-2 has regulatory role in metabolism. Microarray experiments showed, that in PARP-2 silenced HepG2 cells the expression of genes that are involved in cholesterol symthesis increased as compared to the isogenic control. In the liver the PARP-2 is a repressor to the SREBP1 gene, therefore the depletion of PARP-2 inhibits the expression of genes, wich are involved in cholesterol metabolism. In skeletal muscle the PARP-2 plays a major role in mitochondrial biogenesis, therfore we investigated the effects of PARP-2 silencing on gene expression in C2C12 myoblast cells. Microarray experiments showed, that the depletion of PARP-2 resulted in an increase in the expression of the genes of cholesterol biosynthesis. Similarly to HepG2 cells, the depletion of PARP-2 induces the expression of SREBP-1 and consequently the expression of SREBP-1 dependent genes. The inhibition of the activity of PARP-2 by UPF1069 induced the expression of cholesterol biosynthetic genes pointih out the importance of the activity of PARP-2 in the regulation of the expression of SREBP-1. In the *musculus gastrocnemicus* muscle of PARP-2$^{-/-}$ mice the main genes of cholesterol biosinthesis were also increased as compeared to the PARP-2$^{+/+}$ mice culminating in higher cholesterol evels compared in the muscle of PARP-2$^{-/-}$ mice. Supported by grants from OTKA (K108308, K105872), TÁMOP-4.2.2. A-11/1/KONV-2012-0025. P.B. and M.S. are recipients of Bolyai fellowship, the laboratory is supported by the Lendület program of the Hungarian Academy of Sciences.

P-40

Topical and nutritional relevant TSLP regulation via RARγ, RXR and VDR response pathways

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TSLP is an important initiator and trigger of atopic dermatitis (AD) and other atopic diseases. Vitamin A and D derivatives are involved in TSLP regulation. Various combinations of specific retinoic acid receptor (RAR), retinoid X receptor (RXR) and vitamin D receptor (VDR) agonists and antagonists were administered topically to mice. TSLP expression was increased by VDR and RARγ specific agonists and by the natural agonist all-*trans* retinoic acid (ATRA), while an RXR agonist was not active. RXR- and RAR-antagonist in addition RARα-agonists reduced TSLP expression. Strongest activation was found after combination of VDR-RXR (5-times) and especially RARγ-RXR (48-times) agonist treatments. In humans, serum levels of TSLP were not increased in patients with AD vs. healthy individuals while supplementation with tomato juice high in lycopene resulted in significantly reduced serum TSLP levels. We conclude that besides minor physiological relevant VDR-signalling mainly RARγ-RXR pathways in the skin are important relevant triggers for AD-relevant TSLP and that diets rich in selected carotenoids can be beneficial to reduce systemic TSLP levels. In addition we conclude that topical retinoids synthesized by internal or external triggers or applied topically induce TSLP production and are important triggers for atopic dermatitis prevalence. Acknowledgements: RR is a member of the COST projects "Mast Cells and Basophils - Targets for innovative therapies" and "SkinBAD". The work was supported by TÁMOP-4.2.2.A-11/1/KONV-2012-0023 "VÉD-ELEM" project. The project is implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund. In addition this project was funded by OTKA K 109362 research grant.

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Spartan has multiple function at stalled replication fork and acts together with RAD18 in postreplicational repair

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Persisting DNA damage in S-phase can block replicative polymerases and consequently lead to replication stalling. Prolonged stalling of replication fork can leads to fork collapse, double strand breaks and gross chromosomal rearrangements, therefore, genomic instability. Stabilization and resumption of replication forks ensure both the completion of replication and the maintenance and preservation of genomic integrity. DNA damage tolerance pathways (DTT), originally termed as post-replication repair (PRR) processes are involved in avoidance of fatal consequences of persistent stalling of DNA replication. To resolve the block of replication DNA damage tolerance mechanisms bypass the lesion without removing it, thereby rescue the replication fork from collapse and also allow the reinitiation of replication downstream of damage. The two strategy of DNA damage tolerance is TLS and homology dependent repair (HDR) and despite of the fact that these pathways are well conserved in eukaryotes, the exact mechanism of PRR, the activation and concerted action of translesion synthesis polymerases, the timing and mechanisms of the following elongation and replication fork restart is poorly elucidated in human cells. The RAD18 protein is a central molecule of the DDT mechanism known as the Rad6 pathway, many of its components encode parts of the ubiquitin conjugating machinery from yeast to mammals and the activation of this pathway is necessary for survival after induction of various types of DNA damage. It is known,that SPARTAN/C1orf124, is an ubiquitin-PCNA interacting regulator of DNA damage tolerance, provides a higher cellular level of ubiquitylated-PCNA by which it regulates the choice of DNA damage tolerance pathways. Preferential association of Spartan with ubiquitin-modified PCNA protects against PCNA deubiquitylation by ubiquitin-specific protease 1 and facilitates the access of a TLS polymerase to the replication fork. Our findigs support that Spartan acts together with RAD18 and has multiple role in stabilization of stalled replication fork.

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Studies on the multiple sclerosis-associated retrovirus (MSRV) protease

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Human endogenous retroviruses (HERVs) which have integrated their genome into the germ line make up more than 8% of the human genome. The multiple sclerosis-associated retrovirus (MSRV) is the founder member of type W family of HERVs. Although most copies of HERV's have no known function, some already have been shown to perform important cellular functions. For example, MSRV encode functional reverse transcriptase; MSRV envelope protein was repeatedly found to be elevated in the serum of multiple sclerosis (MS) patients, induces inflammation and demyelination, indicating a potential role of MSRV in MS pathogenesis. While, the presence of protease (PR) open reading frame have already been confirmed and the recombinant MSRV PR was expressed, protease of MSRV was not further studied, and its activity was not proved so far. To investigate the MSRV PR, we have analyzed its sequence by *in silico* methods. The presence of the aspartic PR domain was confirmed by data available in domain databases, and based on the results of secondary structure predictions. Automated homology modeling methods were also tested, but the Modeller softver was found to be most effective to build up homology models for MSRV PR. Dimer interface of MSRV PR was analyzed and based on the results of our predictions the MSRV PR has a four-stranded beta-sheet dimer interface, similarly to the Xenotropic murine leukemia virus-related virus (XMRV) protease. Potential posttranslational modifications of the protease were also predicted. To clarify the presence or the absence of MSRV PR activity *in vitro*, a pGEX-4T-3 GST expression vector system was chosen for the expression of MSRV PR, using a synthetic gene based on the published protease sequence. We have expressed the protease in *E. coli* cells to test its activity using oligopeptide substrates representing naturally occurring cleavage sites of various retroviruses. The work was supported by the Hungarian Science and Research Fund (K-101591 to J.T.).

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MAP2K independent activation of the D type MAPK, AtMPK9

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Amongst various protein kinases, members of the mitogen activated protein kinase (MAPK) family play a central role in transducing extracellular mitogen and stress stimuli into cellular responses. Great majority of the MAPKs are activated by a three-tiered kinase cascade composed of MAPK, MAPK activating kinase (MAP2K), and MAP2K activating kinase (MAP3K). Atypical MAPKs possess a distinctive, MAP2K-independent activation process. Comparing the sequences of their activation loop, plant MAPKs could be divided into further two subtypes: those carrying the amino acid motif TEY (A-C group) and those with plant specific TDY phosphorylation motif (D group). The TEY subtype MPKs display the evolutionary conserved common docking (CD) domain, a characteristic feature of classical MAPKs and activated by pertinent MAP2Ks. We studied AtMPK9 as a representative of D group MAP kinase, which does not possesses CD domain and its activation mechanism has not been described, yet. Our data shows that elevated kinase activity of AtMPK9 can be detected both in vitro translated and in vivo expressed protein without involvement of MAP2Ks. Our immunoblot analysis, in vitro kinase assay, and mass spectrometry data suggest that dual-phosphorylation of TDY motif is inevitable for activation of AtMPK9 and the phosphorylation – similarly to certain representatives of atypical MAPKs – is implemented by autocatalytic mechanism.

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Batf3 and Spi-B regulate the embryonic stem cell derived lineage commitment of hematopoietic precursors

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Dendritic cells (DCs) can be used as a promising therapeutical approach of several autoimmune diseases and cancers. There are several well-known methods to generate functional dendritic cells from various progenitors. Serious limitation of these protocols is the strict number of available sources. To resolve this obstacle embryonic stem (ES) cells can serve as an outstanding source for clinical applications due to their unlimited expansion capacity and ability to differentiate into numerous cell types including immune cells. In our study we intend to improve the ES cell-derived developmental processes via overexpressing of lineage determining transcription factors. Here we tested the effects of two DC specific transcription factors (Batf3 and Spi-B) which have been described as important regulators of DC development. Genetically modified ES cell lines were engineered using our doxycycline regulated inducible system. Our flow cytometric analysis revealed that overexpression of Batf3 has a negative, in contrast Spi-B has a positive regulatory effect on the formation of mesodermal and myeloid progenitors. These results demonstrate that a single transcription factor is able to modify the ex vivo DC developmental program. This work was funded by the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 "VÉD-ELEM". TÁMOP 4.2.2.A-11/1/KONV-2012-0023 "VÉD-ELEM" implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.

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Analysis of gene expression in macrophages generated from mice lacking PPARg

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The fatty acid activated transcription factor PPARg has been linked to adipocyte differentiation, macrophage and dendritic cell function and also different diseases. In this study we aim the analysis of transcription regulation effect of PPARg in macrophages. However assigning cellular functions to the receptor in various cell types has been hampered by the lack of appropriate genetic models in which PPARg is completely eliminated from a given cell type or an entire animal. The PPARg null mouse line created by us with the crossing of PPARg(floxed) and Sox2Cre mice have phenotypic abnormalities and they show newborn lethality. To generate appropriate amount of mice we set up embrionic liver cell transplantation method. We lethally irradiated the BoyJ congenic mice (recipients) and transplanted with PPARg-KO donor embrionic liver cells. The repopulation efficiency was monitored 8 weeks after transplantation by flow cytometry. Our previous results suggest the IL-4 could improve the effect of PPARg activation on transciption regulation, so we differentiated unstimulated and IL-4 stimulated macrophages from the bone marrow transplanted mice and treated them with or without the PPARg ligand rosiglitazone (RSG). We used RNA-Seq method for genome wide gene expression analysis. The results were analysed with GeneSpring software and our results show that PPARg could regulate the gene expression in different ways. There is a cluster of genes which respond for RSG treatment with an IL-4 independent manner like Angptl4. This RSG effect is failed in PPARg-KO macrophage samples. Another cluster of genes are regulated in IL-4 dependent manner like FABP4. There is a set of genes which seems to be under active inhibition by PPARg, because show a dramatic increase of expression in the PPARg-KO samples. Our results support the importance of the PPARg in the regulation of genes connected to different pathways like cholesterol biosynthesis, NFkB and interferon signaling. Supported by a grant from the Hungarian Scientific Research Fund (OTKA K100196), and TÁMOP422_2012_0023 VÉD-ELEM implemented through the New Hungary Development Plan co-financed by the European Social Fund and the European Regional Development Fund.

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Regulation of protein phosphatase Z by the Hal3 protein in *Candida albicans*

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Protein phosphatase Z (PPZ) is a fungus specific member of the novel Ser/Thr protein phosphatase family. All known PPZ proteins contain a conserved C-terminal catalytic domain and a variable N-terminal regulatory domain. Previously, our group identified and characterized the protein phosphatase Z of *Candida albicans* (CaPpz1), and reported that this enzyme has a role in the cation homeostasis, cell wall integrity, membrane potential determination and hyphae formation. The specific PPZ inhibitor protein, Hal3 was described in *Saccharomyces cerevisiae*. In a sequence based bioinformatics search we found two Hal3 orthologs in *C. albicans* that we termed CaHal3 and CaCab3. In the present study we investigated if the N-terminal domain of CaPpz1 or any of the Hal3 orthologs were able to modulate the phosphatase activity of CaPpz1 *in vitro*. To answer this question, we constructed expression vectors to produce the full length CaPpz1, the CaPpz1-C-terminal catalytic domain, the CaPpz1-N-terminal regulatory domain and the putative inhibitors (CaHal3 and CaCab3) as fusion proteins in *E. coli*. These proteins were expressed and purified by affinity chromatography. The phosphatase activity of the recombinant CaPpz1 and CaPpz1-C-terminal domain was measured either with p-nitrophenylphosphate or by radiolabeled myosin light chain substrate. The phosphatase assays demonstrated that the full length CaPpz1 as well as its catalytic domain was able to dephosphorylate both of the substrates. The phosphatase activity of the two enzyme forms was inhibited by CaHal3 in a concentration dependent manner, while the CaPpz1-N-terminal or CaCab3 protein had no significant effect. Our result shows that CaHal3 is a potential CaPpz1 regulator in *C. albicans*. Supported by the OTKA grant K108989 and 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' to K.P.

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How could Arc (ARG 3.1) protein regulate memory formation via a fuzzy segment?

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The discovery of intrinsically disordered proteins (IDP) and intrinsically disordered regions (IDR) challenged the traditional structure-function paradigm, which stated that a well-defined 3D structure is required for protein function. These proteins and domains are characterized by an ensemble of structures, which enable versatile roles in different cellular processes (signalling in particular). Fuzzy complexes retain their conformational heterogeneity even in bound form and their structural multiplicity is required for specific biological functions. This feature is crucial in the context of different cellular networks.
Arc/ARG 3.1 is an activity regulated cytoskeletal protein, which is a member of the immediate early gene (IEG) family. Its mRNA is rapidly transcribed after neuronal stimuli and involved in processes of synaptic plasticity, long term potentiation (LTP) and depression (LTD). Arc/Arg3.1 selectively modulates trafficking of AMPA-type glutamate receptors (AMPARs) in neurons by accelerating endocytosis and reducing surface expression. It interacts with specific isoforms of dynamin and endophilin to enhance receptor endocytosis. It has been shown that the 195-214 segment of Arc mediates binding to dynamin 2, but the molecular mechanisms are yet to be revealed. Based on preliminary bioinformatics data, this Arc region forms a fuzzy complex with dynamin 2. Thus we assume that the dynamic properties of this region are critical for interactions. To probe this tenet, we aim to alter such properties and assess their impact on the formation of Arc-dynamin 2 complex formation and modulation of AMPA-R trafficking in vivo. Using IUPred and ANCHOR bioinformatics predictors, we designed Arc variants with different degrees of disorder and altered preference for binding. Our next step is to test the designed clones by different protein binding assays *in vitro* and *in vivo.* These results may give us insight into the molecular mechanisms of learning and memory. Supported by LP2012-41 Lendület Program, Hungarian Academy of Sciences, University of Debrecen, Department of Biochemistry and Molecular Biology.

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Soluble components of the flagellar export apparatus, FliI, FliJ, and FliH do not deliver flagellin, the major filament protein from the cytosol to the export gate

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Flagella, the locomotion organelles of bacteria, extend from the cytoplasm to the cell exterior. External flagellar proteins are synthesized in the cytoplasm and exported by the flagellar type III secretion system. The soluble components of the flagellar export apparatus, FliI, FliH, and FliJ, have been implicated to carry late export substrates in complex with their cognate chaperones from the cytoplasm to the export gate. The importance of the soluble components in the delivery of the three minor late substrates FlgK, FlgL (hook-filament junction) and FliD (filament-cap) has been convincingly demonstrated, but their role in the transport of the major filament component flagellin (FliC) is still unclear. We have used continuous ATPase activity measurements and quartz crystal microbalance (QCM) studies to characterize interactions between the soluble export components and flagellin or the FliC:FliS substrate-chaperone complex. Also, K_d values for the interactions between soluble export component pairs were determined. FliC or FliC:FliS did not influence the ATPase activity of FliI alone or in complex with FliH and/or FliJ suggesting lack of interaction in solution. Immobilized FliI, FliH, or FliJ did not interact with FliC or FliC:FliS as revealed by QCM. The lack of interaction in the fluid phase between FliC or FliC:FliS with the soluble export components, in particular with the ATPase FliI, suggests that cells use different mechanisms for the export of late minor substrates, and the major substrate, FliC. It seems that the abundantly produced flagellin does not require the assistance of the soluble export components to efficiently reach the export gate. This work was supported by the Hungarian Scientific Research Fund (OTKA) grants K104726 and NK108642, and the János Bolyai Research Fellowship of the Hungarian Academy of Sciences.

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Compounds enhancing intracellular cAMP levels increase the expression of transglutaminase 2 in mouse thymocytes

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Previous work in our laboratory has shown that transglutaminase 2 (TG2), a multifunctional protein is induced during in vivo, but not in vitro apoptosis of thymocytes indicating that factors present in the tissue enviroment are requied for the process. Recently our laboratory has found the retinoids and the TGF-β released by engulfing machrophages are involved in the process. Since adenosine and PGE2, which are also produced by macrophages during engulfment of apoptotic cells, trigger the adenylate cyclase pathway, we decided to investigate the possible involvement of the adenylate cyclase pathway in the TG2 induction. Our date indicate the compounds enhancing intracellular cAMP levels, alone or in combination with retinoids and TGF-β can contribute to TG2 expression in dying thymocytes. This study was supported by Hungarian grants from the National Research Fund (OTKA K104228) and the TÁMOP 4.2.2.A-11/1/KONV-2012-0023 „VÉD-ELEM" project co-financed by European Social Fund.

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Macrophages engulfing apoptotic cells produce non-classical retinoids to enhance phagocytosis of apoptotic cells

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Retinoids have been reported to modulate several functions of mononuclear phagocytes, such as proliferation and phagocytosis of Fc-opsonized particles or yeast cells, but their effect on the apoptotic cell uptake has so far not been investigated. Here we report that following treatment of macrophages with various RAR or RXR ligands we detected enhanced apopto-phagocytotic capacity and increased expression of the phagocytosis receptor Mertk, Stabilin 2, TIM4 and CD14, opsonin C1qb, lipid transporter ABCA1 and the integrin β3 co-receptor transglutaminase 2 (TG2) genes. Previously it was reported that apoptotic cells promote their own clearance by triggering the lipid sensing LXR receptor in phagocytes following their uptake. This observation was explained by demonstrating that LXR stimulation upregulates the expression of the phagocytosis receptor Mertk, TG2 and RARα. Since RARα is an LXR target gene, and previously we detected increased expression of retinoic acid (RA) producing RALDH1, 2 enzyme expression in LXR agonist treated macrophages, we hypothesized that LXR receptor activation might initiate RA production and signaling which could contribute to the observed phagocytosis enhancement following LXR activation. Indeed, LXR agonist treatment upregulated phagocytosis related genes, which were partially overlapping with the retinoid induced ones. Blocking RA production during LXR activation attenuated the phagocytosis enhancement and abolished the induction of retinoid dependent genes. Using HPLC technique and RARE lacZ transgenic mice, in which the lacZ expression is dependent on the presence of retinoids, we detected the presence of RA in apoptosing thymus and in peritoneal macrophages of LXR agonist injected mice, respectively. Increasing evidence suggests that impaired phagocytosis of apoptotic cells contributes to the pathogenesis of various autoimmune diseases. Our results identify molecular targets to enhance phagocytic capacity of macrophages.
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Effect of a preparation of vitamins, minerals and trace elements on cardiac gene expression pattern at the transcript level in diabetic rats

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Although multivitamin products are widely used as dietary supplements to maintain health or as special medical food in certain diseases, the effects of these products were not investigated in diabetes mellitus, a major cardiovascular risk factor. Therefore, here we investigated if a preparation of different minerals, vitamins, and trace elements (MVT) for human use affects the cardiac gene expression pattern in experimental diabetes. Two days old male Wistar rats were injected with 100 mg/kg of streptozotocin or its vehicle to induce diabetes mellitus. From week 4, rats were fed with an MVT preparation or vehicle for 8 weeks. Well established diagnostic parameters of diabetes, i.e. fasting blood glucose measurement and oral glucose tolerance test were performed at week 4 and 12 in order to monitor the development of experimental diabetes and the effects of the MVT preparation on glucose tolerance. At week 12, total RNA was isolated from the myocardium and assayed by rat oligonucleotide microarray for 44000 oligonucleotides. Significantly elevated fasting blood glucose concentration and an impaired glucose tolerance were found in streptozotocin-treated rats at week 4. At week 12, both fasting glucose and glucose tolerance were significantly improved by MVT treatment in diabetic rats. On the DNA microarray, 254 genes showed significant expression change due to diabetes or MVT treatment. Diabetes mellitus resulted in significant up- or down-regulation of 122 genes, among which 68 genes changed to the opposite and 2 genes to the same direction due to MVT treatment. In the non-diabetic group, 90 genes showed significant change due to the MVT treatment. This is the first demonstration that MVT treatment significantly alters cardiac gene expression profile both in control and diabetic rats which may be involved in prevention of cardiac pathologies in the presence of diabetes mellitus. Funding: MED_FOOD TECH_08-A1-2008-0275, Baross DA-TECH-07-2008-0041, TÁMOP-4.2.1/B-09/1/KONV-2010-0005, TÁMOP-4.2.2/B-10/1-2010-0012), the Hungarian Scientific Research Fund (OTKA K79167), European Regional Development Fund and VÁTI Hungarian Nonprofit LLC for Regional Development and Town Planning (HURO/0901/137/2.2.2-HU-RO-TRANS-MED). M. Sarkozy holds a 'Jedlik Ányos Predoctoral Fellowship'. This research was realized in the frames of TÁMOP-4.2.4.A/2-11/1-2012-0001 'National Excellence Program'.

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Optimization of individual mature miRNA detection by quantitative PCR

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MicroRNAs (miRNAs) are ~22 nucleotide long non-coding RNAs influencing many cellular processes by their regulatory functions on gene expression. Their biogenesis consists of different, subsequential processing steps during which mature miRNAs are liberated from longer precursor RNA forms. Due to their short size, detection and accurate quantification of mature miRNAs are challenging, nevertheless a number of different techniques have been developed so far. For individual mature miRNA detection, quantitative stem-loop real-time PCR represents a widely used method. Although there are some data on optimization of this technique, there are still many parameters which are not investigated yet. Therefore, we examined several factors influencing the measurements, including amplification efficiencies from total RNA samples and various parameters of the reverse transcription and real-time PCR reactions. Notably, we found that total RNA input can affect the detection and carry over DNA contamination could also mislead the measurements in a sequence specific manner. Additionally, we provided evidence that 3' isomiR species of a particular miRNA can be cross-detected by different qPCR techniques. In summary, we have thoroughly optimized the stem-loop quantitative PCR and provide a detailed, refined protocol for reliable detection of microRNA species by this technique. This work 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/1-11-1-2012-0001 "National Excellence Program" and by the TransRat grant (KMR_12-2012-0112).

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Myosin phosphatase regulates gene expression via mediating arginine methylation in human hepatocarcinoma cells

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The myosin phosphatase (PP1M) holoenzyme is a serine/threonine specific protein phosphatase. It is involved in the regulation of cell contractility via dephosphorylation of the 20 kDa light chain of myosin. The fact that several novel non-cytoskeletal protein substrates of PP1M have been identified indicating the complex function of PP1M in different cellular processes. In PP1M protein phosphatase-1 catalytic subunit is associated with myosin phosphatase target subunit 1 (MYPT1). MYPT1 was found to be localized not only in the cytosol and cytoskeleton but in the nuclei of aortic smooth muscle, primary neuronal as well as of human hepatocarcinoma (HepG2) cells. Our goal was to investigate the nuclear functions of PP1M by determining the subnuclear localization and the interacting proteins of MYPT1. The dominant nuclear protein phosphatase was found to be the PP1 in HepG2 cells. Numerous nuclear MYPT1-interacting proteins were identified such as histone 1, splicing factor proteins as well as the members of the methylosome complex, i. e. protein arginine methyltransferase 5 (PRMT5). In addition PRMT5 was found to be phosphorylated at Thr80 by Rho-associated protein kinase *in vitro,* whereas PP1M diminished the phosphorylation level of this site. Silencing of MYPT1 significantly induced the general symmetric dimethylation (PRMT5 specific methylation) level on arginine3 residues of histone H2A and histone H4 and caused a global change in gene expression. Our data suggests novel physiological roles of PP1M in the nuclear dephosphorylation processes related to the regulation of transcription, RNA splicing and the functions of the methylosome complex. This work was supported by grants from TÁMOP-4.2.2/B-10/1-2010-0024, TÁMOP-4.2.2.A-11/1/KONV-2012-0025, TÁMOP-4.2.4.A/2-11/1-2012-0001, PD OTKA 104878 (B.L.), Mecenatura Fund and Szodoray Fellowship(B.L.).

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Small RNA regulated genes show expression changes during virus infection

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Symptoms caused by plant viruses are responsible for remarkable crop losses. The molecular background of virus-induced disease symptoms have been extensively studied, but poorly understood so far. In plants, RNA silencing (RNAi) is a natural defense mechanism against invading viruses. During this process the replication intermediers of viruses are diced by plant DCL enzyme to 21-24 nt RNA (vsiRNA). VsiRNAs incorporates into AGO complex and this complex can cleave further virus RNAs. As a response to this defense system, viruses have developed silencing suppressors, which repress the plant RNA silencing system. RNAi have a role not only in the defense against pathogen infection but also in the regulation of plant endogenous genes. Endogenous siRNA are involved in developmental processes and regulation of stress response. During virus infection, virus derived small RNAs or RNA silencing suppressor of the virus can interact with host endogenous small RNA pathways and with mRNAs regulated by these pathways and modify their expression. In our experimental system we use two isolates of cucumber mosaic virus (CMV), which cause dramatically different symptoms on tomato. Nt-CMV cause mosaic symptoms, while infection with ScL-CMV, tomato leaves show serious developmental abnormalities, called shoestring symptoms. This symptom is very similar to the phenotype of an RNA silencing mutant (rdr6) tomato. The gene product of RDR6 is essential for the production of trans-acting siRNAs. Based on these facts there is a possibility that the ScL-CMV interfere with the plant endogenous small RNA system or with the expression of leaf polarity genes and may be this lay behind the background of symptoms. Supported by OTKA grant K-106170.

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A homogenous assay to follow-up the aptamer selection procedure

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Aptamers are short, single-stranded oligonucleotides that selectively bind to their cognate molecules. The *in vitro* aptamer selection protocols, referred to as SELEX, fundamentally follow the same iterative procedure to isolate and identify those sequences that bind their molecular targets with the highest affinity from a random oligonucleotide library. In the effective selection process it is expected that the sequence diversity of oligonucleotide library is drastically reduced, thus affinity of aptamer pool increases after each successive selection step. However, a follow-up of the progress of selection is an arduous task with currently employed techniques. Considering the obvious advantages of simple mix-and-measure homogeneous assays, we aimed at developing an Amplified Luminescent Proximity Homogenous Assay (ALPHA) based method to follow the enrichment of the oligonucleotide pool of iterative aptamer selection cycles. Herein, we demonstrate the pertinence of this approach with proof-of-principle studies by using our previously selected apple stem pitting virus coat protein-specific aptamer. The oligonucleotide was PCR amplified by using 5' biotinylated forward and 5' phosphorylated reverse primer. Following purification of the PCR product, the double stranded DNA was converted back into single stranded one by lambda exonuclease treatment. The binding capacity of digested PCR product was evaluated by application of hexahistidine labelled recombinant virus coat proteins. The digested PCR product and virus coat proteins were preincubated then bring into contact with streptavidin donor and anti-Histidine acceptor ALPHA beads. The detected fluorescence signals clearly indicated specific interaction of oligonucleotides and proteins. Furthermore, the obtained data were in concert with the fluorescence measurements gained by using biotinylated aptamer instead of lambda exonuclease treated PCR product. The financial support of ENIAC CAJA4EU is gratefully acknowledged.

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Stress induces cyanide-resistant alternative oxidase activity in *Botrytis cinerea*

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Botrytis cinerea is a fungal pathogen that infects almost all vegetable and fruit crops and annually causes $10 billion to $100 billion in losses worldwide. In fungi, the final steps of this catabolic process take place in mitochondria and lead to the synthesis of the high energy intermediate ATP. A characteristic feature of fungal mitochondria is the presence of alternative oxidase, the activity of which is insensitive to inhibitors of cytochrome C oxidase and the bc1 complex. Cyanide-resistant alternative oxidase, a quinol oxidase localized in the inner mitochondrial membrane is encoded by the nuclear gene *aox* in *Botrytis cinerea*. Fungal alternative oxidase is known to be involved in stress-related cellular responses. Typically, specific activity of the alternative oxidase as well as the level of transcription of the encoding gene increased during stress events. The strain B05.10 was cultured in Gamborg media for 24 h and then transferred to the flasks contained high concentration of D-glucose and sorbitol (osmotic stress), KCl (low water activity stress) and H_2O_2 (oxidative stress). Specific probe of the *aox* fragment was designed and RNA was isolated. Northern blot analysis was carried out for all samples. Cyanide-resistant alternative oxidase participates in the antioxidant defense mechanisms of cells. A key question for fungal AOX is whether it is constitutively expressed or induced under specific conditions. Our expression analysis indicated that *aox* expression rate increased with increasing concentrations of osmotic stress agents (e.g. D-glucose and sorbitol) in *B. cinerea*. Other stress factors (1M KCl and 100 mM H_2O_2) also increased the expression level of *aox* in this fungus.
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Aox sequences as DNA barcode marker for Fungi

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Several cryptic fungal species live in close proximity to each other due to the sympatric speciation. These species may have slightly different environmental demands and different ecological niche. Differences are observed in phenology of the cryptic species. The identification and the determination of ecological specialization of the cryptic species of those fungi which are of agricultural significance, i.e. which infect plants, is important for being able to choose the appropriate strategy of defense against them. This is the same also in the case of *Botrytis cinerea* (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) which is able to attack several economically important plants causing grey rot. *Botrytis cinerea* species complex includes two cryptic species (*B. cinerea* and *B. pseudocinerea*) which could differ in their pathogenicity, fungicide resistance, host variability and genetic diversity. Group I fungi generally display a reduced host range and showed natural resistance against fenhexamid belonging hidroxyanilin. On the basis of classical taxonomic markers, the two related species are very difficult to be distinguished; therefore, their separation is usually performed using molecular methods based on the time-consuming molecular analysis of several markers. Our goal was to find markers, which are suitable for the differentiation. The β-tubulin sequences has been used earlier for differentiate *B. cinerea* from *B. pseudocinerea*. The parsimony analysis of the β-tubulin sequences clearly separated the two *B. cinerea* cryptic species. Testing the nucleotide sequences of the alternative oxidase encoding gene (*aox*), *B. cinerea* and *B. pseudocinerea* strains were clearly differentiated. Moreover, the analysis of the protein sequences of the enzyme with the maximum likelihood method reflected well the taxonomic relationships of the different fungi. Supported by the TÁMOP-4.2.2.A-11/1/KONV-2012-0043 project. A.S. and M. A. were supported by the European Union and Hungary (co-financed by the European Social Fund) in the framework of the TÁMOP-4.2.4.A/ 2-11/1-2012-0001 National Excellence Program.

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Gene expression profile of lineage specific transcription factors in embryonic and adult stem cell-derived dendritic cells

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The application of dendritic cells (DCs) to prime responses to tumor antigens provides a promising approach to cancer immunotherapy. However, only a limited number of DCs can be generated from adult precursors. Pluripotent embryonic stem (ES) cells can be used as a unique source of precursor because this type of cells can be expanded without limit, thus they are considered as a scalable source for clinical applications. The main goal of this study is to compare the gene expression pattern of the mouse embryonic stem (ES) cell- versus bone marrow (BM)-derived DCs. We propose that the altered gene expression of DC affiliated transcription factors in ES-DCs might contribute for the impaired T cell activation capacity of these cells. To investigate the potential role of the putative DC specific transcription factors on ex vivo generated antigen presenting cells, we have monitored the mRNA level of seventeen myeloid/DC specific transcription factors (Irf8, Relb, Egr1, Egr2, Sfpi1 (PU.1), Irf2, Mafb, Tcf4, Maf, Ikzf1, Batf3, Spib, Runx3, Irf4, Id2, Bcl6 and Zbtb46) with real-time quantitative PCR from ES- or BM- derived DCs and their progenitors. Our data revealed that three genes showed a lower expression in ES derived DCs suggesting that upregulation of these factors might modify the phenotype of the ES derived antigen presenting cells. Grant support: TÁMOP 4.2.2.A-11/1/KONV-2012-0023 "VÉD-ELEM" and TÁMOP-4.2.2/B-10/1-2010-0024 project. These projects are co-financed by the European Union and the European Social Fund.

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The role of myosin phosphatase in regulating the SNAP25 protein

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Reversible phosphorylation/dephosporylation is determined by the activity of protein kinases and phosphatases and plays a major role in the regulation of synaptic proteins. The SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) complex is a key element in the process of neurotransmitter release, which is responsible for the connection between the synaptic vesicle and the membrane of the cell, and consists of syntaxin, synaptobrevin and the SNAP25 proteins. The phosphorylation of SNAP25 protein influences the SNARE complex formation and mediates vesicle fusion. One of the potential regulators of the SNARE complex is the myosin phosphatase holoenzyme (PP1M) that consists of a protein phosphatase 1 catalytic subunit (PP1c) and a regulatory subunit (myosin phosphatase targeting subunit, MYPT). The inhibition of myosin phosphatase was presented to decrease, while the ROK increase the neurotransmitter release. Our goal was to investigate the effect of ROK/PP1M on SNAP25 in B50 neuroblastoma cells and rat cortical synaptosomes. We proved that MYPT interacts with ROCK, PP1c and synaptosomal-associated protein of 25 kDa (SNAP25) by immunprecipitation and Far Western analysis. Surface Plasmon Resonance data also suggests a strong interaction between MYPT1 and SNAP25 (K_A= 1.3 x 10^6). Mass spectrometry identified SNAP25 Thr138 residue as a novel ROCK-dependent phosphorylation site. MYPT-silencing resulted in an increased phosphorylation of $SNAP25^{Ser187}$. SNAP25wt but neither $SNAP25^{S187A}$ nor $SNAP25^{T138A}$ was found to be phosphorylated by ROCK using *in vitro* kinase assay. PP1M dephosphorylates SNAP25 at T138 and S187 phosphorylation sites *in vitro*. Our results suggest that ROK/PP1M play a crucial role in the neurotransmitter release by regulating SNAP25 protein phosphorylation. Supported by OTKA PD104878, TÁMOP 4.2.2.A-11/1/KONV-2012-0025.

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COMPARATIVE POPULATION GENOMICS AND PROTEIN STRUCTURAL ANALYSIS TO REVEAL NOVEL FUNCTIONS OF HUMAN TRANSGLUTAMINASE 2

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Transglutaminases catalyse the posttranslational modification of proteins by transamidation of available glutamine residues. Transglutaminase 2 (TGM2) is a unique member of the family with multiple enzymatic activities and interacting partners but its full physiological and pathological role is far from understood. Based on single nucleotide variations (SNV'S) among transglutaminase family members, human TGM2 is under strong evolutionary pressure. TGM2 SNVs are rare and may lead to functionally important changes in protein function. Based on the databases TGM2 does not have any pathological phenotype associated SNV's and homozygous deletion of the mouse TGM2 show no life threatening manifestations. This comparative genomics study between humans, primates and mouse sequences of transglutaminase family members revealed specific regions with stretch of amino acid changes. These linear sequences are mainly in regions of TGM2 outside of so far identified functional sites, suggesting gain of function during evolution to humans. By using bioinformatic and structural analytic tools we have studied the effect of these changes and found novel sites with the potential of functional significance. This might shed light on the functional differences between transglutaminases of these species and their relevance to human physiology and pathology. Grant support: EU FP7 Marie Curie ITN TRANSPATH project, Hungarian Scientific Research Fund (OTKA) and Hungarian Academy of Sciences (MTA).

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Identification of LIM-kinase 2 as a novel PP1 inhibitor in THP-1 leukemic cells

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Phosphorylation of the retinoblastoma protein (pRb) is crucial for cell survival and protein phosphatase-1 (PP1) is involved in the regulation of this process. The basal level of pRb phosphorylation in THP-1 cells is relatively high, which may reflect increased effectiveness of PP1 inhibitory proteins. Hence, we tested the presence of phosphorylated CPI-17, a PP1 inhibitory phosphoprotein in these cells. We found relatively low level of phospho-CPI-17, but several other proteins in higher molecular mass range cross-reacted with the anti-phospho-CPI17 antibody, and their phosphorylation increased markedly upon treatment with calyculin A (CLA), a cell-permeable inhibitor of both PP1 and PP2A. Inhibition of protein kinase C (PKC) has decreased the CLA-induced phosphorylation of the CPI-17-like proteins; while the inhibitor of Rho associated kinase (ROK) was without any effect, suggesting the major role of PKC in phosphorylation these proteins. Search in protein databases based on CPI-17 phosphorylation sequence highlighted known CPI-17-like proteins, such as PHI and KEPI. In addition, LIMK2 showed significant sequence similarity to CPI-17, raising its possible role in PP1 inhibition. To test this hypothesis, Flag-LIMK2 expressed in tsA201 cells was immobilized on Flag-affinity column, and then phosphorylated *in vitro* by PKC. The phosphorylation of LIMK2 at the inhibitory sequence was confirmed by Western blot analysis using anti-phospho-CPI-17 antibody. Next, the phosphatase activity of PP1 was measured in the presence of unphosphorylated as well as phosphorylated Flag-LIMK2 using myosin light chain substrate. Unphosphorylated Flag-LIMK2 inhibited the phosphatase activity of PP1 only slightly, while the phosphorylated Flag-LIMK2 decreased the activity of PP1 markedly in a concentration-dependent manner. Our results suggest that LIMK2 may be a novel inhibitor of PP1 and it may mediate the phosphorylation level of proteins important in the regulation of tumorgenesis. 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' (A.K.). Direct costs of this study were supported by TÁMOP-4.2.2.A-11/1/ KONV-2012-0025 (F.E.) and OTKA K109249.

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Mesenchymal stem cells and macrophages: supportive cells for tissue regeneration

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In recent years it has become clear that the therapeutic potential of mesenchymal stem or stromal cells (MSC) is related not only to their multilineage differentiation capacity but also to their ability to suppress inflammatory and immune responses. They secrete anti-inflammatory factors such as TGF-β and hepatocyte growth factor, they inhibit lymphocyte proliferation via the expression of indoleamine 2, 3-dioxygenase, and they express inhibitory co-stimulatory molecules such as programmed death ligand 1. In addition, MSC modulate the immune system via indirect mechanisms by inducing immune cells to adapt a regulatory function. MSC induce regulatory T cells in vitro and in vivo and affect the differentiation and function of dendritic cells. In recent years it has become clear that MSC also regulate the function of macrophages (MΦ). MSC induce MΦs to adapt an enhanced regulatory phenotype characterized by low pro-inflammatory (IL-12, TNF-α) and high anti-inflammatory cytokine (IL-10) production, high ability to ingest pathogens and apoptotic cells. This effect of MSC is at least partially mediated by soluble mechanisms and prostaglandin E2 has been indicated to be one of the factors involved. At the same time, it also became clear that MΦs contribute to tissue formation, metabolism, homeostasis, and repair. Therefore, improved understanding of MΦ phenotypes and their regulation by MSC may assist in generation of novel therapies based on manipulating MΦ function to improve tissue regeneration and wound healing. This work received financial support from the program nr. KTIA_AIK_12-1-2012-0025 (Hungary).

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Characterization of the interaction between metastasis-associated ezrin and S100A4

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Ezrin belongs to the ERM (ezrin, radixin, moesin) protein family that has a role in organizing the cortical cytoskeleton by linking filamentous actin to the apical membrane of epithelial cells. In its inactive state, an intramolecular interaction is formed between the N-terminal and C-terminal domains, blocking protein-protein interaction sites. Upon activation the unmasked N- and C-terminal domains bind to the plasma membrane and F-actin, respectively. This activation mechanism is associated with specific cellular functions: cell morphology changes, adhesion and migration. Ezrin is highly expressed in a variety of human cancers and promotes metastasis. S100P, a member of the Ca^{2+}-binding EF-hand S100 protein family serves as an activator of ezrin: it binds to its N-terminal domain and unmasks the actin-binding site, thus inducing its activation. Here we characterize the interaction of ezrin with another metastasis-associated protein, S100A4. S100A4 is one of the regulators of the motility of tumor cells by inhibiting non-muscle myosin IIA filament assembly. According to our results, S100A4 binds to the N-terminal domain of ezrin with similar affinity as S100P. We demonstrate that full-length ezrin interacts with F-actin in an S100A4-dependent manner. Additionally, competitive binding studies revealed that the ezrin- and myosin-binding sites overlap on S100A4. We validate the interaction by cell culture-based assays, e.g. co-immunoprecipitation and co-localization. As both proteins play an important role in promoting metastasis, the characterization of their interaction could serve as a basis for the design of novel therapeutic agents. Supported by the Hungarian Scientific Research Fund (OTKA N81950).

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Enhanced PMCA4b expression and modulation of calcium signals in differentiated MCF-7 breast cancer cells

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Plasma membrane Ca^{2+} ATPases (PMCAs) are responsible for the expulsion of increased intracellular Ca^{2+} into the extracellular space, therefore these pumps play an important role in maintaining intracellular Ca^{2+} homeostasis. Increasing evidence suggests the role of intracellular Ca^{2+} homeostasis remodeling in cancer progression. Previous studies showed that the expression of different plasma membrane Ca^{2+} ATPase isoforms was altered in several types of cancer cells. In this study we induced differentiation of MCF-7 breast cancer cells by histone deacetylase inhibitors (HDACi) and by phorbol 12-myristate 13-acetate (PMA), and we examined the expression levels of PMCA isoforms both at mRNA and protein levels. We established an MCF-7 cell line stably expressing the GCaMP2, genetically encoded Ca^{2+} sensor to perform Ca^{2+} signal measurements as a functional assay. We found marked PMCA4b upregulation during short chain fatty acid-induced differentiation that was further enhanced by PMA, while there were no remarkable changes in the expression levels of the other PMCA isoforms. Time course experiments suggest correlation between the enhanced PMCA4b expression and cell differentiation. The FDA-approved epigenetic drugs, suberoylanilide hydroxamic acid (SAHA) and valproate, had similar effects. Immunocytochemical analysis revealed that the upregulated protein was located mostly in the plasma membrane. We tested the characteristics of Ca^{2+} signals evoked by various calcium mobilizing agents, and we found that the Ca^{2+} clearance was faster in differentiated cells after stimulus than in the non-differentiated control cells. PMCA4 protein was also found in normal breast tissue samples, suggesting that this protein plays an important role in the regulation of Ca^{2+} homeostasis of the mammary epithelium. Our results suggest that modulation of PMCA4b expression may contribute to the remodeling of intracellular Ca^{2+} homeostasis during tumor progression. Supported by grants from the Hungarian Scientific Research Fund (OTKA CK 80283, K 101064), the Hungarian Ministry of National Development ("TransRat" KMR_12-1-2012-0112 and KTIA_AIK_12-1-2012-0025), and by the Association pour la Recherche sur le Cancer, France and Inserm.

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Expression and function of NLRC5 in human keratinocytes.

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Keratinocytes represent the first line of defense against pathogens passively as physical barrier and also actively by cytokine and chemokine expression, initiating and regulating inflammation during infection in the skin. For pathogen-derived danger-signal sensing, keratinocytes express pattern recognition receptors, such as the intracellular Nod-like receptors (NLRs). The largest member of the NLR family is NLRC5. Recently, conflicting reports have been presented about the function of NLRC5. NLRC5 mRNA was found to be expressed mainly in human immune-cells, but there is no information about the expression of NLRC5 in keratinocytes. Therefore, we aimed to study the expression of NLRC5 in HPV-immortalized human keratinocyte cell line in the presence or absence of poly(I:C), which is the synthetic analogue of viral double-stranded RNA (dsRNA). We also aimed to study the poly(I:C)-induced secretion of IL-6 proinflammatory cytokine and IL-8 chemokine in NLRC5-silenced keratinocytes. We found that NLRC5 is expressed in human keratinocytes (quantitative real-time PCR). We also found that poly(I:C) significantly induces the expression of NLRC5 in HPV-immortalized keratinocytes. We have also studied the effect of silencing of NLRC5 expression using siRNA on the poly(I:C)-induced IL-6 and IL-8 secretion (ELISA). We found that the NLRC5 silencing significantly decreases the poly(I:C)-induced secretion of these cytokines.
Based on our findings, it is possible that NLRC5 might act as an inflammatory protein and promote inflammatory conditions upon viral infection in human keratinocytes. Financial support: OTKA K-109429, TÁMOP-4.2.2.A-1/1/KONV-2012-0023.

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KUTATÓI ÁLLÁS

Ph.D. végzettséggel rendelkező munkatársat keresünk bioinformatikai kutatási feladatkörre a Szegedi Biológiai Kutatóközpont Biokémiai Intézet Szintetikus és Rendszerbiológiai Egységébe. Az ideális jelentkező programozásban jártas, önálló kutatásra képes és szívesen dolgozna az antibiotikumrezisztencia evolúciójának új aspektusain. A sikeres jelentkezőre kiemelt bér, kiterjedt kollaborációs lehetőségek és motiváló légkör vár. További részletekért lásd:

http://group.szbk.u-szeged.hu/sysbiol/papp-balazs-lab-openpositions.html

Az érdeklődőktől életrajzot, 2 ajánló nevét, elérhetőségét és szakmai tapasztalataik rövid leírását várjuk elektronikus formában.

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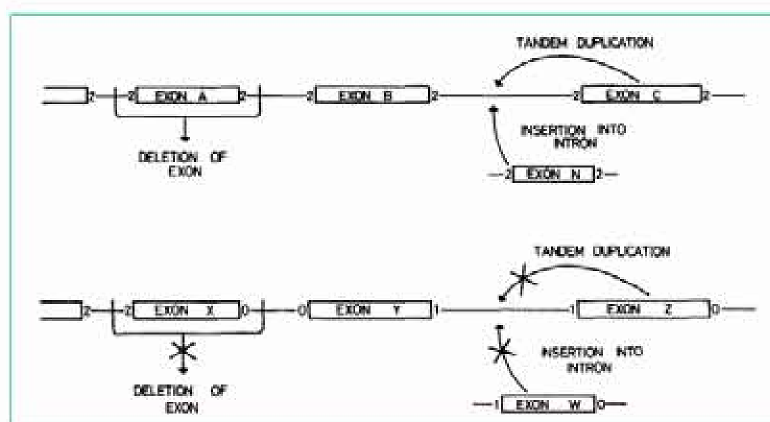

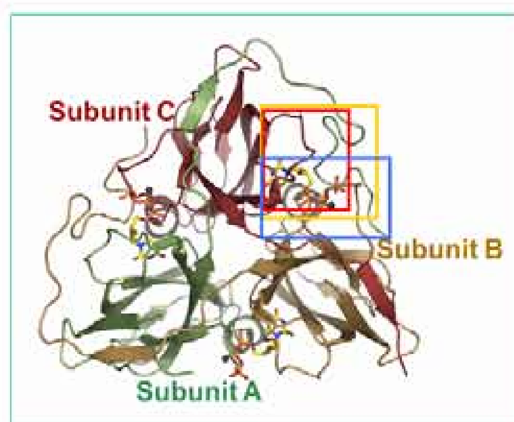

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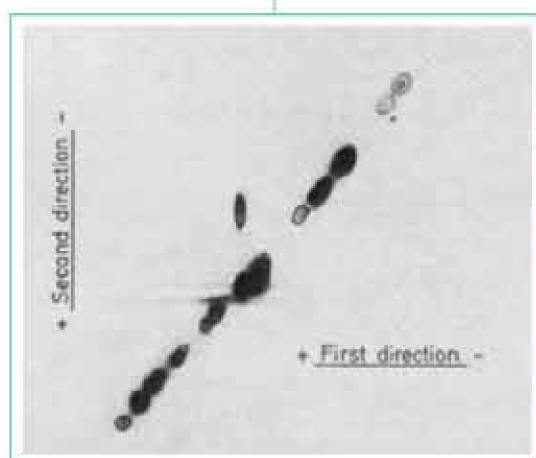

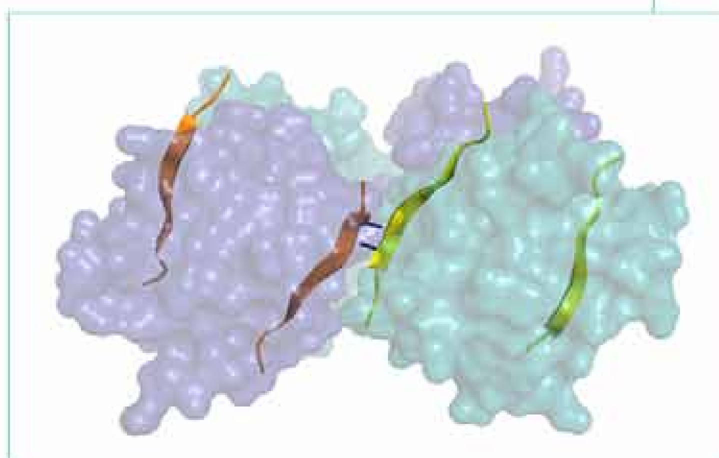

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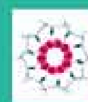

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