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ABSTRACTS

Antimicrobial susceptibility of enterococcal isolates from bryndza sheep cheese

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The occurrence and antibiotic resistance of 310 enterococcal isolates from bryndza sheep cheese samples were investigated. Enterococci were isolated from these samples with the use of bile esculin agar and identified with STREPTO test and EN-COCCUStest (Pliva-Lachema). Namely, a total of 178 *Enterococcus faecium*, 49 *E. faecalis*, 68 *E. durans*, 3 *E. casseliflavus*, 8 *E. saccharominimus*, 8 *E. gallinarum* and 1 *E. hirae* were evaluated for susceptibility to 9 antimicrobial agents (vancomycin, teicoplanin, ampicillin, streptomycin, gentamicin, erythromycin, rifampicin, and nitrofurantoin). Antibiotic resistance was determined by the disk diffusion method. All enterococcal isolates from bryndza sheep cheese were susceptible to vancomycin, teicoplanin, ampicillin, streptomycin, and gentamicin. Several enterococcal isolates were resistant to rifampicin (25%) and erythromycin (26%). Furthermore, a few of isolates were resistant to ciprofloxacin (2%) and nitrofurantoin (1%).

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Identification of novel polymorphisms in the basic fibroblast growth factor gene in Czech population

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Basic Fibroblast growth factor (bFGF) is a member of the fibroblast growth factor family that possesses broad mitogenic and angiogenic activities. This protein has been implicated in several diverse biological processes, such as limb and nervous system development, wound healing, tumor growth and angiogenesis. We investigated possible genetic polymorphism in the promoter, coded sequence and 3' flanking region of the bFGF gene.
Polymorphisms were analyzed by means of heteroduplex analysis and fragments with altered mobility were sequenced. Genotypes were detected by polymerase chain reaction and subsequent restriction with specific endonucleases. Allele frequencies were determined in a sample of healthy Caucasian subjects (n=126). The study was approved by the Committee for Ethics of Medical Experiments on Human Subjects, Faculty of Medicine, Masaryk University, Brno.

Three substitutions (-553T/A, -834T/A and -921C/G) were identified in the promoter region. Linkage disequilibrium was found among these polymorphisms ($P<0.01$). One polymorphism (754 C/G) was found in the intron 1 and one polymorphism (65796 T/C) was uncovered in the polyA signal of the bFGF gene. No polymorphisms were found in the three exons of the bFGF gene. The frequencies of the mutated alleles (-553A, -834A, -921G, 754G and 65796C) were 0.04, 0.05, 0.14, 0.36 and 0.01 respectively. We conclude that the new variants of the bFGF gene appear to be common polymorphisms in the Czech population.

This work was supported by postdoctoral grant 303/05/P523 from the Czech Science Foundation.

Current ethical problems in cell biology

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We selected important ethical conflicts of interest (cf. Berger 2003) in current cell biology and which remain still unresolved: the use of human biologic material, patents for biological material, cloning and use of stem cells, genetic engineering, onset of human life and death, and the use of vertebrate biomodels. New data from cell and

molecular biology cannot facilitate the solution of such problems – on the contrary, they make these solutions more difficult. A solution may be found within the socio-cultural problems. Intensive research is necessary both to accomplish economical and medical benefit and to clarify ethical rules. Thus, essential cell biology seems to be the important part of education for non-life sciences students and students of biology and medicine would touch the social and ethical implications of recent biotechnologies in each knowledge-based economy (cf. Lim 2003).

This work was supported by grant 257/2005 the Czech Ministry of Education.

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Aggregate-prone proteins and human diseases

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A growing number of diseases are caused by glutamine or alanine codon reiteration mutations in different proteins. These mutations increase the aggregation propensity of their target proteins. These diseases include Huntington's disease, a condition caused by the expansion of the polyglutamine stretch in huntingtin, and oculopharyngeal muscular dystrophy, a disease caused by the expansion of the polyalanine stretch in poly-A binding protein nuclear 1 (PABPN1). This article briefly reviews the conditions caused by the expansions of glutamine and alanine stretches, and discusses the possible roles of aggregates and potential therapeutic strategies for these diseases.

Mechanism of antitumor activity of platinum complexes

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Changing the chemical structure of platinum compounds may substantially modulate their DNA binding mode, subsequent processing of DNA damage and consequently the mechanism of biological efficacy of these compounds. These structural modifications may also affect the spectrum of biological activity of the platinum agents and the development of drug resistance, and also their toxicity profile. Hence, a further understanding of how new platinum compounds modify DNA and how these modifications are further processed in cells may lead to further insight of "downstream" effects, initiated through differential protein recognition and repair, that may produce unique biological effects. On the other hand, there is still a gap between these molecular events involving DNA interactions and an understanding as to why platinum anti-cancer drugs are more poisonous to cancer cells than to normal cells. The studies so far performed in this area have implicated multiple systems including several classes of DNA repair, replication, transcription, cell cycle and cell death responses involved in the processes associated with cellular sensitivity to the platinum drugs. It is also likely that many other determinants remain to be identified. Complete knowledge of how modifications of DNA by antitumor platinum compounds and other metal-based drugs affect the components of these pathways should provide a basis for understanding the mechanism of action of platinum drugs and, thereby, a rational basis for the design of new metal-based drugs and to identify optimal treatment strategies.

Binding of mutant and wild type p53 proteins to supercoiled DNA

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DNA sequence-specific interaction via gene transactivation underlies the growth suppressing and apoptotic functions of tumor suppressor wild-type p53 (wtp53) [1,2]. The tumor suppressor p53 gene is mutated in about 50-60 % of all human tumors, rendering it the most frequently mutated single gene in human cancer [3,4]. The substitution of a single amino acid residue within the central DNA-binding domain (CD) is the characteristic feature of the p53 mutational spectrum and affects sequence-specific DNA binding. Loss of p53- sequence-specific DNA binding impaired ability to elicit the same transcriptional response as wtp53 are the hallmarks of mutant p53 (mutp53) proteins. Functional analyses of mutp53 containing cancer cells indicate that in addition to a loss of wtp53 activities, mutp53 proteins often acquire new properties endowing tumor cells with enhanced oncogenic potential ("gain of function")[5]. For example the mutp53-specific DNA and/or protein interactions may be involved in the molecular basis underlying activities of mutp53 proteins.

Majority of the hot spot p53 (the most frequently mutated codons: 175, 245, 248, 273, 282) lost the sequence-specific DNA binding, but proteins can still induce or repress transcription of mutp53-specific target genes (MDR-1, c-myc, telomerase, EGFR and PCNA) [6,7]. So far, the common denominator for mutp53 DNA binding, e.g. the common sequence element, was not found. Emanating from our previous discovery that DNA structure and DNA superhelicity are the important determinants of wtp53- DNA binding, we compared various hot spot mutp53 proteins (Gly245Ser, Arg248Phe and Arg273His) and wtp53 in their binding to supercoiled (sc) DNA.

We shown that wtp53 can bind DNA in a conformation selective manner via its central (CD) or C-terminal (CTD) domains. Full length (fl) p53 binds selectively to supercoiled (sc) DNA regardless of the presence or absence of the p53 consensus sequence (p53CON). The SCS binding was studied using deletion p53 constructs [8] and selective modulation of the fl protein CD and CTD by oxidation agents and monoclonal antibodies [9]. In competition experiments, truncated p53 lacking the C-terminal DNA binding site, or fl p53 with this site blocked by the antibodies, bound sc and relaxed DNAs with similar affinities. On the contrary, fl p53 with the CD inactivated due to oxidation of cysteine residues but with the CTD available for DNA binding, retained the preference for the scDNA. Our results suggest that accessibility of the CTD and oligomerization of the protein are essential for the p53 SCS binding [8,9].

Mutp53 proteins exhibit the ability to interact selectively with non-B DNA elements (e.g., MAR/SAR elements and stem-loop structures). [5,10]. We analyzed the binding of various mutp53 proteins to supercoiled DNA lacking wtp53CON site. Using various DNA-binding assays we show that mutp53 proteins bind selectively and with high affinity to supercoiled DNA and that antibodies mapping to the p53 CTD influence mutp53-scDNA interactions.

We propose that DNA structure-selective binding of mutp53 proteins is the basis for the well-documented interaction of mutp53 with genomic DNA, e.g. MAR elements, and together with recognition of specific DNA sequences also for transcriptional activities mediates by mutp53.

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Mutant p53 binding to secondary DNA structure forming DNA in the Chromatin-context of glioblatomas

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The most frequently mutated single gene in human cancer, the tumor suppressor *p53*, is mutated in about 50-60% of all human and about 20% in brain tumors [1,2]. Expression of mutant p53 proteins (mutp53) in certain tumors correlates with bad prognosis and/or resistance to chemotherapy through accumulation in high amounts in tumor cells. Functional analyses indicate that in addition to a loss of activities normally executed by wt p53, mutp53 proteins often procure new oncogenic properties for tumor cells ("gain of function") [3]. Whereas multiple functions of wild–type p53 (wtp53) in maintaining genomic integrity and protecting cells from different kinds of cellular stress have been analyzed in detail, the molecular basis underlying activities of mutp53 proteins have not been well understood yet.

Despite the loss of sequence-specific DNA binding mutp53 proteins retained the ability to interact selectively with non-B DNA elements (MAR/SAR elements and stem-loop structures) that were shown *in vitro* with isolated DNA and oligonucleotides [3,4]. As this activity might be important for the postulated mutp53 "gain of function", we analyzed the interaction of mutp53 with genomic DNA using chromatin immuno-precipitation (ChIP) *in vivo*.

Human glioblastoma [4-5] is the most frequent primary brain tumor and represents the ultimate and the most malignant state of astrocytoma progression with mutation of *p53* gene as one of the most important genetic alteration [6]. Two cell lines, Onda (273Arg -> Cys) and U251 (273Arg -> His) cells, with the most frequently occurring point mutation in brain cancer - in amino-acid Arg 273 were chosen.

About 150 genomic DNA fragments were obtained from ChIP-assay. Mutp53 DNA-binding sites contain human repetitive elements (Alu elements, long interspersed transposable elements, long terminal repeats, satellite elements, mammalian-wide interspersed repeats), secondary structure DNA forming sequences, and elements with high MAR potential (containing MAR-helix-unwinding motifs, ORI sites, AT-rich blocks). The latter motifs have already been identified as specific targets for mutp53 *in vitro* [3, 7]. In addition, about 50 sequences of genes that are directly connected with cancer, such as suppressor proteins, drug transporters, oncogenes, signaling proteins and chromatin remodeling factors were identified**.** Interestingly, the identified genes are expressed in glioblastoma, pointing out the specificity of the ChIP-assay. Using siRNA technology, we found that mutp53 exerts a strong influence on the expression of several these newly founded genes in U251 cells.

Identification of these genes increases the family of genes up-regulated by mutp53 [8] up to now analyzed only in mutp53 transfected p53-null cell lines e.g., MDR1, c-myc, telomerase, EGFR and PCNA. The latter was also up-regulated in Onda and U251 cells.

We suppose that defining the DNA structure/sequence requirement for mutp53 *in vivo* DNA binding contribute to understand the molecular basic of the mutp53 "gain of function" activities.

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Genetically modifed tumour vaccines

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Gene therapy represents a novel,developing modality of cancertreatment. The effects of therapeutic strategies based on the insertion of immunostimulatory cytokine genes into the genome of tumour cells followed by vaccination with the resulting genetically modified and irradiatedtumour vaccines will be demonstrated and discussed. As a model system for these purposes, human papillomavirus (HPV 16) E6/E7 oncogene-induced murine neoplasms transplanted in syngeneic mice and mimicking human cervical carcinomas were utilized. The aim of the experiments was to investigate whether MHC class I-deficient as well as -proficient tumours can be cured by : (1) MHC class I-deficient/proficient cellular vaccines (cross-reactivity of MHC I-deficient and -proficient tumours, and the effectiveness of the vaccines in the MHC class I-matched and - unmatched tumour / vaccine combinations), 2) vaccines generated by fusion of dendritic and tumour cells, (3) genetically modified, IL-2- / IL-12-producing, MHC class I-deficient / -proficient cellular vaccines, and (4) DNA vaccines. It has been found that the HPV-16-induced, E6/E7 tumour rejectionantigen-carrying, MHC class I- proficient (TC-1) and MHC class I-deficient (MK16) tumours do not cross-react with each other in immunization /.challenge experiments. Vaccines prepared by fusion of MHC class I-proficient tumour cells and dendritic cells inhibit growth of homologous MHC class I-proficient tumour cells, but not growth of their MHC class I-deficient subline. Both, the MHC class I-proficient (TC-1) and -deficient (TC-1/A9) HPV 16-induced tumours could be inhibited with.IL-12-producing,MHC class I-proficient as well as -deficient tumour vaccines. Minimal residual disease after surgery of HPV 16-induced, MHC class I-deficient (MK16) tumours could be cured with IL-2-producing, MHC class I-proficient (TC-1-IL-2) tumour vaccine. The E6/E7 HPV 16 plasmid DNA vaccine could protect against HPV 16-induced, MHC class I-deficient tumours. The importance of these findings for the design of the therapeutic strategies for treatment of the HPV 16-associated human tumours will be briefly discussed.

Age - depending differences in the response of mitochondrial enzymes succinate dehydrogenase and NADH dehydrogenase to T4 after short-term application of thyroxin in the heart

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In our recent experiments we have observed that short-term administration of large dose of thyroxine (T4) to suckling rats produced a marker increase in weight of the heart. The increase was most pronounced during 3rd postnatal week. The aim of the present experiment was to study the incidence of age-depending differences in the response to T4 in two enzymes of the developing heart. In the course of 4 consecutive days to suckling rats at the age from 10th, 15th and 25th postnatal day thyroxine was injected at the dose of 1μg /lg body weight/day. The following enzymes activity were assayed in whole homogenate from the left ventricle and mitochondrial fraction, resp.

1) succinate dehydrogenase (EC 1.3.99.1)
2) NADH dehydrogenase (EC 1.6.99.1)

Succinate dehydrogenase is localized on the internal mitochondrial membrane and is used as a marker of citric-cycle metabolic activity. NADH dehydrogenase is localized on both mitochondrial membranes, on the endoplasmic reticulum and on the other membranes of microsome fraction. In mitochondrial fraction after inhibition of enzymes on internal membranes this enzyme may serve as marker of outher mitochondrial membrane activity. Age-dependent changes in control animals as well as changes after treatment with T4 in case of succinate dehydrogenase in the homogenate were found. In the mitochondrial fraction of T4 caused significant decrease in the first two age groups, in rats 30-days-old no significant changes were occurred. In case of NADH dehydrogenase a marker age-depending difference was observed. In 14-day-old animals were enzyme activities in homogenate and mitochondrial fractions significantly higher in comparison with older unteated animals. Injection of T4 significantly decreased enzyme activity in 19-days-old animals only, both in the homogenate (23%) and mitochondrial fraction (48%).

These results correspond with the changes of cardiocytes at ultrastructural level and promote our suggestions of a more sensitive response to T4 during the third postnatal week of rat's life.

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Nitric Oxide Synthase and Nitric Oxide Importance for Meiotic Maturation of Pig Oocytes

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Nitric oxide synthase (NOS) has been considered as a highly regulated enzyme that can produce nitric oxide (NO) from molecular oxygen and L-arginine. Three isoforms of NOS are known – neuronal, inducible and endothelial. NO is an important signalling molecule that mediates many physiological processes.

The aim of our study was to assess whether NOS is involved in porcine oocytes meiotic maturation by NO production. The expression of NOS was studied in three stages of porcine oocytes meiotic maturation, germinal vesicle (0 h), first meiotic metaphase (24 h) and second meiotic metaphase stage (48 h), using western blotting method. The activity of NOS was observed by measurement of NO metabolites, nitrites and nitrates, with Griess reagents.

Two NOS isoforms were detected in porcine oocytes: inducible and endothelial nitric oxide synthase. These isoforms were detected in all three stages of porcine oocyte meiotic maturation. The results of NO measurement showed that the amount of NO metabolites significantly increased with the stage of meiotic maturation. Our results indicate that the production of NO is sustainable and that NOS is involved in the process of porcine oocyte meiotic maturation by producing NO.

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Solid-Phase microextraction of volatile components from silages

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Volatile components of silages can affect palatability and voluntary intake of these important feeds by animals and moreover, some of them can be carried-over to milk. Volatiles from five samples of grass silages and eight samples of maize silages were therefore analysed by gas chromatography and mass spectrometry (GC-MS) after static headspace solid-phase microextraction (SHS-SPME). Using a polyacrylate fibre, 23 volatile components were detected. Mainly phenolic and terpenic compounds were found in both maize and grass silages. These components were identified by Mass Spectral Library (Xcalibur NIST 98/EPA/NIH), only two of them were identified using standards: 4-ethylphenol and 2-methoxy-4-vinylphenol. Contents of these two components ranged between tens and hundreds mg per kg. Higher content of 4-ethylphenol was found in maize silages, while 2-methoxy-4-vinylphenol prevailed in grass silages.

Adhesion and growth of vascular endothelial and smooth muscle cells on artificial vascular prostheses and protein-coated surfaces

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Clinically used vascular prostheses are constructed as bioinert, *i.e.* not allowing for cellular adhesion and proliferation in order to prevent narrowing of these grafts. In the first set of experiments, we focused on the adhesion and growth of vascular smooth muscle (VSMC) and endothelial (EC) cells in cultures on knitted vascular prostheses made of polyethyleneterephtalate (PET) with or without collagen type I impregnation (VÚP, Joint-Stock Company, Brno). Graft samples were seeded with rat aortic VSMC, bovine pulmonary artery EC of the CPAE line and with human umbilical or saphenous vein EC (HUVEC, HSVEC). VSMC were cultured in the DMEM medium supplemented with 10% of fetal bovine serum (FBS), bovine EC in the MEM medium with 2 mM L-glutamine, Earle`s BSS with 1.5 g/L sodium bicarbonate, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate and 20% of FBS. Human EC were cultured in M199 medium with 20% FBS supplemented

with heparine (40 IU/ml) and ECGS (20ug/ml). The polystyrene (PS) test plates (TPP, Switzerland) or glass coverslips (Menzel-Glaser, Germany) served as standard control surfaces. The cell adhesion to the graft materials was examined in a static cell culture system. Compared to standard materials, the cells adhered to the prostheses in relatively lower number, by a smaller adhesion area and in non-homogenous distribution. VSMC showed higher growth activity than EC and furthermore, VSMC from male donors attached in a higher degree than female-derived ones. The cell attachment to the cultivation material is not direct but mediated through extracellular matrix (ECM) proteins such as collagen. Despite of this, the adhesion of VSMC and EC to the collagen-impregnated PET prostheses was lower than to collagen-free prostheses. Although the vascular cells on the grafts did not reach the final population density found on the standard culture surfaces, the results show that the grafts are not absolutely bioinert and significant restenosis could occur after some time thanks to the uncontrolled cell proliferation and hemocoagulation due to the surface trombogenicity. Higher adhesiveness of artificial materials can be achieved by modification of their surface properties such as ECM proteins deposition. Therefore, in the second set of experiments, inert PS deposited on glass was coated with selected ECM proteins (collagen, fibronectin), seeded with the human EC and introduced into a dynamic system simulating blood flow. Despite of cell detachment observed, the way of biological component introduction appears to be promising. Therefore, it seems to be more appropriate to design the vascular prostheses as bioartificial, *i.e.* allowing confluent, mature EC coverage having natural anti-thrombogenic and anti-immunogenic features, moreover contributing to maintain the VSMC in a non-proliferative contractile phenotype. To find and properly combine the physical and chemical surface properties demands further interdisciplinar investigation.

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Visualisation and long-term observation of living cells

(From micro cinematography to sequential image digitisation; from phase contrast to quantitative fluorescent microscopy)

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It is obvious that for full understanding of basic cellular processes we need information about spatial and temporal distribution of organelles and molecules in living cells. Considering the size of cells, subcellular and molecular structures may be studied only with help of advanced microscopic techniques and digital image capturing devices.

All major producers of microscopes have recently come with devices specifically designed for the purposes of living cell microscopy (e.g. CELL^{R} – The Real-Time Imaging Station for Live Cell Experiments, Olympus). In majority of cases, these systems are based on wide-field fluorescence microscopy or on confocal microscopy, utilizing very sensitive digital cameras and powerful software. They allow to use several new methods which are based on resonance energy transfer (FRET) or photobleaching (FRAP). Using the combination of these approaches we are able to track the fate of individual fluorescent-labeled molecules in the living cells much the same way as we observe dynamic morphology of the same cells in phase-contrast based video microscopy. Microscopy will then display not only morphological data but also biochemical and physiological information. On the other hand, no matter how enthusiastic are we about digital techniques we have to keep in our mind one basic rule: „The best digital images are still obtained by presenting the best possible optical image to the camera". Therefore our goal should always be to prepare perfect digital images as a source of useful scientific information.

Dynamic nature of many cellular processes can only be analyzed if studied in a suitable sequential format. The great advantage of digital image recording is that it can be transformed into sequences. These sequences can then be presented in standard PowerPoint presentations or shared via Internet with the whole scientific community.

The successful use of all these visualization methods presumes adequate handing of many common technical problems associated with long-time observation of living cells. These problems include maintenance of long-term constant temperature (37^{o}C), anti-shock protection, maintenance of image sharpness and reducing the negative influence of light on cells. The special problems represents the construction of the distinct cultivation chambers that on one hand enable the use of the modern microscopic techniques, and, on the other hand, allow the exchange of cultivation media during experiments.

In this presentation possibilities for observation of living cells are discussed in terms of their advantages and disadvantages for the particular study models.

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The control of recessive disorders in cattle

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Inherited disorders are hereditarily caused physical or functional anomalies of the norm with a negative impact on health. The knowledge of the molecular basis of a genetic defect offers the possibility to detect carriers directly at the DNA level early in live and even in embryonic cells. The detection of heterozygous carriers enables their exclusion and consequently, the control and quick improvement of genetic health of the population.

In the paper, the genotyping of complex vertebral malformation (CVM), bovine leukocyte adhesion deficiency (BLAD), deficiency of uridine monophosphate synthase (DUMPS) and bovine citrullinaemia at the gene level was done. In 406 Holstein sires and 146 Czech Simmental sires, mated in the AI programme in the Czech Republic, no one heterozygous sire for DUMPS, bovine citrullinaemia and BLAD was found. Therefore, the measure for eradication of BLAD in the Holstein cattle in the Czech Republic was efficient, as Hradil (1994) reported in the 90´s 65 heterozygous sires from 377 and 4 positive cows from 61.

In this paper, 111 elite Holstein females were analysed, 21 (18.9%) were heterozygotes for CVM, but they were dominant homozygotes for BLAD, DUMPS and bovine citrullinaemia. In the analysed group of young sires, only 4 were heterozygous for CVM, because since 2002 the CVM status of sires used in the AI programme in the Czech Republic must be declared, and the mating of positive sires is heavily restricted.

Resulting from our study, the situation regarding the analysed recessive disorders, except of CVM, seems to be good. Nevertheless, the monitoring of BLAD in young Holstein sires is recommended. The rigorous control of CVM status of Holstein sires and elite females, entering the breeding is required, as the disorder is at present very serious problem.

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Modern Methods of 3-D Reconstructions Cells

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Modern computer technology and digital images acquisition enables highly improve abilities of classical and confocal microscopes. New methods of cells 3-D reconstruction based on series of 2-D images obtained by mentioned type of microscopes are presented.

For confocal microscope the volume reconstruction methods using ray casting which are able to display a transparent or semi-transparent bodies with defined transparency using adaptive filters are explained. These methods are inspired by human vision and they are using principles of 3-D perception. These methods highly improve classical methods of confocal microscope images reconstruction based on detection of maximum intenzity in series of cuts. They are well suited for 3-D display of small bodies like cells with complicated topology.

Following lecture part is devoted to Fourier transform based methods of 3-D reconstruction using classical microscope. These methods are able in many cases to supplant expensive confocal microscope by classical one. These methods are especially suitable for display of opaque bodies but even for semi-transparent bodies they give often satisfactory results.

The last part is devoted to 3-D animation creation based on perspective using. These methods enable totally new approach to investigation of cells as the make possible to view the cell not only from outside but inside too.

Characterization of enterococci in bryndza cheese

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Enterococci are part of lactic acid bacteria, they are Gram-positive, catalase-negative cocci that occur in various dairy and fermented meat products, but their natural habitat is the intestinal tract of human and animals. The presence and growth of enterococci in fermented foods results in organoleptically unique products. They were found in a typical Slovak sheep milk cheese - bryndza in high levels. The genus *Enterococcus* is of increased significance as a cause of nosocomial infections, and this trend is exacerbated by the development of antibiotic resistance, especially to antibiotic vancomycin. Four hundred and twenty bacterial strains isolated on the Slanetz-Bartley agar from bryndza cheese obtained at three periods of the year from different commercial producers have been analysed. Three hundred and thirty one strains were identified to be *Enterococcus* sp. by phenotyping methods. The most frequent species, *E. faecium, E. durans* and *E. faecalis*, were confirmed by PCR using species specific *ddl* primers to D-Ala:D-Ala ligase gene. Screening for v*anA* and v*anB* genes using PCR demonstrated no occurrence of these vancomycin resistance genes in enterococci from bryndza samples. Plate tests also showed no vancomycin resistance of these enterococci. PCR was used for assessment of presence of virulence determinants: *gelE* and *agg* genes as well as some cytolysin genes. Gene *gelE* was found in 20 *E. faecalis* isolates, but only 13 of them showed gelatinase positive phenotype. Seven isolates had five cytolysin genes, but none of the isolates exhibited a positive haemolytic phenotype. Four isolates possessed the *agg* gene.

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Controlled behaviour of vascular smooth muscle cells on ultrathin protein layers

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Modification of the surface of ceramic bone implants by collagen I, improved cell adhesion and proliferation (Brodie et al. 2005). In this study, we tested influence of extracellular matrix protein layers on the behaviour of vascular smooth muscle cells (VSMC). For coating we used successively deposited molecular layers of collagen I (CO I), heparin (H), collagen IV (CO IV), and laminin (La) on polystyrene (PS) non-modified with plasma discharge. We prepared following samples: PS-CO_I, PS-CO_I/La, PS-CO_I/H/CO_IV, PS-CO_I/H/CO_IV/La, PS-CO_I/La/CO_IV, PS- control.

The layers were seeded with rat aortic VSMC (passage 5-6, 17 000 cell/cm^2). Number of initially attached cells 24 hours after seeding was the highest on PS-CO_I/H/CO_IV/La_I, although Student`s test for unpaired data showed no statistical significance in comparison with PS. However, spreading of VSMC on all samples coated by proteins was significantly higher than on the control sample. Spreading area of VSMC on CO_IV as the upper layer (i.e., PS-CO_I/H/CO_IV and PS-CO_I/La/CO_IV), was larger by 432 % and 452 %, respectively, than that on PS. Samples with laminin at the top (i.e., PS-CO_I/La and PS-CO_I/H/CO_IV/La), showed increased spreading by 227% and 222%, respectively, in comparison with PS. On day 7 after seeding, all ECM proteins supported VSMC proliferation (by 25 to 55% compared to PS). Immunocytochemical staining of vinculin depicted assembly of integrin receptors into focal adhesion plaques, which were best developed on layers with CO_IV as the upper coating. Alpha-actin filament bundles were well developed in VSMC on all samples.

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Non-woven PGA and PGA/PVA scaffolds are suitable for tissue engineering

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New composite three-dimensional biodegradable scaffolds from polyglycolic acid and polyvinylalcohol (PGA/PVA scaffolds) or PGA (PGA scaffolds) were developed. The scaffolds were prepared by a wet-laid method, the PGA/PVA scaffolds were subsequently treated with PVA solution (PVA/PGA/PVA scaffolds) and PGA scaffolds with hyaluronic sodium solution (PGA/HA scaffolds) and/or subsequently processed by needle punching (PGA/PVA and PGA/HA scaffolds). Chondrocytes were isolated from rabbit, cultured for 28 days and seeded onto the scaffolds at density of 80 x 10^3 cells/cm^2. Proliferation and viability of chondrocytes were testing using MTT test and confocal microscope.

The absorbance of PVA/PGA/PVA and polystyrene groups were significantly higher compared to the other scaffolds at 24 hours after seeding. After a seven-day cultivation, the chondrocytes showed the highest proliferation rate in polystyrene. Absorbance of PGA/HA scaffolds was significantly lower compared to the absorbance of PGA and PVA/PGA scaffolds.

This study showed the ability of chondrocytes to proliferate on different non-woven scaffolds from PGA and/or PVA and proved their potential for tissue engineering.

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Platinum derivative LA-12 can overcome resistance of HT-29 cells to CDDP

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Platinum derivatives are most frequently, long time-used cytostatic drugs with a wide range of applications. The first treatment with platinum–based cytostatic drug cisplatin (CDDP) was reported in 1975. New platinum [IV] derivative, coded as LA-12, was studied in this work. Effects of the complex on HT-29 cell line derived from human colorectal adenocarcinoma were compared with those of CDDP and oxaliplatinum which are routinely used as a highly potent treatment of various types of malignant tumours. However, the use of these drugs is limited by their negative side effects and development of resistance in the treated cells. LA-12 appears to be very effective, because it is able to elicit a cytotoxic response in cells resistant to both CDDP and L-OHP.

We determined cytotoxicity, measured by MTT metabolic assay; numbers of cells, their viability and induction of apoptosis. CDDP showed the lowest cytotoxicity among studied compounds, where LA-12 was the most cytotoxic one, inhibiting the growth of the cell population and causing a decrease of viability and increase of apoptosis. In contrast, L-OHP decreased cell numbers but not cell viability. Our results suggest that resistance of HT-29 to CDDP and low sensitivity to L-OHP could be overcome by novel platinum derivative LA-12.

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Characterization of *C. reinhardtii* repair-deficient mutants with predicted role in cell cycle response to DNA damage

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The aim of our work was to complete phenotypical characteristics of selected repair-deficient mutant strains included in the collection of UV-sensitive mutants, and to study the connection between DNA repair and the cell cycle regulation. Phenotypic characterisation, genetic and molecular analyses of mutants were carried out. The effect of DNA damage on the progression of selected algal strains *uvs11*, *uvsX1* and *uvsX2* through the cell cycle has been investigated. Repair of DNA lesions was monitored by cell viability. Our results indicate that the *UVS11, UVSX1* and *UVSX2* genes could play a role in a checkpoint control, e.g. block cell cycle progression if DNA is damaged. This suggestion is supported with the fact that survival of damaged cells in tested mutants is restored to nearly wild type level by artificially imposing a G2 block with MBC.

We have also monitored the histone H1 kinase activity, the presence of CDKs and changes in kinase activity after UV irradiation of wild type and the mutant strains. Both control and mutagen treated cultures were analysed for the accumulation of total protein, RNA and the DNA level. The reproductive processes were monitored by observation of nuclear division, protoplast and daughter cell formation in both strains. The results of our experiments could be interpreted by postulating DNA damage checkpoint. The functionally important response of wild type cells to UV irradiation was complete block of the kinase activity of the CDK-like protein and its recovery some hours later. The UV effect on kinase activities was accompanied by a prolongation of the cell cycle due to a delay in mitoses, protoplast fissions, and daughter cell release. In contrast to the wild type, the cells of mutant strains exhibited different response in the course of kinase activities during the cell cycle if compared with untreated cells. Our results suppose that green alga *C. reinhardtii* probably possesses some type of the control mechanism to arrest the cell cycle progression. This "checkpoint" is likely to be activated just at the end of the growth stage, before the cell reproduction. The products of *UVS11, UVSX1* and *UVSX2* genes seem to be a part of the putative signal pathway in cell response to DNA damage.

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Evidence for DPOR presence in selected plant species

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Chlorophylls are one of the most abundant classes of natural pigments, and their biosynthesis is therefore a major metabolic activity in the ecosphere. Two pathways exist for chlorophyll biosynthesis, one takes place in darkness and the other requires continuous light as a precondition. The key process for Chl synthesis is the reduction of protochlorophyllide (Pchlide) [1, 2]. This enzymatic reaction is catalysed with one of two different enzymes – DPOR (dark-operative Pchlide oxidoreductase) or structurally distinct LPOR (light-dependent Pchlide oxidoreductase). DPOR which consists of three subunits encoded by three plastid genes in eukaryotes greening in the dark was subject of our study.

For our analysis we have chosen four conifer species *Pinus sylvestris, Pinus mugo, Larix decidua, Picea abies.* Seeing that chlorophyll biosynthesis using DPOR is limited to germination we have worked with cotyledons from 14-days old seedlings.

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The nucleolar structure and nucleolar proteins as indicators of cell proliferation events in plants

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Cell proliferation is a crucial cellular process which influences development. In plants, meristems are formed by actively proliferating cells, in which the main expression of proliferation is the existence of a cell division cycle. Many cell activities are influenced by the cell proliferation status and cell cycle progression, among them ribosome biogenesis, which is morphologically expressed as the nucleolus. The connection is established through nucleolar proteins, which regulate synthesis and processing of preribosomal precursors and, at the same

time, are targets of various cell cycle regulators, such as certain kinases. Nucleolin is one of these nucleolar proteins, whose level increases with cell proliferation and depends on the cell cycle stages. Not only the levels, but also other important features of the protein, such as its distribution *in situ* in the nucleolus, its phosphorylation and its physiological degradation, depend on these parameters. Furthermore, since the nucleolar structure is highly sensitive to functional variations, distinct nucleolar structures, regarding the nucleolar size and the distribution of nucleolar subcomponents, have been defined for each period of the cell cycle, using synchronized cells. In addition to increase our knowledge on the cellular physiology, these relationships can be used to mark the proliferative state of the cell and the periods of cell cycle.

Contribution of excitatory amino acids to neurodegenerative diseases: do the astrocytes have a key role in the process?

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Excitatory amino acids (EAAs) such as glutamate (Glu) represent brain major neurotransmitters. They are involved in numerous functions based on rapid signalling between cells in neuronal networks. However, besides their role in brain intercellular communication EAAs can also represent potent cytotoxic agents. An excess of EAAs at synaptic cleft is indeed longer known to induce neuronal death, which could be involved in numerous brain diseases such as stroke, trauma, ischemia or slow neurodegenerative processes like Huntington'disease, amyotrophic lateral sclerosis (ALS) or even Alzheimer's disease. Basically, the cellular mechanisms leading to the excessive concentration of Glu in the synaptic space are not known. Recent hypothesis are focused on a deficit in the synaptic removal of the excitatory transmitter from the extracellular space, which primarily involves specific transport systems mainly into the astrocytes surrounding the nerve terminals. Indeed, astrocytes are known to express EAA transporters (EAATs) of the GLT1 and GLAST subtypes, whereas neuronal contribution to natural removal of Glu from the synapse could involve the EAAC1 transporter. Because pharmacological alteration of EAAT activity *in vivo* or *in vitro* or change in the expression of GLT1 gene in human could contribute to neurodegenerative diseases, we focused our studies on understanding regulatory processes of EAA transport with the view to promote neuroprotection strategies through stimulation of EAA transport. We demonstrated that if an excess of extracellular Glu can likely contribute to neuronal death, conversely a depletion of Glu into differentiated astrocytes could also induce neuronal death indirectly as a consequence of a primary alteration of the astrocytes. Glu depletion could indeed induce a sensitization of astrocytes to oxidative stress. Regarding the role of EAAC1, which represents a very active process for Glu removal at least during the earlier stages of brain development, we showed that astrocytes could stimulate neuronal EAAC1 expression and activity. Further data suggested that cholesterol secreted from astrocytes could be one of the factors influencing positively EAAC1 activity. We presently tested the hypothesis of a special vulnerability of specific neuronal populations such as the dopaminergic neurones of the mesencephalon to extracellular Glu exposure and/or intracellular Glu depletion, the degeneration of which is related to Parkinson's disease.

Effects of adenine nucleosides on cultured *Drosophila* cells

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During *Drosophila* embryogenesis, parts of the embryonic epithelium invaginate, giving rise to the so-called imaginal discs; structures that eventually determine the shape of the adult fly. Cells derived from the imaginal wing discs of third instar larvae were previously used to establish the Cl.8+ cell line. In this work, Cl.8+ cells were studied with respect to their motility and their arrangement of the actin microfilament system, whose dynamicity is essential for the morphological changes taking place throughout the *Drosophila* development. Actin assembly and adhesion-related events are also of great interest in the context of apoptosis, as their failures have previously been associated with increased cell death, and cell survival within disc epithelia has been proposed to be promoted through cytoskeletal organization. The morphology and motility of live Cl.8+ cells with or without the counterstaining of nuclei by Hoechst dye were studied using DIC and confocal fluorescence microscopy. A vivid protrusion of filopodia-resembling spikes at the cell edges was observed. To examine the

distribution of F-actin, cells were fixed, permeabilized, stained with fluorescent phalloidin probes, and scrutinized in the confocal microscope.

One of our objectives is a better understanding of the functions of adenosine deaminases, the enzymes catalyzing deamination of adenosine and deoxyadenosine. In the recent years, adenosine deaminases have gained increased attention and have been ascribed an exciting role in insect development. Upon the breakdown of ATP and nucleic acids, tissue levels of adenosine nucleosides increase and reach toxic concentrations. The action of adenosine deaminases seems to provide a protection for growing tissues; an aspect of particularly high value in the *Drosophila*, where metamorphosis involves massive apoptosis and tissue remodelling. A family of adenosine deaminase-related growth factors (ADGFs) have recently been described. They are growth factors with a unique mode of action, as their enzymatic activity is required for their mitogenic function.

The addition of adenine nucleosides to the cell culture media caused dramatic changes in cell morphology and impaired the outgrowth of filopodia. Adenosine and deoxyadenosine appeared to influence the actin cytoskeleton of the Cl.8+ cells in differential manner, as revealed by the resulting changes of the cell shape and the actin microfilament staining.

We have developed an assay facilitating the investigation of signal transduction pathways connecting actin remodelling, cell adhesion, and cell survival.

Effect of chromium exposure and smoking on Lung cancer incidence

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The increased occurrence of lung cancer in residents of Dolný Kubín, the North-Slovakia district with ferrochromium industry, compared to the general population of Slovakia, led us to the study assessing influence of the occupational and environmental exposure to chromium on the lung cancer incidence, respecting also the risk coming from cigarette smoking.

Residents of Dolný Kubín district with the diagnosed lung cancer in 1984-1999 were involved in the study.

The occurrence of lung cancer was significantly higher in people working in ferrochromium industry. The age at the onset of the disease in people exposed to chromium was by 5.5 years lower than in non-exposed.

Smoking was an important risk factor, which has been proved particularly in non-exposed group where 62% were smokers and the onset of the lung cancer in them occurred about 3.4 years earlier than in non-smokers. In exposed groups, no significant effect of smoking was found.

We can conclude, that occupational exposure to chromium was identified as the main risk factor of lung cancer in Dolný Kubín district even overlaying effect of smoking.

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Do statins reduce delayed neuronal death following transient forebrain ischemia in the adult rat hippocampus?

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Brain ischemia is one of the major complications of brain metabolism leading to oxygen deprivation and death of neurons.

Statins might have beneficial effect in several CNS disorders. It seems likely that these effects are based on the antiinflamatory and antiproliferative properties of statins and neuroprotective effect that reduce oxidative stress. Profylactic statins therapy may reduce damage of the brain during ischemia.

We investigated the effect of prophylactic therapy with simvastatin on delayed neuronal death in the rat hippocampus. The rats were given a daily dose of 20mg kg $^{-1}$ of simvastatin orally for 14 days. Stroke was then stimulated by ligation of common carotid arteries for 15 minutes.

The hippocampus is known to be particularly sensitive to anoxic or ischemic injury because of its high metabolic rate. Number of surviving neurons of the hippocampal CA 1 subfield was counted in adult rats after 15 min. global ischemia and 72 hours reperfusion. Synchronously, lipoperoxidation and oxidative modification of neuronal proteins were measured in homogenates of hippocampus of similarly treated rats.

Our morphological findings have demonstrated that prophylactic statin treatment significantly reduced delayed neuronal death after transient forebrain ischemia.

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Effect of food restriction on c-Fos and synaptophysin immunoreactivity in hippocampus aftrer transient brain ischemia in rats

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In order to determine whether food restriction would have an influence on c- Fos protein and synaptophysin production in hippocampal cells after ischemic insult, the immunostaining was employed. Experimental rats were fed by special diet for 8 weeks. They lost 15-20% of their weight. Stroke was then stimulated by ligation of common carotid arteries for 15 minutes. We used positive (ischemised rats without special diet) and negative (non- ischemised, shame-operated rats) controls as well. The time of reperfusion was from 2 hours to 14 days.

Immumoreactivity for Synaptophysin in experimental and positive control rats was transiently increased in the ischemic lesions from 3 to 7 days after celebral ischemia. Thereafter, synaptophysin immunostaining in the CA 1 region of hippocampus gradually decreased. However, the level of synaptophysin stayed increased in CA 3 region of hippocampus until 14 days of reperfusion.

The level of c-Fos immunoreactivity increased after 2 hours of reperfusion in experimental and positive control rats as well. There were not seen any difference between ischemic rats with or without food restriction.

We can conclude that 15 minutes long transient ischemia results in transient increase of synaptophysin in experimental and positive control rats. Immunoreactivity for synaptophysin an c- Fos protein did not show any noticeable change between ischemised rats fed et libitum and ischemised undernourished rats.

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The role of calmodulin in cell cycle

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Calmodulin is a small, highly conserved, Ca^{2+}-binding protein present in all eukaryotic cells. It is one of the mediators of Ca^{2+} signals induced by extracellular stimuli. Several lines of evidence have suggested that calmodulin is essential for eukaryotic cell proliferation. In our previous papers, the intracellular localization of protein calmodulin and cytoskeletal actin in cells of yeast strain *Yarrowia lipolytica* has been studied by indirect immunofluorescence using anti-calmodulin antibody and rhodamine-conjugated phalloidin. We have found that there exist relationships between cytoskeletal components and protein calmodulin. Microtubule disassembly is promoted by calmodulin in the presence of Ca^{2+}. Calmodulin is also implicated in regulation of microfilaments (1, 2). Distribution of calmodulin changes in a typical way during the cell cycle (3). In the unbudded cell calmodulin localizes to a sharp crescent shaped patch on the cortex of the cell. At bud emergence, calmodulin concentrates to the budding portion. All budded cells exhibit bright staining calmodulin dots at the tip of the bud. In small budded cells are staining dots localized at the bud neck, in cells with large buds calmodulin diffuses throughout the entire bud. At cytokinesis, calmodulin is mainly present at the connecting passage between the mother and daughter cells. Double staining experiments were carried out. They have shown that the location of calmodulin dots coincides with that of actin structures. Calmodulin emerges in mentioned parts of the cell always sooner than actin. This fact proved that calmodulin is required for distribution of actin dots. The finding that calmodulin is unconditionally needed for nuclear division was particularly important. Therefore we studied new, intentionally prepared mutants *Y. lipolytica* in our present experiments. We found that opened cell cycle without sufficient level of calmodulin stops in M-phase (4). Inhibition of karyokinesis start caused by the low level of calmodulin is reversible and without consequences, if the blockade takes less than four hours (5). After increase in calmodulin level, the karyokinesis starts in 15 minutes and it passes correctly. But if the blockade of karyokinesis takes more than four hours as a result of extremely low level of intracellular calmodulin, the cell reacts to calmodulin level normalization late (after 2 – 4 hours) and the process of the karyokinesis is very aberrant. However, the cytokinesis is not restored no more. After about 20 hours, we can observe cell formations vary in size in the culture with from two to four nuclei different in size and DNA content. In these cells calmodulin and actin dots are dispersed all over the periphery of cell and their number and size are strongly reduced. *Conclusions:* 1) the accumulation of calmodulin dots at the regions of cell growth suggests that this protein is important for cell polarity development in yeast; 2) calmodulin emerges in mentioned parts of the cell always sooner than actin. It proves that calmodulin is required for distribution of actin structures; 3) calmodulin is unconditionally needed for nuclear division.

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Cathodic PR- and DR- intercellular proteins synthesized in the infected barley leaves – their biochemical function

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The main looses on our second important agricultural cereal are caused with air pathogens. One of them is powdery mildew (*Blumeria graminis* (DC) *Speer f.sp*. hordei). This pathogen attact barley plants in

a dependance of their resistance genes. Development of infection and its progress we can see on various phases of powdery mildew asexual cycle. Plants defend against to pathogen with various system of morphological and molecular strategies. One of them is biosynthesis of PR and DR-proteins (Van Loon and Van Strien 1999). PR-proteins have been classified into 14 groups based on their amino acid sequences and biochemical function. Many of these proteins have antifungal activity (Hlinková and Bobák 2002). Although much is known about some metabolic responses, protein analyses at host-pathogen interaction are focused on acid region. Very week attention is focused on protein synthesis in alkaline region at the period pathogen sporulation time-late phases of pathogen asexual development. In our work, we studied basic protein patterns (C-PAGE) of intercellular fluid isolated from the primary leaves of various barley genotypes carrying different resistance genes from Ml-a locus and infected with two different powdery mildew pathotypes by the period of their sporulation.

Basic PR-proteins in the intercellular fluid were found only in the infected barley cells and near of infection site. All had the same molecular masses independently on resistance genes of the host plants and virulence genes of pathogens. Their molecular mass was from the interval Mr~12- 25 kDa.

Biochemical analyses showed quantitative and qualitative differences by basic intercellular β-1,3-glucanases, chitinases, peroxidases and RN-ases. What was interesting and it is difference compared to acid intercellular patterns, basic PR-proteins were not found in the uninfected leaf part. Low molecular basic PR-proteins did not contained proteins with peroxidase activity. Peroxidases were found only in the region of higher molecular masses and are connected probably with defense reactions. Their expression is activated with membrane degradation products. Higher amount of these enzymes was identified for all gene combinations. Amount of basic β-1,3-glucanases detected with laminarine as a substrate (Pan et al. 1991) did not show quantitative differences – 4 isozymes were found in all basic patterns. Genetic differences were identified with aniline blue (Trudel and Asselin 1989). Isozyme patterns in this case reflected genetic background of host and pathogens. Similar picture we found for basic chitinases. Constitutively were expressed 3 genes and their proteins had molecular mass Mr~68; 55 and 48 kDa. Low molecular chitinase synthesized as a result of pathogenesis had Mr between 20-35kDa. Content of basic β-1,3-glucanases and chitinases is lower compared to acid intercellular patterns. Opposite results in quantitative content gave RN-ases, their amount is three-time higher in basic protein patterns.

We can conclude that the intercellular fluid contains a large amount of basic PR and DR-proteins too which are coding with various gene groups; their biochemical function and content is function of both genetic systems host and pathogen as well as a disease progress. In the intracellular spaces they create protein complexes with various molecular masses. Channels for them can be identifying by FISH method.

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Synthesis of ^{18}F-labelled substance P for positron emission tomography evaluation of two labelling agents [^{18}F] fluorobenzaldehyde and [^{18}F]succinimidyl-fluorobenzoic acid

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Substance P (SP) is an agonist for the NK_1-receptor and is considered to be a neuromodulator or neurotransmitter. Changes in SP have been implicated in Parkinson's disease, arthritis, inflammatory bowel disease and asthma.

The reaction of an oxoamino derivative of SP (which was kindly donated by Dr M. Schottelius and Dr H. J. Wester from the Technical University Munich) with [^{18}F]fluorobenzaldehyde to form an oxime, was investigated as described by Poethko (1). In the production of [^{18}F]fluorobenzaldehyde, DMSO was replaced by 1,3-dimethylimidazolidin-2-one (DMI). The reaction in DMSO led to the formation of substantial amounts of [^{18}F]fluorobenzoic acid due to oxidation of aldehyde group by the solvent. The amount of radioactive by-products was reduced significantly using DMI. The radiochemical yield of purified aldehyde was up to 40%.

After dilution of the reaction mixture with water, [^{18}F]fluorobenzaldehyde was purified by solid-phase extraction on Oasis HLB cartridge. Radioactive impurities were removed by elution with mixtures of water and

organic solvent. Finally, [^{18}F]fluorobenzaldehyde was eluted with methanol. This solution was added to the oxoamino derivative of SP dissolved in 0.5 ml of phosphate buffer pH 2.7 (the oxoamino group was attached to the N-terminus of peptide) and heated at 60ºC for 20 min. Based on HPLC data, the radiochemical yield of the Schiff base formation was 35%. The whole procedure was fully automated with a Zymark robotic system.

Previously, preparation of succinimidyl [^{18}F]fluorobenzate (SFB, for acylation of unmodified SP) led to the radiochemical yield less then 10%. Several reaction steps were optimized. Previously used solid-phase extraction of SFB was replaced by HPLC followed by solid-phase extraction. The overall radiochemical yield with a Zymark robotic system was 24% (10-45%).

The acylation of SP at ε-aminogroup of Lys residue was carried out in borate buffer pH 8.5 in the presence of a catalytic amount of triethylamine at ambient temperature. After HPLC purification the radiochemical yield of acylation varies in the range of 1-15%.

In conclusion ^{18}F-labelled SP can be labelled relaible via two different synthetic routes. Biological studies involving in vivo metabolic stability, and pharmacokinetics are currently underway.

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GFP dedetection in low level signal/noice ratio plant samples

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Green fluorescent protein (GFP) offers a wide range of applications in plant biology (Stewart 2001). GFP represents an efficient tool for the detection/selection of transgenics and also for the improvement of transformation, selection and regeneration protocols for the recovery of stable transgenic plants. GFP has been found to be superior to other marker genes used in plant biology such as GUS or LUC owing to many advantages it displays. Early identification of transgenic cells is crucial point for efficient recovery of transgenic plants and the application of GFP can reduce the amount of material to be handled and analysed through culture and regeneration. GFP green fluorescence allows rapid non-invasive identification of transformed cells and therefore early elimination of non-transformed cells, silencing events or developing chimeras. Moreover, various approaches for quantification of GFP fluorescence have been recently reported with the aim to quantify gene expression and to identify early homozygots (Millwood et al. 2003, Hraška et al. 2005).

On the other hand problems associated with a low signal/low signal-noise ratio could be met quite often. Low levels of background fluorescence of various compounds present in intact, wounded and untransformed tissues and/or in *Agrobacterium* strains do not usually impede the successful GFP fluorescence detection and can be restricted by implementation of suitable filter systems. Choose of the right detection device is an important factor. Another way to circumvent such complications represents the use of proper tissue or plant part(s) because the intensity of visible fluorescence is affected by numerous factors among which chlorophyll content and presence of other flouresencing compounds play an important role.

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Expression of membrane molecules on human normal and leukemic haematopoietic stem/progenitor cells (CD34+)

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We have studied haematopoietic samples of mobilized haematopoietic stem/progenitor cells (peripheral blood, bone marrow, leukapheresis) in order to identify differentiation markers expressed on subsets within these cell

populations. Using the methods of direct and indirect immunomagnetization and immunofluorescence in suspension by help of flow cytometry we have tested expression of CD34 surface molecule and other markers on human normal and pathological cells of patients with hematological malignancies. On leukemic cells from some patients with AML, CML, CML-BC, non T ALL we found coexpression CD34 molecule with other membrane markers. The occurrence of CD34 molecule with HLA-DR, CD11a, CD18, CD33, CD38, CD43, CD44, CD45, CD46, CD47, CD50, CD55, CD58, CD133 and CD184 molecules was detected very frequently, but also coexpression CD34, with CD7, CD11b, CD11c, CD15, CDw17, CD19, CD35, CD53, CD65, CD66, CD71, CD76, CD90, CD117, were found in individual cases. We did not detect on the normal and leukemic cells the occurrence of CD34 with CD1, CD3, CD5, CD8, CDw12, CD20, CD24 and CCR5 molecules. The coexpression of CD34 antigen with other CD markers on progenitor cells and leukemic cells show great heterogeneity and diversity of phenotypes and so further characterization of the CD34 cells is important for better knowledge of differentiation of multipotent precursors.

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Distribution of protein bodies in norway spruce embryos and megagametophytes demonstrated by visualization of globoids by low vacuum scanning electron microscopy

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Organelles for protein storage, known as protein bodies, are commonly found in seeds. In some plants, they contain inclusions − globoids or crystalloids (Pernollet 1978). In previous studies on pine megagametophytes and embryos, globoids and spherosomes, i.e., particles with a higher level of brightness were visualized by low vacuum scanning electron microscopy (SEM) with a backscattered electrons (BSE) detector (Hřib et al. 2003, 2004).

Longitudinal sections through embryos and megagametophytes of uncoated Norway spruce (*Picea abies* /L. / Karst.) dry seeds were made. Protein bodies were demonstrated by the presence of globoids appearing as bright particles in SEM images (Vega TS 5136 LM analytical SEM in a low vacuum mode with BSE, TESCAN Brno; low vacuum, 10-12 Pa; accelerating voltage, 30 kV; SEM magnification, x 3 300), and were analysed by the software package Morphology, which also allowed us to measure the area taken by each globoid (μm^2).

In embryos, two major types of distribution of protein bodies by means of globoid visualization were distinguished. The first included a high percentage of bright spherical particles, >1 μm, in the subapical part of the hypocotyls. Globoids were also located in the cotyledon, with more being present in the top than in the bottom part. In this distribution type, globoids covered 1.79 to 1.87 % of the total image area. In the second type, most of the globoids were accumulated in the central part of the hypocotyls; they took 3.08 to 3.10 % of the total image area. The distribution of globoids in the cotyledon was reverse, i.e., more particles were present in the bottom than in the top part.

In megagametophytes, only one type of distribution was observed, with a higher percentage of globoids located near the cotyledons than near the radicle.

The difference in distribution of protein bodies in the embryo and the megagametophyte and between the embryo and the megagametophyte apparently plays a role in seed germination and early growth of seedlings, as well as in the plant defence mechanism.

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Effect of laser irradiation on the cytoskeleton of protozoa

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The effect of pulse laser irradiation on the cortical microtubular system of the protozoan *Blepharisma* was investigated.

Blepharisma undulans japonicus (phylum *Ciliophora*, genus *Heterotricha*) is a ciliated infusorian, about 150 to 500 μm in size, which produces enveloped protective cysts permitting survival in adverse environmental conditions. To study changes that may be induced by laser irradiation in *Blepharisma* surface structures, namely the cortical microtubular system, both vegetative cells and protective cysts were exposed to a MINILITE II, pulsed Nd:YAG laser (λ = 355 nm; pulse width between 250 and 350 μs). The microtubular system of the cytoskeleton was visualized by indirect immunofluorescence, using TU - 01 primary and SwAM - FITC secondary antibodies, in the irradiated as well as control cells and cysts. Further information on the cytoskeletal microtubules as well as the overall ultrastructure of intact and laser-treated vegetative cells and cysts was obtained by electron microscopy.

Exposure to laser irradiation resulted in damage to the surface of both vegetative cells and cysts. In vegetative cells, cortical microtubules at the site of irradiation became disintegrated and kinetosomes and cilia disappeared, as detected by indirect immunofluorescence. The cells, however, survived this damage. In cysts, indirect immunofluorescence failed to detect any organized microtubular system, but electron microscopy showed occasional microtubules and kinetosomes present in the cytoplasm. In a complete cyst with a thick wall (up to 20 μm), a single dose of laser irradiation produced only mild damage to the wall, while repeated doses resulted in perforation of the wall and spillage of the inner content that led to complete destruction of the cyst. The most sensitive to irradiation was the polar protrusion due to its thinner wall (2-5 μm). This was demonstrated by both visualization methods.

It can be concluded that the protective cyst wall can substitute the microtubular system in maintaining the shape but not in processes facilitating survival of the cyst after serious damage due to repeat laser irradiation.

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The mammalian daily and seasonal clock

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All mammals exhibit an array of daily behavioral, physiological, hormonal, biochemical and molecular rhythms. The circadian rhythms persist even in a nonperiodic environment with a period close, but not equal to, 24 h. Under natural conditions, the rhythms are entrained to the 24 h day by the light-dark (LD) cycle, mostly by the light period of the day. The circadian rhythms are controlled by a pacemaker located in two suprachiasmatic nuclei (SCN) of the hypothalamus. The SCN themselves exhibit rhythms in metabolic, electrical and neural activity, production of peptides and expression od many genes. The SCN rhythmicity is due to the SCN molecular clockwork. At least eight clock genes are thought to be involved in the clockwork by forming interacting transcriptional-translational feedback loops. Although the central clock is located in the SCN in the brain, almost all organs exhibit circadian rhythms and serve as peripheral clocks. About 10 % of all genes in the body are expressed in a cyclic manner.

Daylength, that is, photoperiod, affects many biological variables in mammals, including circadian rhythms, e.g., in locomotor activity and in pineal melatonin production. Rhythmicity of the SCN itself as well as of the peripheral organs is affected by the photoperiod. Recent data indicate that the whole complex molecular clockwork in the mammalian SCN is photoperiod dependent. Thus it appears that the SCN is not only the circadian clock, but also a clock for all seasons.

Role of nitric oxide in meiotic resumption of pig oocytes

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Fully-grown mammalian oocytes are arrested at the diplotene stage of the first meiotic prophase, which is also termed the germinal vesicle (GV) stage. GV-stage-arrested oocytes can spontaneously resume meiosis when they are released from the inhibitory environment of follicles. The mechanisms that promote the resumption of meiosis in mammalian oocytes remain poorly understood.

Nitric oxide (NO) is one of the free radicals that are implicated in a variety of intracellular signaling mechanisms. NO is synthetized from L-arginine by three different nitric oxide synthase (NOS) isoforms: neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). Of the three NOS isoenzymes, ovaries express eNOS and iNOS but not nNOS. It has been suggested that NO-NOS has important physiological roles in a variety of reproductive processes such as follicular development, ovulation, and spermatogenesis.

In the present study we investigated the role of NO-dependent events in meiotic resumption in pig oocytes. Cumulus-oocyte complexes (COC) collected from porcine ovaries were cultured in a modified M199 medium at 39 ^{0}C in humidified atmosphere with 5% CO_2 in air for 24 h. For the study of the role of NOS-derived NO during oocyte GVBD fully grown oocytes were collected and cultured in the medium containing iNOS specific inhibitor aminoguanidine (AG) or non-specific NOS inhibitor L-NAME. After incubation, all oocytes were examined with an inverted microscope and classified as being at the following stages: germinal vesicle, late diakineze, prometaphase, methaphase I and oocytes with degenerative changes or atypical morphology.

The meiotic resumption of the oocytes was significantly blocked by iNOS specific inhibitor AG in a dose-dependent manner. The addition of L-NAME to the culture medium had no significant effect on meiotic resumption and GVBD. Our results suggest that the iNOS-derived NO pathway plays important roles in pig oocyte meiotic maturation, especially in germinal vesicle breakdown. We hypothesize that the iNOS-NO system and eNOS-NO system may play a different role in the regulation of meiotic maturation in pig oocytes.

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Capillary-epithelial relationship during development of the embryonic kidney

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The vascular blood supply of the chick mesonephros develops concurrently with the formation of nephrons. The function of nephrons depends on the establishment of the glomerular blood supply and the initiation of the glomerular filtration. A prerequisite of the tubular transport in the mesonephros is the development of the peritubular portal circulation (Friebová-Zemanová, 1980). The structural differentiation of the interface between the capillary and tubular wall in the 5 – 10 day embryos was studied in association with expression of the selected proliferative markers and membrane bound enzyme activities.

Embryonic kidneys were taken from 5 to 10day old Grey Leghorn chick embryos (Koleč farm). Samples for electron microscopy were fixed with Karnovsky´s solution and embedded in Epon (Glycidether, Serva). Proliferative markers PCNA (Anti-Proliferating Cell Nuclear Antigen, Sigma), VEGF (Anti-Vascular Endothelial Growth Factor, Santa Crus Biotech) and EGF (Anti-Mouse Epidermal Growth Factor, Sigma) were detected by DAKO LSAB®2 System-HRP. Two enzyme markers of the cell surface membrane, alkaline phosphatase and ATP-ase, were demonstrated ultrastructurally. Lead and cerium capture methods were used for detection of alkaline phosphatase activity (AlP) and Schultze – Wollenberger modification of Wachstein-Meisel technique for ATP-ase.

Postinductive organization of nephrons was associated with an expression of PCNA and VEGF in the developing tubules as well as in the endothelium of primitive vessels in the mesonephros of 5 day embryos. Development of the mesonephros included, in addition of the growth of nephrons, the structural differentiation of their segments. Differentiation of the tubular epithelium was accompanied by a subsequent decrease of PCNA-positive cells in 7-day embryos. Simultaneously, the development of the peritubular capillaries was associated with an increase of PCNA-positive endothelial cells. EGF expression was confirmed in cells of the mesonephric blastema, tubules and in the vascular endothelium of the 5-day embryos. The differentiation of the epithelium in the mesonephric tubules was associated with an increase of EGF-positivity.

Differentiation of the epithelium in the proximal tubule of an absorptive type was characterized by formation of the brush border on the luminal surface and endocytotic apparatus in the apical part of the cell. Basolateral cell specialization for transport into the capillary displayed numerous interdigitating microvilli in the irregularly distended intercellular spaces. Membrane bounded activity of AlP was expressed on the microvilli of both the brush border and the basolateral cell surface. Functional specialization of the epithelium in the distal tubule for ion transport was characterized by the formation of the basolateral labyrinth and mitochondrial

accumulation in the adjacent cytoplasmic compartment. This differentiation of the epithelium was associated by expression of the ATP-ase activity on the basolateral cell membrane.

Structural specialization accompanied by expression of membrane enzyme activities was confirmed at the capillary face of the epithelium in the proximal and distal tubules.

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No interference of the isonicotinoyl hydrazone analogs with pyridoxal phosphate enzymes or antitumour activity of doxorubicin

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Pyridoxal isonicotinoyl hydrazone (PIH) and salicylaldehyde isonicotinoyl hydrazone (SIH) represent novel lipophilic iron chelators to be used in the prevention of anthracycline-induced cardiotoxicity. Structural similarity of the PIH hydrolysis product with pyridoxal phosphate coenzyme (PLP) suggests possible interactions with an impact on either catalytic activity or spectral characteristics of the PLP-dependent enzymes. Decarboxylation of L-Tyr-1-^{14}C by DOPA decarboxylase (EC. 4.1.1.28) was used as a model for catalytic studies. It was found out that the enzyme was inhibited by PIH with IC_{50} 2.8 x 10^{-4} M and the inhibition was not reversed in excess of PLP. Expected therapeutic concentrations in plasma, however, do not reach these levels. Influences of PIH on spectral characteristics of PLP-dependent enzymes were investigated in porcine alanine amino transferase (ALT, EC 2.6.1.2). Characteristic spectra of the enzyme's catalytic centre were not modified by incubation with PIH for period of 1 week at 25°C. The interferences of PIH with PLP-dependent enzymes can be regarded as negligible. As the chelators are intended for use in combination therapy with cytostatic drugs, it is crucial that their antitumour properties are not discriminated by the chelators. Both PIH and SIH exerted their own dose-dependent antiproliferative properties in A549 human lung adenocarcinoma cell line as measured by trypan blue exclusion. Addition of 100 microM of PIH or SIH to 0.03 microM doxorubicin (established IC_{50} for A549 cells) and 1 microM (which caused more than 90% cell death) had no effect on doxorubicin (DOX) antiproliferative effects. It was also shown that the chelators did not hamper DOX-induced oxidative stress in A549 cancer cells. Several markers of oxidative stress and cellular damage were followed for this purpose: TBARS formation, GSH contents and LDH leakage. No significant changes compared to the effects of DOX itself were observed after PIH or SIH pretreatment of the A549 cells. It can be concluded that the chelators do not exert any undesirable effects on either PLP-dependent enzymes or cytotoxic activity of DOX and are promising candidates for prevention of anthracycline cardiotoxicity.

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Expression and translocation of major nucleolar proteins in relation to transcriptional activity of nucleolus

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Changes in the expression and cellular localization of abundant nucleolar proteins: C23/nucleolin, upstream binding factor (UBF), B23/nucleophosmin, and fibrillarin were examined in human lymphocytes subjected to the control of nucleolar activity by phytohemagglutinin and actinomycin D. Data suggest that the up-regulation of ribosomal RNA transcription induced by phytohemagglutinin was accompanied by a significant increase in the nucleolar content of C23/nucleolin, UBF, B23/nucleophosmin, whilst the nucleolar content of fibrillarin had a relatively low-variable. An unraveling of multicopy ribosomal gene accompanying the mitogenic stimulation was detected through the immunofluorescence of UBF permanently associated with rDNA. Down-regulation of RNA polymerase I activity induced by actinomycin D, 24 hrs after initiating stimulation, did not influence the expression of C23/nucleolin, UBF, and fibrillarin and up-regulated the expression of B23/nucleophosmin. This

inhibition resulted in the translocation of chaperons C23/nucleolin and B23/nucleophosmin to the nucleoplasm, while UBF and fibrillarin persisted in the nucleolus. The re-clustering of dispersed transcription units of rDNA, induced by ActD, despite the persistence of UBF in the nucleolus contradicts the hypothesis that neo-synthesis of UBF is the main drive for unraveling a multicopy rDNA gene. The translocations of C23/nucleolin and B23/nucleophosmin are discussed in relation to the cellular stress response caused by a genotoxic activity of actinomycin D. The reannealing activity of nucleolin and nucleophosmin is suggested to help drive the genotoxic agent to the nucleolus and diminish the diversity of genotoxic damage as well as inhibit the growth-division activity of cells.

Reduced afinity of tumor suppressor protein p53 to DNA modified by antitumor platinum drugs correlates with their antitumor efficacy

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The trinuclear platinum agent BBR3464, a representative of a new class of anticancer drugs, is more potent than conventional mononuclear cisplatin [*cis*-diamminedichloroplatinum(II)]. BBR3464 retains significant activity in human tumor cell lines and xenografts that are refractory or poorly responsive to cisplatin, and displays a high activity in human tumor cell lines that are characterized by both wildtype and mutant p53 gene. In contrast, on average, cells with mutant p53 are more resistant to the effect of cisplatin. It has been hypothesized that the sensitivity or resistance of tumor cells to cisplatin might be also associated with cell cycle control and repair processes that involve p53. DNA is a major pharmacological target of platinum compounds and DNA binding activity of the p53 protein is crucial for its tumor suppressor function. This study, using gel-mobility-shift assays, was undertaken to examine the interactions of active and latent p53 protein with DNA fragments and oligodeoxyribonucleotide duplexes modified by BBR3464 in a cell free medium and to compare these results with those describing the interactions of these proteins with DNA modified by cisplatin. The results indicate that structurally different DNA adducts of BBR3464 and cisplatin exhibit a different efficiency to affect the binding affinity of the modified DNA to p53 protein. It has been suggested that different structural perturbations induced in DNA by the adducts of BBR3464 and cisplatin produce a differential response to p53 protein activation and recognition and that a 'molecular approach' to control of downstream effects such as protein recognition and pathways of apoptosis induction may consist in design of structurally unique DNA adducts as cell signals.

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Physical and functional interaction between protein p73 and mutant p53

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The p53 and p73 proteins have a high degree of structural identity especially in DNA binding domain, TP73 gene can be translated to many p73 isoforms with different transactivation and DNA binding ability and protein-protein interaction activity. It was shown that the interaction between the tumour supressor protein p53 and the p73 protein is very weak under physiological conditions but mutated p53 proteins gain the ability to interact with p73. The reduced activity of p73 isoforms due to interaction with mutated p53 proteins can affect the ability of p73 to induce apoptosis in response to genotoxic agents.

In our study we analysed the specificity and affinity of binding of particular mutated p53 proteins (285Lys p53, p53beta, 175Ala p53 (P) and 175Ala p53(R)), p73 isoforms beta and delta and assumed complexes of mut p53/p73 to p53 responsive elements using EMSA (electromobility shift-assay). Proteins were in the form of transfected cells lysates or IVTT (in vitro transcribed/translated) proteins.

It seems, that in conditions of *in vitro* EMSA experiments, there are not any physical interactions between chosen p53 mutated proteins and p73 beta or delta isoforms – we did not observed any complex formation.

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Luminometric determination of antioxidant capacity towards individual reactive oxygen species

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The aim of this work was to optimise chemical systems for production of individual reactive oxygen species (ROS) other than peroxyl radicals with the intention of using them as a new tool for evaluating the antioxidative properties of various materials in laboratory conditions. Chemical systems such as hypoxanthine/xanthine oxidase (HX/XO), hydrogen peroxide itself or hydrogen peroxide/ferrous sulphate were employed to produce superoxide anion radical, hydrogen peroxide or hydroxyl radical, respectively. Different ROS scavengers were used to evaluate a contribution of individual ROS to the chemiluminescence (CL) response of respective ROS-generating systems. Results obtained in the CL measurements were compared with the results obtained from electron spin resonance (ESR) spectroscopy measurements.Luminol (0.8mM) dissolved in a borate buffer, pH 9.0 was used as a luminophor in the CL measurements. Superoxide dismutase (SOD; 16U/cuvette), catalase (CAT; 80U/cuvette) or dimethylthiourea (DMTU; 0.8mM) were used as the superoxide anion radical, hydrogen peroxide or hydroxyl radical scavengers, respectively. Furthermore, by reason of comparing the SOD and CAT activity in their active and inactivated states, they were heat inactivated (30 minutes; 90°C) and used in the hydrogen peroxide system measurements. Desferrioxamine (DFO; 10^{-2}-10^{-4} M) was also used in the hydrogen peroxide system as an iron chelator.

In the HX/XO system SOD, CAT and DMTU deepened the CL signal. In the $FeSO_4$/hydrogen peroxide system, only CAT and DMTU deepened the CL signal. In the hydrogen peroxide system SOD and CAT increased and DMTU deepened the CL signal. In the hydrogen peroxide system with various concentrations of DFO, CAT and SOD both in their active and inactivated states increased the CL signal according to the DFO concentration. The active ROS scavengers deepened the CL signal after the increase, while the inactivated ROS scavengers did not.

ESR measurements were performed only in the $FeSO_4$/hydrogen peroxide system. 5,5-dimethyl-pyrroline-N-oxide (DMPO) was used as a spin trap. According to typical ESR spectra, it was proven that $^{\cdot}OH$ was produced in this chemical system.

It can be seen that none of the systems studied in our experiments generated a single free radical, but rather a complex of different reactive oxygen species. Regardless of the fact that the chemical systems are not clear, they are still different and their use lies in their ability to furnish different information. Nevertheless, the CL methods are useful, fast and sensitive tools for determining the antioxidative capacity of biological materials both in research and clinical laboratories.

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Quantification of cell-free fetal DNA in maternal circulation: A new diagnostic tool

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Multiple studies have demonstrated that cell-free DNA of fetal origin circulates in the blood of pregnant women. Elevated concentrations of this DNA in maternal plasma have been found in various complications of pregnancy including preeclampsia. In our study, the concentrations of fetal DNA in maternal plasma have been measured during all trimesters of pregnancy and compared with clinical data.

Fetal SRY-specific DNA quantification was carried out by real-time PCR method on the ABI PRISM 5700 and 7000 machines using the MGB probe and a couple of primers. Cell-free DNAs isolated from plasma of 200 pregnant women were tested. We followed the changes in the concentrations of free fetal DNA in maternal plasma in pregnancies from the 5th to 42nd gestation week. To determine the proportion of fetal DNA in maternal circulation, we used GAPDH/SRY ratio.

We have found that both SRY concentrations and GAPDH/SRY vary in different ways during pregnancy**.** The SRY values in females with clinically diagnosed preeclampsia were not elevated significantly when compared with the pregnancies of the same gestation age (Wilcoxon test, p= 1.00). No significant elevations of cell-free fetal DNA concentrations in maternal circulation have been found in twin pregnancies, in pregnancies with IUGR and hypertension**.** In fetal sex determination, we achieved 96% sensitivity and 91.1% specificity. It has been reported that concentrations of cell-free fetal DNA are elevated before clinical onset of and during

preeclampsia. We examined the preeclamptic patients at the time of diagnosis, twin pregnancies, pregnancies with IUGR and hypertension. No significant elevations of these values in our patients were detected when compared with the control pregnancies of the same gestational age. It is difficult to interpret the variations in the concentration of cell-free fetal DNA during the same pregnancy and the differences among different physiological pregnancies, because the physiological and pathological factors affecting release of fetal DNA into and clearance from maternal circulation are poor understood. Probably, a complex multifactorial trait results in the final concentration of fetal DNA in maternal plasma.

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Possible mechanisms of regulation of programmed cell death in the human ovary

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The apoptosis running under signs of programmed cell death named atresia folliculi was confirmed in the human ovary. This process afflicts developing follicles as well as corpora lutea during fertile period of course with exception of the dominant follicle in ovulatory cycles. But it is well known that accelerated atresia accompanies primary (PA) and secondary amenorrhea (SA). Development of follicles undergoes hypothalamus-hypophysis-ovary axis hormonal control but the complete pathway is much more complicated. In addition immunological reasons in which ovarian components can be the source of an antigen inducing production of circulating autoantibodies may play non-neglectable role in ovarian disorders. Presented study contributes to elucidation of some mechanisms concerning atresia folliculi using procedures for detection of some cytokines, enzymes, and hormone receptors. Ovarian samples obtained during diagnostic laparoscopy were collected (23 patients, aged 2 – 21 yrs) from 15 patients suffering by primary (PA) and 8 by secondary (SA) amenorrhea. Material was fixed in 4 % paraformaldehyde in PBS and processed for light and electron microscopical evaluation. Simultaneously serum hormonal levels of FSH, LH, estradiol, and progesterone were ascertained. Immunohistochemical procedures for detection of estradiol and progesterone receptors, Epidermal (EGF) and basic Fibroblast Growth and Vascular Endothelial Growth Factors (VEGF), Nitric Oxide Synthase-3 (NOS-3), and cleaved Caspase-3 (c-Casp-3) combined by strepatavidine biotin enhancing system and peroxidase labeling were performed. Proliferating Cell Nuclear Antigen was detected as a marker of cell stimulation.

Hypergonadotropic hypogonadism in PA girls was characterized by decreased gonadal function due to the inability of the gonads to respond to pituitary gonadotropins. It is known that this disorder in females has many causes among which are ovarian dysgenesis and abnormalities of the ovarian receptors for the pituitary gonadotropins. Findings of depletion of follicular apparatus in which primary follicles dominated and stimulated underwent signs of apoptosis were observed. Also serum levels of FSH and LH were significantly increased while estrogen and progesterone levels were considerably variable. In SA patients advanced stages of follicles and/or corpora lutea and albicantia were found. Apoptotic nature of cell death in both groups of patients during degradation of follicles confirmed positivity of c-Casp-3 in all their components. Accelerated apoptosis could be associated also with endogenous synthesis of NO known as a proapoptotic factor which was supported by findings of NOS-3. Expression of estrogen receptors was detected in the cytoplasm of granulosa cells and ooplasm but rarely in nuclear localization. This was lower in comparison with non affected ovary. Similar findings concerned progesterone receptors which were never found in nuclei. As well expression of bFGF was only sparse especially in granulosa cells where is believed as necessary for their further differentiation. On the contrary EGF was not affected significantly. Observed lack of VEGF detection could be associated with altered angiogenetic process needed for course of follicular development and endocrine function during physiological oogenesis.

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Plastids as drug targets

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Plastids were acquired through establishment of an endosymbiotic relationship between a eukaryotic host and a photosynthetic prokaryote – a cyanobacterium. Evolution of an endosymbiont into a plastid has been associated with a loss of host cell function resulting in cell growth and viability being dependent upon a functional plastid. Apart from the plastid's own biosynthesis and photosynthesis, plastids perform a wide range of metabolic functions, from haem, fatty acid and aminoacid biosynthesis, nitrate assimilation, etc. to storage function. A reduced but functional plastid genome is retained in nonphotosynthetic residual plastids of holoparasitic flowering plants, some heterotrophic algae, and in protozoan apicomplexan parasites. Besides this plant-like traits are associated with metabolism of aplastidial trypanosomatid parasites, close relatives to euglenoid algae. The plastid's own machineries, being cyanobacterial in origin, offers conventional targets for antibacterial drugs inhibiting plastid functions. Plastids of the photosynthetic flagellate *Euglena gracilis* are extremely sensitive to various chemical and physical agents. These induce irreversible loss of chloroplasts and depletion of chloroplast DNA (ctDNA). We have demonstrated that practically all inhibitors of bacterial protein- and DNA-synthesis affected chloroplasts in *E. gracilis* inducing transformation of green cells to white ones without any loss of cell viability in a process called bleaching. Identification of bleaching agents has taken on additional significance with the discovery of a residual plastid genome contained within the apicoplast of protozoan parasites of the phylum Apicomplexa. Organisms within this phylum such as *Plasmodium, Toxoplasma* and *Eimeria* are major disease causing agents. Bacterial metabolic pathways in the relict plastid of apicomplexan parasites make this organelle a promising new parasite-specific target for therapeutic agents and for drug development. Bleaching in *Euglena* could provide a rapid screening method for these agents.

Analysis of Scotin isoforms expression in neoplastic transformed cells

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Damage of the proteins involved in apoptotic machinery can evoke neoplastic transformation of the cell. Consequently the understanding of apoptosis has provided the basis for novel targeted therapies that can induce death in cancer cells or sensitise them to established cytotoxic agents and radiation therapy. The best-described component of programmed cell death is p53 protein. P53 is known as a „guardian of the genome" and can act as a transcription factor that, in response to cellular stress, binds DNA in a sequence-specific manner and induces the expression of genes liable for specific physiological functions.

Recent research have identified several genes directly inducible by p53 that encode proteins with apoptotic potential, such as Bax, CD95/Fas, Noxa, Pidd, P53AIP, PUMA and most recently Scotin. Scotin is localized in Endoplasmic Reticulum and is involved in apoptosis induced by DNA damage in a p53-dependent manner. On the basis of the full Scotin DNA sequence we predict generation of approximately 16 transcription variants. We have focused on eight of them (1, 5, 6, 8, 9, 11, 14, 16), most frequently occurring in human breast carcinoma tissues.

We monitored the appearance of these isoforms on two levels. Firstly we treated some carcinoma cell lines, with different p53 status, by cytotoxic agents (actinomycin D, roscovitine, camptothecin, vinblastine, doxorubicin, oxaliplatin and cisplatin). 24 hours after the treatment we analysed levels of mRNA of some predicted scotine isoforms. Subsequently we compared the expression of Scotin isoforms in normal and carcinoma tissues.

The aim of this study is to analyse the role of particular Scotin isoforms in p53-dependent / independent induced apoptosis in neoplastic-transformed cells. Our results emphasise the complexity of regulation of Scotin expression and the role of endoplasmic reticulum in response to stress through several apoptotic pathways.

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***Enterococcus faecium* isolated from bryndza cheese and its genetic variability**

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One hundred and seventy-six isolates of *Enterococcus faecium* were recovered from sheep milk cheese bryndza supplied by five different commercial distributers over 10-month period. All *E. faecium* isolates were identified on the genus and species level by phenotypic methods and biochemical sets, and multiplex PCR with species-specific primers for $ddl_{E.\ faecium}$ and *vanA* genes. The isolates were next evaluated for the presence of plasmids and their relatedness: (i) by plasmid profile analysis after restriction endonuclease *Eco*RI and *Hin*dIII digestion

and (ii) using Enterobacterial Repetitive Intergenic Consensus sequence-based polymerase chain reaction (ERIC-PCR).

We have found that 82 (46.6%) isolates out of 176 ones showed the presence of the plasmids, which were distributed in all studied types of bryndza. In one cluster we have generaly found fingerprints of isolates from the same bryndza distributor and from the same period of sampling, utmost from two periods of sampling from the same distributor. The ERIC-PCR clusters were mostly formed with fingerprints of isolates of the same producer and of the same time of sampling. However, we have also found fingerprints of several isolates grouped in the same cluster coming from various producers.

Our results showed that both methods are suitable for distinguishing of enterococci from bryndza cheese. We have proved that none of *E. faecium* isolates possessed *vanA* gene and that there is a considerable genetic variability among *E. faecium* isolates. To our knowledge, this is a first report about both ERIC sequences and plasmid profiles variability studied in enterococci from bryndza cheese.

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Chromosomal aberrations of peripheral Blood lymphocytes (CAPL) as a method for identification of an occupational genotoxic effect in foundry industry

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Genetic toxicity testintg can identify damages of the DNA and chromosome of cell. These processes could result to the initiation and progression of cancer. The damages are usually measured as chromosome aberrations, using for example method cytogenetic analysis of peripheral blood lymphocytes (CAPL).

In foundry technologies, an application of moulding mixtures based on synthetic resins (furan ones included) has been more and more used worldwide. The furan technology is more ecological from both occupational and the residential environments point of view.

Foundry workers in the furan technology are still exposed to SiO_2 (IARC, Group 1 – carcinogenic for human), furan products (Group 2B – possibly carcinogenic to human), to a very low concentrations of formaldehyde (Group 2A –probably carcinogenic to human) and strong-inorganic-acid mists containing sulfuric acid (Group 1); to polycyclic aromatic hydrocarbons (PAHs) (Group 2A), and sometimes to heavy metals (CrVI, Ni, Cd – Group 1).

This study was intended to specify the relationship among an external dose (measured chemical compounds in personal air), internal dose (biological exposure tests in urine) and genotoxic effect (in peripheral blood) in a group of foundry workers and a control group.

Significant differences were found between the control group and the group of foundry workers in their exposure to hazardous substances in occupational air (dust particle exposure; carcinogenic PAHs exposure). In all these parameters, founders were the group with the significantly higher exposure.

For biomarkers in urine, the significant difference was noted between the control group and founders in levels of 1-OH pyrene (metabolite of benzo/a/pyrene) which corresponds to the confirmed different PAHs exposures. The level of pyromucic (furoic) acid - as a marker of the furfural exposure (however, it is also a part of some food additives) - was higher in the control group but the difference was not statistically confirmed.
Although statistically significant differences between parameters of the control group and founders were in their majority found, the values of all above mentioned substances and biomarkers ranged deep below the hygienic exposure limits for occupational environment.

However, cytogenetic analysis of peripheral blood lymphocytes as the exposure and early biological effect test of genotoxic substances divided the observed groups into two significantly different groups. The control group showed 1.31% AB.C. which corresponds to the value for the non-exposed adult population o the Czech Republic, while founders showed 2.79% AB.C. This value is significantly higher compared with the control group and also with the non-exposed adult population of the CR and gives evidence for the increased exposure to genotoxic substances.

Conclusion.

Even though the foundry plant using furan technology has met the limit values of all parameters monitored due to the health protection from the hygienist´s point of view, CAPL - as a group test of the exposure and early biological effect of genotoxic substances - proved that founders belong to the group of workers exposed to a higher exposure of genotoxic substances.

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Ames test and chemical indentification of genotoxic compounds in complex mixture of Ostravian urban air

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Genetic toxicology testing is able to find substances that can damage DNA and chromosomes of cells. These processes could result to the initiation and progression of cancer. Real complex mixtures from environment include except from measured contaminants a lot of unknown chemical components. The interactions among all components are complicated not only on chemical, but even more on the biological level. There is not enough relevant information to estimate resulting biological (genotoxic) effect. So testing of biological effect is an important moment for environmental safety. The most widely used test to identify mutagenic/ genotoxic effect of chemical or their complex mixtures is Salmonella (Ames) test.

Urban air samples taken in Ostrava were tested in Ames test and concurrently chemical analysis of hygienically relevant carcinogenic and mutagenic compounds was carried out.

Two sites of Ostrava were observed during the period of one year (January – December). The first site was the industrial centre of the city (coking plants, chemical works, automobile traffic, local heating systems). The second one was situated in periphery on the windward side of the town (no dominating sources of contamination) in the distance of 11 km as the crow flies from the centre.

The results of chemical analysis (eight polycyclic aromatic hydrocarbons - PAHs, benzene, arsenic, ozone) and results of Ames test were compared using analysis of variance (ANOVA), correlation and regression analysis.

Genotoxic effects in Ames test were higher in the industrial centre of the town in comparison with periphery but not significantly. The same picture was discovered in the results of chemical identifications of followed up substances. Except from ozone, the industrial centre of the town was more polluted with chemicals but differences were not significant. The only significant difference was found in case of benzene (higher value in the industrial centre of the town).

We were interested if there were any correlations among parts of complex mixture of air for both sampled sites and if there was any dominant part of complex mixture for biological (genotoxic) effect. Significant correlations were confirmed for presence of benzo/a/pyrene, PAHs and arsenic in both sites. No observed carcinogenic or mutagenic substances correlated with biological (genotoxic) effect of air samples in Ames test. It was surprised to find that one exception - correlation between measured levels of benzene and biological effect in Ames test in statistically less polluted site – was confirmed.

Conclusion. Results of the study confirmed that interactions among component parts of complex mixture are complicated and resulting biological effect can't be unequivocally replaced with chemical identification of one, even if hygienically serious, contaminant.

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The influence of biochanin A on the meiotic maturation of pig oocytes

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Phytoestrogens are natural plant substances structurally similar to endogenous estrogens. There is evidence, that they influence the maturation of mammalian oocytes (Jung *et al*., 1993; Sun *et al*., 1998; Van Cauweberge *et* Alexander 2000). Although biochanin A is one of the first phytoestrogens connected with reproductive disorders (Bennetts *et al*., 1946), there are no data about its influence on oocytes.

The aim of our study was to verify biochanin A effects on the meiotic maturation of pig oocytes.

The oocytes were aspirated from ovarian follicles and then selected. Fully grown oocytes with compact cumular cells were cultivated for 24 hours to the MI stage of meiotic maturation in M 199 medium under constant conditions of 39 ºC and 5 % CO_2 in the air. Various amounts of biochanin A (0, 20, 30, 40, 50, 60, 80 µg/ml) were added to the medium.

Biochanin A inhibited pig oocyte maturation before germinal vesicle breakdown (GVBD) in a dose-dependent manner from a concentration 30 µg/ml. The addition of 80 µg/ml of biochanin A already acted toxically.

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The assessment of environmental genotoxic risk and protection of human health

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The aim of our study was to quantify chromosomal aberrations in lymphocytes (CA) of clinical pathology personell in relationship of the occupational exposure to carcinogenic chemicals, especially: benzene (and his homological compounds), ethylbenzene, o.m.p.– xylene and formaldehyde. We report here results obtained with some of these markers. The following presentation includes a summary of preliminary results from our study, is considerable that the biomonitoring of the carcinogenic hazard and evaluation of health risk in the groups of clinical pathologists is topical problem. The results show:
1) Formaldehyde (FA) - is meaningful harmful chemical compounds in occupational environment in the workplaces of pathological departments. FA exposure was in the range 0.645 – 5.156 mg.m^3 (+/-10%).
2) As biomarkers were followed CA. Occupational exposure (especially in relationship to FA concentrations) signicantly increased the percentage of cells with CA (i.e. biomarkers of exposure and an early genotoxic effect) in exposed versus control groups.
3) The method of Risk Assessment using the toxic equivalents (TEF) of U.S. Environmental Protection Agency (for long – term genotoxic effect) and the units of carcinogenic risk for FA (IUR) of WHO in the occupation under observation, enabled to estimate the increase of individual risk (ILCR) to be affected by cancer due to occupational exposure to FA during 10-year exposure. The increase of the ILCR - 1E-04 (i.e. 1.0 diseases per 10,000 workers) was estimated already during 10-year exposure.
4) The immmediatelly intervention of the Hygiene Service on the field of hygienic regime in department of pathology, contributed to a decrease of the risk factors of work practises and working conditions.
The study was supported by the project „Special Monitoring of the Health State of Inhabitants in the Ostrava-Karviná Region in Relation to the Environment“.

Localisation of NO production and NOS activity in cucumber protoplast

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Nitric oxide (NO) participates in many important physiological and pathological processes in all type of organism. Recently a signaling role of NO has been demonstrated in plant germination, growth, stomata movement, maturation and senescence. NO is also involved in plant response to various types of biotic and abiotic stress.

Available research findings on the exact sources and localization of NO production in plant cells and organs are still very limited. We used purified protoplast prepared from fresh fully developed leaves of *Cucumis sativa* as a model system for histochemical studies of the production of reactive nitrogen and oxygen species. Cell permeable probes 4,5-diaminofluorescein diacetate (DAF-2DA), its fluoride derivative (DAF-FM DA) and diaminorhodamine diacetate (DAR-DA) were used for real-time bioimaging of NO production in cucumber protoplasts. Dichloroflurescein diacetate (DCF-DA) was used as specific probe for the detection of peroxonitrite formation. The specifity of individual fluorescent probes for different types of RNS and ROS was determined by use of known NO scavengers and nitric oxide synthase (NOS) inhibitors in combination with superoxide

dismutase and catalase. The histochemical localization of NOS was performed by diaphorase staining with NBT and NADPH.

Hydrogen peroxide was detected by the formation of a brown precipitate with diaminobenzidine in the presence or absence of catalase. The protoplast vitality and mortality were assessed by fluorescein diacetate or methylen blue staining, respectively. To complement histochemical studies, we also determined spectrophotometrically NOS activity by oxyhemoglobin method and nitrate/nitrite content with Griess reagent in fresh leaves and protoplast samples.

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Mutagenicity as a criterion of the efficiency of biodegradation of synthetic dyes

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Three to ten per cent of synthetic dyes mostly from textile industry end as the waste polluting surface water. These compounds represent recalcitrant xenobiotics resistant to natural degradation. Besides chemical and physical processes, ecological friendly biodegradation technologies can be used for their removal from the environment. Microorganisms used for bioremediation differ by their biodegradation potential and types of metabolites produced. In our study, a two-step biodegradation process using activated sludge and a ligninolytic fungus was applied. With some dyes, biodegradation can result in generation of mutagenic products and, therefore, the biodegradation efficiency was evaluated by measuring the mutagenicity at the beginning, in the course and at the end of biodegradation process. The purpose was to find out whether, in the case of selected synthetic dyes, metabolic products with a mutagenic activity were not produced during biodegradation with the activated sludge and the fungus *Irpex lacteus*. Further, applicability of Ames test as a tool for evaluation of the efficiency of biodegradation and various ecological risks was checked. Characteristic features of biodegradation were documented using samples containing the dyes where mutagenicity had been detected with the strains *Salmonella typhimurium* His- TA98, TA100, YG1041 and YG1042. These dyes included the azo dye Reactive Orange 16 (RO16, Lachema, Czech Republic) and the anthraquinone dyes Disperse Blue 3 (DB3, Aldrich, USA) and Remazol Brilliant Blue R (RBBR, Sigma, USA). The experiments showed differences between the individual dyes in persisting mutagenicity after biodegradation with the activated sludge. In the case of DB3 and RO16, the mutagenicity decreased twofold whereas, in the case of RBBR, it decreased to undetectable values. After subsequent biodegradation with the ligninolytic fungus, the mutagenicity of all three dyes disappeared completely. In addition to pure dyes, three samples of dye mixtures used for dyeing of textiles in JITEX Pisek, a.s. were analyzed. Similarly, significant differences in mutagenicity after the first and second biodegradation step were observed with those samples. The results show that mutagenicity tests can be used for monitoring the course of biodegradation. In addition, by comparing the changes in mutation potential, both the biodegradation efficiency of removal of xenobiotics from the environment and reduction of the ecological risk can be established.

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UP-regulation of gamma-glutamyltranspeptidase (GGT) and dipeptidyl peptidase IV (DPP-IV) activity in human brain tumors

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Progression of tumors, including those of the brain, depends on a complex interactions of parenchymal and blood vessel cells. In gliomas, these interactions involve up-regulation of several receptor-ligand molecular systems, especially those related to growth factors as well as some enzymatically active molecules.

In this study we examined catalytic activity of GGT and DPP-IV, the important molecular constituents of the normal brain vasculature and its astroglial envelope, in a pilot group of biopsies of human brain tumors of various type and the WHO grade of malignancy (G- I to IV). Activity of both enzymes was found to remarkably vary both within individual biopsy samples and the G-I to IV scale, being highest in some, but not all, glioblastoma multiforme (G-IV) samples. High activity of GGT was also detected in a few ventricle-appendages-derived tumors. Microscopically, both enzymes were localized primarily in the blood vessels. In G-IV gliobastoma multiforme, high enzyme activity was observed also in the in-between tumor parenchyma, rich in the transformed astrocytes endowed often with hypertrophic and GFAP immune positive fibers. The enzymes are supposed to participate in maintenance of redox homeostasis (GGT) of these tumors, remodeling of their vascular bed and supporting invasive growth of tumor cells (DPP-IV).

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Cytokine expression in chronic tonsilitis and oropharyngeal carcinoma

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Chronic inflammation and malignant transformation are biological processes regulated by various cytokines taking part in the process of cell to cell signaling. Although there have been referred data that inflammatory stimuli and immune responses may contribute to the development of some malignancies, the question of the relationship between especially chronic inflammation and carcinogenesis is not yet fully understood. The aim of this study was to determine the expression of some cytokines with the potential role in both processes and together with the evaluation of cell proliferation and death.

Samples from patients suffering from chronic tonsillitis (n=18) and oropharyngeal carcinoma (n=7) were fixed in 4% paraformaldehyde and processed for light and electron microscopical evaluation and immunohistochemical staining. Immunohistochemical procedures were performed for detection of e-NOS and NOS-3 as sources of NO (pro- and anti apoptotic agent). Ki-67 and PCNA were used as proliferative markers, and cleaved caspase-3 (c-casp-3) as apoptotic one. Basic fibroblast (bFGF), epidermal (EGF), transforming (TGF) and vascular endothelial (VEGF) growth factors were followed (cytokines involved in cell cycle regulation). Estrogen (ER) and progesterone (PR) receptors were estimated. The antibody binding was visualized by Vectastain ABC Elite kit and diaminobenzidine as a chromogen.

Findings in chronic tonsillitis revealed frequent apoptotic cells and simultaneously mitotic activity in lymphoid follicles. Cell proliferation and apoptosis occurred sparsely in the interfollicular zone and surface epithelium. The morphological evaluation of cell proliferation and cell death was confirmed by the immunoreactivity to proliferative Ki-67, PCNA and apoptotic c-casp-3 markers. The expression of eNOS was detected in different levels in blood vessels. The highest staining was found in the endothelial lining of the high endothelial veins which exhibited also VEGF. VEGF was present also in basal layer of epithelium, in individual mononuclear and almost regularly in dendritic cells of germinal centers. Ki-67, PCNA and c- casp-3 positive cells were scattered in the oropharyngeal carcinoma malignant tissue. Those findings were confirmed also by the morphological assessment. The positivity of eNOS was regularly observed in the highly vascularized regions of the tumor and it was localized in endothelial cells of capillaries supplying the tumorous masses while NOS-3 was moreover detected in macrophages as well as in some clumps of epithelial cells. Corresponding findings concerned prevalence of VEGF that was expressed even so in suprabasal layers but especially in papilomatous epithelial projections. TGF localization dominated in malignant samples in the epithelium whereas positive cells in the connective tissue were only scarcely distributed. In contrast, chronic tonsillitis was characterized by higher expression of TGF (similarly as EGF and bFGF) in germinal centers of activated follicles. Interesting findings were associated with steroid receptors. While PR expression was detected only in the cytoplasm of epithelial, endothelial and some connective tissue cells as cytosolic form; ER were confirmed besides the cytoplasm relatively often in the nuclei. This could be associated with their effect in transcription. A palette of cytokines detected in stimulated or malignant tissue confirmed conspicuous pathway of cell to cell signaling.

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Molecules related to CD 95 mediated apoptosis in embryonic molar tooth development

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Embryonic tooth development is based on epithelio-mesenchymal interactions. Apoptotis occurs during all stages as shown in early morphogenesis, amelogenesis, dentinogenesis and during eruption. The restricted distribution pattern suggests important roles of apoptosis in formation of tooth buds of appropriate shape, size and correct position in the jaw. Dental apoptosis has been associated with expression of a number of different molecules, however, activation and transduction mechanisms are not known yet.

In this study, candidate molecules engaged in CD 95 mediated apoptosis were investigated using immunohistochemistry. CD95 (Fas/APO-1) belongs to the TNF receptor (TNFR) family. CD 95 ligand binding followed by CD 95 receptor oligomerization leads to formation of the death inducing signal complex. CD 95 mediated apoptosis is considered as a possible machinery involved in cell elimination during tooth development since its role in embryogenesis has been shown, e. g. in bone formation. CD 95 receptor, corresponding death domain (FADD) and CD 95 ligand were detected and correlated.

Caspase activation together with specific DNA fragmentation are two hallmarks of apoptosis. Caspases are present as proenzymes in cell cytoplasm and become activated in a cascade manner. Caspase 3 has a central position in intracellular apoptosis machinery. Procaspase 3 and active caspase 3 were localized and activation of caspase 3 collocated with TUNEL positive cells. As further criteria to confirm apoptosis in these cells, morphological features were evaluated after hematoxylin – eosin staining (H&E).

Field vole (*Microtus agrestis*) represents a valuable model of odontogenesis, due to taxonomical relationship to the mouse, the same tooth formula but different final molar shape and patterning. Embryonic days 13.5 – 15.5 of molar tooth development were evaluated in serial frontal sections. At this stage, a signalling centre of developing molars – primary enamel knot - forms and this structure is gradually eliminated by apoptosis.

Caspase 3 activation strongly correlated with TUNEL positive areas of developing molar tooth germ.

CD 95 receptor was found at all stages under study. Strong expression of CD 95 molecules was detected particularly at the bud stage, where CD 95 positive cells were around the middle axes of the tooth bud. At the cap stage, CD 95 receptor molecules were expressed in the part of primary enamel knot facing reticullum stelatum and in the stalk. The CD 95 positive areas expanded at early bell stage, however, cervical loops remained negative.

CD 95 ligand molecules correlated strongly with CD 95 positive areas, however, strong expression of CD 95 ligand was found in the tip of the growing tooth germ and protruding cervical loop.

FADD showed positive correlation with CD 95 positive areas, however, was found also in other cells, probably due to sharing this domain among other TNF-β receptors.

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Tooth explant cultures in dental apoptosis research

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Odontogenesis involves complementary cellular events such as cell division, migration and death, which overlap in time and are controlled by epithelio-mesenchymal interactions. Temporospatial distribution of eliminated apoptotic cells during normal tooth development has been described at bud, cap and bell stages of molar formation in serial histological sections. Programmed cell death (apoptosis) seems to have a crucial role in morphogenetical processes involved in final tooth shape, size and position arrangement in the jaw. However, any functional confirmation has been still missing.

Thus, a new methodical approach must be applied to find out more about the role of apoptosis in early odontogenesis. Tooth primordia explant culture systems correspond to 3R laboratory practice and allow manipulation and targeted modification of molecular signalling and pathways expected in dental apoptosis.

Cultures can be manipulated in several ways, such as implantation of beads soaked in purified signalling proteins or separation of epithelium and mesenchyme for recombinations. Cells can also be transplanted into specific regions of the explants and their fates followed. DNA (gene constructs) can be electroporated into specific areas of the epithelium and/or mesenchyme to misexpress genes, inhibit protein function using dominant

negatives or inhibit translation using morpholino antisense oligonucleotides. Moreover, normal tooth development can be achieved when explants are transferred to renal capsules.

Exploitation of tooth explant cultures in dental apoptosis research makes major contributions to further investigations focused on molecular pathways playing role in apoptotic cell elimination during early odontogenesis and also possible targeted modulations with respect to emerging molecular dentistry.

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Endoplasmic reticulum quality control and congenital pathology

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Many human diseases are caused by mutations altering the folding pathway and final conformation of a protein. Many conformational diseases are caused by mutations in secretory proteins and leading to metabolic dysfunctions i.e. diabetes, to development and neurological diseases such as Alzheimer's. The endoplasmic reticulum is a processing place for the maturation, folding, transport and storage of proteins, and is also the most prominent intracellular Ca store. Calreticulin (CRT) is an endoplasmic reticulum resident Ca binding chaperone involved in regulation of intracellular Ca homeostasis and endoplasmic reticulum Ca capacity. Calnexin (CNX) is a type I integral membrane chaperone of the endoplasmic reticulum. CRT and CNX, together with ERp57 (a PDI-like protein with thoredoxin domains which is also resident in the endoplasmic reticulum) constitute the CRT/CNX cycle that is responsible for the folding and quality control of newly-synthesized glycoproteins.

Quality control of the endoplasmic reticulum plays a critical role in protein folding, modification and modification of a secretory pathway. As endoplasmic reticulum chaperones, CRT and CNX have similar substrate specificity and share several common features. Yet, surprisingly, mice bearing a disruption in the CRT gene die from a lesion in cardiac development, develop significant metabolic problems whereas CNX-deficient mice are born alive with, yet not understood, neurological problems. Studies with CRT and CNX gene knockout mice and CRT- and CNX-deficient cell lines indicate that CNX is unable to compensate for the loss of CRT and conversely, CRT cannot compensate for the loss of CNX. CRT or CNX deficiency or reduction in the level of ERp57 protein (ERp57 heterozygote mice) leads to development of metabolic disorders as documented by sever changes serum lipids and carbohydrates composition in these animals (unpublished). These observations indicate that CRT, CNX and ERp57, in addition of being involved in maturation of glycoproteins in the endoplasmic reticulum, perform other distinct functions including affecting energy metabolism.

New trends in clinical cytogenetics

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The main topic of cytogenetics is investigation of structure, function and evolution of chromosomes, their behaviour in germ and somatic cells. Human and medical cytogenetics is involved in studies of changes and irregularities of these processes which can lead to structural and numerical changes of genes and chromosomes in cell nuclei. Hereditary chromosomal syndromes are manifestated by mental retardation, single or multiple congenital malformations, infertility, spontaneous abortions etc. The same structural and numerical chromosomal aberrations can appear as acquired ones in malignant cells and it is hypothesized that structural changes are one of the first events in the genome leading to malignant transformation of the cell. Therefore, cytogenetic examination of tumor cells is becoming one of the important part of basic clinical tests in haematology and oncology.

Advantages of molecular over conventional karyotyping is in its higher resolution, amenability to automatization and quality control procedures, direct mapping of aberrations to the genome sequence and shorter reporting time. However, I do not believe that molecular karyotyping will soon replace the classical methods. Essential for successful introduction of this technology to cytogenetic practice are good knowledge of copy number variations in population, robust protocol, quality criteria which define array experiments and reporting guidelines for correct interpretation of the results obtained by different laboratories.

Plant seeds as a suitable model for study of basic principles of aging

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Aging is in humans frequently connected with dietary habits and other aspects of life style. However, plants do not have these aspects of their life and are aging as well. Consequently "Plants present challenges to general theories of biological aging." as stated by Thomas (2002). The search for universal principles of aging in plant models lasted already more than one hundred years (cf. Murín 2001). Our own research with different cultivars of *Vicia faba L.* in this field is lasting almost twenty years (cf. Murín, Mičieta 2001).

Our first aim was to determine individual variability of seeds as manifested in darkening of their testa (Murín, 1988b). It was found as solid marker of aging process graduating from most viable seeds to the dead ones within nine years of storage as evaluated by their germinating rate, root length, chromosomal aberrations and length of mitotic cycle (Murín, 1988a). Apart of classical evaluation of genetic damage by frequency of chromosomal aberrations, impact of aging to manifestation of SCE (sister-.chromatid exchange) frequency was reported by Mičieta (1993). Connection of this irreversible process with changes in the content of free endogenous cytokinins was not confirmed (Murín et al., 1994). Utilizing method of storage effect (cf. Murín, Mičieta 1997b) artificial aging was arranged succesfully (Murín, Mičieta 1997a). This was first step to possible rejuvenation as according to Osborne et al. (1984) "Seed embryos offer a uniquely attractive test-sample for DNA repair in plants." After treatment with mutagen MMS that caused synergic effect of the aging damage of chromosomes and the viability of the seeds generally, significant recovery was obtained thanks to storage effect (Murín et al. 2000). This effect was confirmed also for practical use of enhancement of growth of industrially stored seeds (Murín et al. 2003). All these obtained data can be used for better maintaining of seeds stored in seed-banks. General conclusions about aging can be also reached by summarizing all obtained data as it is in this report.

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Microsatellite markers for the study of paternity in the meadow pipit (*Anthus pratensis*)

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Microsatellites are the polymorphic regions of DNA. They are inherited genetically strictly according to Mendelian laws, so they are very powerful markers to study extra pair copulations, population genetics, ecology or migration of animals. The search for the new microsatellite loci from a ´new´ species usually requires the cloning and sequencing of their DNA. An alternative way is a cross-species amplification of their genomic DNA with primers, which are known in other species usually from the same class.

We collected blood samples of 116 adult individuals (males and females) caught in the Jeseníky Mountains, Northern Moravia, the Czech republic in three years (1999, 2000 and 2001), in the Krkonoše Mountains in the north of the Czech republic (2001 and 2002), and in the Tydal province in central Norway (1998 and 2000). The DNA from blood in the storage buffer was isolated using the phenol – chloroform method. 34 primer pairs were

used for the PCR amplification; these primer pairs were used to study the microsatellite DNA loci in birds: Lox1, Lox2, Lox3, Lox6, Lox7, Lox8, Ase10, Ase13, Ase16, Ase18, Ase21, Ase25, Ase34, Ase37, Ase48, Pca2, Pca4, Pca5, Pca7, Pca9, HrU1, HrU2, HrU3, HrU4, HrU5, HrU6, HrU7, HrU8, FhU2, FhU3, FhU4, Phtr2, Pocc5 and Mcyμ4. The DNA fragments were run through 6% denaturing polyacrylamide gel. The visualization of the DNA fragments was accomplished using silver treatment. At first we used identical PCR conditions as was described in the case of the original species. Secondly, we tested higher annealing temperatures (max. 62 °C) for primer pairs, which showed an unscorable multi-band pattern and lower annealing temperature (min. 48 °C) for primer pairs, which showed no product in PCR reaction.

Only 4 of 34 tested primer pairs were to successfully work in meadow pipit for paternity studies. The fifth primer pair for the locus Lox8 showed a scorable band pattern but we found a high number of the null alleles and the results of paternity, which were in opposition to the other 4 primers. We tried to design new primers for this locus but with the same effect. Finally we found in total four cross-species primers that could be used to study paternity in meadow pipit: Mcyμ4 (5 alleles, probability of exclusion 0,3028), HrU5 (6 alleles, probability of exclusion 0,1909), Ase18 (10 alleles, probability of exclusion 0,6229) and Ase48 (15 regular alleles + 1 null allele with frequency lower than 3,5%, probability of exclusion 0,3028). The probability of exclusion for combination of these four microsatellite loci was 0,9544.

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To the expression of different growth factors, no-synthases. hβ – defensins, estrogen and progesteron receptors in the mucosa of nasal polyposis

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Polyposis nasi is a relatively common disease associated with disorders in relation of immune response. There are known different factors in its etiopatology, confirmed were coincidence with asthma bronchiale, rhinitis and sinusitis. In histological structure of the polypous mucosa, chronic process predominates with an inflammatory infiltration of mononuclears, eosinofils, plasma and mast cells in gely-like extracellular matrix. The aim of this study was to determine the expression of some cytokines, chemokines and hormone receptors with possible potential role in relation to development of those changes.

Samples from human polypous mucosa (15) were obtained during indicated nasal surgery and processed for light and electron microscopical evaluation. Immunohistochemical procedures for detection of Transforming (TGF), Epidermal (EGF), basic Fibrobalst (bFGF) andVascular Endothelial Growth Factors (VEGF) were performed on cryostate, paraffin and deeponized semithin sections. Nitric Oxide Synthases (NOS-2 and 3) were studied as sources of an important signal molecule (NO) involved in regulation of cell cycle and apoptosis. Estrogen and progesterone receptors were estimated together with PCNA (Anti-Proliferating Cell Nuclear Antigen) as possible markers of proliferative activity in the epithelium as well as in connective tissue of the propria mucosae. Detection of defensins (hβD□2 and 3) was performed immmunohistochemically.

TGF was present in the basal portion of the pseudostratified columnar epithelium which covered majority of polyp's surface but a distinctly higher expression was observed at regions of its metaplastic lesions. Also the lining of glands was characterized by different level of immunostaining. While in excretory ducts the reaction product filled almost the whole cytoplasm of epithelial cells, secretory tubules displayed only its sparse distribution. In comparison with expression of EGF which was regularly evident in epithelial cell cytoplasm the findings of bFGF in fibroblast and other mononuclear cells in the propria mucosae were relatively scarce. On the contrary VEGF was detected not only in the endothelial lining of capillaries and small veins especially but also in the preudostratified columnar epithelium and in fibroblasts of the propria mucosae. In addition there were not only fibroblasts which expressed that important angiogenetic factor but also other mononuclear cells as macrophages and with the highest probability also plasma cells. Interesting findings were those which concerned steroid receptors. While progesterone receptor expression was detected only in the cytoplasm of epithelial, endothelial and some connective tissue cells, estrogen receptors were confirmed almost regularly in the nuclei as well. Presence of nuclear receptors can be associated with a direct stimulation of cells by steroid hormones in that hyperproliferative disease. NOS-2 and 3 were demonstrated in macrophages and endothelial cells and in some epithelial cells of glands. Detection of hβD□2 and hβD 3 in covering as well as glandular epithelium confirmed a secretion of those endogenous antibiotic proteins.

Obtained results confirmed multifactorial role of different cytokines, chemokines and hormones in development of the polyposis nasi. Not even production of endogenous beta defensins, however, could avert a disaster of chronic inflammatory process.

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Comparison of antioxidant properties of uric acid and its catabolic products

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In this study we examine antioxidant properties of purine catabolic products – uric acid, allantoin and allantoic acid. In humans and hominoids, a purine catabolic pathway is arrested at the level of uric acid due to the absence of the enzyme urate oxidase that catalyses conversion of uric acid to allantoin in other animal species. Thus, these species have much higher concentrations of uric acid in body fluids compared to other mammals. Some authors regard it as evolutionary advantage over other mammal species. Uric acid is contained both in intracellular milieu and extracellular fluids and is considered to be an important component of organism's antioxidant defenses. It effectively scavenges harmful reactive oxygen species and acts as chelator of free transition metal ions, decreasing so their redox activity and ability to participate in hydroxyl radical-producing Fenton reaction. While antioxidant properties of uric acid are nowadays well described, little is known about its catabolic products. The aim of this study was to compare uric acid with its catabolic products from the point of their antioxidant properties.

We tested scavenging of peroxyl radical, superoxide anion and hydroxyl radical by uric acid, allantoin and allantoic acid. Peroxyl–radical scavenging capacity was measured luminometrically using TRAP method. Scavenging of hydroxyl radical was measured luminometrically; system Fe^{II+}-EDTA/hydrogen peroxide was used as a source of hydroxyl radicals. Scavenging of superoxide was measured colorimetrically using XTT test. For producing of superoxide, system xanthine/xanthine oxidase system was employed. We found that neither allantoin nor allantoic acid scavenged peroxyl radical or hydroxyl radical. Conversely, uric acid effectively scavenged these reactive oxygen species. Neither uric acid nor allantoic acid significantly scavenged superoxide. Allantoin had significant superoxide-scavenging properties compared to control, but there was not observed statistically significant difference between allantoin and uric acid.

Our results imply that neither allantoin nor allantoic acid scavenge peroxyl radical or hydroxyl radical. Superoxide-scavenging activity of allantoin is weak and does not significantly differ from that of uric acid. These results accord with the hypothesis that in humans and hominoids, the arresting of purine catabolism at the level of uric acid and consequent increase in uric acid concentration was profitable due to improvement of organism's antioxidant defenses.

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Stem cells in flatworms: isolation and cultivation

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Cell renewal in multicellular animals follows three different strategies: proliferation of differentiated cells, mitoses and commitment of stem cells with subsequent differentiation, and propagation of a germ line that is separated from the somatic cell lines. Different taxonomic units use different combinations of these strategies. Whereas mammals apply all three pathways, lower metazoans often rely exclusively on stem cells as the only source for all differentiated cells. Flatworms represent an extreme case, using a totipotent stem cell population that is competent for all tissues. This population supplies cells for growth, physiological and reparative regeneration and even for reproduction, sexual as well as asexual (Peter et al. 2001, 2004). This fact has long been known from free living flatworms ("turbellarians") from the taxon Tricladida that are commonly termed planarians. Their extraordinary regenerative capacities led to the discovery of the "neoblast", a proliferative somatic cell competent for the formation of all cell types in the adult animal (Baguna et. al. 1994). The term stands for a rather heterogeneous collection of cells, with a fraction of actual stem cells. Establishing permanent lines of these would create considerable potential for in vitro and in vivo experiments on differentiation and even pave the way for obtaining transgenic animals without using a germ line.

Several protocols for isolating and cultivating such neoblasts were developed with the aim of getting an insight into their heterogeneity and improving former methods of cultivation (Schürmann and Peter 2001). The purely asexual planarian species *Dugesia tahitiensis* is being used for our current experiments. Mechanical tissue disintegration and centrifugation in different types of Percoll gradients yielded highly enriched neoblast preparations and allowed the definition of four tentative subtypes. Rather pure neoblasts well suited for cultivation were obtained by sequential sieving through meshes. They did not adhere to glass or plastic dishes and retained a spherical form even during long culture periods. They did however adhere on matrix layers formed by differentiated planarian cells. In this case they flattened and a few cells occasionally formed temporary processes. When comparing different conditions for culture, isotonic media with foetal bovine serum and glucose as nutrients proved to be best with respect to long-term viability of cells. Until now only primary cultures could be gained. Mitoses were observed until two days of culture. Rather dense cultures could be kept for 4 weeks, without any signs of differentiation visible by light microscopy. A few cells survived, however, even longer. The oldest cultures are now being kept for 25 weeks, with still some viable cells (calcein-AM/propidium iodide). Cultivated neoblasts seem to undergo rather slow apoptosis before entering necrosis. Even then they are visible for a long time. Autolysis appears to be retarded considerably, with no lytic compartment in this cell type. A soluble fraction enhancing mitosis rates up to tenfold was prepared by homogenizing regenerating planarians. Following partial purification of the active component(s) by ultrafiltration, this fraction is presently being purified further and characterized by electrophoresis. Current efforts centre in a detailed description of the putative stem cells surviving extremely long. In addition we try to find out if the fraction mentioned above (either alone or combined with feeder cells or matrix layers) may drive these neoblasts into cell cycle, resulting in mitoses and expanding cultures.

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An asymmetric approach to the radiosynthesis of both enantiomers of α-[^{11}C]methyldopa α-[^{11}C]methyltyrosine for positron emission tomography and their possible applications in neurology and oncology

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In positron emission tomography (PET) α-methyl amino acids can play a dual role: 1. precursors of non-metabolised neurotransmitters (analogues of serotonin, dopamine, tyramine etc) for the study of neurodegenerative diseases; 2. non-metabolised analogues of proteinogenic amino acids for the study of amino acids uptake into normal and cancer cells. The difference in the uptake rates during a PET scan could visualise cancer cells in a human body. Clinical applications of such amino acids are strongly limited due to their poor availability. For the synthesis of the only enantiomerically pure ^{11}C-labelled α-methyl amino acid, α-[^{11}C]methyltryptophan, an industrial procedure was adopted. All attempts to prepare enantiomerically pure α-[^{11}C]methylated tyrosine failed.

We carried out [^{11}C]methylation of metalocomplex synthons derived from protected DOPA or tyrosine. For [^{11}C]methylation, sodium hydroxide (5 mg of fine dry powder) was sealed in a vial, which was flushed with dry nitrogen before addition of a solution of the complex (10 mg) and $^{11}CH_3I$ in 1,3-dimethylimidazolidin-2-one (DMI, 300 μl). After 10 min at 25ºC, a 9% radiochemical yield (decay corrected) of a mixture of the diastereomeric α-[^{11}C]methylDOPA complexes or a 7% radiochemical yield of a mixture of the diastereomeric α-[^{11}C]methyltyrosine complexes was achieved. Individual diastereomers were successfully separated by preparative HPLC, diluted with excess of water and extracted on C_{18} cartridges. Optimisation of the procedure

followed by hydrolysis of the complexes and purification of the enantiomers of α-[^{11}C]methylDOPA and α-[^{11}C]methyltyrosine is underway.

Organization and dynamics of the cell nucleus: formation and maintenance of nuclear speckles

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The existence of the nuclear skeleton is a subject of intensive debate the issue of which is not yet settled. Importantly in this respect, recent results have indicated [1,2,3] that lamins A/C provide a structural scaffold of nuclear domains termed nuclear speckles. These domains are highly enriched in splicing factors. To further substantiate these findings [4], we characterized nuclear speckles both in normal mouse embryonic fibroblasts (+/+ cells) as well as in cells with knock-outed gene for lamin A/C (-/- cells). If the importance of nuclear lamins for the structure of nuclear speckled is confirmed, such a finding would be a strong argument for the existence of nuclear skeleton.

By means of electron microscopy and immunofluorescence microscopy of splicing factors, we showed that morphology of nuclear speckles was the similar in +/+ and -/- cells. The results of the FRAP analysis of recombinant splicing factors with GFP demonstrated that their association with speckles was highly dynamic and was the same in +/+ and -/- cells. We also showed that the apparent association of lamins A/C with nuclear speckles was rather due to a cross reactivity of the given anti-lamin A/C antibody. Our results thus demonstrate that the formation and maintenance of nuclear speckles is independent of the presence of lamin A/C, and argue against an essential role of lamins A/C in the speckle morphology.

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Enhanced efficacy of photodynamic therapy by Imatinib Mesylate on colon adenocarcinoma cell line CT 26

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Photodynamic therapy (PDT) has been described as a promising modality for the cancer treatment that involves the combination of visible light and a photosensitizer. The light sensitive drug (photosensitiser) is localized preferentially in the cancer tissue, then cytotoxic agents generated upon illumination using an appropriate wavelength trigger a cascade of biochemical responses that inactivate cancer cells. The hypericin was used in this work as a photosensitizing agent.

Imatinib mesylate (Glivec, STI571) is a drug used to treat certain types of cancer and it is a first member of a new class of chemotherapy agents, which acts by inhibiting particular tyrosine kinase enzymes, instead of simply inhibiting rapidly dividing cells.

The combination of photodynamic therapy by hypericin and chemotherapy by imatinib was a principal idea of cancer therapy, which gently efficate the apoptosis of metastatic murine colon carcinoma CT26 cells. The study include the different dose of light wavelength, the concentration range of hypericin and imatinib, the different time range of the hypericin and imatinib activated cells damage. The combination of hypericin 10^{-7} to 10^{-8} M with a PDT light dose 4.4 J/cm^2 and 1μM imatinib showed the best pro-apoptotic effect.

In summary, our results suggest the enhanced efficacy of photodynamic therapy by imatinib on the metastatic colon adenocarcinoma cells CT 26.

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The role of DPOR enzyme in chloroplast differentiation

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Dark-operative protochlorophyllide oxidoreductase (DPOR) is an enzyme involved in chlorophyll biosynthesis, precisely in a step of protochlorophyllide (Pchlide) reduction (Armstrong, 1998). D-ring of Pchlide is reduced in two different ways by two different enzymes. DPOR enables light-independent way, but its presence and activity among phototrophs is not uniform. It has been determined in cyanobacteria, algae, non-vascular plants and some gymnosperms, but never in angiosperms (Fujita and Bauer, 2003).

The other way of Pchlide reduction is light-dependent, by LPOR (light-dependent Pchlide oxidoreductase). This mechanism is ubiquitous for all phototrophs and for angiosperms it is the sole Pchlide reducing mechanism. Therefore dark-grown angiosperms fail to synthetize chlorophyll and they accumulate large amounts of Pchlide (Nomata et al., 2005). Pchlide is bound to ternary complex with LPOR and NADPH and in this way is stored in prolamellar body, the main structure of etioplasts (Sperling et al., 1998). Chloroplast differentiation is closely related to Chl formation and plant greening. Development of functional chloroplasts is not exclusively dependent on light. As plants are able to form chlorophyll via DPOR in dark, they also can develop chloroplasts without presence of light (Kutík, 1998). The recent investigation in this field is focused on mechanisms involved in limitation of DPOR synthesis in some non-angiosperms as well as on biochemical and physiological characterization of basic properties of DPOR and comparative study of DPOR with other enzymes (nitrogenase, COR) leading to understanding of evolutionary events.

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Conifer seedlings grown in dark: Are they prepared for photosynthesis?

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The seedlings of many conifers are not etiolated during germination without light, but become green even in absolute darkness. This ability is due to DPOR enzyme, that enables chlorophyll (Chl) synthesis in dark in many phototrophs except angiosperms (Fujita and Bauer, 2003). The activity of DPOR in conifers depends on stage of ontogeny, mostly is limited to young seedlings and level of greening in dark greatly varies among species. Some of the conifers seems to lost the ability of greening in dark, e.g. larch (Walles and Hudák, 1975, Mariani, et al., 1990). But the relation between Chl accumulation and formation of membrane system of chloroplasts and Chl content sufficent for differentiation of functional chloroplasts even in dark remain to be dicovered.

In this study we have focused on disposition to photosynthesis of selected dark-grown conifer seedlings via pigment analysis and observation of photosynthetic apparatus development.

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Membrane activity of some novel *N*-oxides

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Biological activities of three series of newly synthesized *N*-oxides were studied. Individual components in each of the series differed in their alkyl chain lengths, with each particular series having one or two free radical scavenging groups. To determine their biological efficiencies the influence of nitroxides on *Cucumber sativus* were studied. The parameters studied were potassium efflux, chlorophyll content and inhibition of growth of cucumber hypocotyls. These measurements permitted one to determine the intensity of the interaction between membranes and *N*-oxides which is important from the point of view of their potential application as antioxidants. To act as free radical scavengers they should be incorporated into protected membranes in small enough concentrations so as not to cause serious damages.

It was found that, at the concentrations used, particular series of N-oxides influenced the parameters studied in various ways. Series 1 and 2 had almost no influence on chlorophyll content and potassium efflux, with only a slight exertion on the cucumber hypocotyls growth. This influence was significant in the case of series three making this group more membrane-active.

The results obtained seem to point that lipophilicity of a compound and the number of its free radical scavenging groups are not the most important factors in deciding biological activity. They also show that series 1 and 2 may be used as effective antioxidants without damage to the protected object.

Cell cycle regulation as a response to DNA damage in *Chlamydomodas reinhardtii*

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Chlamydomonas reinhardtii, also known as green yeast, is a photosynthesizing single-celled alga, which is widely used as a model organism for the study of many biological phenomenon, including DNA repair and cell cycle. Several *C. reinhardtii* repair-deficient mutants exist which in response to high doses of UV irradiation divide at least once before dying, suggesting that products of such mutant genes are not necessary directly for DNA repair but they are involved in other cell responses to DNA damage, e.g. cell cycle regulation.

The actual mechanism(s), by which response of *Chlamydomonas* cells to UV irradiation is mediated, remains still unclear. The functionally important response of wild type cells to UV irradiation was complete block of the kinase activity of the CDK-like protein and its recovery some hours later. The UV effect on kinase activities was accompanied by a prolongation of the cell cycle due to a delay in mitoses, protoplast fissions, and daughter cell release. In contrast to the wild type, the cells of mutant strains did not show any substantial change neither in the course of kinase activities, nor during the cell cycle if compared with untreated cells.

Our results suppose that green alga *Chlamydomonas reinhardtii* possesses some type of the control mechanism to arrest the cell cycle progression in response to DNA damage. This "checkpoint" is likely to be activated just at the end of the growth stage, before the cell reproduction. The products of *UVS11, UVSX1* and *UVSX2* genes seem to be a part of the putative signal pathway in cell response to DNA damage. The molecular mechanism of cellular response remains elusive, waiting for our future, more detailed, analysis.

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Dynamics of proteins and protein-ligand interactions

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Conformational dynamics and internal motion of proteins play a crucial role in many biological processes such as protein-protein and protein-ligand interactions, protein folding, and enzymatic reactions. Internal mobility of protein is strongly modulated by their intra – and inter-molecular interactions. Nuclear magnetic resonance (NMR) spectroscopy represents a unique and powerful tool to investigate internal dynamics and motions of biomacromolecules spanning a wide range of time scales, ranging from pico- to milliseconds. The lecture will review the basic principles of NMR approach to extract information about the spatial structure and motional

behavior of biomacromolecules using isotope-assisted spectroscopy. As an example, NMR and molecular dynamics study of internal motion changes in Major Urinary Protein I upon interaction with its natural pheromone will be used to demonstrate the basic principles.

Binding of mouse pheromones to major urinary proteins (MUPs) represents a typical example of interactions between lipocalins and small hydrophobic ligands. Previously, based on the analysis of ^{15}N relaxation data, we observed that the backbone flexibility of MUP-I increased slightly upon pheromone binding, in contrast to the decreased flexibility expected for induced-fit interactions. To shed the light on this unusual observation, we have performed an independent study adopting different methodology. Backbone dynamics of mouse major urinary protein I (MUP-I) was studied by ^{15}N NMR relaxation at multiple temperatures for a complex of MUP-I with its natural pheromonal ligand, 2-sec-4,5-dihydrothiazole, and for the free protein. Graphical analysis of the reduced spectral density values provided an unbiased qualitative picture of the internal motions. Quantitative parameters were obtained using a novel method of simultaneous data fitting at multiple temperatures to several models of different complexity. The relaxation data were complemented by the molecular dynamics simulations. Correlation functions and frequency-dependent order parameters were calculated from the simulated motions of the amide NH vectors. Comparison of the experimental and simulated order parameters and the information about slow conformational exchanges provided a picture of the molecular motions and offered an explanation for the observed difference in the dynamics of the free and bound MUP-I.

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In vitro study of a potential photosensitiser indocyanine green

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The present study investigates the cytoxicity and phototoxicity properties of a potential photosensitiser, indocyanine green (ICG) in vitro.

In the first series of experiments we studied the cytotoxic effect of ICG on HeLa cells after adding ICG to the cells immediately after the seeding at concentrations of ICG ranging from 206 to 272 μM. Cytotoxicity was evaluated 24 hours after the addition of ICG to the cells by means of MTT assay. A statistically significant cytotoxic effect in comparison with the control was found beginning at concentration of 206 μM ($P< 0,001$). In the second series we studied the cytotoxic effect of ICG on HeLa cells when ICG was added to the cells 24 hours after the seeding at concentration of ICG 206 – 242 μM. No cytotoxic effect was detected.

Based on the above results of cytotoxicity tests we chose lower concentrations of ICG for testing ICG phototoxicity. We studied the proliferation curve of HeLa cells under a combined effect of ICG at concentration of 94 and 194 μM and laser irradiation (360 mW, 830 nm) at densities of 0,24 and 60 J/cm^2 at the time intervals of 1 and 24 hours after irradiation. To improve attachment of adherent HeLa cells ICG was added 24 hours after the seeding.

No phototoxic effect was detected in any of the experiments at the time period of 1 hour after irradiation. At a concentration of 94 μM ($P^{24} = 0,028$; $P^{60} < 0,01$) and 194 μM ICG ($P^{24} = 0,038$; $P^{60} = 0,023$) a phototoxic effect can be found at irradiation density of 24 and 60 J/cm^2 after the time period of 24 hours.

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Biological effects of benzo[c]phenanthridine alkaloids

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Quaternary benzo[c]phenanthridine alkaloids (QBA) are group of natural products, which are discussed as potencial cytostatics for cancer treatment. QBA are relatively small group of isoquinoline alkaloids that have phenylalanin as their precursor. They are distributed in a number of plant species of *Papaveraceae, Fumariaceae* and *Rutaceae* families. Sanguinarin and chelerythrin are the two best known QBA and their biological activities have been widely studied (1,2,3). In addition to them about 10 other QBA differing only by substitution on the aromatic rings have been isolated as a minor components. The informations about biological effects of this minor QBA are rare.

In this study the differential cytotoxicity of sanguinarine (SA), chelerythrine (CHE) and their minority derivates sanguirubine (SR), chelirubine (CHR) and macarpine (MA) was assessed *in vitro* by MTT assay. One normal (skin fibroblasts) and 3 tumour cell lines (HeLa; A 431; HL-60) were used as a model object. After 72 hours, MTT assay was performed to evaluate the in vitro cytotoxicity induced by tested alkaloids. Our results show that HL-60 and skin fibroblasts were the most sensitive cell culture lines, while the carcinoma cell lines HeLa and A-431 appeared much more resistant. For apoptosis detection morphology of nuclei of alkaloid treated HL-60 cells after DAPI staining was studied and flow cytometric analysis using Annexin V-FITC and PI was applied. All tested alkaloids appeared to be inductors of apoptosis.

Based on our observations we concluded that alkaloids tested in this study show strong antiproliferative and proapoptotic effect in vitro.

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Are nucleoli useful markers of various cell states?

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The present knowledge on nucleoli, their structural organization and shape might provide very useful information not only on the nucleolar function but also on the cell biology in respect of their proliferative, resting, maturation and dying state at the single cell level. However, such approach requires a very carefully selected methods for visualization of nucleoli and their functional compartments.

Modulation of cytokinetics by intervention in arachidonic acid metabolism during differentiation of leukaemic cell HL-60

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Metabolism of arachidonic acid (AA) plays a significant role in the regulation of many important biological processes. It was demonstrated that its inhibition can delay carcinogenesis and decrease proliferation of various cancer cell populations (1, 2).

The main objective of our study was to describe consequences of modulation of AA metabolism during induced differentiation of leukaemic cells. The changes of cytokinetic parameters and involved signalling pathways were studied. As cellular model for this study the human leukaemic promyelocytic cell line HL-60 cultivated in serum-free conditions for 24 and 72 h were used. Monocytic differentiation was induced by 1α, 25-dihydroxyvitamin D3 (VitD3) itself or by its co-treatment with transforming growth factor-β1 (TGF-β1). The AA metabolism was modulated with specific 5-lipoxygenase (5-LPO) inhibitors AA-861 and MK-886.

Any of the treatment used did not affect the viability of cell population, but slightly decreased the proliferation. Parameters of cell death detected by evaluation of subG_0/G_1 population, expression of caspase-3 and -8 were not significantly influenced. Nevertheless, 5-LPO inhibitors significantly potentiated differentiation

induced by VitD3 (detected by CD14 and CD11b, NBT test). VitD3 and TGF-β1 remarkably increased expression of 5-LPO and this effect was further potentiated by treatment with both 5-LPO inhibitors. AA-861 time dependently enhanced expression of transcription factor c-Jun. The changes of expression level of anti-apoptotic protein Mcl-1 were demonstrated, whereas Bcl-2 expression was not affected.

The increased expression of Mcl-1 and inhibitor of NF-κB activity - IκB correlated with potentiated differentiation. This explains the unchanged expression of Bcl-2, which is the primary target for NF-□B transactivation. Although inhibitors MK-886 and AA-861 did not influenced expression of 5-LPO, they could potentiate the effects of monocytic differentiation inductors. The results showed that the activity of 5-LPO can significantly regulate monocytic differentiation.

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The universal probe library for qPCR

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Universal ProbeLibrary is new technology which redefining and revolutionising real-time qPCR assays. There is no need waiting for prevalidated, specific probes. Specific real-time qPCR assays can be designed in 30 seconds and prices for this type of specific hydrolysis probes are comparable to SYBRGreen I. One standard PCR protocol can be used with dedicated master mix on any real-time PCR instrument.

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Primers and probes for the specific intron spanning assay are designed in the web-based Assay Design Center at *www.universalprobelibrary.com* .

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The unique combination of software and 165 prevalidated probes enables the design of over two million real-time PCR assays. Over one half million of these are intron-spanning assays.

Level of infereron-gamma after melanoma devitalization in the MeLiM animal model

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Numerous cytokines are important as regulators of tumour growth influencing either the immune response to neoplastic cells or directly the tumour cell behaviour. Interferon-gamma (IFN-gamma) plays physiologically important roles in this respect promoting innate and adaptive immunity. It has a major role in immune control by increasing the expression of HLA class I antigens and regulation of the Th1 immune response [1,2]. Recently, a role for IFN-gamma in preventing development of primary and transplanted tumours has been identified [3].

Malignant melanoma is the most serious malignancy of the skin with increasing incidence throughout the world by approximately 5% per year. The MeLiM (*Me*lanoma-bearing *Li*bechov *M*inipig) strain has been established as a suitable animal model of this cancer disease due to histopathological [4] and biochemical [5] similarities with human melanoma. In this study, plasma levels of IFN-gamma were detected in the MeLiM model using sandwich ELISA kit (Biosource, USA). Piglets with progressing multiple skin melanoma were chosen. One of tumours was surgically ischaemized (devitalized) and IFN-gamma was quantified in several intervals thereafter. We found increased levels of IFN-gamma after melanoma devitalization. This observation

suggests activation of Th1 immune response and correlates well with increased tumour lymphocyte infiltration we ascertained after the treatment [6].
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Cellular factors in retrovirus infection

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The restriction factors are positively selected factors that can stop and/or eliminate retroviral infection. Murine leukaemia virus-related proviruses in humans might be the remnants of such elimination. The resistance of human cells to some murine viruses indicates that there is a phylogenetic relationship between the non-permissiveness factors. Both known non-permissivness factors (TRIM 5□, APOBEC3G) developed as a protective mechanism against retroviruses culminating in the control of zoonotic infections. Genes involved in the establishment of non-permissiveness to retroviral infection such as TRIM 5□ or APOBEC3G, in which mutations required for making them resistant to HIV have been introduced, might be employed for gene therapy directed against HIV and other retroviruses.

Stem cells and regenerative medicine

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Cell replacement therapy is a promising treatment for many intractable diseases and its current research opens the new medical field – Regenerative medicine, the aim of which is to restore damaged vital body functions. A stem cell is a special kind of cell that has a unique capacity to renew itself and to give rise to specialized cell types. Potential benefit of the treatment using stem cells can therefore be compared to use of antibiotics. An opportunity to use either adult or embryonic stem cells led us to explore their similarities and differences, but it also raised important ethical and safety issues.

Current neurotransplantation research focuses on the potential of neural grafts to replace damaged cell populations, the production of missing transmitters and neuroactive substances as well as on the delivery of growth factors such as BDNF, GDNF or NGF. The limited regenerative capacity of the adult central nervous system (CNS) remains a major stumbling block in the development of effective therapies for neurodegenerative diseases such as Parkinson's disease, multiple sclerosis, Alzheimer's disease and affective disorders. Neural as well as non-neural stem cell (SC) therapy might overcome the low regenerative capacity in the human CNS. There is, however many ethical issues which must be solved, before we can use human stem cells obtained form embryonic or fetal tissue, and particularly the nuclear transfer (i.e. so-called therapeutic cloning). As long as the beneficial effect is going to be shown, the use of these cells for treatment of patients will be inevitable.

Embryonic stem cells (ESC) and bone marrow stromal cells (MSC) are pluripotent progenitor cells that have the capacity to migrate towards lesions and induce or facilitate site-dependent differentiation in response to environmental signals. In our studies, we examined the behavior the rat fetal cells, mouse ESC and rat or human MSC grafted to injured rat brain and spinal cord. We studied whether these cells are capable of survival and if they participate in lesion repair, differentiate into neurons and astrocytes, prevent scar formation or promote neurogenesis. The fate of mouse ESC and rat or human MSC was further studied in vivo, using cells labeled in culture with magnetic iron-oxide nanoparticles. In vivo MR imaging was used to track their fate; electron microscopy and staining for iron confirmed the presence of nanoparticles inside the cells. The labeled ESC as well as MSC preferentially migrated into the lesion. In the case of MSC, only a few of the cells

differentiated to neurones and glia, while many implanted ESC stained for neuronal and astrocyte markers. Five weeks after cell injection, treated animals showed significant recovery of hind limb sensitivity, an increase of the spare white and grey matter and significant improvement in BBB scores. While the embryonic (and also fetal) SC can replaced damaged neurones, the undifferentiated adult stem cells as MSC, can improved the neurological outcome by the production of regeneration promoting growth factors and cytokines.

Spinal cord injury results in the formation of complex scar tissue that prevents regenerating axons from transversing the lesion and results in paraplegia and tetraplegia. Inducing the axons of the spinal tracts to regenerate requires the removal of the scar tissue and the development of methods that reform the tissue structure to a suitable physicochemical structure. Polymer matrices show neuroinductive and neuroconductive properties and have the potential to repair tissue defects by promoting glial and axonal regrowth. Their chemical and physical properties can be tailored to a specific use, and in the future they may be used in human medicine as 3D carriers for stem cell implantation. Recently, we used polymer hydrogels in which ESC or MSC were grown in vitro and found improved spinal cord regeneration.

It is evident that there may be various ways in which SC may interact with the host CNS tissue. We do not yet know enough about the multipotency of different SC types, about SC fate in the host CNS or how SC are capable of making new functional connections. The current studies already demonstrate the immense potential of SC as a therapeutic tool not only in the treatment of neurological disorders, but also in wide range of other diseases.

ER-to-cell surface signalling pathways in cells differentially expressing calreticulin

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Calreticulin is a Ca^{2+}-binding protein of the SR/ER, from where it acts as a chaperone, affects Calcium homeostasis, gene expression and cell adhesion. Calreticulin is involved in transcriptional control via the calcineurin/GATA-4/NFAT-3 and calmodulin/CaMK II pathways. Cell adhesion to extracellular matrix can generate transmembrane signals important for cell survival and migration. In a variety of cell types integrin stimulation by ECM proteins, such as fibronectin, leads to changes in intracellular protein tyrosine phosphorylation. In fibroblasts, protein tyrosine phosphorylation leads to focal adhesion kinase, vinculin and paxillin co-localizing at the site of cell attachment to the ECM. Focal adhesion kinase is an important signaling molecule that controls multiple cellular processes by regulating the assembly of multiprotein complexes. Interaction between focal adhesion kinase and paxillin is critical for the activation of signaling cascades involved in cell survival and motility. In fibroblasts over- or underexpressing calreticulin the differences in the adhesive properties of the two cell types are related to the calmodulin and CaMKII pathway. The inhibition of the pathway using calmodulin inhibitor, W7, and CaMK II inhibitor, KN-62, caused the weekly adhesive calreticulin underexpressing cells to behave like the calreticulin overexpressers, by increased spreading and adhesiveness on the substrata. Increased levels of focal adhesion kinase, paxillin and fibronectin, as well, phosphorylated focal adhesion kinase and phosphorylated paxillin were observed using western blot analysis and immunohistochemistry. Regarding fibronectin, we propose that calreticulin, *via* its Ca^{2+}-homeostatic effects, may affect fibronectin synthesis and matrix assembly by modulating fibronectin gene expression, and also by influencing formation and/or stability of cellular adhesions, both of which are instrumental in matrix assembly and remodeling. Interestingly, it appears that calreticulin level of expression modulates, besides calmodulin/CaMKII pathway, also c-src activity in a manner that allows cells to be better able to spread on the substratum.

Morphology of perineal lacerations and episiotomy – a light miscoscopic study

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Evidence-based medicine does not support al the maternal benefits traditionally ascribed to routine episiotomy. The range of obstetric perineal injury was suggested to be influenced by concurrent vaginal infection and inflammation of the wall of vagina. The aim of this pilot study was to design a histological protocol for quantitative assessment of rupture proneness of perineum at the time of vaginal birth.

We analyzed five samples of wall of the vagina (10×5×5 mm), taken away from the edge of a rupture which ocurred during the labour, and one control sample taken away from the edge of routine episiotomy. All patients

gave their informed consent. We performed a multistage systematic random sampling at the level of tissue block and at the level of serial sections. We used HE and green trichrome stains to examine the overall morphology of the sample and immunohistochemistry in order to detect CD68+ cells infiltrating the vagina wall.

Two parameters were found to be suitable for quantitative assessment of inflammatory reaction. Numerical density of CD68+ cells (monocytes/macrophages, neutrophils, and large lymphocytes) were estimated by physical disector. Volume of haemorrage were estimated with use of Cavalieri principle.

Stereological quantification of both parameters was designed as unbiased, therefore it required random sampling. The approach presented will become a part of a clinical study in order to test the hypotheses whether vaginal infection diagnosed according to the vaginal culture (1) is related to the severity of obstetric peirineal injury, and (2) is correlated with microscopic morphology of the tissue sample. The results of microscopic study have to be correlated with obstetrician's clinical experience.

This study was supported by the project MSM4977751303.

New approaches in mutagenesis in Czech Republic

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Mutagenic effects of various xenobiotics and their complex mixtures in the environment is a major area of an interest of the Czech and Slovak Environmental Mutagen Society (CSEMS). The society has more than 30 years tradition in organizing and supporting various activities related to the studies of genotoxic, mutagenic and carcinogenic effects of chemical compounds, particles and ionizing radiation. The results achieved by various scientific groups in Czech and Slovak Republics are presented every year in Brno during annual meetings of the society as traditional exchange of experiences and discussion forum about consequences of the findings for human health. Simultaneously, major trends in genetic toxicology and mutagenesis are presented by leading scientists visiting international meetings.

The current research in the area of environmental mutagenesis may be divided in several steps covering different stages of a multi-step process of chemical carcinogenesis. As a first step, the relevant method of external exposure measurement involving stationary and also personal exposure monitoring are required for high quality studies. The next step is the measurement of internal or biological effective dose by the analysis of compounds and/or their metabolites in biological fluids, protein and DNA adducts. Particularly DNA adduct analysis based on the application of advanced scientific technologies such as the ^{32}P-postlabelling assay is of high importance since it informs on the interaction of chemical with target molecules. The biological effect of the exposure may be assessed by cytogenetic analysis, the only validated methods among biomarkers of the effect. It was demonstrated that together with conventional cytogenetic analysis of peripheral lymphocytes having long-term tradition in the hygiene service in the Czech and Slovak Republics, fluorescence *in situ* hybridization (FISH) enabling to detect translocations as a most stable aberrations is of increasing importance for the analysis of clastogenic effects of various exposures. These effects may be modified by factors of individual susceptibility including genetic polymorphisms for xenobiotic metabolizing enzymes, DNA repair and further enzymes, which should be also included in well conducted studies. Finally, epidemiologic studies dealing for example with the outcome of pregnancies, changes of immunity, fertility and morbidity in the areas with increased air pollution by genotoxic pollutants also belongs to the area of CSEMS.

The activities of CSEMS are well recognized by European Environmental Mutagen Society (EEMS) as indicated by the fact that our society organized repeatedly in past EEMS Annual Meetings. The next EEMS Meeting will be held in 2006 again in Praha (http://www.EEMS2006.org).

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Molecular determination of sex and paternity of nestlings in populations of the meadow pipit (*Anthus pratensis*)

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The aims of our study were to determine nestlings sex ratio and frequency of extra-pair paternity (EPP) in several populations of meadow pipit (*Anthus pratensis*) breeding in Tydal province in the Central Norway (1998, 2000), and in the Jeseníky Mountains (1999, 2001) and the Krkonoše Mountains (2001, 2002) in the Czech Republic.

The meadow pipit is a small passerines building the nests on the ground in open habitats of the West Palearctic. Pipit nestlings do not show any feature of external sexual dimorphism. Their sex was determined by using DNA sexing technique. This method is based on finding of the first W-linked avian gene CHD1 (*Griffiths and Tiwari 1995*). This gene has become a universal marker for DNA sexing in birds. It exists in very similar copies (CHD-Z, CHD-W) differing in the length of introns and therefore the results show two DNA fragments in females (ZW), but only single one in males (ZZ). The frequency of EPP was detected by analysing the microsatellite DNA. Microsatellites are short nucleotide sequence motifs that frequently occur as randomly dispersed repetitive elements in avian genome and have become a genetic marker for population analysis in birds (*Ellegren 1992*). The parentage was determined by the comparison of DNA fragments of nestlings, their mother and their putative father

DNA from blood and tissue samples was obtained by the phenol-chloroform extraction. The sex of nestlings was determined by PCR amplification of CHD gene using primers P2 and P8 (*Griffiths et al. 1998*). The avian paternity primers Ase18, Ase48 (*Richardson et al. 2000*), Hru5 (*Primmer et al. 1995*) and Mcyμ4 (*Double et al. 1997*) were used to detect the occurence of EPP. The sex of nestlings and EPP were determined by vizualizing DNA fragments on 6% denaturing polyacrylamide gel after silver staining.

Results indicate that during the study period (1998 – 2002) the average sex ratios in observing populations of meadow pipits were 0,7M:1,0F in the Jeseníky Mountains; 1,5M:1,0F in the Krkonoše Mountains; and 0,9M:1,0F in Norway. Our results also show that during the same period the average frequencies of EPP in these populations were 43,9% in the Jeseníky Mountains; 40,9% in the Krkonoše Mountains; and 18,3% in Norway.

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Prenatal differentiation of the rat intestinal epithelium with respect to programmed cell death

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Morphological differentiation and rebuilding of intestinal epithelium of laboratory rat between 16th and 21st day of prenatal development was studied. During maturation non-differentiated pseudostratified epithelium is transformed into simple epithelium.

Rat embryos were processed for light and electron microscopy; semithin sections stained by toluidin blue and paraffin serial sections stained by hematoxylin-eosin were employed. The evaluation of apoptosis by the activated caspase-3 as the key enzyme of executing phase of apoptosis was proved.

On the 16th day the mucosa of small and large intestine is covered by pseudostratified epithelium and the lumen is narrow, slit-like, often is invisible. The submicroscopic pictures reveal epithelial cells with a plane surface without microvilli and with irregular protrusions of apical membrane. From 17th day the epithelium undergoes an intensive rebuilding with formation of indifferent primitive folds. In the electron microscopy we find in this epithelium many secondary lumina corresponding with intraepithelial vacuoles in semithin sections. Up to the 19th day first mucosal folds appear subsequently rearranging to primitive intestinal villi. In the large intestine from 19th day onward we observed differentiation of secondary folds often with the groups of separated cells on their tips. These folds by their form remind non-differentiated intestinal villi. From 20th day first crypts are differentiating between these folds. In accordance with formation of primitive folds, villi and crypts we observed some signs of the programmed cell death. In early stages (18th day) of rebuilding of small intestine we observed particular cells separated from the epithelium or small groups of cells with narrow apical surface withouth microvilli, cells with disintegrated cytoplasm, especially with the loss of matrix density. In the cells

dilated mitochondria are often found. In some cells deformed nucleus with condensed chromatin and with deposits of apoptotic bodies is found. We observed some exfoliated cells or their component in the intestinal lumen. From 20th day we observed signs of cell death in whole groups of epithelial cells. These groups of cells have a non-damaged apical surface but their cytoplasm was typically vacuolised with dilated mitochondria and perinuclear cisternae. In these groups of cells no signs of apoptotic bodies were observed. With the intensive rebuilding of large intestine we observed in electron microscopy frequent release of cells, small cell groups or fragments of cells from intercellular junctions and their extrusion into the intestinal lumen. The apoptotic bodies are scantly visible.

In connection with observed signs of transformation and programmed cell death the activated caspase-3 was proved as the key enzyme of executing phase of apoptosis. We observed the strong positivity of caspase-3 on the luminal surface of small intestinal epithelium from 17th day. This positivity evidently decreased from 20th day. In the large intestine (colon) positive findings of caspase-3 were detected in small deposits from 18th day of prenatal development. On the basis of these results we could demonstrate a partial participation of apoptosis in programmed cell death during prenatal formation of intestinal mucous membrane.

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From oxidative DNA damage to molecular epidemiology

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Reactive forms of oxygen are released during normal respiration, by the oxidative burst of the macrophages in response to infection, and by a variety of exogenous environmental agents. Oxidative stress, caused by imbalance between the production of reactive forms of oxygen and their elimination, leads to oxidative damage of biomolecules. It is generally accepted, that oxidative stress is involved either as cause or effect in a variety of human degenerative diseases. The antioxidant hypothesis proposes that natural antioxidants in fruits and vegetables scavenge free radicals before they can cause damage. To test the hypothesis a reliable biomarker for *in vivo* assessment of oxidative damage is needed which can be correlated with antioxidant levels on one hand and with disease on the other. Oxidative damage to lymphocyte DNA can be measured by the comet assay or by HPLC. The presentation gives a brief review about the methods and their application both in *in vitro* and in *in vivo* studies dealing with mutagenesis and carcinogenesis. Molecular epidemiological studies in Slovakia concerning different disease states, ageing, role of diet and antioxidant supplementation in prevention are reviewed as well.

SERMs from food and plants - an alternative in hormone replacement therapy?

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Plant derived substances to a differing extent can exhibit phyto-SERM like properties and represent therefore from a pharmacological perspective a highly interesting group of compounds. They even may be useful in the treatment of menopausal complaints or at least represent innovative lead structures for chemicals applicable in menopausal health. However, from the perspective of a molecular endocrinologist a lot more research towards the understanding of the molecular mode of organ-specific function of phytoestrogens is needed before those far ranging conclusions can finally be reached.

Autophagy is preferred pathway of camptothecin-induced programmed cell death of v-*myb*-transformed monoblasts

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A damage of programmed cell death pathways is a frequent event during malignant transformation, and it can significantly modify the response of cancer cell to a therapy. In this study, we analysed programmed cell death in chicken v-*myb*-transformed BM2 and human U937 promonocytes induced by arsenic trioxide, cycloheximide and camptothecin. The presence of functional v-Myb protein pre-determined the BM2 cells to autophagic pathway of programmed cell death in response to the DNA-damaging agents. The absence of transactivating v-Myb protein allowed activation of cell death pathway marked by mitochondrial membrane depolarization and resulting in necrosis. The fact that the antiapoptotic function of the Myb protein can be overcome by the induction of alternative cell death pathway suggests that understanding of molecular mechanisms of autophagy could improve therapy of cancer cells suffering from defective apoptosis.

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Expression of MRP4 and MRP5 genes is related to the PMEDAP resistance in SD lymphoma

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Multidrug resistance is associated with chemotherapy of malignant diseases. Recent studies showed, that two structurally similar members of MRP family, MRP4 and MRP5, are involved in a transport of nucleosides and their structural analogues – potential anticancer drugs.

Drug: 9-[2-(phosphonomethoxy) ethyl]-2,6-diaminopurine (PMEDAP).

Tumour: Spontaneous transplantable T-cell lymphoma of SD/Cub rats.

Protocol of treatment: The group of SPraha-Dawley (SD/Cub) rats was inoculated with 10^6 lymphoma cells and after 3 days PMEDAP (s.c., 5 mg/kg) was administered in 24-hour intervals for 5 consecutive days; afterwards the experimental animals were left untreated for two days. This time schedule lasted three weeks. Four hours after the last PMEDAP dose the animals were autopsied and lymphomas were weighted. Suspension of lymphoma cells (10^6 cells) from PMEDAP treated tumours was than used for next passage into young healthy rats. Remaining lymphoma cells served for RQ-RT-PCR examination of the MRP4 and MRP5 gene expression. The control animals were given with placebo in the same way. To achieve the *in vivo* long lasting drug administration the experiment continued for 4 passages according to the described protocol.

Results: Expression of both MRP4 and MRP5 genes in treated and untreated SD lymphomas were compared to lymphoma weight for every passage. Even though the weight of PMEDAP treated tumours in the 1st passage was significantly decreased compared with untreated lymphomas, expression of the MRP4 and MRP5 genes in tumour cells significantly increased. Continuously treated tumours acquired resistance to PMEDAP treatment in the 2ndpassage; they grew identically as untreated tumours. The increased MRP4 gene expression persisted in the two next passages. On the other hand, the increased expression of the MRP5 gene lasted only in the first and second passage but in the next two passages decreased on the level of the control (untreated) lymphoma cells.

These results show that both MRP4 and MRP5 genes are involved in the induction of resistance PMEDAP treatment in SD lymphoma but most probably in the different mode.

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Phosphorylation of p53 in MOLT-4 leukemia cells after treatment with valproic acid

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Objective: Our work focuses on evaluation of changes of gene expression in human leukemia MOLT-4 cells after treatment with histonedeacetylases inhibitor valproic acid (VA). We are mainly interested in the expression of tumor suppressor protein p53, which can induce cell cycle arrest or apoptosis. The main target for p53-

induced cell cycle arrest seems to be the p21 gene. This induction is promoted by chromatin remodeling, which follows acetylation of histones H3 and H4.

Materials and methods: The influence of VA has been assessed by clonogenic survival assay. Apoptosis has been measured by flow-cytometry. Western blotting has been used for determination of histone acetylation and expression of p53.

Results: VA in low concentrations (2, 4 mM) causes elevated expression of p53 and p53 phosphorylation at serin 392 in a period from 2 to 4 hours of treatment. When the cells were incubated three days with VA, EC50 determined by clonogenic survival assay was 1.76 mM. In the case of continuous exposure (14 days) to VA EC50 decreased to 0.625 mM. The percentage of apoptic cells determined with flow-cytometric analysis (subG1 peak) increased to 37% after 72 hours of treatment with 4 mM VA.

Conclusion: Our results show that VA in low concentrations induces in MOLT-4 leukemia cells expression of p53 in early period of treatment and leads the cells to apoptosis.

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Quartz and Carcinogenicity Risk on the Workplaces of the Quarrying and Stone Processing

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According to the IARC/WHO scientific publication from 1997, the crystalline silica and cristobalite are classified in Group 1, i.e. the substance carcinogenic for humans (especially occurence of pulmonary and other malignancies). The evaluation and assessment of the carcinogenic risk in the occupationally exposed group (in Czech Republic) is medical and legislative problem. Which is significant from the view of health status and socioeconomic conditions for workers of the quarrying and stone processing. From the evaluation of results, it is possible to deduce the following conclusions:

These results suggested, that quartz play an main role as determinant of the genotoxic (carcinogenic) potencial in complex mixture of fibrogenic respirabile airborne particles in ambient air at the workplaces of : Quarrying and stone processing (of the respirabile dust concentration 0,18 –2,16 mg/m^3, quartz-containing in the dust 30%).

The comparision of the chromosomal aberrations levels (%AB.C.) in peripheral lymphocytes of workers professionally exposed to industrial dust (with content of quartz) quarrying and stone processing demonstrates, the existence of differences versus control level of %AB.C in the Czech Republic. The frequency of chromosomal aberrations were significantly higher in exposed groups, than in the control.

The results indicate a significant increasing in the formation of chromosomal aberration (%AB.C.) in the group of workers professionally exposed to dust with content of quartz, in the early period of exposure (about 30% HAE). The results show evidence of high dose non – linearity of chromosomal aberration dose-response.

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Newly emerging viruses – a new threat to mankind?

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Within the last 50 years, several viruses have emerged, which were never heard of before. Some of them are extremely dangerous, killing 50–80% of infected people within 2 weeks. The group of new viruses is heterogeneous, it encompasses families of Filo-, Arena-, Hanta-, Retro-, and Coronaviruses. Most of them are brand new to the civilized world. There also exist old-new viruses which are mutants of previously known ones and still others are just migrants supported by global climatic changes.

Our talk will concentrate on the following questions: Where are they coming from? What diseases are they causing? How many victims they claim? What are their routes of transmission? Can they conceivably exterminate mankind or at least uproot civilization? Can they serve as weapons in the hands of armies or of terrorists? Is any defense being organized?

We will see that the new viruses are indeed a real phenomenon. They have newly emerged mainly as a consequence of human greediness, which is destroying tropical forests. Their successful spreading is stimulated

by poverty, suffering and ignorance. The future is unpredictable. There are certainly still thousands of other viruses in store, which one day can get loose and attack man. Their virulence and efficiency of transmission can escalate. From time to time, they are likely to cause serious problems, mainly if they strike us unprepared.

The circadian clock output pathways in termites

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Termites exhibit some rhythmic activities that are probably regulated by a clock mechanism. Their foraging behavior is controlled by as important environmental inputs the thermo- and photoperiods affect colony activities, especially in the autumn and spring and the circadian rhythm of locomotory and other activities changes with the age of workers.

The circadian clocks of insects include (1) input pathway that transmits environmental signals such as light to the (2) core endogenous oscillator, and (3) one or more output pathways that carry time signals from this oscillator to the peripheral physiological systems. The undecapeptide corazonin and the octapeptide pigment-dispersing hormone (PDH) may be involved in the output pathway.

We used antisera against these neuropeptides for immunohistochemical detection of crazonin-like and PDH-like positive cells in the central nervous system in termites. We examined representatives of all families except Serritermitidae and in case of large families we extended our research to subfamily level. Here is a list of species that were examined and their affiliation to families and subfamilies:

MASTOTERMITIDAE: *Mastotermes darwiniensis* (Froggat)
KALOTERMITIDAE: *Neotermes castaneusss*
TERMOPSIDAE: *Zootermopsis angusticollis*
HODOTERMITIDAE: *Hodotermes mossambicus*
TERMITIDAE: Nasutitermitinae: *Nasutitermes costalis (*Holmgren)
Macrotermitinae: *Macrotermes abonarius* (Hagen)
Macrotermes jeanneli
Odontotermes feae (Wasmann)
Prorhinotermitinae: *Prorhinotermes simplex*
Heterotermitinae: *Reticulitermes flavipes*

Our study showed that the distribution of the corazonin and PDH-positive neurons varies both in number and localization within Isoptera more than in other Polyneoptera at the order level. This diverse distribution suggests flexibility in the evolution of the clock or functional differences in the role of corazonin and PDH.

Ontogeny of reactive nitrogen species production in pig

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Reactive nitric species (RNS) play important role in the defence of organism against potential pathogens. In the present work there was established the ability of porcine neutrophilic granulocytes to produce produce RNS during ontogenetic development.

Peripheral blood from 7 (55th day of gestation) and 5 (92th day of gestation) fetuses and 8 (1, 3, 8, 17, 31, 41 days after birth) postnatal piglets was used in this experiment.

For detection of NO and other RNS production by neutrophils diaminofluorescein diacetate and dihydrodichlorofluorescein diacetate were used (Crow et al., 1997; Kojima et al., 1998). Levels of plasma nitrites/nitrates which originates as a product of degradation of NO were detected using the Griess reagent assay (Sun et al., 2003). Aditionaly measurement of protein tyrosine nitration in neutrophilic granulocytes was provided by immunohistochemistry (Viera et al., 1999).

It was found that ability of neutrophils to RNS species develops during the ontogeny. While spontaneous RNS production is significantly higher in fetuses it decrease during the subsequent early postnatal development. Spontaneous RNS production is strongly influenced by weaning. Fetal neutrophils are not able to effectively

increase the production of RNS after stimulation by PMA. The capability of neutrophils to produce RNS after stimulation by PMA increase during the postnatal development.

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Molecular cytogenetic analyses of malignant brain tumour cells

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The most frequent tumours of the central nervous system include so-called diffuse gliomas. It is a heterogeneous group of tumours with various histological subtypes that differ in their response to treatment and in the prognosis of the disease. This group includes so-called astrocytomas that are further divided into low-grade astrocytoma (WHO grade II), anaplastic astrocytoma (WHO grade III), and glioblastoma multiforme (WHO grade IV). It also includes oligodendroglioma and oligoastrocytoma (WHO grade II–IV). Nowadays the treatment of diffuse gliomas (surgical intervention, actinotherapy, chemotherapy) is very problematic, because the differentiation of glial subtypes is based solely on nuclear and cellular morphology. This evaluation is subjective, and various subtypes cannot be distinguished using specific immunohistochemical markers only. Therefore, new diagnostic and prognostic indicators must be established to enable stratification of the treatment and to help reduce morbidity and mortality of patients. Recently, the subclassification of patients according to findings of specific chromosomal aberrations by molecular-cytogenetic techniques was used for larger cohorts of patients.

For detection of the most frequent chromosomal changes in glial cells (deletion of tumor-suppressor genes p53, p16 and RB1, deletion of 1p36 and/or 19q13.3, amplification of EGFR gene, trisomy 7 and monosomy 10) we used interphase fluorescence in situ hybridization (I-FISH) with locus-specific and/or α-satellite probes (Abbott Vysis™). We examined 27 patients with different types of gliomas (8x low-grade astrocytoma, 5x anaplastic astrocytoma, 7x glioblastoma, 1x low-grade oligodendroglioma, 6x anaplastic oligodendroglioma). The results of I-FISH analyses were correlated with morphological and clinical findings.

Molecular-cytogenetic analyses were successfull in 23 patients. In four cases I-FISH was found uninformative, due to non-adequate tissue specimen. In most patients results of molecular cytogenetic analyses were in agreement with histological and clinical findings. In one patient with original diagnosis low-grade astrocytoma (grade II) we found bialelic deletion of *p16* gene, which is connected with worse prognosis. In one patient with original diagnosis of high-grade astrocytoma (grade III-IV) we proved amplification of *EGFR* gene - typical aberration of primary glioblastoma multiforme (grade IV). In other patient with anaplastic astrocytoma (grade III) we did not proved any structural genetic aberrations (only aneuploidy was found) - this finding is considered as marker of better prognosis. In six patients with anaplastic oligodendroglioma combined deletion of 1p36 and 19q13.3 was found, which could predict good response to chemotherapy for these patients. I-FISH is a powerful tool for surveying chromosomal abberations in tumour cells. A systematic molecular cytogenetic analysis may advance diagnosis, grading and classification of brain tumours, hopefully improve treatment and lower mortality of the patients.

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Role of extra-cellular matrix proteins in determining cell fate

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Proliferation of animal cells is a highly conserved process tightly controlled by the interplay between growth-promoting and growth-limiting signals whose operation results is a timed progression through the cell cycle (1). Signals which limit cell cycle advance are critically important for the control of cell number and the maintenance of tissue homeostasis both through restraints on cell proliferation and through the induction of programmed cell death (2). The molecular machinery that controls cell-cycle progression is based on the sequential activity of a family of protein kinases known as cyclin-dependent kinases or CDKs (3). Considering the importance of CDKs in cellular proliferation, it is not surprising that their activity is exquisitely regulated. One of the key players in this process is the cyclin-dependent kinase inhibitor p21 (4). This blocks progression through the cell cycle by forming ternary complexes with cyclin-dependent kinases, thus inhibiting their enzymatic activity (5).

Extracellular matrix (ECM) proteins, which have important functions in providing structural integrity to tissues (6), have a seminal role in the control of cell cycle progression and cellular growth (7). These functions rely on spatio-temporal expression of adhesive as well as anti-adhesive components of the ECM proteins (8). ECM proteins like fibronectin, collagen and laminin are best characterized, though other types of proteins including mammalian lectins, also function as modulators of cell adhesion. Selectins mediate cell-cell interactions (9), whereas galectins (10), animal lectins that specifically bind β-galactoside residues, were implicated as modulators of cell-matrix interactions. While lacking a signal peptide and found mainly in the cytosol, galectins are externalized by an atypical secretory mechanism (11) to regulate cell growth, cell transformation, embryogenesis, and apoptosis (10).

We have recently shown that cells adhere and spread when cultured on immobilized galectin-8 (12), an ECM protein, which is a member of the galectin family (13-15). Upon secretion, galectin-8 binds to a subset of cell surface integrins (16) and triggers a unique signal transduction pathway (17) that promotes cell adhesion and spreading.

While immobilized galecin-8 functions as an ECM protein and promotes cell adhesion, soluble galectin-8 inhibits cell growth. We therefore set to determine the molecular elements involved in the growth regulation modulated by galctin-8. In this presentation we will provide evidence that soluble galectin-8 inhibits cellular growth by activating both Jun kinase (JNK) and protein kinase B (PKB, also known as Akt) which promote the accumulation of the cyclin-dependent kinase inhibitor p21. Furthermore, when the expression of p21 is inhibited, galectin-8 drives the cells into an apoptotic process. These results implicate soluble galectin-8 as a potential modulator of cell growth through up regulation of genes encoding for inhibitors of cell cycle progression (18).

Our findings combined with our previous studies (15-17) reveals that galectin-8 can act in three different modes, depending on the cellular context and the extracellular environment. When it is immobilized it interacts with high affinity receptors to promote cell adhesion, spreading and cell migration (15,17). When galectin-8 is present as a soluble ligand it interacts with receptors that induce the accumulation of cyclin-dependent kinase inhibitors, represented by p21 that attenuate the rate of DNA synthesis and induce a cytostatic effect. The third, pro-apoptotic mode of action of galectin-8 is exhibited under conditions which prevent the accumulation of p21, or following a sustained deprivation of growth factors. These different modes of action of galectin-8 are mediated by different signalling pathways, with the PI3K and MAPK being the predominant mediators of cell motility induced by immobilized galectin-8; with JNK and p21 being key players in mediating the cytostatic effects, and with JNK and caspases contributing to the pro-apoptotic effects of this lectin. In view of the cytostatic effects of galectin-8, no wonder that a number of tumor cells, such as malignant colon tissues (19), attenuate the expression of this protein. Still, an interesting question is how galectin-8 is beneficial to invasive prostate carcinomas (20) and other tumors (21) that highly express this lectin. Hence, the cellular cues that dictate which mode of action of galectin-8 is being operative under physiological or pathological conditions will be discussed.

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Photomicrography of fast-moving cells

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Attempts to photograph fast-moving cells in the convetional laboratory microscope, some problems can be encountered. The simplest solution is the use of direct sunlight for microscope illumination (Žižka and Žižka Jr. 2001). Another approach involves the use of strong light sources (strong halogen lamps, mercury and xenon discharge lamps, etc. - Brocksch 1994) or special electronic flashes (Hýsek and Žižka 1995, Patterson 2003). An interesting approach is the use of high-sensitivity film (e.g. 800 or 1600 ASA). We here report on a completely new solution of this problem that makes use of a novel microscope illumination (LED diode), and its use in microbiology.

Native specimens from the pond near the Hostivice township were viewed under a Lambda DN 45 - BH 51 laboratory microscope (Lambda Praha, formerly Meopta Praha, Czechoslovakia) equipped with a new LED diode white-light illumination system preserving the Köhler principle (Abbe´s condenser with laterally-extensible aperture iris diaphragm). Images were recorded with Lambda FS - 1 microphotography equipment fitted with Minolta X - 300 S camera or Praktica VLC body (35 mm film Fuji Color Superia 200 or 400).

Bacteria, algae and protozoans were observed under a standard laboratory microscope with the new LED diode illlumination system. Our main work was focused on photographing flagellates and infusorians, which are much faster-moving than bacteria and algae. The internal structures observed in the flagellates including plastids, pyrenoids, vacuoles and granules, the infusorian structures recorded were, e.g. cytostome, food vacuole, granules, etc.

Comparison of our results with the data of other authors and with our previous results shows that when we using standard light field or relief oblique illumination, the power output of the LED diode is quite sufficient and makes it possible to photograph even highly motile microorganisms.

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Autofluorescence of wood-rotting fungus *Fomes fomentarius*

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Autofluorescence (primary fluorescence) of fungi has as yet been studied relatively rarely (e.g. Arcangeli *et al.* 2000, Sedarati *et al.* 2003), most studies having a medical character (e.g. Mann 1983, Elston 2001). We have studied the autofluorescence of the wood-rotting fungus *Fomes fomentarius* parasitizing on its natural host, the birch *Betula pendula.*

The study was performed on fruiting bodies of *F. fomentarius* with parts of the *B. pendula* wood collected in the Krč forest in Praha outskirts. Native specimens were observed in fluorescence microscopes Jenalumar Zeiss and Fluoval 2 Zeiss at a blue excitation (filters: B228g, B226g, B223g and KP490) or a green excitation (filter G241), using appropriate beam-splitters 510 and 570 and recommended barrier filters G247 and R276. The images were recorded by a Zeiss microphotographic device on Agfa Vista 400 ASA or Kodak Max 400 ASA films.

We observed the core of fresh *F. fomentarius* fruiting bodies in fluorescent microscopes. When excited with blue light, they featured thin-walled generative hyphae with septa and clamps, some of which emitted a strong yellow, wellow-green to yellow-red autofluorescence. In addition, we found yellow-red fluorescing thick-walled skeletal hyphae and strongly fluorescent thick-walled pith sets. On using green excitation, intensive red fluorescence was observed not only in all these fruiting body components but also in the rest of the wood material.

As compared with the autofluorescence of other microorganisms shown by, e.g., Arcangeli *et al.* (2000), Elston (2001) or Coombs and Franco (2003), the *F. fomentarius* autofluorescence was many times more intensive, its intensity being comparable with the autofluorescence of lysed hyphae of the fungus *Trametes versicolor* (Sedarati *et al.* 2003).

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