MEETINGS AND ABSTRACTS OF THE CZECHOSLOVAK BIOLOGICAL SOCIETY 2004

SCHŮZE BRNĚNSKÉ POBOČKY ČESKOSLOVENSKÉ BIOLOGICKÉ SPOLEČNOSTI V ROCE 2004

Členská schůze 12. ledna 2004

(Schůze konaná ve spolupráci s Českou anatomickou společností a Laboratoří genetiky a embryologie Ústavu živočišné fyziologie a genetiky AV ČR v Brně)

R. J. Radlanski (Abteilung Experimentelle Zahn-, Mund- und Kieferheilkunde/Orale Strukturbiologie, Universitätsklinikum Benjamin Franklin, Klinik und Poliklinik für Zahn-, Mund- und Kieferheilkunde, Freie Universität Berlin, Bundesrepublik Deutschland): **Human craniofacial and dental development: What do we not know**.

Členská schůze 10. března 2004

P. Dvořák (Laboratoř molekulární embryologie, Agronomická fakulta MUZL v Brně; Oddělení molekulární embryologie, Ústav experimentální mediciny AV ČR a Centrum buněčné terapie a tkáňových náhrad, 2. lékařská fakulta UK v Praze): **Lidské embryonální kmenové buňky**.

J. Pacherník (Laboratoř molekulární embryologie, Agronomická fakulta MUZL v Brně a Centrum buněčné terapie a tkáňových náhrad, 2. lékařská fakulta UK v Praze): **Růst embryonálních kmenových buněk na definovaných vrstvách biologických makromolekul**.

J. Kroupová (Laboratoř molekulární embryologie, Agronomická fakulta MUZL v Brně): **Značení a kultivace embryonálních kmenových buněk na hydrogelových nosičích**.

Členská schůze 1. dubna 2004

7. Babákova přednáška

(Schůze konaná ve spolupráci s Hlavním výborem Československé biologické společnosti a Biologickým ústavem Lékařské fakulty MU v Brně)

M. V. Nermut (National Institute for Biological Standards and Control, South Mimms, Potters Bar, Herts, United Kingdom): **HIV the Smart Killer**. **Structural Aspects of HIV Assembly**.

Členská schůze 14. dubna 2004

J. Fajkus (Laboratoř molekulárních komplexů DNA, Biofyzikální ústav AV ČR v Brně a Laboratoř funkční genomiky a proteomiky, Přírodovědecká fakulta MU v Brně): **Rozumíme biologii telomer**?

M. Skleničková (Laboratoř molekulárních komplexů DNA, Biofyzikální ústav AV ČR v Brně): **Jak vypadají telomery**?

Z. Kunická[1], *R. Hájek*[2], *J. Fajkus*[3] ([1]Laboratoř funkční genomiky a proteomiky, Přírodovědecká fakulta MU v Brně, [2]Interní hematoonkologická klinika, Lékařská fakulta MU a FN v Brně a [3]Laboratoř

molekulárních komplexů DNA, Biofyzikální ústav AV ČR v Brně): **Stanovení telomerázové aktivity u mnohočetného myelomu pomocí RQ-TRAP**.

M. Kuchař[1], M. Horáková[1], L. Krejčí[1], D. Zachová[1], J. Fajkus[2] ([1]Laboratoř funkční genomiky a proteomiky, Přírodovědecká fakulta MU v Brně a [2]Laboratoř molekulárních komplexů DNA, Biofyzikální ústav AV ČR v Brně): **Využití dvojhybridního systému pro studium telomerových proteinů**.

P. Schrumpfová[1], G. Rotková[1], M. Kuchař[1], L. Skřišovská[1], T. Kubičárová[2], J. Fajkus[2] ([1]Laboratoř funkční genomiky a proteomiky, Přírodovědecká fakulta MU v Brně a [2]Laboratoř molekulárních komplexů DNA, Biofyzikální ústav AV ČR v Brně): **Charakterizace Myb-like proteinů *Arabidopsis thaliana* s afinitou k telomerové DNA, které patří do nové skupiny SMH proteinů**.

Členská schůze 12. května 2004

(Schůze konaná ve spolupráci s Českou anatomickou společností a Anatomickým ústavem Lékařské fakulty MU v Brně)

L. Páč, M. Buchtová, Z. Knotek[+]* (Anatomický ústav, Lékařská fakulta MU v Brně, *Ústav anatomie, histologie a embryologie a [+]Klinika chorob ptáků, plazů a drobných savců, Fakulta veterinárního lékařství VFU v Brně): **Senzitivní tělíska z kůže nosu želvy *(Agrionemys horsfieldi)***.

L. Horáčková (Anatomický ústav, Lékařská fakulta MU v Brně): **Mezinárodní expedice v nekropoli Nové říše – Sakkára 2004 (Egypt)**.

M. Račanská, A. Shbat, L. Prokeš[+]* (Anatomický ústav, Lékařská fakulta MU v Brně, *Katedra antropologie a [+]Laboratoř atomové spektrochemie, Přírodovědecká fakulta MU v Brně): **Antropologická analýza kosterních pozůstatků biskupa Jana Filipce (1431–1509)**.

Členská schůze 29. září 2004

(Schůze konaná ve spolupráci s Českou fyziologickou společností k 25. výročí úmrtí profesora MUDr. Vladislava Kruty, DrSc. (1908–1979), dlouholetého přednosty Fyziologického ústavu Lékařské fakulty MU v Brně)

O životě a díle prof. V. Kruty hovořili V. Kruta (Paříž), P. Bravený, B. Fišer, Z. Franc a M. Kukleta (Brno), J. Mareš, H. Rašková a R. Rokyta (Praha).

Členská schůze 13. října 2004

M. Hanáková, P. Nikolová, P. Peťovská, M. Vilémová, A. Oltová, Š. Prášilová, I. Grochová, Z. Kalina, R. Gaillyová (Oddělení lékařské genetiky FN Brno – pracoviště Dětská nemocnice): **Postnatální diagnostika chromosomálních aberací na OLG FN Brno v letech 1999–2003**.

P. Nikolová, P. Peťovská, M. Hanáková, M. Vilémová, A. Oltová, Z. Kalina, Š. Prášilová, H. Filková, M. Pešáková, D. Žežulková, P. Kuglík, B. Ravčuková, R. Gaillyová (Oddělení lékařské genetiky FN Brno – pracoviště Dětská nemocnice): **Cytogenetické nálezy u pacientů s poruchou fertility vyšetřených na OLG FN Brno v letech 1999–2003, vybrané kazuistiky**.

P. Peťovská, P. Nikolová, M. Hanáková, M. Vilémová, A. Oltová, H. Filková, D. Žežulková, M. Pešáková, P. Kuglík, Š. Prášilová, J. Šoukalová, R. Gaillyová (Oddělení lékařské genetiky FN Brno – pracoviště Dětská nemocnice): **Vrozené chromosomální aberace u dětí vyšetřených na OLG FN Brno v letech 1999–2003, vybrané kazuistiky**.

Členská schůze 10. listopadu 2004

M. Vojtíšková (Biofyzikální ústav AV ČR v Brně): **Reparační mechanismy lidského genomu**.

I. Sedlářová, K. Stehlíková, V. Brabec, M. Vojtíšková* (Biofyzikální ústav AV ČR a *Katedra molekulární biologie a genetiky, Přírodovědecká fakulta MU v Brně): **Výhody bezbuněčného RTS systému při expresi reparačního hXPA proteinu**.

M. Hyršová, E. Janovská, V. Brabec, M. Vojtíšková* (Biofyzikální ústav AV ČR a *Katedra mikrobiologie, Přírodovědecká fakulta MU v Brně): **Interakce cis-platiny s plasmidovou DNA pUC19 v buňkách *E. coli* a možnosti reparace**.

Členská schůze 8. prosince 2004

(Schůze věnovaná prezentaci výsledků práce studentů doktorského týmu projektu č. 523/03/H076 GA ČR z Ústavu genetiky Agronomické fakulty MZLU v Brně)

J. Dvořák (Ústav genetiky, Agronomická fakulta MZLU v Brně): **Doktorské granty GAČR**.

K. Civáňová (Ústav genetiky, Agronomická fakulta MZLU v Brně): **Mikrosatelity vs SNP polymorfismy, metodiky detekce a jejich využití**.

L. Přívětivá (Ústav genetiky, Agronomická fakulta MZLU v Brně): **Identifikace živočišného druhu a konkrétního kusu v masných výrobcích při využití PCR**.

Z. Vykoukalová (Ústav genetiky, Agronomická fakulta MZLU v Brně): **Detekce mutací v genomu hospodářských zvířat využitím přímého sekvenování**.

J. Verner (Ústav genetiky, Agronomická fakulta MZLU v Brně): **Mutace genů ovlivňující užitkovost – Analýza genu MYOD1 a asociace s masnou užitkovostí u prasat**.

T. Kaliská, D. Krekáč (Laboratoř molekulární embryologie, Agronomická fakulta MZLU v Brně): **Regulačné proteíny v proliferujúcich a diferencujúcich bunkách embryonálneho pôvodu**.

16. prosince 2004

Symposium Aktuální otázky bioklimatologie zvířat 2004

(Uspořádala Česká bioklimatologická společnost při RČVS – Sekce bioklimatologie zvířat, Ústav výživy, dietetiky, zoohygieny a vegetabilních potravin Fakulty veterinární hygieny a ekologie VFU Brno ve spolupráci s Ústřední komisí pro ochranu zvířat a Výzkumným ústavem živočišné výroby v Praze a Brněnskou pobočkou Československé biologické společnosti)

ABSTRACTS

J. Fajkus (Institute of Biophysics, Academy of Sciences of the Czech Republic and Department of Functional Genomics and Proteomics, Masaryk University Brno): **Do we understand telomere biology?**
fajkus@ibp.cz

Although the specific nature of chromosome ends has been observed already in the first half of the 20th century, their molecular structure and a basic mechanism of their maintenance by a special nucleoprotein complex of telomerase has been recognized only about two decades ago. The massive explosion of interest in telomere biology has been driven by findings of association between telomerase activation and immortalisation of cancer cells. Current research in telomere biology can show off not only interesting findings of basic research, but also promising applications in diagnostics and therapy of cancer, as well as possible use of telomerase activation for rejuvenation of cells and tissues e.g., for transplantation purposes. Due to the focused effort of numerous research teams, today's picture of telomere metabolism shows increasing complexity and interesting connections of telomere metabolism with processes of DNA replication, recombination and repair which are mediated by protein factors common to these processes. Further, alternative (telomerase independent) pathways

of telomere maintenance have been found not only in insects or yeasts, but also in human cells. The present postgenomic era enables us to choose the optimal model for studying specific phenomena and rapidly transfer the knowledge gained in the experimentally best accessible system to other systems using applications of bioinformatics. Current telomeric research faces now the problem of elucidation of telomere dynamics during development and differentiation of multicellular organisms. The useful models for such studies are higher plants which offer a possibility of regeneration of manipulated cells without ethical concerns. Moreover, our recent findings show that nodal points in phylogenesis of plant groups within the order *Asparagales* are associated with substantial switch points in telomere and telomerase structure. To further extend our understanding of interrelationships among factors governing telomere metabolism, the present focus of our research is to analyse molecular evolution and developmental regulation of plant telomerases, telomeric DNA and associated proteins. To achieve this goal, common biochemical and molecular biology techniques are being combined with proteomic and structural biology approaches as well as with *in situ* microscopy techniques.

Supported by the Grant Agency of the Czech Republic (204/02/0027), Grant Agency of the Czech Ministry of Health (NC7043-3), the Grant Agency of the Academy of Sciences of the Czech Republic (S5004010) and the institutional support (AV0Z5004920 and MSM143100008).

M. Skleničková (Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno): **How do telomeres look like?** sklemar@ibp.cz

The maintenance of stable telomere lengths is essential for indefinite cellular proliferation of germ line and tumour cells. In most cases, telomere synthesis is performed by nucleoprotein enzyme complex of telomerase. Analysis of telomeres at the level of one cell enables to visualise telomeres of individual chromosome arms, analyse their lengths and detect changes that may be connected to some specific feature of the cancer disease or its prognosis.

Several *in situ* techniques, useful for the visualisation of telomeres will be presented. Fluorescent *in situ* hybridisation (FISH) is the basic technique of detection and visualization DNA sequences at the level of single cell. In telomere research classical FISH on metaphase spreads with DNA or PNA probes may be performed. FISH on extended DNA fibres (fiber-FISH) is high resolution *in situ* method. Extended DNA fibres (EDF) are obtained from interphase nuclei, so mitotic activity of cells is not required. Some results show that fiber-FISH enables DNA mapping in a range of 1-400kb, thus connecting and overlapping interphase FISH mapping with conventional and pulsed-field gel electrophoresis.

Special attention will be paid to the technique PRINS (Primed *in situ* labelling), which was developed for the detection of repetitive sequences such as centromeres and telomeres. PRINS utilizes unlabeled oligonucleotide probe, which after hybridization serves as a primer for DNA-polymerase for chain elongation using labelled nucleotides. Because the probe is unlabeled, its non-specific hybridization does not give rise to a signal, and high probe concentration may be used. Modification of PRINS, dideoxy-PRINS, is applicable for sequences, which lack one or more of the four bases of DNA. The lacking nucleotide(s) is then added in the form of dideoxynucleotide. In the case of non-specific hybridization or non-relevant priming from random breaks in chromosomal DNA, the incorporated dideoxynucleotide(s) stop(s) the chain polymerization and the background doesn't arise. Dideoxy-PRINS is thus very suitable for detailed analysis of human as well as *Arabidopsis*-type plant chromosomes.

Supported by the Grant Agency of the Czech Ministry of Health (NC7043-3), the Grant Agency of the Academy of Sciences of the Czech Republic (S5004010) and the institutional support (AV0Z5004920).

Z. Kunická[1], R. Hájek[2] and J. Fajkus[1,3] ([1]Department of Functional Genomics and Proteomics, Masaryk University Brno, [2]Department of Internal Medicine – Hematooncology, University Hospital Brno and [3]Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno): **Detection of telomerase activity of multiple myeloma cells by RQ-TRAP**.

DNA replication of linear eukaryotic chromosomes results in shortening of the telomeres due to incomplete replication of the 3´ end of the parental strand. Telomerase, a specialised reverse tran-

scriptase, adds a short, tandemly repeated DNA sequence motif to chromosome ends. Telomerase is repressed in most human somatic cells and in the absence of telomere-elongating activity the number of cell doublings is limited. Germline and tumour cells overcome this limitation by the expression of telomerase. It has been shown that telomerase activity is present in 80–90 % tumour samples. Thus, the telomerase is the most universal tumour marker in oncology diagnostics.

Multiple myeloma (MM) is an incurable disease of the plasma cells. In MM, the B cells are damaged and give rise to too many plasma cells (myeloma cells). Mature myeloma cells may fail to activate the immune system and may produce substances that decrease the body's normal immune response. Unlimited proliferation of myeloma cells inhibits normal bone-forming cells. Production of substances that activate bone-resorbing cells, osteoclasts, is increased. Increased activity of osteoclasts causes bone destruction by osteolytic lesions.

Telomerase activity was detected in CD 138+ multiple myeloma cells obtained by immunomagnetic separation – MACS (magnetic-activated cell separation) from patient´s bone marrow or in standard multiple myeloma cell line ARH 77.

For telomerase activity detection, the TRAP (telomeric repeat amplification protocol) method is usually used, which is the primary technique for sensitive detection telomerase activity. This method requires a relatively time-consuming analytical phase, involving polyacrylamide gel electrophoresis and densitometric analysis. We have adapted the TRAPeze XL kit for use in real-time quantitative TRAP (RQ-TRAP). The kit applies Amplifluor™ primers for detection of both amplified telomerase products and internal PCR control labelled with fluorescein and sulforhodamine, respectively. We demonstrate that the kit can also be used for RQ-TRAP assays to detect amplification of telomerase products and the control simultaneously using the fluorescein (FAM) and rhodamine (ROX) channels of the Rotor-Gene(tm) 3000 instrument.

The activity of MM cells was measured in parallel using the polyacrylamide gel electrophoresis (PAGE) pattern of products and three replicates (for each number of cells) tested by RQ-TRAP. Our analysis demonstrated that both "classic" TRAP and Real Time TRAP have similar results. However, RQ-TRAP saves time and consumables necessary for post-amplification procedures and offers higher sensitivity and reliability in quantifying of telomerase activity.

Supported by the Grant Agency of the Czech Ministry of Health (NC7043-3), the Grant Agency of the Academy of Sciences of the Czech Republic (S5004010) and the institutional support (AV0Z5004920 and MSM 143100008).

M. Kuchař[1], M. Horáková[1], L. Krejčí[1], D. Zachová[1], J.Fajkus[1,2] ([1]Department of Functional Genomics and Proteomics, Masaryk University Brno and [2]Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno): **The use of yeast two-hybrid system for a study of telomere-associated proteins**.
mkuchar@ibp.cz

Telomere-binding proteins participate in forming functional structure of chromosome ends and regulating telomerase action.

For the study of the telomere-associated proteins, the yeast two-hybrid system has been used. This system is based on two physically separated domains of a transcription factor, the DNA binding domain (responsible for binding to the promoter) and the activating domain (activating expression of a reporter gene). These domains are able to come into close physical proximity only via interaction of proteins fused to each of the domains.

In case of our study, the yeast forward two-hybrid system has been used to identify protein-protein interactions between two telomere-binding proteins, to detect multimerisations of some telomere-binding proteins, and to find other proteins interacting with a known telomere-binding protein.

Numerous interacting telomere-associated proteins from various organisms have been found. For example, *Schizosaccharomyces pombe* spRap1p (Q96TL7) interacts with telomere-binding protein Taz1p (NP_594047) which enables spRap1p binding to telomere via this interaction. Another protein-protein interaction was observed between Ku70p and Ku80p in humans (Ku70 = P12956, Ku80 = P13010), budding yeast (Ku70 = P32807, Ku80 = Q04437), *Arabidopsis thaliana* (Ku70p = NP_564012, Ku80p = AAG44851) and other organisms.

However, homologous proteins from different organisms (orthologs) do not always interact in the same way. Human hEst1Ap (KIAA0732) and hEst1Bp (KIAA1089) bind directly telomerase catalytic subunit hTERTp (AAC51672), whereas *Saccharomyces cerevisiae* analogue Est1p (S51454) binds telomerase via its RNA subunit TLC1 (U14595). This fact leads to an idea, that in the case of the used (two-hybrid) system failure, a system modification as an n-hybrid system can be used. The situation is further complicated by the fact, that in our model system, *Arabidopsis thaliana*, the orthologs of human, yeast or protozoan telomere-binding proteins have more than just one candidate paralogs identified by bioinformatic approaches. Therefore, the two hybrid assay constitutes one of the important screening techniques for functional activity of the candidate proteins. Examples of the results obtained will be presented.

Supported by the Grant Agency of the Czech Republic (204/02/0027) and the institutional support (AV0Z5004920 and MSM 143100008)

P. Schrumpfová[1], G. Rotková[1], M. Kuchař[1], L. Skřišovská[1], T. Kubičárová[1,2] and J. Fajkus[1,2] ([1]Department of Functional Genomics and Proteomics, Masaryk University Brno and [2]Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno): **Characterization of two telomeric sequence binding myb-like protein from *Arabidopsis thaliana* that are members of novelestablished SMH-type gene family**. schpetra@centrum.cz

Telomeres are nucleoprotein structures protecting the eukaryotic chromosome end from degradation and end-to-end fusion. Using a genomic approach, two *Arabidopsis thaliana* genes coding for candidate myb-like telomere binding proteins were cloned and expressed in *E. coli.* Both proteins termed as AtTBP2 (acc. nos. T46051 (protein database), GI:638639 (nucleotide database) 295 AA, 32 kDa, pI 9.53) and AtTBP3 (BAB08466, GI:9757879; 299 AA, 33kDa, pI 9.88), are new members of recently establishes gene family containing Single myb histone 1(Smh) type of genes, that are encoding proteins with unique triple motif structure of an N-terminal myb-like domain, central region with homology to the conserved H1/H5 domain and coiled-coil domain near the C-terminus. Sooner entitled proteins by our laboratory like AtTBP2 and AtTBP3 are in now databases termed as AtTRB3 (new acc. no NP_190554.1 (protein database), GI: 30693241(nucleotide database) and AtTRB2 (NP_201559.1, GI: 30698321). We found out that both proteins that expressed in various Arabidopsis thaliana tissues and the genes coding these two proteins maps to chromosome 5 (attbp2) and chromosome 3 (attbp3). Gel-retardation assays revealed that each of the two proteins is able to bind the G-rich strand and double-stranded DNA of plant telomeric sequence, the latter being a better substrate for binding by both proteins, with an affinity proportional to a number of telomeric repeats. Substrates bearing a non-telomeric DNA sequence positioned between two telomeric repeats were bound with efficiency depending on the length of interrupting sequence. A modified G-rich telomeric oligonucleotide, in which all G-positions are substituted with 7-deaza-8-aza-G (pyrazolo [3, 4-d] pyrimidine, PPG), shows reduced AtTBP3 binding even in the absence of competitor DNA, while the interaction with AtTBP2 is similar compared to the unmodified tetramer telomeric sequence. The ability to bind variant telomere sequences decreased with sequence divergence from the Arabidopsis thaliana telomeric DNA. None of the proteins alone or their mixture affects telomerase activity in vitro in telomerase activity assay (TRAP).

Supported by the Grant Agency of the Czech Republic (204/02/0027) and the institutional support (AV0Z5004920 and MSM 143100008)

M. Hanáková, P. Nikolová, P. Peťovská, M. Vilémová, A. Oltová, V. Vránová, D. Žežulková, H. Filková, P. Kuglík, Š. Prášilová, I. Grochová, Z. Kalina, R. Gaillyová (Department of Medical Genetics, Faculty Children's Hospital Brno): **Postnatal diagnostics of chromosomal aberrations in Department of Medical Genetics of the Faculty Children's Hospital Brno in the period of 1999–2003**.

During 1999–2003 years 7,102 patients were examined by the use of classical cytogenetic (G, C, NOR – chromosome banding) and molecular cytogenetic (FISH, SKY, CGH) methods in Department of Medical Genetics of the Faculty Children's Hospital Brno. Pathological karyotypes were found in 881 cases (12%).

The patients were divided into 6 groups: female dysfertility, male dysfertility, gamete donors, suspected congenital chromosomal aberrations in the children, other adults and acquired chromosomal aberrations. The three groups, namely gamete donors, other adults and acquired chromosomal aberrations are presented in details in this report.

The group of gamete donors involved 261 patients and 21 pathologies (8%) were observed. Mosaics of X aneuploidy were found in 19 cases (90%), balanced translocation in 1 case (5%), and mosaic of Klinefelter´s syndrome in 1 case (5%). The most of pathologies were female's ones. There was presented chosen case of potential octets donor with balanced t (2; 5) in the karyotype.

1,092 patients were examined in group of other adults. The group included physically and psychically affected people and people with genetic load in the family older than 18. There were found 147 pathologies (14%). Numerical aberrations were found in 50 cases (34%), the most frequent findings were mosaics of X aneuploidy (40%). Structural aberrations were found in 92 cases (63%), the most frequent findings were balanced translocations (60%). The group of combination of numerical and structural aberrations was represented by 5 cases (3%). There was presented a chosen case of patient with the partial deletion of the short arms of chromosome 9.

599 individuals with 162 found pathologies (27%) were in the group of acquired chromosomal aberrations that included patients in occupational hazard, chosen medical therapy and other chosen indications.

P. Nikolová, P. Peťovská, M. Hanáková, M. Vilémová, A. Oltová, Z. Kalina, Š. Prášilová, H. Filková, M. Pešáková, D. Žežulková, P. Kuglík, B. Ravčuková, R. Gaillyová (Department of Medical Genetics, Faculty Children's Hospital Brno): **Cytogenetic findings in dysfertile patients examined in Department of Medical Genetics of the Faculty Children's Hospital Brno in the period of 1999–2003**.

Male dysfertility. There were examined 1,781 dysfertile men in the period of 1999–2003. Pathological karyotype was found in 4.7% of them. The structural aberrations made up 50.6% of all pathological findings. The most frequent were translocations (67%). The numerical aberrations made up 43.4% of all pathological findings. The most frequent was Klinefelter´s syndrome (70%). The combinations of both aberrations made up 6%. There were three cases, in the male dysfertility group, presented in detail:

man examined for primary sterility and azoospermia; karyotype: 46,X,del(Y),

man examined for primary sterility and aspermia; karyotype: 45,X/46,X,idic(Y),

man examined for secondary sterility and oligoasthenospermia; karyotype: 46,X,Yqh+,t(9;21) (p24.3;q22.3).

Female dysfertility. There were examined 1,864 dysfertile women in the period of 1999–2003. Pathological karyotype was found in 15.8% of them. The structural aberrations made up 6.4% of all pathological findings. The most frequent were translocations (79%). The numerical aberrations made up 91.9% of all pathological findings. The most frequent were mosaics of X aneuploidy (96.3%). The combinations of both aberrations made up 1.7%. There were two cases, in the female dysfertility group, presented in detail:

woman examined for infertility; karyotype: 47,XX,+8/ 46,XX and

woman with diagnosed Turner´s syndrome, who gave birth to a healthy baby with normal karyotype.

P. Peťovská, P. Nikolová, M. Hanáková, M. Vilémová, A. Oltová, H. Filková, D. Žežulková, M. Pešáková, P. Kuglík, Š. Prášilová, Z. Kalina, J. Šoukalová, R. Gaillyová (Department of Medical Genetics, Faculty Children's Hospital Brno): **Congenital chromosomal aberrations in children examined in Department of Medical Genetics of the Faculty Children's Hospital Brno in the period 1999–2003**.

Authors examined 1,637 children, 0–18 years old, with suspected congenital chromosomal aberrations in the period 1999–2003. 1,449 children showed normal karyotype and 188 remained various pathological findings (11.5%). Following cytogenetic and molecular-cytogenetic methods were used in this study: classical G – banding for examination of karyotype, FISH and CGH or spectral karyotyping for verifications of findings.

Numerical aberrations represented 119 cases (63.3%), structural aberrations 61 cases (32.4%) and combinations of both groups 8 cases (4.3%).

The most frequent numerical abnormalities were trisomy of 21 (Down´s syndrome), aneuploidy of X (Klinefelter´s syndrome, Turner´s syndrome and mosaics of X aneuploidy) and mosaics of marker chromosome. There were presented two cases in details: the boy with benign brain dysfunction and orthopaedic problems, karyotype 48, XXYY; and the girl with hearing loss, in which 60% mosaics of marker chromosome was founded.

The most frequent structural aberrations were balanced and unbalanced translocations. There were presented chosen case of the psychomotoric retarded Vietnamese girl with finding of complex karyotype with transformations of chromosomes 2,5,10. The second presented case was stigmatized newborn with finding of partial trisomy of chromosome 11 in the region 11p15.4–11pter.

M. Hyršová[1], E. Janovská, V. Brabec, M. Vojtíšková (Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno and [1]Department of Microbiology, Faculty of Sciences, Masaryk University Brno):
Interaction of plasmid DNA pUC19 with cisplatin in *E. coli* cells and possibilities of the reparation.

The initial biologically relevant event for the anticancer activity of cisplatin is associated with the formation of certain kinetically stable cisplatin-DNA adducts. The major product is the DNA lesion between two intra-stranded adjacent guanines, and the inter-strand G to G crosslink at GpC sequence as the minor adduct. Successful application of cisplatin in a treatment of tumour cells can be restricted by inner or induced resistance of the cell.

Cytotoxicity of cisplatin probably results from its ability to form DNA covalent adducts on one hand and on the other hand these adducts can be removed a repair mechanism of cells. One of them is nucleotide excision repair (NER). NER is the primary DNA repair pathways in cells capable of removing extensive variety bulky lesions, induced by chemicals and radiation with varying efficiencies. NER is a highly conserved process and in bacteria and eukaryotes can be viewed in four steps: (1) damage recognition and strand verification; (2) dual incisions; (3) excision and repair synthesis; and (4) DNA ligation. In *E. coli* cells, the first three steps of this process are carried out by an ensemble of three proteins termed UvrABC endonuclease encoded by the uvrA, uvrB, and uvrC genes in an ATP hydrolysis-dependent series of reactions.

Aim of the work was to study on plasmid DNA efficiency of NER in E. *coli* cells after cisplatin treatment. For this, authors have used the set of isogenic repair-proficient or repair-deficient *E. coli* strains, derived from the parent WP2 wt (wild-type) strain to single WP2 (uvrA) or double WP100 (uvrA recA) mutants (*Janovská et al, J. Inorg. Biochem. 90, 2002, 155 - 158*) and provided transformation with plasmid DNA pUC19. UV drop test and colony-forming ability surprisingly showed higher sensitivity to cisplatin in the repair-proficient strain WP2 (pUC19) carrying plasmid in compare to cisplatin resistant WP2 wt strain. Survival of the repair-deficient strains WP2(uvrA) and WP100 was not influenced by the attendance of plasmid DNA and these *E. coli* strains deficient in excision and recombination repair were considerably more sensitive to cisplatin than their wild-type parent. Isolated samples plasmid DNA pUC19 after in situ cisplatin treatment in isogenic *E. coli* strains were cleaved with BamHI (the detection of intra-stranded GpG adducts) and analyzed by alkaline agarose gel electrophoresis (the demonstration of inter-stranded cross-links). We found preferential formation of inter-stranded cross-links by cisplatin during *in situ* modification of plasmid DNA pUC19 in *E. coli* cells in agreement with results of in *vitro* experiments (*Vrána et al, Nucleic Acids Res. 24, 1996, 3918 -3925*). Experiments are in progress to obtain more detailed information about cellular processing of plasmid DNA modified by cisplatin in dependency on host *E. coli* strains.

I. Sedlářová[1], K. Stehlíková, V. Brabec, M. Vojtíšková (Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno and [1]Department of Molecular Biology and Genetics, Faculty of Sciences, Masaryk University Brno):
Advantages of cell-free rapid translation system (RTS) for the expression of hXPA repair proteins.

Cisplatin is a widely used chemotherapeutic agent. It reacts with nucleophilic bases in DNA and forms different kind of adducts. The presence of these adducts in DNA through to be responsible for

the therapeutic effects. On the other hand some tumour cells develop resistance to cisplatin and these processes have been subject of intense research because resistance is a major obstacle for the clinical use of this class of drugs.

It has been shown that in the mechanism of antitumor action of platinum drugs, nucleotide excision repair (NER) plays an important role. Although the protein components have been identified and the NER reaction has been reconstituted *in vitro,* the biochemical process through which the global genomic repair pathway is initiated and recognises damaged DNA is still poorly understood. The XPA protein has an essential function in NER, where it works in co-ordination with other proteins of this repair pathway. The *xpa* gene localised on human chromosome 9q22.3 encodes the XPA polypeptide of 273 amino acids (Mr 31 kDa) and contains the zinc finger motif that is necessary for the function of the gene product.

In order to *in vitro* study the structural mechanism of the XPA protein towards cisplatinum modified DNA we were looked for a fast and efficient method for the high production of recombinant proteins and we introduced new procedures for the expression and purification of the hXPA protein by used the rapid translation system (RTS). Recombinant plasmid pET15b.XPA (*Missura et al, EMBO J. 20, 200, 3554–3564*) with cloned *xpa* cDNA was used as DNA template for *in vitro* cell-free protein expression on the basis of *E. coli* lysates in the specific device of the RTS 500 instrument (Roche /*The RTS website: www.proteinexpression.com/*). During reaction, transcription and translation take place simultaneously and reaction substrates are continuously supplied through a semi-permeable membrane from the feeding store. The RTS expressed recombinant hXPA protein with N– terminal His_6-tag in one ml of reaction mixture was next purified on Ni^{2+}NTA agarose and by hydroxyapatite chromatography. Purified hXPA samples (in yields up to 0.5 mg) were characterised by SDS-gel electrophoresis, UV spectroscopy, Bradford assays and tested for the recognition of DNA damage caused by cisplatin under different reaction conditions (time, temperature, concentrations, etc.). Next experiments showed that hXPA protein prepared by RTS protocol binds more effectively to cisplatin modified DNA compared with recombinant hXPA protein expressed in *E. coli* cells.

Advantages of the RTS method consist in wide possibilities to prepare a protein in short time under definable reaction conditions and in the high yield with sufficient quality as well as in the elimination of large volume cultivated bacterial cells.

Supported by the Grant Agency of Academy of Sciences of the Czech Republic (Grant A500410).

K. Civáňová, A. Knoll (Department of Genetics, Faculty of Agriculture, Mendel University of Agriculture and Forestry Brno): **Microsatellites versus SNP polymorphisms, methods of detection and their utilization**

Although the highly informative, multi-allelic microsatellite markers (MS) have dominated as a markers of choice in many research fields in biology (genome mapping, population studies, parentage and kinship, forensic identification), an increasing importance is now given to merely biallelic base substitutions of lower heterozygosity – single nucleotide polymorphisms (SNPs), which have been proposed as the next generation of markers for the identification of loci associated with complex diseases and which allow the unification of the candidate gene approach and association-based fine mapping to identify genes of interest.

Our main challenge is to use the convenient and most reliable methods to find out genetic differences among animal individuals, to assess their influence on the quantitative trait loci and the production traits, or eventually, use them for parentage or individual testing. The progressive recent technologies in nucleotide polymorphism detection take note of need to analyze the big amount of samples in one test tube as quickly as possible. This advantage offer mainly technologies of multiplex PCR and DNA pooling.

The one of progressive methods for SNP analysis which we would like to introduce in our laboratory is SNaPshot minisequencing. The method could multiplex and interrogate up to 10 single-base primer-extensions in one reaction. To verify the usage of this method for SNP analysis in porcine genome we have chosen seven porcine genes which are supposed to influence the meat production in pig (*IGF2*, *FOS*, *MC4R*, *DGAT1*, *MYF4*, *MYF3*, and *MC3R*). The specific known polymorphisms of

these genes are analyzed by SNaPshot that is actually the di-deoxy chain-terminating DNA-sequencing technology that combines the specificity of nucleotide incorporation by DNA polymerase and the sensitivity of fluorescence. The SNaPshot multiplex system enables capillary electrophoresis instruments (ABI Prism 3100 genetic analyzer, Applied Biosystems) to perform SNP analyses from high throughput SNP validation to SNP scoring. The used size standard (GeneScan 120 LIZ) eliminates the need for manual allele calls of the multiplexed samples when used with GeneMapper v.3.1 software.

As a result, we confirmed the convenience of usage of this method and we find it very time saving, reliable and efficient with reproducible results. This complete system contains everything required for precise and cost-effective SNP analysis and could replace the contemporary methods used in routine testing.

Supported by Projects of the Grant Agency of the Czech Republic 523/03/H076 and MŠMT ČR FRVŠ 170/2004.

L. Přívětivá, L. Putnová, P. Stehno, J. Dvořák (Department of Genetics, Faculty of Agriculture, Mendel University of Agriculture and Forestry Brno): **Identification of the species and individual animals in the processed meat products by PCR – based analyses.**

The aim of the work was to establish and optimize the molecular-genetic methods, which could be used in the Laboratory of Applied Molecular Genetics (LAMGen) at the Department of Genetics MZLU Brno for the monitoring of food products safety.

During the work, the methods of PCR reactions for the detection of cattle, porcine and chicken tissues were optimized and the presence of declared tissues in meat products from the markets was proved. Throughout the identification of individuals in the meat mixture products (after the different technological interventions), we were able to confirm the presence of the concrete head of animal. When accumulating several individuals in the meat mixture, allele composition is more numerous and variable. In this case, it can happen that the alleles, responding to the individual DNA profile, could be found even if a specific individual is not physically present in the mixture. In the meat products, made from animals with available results of fragment analysis, we confirmed the presence of microsatellite alleles corresponding to the specific individuals' data logging. On the other hand, in the products, containing tissues from the animals without available results of fragment analysis, it was in addition possible to identify the microsatellite alleles, which missed in output data logging of analyzed animals.

Authors concluded, that in the meat products, made from the mixture of large number of animals, the presence of the specific individual can be only excluded, not confirmed.

Supported by the Grant Agency of the Czech Republic (Project 523/03/4076).

Z. Vykoukalová, A. Knoll (Department of Genetics, Faculty of Agriculture, Mendel University of Agriculture and Forestry Brno): **Detection of polymorphisms in farm animals using direct sequencing of PCR product**.

A key step in all strategies for causative gene identification is the resequencing of candidate genes or other genomic regions of interest in phenotype divergent animals to identify those single nucleotide polymorphisms (SNPs) associated with a certain phenotype. DNA sequencing is the determination of all or part of the nucleotide sequence of a specific deoxyribonucleic acid (DNA) molecule. This method depends on the enzymatic synthesis of labelled DNA, using special modified nucleotides called dideoxynucleotides to terminate the elongating strand. The automated cycle sequencing in combination with a fluorescent labelled primers or ddNTPs is recently mostly used method. We performed the direct sequencing of PCR products using ABI PRISM 310 and ABI PRISM 3100-*Avant* Genetic Analyzer (Applied Biosystems). The raw sequencing data were processed using DNA Sequencing analysis Software Ver. 5.1. The SeqScape Software Ver. 2.1 is efficient tools for large-scale multiple alignment of sequences of different animals.

The aim of the study was the determination of the porcine *MYF6* gene structure by direct sequencing of the PCR product. A fragment of this gene was amplified using primer pair designed from aligned cDNA sequence of the human and mouse orthologous gene. Length of the sequenced fragment was 379 bp. We detected four polymorphisms in this fragment: C/A in position 128, C/G in position 161, A/G in position 192 a C/T in position 282. All these polymorphisms were located in the intron.

Resequencing approach was applied to detection of polymorphisms in selected genes: *TNFA* (tumour necrosis factor alpha), *ADRB3* (beta-3 adrenergic receptor) and *SLC6A14* (solute carrier family 6, member 14). DNA sequences of Large White, Landrace, Piétrain, Meishan and Wild pig were aligned. According to exon-intron structure and translation analysis was determined that none of founded polymorphisms were connected to amino acid change in *TNFA* and *SLC6A14* genes. In *ADRB3* four SNPs cause amino acid changes. Furthermore three silent mutation and six intronic SNPs were found.

Supported by the Grant Agency of the Czech Republic (Project 523/02/D026).

Compiled and revised by S. Čech