CEITEC | Central European Institute of Technology
BRNO | CZECH REPUBLIC

External Newsletter Q3/2014

Dear friends of CEITEC,

We are very happy to have ceremonially opened our new CEITEC building on the Masaryk University campus, in a great celebration of our future potential. Also, we have just concluded the second full-scale scientific evaluation in CEITEC's short history. Here, 24 international scientists spent three intense days reviewing our science to produce useful recommendations intended to encourage our development towards European standards of excellence.

You can read about these two important events and much more in this newsletter.

Best wishes,
Markus Dettenhofer
CEITEC Executive Director

CONTENT

3 CEITEC Opened New Buildings
Symposium on Frontiers in Cryo-Electron Microscopy

4 Foreign Experts Confirm High Level of Research at CEITEC
CEITEC Remote Mobile Laboratory Wins Gold Medal at the International Engineering Fair in Brno
CEITEC Nano Research Infrastructure Ranked in MERIL Database

5 Awards
Information Day for Companies at CEITEC MU

6 Events

7 Interview with CEITEC Scientist
Jan Novotný, Materials Characterization and Advanced Coatings Research Group

10 Selected Recent Publications

In Memoriam - Prof. MUDr. Robert Kuba, PhD

On 6/10/2014, Prof. MUDr. Robert Kuba, PhD, suddenly and unexpectedly died at the age of 45. He was a professor of neurology, working at Masaryk University, the First Neurological Clinic at St. Anne's University Hospital in Brno and as principal investigator at CEITEC in the Behavioural and Social Neuroscience research group in the Brain and Mind research programme. He was a very experienced neurologist and had been the senior consultant in the clinic. He dealt with all the areas of epileptology, from idiopathic generalised syndrome to pharmacoresistant epilepsy and epilepsy surgery. He enjoyed teaching and guiding students – whether undergraduates, doctoral students, or on a European course in epilepsy surgery and on epileptology courses at the Brno Epilepsy Centre. He was an internationally recognised epileptologist, the author of a range of notable publications, an exemplary husband and father of two children, and a good friend to his colleagues.

CEITEC Opened New Buildings

Two new pavilions for scientific teams focussing on research into biologically significant molecules, evolution and the genetic equipment of plants, as well the human mind and people's health, were opened on September 12 by CEITEC at the Bohunice University Campus of Masaryk University. Scientists can now begin to make use of first rate laboratories and unique equipment that can be found in only a few workplaces in the world. Moreover, the building was awarded Building of the Year 2014 for its high-tech architecture.

More information and a photogallery can be found HERE.

Foto: © Jiří Salik Sláma

Symposium on Frontiers in Cryo-Electron Microscopy

On September 5, installation of the Cryo-Electron Microscopy and Tomography facility in the new building of CEITEC was completed and opened for full operation. At this occasion, a one-day symposium on Frontiers in Cryo-Electron Microscopy was held. Part of the symposium was tours of the facility with demonstration of operation of the newly installed microscopes including a unique FEI Titan Krios transmission electron microscope. This microscope is capable of imaging purified biomolecules to near-atomic resolution or visualising intracellular compartments and macromolecular complexes in the cell to recently unimaginable detail in 3D.

More information and photos from the event can be found HERE.

Foreign Experts Confirm High Level of Research at CEITEC

Several groups ranking among the best in the world and more than half fully comparable with the remainder of Europe. That is the overall result of the demanding evaluation of our research groups carried out in CEITEC by a group of international experts.

More than twenty scientists from renowned institutions around the world travelled to Brno to assess for a second time the results of the work of experts from CEITEC. Over the course of three days (September 22-24) the foreign evaluators met personally with all CEITEC's scientists and examined first and foremost the progress they had made in their work over the last two years.

More information can be found HERE and HERE.

CEITEC Remote Mobile Laboratory Wins Gold Medal at the International Engineering Fair in Brno

Participation by CEITEC BUT scientists for the first time at the International Engineering Fair brought them a gold medal. This mobile laboratory intended for rapid contactless chemical analysis, which was presented at the Brno University of Technology stand, was assessed by the international evaluation commission as this year's most innovative exhibit created in contractual cooperation between a company and a research organisation.

More information about the instrument and its features can be found in an interview with a member of the research team, Jan Novotný, on page 7 of this newsletter or in a press release HERE.

CEITEC Nano Research Infrastructure Ranked in MERIL Database

The CEITEC Nano Research infrastructure has successfully passed the evaluation of the European Science Foundation and has been ranked among European research infrastructures in the MERIL database (Mapping of the European Research Infrastructure Landscape). We have thus joined other European Micro and Nanotechnology facilities (e.g. KIT, CRANN, ETH), that operate on the principle of open and transparent access. The database is primarily used to establish collaboration, to present services to potential users, and serves as a basis for mapping in the ESFRI research infrastructures in the EU. Last but not least it is confirmation that the given research infrastructure meets European criteria.

More on the MERIL database can be found HERE.

AWARDS

Prof. Jiří Šponer, Structure and Dynamics of Nucleic Acids research group leader, was awarded the **Praemium Academiae**. This award is given to scientific personalities who are leaders in their field and is as well a scientific grant, which provides financial and moral support. Prof. Šponer was honoured for his work, which seeks to reveal the evolution, structure and function of DNA and RNA, using computational methods.

Prof. Ivan Rektor, Brain and Mind research programme coordinator, was given the ILAE – CEA **European Epilepsy Education Award** 2014 during the 11th European Congress on Epileptology in Stockholm on Sunday, June 29. The award is given in recognition of outstanding contributions to European epilepsy education. During his career, Prof. Rektor organised several congresses and has lectured among others in Vietnam, Mongolia, Turkey, Nicaragua and in Seville, Lisbon, Copenhagen, Vienna and Paris. The award, which consists of a diploma, is made no more than once every two years and to a maximum of one awardee on every occasion.

Dr. Marek Mráz from Medical Genomics research group received the **Discovery Award**, granted to young researchers under 40 who have contributed to an extraordinary shift in the field of medicine and pharmacy. The award was established to support and encourage brave and innovative achievements that will benefit patients, improve their quality of life and also contribute to the innovative potential of the Czech healthcare and pharmacy. Dr. Mráz was awarded for his publication "miR-150 influences B-cell receptor signaling in chronic lymphocytic leukemia by regulating expression of GAB1 and FOXP1" in the journal Blood – more information can be found on page 13.

EVENTS

Information day for companies at CEITEC MU

WHEN: November 27, 2014

WHERE: Bohunice University Campus, Kamenice 5, Brno

CEITEC and Masaryk University Technology Transfer Office would like to invite you to an information day for companies at CEITEC MU. The meeting aims to extend and support the cooperation of CEITEC MU core facilities with the commercial sector. After an introductory word from Structural Biology research programme coordinator Vladimír Sklenář and business development manager Pavel Indruch, eight core facilities available at CEITEC MU will be introduced, for which guided tours will follow.

The information day will be held in Czech.

More information and the registration can be found HERE.

Current State-of-Art to Study Biomolecular Interactions and Assemblies in Life Sciences

WHEN: November 10, 2014

WHERE: Mendel Museum, Augustinian Abbey, Mendlovo nám. 1a, Brno

We would like to cordially invite you to the upcoming workshop organised by the Biomolecular Interaction and Crystallisation core facility. The workshop will be dedicated to various biophysical techniques used for the study of biomolecular interactions, protein production and purification issues and is intended for researchers, companies' representatives and life sciences students. The programme of the workshop will consist of eight lectures given by outstanding speakers.

More information and the registration can be found HERE.

Structural Biology Seminar Series

You are cordially invited to the CEITEC Structural Biology Seminar Series organized by Structural Biology research programme coordinator, Prof. Vladimír Sklenář. The lectures have been organized every month since January 2013. Forthcoming lectures will be held on:

- November 20
  Dr. Dieter Blaas, The Max F. Perutz Laboratories
- December 11
  Emanuele Buratti, International Centre for Genetic Engineering and Biotechnology

More information can be found HERE.

Conference "Young Scientists: Balancing Career and Family"

WHEN: November 27, 2014

WHERE: Barceló Brno Palace, Šilingrovo nám. 2, Brno

WORK - LIFE
BALANCE

We cordially invite all employees in the field of science and research to this conference focusing on supporting young scientists.

The objective of the conference is to present good practice examples and offer space for discussion on suitable forms of support for PhD students and post-docs by employers and other institutions (such as providers of grant schemes or lawmakers), with an emphasis on balancing their family and professional life.

More information and the registration can be found HERE.

For other interesting events visit our webpage.

INTERVIEW WITH CEITEC SCIENTIST

Jan Novotný, PhD.

Research Group – **Materials Characterization and Advanced Coatings**
Research Programme – **Advanced Nanotechnologies and Microtechnologies**

At the International Engineering Fair with your team you won the Gold Medal for the best innovative exhibit arising from cooperation between a company and a research organization. Congratulations! What does this prize mean for you?

I have to say that it means a lot to us, as it is the outcome of the repeated efforts of many people. Research and development, which often works on a trial and error basis with many smaller failures, it is easy to reach a point at which you can no longer see the light at the end of the tunnel. Such an award doubles the pleasure, both as confirmation that our work has a point to it and that someone other than us is able to see that.

You gained the award for the rLIBS instrument. What exactly is rLIBS and how does it work?

rLIBS is the working name for a mobile laboratory intended for contactless elemental analysis of substances in the field, at distances of up to 20 m. The basis of the device is the modern analytical method LIBS (Laser-Induced Breakdown Spectroscopy). It makes use of a targeted laser beam by which the instrument vaporises a small amount of the investigated material (of the order of micrograms), forms a microplasma and then with the aid of atomic emission spectroscopy identifies individual chemical elements present. Put simply, we can say that with the help of a single laser pulse into a distant object we are able to determine its elemental composition.

What are the greatest advantages of this piece of equipment?

The advantages follow from the fact that analyses of elemental composition can be carried out directly in the field and samples do not need to be transported into the laboratory. Among the main advantages is the possibility of remote analysis, from up to 20 m, as well as rapidity, the ability to analyse samples of all states of matter and sizes without special preparation and the ability to determine the majority of the elements of the periodic table.

What kinds of real-world applications are there for the rLIBS?

There is a broad range of potential applications for the mobile laboratory: product quality control in production companies, sorting lines, detecting contamination in water, air, soil and plants; analysing minerals, building material, archaeological finds, forensic analyses, etc. It needs to be said that the rLIBS was made to be universal – for testing various applications in the field. For each concrete purpose it would be possible, and advisable, to develop a more specialised version – which would normally be both smaller and cheaper.

The scope of your research is much wider, however. What else have you been working on?

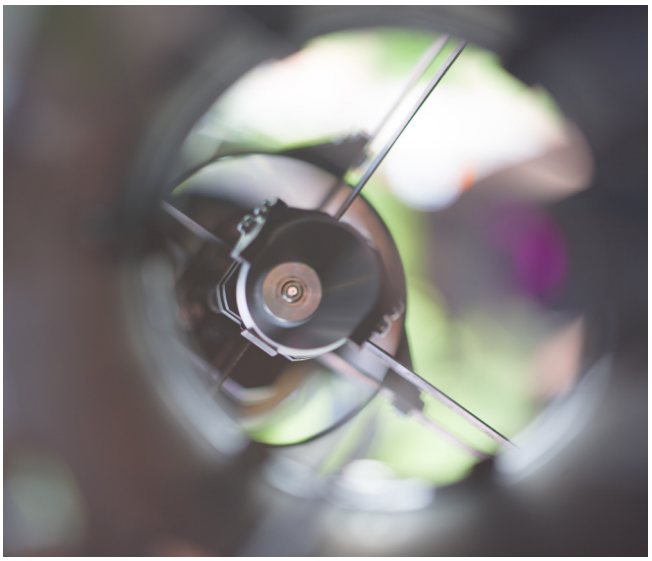
The rLIBS and remote analysis of materials is one of the outcomes of our research into LIBS methods. We are dealing both with the study of the basic principles of this method and with experimental research into applications, and in recent years also with the development of instrumentation – such as for example a specialised interaction chamber. We are also closely cooperating with the computed microtomography laboratory. By combining both techniques we can for example identify very effectively the chemical composition of samples in 3D.

Talking specifically about myself, currently I am spending part of the year at a Laser laboratory at the University of Málaga. I am working on the very interesting and up-to-date topic of LIBS detection of individual nanoparticles and their adsorbates in connection with optical catapulting and with optical manipulation – the practical outcome of which could be among other things the use of LIBS for rapid diagnostics of contamination or on-line evaluation of the functioning and effectiveness of filters.

Jan Novotný, PhD.

After graduating from the sports high school in his home town of Vrbno pod Pradědem he began to study for a master's degree in physics at the Institute of Physical Engineering at Brno University of Technology.

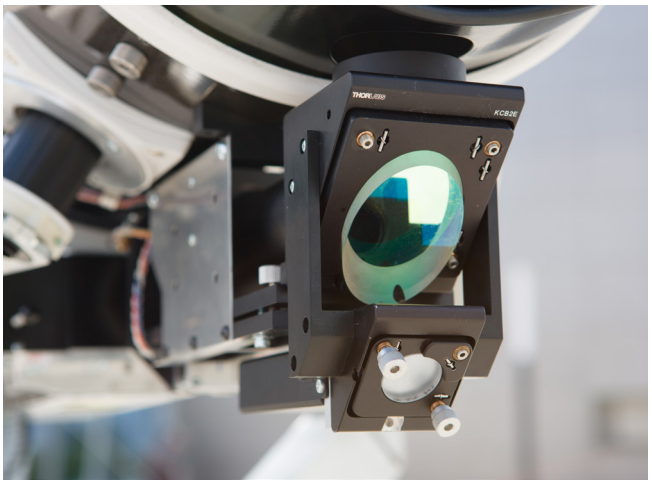
His diploma work on the theme of the automatic focussing of laser beams was produced in the group under Prof. Kaiser and he defended it in 2007. He continued to work in the laser spectroscopy laboratory for his PhD which he completed in 2012 by defending his dissertation on the theme of remote laser spectroscopy. Currently he is a post-doc employed at the Central European Institute of Technology (CEITEC), where in the Material Characterisation and Advanced Coatings group under Professor Kaiser he has continued to concern himself with research in the field of Laser Induced Breakdown Spectroscopy (LIBS).



As you said, you are currently on a 3-month placement at the University of Malága in Spain. Could you compare conditions for science there and in CEITEC?

The research laboratory at the University of Malága, like ours in CEITEC, is looking at the LIBS method of laser spectroscopy. It has a rather longer history as is shown in its greater body of experience and publication activity. It could be described as a leader in the field of LIBS remote spectroscopy, and so was an obvious choice as a workplace for my placement.

When it comes to comparing conditions for science in both workplaces, given my relatively brief experience, it is not possible to generalise. I can rather evaluate the practical running of the laboratory, its excellent equipment, the level of engagement of colleagues here and their constructive approach to resolving practical problems, all of which have made a very positive impression on me.



How is your foreign experience influencing your own research?

Foreign placements in general are excellent for professional development. For me personally they are a source of inspiration for further directions for research, allowing me in a natural manner to gain new professional contacts for initiating cooperation between universities, offering valuable know-how and providing a different perspective on problems that are yet to be solved – whether scientific or rather professional/organisational. For example my actual placement in Malága, thanks to local conditions, made it possible for me to quickly become involved in all the mentioned areas of the preparation, manipulation and LIBS analysis of particles of dimensions of the order of tens of nanometres. The research team in Malága is made up mostly of chemists, while the core of our laboratory is rather drawn from physicists, opticists and designers. From this arises the difference in the spectrum of activities which the two laboratories are involved in, and also it explains why such cooperation is so effective.

Given that that due to the limited time available during a placement like this, there is not much more than the chance to initiate such research, I would like to transfer the solving of this area also to our laboratory.

You are a parent as well as a scientist. How difficult is it for a young scientist to combine family and working life?

Sometimes it is tough, as both are pretty demanding on time. It is not so rare for me to neglect one in favour of the other. However despite being so demanding, both parenthood and science are immensely enriching, and so it is necessary to constantly try to combine them, to seek compromises, and of course to prioritise wisely.
As a relatively new father I could not imagine that I would leave my family behind for several months while on placement, and so we all went together. My wife and son have a chance that way to enjoy a new pleasant environment and I can get on with my work while still having them with me.

What was the main reason for you becoming a scientist? Who or what gave you the greatest motivation?

To be honest it was never part of my plans. I was never one of the young scientists, the sort that made perpetual motion machines from Merkur in childhood. Although physics always interested me, terms such as science and research were rather foreign to me. This changed once I got to university, largely due to the study environment and the possibilities offered by the Institute of Physical Engineering at BUT, and which are now further enhanced by the founding of CEITEC. There among other things I entered the world of laser spectroscopy and began to gradually get to know what science involves. You get to be in close contact with physics – experiencing first hand rules that previously you had only read about, and you get the chance to discover new ones. It opens doors to the world, you get to work with high-tech equipment, you have room to realise your own ideas and theories, and you continually learn new things. It really enthuses you. And even if it isn't always as wonderful as it might seem, your work itself can easily also be your hobby and at the same time the focus of professional fulfilment.

And a classical question to end with – what are your ambitions and further scientific plans within CEITEC?

I would like to continue with experimental and applied research in the area of LIBS, since I am convinced that this method has much to offer in the future and that there is much unexplored potential.

A natural continuation or outcome of our work to date would be the founding of a start-up, the main business of which would be practical applications, developing the equipment and its transfer to industry.

Thank you for the interview!

SELECTED RECENT PUBLICATIONS

ADVANCED NANOTECHNOLOGIES AND MICROTECHNOLOGIES

Chemical Communications

Convergent and divergent two-dimensional coordination networks formed through substrate-activated or quenched alkynyl ligation

Čechal J., Kley C., Kumagai T., Schramm F., Ruben M., Stepanow S., Kern K.

Research Group: Fabrication and Characterisation of Nanostructures

Abstract: Metal coordination assemblies of the symmetric bi-functional 4,4′-di-(1,4-buta-1,3-diynyl)-benzoic acid are investigated by scanning tunnelling microscopy on metal surfaces. The formation of long-range ordered, short-range disordered and random phases depends on the competition between the convergent and divergent coordination motifs of the individual functional groups and is crucially influenced by the substrate.

Materials and Design

Finite element analysis of bone loss around failing implants.

Wolff J., Narra N., Antalainen A., Valášek J., **Kaiser J.**, Sándor G., Marcián P.

Research Group: Materials Characterization and Advanced Coatings

Abstract: Dental implants induce diverse forces on their surrounding bone. However, when excessive unphysiological forces are applied, resorption of the neighbouring bone may occur. The aim of this study was to assess possible causes of bone loss around failing dental implants using finite element analysis. A further aim was to assess the implications of progressive bone loss on the strains induced by dental implants.

Between 2003 and 2009 a total of 3700 implant operations were performed in a private clinic. Ten patients with 16 fixtures developed severe marginal bone defects. Finite element analysis was used to assess the effective strains produced at the bone-implant interface under unidirectional axial loading. These simulations were carried out on 4 specific implant types – Camlog Plus, Astra Osseo Speed, Straumann BL and Straumann S/SP. All implant types exhibited degraded performance under circular and horizontal bone loss conditions. This is evidenced by increased distribution of pathological strain intensities (>3000 με), in accordance with the mechanostat hypothesis, in the surrounding bone. Among the implants, the Camlog design seemed to have performed poorly, especially at the chamfer in the implant collar (>25000 με). Implants are designed to perform under nearly ideal conditions from insertion till osseointegration. However, when the surrounding bone undergoes remodelling, implant geometries can have varied performance, which in some cases can exacerbate bone loss. The results of this study indicate the importance of evaluating implant geometries under clinically observed conditions of progressive bone loss.

ADVANCED MATERIALS

Ceramics International

Consolidation of nanoparticle suspensions by centrifugation in non-porous moulds

Trunec M., Mišák J.

Research Group: Advanced Ceramic Materials

Abstract: Alumina and zirconia nanosuspensions with a mean particle size of 100 nm and 15nm, respectively, were consolidated by centrifugal compaction in non-porous moulds. The nano suspensions consolidated by high-speed centrifugation were deposited irregularly, resulting in a powder deposit with density profile. The homogeneity of the powder deposits was described and homogeneous and well packed deposit regions were identified. Plate-like bodies were prepared from the homogeneous regions of the deposit. The advantage of regular and dense nanoparticle packing by centrifugal compaction was demonstrated by fabricating transparent alumina and tetragonal zirconia ceramics. The transparent alumina had an in-line transmission of 55% in the visible light at a thickness of 0.8mm. The transparent tetragonal zirconia reached a dense microstructure with an average grain size of 65 nm and an in-line transmission of 25% at a thickness of 0.5mm.

STRUCTURAL BIOLOGY

Molecular Cell

Molecular Basis for Coordinating Transcription Termination with Noncoding RNA Degradation

Tudek A., Porrua O., **Kabzinski T.**, Lidschreiber M., **Kubicek K.**, **Fortova A.**, Lacroute F., **Vanacova S.**, Cramer P., **Stefl R.**, Libri D.

Research Group: Structural Biology of Gene Regulation

Abstract: The Nrd1-Nab3-Sen1 complex controls pervasive transcription in yeast by terminating transcription of cryptic transcripts and directing these RNAs to degradation by the exosome. Tudek et al. show that the same domain of Nrd1p mediates mutually exclusive interactions with RNAPII or the exosome cofactor TRAMP to coordinate termination and RNA degradation.

Chemical Science

Azidophenyl as a click-transformable redox label of DNA suitable for electrochemical detection of DNA–protein interactions

Balintová J., **Špaček J.**, Pohl R., Brázdová M., **Havran L.**, **Fojta M.**, Hocek M.

Research Group: Structure and Interaction of Biomolecules at Surfaces

Abstract: A new approach for the detection of DNA-protein binding has been proposed. The technique uses electrochemically active azidophenyl group, attached to cytosine or 7-deazaadenine nucleobases incorporated into a DNA probe. 5-(4-Azidophenyl)-2´-deoxycytidine and 7-(4-azidophenyl)-7-deaza-2´-deoxyadenosine nucleosides were prepared by aqueous-phase Suzuki cross-coupling and converted to nucleoside triphosphates (dNTPs) which served as substrates for incorporation into DNA by DNA polymerase. The azidophenyl-modified nucleotides and azidophenyl-modified DNA yield a strong voltammetric signal at –0.9 V due to reduction of the azido function. The Cu-catalyzed click reaction of azidophenyl-modified nucleosides or azidophenyl-modified DNA with 4-nitrophenylacetylene give nitrophenyl-substituted triazoles, exerting a reduction peak at –0.4 V in voltammetry. The click reaction with phenylacetylene yield electrochemically silent phenyltriazoles. Thus, conversion of the azidophenyl moiety to the triazole derivatives can be monitored selectively by voltammetry. The transformation of the azidophenyl label to nitrophenyltriazole was used for electrochemical detection of p53 protein-DNA interactions. Our study has shown that azidophenyl groups in the parts of the DNA not shielded by the bound protein protein were transformed to nitrophenyltriazoles, whereas those covered by the protein were not.

GENOMICS AND PROTEOMICS OF PLANT SYSTEMS

Journal of Experimental Botany

Identification of AHK2- and AHK3-like cytokinin receptors in Brassica napus reveals two subfamilies of AHK2 orthologues

Kuderová A., Gallová L., **Kuricová K.**, **Nejedlá E.**, **Čurdová A.**, Micenková L., Plíhal O., Šmajs D., Spíchal L., **Hejátko J.**

Research Group: Functional Genomics and Proteomics of Plants

Abstract: Cytokinin (CK) signalling is known to play key roles in the regulation of plant growth and development, crop yields, and tolerance to both abiotic stress and pathogen defences, but the mechanisms involved are poorly characterized in dicotyledonous crops. Here the identification and functional characterization of sensor histidine kinases homologous to *Arabidopsis* CK receptors AHK2 and AHK3 in winter oilseed rape are presented. Five HASE-containing His kinases were identified in *Brassica napus* var. Tapidor (BnCHK1–BnCHK5) by heterologous hybridization of its genomic library with gene-specific probes from *Arabidopsis*. Using a bioinformatic approach and cDNA cloning, the precise gene and putative protein domain structures were determined. Based on phylogenetic analysis, four *AHK2 (BnCHK1–BnCHK4)* homologues and one *AHK3 (BnCHK5)* homologue were defined. It is further suggested that *BnCHK1* and *BnCHK3*, and *BnCHK5* are orthologues of *AHK2* and *AHK3*, originally from the B. *rapa* A genome, respectively. *BnCHK1*, *BnCHK3*, and *BnCHK5* displayed high affinity for *trans*-zeatin (1–3 nM) in a live-cell competitive receptor assay, but not with other plant hormones (indole acetic acid, GA3, and abscisic acid), confirming the prediction that they are genuine CK receptors. It is shown that BnCHK1 and BnCHK3, and BnCHK5 display distinct preferences for various CK bases and metabolites, characteristic of their AHK counterparts, AHK2 and AHK3, respectively.

GENOMICS AND PROTEOMICS OF PLANT SYSTEMS

Journal of Chromatography A

Double opposite end injection capillary electrophoresis with contactless conductometric detection for simultaneous determination of chloride, sodium and potassium in cystic fibrosis diagnosis.

Kubáň P., **Greguš M.**, Pokojová E., Skřičková J., **Foret F.**

Research Group: Bioanalytical Instrumentation

Abstract: A novel approach for diagnosis of cystic fibrosis is presented. A simple and fast procedure to obtain sweat sample was developed. It consists of repeatedly wiping the skin of the forearm with deionized water moisturized cotton swab and extraction in 1 mL of deionized water. Double opposite end injection capillary electrophoresis with contactless conductometric detection is used for the analysis of the extract. Chloride, sodium and potassium as the three target ions that participate in the ion transfer across the cellular membranes, and are affected by CF, are simultaneously determined in approximately 3 min in a background electrolyte containing 20 mM 2-(N-morpholino)ethanesulfonic acid, 20 mM L-histidine and 2 mM 18-crown-6. By using the target ion ratios rather than the concentrations of each individual ion combined with principal component analysis, the diagnosis of CF can be made more accurately and greatly reduce the number of false positive or negative results as is often the case when single ion (chloride) is analyzed.

Cell Host Microbes

Nonsense-mediated mRNA decay modulates TNL immune receptor levels and plant innate immunity

Gloggnitzer J., Akimcheva S., Srinivasan A., Kusenda B., Riehs N., Stampfl H., Bautor J., Dekrout B., Jonak C., Jiménez-Gómez J.M., Parker J.E., **Riha K.**

Research Group: Plant Molecular Biology

Abstract: Nonsense-mediated mRNA decay (NMD) is a conserved eukaryotic RNA surveillance mechanism that degrades aberrant mRNAs. NMD impairment in *Arabidopsis* is linked to constitutive immune response activation and enhanced antibacterial resistance, but the underlying mechanisms are unknown. Here we show that NMD contributes to innate immunity in *Arabidopsis* by controlling the turnover of numerous TIR domain-containing, nucleotide-binding, leucine-rich repeat (TNL) immune receptor-encoding mRNAs. Autoimmunity resulting from NMD impairment depends on TNL signaling pathway components and can be triggered through deregulation of a single TNL gene, RPS6. Bacterial infection of plants causes host-programmed inhibition of NMD, leading to stabilization of NMD-regulated TNL transcripts. Conversely, constitutive NMD activity prevents TNL stabilization and impairs plant defense, demonstrating that host-regulated NMD contributes to disease resistance. Thus, NMD shapes plant innate immunity by controlling the threshold for activation of TNL resistance pathways.

MOLECULAR MEDICINE

Leukemia

Detailed analysis of therapy-driven clonal evolution of TP53 mutations in chronic lymphocytic leukemia

Malcikova J., **Stano-Kozubik K.**, **Tichy B.**, **Kantorova B.**, **Pavlova S.**, **Tom N.**, **Radova L.**, Smardova J., **Pardy F.**, **Doubek M.**, Brychtova Y., **Mraz M.**, **Plevova K.**, Diviskova E., Oltova A., **Mayer J.**, **Pospisilova S.**, **Trbusek M.**

Research Group: Medical Genomics, Molecular Oncology I - Hematooncology & Genomics Core Facility

Abstract: In chronic lymphocytic leukemia (CLL), the worst prognosis is associated with TP53 defects with the affected patients being potentially directed to alternative treatment. Therapy administration was shown to drive the selection of new TP53 mutations in CLL. Using ultra-deep next-generation sequencing (NGS) we performed a detailed analysis of TP53 mutations' clonal evolution. We retrospectively analyzed samples assessed as TP53-wild-type (wt) by FASAY from 20 patients with a new TP53 mutation detected in relapse and 40 patients remaining TP53-wt in relapse. Minor TP53-mutated subclones were disclosed in 18/20 patients experiencing later mutation selection, while only one minor-clone mutation was observed in those patients remaining TP53-wt (n=40). We documented that (i) minor TP53 mutations may be present before therapy and may occur in any relapse; (ii) the majority of TP53-mutated minor clones expand to dominant clone under the selective pressure of chemotherapy, while persistence of minor-clone mutations is rare; (iii) multiple minor-clone TP53 mutations are common and may simultaneously expand. In conclusion, patients with minor-clone TP53 mutations carry a high risk of mutation selection by therapy. Deep sequencing can shift TP53 mutation identification to a period before therapy administration, which might be of particular importance for clinical trials.

MOLECULAR MEDICINE

Blood

miR-150 influences B-cell receptor signaling in chronic lymphocytic leukemia by regulating expression of GAB1 and FOXP1

Mraz M., Chen L., Rassenti L.Z., Ghia E.M., Li H., Jepsen K., Smith E.N., Messer K., Frazer K.A., Kipps T.J.

Research Group: Medical Genomics

Abstract: We examined the microRNAs (miRNAs) expressed in chronic lymphocytic leukemia (CLL) and identified *miR-150* as the most abundant, but with leukemia cell expression levels that varied among patients. CLL cells that expressed ζ-chain-associated protein of 70 kDa (ZAP-70) or that used unmutated immunoglobulin heavy chain variable (IGHV) genes, each had a median expression level of *miR-150* that was significantly lower than that of ZAP-70-negative CLL cells or those that used mutated IGHV genes. In samples stratified for expression of *miR-150*, CLL cells with low-level *miR-150* expressed relatively higher levels of forkhead box P1 (FOXP1) and GRB2-associated binding protein 1 (GAB1), genes with 3' untranslated regions having evolutionary-conserved binding sites for *miR-150*. High-level expression of *miR-150* could repress expression of these genes, which encode proteins that enhance B-cell receptor signaling, a putative CLL-growth/survival signal. Also, high-level expression of *miR-150* was a significant independent predictor of longer treatment-free survival or overall survival, whereas an inverse association was observed for high-level expression of GAB1 or FOXP1 for overall survival. This study demonstrates that expression of *miR-150* can influence the relative expression of GAB1 and FOXP1 and the signaling potential of the B-cell receptor, thereby possibly accounting for the noted association of expression of *miR-150* and disease outcome.

BRAIN AND MIND RESEARCH

Progress in Neuro-psychopharmacology & Biological Psychiatry 2014

Repetitive transcranial magnetic stimulation reduces cigarette consumption in schizophrenia patients

Prikryl R., **Ustohal L.**, **Prikrylova Kucerova H.**, **Kasparek T.**, Jarkovsky J., **Hublova V.**, **Vrzalova M.**, **Ceskova E.**

Research Group: Applied Neuroscience

Abstract:

INTRODUCTION

High-frequency repetitive transcranial magnetic stimulation (rTMS) over the left dorsolateral prefrontal cortex (DLPFC) seemed to decrease tobacco consumption and craving in nicotine-dependent people without psychiatric disorder or otherwise healthy people. Even if the prevalence of cigarette smoking in schizophrenia patients is high and estimated to be between 45% and 88%, this technique has not been systematically studied in this indication in schizophrenia yet.

THE AIM OF THE STUDY:

The aim of this study was to test the ability of high-frequency (10Hz) rTMS over the left DLPFC to decrease cigarette consumption in schizophrenia patients.

METHODS:

The study included 35 male schizophrenia patients on stable antipsychotic medication. The patients were divided into two groups: the first (18 patients) were actively stimulated and the second (17 patients) underwent sham (placebo) stimulation. The sham rTMS was administered using a purpose-built sham coil that was identical in appearance to the real coil and made the same noise but did not deliver a substantial stimulus. The rTMS was administered at the stimulation parameters: location (left dorsolateral prefrontal cortex: DLPFC), intensity of magnetic stimulation in % of motor threshold (110%), stimulation frequency (10Hz), number of trains (20), single train duration (10s), inter-train interval (30s), and total number of stimulation sessions (21). In each stimulation session, 2000 TMS pulses were given, with a total of 42,000 pulses per treatment course. Patients noted the number of cigarettes smoked in the 7days before treatment, during the whole stimulation treatment (21 days), and again for a 7-day period after treatment.

RESULTS:

Cigarette consumption was statistically significantly lower in the actively stimulated patients than in the sham rTMS group as early as the first week of stimulation. No statistically relevant correlations were found in the changes of ongoing negative or depressive schizophrenia symptoms and the number of cigarettes smoked.

CONCLUSION:

High-frequency rTMS over the left DLPFC has the ability to decrease the number of cigarettes smoked in schizophrenia patients.

BRAIN AND MIND RESEARCH

BioMed Research International 2014

Inflammatory Profiling of Schwann Cells in Contact with Growing Axons Distal to Nerve Injury

Dubový P., **Klusáková I.**, **Hradilová Svíženská I.**

Research Group: Cellular and Molecular Neurobiology

Abstract: Activated Schwann cells distal to nerve injury upregulate inflammatory mediators, including cytokines. The goal of the present study was to investigate expression of proinflammatory (IL-1β, TNFα) and anti-inflammatory cytokines (IL-4, IL-10) in activated Schwann cells in relation to growing axons distal to crush injury of rat sciatic nerves. Seven days from sciatic nerve crush, transverse cryostat sections were cut 5 mm distal to lesion and incubated for double immunostaining to indicate Schwann cells (GFAP or S100b) and individual investigated cytokines or to demonstrate growing axons (GAP43). The Schwann cells of naïve sciatic nerves and those removed from sham-operated rats displayed similar weak immunoreactivity for the investigated cytokines. In contrast, increased intensity of cytokine immunofluorescence was found in Schwann cells distal to crush lesion. The cytokine positive Schwann cells were found in close contact with growing axons detected by immunostaining for GAP43. The results of immunohistochemical analysis distal to nerve crush injury suggest that inflammatory profiling of Schwann cells including upregulation of both pro- and anti-inflammatory cytokines does not prevent growth of axons distal to nerve crush injury.

MOLECULAR VETERINARY MEDICINE

PROTIST

Hemolivia and Hepatozoon: Haemogregarines with Tangled Evolutionary Relationships

Kvičerová J., Hypša V., **Dvořáková N.**, Mikulíček P., Jandzik D., Gardner M.G., Javanbakht H., Tiar G., **Široký P.**

Research Group: Molecular Bacteriology

Abstract: The generic name *Hemolivia* has been used for haemogregarines characterized by morphological and biological features. The few molecular studies, focused on other haemogregarine genera but involving *Hemolivia* samples, indicated its close relationship to the genus *Hepatozoon*. Here we analyze molecular data for *Hemolivia* from a broad geographic area and host spectrum and provide detailed morphological documentation of the included samples. Based on molecular analyses in context of other haemogregarines, we demonstrate that several sequences deposited in GenBank from isolates described as *Hepatozoon* belong to the *Hemolivia* cluster. This illustrates the overall difficulty with recognizing *Hemolivia* and *Hepatozoon* without sufficient morphological and molecular information. The close proximity of both genera is also reflected in uncertainty about their precise phylogeny when using 18S rDNA. They cluster with almost identical likelihood either as two sister taxa or as monophyletic *Hemolivia* within paraphyletic *Hepatozoon*. However, regardless of these difficulties, the results presented here provide a reliable background for the unequivocal placement of new samples into the *Hemolivia/ Hepatozoon* complex.

Chromosome Research

The frequency of precocious segregation of sister chromatids in mouse female meiosis I is affected by genetic background

Danylevska A., **Kovacovicova K.**, **Awadova T.**, **Anger M.**

Research Group: Mammalian Reproduction

Abstract: Mammalian female gametes frequently suffer from numerical chromosomal aberrations, the main cause of miscarriages and severe developmental defects. The underlying mechanisms responsible for the development of aneuploidy in oocytes are still not completely understood and remain a subject of extensive research. From studies focused on prevalence of aneuploidy in mouse oocytes, it has become obvious that reported rates of aneuploidy are strongly dependent on the method used for chromosome counting. In addition, it seems likely that differences between mouse strains could influence the frequency of aneuploidy as well; however, up till now, such a comparison has not been available. Therefore, in our study, we measured the levels of aneuploidy which has resulted from missegregation in meiosis I, in oocytes of three commonly used mouse strains—CD-1, C3H/HeJ, and C57BL/6. Our results revealed that, although the overall chromosomal numerical aberration rates were similar in all three strains, a different number of oocytes in each strain contained prematurely segregated sister chromatids (PSSC). This indicates that a predisposition for this type of chromosome segregation error in oocyte meiosis I is dependent on genetic background.

MOLECULAR VETERINARY MEDICINE

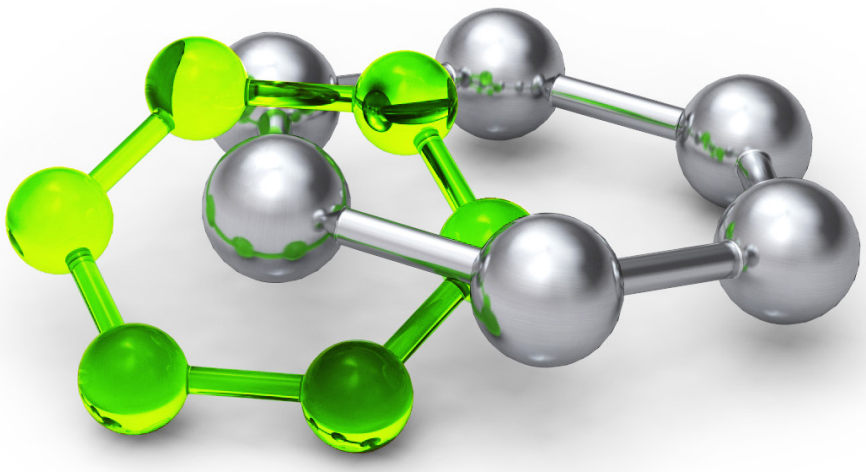
Chromosome Research

Illegitimate recombination between T cell receptor genes in humans and pigs (Sus scrofa domestica)

Musilova P., **Drbalova J.**, **Kubickova S.**, **Cernohorska H.**, Stepanova H., **Rubes J.**

Research Group: Animal Cytogenomics

Abstract: T cell receptor (TCR) genes (*TRA/TRD*, *TRB* and *TRG*) reside in three regions on human chromosomes (14q11.2, 7q34 and 7p14, respectively) and pig chromosomes (7q15.3-q21, 18q11.3-q12 and 9q21-22, respectively). During the maturation of T cells, TCR genes are rearranged by site-specific recombination. Occasionally, interlocus recombination of different TCR genes takes place, resulting in chromosome rearrangements. It has been suggested that the absolute number of these "innocent" *trans*-rearrangements correlates with the risk of lymphoma. The aims of this work were to assess the frequencies of rearrangements with breakpoints in TCR genes in domestic pig lymphocytes and to compare these with the frequencies of corresponding rearrangements in human lymphocytes by using fluorescence in situ hybridization with chromosome painting probes. We show that frequencies of *trans*-rearrangements involving *TRA/TRD* locus in pigs are significantly higher than the frequency of translocations with breakpoints in *TRB* and *TRG* genes in pigs and the frequencies of corresponding trans-rearrangements involving *TRA/TRD* locus in humans. Complex structure of the pig *TRA/TRD* locus with high number of potential V(D)J rearrangements compared to the human locus may account for the observed differences. Furthermore, we demonstrated that *trans*-rearrangements involving pig *TRA/TRD* locus occur at lower frequencies in γδ T cells than in αβ T lymphocytes. The decrease of the frequencies in γδ T cells is probably caused by the absence of *TRA* recombination during maturation of this T cell lineage. High numbers of innocent trans-rearrangements in pigs may indicate a higher risk of T-cell lymphoma than in humans.



Please send us your comments and ideas to **pavla.vyhnankova@ceitec.cz**