BIOKÉMIA

A Magyar Biokémiai Egyesület internetes folyóirata

XL. évfolyam 3. szám

2016. augusztus

Annual Meeting of the Hungarian Biochemical Society

Szeged

August 28-31, 2016

Final Program & Abstract Book

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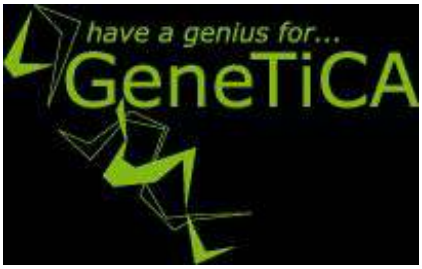

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XL. ÉVFOLYAM 3. SZÁM **2016. augusztus**

TARTALOMJEGYZÉK

Final Program & Abstract Book of the Annual Meeting of the Hungarian Biochemical Society (HBS), 2016

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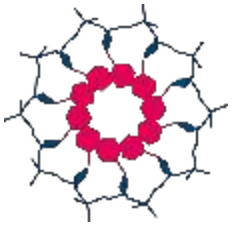
Annual Meeting of the Hungarian Biochemical Society



Szeged

August 28-31, 2016

Final Program & Abstract Book

WELCOME MESSAGE

Dear Colleague,

On behalf of the Organizing Committee, we warmly welcome you at the Annual Meeting of the Hungarian Biochemical Society held in Szeged, August 28-31, 2016. As you know, Szeged has a rich history of groundbreaking results in biochemistry. Nowadays, too, original research is pursued in diverse fields of biochemistry and molecular biology at several institutes in Szeged. Needless to mention, Szeged is a great city with lots of sunshine and attractions. Do not miss this unique occasion to taste Hungarian biochemistry - and the famous Szeged fisherman's soup as well.

The topics of the Congress are the following: *Lipids and membranes in action, Genome organization, maintenance, functional genomic, Bioinformatics, synthetic biology, genome engineering, biotechnology; Molecular signaling, cell-cell communication, cell death and differentiation; Molecular basis of disease and therapy, stem cells, immunity and inflammation; Regulation of gene expression, regulatory RNA, epigenetics; Protein structure, function and modeling; Translational medicine*

The program includes plenary and award lectures, 8 sessions of oral presentations and 2 poster sessions. The section chairs and the scientific organizing committee finalized the program based on the submitted abstracts. The Congress is organized in collaboration with Congress and Hobby Service Ltd. The announcement and all relevant information can be found at http://www.congress-service.hu/2016/biokemia/index.html.

The final program and the abstracts are published in the 3rd issue of Biokémia, the official journal of the society and can be found at www.mbkegy.hu.

We hope that you will join us and share your latest results with us.

On behalf of the organizers:

Imre M. Boros	***László Buday***	***Mihály Kovács***
Chair of the Conference	***RCNS HAS Budapest,***	***Eötvös University Budapest***
University of Szeged	***President of HBS***	***Secretary General of HBS***

GENERAL INFORMATION

Congress venue

IH Event Center

6721 Szeged, Felső Tiszapart 2.

IH Event Center is one of the newest conference venues of Szeged. It is situated directly on the bank of River Tisza, next to Hotel Novotel****, a ten-minute walk from the city centre.

Scientific Organizing Committee

Tamás Csont, Szeged University
Lajos Haracska, BRC Szeged
György Pósfai, BRC Szeged
Mária Szűcs, Szeged University
László Vigh, BRC Szeged
Mihály Kovács, ELTE Budapest, secretary general of HBS
László Buday, RCNS Budapest, president of HBS
Imre Boros, Szeged University, vice president of HBS, chair of organizing committee

Secretary of the Organizing Committee

Angela Cakó
e-mail: mbke.conference2016@gmail.com
phone: +36 (62) 544-887

Congress office

Congress & Hobby Service Ltd.
H-6701 Szeged, Hungary
POBox 1022
phone: +36-62-484-531
fax: +36-62-450-014
e-mail: info@congress-service.hu

Information on the meeting can be found at http://www.congress service.hu/2016/biokemia/index.html.

Registration opening hours at the venue

28th August 15:00–18.30
29th August 08:00–18:30
30th August 08:00–18:30
31st August 08:00–13:00

Phone (only available on 28-31 August, 2016): +36-30/977-4007

Registration
All participants wishing to attend the conference should register online.

Registration fees (without VAT)		**Late fee after 15 June**
For non-members	Registration fee for junior researchers from education and other non-profit institutions*	52 000 Ft
	Registration fee for non junior researchers from education and other non-profit institutions	63 000 Ft
	Registration fee for participants from industry and other parts of the business sphere	68 000 Ft
For HBS members	Registration fee for junior researchers from education and other non-profit institutions*	33 000 Ft
	Registration fee for non junior researchers from education and other non-profit institutions	41 000 Ft
	Registration fee for participants from industry and other parts of the business sphere	46 000 Ft

* Junior researcher: university or Ph.D. student or researcher under 30.

Food and drink costs (25.000 HUF) will be listed separately on the invoice.
Accommodation is not included in the registration fee.
VAT is not included in the registration fees.

Registration fee includes coffee breaks, lunches, 1st and 3rd day dinners, conference materials, conference attendance, and admission to the exhibition.

Technical information for poster presenters

Posters will be presented in paper format. The size of the usable work area on poster boards: 120 cm height, 100 cm width.

Social programs
Welcome reception *(sponsored by Bio-Science Ltd.)*
*Hotel Novotel*****
Sunday, 28 August, 19:00 h
The price is included in the registration fee.
All Participants are invited to attend.

Optional Shipping tour and Fishermen's party
Traditional Fishermen's Inn of Szeged
Monday, 29 August, 19:00 h

During the journey you can enjoy the beautiful view of the River Tisza by ship which will land at the traditional Fisherman's Inn of Szeged that is located at the bank of the river. The richly flavored and delicious fisherman's soup – a specialty of Szeged – the beautiful natural environment of the bank of the river will provide a unique atmosphere of the evening. Price of the program: 7800 Ft

Menu:

- *„Old Kőrössy" fish soup from carp and catfish fillet*
- *Fish cracklings „a'la Makó" with breaded onion rings*
- *Apple crépes with chocolate and caremel dressing a'la Tápé*
- *Drinks: 1 glass champagne, 1 glass wine or beer and 1 bottle of mineral water*

Closing banquet
Tisza River Cafe Club
Tuesday, 30 August, 19:00 h
All Participants are invited to attend the Closing banquet.The price is included in the registration fee.

Confirmation
Upon receipt of your registration you will receive an automatic confirmation reply.

Payment
After registering for the Congress and receiving the confirmation, please arrange your payment. All payments should be made in HUF and all bank charges will be charged to the payer. Also, please indicate your name, the name of the event and your registration number on the transfer.

Cancellation of Congress Participation
In the case of cancellation of the participation by 28 July 2016, the registration fee minus a processing fee of 2500 HUF+VAT will be refunded. After this date, no refund would be made. Please, notify the Congress Office of your cancellation in writing.

Modification of Congress Participation
In the case of cancellation or modification of any of the dates of your invoice, it will mean a handling charge of 2500 HUF+VAT. After the congress, handling charge raises to 4000 HUF+VAT. Please, notify the Congress Office of your modification in writing.

Liability, insurance
The participants take part on all excursions at their own risk. The registration fee does not include any kind of insurance. The Organizing Committee and the Congress Office can not accept liability for personal accidents, losses or damage. Health, baggage or accident insurance is recommended.

PROGRAM AT A GLANCE

28th August, 2016

14:00 - *Arrival and registration*

Session I. Opening plenary session
Chairs: György Keserű, László Buday, Mihály Kovács, Imre M. Boros

15:50-16:00 **Opening remarks**: Imre M. Boros, László Buday

16:00-16:40 **E1-1 FEBS National Congress Lecture**
On the road to eliminating tumor initiating cells in breast cancers: Are we there yet
Prof. Michael Lisanti

16:40-17:10 **E1-2 Tankó Award Lecture**
The expanded chemical space of DNA: moonlighting roles of unorthodox bases
Beáta Vértessy

17:10-17:40 **E1-3 Bio-Science Award Lecture**
Structural characterization of the native RSK1 signalosome and its misregulation in malignant melanomas
Gergő Gógl

17:40-18:00 **E1-4 Tankó Award Lecture**
Remembrance of things Past
Mária Wollemann

19:00 - ***Welcome party by Bio-Science Ltd. in Hotel Novotel***

29th August, 2016

Session II. Translational medicine
Chairs: Péter Arányi, György Keserű

09:00-09:20 E2-1 **Polypharmacology approach for a fibrotic disorder: Improved anti-fibrotic efficacy with dual-target inhibitors of peripheral CB1R and inducible nitric oxide (iNOS) in liver fibrosis**
Resat Cinar

09:20-09:40 E2-2 **DNA hypermethylation pushes immune escape through downregulation of antigen presentation genes in breast cancer**
Balázs Győrffy

09:40-10:00 E2-3 **Next generation sequencing in the diagnosis of rare genetic diseases**
Mária Judit Molnár

10:00-10:20 E2-4 **Extranuclear effects of PARP activation: Relevance in cardiovascular diseases**
Ferenc Gallyas

10:20-10:40 E2-5 **Restoration of altered redox balance in rats by short term metformin treatment: Relevance to aging**
Syed Ibrahim Rizvi

11:40 -11:00 **Coffee Break**

Session III. Molecular basis of disease and therapy, stem cells, immunity and inflammation
Chair: István Szatmári

11:00 -11:30 E3-1 **HOFI/SH3PXD2B, a regulator of tumor development**
Árpád Lányi

11:30-12:00 E3-2 **The Emerging Role of Ring1 and YY1 Binding Protein in Stem Cell Differentiation**
Melinda Pirity

12:00-12:20 E3-3 **E3-deficiency mediated by the pathogenic mutations of the human dihydrolipoamide dehydrogenase (hE3): elucidation of the molecular pathomechanism by a multifaceted structural approach**
Attila Ambrus

12:20-12:40 E3-4 **Enhanced dendritic cell differentiation from pluripotent stem cells by ectopic expression of Runx3**
Pál Botó

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

Session IV. Regulation of gene expression, regulatory RNA, epigenetics
Chair: Imre M. Boros

14:00 -14:20 E4-1 **Transcriptional outcomes in response to DNA damage**
Tibor Pankotai

14:20-14:40 E4-2 **SUMOylation regulates light-induced signaling in Arabidopsis thaliana**
András Viczián

14:40-15:00 E4-3 **Intrinsic protein disorder in histone lysine methylation**
Ágnes Tantos

15:00-15:20 E4-4 **Impact of insulin-like growth factor II (IGF II) gene inhibition with IGF II antisense oligomer during the development of rat hepatocarcinogenesis**
Miltu Kumar Ghosh

15:20-15:40 E4-5 **In search for natural small molecule modulators of breast cancer**
Péter Bai

Technical presentation I.

15:40-15:50 C1-1 **Together or alone – Application of sgRNA or crRNA-tracrRNA complex in CRISPR-Cas9 systems (The Alt-R CRISPR-Cas9 system of Integrated DNA Technologies)**
Éva Kerekes, Bio-Science Ltd., Budapest

15:50-16:05 C1-2 **Monitoring protein interactions in living cells using the luminescent NanoLuc® Binary Technology**
Zsolt Somlai, Bio-Science Ltd., Budapest

Poster Session I.

16:05-18:00 P1 - P35

19:00 - ***Shipping Tour and dinner at Fisherman's Restaurant***

30th August, 2016

Session V. Lipids and membranes in action

Chairs: János Szöllősi, László Vigh

09:00-09:25 E5-1 **New fluorescence-based functional assays for organic anion transporting polypeptides (OATPs), transporters involved in drug ADME-Tox**
Csilla Laczka

09:25-09:50 E5-2 **Interplay between membrane and storage lipid metabolism during heat stress management in fission yeast**
Gábor Balogh

09:50-10:15 E5-3 **HER2, the epitome of membrane proteins targeted in cancer therapy**
György Vereb

10:15-10:40 E5-4 **Membrane nanotube networks between B cells, conductors of humoral immune response: on the road to reveal the factors controlling their growth, reaction and function**
János Matkó

10:40 -11:00 **Coffee Break**

Session VI. Bioinformatics, synthetic biology, genome engineering, biotechnology

Chair: György Pósfai

11:00-11:25 E6-1 **Protein complex prediction through proteome-wide simulations**
Attila Csikász-Nagy

11:25-11:50 E6-2 **SignaLink 3, a curated and integrated signaling resource for tissue-specific network modeling and detailed analysis of signaling pathways**
Dávid Fazekas

11:50-12:15 E6-3 **Detrimental effect of knock-outs in yeast is partially explained by maladaptive transcriptional response**
Károly Kovács

12:15-12:40 E6-4 **pORTMAGE genome engineering systematically compares mutational effects across bacterial species**
Gábor Apjok

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

Session VII. Genome organization, maintenance, functional genomics

Chairs: Lajos Haracska, Mihály Kovács

14:00-14:20 E7-1 **Super-enhancers and person-to-person genetic variability in the context of the 1000 Genomes Project**
Bálint L. Bálint

14:20-14:40 E7-2 **Regulation of FAN1 nuclease activity**
Péter Burkovics

14:40-15:00 E7-3 **Motor enzymatic activities controlling the initiation and outcome of homologous recombination**
Mihály Kovács

15:00-15:40 E7-4 **Deoxynucleotide metabolism and genome integrity**
Eszter Judit Szabó

15:40-16:00 E7-5 **Accurate mutation detection in whole genome sequences of multiple isogenic samples with IsoMut**
Dávod Szüts

Technical presentation II.

16:00-16:20 C2-1 **BLI and the many ways to enhance your biomolecular binding kinetic and quantification assays capabilities**
Renata Gronczewska Fortebio, Division of Pall Life Science

16:20-16:35 C2-2 **Automated solutions in biochemical research workflows**
András Dékány
GeneTiCA Ltd., Tiszanána

Poster Session II.

16:00-18:00 P36- P70

19:00- ***Conference Banquet, Tisza River Cafe Club & Restaurant***

31st August, 2016

Session VIII. Protein structure, function and modeling

Chairs: Tamás Csont, László Nyitray

09:00 – 09:30 E8-1 **Molecular recognition with peptidic foldamers: targeting proteins and membrane**
Tamás Martinek

09:30-09:50 E8-2 **Are the phosphopantothenoylcysteine decarboxylase subunits moonlighting proteins in Candida albicans?**
Viktor Dombrádi

09:50-10:10 E8-3 **Structural snapshots and mechanistic insights unveil the enzymatic cycle of Plasmodium CCT**
Gergely Nándor Nagy

10:10-10:25 E8-4 **Reconstitution of the Drosophila protein phosphatase 4 holoenzyme**
Zoltán Lipinszki

10:25-10:40 E8-5 **A unique feature of the Drosophila APC/C**
Ágota Nagy

10:40 -11:00 **Coffee Break**

Session IX. Molecular signaling, cell-cell communication, cell death and differentiation

Chair: László Buday

11:00 -11:25 E9-1 **Pathogenic mutations of the vasopressin V2 receptor**
László Hunyady

11:25-11:50 E9-2 **Signals derived by engulfing macrophages regulate the transglutaminase 2 expression in dying thymocytes**
Zsuzsa Szondy

11:50-12:10 E9-3 **Differentiating human beige adipocytes secrete cytokines ("batokines")**
Endre Károly Kristóf

12:10-12:25 E9-4 **PKC mediated phosphorylation of TIMAP regulates PP1c activity in endothelial cells**
Anita Boratkó

12:25-12:40 E9-5 **Testis-specific glutamate dehydrogenase is essential in the development of spermatid mitochondria**
Rita Sinka

12:50 -13:00 ***Closing ceremony, Best Presentation Awards***

Chairs: Imre M. Boros, László Buday

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

SCIENTIFIC PROGRAM

28th August, 2016

14:00 - Arrival and registration

Session I. Opening plenary session

Chairs: György Keserű, László Buday, Mihály Kovács, Imre M. Boros

15:50-16:00 **Opening remarks**
Imre M. Boros and László Buday

16:00-16:40 E1-1
FEBS National Congress Lecture
On the road to eliminating tumor initiating cells in breast cancers: Are we there yet
Prof. Michael Lisanti
Director of the Manchester Breakthrough Breast Cancer Research Unit; Professor of Cancer Biology and Director of the Manchester Centre for Cellular Metabolism (MCCM), Manchester, U.K.

Introduction by György Keserű, President of the Drug-Biochemistry Section of HBS

16:40-17:10 E1-2
Tankó Award Lecture
The expanded chemical space of DNA: moonlighting roles of unorthodox bases
Beáta Vértessy
Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Budapest; Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

Introduction and Award presented by Mihály Kovács, General Secretary of HBS and László Buday, President of HBS

17:10-17:40 E1-3
Bio-Science Award Lecture
Structural characterization of the native RSK1 signalosome and its misregulation in malignant melanomas
Gergő Gógl
Institute of Enzymology, Hungarian Academy of Sciences, Budapest; Department of Biochemistry, Eötvös Loránd University, Budapest

Introduction and Award presented by Ágnes Tátrai, CEO of the Bio-Science Ltd. and László Buday, President of HBS

17:40-18:00 E1-4

Tankó Award Lecture

Remembrance of things past

Mária Wollemann
Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

Introduction and Award presented by Imre M. Boros, Vice President of HBS and László Buday, President of HBS

19:00 - Welcome party by Bio-Science Ltd. in Hotel Novotel

29th August, 2016

Session II. Translational medicine

Chairs: Péter Arányi, György Keserű

09:00-09:20 E2-1

Polypharmacology approach for a fibrotic disorder: Improved anti-fibrotic efficacy with dual-target inhibitors of peripheral CB1R and inducible nitric oxide (iNOS) in liver fibrosis

Resat Cinar
Laboratory of Physiological Studies, National Institute on Alcohol Abuse and Alcoholism, National Institute of Health, Rockville, MD, U.S.A.

09:20-09:40 E2-2

DNA hypermethylation pushes immune escape through downregulation of antigen presentation genes in breast cancer

Balázs Győrffy
Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

09:40-10:00 E2-3

Next generation sequencing in the diagnosis of rare genetic diseases

Mária Judit Molnár
Institute of Genomic Medicine and Rare Disorders, Semmelweis University, Budapest

10:00-10:20 E2-4

Extranuclear effects of PARP activation: Relevance in cardiovascular diseases

Ferenc Gallyas
Department of Biochemistry and Medical Chemistry, University of Pécs, Medical School, Pécs

10:20-10:40 E2-5

Restoration of altered redox balance in rats by short term metformin treatment: Relevance to aging

Syed Ibrahim Rizvi
Department of Biochemistry, University of Allahabad, India

11:40 -11:00 Coffee Break

Session III. Molecular basis of disease and therapy, stem cells, immunity and inflammation

Chair: István Szatmári

11:00 -11:30 E3-1

HOFI/SH3PXD2B, a regulator of tumor development

Árpád Lányi

Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen

11:30-12:00 E3-2

The emerging role of ring1 and YY1 binding protein in stem cell differentiation

Melinda Pirity

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

12:00-12:20 E3-3

E3-deficiency mediated by the pathogenic mutations of the human dihydrolipoamide dehydrogenase (hE3): elucidation of the molecular pathomechanism by a multifaceted structural approach

Attila Ambrus

MTA-SE Laboratory for Neurobiochemistry, Department of Medical Biochemistry, Semmelweis University, Budapest

12:20-12:40 E3-4

Enhanced dendritic cell differentiation from pluripotent stem cells by ectopic expression of Runx3

Pál Botó

Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen

13:00 -14:00 Lunch

Session IV. Regulation of gene expression, regulatory RNA, epigenetics

Chair: Imre M. Boros

14:00 -14:20 E4-1

Transcriptional outcomes in response to DNA damage

Tibor Pankotai

Department of Biochemistry and Molecular Biology, University of Szeged, Szeged

14:20-14:40 E4-2

SUMOylation regulates light-induced signaling in Arabidopsis thaliana

András Viczián

Plant Biology Institute, Biological Research Centre, Hungarian Academy of Sciences, Szeged

14:40-15:00 E4-3

Intrinsic protein disorder in histone lysine methylation

Ágnes Tantos

Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

15:00-15:20 E4-4
Impact of insulin-like growth factor II (IGF II) gene inhibition with IGF II antisense oligomer during the development of rat hepatocarcinogenesis
Miltu Kumar Ghosh
Department of Pharmacy, NSHM Knowledge Campous, India

15:20-15:40 E4-5
In search for natural small molecule modulators of breast cancer
Péter Bai
Department of Medical Chemistry, Faculty of Medicine, University of Debrecen; MTA-DE „Lendület" Laboratory of Cellular Metabolism, University of Debrecen; Research Center for Molecular Medicine, University of Debrecen, Debrecen

Technical presentation I.

15:40-15:50 C1-1
Together or alone – Application of sgRNA or crRNA-tracrRNA complex in CRISPR-Cas9 systems (The Alt-R CRISPR-Cas9 system of Integrated DNA Technologies)
Éva Kerekes
Bio-Science Ltd., Budapest

15:50-16:05 C1-2
Monitoring protein interactions in living cells using the luminescent NanoLuc® Binary Technology
Zsolt Somlai
Bio-Science Ltd., Budapest

Poster Session I.

16:05-18:00 P1 - P35

19:00 - Shipping Tour and dinner at Fisherman's Restaurant

30th August, 2016

Session V. Lipids and membranes in action

Chairs: János Szöllősi, László Vigh

09:00-09:25 E5-1
New fluorescence-based functional assays for organic anion transporting polypeptides (OATPs), transporters involved in drug ADME-Tox
Csilla Laczka
Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

09:25-09:50 E5-2
Interplay between membrane and storage lipid metabolism during heat stress management in fission yeast
Gábor Balogh
Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

09:50-10:15 E5-3
HER2, the epitome of membrane proteins targeted in cancer therapy
György Vereb
Department of Biophysics and Cell Biology, and MTA-DE Cell Biology and Signaling Research Group, University of Debrecen, Debrecen

10:15-10:40 E5-4
Membrane nanotube networks between B cells, conductors of humoral immune response: on the road to reveal the factors controlling their growth, reaction and function
János Matkó
Department of Immunology, Eötvös Loránd University, Faculty of Science, Budapest

10:40 -11:00 Coffee Break

Session VI. Bioinformatics, synthetic biology, genome engineering, biotechnology

Chair: György Pósfai

11:00-11:25 E6-1
Protein complex prediction through proteome-wide simulations
Attila Csikász-Nagy
Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest; Randall Division of Cell and Molecular Biophysics, New Hunt's House, King's College London, U.K.

11:25-11:50 E6-2
SignaLink 3, a curated and integrated signaling resource for tissue-specific network modeling and detailed analysis of signaling pathways
Dávid Fazekas
Department of Genetics, Eötvös Loránd University, Budapest

11:50-12:15 E6-3
Detrimental effect of knock-outs in yeast is partially explained by maladaptive transcriptional response
Károly Kovács
Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

12:15-12:40 E6-4
pORTMAGE genome engineering systematically compares mutational effects across bacterial species
Gábor Apjok
Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

13:00 -14:00 Lunch

Session VII. Genome organization, maintenance, functional genomics

Chairs: Lajos Haracska, Mihály Kovács

14:00-14:20 E7-1

Super-enhancers and person-to-person genetic variability in the context of the 1000 Genomes Project

Bálint L. Bálint
Department of Biochemistry and Molecular Biology, Genomic Medicine and Bioinformatic Core Facility, University of Debrecen, Debrecen

14:20-14:40 E7-2

Regulation of FAN1 nuclease activity

Péter Burkovics
Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged

14:40-15:00 E7-3

Motor enzymatic activities controlling the initiation and outcome of homologous recombination

Mihály Kovács
Department of Biochemistry, Eötvös Loránd University, Budapest

15:00-15:40 E7-4

Deoxynucleotide metabolism and genome integrity

Eszter Judit Szabó
Institutes of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; Department of Applied Biotechnology and Food Sciences, Budapest University of Technology and Economics, Budapest

15:40-16:00 E7-5

Accurate mutation detection in whole genome sequences of multiple isogenic samples with IsoMut

Dávid Szüts
Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

Technical presentation II.

16:00-16:20 C2-1

BLI and the many ways to enhance your biomolecular binding kinetic and quantification assays capabilities

Renata Gronczewska
Fortebio, Division of Pall Life Science, Menlo Park, CA, U.S.A

16:20-16:35 C2-2

Automated solutions in biochemical research workflows

András Dékány
GeneTiCA Ltd., Tiszanána

Poster Session II.

16:00-18:00 P36- P70

19:00- Conference Banquet, Tisza River Cafe Club & Restaurant

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31st August, 2016

Session VIII. Protein structure, function and modeling

Chairs: Tamás Csont, László Nyitray

09:00–09:30 E8-1
Molecular recognition with peptidic foldamers: targeting proteins and membrane
Tamás Martinek
Institute of Pharmaceutical Analysis, University of Szeged, Szeged

09:30-09:50 E8-2
Are the phosphopantothenoylcysteine decarboxylase subunits moonlighting proteins in Candida albicans?
Viktor Dombrádi
Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen

09:50-10:10 E8-3
Structural snapshots and mechanistic insights unveil the enzymatic cycle of Plasmodium CCT
Gergely Nándor Nagy
Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Budapest; Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

10:10-10:25 E8-4
Reconstitution of the Drosophila protein phosphatase 4 holoenzyme
Zoltán Lipinszki
Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

10:25-10:40 E8-5
A unique feature of the Drosophila APC/C
Ágota Nagy
Department of Genetics, University of Szeged, Szeged

10:40 -11:00 Coffee Break

Session IX. Molecular signaling, cell-cell communication, cell death and differentiation

Chair: László Buday

11:00 -11:25 E9-1

Pathogenic mutations of the vasopressin V2 receptor

László Hunyady

Department of Physiology, Faculty of Medicine, Semmelweis University and MTA-SE Laboratory of Molecular Physiology, Budapest

11:25-11:50 E9-2

Signals derived by engulfing macrophages regulate the transglutaminase 2 expression in dying thymocytes

Zsuzsa Szondy

Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen

11:50-12:10 E9-3

Differentiating human beige adipocytes secrete cytokines ("batokines")

Endre Károly Kristóf

Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen

12:10-12:25 E9-4

PKC mediated phosphorylation of TIMAP regulates PP1c activity in endothelial cells

Anita Boratkó

Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen

12:25-12:40 E9-5

Testis-specific glutamate dehydrogenase is essential in the development of spermatid mitochondria

Rita Sinka

Department of Genetics, University of Szeged, Szeged

12:50 -13:00 **Closing ceremony, Best Presentation Awards**

Chairs: Imre M. Boros and László Buday

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

14:00 *Departure*

ABSTRACTS OF THE LECTURES

E1 - Opening plenary session

E1-1
On the road to eliminating tumor initiating cells in breast cancers: Are we there yet?

Michael Lisanti[1,2]

[1]The Breast Cancer Now Manchester Research Unit, Institute of Cancer Sciences, Cancer Research U.K. Manchester Institute, University of Manchester, Manchester, U.K.; [2]The Manchester Centre for Cellular Metabolism (MCCM), Institute of Cancer Sciences, Cancer Research UK Manchester Institute, University of Manchester, Manchester, U.K.

The lecture will discuss the possibility of treating cancer like an infectious disease, by repurposing certain classes of FDA-approved antibiotics. Interestingly, mitochondria are originally derived from aerobic bacteria that were engulfed by eukaryotic cells and adapted over millions of years of evolution. This is known as the 'Endo-symbiotic Theory of Mitochondrial Evolution'. As a consequence, certain antibiotics target mitochondrial protein translation as a manageable side effect. We have recently proposed to harness this side effect and to re-purpose it as a therapeutic effect to target breast CSCs. In accordance with this strategy, we have already experimentally identified several different classes of mitochondrial-targeted antibiotics that could be used halt the proliferation of CSCs. These antibiotics included azithromycin, doxycycline, and atovaquone, as well as several others. Thus, targeting mitochondrial biogenesis, with FDA-approved antibiotics, may be a new effective strategy for inhibiting mitochondrial function in CSCs.

[1] Martinez-Outschoorn UE, Sotgia F, Lisanti MP. (2015) Caveolae and signalling in cancer. Nat Rev Cancer 15(4): 225-37.
[2] Killock D. (2015) Drug therapy: Can the mitochondrial adverse effects of antibiotics be exploited to target cancer metabolism? Nat Rev Clin Oncol 12(4): 190.
[3] Martinez-Outschoorn UE, Peiris-Pagès M, Pestell RG, Sotgia F, Lisanti MP. (2016) Cancer metabolism: a therapeutic perspective. Nat Rev Clin Oncol *In Press*.
[4] Fiorillo et al. (2015) Repurposing Atovaquone: Targeting mitochondrial complex III and OXPHOS to eradicate cancer stem cells. Oncotarget 7(23): 34084-99.

E1-2
The expanded chemical space of DNA: moonlighting roles of unorthodox bases

Beáta G. Vértessy[1,2]

[1]Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Budapest; [2]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

Maintenance of genome integrity and fidelity of DNA replication are of key importance for living organisms. DNA damage recognition and repair pathways and the regulation of nucleotide pools act hand in hand to prevent DNA mistakes and preserve the correct genomic information. Due to the vital significance of these pathways, they also offer numerous targets to fight harmful cells, either pathogenic microbes or tumor cells. Inhibition of key proteins within pathways responsible for genome integrity is a frequently used chemotherapeutic strategy against infectious diseases and many forms of cancer [1, 2]. Recently, it has been also observed that the chemical composition of DNA is much more complex than the traditional four-base alphabet, and several unusual bases are not just simple mistakes that need to be repaired but also present epigenetic signals. A clear and objective description of the true chemical composition of genomic DNA is hindered by lack of sequencing methods that are directly deciphering the actual bases, not just simplify these into the A, T, G, C context. Our research is focused on the physiological roles and metabolism of uracil-DNA that is also a causative factor in thymine-less cell death, an oft-used anticancer clinical strategy. To identify the molecular mechanism of thymine-less cell death and to analyze the intriguing roles of uracil-DNA, we also develop novel tools to pinpoint uracil residues in DNA in tumor cells of varied genetic background [3, 4]. These results allow an insight into communication between BER and MMR pathways of DNA repair. Funding: OTKA NKFIH K109486 and ICGEB CRP/HUN14-01.

[1] Nyíri K, Vértessy BG (2016) BBA - General Subjects *in press*.
[2] Wilson PM et al (2014) Nat Rev Clin Oncol 11: 282-298.
[3] Horváth A, Vértessy BG (2010) Nucleic Acids Res 38(21):e196.
[4] Róna G et al, Vértessy BG (2016) Nucleic Acids Res 44(3):e28.

E1-3
Structural characterization of the native RSK1 signalosome and its misregulation in malignant melanomas

Gergo Gogl[12], Anita Alexa[1], Peter Sok[1], Laszlo Nyitray[2], Attila Remenyi[1]

[1] MTA Lendület Fehérje Kölcsönhatás Kutatócsoport, Hungarian Academy of Sciences, Institute of Enzymology, Budapest; [2] ELTE, Department of Biochemistry, Budapest

Mitogen-activated protein kinases (MAPK) exert their regulatory role to a great extent by turning on downstream protein kinases. These MAPK activated protein kinases (MAPKAPK) all contain a calcium/calmodulin dependent protein kinase domain and a C-terminal MAPK binding linear motif. Ribosomal S6 kinase 1 (RSK1) is one of the downstream effector kinase of the extracellular signal regulated kinase (ERK) pathway controlling cell growth. Here we present the structural characterization of the ERK2-RSK1 signaling complex. Combination of crystallography, molecular dynamics and small angle X-ray scattering studies shed light on different structural states of the inactive and active complex. The C-terminal tail of RSK1 is also targeted by S100B, a calmodulin like calcium binding protein, which is highly expressed in malignant melanoma. We have shown that S100B directly competes with ERK2 and therefore acts as an RSK1 inhibitor inside melanoma cells. S100B binding induces structural reorganization of RSK1 and the two proteins form a fuzzy complex. The newly discovered crosstalk between MAPK and Ca-signaling pathways - mediated through RSK1 - highlights the central role of MAPK-MAPKAPK heterodimeric complexes as signaling integrators, which respond to upstream signals as complex information processing hubs.

E1-4
Remembrance of things past

Mária Wollemann
Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

I was born in 1923 in Budapest. I started my medical university studies in Szeged in 1941. At that time, there was a more liberal and progressive faculty in Szeged than in Budapest having Albert Szent-Györgyi as a rector and lecturer and also Miklós Jancsó, György Ivanovich, Brunó Straub and István Rusznyák. I finished my medical studies in Budapest in 1947 and entered the Medical Chemical Institute in Szeged in the same year and continued in Budapest headed by Straub till 1948. I entered the newly established Biochemical Institute of the Hungarian Academy of Sciences in 1949 directed by Imre Szörényi. I moved to the National Scientific Institute of Neurosurgery in 1954 where I was working for 15 years investigating enzyme activities of freshly operated brain tumors. Meanwhile, I visited foreign laboratories for half years in Berlin and Paris and a year in New York. My book on „Biochemistry of Brain Tumours" was edited not only by Akadémiai Kiadó in Budapest, but also by MacMillan Press Ltd. London, Park Press Baltimore, Mir Press Moscow and even translated in Japan. It was also accepted as a thesis of Doctor of Medicine. I entered the Institute of Biochemistry of the Biological Research Center in Szeged in 1971 as a head of the membrane group doing β-adrenergic receptor research in heart. I was the deputy director in 1978 and the director of the institute in 1979-1983. I officially retired as a scientific advisor in 1985, but ever since I have continued working on my favorite subject, the opioid receptors. My group pioneered the purification to homogeneity one subtype of the kappa opioid receptor from frog brain and produced a monoclonal antibody against it which showed the presence of the kappa subtype in the neurons and also in the glial cells. I published 135 scientific papers, among them 65 after my retirement and received 1302 citations to date. As I previously professed „The aim of my life is to study the Nature" (Acta Biologica Hung.54 (2) 239-144, 2OO3).

E2 - Translational medicine

E2-1
Polypharmacology approach for a fibrotic disorder: Improved anti-fibrotic efficacy with dual-target inhibitors of peripheral cannabinoid CB1 receptor (CB_1R) and inducible nitric oxide (iNOS) in liver fibrosis

Resat Cinar, Malliga Iyer, George Kunos
Laboratory of Physiological Studies, National Institute on Alcohol Abuse and Alcoholism, National Institute of Health, Rockville, MD, U.S.A.

Liver fibrosis is a multi-factorial disease which lacks effective treatment. For multifactorial diseases, the conventional pharmacological approach based on the 'one disease/one target/one drug' paradigm may limit therapeutic efficacy and could be improved by simultaneously hitting multiple therapeutic targets. When applied to liver fibrosis, such an approach could aim at both preventing its progression and reversing the process, leading to improved liver function. This could best be achieved by engaging targets involved both in the fibrotic process itself and in the underlying pathologic conditions that predispose to liver fibrosis. One such target is the endocannabinoid/CB_1R system. Overactivity of the CB_1R induces profibrotic gene expression and also promotes pathologies that predispose to liver fibrosis, such as obesity/diabetes, alcoholic liver disease and viral hepatitis. Another potential therapeutic target involved in both fibrosis and its underlying

pathologies is iNOS. In order to improve the efficacy and safety of antifibrotic medications, we have designed an orally bioavailable, dual-target compound, which acts as a potent and peripherally selective CB_1R antagonist, and also accumulates in liver where it is metabolized to release an iNOS-inhibitory leaving group, resulting in a significant reduction in hepatic iNOS activity. In mouse models of fibrosis induced by CCl_4 or bile-duct ligation, the hybrid CB_1R/iNOS antagonist surpassed the antifibrotic efficacy of the CB_1R antagonist rimonabant at doses causing equal CB_1R blockade and, unlike rimonabant, did not induce anxiety-like behaviors due to its lack of CB_1R occupancy in the CNS, as documented using CB_1R PET. The hybrid inhibitor also targeted uniquely iNOS-mediated profibrotic pathways. Thus, dual-target peripheral CB_1R/iNOS antagonists are both safe, efficacious and have therapeutic potential in liver fibrosis. The study is supported by intramural funds of the NIAAA/NIH.

E2-2

DNA hypermethylation pushes immune escape through downregulation of antigen presentation genes in breast cancer

Györffy Balázs
Lendület Onkológiai Biomarker Group, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

HLA class I downregulation/loss is a major mechanism of immune escape. Tumors with low MHC1 expression have higher risk of relapse in HER2+ and triple-negative breast cancer (BC). We aimed to investigate the contribution of DNA methylation defects to HLAs and B2M downregulation/loss among BC subtypes. Gene-expression and methylation data were retrieved for 970 breast cancer sample from TCGA. We defined HLA (classical A, B, C and non-classical G) and B2M expression by mRNA expression. For each of these genes, the methylation of 19,956 genes were compared between high and low tertile groups in each breast cancer subtype (HER2+, n=290; ER-HER2- (TN), n=201; ER+HER2-; n=479). To reduce false discovery rate, only results with p<1E-05 were considered significant. The HLA-C gene was hypermethylated in the low tertile group (p=3.7E-05 and p=1.5E-05 and p=5.0E-12 in HER2+, TN and ER+HER2- groups respectively). B2M, HLA-A and HLA-B were also significantly hypermethylated in the low compared to high tertile group in each BC subtype. HLA-G expression was not significantly correlated to methylation. Overall, except for HLA-G, a plethora of genes showed differential methylation between low and high tertile groups. In particular, several genes involved in antigen presentation and immune function were co-hypermethylated. The 25 top-ranked genes consistently hypermethylated in each BC subtype in the low HLA-A group included HLA-A, HLA-B, HLA-E, HLA-F, B2M, TAP1, PSMB8 (antigen presentation) and IFIT3, IRF1, IFNAR1 (interferon-signaling). These findings suggest that epigenetic defects (hypermethylation) contributes significantly to HLAs and B2M loss/downregulation in all breast cancer subtypes. Several genes involved in the antigen presentation function were co-hypermethylated, partially due to co-localization on chromosome 6p21.3, suggesting a coordinate contribution to immune escape. Preclinical studies show that HLA expression can be recovered by treatment with DNA methylation inhibitors. Our data provide a rationale for testing DNA-demethylating agents in combination with immune checkpoint inhibitors in breast cancer.

E2-3

Next generation sequencing in the diagnosis of rare genetic diseases

Mária Judit Molnár
Institute of Genomic Medicine and Rare Disorders, Semmelweis University, Budapest

Not submitted.

E2-4
Extranuclear effects of PARP activation: Relevance in cardiovascular diseases
Eniko Hocsak, Nikoletta Kalman, Boglarka Racz, Balazs Sumegi, Ferenc Gallyas Jr.
Department of Biochemistry and Medical Chemistry, University of Pecs, Medical School, Pécs

Oxidative stress induces DNA breaks and poly(ADP-ribose) (PAR) polymerase (PARP)-1 activation, which facilitates mitochondrial damage, malevolent alteration of signaling pathways, reactive oxygen species (ROS) production, and cell death. Here, we show the mechanism by which PARP-1 influences these processes via PARylation of activating transcription factor (ATF)4 responsible for mitogen activated protein kinase (MAPK) phosphatase (MKP)-1 expression, thereby MAPK down-regulation. PARP inhibitor or silencing of PARP induced MKP-1 expression by an activating transcription factor (ATF)4-dependent way, and inactivated c-Jun N-terminal kinase (JNK) and p38 MAP kinases. Additionally, it induced ATF4 expression and binding to cAMP-response element (CRE) leading to the activation of ATF4-MKP-1-MAPK pathway. In contrast, PARP-1 activation induced PARylation and diminished binding to CRE sequence of ATF4. In oxidative stress, PARP inhibition reduced ROS induced cell death, suppressed mitochondrial ROS production and protected mitochondrial membrane potential on an AFT4 and MPK-1 dependent way. Basically identical results were obtained in WRL-68, A-549 and T24/83 human cell lines indicating that the aforementioned mechanism can be universal. We provide the first description of PARP-1-ATF4-MKP-1-JNK/p38 MAPK retrograde pathway, which is responsible for the regulation of mitochondrial integrity, ROS production and cell death in oxidative stress, and represents a new mechanism of PARP in cancer therapy by modulating JNK-dependent cancer stem cells development. The PARP-1-ATF4-MKP-1-JNK/p38 MAPK pathway represents a novel mechanism by which PARP inhibitors protect cells in oxidative stress related diseases.

E2-5
Restoration of altered redox balance in rats by short term metformin treatment: Relevance to aging
Syed Ibrahim Rizvi, Geetika Garg, Sandeep Singh, Abhishek Kumar Singh
Department of Biochemistry, University of Allahabad, Allahabad 211002, India

The biguanide drug, metformin is a drug of choice for the management of type 2 diabetes. Recent studies show that metformin also acts as a calorie restriction mimetic, although the molecular mechanisms have not yet been unequivocally established. The present study aims to examine whether the short term metformin treatment can provide protective effects against oxidative stress in young and old age rats. Young (age 4 months) and old (age 24 months) male Wistar rats were treated with metformin (300 mg/kg b.w.) for 4 weeks. At the end of the treatment period, an array of biomarkers of oxidative stress were evaluated including plasma antioxidant capacity measured in terms of ferric reducing antioxidant potential (FRAP), Reactive Oxygen species (ROS), lipid peroxidation (MDA), reduced glutathione (GSH), total plasma thiol (SH), plasma membrane redox system (PMRS), protein carbonyl (PCO), advanced oxidation protein products (AOPP) and advanced glycation end products (AGEs) in control and experimental groups. Metformin treatment resulted in increase in FRAP, GSH, SH and PMRS activities in both age groups compared to respective controls. On the other hand treated groups exhibited significant reductions in ROS, MDA, PCO, AOPP and AGEs levels. Except for FRAP and protein carbonyl, the effect of metformin on all other parameters was more pronounced in old aged rats. Metformin caused a significant increase in PMRS activity in young rats, however the effect was less pronounced in old rats. The result of this study emphasizes that short term metformin treatment can restore antioxidant status in old age rats. The findings add to the growing evidence for the putative anti-aging role of metformin.

E3 - Molecular basis of disease and therapy, stem cells, immunity and inflammation

E3-1
HOFI/SH3PXD2B, a regulator of tumor development
Árpád Lányi
Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen

HOFI (homologue of FISH, SH3PXD2B) is an adapter protein with a lipid-binding N-terminal phagocyte oxidase domain followed by four tandem SH3-domains. Lack of HOFI in mice causes abnormal bone development, cardiovascular disease, spontaneous glaucoma, deafness and an almost complete lack of visceral adipose tissue. We and others have recently shown that HOFI is required for the development of epidermal growth factorinduced membrane ruffles and lamellipodia via complexes that contain epidermal growth factor-receptor, c-Src and cortactin, all promoting cellular movement and implicated in tumor progression. Based on the complex phenotype of the HOFI-knock out mice and HOFI's involvement in the regulation of cell motility, we hypothesized that HOFI may be a regulator of tumor progression. To test this, we set up several in vivo tumor models addressing the contribution of HOFI to the develop-

ment of a functional tumor-associated stroma. In this talk, I present data strongly suggesting the pro-tumorigenic effect of HOFI by a complex mechanism that includes regulation of endothel cell functions, cell proliferation and the immune compartment of tumor-associated stroma. This work was supported by grants from the Hungarian Research Fund OTKA K 109444 (Hungarian Academy of Science), and by the Romanian Ministry of Education, Executive Agency For Higher Education, Research, Development and Innovation Funding, PNCDI II, project no. 119/2014. PN-II-PT-PCCA-2013-4 1583.

E3-2

The emerging role of Ring1 and Yy1 binding proteins in stem cell differentiation

Gergo Kovacs, Eniko Sutus, Surya Henry, Viktoria Szabo, Melinda K Pirity

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

Polycomb Repressive Complexes (PRCs) are transcriptional repressors that control stem cell identity, differentiation and tumorigenesis. Their activity is divided between two multi-protein complexes called PRC1 and PRC2. The notion of the polycomb recruitment is that there is a strict hierarchical order, where PRC2 recruits PRC1 to initiate chromatin compaction and gene repression. From past years this hypothesis was turned upside-down by showing that the recruitment of PRC1 can occur also independently of PRC2. Recent findings also described the presence of alternate, canonical and non-canonical PRCs, which opened up a number of interesting new questions in this field. Polycomb-group Ring1 and Yy1 Binding Protein (Rybp) are newly discovered components of the recently identified non-canonical PRC1 complex (nc-PRC1) and the role of Rybp in this context is unknown. Work from our laboratory has demonstrated that Rybp itself is essential for mouse embryonic development since *rybp homozygous null (KO)* mouse embryos die at the time of implantation. Furthermore*, rybp KO* embryonic stem (ES) cells have limited developmental potential; they can not form beating cardiomyocytes (CMCs) and mature neuronal cell types *in vitro*. Our findings demonstrate that Rybp is essential for embryonic development, the proper execution of differentiation and its function can not be compensated by other PRC1-related complexes. Current results and possible mechanisms of Rybp action in PcG recruitment, lineage commitment during embryonic development and disease conditions will be addressed. This work was supported by TÁMOP-4.1.1.C-13/1/KONV-2014-0001 and TÁMOP-4.2.6.A-15/1-2015-0002.

E3-3

E3-deficiency mediated by the pathogenic mutations of the human dihydrolipoamide dehydrogenase (hE3): elucidation of the molecular pathomechanism by a multifaceted structural approach

Attila Ambrus[1], Eszter Szabo[1], Reka Mizsei[1], Junjie Wang[2], Zsofia Zambo[1], Agnes Hubert[1], Balint Nagy[1], Beata Torocsik[1], Manfred S. Weiss[3], Frank Jordan[2], Vera Adam-Vizi[1]

[1]MTA-SE Laboratory for Neurobiochemistry, Department of Medical Biochemistry, Semmelweis University, Budapest; [2]Department of Chemistry, Rutgers University, Newark, NJ, U.S.A.; [3]Macromolecular Crystallography, Helmholtz-Zentrum Berlin, Berlin, Germany

The α-ketoglutarate dehydrogenase complex (KGDHc) represents a rate-limiting step in the Krebs cycle catalyzing the oxidative decarboxylation of α-ketoglutarate while generating succinyl-CoA and NADH. The KGDHc is one of the major generators of oxidative stress in the mitochondrion under pathological conditions; malfunctioning of and reactive oxygen species (ROS) generation by KGDHc are implicated in the progression of senescence/aging, neurodegenerative diseases, ischemia-reperfusion, hypoxia- and glutamate-induced cerebral damage, E3-deficiency, among others. ROS generation by KGDHc is attributed to the homodimeric flavoenzyme E3 component, which is also part of the pyruvate dehydrogenase complex (PDHc) and selected other dehydrogenase complexes. Pathogenic mutations of hE3 (h for human) lead to an inherited, often lethal disease known as E3-deficiency; the clinical course of E3-deficiency is greatly diversified and often involves cardiological and/or neurological symptoms. Selected pathogenic mutations of hE3 stimulate the ROS generation by hE3 and hKGDHc or impair the recruitment of hE3 to the harboring complexes, which are likely to be important factors in the respective molecular pathogenesis. Structural alterations of the 14 pathogenic variants of hE3 and their role in the respective molecular pathogenesis have been investigated in our laboratory by a multifaceted structural approach, which involves CD spectroscopy, MD simulation, H/D-exchange mass spectrometry, electron microscopy and x-ray crystallography. The biophysical approaches are accompanied by biochemical assays for assessing *i.,* FAD-content, *ii.,* superoxide- or H_2O_2-generating capacities and enzymatic activities in the forward or the reverse catalytic directions as a function of pH, *in vivo* affectors or reconstructing with other recombinant hPDHc or hKGDHc components (E1, E2), and *iii.,* monomerization *via* calibrated size-exclusion chromatography coupled to nano-LC MS. Funding*:* OTKA, NAP, MTA.

E3-4
Enhanced dendritic cell differentiation from pluripotent stem cells by ectopic expression of Runx3

Pal Boto[1], Erika Takacs[1], Istvan Szatmari[1]

[1]*Stem Cell Differentiation Laboratory, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen*

Dendritic cells (DCs) are professional antigen-presenting cells (APCs) of the immune system. Their major functions related to the immune responses are clearly proven. They are required for the capturing, processing and presentation of antigens on the cell surface, thus together with co-stimulatory molecules can regulate the immune responses. Furthermore, one additional key role of DCs is the maintenance of B cell functions and recall responses. Thus, DCs are critical in the establishment of immunological memory. Clinical applications of DCs related to prime responses to tumor antigens could act as a key tool of cancer immunotherapies. Well known issue of the current protocols is that only a limited number of DCs can be obtained from adult precursors. In contrast, embryonic stem (ES) cells could serve as unlimited source of DC generation. The major challenge is to achieve the properly governed differentiation because immaturation and impaired functional characteristics are common traits of these ES derived cells. Consistent with this, our results indicated that ES derived DCs showed less mature cells compared to the bone-marrow (BM) derived DCs. This finding led us to examine the gene expression profile of ES and BM derived DCs. Quantification of 17 DC specific transcription factors revealed that three of these, namely Runx-3, Spi-B and Irf4 showed lower expression in ES derived DCs. In the light of our results we tested the effects of these three transcription factors in developing mouse ES-DCs with an isogenic expression screen. Our results revealed that forced expression of Irf4 in ES-DC negatively modulates, but Spi-B and Runx3 are both enhancers of the early myeloid commitment. Moreover, overexpression of Runx3 improved the maturation as well as the T cell activation capacity of ES derived DCs.

E4 - Regulation of gene expression, regulatory RNA, epigenetics

E4-1
Transcriptional outcomes (fates) in response to DNA damage

Zsuzsanna Ujfaludi[1], Barbara Borsos[1], Celine Bonhomme[2], David Chen[3], Evi Soutoglou[2], Tibor Pankotai[1]

[1]*Department of Biochemistry and Molecular Biology, University of Szeged, Szeged;* [2] *Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS, UMR 7104, Université de Strasbourg, INSERM U964, Illkirch, France ;* [3] *Division of Molecular Radiation Biology, Department of Radiation Oncology, University of Texas Southwestern Medical Center, Dallas, Texas, U.S.A.*

Various types of DNA damage interfere with key vital processes which use DNA as a template, like replication and transcription. Upon large amount of genotoxic impacts, transcription is over-activated and probably results in the activation of several DNA damage recognition processes. During transcription, numerous components of the transcription machinery may act as a platform to recruit repair proteins at break sites. In contrast to that, when DNA damage occurs at a transcribing unit, it leads to transcriptional block. This multistep process involves several kinases and the ubiquitin ligases like NEDD4 and CUL3 leading to proteasome dependent degradation of RNA polymerase II (RNAPII) which happens at the site of the damage. Finally, at the break site ddRNA (a new class of noncoding RNA) production could be observed by controlling the DDR activation at sites of DNA damage. Taken together these results support an uncharacterized function of RNAPII complexes which allow the recognition of DNA damages and like this enhance cell survival following DNA damage. This work was supported by OTKA-PD [112118], and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

E4-2
SUMOylation regulates light-induced signaling in Arabidopsis thaliana

András Viczián[1] Ari Sadanandom[2], Péter Bernula[1], Éva Ádám[1], Beatriz Orosa[2], László Kozma-Bognár[1], Ferenc Nagy[1,3]

[1]*Plant Biology Institute, Biological Research Centre, Szeged;* [2]*School of Biological and Biomedical Sciences, University of Durham, Durham;* [3]*Institute of Molecular Plant Science, School of Biology, University Edinburgh, Edinburgh*

The red/far-red light absorbing photoreceptor phytochrome-B (phyB) cycles between the biologically inactive (Pr, λ_{max}=660nm) and active (Pfr, λ_{max}=730 nm) forms and functions as a light quality and quantity controlled switch to regulate photomorphogenesis in Arabidopsis. At the molecular level, phyB interacts in a conformation-dependent fashion with a battery of downstream regulatory proteins, including PHYTOCHROME INTERACTING FACTOR (PIF) transcription factors, and by modulating their activity/abundance it alters expression patterns of genes underlying photomorphogenesis. We found

that phyB protein is posttranslationally modified by covalently attached SUMO (Small Ubiquitin-like Modifier) molecules (SUMOylated); the accumulation of SUMOylated phyB is enhanced by red light and displays a diurnal pattern in plants grown under light/dark cycles. Our data demonstrate that (i) transgenic plants expressing the non-SUMOylated mutant phyB^{Lys996Arg}-YFP photoreceptor are hypersensitive to red light, (ii) light-induced SUMOylation of the mutant phyB is drastically decreased as compared to phyB-YFP and (iii) SUMOylation of phyB inhibits binding of PIF5 to phyB Pfr. In addition, we found that PIF3 which plays a key role in the initiation of photomorphogenesis is also SUMOylated. The non-SUMOylated mutant PIF3-YFP triggers hyposensitive growth responses indicating that this molecule has higher biological activity than its wild type counterpart. Taken together, we conclude that SUMOylation of phyB negatively, whereas SUMOylation of PIF3 positively regulates light signaling. Interestingly, these conclusions mean that the SUMOylation of these molecules decreases their activity, resulting in opposite biological effects. Our observations reveal how a certain posttranslational modification can fine tune the activity of different components in the same signal transduction pathway. Work was supported by BBSRC (BB/K006975/1) and OTKA (K-108559, NN 110636) grants and by the Bolyai János Scholarship of the Hungarian Academy of Sciences.

E4-3

Intrinsic protein disorder in histone lysine methylation

Tamás Lázár[1,2], Éva Schád[1], Beáta Szabó[1], Tamás Horváth[1], Attila Mészáros[1], Péter Tompa[1,3,4], Ágnes Tantos[1]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2]Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest; [3]VIB Structural Biology Research Center Brussels; [4]Vrije Universiteit Brussel, Brussels

Histone lysine methyltransferases (HKMTs) catalyze mono-, di- and trimethylation of lysine residues, resulting in a regulatory pattern that controls gene expression. Their involvement in many different cellular processes and diseases makes HKMTs an intensively studied protein group, but scientific interest so far has been concentrated mostly on their catalytic domains. We set out to analyze the structural heterogeneity of human HKMTs and found that many contain long intrinsically disordered regions (IDRs) that are conserved through vertebrate species. Our predictions show that these IDRs contain several linear motifs and conserved putative binding sites that harbor cancer-related SNPs. Although there are only limited data available in the literature, some of the predicted binding regions overlap with interacting segments identified experimentally. The importance of a disordered binding site is illustrated through the example of the ternary complex between MLL1, menin and LEDGF/p75. Our suggestion is that intrinsic protein disorder plays an as yet unrecognized role in epigenetic regulation, which needs to be further elucidated through structural and functional studies aimed specifically at the disordered regions of HKMTs. Funding: Odysseus grant G.0029.12 from Research Foundation Flanders, Korean-Hungarian Joint Laboratory grant from Korea Research Council of Fundamental Science and Technology, OTKA grant PD-OTKA 108772, the Bolyai János Research Scholarship of the Hungarian Academy of Sciences and MedInProt Protein Science Research Synergy Program.

E4-4

Impact of insulin-like growth factor II (IGF II) gene inhibition with IGF II antisense oligomer during the development of rat hepatocarcinogenesis

Miltu Kumar Ghosh[1], Biswajit Mukherjee[2]

*[1]Department of Pharmacy, NSHM Knowledge Campous, India ; [2]*Department of Pharmaceutical Technology, Jadavpur University, India

Background: Hepatocellular carcinoma (HCC) is a multistep complex process, caused by many of genetic alteration. Insulin-like growth factors and their receptor have been widely implicated to HCC. Insulin-like growth factor-II (IGF-II) is a mitogenic polypeptide, found in various fetal and neonatal tissues of humans and rats and expresses in HCC. Here we investigated anticancer potential of phosphorothioate antisense oligonucleotides (ASOs) against three coding exons (exon-1/exon-2/ exon-3) of IGF-II messenger ribonucleic acid in rat hepatocarcinogenesis model. **Methods:** During diethylnitrosamine and 2-acetylaminofluorene induced hepatocarcinogenesis, rats were treated with ASOs. Various biochemical and histological studies were conducted. **Results:** About 40% of carcinogen treated rats, which received two oligomers (against exon-1 or-3) did not show any hepatic lesion, hyperplastic nodule or tumor and remaining 60% of those rats showed lesion incidence and had about 59% and 55% reductions in the numbers of hepatic altered foci, respectively. Reductions in the total lesion-area when compared with carcinogen control rats were 64% and 53%, respectively for the animals treated with carcinogen and received the ASOs against exon-1/-3. Fluorescein isothiocyanatela–beled ASO reached in the hepatocytes in 2 h. No predominant IGF-II overexpression was observed in case of rats treated with the two ASOs. Treatment of the antisense IGF-II oligomers in carcinogen treated rats show better hepatocellular integrity along with several preneoplastic/neoplastic marker

isoenzyme/enzyme modulations. **Conclusions:** Two of the three antisense oligomer-types effectively controlled IGF-II overexpression, causing the delay of the development and/or progress of hepatic cancer in rats. **Funding:** The work has been carried out with the fund granted to Dr. Biswajit Mukherjee as a major research project from Indian Council of Medical Research, Govt. of India (grant no. 58/7/2009-BMS).

E4-5

In search for natural small molecule modulators of breast cancer

Edit Mikó[1,2*], András Vida[1,2*], Tamás Fodor[1], Tünde Kovács[1], Gyula Újlaki[1], Patrik Kovács[1], Bence Lovas[1],Zoltán Hujber[4], György Trencsényi[5], Anna Sebestyén[4], Péter Bai[1,2,3]

[1]Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen; [2]MTA-DE Lendület Laboratory of Cellular Metabolism, University of Debrecen, Debrecen; [3]Research Center for Molecular Medicine, University of Debrecen, Debrecen; [4]1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest; [5]Department of Medical Imaging, Division of Nuclear Medicine and Translational Imaging, University of Debrecen, Debrecen

* equal contribution

Warburg metabolism is a characteristic change in the metabolism of cancer cells that was first described by Otto Warburg in the 1920's. Originally, Warburg suggested that tumor cells suppress mitochondrial oxidation even in the presence of oxygen. In fact, Warburg metabolism is way more complex than the original observation; it is now viewed as a complex rearrangement of metabolism to support highly proliferating cells (e.g. tumor cells) by providing key metabolites to support cell division. It should be noted that the actual metabolic changes are tissue/tumor specific, similarly to the molecular mechanism that brings about these changes. Reverting or enhancing Warburg metabolism in susceptible tumors impacts on tumorigenesis, chemosensitivity and the overall pathology of the tumors. Our aim is to identify small molecule modulators of Warburg metabolism and breast cancer pathology. We have established a phenotyping pipeline for the identification of such (metabotropic) small molecule modulators. The basis of selection is a capability of the chemicals to slow down the proliferation of multiple established breast cancer cell lines (SRB assay, videomicroscopy). Metabolic changes were characterized by oximetry (Seahorse), RT-qPCR and metabolite analysis. We realized that such metabotropic molecules exert non-metabolic effects too and can impact on other hallmarks of cancer as well. By extending our experimentation we observed changes in oxidative stress and the ENT transition as well. The molecules that performed well in the in vitro tests were further characterized in in vivo tumor grafting models. The treatment of tumor-bearing mice with the small molecule metabolites changed the behavior of the tumor grafts. These tumors were less invasive, showed less characteristic Warburg and ENT features. Funding: NKFIH (OTKA, K108308, K105872, C120732, C129074), TÁMOP-4.2.2. A-11/1/KONV-2012-0025, the Momentum fellowship of the Hungarian Academy of Sciences and the University of Debrecen.

C1 - Technical presentation I.

C1-1

Together or alone – Application of sgRNA or crRNA-tracrRNA complex in CRISPR-Cas9 systems (The Alt-R CRISPR-Cas9 system of Integrated DNA Technologies)

Éva Kerekes

Bio-Science Ltd., Budapest

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is the most potent and precise genome editing tool of the latest years of the molecular biology and biotechnology. The application is derived from the prokaryotic genom, and in its native form it works as a powerful immune system against foreign DNA. CRISPR-Cas9 generates double-stranded breaks in DNA, which is repaired by non-homologous end-joining or homology-directed recombination, resulting in a modified sequence. While non-homologous end joining frequently causes small nucleotide insertions or deletions, homology directed repair could be used to generate specific nucleotide changes. Based on these repair mechanisms, CRISPR-Cas9 was originally used to „knock-out" specific target genes, but alterations in Cas9 enzyme have expanded the application of the system to selectively activate or inactivate target genes. Therefore, due to the simplicity, versatility and efficiency of the tool, it has become one of the most popular approaches for genome engineering of the recent years. CRISPR-Cas9 applies two RNAs – crRNA and tracrRNA (CRISPR and trans-activating RNA), and a Cas9 endonuclease. Many manufacturers have started to produce the CRISPR-Cas9 tool as a two-component system: combining crRNA and tracrRNA into a single guide RNA (sgRNA) or guide RNA (gRNA). However, new findings have shown that the optimization of the length of the crRNA and tracrRNA improves the targeting of Cas9 to dsDNA targets, and in many cases, the application of the crRNA-tracrRNA comlex elicits less toxicity and innate immune response, besides more potent than single guide RNAs.

C1-2
Monitoring protein interactions in living cells using the luminescent NanoLuc® Binary Technology
Zsolt Somlai
Bio-Science Ltd., Budapest

Protein:protein interactions are essential to the cellular signal transduction pathways. Although numerous approaches exist to monitor these in vitro, methods for intracellular, moreover for live-cell detection have been more limited. NanoLuc® Binary Technology (NanoBiT™) from Promega is a two-subunit system based on NanoLuc® luciferase that can be used for intracellular detection of protein:protein interactions. Large BiT (LgBiT; 18kDa) and Small Bit (SmBiT; 11 amino acids) subunits are fused to proteins of interest, and when expressed, the interaction brings the subunits into close proximity to form a functional enzyme that generates a very bright, luminescent signal. NanoBiT™ is extremely bright being >1000 fold brighter than split firefly luciferase at 37 °C. This ensures that fusion partners can be expressed at very low levels, minimizing potential artifacts. Unlike related approaches where an enzyme or protein is segmented into two fragments, LgBiT was independently optimized for structural stability and SmBiT was selected from a peptide library specifically for the interaction application. The result is a subunit pair that weakly associates yet retains a bright luminescent signal. In contrast to many protein complementation assays, the LgBiT:SmBiT interaction is reversible and can detect rapidly dissociating or associating proteins. Dynamics can be followed in real-time inside living cells for 1-2 hours using a nonlytic detection reagent. The NanoBit™ system can also be used for short-term end-point detection. NanoBit™ truly offers experimental flexibility and is validated in 96-, 384- and 1536-well formats. Advantages over segmented protein systems include greater sensitivity, fusion protein expression at or near physiological levels, reversibility, fusion to a peptide or a small, structurally stable protein domain, real-time measurement using a nonlytic assay format, and subunits with reduced affinity for spontaneous association.

E5 - Lipids and membranes in action

E5-1
New fluorescence-based functional assays for Organic Anion Transporting Polypeptides (OATPs), transporters involved in drug ADME-Tox
Izabel Patik, Virág Székely, Daniella Kovacsics, Orsolya Német, Szilárd Tóth, Tímea Windt, Éva Bakos, Csilla Laczka
Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

The *in vivo* fate and effectiveness of a drug highly depends on its absorption, distribution, metabolism, excretion and toxicity (ADME-Tox) properties. Plasma membrane proteins that mediate transmembrane movement of clinically applied molecules are key determinants of drug ADME. Organic Anion Transporting Polypeptides (OATPs) are membrane proteins facilitating the uptake of various organic compounds, including bile acids, bilirubin, steroid and thyroid hormones. Besides their physiological role in bile acid or hormone homeostasis, several OATPs (OATP1A2, 1B1, 1B3 and 2B1) transport clinical molecules (statins, antivirals and chemotherapeutics). Altered function of these OATPs due to drug-drug interactions or genetic mutations leads to altered efficacy of medications or even toxicity. Therefore, investigation of the interaction between a new molecular entity and several members of the OATP family is recommended by international regulatory agencies. Additionally, OATPs show altered expression in various cancers that may result in modified drug sensitivity of tumors. Hence these proteins are promising targets of anti-cancer therapy. In spite of their clinical relevance, our knowledge about the substrate specificity and transport mechanism of OATPs is incomplete. The exact contribution of OATPs to anti-cancer drug sensitivity is also not well understood. This is due to the lack of proper assays for functional tests of OATPs. Therefore, we have developed fluorescence-based *in vitro* functional assays for the investigation of the poorly characterized OATP members, as well as for OATPs involved in pharmacokinetics, OATP1A2, 1B1, 1B3 and 2B1. The newly developed methods open the avenue for large scale OATP drug interaction testing as well as for the clarification of the role of OATPs in chemotherapy response. The authors are grateful for funding by OTKA (K 109423) and MedInProt. Csilla Laczka is a recipient of the János Bolyai Scholarship of the Hungarian Academy of Sciences.

E5-2
Interplay between membrane and storage lipid metabolism during heat stress management in fission yeast
Mária Péter, Attila Glatz, Péter Gudmann, Imre Gombos, Zsolt Török, Ibolya Horváth, László Vígh, Gábor Balogh
Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

One of the most important environmental stressors is temperature fluctuation. The heat stress response (HSR) is initiated by membrane-localized temperature sensors that cause signal transduction events to alter transcriptional, translational and post-translational control processes. Tightly linked to the HSR,

the maintenance of correct membrane fluidity is of critical importance especially in poikilothermic organisms. Here we present the first in-depth lipidomic analysis of the fission yeast *Schizosaccharomyces pombe* in response to mild heat stress (HS; 40 °C, 1 h). We could identify several components of the homeoviscous membrane adaptation to be effectively working in the wild type cells. In response to HS, *S. pombe* is able to increase membrane rigidity acting against the heat-induced hyperfluidization via decreasing fatty acid (FA) unsaturation, reducing short chain species or elevating the ratio of bilayer forming versus non-bilayer forming structural lipids. Genetic manipulation of triglyceride (TG) content alone or in combination with HS gave rise to distinct lipidomic fingerprints in *S. pombe* cells. We demonstrate that, although not crucial at normal growth temperature, the potential for TG formation becomes important under stress conditions. Upon heat challenge, the complete absence of TG production in the dga1Δplh1Δ double knockout cells resulted in long-lasting growth arrest, significant differences in membrane lipid composition and enhanced signaling lipid generation. The calculation of lipid fluxes demonstrates very rapid membrane remodeling. The production of TG in response to HS is not merely a „passive way" for energy storage, but it actively contributes to the coping mechanism by accommodating unsaturated FAs from structural lipids. Expanding the versatile role of LDs, our data fit well to the suggested holdase chaperone function; the HS-induced LD accumulation itself may serve as a reservoir for unfolded proteins during stress.

E5-3

HER2, the epitome of membrane proteins targeted in cancer therapy

György Vereb, Gábor Tóth, Árpád Szöőr, Tamás Lajtos, László Simon, László Ujlaky-Nagy, Barabara Zsebik, János Szöllősi

Department of Biophysics and Cell Biology, and MTA-DE Cell Biology and Signaling Research Group, Debrecen

Since HER2, an EGFR family member, is differentially expressed in tumors, it is an ideal target of antitumor therapy. Trastuzumab, a humanized anti-HER2 antibody was the first of its kind to be used against solid tumors. In spite of considerable success, resistance also occurs, and a major cause is the massive extracellular matrix evolved by tumors. Considering that the antibody can mainly act by down-regulating HER2 or by recruiting ADCC, various biochemical and immunological approaches can be proposed for decreasing resistance. (1) Since HSP-90 serves to retain HER2 in an inactive conformation, the geldanamycin derivative 17-AAG can be used to enhance HER2 dimerization, consequential down-regulation, and thus to decrease proliferation of trastuzumab resistant breast cancer cells. (2) The myxobacterial antibiotic archazolid interferes with the recirculation of HER2, resulting in its retention in phagolysosomes and autophagosomes, decreases HER2 phosphorylation, and inhibits the growth of xenograft tumors. Yet, its bioavailability needs to be improved. (3) Trastuzumab can also be combined with other anti-HER2 antibodies, such as pertuzumab. This increases the resistance-free period in tumor-bearing mice. The background of this beneficial, additive effect is that the maximal approved clinical doses of either antibody alone do not saturate ADCC. Thus, it is recommended that both in the adjuvant and neoadjuvant setting the two antibodies are applied in combination. (4) Finally, a chimeric antigen receptor (CAR) containing trastuzumab scFv for MHC unrestricted target recognition, and important motifs of the T cell receptor and co-signaling molecules, when transduced into naive T cells, enables the destruction of HER2 positive tumors by the CAR T cells that perform active reconnaissance, even when the therapeutic antibody cannot anymore penetrate the extracellular matrix with passive diffusion. Supported by TÁMOP 4.2.2.A-11/1/KONV-2012-0025 and OTKA NK 101337.

E5-4

Membrane nanotube networks between B cells, conductors of humoral immune response: on the road to reveal the factors controlling their growth, retraction and function

Anikó Osteikoetxea-Molnár[1], Eszter A.Tóth[1], Edina Szabó-Meleg[2,4], Ádám Oszvald[1], Tamás Bozó[3], Miklós Kellermayer[3], Miklós Nyitrai[2,4], László Nyitray[7], Imre Derényi[5], Gábor Balogh[6], Mária Péter[6], László Vígh[6], János Matkó[1]

[1]Eötvös Loránd University, Faculty of Science, Department of Immunology, Budapest; [2]University of Pécs, Medical School, Department of Biophysics, Pécs; [3]Semmelweis Medical University of Budapest, Department of Biophysics & Radiobiology, Budapest; [4]MTA-PTE Nuclear-Mitochondrial Interactions Research Group, Pécs; [5]Department of Biological Physics, Eötvös Lorand University, Budapest; [6]Biological Research Centre, Hungarian Academy of Sciences, Szeged; [7]Eötvös Loránd University, Faculty of Science, Department of Biochemistry, Budapest

Membrane nanotubes are transient long-distance connections between cells that can facilitate intercellular communication. These tethers form in two ways: either as an actin-driven protrusion growing towards a nearby cell, or cells draw out NTs as they subsequently move apart after a previous contact (e.g. synapsis or following cell division). Traffic of bacteria, vesicles, calcium ions, viral proteins, prions, cytoplasmic molecules, organelles and mem-

brane anchored proteins have all been observed along NTs either inside or along their surface membrane [1, 2]. Much less is known, however, about how the external factors [3] or the membrane lipid constituents control the TNT growth. We pointed out here essential factors that govern growth and retrieval of nanotubes: B cells can form tunneling nanotubes (TNT) only if their cell surface integrins specifically interact with a selective component of the extracellular matrix (e.g. fibronectin or laminin) followed by cell spreading. Cytoplasmic free [Ca^{2+}]/actin polymerization-depolymerization equilibrium/ myosin 2A activity axis was also shown as a critical drive in control of TNT formation/ retraction by B cells. Dynamic changes in membrane lipid composition (especially in conical shaped and raft lipids) can also govern TNT formation. The nanotubular connections between B cells were sensitive to environmental mechanical stresses, as well as to changes in the membrane fluidity and molecular order of the lipid bilayer. An extensive transport of immunregulatory membrane molecules (MHC-peptide complexes or B7 family costimulatory proteins) was shown between the connected B cells, influencing the efficiency of antigen presentation and T cell activation. In addition, transport of mitochondria via TNTs may control the death/survival equilibrium of B cells. Further studies demonstrating *in vivo* significance of TNTs are undergoing on *ex vivo* mouse lymph nodes, using 2 photon microscopy. Support: National Research & Innovation Development Agency (NKFIH)/OTKA K104971 project.

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E6 - Bioinformatics, synthetic biology, genome engineering, biotechnology

E6-1

Protein complex prediction through proteome-wide simulations

Attila Csikász-Nagy[1,2], Simone Rizzetto[3], Corrado Priami[3]

[1]Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest; [2]Randall Division of Cell and Molecular Biophysics, New Hunt's House, King's College London, London, U.K.; [3]The Microsoft Research-University of Trento Centre for Computational Systems Biology, Rovereto, Italy

Multiple copies of each protein are present in cells and some of these could be involved in multiple complexes, thus it is a challenging task to identify protein complex compositions and abundances of all possible complexes. We introduce an integrative simulation based computational approach that enables us to predict protein complexes together with their abundances from existing data sources on protein-protein and domain-domain interactions and protein abundances. The simulations show consistent protein complex compositions with manually curated data and can also predict the abundances of various alternative forms of the complexes [1]. The updated version of the tool incorporates data on protein localization and tissue-specific protein abundances to improve and enable a wider range of predictions. The tool will be shortly available for the community to test various perturbations on the complexome. As an example, we show how perturbations by drugs can influence the composition and abundance of protein complexes. We acknowledge funding through the Pázmány University grant KAP16-71009-1.2-ITK.

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E6-2

SignaLink 3, a curated and integrated signaling resource for tissue-specific network modeling and detailed analysis of signaling pathways

Dávid Fazekas[1,*], Dénes Türei[1,2,3,*], Kitti Csályi[1,4,*], Dezső Módos[1,5], Tamás Kadlecsik[1], János Kubisch[1], Dániel Veres[2], Réka E. Tóth[1],Zoltán Dúl[1,6], Benedek Thäler[2,7], Dávid Gyurkó[2], Máté Pálfy[1,8], Bernadette Hotzi[1], Viktor A. Billes[1], Mihály Koltai[9,10], Máté Szalay-Bekő[1, 2], Illés J. Farkas[9], Katalin Lenti[1,5], Máté Varga[1], Tibor Vellai[1], Péter Csermely[2], Tamás Korcsmáros[1,11,12]

[1]Department of Genetics, Eötvös Loránd University, Budapest; [2]Department of Medical Chemistry, Semmelweis University, Budapest; [3]EMBL-EBI, UK; [4]Max-Delbrück-Centrum Für Molekulare Medizin Berlin-Buch, Germany; [5]Department of Morphology and Physiology, Semmelweis University, Budapest; [6]King's College London, U.K.; [7]Faculty of Electrical Engineering and Informatics, Budapest University of

*Technology and Economics, Budapest; [8]Max Planck Institute of Molecular Cell Biology and Genetics, Germany; [9]Statistical and Biological Physics Group of the Hungarian Academy of Sciences, Hungary; [10]Center for Molecular Biology, Universitat Heidelberg, Germany; [11]TGAC, The Genome Analysis Centre, U.K.; [12]Gut Health and Food Safety Programme, Institute of Food Research, UK; *equal contributions*

Eukaryotic signaling networks are built of dynamically intertwined signaling pathways regulated by context-specific enzymes, transcription factors and microRNAs. Interestingly, most of the databases contain only a few layers of the signaling network and only a few provide important details on the interactions, such as effect, enzymatic type or tissue localization. These details would be highly important for advanced and context specific network modeling and for network based pharmacological attempts. As a continuation of the past eight years of signaling database development, here we introduce SignaLink 3, a comprehensive resource to provide integrated, detailed and reliable data in a user-friendly environment. The major improvements of the 3rd version are: (1) Literature curation of pathways updated with hundreds of new interactions and references; (2) Four new curated pathways: Toll-like receptor, HIPPO, GPCR and Intracellular immune pathways; (3) Tissue and subcellular localizations for proteins allowing tissue-specific filtering of interactions; (4) Molecular background of interactions (binding, phosphorylation, etc.) added; (5) Drug target, disease and cancer type annotation for each protein. The website of SignaLink 3 gives an opportunity to interactively browse the database content. For each protein regulators are listed by layers (post-translational, transcriptional and post-transcriptional), and presented in an interactive network visualization. Various annotations for proteins and interactions are also displayed. Hyperlinks to other resources are available, while SignaLink protein datasheets are also linked from other webpages, such as UniProt, WormBase and FlyBase. We developed a BioMART-like customizable download page, where users can easily select and combine the species, pathways, layers and download data in six standard formats: CSV, BioPAX, SBML, PSI-MI tab or PSI-MI XML and Cytoscape. Alternatively, the database can be installed to a local MySQL server.

E6-3

Detrimental effect of knock-outs in yeast is partially explained by maladaptive transcriptional response

Károly Kovács[1], Zoltán Farkas[1], Djordje Bajic[2], Dorottya Kalapis[1], Patrick Kemmeren[3], Frank C. P. Holstege[3], Richard Notebaart[4], Andreea Daraba[1], Zoltán Bódi[1], Csaba Pál[1], Balázs Papp[1]

[1]Biological Research Centre, Hungarian Academy of Sciences, Szeged; [2]Logic of Genomic Systems Lab at Centro Nacional de Biotecnología, CSIC, Madrid¸ [3]Molecular Cancer Research, University Medical Center Utrecht, Utrecht, The Netherlands; [4]Radboud University, Nijmegen, The Netherlands

The fitness impact of gene deletion is generally assumed to arise from the loss of specific molecular functions carried out by the deleted gene. Here we propose an alternative model by which gene loss evoke stereotypic gene expression responses that represent maladaptive solutions to gene loss and hence incur an extra fitness cost. To systematically test the plausibility of this scenario, we analyzed the transcriptome profiles of ~1400 single-gene deletion Saccharomyces cerevisiae strains. We observed that the extent of transcriptional reprogramming was strongly correlated with the fitness contribution of the disrupted gene, even when growth-rate related expression changes were accounted for. This pattern is consistent with maladaptive transcriptional reprogramming for three reasons. First, gene deletions resulted in global expression alterations mostly involving transcripts with no functional connections to the perturbed cellular subsystems. Second, altered expression of dosage sensitive genes correlated with the fitness impact of gene deletion. Third, genes specifically providing functional backup were only very rarely upregulated in the corresponding gene deletion backgrounds, indicating a lack of adaptive reprogramming specific for the perturbed gene. To directly test the maladaptive model, we experimentally restored the decreased expression level of haploinsufficient genes and showed that the restoration can ameliorate the fitness defect. We conclude that the fitness impact of gene loss does not purely result from the loss of the disrupted function, but maladaptive genome-wide expression responses can also contribute to the fitness cost. Our work has far-reaching consequences for interpreting results of genetic perturbation studies. This work was funded by The Wellcome Trust and the Hungarian Academy of Sciences Postdoctoral Fellowship Programme (Postdoc2014-85).

E6-4

pORTMAGE genome engineering systematically compares mutational effects across bacterial species

Gábor Apjok**,** Ákos Nyerges, Bálint Csörgő, Viktória Lázár, Csaba Pál

Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

Bacterial resistance to clinically applied antibiotics is emerging as a world-wide crisis, while the develop-

ment of new drugs to treat resistant bacteria is falling behind. Recent laboratory evolution studies show that evolution of resistance towards a single drug frequently alters fitness to multiple other antimicrobial agents at the same time. This phenomenon could potentially inform future therapeutic strategies to mitigate resistance evolution. However, all systematic studies have focused on a single species and as a consequence, the evolutionary conservation of the molecular mechanisms underlying antibiotic resistance remains unknown. We recently developed a broad-host multiplex genome editing system, termed pORTMAGE, which allows rapid and efficient genome engineering on a wide variety of bacteria without producing any observable off-target effects. This advance allows us to integrate key antibiotic resistance mutations into the genome of clinically relevant strains, as we demonstrated in two evolutionary related species, *Escherichia coli* and *Salmonella enterica*. As a second step, we systematically compared mutational effects and charted resistance interactions by utilizing high-throughput phenotypic assays. Our results indicate that mutational changes generally have similar effects on antibiotic resistance, despite over 100 million years of evolutional divergence between the two species. However, the phenotypic effects of certain canonical mutations varied extensively, which gives insight into the governing rule and conservation of evolutionary trade-offs in nature.

E7 - Genome organization, maintenance, functional genomics

E7-1

Super-enhancers and person-to-person genetic variability in the context of the 1000 Genomes Project

Dóra Bojcsuk, Lilla Ozgyin, Bálint L. Bálint
Department of Biochemistry and Molecular Biology, Genomic Medicine and Bioinformatic Core Facility, University of Debrecen, Debrecen

Understanding the person-to-person variability in healthy and diseased states became a possibility by using the unprecedented amount of available genomic data. Super-enhancers seem to be key elements of transcription regulation both in cancer and healthy states. We have investigated the mechanisms that drive transcription in the MCF-7 cancer cell line and in the LCL cell lines derived from a family of the 1000 Genomes project. Interestingly, subsets of estrogen receptor (ER) dependent super-enhancers bind ER even without the estrogen signal (estradiol, E2). These binding sites will anchor the Mediator complex upon ligand activation and will serve as organizers of later events of the transcription. In the cell lines of the 1000 Genomes project, investigating a specific family gives us the opportunity to better understand the genetic differences that are the basis of person-to-person differences in gene expression. The cell line specific master regulator transcription factor of LCL cells is PAX5. We have mapped the binding sites of PAX5 and the PAX5 super-enhancers in parents and a child of a well-characterized family. We have found that the inter-family variability of TF binding sites is significant; roughly 15% of binding sites might behave in a person specific manner. Interestingly, these differences can be explained by an allele specific binding of the TF to some genetic positions in many cases. The allelic differences might be explained only in a small subset by known SNP-s. Usually the known SNP-s localize between TF binding sites. Some allele specific differences in TF binding could be correlated with allele specific transcription. Our results can bring a better understanding of how super enhancers work in the context of person-to–person genetic variability. Funding: Internal Research University Grant entitled „Dissecting the genetic and epigenetic components of gene expression regulation in the context of the 1000 Genomes Project".

E7-2

Regulation of FAN1 nuclease activity

Peter Burkovics, Szilvia Juhasz, Lili Dome, Agnes Toth, David Balogh, Lajos Haracska
Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences,Szeged

Ensuring the maintenance of the genome during replication is a hard challenge for every cell. In this respect, interstrand crosslinks (ICLs) are one of the most dangerous damages because they inhibit unwinding of the strands and, as a consequence, DNA synthesis opposite the damage on both the leading and the lagging strands. Chemotherapeutic agents take advantage of this extremely toxic property of ICLs. In higher order eukaryotes such as humans, a special ICL repair system evolved, which was named Fanconi anemia (FA) after the genetic disorder caused by the failure of this repair pathway. Following the formation of an ICL, the FA pathway is activated via damage recognition executed by the Fanconi core complex, which activates the FANCL ubiquitin ligase protein. This ligase ubiquitylates the FANCD2 and FANCI proteins. As a result of the activity of the ubiquitylated FANCD2, the ICL is removed by special endonucleases, and the resulting double-strand break is repaired by homologous recombination. Interestingly, there are several endonucleases involved in ICL repair, but the exact mechanism of how they act is still unclear. The absence of some of them, for example the depletion of SLX4, causes Fanconi anemia in humans. FAN1 was a candidate for FA pathway membership, but none of the unassigned FA patients had *fan1* mutations. Furthermore, two patients having *fan1* mutations displayed no symptoms of the classical FA

phenotype. However, patients with the kidney disorder karyomegalic interstitial nephritis (KIN), which is linked to FAN1 failure, show genome instability, progressive kidney failure, and inclination to cancer. Based on these observations, we hypothesize that FAN1 has an important function in the maintenance of genome integrity, but its function has to be either independent from the FA core complex or redundant. Here we characterize the action of FAN1 using cell biological and biochemical methods, and clarify its function in ICL repair and in ensuring the maintenance of the human genome.

E7-3

Motor enzymatic activities controlling the initiation and outcome of homologous recombination

Gábor Harami, Máté Gyimesi, Veronika Ferencziová, Máté Martina, Mihály Kovács

ELTE-MTA Momentum Motor Enzymology Research Group, Department. of Biochemistry, Eötvös Loránd University, Budapest

Homologous recombination (HR) is a ubiquitous process that enables error-free repair of broken DNA molecules in somatic cells, and also aids the formation of new genetic combinations and proper chromosome segregation during meiosis. Our recent experiments on bacterial and eukaryotic recombinases, helicases and topoisomerases are aimed at determining (i) how the potentially harmful illegitimate (non-allelic) recombination events are selectively prevented, and (ii) how the outcome of the dissolution or resolution of joint DNA molecules is directed towards crossover or non-crossover pathways, depending on the biological context of HR events. We detected significant mechanistic differences between bacterial and eukaryotic systems regarding the formation of recombinase-nucleoprotein filaments, and also the helicase-catalyzed disruption of DNA strand invasions formed by recombinases. We will also discuss recent insights into the pathway choice between alternative DNA repair pathways and joint molecule processing outcomes, probed in C. elegans and zebrafish systems. Funding: MTA LP2011-006/2011, NKFIH K-116072, NKFIH ERC_HU 117680, H2020-MSCA-IF-2014 657076.

E7-4

Deoxyribonucleotide metabolism and genome integrity

Rita Hirmondó[1], Judit Eszter Szabó[1,2], Beáta Vértessy[1,2], Judit Tóth[1]

[1]Institutes of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2]Department of Applied Biotechnology and Food Sciences, Budapest University of Technology and Economics, Budapest

Cells maintain a fine-tuned concentration balance in the pool of dNTPs. The perturbation of this balance results in increased mutation frequencies. Members of the dUTPase superfamily play an important role in the maintenance of the pyrimidine nucleotide balance and of genome integrity [1]. These enzymes generate dUMP, the obligate precursor for *de novo* dTTP biosynthesis, from either dUTP (monofunctional dUTPase, Dut) or dCTP (bifunctional dCTP deaminase/dUTPase, Dcd-dut). In addition, the elimination of dUTP by these enzymes prevents uracil incorporation into DNA. These two outcomes have been thought to be interdependent. The bifunctional Dcd-duts are cooperatively regulated by dTTP, however, the manifestation of allosteric behavior within the same trimeric protein architecture of dUTPases has been a question of debate for decades. Here we show by *in vitro* enzymatic analysis and structural comparison that subunits of dUTPase work independently in contrast to Dcd-duts [2]. We also show that lack of allosteric regulation in dUTPase is related to the functional adaptation to more efficient dUTP hydrolysis, which is advantageous rather in uracil-DNA prevention than in the maintenance of dNTP pool balance. In agreement with this, we show that in *dut* mutant mycobacteria, the dNTP balance and the mutational spectrum remained unchanged, but the uracil content of DNA and the mutation rate increased in parallel with the *in vitro* enzyme activity-loss. Conversely, *dcd-dut* mutations resulted in perturbed dNTP balance and markedly changed mutational spectrum, but did not increase the uracil content of DNA. Thus, dNTP balance and the prevention of DNA uracilation are decoupled and separately brought about by the Dcd-dut and Dut enzymes, respectively. We propose that the enzymatic properties manifested in DCD-duts and dUTPases represent instances of adaptation to the distinct roles of dUMP production for dTTP synthesis and dUTP elimination for uracil-DNA avoidance, respectively. Funding: NKFIH K 115993, NK 84008, K109486; ICGEB CRP/HUN14-01.

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E7-5

Accurate mutation detection in whole genome sequences of multiple isogenic samples with IsoMut

Orsolya Pipek[1], Dezső Ribli[1], János Molnár[2], Ádám Póti[2], Marcin Krzystanek[3], András Bodor[1], Gábor E. Tusnády[2], Zoltán Szállási[3,4], István Csabai[1], Dávid Szüts[2]

[1]*Department of Physics of Complex Systems, Eötvös Loránd University, Budapest;* [2]*Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest;* [3]*Center for Biological Sequence Analysis, Department of Systems Biology, Technical University of Denmark, Lyngby, Denmark;* [4]*Computational Health Informatics Program (CHIP), Boston Children's Hospital, Boston, USA, and Harvard Medical School, Boston, U.S.A.*

Exposure to DNA damaging chemicals or the failure of DNA repair processes can lead to an accumulation of mutations in the genomes of somatic cells. Detection of somatic mutations is one of the main goals of next generation sequencing. However, most of the routinely used mutation calling algorithms are not optimized for the analysis of genomic DNA isolated from frequently used non-human experimental model systems, where reliable databases of common variations among individuals are not available. We designed the IsoMut algorithm and software tool that can be readily adapted to experimental scenarios where the goal is the identification of experimentally induced mutations in multiple isogenic samples. We showed that IsoMut, when tuned correctly, decreases the false positive rate compared to conventional tools in a 30 sample experimental setup; and detects not only single nucleotide variations, but short insertions and deletions, as well. We have successfully used IsoMut to detect the mutagenic effect and determine the detailed mutagenic spectrum of various physical and chemical mutagens in cultured chicken DT40 cells. IsoMut is available for download online at https://github.com/genomicshu/isomut. This work was supported by Momentum Grant LP2011-015 of the Hungarian Academy of Sciences.

C2 - Technical presentation II.

C2-1

BLI and the many ways to enhance your biomolecular binding kinetic and quantification assays capabilities

Renata Gronczewska

Business Development Sales Manager, Fortebio, Division of Pall Life Science, Menlo Park, CA, U.S.A

Biolayer Interferometry (BLI) is a powerful technique that enables direct measurement of biomolecular interactions in real time without the need for labeled reagents. Here we describe the analysis of a high-affinity binding interaction between a monoclonal antibody and purified antigen using BLI. A simple Dipand-Read™ format in which biosensors are dipped into microplate wells containing purified or complex samples provides a highly parallel, user-friendly technique to study molecular interactions. A rapid rise in publications citing the use of BLI technology in a wide range of applications, from biopharmaceutical discovery to infectious diseases monitoring, suggests broad utility of this technology in the life sciences.

C2-2

Automated solutions in biochemical research workflows

András Dékány
GeneTiCA Ltd., Tiszanána

Not submitted.

E8 - Protein structure, function and modeling

E8-1

Molecular recognition with peptidic foldamers: targeting proteins and membrane

Tamás A. Martinek
Institute of Pharmaceutical Analysis, University of Szeged, Szeged

Solvent exposed flat regions are responsible for many of the known protein–protein and protein-membrane interaction interfaces [1]. It is promising to construct artificial protein mimetic sequences, which fold (foldamers) and are able to cover these surfaces with programmable anchor points [2]. Peptidic foldamers are known to form compact secondary structures, which can be controlled through the stereochemical pattern along the peptidic backbone [3]. It will be shown how these foldamer secondary structures can be tuned for recognizing biomolecular targets. We present multivalent helical foldamer-dendrimer conjugates that recognize and inhibit the neurotoxic oligomeric β-amyloid (Aβ) [4], moreover, they can mimic the molecular recognition properties of the anti-Aβ monoclonal antibodies in a capture ELISA. As an extension of this approach, we demonstrate that hot regions of a protein can be mapped by using a folded fragment library displaying protruding proteinogenic side chains. The weak binder folded fragments can be linked together in presence of the target protein template with a dynamic covalent library (DCL) approach to improve affinity. We also present the design and the biological effects of the foldameric analogs of anginex, a 33-mer antiangiogenic peptide with a tendency to form β-sandwich. The effects of the β-amino acid substitutions on the β-sheet structure and the bioactivity will be discussed [5, 6].

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E8-2

Are the phosphopantothenoylcysteine decarboxylase subunits moonlighting proteins in *Candida albicans*?

Katalin Petrényi[1], Cristina Molero[2], Zoltán Kónya[1], Ferenc Erdődi[1], Joaquin Ariño[2], Viktor Dombrádi[1]
[1]Department of Medical Chemistry, Faculty of Medicine, University of Debrecen; [2]Institut de Biotecnologia i Biomedicina and Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Spain

The *SIS2/HAL3* gene was discovered as a suppressor of the *sit4* mutation and a regulator of salt tolerance in *Saccharomyces cerevisiae*. Both functions are justified by the role of Hal3 as inhibitor of the fungus specific protein phosphatase Z1 (Ppz1). Later on, it was shown that Hal3 (and its paralog Vhs3) are moonlighting proteins that, together with Cab3, form an essential heterotrimeric phosphopantothenoylcysteine decarboxylase (PPCDC) enzyme, which catalyzes an essential step of CoA synthesis. In *Candida albicans* sequence database we identified two putative PPCDC subunits that were termed CaHal3 and CaCab3 on the bases of their structural similarity to their *S. cerevisiae* counterparts. By tetrad analysis of *S. cerevisiae* mutant strains overexpressing the *Candida* proteins we proved that they were indeed essential PPCDC subunits, since the expression of CaCab3 and CaHal3 rescued the *cab3* and the *hal3 vhs3* mutations, respectively. By pull down analysis and phosphatase assays we demonstrated that both of the bacterially expressed *Candida* proteins were able to bind and inhibit the *C. albicans* Ppz1 (CaPpz1). The phosphatase related functions of the *C. albicans* proteins were tested by overexpressing them in additional *S. cerevisiae* mutants. We found that, contrary to structural predictions, only CaCab3 and not CaHal3 complemented the absence of authentic ScHal3 in functional tests like (i) tolerance to LiCl or (ii) hygromycin B, (iii) the requirement for external K^+ concentrations in the *hal3* mutant, and (iv) growth in the MAP kinase deficient *slt2* mutant. From these results we concluded that both CaHal3 and CaCab3 retained their PPCDC related function, and both of them are potential CaPpz1 inhibitors *in vitro*. However, under *in vivo* conditions only CaCab3 acts as a phosphatase regulator, suggesting that in *C. albicans* only CaCab3 is a moonlighting protein. Our work was supported by OTKA grant K108989 to VD and MINECO grant BFU2014-54591-C2-1-P to JA.

E8-3

Structural snapshots and mechanistic insights unveil the enzymatic cycle of Plasmodium CCT

Gergely N. Nagy[1,2], Fanni Hajdú[1,2], Lívia Marton[2,3], Ewelina Guca[4], François Hoh[5], Henri Vial[4], Rachel Cerdan[4], Beáta G. Vértessy[1,2]

[1]Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Budapest; [2]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [3]Doctoral School of Multidisciplinary Medical Science, University of Szeged, Szeged; [4]Dynamique des Interactions Membranaires Normales et Pathologiques, UMR 5235, CNRS, UM, Montpellier, France; [5]Centre de Biochimie Structurale, INSERM U1054, CNRS UMR 5048, Montpellier, France

Malaria remains to be a major biomedical challenge necessitating intense research into potential novel drug targets. The parasite *Plasmodium falciparum* in its erythrocytic stage relies exclusively on the *de novo* biosynthesis of phospholipids. Accordingly, CTP:phosphocholine cytidylyltransferase (CCT) that catalyzes a rate-limiting step in phosphatidylcholine biosynthesis is a validated antimalarial target [1, 2]. Here we provide structural insights into the enzymatic cycle of *Pf*CCT with a series of apo and holo structures that allow the identification of conformational changes upon catalysis that is yet unprecedented among CCTs. In addition, the presented biochemical characterization of key active site residues and active site residue variations of the *Plasmodium* and mammalian CCT offer a comprehensive overview on general catalytic mechanism of the CCT enzyme family. While many mechanistic aspects remains unresolved, our results constitute a missing link that connects the autoinhibitory and catalytic mechanism of this regulatory enzyme. Supported by the Hungarian Scientific Research Fund [OTKA K115993, NK 84008, K109486]; the MedinProt program of the Hungarian Academy of Sciences; the International Centre for Genetic Engineering and Biotechnology [ICGEB CRP/HUN14-01] and the European Commission FP7 Biostruct-X project [contract No. 283570]. A Hungarian-French Bilateral Science and Technology Collaborative grant (TÉT HU_FR 14) that covered collaborative travel expenses is also acknowledged.

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E8-4

Reconstitution of the *Drosophila* Protein Phosphatase 4 holoenzyme

Zoltán Lipinszki

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

Reversible protein phosphorylation is the most common ways to rapidly regulate the activity, alter subcellular localization, control half-life or mediate interactions of proteins with other biomolecules. Therefore this post-synthetic modification is fundamental in coordinating cell cycle progression in eukaryotes. Although roles of the protein kinases responsible for protein phosphorylation during cell division have long been recognized, the functions of the multiple protein phosphatases that reverses these events presents one of the major remaining challenges of the field of mitosis. Our lab is investigating Protein Phosphatase 4 (PP4), an evolutionarily conserved enzyme that has an essential, but relatively unexplored role in mitosis. PP4 belongs to the PP2A family of Ser/Thr phosphoprotein phosphatases, and similarly to PP2A it comprises one catalytic (PP4c), one structural (R2) and one regulatory 3 (R3) subunits. While crystallography has revealed the structure of the heterotrimeric PP2A, nothing is known about the physical organization of PP4. Having the 3D model of PP4 would allow us to gain insight into its intermolecular architecture, discover binding surfaces (e.g. for the substrates) and identify amino acids and post-synthetic modifications required for the function and assembly of the holoenzyme. Moreover, purified PP4 would be extremely useful to develop an *in vitro* dephosphorylation assay, which would facilitate the identification of specific inhibitors and could also be used for enzymatic reactions. Therefore we aim to reconstitute the *Drosophila* PP4 holoenzyme from purified recombinant proteins by employing various heterologous protein expression/purification systems. Although challenging, we have made a good progress in this project, which I am going to present in detail. This work is supported by the National Research, Development and Innovation Office (OTKA PD 115404) and the Hungarian Academy of Sciences (János Bolyai Research Fellowship).

E8-5

A unique feature of the *Drosophila* APC/C

Ágota Nagy[1], Judit Horváth[2], Olga Nagy[2], Margit Pál[2], Levente Kovács[2], Péter Deák[1,2]

[1] Department of Genetics, University of Szeged, Szeged; [2]Biological Research Centre, HAS, Szeged

The Anaphase Promoting Complex (APC/C) plays a key role in controlling the protein level of cell cycle regulators during mitosis and G1 phase. As an E3 enzyme, this large complex determines the specificity of the ubiquitin-dependent destruction of target

proteins. The APC/C contains at least 13-15 subunits, many of which were identified and characterized by biochemical and genetic means from several model organisms. We have shown recently that one of these subunits, Apc11 interacts with Mr/Apc2, and they together form a binding site for Vihar, the E2-C type ubiquitin conjugating enzyme in *Drosophila*. One of the smallest subunit of the APC/C, the Cdc26 was characterized in yeast and human cells, but not in *Drosophila*. Recently, our group identified not one, but two putative Cdc26 proteins in *Drosophila melanogaster*, which is unique among eukaryotes examined so far. The two proteins are different in size and amino acid sequence, but both contain a highly conserved, short N-terminal region of 20 amino acids. Our aim is to characterize these two subunits through genetic analysis. Genetic characterization of P element insertion alleles of one of the genes, the *DmCdc26*, revealed pupal lethality. Larval brain preparations of these mutants show mitotic arrest characteristic to the loss of function phenotypes of other essential APC/C subunits. Also, the human Cdc26 protein can rescue this phenotype, which suggests functional conservation. The other gene proved to be nonessential, but it could complement the loss of function phenotype of *DmCdc26*, therefore it was dubbed by us as *DmCdc26-like*. These findings suggest that the APC/C in Drosophila is somewhat different from those in yeast and human cells and may warrant further analysis. We are investigating the genetic and biochemical relationship between these proteins and the other subunits of the APC/C. The study is funded by the Hungarian Research Fund K116372.

E9 - Molecular signaling, cell-cell communication, cell death and differentiation

E9-1

Pathogenic mutations of the vasopressin V2 receptor

László Hunyady, András Sziráki, László Erdélyi
Department of Physiology, Faculty of Medicine, Semmelweis University and MTA-SE Laboratory of Moleular Physiology, Budapest

Pathogenic mutations of the vasopressin V2 receptor include loss of function mutations, which cause nephrogenic diabetes insipidus, and gain of function mutations, which cause nephrogenic syndrome of inappropriate antidiuresis (NSIAD). Loss of function mutations of the V2 receptor can cause nephrogenic diabetes insipidus either by interfering with the expression of the receptor or by impairing its binding and/or signaling function. We have investigated the pathomechanism of disease causing V2 receptor mutations, by expressing the receptor in eukaryotic expression systems. We have tested the surface expression, the cAMP response and the ß-arrestin binding of the expressed receptors to analyze their function. We have published recently that the N321K missense mutation of the V2 receptor causes impaired agonist sensitivity of the receptor, and the receptor has different agonist sensitivity to various peptide antagonists. Mutations can also cause conformational changes, which lead to endoplasmic reticulum retention, and impaired surface expression of the receptor. We have recently identified a mutation, which cause nephrogenic diabetes insipidus, due to the intracellular retention and impaired surface expression of the receptor. We have shown that pretreatment of the cells with tolvaptan, a membrane permeant V2 receptor antagonist, partially restores the function of this V2 receptor mutant. Our data suggest that tolvaptan functions as a pharmacological chaperone, which stabilizes the conformation of the mutant V2 receptor leading to its increased surface expression. Our data demonstrate that that understanding of the pathomechanism of disease causing G protein coupled receptor mutations may lead to individual treatments for the patients. This work was supported by National Research, Innovation and Development Office grants (OTKA 100883 and 116954).

E9-2

Signals derived by engulfing macrophages regulate the transglutaminase 2 expression in dying thymocytes

Katalin Sándor, Éva Garabuczi , Bence Dániel, Zsuzsa Szondy
Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen

Transglutaminase 2 (TGM2) is a multifunctional protein known to be associated with the *in vivo* apoptosis program. Previous studies in our laboratory have shown that TGM2 is strongly induced in dying thymocytes *in vivo*, however, the protein expression does not increase if thymocytes die *in vitro* indicating that factors present only in the tissue environment contribute to this molecular event. Here we show that three compounds, retinoids, TGF-β and adenosine, released by macrophages engulfing apoptotic cells, contribute together to the upregulation of *Tgm2* in dying thymocytes. The existence of TGF-β and retinoid responsive elements in the promoter region of *Tgm2* has already been reported, but the intergenic regulatory elements participating in the regulation of *Tgm2* have not yet been identified. Here we used publicly available results from DNase I hypersensitivity analysis followed by deep sequencing and chromatin immunoprecipitation followed by deep sequencing against CCCTC-binding factor, H3K4me3, H3K4me1 and H3K27ac to map a putative regulatory element set for *Tgm2* in thymocytes. By measuring eRNA expressions of these putative enhancers in retinoid, rTGF-β or dibutiryl cAMP-

exposed thymocytes we determined which of them are functional. By applying ChIP-qPCR against SMAD4, retinoic acid receptor, retinoid X receptor, cAMP response element binding protein, P300 and H3K27ac under the same conditions, we identified two enhancers of *Tgm2*, which seem to act as integrators of the TGF-β, retinoid and adenylate cyclase signaling pathways in dying thymocytes. Our study describes a novel strategy to identify and characterize the signal specific functional enhancer set of a gene by integrating genome-wide datasets and measuring the production of enhancer specific RNA molecules. This work was supported by OTKA T104228, NK105046 and the TÁMOP 4.2.2.A 11/1/KONV-2012-0023 „VÉD-ELEM" project.

E9-3

Differentiating human beige adipocytes secrete cytokines („batokines")

Endre Kristóf[1], Ágnes Klusóczki[1], Zsolt Combi[1], Roland Veress[1], Ferenc Győry[2], Szilárd Póliska[1], Zsolt Bacsó[3], László Fésüs[1]

University of Debrecen, Debrecen [1]Department of Biochemistry and Molecular Biology; [2]Department of Surgery; [3]Department of Biophysics and Cell Biology

Several studies highlighted the strong negative correlation between obesity and metabolically active brown adipose tissue amount in adult humans. There are at least two types of thermogenic fat depots, classical brown and beige, which have different origins and tissue distribution. Beige adipocytes contribute to the regulation of systemic metabolic homeostasis not exclusively by thermogenesis and mitochondrial uncoupling [1]. In the present study, we investigated the secretion of „batokines" by primary human brown and beige adipocytes and by human adipose tissue samples. Adipose-derived mesenchymal stem cells obtained from herniotomy (abdominal subcutaneous) or thyroid surgery („deep neck" and cervical subcutaneous) and a human preadipocyte cell line (SGBS) were differentiated into white, brown (by BMP7 treatment) or beige (by irisin administration or by a previously described cocktail) adipocytes [2]. Cytokine gene expression in browning adipocytes was determined by RNA sequencing. Conditioned differentiation media was collected during the replacement of the adipogenic cocktails and secreted IL-6, IL-8, TNFα, MCP-1 and IL1-ß were measured by ELISA. IL-6, MCP-1 and IL-8 secretion was significantly higher by beige compared to white adipocytes. In contrast to BMP7 administration (when classical brown adipocyte differentiation occurs), irisin treatment (which induces beige adipocyte differentiation) resulted in an increased total IL-6, MCP-1 and IL-8 production. Media collected daily or after three day periods contained the same amount of IL-6 depending only on the phase of differentiation. This suggests that adipocytes adjusted their production of the cytokine to reach an optimal level in the medium. In the heterogeneous population of differentiating adipocytes, IL-6 containing vesicles could be visualized mostly in beige cells by Laser-scanning cytometry. In the future we intend to explore the functional significance of the produced „batokines".

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E9-4

PKC mediated phosphorylation of TIMAP regulates PP1c activity in endothelial cells

Anita Boratkó, Csilla Csortos

Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen

TGF-β inhibited membrane-associated protein (TIMAP) has been considered as an endothelial cell type predominant member of the myosin phosphatase targeting (MYPT) family of the regulatory subunits of protein phosphatase 1 (PP1). Phosphorylation state of TIMAP has a remarkable effect on PP1c activity and endothelial barrier function. Here we present evidence for a previously unidentified phospho-site in TIMAP. We detected specific interaction between TIMAP and the α isoform of PKC in endothelial cells by immunoprecipitation, further, wild type recombinant TIMAP was phosphorylated by PKC in in vitro kinase assay. To identify the concerned side chain(s), several truncated or Ser-Ala recombinant mutants of TIMAP were created. We found that PKC phosphorylates TIMAP only on Ser331 side chain and there is no crosstalk with the previously identified PKA/GSK-3β pathway. Phosphorylation of TIMAP upon PKC activation in endothelial cells results in translocation of TIMAP to the membrane that fails in PKC depleted cells. Interaction between TIMAP and phospho-ERM was described earlier, but Ser331 phosphorylated TIMAP showed decreased binding to ERM. Accordingly, phospho-ERM level in the membrane fraction of S331D TIMAP mutant transfected cells increased, but the S331A mutant overexpressing endothelial cells had lower phospho-ERM level. Consistent with the phospho-ERM level, electric cell substrate impedance sensing (ECIS) measurements showed that the S331A mutation of TIMAP resulted in faster recovery from the PMA treatment. Thus, phosphorylation of TIMAP on Ser331 by PKC represents a new mechanism of endothelial barrier regulation through the inhibition of phospho-ERM dephosphorylation.

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E9-5

Testis-specific glutamate dehydrogenase is essential in the development of spermatid mitochondria

Viktor Vedelek[1], Barbara Laurinyecz[1], Attila L. Kovács[2], Gábor Juhász[2], Rita Sinka[1]

[1]Department of Genetics, University of Szeged, Szeged; [2]Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest

Mitochondria are essential organelles of developing spermatids in Drosophila, which undergo dramatic changes in size and shape after meiotic division, where mitochondria localized in the cytoplasm, migrate near the nucleus, aggregate, fuse and create the Nebenkern. During spermatid elongation the two similar mitochondrial derivatives of the Nebenkern start to elongate parallel to the axoneme. One of the elongated mitochondrial derivatives starts to lose volume and becomes the minor mitochondrial derivative, while the other one accumulates paracrystalline and becomes the major mitochondrial derivative. Proteins and intracellular environment that are responsible for cyst elongation and paracrystalline formation in the major mitochondrial derivative need to be identified. We present our results of the investigation of the function of the testis specific glutamate dehydrogenase during spermatogenesis. We show that a *Minos* element insertion in the glutamate dehydrogenase, *big bubble 8* (*bb8)* gene, causes recessive male sterility. We demonstrate *bb8* mRNA enrichment in spermatids and the mitochondrial localization of Bb8 protein during spermatogenesis. We report that megamitochondria develop in the homozygous mutant testes, in elongating spermatids. Ultrastructural analysis of the cross section of elongated spermatids shows enlarged mitochondria and the production of paracrystalline in both major and minor mitochondrial derivatives. Our results suggest that the Bb8 protein and presumably glutamate metabolism have a crucial role in the normal development and establishment of the identity of the mitochondrial derivatives during spermatid elongation. Supported by EMBO Installation Grant No.1825, OTKA NF 101001. R.S. is a Bolyai Fellow of the Hungarian Academy of Sciences.

ABSTRACTS OF THE POSTERS

P - Posters

P1

Increased expression of MCT4 and GLUT-1 in early events of spheroid formation of adenocarcinomic epithelial cells A549

Róbert Alföldi, Liliána Z. Fehér, Gábor J. Szebeni, László G. Puskás
Avidin Ltd., Szeged

Monolayer cell cultures (2D) can not adequately represent the growth and microenvironment conditions of three-dimensional (3D) tumors, resulting in false interpretation of molecular events and responses to chemotherapeutic interventions of solid tumors in vivo. Anaerobic conditions within an expanding spheroid, similar to in vivo conditions, cells are competing for nutrients and oxygen resulting in activated expression of metabolic enzymes in order to ensure an adequate supply of energy for cell proliferation. On the other hand cells growing in 2D cultures no such competition and no selective pressure exist. Therefore, in vitro multicellular tumor spheroid models provide a more reliable tool for drug screening and target identification. We established a screening strategy where small spheroids of A549 adenocarcinomic epithelial cells are studied. Induction of ITGA6 a marker in cellular polarity in 3D cultures could not be confirmed in our early formed spheroids. When these small colonies of cells are propagated under levitating conditions, nutrient and oxygen limitation is not relevant, therefore hypoxic induction of transcription machinery has not been initiated. We found that expression of genes coding for glycolytic enzymes PGK1, ALDOA1 and LDHA, some of the main indicators of cancer glycolytic pathway, was not changed significantly. In contrast, elevation of lactate transporter MCT4 and glucose transporter GLUT1 mRNA could be detected in 3D propagated cells. Interestingly, the other type of lactate transporter, MCT-1 expression was unaltered, therefore specific inhibition of MCT4 might prevent spheroid formation and growth. Our strategy for screening novel targets of early events of spheroid formation might identify proteins that may be promising pharmacological targets for cancer chemotherapy.

P2

Effect of novel protein phosphatase regulatory molecules on the adipogenic differentiation of mesenchymal stem cells

Bálint Bécsi[1,2], Zoltán Kónya[2], Ferenc Erdődi[1,2]
[1]MTA-DE Cell Biology and Signaling Research Group; [2]Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen

Mesenchymal stem cells (MSCs) provide an excellent cellular model to investigate the mechanism of the adipogenic differentiation of adult stem cells. The adipogenesis is stimulated by members of two families of transcriptional factors: the different isoforms of the enhancer binding proteins (C/EBPs) and the peroxisome proliferator-activated receptors (PPARs). The activity of these proteins is largely dependent on their phosphorylation state. The extent of protein phosphorylation is determined by the activity ratio of the phosphorylating protein kinases and the dephosphorylating protein phosphatases. We investigated the effect of two novel protein phosphatase regulatory compounds on the adipogenic differentiation of MSCs. The green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) was earlier identified as a phosphatase inhibitor in vitro at high concentration, but in vivo at low concentration it is a 67-kDa Laminin Receptor (67LR) agonist and activates protein phosphatase-2A (PP2A) through the 67LR/protein kinase A (PKA) pathway. During the adipogenic differentiation EGCG suppressed the lipid deposition as well as decreased the expression of the active form of the major transcriptional factors. In contrast, the acetylated bromobenzyl-selenoglycoside (BBSG), which has a phosphatase activatory effect on both protein phosphatase-1 (PP1) and PP2A, induced lipid droplet formation in the cytosol of the differentiating MSCs and enhanced the expression of the key transcriptional factors. These data suggest that the treatment of MSCs with phosphatase regulatory molecules influences adipogenic differentiation. Our hypothesis is that EGCG exert their effect through 67LR. Furthermore, we assume that BBSG can penetrate across the cell membrane and acts intracellularly, but the molecular mechanism is yet to be investigated. This work was supported by Hungarian Science Research Fund (OTKA K109249).

P3

Cannabinoid and opioid signaling is reduced in different brain regions of a rat model of schizophrenia

Edina Szűcs[1], Szabolcs Dvorácskó[1], Csaba Tömböly[1], Alexandra Büki[2], Gabriella Kékesi[2], Gyöngyi Horváth[2], Sándor Benyhe[1]
[1]Institute of Biochemistry, Biological Reserch Centre, Hungarian Academy of Sciences, Szeged; [2]Department of Physiology, Faculty of Medicine, University of Szeged, Szeged

Clinical reports suggest that many patients with schizophrenia are less sensitive to pain than others. Studies have implicated both the endocannabinoid and opioid systems in the pathophysiology of schizophrenia. Animal models do not interpret

schizophrenia completely, but they can model a number of symptoms of the disease. We investigated changes in the signaling properties of the mu-opioid (MOP) receptor and cannabinoid receptors in different brain regions, some of them are involved in the pain transmission. Our goal was to compare the transmembrane signaling mediated by MOP and cannabinoid receptors in control rats and in a recently developed rat model of schizophrenia. Regulatory G-protein activation via MOP receptors were measured in [^{35}S]GTPγS binding assays in the presence of a highly selective MOP receptor peptide agonist, DAMGO. It was found that the MOP receptor mediated activation of G-proteins was substantially lower in membranes prepared from the „schizophrenic" model rats than in control animals. The potency of DAMGO to activate MOP receptor was also decreased in all brain regions studied. Cannabinoid signaling was triggered by the non-selective agonist ligand WIN-55,212. The highest stimulation was found in the cerebellar membranes, moreover an extremely significant decrease in G-protein activation was observed in the cerebellum of the model animals. In our rat model of schizophrenia, MOP receptor and cannabinoid receptor mediated G-proteins have a reduced stimulatory activity compared to membrane preparations taken from control animals.

P4

Probing the epigenetic background of Huntington's disease using histone PTM mimics

Nóra Zsindely, Júlia Varga, László Bodai
Department of Biochemistry and Molecular Biology, University of Szeged, Szeged

Huntington's disease (HD) is a devastating autosomal dominant neurodegenerative disorder that primarily affects striatal neurons. HD is caused by expansion of a polymorphic CAG repeat in the first exon of the huntingtin (*htt*) gene that translates to a pure polyglutamine repeat region. While in healthy individuals the polyglutamine repeat have less than 36 glutamine residues, mutant alleles with more than 39 glutamines cause HD with complete penetrance. Mutant Htt proteins with an expanded polyglutamine repeat form intracellular aggregates and participate in aberrant protein-protein interactions that lead to a multifaceted pathogenesis. We and others have shown that mutant *htt* binds and inhibits some histone acetyltransferases thereby inducing epigenetic changes and transcriptional dysregulation. In this study, we aim to determine whether specific histone marks have a critical role in HD pathogenesis and/or progression using a *Drosophila* model of the disease. For this purpose, we introduced point mutations mimicking post-translation modifications of specific lysine residues in the *His3.3A* and *His4r* variant histone genes *in vitro* and used these constructs to generate transgenic flies. We show by immunoblots that FLAG-tag labeled histone PTM mimic proteins can be expressed in the nervous system. Immunofluorescence microscopy revealed that FLAG-tagged His3.3A is localized to the nucleus and is incorporated in chromosomes. Preliminary data indicate that histone PTM mimic transgenes expressed in the nervous system can exert functional effects. Funding: NKFI grant 112294.

P5

Canonical elements drive super-enhancer formations

Dóra Bojcsuk[1], Gergely Nagy[1,2], Bálint L. Bálint[1]
[1]Department of Biochemistry and Molecular Biology, Genomic Medicine and Bioinformatic Core Facility, University of Debrecen, Debrecen, [2]Department of Biochemistry and Molecular Biology, MTA-DE „Lendulet" Immunogenomics Research Group, University of Debrecen, Debrecen

Super-enhancers (SEs) are well-studied regulatory units of the genome since *Lovén et al*. reported this phenomenon in 2013 [1]. SEs were defined as clusters of outstandingly active enhancers, which regulate cell-type specific genes. In this study, we examined the formation of estrogen receptor driven super-enhancers in MCF-7 cell line. We found that in the lack of stimulation, the future super-enhancers were represented by one or few ChIP-seq peak(s) (called mother enhancers), and upon estradiol treatment, the appearing further peaks (named daughter enhancers) were recruited nearby to form eventually super-enhancers. Mother enhancers always possessed a canonical binding element, while the subsequent binding sites, if they did, had poor ones. We also investigated the simultaneous presence of MED1, BRD4, P300 transcription factors (TFs), signal of DNase I hypersensitivity and the active histone mark H3K27ac. Interestingly, all of them are specific for mother enhancers. We were curious whether the mother and daughter enhancers are specific for other TFs, so we examined further TFs, such as FoxA1, AP2γ, AR, RAR, VDR and JUNB in other cell types. All these TFs showed similar patterns as obtained for estrogen receptor, which indicates that we found a general phenomenon. Although numerous studies suggested that in the DNA binding of transcription factors, protein-protein interactions are more important than the presence of canonical elements, based on our results it seems that certain response elements themselves are able to guide transcription factors, and interestingly, not only to the given site, but also to neighboring regions and typically each super-enhancer is dominated by a single transcription factor, which recruits the further proteins primarily to the canonical elements. The project is funded by Internal Research University Grant entitled „Dissecting the genetic and epigenetic components

of gene expression regulation in the context of the 1000 Genomes Project".

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P6

The presence of HOFI promotes tumor progression

Eszter Boldizsár[1], Adrienn Gyöngyösi[1], Attila Pap[2], Szilvia Török[3], Balázs Döme[3], László Nagy[2], Árpád Lányi[1]

[1]University of Debrecen, Department of Immunology, Debrecen; [2]University of Debrecen, Department of Biochemistry and Molecular Biology, Debrecen; [3]National Koranyi Institute of TB and Pulmonology, Department of Tumor Biology, Budapest

HOFI (homologue of FISH, SH3PXD2B) is a typical adapter protein with an N-terminal phagocyte oxidase homology (PX)-domain followed by a set of four SH3-domains. Its involvement in the regulation of cell migration, the development of functional podosomes and lamellipodia suggested that it may play a role in tumor progression. The fundamental role of HOFI in the regulation of mammalian development strongly suggests its involvement in the development of a functional tumor-associated stroma (TAS). TAS is considered to be a major contributing factor to tumor progression. In this work, first we investigated the role of HOFI in the regulation of tumor cell proliferation both in vitro and in vivo. Next, we studied the contribution of HOFI in the organization of TAS in various murine models. We found decreased proliferation in vitro and diminished growth in SCID mice when HOFI expression was silenced in A2058 human melanoma cells. In addition, subcutaneous injection of B16-F10 murine melanoma cells into HOFI-deficient animals resulted in a significant reduction of tumor growth compared to wild type animals. To examine the contribution of HOFI to the immune compartment of the TAS we also injected B16-F10 cells into bone marrow chimeras that received either wild-type or HOFI-deficient bone marrow. Interestingly, tumor growth was also impaired in chimeras, receiving HOFI-deficient bone marrow suggesting a role for HOFI in tumor-specific immunosurveillance. Taken together, HOFI appears to be an intrinsic tumor promoting factor controlling the growth rate of transformed cells as well as extrinsic, indirect regulator of TAS. Based on our bone marrow transfer experiments, HOFI should control one or more subsets of tumor-infiltrating immunocytes involved in anti-tumor responses. This work was supported by the Hungarian Research Fund OTKA K 109444.

P7

Beyond initiation: Human P53 plays role in transcription elongation

Barbara Nikolett Borsos[1], Ildikó Huliák[1], Zsuzsanna Ujfaludi[1], Imre Miklós Boros[1,2], Tibor Pankotai[1]

[1]Department of Biochemistry and Molecular Biology, University of Szeged, Szeged; [2]Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

The P53 tumor suppressor regulates the transcription initiation of selected genes by binding to specific DNA sequences at their promoters. Here we report a novel role of P53 in transcription elongation in human cells. Upon transcription elongation blockage P53 is associated with genes, which have not been reported earlier as its direct targets. P53 could be co-immunoprecipitated with active forms of RPB1, highlighting its association with the elongating RNAPII. During normal transcription cycle, P53 and RPB1 localized at distinct regions of selected non-canonical P53-target genes and this pattern of localization was changed upon transcription elongation block. Additionally, transcription elongation block induced the ubiquitylation and the proteosomal degradation of RPB1. Finally, we showed that the transcription block induced RPB1 degradation is mediated by P53. Our results reveal a novel role of P53 in human cells during transcription elongation blockage that might serve to facilitate the removal of RNAPII from DNA.

P8

Competition of apoptotic and necrotic cells for uptake by bone marrow-derived macrophages

Zsófia Budai, Zsolt Sarang, Zsuzsa Szondy
Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen

One of the major roles of professional phagocytes is the removal of dead cells. The uptake of apoptotic cells is well described as the final stage of programmed cell death including the sensing of corpses via „find me" signals, the recognition of apoptotic bodies via „eat me" signals, and the internalization mechanism. Without prompt and effective phagocytosis uncleared corpses can undergo secondary necrosis promoting inflammation and autoimmunity. In contrast to the apoptotic cell phagocytosis, much less is known about the clearance of necrotic cell debris. Since necrotic and apoptotic cell surface shares, at least partially, the same molecules the question arose which cell type is the preferred pray of macrophages, when both cell types are present and how effective is the engulfment of necrotic and apoptotic cells? We studied the phagocytosis of necrotic and apoptotic thymocytes and NB4 cells by mouse bone marrow-derived macrophages and measured the phagocytosis by two- or three-color flow cytometry and fluorescent microscopy. In our

experiments macrophages showed slightly higher preference for engulfing apoptotic cells and apoptotic cells were similarly effective competitors as necrotic cells. We found that prefeeding of macrophages with either apoptotic or necrotic cells does not influence subsequent phagocytic preference. Our result might provide insights into the so far not studied aspects of necrotic cell uptake. Funding: OTKA K 104228 to Zsuzsa Szondy and Internal research grant of University of Debrecen to Zsolt Sarang.

P9

Biochemical activities of the Wiskott-Aldrich syndrome homology region 2 domains of Sarcomere Length Short protein

Mónika Ágnes Tóth[1], Péter Gaszler[1], Judit Viktória Fórizs[1], Andrea Teréz Vig[1], Ede Migh[2], József Mihály[2], Beáta Bugyi[1]

[1]Medical School, Department of Biophysics, Pécs; [2] Institute of Genetics, Hungarian Academy of Sciences, Biological Research Centre, Szeged

Drosophila melanogaster Sarcomere Length Short (SALS) is a recently identified Wiskott - Aldrich syndrome protein homology 2 (WH2) domain protein involved in skeletal muscle thin filament regulation. SALS was shown to be important for the establishment of the proper length and organization of sarcomeric actin filaments. Here we present the detailed characterization of the biochemical activities of the tandem WH2 domains of SALS (SALS-WH2). Our results revealed that SALS-WH2 binds both monomeric and filamentous actin and shifts the monomer : filament equilibrium towards monomeric actin. In addition, SALS-WH2 can bind to but fails to depolymerize phalloidin-, or jasplakinolide-bound actin filaments. These interactions endow SALS-WH2 with two major activities in the regulation of actin dynamics: SALS-WH2 sequesters actin monomers into non-polymerizable complexes and enhances actin filament disassembly by severing, which is modulated by tropomyosin. We also show that profilin does not influence the activities of the WH2 domains of SALS in actin dynamics. In conclusion, the tandem WH2 domains of SALS are multifunctional regulators of actin dynamics. Our findings suggest that the activities of the WH2 domains do not reconstitute the presumed biological function of the full-length protein. Consequently, the interactions of the WH2 domains of SALS with actin must be tuned in the cellular context by other modules of the protein and/or sarcomeric components for its proper functioning. This research was supported by grants from the Hungarian Science Foundation OTKA K109689 (to BB) and from the National Innovation Office „Baross Gábor" Program (REG-DD-09-1-2009-0009 Tirfm 09 (to BB)). 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.

P10

Intramolecular interactions regulate lipid binding of the scaffold protein Tks4

Anna Cserkaszky[1], László Radnai[1], Kitti Koprivanacz[1], Balázs Merő[1], Bálint Szeder[1], Gyöngyi Kudlik[1], Gergő Gógl[2], László Nyitray[2], László Buday[1]

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

The scaffold protein Tks4 is a member of the p47phox-related organizer superfamily. It plays a role in cell motility by being essential for the formation of actin-rich membrane protrusions like podosomes and invadopodia of normal and cancer cells, respectively, and is regulated by the epidermal growth factor (EGF) signaling pathway. EGF induces the translocation of Tks4 from the cytoplasm to the plasma membrane. Tks4 and the evolutionarily-related protein p47phox share many similarities in their N-terminal region: a phosphoinositide-binding PX domain is followed by two Src Homology-3 domains (so called „tandem SH3") and a proline-rich region (PRR). Tks4 contains two additional SH3 domains and several other PRRs. It is known that intramolecular interactions are necessary for the autoinhibited state of p47phox: the tandem SH3 domain binds the PRR while the second SH3 domain interacts with the PX domain preventing its membrane binding. Based on the conserved structural features of p47phox and Tks4 and the fact that an intramolecular interaction between the third SH3 and the PX domains of Tks4 has also been reported, we hypothesized that Tks4 is also capable of autoinhibition. In this study we aimed to identify the intramolecular interactions within the N-terminal part of the Tks4 protein that are necessary for the presumable autoinhibited conformation. We showed by fluorescence-based titrations and Small Angle X-ray Scattering that the tandem SH3 domain of Tks4 binds the PRR and that the PX domain interacts with the third SH3 domain. Phosphatidylinositol-3-phosphate (PI3P) and phosphatidic acid (PA) were identified as the main binding partners of the PX domain by lipid binding assays. The presence of the tandem SH3 together with the PRR effectively inhibited PA binding while PI3P binding was less affected even when the third SH3 domain was also present. These findings further support our hypothesis. This work was supported by OTKA K83867, LENDÜLET and MedInProt grants.

P11

Identification of possible predictive biomarkers of infliximab in rheumatoid arthritis

Mária Csumita[1], Szilárd Póliska[1,2], Zoltán Szekanecz[3], Gábor Zahuczky[2]

[1]Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen; [2]UD-GenoMed Medical Genomic Technologies Ltd., Debrecen; [3]Department of Rheumatology, Faculty of Medicine, University of Debrecen, Debrecen

Tumor necrosis factor alpha (TNFα), a proinflammatory cytokine, is a central factor in inflammatory immune response [1]. There are several monoclonal antibody based anti-TNFα biologic agents are used in the therapy of inflammatory conditions, such as rheumatoid arthritis (RA); e.g. infliximab, adalimumab and etanercept, among others but the efficacy of these are different [2]. A significant percentage, approximately 30% of RA patients fail to respond to biologic therapy and it was also shown that efficacy may decline following cycling to a second TNF inhibitor [3]. Therefore, identification of biomarkers that can predict the efficacy of an anti-TNFα therapy could help the clinical decision. Previously, we have identified potential gene expression biomarkers, which discriminate between responder and non-responder RA patients receiving infliximab therapy [4]. In this study, we planned to find correlation between gene expression panel, protein immunoblot pattern and serum levels of cytokine and anti-drug-antibody (ADA) levels, as production of ADA can negatively affect the response of the biological therapy and cause secondary failure. In our preliminary study, we measured TNFα, infliximab and anti-infliximab antibody levels from serum samples using specific ELISA kits. ADA status (positive/negative) was correlated with gene expression data and a list of genes was identified which showed altered expression levels between ADA+ and ADA- patients. We demonstrated that a different set of genes for example Lactotranferrin (LTF) changed during the first two weeks of infliximab therapy in ADA+ and ADA- patients. We detected LTF in a serum samples using western blot analytical technique. Our results suggest that combination of genomic and proteomic biomarkers are needed to develop a co-diagnostic tool which can help the clinical decision. Funding: UD-GenoMed Medical Genomic Technologies Ltd.

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P12

Alterations in corneal parameters and protein profile in tears of type 1 diabetes mellitus patients

Eszter Deák[1,2], Éva Csősz[2], Adrienne Csutak[1]

[1]Department of Ophthalmology, Faculty of Medicine, University of Debrecen, Debrecen; [2]Department of Biochemistry and Molecular Biology, Proteomics Core Facility, Faculty of Medicine, University of Debrecen, Debrecen

Background: Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes mellitus and is currently the leading cause of blindness in the economically active population in developed countries. The initially latent disease could lead to vision loss without any symptoms initially. Timely diagnosis and therapy, however, can significantly decelerate its progress, necessitating regular DR screening or appropriate follow-up in all patients with diabetes. **Purpose**: The aim of this study was to compare the tear protein profile and the quantified epithelial, stromal and endothelial cell density and subbasal nerve morphology in young patients with type 1 diabetes mellitus with and without diabetic retinopathy. **Methods**: 19 young patients (mean age: 24.8±10.3 years) with type 1 diabetes, with (n=10) and without (n=9) retinopathy and 19 age-matched healthy control subjects (mean age: 24.9±10.1 years) underwent tear sampling and corneal confocal microscopy (CCM). **Results**: We found significant difference between the diabetic and healthy subjects in tear protein profile and also in corneal parameters, such as epithelial, stromal, endothelial cell density and also nerve fibre specific parameters. The corneal parameters determined in a previous study were measured again after 2 years follow-up in type 1 diabetes patients with and without retinopathy and were compared to the parameters measured in control subjects. **Conclusions**: Combining the tear analysis with the corneal confocal microscopy under subclinical conditions appears to have considerable utility as an efficient biomarker pairing, which can help to identify the early corneal cellular and small nerve fibre pathology in young patients with type 1 diabetes without retinopathy showing increasing tendency in severity in patients with retinopathy. **Acknowledgement**: The research was partially supported by the project TAMOP4.2.2.A11/1 KONYV20120045 and co-financed by the OTKA Grant 108643.

P13

Novel substrates reveal a central role of MAP kinase signaling in environmental adaptation of plant growth

Magdaléna Dőry[1], Zoltán Doleschall[2], Szilvia K. Nagy[3], Katalin Jäger[1], Tamás Mészáros[3,4], Beáta Barnabás[1], Róbert Dóczi[1]

[1]*Department of Plant Cell Biology, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár;* [2]*Department of Pathogenetics, National Institute of Oncology, Budapest;* [3]*Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest;* [4]*Research Group for Technical Analytical Chemistry, Hungarian Academy of Sciences, Budapest University of Technology and Economics, Budapest*

Unlike in animal development – where organs are formed early in embryogenesis and their further development is basically independent of environmental factors – organogenesis and organ development in sessile plants flexibly respond to changing environmental conditions. An extreme example of this developmental flexibility is bonsai cultivation, such techniques lead to the most extreme forms of environmentally induced plant dwarfism. The importance of stress-induced growth retardation in plants is difficult to overemphasize: plant biomass is humankind's most important primary resource. The mitogen-activated protein kinase (MAPK) phosphorylation cascades are well-conserved signaling modules in all eukaryotes, and they have been mainly associated with stress signaling in plants. Using genetic approaches, we have found that a MAPK module participates in meristem regulation. Knock-out mutant plants display increased rates of leaf primordia development, implying increased meristem activity. In contrast, overexpression leads to severe dwarfism, associated with the collapse of meristems. In good agreement with these findings, we provide biochemical evidence that several well-known meristem regulators, including transcription factors and hormone transporters are phosphorylated by MAPKs. Taken together, our results imply that besides induction of defence responses, environmentally-activated MAPK signaling participates in linking external cues to developmental regulation. Modulation of key meristem regulatory proteins by post-translational modifications provides a novel mechanism for the environmental plasticity of plant growth. This work was financially supported by OTKA grant NN 114511. RD is a Bolyai Fellow of the Hungarian Academy of Sciences.

P14

Selection of dynamic ensembles to achieve maximum correspondence to experimental NMR data – an extension for the CoNSEnsX server

Dániel Dudola, Zoltán Gáspári
Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest

Generation of dynamic structural ensembles that are compatible with experimentally determined mobility parameters is a fruitful approach for understanding subtle details of protein function. There are multiple methods to obtain such ensembles; the two most important ones are ensemble-restrained molecular dynamics and the application of a selection step from a large pool of conformers. Nevertheless, for the evaluation of such ensembles in terms of their correspondence to experimental parameters – both those used for the generation of the ensemble and those that were not – there are very few standardized tools available. Here we report a complete recoding of our previously published approach, CoNSEnsX (compliance of NMR-derived structural data to experimental parameters) providing both enhanced functionality and enhanced user interface [1]. In particular, the new tool is capable of handling multiple independent residual coupling data sets and can perform a superposition of the conformers submitted. The functionality of the new software is demonstrated on structural ensembles of ubiquitin and the PDZ domains of PSD-95. The incorporation of a simple selection approach, its uses and evaluation will also be presented.

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P15

Investigating the role of Hsp27 in neuroinflammation and apoptosis using a mouse model of fetal alcohol syndrome

Brigitta Dukay, Melinda E. Tóth, László Vígh, Miklós Sántha
Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

Heat shock proteins (Hsps) are molecular chaperones upregulated in response to different forms of cellular stresses. Evidence suggests that Hsps have protective function in various disease conditions and they are involved in the regulation of several cellular processes. Here we studied the effect of Hsp27 against ethanol induced neurodegeneration in a mouse model of fetal alcohol syndrome, using our previously generated transgenic strain that expresses high level of Hsp27 in brain vessels and neurons. To induce neuronal damage, 7 day-old mice were treated with 5 g/kg ethanol injection. This single

day of ethanol treatment resulted in a dramatic cell death in the cortex, thalamus and striatum. Reduced number of apoptotic cells were counted in the brain of Hsp27 transgenic animals compared to wild-types, however, the result was not statistically significant. mRNA levels of pro-inflammatory cytokines (TNFα, IL-1β), markers of reactive astrogliosis and microgliosis (GFAP and Iba1) were increased by twofold in the wild-type animals 24 hours after the ethanol treatment. Iba-1 mRNA level was also doubled in the brain of non-treated Hsp27 transgenic animals; however it did not show any further increase after ethanol treatment. The level of TNFα, IL-1β and GFAP were not increased in response to Hsp27 overexpression alone, but they showed a much higher (10-, 7- and 4.5 fold) increase in the ethanol treated transgenic mice compared to ethanol treated wild-type group. Microglia activation was demonstrated by Iba1 immunostaining in the ethanol treated animals (both transgenic and wild-type) but not in untreated Hsp27 transgenic mice, despite the increased Iba1 mRNA level. Our results show that Hsp27 can promote the expression of pro-inflammatory cytokines and GFAP in the brain *in vivo*, under acute brain injury, suggesting a pivotal role in the regulation of inflammatory responses. This study was supported by the Hungarian Scientific Research Fund OTKA, Grant No. OTKA NN- 111006.

P16

Novel role of Tks4 scaffold protein in modulating adipogenic and osteogenic differentiation of Mesenchymal Stem Cells

Virag Vas[1], Metta Dülk[1], Gyöngyi Kudlik[1], Anna Fekete[1], Dávid Ernszt[2,3], Krisztián Kvell[2,3], Balázs L. Merő[1], Bálint Szeder[1], László Radnai[1], Dalma E. Csécsy[1], Tamás Kovács[1], Ferenc Uher[4], Árpád Lányi[5], László Buday[1,6]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pecs, Pécs; [3]Szentagothai Research Center, University of Pecs, Pécs;, [4]Stem Cell Biology, National Blood Service, Budapest; [5]Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen; [6]Department of Medical Chemistry, Semmelweis University Medical School, Budapest

Tks4 (tyrosine kinase substrate with 4 SH3 domains) scaffold protein has recently been found to be expressed in Mesenchymal Stem Cells (MSCs), but its biological significance remains unknown. Dysfunction of Tks4 causes a genetic disease called Frank-ter Haar syndrome (FTHS) (OMIM:249420), with a wide variety of defects including of mesenchymal tissues (bone, fat and cartilage) throughout both embryogenic and postnatal development. Here, we were testing the hypothesis that deletion of Tks4 affects the differentiation potential of MSCs. Therefore we generated Tks4-/- mice on C57BL/6 background and characterized the phenotype of fat and bone tissue of the adult mice. Furthermore, we isolated bone marrow MSCs from wild type and Tks4-/- mice and challenged their differentiation potential in vitro. We found that the Tks4-/- mice have lipodystrophy, showed highly reduced fat weight. In addition, the Tks4-/- mice have shortened long bones and craniofacial abnormalities. The *in vitro* experiments showed that Tks4-/- MSCs had reduced ability to differentiate into osteogenic and adipogenic lineages compared to wild type MSCs. We demonstrated by Western blot that a major adipogenic transcription factor isoform, PPARγ2 and a key osteogenic transcription factor, RunX2 were down regulated in knock-out cells during adipogenic or osteogenic induction, respectively. We studied further the impaired adipogenic differentiation of Tks4-/- MSCs with a lipidomic TaqMan array and concluded that the expression of a group of transcription factors, sterol metabolism, fatty acid metabolism and lipid droplet formation genes were reduced in Tks4 mutant MSCs. Overall, our data revealed a novel function of Tks4 protein in the MSCs differentiation process and may serve one possible explanation for why Tks4 deficient mice and FTHS patients display defects in mesenchymal originated tissue.

P17

Genome-wide mapping of COUP-TFII and ERα co-occupancy in breast cancer cells

Edina Erdős, Dóra Bojcsuk, Bálint László Bálint

Faculty of Medicine, Department of Biochemistry and Molecular Biology,Genomic Medicine and Bioinformatic Core Facility, University of Debrecen, Debrecen

Chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) is an orphan nuclear receptor having no identified ligands. Its expression level has been shown to be associated with the prognosis and outcome of breast cancer, and correlates with lymph node and estrogen receptor (ERα) status. Gene regulation processes controlled by COUP-TFII are unknown, therefore our aims were to investigate the COUP-TFII binding events alone and together with ERα by genomic and bioinformatic approaches in breast cancer cells. COUP-TFII and ERα ChIP-seq data derived from MCF-7 cell line were collected from NCBI Sequence Read Archive and were reanalyzed by our bioinformatic pipeline. Then, we separated the common and individual COUP-TFII and ERα binding sites. These sites were classified based on the binding of the pioneer factors of ERα such as FoxA1 and AP2γ, and we found that unique ERα binding sites contain only ERE motif in the absence of pioneer factor binding. Common

COUP-TFII/ERα binding sites showed the presence of FoxA1 and AP2γ as well as DNase I hypersensitivity. In contrast, FoxA1 or AP2γ together with COUP-TFII did not show DNA accessibility. Some of the unique COUP-TFII binding sites showed opened chromatin without ERα binding. Finally, pathway analysis demonstrated that some of the resulted signaling pathways are regulated by COUP-TFII in MCF-7. Taken together, these data suggest that COUP-TFII might have a key role in ERα enhancer activation, therefore, in our future studies, we would like to investigate the role of COUP-TFII in relation to the progression and metastasis of breast cancer by using genome-wide approaches. The project is funded by Internal Research University Grant entitled „Dissecting the genetic and epigenetic components of gene expression regulation in the context of the 1000 Genomes Project".

P18

Identification of novel therapeutic targets in patients with increased intracranial brain pressure and edema at the single cell level

Nóra Faragó [1,2,3], Ágnes Katalin Kocsis [1], Csilla Braskó[1], Márton Rózsa[1], Judith Baka[1], Balázs Kovács[1], Katalin Mikite[1], Viktor Szemenyei[1], Gábor Molnár[1], Attila Ozsvár[1], Ágnes Zvara[2], Pál Barzó [4], László G. Puskás[2,3], Gábor Tamás[1]

[1]MTA-SZTE Research Group for Cortical Microcircuits of the Hungarian Academy of Sciences, Department of Physiology, Anatomy and Neuroscience, University of Szeged, Szeged; [2]Laboratory of Functional Genomics, Department of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged; *[3]Avidin Ltd., Szeged; [4]Department of Neurosurgery, University of Szeged, Szeged*

Identification of cell type specific molecular events having important role in edema formation and resolution could result in better understanding of associated brain insults and could provide novel therapeutic targets. To reveal population specific cellular responses to edema and increased intracranial pressure, that might remain undiscovered in samples formed by a multitude of cell types using classical genomic analysis, we combined single cell electrophysiology, anatomy and digital PCR in neurons identified in situ on tissue samples collected from patients. Assessment of mRNA copy number and corresponding functional changes verified significant differential expression of HMOX1, SOD2, CACNA1B, KCNN4, KCNS1, SCN3B, AQP1, HTR5A and CRH in pyramidal cells and in fast spiking interneurons revealing cell type specific quantitative changes of known and potential targets in the therapy. Pharmacological and immunohystochemical results confirmed a functionally detectable presence of KCNN4 and KCa3.1 channels in pyramidal cells in edema, suggesting their neuroprotective functions. Dendritic spine density measurements uncovered a significant drop in both pathophysiological conditions relative to control in pyramidal cells but not in basket cells, indicating a specific loss of excitatory synapses, resulting lower synapse numbers that might contribute to higher input resistances measured in edema.

P19

Functional investigation of the unstructured N-terminal domain of *Candida albicans* protein phosphatase Z1

Krisztina Szabó, Zoltán Kónya, Viktor Dombrádi, Ilona Farkas

Department of Medical Chemistry, School of Medicine, University of Debrecen, Debrecen

Candida albicans protein phosphatase Z1 (CaPpz1) is a fungus specific Ser-Thr phosphatase involved in the regulation of cation homeostasis, cell wall biosynthesis, stress response, hyphal growth and virulence. CaPpz1 is composed of a conserved C-terminal catalytic domain and a variable, intrinsically unstructured N-terminal domain that has four potential protein binding sites. To test their role, CaPpz1 mutants were generated that lack one of these regions. CaPpz1 del1-16, del25-43, del67-108, del120-142, and wild type CaPpz1 were expressed in *E. coli* with a GST-tag. Fusion proteins were purified and the tag was removed by Prescission protease treatment. The phosphatase activity of recombinant proteins was assayed with ^{32}P-myosin light chain substrate. The *in vitro* measurements revealed that the del1-16 mutation significantly decreased the specific activity of the enzyme. The *in vivo* analysis of mutations was performed by complementation of *Saccharomyces cerevisiae ppz1* cells that lack the endogenous *PPZ1* gene. *ppz1* cells were transformed with low copy number YCplac111 vectors that contained the coding region of *CaPPZ1* wild type or mutant genes downstream of the *S. cerevisiae PPZ1* promoter. The del67-108 and del120-142 proteins acted similarly to wild type CaPpz1 by complementing the caffeine sensitivity of the *ppz1* strain. However, the del1-16 and del25-43 mutants did not diminish caffeine sensitivity. The del1-16 mutant did not compensate LiCl tolerance of the *ppz1* cells either. On the other hand, CaPpz1 del25-43, del67-108, del120-142 and wild type CaPpz1 partially complemented the LiCl phenotype. Therefore, the peptide regions 1-16 and 25-43 in the N-terminal domain of CaPpz1 seem to be necessary for the normal physiological functions of CaPpz1, due to their role in phosphatase activity and/or in protein-protein interactions. Our work was supported by OTKA grant K108989.

P20

Dynamic protein structural ensembles based on NMR data: methdology & applications

Zoltán Gáspári, Bertalan Kovács, Zita Harmat
Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest

Protein NMR is the most powerful technique to study protein internal dynamics at the atomic level at multiple time scales. Combination of NMR-derived parameters with molecular dynamics calculations can lead to atomic-level ensemble-based structural models that are consistent with the experimentally determined mobility. We have implemented different restraining schemes in GROMACS [1, 2] and performed calculations on different proteins for which experimental data are available [3, 4]. The obtained ensembles can be used to understand ligand binding and can also yield clues of selected catalytic mechanisms.

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P21

Coordination of actin-microtubule dynamics by DAAM formin

Péter Gaszler, Judit Viktória Fórizs, Andrea Teréz Vig, Beáta Bugyi

[1] Department of Biophysics, Medical School, University of Pécs, Pécs

The proper functioning of cells relies on the dynamic remodeling of their actin and microtubule cytoskeleton. Recent studies indicate that these cytoskeletal elements do not function individually, but their structure and dynamics are co-regulated by associated proteins. DAAM, the member of the formin family protein has a well-established role in actin dynamics regulation. It possesses two conserved formin homology domains (FH1-FH2), which catalyze actin nucleation and processively mediate filament elongation. In this work, we analyzed the possible role of DAAM in the coordination of actin-microtubule cytoskeleton by fluorescence spectroscopy, TIRF microscopy and protein biochemical approaches. We found in centrifugation-based sedimentation experiments, that DAAM can organize individual actin filaments, as well as microtubules into higher-order bundled structures. Interestingly, we found that DAAM can physically link together the actin and microtubule networks by visualizing single polymers in TIRF microscopy. We also investigated the importance of the different regions of DAAM in these activities. We showed that the conserved FH2 domain is sufficient and necessary for actin bundling, but it fails to bundle microtubules. The C-terminal elements, downstream to the FH domains are essential for microtubule bundling and the co-regulation of the two polymer networks. In conclusion, we identified a novel role of DAAM as a possible regulator of the functional coordination between the actin and microtubule cytoskeleton.

P22

Size-Dependent Impact of Silver Nanoparticles on Multidrug Resistant Breast Cancer Cells

Mohana Krishna Gopisetty[1], Dávid Kovács[1], Nóra Igaz[1], Zoltán Kónya[2,3], Imre Miklós Boros[1,4], Mónika Kiricsi[1]

[1]Department of Biochemistry and Molecular Biology, University of Szeged, Szeged; [2]Department of Applied and Environmental Chemistry, University of Szeged, Szeged; [3]MTA-SZTE Reaction Kinetics and Surface Chemistry Research Group, Szeged; [4]Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

Multidrug resistance (MDR) is defined as the ability of cancerous cells to evade structurally and functionally unrelated chemotherapeutic drugs, which phenomenon renders the general tumor therapy regimens ineffective. MDR is largely attributed to the overexpression of P-glycoprotein (Pgp) [1], a membrane localized ATP-driven efflux protein, member of the ATP-binding cassette (ABC) transporter family. Previous findings of our research group suggested the possible role of approximately 30 nm sized silver nanoparticles (AgNPs) in inhibiting ABC transporters in multidrug resistant Colo 320 colon cancer cells [2]. Therefore, the present work was designed to elucidate the size-dependent impact of AgNPs on Pgp overexpressing drug resistant MCF7-KCR breast cancer cells. Citrate coated quasi-spherical AgNPs of three different sizes (approx. 4 , 17 and 72 nm) have been synthesized and characterized by electron microscopy, dynamic light scattering and UV/VIS absorption. Their toxicity, Pgp inhibiting activity and the underlying molecular mechanisms have been examined in MCF7-KCR cells by viability assays, Western blotting and by

fluorescent staining methods. Our results revealed a marked difference in cytotoxicity as well as in Pgp inhibiting potential between differently sized AgNPs, as the largest AgNPs resulted to be the least toxic but showed the greatest inhibitory potential on efflux activity of Pgpcompared to small and medium sized AgNPs. JC-1 staining and cytochrome C release experiments indicated that the size-dependent inhibition of Pgp was not the direct result of mitochondrial dysfunction and of the toxicity induced by AgNPs. Detailed study of the mechanism behind the Pgp inhibition by these differently sized nanoparticles is our plan in future.

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P23

Selection of proBNP specific aptamers

Ákos Harkai[1], Zoltán Tolnai[1], Judit András[1], Anna Gyurkovics[1], Éva Nagyné Scholz[1], Zsuzsanna Szeitner[1], Tamás Mészáros[1,2]

[1]Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest; [2]MTA-BME Research Group for Technical Analytical Chemistry, Budapest University of Technology and Economics, Budapest

B-type Natriuretic Peptide (BNP, 32aa) is one of the most commonly used biomarkers for diagnosis of heart failure (HF). This biologically active peptide hormone originates from the myocardial tissue and influences the fluid and electrolyte homeostasis of blood, thus responsible for the balanced circulation [1]. It has been demonstrated by numerous clinical studies that not only BNP, but also the components of its biosynthetic pathway, such as proBNP (108aa) and NT-proBNP (76aa) are present at elevated levels in systemic circulation of HF patients [2, 3]. Presently, all BNP detecting methods rely on selective antibodies. We aimed at selecting proBNP specific DNA aptamers to aid development of novel BNP detecting devices. The word „aptamer" means fitting component, expressing the main feature of these single-stranded oligonucleotides, namely selective binding to target molecules ranging from small molecules to proteins. We use Systematic Evolution of Ligands by EXponential enrichment (SELEX) to select proBNP specific aptamers. The main feature of the applied toggle-SELEX is the alternation of target molecules during the selection procedure to increase the selectivity of obtained aptamers [4]. We used an N-terminal peptide of proBNP and *in vitro* translated, GST tagged proBNP protein as targets of selection. Following SELEX, we determined the sequences of 96 oligonucleotides and studied their sequences by *in silico* analysis. The results showed that there were more repeating aptamer sequences and molecular motifs as well, suggesting the success of selection. Currently, we carry out *in vitro* functional studies to verify the specificity and applicability of the selected aptamers.

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P24

ZBTB1 as a regulator of DNA damage tolerance

Lili Hegedűs, Kata Dudás, Lajos Haracska, Péter Burkovics

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

DNA damage can occur due to several exogenous and endogenous factors. When the cell cycle reaches the S phase, DNA lesions can block the replication fork because the replicative polymerase can not incorporate nucleotides opposite the damaged sites. This may lead to the generation of double-strand breaks, genomic instability, or cell death. The Rad18-dependent DNA damage tolerance (DDT) pathway can rescue the stalled replication fork because it can ubiquitylate the PCNA which induces the recruitment of a translesion (TLS) polymerase to the damaged site. Translesion polymerases can ensure the continuation of replication because they are able to insert nucleotides opposite the damaged sites. These polymerases frequently make errors and generate point mutations. PCNA can also be polyubiquitylated by the Mms2, Ubc13, and HLTF/SHPRH proteins initiating the template switch mechanism, in which the newly synthetized DNA strand serves as a template for replication. Recently, ZBTB1 was described as an upstream regulator of the Rad18-dependent DNA damage tolerance pathway. Its association with KAP1 leads to the relaxation of the chromatin at the damaged site, thus increasing the access of Rad18 and promoting the monoubiquitylation of PCNA. BRCA1 was also described as an upstream regulator of the DDT

pathway. It can recruit Rad18 and TLS polymerases to the damaged site, but the real function of BRCA1 in the regulation of Rad18 is unknown yet. In our study, we examined the regulatory role of ZBTB1 in the DDT pathway and its relationship with the BRCA1 protein. With localization studies and cell survival experiments, we proved that ZBTB1 and BRCA1 act together at the stalled replication fork and regulate the recruitment of Rad18 and polymerase η to the damaged site.

P25

Dynamic structural ensembles of the third PDZ domain of PSD-95

Anett Hinsenkamp, Zoltán Gáspári
Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest

PSD-95 plays a crucial role in organizing the dynamic protein network of the postsynaptic density. PSD-95 is composed of three PDZ, one SH3 and one GK domains. The third PDZ domain of PSD-95 has an additional alpha helix on its carboxy terminus. Although it is relatively far from the peptide binding site, truncating the helix leads to decreased ligand binding affinity. The change in binding affinity is believed to be a consequence of internal molecular allostery modulated via delocalized conformational entropy mechanism [1]. To understand the molecular process of allostery, it is essential to generate structural models that reflect the internal dynamics of the free and peptide-bound forms of the domain at the atomic level. Ensembles of the third PDZ domain of PSD-95 were calculated by molecular dynamics simulations restrained by backbone and side-chain order parameters derived from heteronuclear NMR relaxation data [1]. Our preliminary results confirm the importance of including both the backbone and side-chain dynamics in molecular representations. Detailed analysis of the ensembles with respect to possible allosteric routes will be presented. This work was supported by KAP15-082-1.2-ITK.

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P26

Cloning, expression and purification of the E1 and E2 components of the human alpha-ketoglutarate dehydrogenase complex for structural studies

Ágnes Hubert, Bálint Nagy, Zsófia Zámbó, Eszter Szabó, Vera Ádám-Vizi, Attila Ambrus
Department of Medical Biochemistry, Semmelweis University, Budapest

The human alpha-ketoglutarate dehydrogenase complex (hKGDHc) is a rate–limiting enzyme in the Krebs cycle and has clinical implications in hypoxia and various neurodegenerative disorders, including Alzheimer's disease. The atomic structure of the hE3 subunit and the subunit stoichiometry of the hKGDHc have already been revealed using recombinant subunits. However, the overall 3D architecture of the complex and its potential alterations under various pathologically relevant conditions (e.g. pathogenic mutations, acidosis) are still obscure due mainly to restricted amounts and purity of the isolated hE1 and hE2 components. The main objective of this project is to produce highly purified hE1 and hE2 in adequate amounts for cryo-electron microscopy (cryo-EM). Genes coding for hE1 and hE2 were codon-optimized for *E. coli* expression, cloned into a pET52b+ vector and expressed in *E. coli* BL21(*DE3*) with two or one Twin-Strep affinity fusion tags, respectively. Both hE1 and hE2 have been successfully expressed to the soluble phase in considerable amounts following expression optimization. hE2 was purified using affinity chromatography in a single step; the purified hE2 component exhibited a higher order oligomerization state (likely a 24-meric structure) as expected according to negative stain electron microscopy. The optimization of the hE1 purification is underway. This project is supported by the Hungarian Brain Research Program.

P27

Non-radioactive *in vitro* kinase activity determination by pIMAGO nanoparticles

Brigitta M. Kállai[1], Szilvia K. Nagy[1], Anna Gyurkovics[1], Tamás Mészáros[1,2]
[1]Semmelweis University, Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Budapest, [2]Research Group for Technical Analytical Chemistry of the Hungarian Academy of Sciences, Budapest

The plant mitogen-activated protein kinases (MPK) have an important role in the conversion of the incoming environmental signals into specific cell responses. The *in vitro* activity of these kinases is measured by using myelin basic protein (MBP) and radioactively labeled ATP. Recently, a nanoparticle-based phosphoprotein detection technique, termed pIMAGO was published as a reasonable alternative for kinase activity determination [1]. The nanoparticles are composed of dendrimers, which are functionalized with titanium ions and biotin groups. The titanium ions provide the selective binding to phosphate groups, while the biotin groups allow the signal amplification by different means of detection. The system was tested on a few mammalian protein kinases and their substrates, even in a multiplexed imaging method [1, 2]. The aim of our work was to prove the feasibility of pIMAGO in plant kinase research by using a panel of plant MPK substrates. First, we used the AtMPK9, which is activated

through intramolecular autophosphorylation as we previously demonstrated by using radioactive ATP [3]. Although the results obtained by using pIMAGO were in concert with our previous data, there was a significant background that complicated the evaluation. We optimized the system by testing different gel separation and transfer circumstances and manage to decrease the background and increase the reproducibility. Importantly, we could detect the phosphorylation of the MBP protein as well. Since none of the fluorescent dye-based phosphoprotein detecting methods is suitable for labeling of phosphoproteins of high pI value, the pIMAGO might be more generally applicable than most of the radioactivity-free protein kinase activity determining approaches. This work was supported by the OTKA NN-111085.

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P28

Proteomics examination of OSCC-specific salivary biomarkers in a Hungarian population

Éva Csősz[1], Péter Lábiscsák[1], Bernadett Jakob[1], Gergő Kalló[1], Miklós Emri[2], Ildikó Tar[3], Mária Fera[4], József Tőzsér[1], Csongor Kiss[5], Ildikó Márton[4]
University of Debrecen, Faculty of Medicine, Departments of [1]Biochemistry and Molecular Biology; [2] Nuclear Medicine; [5]Department of Pediatrics; University of Debrecen, Faculty of Dentistry, Departments of [3] Periodontology; [4]Restorative Dentistry, Debrecen

Oral squamous cell carcinoma (OSCC) accounts for about 90% of malignant oral lesions being the 6th most common malignancy worldwide. Diagnostic delay may contribute to dismal survival rate in many cases; therefore, there is a need for developing specific and sensitive biomarkers to improve early detection. Hungarian population occupies the top places of statistics regarding OSCC incidence, thus we aimed at finding potential salivary protein biomarkers suitable for the Hungarian population. In this study, 14 proteins previously reported in the literature as significantly elevated in saliva of OSCC patients were examined. In case of IL-1α, IL-1β, IL-6, IL-8, TNF-α and VEGF, the salivary concentrations were determined using Luminex-based multiplex immunobead assay. In case of catalase, profilin-1, S100A9/MRP14, CD59, galectin-3-bindig protein, CD44, thioredoxin and keratin-19 SRM based targeted proteomic method was developed and optimized, and the relative amount of the proteins was determined in the saliva of OSCC patients and controls. According to our results, the levels of IL-6, TNF-α, protein S100A9 and thioredoxin were significantly elevated in the saliva of OSCC patients compared to the controls. In order to verify the results obtained by Luminex and SRM analyses, ELISA experiments were carried out on a larger cohort of OSCC patients and controls, and the salivary concentration of IL-6, protein S100A9 and thioredoxin was determined. Based on the verification, IL-6 and protein S100A9 seems to be useful protein biomarkers for OSCC detection in the Hungarian population. This work was supported by the Hungarian Scientific Research Fund OTKA K105034.

P29

Cleaning and stabilizing the genome of Escherichia coli BL21 by genome-wide abolishment of mobile genetic elements

Kinga Umenhoffer[1], Gábor Draskovits[1], Ákos Nyerges[1], Bálint Csörgő[1], István Nagy[2], Balázs Bogos[1,3], Tamás Fehér[1], Edit Timár[1], Ildikó Karcagi[1], Csaba Pál[1], György Pósfai[1]
[1]Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged; [2]Sequencing Platform, Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged; [3]Theoretical Biology Unit, Institute for Integrative Biology, Department of Environmental System Science, ETH Zurich, Zurich, Switzerland

Aim: One of the major goals of synthetic biology is to engineer flexible and predictable biological cellular chasses for useful applications. The reduction of biological complexity might offer a solution for increased cellular stability and programmability, either through genome streamlining or the assembly of fully synthetic genomes with refactored sets of genes. In previous work, we reduced the Escherichia coli K-12 genome towards a simplified biological chassis and demonstrated the feasibility of systematic genome streamlining [1]. The reduced genome strain showed increased genome stability as a host, due to removal of prophages, IS elements and error-prone DNA polymerases [2, 3] . Building on this advance, our new aim was to rapidly refactor the genome of E. coli BL21(DE3), an industrially important organism, in a similar, but more efficient manner. **Results:** Removal of prophages has been achieved by directed genome shuffling between the clean-genome K-12 and BL21(DE3). Shuffling resulted in a hybrid genome strain retaining all the advantageous features of BL21. To inactivate all the

remaining IS elements, we took advantage of the robustness of Multiplex Automated Genome Engineering [4] combined with CRISPR/Cas9-aided selection. This novel genome editing workflow enabled rapid mass-inactivation of mobile IS elements, without a priori knowledge of their exact genomic location/direction. **Conclusions:** The engineered strain offers some advantageous properties for daily laboratory work and displayed excellent recombinant protein expression properties and increased genomic stability. This research was supported by State of Hungary OTKA PD 106 231.

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P30

Protein intrinsic disorder in the synaptome: a comparative study

Annamária Kiss-Tóth, Zoltán Gáspári
Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest

The synaptome, i.e. the proteins in the synapses form an intricate network both at the pre- and the postsynaptic sides. Understanding the organization and possible dynamic nature of this network requires detailed characterization of the set of synaptic proteins. In this study, we primarily focus on the extent and distribution of intrinsic protein disorder in these proteins. Besides providing linear motifs as recognition sites, longer disordered segments might confer the flexibility for structural adaptation of several scaffold proteins in the postsynaptic density. Care is taken to distinguish between genuinely disordered segments and possible oligomeric fibrillar motifs like coiled coils [1]. The analysis is done on multiple data sets in a comparative manner, besides the full synaptome [2], a subset with annotated function in synaptic transmission, as well as pre- and postsynaptic proteins are investigated separately. In addition, orthologous protein sets from great apes have also been selected for investigation. Protein sets used as reference include the full human proteome and the well-anotated human immunome [3]. This work was supported by KAP15-057-1.1-ITK.

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P31

Human SGBS preadipocyte cell line can serve as a model for beige differentiation

Ágnes Klusóczki[1], Endre Kristóf[1], Quang-Minh Doan-Xuan[2], Zsolt Bacsó[2], Péter Bai[3], László Fésüs[1]
[1]Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen; [2]Department of Biophysics and Cell Biology, University of Debrecen, Debrecen; [3]Department of Medical Chemistry, University of Debrecen, Debrecen

White adipose tissue is specialized for energy storage, brown adipocytes contain a high amount of mitochondria which express uncoupling protein 1 (UCP1) and its main function is thermogenesis and energy expenditure. Recent studies have discovered a second type of thermogenic fat cells, known as beige adipocytes, which are generated within the white adipose tissue in response to cold or adrenergic stimulation. The human Simpson-Golabi-Behmel syndrome (SGBS) preadipocyte cell line provides a unique and useful tool for studies of human adipocyte biology [1]. Irisin was discovered as a myokine, which induced a beige differentiation of subcutaneous white adipose tissue in mouse models and a beige adipogenic program in differentiating human primary subcutaneous preadipocytes [2]. BMP7 is a paracrine/autocrine mediator which shifts the preadipocytes to differentiate into classical brown adipocytes. Our results suggest that a previously described brown adipocyte differentiation protocol (long-term Rosiglitazone treatment) could be successfully used to induce browning of SGBS cells which follows very likely the beige pathway. Irisin treatment resulted in a significant upregulation of UCP1 and TBX1 genes in human SGBS adipocytes and did not influence the expression of the classical brown adipocyte marker, ZIC1. BMP7 moderately induced a classical brown phenotype in differentiating SGBS cells. The continuous Rosiglitazone or Irisin treatment can induce a beige phenotype in differentiating SGBS cells which contained more UCP1 protein and possessed a higher oxygen consumption rate than white cells. Thus, SGBS cells can be shifted into both white and beige adipocytes. In the future we aim to investigate if browning of SGBS cells can be maintained or may become masked in response to long-term differentiation.

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P32
Selenoglycosides: novel activators of protein phosphatase-1 and -2A

Zoltán Kónya[1], Bálint Bécsi[1,2], Andrea Kiss[1], László Szilágyi[4], Katalin E. Kövér[3], Ferenc Erdődi[1,2]
[1]Department of Medical Chemistry, [2]MTA-DE Cell Biology and Signaling Research Group, Faculty of Medicine, University of Debrecen, Debrecen; [3]Department of Organic Chemistry, [4]Department of Inorganic and Analytical Chemistry, Faculty of Science and Technology, University of Debrecen, Debrecen

Protein phosphatase-1 (PP1) and -2A (PP2A) are responsible for the dephosphorylation of more than 90% of the phosphoserine/threonine containing proteins, thereby they regulate numerous physiological processes. In many cases initiation of certain signaling events is coupled with PP1 and PP2A activation, therefore, identification of phosphatase activators may provide possibility to influence signalling pathways with „ drug candidate" molecules. We found that tetra-O-acetylation of seleno-glucopyranoses results in compounds which are able to increase the activity of both PP1 and PP2A. The goal of our present study was to further investigate the structural background of this phosphatase activation assaying 16 selenoglycoside derivatives. Benzyl-tetra-O-acetyl-1-seleno-ß-D-glucopyranoses increases PP1 and PP2A activity by 2-fold, however, substitution of –Br, or -CF_3 in the benzene ring led to more enhanced activation. Non-acetylated selenoglucopyranose and the acetylated ones with -NO2 substitution within the benzyl or pyridyl rings were without effect. Bromo-benzyl-tetra-O-acetyl-1-seleno-ß-D-glucopyranose inhibited the interaction of PP1c catalytic subunit with MYPT1 regulatory subunit, suggesting that activatory selenoglycosides may bind to the surface of PP1c which interacts with the consensus PP1c binding sequence present in many regulatory proteins. We verified the interaction of selenoglycosides with PP1 by microscale thermophoresis (MST) within the K_D range of 2.56-115 µM. In summary, we identified selenoglycosides as a family of compounds that might serve as possible „lead" molecules for the development of more effective phosphatase activators. This work was sponsored by OTKA K109249, K105459 and PD111715.

P33
***Leishmania tarentolae* as a platform for recombinant protein expression**

Klaudia Kopper[1], Judit András[2], Tamás Mészáros[2,3]
[1]University of Technology and Economics, Department of Applied Biotechnology and Food Science, Budapest; [2]Semmelweis University, Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Budapest; [3]Research Group for Technical Analytical Chemistry of the Hungarian Academy of Sciences, Budapest

Due to the rapidly increasing need for the production of eukaryotic recombinant proteins for scientific and therapeutic purposes, there is a great demand for expression systems, which can couple the high protein yield with mammalian-like posttranslational protein modifications. The eukaryotic protozoan parasite of the white-spotted wall gecko (*Leishmania tarentolae)* was used to develop the so called LEXSY protein expression system by Jena Bioscience. According to the manufacturer, the LEXSY satisfies the above mentioned requirements since the general glycosylation characteristics of *Leishmania* species are very similar to those of mammals. Further advantages of LEXSY are its bacterial-like cultivation and its ability to produce cytosolic, membrane and secretory proteins without the formation of inclusion bodies [1]. We aimed at producing the osteogenic human bone morphogenic protein 2 (BMP-2) and its antagonist (Noggin), two glycosylated, extracellular, human proteins by LEXSY. *Leishmania* holds a unique mRNA processing, which involves trans-splicing steps. The mRNA maturation depends on repetitive and homopolymeric intergenic regions [2]; therefore, the LEXSY expression vectors also must contain these elements. The repetitive sequences are prone for recombination, and according to our experience, the LEXSY vectors are instable in commonly applied bacterial host strains. We tested the Stable Competent *E. coli* cells (New England BioLabs) for propagation of LEXSY vector constructs and successfully isolated intact vectors for transfection of *Leishmania* cells. The transfection of cells is followed by either polyclonal or clonal selection of positive clones. Presently, we compare the efficiency of the two approaches to establish a straightforward protocol for protein expression by LEXSY. This work was supported by National Research, Development and Innovation Fund (VKSZ_14-1-2015-0004).

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P34

The SH3 domain of Caskin1 binds to lysophosphatidic acid suggesting a direct role for the lipid in intracellular signaling

Kitti Koprivanacz[1], Balázs Besztercei[1], Tünde Juhász[1], László Radnai[1], Balázs Merő[1], Orsolya Tőke[2], Judith Mihály[3], László Buday[1], Károly Liliom[1]

[1]Institute of Enzymology, [2]Institute of Organic Chemistry; [3]Institute of Materials and Environmental Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

„ Src homology 3" or SH3 domains consist one of the most common protein domains in signal transduction, generally characterized by their binding to prolin-rich sequences on other signaling proteins [1, 2]. Caskin1, a scaffold protein regulating cortical actin filaments and enriched in neural synapses in mammals [3], has an atypical SH3 domain, the prolin-rich interacting partner protein of it could not be identified yet. Based on previous reports that several SH3 domains are able to bind phospholipids [4], we sought for lipid interacting partners of the SH3 domain of human Caskin1. We investigated the signaling-born lysophospholipid mediators sphingosine-1-phosphate and lysophosphatidic acid [5, 6], well-known first messengers activating G protein-coupled receptors [7, 8], but with mostly unknown intracellular protein targets, as potential binding partners for this SH3 domain. Here, we provide evidence that the SH3 domain of human Caskin1 selectively binds to lysophosphatidic acid in a biphasic manner in vitro. The binding strength and stoichiometry depend on the association-state of the lipid, as well as on the molecular subspecies determined by its fatty acid moiety. This in vitro observed interaction allows enhanced binding of Caskin1's SH3 domain to membranes if lysophosphatidic acid accumulates locally due to elevated production in signaling, fulfilling a second messenger-like role for lysophosphatidic acid. Our results suggest that the SH3 domain of human Caskin1 is a lipid binding domain rather than a prolin-rich motif interacting domain.

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P35

Internal motion of the PDZ1-PDZ2 tandem of PSD-95 represented by dynamic structural ensembles

Bertalan Kovács, Zoltán Gáspári

Pázmány Péter Catholic University, Faculty of Information Technology and Bionics, Budapest

The PSD-95 (post synaptic density protein 95) is an intracellular protein found in the postsynaptic part of neurons. It plays an important role in organizing signal transduction processes. As a major scaffold protein, it anchors membrane receptor proteins in the postsynaptic membrane. Its first two PDZ domains, near the N-terminus, form a structurally and functionally independent subunit often referred to as a PDZ tandem. The PDZ1-PDZ2 tandem of PSD-95 has been proved to be rigid in its apo state; however, ligand binding to both domains induces elevated interdomain mobility [1]. The present study aims at the elucidation of the change in the internal motion of PDZ tandem upon ligand binding by means of dynamic structural ensembles. Structural ensembles are generated by restrained molecular dynamic simulation, where experimental NMR parameters [2], such as NOEs and Lipari-Szabo model-free order parameters (S^2) are imposed on the protein models. Detailed analysis of the ensembles is expected to shed light on the structural and dynamical rearrangements at the domain level leading to changes in interdomain mobility.

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P36

Rybp is required for the terminal differentiation of neural lineages

Gergo Kovacs, Eniko Sutus, Viktoria Szabo, Melinda K Pirity

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

Polycomb Group (PcG) family member Ring1 and Yy1 Binding Protein (Rybp) regulates diverse biological functions through both Polycomb-dependent and -independent mechanisms. We have previously reported that lack of Rybp causes early embryonic

lethality in mice and that a portion of heterozygotes does not have cerebellum, exhibit exencephaly and disorganized neurocortex. These studies demonstrated the essential *in vivo* role of Rybp in the development of the central nervous system (CNS). In this work, we used wild type *(rybp$^{+/+}$)* and *rybp null* mutant *(rybp$^{-/-}$)* mouse embryonic stem (ES) cells and differentiated them towards neural lineages *in vitro* with the purpose to uncover underlying molecular events that are responsible for the *in vivo* phenotypic changes. By using histological, gene expression and protein localization analyses we revealed that: *rybp$^{-/-}$* ES cells are able to differentiate to all three major germ layers and develop towards neural stem cells (NSCs) and neural precursors (NPCs), but they can not form matured neurons and glias. Furthermore, lack of *rybp* coincided with altered gene expression of key neural markers and transcription factors including Pax6 and Plagl1 pinpointing a possible transcriptional circuit among Rybp and these genes. Together, these findings support critical roles for Rybp in neural lineage commitment. Revealing the genetic underpinnings of differentiation has important implications not only for understanding neurological diseases, learning and memory but also for the possibility of neural repair through genetic reprogramming of non-neural cells to a neurogenic fate. This work was supported by TÁMOP-4.1.1.C-13/1/KONV-2014-0001 and TÁMOP-4.2.6.A-15/1-2015-0002.

P37

Investigation the protein family occurring most abundantly in the Drosophila sperm

Barbara Laurinyecz[1], Csilla Slezák[1], Viktor Vedelek[1], Attila Lajos Kovács[2], Gábor Juhász[2], Rita Sinka[1]

[1]Department of Genetics, University of Szeged, Szeged; [2]Department of Anatomy, Cell and Developmental Biology, Eötvös Loránd University, Budapest

Drosophila sperm reaches an extraordinary longevity with 1.8 mm at the end of spermatogenesis. A mature sperm contains acrosome, elongated nuclei and the long tail part, which consists of axoneme and two elongated mitochondrial derivatives. Elongation of spermatids is driven by the extension of mitochondria and the microtubule array surround them. Organization of the mitochondrial derivatives and the function of them in the mature sperms are not fully known. Proteomic studies revealed that the Sperm-leucyl-aminopeptidase (S-Lap) protein family, with all eight members of it, is an abundant constituent of the mature sperm, however, their functions are unknown [1]. We have identified mutations in four S-Lap genes with male-sterile phenotype and characterized them genetically and morphologically. We used the CRISPR-Cas9 method to induce mutation in the remaining four S-Lap genes. In spite of their similarity, there is no redundancy between the investigated S-Lap genes, and they should have important single role during spermatogenesis. We proved that the S-Lap proteins localized to the mitochondria and found that S-Lap mutants show mitochondrial structural abnormalities. We measured leucyl aminopeptidase activity and found that the lack of a single S-Lap protein does not affect the testis-specific enzyme activity, what confirms the assumption that the diverged members of the protein family gained new function during spermatogenesis. Funding: OTKA NF 101001, MTA Bolyai János Kutatási Ösztöndíj.

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P38

Inhibition profiling of retroviral protease inhibitors using an HIV-2 modular system

Mohamed Mahdi, Zsófia Szojka, János András Mótyán, József Tőzsér

Laboratory of Retroviral Biochemistry, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen

Retroviral protease inhibitors (PIs) are fundamental pillars in the treatment of HIV infection and AIDS. Currently used PIs are designed against HIV-1, and their effect on HIV-2 is understudied. Using a modular HIV-2 protease cassette system, inhibition profiling assays were carried out for protease inhibitors both in enzymatic and cell culture assays. Moreover, the treatment-associated resistance mutations (I54M-L90M) were introduced into the modular system, and comparative inhibition assays were performed to determine their effect on the susceptibility of the protease. Our results indicate that darunavir, saquinavir, indinavir and lopinavir were very effective HIV-2 protease inhibitors, while tipranavir, nelfinavir and amprenavir showed a decreased efficacy. I54M-L90M double mutation resulted in a significant This is the first study to characterize in-depth the susceptibility of HIV-2 protease to all of the inhibitors, using a standardized protocol that allows for the comparative analysis of results obtained from enzymatic assays and those measured in cell culture

P39

A Serine protease inhibitor is involved in cellular response upon UV irradiation

Hajnalka Majoros[1], Zsuzsanna Újfaludi[1], Zita Nagy[3], Barbara Nikolett Borsos[1], Viktória Vivien Hudacsek[1], Frederic Coin[3], Tibor Pankotai[1], Imre Miklós Boros[1,2]

[1]*Department of Biochemistry and Molecular Biology, University of Szeged, Faculty of Science and Informatics, Szeged;* [2]*Institute of Biochemistry, Biological Research Centre, Szeged;* [3]*Department of Functional Genomics and Cancer, Institute of Genetics and Molecular and Cellular Biology (IGBMC), Illkirch, France*

Ultraviolet light induced DNA damage response is an extensively studied process because improper or delayed repair of the errors could lead to cancerous malformations. Although several proteins involved in DNA damage repair have been identified, the entire repair cascade and the protein-protein interactions remain to be explored. In a pilot experiment, we identified a Serpin as one of the most dramatically upregulated gene in keratinocytes following UV irradiation. Serpin consist of a very heterogenic serine protease inhibitor protein that has several functions. However, their role in DNA repair has not been reported yet. To learn about the possible biological function of the Serpin of interest, we used immortalized keratinocyte (HkerE6SFM, HaCaT), melanoma (A375) and osteo-sarcoma (U2OS) cell lines. Firstly, we analyzed the expression of the examined gene in a time-dependent manner (2h, 8h, and 24h) upon UV irradiation. We found that both the mRNA and the protein levels of the studied Serpin family member increased upon UV irradiation. Additionally, the UV treatment in U2OS cells resulted in the transport of this specific Serpin from cytoplasm to the nucleus in a time-dependent manner (2-4h after UV treatment). Considering the DNA damages upon UV irradiation we tested whether the Serpin protein participates in the Nucleotide Excision Repair or it is involved in the regulation of any known DNA repair pathways. Applying oxidative stress the examined Serpin protein level was also elevated in the nucleus, but was not changed upon DNA double-strand break inductions. Additionally, with immunostaining and LacO-tethering techniques, we observed co-localization between the studied protein and the NER factor XPB (Xeroderma pigmentosum type B) protein in U2OS cells one and two hours after UV irradiation. We conclude that this Serpin protein could be a potential player in the UV and oxidative stress induced DNA repair responses. Fund: TÁMOP-4.2.2-08/1-2008-0001 and OTKA PD112118.

P40

Involvement of deubiquitylating enzymes in the maintenance of gut homeostasis in *Drosophila*

Navodita Maurice[1], Levente Kovács[1], Péter Deák[1,2]

[1]*Institute of Biochemistry, Biological Research Centre, HAS, Szeged;* [2]*Department of Genetics, University of Szeged, Szeged*

The gut of all Eumetazoans is a fascinating organ partaking in food digestion, metabolism and physiology modulation. The gut epithelium undergoes continuous renewal by the activity of intestinal stem cells and *Drosophila melanogaster* has long been a powerful genetic model to study stem cell biology. The pluripotent intestinal stem cells renew the midgut epithelium of *D. melanogaster* every 1–2 weeks [1]. ISCs produce enteroblasts which either differentiate into absorptive enterocytes or secretory entero-endocrine cells. Ubiquitylation is a post-translational modification in which ubiquitin is attached to the substrate protein by acting as a signal for protein degradation via proteasome and alters cellular location of proteins, affects their activity and modulates protein-protein interactions. Ubiquitylation is a reversible process kept in equilibrium by the ubiquitylating and deubiquitylating enzymes (DUBs) [2]. DUBs are proteases removing ubiquitins from the ubiquitylated substrates and participate in the functioning of several signaling pathways. Since studies on the role of ubiquitylation in regulation of *Drosophila* gut integrity have not been conducted yet we aimed a comprehensive study to track the involvement of deubiquitylating enzymes or DUBs in gut homeostasis maintenance. We used transgenic RNA interference targeting all the *Drosophila* DUBs and monitored gut leakage in adult females by using bromophenol blue as an indicator. Our DUBs screening results gave positive results with some lines. The study was funded by the Hungarian Research Fund K116372.

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P41

Structural background of the regulation of Abl family kinases by SH3 domain tyrosine phosphorylation

Balázs Merő[1], László Radnai[1], Ibolya Leveles[1,2], Orsolya Tőke[3], Gergő Gógl[4], Bálint Szeder[1], Gréta Kuzma[1], Anna Fekete[1], Anna Cserkaszky[1], László Nyitray[4], Beáta G. Vértessy[1,2], László Buday[1]

[1]*Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest;* [2]*Department of Applied Biotechnology and Food Sciences; Budapest University of Technology and Economics, Budapest,* [3]*Institute of Organic Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest;* [4]*Department of Biochemistry, Eötvös Loránd University, Budapest*

Src homology 3 (SH3) domains mediate protein-protein interactions in eukaryotes. They bind short, proline rich linear motifs within intrinsically unstructured regions of partner proteins. Phosphorylation at different conserved tyrosine residues of SH3 domains might regulate their interactions. In this work, we investigated the structural and functional consequences of tyrosine phosphorylation within the SH3 domains of Abl1 and Abl2 non-receptor tyrosine kinases *in vitro*. The N-terminal region of Abl kinases share a common domain architecture: one SH3 domain is followed by an SH2 and a kinase domain. The SH3 domain plays an important role in the autoinhibition of the kinase by binding to the linker region connecting the SH2 and kinase domains. Disruption of this interaction leads to kinase activation. This might be the result of tyrosine phosphorylation within the ligand binding groove of the SH3 domain. We successfully phosphorylated the SH3 domains of Abl1 and Abl2 *in vitro* that resulted in the inhibition of ligand binding. We also solved crystal structures of both phosphorylated SH3 domains at atomic resolution. By comparing these structures to their non-phosphorylated counterparts, no large scale structural rearrangements were observed. However, our crystal structures indicated a possible monomer-dimer equilibrium that might be regulated by tyrosine phosphorylation. Therefore, we investigated whether phosphorylation affects the oligomeric state of SH3 domains by size-exclusion chromatography and small angle X-ray scattering. Both methods confirmed the existence of a monomer-to-dimer transition upon phosphorylation. This, together with the inhibition of ligand binding might be important features in the activation mechanism of the full-length kinases *in vivo*. This work was supported by grants from the Hungarian Scientific Research Fund (OTKA K 83867) and from the Hungarian Academy of Sciences („LENDÜLET" and the MTA Postdoctoral Fellowship Program).

P42

Intrinsically disordered N-terminal tail of DR0550 Nudix hydrolase responsible for stress tolerance as well as RNA binding

Attila Mészáros[1], Ágnes Tantos[1], Tamás Horváth[1], Péter Tompa[1,2]

[1]Institute of Enzymology, Research Centre of Natural Sciences, Hungarian Academy of Sciences, Budapest; [2]Vlaams Institute for Biotechnology, Structural Biology Research Center, Institute of Enzymology, Brussel, Belgium

Proteins of the Nudix hydrolase superfamily are widely distributed among all classes of organisms and they have a typical pyrophosphatase activity [1]. One of the Nudix hydrolases, called DR0550 in *Deinococcus radiodurans* contains significant intrinsically disordered regions on the N- and C-terminus that are not present in non-extremophile homologues [2]. IDPs play a crucial role in many processes of living organisms due to their specific structure and ensuing specific features, including chaperone function [3]. DR0550 Nudix hydrolase was chosen to examine with the aim of shedding light on the potential chaperone function of its disordered segments. Our hypothesis is that we can measure significant differences between the stress-tolerance of the wild type and mutant enzymes due to the presumed intramolecular chaperon activity of the disordered tails. We created different mutants (delta-C, delta-N, N-term, scramled) and examined the stress-tolerance of the wild type and the mutant Nudix forms. It became evident that the C-terminal region is indispensible for the proper folding of the protein, but the N-terminal region can be removed without significant activity loss. To understand the molecular mechanism of the intramolecular chaperon activity the structures of the different protein constructs were investigated by many spectrofotometric techniques (CD, DSF, SAXS, FT-IR) as well as by bioinformatic analysis (DMD). We also found a new short linear motif on the N-terminal tail of the wild type Nudix which can bind RNA in contrast with the mutant forms of the protein. The interaction with RNA occurs via this new motif. In the future, we aim to investigate this motif to characterize protein-RNA interaction as well as shedding light its role in living cells. This work was supported by Hungarian-Korean Joint Laboratory Program for Biosciences.

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P43

Dissecting the chaperone activity of intrinsically disordered plant dehydrin ERD14 in vivo

Nikoletta Murvai[1], Bianka Szalaine Agoston[1,2], Zoltan Magos[1], Anna Felmery[1], Agnes Tantos[1], Beata Szabo[1], Denes Kovacs[3], Andras Lang[2], Andras Perczel[2], Lajos Kalmar[1], Peter Tompa[1,3]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2]Institute of Chemistry, Eötvös Loránd University, Budapest; [3]VIB Department of Structur-

al Biology, Vrije Universiteit Brussel, Brussels, Belgium

Intrinsically disordered stress proteins, such as Arabidopsis thaliana ERD14, can protect client proteins and cells under dehydration stress conditions. ERD14 enhances viability of E. coli cells following heat stress, primarily by protecting its proteome. In-cell NMR experiments show that this activity is associated with a largely disordered structural state, in which short sequence elements (K-segments) transiently sample helical conformations in vitro and engage in partner binding in vivo [1]. Deletion of these segments impairs cellular activity, whereas scrambling the entire sequence completely abolishes cell protection. We identified dozens of potential client proteins by mass-spectroscopy in cross-linking pull-down experiments, several of which are essential for cell viability. From these, ERD14 protects citrate synthase in vitro under the same stress conditions applied in vivo. Our data suggest that chaperone activity is compatible with structural disorder in vivo.

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P44

Curcumin and its analogue induce apoptosis in leukemia cells and have additive effects with bortezomib in cellular and xenograft models

Lajos István Nagy[1], Liliána Z. Fehér[1], Gábor J. Szebeni[1], Márió Gyuris[1], Róbert Alföldi[1], Béla Ózsvári[1], László Hackler Jr.[1], Árpád Balázs[1], Iván Kanizsai[1], László G. Puskás[1,2]

[1]AVIDIN Ltd., Szeged, Hungary; [2]Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged

Combination therapy of bortezomib with other chemotherapeutics is an emerging treatment strategy. Since both curcumin and bortezomib inhibit NF-κB, we tested the effects of their combination on leukemia cells. To improve potency, a novel Mannich-type curcumin derivative, C-150 was synthesized. Curcumin and its analogue showed potent anti-proliferative and apoptotic effects on the human leukemia cell line, HL60 with different potency but similar additive properties with bortezomib. Additive anti-proliferative effects were correlated well with LPS-induced NF-κB inhibition results. Gene-expression data on cell cycle and apoptosis related genes, obtained by high-throughput QPCR, showed that curcumin and its analogue act through similar signaling pathways. In correlation with in vitro results, similar additive effect could be observed in SCID mice inoculated systemically with HL60 cells. C-150 in a liposomal formulation given intravenously in combination with bortezomib was more efficient than either of the drug alone. As our novel curcumin analogue exerted anticancer effects in leukemic cells at submicromolar concentration in vitro and at 3 mg/kg dose in vivo, which was potentiated by bortezomib it holds a great promise as a future therapeutic agent in the treatment of leukemia alone or in combination.

P45

***Arabidopsis thaliana* protoplast transfection mediated by polyethylenimine (PEI) nanovector**

Szilvia Krisztina Nagy[1], Tamás Mészáros[1,2]

[1]Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest; [2]MTA-BME Research Group of Technical Analytical Chemistry, Budapest

Transient gene expression in eukaryotic cells is a valuable alternative of establishment of stable protein overexpressing cell lines. This approach is often advantageous since the proteins of interest can be obtained in a relatively simple and rapid manner. Transformation of cells with exogenous DNA is indispensable step of this process. Polyethylenimine (PEI) is one of the most common reagents for transfections due to its simple use and high transfection efficiency. Similarly to many transfection reagents, PEI forms nanoparticles upon addition of DNA constructs and penetrates the cell membrane. PEI is commonly applied for transformation of various eukaryotic cells, but only a single study was carried out to investigate its applicability for transfection of plant protoplast cells [1]. This demonstrated that PEI can effectively transfer DNA into protoplasts. Most of the plant sciences laboratories use the well-established PEG-mediated protoplast transformation method to perform transient expression [2]. Here, we present a comparative study to assess the effectiveness of these two protoplast transforming methods. We used Columbia (Col-0) *Arabidopsis thaliana* root-cell suspension culture derived protoplast for our analysis. We prepared PEI/DNA nanovectors using different ratios of PEI (with molecular weight of 25 kDa) and GFP expressing plasmid (pBSK-GFP). We compared the transfection efficiencies by observing the fluorescence signal and counting the transformed protoplasts. According to the obtained results, PEI-based transformation is a rational alternative of the generally applied PEG-based protoplast transfection. This work was supported by the OTKA NN-111085.

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P46

Genomic determinants of molecular phenotype differences between B-lymphobastoid cells of a CEU trio

Lilla Ozgyin[1], Attila Horváth[2], Bálint L. Bálint[1]

[1]Genomic Medicine and Bioinformatics Core Facility, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen; [2]MTA-DE Lendület Immunogenomics Research Group, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen

In our research, we are focusing on the extent of variability in different molecular phenotypes including genome-wide active histone signal, Pax-5 master regulator transcription factor binding and steady-state mRNA levels in connection with personal genomic variability. Our model cells are immortalized B-cells from an apparently healthy Utah trio of European origin. Making use of our in-house ChIP-seq and RNA-seq data and publicly available personal genome sequences we found that differentially occupied cis regulatory sites (i.e. enhancers) are rarely affected by motif-disrupting SNPs either in Pax-5, or in collaborating transcription factor motifs (0.4% of Pax-5 peaks). However, we found that the loss or gain of both Pax-5 and active histone mark peaks correlate at larger regions (average size is 206 kb) with clear boundaries. These regions are associated with topologically associated domains and partially overlap with super-enhancers. Also, differential Pax-5 binding results in differential histone acetylation and a corresponding change in the expression of one or more proximal genes. Allele-specific binding analysis using heterozygous single nucleotide variants showed that in case of binding loss, most signals come from one parental allele. By applying genome-wide technologies we have the necessary tools to extend our understanding of molecular phenotype differences between individuals, thus paving the way for precision medicine. The project is funded by an Internal Research University Grant. BLB is a Szodoray fellow at the Medical Faculty of the University of Debrecen.

P47

The role of TAF10/TAF10b containing complexes in *Drosophila melanogaster*

Zoltan Pahi[1], Zsuzsanna Kiss[1], Orbán Komonyi[1], Barbara N. Borsos[1], Laszlo Tora[3], Imre M. Boros[1,2], Tibor Pankotai[1,2]

[1]Department of Biochemistry and Molecular Biology, University of Szeged, Szeged; [2]Institute of Biochemistry, Biological Research Centre, HAS, Szeged; [3]Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France

In eukaryotes, the TFIID complex is required in preinitiation complex assembly for positioning the RNA polymerase II around the transcription start sites of different core promoters. On the other hand, histone acetyltransferase complexes like SAGA and ATAC, modulate transcription at several steps through modifications of specific core histone residues. In this study, we investigated the function of *Drosophila melanogaster* (d)TAF10 and TAF10b proteins, which are subunits of dTFIID and dSAGA, respectively. We generated a mutation which eliminated the production of both *Drosophila* TAF10 orthologues. The simultaneous deletion of the two *dTaf10 and dTaf10b* genes impairs the recruitment of a dTFIID subunit dTAF5 to polytene chromosomes, while binding of another TFIID subunit, dTAF1 and RNAPII seems not to be affected. The lack of both dTAF10 and dTAF10b proteins results in failures in the larval-pupal transition during metamorphosis and in the transcriptional reprogramming at this developmental stage. Although the dTAF10b is part of dSAGA complex, the microarray analysis showed that mutations either in dATAC subunit (*dAada2a^{d189}*) or in *dTaf10* genes resulted in similar changes in the steady state mRNA levels. This observation suggests that dTAF10/dTAF10b containing complexes and dATAC take part in similar biological pathways. Importantly, the phenotype resulting from *dTaf10*+*dTaf10b* mutation can be rescued by ectopically added ecdysone, suggesting that dTAF10- and/or dTAF10b- containing complexes are involved in the expression of ecdysone biosynthetic genes. Our data support the idea that the presence of dTAF10 proteins in dTFIID and/or in dSAGA is required only at specific developmental steps. We propose that distinct forms of dTFIID and/or dSAGA exist during Drosophila metamorphosis, wherein the different TAF compositions could serve in targeting the RNAPII at different developmental stages and tissues.

P48

Alterations of signaling pathways in bone tissue of PACAP KO mice

Andrea Pálfi[1], Vince Szegeczki1, Bernadett Czibere[1], Gergő Józsa[2], Balázs Fülöp[2], Andrea Tamás[2], Dóra Reglődi[2], Róza Zakány[1], Tamás Juhász[1]

[1]Department of Anatomy, Histology and Embryology, Faculty of Medicine, University of Debrecen, Debrecen; [2]Department of Anatomy, PTE-MTA „Lendület PACAP Research Team", Pécs

PACAP (pituitary adenylate-cyclase activating polypeptide) is a 38 amino acid long neuropeptide, present in nervous system and various peripheral tissues, including bone. We found an enhancing effect of PACAP in osteogenesis in UMR-106 cells. In contrast with this, the macroscopical skeletal morphology of PACAP KO mice does not exhibit any significant alterations. The role of Notch signaling in bone formation is well established; therefore we aimed to investigate a presumable connection of PACAP-Notch signaling pathways both in PACAP deficient mice and in UMR-106 cell line. We administrated PACAP 1-38 (100 nM) as a PAC1 receptor agonist and DAPT (5 μM) as a Notch inhibitor to the medium of cell cultures. In the bones of PACAP KO mice, lower calcification was detected with Alizarin red and von Kossa staining, although the expression and activity of alkaline phosphatase was higher than in the bones of control animals. Expression of Notch receptors was decreased in KO mice, but the protein level of ligands (DLL1, DLL3, DLL4, Jagged1) was significantly elevated. Similar elevation was observed in the protein expression of Notch-target molecules (TACE, Numb, CSL and Adam9). In UMR-106 cells, in vitro model of ossification, the protein expression of Notch 1 and 2 receptors elevated in the presence of DAPT. The protein expression of Notch ligands (DLL1, Jagged1) and target molecules (TACE, Numb) increased by PACAP treatment. The transcription factor NFATc1 can be a target of both Notch and PACAP signaling, its expression showed an elevation after PACAP administration. Our results suggest that Notch and PACAP signaling pathways are in connection in bone, but exploration of their exact role in bone formation requires further experiments. Supported by: TÁMOP 4.2.1.B-10/2/KONV-2010-002, GOP-1.1.1-11-2012-0197, MTA-PTE „Lendület", RH/751/2015. T.J. was supported by Bolyai János Scholarship, Szodoray Lajos Fund.

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Interaction of TIMAP-PP1c complex with merlin

Anita Boratkó, Margit Péter, Csilla Csortos

Department of Medical Chemistry, Faculty of Medicine, University of Debrecen

TIMAP (TGFβ-inhibited membrane-associated protein) is a highly expressed protein in endothelial cells compared to other cell types. The interaction of TIMAP with the catalytic subunit of protein phosphatase 1 (PP1c) was characterized, and we showed, that TIMAP is involved in the endothelial barrier maintenance through the regulation of ERM (ezrin-radixin-moesin) phosphorylation level. Merlin (moesin-ezrin-radixin like protein), which was primarily recognized as a tumor suppressor, shares its domain organization with the ERM proteins. Ser518 phosphorylation of merlin modulates its intra- and intermolecular associations and, consequently, the biological activity of the protein. Phosphorylated merlin is thought to have an open form, while the dephosphorylated protein is presumably in a closed conformation. We identified a protein-protein interaction between TIMAP-PP1c and merlin using pull-down and identified the FERM domain of merlin and the N-terminal part of TIMAP as critical regions of the interaction. In endothelial cells, co-immunoprecipitation of merlin with TIMAP-PP1c was also shown, but there was no detectable interaction between merlin and PP1c in TIMAP depleted cells. Furthermore, we detected that TIMAP is able to bind phospho-Ser518-merlin. To test whether PP1c is responsible for dephosphorylation of merlin, endothelial cell lysates were treated with specific phosphatase inhibitors. In the presence of tautomycetin, a potent PP1 inhibitor, the phosphorylation level of merlin was elevated implying that a type-1 phosphatase dephosphorylates phospho-Ser518 merlin. Results of immunofluorescent staining and subcellular fractionation of TIMAP depleted and non-siRNA treated endothelial cells strongly suggest that the TIMAP-PP1c complex regulates dephosphorylation of phospho-Ser518-merlin. This work was supported by grant PD116262 from the Hungarian Science Research Fund (AB).

P50

Blocking Chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) is protective in experimental Crohn's disease: proinflammatory role of eosinophils

Balázs Radnai[1,2], Eva Maria Sturm[2], Angela Jačan[2], Katharina Jandl[2], Sandra Labocha[3], Nerea Ferreirós[3], Magdalena Grill[2], Carina Hasenoehrl[2], Gregor Gorkiewicz[4], Günther Marsche[2], Ákos Heinemann[2], Christoph Högenauer[4], Rudolf Schicho[2]

[1]Institute of Biochemistry and Medical Chemistry, Medical School, University of Pécs, Pécs; [2]Institute of Experimental and Clinical Pharmacology, Medical University of Graz, Graz, Austria; [3]Institute of Clinical Pharmacology, Goethe University, Frankfurt/Main, Germany; [4]Institute of Pathology, Medical University of Graz, Graz, Austria; [5]Division of Gastroenterology and Hepatology, Department of

Internal Medicine, Medical University of Graz, Graz, Austria

Aims: In our previous study [1] we investigated the role of prostaglandin D_2 (PGD_2) receptor CRTH2 in experimental ulcerative colitis (UC) and in UC patients and we found proinflammatory characteristics of CRTH2. In the current report, we aimed to clarify the role of CRTH2 in experimental Crohn's disease (CD; induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS) in mice) and in CD patients. **Results:** Compared to control subjects, we measured elevated serum concentrations of PGD_2 and Δ^{12}-PGJ_2, and higher amounts of CRTH2 in the colonic tissue of CD patients. The findings were suggestive of a critical involvement of the PGD_2/CRTH2 axis in human CD. Both in CD patients and in TNBS-colitic mice, we detected CRTH2-positive cells, including eosinophils in the mucosa of inflamed colon. The CRTH2 antagonist OC-459 protected against TNBS-induced colitis in mice, and it reduced inflammation scores and proinflammatory cytokine (TNF-α, IL-1β, IL-6) production. In contrast, MK0524, an antagonist of the second PGD_2 receptor, D-type prostanoid receptor (DP), failed to do so. By measuring migration of eosinophils upon OC-459 treatment, we found a reduced influx to the colon *in vivo*. Chemotaxis induced by CRTH2 agonism in human eosinophils *in vitro* was inhibited, suggesting a prominent proinflammatory role for CRTH2-expressing eosinophils in colitis. To get a clear picture of the role of eosinophils in experimental CD, we used eosinophil-depleted ΔdblGATA knockout mice and IL-5 transgenic mice with eosinophilia in the same model. ΔdblGATA KO mice were found to be less sensitive to colitis, however, IL-5 transgenic mice showed more severe signs of inflammation as compared to their wild type littermates. **Conclusion:** CRTH2 might be a new pharmacological target in CD therapy and may act at least partially via CRTH2-positive eosinophils, which seem to play a significant proinflammatory role in this disease. **Acknowledgement:** This work was supported by the Austrian Science Fund FWF P25633.

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P51

PARP-1 downregulation protects macrophages from H_2O_2 induced cell death

Zsolt Regdon, László Virág
Department of Medical Chemistry, University of Debrecen, Faculty of Medicine, Debrecen

Macrophages (MFs) represent a heterogeneous group of cells of the innate immune system. Their main functions cover but are not limited to first response to pathogens, induction and resolution of inflammation, clearing of pathogens and cell debris and maintaining tissue homeostasis. Upon LPS stimulation MFs display increased migration, phagocytosis and cytokine release. Poly(ADP-ribose) polymerases (PARPs) are a family of enzymes catalyzing ADP-ribose transfer to target proteins. Posttranslational modification of these proteins results in modulation of chromatin structure, transcription, replication and DNA repair. PARP-1 also acts as a cofactor of NF-κB, the master regulator of inflammation. Oxidative stress-induced PARP-1 hyperactivation may lead to cell dysfunction and necrotic cell death, as demonstrated in various cellular systems. Here, we have investigated the possible role of PARP1 in oxidative stress adaptation of MFs in RAW264.7 macrophage-like cell line and primary mouse bone marrow-derived macrophages (BMDM). Preconditioning of cells with LPS attenuated secretion of TNF-α following a secondary LPS stimulus. Furthermore, LPS preconditioned MFs proved more resistant to H_2O_2 induced cell death (as indicated by MTT and LDH assays). Moreover, we observed that LPS preconditioning resulted in downregulation of PARP1 (mRNA and protein), which is likely to be the underlying mechanism for both observations. Experiments are ongoing to establish the causal link between PARP-1 downregulation and altered responses of LPS-preconditioned macrophages. We also plan to identify the molecular mechanism of LPS-induced PARP-1 downregulation.

P52

Consequences of loss of retinol saturase enzyme in mice

Tibor Sághy, Zsolt Sarang, Zsuzsa Szondy,
Signaling and Apoptosis Research Group, Division of Dental Biochemistry, Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen

Retinoids acting via retinoid (RAR) and retinoid X (RXR) receptors have been reported to modulate several functions of mononuclear phagocytes. Previously, we have found that retinoids also enhance clearance of apoptotic cells. We have also reported that engulfing macrophages express retinoic acid producing RALDH enzymes and are capable of producing retinoids, most likely dihydro-RA derivative. In apoptosing thymus and in bone marrow macrophages, following LXR agonist or ATRA treatment, we detected increased expression of the retsat enzyme. Using knock out mouse strain we found that retsat deficient mice develop splenomegaly at old age and produce anti-nuclear and anti-DNA antibodies. In the spleen of the KO mice we detected increased number of active caspase 3 positive cells indicating that clearance of dead cells might be

delayed. Our results indicate that dihydro-retinoids might be involved in clearance of apoptotic and loss of retsat enzyme leads to development of autoimmune disease.

P53

The role of miR-212/132 and calcineurin axis in uremic left ventricular hypertrophy and dysfunction

Márta Sárközy[1], Márton Pipicz[1], Renáta Gáspár[1], Gergő Szűcs[1,2], Kamilla Gömöri[1], Andrea Siska[3], Imre Földesi[3], Krisztina Kiss[4], Péter Bencsik[4], Nóra Zsindely[5], László Bodai[5], Csaba Csonka[1], Thomas Thum[6], Sándor Bátkai[6], Tamás Csont[1]

[1]Metabolic Diseases and Cell Signaling Group, Department of Biochemistry, Faculty of Medicine, University of Szeged, Szeged; [2]Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Szeged; [3]Department of Laboratory Medicine, Faculty of Medicine, University of Szeged, Szeged; [4]Cardiovascular Research Group, Department of Biochemistry, Faculty of Medicine, University of Szeged, Szeged; [5]Department of Biochemistry and Molecular Biology, University of Szeged, Szeged; [6]Hannover Medical School, IMTTS, Hannover, Germany

Background: The prevalence of uraemia is continuously increasing in developed countries. Uremic cardiomyopathy characterized by left ventricular hypertrophy and diastolic dysfunction is a common cardiovascular complication of uraemia; however, the underlying molecular mechanisms are not clear. The miR-212/132 cluster has already been implicated in the development of left ventricular hypertrophy via modulation of the calcineurin pathway in TAC mice. **Purpose:** Therefore, here we investigated the effect of uraemia on the myocardial expression of miR-212/132 cluster and the calcineurin pathway. **Methods:** Uraemia was induced by 5/6 nephrectomy in male Wistar rats. Eight weeks later serum urea and creatinine levels were measured and transthoracic echocardiography was performed. Then, RNA was isolated from left ventricles of nephrectomised and sham-operated rats and expression of miR-212 and miR-132, as well as components of the calcineurin pathway including atrogene-1 and MCIP1.4 was measured by qRT-PCR. **Results:** In the nephrectomised group, serum urea and creatinine levels were significantly higher proving the development of uraemia. In the uremic group, left ventricular anterior and septal walls were significantly thicker, e' was significantly decreased and E/e' was significantly increased referring to left ventricular hypertrophy and diastolic dysfunction. In the uremic group, heart weight/body weight ratio was also significantly elevated as compared to the control group. In the uremic group, miR-212 was significantly overexpressed. Moreover, atrogene-1 showed significant down-regulation and MCIP1.4 showed significant up-regulation in the uremic group. **Conclusions:** Myocardial overexpression of miR-212 and subsequent modulation of the calcineurin pathway might play a role in the development of uraemia induced cardiac hypertrophy and diastolic dysfunction.

P54

Ncb5or flavoheme reductase – a potential alternative entry from the cytosol into microsomal electron transfer chains

Farkas Sarnyai[1], Veronika Zámbó[1], Éva Kereszturi[1], Mónika Tóth[1], Gábor Lotz[2], Miklós Csala[1]

[1]Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest; [2]Second Department of Pathology, Semmelweis University, Budapest

Cytochrome b5 (b5) and Cyb5 reductase (b5R) are integral membrane proteins of the endoplasmic reticulum (ER). They contain heme and FAD prosthetic groups, respectively, and they channel the electrons of cytosolic NAD(P)H towards microsomal monooxygenase enzymes involved in acyl-CoA desaturation and drug metabolism. NADH cytochrome b_5 oxidoreductase (Ncb5or) possesses two redox domains, which are homologous to b5 and b5R, but the protein lacks any membrane anchor. On the basis of the domain structure and the marked alterations in lipid metabolism found in Ncb5or (-/-) mice it was proposed that the soluble enzyme might be involved in fatty acid desaturation. However, the cytosolic or ER localization of the protein has not yet been unequivocally elucidated, and hence it was ambiguous whether the enzyme utilizes cytosolic or microsomal NADPH. Computational tools were used to identify any ER-targeting signals in human Ncb5or and to predict the subcellular localization of the protein. None of the applied tools revealed signal peptide or ER-retention signal in the polypeptide, and the most likely topology of Ncb5or protein was unequivocally predicted to be cytoplasmic. Green fluorescent EGFP-Ncb5or fusion protein was expressed in transiently transfected human HEK293T cells and detected by fluorescent microscopy. The location of endogenously expressed Ncb5or was assessed in HEK293T cells by two methods. Cells were harvested and homogenized to separate the subcellular fractions by differential centrifugation. Ncb5or and specific marker proteins of various cellular organelles were detected by Western blot. In addition, the endogenous protein was also visualized by using in vitro immunocytochemistry. Subcellular fractions of rat livers were also isolated and analyzed for the presence of Ncb5or protein. Purity of the generated cell fractions was confirmed by immunoblot with characteristic

marker proteins of the organelles. Ncb5or could only be detected in the cytosolic fractions of HEK293T cells and rat livers by using Westren blot. EGFP-Ncb5or fusion protein was detected in the cytoplasm of the cells, and it was not co-localized with fluorescent markers labeling either the nucleus or the ER. Similar location of endogenous Ncb5or protein was observed by immunocytochemistry. Our results clearly prove that Ncb5or is located in the cytoplasm in cultured cells and in liver *in vivo*. Therefore, the utilization of ER luminal reducing equivalents by this enzyme can be ruled out. Further research is needed to confirm the putative role of Ncb5or in fatty acid desaturation, which in turn will help to understand the contribution of this novel protein to the protection of pancreatic β-cells against lipotoxicity.

P55

Adaptive evolution of complex innovations through stepwise metabolic niche expansion

Balázs Szappanos[1], Jonathan Fritzemeier[2], Bálint Csörgő[1], Viktória Lázár[1] , Xiaowen Lu[3] , Gergely Fekete[1] , Balázs Bálint[4] , Róbert Herczeg[4] , István Nagy[4,5] , Richard A. Notebaart[3] , Martin J. Lercher[2], Csaba Pál[1], Balázs Papp[1]

[1]Synthetic and Systems Biology Unit, Institute of Biochemistry, Biological Research Centre of the Hungarian Academy of Sciences, Szeged; [2]Department for Computer Science, Heinrich Heine University, Düsseldorf, Germany; [3]Department of Bioinformatics (CMBI), Radboud University Medical Centre, Nijmegen, The Netherlands; [4]SeqOmics Biotechnology Ltd, Mórahalom; [5]Sequencing Platform, Institute of Biochemistry, Biological Research Centre, Hungarian Academy of Sciences, Szeged

A central challenge in evolutionary biology concerns the mechanisms by which complex metabolic innovations requiring multiple mutations arise. Here, we propose that metabolic innovations accessible through the addition of a single reaction serve as stepping stones towards the later establishment of complex metabolic features in another environment. We demonstrate the feasibility of this hypothesis through three complementary analyses [1]. First, using genome-scale metabolic modeling, we show that complex metabolic innovations in Escherichia coli can arise via changing nutrient conditions. Second, using phylogenetic approaches, we demonstrate that the acquisition patterns of complex metabolic pathways during the evolutionary history of bacterial genomes support the hypothesis. Third, we show how adaptation of laboratory populations of Escherichia coli to one carbon source facilitates the later adaptation to another carbon source. Our work demonstrates how complex innovations can evolve through series of adaptive steps without the need to invoke non-adaptive processes. Supported by the „Lendület" Program of the Hungarian Academy of Sciences and The Wellcome Trust (B.P. and C.P.), European Research Council (C.P.), the Hungarian Scientific Research Fund PD 109572 (B.C.), 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" (B.S.).

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P56

Spiegelmer development for cardiac troponin diagnostics

Zsuzsanna Szeitner[1], Anna Doleschall[1], Gergely Lautner[2], Róbert Gyurcsányi[2], Katalin Keltai[3], Marina Varga[4], Tamás Mészáros[1]

[1]Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest; [2]Department of Inorganic and Analytical Chemistry, University of Technology and Economics, Budapest; [3] Kútvölgyi Health Center and Hospital, Semmelweis University, Budapest; [4]Department of Transplantation and Surgery, Semmelweis University, Budapest

In recent years, antibodies, the most generally applied receptor molecules in protein detecting devices, have been rivaled by appearance of short single stranded oligonucleotides with discriminative molecular recognition and binding capacity. These are superior to antibodies in many ways; they are in vitro selected, chemically synthesized and insensitive to chemical and physical conditions. However, their use is hampered due to their susceptibility to enzymatic degradations. Spiegelmers are artificial oligonucleotides named for being a mirror image of natural D-oligonucleotides. Due to their L-nucleotides, they are highly resistant to nuclease degradation and currently being tested in clinical trials as potential drugs. We aimed at producing human cardiac troponin I (cTnI) specific oligonucleotides to provide alternative receptors for biosensor development. The elevated troponin I levels are highly specific for cardiac injury, thus various antibody based systems have been developed for fast and sensitive detection of myocardial infarction. Our results suggest that protein selective Spiegelmers can be effectively selected by rational identification of protein epitopes and high-throughput screening of isolated candidates. The results of surface plasmon resonance measurements demonstrated that the characterized oligonucleotide binds to cTnI with low nanomolar affinity. To test the applicability of

the selected Spiegelmer in sandwich ELISA based assays, we developed an Amplified Luminescent Proximity Homogenous Assay using our receptor and a commercial cTnI selective antibody. The obtained data corroborated our assumption; the developed Spiegelmer could detect purified human troponin complex and recombinant troponin I protein even in such a complex protein matrix as blood serum. These findings indicate that Spiegelmers could be reasonable alternatives of antibodies in diagnostics. Funding: National Research, Development and Innovation NKFIA-VKSZ_14-1-2015-0004 grant.

P57

Regulation of de- and remyelination in the central nervous system

Gabor T. Szilagyi[1], Arkadiusz M. Nawrocki[2], Janos Schmidt[1], Ferenc Gallyas Jr.[1], Zsolt Illes[2]

[1]Department of Biochemistry and Medical Chemistry, University of Pécs, Medical School, Pécs; [2]Department of Neurology, University of Southern Denmark, Odense, Denmark

Despite of substantial basic and clinical research, two major questions are still elusive in the pathogenesis of multiple sclerosis (MS); (i) why susceptibility of oligodendrocyte subsets to death stimuli varies, and (ii) why oligodendrocyte precursor cells in affected areas fail to replace eliminated oligodendrocytes. To answer these questions, we performed proteomic analysis in a cuprizone-induced demyelination model, the degenerative animal model of MS, after 4 weeks of demyelination, and 2 or 14 days of remyelination. We performed nano-liquid chromatography coupled nano-electronspray ionisation mass spectrometry analysis after isobaric tags for relative and absolute quantitation labeling of lysyl endopeptidase-digested peptides from corpus callosum proteins. We were able to identify about 6000 proteins, out of which 828 changed its steady state level and/or post-translational modification status during de- and remyelination. To indicate the importance of kinase signaling mechanisms of these processes, protein phosphorylation was found to be the most substantial among the observed changes. Additionally, we found significant differences between the groups among proteins involved in apoptosis inducing factor- and caspase-mediated apoptotic processes, and negative regulation of nuclear factor kappa B. Presently we are comparing the results on the cuprizone model with liquor proteomics data of 98 patients with MS. We are performing pathway analyses on the significant proteins common in the two systems and also aim to identify pathogenesis-related biomarkers in MS. This study was supported by grants from OTKA NN-109841 and the Lundbeck Foundation.

P58

Application of SV40 large T antigen-based *in vitro* replication for monitoring translesion DNA synthesis in cellular extracts

Zoltán Szeltner, Ádám Póti, Dávid Szüts

Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest

DNA damage tolerance via translesion synthesis is important in bypassing DNA lesions during replication and contributes to genome stability. In this study, we tested the applicability of an SV40 large T antigen-based in vitro replication system to the investigation of replicative lesion bypass. Here we show that plasmids containing synthetic cys-syn cyclobutane pyrimidine dimer photoproducts are replicated with equal efficiency to lesion-fee plasmids in vitro. By applying both Sanger- and new generation sequencing we demonstrate that translesion synthesis is a frequently used lesion bypass pathway in soluble cytosolic extracts of Hela cells, providing almost completely error-free bypass on cys-syn cyclobutane pyrimidine dimer lesions. This suggests the involvement of polymerase η in the process. Acknowledgement: Momentum Grant of the Hungarian Academy of Sciences LP2011-15.

P59

Drosophila as a new tool to study the chromatin structural changes activated by DNA damages

Diána Szűcs, Árpád Varga, Evelin Viszinger, Ivett Berzsenyi, Bence Ollé, Barbara Nikolett Borsos, Zoltán Gábor Páhi, Hajnalka Majoros, Zsuzsanna Újfaludi, Tibor Pankotai

[1]Genome integrity and DNA repair Group, Department of Biochemistry and Molecular Biology, University of Szeged, Szeged

In eukaryotic cells, any processes which involve DNA have to take place in the context of chromatin structure, which affects the probability of the damaging agents to cause DNA breaks and the recruitment of the repair proteins. The improper repair or persistence of breaks leads to genome instability, which could result in tumor formation. Our goal is to understand what makes cells able to recognize the appearance of DNA break and how the chromatin structure could change around the break. The answers to these questions will provide information on whether specific chromatin structures predispose sites for DNA break and whether memory of previous break is retained in the chromatin structure. We started to setup human cell culture-based and Drosophila *in vivo* experimental systems by which we could study how unique histone post-translational modifications (PTMs) could affect the DNA repair. We take advantage of the *Drosophila* model system where we delete the endogenous histone cluster

and we substitute it with mutant histones which permits or mimics unique histone PTMs. We have already started to mutate histone genes and screen 50 different histone PTMs. We will use these flies to check the DNA repair kinetics in those animals which consist of only the mutated histones. Using the Drosophila and the human cell culture based system we have already identified new H3 and H4 histone PTM candidates that play role in chromosomal rearrangement which could influence the DNA repair processes. The system developed in our laboratory would help in understanding the mechanisms, which give rise to frequent chromosomal break points often detected in tumors. Progress in integrating the chromatin dimension in DNA repair will help to understand how DNA damage may impact on genome stability. These results would also help identifying new key targets in DNA damage repair and the final goal of the project is to find potential biomarkers which could be used in anticancer therapies. Supported by OTKA-PD [112118] and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

P60

PARP inhibition initiates the formation of mitochondria-associated mTOR-phospho-ATM NEMO-Akt cytoprotective signalosome in oxidative stress

Antal Tapodi, Enikő Hocsák, Dóra Virág, Ferenc Gallyas, Balázs Sümegi
Department of Biochemistry and Medical Chemistry, University of Pécs, Medical School, Pécs

Cytoprotection of inhibition of poly-ADP-ribose polymerase 1 (PARP1) in oxidative stress is mediated by preservation of NAD^+ and ATP level of the cell. Previously, we proved that PARP1 inhibition also activates phosphorylation of Akt, which effect contributes to the cytoprotective effect of PARP inhibition. However, it has not yet been revealed how the nuclear signal of PARP inhibition might be transported to the cytoplasm leading to Akt activation. Here, we demonstrate that another DNA break sensor ATM has a pivotal role in the signal translocation and cytoprotection induced by PARP inhibition in oxidative stress. Several studies have proved that ATM is a substrate for PARP and here we establish that the interaction of ATM and PARP is increased in oxidative stress. It is also proved that ATM is exported from the nucleus to the cytoplasm by interacting with NEMO/IKKγ in a Ca^{2+} dependent mechanism. This study establishes first that interaction of ATM and NEMO is increased by PARP inhibition; furthermore, phospho-ATM migrates to cytosol in NEMO dependent manner and forms a mitochondria-associated mTOR-phospho-ATM-NEMO-Akt cytoprotective signalosome in oxidative stress. However, the protective effect of this signalosome induced by PARP inhibition in oxidative stress was completely attenuated by sequenced suppression of ATM NEMO or mTOR, respectively, by siRNA. Taken together, these data suggest that PARP inhibition initiates the formation of mitochondria-associated mTOR-p-ATM-NEMO-Akt cytoprotective signalosome in oxidative stress.

P61

Flotillin-1 is an interacting partner of protein phosphatase 2A

Zsófia Thalwieser, Előd Kovács, Csilla Csortos, Anita Boratkó
Department of Medical Chemistry, University of Debrecen, Faculty of Medicine, Debrecen

Protein phosphatase 2A (PP2A), one of the main phospho-Ser/Thr specific phosphatases in mammalian cells plays an important role in the regulation of cell cycle, signal transduction, cell differentiation, cytoskeletal remodeling and even cellular dysfunction. A typical PP2A holoenzyme contains a scaffold A subunit, a catalytic C subunit and one of the many possible regulatory B subunits, which are assigned into B, B′, B″ and B‴ families. The variable B subunit(s) influences substrate specificity and/or subcellular localization of a given PP2A holoenzyme. Our goal was to identify new interacting partners or substrate proteins of the PP2A–B55α holoenzyme in endothelial cells. The coding sequence of B55α was amplified using specific primers and cloned into pGEX-4T-2 vector. Recombinant protein expression was optimized and to identify new interacting partner of PP2A-B55 in endothelial cells, GST pull-down assay was performed. Flotillin-1 protein was identified by LC-MS/MS analysis and the interaction was confirmed by Western blot analysis of the pull-down samples using anti-flotillin-1 antibody. Bacterial expression construct of flotillin-1 was also created and interaction of GST-flotillin-1 with the A, C and B55 subunit of PP2A holoenzyme was shown by pull down assay. Immunoprecipitation experiments were utilized to verify the interaction of the endogenous proteins in endothelial cells. Immunofluorescent staining of flotillin-1 and B55 showed co-localization of the proteins in the cytosol of the cells. Interestingly, the co-localization pattern suggests that the proteins are associated with the intermedier filaments of the endothelial cells. Accordingly, we showed that both PP2A and flotillin-1 interact with vimentin. Our further plans are to characterize the PP2A – flotillin-1- vimentin interaction and to test whether flotillin-1 is a substrate of the PP2A. This work was supported by grant PD116262 from the Hungarian Scientific Research Fund.

P62

Influence of transglutaminase 2 activities on aggresomes and formation of covalently cross-linked protein polymers in a cellular model of Huntington's disease

Kiruphagaran Thangaraju[1], Róbert Király[1], László Fésüs[1,2]

[1]Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen; [2]Stem Cell, Apoptosis and Genomics Research Group of Hungarian Academy of Sciences, University of Debrecen, Debrecen

Huntington's disease (HD) is a neurodegenerative disorder caused by pathological expansion of polyglutamine repeats in the N-terminal region of huntingtin protein (htt) leading to the formation of neuronal inclusions. Transglutaminase 2 (TG2) is a ubiquitous, multifunctional protein with various activities; the best studied function is its Ca^{2+}-dependent transamidase activity leading to protein crosslinking or amine incorporation. It also has isopeptidase activity when the previously formed isopeptide or amide bonds are cleaved. The activity, expression and amounts of TG2 is increased in HD and the conditions favoring the activation of this enzyme like high Ca^{2+} concentration and low GTP levels have been also observed. These resulted in a hypothesis that TG2 has a pathogenic role in HD. Several studies demonstrated that mutant htt with polyglutamine expansion is a substrate and interacting partner of TG2. Contrary, *in situ* study reported that TG2 only selectively modifies mutant huntingtin associated proteins and it does not contribute to the mutant huntingtin crosslinking and aggregate formation. Therefore, our goal is to confirm or reject the patho-biochemical role of TG2 by using *in situ* cell models. The *in situ* effect of TG2 on aggregate formation was studied in PC12 neuronal rat cells, which express tetracycline inducible GFP-tagged exon 1 fragment of htt gene with 74 glutamine repeats and stably transfected with human wild type TG2 and TG2 variants, which have either transamidase or isopeptidase activity or no activity. In the presence of tetracycline and autophagy or proteasome inhibitors, high molecular weight polymers of the exon 1 fragment appeared in cells transfected with TG2 wild type and variants with transamidase activity compared to cells without TG2. Moreover, cells expressing TG2 variant with only isopeptidase activity showed less polymers. Our initial results suggest that TG2 crosslinking activity is involved in huntingtin exon 1 fragment crosslinking and further experiments are in progress to validate the exact role of TG2 in HD. Research grants from the University of Debrecen (RH/885/2013), OTKA NK 105046 and EU FP7-ITN TRANSPATH.

P63

A novel, modified asymmetric PCR aids the aptamer selection procedure

Zoltán Tolnai, Éva Scholz, Judit András, Zsuzsanna Szeitner, Tamás Mészáros

Department of Medical Chemistry, Molecular Biology and Pathobiochemistry, Semmelweis University, Budapest

Aptamers are oligonucleotides that can bind to their targets with great selectivity and specificity similar to antibodies. In the last decades, the in vitro selection of aptamers from random DNA library was improved with several different methods. However, the efficient amplification and conversion of the dsDNA to ssDNA between the selection steps is not fully solved yet. The amplification of the by-products by PCR and the loss of sequences during production of ssDNA hinder the selection. We aimed at developing a new, modified asymmetric PCR to circumvent this shortcoming of aptamer generation. The key of our method is the addition of a 3' terminal blocked reverse primer to the PCR mixture. On one hand, the modification of reverse primer blocks the synthesis of the complementary strand, thus directly provides ssDNA for the next selection cycle. On the other hand, this approach dramatically decreases the nonspecific annealing and consequently, the formation of PCR by-products. We confirmed our findings by analyzing the PCR products by acrylamide gel electrophoresis. According to the results of this analysis, the novel approach worked equally efficiently with using unique oligonucleotide and complex ssDNA library as template of the reaction. We also studied whether the modified asymmetric PCR evades dsDNA to ssDNA conversion step of aptamer selection. To this end, we applied our previously published Alphascreen-based method by amplifying virus protein selective aptamer completed DNA library. The obtained data corroborated our hypothesis, enrichment of DNA library can be analyzed without conversion of dsDNA. Currently, we are studying the effect of asymmetric PCR on the sequence diversity of ssDNA library by Next-Generation Sequencing**.**

P64

Newly identified PP1 inhibitory functions of LIM kinase-2

Emese Tóth, Ferenc Erdődi, Andrea Kiss

Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, Debrecen

Retinoblastoma protein (pRb) plays a key role in the proliferation and survival of cells. We showed earlier, that myosin phosphatase (MP) including protein phosphatase-1 (PP1) catalytic subunit and MP target subunit (MYPT1) also takes part in the dephosphorylation of pRb. In THP-1 leukemic cells, we detected relatively high pRb phosphorylation, which may refer to increased level of MP inhibitory pro-

teins. Therefore, we checked the presence of phosphorylated CPI-17 (a MP inhibitory protein) in THP-1 cells. We found relatively low level of phospho-CPI-17, but several other proteins were cross-reacted with anti-phospho-CPI-17 antibody in higher molecular mass range. Phosphorylation state of these proteins increased notably in the presence of calyculin-A, applied in a PP2A inhibitory concentration. Increased phosphorylation of these proteins was attenuated by protein kinase-C (PKC), but not by Rho-kinase inhibitors, which suggests PKC-dependent phosphorylation in this process. Search in protein databases highlighted that LIMK2 shows significant sequence similarity to the inhibitory phosphorylation site of CPI-17 at the C terminal region, raising its possible role in PP1 inhibition. To test our hypothesis, Flag-LIMK2 was overexpressed in tsA201 cells, immobilized to Flag-beads and phosphorylated *in vitro* by PKC. Phosphorylation of LIMK2 at the CPI-17-like site was confirmed by Western blot. Phosphatase activity of PP1 was measured in the presence of phosphorylated and unphosphorylated Flag-LIMK2, using myosin light chain substrate. Phosphorylated Flag-LIMK2 markedly decreased the activity of PP1 in a concentration-dependent manner, while unphosphorylated protein had only slight effect. Our results suggest that – besides its kinase function - LIMK2 may be a novel inhibitor of PP1 and may regulate phosphorylation level and activity of proteins important in the proliferation of leukemic cells. This research was supported by OTKA K109249 and PD111715.

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Generation and analysis of mutations of the *Drosophila Hat1* gene

Júlia Varga[1], Nóra Zsindely[2], László Bodai[1]
Department of Biochemistry and Molecular Biology, University of Szeged, Szeged

The aim of our study was to characterize the type B histone acetyltransferase encoded by the *CG2051* gene (*dHat1*) in *Drosophila*. dHat1 is the only *Drosophila* orthologue of cytoplasmic Hat1 proteins, which are involved in acetylation of newly synthesized histone H4 on K5 and K12 residues. To be able to study the function of dHat1 we generated deletions by imprecise excision of the P{EPgy2}CG2051[EY21697] transposon inserted in the first intron of the *CG2051* gene. The P{EPgy2} element was remobilized by Δ2-3 transposase and revertants were selected based on loss of the *mini-white* marker gene present on the transposon. Deletion carrying revertant lines were identified by performing PCR reactions with primers straddling the *CG2051* gene. The extent of the largest deletion was determined by capillary sequencing. *Df(3R)CG2051[Δ57]* removes 80% of the coding region of *CG2051* including the HAT domain; but does not affect neighboring genes. As acetylation mediated by orthologs of dHat1 was reported to target histone H4 and precede H4 deposition we decided to identify the targets of dHat1. We found by immunoblot analysis that dHat1 is responsible for the majority of H4K5 acetylation in embryos. Surprisingly, however, *Df(3R)CG2051[Δ57]* is homozygous viable and fertile indicating that dHat1 is not essential for *Drosophila* development and reproduction. In fecundity assays we found that loss of *CG2051*did not reduce the number of eggs laid by female flies, however, its overexpression resulted in elevated egg laying. To analyze the influence of loss of dHat1 on chromatin organization we examined the effects of partial loss of *CG2051* on position-effect variegation of the w[m4] allele, in which the *white* gene is juxtaposed with heterochromatin. Based on our findings *Df(3R)CG2051[Δ57]* is a dominant suppressor of w[m4] variegation suggesting that dHat1 may play a role in the formation and/or maintenance of closed chromatin. Financial support: NKFI 112294.

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***In vitro* analysis of fast evolving Drosophila telomeric proteins and their potential role in species formation**

Balázs Vedelek, Bence László Csanád, Borbála Böröcz, András Blastyák, Imre Miklós Boros
Department of Biochemistry and Molecular Biology, University of Szeged, Szeged

The most important functions of a cell are usually performed by highly conserved proteins. Any mutation in these essential sequences could compromise the survival of the organism. In some cases however, the cells are seemingly tempting fate; they fulfill these important functions by fast evolving proteins. In order to resolve this contradiction we assume that these proteins may have an additional function: they may contribute to species formation. The separation of a species into two subspecies can be beneficial; the new species often utilize the sources of an environment more efficiently than the ancestor one. Variety of barriers could aid the speciation process. For instance post-zygotic barriers are often genes with important function and rapid evolution that might result in impaired cell functions in hybrids. In Drosophila telomere maintenance is performed by a hypothetical complex the Terminin, which is believed to consist of HOAP, HipHop, Ver, DTL/Moi and HP1 subunits [1]. Curiously, HP1 is evolutionary conserved, while terminin proteins manifest an accelerated rate of evolution. However, the existence of Terminin complex so far is not proven experimentally, we showed that Ver and DTL as well as HOAP, HipHop and HP1 forms two subcomplexes [2]. In order to study Terminin proteins potential role in speciation we used biochemical approaches. We reconstituted *Drosophila melanogaster* terminin sub-complexes by coexpressing its subunits in bacteria and chose one protein of each

subcomplex to replace them with their orthologs from the closely related *D. yakuba*. We found that Ver from *D. yakuba* and DTL from *D. melanogaster* could form a stable hybrid complex while HipHop from *D. yakuba* was unable to form a subcomplex with HOAP and HP1 from *D. melanogaster in vitro*. This suggests rapid co-evolution among these proteins. Therefore in a hybrid organism a functional complex may not form, implying a role in the post-zygotic isolation of species. Funding: OTKA K 100969.

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Stage-specific transcriptome analysis in Drosophila testis

Viktor Vedelek[1], Gábor Grézal[2], Barbara Lauri nyecz[1], Bence Kovács[1], Imre Boros[2], László Bodai[2], Rita Sinka[1]

University of Szeged [1]Department of Genetics, [2]Department of Biochemistry and Molecular Biology, Szeged

Next-generation sequencing methods, such as RNA-sequencing is a versatile tool to describe the developmental specific expression of genes in model organisms, such as *Drosophila melanogaster*. To gain a better understanding of cellular differentiation in Drosophila testis, we applied this method to analyze the testis specific transcriptome. We isolated the apical region, which contains stem cells and developing spermatocytes; the middle piece with meiotic cysts enrichment and the distal parts of the testis with elongated post-meiotic spermatids by dissecting and cutting wild type testis. Total RNA were isolated and analyzed by next generation sequencing, using Illumina MiSeq system. We conducted a comprehensive analysis of previous microarray data and our RNA-seq data. We quantified the significant stage specific transcripts by real-time quantitative polymerase chain reactions and *in situ* hybridization. This new approach was able to identify several new, stage specific transcripts during spermatogenesis, which could help us to identify genes responsible for the organization of post-meiotic elongation and individualization of spermatids. Supported by OTKA NF 101001. R.S. is a Bolyai Fellow of the Hungarian Academy of Sciences.

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Deletion of cyclophilin D enhances cholesterol biosynthesis in mouse liver

Péter Balázs Jakus[1], Nikoletta Kálmán[1], Csenge Antus[1], Krisztián Erős[1], Anikó Takátsy[1], Edit Pollák[2], Zoltán Hegedűs[1], István Nagy[3], Balázs Veres[1]

[1]University of Pécs, Medical Faculty, Department of Biochemistry, Pécs; [2]University of Pécs, Faculty of Sciences, Department of Comparative Anatomy and Developmental Biology, Pécs; [3]SeqOmics Biotechnology Ltd., Mórahalom

Cyclophilin-D (CypD) is a mitochondrial matrix protein with a crucial regulatory role in mitochondrial permeability transition pore (PTP) opening. PTP opening causes the collapse of mitochondrial membrane potential leading to cell death and reactive oxygen species formation. The importance of PTP opening has been implicated in various pathological conditions, however the exact physiological role is still obscure. CypD was recently identified as a new partner of mitochondria-associated endoplasmic reticulum membranes (MAMs) and demonstrated a new role for MAMs in the control of insulin's action in the liver [1]. Moreover, deletion of CypD impairs β-oxidation and promotes glucose metabolism [2] indicating a more general metabolic regulatory role. In our study, total mRNA screening of liver from CypD KO mice showed significantly increased expression of genes involved in cholesterol biosynthesis compared to wild-type animals. Gene expression-based pathway analysis revealed more than 20 overexpressed genes in the cholesterol biosynthesis superpathway, however serum cholesterol levels, body fat composition (measured by NMR) and histological examinations did not show significant changes between wild type and CypD KO animals. Ultrastructural analysis by electron microscopy however, showed alterations in the organization of endoplasmic reticulum and mitochondria, suggesting a MAM-mediated transcriptional regulation, possibly through the insulin-induced gene (Insig), an endoplasmic reticulum membrane-embedded sterol sensor that regulates the cellular accumulation of sterols. The exact mechanism and the possible fate of cholesterol remain elusive and need further investigations.

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The CT region of DAAM has a supporting role in FH2-mediated actin dynamics regulation

Péter Gaszler[1], Andrea Vig[1], Judit Viktória Fórizs[1], Mónika Ágnes Tóth[1], István Földi[2], József Mihály[2], Beáta Bugyi[1]

[1]University of Pécs, Medical School, Department of Biophysics, Pécs; [2]Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged

DAAM is a DRF formin protein essential for the regulation of actin cytoskeleton dynamics in diverse biological processes. The conserved FH1-FH2 domains of DAAM catalyze actin nucleation and processively mediate filament elongation. These activities of FH2 are autoregulated by the N- and C-terminal regions. The C-terminal DAD-CT of formins was suggested recently to directly regulate actin dynamics, as well. We analyzed the role of DAD-CT region of Drosophila DAAM in its interaction with actin by in vitro biochemical and in vivo genetic approaches. We found that DAD-CT can bind actin and enhance the nucleating activity of FH2, through the CT region. Despite of being able to bind actin, DAD-CT does not significantly influence actin assembly in the absence of the FH2 domain. Analysis of the I732A loss-of-function mutation in FH2 and FH2-DAD-CT revealed that DAD-CT can compensate for the compromised actin-binding of the FH2 domain. However, the actin nucleation, elongation and barbed end capping activity of the FH2 domain can not be restored by the presence of DAD-CT. Consistently, in vivo data show that the CT region is not essential, yet contributes to DAAM-mediated filopodia formation in primary neurons. In conclusion, DAAM DAD-CT possesses an FH2-dependent function in actin dynamics. Our data suggest that the FH2 region is the core actin-interacting element of DAAM and DAD-CT has only a supporting role in actin nucleation, presumably through stabilization of nucleation intermediers. Supported by grants from the Hungarian Science Foundation (OTKA) Grants K109689 (to BB), K109330 (to JM) and by the National Innovation Office „Baross Gábor" Program (REG-DD-09-1-2009-0009 Tirfm 09 (to BB)). 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".

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Decreased PMCA4b levels in the red blood cells of healthy individuals is connected to a minor haplotype in the ATP2B4 gene

Boglárka Zámbó[1], György Várady[1], Rita Padányi[2], Edit Szabó[1], Adrienn Németh[1], Ágnes Enyedi[2], Balázs Sarkadi[1,3]

[1]Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest; [2]II. Department of Pathology, Faculty of Medicine, Semmelweis University, Budapest; [3]MTA-SE Molecular Biophysics Research Group, Department of Biophysics and Radiation Biology, Semmelweis University, Budapest

Aims: We have developed a flow cytometry method to measure quantitatively the levels of several membrane proteins in red blood cells (RBCs) [1]. We have screened the PMCA4b protein levels in a large number of healthy volunteers, and observed a significant heterogeneity in the expressions. We wished to compare the functionality of the proteins and find the potential genetic background which leads to reduced PMCA4b protein levels. **Results:** In subjects with low PMCA4b expression, Western-blot analysis of the RBC membrane proteins revealed that the molecular mass of the PMCA4b protein was unchanged, and we found no compensation by other PMCA4 variants. We confirmed that the reduced PMCA4b levels resulted in an impaired calcium pump function in Fluo-4 loaded RBCs. In order to explore the genetic background of the reduced expression, we sequenced the ATP2B4 gene in individuals with low PMCA4b level. Interestingly, we found no alterations which lead to amino acid changes, while we found a haplotype in the first part of the gene, consisting of 25 SNPs. We set up TaqMan based genotyping for a tagging SNP to screen a larger cohort, and found that the reduced PMCA4b protein level in RBCs correlated with the minor variant of this haplotype in 176 samples ($p<0.001$; MAF=0.1; CT 25%, TT 50% reduction). **Conclusions:** We found a haplotype in the ATP2B4 gene which causes significantly reduced protein levels in the RBC membrane. This haplotype is overlapping the recently described promoter region of the ATP2B4 gene, and decreased protein expression is probably related to alterations in the binding of transcription factors in this region. Interestingly, while we found these alterations in healthy volunteers, recent GWA studies indicate that SNPs of this haplotype result in a decreased mean corpuscular haemoglobin concentration (MCHC, [2]) in the RBCs, and may be protective against malaria infection [3]. **Acknowledgment:** This work has been supported by OTKA K115375.

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LIST OF PARTICIPANTS

Alföldi Róbert
Avidin Ltd.
6726 Szeged, Alsókikötő sor 11.
e-mail: r.alfoldi@avidinbiotech.com

Ambrus Attila
Semmelweis University
Faculty of Medicine
Department of Medical Biochemistry
1094 Budapest, Tűzoltó utca 37-47.
e-mail: ambrus.attila@med.semmelweis-univ.hu

András Judit
Semmelweis University
Department of Medical Chemistry, Molecular Biology and Pathobiochemistry
1094 Budapest, Tűzoltó utca 37-47.
e-mail: andras.judit@med.semmelweis-univ.hu

Apjok Gábor
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: agabor723@gmail.com

Balikó Gabriella
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: baliko.gabriella@brc.mta.hu

Bálint L. Bálint
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
Center for Clinical Genomics and Personalized Medicine
4032 Debrecen, Nagyerdei krt. 98.
e-mail: lbalint@med.unideb.hu

Balogh Gábor
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: baloghg@brc.hu

Bay Péter
University of Debrecen
Faculty of Medicine
Department of Medical Chemistry
4032 Debrecen, Egyetem tér 1.
e-mail: baip@med.unideb.hu

Bécsi Bálint
University of Debrecen
Faculty of Medicine
Department of Medical Chemistry
4032 Debrecen, Egyetem tér 1.
e-mail: bbalint@med.unideb.hu

Benkő Szilvia
University of Debrecen
Faculty of Medicine
Department of Physiology
4032 Debrecen, Nagyerdei krt. 98.
e-mail: szbenko@gmail.com

Benyhe Sándor
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: benyhe@brc.hu

Bodai László
University of Szeged
Faculty of Science and Informatics
Department of Biochemistry and Molecular Biology
6726 Szeged, Közép fasor 52.
e-mail: bodai@bio.u-szeged.hu

Bojcsuk Dóra
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
4032 Debrecen, Egyetem tér 1.
e-mail: bojcsuk.dora@med.unideb.hu

Boldizsár Eszter
University of Debrecen
Faculty of Medicine
Department of Immunology
4032 Debrecen, Egyetem tér 1.
e-mail: mandarin0390@gmail.com

Boratkó Anita
University of Debrecen
Faculty of Medicine
Department of Medical Chemistry
4032 Debrecen, Egyetem tér 1.
e-mail: boratko@med.unideb.hu

Boros Imre Miklós
University of Szeged
Faculty of Science and Informatics
Department of Biochemistry and Molecular Biology
6726 Szeged, Közép fasor 52.
e-mail: borosi@bio.u-szeged.hu

Borsos Barbara
University of Szeged,
Faculty of Science and Informatics
Department of Biochemistry and Molecular Biology
6726 Szeged, Közép fasor 52.
e-mail: riddles86@gmail.com

Botó Pál
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
4032 Debrecen, Nagyerdei krt. 98.
e-mail: boto.pal@med.unideb.hu

Budai Zsófia
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
4032 Debrecen, Egyetem tér 1.
e-mail: budai.zsofia@med.unideb.hu

Buday László
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: buday.laszlo@ttk.mta.hu

Bugyi Beáta
University of Pécs
Medical School
Department of Biophysics
7624 Pécs, Szigeti út 12.
e-mail: beata.bugyi@aok.pte.hu

Burkovics Péter
Biological Research Centre, HAS
Institute of Genetics
6726 Szeged, Temesvári krt 62.
e-mail: burkovicsp@gmail.com

Cinar Resat
National Institute of Health
National Institute on Alcohol Abuse and Alcoholism
Neuroendocrinology Section
5625 Fishers lane, Rockville, MD 20852, U.S.A.
e-mail: resat.cinar@nih.gov

Cserkaszki Anna
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1171 Budapest, Magyar tudósok körútja 2.
e-mail: anna.cserkaszky@gmail.com

Csikász-Nagy Attila
Pázmány Péter Catholic University
Faculty of Information Technology & Bionics
Laboratory of Structural Biology and Proteomics
1083 Budapest, Práter u. 50/A.
e-mail: csikasz-nagy.attila@itk.ppke.hu

Csumita Mária
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
4032 Debrecen, Nagyerdei krt. 98.
e-mail: csumita.maria@med.unideb.hu

Deák Eszter
University of Debrecen
Faculty of Medicine
Department of Ophthalmology
4032 Debrecen, Nagyerdei krt. 98.
e-mail: deak.eszter@med.unideb.hu

Deák Péter
University of Szeged
Faculty of Science and Informatics
Department of Genetics
Faculty of Science and Informatics
6726 Szeged, Közép fasor 52.
e-mail: deakp@bio.u-szeged.hu

Dékány András
GeneTiCA Ltd.
3385 Tiszanána, Bem út 35.
e-mail: dekany@genetica.hu

Dóczi Róbert
Centre for Agricultural Research, HAS
Agricultural Institute
Department of Plant Cell Biology
2462 Martonvásár Brunszvik u. 2.
e-mail: robert.doczi@gmail.com

Dombrádi Viktor
University of Debrecen
Faculty of Medicine
Department of Medical Chemistry
4032 Debrecen, Egyetem tér 1.
e-mail: dombradi@med.unideb.hu

Dudola Dániel
Pázmány Péter Catholic University
Faculty of Information Technology and Bionics
1083 Budapest, Práter u. 50/A
e-mail: dudola.daniel@itk.ppke.hu

Dukay Brigitta
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt 62.
e-mail: dukay.brigi@gmail.com

Dülk Metta
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: dulk.metta@ttk.mta.hu

Erdődi Ferenc
University of Debrecen
Faculty of Medicine
Department of Medical Chemistry
4032 Debrecen, Egyetem tér 1.
e-mail: erdodi@med.unideb.hu

Erdős Edina
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
4032 Debrecen, Egyetem tér 1.
e-mail: edina.erdos@med.unideb.hu

Faragó Nóra
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt 62.
e-mail: farago.nora@gmail.com

Farkas Ilona
University of Debrecen
Faculty of Medicine
Department of Medical Chemistry
4032 Debrecen Egyetem tér 1.
e-mail: farkas@med.unideb.hu

Fazekas Dávid
Eötvös Loránd University
Department of Genetics
1117 Budapest, Pázmány Péter sétány 1/c
e-mail: fazekas@netbiol.elte.hu

Fehér Tamás
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt 62.
e-mail: fehert@brc.hu

Fésüs László
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Medicine
4032 Debrecen, Egyetem tér 1.
e-mail: fesus@med.unideb.hu

Gallyas Ferenc
University of Pécs
Medical School
Biochemistry and Medical Chemistry
7624 Pécs, Szigeti út 12.
e-mail: ferenc.gallyas@aok.pte.hu

Gáspári Zoltán
Pázmány Péter Catholic University
Faculty of Information Technology & Bionics
1083 Budapest Práter u. 50/A.
e-mail: gaspari.zoltan@itk.ppke.hu

Gaszler Péter
University of Pécs
Medical School
Department of Biophysics
7624 Pécs, Szigeti út 12.
e-mail: gaszlerpeter@gmail.com

Glász-Bóna Annamária
Izinta Ltd.
1121 Budapest, Konkoly-Thege Miklós út 29/33
e-mail: info@izinta.hu

Glatz Attila
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt 62.
e-mail: glatz.attila@brc.mta.hu

Gógl Gergő
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: gogl.gergo@gmail.com

Ghosh Miltu Kumar
NSHM Knowledge Campous
Department of Pharmacy
India
e-mail: miltu.pharm@gmail.com

Gopisetty Mohana Krishna
University of Szeged
Faculty of Science and Informatics
Department of Biochemistry and Molecular Biology
6726 Szeged, Közép fasor 52.
e-mail: jaisairam205@gmail.com

Gronczewska Renata
ForteBio, Division of Pall Life Science
Menlo Science & Technology Park
1360 Willow Road, Suite 201, Menlo Park, CA
94025, U.S.A.
e-mail: renata_gronczewska@europe.pall.com

Györfy Zsuzsanna
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: gyorfyzs@brc.hu

Győrffy Balázs
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: gyorffy.balazs@ttk.mta.hu

Haracska Lajos
Biological Research Centre, HAS
Institute of Genetics
6726 Szeged Temesvári krt. 62.
e-mail: haracska@brc.mta.hu

Harkai Ákos
Semmelweis University
Medical School
Department of Medical Chemistry, Molecular Biology and Pathobiochemistry
1085 Budapest, Üllői út 26.
e-mail: akos.harkai@gmail.com

Hegedűs Lili
Biological Research Centre, HAS
Institute of Genetics
6726 Szeged, Temesvári krt 62.
e-mail: lilidome@gmail.com

Henry Surya
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: henry.surya@brc.mta.hu

Hinsenkamp Anett
Pázmány Péter Catholic University
1083 Budapest, Práter u. 50/A
e-mail: hinsenkamp.anett@itk.ppke.hu

Horváth Ibolya
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62
e-mail: horvath.ibolya@brc.mta.hu

Hubert Ágnes
Semmelweis University
Faculty of Medicine
Department of Medical Biochemistry
1094 Budapest, Tűzoltó u. 37-47
e-mail: hubertagi@gmail.com

Hunyady László
Semmelweis University
Faculty of Medicine
Department of Physiology
1094 Budapest, Tűzoltó utca 37-47.
e-mail: hunyady.laszlo@med.semmelweis-univ.hu

Kállai Brigitta
Semmelweis University
Faculty of Medicine
Department of Medical Chemistry, Molecular Biology and Pathobiochemistry
e-mail: kallai.brigitta@med.semmelweis-univ.hu

Kalló Gergő
University of Debrecen
Department of Biochemistry and Molecular Biology
4032 Debrecen Nagyerdei krt. 98.
e-mail: kallogergo89@gmail.com

Kálmán Nikoletta
University of Pécs
Medical School
Department of Biophysics
7624 Pécs, Szigeti út 12.
e-mail: knikoletta@gmail.com

Karcagi Ildikó
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62
e-mail: karcagi.ildiko@brc.mta.hu

Kerekes Éva
Bioscience Ltd.
1119 Budapest, Andor u. 47-49.
e-mail: kerekes.eva@bio-science.hu

Kiss-Tóth Annamária
Pázmány Péter Catholic University
1083 Budapest, Práter u. 50/A
e-mail: kiss-toth.annamaria@itk.ppke.hu

Klusóczki Ágnes
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
4032 Debrecen, Egyetem tér 1.
e-mail: klusoczkiagnes@gmail.com

Kónya Zoltán
University of Debrecen
Faculty of Medicine
Department of Medical Chemistry
4032 Debrecen, Egyetem tér 1.
e-mail: konya.zoltan@med.unideb.hu

Kopper Klaudia
University of Technology and Economics
Department of Applied Biotechnology and Food Science
1111 Budapest, Műegyetem rkp. 3.
e-mail: klaudia.kopper@gmail.com

Koprivanacz Kitti
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: koprivanacz.kitti@ttk.mta.hu

Kovács Bertalan
Pázmány Péter Catholic University
1083 Budapest, Práter u. 50/A
e-mail: kovacs.bertalan@itk.ppke.hu

Kovács Elek Gergő
University of Debrecen
Faculty of Medicine
Department of Physiology
e-mail: kegergo@gmail.com

Kovács Gergő
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62
e-mail: gergokovacs.greg@gmail.com

Kovács Károly
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62
e-mail: kkaroly@gmail.com

Kovács Mihály
Eötvös Loránd University
Department of Biochemistry
1117 Budapest, Pázmány Péter sétány 1/C.
e-mail: mihaly.kovacs@ttk.elte.hu

Kovács Tamás
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1094 Budapest, Viola utca 16-18.
e-mail: kovitomi.elte@gmail.com

Kőszegi Balázs
University of Pécs
Medical School
Department of Biophysics
7624 Pécs, Szigeti út 12.
e-mail: balazs.koszegi@aok.pte.hu

Kristóf Endre Károly
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
4010 Debrecen, Egyetem tér 1.
e-mail: kristof.endre@med.unideb.hu

Kudlik Gyöngyi
Research Centre for Natural Sciences, HAS
Budapest, Magyar tudósok körútja 2.
e-mail: gyongyi0888@gmail.com

Kurucz Anita
Semmelweis University
Faculty of Medicine
Medical Chemistry & Molecular Biology &
Pathobiochemistry
1094 Budapest, Tűzoltó u. 37-47.
e-mail: kurucz.anita@med.semmelweis-univ.hu

Laczka Csilla
Research Centre for Natural Sciences, HAS
Institute of Enzymology
Budapest, Tűzoltó u. 37-47.
e-mail: laczka.csilla@ttk.mta.hu

Lányi Árpád
University of Debrecen
Faculty of Medicine
Department of Immunology
4032 Debrecen, Egyetem tér 1.
e-mail: alanyi@med.unideb.hu

Laurinyecz Barbara
University of Szeged
Faculty of Science and Informatics
Department of Genetics
6726 Szeged, Közép fasor 52.
e-mail: barbara@bio.u-szeged.hu

Leipoldné Víg Andrea
University of Pécs
Faculty of Medicine
Department of Biophysics
7624 Pécs, Szigeti út 12.
e-mail: andrea.vig@aok.pte.hu

Lisanti Michael
University of Manchester
Institute of Cancer Sciences
Manchester, U.K.
e-mail: michael.lisanti@ics.manchester.ac.uk

Lipinszki Zoltán
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: lipinszki.zoltan@brc.mta.hu

Mahdi Mohamed
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
4010 Debrecen Egyetem tér 1.
e-mail: mohamed@med.unideb.hu

Majoros Hajnalka
University of Szeged
Faculty of Science and Informatics
Department of Biochemistry and Molecular Biology
6726 Szeged, Közép fasor 52.
e-mail: majoroshajnalka@bio.u-szeged.hu

Martinek Tamás
University of Szeged
Department of Pharmaceutical Analysis
6720 Szeged, Somogyi u. 4.
e-mail: martinek@pharm.u-szeged.hu

Matkó János
Eötvös Loránd University
Faculty of Science
Department of Immunology
1117 Budapest, Pázmány Péter sétány 1/C.
e-mail: janos.matko@ttk.elte.hu

Maurice Navodita
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: navoditageorge@gmail.com

Merő Balázs
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: mero.balazs@ttk.mta.hu

Mészáros Attila
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: meszaros.attila@ttk.mta.hu

Mészáros Tamás
Semmelweis University
Faculty of Medicine
Department of Medical Chemistry
1094 Budapest, Tűzoltó u. 37-47.
e-mail: meszaros.tamas@med.semmelweis univ.hu

Murvai Nikoletta
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: murvai.nikoletta@ttk.mta.hu

Nagy Ágota
University of Szeged
Department of Genetics
6726 Szeged, Közép fasor 52.
e-mail: nagota@brc.hu

Nagy Gergely Nándor
Budapest University of Technology and Economics
Department of Applied Biotechnology and Food Science
1111 Budapest, Szent Gellért tér 4.
e-mail: nagy.gergely@ttk.mta.hu

Nagy Lajos István
Avidin Ltd.
6726 Szeged, Alsó kikötő sor 11.
e-mail: lajos@avidinbiotech.com

Nagy Szilvia Krisztina
Semmelweis University
Faculty of Medicine
Department of Medical Chemistry, Molecular Biology and Pathobiochemistry
1085 Budapest, Üllői út 26.
e-mail: nagy.szilvia@med.semmelweis-univ.hu

Nyitray László
Eötvös Loránd University
Department of Biochemistry
1117 Budapest, Pázmány Péter sétány 1/C.
e-mail: nyitray@elte.hu

Ozgyin Lilla
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
4032 Debrecen, Nagyerdei krt.98.
e-mail: ozgyin.lilla@med.unideb.hu

Ozsvári Béla
University of Manchester
Intitute of Cancer Sciences
CRUK Manchester Institute
Manchester, U.K.
e-mail: bela.ozsvari@ics.manchester.ac.uk

Páhi Zoltán Gábor
University of Szeged
Faculty of Science and Informatics
Department of Biochemistry and Molecular Biology
6726 Szeged, Közép fasor 52.
e-mail: pahizol@gmail.com

Pálfi Andrea
University of Debrecen
Faculty of Medicine
4032 Debrecen Nagyerdei krt. 98.
e-mail: p_andi@hotmail.hu

Pankotai Tibor
University of Szeged
Faculty of Science and Informatics
Department of Biochemistry and Molecular Biology
6726 Szeged, Közép fasor 52.
e-mail: pankotai@bio.u-szeged.hu

Péter Margit
University of Debrecen
Faculty of Medicine
Department of Medical Chemistry
4032 Debrecen, Egyetem tér 1.
e-mail: peter.margit@med.unideb.hu

Pintér Lajos
Biological Research Centre, HAS
Institute of Genetics
6726 Szeged, Temesvári krt. 62.
e-mail: pinterl@brc.hu

Pirity Melinda
Biological Research Centre, HAS
Institute of Genetics
6726 Szeged, Temesvári krt. 62.
e-mail: pirity.melinda@brc.mta.hu

Pósfai György
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: posfai@brc.hu

Radnai Balázs
University of Pécs
Medical School
Institute of Biochemistry and Medical Chemistry
7624 Pécs, Szigeti u. 12.
e-mail: balazs.radnai@aok.pte.hu

Radnai László
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: radnai.biokemia@gmail.com

Regdon Zsolt
University of Debrecen
Faculty of Medicine
Department of Medical Chemistry
4032 Debrecen Egyetem tér 1.
e-mail: regdon.zsolt@med.unideb.hu

Reményi Viktória
Izinta Ltd.
1121 Budapest, Konkoly-Thege Miklós út 29/33.
e-mail: info@izinta.hu

Rizvi Syed Ibrahim
University of Allahabad
Department of Biochemistry
Allahabad 211002, India
e-mail: sirizvi@gmail.com

Romanovics Szandra
UD-GenoMed Medical Genomic Technologies Kft.
4032 Debrecen, Nagyerdei krt. 98.
e-mail: romanovics.szandra@ud-genomed.hu

Röthler Bálint
Biotech Hungary Ltd.
1221 Budapest, Kenderike u. 1.
e-mail: stankovic@biotech-hungary.hu

Sághy Tibor
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
Section of Dental Biochemistry
4032 Debrecen, Nagyerdei krt.98.
e-mail: saghy91@gmail.com

Sárközy Márta
University of Szeged
Faculty of Medicine
Department of Biochemistry
6720 Szeged
Dóm tér 9.
e-mail: martasarkozy@gmail.com

Sarnyai Farkas
Semmelweis University
Faculty of Medicine
Department of Medical Chemistry
1428 Budapest, Pf.2.
e-mail: sarnyai.farkas@med.semmelweis-univ.hu

Simon István
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: simon.istvan@ttk.mta.hu

Sinka Rita
University of Szeged
Faculty of Science and Informatics
Department of Genetics
6726 Szeged, Közép fasor 52.
e-mail: rsinka@bio.u-szeged.hu

Somlai Zsolt
Bioscience Ltd.
1119 Budapest, Andor u. 47-49.
e-mail: somlai.zsolt@bio-science.hu
Stankovic Mona
Biotech Hungary Ltd.
1221 Budapest, Kenderike u. 1.
e-mail: stankovic@biotech-hungary.hu

Sümegi Balázs
University of Pécs
Faculty of Medicine
Department of Biochemistry and Medical Chemistry
7624 Pécs, Szigeti út 12.
e-mail: balazs.sumegi@aok.pte.hu

Szabó Judit Eszter
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: szabo.judit.eszter@ttk.mta.hu

Szakács Tibor
ABL&E-JASCO Magyarország Kft.
1116 Budapest, Fehérvári út 130.
e-mail: ablehun@ablelab.com

Szappanos Balázs
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: szbalazs@brc.hu

Szatmári István
Faculty of Medicine
University of Debrecen
Department of Biochemistry and Molecular Biology
4010 Debrecen, Egyetem tér 1.
e-mail: szatmari@med.unideb.hu

Szeder Bálint
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: szeder.balint@ttk.mta.hu

Szeitner Zsuzsanna
Semmelweis University
Faculty of Medicine
Department of Medical Chemistry, Molecular
Biology and Pathobiochemistry
1094 Budapest, Tűzoltó u. 37-47.
e-mail:
szeitner.zsuzsanna@med.semmelweis-univ.hu

Szeltner Zoltán
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar Tudósok körútja 2.
e-mail: szeltner.zoltan@ttk.mta.hu

Szondy Zsuzsa
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
Section of Dental Biochemistry
4032 Debrecen, Nagyerdei krt. 98.
e-mail: szondy@med.unideb.hu

Szöllősi János
University of Debrecen
Department of Biophysics and Cell Biology
4032 Debrecen, Egyetem tér 1.
e-mail: szollo@med.unideb.hu

Szűcs Diána
University of Szeged
Faculty of Science and Informatics
Department of Biochemistry and Molecular Biology
6726 Szeged, Közép fasor 52.
e-mail: szdtait@gmail.com

Szűcs Mária
HBS Biokémia journal
e-mail: szucs.maria@brc.mta.hu

Szüts Dávid
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: szuts.david@ttk.mta.hu

Tantos Ágnes
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: tantos.agnes@ttk.mta.hu

Tapodi Antal
University of Pécs
Faculty of Medicine
Department of Biochemistry
7624 Pécs, Szigeti út 12.
e-mail: antal.tapodi@aok.pte.hu

Tátrai Ágnes
Bioscience Ltd.
1119 Budapest, Andor u. 47-49.
e-mail: tatrai.agnes@bio-science.hu

Thalwieser Zsófia
University of Debrecen
Faculty of Medicine
Department of Medical Chemistry
4032 Debrecen, Egyetem tér 1.
e-mail: kiswieser@gmail.com

Thangaraju Kiruphagaran
University of Debrecen
Faculty of Medicine
Department of Biochemistry and Molecular Biology
4032 Debrecen, Egyetem tér 1.
e-mail: kiruphagaran.thangaraju@med.unideb.hu

Tolnai Zoltán János
Semmelweis University
Faculty of Medicine
Department of Medical Chemistry
1094 Budapest, Tűzoltó u. 37-47.
e-mail: tolnai.zoltan@med.semmelweis-univ.hu

Tóth Emese
University of Debrecen
Faculty of Medicine
Department of Medical Chemistry
4032 Debrecen, Egyetem tér 1.
e-mail: toth.emese@med.unideb.hu

Tóth Erzsébet Melinda
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: toth.e.melinda@gmail.com

Török Zsolt
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: torok.zsolt@brc.mta.hu

Varga Júlia
University of Szeged
Faculty of Science and Informatics
Department of Biochemistry and Molecular Biology
6726 Szeged, Közép fasor 52.
e-mail: sajjul@freemail.hu

Vas Virág
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: vas.virag@ttk.mta.hu

Vedelek Balázs
University of Szeged
Faculty of Science and Informatics
Department of Biochemistry and Molecular Biology
6726 Szeged, Közép fasor 52.
e-mail: balazsvedelek@gmail.com

Vedelek Viktor
University of Szeged
Faculty of Science and Informatics
Department of Genetics
6726 Szeged, Közép fasor 52.
e-mail: ugu.omail@gmail.com

Vereb György
University of Debrecen
Department of Biophysics and Cell Biology
4032 Debrecen, Egyetem tér 1.
e-mail: vereb2014@gmail.com

Veres Balázs
University of Pécs
Faculty of Medicine
Department of Biochemistry
7624 Pécs, Szigeti út 12.
e-mail: balazs.veres@aok.pte.hu

Vértessy G. Beáta
BME VBK
Department of Applied Biotechnology and Food Science
1111 Budapest, Műegyetem rkp. 3.
e-mail: vertessy.beata@ttk.mta.hu

Viczián András
Biological Research Centre, HAS
Institute of Plant Biology
6726 Szeged, Temesvári krt. 62.
e-mail: aviczian@brc.hu

Vígh László
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: vigh@brc.hu

Virág László
University of Debrecen
Faculty of Medicine
Department of Medical Chemistry
4032 Debrecen, Egyetem tér 1.
e-mail: lvirag@med.unideb.hu

Wollemann Mária
Biological Research Centre, HAS
Institute of Biochemistry
6726 Szeged, Temesvári krt. 62.
e-mail: wolleman@brc.hu

Zámbó Boglárka
Research Centre for Natural Sciences, HAS
Institute of Enzymology
1117 Budapest, Magyar tudósok körútja 2.
e-mail: zambo.boglarka@gmail.com

Zámbó Veronika
Semmelweis University
Department of Medical Chemistry, Molecular Biology and Pathobiochemistry
1094 Budapest, Tűzoltó utca 37-47.
e-mail: zambo.veronika@med.semmelweis-univ.hu

AUTHORS' INDEX

Ádám É. E4-2
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Dukay B. P15
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Glatz A. E5-2
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Jakob B. P28
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Kadlecsik T. E6-2
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Kalmar L. P43
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Karcagi I. P29
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Korcsmáros T. E6-2
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Kovács T. E4-5, P16
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Kozma-Bognár L. E4-2
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Kunos G. E2-1
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Lipinszki Z. E8-4
Lisanti M. E1-1
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Mahdi M. P38
Majoros H. P39, P59
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Martina M. E7-3
Martinek T. E8-1
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Merő B. P10, P16, P34, P41
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Mizsei R. E3-3
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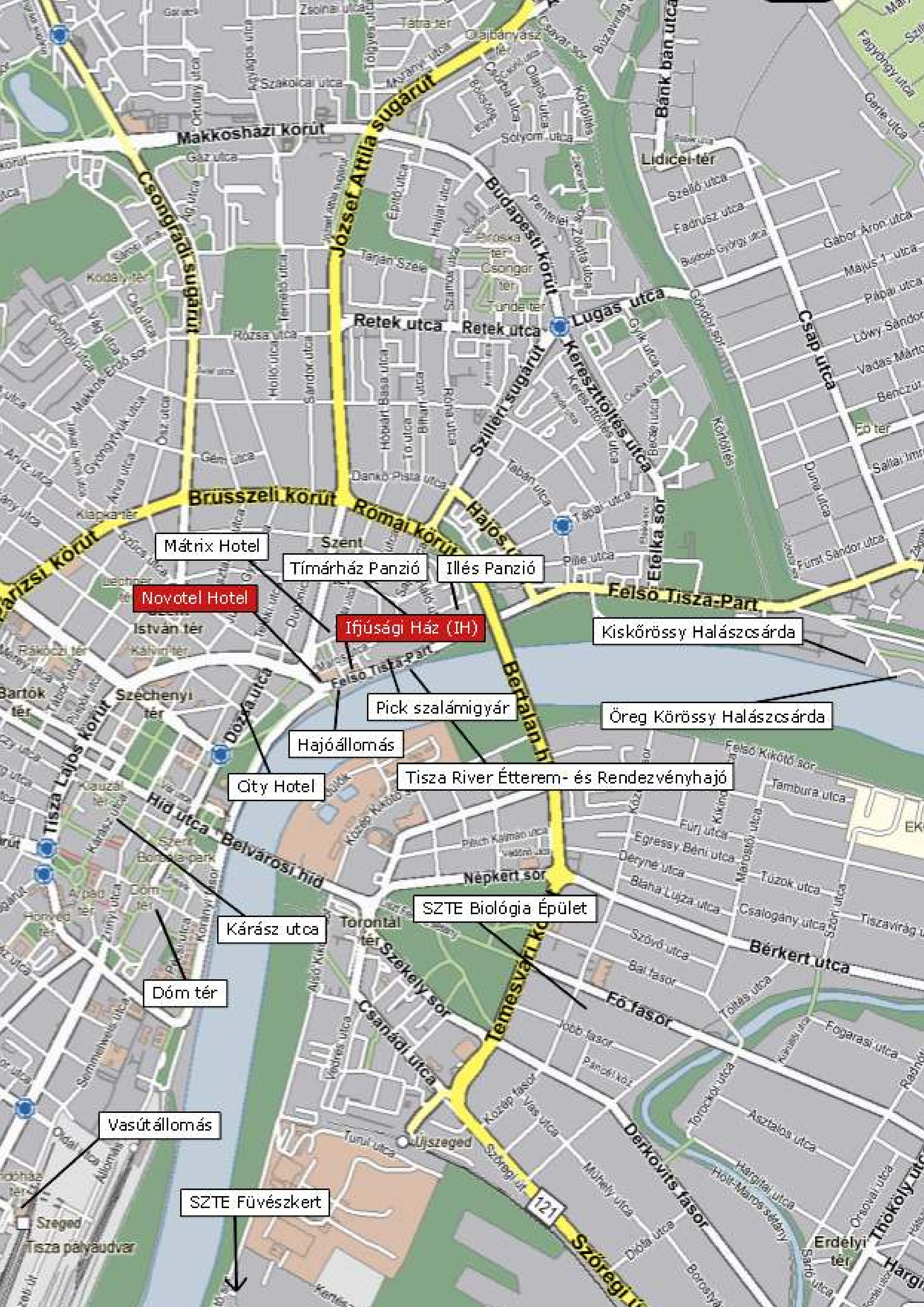

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Dóm tér
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Fotók:
Szegedi Tudományegyetem Füvészkert
6726 Szeged, Lövölde u. 42.
Web: www.fuveszkert.u-szeged.hu