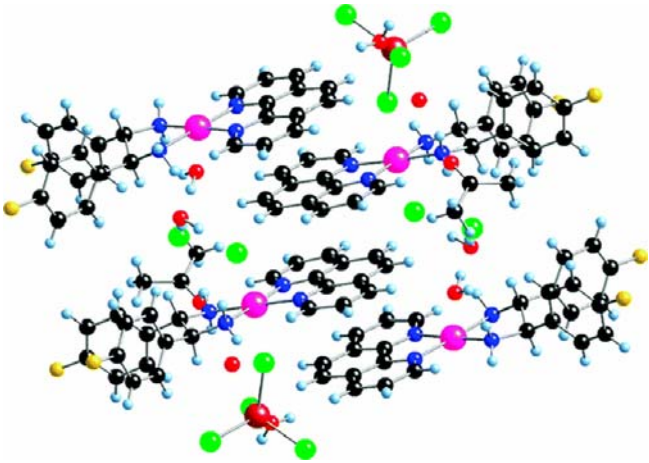
INSTITUTE OF BIOPHYSICS

ACADEMY OF SCIENCES OF THE CZECH REPUBLIC



RESEARCH REPORT 2009

IBP AS CR, BRNO 2009

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Introduction

The year 2009 was unfavourable for the Institute of Biophysics (IBP) Academy of Science of the Czech Republic (AS CR), v.v.i. Last year was critical for several reasons. There is no doubt that the principal cause of negative factors affecting our Institute was the economic depression, which was reflected in the relationships between structures of the society and gave rise to a social crisis, which enhanced existing contradictions and generated new problems. One of the issues intensified by the depression was the reform of research and development, which included not only positive measures but also some contentious issues, such as the evaluation methodology. During the implementation of the reform in the first half of the year under the conditions of growing depression the budget of the AS CR for 2010 – 2012 was drastically reduced. The budget was decreased to half of the year's level. Expectations of budget cuts led to a reassessment of the financial outlook and the possibilities of development in individual institutes of the AS CR. The reassessment resulted in the termination of preparations of a regional project for the Operational Programme Research and Development for Innovations (OP VaVpI) and indirectly in the termination of participation of the IBP in the CEITEC project. The management as well as both Boards of the Institute assessed the risks associated with the preparation and/or implementation of these activities as disproportionately high.

The budget of the AS CR was adjusted after repeated negotiations between the management and the government. The budget of the AS CR was decreased by 10%, which corresponded to cutting the funds in other resorts and thus the reduction was directly transferred to all institutes. The budget cuts for the year 2010 by 10% lead in our Institute to non-standard measures. The measures did not take into account the evaluation since they had to be taken before the evaluation was performed. The measures consisted in the reduction of the workload and some researchers had to be dismissed, which affected nearly all departments. A reduced budget for 2010 was drawn up by the management of the institute, discussed and approved by the IBP Board.

In 2009 the institutional evaluation of individual departments was carried out using the previous year algorithm but its results were applied only to setting the bonuses of department heads and their subordinates from the funds of the Institute. Here it has to be noted that the performance level of

the Institute is surprisingly high and the team performances are very close except for the team of J. Šponer, whose efficiency is the highest. All other teams reached approximately the same value with a standard deviation less than 10% (in the past the differences in performance were many times higher). Using "higher resolution", we would have to list next four departments (those of V. Brabec, A. Kovařík, A. Kozubík and B. Vyskot) then the department of M. Fojta and finally the final department cluster (S. Kozubek, A. Lojek, M. Vorlíčková).

An increase in citation numbers is significant in several departments (A. Kovařík, A. Kozubík, A. Lojek, M. Vorlíčková, B. Vyskot), a high level of citation numbers is maintained in the departments of V. Brabec, M. Fojta and J. Šponer. A growth in cumulative IF is evident in the departments of S. Kozubek, S. Kozubík and A. Lojek. This year the two latter departments reached their record levels of cumulative IF. The department led by A. Kozubík produced most publications (27) for the year 2009, at least eight of which were prepared basically inside this department and seven publications have IF>5. A mention must be made of the joint laboratory of the IBP and Mendel University of Agriculture and Forestry (headed by B. Brzobohatý), where the participation of the laboratory (IBP is involved in three high quality publications) is proportional to IBP's share. Under normal conditions the subsidies for the laboratory would have increased.

The evaluation of departments introduced in 2005 was useful and resulted in a growth of the performance of the Institute as a whole as well as performance of nearly all departments. It was a stabilizing element necessary for the development of the Institute. The Institute management and the IBP Board guaranteed its implementation. This year we had to abandon its application due to external, highly non-standard influences.

Obviously the future of the AS CR will depend, to a certain degree, on the new Research and Development Council of the Government of the Czech Republic (R&D Council). The evaluation methodology developed by the R&D Council, which originally should have been used for allocation of funds for research and development has been and still is criticized not only on the part of the AS CR. In accordance with the decision of Prime Minister and Chairman of the R&D Council, Jan Fischer, the methodology should be newly designed and should include the comments of the AS CR. Besides, AS CR committees and experts from abroad will carry out a comprehensive

evaluation of all institutes in 2010 and in the first half of 2011. Its results, however, will be available only in the second half of 2011. Therefore it will be necessary to carry out our own institutional evaluation in 2010 and in case of considerable disproportions financial resources of individual departments will have to be adjusted according to the evaluation results. When the results of teams will be roughly equal, it will be more appropriate to wait for the results of academic evaluation, which will provide assessment of particular sections (in our case departments) of the Institute. According to the academic evaluation and application of its results in allocating funds among institutes it will be necessary to adapt our own concept of further stimulation of research activities of the Institute or even to conceive a new one.

As mentioned above, further development of the Institute will have to be specified in the near future in compliance with the results of academic evaluation as well as the overall situation in this country. It is characteristic of the AS CR that its primary mission is to conduct basic research, i.e. to develop scientific knowledge at international level. The mission also states that the AS CR also fosters collaborations between applied research and industry, which has been so far interpreted in the manner that it may or may not be realized. Our Institute is nearly exclusively focused on a high-quality basic research and in recent years it has been improving its position in this field. The IBP has been assessed very positively by the Academy Evaluation Committee and it is renowned as one of the best institutes of the AS CR. Based on the graphical outputs from the ASEP publication database, created by the library of the AS CR, the IBP performance has risen from the cumulative IF = 197 in 2005 (similar figures were also reached in 2000-2004) to IF = 445 in 2009 (similar figures were also reached in 2006-2008). Moreover if these figures are proportionally compared with the subsidy provided by the AS CR, then the IBP is the most productive institute of the 5. section of the AS CR (in the sense of cumulative IF). Owing to the fact that high-quality basic research necessitates a long tradition, it would be highly desirable that the IBP continues to exist as a high-quality institute for basic research.

There has been a considerable external pressure to interlink basic research with the application sphere. It is obvious from the measures of the reform which transfers financial funds towards applied and industrial research (e.g. foundation of the Czech Technology Agency, programmes of the Ministry of Industry and Trade). Participation in the Operational programme OP

VaVpI requires a certain degree of collaboration with the application sphere, which would mean that the Institute would not be able to prepare a project for the 2. priority axis. One of the causes of our withdrawal from the CEITEC project was the impossibility to obtain a guarantee of funding from sources outside the state budget. If the future emphasis is shifted to the applied research and cooperation with industry, our Institute will have to include this focus in the concept of its further development. Naturally the quality of basic research will suffer to the detriment of the whole society.

Stanislav Kozubek

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DNA and glutathione interactions in cell-free media of asymmetric platinum(II) complexes cis- and trans-[$PtCl_2$(isopropylamine)(1-methylimidazole)]: relations to their different antitumor effects

The global modification of mammalian and plasmid DNAs by the novel platinum compounds *cis*- [$PtCl_2$(isopropylamine)(1-methylimidazole)] and *trans*- [$PtCl_2$(isopropylamine)(1-methylimidazole)] and the reactivity of these compounds with reduced glutathione (GSH) were investigated in cell-free media using various biochemical and biophysical methods. Earlier cytotoxicity studies had revealed that the replacement of the NH_3 groups in cisplatin by the azole and isopropylamine ligands lowers the activity of cisplatin in both sensitive and resistant cell lines. The results of the present work show that this replacement does not considerably affect the DNA modifications by this drug, recognition of these modifications by HMGB1 protein, their repair, and reactivity of the platinum complex with GSH. These results were interpreted to mean that the reduced activity of this

analog of cisplatin in tumor cell lines is due to factors that do not operate at the level of the target DNA. In contrast, earlier studies had shown that the replacement of the NH_3 groups in the clinically ineffective trans isomer (transplatin) by the azole and isopropylamine ligands results in a radical enhancement of its activity in tumor cell lines. Importantly, this replacement also markedly alters the DNA binding mode of transplatin, which is distinctly different from that of cisplatin, but does not affect reactivity with GSH. Hence, the results of the present work are consistent with the view and support the hypothesis systematically tested by us and others that platinum drugs that bind to DNA in a fundamentally different manner from that of conventional cisplatin may have altered pharmacological properties.

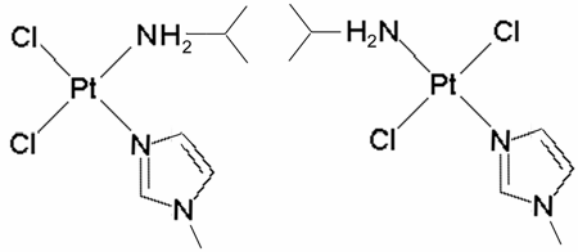

Figure 1: Structures of *cis*-[$PtCl_2$(isopropylamine)(1-methylimidazole)] and *trans*-[$PtCl_2$(isopropylamine)(1-methylimidazole)].

Factors affecting DNA-DNA interstrand cross-links in the antiparallel 3'-3' sense: A comparison with the 5'-5' directional isomer

A study of the unusual 3'–3' 1,4-GG interstrand cross-link (IXL) formation in duplex DNA by a series of polynuclear platinum anticancer complexes has been performed. To examine the effect of possible preassociation through charge and hydrogen-bonding effects the closely related compounds [{*trans*-$PtCl(NH_3)_2$}$_2$(μ-*trans*-$Pt(NH_3)_2$-{NH_2 $(CH_2)_6NH_2$}$_2$)]$^{4+}$ (BBR3464, **1**), [{trans-$PtCl$ $(NH_3)_2$}$_2$(μ-NH_2 $(CH_2)_6$-NH_2)]$^{2+}$ (BBR3005, **2**), [{*trans*-$PtCl(NH_3)_2$}$_2$(μ-H_2N $(CH_2)_3NH_2$ $(CH_2)_4$)]$^{3+}$ (BBR3571, **3**) and [{*trans*-$PtCl$ $(NH_3)_2$}$_2$-{μ-H_2N $(CH_2)_3N$ $(COCF_3)$ $(CH_2)_4$}]$^{2+}$ (BBR3571-$COCF_3$, **4**) were studied. Two different molecular biology approaches were used to investigate the effect of DNA template upon IXL formation

in synthetic 20-base-pair duplexes. In the "hybridisation directed" method the monofunctionally adducted top strands were hybridised with their complementary 5'-end labeled strands; after 24 h the efficiency of interstrand cross-linking in the 5'–5' direction was slightly higher than in the 3'–3' direction. The second method involved "postsynthetic modification" of the intact duplex; significantly less crosslinking was observed, but again a slight preference for the 5'–5' duplex was present. 2D [^{1}H,^{15}N] HSQC NMR spectroscopy studies of the reaction of [^{15}N]-**1** with the sequence 5'-d{TATACATGTATA}$_2$ allowed direct comparison of the stepwise formation of the 3'–3' IXL with the previously studied 5'–5' XL on the analogous sequence 5'- d(ATATGTACATAT)$_2$. Whereas the preassociation and aquation steps were similar, differences were evident at the monofunctional binding step. The reaction did not yield a single distinct 3'–3' 1,4-GG IXL, but numerous crosslinked adducts formed. Similar results were found for the reaction with the dinuclear [15N]-**2**. Molecular dynamics simulations for the 3'–3' IXLs formed by both **1** and **2** showed a highly distorted structure with evident fraying of the end base pairs and considerable widening of the minor groove.

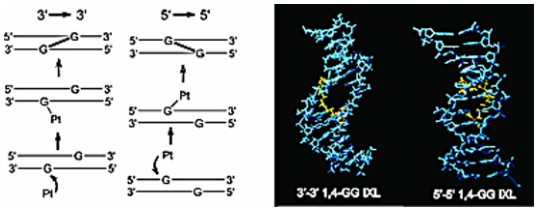

Figure 2: Left panel: A schematic showing the directionality of the two possible DNA–DNA interstrand cross-links (3'–3' and 5'–5') that multinuclear platinum complexes can form. Right panel: Snapshots of the molecular dynamics simulations run on the 3'–3' 1,4-GG and 5'–5' 1,4-GG interstrand cross-links formed by trinuclear platinum complex BBR3464. The 3'–3' 1,4-GG interstrand crosslink is highly distorted with obvious widening of the minor groove and fraying of the base pairs. The 5'–5' 1,4-GG interstrand crosslink is much less distorting with the duplex maintaining its integrity.

Energetics, conformation, and recognition of DNA duplexes modified by methylated analogues of $[PtCl(dien)]^+$

In early studies of empiric structure–activity relationships, monodentate Pt^{II} complexes were considered to be biologically inactive. Examples of such inactive monodentate Pt^{II} compounds are $[PtCl(dien)]^+$ (dien=diethylentriamine) and $[PtCl(NH_3)_3]^+$. DNA is considered the major biological target of platinum compounds. Thus, monodentate DNA binding of Pt^{II} compounds was previously expected to display insignificant biological effects because it was assumed to affect DNA conformation and downstream cellular processes markedly less than the cross-links of bifunctional Pt^{II} complexes. More recently it was shown that some monodentate Pt^{II} complexes do exhibit biological effects; the active monodentate Pt^{II} complexes commonly feature bulkier amine ligands than the hitherto used dien or NH_3 groups. We were therefore interested in determining whether a simple but marked enhancement of the bulkiness of the dien ligand in monodentate $[Pt(NO_3)(dien)]^+$ by multiple methylation of this ligand affects the early phases in which platinum compounds exert their biological activity. More specifically, the goals of this study, performed in cell-free media, were to determine how the modification of DNA duplexes by methylated analogues of $[Pt(NO_3)(dien)]^+$ affects their energetics and how the alterations of this biophysical parameter are reflected by the recognition of these duplexes by DNA polymerases and the DNA repair system. We have found that the impact of the methylation of $[Pt(NO_3)(dien)]^+$ on the biophysical properties of DNA (thermodynamic, thermal, and conformational properties) and its biochemical processes (DNA polymerization and the repair of DNA adducts) is remarkable. Hence, we conclude that monodentate DNA binding of Pt^{II} compounds may considerably affect the biophysical properties of DNA and consequently downstream cellular processes as a result of a large increase in the bulkiness of the nonleaving ligands in this class of metal complex.

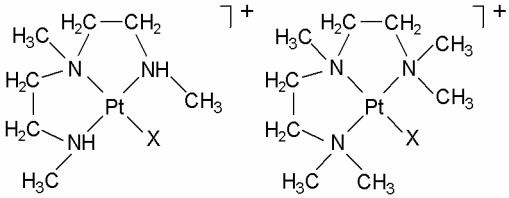

Figure 3: Structures of $[Pt(NO_3)(dien\text{-}Me_3)]^+$ and $[Pt(NO_3)(dien\text{-}Me_5)]^+$.

DNA interactions of dinuclear Ru^{II} arene antitumor complexes in cell-free media

The new dinuclear ruthenium drugs - in which two {(η^6-p-isopropyltoluene)RuCl[3-(oxo-κO)-2-methyl-4-pyridinonato-κO_4]} units were linked by flexible chains of different length [$(CH_2)_n$ (n = 4, 6, 8, 12)] - were found to exert promising cytotoxic effects in human cancer cells. DNA modifications by these new dinuclear Ru^{II} arene compounds, which differed in the length of the linker between the two Ru^{II} centers, were examined by biochemical and biophysical methods. The complexes bind DNA forming intrastrand and interstrand cross-links in one DNA molecule in the absence of proteins. An intriguing aspect of the DNA-binding mode of these dinuclear Ru^{II} compounds is that they can crosslink two DNA duplexes and also proteins to DNA—a feature not observed for other antitumor ruthenium complexes. Thus, the concept for the design of interhelical and DNA–protein cross-linking agents based on dinuclear Ru^{II} arene complexes with sufficiently long linkers between two Ru centers may result in new compounds which exhibit a variety of biological effects and can be also useful in nucleic acids research.

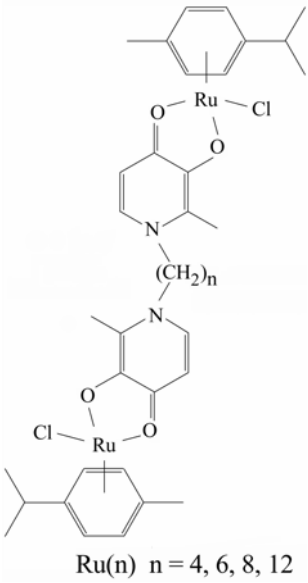

Figure 4: Structures of the dinuclear Ru^{II} arene complexes and their abbreviations.

Mechanistic studies of the modulation of cleavage activity of topoisomerase I by DNA adducts of mono- and bi-functional Pt^{II} complexes

Using electrophoresis and replication mapping, we show that the presence of DNA adducts of bifunctional antitumor cisplatin or monodentate [PtCl(dien)]Cl (dien = diethylenetriamine) in the substrate DNA inhibits eukaryotic topoisomerase 1 (top1) action, the adducts of cisplatin being more effective. The presence of camptothecin in the samples of platinated DNA markedly enhances effects of Pt–DNA adducts on top1 activity. Interestingly, the effects of Pt–DNA adducts on the catalytic activity of top1 in the presence of camptothecin differ depending on the sequence context. A multiple metallation of the short nucleotide sequences on the scissile strand, immediately downstream of the cleavage site impedes the cleavage by top1. On the other hand, DNA cleavage by top1 at some cleavage sites which were not platinated in their close proximity is notably enhanced as a consequence of global platination of DNA. It has been suggested that this enhancement of DNA cleavage by top1 may consist in its inability to bind to other cleavage sites platinated in their close neighborhood; thus, more molecules of top1 may become available for cleavage at the sites where top1 normally cleaves and where platination does not interfere.

Studies of the mechanism of action of platinum(II) complexes with potent cytotoxicity in human cancer cells

The biological activity of 12 platinum(II)-based DNA intercalators of the type $[Pt(I_L)(A_L)]^{2+}$, where I_L is an intercalating ligand (1,10-phenanthroline or a methylated derivative) and A_L is an ancillary ligand (diamino-cyclohexane, diphenylethylenediamine or 1,2-bis(4-fluorophenyl)-1,2-ethylenediamine) was examined. The chiral compounds and the racemic compounds were tested against a panel of human cancer cell lines, with a number of complexes displaying activity significantly greater than that of cisplatin (up to 100-fold increase in activity in the A-427 cell line). The activity of the complexes containing diphenylethylenediamine and 1,2-bis(4-fluorophenyl)-1,2 ethylenediamine was significantly lower compared to the complexes containing diaminocyclohexane. Further in vitro testing, such as DNA unwinding, competition assays, and DNase I footprinting, was conducted on the most active compound [(5,6-dimethyl-1,10-phenanthro-line)(1*S*,2*S*-diaminocyclohexane)platinum(II)]$^{2+}$ and its enantiomer to provide information about the mechanism of action. These complexes

display activity in cisplatin resistant cell lines, have higher cellular uptake than cisplatin, and do not activate caspase-3 as cisplatin does, indicating that these complexes exhibit a different mechanism of action.

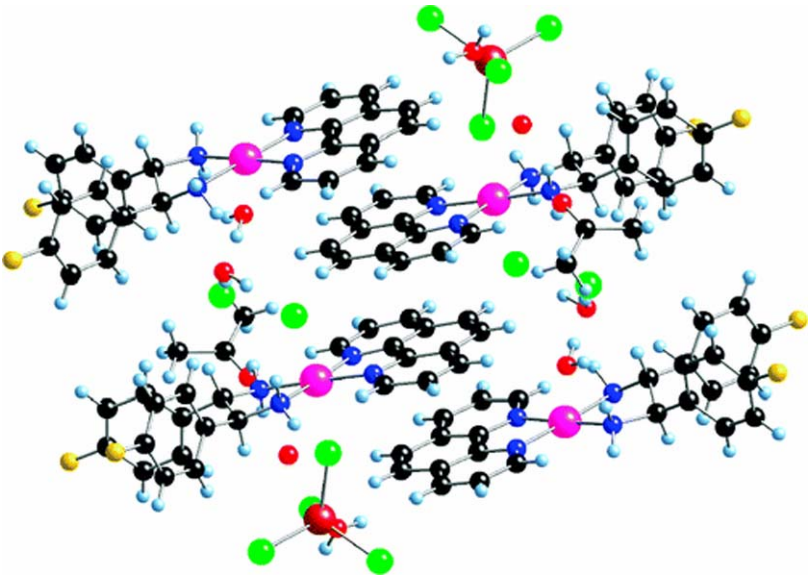

Figure 5: Structures of the platinum(II)-based DNA intercalating complexes.

Oligopyridine–ruthenium(II)–amino acid conjugates: synthesis, characterization, DNA binding properties and interactions with the oligonucleotide duplex d(5-CGCGCG-3)$_2$

The binding properties of diastereomeric oligopyridine–ruthenium(II)–amino acid conjugated complexes of the general formulas Λ- and Δ-$[Ru(bpy)_2(4,4'(CO_2Y)_2\text{-bpy})]^{2+}$, where Y = L-AlaCONH$_2$, L-LysCONH$_2$, L-HisCONH$_2$, L-TyrCONH$_2$ with calf-thymus DNA and the oligonucleotide duplex d(5-CGCGCG-3′)$_2$, were investigated by means of circular dichroism (CD), NMR spectroscopy and DNA thermal denaturation (T_m) curves were studied. CD and T_m data indicate that all diastereomeric complexes bind to the DNA major groove, Δ-diastereomers in a similar

manner, while Λ-diastereomers in dependence of the nature of the amino acid. NMR studies of d(5′CGCGCG-3′)$_2$, and the complexes Δ- **1**, Δ- **2**, Λ- **1** and Λ- **2** indicate that Δ- **1** and Δ- **2** were bound having the ancillary bpy ligands towards the DNA groove, while the corresponding Λ- **1** and Λ- **2** were orientated in a similar way, facing the ligand 4,4′-$(CO_2Y)_2$bpy towards the DNA major groove. Photoinduced DNA cleavage was observed in all cases studied, which take place through singlet oxygen production. Δ- **4** and Λ- **4** show the lower photoinduced cleavage yield, probably because the singlet oxygen ($^1O_2$) oxidizes not only the DNA phosporodiesteric bonds but the tyrosine's phenolic OH bond as well.

Granted projects

GA AS CR 1QS500040581, Metallodrugs, design and mechanism of action. Principal investigator: O. Vrána, 2005 - 2009

HHMI (USA), INTNL 55005613, Platinum and ruthenium compounds. From DNA damage to cancer chemotherapy. Principal investigator: J. Kašpárková, 2006 - 2010

Ministry of Education, Youth and Sports CR, ME, LC06030, Center of Basic Research, Biomolecular Center. Co-principal investigator: V. Brabec, 2006 - 2010

GA AS CR KAN200200651, Nanoparticle and supramolecular systems for targeted transport of therapeutic drugs. Co-principal investigator: V. Brabec, 2006 - 2010

GA AS CR IAA400040803, Mechanistic studies related to targeted cancer chemotherapy with light-activated platinum and ruthenium antitumor agents. Principal investigator: J. Kašpárková, 2008 - 2011

Ministry of Education, Youth and Sports CR, Kontakt, ME08017, Platinum metal complexes as DNA-protein cross-linking agents. Principal investigator: V. Brabec, 2008 - 2010

Ministry of Education, Youth and Sports CR, COST, OC08003, Structure, recognition and processing of DNA damage by antitumor metal-based drugs. Principal investigator: V. Brabec, 2008 - 2011

GA CR 30109/H004, Molecular and structural biology of selected antitumor drugs. From mechanistic studies to chemotherapy of tumors. Principal investigator: V. Brabec, 2009 - 2012

AS CR Program Support to project of international collaboration of AS CR M200040901, Antitumor transition metal-based complexes. From mechanistic studies to cancer chemotherapy. Principal investigator: J. Kašpárková, 2009 - 2013

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Triantafillidi, K., Karidi, K., Malina, J., Garoufis, A.: *Oligopyridine–ruthenium(II)–amino acid conjugates: synthesis, characterization, DNA binding properties and interactions with the oligonucleotide duplex d(5-CGCGCG-3)*$_2$*.* Dalton Trans., 2009, 6403-6415.

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Within the DCBMO, two partially autonomous research groups were involved in specifically oriented research. The group **"Analysis of proteins important in biomedicine"** leaded by Prof. Emil Paleček dealed mainly with peptides and proteins and particularly with their properties at electrically charged surfaces (mostly concentrated in field II, as specified below). The research was oriented toward a new method of electrochemical analysis based on the ability of proteins to catalyze hydrogen evolution at mercury electrodes. Such electrocatalysis is manifested by the so-called peak H, yielded by constant current chronopotentiometric stripping method. Peak H differs from the previously studied electrochemical signals of proteins particularly (i) by its ability to detect proteins down to nanomolar and subnanomolar concentrations and (ii) by its high sensitivity (a) to local and global changes in protein structures and (b) to protein redox states. In 2009 a considerable progress in electrochemical analysis of proteins, and particularly in the studies of changes in the protein structure at electrically charged surfaces was achieved. In addition, the group focused on the electrochemical analysis and chemical modification of polysaccharides.

The group **"Physics and Physical Chemistry of Biopolymers"** leaded by Dr. František Jelen was oriented towards (a) interactions of nucleic acids components with metal ions, such as copper; (b) development of electrochemical methods for microanalysis of nucleic acids components, their metabolites and analogues; (c) application of elimination voltammetry (EVLS) in analysis of nucleic acids. EVLS in connection with the stripping procedure proved useful for both qualitative and quantitative microanalysis of purine derivatives, and can reveal details of studied electrode processes. Activities of the group came mainly under the field I.

Summary of the results:

In 2009 the Department of Biophysical Chemistry and Molecular Oncology pursued research concentrated to three main fields (see below). Despite existence of the above mentioned groups, many results arose from collaboration of scientists through the whole Department.

Field I: Electrochemistry of natural, synthetic and chemically modified nucleic acids and their components, development of electrochemical DNA sensors and their applications in detection of DNA damage, DNA hybridization and in molecular diagnostics

Field II: Properties of peptides, proteins and polysaccharides at electrically charged surfaces, application of electrochemistry in development of novel micromethods for protein and polysaccharide analysis

Field III: Structure and interaction of DNA and proteins in oncological research, especially with respect to the p53-family proteins

Research in the Field I included systematic studies of the behavior of nucleic acids components, their metabolites, metal complexes, synthetic oligo-nucleotides (ODNs), chemically modified or damaged DNAs and their complexes with biologically active compounds at electrodes. The studies were oriented towards novel techniques of electrochemical DNA labeling and development of new bioanalytical and diagnostic approaches applicable in practical biosensing.

Adsorption and two-dimensional condensation of 5-methylcytosine

Purine and pyrimidine derivatives occurring in nucleic acids posses an extraordinary high ability of self-association at the electrode surface and can form there by a two-dimensional (2D) condensation a monomolecular compact film (self-assembled monolayer - SAM). The effects of methyl substituent on the 2D condensation were studied using the 5-methylcytosine molecule which is involved in gene silencing and has a great biological impact. At acid pHs, 5-methylcytosine forms at the mercury electrode a physisorbed self-assembled 2D layer at potentials close to the potential of electrocapillary maximum. From the temperature dependence of the electrode double layer capacitance, the standard Gibbs energy of adsorption ($\Delta G_m \tilde{=} .7$ kJmol^{-1}), lateral interaction coefficient of the Frumkin adsorption isotherm (ac=2.05) and area occupied by one molecule (A=1.31 nm^2) in the 2D layer were determined. Measurements performed on a single-crystal Au(111) surface show that the 2D condensation can take place on other substrates as well.

Improved electrochemical detection of purine nucleobases at mechanically roughened edge-plane pyrolytic graphite electrode

Mechanically grinded edge-plane pyrolytic graphite electrode (g-PGEe) was applied in voltammetric analysis of purine nucleobases, acid-hydrolyzed synthetic oligodeoxynucleotides and a nonhydrolyzed plasmid DNA. Properties of the mechanically grinded electrodes in these analytical

applications were compared with some other carbon electrode types. We show that the electrode surface grinding with 15-μm SiC particles resulted in a remarkable improvement of oxidation signals of purine bases with no addition of copper ions. Addition of the copper ions, causing a strong enhancement of the purine oxidation responses at fine-polished carbon electrodes, had only small effect on the purine signals at the g-PGEe. On the other hand, the g-PGEe appeared less suitable for the ex situ AdTS voltammetric measurements of nonhydrolyzed plasmid DNA, compared to freshly peeled basal plane pyrolytic graphite electrode.

Improved sensitivity and selectivity of uric acid voltammetric sensing with mechanically grinded carbon/graphite electrodes

Determination of uric acid (UA) levels in body fluids is important for diagnostics and prevention of severe metabolic disorders. Electrochemical determination of the UA relies on an oxidation signal measurable at different carbon-based electrodes. Improvement of the UA electrochemical sensing has usually been attained via various modifications of the electrode surfaces. We showed that a strong enhancement of the UA oxidation signal can be reached by a simple mechanical grinding of the surfaces of glassy carbon or edge plane-oriented pyrolytic graphite electrodes with SiC particles of an optimum size 15μm. In contrast to fine polished electrodes (finally with 1-μm particles), the grinded ones exhibited an excellent separation of oxidation signals of ascorbic acid, dopamine (representing most important natural interferents in UA determination), xanthine and hypoxanthine (precursors of UA in purine catabolism), making it possible to detect these substances in a mixture. Enhancement of UA and dopamine (DA) oxidation signals at the grinded electrodes allowed their easy detection at nanomolar levels in up to 104-fold excesses of ascorbic acid. Due to a strong adsorption at the electrode surface, nanomolar concentrations of UA and DA can be determined by ex situ voltammetry. Similarly, strong enhancement of oxidation signals was observed for purine nucleobases, guanine and adenine. The grinded electrodes have been tested in analysis of real clinical samples of human serum or urine. An excellent agreement between electrochemical and routine biochemical determination of UA in the biological samples is demonstrated.

Simultaneous electrochemical monitoring of metabolites related to the xanthine oxidase pathway using a grinded carbon electrode

Using a mechanically grinded pyrolytic graphite electrode in edge orientation, a sensitive electrochemical method was developed for simultaneous determination of uric acid (UA), xanthine (XAN), hypoxanthine (HYP) (products of purine catabolism in human), allopurinol (ALO), and oxypurinol (OXY) (a drug used in treatment of purine catabolism disorders and its metabolite, respectively). It is demonstrated that differential pulse voltammetry in connection with this electrode can serve as a simple and efficient tool for monitoring transformation of purine catabolites (HYP → XAN →UA) catalyzed by xanthine oxidase (XO) as well as inhibition of this pathway by ALO being enzymatically converted to OXY. Our protocol is based on direct electrochemical measurement of oxidation peaks for each of the substances during in vitro reactions in a single detection step by the same electrode system. In addition, we show that the proposed electrochemical technique can be applied to parallel detection of metabolites involved in the XO pathway excreted in urine without any pretreatment of the clinical samples.

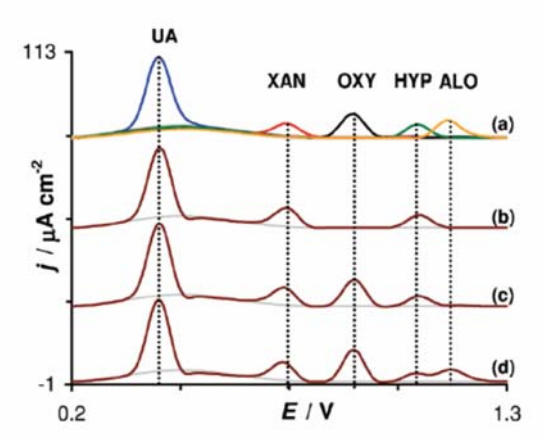

Figure 1: Electrochemical responses of uric acid (UA), xanthine (XAN), hypoxanthine (HYP), allopurinol (ALO) and oxypurinol (OXY) at a grinded graphite electrode (from top to bottom: individual substances and mixtures UA+XAN+HYP, UA+XAN+HYP+OXY, UA+XAN+HYP,+OXY+ALLO).

Voltammetric study of adenine complex with copper on mercury electrode

The determination of adenine (Ade), adenosine (Ado) and hydrolyzed adenosine (h-Ado) in the presence of copper(II) ions is described by cyclic (CV) and elimination voltammetry with linear scan (EVLS) in connection with adsorptive stripping technique. Signals of adenine and copper-adenine complex were measured on a hanging mercury drop electrode (HMDE) in buffered solutions with different pH. The differences in electrochemical behavior of Ade and Ado were found not only in dependence on the presence of copper ions, scan rate, Ade concentration and pH, but also on the accumulation time and potential where a copper-Ade complex is formed. A deeper evaluation of voltammetric responses was carried out by EVLS. The EVLS function E4 eliminating charging and kinetic current components and conserving the diffusion current component was capable of enhancing the current sensitivity of CV peaks and of detecting electron transfer in adsorbed state. The irreversible electrode process of a totally adsorbed electroactive species is indicated by means of a peak-counterpeak signal. Our results show that EVLS in connection with the adsorptive stripping procedure is not only a useful tool for both qualitative and quantitative microanalysis of Ade but also for revealing certain details in electrode processes.

Detection of abasic sites in DNA by electrochemical, immuno-electrochemical and acoustic methods using OsO_4, 2,2 '-bipyridine as a probe for unpaired thymine residues

We report on comparative analysis of the detection of DNA damage modeled by presence of abasic sites (AP) at defined positions using the chemical modification of the DNA by the complex of osmium tetroxide-2,2-bipyridine (Os,bipy) that selectively binds to unpaired thymine residues in the damaged DNA. AP were detected by electrochemical detection (EC) of the Os,bipy-thymine adducts, by immunoelectrochemical (IE) and by thickness shear mode acoustic methods (TSM). EC method of detection can perfectly distinguish between the number of AP. IE and TSM methods were of comparable sensitivity.

End-labeling of peptide nucleic acid with osmium complex. Voltammetry at carbon and mercury electrodes

Peptide nucleic acid (PNA), the DNA mimic with electrically neutral pseudopeptide backbone, is intensively used in biotechnologies and particularly in single-base mismatch detection in DNA hybridization sensors. We propose a simple method of covalent end-labeling of PNA with Os,bipy. Os,bipy-modified PNA (PNA-Os,bipy) produces voltammetric stripping peaks at carbon and mercury electrodes. Peak potential (Ep) of one of the anodic peaks of PNA-Os,bipy at the pyrolytic graphite electrode (PGE) differs from Ep of the reagent, allowing PNA-Os,bipy analysis directly in the reaction mixture. At the hanging mercury electrode (HMDE) the PNA-Os,bipy yields a catalytic peak Catp, in addition to the redox couples. Using Catp it is possible to detect purified PNA–Os,bipy down to 1 pM concentration at accumulation time 60 s. To our knowledge this is the highest sensitivity of the electrochemical detection of PNA.

Electrochemical DNA detection based on the polyhedral boron cluster label

Polyhedral boron clusters are proposed as new, chemically and biologically stable, versatile redox labels for electrochemical DNA hybridization sensors. Selective and sensitive detection of the redox labeled DNA-probe was achieved by means of covalently attached electroactive marker 7,8-dicarba-nido-undekaborate group. A nanomolar concentration of boron cluster-labeled DNA was recognized. High specificity of the analysis with the boron cluster-labeled DNA probe, including detection of single base mismatch, was demonstrated. The above findings, together with proposed earlier use of metallacarboranes as an electrochemical label for biomolecules opens the door for a “multicolor” electrochemical coding of DNA with boron clusters and simultaneous detection of several DNA targets.

Detection of single nucleotide polymorphisms in p53 mutation hotspots and expression of mutant p53 in human cell lines using an enzyme-linked electrochemical assay

An enzyme-linked electrochemical technique for single nucleotide polymorphism (SNP) typing in the p53 tumor suppressor gene is presented. The technique is based on a DNA polymerase-catalyzed extension of

a primer hybridized to a target DNA strand upstream (5'→3') to the SNP site by one nucleotide bearing a biotin tag. Under optimized conditions, efficient incorporation of the biotinylated nucleotide occurs only in the case of complementarity between the first nucleotide in single-stranded 5'-overhang of the target strand. The introduced biotin tag is detected after capture of the primer extension products at magnetic beads bearing oligoT strands via oligoA adaptors at 5'-ends of the primer, binding of streptavidin-alkaline phosphatase conjugate and enzymatic conversion of 1-naphthyl phosphate into 1-naphthol which is determined electrochemically at carbon electrodes. In addition to model studies with synthetic oligonucleotides, we report on detection of mutant p53 expression in human cell lines using reverse transcription-PCR technique combined with amplified primer extension and the magnetic beads-based electrochemical assay.

Tetrathiafulvalene-labelled nucleosides and nucleoside triphosphates: synthesis, electrochemistry and the scope of their polymerase incorporation into DNA

The title 5-substituted pyrimidines (U and C) and 7-substituted 7-deazapurines (7-deazaA and 7-deazaG) bearing tetrathiafulvelene (TTF) attached through an acetylene linker have been prepared by Sonogashira cross-coupling of the corresponding 5- or 7-iodo derivatives of nucleosides with 2-ethynyltetrathiafulvalene. Their subsequent triphosphorylation gave the corresponding nucleoside triphosphates (dNTPs). Square-wave voltammetry of the TTF-labeled nucleosides and nucleotides showed two peaks, one at 0.2–0.3 V and the other at around 0.65 V (vs. Ag|AgCl|3 M KCl), which correspond to two reversible one-electron redox.

Base-modified DNA labeled by [Ru(bpy)(3)](2+) and [Os(bpy)(3)](2+) complexes: construction by polymerase incorporation of modified nucleoside triphosphates, electrochemical and luminescent properties, and applications

Modified 2'-deoxynucleoside triphosphates (dNTPs) bearing $[Ru(bpy)_3]^{2+}$ and $[Os(bpy)_3]^{2+}$ complexes attached via an acetylene linker to the 5-position of pyrimidines (C and U) or to the 7-position of 7-deazapurines (7-deaza-A and 7-deaza-G) have been prepared in one step by aqueous crosscouplings of halogenated dNTPs with the corresponding terminal acetylenes. Polymerase incorporation by primer extension using Vent (exo-)

or Pwo polymerases gave DNA labeled in specific positions with Ru^{2+} or Os^{2+} complexes. Square-wave voltammetry could be efficiently used to detect these labeled nucleic acids by reversible oxidations of $Ru^{2+/3+}$ or $Os^{2+/3+}$. The redox potentials of the Ru^{2+} complexes (1.1–1.25 V) are very close to that of G oxidation (1.1 V), while the potentials of Os^{2+} complexes (0.75 V) are sufficiently different to enable their independent detection. On the other hand, Ru^{2+}-labeled DNA can be independently analyzed by luminescence. In combination with previously reported dNTPs bearing ferrocene, aminophenyl, and nitrophenyl tags, the Os-labeled dATP has been successfully used for "multicolor" redox labeling of DNA and for DNA minisequencing.

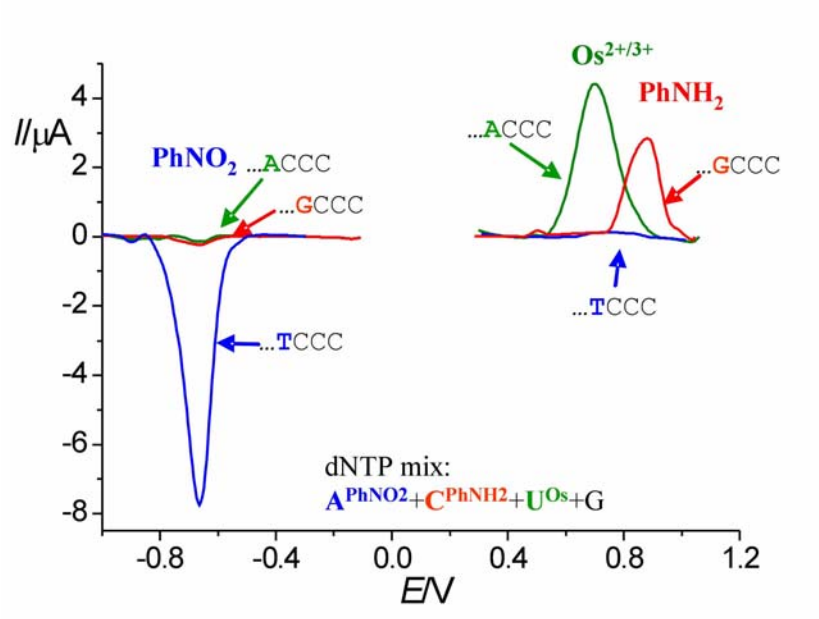

Figure 2: Electrochemical DNA minisequencing using nucleotides labeled with nitrophenyl (PhNO2), aminophenyl (PhNH2) and $[Os(bpy)_3]^{2+}$ (Os).

Ex situ voltammetry and chronopotentiometry of doxorubicin at a pyrolytic graphite electrode: redox and catalytic properties and analytical applications

Ex situ (adsorptive transfer stripping) electrochemical techniques in connection with basal-plane PGE have been applied to the study of redox and catalytic properties of doxorubicin (DOX). Cyclic and square-wave voltammetry and constant current chronopotentiometric stripping (CPS) analysis were used to follow reversible reduction of DOX quinone moiety around -0.5 V and its coupling to catalytic oxygen reduction. CPS was for the first time used for sensitive ex situ determination of the DOX using the catalytic signal around -0.5 V in the presence of oxygen, allowing detection of femtomole amounts of DOX. We show that specific interaction of DOX with double-stranded DNA can easily be monitored using the catalytic CPS signal.

Indicator-based and indicator-free magnetic assays connected with disposable electrochemical nucleic acid sensor system

An indicator-based and indicator-free magnetic assays connected with a disposable pencil graphite electrode (PGE) were successfully developed, and also compared for the electrochemical detection of DNA hybridization. The oxidation signals of echinomycin (ECHI) and electroactive DNA bases, guanine and adenine, respectively were monitored in the presence of DNA hybridization by using differential pulse voltammetry (DPV) technique. The biotinylated probe was immobilized onto the magnetic beads (magnetic particles, microspheres) and hybridization with its complementary target at the surface of particles within the medium was exhibited successfully using electrochemical sensor system. For the selectivity studies, the results represent that both indicator-based and indicator-free magnetic assays provide a better discrimination for DNA hybridization compared to duplex with one-base or more mismatches. The detection limits (S/N = 3) of the magnetic assays based on indicator or indicator-free systems were found in nM concentration level of target using disposable sensor technology with good reproducibility. The characterization and advantages of both proposed magnetic assays connected with a disposable electrochemical sensor are also discussed and compared with those methods previously reported in the literature.

In the Field II the work included basic studies of electrochemical behavior of peptides and proteins. Efficient, highly sensitive electrochemical techniques suitable for monitoring protein denaturation and determination of redox state, interactions of apoproteins with their cofactors. Potential-dependent changes in the structure of a protein adsorbed at mercury surface were observed for the first time. Osmium tetroxide complexes were introduced as new tools for electrochemical analysis of polysaccharides and new procedures for electrode pretreatment were applied.

Electrochemical determination of thioredoxin redox states

Thioredoxin (TRX) is a general protein disulfide reductase with a large number of biological functions, including its roles in human diseases. The TRX redox mechanism is based on reversible oxidation of two cysteine thiol groups to a disulfide, accompanied by the transfer of two protons. Using constant-current chronopotentiometric stripping analysis (CPSA) and the electrocatalytic TRX peak H, we have determined redox states of TRX at submicromolar TRX concentrations. A concentration of 1 nM TRX produces a well-developed peak H at moderate accumulation time without stirring. On the basis of this peak, interactions of 4-hydroxy-2-nonenal (HNE, product of lipid peroxidation) with TRX and the formation of TRX-HNE adducts were studied. CPSA of TRX at a carbon electrode is less sensitive and does not discriminate between reduced and oxidized forms of TRX.

Ionic strength-dependent structural transition of proteins at electrode surfaces

Using constant current chronopotentiometry we showed that in 50 mM sodium phosphate (pH 7) bovine serum albumin and some other proteins were not significantly denatured at a bare mercury electrode while at higher phosphate concentrations they underwent electric field-driven denaturation on the electrode surface.

Voltammetry of Os(VI)-modified polysaccharides at carbon electrodes

We show that polysaccharides (PSs, such as dextran and mannan) can be chemically modified by Os(VI) complexes, yielding electroactive adducts. Os(VI) complexes with different ligands (e.g., temed and 2,2'-bipyridine) produced at pyrolytic graphite electrodes redox couples at different potentials suitable for "multicolor" labeling of PSs and for studies of ligand exchange kinetics. PS-Os(VI)L adducts can be determined not only in their purified forms but also in the reaction mixtures.

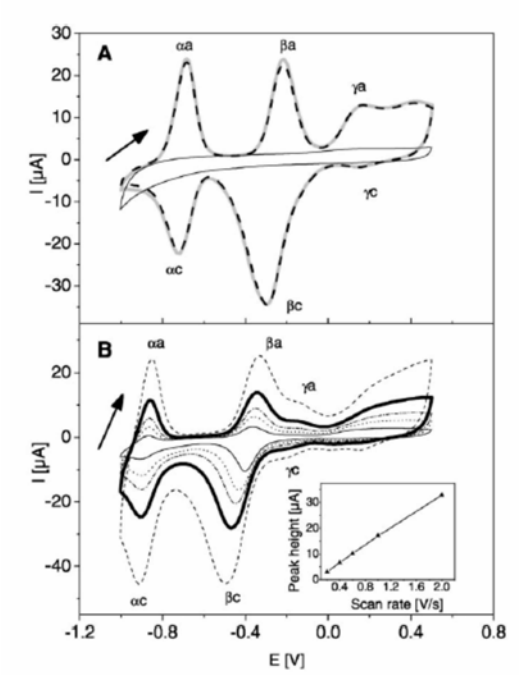

Figure 3: Electrochemical responses of Os(VI),L-modified dextran at a carbon electrode.

Electrochemical renewal of stationary mercury drop or meniscus electrodes

We show that a liquid mercury electrode surface can be electrochemically renewed without mechanical detachment of the drop. Voltammetric experiments with a mechanically renewed stationary (hanging) mercury drop or meniscus electrode (SME) and an electrochemically renewed SME are compared. The measurements were performed with two surface active organic depolarizers, i.e., 2-aminoanthraquinone and dithiothreitol and surface inactive $Cd(NO_3)_2$. The results show that efficient purely electrochemical renewal of the electrode surface of SME for voltammetric purposes is possible.

Interaction of biomacromolecules with surfaces viewed by electrochemical methods

The electrocatalytic evolution of hydrogen on mercury electrodes by organic molecules indicates, when followed with chronopotentiometric stripping method by the „peak H“, that when the stripping current is changed, the mechanism of the electrode process changes. The changes become the more prominent the larger is the catalyzing organic molecule. We explain this phenomenon by dynamic interaction of the catalyzing molecule with the electrode surface.

In the Field III, the studies on structure and interactions of the proteins involved in important signaling pathways were continued. Modulation of gene expression in U251 glioblastoma cells by binding of mutant p53 R273H to intronic and intergenic sequences was described. Effects of DNA supercoiling on DNA binding by BRCA1 protein was reported for the first time. Bilateral changes in IL-6 protein, but not in its receptor gp130, in rat dorsal root ganglia following sciatic nerve ligature, were observed.

Modulation of gene expression in U251 glioblastoma cells by binding of mutant p53 R273H to intronic and intergenic sequences

Missense point mutations in the TP53 gene are frequent genetic alterations in human tumor tissue and cell lines derived thereof. Mutant p53 (mutp53) proteins have lost sequence-specific DNA binding, but have retained the ability to interact in a structure selective manner with non-B DNA and to

act as regulators of transcription. To identify functional binding sites of mutp53, we established a small library of genomic sequences bound by p53 R273H in U251 human glioblastoma cells using chromatin immunoprecipitation (ChIP). Mutp53 binding to isolated DNA fragments confirmed the specificity of the ChIP. The mutp53-bound DNA sequences are rich in repetitive DNA elements, which are dispersed over non-coding DNA regions. Stable down-regulation of mutp53 expression strongly suggested that mutp53 binding to genomic DNA is functional. We identified the PPARGC1A and FRMD5 genes as p53 R273H targets regulated by binding to intronic and intra-genic sequences. We propose a model that attributes the oncogenic functions of mutp53 to its ability to interact with intronic and intergenic non-B DNA sequences and modulate gene transcription via re-organization of chromatin.

The central region of BRCA1 binds preferentially to supercoiled DNA

BRCA1 is a multifunctional tumor suppressor protein with implications in regulating processes, such as cell cycle, transcription, DNA repair, and chromatin remodeling. The function of BRCA1 likely involves interactions with a vast number of proteins and likewise DNA. To this date there is only fragmentary evidence about BRCA1 binding to DNA. In this study, we provide detailed analyses of various BRCA1 protein constructs binding to linear and supercoiled (sc) DNAs. We demonstrate that the central region of human BRCA1 binds strongly to negatively sc plasmid DNA at a native superhelix density, as evidenced by electrophoretic retardation of sc DNA in agarose gels. At relatively low BRCA1:DNA ratios, binding of BRCA1 to sc DNA results in the appearance of one or more retarded DNA bands on the gels. After removal of BRCA1, the original mobility of the sc DNA is recovered. BRCA1 proteins at higher concentrations also bind to the same DNA but in linear state, leading to formation of a smeared retarded band. Our experiments not only demonstrate a preference for BRCA1 binding to sc DNA, but also show that the central region may contain at least two efficient DNA binding domains with strong affinity for sc DNA. The biological implications of the novel DNA binding activities of BRCA1 are discussed.

Bilateral changes in IL-6 protein, but not in its receptor gp130, in rat dorsal root ganglia following sciatic nerve ligature

Local intracellular signaling cascades following peripheral nerve injury lead to robust axon regeneration and neuropathic pain induction. Cytokines are classic injury-induced mediators. We used sciatic nerve ligature (ScNL) to investigate temporal changes in IL-6 and its receptor gp130 in both ipsilateral and contralateral lumbal (L4-L5) dorsal root ganglia (DRG). Rats were operated aseptically on unilateral ScNL and allowed to survive for 1, 3, 7, and 14 days. Immunohistochemistry and Western blot analysis were used to determine levels of IL-6 and gp130 in DRG. A distinct increase in immunostaining for IL-6 was found in the neuronal cell bodies of sections through both ipsilateral and contralateral DRG at 1 and 3 days after operation. After 7 and 14 days, the DRG sections displayed only a moderate elevation in immunostaining when compared with sections of naïve DRG. The levels of IL-6 protein increased in both ipsilateral and contralateral lumbal DRG following peripheral nerve injury. The elevation of IL-6 protein was significant in both ipsilateral and contralateral DRG 1, 3, 7, and 14 days after operation. On the other hand, the levels of gp130 receptor did not change significantly. The data provide evidence for changes in IL-6 levels not only in the DRG associated with the damaged nerve but also in those unassociated with nerve injury during the experimental neuropathic pain model.

Granted projects

GA AS CR IAA500040701, Interactions of wild type and mutant p53 proteins with damaged DNA and their roles in cellular response to anticancer chemotherapy. Principal investigator: M. Fojta, 2007 - 2010

GA AS CR IAA400040901, DNA labeling with redox markers for electrochemical sensing. Applications in analysis of nucleotide sequences and molecular diagnostic. Principal investigator: M. Fojta, 2009 - 2013

GA AS CR IAA400040804, Application of electrochemical methods focused on the microanalysis of nucleic acids bases and oligonucleotides. Principal investigator: F. Jelen, 2008 - 2010

AS CR M200040904, Complex interaction of oncology-related important transcription factors with target DNA in vitro and in vivo. Principal investigator: V. Brázda, 2009 - 2012

GA AS CR KAN400310651, Nanotechnologies for protein and gene diagnostics. Principal investigator: F. Foret, Principal co-investigator: E. Paleček, 2006 - 2010

GA AS CR KAN200040651, Electrochemical and optical analysis of biomacromolecules at the microelectrodes modified by an electroactive material nanolayer. Principal investigator: S. Hasoň, 2006 - 2010

GA AS CR 1QS500040581, Metallodrugs, design and mechanism of action Principal investigator: O. Vrána, Principal co-investigator: M. Fojta, 2005 - 2009

GA CR 203/09/0317, Construction of novel functional nucleic acids for applications in chemical biology, catalysis and self assembly. Principal investigator: M. Hocek, Principal co-investigator: M. Fojta, 2009 - 2013

GA CR 301/10/1211, Transcriptional activities of wild-type and mutant p53, decision between cell proliferation, cell cycle arrest and apoptosis. Principal investigator: V. Brázda, 2009 - 2012

GA CR 204/08/1570, In vitro and in silico identification of non-canonical DNA structures in genomic DNA sequences. Principal investigator: M. Brázdová, Principal co-investigators: M. Lexa, O. Fučík, 2008 - 2010

GA CR 203/07/1195, Analysis of DNA structure and interactions using electrochemical techniques and chemical probes. Novel techniques and sensors for DNA damage detection. Principal investigator: M. Fojta, 2007 - 2009

GA CR 204/07/P476, Interactions of p73 protein and its isoforms with DNA. Influences of DNA supercoiling, conformation and anticancer drugs. Principal investigator: H. Pivoňková, 2007 - 2009

GA CR 301/07/P160, Study of posttranslation modification of the tumor suppressor protein p53, its homologues and interacting proteins in human cancer cell lines. Principal investigator: E. Brázdová-Jagelská, 2007 - 2009

GA CR 202/07/P497, Interactions of proteins with surfaces. New biophysical methods of analysis of tumor suppressor p53. Principal investigator: V. Ostatná, 2007 - 2009

GA CR 202/08/1688, Utilization of physical methods of investigation of nucleic acid and protein adsorption at interfaces in medical diagnosis and biocompatibility study. Principal investigator: V. Vetterl, 2008 - 2010

GA AS CR IAA400040903, Interfacial and electrochemical behavior of synthetic oligonucleotides: effects of nucleotide sequence, conformation and chemical modification. Principal investigator: L. Havran, 2009 - 2011

GA CR 203/08/P598, Electrochemical tools for detection of point mutations and polymorphisms in DNA. Principal investigator: P. Kostečka, 2008 - 2010

Ministry of Education, Youth and Sports of the CR - Research centre LC06035, Centre of biophysical chemistry, bioelectrochemistry and bioanalysis. New tools for genomics, proteomics and biomedicine. Coordinator: M. Fojta, 2006 - 2010

Ministry of Education, Youth and Sports of the CR - ME09038, Interactions of proteins and peptides with surfaces. New tools for biomedicine. Principal investigator: E. Paleček, Principal co-investigator: J. Wang, 2009 - 2012

Ministry of Education, Youth and Sports of the CR - 1 M0528, Stomatological Research Centre. Principal investigator: J. Vaněk, Co-investigator and Guarantor at IBP: V. Vetterl, 2005 - 2009

6FP EU Integrated Project No. 502983, Mutant p53 as a target for improved cancer treatment. Coordinator: K. Wiman (Karolinska Institute, Stockholm, Sweden); Principal co-investigator: E. Paleček, 2004 - 2009

Marie Currie TOK No. 42708, Interactions of Nucleic Acids and Proteins at Interfaces - Fundamentals and Applications. Principal investigator: J. Radecki (Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences in Olsztyn, Poland), Principal co-investigator: E. Paleček, 2007 - 2010

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PhD. thesis defended in 2009

Ing. Jan Vacek, PhD., Recent approaches in electrochemical analysis of damage, hybridization, and interactions of DNA

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Cell culture-induced gradual and frequent epigenetic reprogramming of invertedly repeated tobacco transgene epialleles (Aleš Kovařík)

The ability of mature plant cells to regenerate a whole organism is probably the most remarkable growth attribute of plant cells that distinguishes them from mammalian cells. The basis of such a capacity in plants lies in the availability of undifferentiated cells that can subsequently differentiate into all the cell types present in a mature organism. The exact molecular processes involved in the maintenance and/or induction of cell undifferentiation in plants are still poorly understood. Here we tested the hypothesis that the dedifferentiation process induces epigenetic reprogramming of promoters influencing gene activity. Using a two component transgene system involving two epiallelic variants of the invertedly repeated silencing locus (1) we have studied stability of trans-silencing interactions in cell culture and regenerated plants (Fig. 1). In parental hybrids the posttransriptionally but not transcriptionally silenced

epiallele of locus 1 trans-silenced and trans-methylated target locus 2. Expression and methylation of both silenced (Lo1/Lo2) and non-silenced (Lo1E/Lo2) hybrids were stable over several generations in plants. However, in early Lo1E/Lo2 callus decreased expression of the nptII reporter gene was observed while the Lo1/Lo2 remained silenced. Analysis of small RNA species and coding region methylation suggested that the nptII genes were silenced by a PTGS mechanism in both cultures. Expression changes were correlated with changes in methylation status of the 35S promoter at the silencing locus 1: the PTGS variant in Lo1/Lo2 line acquired methylation while the TGS epiallele in Lo1E/Lo2 line showed reduced methylation compared to the parental plant. Bisulfite genomic sequencing of locus 1 revealed molecules with no, intermediate and high level of methylation (Table 1). These data indicated that a cell culture process brought two epialleles of the silencer locus to the same epigenetic ground characterized by high epilallelic diversity. In regenerated plants about 75% of Lo1E/Lo2 individuals returned to the original non-silenced phenotype while 25% of individuals were silenced. From Lo1/Lo2 callus, 25% of regenerated plants showed increased expression whereas 75% of individuals remained silenced. The results demonstrated sensitivity of transgenes containing inverted structures towards epigenetic changes imposed by cell culture. We propose that many examples of tissue culture-induced phenotypic variability might originate from epigenetic alterations at repeated loci influencing their transcription status.

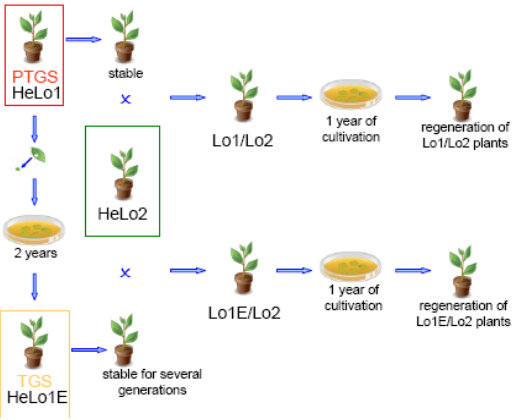

Figure 1: Scheme of the experimental strategy. HeLo1, plant line hemizygous for the transgenic locus 1, expression of the nptII reporter transgene silenced post-transcriptionally; HeLo1E, plant line hemizygous for the transgenic locus 1E, expression of the nptII reporter transgene silenced transcriptionally; HeLo2, plant line hemizygous for the transgenic locus 2, active expression of the nptII reporter transgene; Lo1/Lo2, posttranscriptionally silenced hybrid plant line combining locus 1 and locus 2; Lo1E/Lo2, hybrid plant line combining locus 1E and locus 2, active expression of the locus 2 nptII transgene.

Sample/methylation motif	mCG (%)		mCHG (%)		mCHH (%)	
	mean[a]	range[b]	mean	range	mean	range
Parental TGS Lo1/Lo2 plant	1	0-8	0	0-0	1	0-1
Disc of leaf (green tissue)	0	0-0	2	0-14	0	0-3
Early callus (4 weeks)	0	0-0	2	0-0	5	0-11
Advanced callus (12 months)	48	0-83	51	0-88	34	22-44
Non-silenced regenerant (#2)	90	83-100	75	57-100	34	22-44

Table 1: Summary of bisulfite sequencing analysis of the 35S promoter residing silencing locus (Lo1) in Lo1/Lo2 parental plant, derived calli and a regenerated plant.

[a]Percent methylation at CG, CHG and CHH contexts was calculated from the clones (Fig. 5) and expressed as a mean.
[b]Methylation homogeneity between the clones is indicated by a range.

Making a functional diploid: from polysomic to disomic inheritance

(Roman Matyášek)

Polyploids may arise through chromosome duplication within a species (autopolyploidy) or in association with interspecific hybridisation (allopolyploidy). How do autopolyploid animal and plant species that have undergone one or more rounds of whole genome duplication become established in nature? An immediate difficulty concerns the transition from polysomic inheritance, where a chromosome can combine at meiosis with one of several partners (homologues), to disomic inheritance, where specific chromosome pairs form and segregate regularly. One little understood feature of polyploid speciation is the transition from polysomic to disomic inheritance, and much recent attention has focused on the role of pairing genes in this process. Using computer simulations we study the effects of mutations, chromosomal inversions, chiasma, neofunctionalisation,

subfunctionalisation and selection on the evolution of disomic inheritance in a polyploid over 10,000 generations. We show that: (i) the evolution of pairing genes is not essential for the establishment of disomic inheritance, since genetic drift, coupled with a threshold for homologue pairing fidelity, is sufficient to explain the transition from polysomic to disomic inheritance; (ii) high rates of recombination increase the number of generations required for disomic inheritance to become established; (iii) both neofunctionalisation and subfunctionalisation speed up the transition to disomic inheritance. The data suggest that during polyploid species establishment, selection will favour reduced chiasma number and/or more focused distribution. The data also suggest a new role for subfunctionalisation in that it can drive disomc inheritance. The evolution of subfunctionalisation in genes across the genome will then act to maintain genes in syntenic blocks and may explain why such regions are so highly conserved.

Granted projects

GA CR 521/07/0116, Dynamics of repetitive DNA sequences in polyploid genomes. Principal investigator: A. Kovařík, 2007 - 2009

MSMT/EGIDE, MEB020823, Analyses of ribosomal genes during the stabilisation of an allopolyploid species, Brassica napus. Principal investigator: A. Kovařík, 2008 - 2009

GA CR 206/09/1751, The impact of genomic shock associated with interspecific hybridization and polyploidization on evolution of rDNA loci in young invasive weeds. Principal investigator: R. Matyášek, 2009 - 2013

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PhD. thesis defended in 2009

Mgr. Martina Nešpor-Dadejová, PhD., Epigenetic regulation of ribosomal RNA genes expression in synthetic and natural allopolyploids

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The histidine kinases CYTOKININ-INDEPENDENT1 and ARABIDOPSIS HISTIDINE KINASE2 and 3 regulate vascular tissue development in Arabidopsis shoots

The development and activity of the procambium and cambium, which ensure vascular tissue formation, is critical for overall plant architecture and growth. However, little is known about the molecular factors affecting the activity of vascular meristems and vascular tissue formation. Here we show that the histidine kinase CKI1 and the cytokinin receptors AHK2 and AHK3 are important regulators of vascular tissue development in Arabidopsis shoots. Genetic modifications of CKI1 activity in Arabidopsis causes dysfunction of the two-component signaling pathway and defects in procambial cell maintenance. CKI1 overexpression in protoplasts leads to cytokinin-independent activation of the two-component phosphorelay, and intracellular domains are responsible for cytokinin-independent activity of CKI1. CKI1 expression is restricted to vascular tissues in inflorescence stems, and CKI1 forms homodimers both in vitro and in planta. Loss-of-function ahk2 and ahk3 mutants and plants with reduced levels of endogenous cytokinins show defects in procambium proliferation and an absence of secondary growth. CKI1 partially rescues ahk2 ahk3 phenotypes in vascular tissue, while the negative mutation CKI1H405Q further

accentuates mutant phenotypes. These results indicate that the cytokinin-independent activity of CKI1 and cytokinin-induced AHK2 and AHK3 are important for vascular bundle formation in Arabidopsis.

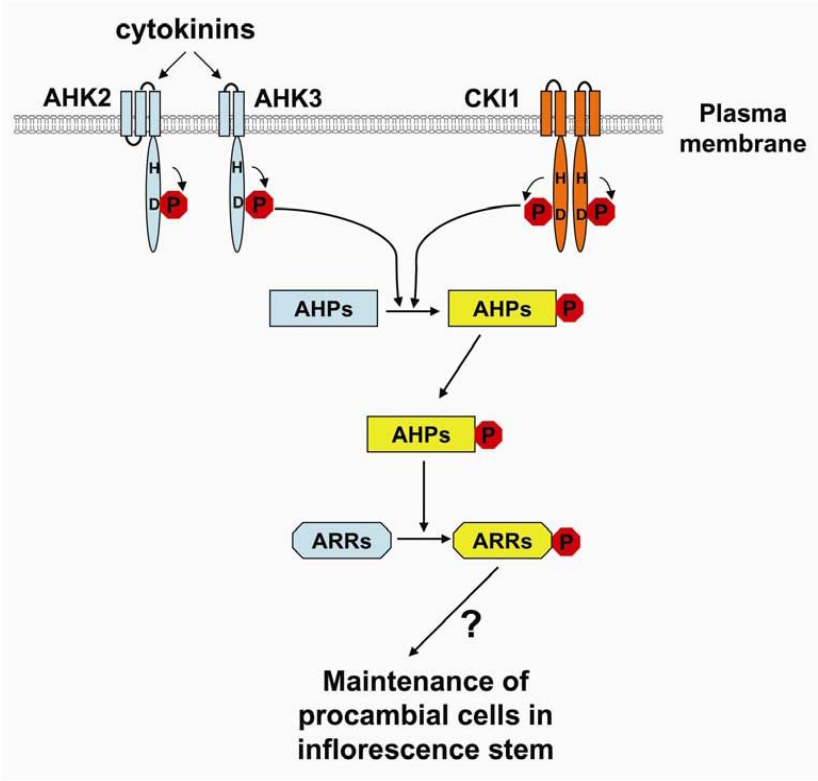

Figure 1: Interaction of cytokinin signaling pathway with histidine kinase CYTOKININ-INDEPENDENT1 (CKI1) in the vascular bundle development – a proposed model. We have shown that both the cytokinin-independent histidine kinase activity of CKI1 and cytokinin-activated histidine kinase activity of cytokinin receptors AHK2 and AHK3 are needed for regulation of procambium proliferation and/or the maintenance of its identity in Arabidopsis inflorescence stems.

Cytokinins modulate auxin-induced organogenesis in plants via regulation of the auxin efflux

Postembryonic de novo organogenesis represents an important competence evolved in plants that allows their physiological and developmental adaptation to changing environmental conditions. The phytohormones auxin and cytokinin (CK) are important regulators of the developmental fate of pluripotent plant cells. However, the molecular nature of their interaction(s) in control of plant organogenesis is largely unknown. Here, we show that CK modulates auxin-induced organogenesis (AIO) via regulation of the efflux dependent intercellular auxin distribution. We used the hypocotyl explants-based in vitro system to study the mechanism underlying de novo organogenesis. We show that auxin, but not CK, is capable of triggering organogenesis in hypocotyl explants. The AIO is accompanied by endogenous CK production and tissue-specific activation of CK signaling. CK affects differential auxin distribution, and the CK mediated modulation of organogenesis is simulated by inhibition of polar auxin transport. CK reduces auxin efflux from cultured tobacco cells and regulates expression of auxin efflux carriers from the PIN family in hypocotyl explants. Moreover, endogenous CK levels influence PIN transcription and are necessary to maintain intercellular auxin distribution in planta. Based on these findings, we propose a model in which auxin acts as a trigger of the organogenic processes, whose output is modulated by the endogenously produced CKs. We propose that an important mechanism of this CK action is its effect on auxin distribution via regulation of expression of auxin efflux carriers.

Granted projects

MEYS 1M06030, Functional genomics and proteomics for crop improvement. Principal investigator: B. Brzobohatý, 2006 - 2011

MEYS LC06034, Regulation of morphogenesis of plant cells and organs. Principal investigator: E. Zažímalová, Co-principal investigator: B. Brzobohatý, 2006 - 2010

GA AS CR IAA600040701, Proteome dynamics in response to increased cytokinin levels in Arabidopsis. Principal investigator: B. Brzobohatý, 2007 - 2010

GA CR 206/09/2062, The role of cytokinins and polyamines in heat stress response and thermotolerance in tobacco and Arabidopsis plants. Principal investigator: R. Vaňková, Co-principal investigator: B. Brzobohatý, 2009 - 2013

GA CR 204/09/P289, Subcellular compartmentation of reversible O-glucosylation in the homeostasis of active cytokinin levels. Principal investigator: S.N. Kiran, 2009 - 2011

NAAR QI91A229, Application of conventional and molecular genetic approaches for the development of grain legumes resistant to viral and fungal pathogenes and insect pests. Principal investigator: M. Griga, 2009 - 2013

Publications

Hejátko, J., Ryu, H., Kim, G.-T., Dobešová, R., Choi, S., Choi S.M., Souček, P., Horák, J., Pekárová, B., Palme, K., Brzobohatý, B., Hwang, I.: *The histidine kinases CYTOKININ-INDEPENDENT1 and ARABIDOPSIS HISTIDINE KINASE2 and 3 regulate vascular tissue development in Arabidopsis shoot.* Plant Cell, 21, 2009, 2008-2021.

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PhD. thesis defended in 2009

Mgr. Radka Dopitová, PhD., Functional analysis of active site of maize beta-glucosidase Zm-p60.1

Mgr. Gabriela Lochmanová, PhD., Dynamics of proteome changes in response to directed modification of plant hormone cytokinin levels

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HP1 protein and epigenetics of nucleoli

We utilized immunofluorescence combined with high-resolution Nipkow disc-based confocal microscopy to visualize the localization of some epigenetic factors in control and SUV39h deficient cells. In addition, the cells were treated with TSA, an inhibitor of histone deacetylases (HDACi). HP1 subtypes were shown to localize at the periphery of the nucleoli, associating with the clusters of pericentromeric heterochromatin called chromocenters. Thus, we determined how SUV39h deficiency and HDACi influenced the presence of HP1 and H3K9 di- and tri-methylation at the chromocenters. This was then compared to similar epigenetic marks within nucleoli. Analyses of HP1 subtypes confirmed different interphase patterns in SUV39h (wt) and SUV39h (dn) cells (Fig. 1 and 2a-d). For example, in the control cells, HP1 alpha was strictly associated with chromocenters,

while HP1 beta was observed not only in the chromocenters, but also in the chromatin-poor regions in close proximity to the chromocenters (Harničarová Horáková et al., 2009). HP1 gamma associated equally with chromocenters and the surrounding chromatin in SUV39h (wt) cells. As expected, SUV39h deficiency and HDACi in wild type cells significantly reduced the levels of all HP1 subtypes at the chromocenters (Harničarová-Horáková et al.,2009).

Following elucidation of HP1 nuclear pattern, we examined the presence of all HP1 subtypes inside the nucleoli, which consist of fibrillar centers (FC), dense fibrillar components (DFC), and granular components (GC). Fibrillarin (ribose 2'-O-methylase), which specifically interacts with small nucleolar RNAs (snoRNAs), is located in DFC. In both SUV39h (wt) and SUV39h (dn) cells, the HP1 alpha and HP1 gamma subtypes were associated with fibrillarin-positive regions to smaller extent, when compared with HP1 beta that strictly co-localized with fibrillarin (Fig. 1). This nucleolus-associated location was observed for HP1 beta, not only in the mouse model studied, but also to a lesser extent in human small lung carcinoma A549 cells and mouse fibroblasts lacking the LMNA gene that encodes A-type lamins (published by Harničarová-Horáková et al., Chromosoma 2009). The association of fibrillarin-positive regions with HP1 beta was also confirmed in SUV39h (wt) and SUV39h (dn) cells transiently expressing GFP- HP1 beta (Fig. 1). Indeed, we observed a high density of GFP-HP1 beta protein in fibrillarin-positive regions of the nucleoli. In addition to the influence of Suv39h deficiency on the epigenetics and structure of nucleoli, we studied whether HDACi can influence the presence of select epigenetic markers inside the nucleoli. Both the absence of SUV39h and HDACi decreased H3K9me2 inside the nucleoli, and these changes were more pronounced after TSA treatment of SUV39h (dn) cells. On the other hand, we observed that in the fibrillarin-positive region, H3K9me3 and HP1 beta levels were relatively stable compared to the chromocenters of identical cells. This implies that both Suv39h deficiency and HDAC inhibition have a subtle impact on some epigenetic profiles and epigenetic stability of DFC where fibrillarin is located.

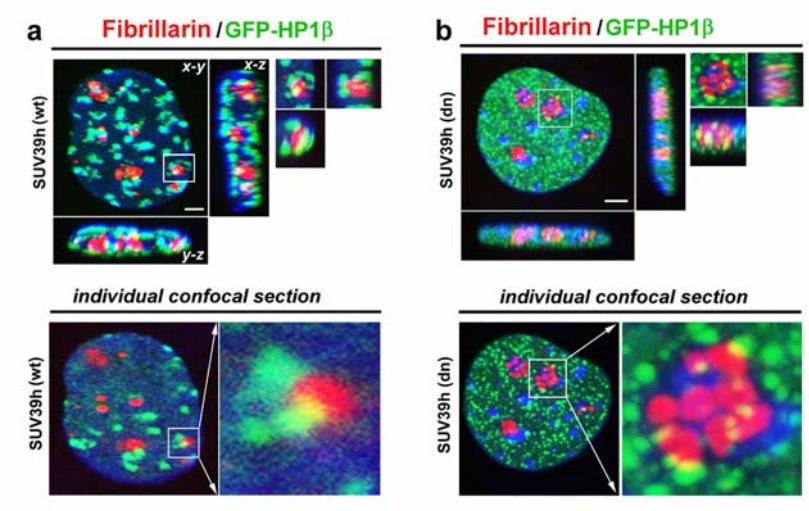

Figure 1: SUV39h (wt) and SUV39h (dn) cells expressing GFP-HP1 beta and fibrillarin localization. a] SUV39h (wt) b] SUV39h (dn) fibroblasts transiently expressing GFP-HP1 beta (green), subsequently stained by antibody against fibrillarin (red). In both the cases tested, fibrillarin co-localized with HP1 beta foci. Scale bars represent 1 micrometer.

Optimization of FRAP technique and DNA repair studies

We have study dynamic properties of chromatin-related proteins, including HP1 subtypes (Fig. 2), histone demethylase JMJD2b, beta-catenin, BMI1, TRF1 or c-MYC in Suv39h (wt), (dn) fibroblasts and in LMNA deficient cells. In selected experimental systems, we analyzed dynamics of chromatin-related proteins and we would like to find the factors that are responsible for kinetics properties of individual proteins. FARP technique was sufficiently optimized in our laboratory; therefore, we would like to continue with such experiments.

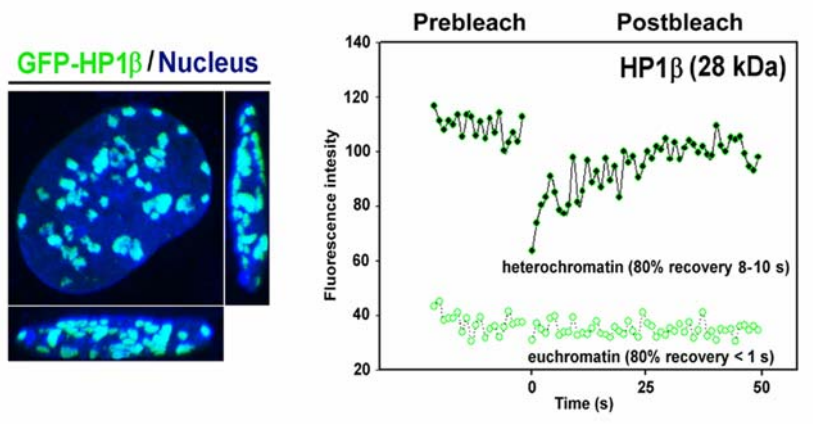

Figure 2: Example of HP1β kinetics in euchromatin and heterochromatin in (wt) mouse fibroblasts. As observed by other authors (Cheutin et al., 2003), there is distinct kinetics between HP1 β accumulated in euchromatin and HP1β that binds to heterochromatin regions. By the use of GFP-HP1 β plasmid we were able to optimize FRAP technique in our laboratory.

In other experiments, double strand breaks (DSBs) were induced by 355 nm UV laser and, following local DNA damage, the presence of phosphorylated H2.AX (gamma H2A.X) was detected as a marker of DSBs (Fig. 3). U2OS cells stably expressing GFP-BMI1 were cultivated under standard conditions and in these cells we analyzed recruitment of Polycomb group (PcG)-related protein BMI1 to DSBs. At 70% confluence, the cells were sensitized with 10 microM BrdU, 16-18 h before local irradiation. Cells were irradiated by UV laser (355 nm). We irradiated half of the nuclei or strips of nuclei by 80% laser output, not reduced at AOTF. The following settings were used: 512 × 512 pixels, 400 Hz, bidirectional mode, 64 lines, zoom >5-10. Irradiated cells were fixed in 4% paraformaldehyde and phosphorylated histone H2A.X (gamma H2AX) was detected with rabbit polyclonal antibody to gammaH2A.X (phospho S139) (Abcam, #ab2893) in immunofluorescence and confocal microscopy.

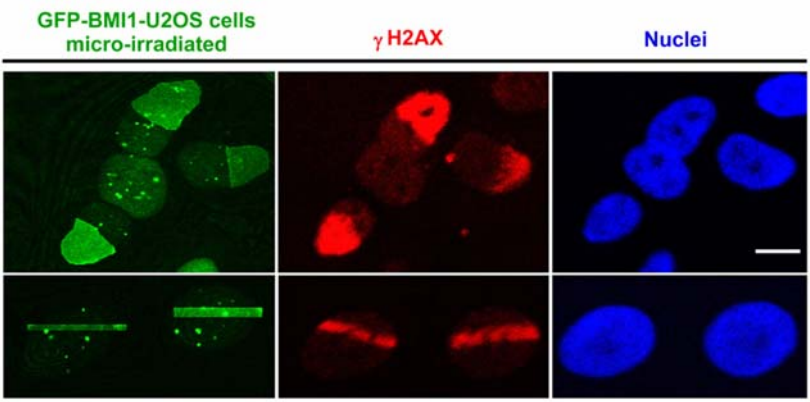

Figure 3: Relationship of BMI1 protein to DSBs induced by micro-irradiation was tested. (A) GFP-BMI1-U2OS (green) cells were micro-irradiated by UV laser (355 nm) and the induction of DSBs was verified using antibody against gammaH2AX (red areas or strips). Bar represents 20 µm. Cells were counterstined by Hoechst 33342.

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Higher-order chromatin structure in DSB induction, repair and misrepair

Cell survival and maintenance of genome integrity are dependent on the efficient and accurate repair of DNA double-strand breaks (DSBs) mediated by exogenous agents but also during execution of DNA function. Cellular response to DNA DSBs consists of complex signaling network that coordinates initial recognition of the lesion with the induction of its repair. However the access of enzymes and regulatory factors to the sits of DSBs is hampered by highly complicated three-dimensional structure of chromatin especially in its condensed regions. The loosening of chromatin in the vicinity of DSBs is necessary to recruit and maintain activities of repair proteins. Our results show that this chromatin loosening results in local changes in chromatin structure and can influence the probability of mutual interaction of DSBs. DSBs repair takes place, in principle, at the sites of their induction; however, as a consequence of chromatin decondensation, some DSBs protrude into a sparse chromatin (chromatin holes) where they could potentially cluster and form complex lesions that are repaired only with difficulties and pose an increased risk of chromosomal translocation formation. According to the proposed model (Falk et al.,2010), the DSB repair may significantly change the probabilities of DSB interactions derived from nuclear distances of the damaged loci, in the dependence of local higher-order chromatin structure (Fig. 4).

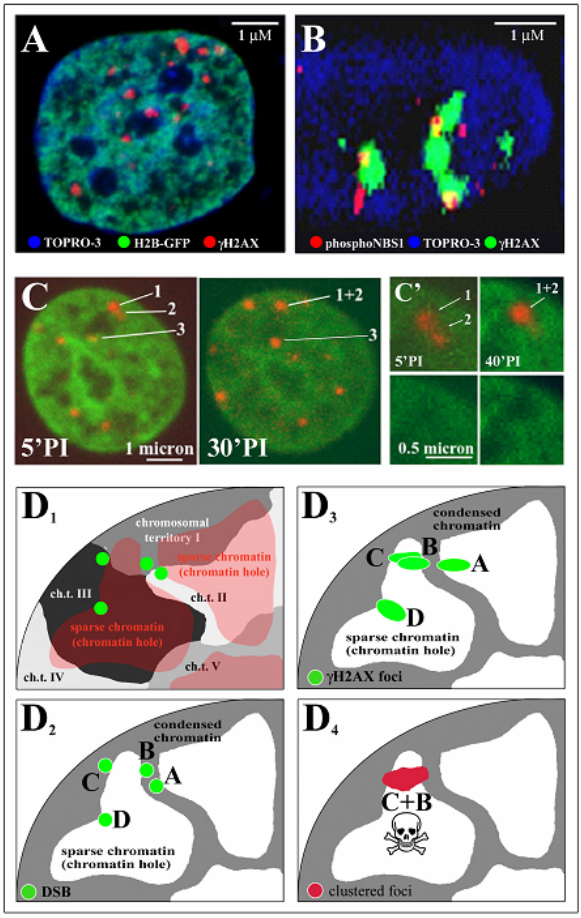

Figure 4: A model showing the relationships between higher-order chromatin structure, DSB repair and formation of chromosomal translocations. (A) x–y central slice through a spatially fixed MCF7 cell nucleus shows the location of γH2AX foci (red), induced with 1.5 Gy of γ-rays, relative to dense and sparse chromatin domains, 10 min PI. Chromatin density is reflected by the intensity of H2B-GFP (green) and TOPRO-3 (blue) staining. Dark areas represent nucleoli and ''chromatin holes''. (B) Clustering of gH2AX foci shown in 3D space (x-z plane) in spatially fixed normal human fibroblasts irradiated with 3 Gy of γ-rays. γH2AX foci (green) observed 2 h PI; NBS1 (red), chromatin (TOPRO-3, blue). (C) Central slices (0.4 mminx–y plane) of human MCF7 cells double-transfected with 53BP1-RFP and H2B-GFP proteins, irradiated with a dose of 1.5 Gy of γ-rays, displayed at 5 min and 30 min PI, show re-localization of 53BP1 foci 2 and 3 (red) from dense to sparse chromatin. After this relocalization, focus 2 formed a cluster with focus 1; this cluster persisted until the end of observation (40 min PI, enlarged detail at panel C0).

Chromatin density, green (H2B-GFP). (D) Proposed model of the relationship between higher-order chromatin structure, DSB repair and formation of chromosomal translocations. (D1) Schematic location of chromosomal territories that could be subject to chromatin exchange during DSB repair. (D2) The higher-order chromatin structure and Brownian movement of chromatin determine the original radius of mutual DSB (γH2AX foci, green) interactions. Heterochromatin between A and B prevents their mutual interaction. (D): chromatin decondensation at sites of DSBs induced at the boundary of eu- and heterochromatin can significantly increase (foci B and C) or decrease (foci A and B) the original probability of interactions between DSBs. (D4) Foci B and C are at the highest risk of chromatin exchanges despite the shortest nuclear distance being between foci A and B. (Figure is adopted from Falk et al.,2010).

Molecular mechanisms of the cell death in leukemia cells caused by DNA damage during exposure of cells to fractionated irradiation in vitro

We studied the effect of fractionated irradiation with γ-rays on ability of human cells of lymphocytic leukemia MOLT4 to repair DNA DSBs. Our results show that many cells are not able to accomplish repair of all induced DNA DSBs before application of the next radiation dose. The long presence of unrepaired DSBs induced by the fractionated irradiation represents long-term genotoxic stress leading to elimination of cells with this damage. Process elimination of cells containing non repaired DSBs after irradiation depends on the mode of radiation dose delivery. In single irradiation with the high radiation dose inducing high number of DNA DSBs, the prevailing process of cell elimination is apoptosis. During fractionated irradiation with lower doses, three different processes participate on elimination of cells with unrepaired DSBs - apoptosis, genotoxic-stress-induced cellular senescence and adaptation to active checkpoint G2 when the cells enter the mitoses and majority of them die during this process. Our results are the first showing induction of massive cellular senescence and active checkpoint adaptation of leukemia cells exposed to long-term DNA damage during fractionated irradiation used for radiotherapy of tumors.

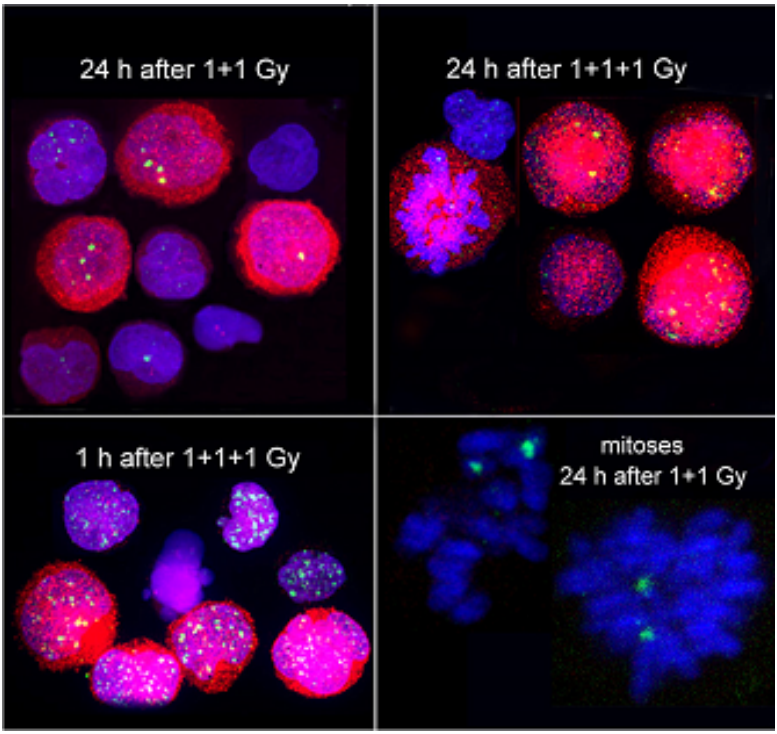

Figure 5: Cyclin B1 expression in Molt 4 cells arrested in G2 phase during repeated irradiation with the doses of 1 Gy in the interval of 24 h indicates that the cells are entering the mitosis in spite of unrepaired DSBs in their DNA Representative projection of Molt 4 cells expressing high level of cyclin B1 (red) at the time 24 h after the doses of 2 x 1 Gy; 1 and 24 h after 3 x 1 Gy. Non repaired DSBs (green) are seen also at some chromosomes (blue) during mitosis. After the single doses of 1 Gy and 3 Gy, as well as after the cumulative dose 4 x 1 Gy, cells with high level of cyclin B1 were observed neither 24 nor 48 h PI. 1h after IR with the third dose, the cells contained high number of DSBs, represented by green foci of γ-H2AX, however 23 h later, the number of these foci decreased indicating that cells repaired DNA damages in spite of their presence at the boundary of mitoses. The cells with high level of cyclin B1 (red) were determined after immunodetection by counting on microscopic slides in visual field of confocal microscope among 500 cells per sample.

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DNA topoisomerase IIα and HMGB proteins: a search for a common link

We have continued in our recently published studies dealing with the effect of HMGB1/2 proteins on the activity and cellular expression of human topoisomerase IIα (Štros et al: Nucleic Acids Res. 2007, 2009). Our results pointed out a correlation between over-expression of topoisomerase IIα and HMGB1/2 in human cancer cell lines. Our results also provided evidence that mechanism of HMGB1-mediated stimulation of activity of topoisomerase IIα was due to enhanced DNA cleavage which was further promoted by topo II poisons. Higher cellular expression (and activity) of topoisomerase IIα by HMGB1/2 proteins in Rb-minus cells, as well as the stimulatory effect of HMGB1 on activity of topoisomerase IIα, could have clinical relevance in respect to prognosis of patients treated with topoisomerase II poisons. This idea is supported by the fact that HMGB1/2 proteins are frequently reported to be over-expressed in cancer, and Rb deletions are observed in most tumors (the project is also conducted in collaboration with Š. Pospíšilová from Center of Molecular Biology and Gene Therapy, University Hospital Brno.

Modulation of telomerase activity by HMGB1 in mouse and human cells

Eukaryotic chromosome stability relies on the presence of intact chromosome ends or telomeres. Telomeres are formed by a special chromatin structure that protects chromosome termini from recombination and degradation, thus preventing end-to-end chromosome fusions and other chromosomal aberrations. Telomeres are replicated by telomerase. Here we have demonstrated that HMGB1 could interact in vitro with telomerase catalytic component TERT and RNA component TR. However, once telomerase is formed, the interaction of HMGB1 with telomerase seems to be weak or undetectable. In support, only a weak telomerase activity could be detected upon immunoprecipitation of cellular lysates with α-HMGB1 antibody. As reconstitution of telomerase has previously been reported in vitro with only TERT and TR (rabbit reticulocyte lysate), we have initiated experiments aiming at understanding of a possible modulatory effect of HMGB1 on telomerase assembly in vitro. We have also demonstrated a stimulatory effect of recombinant HMGB1 on the activity of telomerase in cellular lysates, explaining our previous findings revealing decreased activity of telomerase in HMGB1 knockouted mouse embryonic fibroblasts or human MCF-7 cells with inhibited HMGB1 expression.

Single-Myb-histone proteins from Arabidopsis thaliana: a quantitative study of telomere-binding specificity and kinetics

Proteins that bind telomeric DNA modulate the structure of chromosome ends and control telomere function and maintenance. It has been shown that AtTRB (Arabidopsis thaliana telomererepeat-binding factor) proteins from the SMH (single-Mybhistone) family selectively bind double-stranded telomeric DNA and interact with the telomeric protein AtPOT1b (A. thaliana protection of telomeres 1b), which is involved in telomere capping. In the present study, we performed the first quantitative DNA-binding study of this plant-specific family of proteins. Interactions of full-length proteins AtTRB1 and AtTRB3 with telomeric DNA were analysed by electrophoretic mobility-shift assay, fluorescence anisotropy and surface plasmon resonance to reveal their binding stoichiometry and kinetics. Kinetic analyses at different salt conditions enabled us to estimate the electrostatic component of binding and explain different affinities of the two proteins to telomeric DNA. On the basis of available data, a putative model explaining the binding stoichiometry and the protein arrangement on telomeric DNA has been generated.

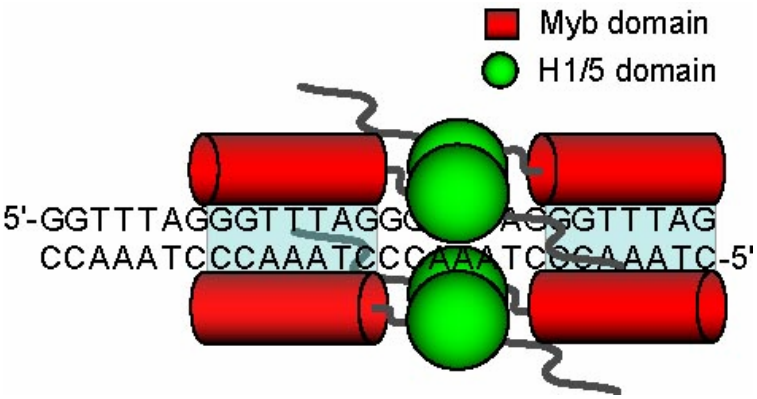

Figure 6: Speculative model of interaction of AtTRBs with telomeric DNA Both homo- and hetero-dimers of AtTRB may participate in the interaction with telomeric DNA. (Hofr et al., Biochem. J. 2009)

AtTRB1, a telomeric DNA-binding protein from Arabidopsis, is concentrated in the nucleolus and shows highly dynamic association with chromatin. AtTRB1, 2 and 3 are members of the SMH (single Myb histone) protein family, which comprises double stranded DNA-binding proteins that are specific to higher plants. They are structurally conserved, containing a Myb domain at the N-terminus, a central H1/H5-like domain and a C-terminally located coiled-coil domain. AtTRB1, 2 and 3 interact through their Myb domain specifically with telomeric double-stranded DNA in vitro, while the central H1/H5-like domain interacts non-specifically with DNA sequences and mediates protein-protein interactions. We showed that AtTRB1, 2 and 3 preferentially localize to the nucleus and nucleolus during interphase. Both the central H1/H5-like domain and the Myb domain from AtTRB1 can direct a GFP fusion protein to the nucleus and nucleolus. AtTRB1–GFP localization is cell cycle-regulated, as the level of nuclear-associated GFP diminishes during mitotic entry and GFP progressively re-associates with chromatin during anaphase/telophase. Using fluorescence recovery after photobleaching and fluorescence loss in photobleaching, we determined the dynamics of AtTRB1 interactions in vivo. The results reveal that AtTRB1 interaction with chromatin is regulated at two levels at least, one of which is coupled with cell-cycle progression, with the other involving rapid exchange.

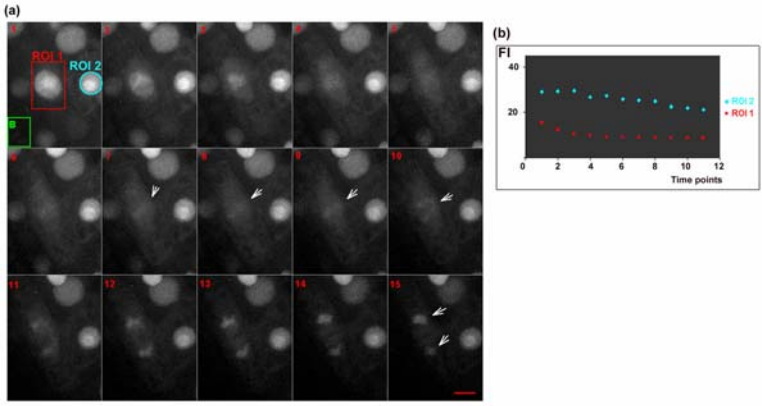

Figure 7. AtTRB1 dynamics during mitosis. (a) Time-lapse images of A. thaliana C24 plants expressing GFP–AtTRB1 followed during the cell cycle at 1 min intervals. GFP–AtTRB1 is re-localized from the nucleolus and nucleus at the beginning of mitosis (1–3); a detectable level of GFP–AtTRB1 associated with mitotic chromatin persists (arrowed in 7–10) and re-integrates into newly formed nucleoli (arrowed in 15). Background fluorescence intensity was measured in the boxed area marked B. Scale bar = 5 lm. (b) Estimation of fluctuation in GFP-AtTRB1 fluorescence intensity in the mitotic region (ROI 1, red line) and the control region (ROI 2, blue line) in scans 1-11. The fluorescence intensity in the mitotic region (ROI 1, red line) decreases rapidly at the beginning of mitosis, and it becomes stabilized over the following stages. Overall sample bleaching is approximately 30% (blue line). Fluorescence intensity measurements were performed using IMAGEJ software (Dvořáčková et al., Plant J. 2010).

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In 2009, we made further progress in our studies aimed at evaluation of the hematopoiesis-modulating effects of adenosine receptor agonists and cyclooxygenase-2 inhibitors, as well as at understanding their mechanisms. An inhibitor of cyclooxygenase-2, meloxicam, has been shown to increase the production of erythropoietin (EPO) (Table 1) and to elevate numbers of hematopoietic progenitor cells committed to erythroid cell lineage (burst-forming units, BFU-E) in mice irradiated with a sublethal dose of 4 Gy of gamma-rays. This is an interesting finding because previous findings concerned predominantly stimulatory effects of cyclooxygenase inhibitors on granulopoiesis. The results may have impact also in clinical practice if cyclooxygenase-2 inhibitors will be used for treatment of myelosuppression of various etiology.

Unirradiated control mice		
48.3 ± 18.1		
Irradiated mice		
Time interval after injection (hours)	Saline-treated controls	Meloxicam-treated mice
6	206.0 ± 86.6*	433.1 ± 87.6#
12	186.3 ± 16.1*	421.4 ± 82.6#

Table 1: Serum concentrations of EPO (pg/ml) in 4 Gy-irradiated mice after administration of meloxicam in a single dose on day 3 after irradiation.

Mice were administered saline or meloxicam in a single dose on day 3 after irradiation. Serum concentration of EPO was determined 6 and 12 hours after the injection. Data are given as means ± S.E.M. Five animals per group were used. * - P<0.05 vs. unirradiated control mice; # - P<0.05 vs. irradiated saline-treated mice.

An adenosine A3 receptor agonist, IB-MECA, was tested for its ability to induce the growth of colonies growing from hematopoietic progenitor cells for granulocytes and macrophages (GM-CFC) in suspension of normal mouse bone marrow cells in vitro and to potentiate stimulatory effects of hematopoietic growth factors (interleukin-3 /IL-3/, stem cell factor /SCF/, granulocyte-macrophage colony-stimulating factor /GM-CSF/, and granulocyte colony-stimulating factor /G-CSF/) on these cells. Whereas IB-MECA alone induced no GM-CFC colony growth, a significant increase in GM-CFC numbers has been observed when IB-MECA was added to the cultures concommitantly with IL-3, SCF, or GM-CSF. These findings give evidence of a significant role played by selective activation of adenosine A3 receptors in the regulation of the growth of granulocyte/macrophage hematopoietic progenitor cells.

Granted projects

ME CR LC06027, Monoclonal gammapathy and multiple myeloma, basic research centre. Principal investigator: R. Hájek, Principal co-investigator: E. Bártová, 2006 - 2010

ME CR ME919, Mapping of fragile sites in human genome. Principal investigator: E. Bártová, 2007 - 2011

GA AS CR IAA500040802, New mechanisms of the oncoprotein functions in the genesis of promyelocytic leukemia. Principal investigator: M. Falk, 2008 - 2011

ME CR LC535, Dynamics and organization of chromosomes in the cell cycle under standard and pathological conditions. Principal investigator: I. Raška, Principal co-investigator: S. Kozubek, 2006 - 2010

GA CR P302/10/1022, Changes in chromatin structure at sites of DNA double-strand breaks and their necessity for DSB repair. Principal investigator: S. Kozubek, 2010 - 2012

ME CR LC06004, Integrated research of the plant genome. Principal investigator: B. Vyskot, 2005 - 2010

GA AS CR IAA600040505, The mechanisms of the creation and reduction of telomeres independent of telomerase. Principal investigator: J. Fajkus, 2005 - 2009

GA CR 204/08/1530, Explanation of the role of HMGB1 protein in keeping the genome stability. Principal investigator: M. Štros, 2008 - 2010

GA AS CR IAA500040801, Telomeres and telomerase: from molecular biology to structural biology. Principal investigator: E. Sýkorová, 2008 - 2012

GA CR 521/09/1912, Telomeres of algae. Principal investigator: E. Sýkorová, 2009 - 2012

GA CR 306/08/0158, Activation of adenosine receptors combined with cyclooxygenase inhibition in modulation of radiation-induced myelo-suppression. Principal investigator: M. Hofer, 2008 - 2012

LC06027, Center of basic research for monoclonal gamapathy of multiple myeloma. Principal investigator: R. Hájek, Principal co-investigator: E. Bártová, 2006 - 2010

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PhD. thesis defended in 2009

Mgr. Gabriela Galiová, PhD., Spatial nuclear arrangement of the genome in humans, higher primates and another animal species

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Department of Cytokinetics focuses on the research in the field of cellular signaling and physiology relevant to cancer and potential role of lipids and their derivatives in these processes. In particular, the effects of environmental substances, such as lipid nutrition components (essential polyunsaturated fatty acids and butyrate) and xenobiotics (cytostatics and environmental organic pollutants) on regulation of cytokinetics, i.e. cell proliferation, differentiation and apoptosis are studied. Using both tumor and non-tumorigenic cells, new types of interactions of lipid dietary

components, anticancer drugs (non-steroidal anti-inflammatory drugs-NSAIDs, cytostatics) or selected environmental pollutants (polycyclic aromatic hydrocarbons, PCBs, dioxins) with physiological regulators of cytokinetics are being investigated. Attention is being paid especially to tumor necrosis factor (TNF) family, tumor growth factor (TGF) family, fibroblast growth factor (FGF) and Wnt/beta-catenin pathway signaling. The results are exploited in cancer prevention/therapy and in ecotoxicology.

Cellular and molecular physiology of lipids (Jiřina Hofmanová)

In the self-renewing tissue of the colon the, a strict control of the balance between proliferation, differentiation, and apoptosis in the crypts is highly important. These processes are affected by many exogenous as well as endogenous agents, which may operate together in the colonic lumen. Among nutritional compounds, especially essential polyunsaturated fatty acids (PUFAs) of ω-6 and ω-3 types, and short-chain fatty acid butyrate produced from fiber were shown to affect the behavior of both normal and cancer colon cell populations. Moreover, these fatty acids may influence the effects of endogenous factors regulating cell growth, differentiation and apoptosis (cytokines, growth factors, apoptotic inducers). Nutritionally induced changes in cellular and tissue fatty acid composition may also result in altered sensitivity to chemo- and radiotherapy. Thus, lipids are capable of influencing a number of pathophysiological processes and modifying the effects of the drugs. Undoubtedly, the action of these compounds is complex and it involves a number of integrated signaling pathways that need to be elucidated.

In our group we investigated the effects of model PUFAs such as arachidonic acid (AA, 20:4, ω-6) and docosahexaenoic acid (DHA, 22:6, ω-3), sodium butyrate (NaBt), and apoptotic inducers of tumor necrosis factor (TNF) family (especially TNF related apoptosis-inducing ligand - TRAIL) during colon carcinogenesis using epithelial cell lines derived from human fetal colon (FHC), adenoma (AA/C1, RG/C2), carcinoma (HT-29, HCT-116), and lymph node metastasis (SW620). Importantly, we studied the mechanisms of the effects of these compounds either alone or in mutual combinations.

i/ Interaction of PUFAs and butyrate

We verified the hypothesis suggesting modulation of the effects of sodium butyrate (NaBt) by ω-3 or ω-6 polyunsaturated fatty acids (PUFAs). Comparing the responses of human colon epithelial cell lines of fetal (FHC)

and adenocarcinoma (HT-29, HCT-116) origin, we detected significant differences in proliferation, differentiation, and apoptotic response to the treatment of NaBt, AA or DHA, and their combinations. While in FHC and HT-29 cells NaBt induced G0/G1 arrest, differentiation, and low level of apoptosis, a G2/M arrest, no differentiation, and a high degree of apoptosis were detected in HCT-116 cells. Moreover, in FHC cells, a significant potentiation of apoptosis accompanied with an increased arrest in the cell cycle, cell detachment, and decrease of differentiation was detected after combined treatment with NaBt and both PUFAs. Changes in cytokinetics induced by fatty acids were accompanied with membrane lipid unpacking, reactive oxygen species production, and decrease of mitochondrial membrane potential. Detection of caspase-3 activation and dynamic modulation of Mcl-1 protein expression imply their possible role in both cell differentiation and apoptotic response. Our results support the concept of modulation of NaBt effects by PUFAs, especially of ω-3 type, in colonic cells in vitro with diverse impact in cell lines derived from normal or neoplastic epithelium.

ii/ The role of PUFAs in modulation of TRAIL-induced apoptosis in colon cancer cells

We continued our studies focused on elucidation of the possible role of DHA in sensitization of colon cancer cells to the apoptotic effects of TRAIL. As our previous results demonstrated the ability of DHA to increase the sensitivity of HT-29 human colon cancer cells to TRAIL-induced apoptosis, we also aimed to investigate the response to these agents in other colon cancer cell lines that are otherwise relatively resistant to TRAIL (e.g. SW620). Moreover, selected combinations of TRAIL and DHA were tested using non-tumor human colon adenoma cell lines. In these cells, we did not observe any stimulating effect of DHA on TRAIL-induced cell death.

iii/ The molecular mechanisms of TRAIL effects in colon epithelial cells

In order to determine the role of particular molecules in regulation of colon cancer cell resistance to TRAIL-induced cell death, mechanistic studies were performed in different colon cancer cell lines, especially focused on the role of anti-apoptotic Mcl-1 protein, and selected pro-survival pathways such as MEK/ERK and PI3K/Akt. We examined the changes of anti-apoptotic Mcl-1 protein level following TRAIL treatment in human cell lines representing different stages of colon carcinogenesis-adenocarcinoma (HT-29, HCT116) or secondary metastasis (SW620), or cell line derived from fetal colon (FHC). While TRAIL was capable of triggering anti-

apoptotic signaling leading to significant early ERK-mediated transcriptional up-regulation of Mcl-1 in selected colon adenocarcinoma cell lines, none or minimal effects were demonstrated in cell lines derived from colon lymph node metastasis or fetal colon, respectively. We showed that it is essential to consider the dynamics of modulation of Mcl-1 level and the balance between TRAIL-induced pro- and anti-apoptotic pathways when predicting the response of cells in different stages of cancer development, and designing the anticancer therapy using TRAIL.
The mechanisms of the decreased sensitivity to TRAIL observed in HT-29 colon adenocarcinoma cell line and FHC fetal cell line, during non-adherent cell cultivation as compared to adherent one were investigated in detail. We focused on the pro-survival (PI3/Akt, MAPK/ERK and NF-κB) pathways, their activation and connection with increased phosphorylation of focal adhesion kinase (FAK) during non-adherent cultivation using inhibitors of particular kinases. Our results suggested that the downstream activation of PI3/Akt pathway by phosphorylated FAK kinase appears to be responsible for the decrease in TRAIL-induced apoptosis during non-adherent cell cultivation.

Growth factors in cancer cell signaling (Karel Souček)

Multifunctional cytokines from Transforming Growth Factor-β family play crucial role in regulation of cytokinetics (proliferation, differentiation and apoptosis) in various types of normal and transformed cells. One of the divergent members of TGF-β family is Growth/Differentiation Factor -15 (GDF-15). Functional role of GDF-15 is not known in all details. On one hand its increased expression is often associated with effects of various chemopreventive compounds; on the other hand it also follows progression of some cancer diseases. Our recent study demonstrated that expression of GDF-15 is not essential for anti-proliferative effects of nonsteroidal anti-inflammatory drugs (NSAIDs). However we were able to demonstrate that sensitivity of the cells to the antiproliferative effects of NSAIDs depends on deregulation of PKB/Akt signaling pathway.

In 2009 we continued with studies focused on (1) functional role of GDF-15, (2) role of TGF- β in pathological plasticity of epithelial cells, (3) mechanisms of neuroendocrine differentiation (NED) of prostate cancer. Studies investigating GDF-15 have shown modulation of differentiation of osteoclasts. Our studies proved novel properties of GDF-15 which can help to clarify its role in cancer progression. TGF-β cytokines belongs to the

important inductors of epithelial-mesenchymal transition (EMT). This process is crucial for dissemination of cancer cells and metastasis development. In our work, we established novel application of cellular impedance analysis for dynamic, non-invasive monitoring of EMT in real-time. Application of such an approach opens a new opportunity for study of this important process in vitro. NED of epithelial prostate cancer cells is phenomena clearly associated with cancer progression. However, mechanisms controlling differentiation of prostate epithelial cells have remained poorly characterized. Surprisingly we described that induction of neuroendocrine phenotype by androgen ablation is associated with acquisition of senescent phenotype. Increase of SA-β-gal activity (general marker of senescence) by cell density is reversible process, but when induced by androgen ablation, the increased activity is permanent. Mechanisms driving this process are currently under investigation. The successful completion of these studies will help to understand mechanisms of cancer progression and reveal innovative strategies for treating prostate cancer in terminal stages.

Molecular mechanisms of Wnt signaling (Vítězslav Bryja)

We studied biochemical and biological properties of canonical and non-canonical Wnt signaling. We have identified novel and surprising role of the Lrp6 receptor in the non-canonical Wnt signaling pathway. Using in vitro techniques, and Xenopus and mouse in vivo models, we identified Lrp6 as physiologically relevant negative regulator of non-canonical Wnt signaling. Loss of Lrp6 leads to the overactivation of non-canonical Wnt signaling pathway, which then results in the developmental defects associated with non-canonical Wnt signaling such as defects in the neural tube closure or in the gastrulation movements. In collaboration with other groups we studied the role of Wnt signaling in the differentiation of stem cells. We were able to show (using Wnt5a-deficient embryonic stem cells) that Wnt5a is required for endothelial differentiation of ES cells and that Wnt/β-catenin signaling acts as a negative regulator of the neural differentiation of ES cells (using Wnt1- and Lrp6-deficient ES cells). Further we focused on the role of Wnt signaling in neural stem cells. We were able to show that canonical Wnt signaling controls the decision between glial and neuronal fates, and that the inhibition of the canonical Wnt signaling promotes gliogenesis in P0 neural stem cells. In neural stem cells we have identified a novel protein CXXC5, which can bind Dishevelled, a key mediator of Wnt signaling, and inhibit its function in the Wnt cascade.

Mechanisms of fibroblast growth factor signaling (Pavel Krejčí)

Fibroblast growth factor receptor 3 (FGFR3) is a transmembrane tyrosine kinase that serves as a receptor for the members of fibroblast growth factor (FGF) family and functions in many biological processes including cell proliferation, differentiation, migration and survival. To date, activating mutations in FGFR3 have been associated with several human disorders such as skeletal dysplasias, multiple myeloma, and cervical and bladder carcinomas. There is no treatment available for achondroplasia at present, thus inciting the development of novel approaches to target FGFR3. Therefore the research was focused in this direction.

Experiments aimed on discovery of novel small molecule inhibitors of FGFR3 signaling: We recently developed a chondrocyte-based high-throughput screening assay for identification of novel inhibitors of FGFR3 signaling in a chondrocyte environment. Using this assay, we identified a novel, potentially therapeutic inhibitor of FGFR3. This work was also a subject of recently approved patent application (PCT/US09/54340: Methods of Inhibiting FGFR3 Signaling).

Experiments aimed to determine yet unknown phenotypes of FGFR3 signaling in disease: We characterized a novel cellular senescence-like phenotype induced by FGFR3 signaling in chondrocytes. For the first time we described the molecular and cellular features of a premature senescence induced by aberrant FGFR3 signaling in chondrocytes, and discussed similarities between the known effects of FGFR3 signaling in skeletal dysplasias and oncogene-induced cellular senescence.

During our analysis of FGF signaling in the proliferating chondrocytes we have summarized recent progress in the field of FGF biology and human disease. This work resulted in two reviews – one focusing on biology of height molecular weight FGF2 and second focusing on the involvement of individual members of the FGF family in human diseases.

Cellular and molecular toxicology (Jan Vondráček)

The principal aim of our studies is to contribute to understanding of effects of environmental organic pollutants at molecular and cellular level, which might be linked to carcinogenesis, reproductive or developmental impairment. At the same time, these toxicological data help us to

understand the physiological role of key signaling proteins that are affected by environmental toxicants, and their functional interactions with other signaling pathways. A principal protein, which is responsible for the action of toxic compounds, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and dioxins, is the aryl hydrocarbon receptor (AhR). Nevertheless, many persistent organic pollutants that fail to activate this transcription factor may also exert significant toxic effects within an organism. Therefore, in 2009, we further concentrated both on the role of AhR in regulation of key metabolic enzymes responsible for the mutagenicity of PAHs and on the effects of non-dioxin-like compounds on intercellular communication. We characterized the impact of a model non-dioxin-like PCB 153 on key proteins involved in formation of gap junctions or adherents junctions. Moreover, we found that PCB 153 may affect not only degradation of gap and adherents junction constituents, such as connexin 43, E-cadherin or β-catenin, but also the signaling function of the latter one, which is an integral part of canonical Wnt signaling pathway. We characterized the role of AhR in the regulation of expression and activities of critical enzymes participating in metabolic activation of PAHs in rat liver progenitor cells, including both CYP1 family enzymes (CYP1A1, CYP1A2, CYP1B1), cytosolic aldo-keto reductases (e.g. AKR1C9) or NQO1. In collaboration with the Centre of Molecular Medicine (Institute of Virology, Slovak Academy of Sciences), we participated in a study that described previously unknown interactions of AhR and hypoxia-inducible factor-1 in regulation of carbonic anhydrase IX, a model target of HIF-1. The present results these results contribute to our understanding of the possible mechanisms of toxic actions of non-dioxin-like PCBs at cell membrane, which might be of relevance to their tumor promoting properties. The interactions of AhR with NRF2 or HIF-1 transcription factors shed further light on the multifaceted effects of AhR ligands on transcriptional regulation in vertebrates.

Mechanisms of the effects of platinum derivatives (Alois Kozubík)

The platinum(II) derivatives (e.g. cisplatin, oxaliplatin) are used in the therapy of many solid cancers, but their negative side effects and cell resistance limit their therapeutic application. Therefore, there is a need for introduction and characterization of the effects of new platinum-based compounds capable of overcoming at least some of these limitations. In this respect, LA-12, a novel Pt(IV) compound with bulky hydrophobic ligand adamantylamine, is a very promising candidate known to exert cytotoxic effect, and no cross-resistance in a panel of cisplatin-sensitive and resistant

cell lines of different origin. We have previously shown the ability of LA-12 to overcome acquired and intrinsic resistance to conventionally used cisplatin in ovarian cancer cells. We also demonstrated the significantly higher efficiency of LA-12 compared to cisplatin and oxaliplatin in inhibition of proliferation, and triggering cell death in colorectal cancer cell lines. Therefore, we further focused our attention to more-detailed examination of mechanisms of LA-12-induced cell cycle modulation, and apoptosis in these cancer cell types. The LA-12 response was particularly studied in HT-29 human colon adenocarcinoma cell line, grown in various degrees of confluence, and we compared LA-12-induced effects with those exerted by cisplatin and oxaliplatin. Importantly, we showed that LA-12 is able to overcome confluence-dependent resistance of HT-29 cells, which was observed in other platinum compounds. Our results highlight the selective advantage of application of LA-12, compared with several platinum(II)-based drugs, in treatment of solid tumors, including the slowly growing types.

In addition to being used as a single agent, our further work supports the role of LA-12 or cisplatin as important candidates to be used in combination therapy with TRAIL. TRAIL is a cytokine which can selectively trigger apoptosis in various cancer cell types. However, the resistance of some cancer cells TRAIL may hamper the previous expectations resulting from the unique killing abilities of this cytokine. The platinum-based drugs have been suggested as interesting agents capable of sensitizing the resistant cancer cells to TRAIL-induced apoptosis. We showed that platinum(IV) complex LA-12 or cisplatin enhanced killing effects of TRAIL in human colon HCT-116 cells and prostate cancer cells PC-3, which was associated with stimulation of activity of initiator caspase-8 and effector caspase-3, and overall apoptosis. Our results highlight the striking ability of LA-12 to sensitize the cancer cells to TRAIL-induced apoptosis even when applied in significantly lower doses compared to cisplatin. Molecular mechanisms responsible for the effects observed are currently under investigation in our laboratory. Our observation will help to improve therapeutical approaches to cancer diseases in terms of more efficient killing of cancer cells, while minimizing the side effects of the therapy.

Granted projects

GA AS CR 1QS500040507, Lipid nutrition compounds-modulation of their effects and possibilities of practical application. Principal investigator: A. Kozubík, 2005 - 2009

PLIVA-LACHEMA - Project LA-12, Principal investigator: A. Kozubík, 2006 - 2009

GA CR 301/07/1557, Novel anticancer platinum complexes - mechanisms of their action and innovative chemotherapy. Principal investigator: A. Kozubík, 2007 - 2011

GA CR 524/07/1178, Importance of cell lipid changes during differentiation and apoptosis of colon epithelial cells. Principal investigator: J. Hofmanová, 2007 - 2011

ESF - MEYS CZ.1.07/2.3.00/09.020, Expanding the qualification competencies of doctoral study graduates at FMD UP, Principal co-investigator: J. Hofmanová, 2009 - 2011

GA CR 303/09/H048, Molecular mechanisms of selected pathological processes in the cell, Principal co-investigator: J. Hofmanová, 2009 - 2012

GA CR 204/07/0834, Role of transforming growth factor-beta in regulation of proliferation, differentiation and apoptosis in prostate and colon cancer. Principal investigator: K. Souček, 2007 - 2009

GA CR 310/07/0961, The role of environmental pollutants in mechanisms regulating development of prostate carcinoma, Principal co-investigator: K. Souček, 2007 - 2010

MZD 9600-4, Modulation of signaling pathways leading to neuroendocrine differentiation in prostate cancer. Principal investigator: K. Souček, 2008 - 2011

MZD 9956-4, Significance of asporin and other extracellular matrix proteins in invasive carcinomas of breast and prostate, Principal co-investigator: K. Souček, 2008 - 2011

GA CR 524/09/1337, Interactions of Wnt and Ah receptor signaling in regulation of functions of liver cells. Principal investigator: J. Vondráček, 2009 - 2011

GA CR 305/09/1526, The role of extracellular matrix-mediated cell adhesion in maintenance of colonic tissue homeostasis and in colon carcinogenesis. Principal investigator: M. Hýžďalová, 2009 - 2011

GA CR 204/09/J030, The role of beta-arrestin in achieving Wnt-signaling specificity. Principal investigator: V. Bryja, 2009 - 2011

GA CR 204/09/H058, Intercellular signaling in development and disease, Principal co-investigator: J. Vondráček, 2009 - 2012

MU Rektor‘s Programme for Students‘ Creative Activity Support MUNI/31/C0008/2008, Differences of adhesive properties and anoikis regulation in colon cells during interaction of fatty acids with cytokines of TNF family. Principal investigator: L. Kočí, 2008 - 2009

MU Rektor’s Programme for Students‘ Creative Activity Support MUNI/31/C0005/2008, Role of TGF beta family proteins in the cytokinetics of prostate and colon cancer cells. Principal investigator: E. Lincová, 2008 - 2009

MU Rektor‘s Programme for Students‘ Creative Activity Support MUNI/31/A0004/2008, Modulation possibilities of cytokine IL-6 signal transduction in prostate epithelial cells. Principal investigator: A. Staršíchová, 2008 - 2009

MU Rektor′s Programme for Students′ Creative Activity Support MUNI/31/A0003/2008, Possibilities in modulation of neuroendocrine differentiation of prostate cancer cells. Principal investigator: Z. Pernicová, 2008 - 2009

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PhD. thesis defended in 2009

Mgr. Lenka Umannová, PhD., Mechanisms of action of toxic aromatic compounds *in vitro*

Mgr. Lenka Stixová, PhD., The role of p38 MAPK in regulation of monocytic differentiation induced by 1 alfa,25-dihydroxyvitamin D3 and 5-lipoxygenase inhibitors in human leukemia cells

FREE RADICAL PATHOPHYSIOLOGY

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The effects of H1-antihistamines on the nitric oxide production by RAW 264.7 cells with respect to their lipophilicity

H1-antihistamines are known to be important modulators of inflammatory response. However, the information about the influence of these drugs on reactive nitrogen species generation is still controversial. We investigated the effects of selected H1-antihistamines on nitric oxide production by lipopolysaccharide-stimulated murine macrophages RAW 264.7, measured as changes in inducible nitric oxide synthase (iNOS) protein expression in cell lysates by Western blotting and nitrite formation in cell supernatants using the Griess reaction. In pharmacological non-toxic concentrations, H1-antihistamines significantly inhibited nitrite accumulation that was not caused by the scavenging ability of drugs against nitric oxide, measured amperometrically. The degree of inhibition of nitrite accumulation positively correlated with the degree of tested lipophilicity, measured by reversed-phase thin layer chromatography. Furthermore, H1-antihistamines differentially modulated the iNOS protein expression. In conclusion, the

modulation of nitric oxide production could be caused by the downregulation of iNOS protein expression and/or the iNOS protein activity.

Carvedilol and adrenergic agonists suppress the lipopolysaccharide-induced NO production in RAW 264.7 macrophages via the adrenergic receptors

The interaction of adrenergic agonists and/or antagonists with the adrenergic receptors expressed on immunologically active cells including macrophages plays an important role in regulation of inflammatory responses. We determined the effects of carvedilol, a unique vasodilating beta-adrenergic antagonist, and endogenous adrenergic agonists (adrenalin, noradrenalin, and dopamine) and/or antagonists (prazosin, atenolol) on lipopolysaccharide-stimulated nitric oxide (NO) production from murine macrophage cell line RAW 264.7. The production of NO was determined as the concentration of nitrites in cell supernatants (Griess reaction) and inducible nitric oxide synthase (iNOS) protein expression (Western blot analysis). Scavenging properties against NO were measured electrochemically. Carvedilol in a concentration range of 1, 5, 10 and 25 μM inhibited iNOS protein expression and decreased the nitrite concentration in cell supernatants. Adrenalin, noradrenalin or dopamine also inhibited the iNOS protein expression and the nitrite accumulation. Prazosine and atenolol prevented the effect of both carvedilol and adrenergic agonists on nitrite accumulation and iNOS expression in lipopolysaccharide-stimulated cells. These results, together with the absence of scavenging properties of carvedilol against NO, imply that both carvedilol and adrenergic agonists suppress the lipopolysaccharide-evoked NO production by macrophages through the activation and modulation of signaling pathways connected with adrenergic receptors.

GROUP OF PATHOPHYSIOLOGY OF FREE RADICALS IN CELL INTERACTIONS

GROUP LEADER
MILAN ČÍŽ

Oxidative modification of collagen influences breast cancer stem cell response to HNE

Breast cancer represents leading cause of mortality and morbidity in women, mostly due to property of primary tumor to metastasize. It was revealed recently that metastases comprise a fraction of stem-like cells, denoted as cancer stem cells (CSCs), usually located in the bone marrow. CSCs are of great importance in cancer biology as they are involved in blood vessel formation, promotion of cell motility and resistance to therapies and especially to metastasis development. One of the important factors influencing the stem cell destiny is their microenvironment and their interaction with extracellular matrix (ECM). Taking together the role of ECM in determining cell destiny and the involvement of lipids, lipid metabolism and lipid peroxidation in breast cancer development, we wanted to investigate the interactions between ECM and the growth regulating lipid peroxidation product 4-hydroxynonenal (HNE) on breast cancer stem cells. Our results indicate that oxidative modification of ECM collagen influences CSC growth, morphology and reaction to extracellular oxidative stress mediated by HNE and the growth inhibiting effects of this aldehyde. This is of importance as oxidative modification of ECM proteins could occur during local inflammation and during chemotherapies which cause lipid peroxidation. These modifications could be toxic for cancer and change gene expression, motility or stage of differentiation of malignant cells eventually maintaining oxidative homeostasis that could act against cancer.

Comparison of the antioxidant properties of vegetables using various methods

The present study investigates the antioxidant properties of selected vegetables, using the total peroxyl radical-trapping parameter (TRAP), oxygen radical absorbance capacity (ORAC) and hydroxyl radical averting capacity (HORAC) methods. ORAC, TRAP and HORAC values well correlated with polyphenol content. A good correlation was found also

between the methods for measuring antioxidant capacity. Nevertheless, ORAC has been found to be the most sensitive method to measure chain breaking antioxidant activity. Although we have found a good correlation between TRAP, ORAC and HORAC, using more than one antioxidant assay is recommended for more detailed understanding the principles of antioxidant properties of samples.

GROUP OF FREE RADICALS IN REGULATION OF CELL PHYSIOLOGY

GROUP LEADER
LUKÁŠ KUBALA

Modulation of arachidonic and linoleic acid metabolites in myeloperoxidase-deficient mice during acute inflammation

Acute inflammation is a common feature of many life-threatening pathologies, including septic shock. One hallmark of acute inflammation is the peroxidation of polyunsaturated fatty acids forming bioactive products that regulate inflammation. Myeloperoxidase (MPO) is an abundant phagocyte-derived hemoprotein released during phagocyte activation. Here, we investigated the role of MPO in modulating biologically active arachidonic acid (AA) and linoleic acid (LA) metabolites during acute inflammation. Wild-type and MPO-knockout (KO) mice were exposed to intraperitoneally injected endotoxin for 24 h, and plasma LA and AA oxidation products were comprehensively analyzed using a liquid chromatography-mass spectrometry method. Compared to wild-type mice, MPO-KO mice had significantly lower plasma levels of LA epoxides and corresponding LA- and AA-derived fatty acid diols. AA and LA hydroxy intermediates (hydroxyeicosatetraenoic and hydroxyoctadecadienoic acids) were also significantly lower in MPO-KO mice. Conversely, MPO-deficient mice had significantly higher plasma levels of cysteinyl-leukotrienes with well-known proinflammatory properties. In vitro experiments revealed significantly lower amounts of AA and LA epoxides, LA- and AA-derived fatty acid diols, and AA and LA hydroxy intermediates in stimulated polymorphonuclear neutrophils isolated from MPO-KO mice. Our results demonstrate that MPO modulates the balance of pro- and anti-inflammatory lipid mediators during acute inflammation and, in this way, may control acute inflammatory diseases.

A myeloperoxidase promoter polymorphism is independently associated with mortality in patients with impaired left ventricular function

Circulating levels of myeloperoxidase (MPO) predict adverse outcome in patients with impaired left ventricular (LV) function. The MPO -463 G/A promoter polymorphism (rs 2333227) regulates MPO transcription, with the

G allele being linked to increased protein expression. The aim of this study was to assess the prognostic information derived from the -463 G/A MPO polymorphism on outcomes of patients with impaired LV function. The -463 G/A promoter MPO genotype as well as MPO plasma levels were determined in 116 patients with impaired LV function. Patients were prospectively followed for a median of 1050 days. The GG genotype was associated with a decrease in overall survival (chi(2) 5.80; p=0.016). This association remained after multivariate adjustment for plasma levels of NT-proBNP, creatinine, hsCRP, and MPO; leukocyte count; and LV function (hazard ratio 3.16 (95% CI 1.17-8.53), p=0.024) and for classical cardiovascular risk factors (hazard ratio 2.88 (95% CI 1.13-7.33), p=0.026). Interestingly, we observed no association of the MPO polymorphism with total MPO protein concentration or MPO activity in plasma. The -463 G/A MPO polymorphism is linked to adverse clinical outcome of patients with impaired LV function. Further studies are needed to elucidate the value of this polymorphism for risk stratification.

The effect of different molecular weight hyaluronan on macrophage physiology

Hyaluronan, a linear glycosaminoglycan, is an abundant component of extracellular matrix. In its native form, the high-molar-mass hyaluronan polymers have an array of structural and regulatory, mainly anti-inflammatory and anti-angiogenic, functions. In contradiction, the biological effects of fragmented low molecular weight hyaluronan are suggested to be pro-angiogenic and pro-inflammatory. The effects of highly purified pharmacological grade hyaluronan of defined molecular weights 11, 52, 87, 250 and 970 kilodaltons were tested on mouse macrophage cell lines RAW 264.7 and MHS. The surface expression of CD44 and Toll-like receptor 2, surface receptors for hyaluronan, was determined by flow cytometry. Activation of macrophages was determined based on nitric oxide and tumour necrosis factor alpha production, inducible nitric oxide synthase expression, and the activation of the nuclear factor kappa B transcriptional factor. Both macrophage cell lines expressed CD44 and Toll-like receptor 2, which were significantly increased by the pre-treatment of macrophages with bacterial lipopolysaccharide. Hyaluronan of any molecular weight did not activate production of nitric oxide or tumour necrosis factor alpha in any mouse macrophage cell lines. Correspondingly, hyaluronan of any tested molecular weight did not stimulate nuclear factor kappa B activation. Similarly, hyaluronan of any molecular weight neither exerted stimulatory

nor inhibitory effects on macrophages pre-treated by lipopolysaccharide. Interestingly, the data does not support the current view of low molecular weight hyaluronan as a pro-inflammatory mediator for macrophages. Further studies are necessary to clarify the effects of different molecular weight hyaluronan on phagocytes.

The comparison of impedance-based method of cell proliferation monitoring with commonly used metabolic-based techniques

Determination of cell numbers is a crucial step in studies focused on cytokinetics and cell toxicity. The impedance-based analysis employing electronic sensor array system xCELLigence System allowing label-free dynamic monitoring of relative viable adherent cell amounts was compared with the most utilized methods for relative quantification of viable cell numbers based on a determination of cellular metabolism. In this study, colorimetric assay based on reduction of tetrazolium salt (MTT) by mitochondrial enzymes and chemiluminiscent assay based on intracellular adenosine triphosphate (ATP) determination were compared with the impedance-based system. Cell morphology was compared by microscopic evaluation. Normal human epidermal keratinocytes (NHEK) and normal human dermal fibroblasts (NHDF), together with 3T3 mouse fibroblast and HaCaT keratinocyte cell lines were employed. The progress of cell growth curves obtained by different methods during 72 hours reflected cell type and cell seeding densities. The impedance-based method was found to be applicable for the determination of the cell proliferation of 3T3 fibroblasts, HaCaT and NHDF, since the comparison of this method with ATP and MTT determinations showed a comparable results. In contrast, the proliferation of NHEK measured by the impedance-based method did not correlate with other methodological approaches. This could be accounted to the specific morphological appearance of these cells. The study shows the impedance-based detection of viable adherent cells is a valuable approach for cytokinetics and pharmacological studies. However, the specific morphological characteristics of cell lines have to be considered employing this method for determination of cell proliferation without using other reference methods.

Granted projects

GA CR 524/07/1511, Interactions between collagen, platelets and neutrophils with respect to wound healing. Principal investigator: M. Číž, 2007 - 2009

GA CR 524/08/1753, The influence of L-arginine and its analogues on the generation of reactive oxygen and nitrogen species by professional phagocytes. Principal investigator: A. Lojek, 2008 - 2012

GA CR 305/08/1704, Role of hyaluronan of different molecular weight in the course of inflammation. Principal investigator: L. Kubala, 2008 - 2010

GA CR 204/07/P539, The role of uric acid in endothelial dysfunction. Principal investigator: I. Papežíková, 2007 - 2009

MEYS - Kontakt MEB 0808106, The role of platelets, neutrophils and components of extracellular matrix in inflammation. Principal investigator: A. Lojek, 2008 - 2009

AS CR M200040908, international collaboration, Role of myeloperoxidase in the regulation of platelets physiology. Principal investigator: L. Kubala, 2009 - 2011

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Vištejnová, L., Dvořáková, J., Hašová, M., Muthny, T., Velebný, V., Souček, K., Kubala, L.: *The comparison of impedance-based method of cell proliferation monitoring with commonly used metabolic-based techniques*. Neuroendocrinology Letters, 30 (Suppl), 2009, 121-127.

PhD. thesis defended in 2009

Mgr. Roman Konopka, PhD., Role of reactive oxygen species in cell differentiation

Mgr. Jana Moravcová, PhD., Pharmacological modulation of the nitric oxide production in the process of inflamation

STRUCTURE AND DYNAMICS OF NUCLEIC ACIDS

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We have carried out a wide range of investigations of structural dynamics and molecular interactions of nucleic acids, using a variety of methods such as long time-scale explicit solvent molecular dynamics (MD) simulations, quantum chemistry calculations and bioinformatics.

Explicit solvent molecular dynamics simulations represent a computational tool that allows to complement experimental studies of nucleic acids molecules by providing some specific insights not easily derivable by the experiments (e.g., describe flexibility of functional RNA segments, delineate effects of various base substitutions and predict details of hydration and monovalent cation binding to RNA). However, the simulations are limited by time scale of the simulations and by quality of the utilized model, i.e., the molecular mechanical force field. We performed an extensive molecular dynamics study (0.6 μs in total) on three A-RNA duplexes where we studied dependence of the A-RNA geometry on base sequence, molecular mechanical force field and salt strength conditions. The simulations revealed surprising dependence of the canonical A-RNA double helix conformation on the base sequence, which may contribute to fine tuning of A-RNA properties such as elasticity. The results also revealed

that the Parmbsc0 AMBER force field makes the A-RNA duplex more compact in comparison to the Parm99 force field (Fig. 1) by preventing temporary sugar phosphate substates with α/γ trand/trans configurations.

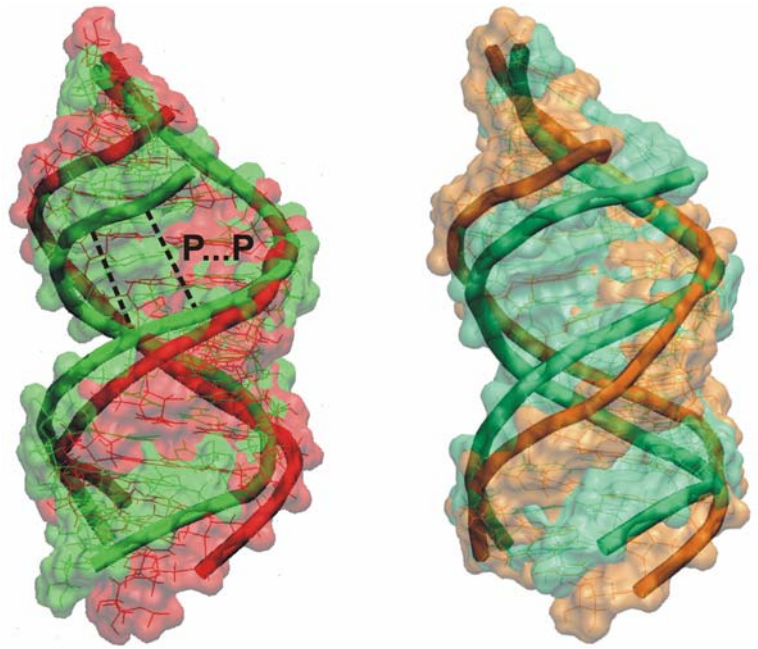

Figure 1: Left - Superposition of duplexes with alternating -CG- sequence simulated with the same force field (parmbsc0) but using different ionic strength. Red duplex was simulated in net-neutralizing Na+ atmosphere while the green one was simulated with excess salt KCl resulting in more compact geometry. Right - Superposition of duplexes with alternating -CG- sequence simulated in net-neutralizing Na+ atmosphere but using different force fields. Duplex simulated with parm99 is in orange while duplex simulated with parmbsc0 is in green. Two of six measured Phosphate/Phosphate distances (P...P) are represented by black dashed lines.

The stabilization of the A-RNA helices caused by the Parmbsc0 force field includes visible reduction of the major groove width, increase of the base pair roll, larger helical inclination and small increases of twist. Therefore, the Parmbsc0 shifts the simulated duplexes more deeply into the A-form. The reduction of the major groove width is also observed in excess salt KCl

simulations (Fig. 1), again accompanied by larger roll, inclination and twist. In addition, the simulations revealed that the effect of the force field and salt condition is sequence-dependent.

We continued in our molecular dynamics studies of DNA quadruplex (G-DNA) molecules. We carried out an extensive set (more than 1.5 μs in total) of MD simulations of two G-DNA molecules: the antiparallel d(G4T4G4)2 dimeric quadruplex with diagonal loops and the parallel-stranded human telomeric monomolecular quadruplex d[AGGG(TTAGGG)3] with three propeller loops (Fig. 2).

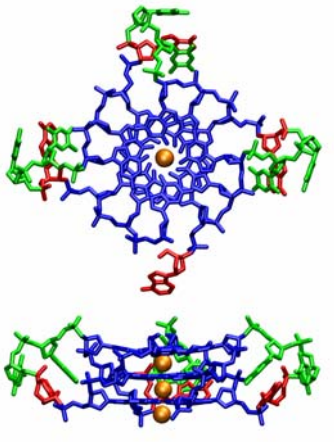

Figure 2: The crystal structure of the human telomeric monomolecular quadruplex d[AGGG(TTAGGG)3] with three propeller loops. Orange balls represent channel K+ ions; nucleotides are shown using green (thymine), red (adenine) and blue (guanine) color. Top – top view, bottom – front view (for higher clarity adenine at the 5' end is not shown).

We also complemented our MD simulations with Locally Enhanced Sampling simulations and free energy calculations. The main purpose of the study was testing of the capability of the MD simulation technique to describe single-stranded topologies of G-DNA loops, which present a very challenging task for computational methods. We also tested several versions of the AMBER force field parameters (Parm99, Parmbsc0, and a version of modified glycosidic χ torsion profile) and the CHARMM force field. However, we found that none of the presently available force fields is accurate enough in describing the G-DNA loops. The imbalance is best seen

for the propeller loops of the human telomeric quadruplex, as their experimental structure is lost within a few ns of standard simulations with all force field versions. Among them, parmbsc0 provides results which are clearly closest to the experimental target values but still not in full agreement. This confirms that the recent improvement of the γ torsional profile penalizing the γ trans substates in the parmbsc0 force field was a step in the right direction, albeit not sufficient to treat all imbalances. The modified χ torsion parameters rigidify the studied systems but do not change the ultimate outcome of the present simulations.

Our results confirm that G-DNA loops represent one of the most difficult targets for molecular modeling approaches and should be considered as reference structures in any future studies aiming to develop or tune nucleic acids force fields.

In cooperation with foreign laboratories, we have carried out molecular dynamics simulations of several RNA systems, namely Hairpin ribozyme, RNA motif called Kink-turn and internal RNA loop with tandem G/A base pairs.

We have also continued extensive quantum chemical studies of key interactions in nucleic acids. Trans Hoogsteen/sugar edge (H/SE) RNA base pairs form one of the six families of RNA base pairs that utilize the 2'-hydroxyl group of ribose for base pairing and play key roles in stabilizing folded RNA molecules (Fig. 3).

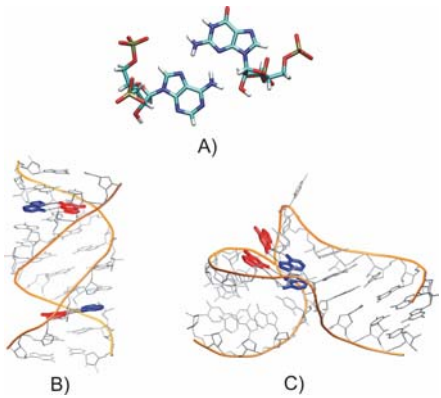

Figure 3: The trans H/SE (sheared) A/rG pair (a) occurs in major recurrent RNA motifs such as Loop E (b) and K-turn (c).

We have carried out a detailed quantum chemical characterization of the intrinsic structures and interaction energies of this base pair family, along with the evaluation of solvent screening effects via continuum solvent approach. We have computed DFT-optimized geometries and MP2 interaction energies for all ten crystallographically identified members of the family, for a representative set of them using the Complete Basis Set extrapolation. For six out of the ten base pairs, we had to apply geometrical constraints to keep geometries relevant to RNA. We have confirmed that the remaining, hitherto undetected, possible members of this family do not have appropriate steric features required to establish stable base pairing in the trans H/SE fashion. The interaction patterns in the trans H/SE family are very diverse with gas phase interaction energies within the range from -1 kcal/mol to -17 kcal/mol. Except of the C/rC and G/rG H/SE base pairs, the interaction energy is roughly evenly distributed between the electrostatic and electron correlation components. Thus, in the trans H/SE base pairs the relative importance of the electron correlation is noticeably smaller than in the cis WC/SE or cis and trans SE/SE base pairs, but still larger than in canonical base pairs. For two trans H/SE base pairs, namely C/rC and G/rG, the Hartree-Fock component of stabilization clearly dominates the binding, due to the favorable orientation of the molecular dipoles of the nucleobases. The trans H/SE A/rG basepair is the intrinsically most stable member of this family. This base pair is also known as the sheared AG base pair and belongs to the most prominent RNA base pairs utilized in molecular building blocks of functional RNAs. For all trans H/SE base pairs we have identified, besides the conventional base pairing, viable alternative structures stabilized via amino-acceptor interactions. In the QM calculations these amino acceptor complexes appear equally stable to those with common H-bonds and, more importantly, the switch to amino-acceptor interaction would not require any significant geometrical rearrangement of the base pairs.

We have also carried out a set of ultra-accurate energy computations on selected RNA base pairs and uracil dimer stacks with steric clashes. Such calculations play major role in verification and parametrization of more simple computational methods.

Structured RNA molecules form complex 3D structures stabilized by multiple interactions involving the nucleotide base, sugar, and phosphate moieties. A significant percentage of the bases in the 3D structures of 16S and 23S ribosomal RNA (rRNA) hydrogen-bond with phosphates of other nucleotides. By extracting and superimposing base-phosphate (BPh)

interactions from a reduced-redundancy subset of 3D structures from the Protein Data Bank (PDB), we have identified recurrent phosphate binding sites on the RNA bases. In addition, we have carried out quantum chemical calculations on model systems representing each BPh interaction. The calculations show that the centers of each cluster correspond to energy minima on the potential energy hypersurface. We have compared the 3D structures of the 16S and 23S rRNAs of *E. coli* and *T. thermophilus* to identify conserved BPh interactions. We have found that most conserved BPh interactions occur in hairpin, internal, or junction loops or as part of tertiary interactions. Bases which form BPh interactions that are conserved in the 3D structures are also coserved in rRNA sequence alignments. Furthermore, bases making BPh interactions are more conserved than bases not making such interactions, even after adjusting for other interactions being made. Thus, BPh interactions have a selective effect on RNA sequence, and so are relevant to RNA bioinformatics.

Granted projects

GA AS CR IAA400040802, Structure, dynamics and reaction mechanism of catalytic RNA. Principal investigator: J. Šponer, 2008 - 2011

GA AS CR 1QS500040581, Metallodrugs, design and mechanism of action. Principal investigator: O. Vrána, Principal co-investigator: J. Šponer, 2005 - 2009

GA AS CR IAA400550701, Structure and dynamics in complexes of solvated biomolecules. Principal investigator: J.E. Šponerová, 2007 - 2009

ME CR LC06030, Biomolecular Center. Principal investigator: V. Sklenář, Principal co-investigator: J. Šponer, 2006 - 2010

GA CR 203/09/1476, Structural dynamics, molecular interactions and function of key RNA motifs. Principal investigator: J. Šponer, 2009 - 2012

GA CR 203/09/H046, Biochemistry on the crossroad from in silico to in vitro. Principal investigator: M. Otyepka, Principal co-investigator: J. Šponer, 2009 - 2012

GA AS CR KJB400040901, Computational study of RNA multiple junctions localized in functionally important sites of the ribosome. Principal investigator: K. Réblová, 2009 - 2011

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PhD. thesis defended in 2009

Maryna V. Krasovska, PhD., Structural dynamics of the catalytic RNA: computational study of the hepatitis delta virus ribozyme

CD Spectroscopy of Nucleic Acids

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Arrangements of human telomeric DNA quadruplex in physiologically relevant K+ solutions

Telomeres play an important role in cellular aging and cancer. Guanine-rich strands of telomeric DNA form qudraplexes, which are pivotal elements for maintaining telomere integrity and controlling cancer cell proliferation. The arrangement of the human telomeric quadruplex in physiologically relevant conditions has not yet been unambiguously determined. Distinct quadruplex structures were observed by various methods. We have shown that the arrangement of the telomeric DNA is polymorphous: The core quadruplex sequence G3(TTAG3)3 forms an antiparallel quadruplex of a basket type in solution containing either K+ or Na+ ions. Analogous sequences extended by flanking nucleotides (studied in other laboratories) form a mixture of the antiparallel and hybrid, so called (3 + 1), quadruplexes in K+-containing solutions. We have, however, shown that long telomeric DNA behave in the same way as the basic G3(TTAG3)3 motif: They fold into an antiparallel quadruplex structure. Both G3(TTAG3)3 and long telomeric DNA are also able to adopt the (3 + 1) quadruplex structure: Molecular crowding

conditions, simulated e.g. by ethanol, induce a slow transition of the K+-stabilized antiparallel quadruplex into the hybrid quadruplex structure and then into a parallel quadruplex. Most importantly, we demonstrate that the same transitions can be induced even in aqueous, K+-containing solution by increasing the DNA concentration. This is why distinct quadruplex structures were detected for AG3(TTAG3)3 by X-ray, nuclear magnetic resonance and circular dichroism spectroscopy: Depending on DNA concentration, the human telomeric DNA can adopt the antiparallel quadruplex, the (3 + 1) structure, or the parallel quadruplex in physiologically relevant concentrations of K+ ions.

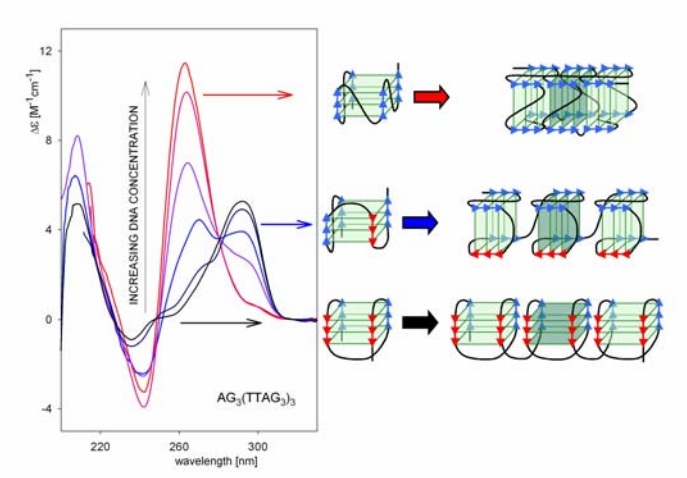

Figure 1: Polymorphism of human telomeric DNA quadruplex. CD spectra corresponding to particular quadruplex arrangements of a human telomeric sequence. On the right there are schematic drawings of particular, concentration dependent quadruplex types of a basic quadruplex unit G3(TTAG3)3 and of a long telomeric DNA chain.

Influence of guanine for adenine mutation in the human G3(TTAG3)3 telomere DNA on its quadruplex folding

DNA of all living organisms is constantly exposed to damages by endogenous oxidation, hydrolysis, and replication errors as well as exogenous genotoxic chemicals and physical agents that lead to mutations. One of frequent spontaneous point mutations is a guanine for adenine

conversion. We have studied the formation and structural properties of quadruplexes of the human telomeric DNA sequence G3(TTAG3)3, in which each guanine base was successively replaced by an adenine base. None of these single base substitutions hindered the formation of antiparallel quadruplexes, as shown by circular dichroism, gel electrophoresis, and UV thermal stability measurements in NaCl solutions. Effect of substitution did differ, however, depending on the position of the substituted base. The A-for-G substitution in the middle quartet of the antiparallel basket scaffold led to the most distorted and least stable structures and these sequences preferred to form bimolecular quadruplexes. Unlike G3(TTAG3)3, no distinct structural changes were observed for intramolecular quadruplexes of the A-containing G3(TTAG3)3 analogs when sodium ions were replaced by potassium ions. Their basic quadruplex topology remained the same in both salts. As in vivo missincorporation of A for a G in the telomeric DNA is possible and potassium is a physiological salt, these findings may have biological relevance.

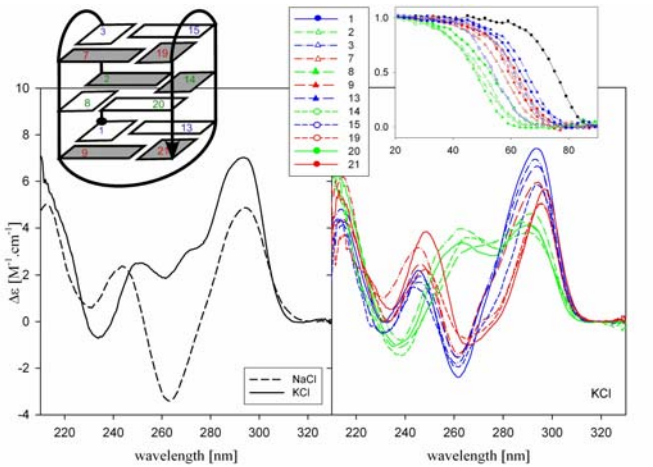

Figure 2: Damage to telomeric DNA sequence by an A for G mutation at particular positions of its quadruplex structure. Left: CD spectra of G3(TTAG3)3 under physiological Na + or K + ions concentrations and a schematic drawing of the G3(TTAG3)3 quadruplex in NaCl (syn- geometries of guanines are shadowed). Right: CD spectra reflecting structures of G3(TTAG3)3 analogs containing A instead of particular G´s and their stability in the presence of K + ions differ from the structure and stability of the intact sequence.

Circular dichroism and conformational polymorphism of DNA

We have reviewed studies that provided important information about conformational properties of DNA using circular dichroic (CD) spectroscopy. The conformational properties include the B-family of structures, A-form, Z-form, guanine quadruplexes, cytosine quadruplexes, triplexes and other less characterized structures. CD spectroscopy is extremely sensitive and relatively inexpensive. This fast and simple method can be used at low as well as high DNA concentrations and with short as well as long DNA molecules. The samples can easily be titrated with various agents to cause conformational isomerizations of DNA. The course of detected CD spectral changes makes possible to distinguish between gradual changes within a single DNA conformation and cooperative isomerizations between discrete structural states. It enables measuring kinetics of the appearance of particular conformers and determination of their thermodynamic parameters. In careful hands, CD spectroscopy is a valuable tool for mapping conformational properties of particular DNA molecules. Due to its numerous advantages, CD spectroscopy significantly participated in all basic conformational findings on DNA.

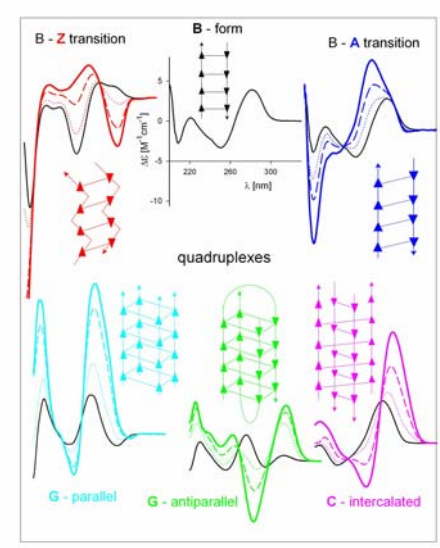

Figure 3: Circular dichroism and conformational polymorphism of DNA. CD spectra of particular DNA arrangements.

Granted projects

GA AS CR A100040701, Biophysical properties of biologically and medically important regions of human DNA. Principal investigator: M. Vorlíčková, 2007 - 2011

GA CR 204/07/0057, Tetraplexes in the human genome. Principal investigator: M. Vorlíčková, 2007 - 2009

GA CR 202/07/0094, Biophysics and bioinformatics of human genome regions with an extreme nucleotide distribution. Principal investigator: J. Kypr, 2007 - 2009

IGA MZ CR NR 9147-3/2007, Pathological microsatelite expansion in the human genome. Principal investigator: J. Kypr, 2007 - 2009

GA AS CR IAA500040903, Biophysics and bioinformatics of genome DNA fragments rich in guanine and adenine bases. Principal investigator: J. Kypr, 2009 - 2013

Publications

Kypr, J., Kejnovská, I., Renčiuk, D., Vorlíčková, M.: *Circular dichroism and conformational polymorphism of DNA*. Nucleic Acids Res., 37, 2009, 1713-1725.

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PhD. thesis defended in 2009

Mgr. Daniel Renčiuk, PhD., Relationship between DNA primary structure and conformation of biologically important regions of the human genome

PLANT DEVELOPMENTAL GENETICS

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We analysed genomic differences between mammals and angiosperms, two groups for which the most extensive genomic data from multiple species exist, and suggests that their genomes are undergoing radically different modes of evolution. The timing of the split between these groups is controversial, but current estimates suggest that it occurred 1000–2000 million years ago. Given their very long period of independent evolution, major differences in genome organization and evolution between the groups are to be expected. Nevertheless, exploring these differences can shed light on factors shaping the genomes of mammals and angiosperms. At the whole genome level (e.g., organization of DNA in the chromosome, diversity in chromosome number and genome size) there are substantial differences between mammals and angiosperms. Recombination plays a role in genome evolution because of its involvement in, for example, genomic rearrangements (chromosomal fusions, inversions and translocations), insertions (including organellar DNA), and repair and deletions of DNA sequences. Much evidence suggests that recombination rates are higher and activity more variable in angiosperms than in mammals, thus leading to differences in genome structure and long-term stability. The higher

recombination frequencies are reflected in the greater number of translocations that can occur during species divergence and higher linkage map recombination frequencies reported in angiosperms compared with mammals. Differences in recombination frequencies are also reflected in different frequencies of illegitimate DNA insertions into the genome via recombination.

In both angiosperms and mammals the most significant and abundant mobile elements are retrotransposons, which are major determinants of genome structure and evolution. Angiosperms contain predominantly LTR retrotransposons belonging to the copia and gypsy superfamilies. Within these there is massive diversity, with thousands or tens of thousands of elements contributing up to 80% of the genome in some species. LTR retrotransposons are less abundant, diverse and active in mammals. Instead the non-LTR retrotransposon classes LINEs (long interspersed nuclear elements) and non-autonomous SINEs (short interspersed nuclear elements) predominate. Angiosperms have higher background levels of retrotransposition than mammals, often caused by bursts of activity associated with hybridization, polyploidy. The sequestration of a germ line early in mammalian development means that there are relatively few cell divisions leading to gamete formation, particularly in oogenesis. By contrast, there is no sequestration of the germ line in angiosperms; instead, gametes are formed from somatic cells in the apical meristems. Even ephemeral species such as A. thaliana with short generation times (7 weeks) undergo many hundreds of divisions between the seeds of one generation and those of the next. For the majority of angiosperms this number is likely to be order(s) of magnitude larger. Because the number of mutations and cell divisions are positively correlated, there are many more opportunities for mutations to arise compared with mammals. Furthermore, whereas the mammalian germ line is largely protected from the environment, the angiosperm germ line is vulnerable to environmental stresses that can also stimulate mutations and retrotransposition.

Different life strategies might drive genomic differences between angiosperms and mammals (Fig. 1). Mammals are capable of high levels of mobility, enabling them to find food and mates and escape disease, predation and adverse conditions. Associated with this is a highly complex, yet constrained pattern of development. In contrast, the sessile nature of angiosperms means that they cannot readily escape adverse conditions, herbivores and poor environmental conditions or attract pollinators. Instead their survival depends on being able to respond to adverse conditions

through biochemical complexity and developmental plasticity, the tool kit for plant survival. This is reflected in the large number of genes (perhaps 25% of the total) involved in the production of secondary metabolites.

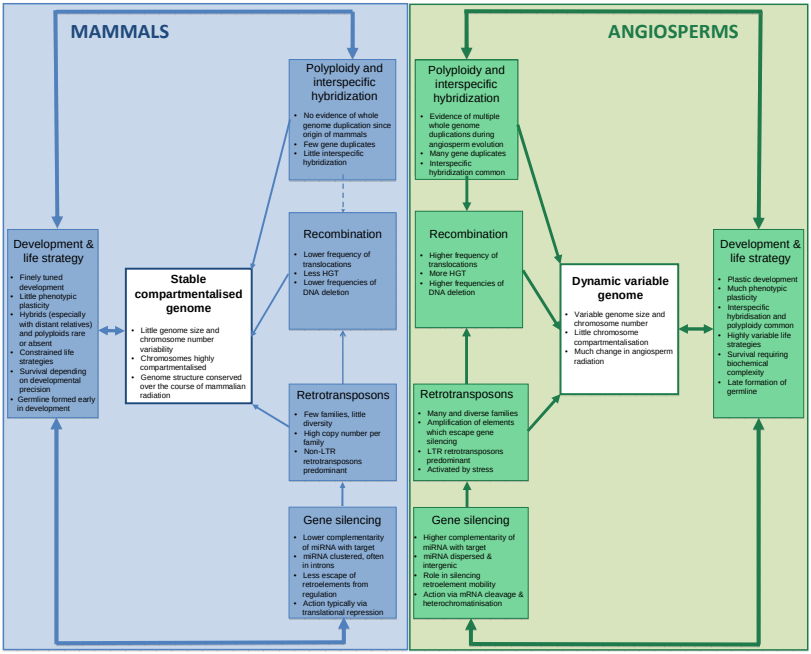

Figure 1: Different interrelationships and their relative strengths, represented by the direction and thickness of the arrows, between mechanisms generating genomic change and the life strategy options and developmental constraints in mammals and angiosperms. HGT refers to Horizontal Gene Transfer, the integration of DNA from sources outside of the nucleus.

The origin and evolution of sex chromosomes have interested evolutionary biologists for a long time. Although sex chromosomes evolve from a pair of autosomes, over time they become different, both from each other and the autosomes, in gene content and structure. While sex chromosomes in most mammals are ancient, sex chromosomes in some fish, insects and dioecious plants are evolutionarily young. Despite the different ages of sex chromosomes in different taxonomic groups, they probably follow similar evolutionary trajectories with discrete identifiable stages. Due to a stepwise

loss of recombination between the X and Y chromosomes, some processes of Y degeneration start to occur. One of important processes acting in non-recombining regions is gene degeneration. Degeneration could be a consequence of TE accumulation. The random inactivation model suggests that the process of gene inactivation is triggered by the disruption of promoter regions by TE insertion. TE insertions can lead to an epigenetic phenomenon, or global changes in chromatin status (heterochromatization).

We performed a comprehensive analysis of repetitive DNA distribution on sex chromosomes in Silene latifolia (Fig. 2). Most TEs are distributed uniformly along both the X and Y chromosomes. Two exceptions are Retand elements, which are localized at subtelomeres and Ogre-like elements, which are present on whole X chromosome but restricted to the PAR region of the Y chromosome. Tandem repeats colonize the centromeres (STAR-C) and subtelomeres (X-43.1) of X chromosome, whereas in the Y chromosome STAR-C and STAR-Y are located in the middle of both arms and X-43.1 is at the subtelomere of the q-arm. Telomere-like sequences are present also in centromeres of the X and Y chromosomes. It is evident that the Y chromosome has a different composition and localization of repetitive DNA compared with X chromosome and autosomes. The presence of sex chromosomes and their tendency to accumulate repetitive DNA gives this dioecious species evolutionary potential different from what one might expect in the hermaphroditic species. The content of repetitive DNA may have a role in phenotypic features.

The study of the molecular structure of young heteromorphic sex chromosomes of plants has shed light on the evolutionary forces that control the differentiation of the X and Y during the earlier stages of their evolution. We have used the model plant Rumex acetosa, a dioecious species with multiple sex chromosomes, 2n = 12 +XX female and 2n = 12 + XY1Y2 male, to analyse the significance of repetitive DNA accumulation during the differentiation of the Y. A bulk segregant analysis (BSA) approach allowed us to identify and isolate random amplified polymorphic DNA (RAPD) markers linked to the sex chromosomes. From a total of 86 RAPD markers in the parents, 6 markers were found to be linked to the Ys and 1 to the X.

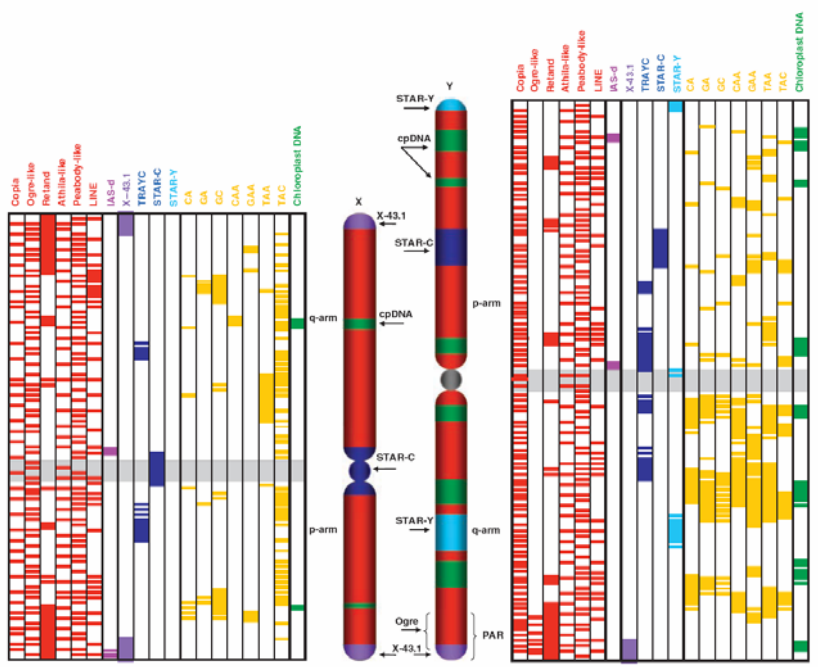

Figure 2: Schematic map of sex chromosomes of Silene latifolia with distribution of various types of repetitive DNA sequences—TEs (red), tandem repeats (blue), microsatellites (yellow) and chloroplast DNA (green). The patterns of elements distribution are derived from FISH data.

Two of the Y-linked markers represent two AT-rich satellite DNAs (satDNAs), named RAYSII and RAYSIII, that share about 80% homology, as well as with RAYSI, another satDNA of R. acetosa. Fluorescent in situ hybridization demonstrated that RAYSII is speciWc for Y1, whilst RAYSIII is located in different clusters along Y1 and Y2 (Fig. 3). The two satDNAs were only detected in the genome of the dioecious species with XX/XY1Y2 multiple sex chromosome systems in the subgenus Acetosa, but were absent from other dioecious species with an XX/XY system of the subgenera Acetosa or Acetosella, as well as in gynodioecious or hermaphrodite species of the subgenera Acetosa, Rumex and Platypodium. Phylogenetic analysis with diVerent cloned monomers of RAYSII and RAYSIII from both R. acetosa and R. papillaris indicate that these two satDNAs are completely separated from each other, and from RAYSI, in

both species. The three Y-specific satDNAs, however, evolved from an ancestral satDNA with repeating units of 120 bp, through intermediate satDNAs of 360 bp. The data therefore support the idea that Y-chromosome differentiation and heterochromatinization in the Rumex species having a multiple sex chromosome system have occurred by different amplification events from a common ancestral satDNA. Since dioecious species with multiple XX/XY1Y2 sex chromosome systems of the section Acetosa appear to have evolved from dioecious species with an XX/XY system, the amplification of tandemly repetitive elements in the Ys of the section Acetosa is a recent evolutionary process that has contributed to an increase in the size and differentiation of the already non-recombining Y chromosomes.

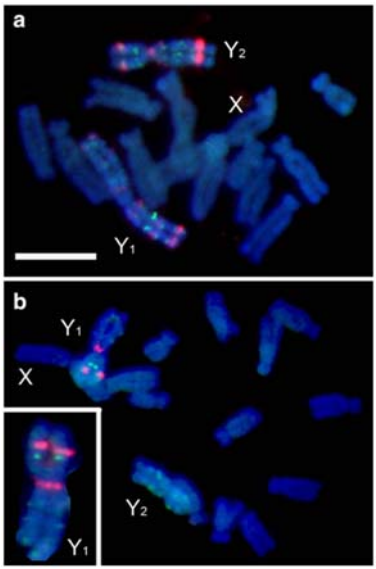

Figure 3: Chromosomal distribution of RAYS tandem repeats in R. acetosa analysed with bicolour FISH. Mitotic metaphase chromosomes of male R. acetosa (counterstained by DAPI, blue) were hybridised with (a) RAYSI (red signals, Cy3 labeled) and RAYSIII (green signals, SpectrumGreen labeled), (b) RAYSII (red signals) and RAYSIII (green signals). The X, Y1 and Y2 chromosomes are indicated, bar = 10 µm.

The chromosomal rearrangements are often considered to be the main mechanism leading to the suppression of recombination between X and Y chromosomes. There are, however, some data indicating that also other

mechanisms can play important role in this process. According to our hypothesis, the recombination between X and Y chromosomes can be at the beginning of sex chromosome evolution realized also via epigenetic modification of the non-recombining part of the Y chromosome. It is, therefore, possible to expect that each Y-chromosome has suppressed recombination ability to any of the X chromosomes and moreover to any other Y chromosome. In the case that inversions would be the only mechanism preventing X/Y recombination, some level of recombination between Y chromosomes should be present in X/Y non-recombining region.

In order to test recombination ability of the Y chromosomes, we have prepared Silene latifolia plants possessing one chromosome X and two genetically distinguishable Y chromosomes. Presence of the X chromosome is necessary as the plants carrying Y chromosomes only are non-viable. Recombination frequency was assessed between four molecular markers spread over the Y chromosome and between sexual phenotype and each of these molecular markers. Results showed that no recombination occurred between markers located in the non-recombining region of the Y chromosomes. Surprising results brought the study of the segregation of the pseudoautosomal molecular marker PAR2. Results indicate that there is present a small frequency of recombination between any of the Y chromosomes and the X chromosome but no recombination between the Y chromosomes was found. In this case, the role of inversions can be ruled out as both the Y chromosomes are able to recombine with the X chromosome. In the case of population specific inversion(s) on the Y chromosome (s), the Y chromosomes should significantly differ in recombination frequency with the X. Our study also brought new light in the issue of sex ratio inheritance and in the issue of sex specific expression. Results based on the study of the segregation of the X chromosome coming from XYY plant indicate that generally observed advantage of the pollen tubes carrying X chromosome does not apply to the pollen tubes that apart of the X chromosome carry also Y chromosome. From these results, it is also possible to deduce that sex specific expression (i.e., the Y chromosome caused difference in expression patterns and phenotype) is present already at the stage of pollen tube.

Males and females of animal species often differ in many morphological and behavioral traits. Recent studies have revealed that sexual dimorphism in animals is also common at the level of gene expression. In mammals, this expression difference between sexes is apparent not only at the later stages when the sexual phenotype is controlled by the action of sexual hormones

produced by gonads, but also in the pregonadal stage - i.e. before the initiation of gonadal development. In plants with separate sexes, sexual dimorphism seems to be much less pronounced than in animals. Among vascular plants displaying sexual dimorphism, Silene latifolia is the most studied species. So far, all the known S. latifolia sexually dimorphic traits are of quantitative character. The most prominent sexually dimorphic trait is flower number, with males producing several times more flowers than females. On the other hand, females usually produce more biomass, mainly because of formation of heavier stems, larger leaves and flowers. Many of these traits were confirmed to be genetically determined because either they have been QTL-mapped or display a response to genetic selection experiments.

With the aim to search for genes specifically expressed in male plants, we went through S. latifolia ESTs described as preferentially or specifically expressed in male flowers. As most of these ESTs were isolated before Arabidopsis thaliana genome sequencing and annotation, their homologies to any plant genes were mostly unknown. Using database search combined with phylogenetic analysis, we unambiguously identified A. thaliana orthologues of nine ESTs from S. latifolia. Six of these A. thaliana genes are expressed ubiquitously, which is in a disagreement with the reported expression of their S. latifolia orthologues. Results show that only six of them are expressed exclusively in male flower buds, twelve ESTs are expressed in leaves and flower buds of both sexes, and two ESTs start to be expressed in male flower buds earlier than in female flower buds. Importantly, we have also found one EST (Men470) that is specifically transcribed in male plants and one EST (CCLS79.1) that is transcribed specifically in female plants. RT-PCR performed on samples from bulks of six different male and female individuals did not differ from the results observed on single-plant analysis. These results clearly show that the male and female S. latifolia plants differ in their gene expression even before the initiation of flowering; this situation is similar to the pregonadal stage in mammals. The described ESTs are not only the first qualitative differences between the sexes at the vegetative stage, but also the first described sequences in plants connected with the sexual dimorphism before flowering at all. Our results implicate a possible route of the evolution of the sexual dimorphism in S. latifolia. The initial stages of the sexual dimorphism evolution are driven by the presence of the non-recombining region that attracts sexually-antagonistic genes. Candidate QTLs for these sexually antagonistic genes on the X chromosome have been already found, and the Y chromosome also most likely contains genes that evolved via this

mechanism. Genes with sex-preferential or sex-limited expression are expected to evolve later on. These genes should be differentially expressed at the initiation of flowering, when the sex-determining genes are active, as their expression is expected to be controlled by the sex-determination genes.

Granted projects

GA CR 204/09/H002, Plant developmental biology. Principal investigator: B. Vyskot, 2009 - 2012

MAYS LC06004, Integrative studies of plant genome. Principal investigator: B. Vyskot, 2006 - 2010

GA CR 521/08/0932, Horizontal gene transfer in plants. Principal investigator: B. Janoušek, 2008 - 2011

GA AS CR IAA600040801, Early phases of evolution of sex chromosomes: comparative study of *Silene otites, S. colpophylla* and *S. latifolia.* Principal investigator*:* B. Janoušek, 2008 - 2011

GA AS CR KJB600040801, Developmental pathways involved in the gynoecium suppression in dioecious plants. Principal investigator: J. Žlůvová, 2008 - 2010

GA AS CR M200040902, international collaboration, Structure and function of plant sex chromosomes. Principal investigator: B. Vyskot, 2009 - 2012

GA CR 522/09/0083, Isolation of genes linked to sex chromosomes and their use to study evolution of sex chromosomes in plants. Principal investigator: R. Hobza, 2009 - 2013

GA AS CR KJB600040901, Silene vulgaris as a model for comparative genomics. Principal investigator: R. Hobza, 2009 - 2011

GA AS CR M200040905, international collaboration, Genus Silene as a model for mating systems and adaptation mechanisms evolution - from ecology to genomics. Principal investigator: R. Hobza, 2009 - 2011

Publications

Bernasconi, G., Antonovics, J., Biere, A., Charlesworth, D., Delph, L.F., Filatov, D., Giraud, T., Hood, M.E., Marais, G.A.B., McCauley, D., Pannell, J.R., Shykoff, J.A., Vyskot, B., Wolfe, L., Widmer, A.: *Silene as a model system in ecology and evolution.* Heredity, 103, 2009, 5-14.

Kejnovský, R., Hobza, R., Čermák, T., Kubát, Z., Vyskot, B.: *The role of repetitive DNA in structure and evolution of sex chromosomes in plants*. Heredity, 102, 2009, 533-541.

Kejnovský, E., Leitch, I.J., Leitch, A.R.: *Contrasting evolutionary dynamics between angiosperm and mammalian genomes*. Trends in Ecology and Evolution, 24, 2009, 572-582.

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Marková, M., Vyskot, B.: *New horizons of genomic in situ hybridization (GISH).* Cytogenetic and Genome Research, 126, 2009, 368-375.

PhD. thesis defended in 2009

RNDr. Michaela Marková, PhD., A study of the evolution of the genus *Silene*

Prestigious International Project

HHMI, INTNL 55005613, Platinum and ruthenium compounds. From DNA damage to cancer chemotherapy. Principal investigator: J. Kašpárková, 2005 - 2010

Prestigious National Projects

ME, LC535, Center of Basic Research, Dynamics and organization of chromosomes during the cell cycle. Principal investigator: I. Raška. Principal co- investigator: S. Kozubek, 2005 - 2009

ME, 1M0021622409, Center of Applied Research, Stomatological research center. Principal investigator: J. Vaněk, Principal co-investigator: V. Vetterl, 2005 - 2009

ME, LC06035, Center of Basic Research, Center of biophysical chemistry, bioelectrochemistry and bioanalysis. New instruments for genomics, proteomics and biomedicine. Principal investigator: M. Fojta, 2006 - 2010

ME, LC06004, Center of Basic Research, Integration of research activities to study the plant genome. Principal investigator: B. Vyskot, 2006 - 2010

ME, LC06030, Center of Basic Research, Biomolecular Center, Principal co-investigators: V. Brabec, J. Šponer, 2006 - 2010

ME, LC06027, Center of Basic Research for Monoclonac Gamapathy and Multiple Myeloma, Principal co-investigator: E. Bártová, 2006 - 2010

Teaching activities - semestral courses (lectures, seminars, practical classes)

Masaryk University, Brno

Viktor Brabec: Seminar of the Department of molecular biophysics and pharmacology

Břetislav Brzobohatý: Structure and function of proteins

Milan Číž: Free radicals in animal physiology

Jiří Fajkus, Miloslava Fojtová: Structure and function of eukaryotic chromosomes

Jiří Fajkus: Seminar of the Department functional genomics and proteomics

Jiří Fajkus, Eva Sýkorová: Analysis of chromatin structure (practical training)

Jiří Fajkus: Applied genomics and proteomics

Miroslav Fojta, Emil Paleček: Chemical properties, structure and interactions of nucleic acids

Jiřina Hofmanová, Alois Kozubík: Health risks

Jiřina Hofmanová, Alois Kozubík: Genotoxicity and carcinogenesis

Jiřina Hofmanová, Jiřina Procházková, Karel Souček, Jan Vondráček, Pavel Krejčí: Molecular physiology of animals

Jiřina Hofmanová, Alois Kozubík, Jiřina Procházková: Special methods of animal physiology

Eduard Kejnovský, Roman Hobza: Evolutionary genomics

Aleš Kovařík: Special methods of microorganism analysis

Stanislav Kozubek, Eva Bártová: Molecular physiology of the genome

Stanislav Kozubek, Martin Falk: Radiation biophysics

Alois Kozubík, Jiřina Hofmanová: Physiology of cell systems

Alois Kozubík, Jiřina Hofmanová, Jiřina Procházková, Karel Souček, Jan Vondráček: Modern methods of cell biology

Lukáš Kubala: Special physiology of blood

Antonín Lojek: Immunology

Olga Nováková: Biophysics - seminar

Jiřina Procházková: Scientific work methodology

Karel Souček, Eva Lincová: Analytical cytometry

Karel Souček: Journal club - cancer biology

Naďa Špačková: Structure and dynamics of nucleic acids

Jiří Šponer: Introduction into molecular biophysics

Jiří Šponer: Molecular interactions and their role in biology and chemistry

Vladimír Vetterl: Introduction to molecular biophysics

Oldřich Vrána: Experimental methods of biophysics

Vladimír Vetterl, František Jelen: Bioelectrochemistry 1

Vladimír Vetterl, František Jelen: Bioelectrochemistry 2

Vladimír Vetterl: Physical properties of biopolymers

Jan Vondráček: Applied chemistry and biochemistry

Jan Vondráček: Physiology of pharmaceuticals and toxic compounds

Boris Vyskot: Developmental genetics

Boris Vyskot: Special seminar in English

Jitka Žlůvová: Evolutionary developmental genetics of plants

Palacký University Olomouc

Viktor Brabec: Biophysical seminar

Jana Kašpárková, Viktor Brabec: Molecular biophysics of mutagens, cancerogens and cytostatics

Jana Kašpárková: Molecular biophysics

Jiří Šponer: Structure and dynamics of nucleic acids

Boris Vyskot: Developmental biology and genetics

Boris Vyskot: Epigenetics

Mendel University of Agriculture and Forestry in Brno

Břetislav Brzobohatý: Molecular plant physiology

Roman Hobza: Gene engineering I

Boris Vyskot: Gene engineering II

University of Veterinary and Pharmaceutical Sciences Brno

Eduard Kejnovský, Roman Hobza: Structure and evolution of genomes

Comenius University in Bratislava

Veronika Ostatná: Medicinal physics 1

Veronika Ostatná: Medicinal physics 2

Charles University in Prague

Jiří Šponer: Structure and dynamic of nucleic acids

University of South Bohemia in České Budějovice

Eduard Kejnovský, Roman Hobza: Evolutionary genomics

University of Ostrava

Boris Vyskot: Developmental biology

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Standard services of the Center of Information Technologies (CIT) include maintenance of the local area network (LAN), the connection of the IBP LAN to Brno Academic Computer Network and to the Internet, maintenance of exchange and IP telephony, maintenance of the IBP e-mail server, including antivirus and antispam systems, maintenance of the IBP web server including design and data update, development and maintenance of computer hardware and software jointly used by all laboratories (servers, graphic workstations, PCs with connected scientific instruments) running under UNIX, MS Windows 2000/XP/Vista/7. CIT also provides consulting services for individual scientists.

Library – a part of CIT takes care of online access to scientific journals over Internet, manages subscriptions to scientific informational resources, manages information exchange among libraries, takes care of printed versions of journals and books in the IBP and arranges access of users to them. Library also collects and archives research results of scientists of the IBP.

Main attention of CIT was devoted to the security issues. Security patches were installed in time and antivirus databases were regularly updated. All e-mails are monitored at the server by a virus scanner together with special software designed to detect and defang dangerous elements inside e-mail messages (dangerous attachments are renamed, so that they cannot be run automatically on PC). In addition, e-mails are scanned by antispam system.

In the 2009 there was built EDUROAM (http://www.eduroam.org/, http://www.eduroam.cz/) network at the IBP. There were installed three access points and a radius server to manage user access to the network. The radius server was connected to the network of radius servers of academic

institutions of the whole world to enable user roaming. A visitor with EDUROAM account created in his home institution can use his account to connect to WiFi (or wired) network at the IBP and all other academic institutions connected to the EDUROAM network.

COST Working Group Meeting of D39/04/06 "Structure, recognition, and processing of DNA damage by antitumour metal-based compounds"

The workshop was organized by Prof. V. Brabec (a local organizer) from the Institute of Biophysics of the AS CR, v.v.i. in Hotel Mediterraneo, Palermo, Italy (June 30 to July 1, 2009). 17 scientists came from several countries including Czech Republic, France, Germany, Israel, Italy and UK. Members of the laboratories involved in this COST working group presented overviews of the activities and major achievements. The leaders of the groups also reported about other activities within their working groups, such as short term scientific missions and joint publications. Each contribution was followed by a discussion in which mostly young fellows reported in more detail on their results. It was concluded that the research in next period should be focused on the following major topics: (a) Novel DNA binding platinum, ruthenium and osmium compounds, (b) Structural changes induced by platinum, ruthenium and osmium complexes in DNA and proteins, (c) Mechanism, recognition and repair of DNA metallation, (d) New methods and techniques for metal-DNA interactions, (e) Pharmacological activity and drug design, (f) Transport of metallodrugs to tumor cells, (g) Reactions of metallodrugs with cancer cell extracts.

XXXIXth Annual Meeting of the European Society for New Methods in Agricultural Research
Conference focus: "Genomics and Proteomics in Plant and Animal Breeding"

The XXXIXth Annual Meeting of the European Society for New Methods in Agricultural Research (ESNA) with conference focus on "Genomics and Proteomics in Plant and Animal Breeding" was organized by the Mendel University in Brno including the Laboratory of Plant Molecular Biology, a joint research and teaching laboratory of the Mendel University in Brno and the Institute of Biophysics AS CR, v.v.i. The meeting took place at the conference centre of the Mendel University in Brno (August 25-29, 2009). The meeting brought together 169 participants from Belarus, Bulgaria, Czech Republic, Germany, Greece, Hungary, Italy, Poland, Romania, Russia, Serbia, Swiss, Turkey and Ukraine involved in development and application of new methods and approaches in agricultural research in working groups: (1) Food Preservation and Safety, (2) Advanced Methods in Animal Sciences, (3) Soil-Plant Relationships, (4) Plant Science and

Biotechnology, (5) Quality of Agro-Ecosystems and (6) Plant/Crop Protection and Biotechnology. Invited lectures were presented by world-recognized specialists in the above mentioned areas, including Prof. Marc van Montagu, Prof. Ann Depicker, Prof. Colin Turnbull, Prof. Klaus Palme, Prof. Wendy Harwood and others.

37th Annual Meeting of the European Radiation Research Society

The conference was organized by the Society for Radiobiology and Crisis Planning of the Jan Evangelista Purkyne Czech Medical Society. Co-organizers of the conference were the Institute of Biophysics, v.v.i., Academy of Sciences of the Czech Republic, Brno, and the Society of

Radiation Oncology, Biology and Physics of the Jan Evangelista Purkyne Czech Medical Society. Michal Hofer from the Institute of Biophysics, v.v.i., served as a co-chairman of the Scientific Committee of the conference. The event was held in hotel Diplomat, Prague, August 26-29, 2009. Conference topics were Radiation Physics, DNA repair, Molecular targets for radio-enhancement, radiation sensitivity, Genetic instability, bystander effect and radio-adaptation, Stem cells, Radiation carcinogenesis, Biological dosimetry, Radiation protection, accidents and consequence management, Pharmacological modulation of radiation damage, Radiation oncology, Normal tissue damage, Non-ionizing radiation, Radioecology, Heavy ions. The list of participants comprises 182 investigators from the Czech Republic, Germany, Austria, Ireland, Poland, Sweden, Korea, Russia, United Kingdom, Belgium, Italy, Ukraine, Moldova, U.S.A., Armenia, Japan, Finland, Hungary, Norway, France, Belarus, and Slovakia.

Analytical Cytometry V

In September 5-8, 2009, the traditional International Conference on "Analytical Cytometry V" – an official conference of the Czech Society for Analytical Cytology (CSAC) was organized in Olomouc. v.v.i., under the leadership of the Inst. of Exp. Botany of the AS CR, v.v.i. (Assoc. Prof. J. Doležel). The principal goal of this Conference was to create a platform for meeting and exchanging ideas in the field of advanced technologies of analytical cytometry (e.g. flow cytometry and cell sorting, laser scanning confocal microscopy, etc.). The number of participants (over 150. Principally from the CR but also from abroad), the high level of professionalism not only of foreign invited speakers, and the quality of discussion confirmed the rapidly growing level of these meetings. These outcomes were significantly augmented by the members of the Council of the CSAC and members of the organizing committee (Assoc. Prof. J. Hofmanová, Assoc. Prof. A. Kozubík, and Assoc. Prof. S. Kozubek) from the Institute of Biophysics of the AS CR, v.v.i., which was one of the main co-organizers of this Conference.

EMBO Workshop on "Apoptosis, Mitochondria and Cancer"

The prestigious EMBO Workshop on "Apoptosis, Mitochondria and Cancer" (MAC'09) took place in the Institute of Molecular Genetics of the AS CR, v.v.i. on October 1-3, 2009. This conference was attended by approx. 135 delegates (35 from the CR and 100 from all over the world including countries such as New Zealand, Australia, Germany, France, Japan, USA, Mexico, and Canada). Invited speakers were leaders of the research area. Besides the possibility of obtaining current information from the dynamically developing discipline and the possibilities of starting new contacts, the Workshop represented an ideal opportunity for self-presentation of Institutions of the AS CR including IBP. On the organization of the conference there participated a three-member committee, co-created by Prof. J. Neužil (principal organizer, Institute of Botany of the AS CR, v.v.i.), Assoc. Prof. A. Kozubík (Institute of Biophysics of the AS CR, v.v.i.), and Dr. L. Anděra (Institute of Molecular Genetics of the AS CR, v.v.i.).

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