CEITEC news

EXTERNAL NEWSLETTER 03/2012

External electronic Newsletter, October 2012

Dear Friends,

On **24 September** 2012, the ceremonial laying of foundation stones officially launched the construction of CEITEC - Central European Institute of Technology. The importance of this event was emphasized in person by the Minister of Education, Youth and Sports of the Czech Republic and other distinguished speakers. We are very pleased to have this support and we are fully committed to fulfilling the high expectations.

We firmly believe the great efforts that have been invested into the establishment of CEITEC will soon have real, tangible results. This can be seen, for example, in the new CEITEC laboratories dedicated to our most important task of establishing CEITEC as an internationally recognized centre of excellence.

The ceremonial hammering of the foundation stones of CEITEC

Martin Bareš, Acting Executive Director

Content

Ceremonial laying of the foundation stones of CEITEC

The construction of CEITEC was officially launched at the Brno University of Technology (BUT) Campus at Pod Palackého vrchem. The ceremony was officially opened by the Minister of Education, Youth and Sports, Mr Petr Fiala. After the hammering of the stones of CEITEC MU and BUT at Pod Palackého Vrchem the participants and the stone of CEITEC MU moved to the University Campus Bohunice, where the stone was laid in a special ceremony.

From the left: Mr Jiří Václavek, moderator; Mr Petr Fiala, the Minister of Education, Youth and Sports

Development will take place simultaneously on two sites in Brno: on the University Campus of Masaryk University (MU) at Bohunice, and on the Brno University of Technology (BUT) Campus at Pod Palackého vrchem. The new facilities will house 25 000 m^2 of scientific laboratories with cutting-edge equipment including 10 Core Facilities to be used by scientists from different disciplines. Thanks to the access students in selected fields will have to state-of-the-art technology, data and practices, the new laboratories will also boost the quality of master's and bachelor's degree programmes. Young researchers will have the opportunity to work with leading scientific teams during their studies. Access to these technologies will also be open to corporate entities involved in joint innovative projects. Researchers will have moved into the new laboratories by the end of 2014.

We would like to thank all participants, speakers: the Minister of Education, Youth and Sports, Mr Petr Fiala; the Rector of Masaryk University, Mr Mikuláš Bek; the Rector of the Brno University of Technology, Mr Karel Rais; the Governor of the South Moravian Region, Mr Michal Hašek; the first Deputy Mayor of the City of Brno, Mr Robert Kotzian; the Executive Director of CEITEC, Mr Martin Bareš; the Scientific Director for Life Sciences, Mr Jaroslav Koča and the Scientific Director for Material Sciences, Mr Radimír Vrba; and those involved in the organisation of the event.

Photo gallery can be found **HERE**, press release can be found **HERE**.

EMBO Gold Medal 2012 recognises CEITEC researcher Jiří Friml

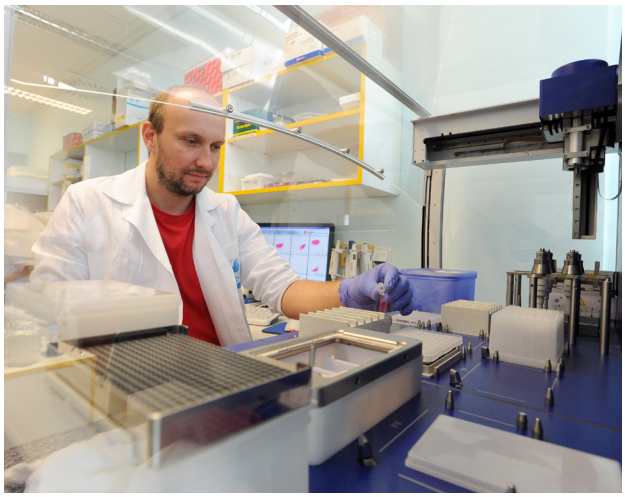
EMBO announced Jiří Friml of the Department of Plant Systems Biology, VIB and Ghent University, Belgium and Central European Institute of Technology, Masaryk University, Brno, Czech Republic as the winner of the 2012 EMBO Gold Medal. Friml receives the award for defining how the plant hormone auxin functions to regulate plant development. He was also recognized for his work showing how the auxin-governed molecular processes optimise adaptation of plant development and growth to ever-changing environmental conditions. More information about EMBO and the prize can be found **HERE**.

Jiří Friml

CEITEC scientist Boris Tichý receives the prestigious Discovery Award

The Discovery Award rewards young Czech scientists under 40 who conduct research in medicine and pharmaceutics. On Wednesday 20 June, the results were announced and awards given for innovation for 2010 and 2011 at Charles University in Prague. Boris Tichý's team from CEITEC´s Molecular Medicine Research Programme was one of three winners.

The projects were assessed by a committee of eleven renowned experts who have been active a long time in the field of health services. Dr. Boris Tichý, Head of the Core Facility – Genomics together with his team received the prize of 100,000 CZK for the project of Improved molecular diagnostics and prognostic stratification of chronic lymphocytic leukemia and other lymphoproliferation. The following collaborators from the Molecular Medicine programme are a part of the team: its Coordinator Prof. Šárka Pospíšilová, Prof. Michael Doubek and Dr. Jitka Malčíková.



Boris Tichý

Changes in the management of CEITEC

Tomáš Hruda

On the 1 September there was a change in the position of Executive Director. The previous Executive Director, Tomáš Hruda, accepted the offer of the position of the Deputy Minister for Research and Higher Education. We would like to thank Tomáš Hruda for all the superb work and enthusiasm he has put into the establishment of the scientific centre of excellence and we also wish him all the best at the Ministry of Education, Youth and Sports.

Martin Bareš was elected Tomáš Hruda's successor and is currently acting executive director at CEITEC. He has worked in the field of neurology at St. Anne´s University Hospital in Brno. From 2009 he has worked as a Professor at the First Department of Neurology, Faculty of Medicine, Masaryk University; in September 2011 he was appointed Vice-rector for development at Masaryk University. Responsibility for the CEITEC project, besides others, is also a part of this position. Within CEITEC Martin Bareš also works as the principal investigator in Research Programme Brain and Mind Research.

From 1 November 2012 Martin Bareš will be replaced by new international management.

Martin Bareš speaking during the ceremonial laying of the foundation stones of CEITEC

CEITEC and Imperial College London become strategic partners

Imperial College London joined the family of CEITEC strategic partners over summer. A Memorandum of Understanding between the two parties was signed in August and covers the area of nanosciences and nanotechnologies. The leading person on the CEITEC side is Prof. Tomáš Šikola, Coordinator of the Advanced Nanotechnologies and Microtechnologies Research Programme and on Imperial side Prof. Stefan Maier, co-director of the Centre for Plasmonics and Metamaterials. The cooperation will result in joint research projects, training and academic exchanges of PhD students, PostDocs as well as faculty members and exchanges of expertise in the fabrication and characterisation of nanostructures.

Imperial College London

Other CEITEC strategic partners include the European Molecular Biology Laboratory or Elettra synchrotron in Trieste.

Joint project with ETH Zurich successfully completed in Brno, cooperation set to carry on

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

ETH Zurich representatives in Brno

The main goal of the project 'Exchange of Experience and Transfer of Best Practices in Operation of Centres of Scientific Excellence between ETH Zurich and CEITEC' was to establish closer co-operation between ETH Zurich and CEITEC.

Ten CEITEC researchers from various research programmes visited ETH Zurich and University of Zurich in May and July 2012. They were accompanied by CEITEC representatives from the grant office, technology transfer, evaluation, core facilities, financial management, public procurement, public relations and human relations who could meet their counterparts and obtain new skills for their future work.

Following these visits, two researchers and nine experts from Zurich representing the above mentioned areas arrived in Brno between 24 and 26 September 2012. The main objective of this visit was to further strengthen their previous contacts, to share know-how and to discuss future cooperation.

All the meetings proved to be successful and thank to this common project, new research collaboration will start and all above mentioned research support services will be improved in CEITEC.

This project was supported by a grant from Switzerland through the Swiss Contribution to the enlarged European Union. You can find more HERE.

A new arrival – a unique microtomography station at BUT

Even the tinyest, invisible cracks or material defects can affect the safety of an airplane or the accuracy of a dental implant in the jaw, these can now be discovered using a 'v|tome|x' microtomographic station from GE Phoenix. This unique device was installed by trained specialists at the Central European Institute of Technology – Brno University of Technology (CEITEC BUT) laboratories. This is the only device of its kind in the Czech Republic, capable of measuring samples of such size. The device will also be used in medical diagnostics where it will help in areas such as prolonging the life of an artificial joint. More information about microgomography station and its usage can be found **HERE**.

Conference invitation - Cell Interaction with Surfaces

We are pleased to invite you to the CEITEC international conference about Cell Interaction with Surfaces, which will be held on 22-23 October 2012 in Masaryk University Congress Centre, Komenského nám. 220/2, Brno.

The thematic sessions will be following:

- Cell-surface interactions and axon regeneration, bio-compatibility cells with surfaces
- Surface antigens of tumor cells – relevance for medicine
- Structure observation and surface imaging methods (TEM) and AFM/SPM observing/imaging of cells
- Biosensors and cell-applications, cell-receptors
- Advanced light microscopy techniques for live cell dynamics evaluation
- Bio-applications of Plasma Technologies

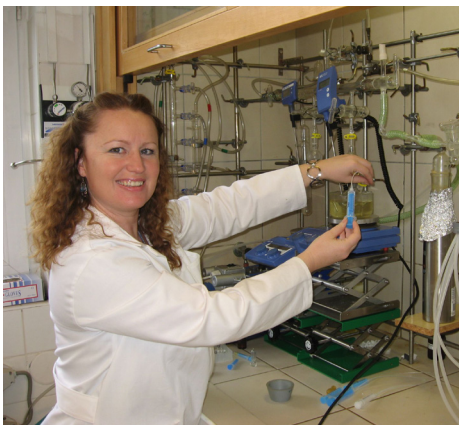
If you want to take part in this interesting conference, please send your name, institution and city to e-mail tomas.havlik@ceitec.vutbr.cz.

More information can be found **HERE**.

The event has been supported by the EU Seventh Framework Programme under the 'Capacities' specific programme (Contract No. 286154 – SYLICA).

Interview with CEITEC scientist

Lucy Vojtová



CEITEC Brno University of Technology, Czech Republic

Research Programme: Advanced Materials
Research Group: Advanced Polymers and Composites

Lucy Vojtová has been working for CEITEC since August 2011 and at the beginning of this year took full-time employment. She leads the research, preparation and characterisation of special polymeric materials and biomaterials at the CEITEC RG 2-3 Advaneced Polymers and Composites. She is the author of many articles in prestigious international journals, patents and numerous presentations at international conferences. Apart from her scientific activities she lectures to both experts and general public and leads bachelor, diploma and dissertation theses. Her students have won a number of awards.

Your team, headed by Prof. Josef Jančář[1], has recently succeeded in the preparation of hydrogel which could in the future function like 'glue' to repair fractured bones. How does this material function in a human body exactly?

The advantage of this hydrogel is the fact that it can be injected into the patient´s body with a syringe. It is a water solution of polymers, which solidifies at the temperature of the human body and the required shape is created only at this temperature, so it is in the body where the 3D structure is created. Therefore, any cavities (even irregular) can be filled because this hydrogel absorbs water and imitates human tissue, nutrients and medicinal substances can enter the body with it. We are also planning to use it as a controlled carrier of drug, eventually stem cells. We can set their gradual release over periods of time, e.g. for the period of two months or even two years, and the treatment is always tailor-made for the patient. In addition, this hydrogel will start to disintegrate in a controlled way into nontoxic, biodegradable products in the body at a time set in advance. With these biomaterials we can see the future of solving common as well as comminuted fractures.

[1] Prof. Josef Jančář is the Research Group Leader of Advanced Polymers and Composites in CEITEC and the Director of the Institute of Materials Chemistry, Faculty of Chemistry, BUT

With your team you are also engaged in the preparation of several other kinds of biomaterials, e.g. implants for worn cartilages.

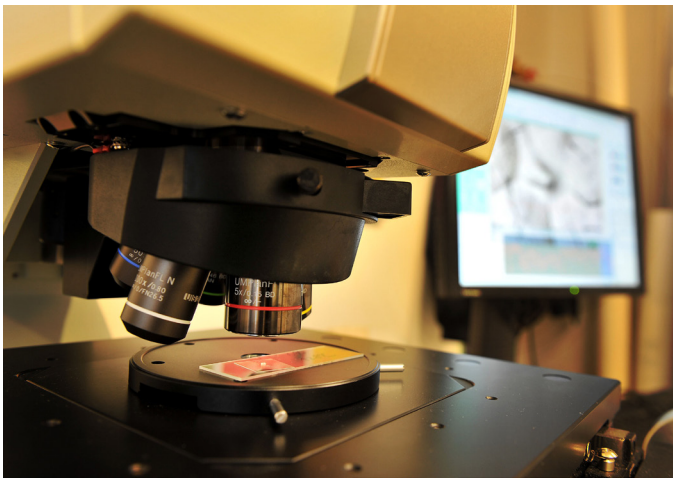
Yes, scaffolds are carriers of cells for tissue engineering, in our case for implants into bones, joints and cartilages. These 3D porous materials based on collagen are put into the patient's body surgically (they are not injected). They can be tailor-made for the patient. We are planning to use scaffolds for major defects of bones instead of the existing bone graft, further also for damaged joints and deformed cartilages. In their chemical composition, they are similar to the damaged tissue so they adjust to the live organism.

Have you tested these materials in practice?

Not on people yet, but we have done several testing of scaffolds on experimental animals. We cooperate with veterinary surgeons from the Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno. The materials are implanted into animals, on which serious diseases of joints and bones have been simulated, which humans suffer from, e.g. injuries of ligament and meniscus, damage of joint cartilage or breaks of long bones. The implants are first filled with stem cells by the technique of stem engineering, which helps to regenerate the damaged tissue. So, we also cooperate with e.g. the Institute of Experimental Medicine AS CR in Prague, Masaryk University in Brno or the National Tissue Center. The created polymeric implant is inserted in the area of affected tissue, where it gives an impulse to other cells to start changing into the cells of cartilages or bones. Unlike most of the materials used up until now, the cellular polymeric carrier disintegrates into nontoxic products the moment the defect is successfully treated, in the time given in advance, so there is no trace left after it in the organism.

How successful were the tests?

Tests on mini pigs have shown that it really works. Thanks to using the new implant, defects of a thighbone have been healed with new bone tissue much faster than normal. Tests on cartilage have also been successful, a piece of joint cartilage was removed, our material with stem cells was inserted and after sixteen weeks a new smooth cartilage grew in the area of the defect. Normally, the cartilage does not have the function to heal, mostly only a scar appears on it. Five-year-long testing on animals has proven successful, that is why we are also preparing clinical testing on people. We are also trying to cooperate actively with the public as well as private sectors.



How long have you been engaged in the research of these biomaterials and how did you get involved in this research?

I have been engaged in biomaterials for nearly 12 years. First, I started to deal with biomaterials during a three-year-long post-doctoral stay at the American Columbia University in New York. This stay followed a three-month-long stay at the University of Connecticut, where I managed to get in, thanks to Professor Josef Jančář, my leader and the Director of the Institute of Materials Chemistry, Faculty of Chemistry, BUT. In New York, we were working on a sensor for diabetics connected with a wireless patient's watch showing the volume of glucoses in blood. This way the watch informs the diabetic when they should take another dose of insulin. It was an approximately 0.5 mm thin biosensor coated with hydrogel, indecomposable in a body, which was implanted under the skin in the area of the abdomen.

And what was your stay in the USA like?

There were 23 nationalities in one group and only one of us was American at the Columbia University. The fact, that we came from different places, made us all try harder, because we were all there for ourselves, so we really worked hard. It was a very competitive environment, most of us worked up to 16 hours a day, including weekends. Of course you can't keep this pace up for a long time, people take it in shifts all the time. The life there is generally faster and in many aspects more demanding, on students too. For instance companies paying school tuitions for eligible students, who they choose according to their results at the beginning of their studies, is standard. Of course, it has to be paid back by the student. Student loans, which the students pay off after they start earning, are common too. For example the Columbia University is expensive but it is such a prestigious school that it opens doors to other high-quality employment.

And do you think this is what brings them results, why they are successful?

Yes, but it is also about the ways and possibilities of work. Instruments are used 24/7 there, they are not switched off at all so it makes the work much faster. The intellectual property protection is also very important for the Americans, they produce one patent following another. For example the Columbia University has its own Patent Office, I have also been assigned a patent representative who wrote the patent for me from my publication. In our country it is more complicated and time consuming, finally I wrote and translated the patent myself. The Americans also produce a lot of publications, often with early results. Due to the fact that there are many more outputs statistically, the chance of success is higher.

What have you gained from this experience?

Apart from priceless work experience it was mainly enthusiasm and this is what students in our country sometimes lack. That's why I introduced Group meetings in my team of students four years ago, in which each of them presents their work and outputs. These debate bees were very successful with students. They share their work projects with each other and this way have an idea what the others are working on. This way they extend their horizons and cooperate and motivate each other, which is the basis for success. They also learn to make a presentation and have discussions in English, they learn to plan and schedule their work, that is what most students can't do well yet. In the USA these bees were held every week, each student's turn came about once a month. For the students to have the outputs and something to present, they must work really hard because a month is quite a short period in research. I myself lead 7 doctoral students and we are going to take on another three from September on, so I am going to introduce these bees again, because they have been interrupted during the period of my maternity leave.

And do you cooperate with abroad?

We are just starting to; students from abroad have been getting in touch with us. We keep permanent relations with the Columbia University and the University of Massachusetts, also in Sweden, Italy, Poland and Germany, but there are also contacts from India, China and Russia. For the time being unfortunately we have not got enough space so we are waiting for the new buildings in CEITEC to be completed and to gain new premises for other recruits.

Do you expect CEITEC to become closer to foreign centres?

Yes, I expect a similar approach from CEITEC as it works abroad. We will move in laboratories which we have designed for ourselves and which comply with our requirements. We will buy specialized instruments, which will also be used 24/7 and necessary materials which are mostly very expensive. But it is all mainly about people and I am not sure whether they will be willing to work in shifts. In the USA we had no families and we found friends mainly at work, so in the end we spent nearly all our free time there. The work fulfilled us because that was the reason why we were there. I believe that if there was a community of students created, who would be in the same situation, the work would intensify and we will have many more outputs. And this is what CEITEC is planning for so I hope that it will take off.

Lastly I would like to ask how you manage to balance motherhood and work? You have a small son and full-time employment.

Mainly thanks to the grandmother and grandfather. I started part-time employment six months after the birth, and the full--time one after one year. By then I wanted to work, I needed to use my brain, which gets lazy quickly. Moreover, scientific work is a bit of a nunnish devotion. One has to like it and enjoy it. The topics are challenging for me. It is a challenge to do and discover something new, more perfect. I also enjoy working with students, teaching them to think in a scientific way, which they cannot do well at the beginning. When they are not motivated daily, they lose motivation quickly and if they have not got it, not everybody has the necessary self-discipline in them. I also want to maintain my team and bring new recruits into CEITEC.

CEITEC is also trying to establish a kindergarten for its employees. Would you welcome it?

Hundred percent yes, it is a great thing, mainly the option of flexibility. If I want to be with my son, I go to work for arranged meetings and consultations and I do the work itself from home at nights, in my second shift. Kindergarten will definitely help, also lately there has been a problem getting places for children in state kindergartens. And I know that foreign employees would definitely welcome it too.

Last question: what advice would you give to young beginning scientists so that they can start their career in the right way?

Ideally, to get involved in a working team and cooperate on an interesting project which e.g. CEITEC is. It is important to find one´s own position and focus and gradually write also your own projects and create your own team. Language ability, hardworking, self-discipline and mainly patience are vital. Nothing comes immediately in science, but sooner or later the results will come.

Selected CEITEC publications

Journal of Hazardous Materials

Phytochelatin synthase activity as a marker of metal pollution

Zitka, O.; Krystofova, O.; Sobrova, P.; Adam, V.; Zehnalek, J.; Beklova, M.; Kizek, R.

CEITEC Research Group: **Submicron Systems and Nanodevices**
Research Programme 1: **Advanced Nanotechnologies and Microtechnologies**

Summary

The synthesis of phytochelatins is catalyzed by γ-Glu-Cys dipeptidyl transpeptidase called phytochelatin synthase (PCS). Aim of this study was to suggest a new tool for determination of phytochelatin synthase activity in the tobacco BY-2 cells treated with different concentrations of the Cd(II). After the optimization steps, an experiment on BY-2 cells exposed to different concentrations of $Cd(NO_3)_2$ for 3 days was performed. At the end of the experiment, cells were harvested and homogenized. Reduced glutathione and cadmium (II) ions were added to the cell suspension supernatant. These mixtures were incubated at 35 °C for 30 min and analysed using high performance liquid chromatography coupled with electrochemical detector (HPLC-ED). The results revealed that PCS activity rises markedly with increasing concentration of cadmium (II) ions. The lowest concentration of the toxic metal ions caused almost three fold increase in PCS activity as compared to control samples. The activity of PCS (270 fkat) in treated cells was more than seven times higher in comparison to control ones. Km for PCS was estimated as 2.3 mM.

Applied Physics Letters

Controlled faceting in (110) germanium nanowire growth by switching between vapor-liquid-solid and vapor-solid- solid growth

Kolíbal, M.; Kalousek, R.; Novák, L.; Vystavěl, T.; Šikola, T.

CEITEC Research Group: **Fabrication and Characterisation of Nanostructures**
Research Programme 1: **Advanced Nanotechnologies and Microtechnologies**

Summary

We show that the hexagonal cross-section of germanium nanowires grown in the h110i direction by physical vapor deposition is a consequence of minimization of surface energy of the collector droplet. If the droplet is lost or solidified, two {001} sidewall facets are quickly overgrown and the nanowire exhibits a rhomboidal cross-section. This process can be controlled by switching between the liquid and solid state of the droplet, enabling the growth of nanowires with segments having different cross-sections. These experiments are supported by in-situ microscopic observations and theoretical model.

Spectrochimica Acta Part B

Fast identification of biominerals by means of stand-off laser-induced breakdown spectroscopy using linear discriminant analysis and artificial neural networks

Vítková, G.; Novotný, K.; Prokeš, L.; Hrdlička, A.; Kaiser, J.; Novotný, J.; Malina, R.; Prochazka, D.

CEITEC Research Group: **X-ray Micro CT and Nano CT**
Research Programme 1: **Advanced Nanotechnologies and Microtechnologies**

Summary

The goal of this paper is to compare two selected statistical techniques used for identification of archeological materials merely on the base of their spectra obtained by stand-off laser-induced breakdown spectroscopy (stand-off LIBS). Data processing using linear discriminant analysis (LDA) and artificial neural networks (ANN) were applied on spectra of 18 different samples, some of them archeological and some recent, containing 7 types of material (i.e. shells, mortar, bricks, soil pellets, ceramic, teeth and bones). As the input data PCA scores were taken. The intended aim of this work is to create a database for simple and fast identification of archeological or paleontological materials in situ. This approach can speed up and simplify the sampling process during archeological excavations that nowadays tend to be quite damaging and timeconsuming.

Scripta Materialia

Rapid sintering of crack-free zirconia ceramics by pressure-less spark plasma sintering

Salamon, D.; Maca, K.; Shen, Z.

CEITEC Research Group: **Advanced Ceramic Materials**
Research Programme 2: **Advanced Materials**

Summary

Heating of a ceramic green body is a key step in sintering. We have created inside a spark plasma sintering apparatus pressureless sintering conditions that allow homogeneous and extremely rapid heating. Dense and crack-free zirconia ceramic was sintered at heating rates of up to 500 $^{o}C\ min^{-1}$ and dwell times of 2 min. This extremely fast sintering process is accompanied by extremely rapid grain growth, indicating a non classical sintering mechanism. No grain size gradients were observed inside the sintered zirkonia ceramics.

Genes and Development

Serine phosphorylation and proline isomerization in RNAP II CTD control recruitment of Nrd1

Kubicek K.; Cerna H.; Holub P.; Pasulka J.; Hrossova D.; Loehr F.; Hofr C.; Vanacova S.; Stefl R.

CEITEC Research Group: **Protein-RNA interactions**
Research Programme 3: **Structural Biology**

Summary

Recruitment of appropriate RNA processing factors to the site of transcription is controlled by post-translational modifications of the C-terminal domain (CTD) of RNA polymerase II (RNAP II). Here, we report the solution structure of the Ser5 phosphorylated (pSer5) CTD bound to Nrd1. The structure reveals a direct recognition of pSer5 by Nrd1 that requires the cis conformation of the upstream pSer5-Pro6 peptidyl-prolyl bond of the CTD. Mutations at the complex interface diminish binding affinity and impair processing or degradation of noncoding RNAs. These findings underpin the interplay between covalent and noncovalent changes in the CTD structure that constitute the CTD code.

Current Biology

ABP1 and ROP6 GTPase Signaling Regulate Clathrin-Mediated Endocytosis in Arabidopsis Roots

Chen, X.; Naramoto, S.; Robert, S.; Tejos, R.; Löfke, Ch.; Lin, D.; Yang, Z.; Friml, J.

CEITEC Research Group: **Developmental and Cell Biology of Plants**
Research Programme 4: **Genomics and Proteomics of Plant Systems**

Summary

The dynamic spatial and temporal distribution of the crucial plant signaling molecule auxin is achieved by feedback coordination of auxin signaling and intercellular auxin transport pathways. Developmental roles of auxin have been attributed predominantly to its effect on transcription; however, an alternative pathway involving AUXIN BINDING PROTEIN1 (ABP1) has been proposed to regulate clathrin-mediated endocytosis in roots and Rho-like GTPase (ROP)-dependent pavement cell interdigitation in leaves. In this study, we show that ROP6 and its downstream effector RIC1 regulate clathrin association with the plasma membrane for clathrin-mediated endocytosis, as well as for its feedback regulation by auxin. Genetic analysis revealed that ROP6/RIC1 acts downstream of ABP1 to regulate endocytosis. This signaling circuit is also involved in the feedback regulation of PIN-FORMED 1 (PIN1) and PIN2 auxin transporters activity (via its constitutive endocytosis) and corresponding auxin transport-mediated processes, including root gravitropism and leave vascular tissue patterning. Our findings suggest that the signaling module auxin–ABP1–ROP6/RIC1–clathrin–PIN1/PIN2 is a shared component of the feedback regulation of auxin transport during both root and aerial development.

Nature Communications

ER-localized auxin transporter PIN8 regulates auxin homeostasis and male gametophyte development in Arabidopsis

Ding, Z.; Wang, B.; Moreno, I.; Dupláková, N.; Simon, S.; Carraro, N.; Reemmer, J.; Pěnčík, A.; Chen, X.; Tejos, R.; Skůpa, P.; Pollmann, S.; Mravec, J.; Petrášek, J.; Zažímalová, E.; Honys, D.; Rolčík, J.; Murphy, A.; Orellana, A.; Geisler M.; Friml, J.

CEITEC Research Group: **Developmental and Cell Biology of Plants**
Research Programme 4: **Genomics and Proteomics of Plant Systems**

Summary

Auxin is a key coordinative signal required for many aspects of plant development and its levels are controlled by auxin metabolism and intercellular auxin transport. Here we find that a member of PINauxin transporter family, PIN8 is expressed in male gametophyte of Arabidopsis thaliana and has a crucial role in pollen development and functionality. Ectopic expression in sporophytic tissues establishes a role of PIN8 in regulating auxin homoeostasis and metabolism. PIN8 co-localizes with PIN5 to the endoplasmic reticulum (ER) where it acts as an auxin transporter. Genetic analyses reveal an antagonistic action of PIN5 and PIN8 in the regulation of intracellular auxin homoeostasis and gametophyte as well as sporophyte development. Our results reveal a role of the auxin transport in male gametophyte development in which the distinct actions of ER-localized PINtransporters regulate cellular auxin homoeostasis and maintain the auxin levels optimal for pollen development and pollen tube growth.

Phytochemistry

Retargeting a maize β-glucosidase to the vacuole – evidence from intact plants that zeatin-O-glucoside is stored in the vacuole.

Kiran, N.S.; Benková, E.; **Reková, A.**; Dubová, J.; Malbeck, J.; Palme, K.; **Brzobohatý, B.**

CEITEC Research Group: **Developmental and Production Biology – Omics Approaches**
Research Programme 4: **Genomics and Proteomics of Plant Systems**

Summary

The activity of the plant hormone cytokinin is regulated by the complex interplay of its metabolism, transport, stability and cellular/tissue localization. Active cytokinin levels are determined in part by their differential distribution of cytokinin metabolites across different subcellular compartments. The maize β-glucosidase Zm-p60.1 specifically cleaves zeatin-O-glucoside, the storage/transport form of zeatin. We successfully retargeted the plastid-localized Zm-p60.1 to the vacuole by using modular heterologous signal peptides. We showed in transgenic tobacco plants that the enzyme is active in at least two compartments (chloroplast and vacuole) with vastly differring biochemical environments. Plants that express Zm-p60.1in the vacuole cannot store zeatin-O-glucoside, thus proving that the vacuole is the storage organelle for zeatin-O-glucoside. Physiological tests showed that vacuole-localized Zm-p60.1 restores zeatin-induced root-growth inhibition. We have shown that Zm-p60.1 is a robust molecular tool and can be used to explore cytokinin metabolism *in planta*.

Atherosclerosis

The molecular basis of familial hypercholesterolemia in the Czech Republic: Spectrum of LDLR mutations and genotype-phenotype correlations

Tichy, L.; **Freiberger, T.**; Zapletalova, P.; Soska, V.; Ravcukova, B.; **Fajkusova, L.**

CEITEC Research Group: **Inherited Diseases I – Genetic Research; Molecular Immunology and Microbiology**
Research Programme 5: **Molecular Medicine**

Summary

Familial hypercholesterolemia (FH), a major risk for coronary heart disease, is predominantly associated with mutations in the genes encoding the low-density lipoprotein receptor (LDLR) and its ligand apolipoprotein B (APOB).
Results: In this study, we characterize the spectrum of mutations causing FH in 2239 Czech probands suspected to have FH. In this set, we found 265 patients (11.8%) with the APOB mutation p. (Arg3527Gln) allelic variants were detected: 70.1 % of these variants were DNA substitutions, 16.5 % small DNA rearrangements, and 13.4 % large DNA rearrangements. Fifty five variants were novel, not described in other FH populations. For lipid profile analyses, FH probands were divided into groups [patients with LDLR mutation (LDLR+), with the APOB mutation (APOB+), and without a detected mutation (LDLR-/APOB-)], and each group into subgroups according to gender. The statistical analysis of lipid profiles was performed in 1722 probands adjusted for age in which biochemical data were obtained without FH treatment (480 LDLR+ patients, 222 APOB+ patients, and 1020 LDLR-/APOB- patients). Significant gradients in i) total cholesterol (LDLR+ patients > APOB+ patients = LDLR-/APOB- patients) ii) LDL cholesterol (LDLR+ patients > APOB+ patients = LDLR-/APOB- patients in men and LDLR+ patients > APOB+ patients > LDLR-/APOB- patients in women), iii) triglycerides (LDLR-/APOB- patients > LDLR+ patients > APOB+ patients), and iv) HDL cholesterol (APOB+ patients > LDLR-/APOB- patients = LDLR+ patients) were shown.

Mycological Progress

RNA secondary structure, an important bioinformatics tool to enhance multiple sequence alignment: a case study (Sordariomycetes, Fungi)

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Research Programme 5: **Molecular Medicine**

Summary

In a case study of fungi of the class Sordariomycetes, we evaluated the effect of multiple sequence alignment (MSA) on the reliability of the phylogenetic clades. We compared two main approaches for constructing MSA based on (1) the knowledge of the secondary (2D) structure of ribosomal RNA (rRNA) genes, and (2) automatic construction of MSA by four alignment programs characterized by different algorithms and evaluation methods, CLUSTAL, MAFFT, MUSCLE, and SAM. In the primary fungal sequences of the two functional rRNA genes, the nuclear small and large ribosomal subunits (18 S and 28 S), we identified four and six, respectively, highly variable regions, which correspond mainly to hairpin loops in the 2D structure. These loops are often positioned in expansion segments, which are missing or are not completely developed in the Archaeal and Eubacterial kingdoms. Proper sorting of these sites was a key for constructing an accurate MSA. We utilized DNA sequences from 28 S as an example for one-gene analysis. Five different MSAs were created and analysed with maximum parsimony and maximum likelihood methods. The phylogenies inferred from the alignments improved with 2D structure with identified homologous segments, and those constructed using the MAFFT alignment program, with all highly variable regions included, provided the most reliable phylograms with higher bootstrap support for the majority of clades. We illustrate and provide example demonstrating that re-evaluating ambiguous positions in the consensus sequences using 2D structure and covariance is a promising means in order to improve the quality and reliability of sequence alignments.

Nucleic Acids Research

Structure and mechanical properties of the ribosomal L1 stalk three-way junction

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Summary

The L1 stalk is a key mobile element of the large ribosomal subunit which interacts with tRNA during translocation. Here, we investigate the structure and mechanical properties of the rRNA H76/H75/H79 three-way junction at the base of the L1 stalk from four different prokaryotic organisms. We propose a coarse-grained elastic model and parameterize it using large-scale atomistic molecular dynamics simulations. Global properties of the junction are well described by a model in which the H76 helix is represented by a straight, isotropically flexible elastic rod, while the junction core is represented by an isotropically flexible spherical hinge. Both the core and the helix contribute substantially to the overall H76 bending fluctuations. The presence of wobble pairs in H76 does not induce any increased flexibility or anisotropy to the helix. The half-closed conformation of the L1 stalk seems to be accessible by thermal fluctuations of the junction itself, without any long-range allosteric effects. Bending fluctuations of H76 with a bulge introduced in it suggest a rationale for the precise position of the bulge in eukaryotes. Our elastic model can be generalized to other RNA junctions found in biological systems or in nanotechnology.

Pharmacology, Biochemistry and Behavior

Differential effects of modafinil, methamphetamine, and MDMA on agonistic behavior in male mice

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Research Programme 6: **Brain and Mind research**

Summary

The aim of the present study was to compare the behavioral effects of modafinil an atypical psychostimulatory acting and cognitive-function improving drug, with the effects of the psychostimulants methamphetamine (MET) and MDMA (3,4-methylenedioxymeth-amphetamine, or "ecstasy") in a model of mouse agonistic behavior. This model enables the observation of ethologicallv well defined sociable, timid, aggressive, and locomotor behavioral acts and postures. Singly-housed male mice (isolates) were separated into 4 groups. The observations were performed in 4 sessions. 1 week apart. In each interaction, singly-housed mice were paired with non-aggressive group-housed partners for 4 min in a neutral environment. The isolates received, in a Latin square design, either a) a vehicle or modafinil at doses 2.0, 10.0. or 50.0 mg/kg; or b) a vehicle or MET at doses 1.0, 5.0, or 10.0 mg/kg: or c) a vehicle or MDMA at doses 2.5, 10.0, or 30.0 mg/kg. The isolates were categorized as timid or aggressive according to their behavior in the control interaction(vehicle pre-treatment). Elements of locomotor, sociable, aggressive, and timid behavior were evaluated (one-way ANOVA).
In the aggressive mice, no change in the sum of aggressive behavior was measured following modafinil administration, while both methamphetamine and MDMA produced dose-dependent inhibition of aggression ($p<0.01$). The substantial difference in the tested drug effects on agonistic behavior was an increased occurrence of sociable acts ($p<0.01$) accompanied by a simultaneous increase of timid acts ($p< 0.01$) recorded after MDMA but not after modafinil or methamphetamine administration. In the timid mice, at least some doses of modafinil decreased timidity ($p< 0.01$) and increased aggression ($p<0.01$) with no effect on sociability. Administration of MDMA increased timid activities ($p<0.01$). Both MDMA and MET decreased sociability ($p<0.01$).

Movement Disorders

Impairment of Brain Vessels May Contribute to Mortality in Patients With Parkinson's Disease

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Research Programme 6: **Brain and Mind research**

Summary

Background: The effect of brain-vessel pathology on mortality in 57 consecutive PD patients was studied.
Methods: Baseline clinical, neuropsychological, ultrasonographic (US), and MR data obtained from patients who died (n = 18) during a 4-year folow-up period were compared with the data of patients who survived.
Results: US/MRI data displayed a more-severe vascular impairment in deceased patients. Differences were significant between both groups with respect to age, clinical and cognitive status, intima-media thickness, and resistance index (indicators of large and small vessel impairment). The sum score of white-matter hyperintensities was significantly higher among decedents. A cluster analysis displayed two clusters that differed in the two parameters (i.e. in age and in sum score).
Conclusions: This study provides evidence that comorbid atherosclerosis and otherwise subclinical impairment of brain vessels may contribute to mortality in PD. The vascular pathology may act in association with other comorbidities on the terrain of progressive neurodegenerative pathology. © 2012 Movement Disorder Society
Key Words: cerebrovascular disease; Parkinson's disease; MRI; ultrasound

BMC Neuroscience

Enhancement of musculocutaneous nerve reinnervation after vascular endothelial growth factor (VEGF) gene therapy

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Research Programme 6: **Brain and Mind research**

Summary

Background: Vascular endothelial growth factor (VEGF) is not only a potent angiogenic factor but it also promotes axonal outgrowth and proliferation of Schwann cells. The aim of the present study was to quantitatively assess reinnervation of musculocutaneous nerve (MCN) stumps using motor and primary sensory neurons after plasmid phVEGF transfection and end-to-end (ETE) or end-to-side (ETS) neurorrhaphy. The distal stump of rat transected MCN, was transfected with plasmid phVEGF, plasmid alone or treated with vehiculum and reinnervated following ETE or ETS neurorrhaphy for 2 months. The number of motor and dorsal root ganglia neurons reinnervating the MCN stump was estimated following their retrograde labeling with Fluoro-Ruby and Fluoro-Emerald. Reinnervation of the MCN stumps was assessed based on density, diameter and myelin sheath thickness of regenerated axons, grooming test and the wet weight index of the biceps brachii muscles.
Results: Immunohistochemical detection under the same conditions revealed increased VEGF in the Schwann cells of the MCN stumps transfected with the plasmid phVEGF, as opposed to control stumps transfected with only the plasmid or treated with vehiculum. The MCN stumps transfected with the plasmid phVEGF were reinnervated by moderately higher numbers of motor and sensory neurons after ETE neurorrhaphy compared with control stumps. However, morphometric quality of myelinated axons, grooming test and the wet weight index were significantly better in the MCN plasmid phVEGF transfected stumps. The ETS neurorrhaphy of the MCN plasmid phVEGF transfected stumps in comparison with control stumps resulted in significant elevation of motor and sensory neurons that reinnervated the MCN. Especially noteworthy was the increased numbers of neurons that sent out collateral sprouts into the MCN stumps. Similarly to ETE neurorrhaphy, phVEGF transfection resulted in significantly higher morphometric quality of myelinated axons, behavioral test and the wet weight index of the biceps brachii muscles.
Conclusion: Our results showed that plasmid phVEGF transfection of MCN stumps could induce an increase in VEGF protein in Schwann cells, which resulted in higher quality axon reinnervation after both ETE and ETS neurorrhaphy. This was also associated with a better wet weight biceps brachii muscle index and functional tests than in control rats.

Epilepsy and Behavior

Postictal psychosis and its electrophysiological correlates in invasive EEG: A case report study and literature review

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CEITEC Research Group: **Behavioural and Social Neuroscience**
Research Programme 6: **Brain and Mind research**

Summary

We identified two patients with medically refractory temporal lobe epilepsy, from whom intracranial EEG recordings were obtained at the time of postictal psychosis. Both patients had mesial temporal epilepsy associated with hippocampal sclerosis. In both patients, the postictal psychosis was associated with a continual "epileptiform" EEG pattern that differed from their interictal and ictal EEG findings (rhythmical slow wave and "abortive" spike-slow wave complex activity in the right hippocampus and lateral temporal cortex in case 1 and a periodic pattern of triphasic waves in the contacts recording activity from the left anterior cin-gulate gyrus). Some cases of postictal psychosis might be caused by the transient impairment of several limbic system structures due to the "continual epileptiform discharge" in some brain regions. Case 2 is the first report of a patient with TLE in whom psychotic symptoms were associated with the epileptiform impairment of the anterior cingulate gyrus.

International Journal of Psychophysiology

Intracerebral recordings of the Bereitschaftspotential demonstrate the heterogeneity of its components

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Research Programme 6: **Brain and Mind research, *Psychophysiology***

Summary

Though consisting of early and late components, the evoked potential preceding a voluntary movement (Bereitschaftspotential - BP) is often considered as a unitary phenomenon. By analyzing intracerebrally recorded BP we attempted to demonstrate that the components are electrophysiological correlates of separate operations, The BPs recorded in 42 epilepsy surgery candidates (28 men. 14 women; aged from 18 to 49 years) during self-paced clenching movements of" the hand opposite to the explored hemisphere were investigated in the study. Microdeep intracerebral 5 to l5-contact electrodes were used. The averaged curves were calculated from approximately 30 trials in each case. All the records were taken with a binaural reference. The total number of explored brain regions was 235; the event-related premovement potentials were observed in 121 of them. Three types of pre-movement responses were observed; (i) the BP with both components; (ii), the BP with the early component only; and (iii) the BP with the late component only. The generators of the early one-component BP were demonstrated in two frontal cortical areas (precentral and middle frontal gyri) and in the parietal area known to be involved in action planning and decision making (pre-cuneus). Some structures generating the early one-component BP were activated during movement; the others were without motor responsiveness. The results suggest a separate elaboration of functional task items in some and their integration in other brain structures, and the existence of volitional mechanisms of different hierarchical character.

Cell Cycle

Lack of response to unaligned chromosomes in mammalian female gametes

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Research Programme 7: **Molecular Veterinary Medicine**

Summary

Chromosome segregation errors are highly frequent in mammalian female meiosis, and their incidence gradually increases with maternal age. the fate of aneuploid eggs is obviously dependent on the stringency of mechanisms for detecting unattached or repairing incorrectly attached kinetochores. In case of their failure, the newly formed embryo will inherit the impaired set of chromosomes, which will have severe consequences for its further development. Whether spindle assembly checkpoint (SAC) in oocytes is capable of arresting cell cycle progression in response to unaligned kinetochores was discussed for a long time. It is known that abolishing SAC increases frequency of chromosome segregation errors and causes precocious entry into anaphase; SAC, therefore, seems to be essential for normal chromosome segregation in meiosis I. However, it was also reported that for anaphase-promoting complex (ApC) activation, which is a prerequisite for entering anaphase; alignment of only a critical mass of kinetochores on equatorial plane is sufficient. this indicates that the function of SAC and of cooperating chromosome attachment correction mechanisms in oocytes is different from somatic cells. to analyze this phenomenon, we used live cell confocal microscopy to monitor chromosome movements, spindle formation, ApC activation and polar body extrusion (pBe) simultaneously in individual oocytes at various time points during first meiotic division. our results, using oocytes from aged animals and interspecific crosses, demonstrate that multiple unaligned kinetochores and severe congression defects are tolerated at the metaphase to anaphase transition, although such cells retain sensitivity to nocodazole. this indicates that checkpoint mechanisms, operating in oocytes at this point, are essential for accurate timing of ApC activation in meiosis I, but they are insufficient in detection or correction of unaligned chromosomes, preparing thus conditions for propagation of the aneuploidy to the embryo.