ACADEMY OF SCIENCES OF THE CZECH REPUBLIC

INSTITUTE OF BIOPHYSICS

RESEARCH REPORT

2004

Brno 2005

CONTENTS

I. INTRODUCTION

The year 2004 proceeded, above all, in token of the evaluation of 5-year research activities and the proposal of the Institutional Research Plan for the period 2005 - 2010. The Academy Council named evaluation committees for the respective scientific divisions. The evaluation of the Institute took place in June 14 under the participation of members of the Academy commission for the 2nd scientific division (Dr. J. Velemínský, DSc. - vice-president of the Academy, Ing. P. Ráb, DSc. as a chairman, Prof. Dr. S. Zadražil, DSc. as a reporter, Doc. Dr. J. Konvalinka CSc. and Dr. B. Vojtěšek, DSc. as members) and referees Prof. Dr. F. Azorín (Spain), Prof. Dr. G. Natile (Italy), Dr. T.M. Jovin (Germany) and Doc. Ing. J. Doležel (Czech Republic). Based on documents prepared by the Institute, reviews worked out by the referees as well as on facts presented by the Management at the evaluation, the Academy Commission ranked the Institute of Biophysics to the highest Ia category. The Institute obtained the 1st place in the 5th section of biological and medical sciences.

In the year 2004, the Academy Council, AS CR, amended rules for categorization of researchers to qualification degrees. The internal norm 3/2004 then introduced two categories of the junior researchers: 3a (postdoctoral fellow) and 3b (associated scientist/scholar). The respective categorization according to the research praxis was realized at the IBP from September 1, 2004.

The Institute Research Council included the same members as in 2003.

As in previous years, the Institute organized "*The Open Door Day*" (November 11 - 12) on the occasion of the Days of Science; the laboratories of the Institute were visited by 82 visitors.
Research activities were also popularized in journals (3 articles), in 2 lectures for the Czechoslovak Biological Society (Brno, April 14) and at the international symposium "*Protection of the ozon layer - chalenges and perspectives*" (Praha, November 19).

The following researchers were awarded for their scientific activities or obtained memberships in research institutions:

- *B. Vyskot* was awarded the Ist order Medal given by the Ministry of education, youth and sports of the CR as an evaluation of his activities in Accreditation Commission
- *A. Kovařík* was given the Prize of the president of the Grant Agency of the CR for the successful solving the project 521/01/0037
- *J. Kašpárková* and *N. Špačková* obtained the Prize of Otto Wichterle for young scientists of the Academy
- *R. Hobza* was nominated for the award "Česká hlava" in the category Doctorandus
- *R. Hobza, M. Falk* and *A. Vaculová* got the Prize of the Institute of Biophysics
- *J. Kypr* has been ranked to "Top 100 Scientists 2005", selected by the International Biographical Centre, Cambridge, UK
- *J. Fajkus* was nominated for the Prize of 15th International Chromosome Conference (September 5 – 10, 2004, Brunel University, London, UK)
- *E. Sýkorová* won the Prize of the Gregor Mendel Genetic Society
- *V. Brázda* obtained Nature Cell Biology Award for the poster presented at the 4th Dubrovnik Signaling Conference, Cavtat, Croatia, May 21 - 30, as well as the Young Investigator Award at the 2th HUPO Congress, Beijing, China (October 21 - 28)

Building and reconstruction activities:

The Academy Council supported financing of complex reconstruction works in the area of the Institute. The first phase of reconstruction started in 2000 and was completed by the end of 2003. As a result, a new laboratory space could be allocated to three research groups. In the year 2004 necessary static adjustments of the main building, reconstruction of isotope laboratories, reconstruction of the garden, construction of new garages and extension of the parking took place.

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II. SCIENTIFIC ACTIVITIES

Individual Laboratories are grouped into five Programs:

I. Biophysical Chemistry of Macromolecules

Laboratory of Biophysical Chemistry and Molecular Oncology - LBCMO
Prof. RNDr. Emil Paleček, DrSc.

Laboratory of Physics of Biomacromolecules - LBP
Prof. RNDr. Vladimír Vetterl, DrSc.

Laboratory of Structure and Dynamics of Nucleic Acids - LSDNA
doc. RNDr. Jiří Šponer, DrSc.

II. Biophysics of Nucleic Acid Complexes

Laboratory of Molecular Biophysics and Pharmacology - LMBP
Prof. RNDr. Viktor Brabec, DrSc.

Laboratory of DNA Molecular Complexes - LDMC
doc. RNDr. Jiří Fajkus, CSc.

Laboratory of Analysis of Chromosomal Proteins - LACP
RNDr. Michal Štros, CSc.

III. Biophysics and Bioinformatics of Genomes

Laboratory of CD Spectroscopy of Nucleic Acids - LSNA
doc. RNDr. Michaela Vorlíčková, DrSc.

Laboratory of DNA Biophysics and Bioinformatics of Genomes - LDBGB
RNDr. Jaroslav Kypr, CSc.

Laboratory of Molecular Epigenetics - LME
RNDr. Aleš Kovařík, CS

IV. Molecular Cytology and Cytogenetics

Laboratory of Molecular Cytology and Cytometry - LMCC
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Laboratory of Plant Development Genetics - LPDG
Prof. RNDr. Boris Vyskot, DrSc.

Laboratory of Plant Development Molecular Analysis - LMAPD
doc. RNDr. Břetislav Brzobohatý, CSc.

V. Kinetics of Cell Populations

Laboratory of Cytokinetics - LC
doc. RNDr. Alois Kozubík, CSc.

Laboratory of Pathophysiology of Free Radicals - LFRP
doc. RNDr. Antonín Lojek, CSc.

Laboratory of Experimental Hematology - LEH
MUDr. Michal Hofer, CSc.

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Laboratory of Computers and Information Systems - LCIS

RNDr. Josef Jursa, CSc.

Research projects were supported by grants:

Institutional Research Plan Z5004920

„Biophysical Properties of Living Systems and their Changes under the Influence of Environmental Factors“

In addition, IBP participated in two research plans of Universities in co-operation with Masaryk University - Faculty of Sciences and Faculty of Informatics.

Grant Agency of the Academy of Sciences of Czech Republic,

- 12 standard grants, 6 junior grants
- Program „The Support of the Targeted Research and Envelopment“, 4 grants
- Project „Development of Basic Science Research in the Key Areas of Science“, 3 grants

Grant Agency of the Czech Republic

- 25 individual grants, in 23 of these scientists of IBP were as principal investigators, in two grants as partial investigators
- 8 postgraduate grants
- 1 doctoral grant

Grant Agencies of Ministries of the Czech Republic

- Ministry of Health, CR, - 5 grants, in 4 of these scientists of IBP participated as principal investigators
- Ministry of Agriculture, CR, - 1 grant (co-investigator from IBP)
- Ministry of Education, Youth and Sports,
  - Program „Research Centres“ - 2 partial investigators from IBP
  - 5 grants under the Program „COST“
  - 7 grants onder the Program „KONTAKT“

Foreign Grant Agencies

12 grants

PROGRAM I

BIOPHYSICAL CHEMISTRY OF MACROMOLECULES

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As well as in the previous years, in 2004 the work in LBCMO was concentrated mainly to two research fields:

Field I. Properties of nucleic acids and proteins at surfaces and their application in DNA biodetectors.
Field II. Structure and interaction of DNA and proteins in oncological research especially with respect to the protein p53.

Work in the field I was focused on electrochemistry of nucleic acids and electrochemical sensors for DNA hybridization and DNA damage as tools potentially applicable in routine diagnostics. A considerable progress was attained in electrochemical analysis of proteins, their interactions and modification with chemical probes (these topics represent a junction towards the field II). The research focused on applications of electrochemical techniques in environmental analysis and in analysis of biological material was continued as well.

Surface-attached molecular beacons light the way for DNA sequencing
Rapid testing of DNA and RNA nucleotide sequences is required for various research protocols including wide-scale genetic testing, diagnostics, fast detection of biological warfare agents, environmental testing and forensic medicine. At present many laboratories are interested in research and development of an inexpensive, easy-to-use, fast-response device for this purpose. Various methods based on acoustic, electronic and optical detection of the DNA hybridization event have been reported.

Electrochemical Detection of DNA Triplet Repeat Expansion
Hereditary neurodegenerative diseases are connected with expansion of trinucleotide repetitive sequences in genomic DNA. Molecular diagnosis of these diseases is based on determination of the triplet repeat length. Currently used methods involve PCR amplification followed by electrophoretic determination of the amplicon size. We propose a novel electrochemical technique based on hybridization of target DNA (tDNA) immobilized at magnetic beads with a reporter probe (RP) complementary to the triplet repeats (12 units per RP). The biotin-labeled RP is detected via an enzyme-linked electrochemical assay involving binding of streptavidin-alkaline phosphatase conjugate and transformation of electroinactive 1-naphthyl phosphate to

electroactive 1-naphthol. Pyrimidine residues within sequences flanking the homopurine (GAA)n repeat in tDNA are premodified with osmium tetroxide, 2,2'-bipyridine (Os,bipy) introducing electroactive labels in tDNA. Length of the triplet expansion is calculated from the ratio of the intensities of electrochemical signals of hybridized RP/tDNA-Os,bipy. The normalized signal increases linearly with the repeat length between 0 and about 200 triplet units, allowing for discrimination between normal, pre-mutated and mutated alleles. Application of this method for detection of asymptomatic heterozygous carrier of expanded alleles is demonstrated.

Voltammetric determination of adenine, guanine and DNA using liquid mercury free polished silver solid amalgam electrode

Liquid mercury free polished silver solid amalgam electrode (p-AgSAE) proved experimentally as suitable substitute for the hanging mercury drop electrode in cathodic stripping voltammetric analyses of purine bases and of acid treated DNA. The analyses are done in alkaline solution in the presence of copper(II) ions. With p-AgSAE using direct current voltammetry the limits of detection for purine bases as well as for two types of DNA were on nanomol level. Low levels of RSD (1 - 3 %, n = 11) of repeated measurements confirmed that the used mode of electrochemical regeneration of p-AgSAE was sufficient, and it provided reliable measurement results. The mentioned working electrode is suitable for a sensitive electrochemical detection of acid-hydrolyzed DNA, and may be applied in DNA hybridization assays as an alternative to working electrodes based on liquid mercury (HMDE, meniscus AgSAE).

Microanalysis of DNA by stripping transfer voltammetry

A cathodic stripping transfer voltammetric procedure for trace determination of DNA and its components was described. The method is based on the DNA acid hydrolysis with subsequent electrochemical determination of released purine bases. In the first step, DNA is hydrolyzed for 30 min in 0.5 M perchloric acid at 75 °C. The electrochemical step involves generation of Cu(I)-purine base complex on a mercury electrode surface, transfer of electrode with accumulated complex into supporting electrolyte where voltammetric measurement is performed. Analysis is carried out in 14-µl drop volume (two-electrode connection) or in 30-µl drop (three-electrode connection) on a platinum plate, which is used as a counter electrode. Blank electrolyte contains 0.05 M borate buffer, pH 9.2 with 6.3 µM Cu(II). We could observe voltammetric signal at hydrolyzed nucleosides, nucleotides, ODN, and DNA containing purine bases.

We are able to accumulate under the controlled potential and determine subnanomolar concentration of DNA corresponding to the amount of 200 pg of DNA.

Electrochemical detection of specific DNA sequences from PCR amplicons on carbon and mercury electrodes using Meldola's blue as an indicator

The electrochemical parameters for (7-dimethyl-amino-1,2- benzo-phenoxazinium) Meldola's Blue (MDB), on binding to DNA at both a hanging mercury drop electrode (HMDE) and carbon paste electrode (CPE) are described. MDB, which interacts with immobilized calf thymus DNA, was detected using double stranded DNA modified HMDE or CPE (dsDNA modified HMDE or CPE), bare HMDE or CPE and single stranded DNA modified HMDE or CPE (ssDNA modified HMDE or CPE) in combination with adsorptive transfer stripping voltammetry (AdTSV) techniques and decreased peak currents were observed. The discrimination of dsDNA and ssDNA and detection of hybridization between synthetic oligonucleotides were determined from changes in the voltammetric peak of MDB. With the help of the planar phenoxazine ring, MDB was found to be intercalating between the base pairs of dsDNA. Several factors affecting the DNA immobilization, hybridization and indicator accumulation were investigated. The partition coefficient was also obtained from the signal of MDB with a dsDNA modified glassy carbon electrode (GCE). Specific DNA sequences from PCR amplicons were detected based on changes in the MDB reduction signal at the CPE. These results demonstrated that MDB could be used as an electroactive hybridization label for DNA biosensors.

Sensitive electrochemical determination of unlabeled MutS protein and detection of point mutations in DNA

MutS protein plays an important role in the DNA repair system in prokaryotic and eukaryotic cells; it recognizes unpaired and mispaired bases in duplex DNA and can be used for detection of point mutations in vitro. We have shown that small amounts of this protein can be detected electrochemically at mercury and carbon electrodes without any labeling. Using the constant current stripping analysis (CPSA) and mercury electrodes, tens of attomoles of this protein can be detected. The sensitivity of the determination at carbon electrodes is by more than 3 orders of magnitude lower. Using biotinylated DNA duplexes attached to magnetic beads single base mismatches and insertion/deletions were recognized

by MutS. Picogram amounts of this protein were detected by CPSA after MutS releasing from the beads.

Electroactivity of avidin and streptavidin. Avidin signals at mercury and carbon electrodes respond to biotin binding

Avidin and streptavidin were studied by phase-sensitive a.c. and cyclic voltammetry as well as by constant current chronopotentiometry at mercury (in alkaline media) and carbon electrodes (in acid medium). In contrast to the generally accepted belief that these proteins are electroinactive, we observed various electrochemical responses at these electrodes. Both proteins produced peaks due to oxidation of tyrosine and tryptophan residues at carbon electrodes and a catalytic peak H at a hanging mercury drop electrode. At the latter electrode avidin produced phase-in a.c. voltammetric and cyclic voltammetric peaks close to -0.6 V (peak S) which were assigned to Hg-S interactions, involving cystine/cysteine residues. In cobalt containing solution avidin produced a characteristic catalytic double wave requiring presence of cystine/cysteine residues in the protein molecule. Streptavidin, which does not contain these residues, yielded neither the catalytic double wave nor peak S. All the above avidin signals responded to biotin binding; peak S increased (up to 4 biotin molecules bound) while other avidin signals decreased as a result of biotin binding. A tentative scheme of interfacial behavior of avidin and avidin-biotin complex, depending on the electrode charge, was suggested.

Sensitive electrochemical detection of native and aggregated α-synuclein protein involved in Parkinson´s disease

The aggregation of α-synuclein, a 14 kDa protein, is involved in several human neurodegenerative disorders, including Parkinson´s disease. We studied native and *in vitro* aggregated α-synuclein by circular dichroism (CD), atomic force microscopy (AFM) and electrochemical methods. We used constant current chronopotentiometric stripping analysis (CPSA) to measure hydrogen evolution catalyzed by α-synuclein (peak H) at hanging mercury drop electrodes (HMDE) and square wave stripping voltammetry (SWSV) to monitor tyrosine oxidation at carbon paste electrodes (CPE). To decrease the volume of the analyte, most of the electrochemical measurements were performed by adsorptive transfer (medium exchange) from 3-6 μL drops of α-synuclein samples. With both CPE and HMDE we observed changes in electrochemical responses of α-synuclein corresponding to protein fibrillization detectable by CD, fluorescence and AFM.

Aggregation-induced changes in peak H at HMDE were relatively large in strongly aggregated samples, suggesting that this electrochemical signal may find use in the analysis of early stages of α-synuclein aggregation. This assumption was documented by marked changes in the peak H potential and height in samples withdrawn at the end of the lag and the beginning of the elongation phase. Native α-synuclein can be detected down to subnanomolar concentrations by CPSA.

Derivatisation of peptides with osmium tetroxide, 2,2′-bipyridine: capillary electrophoretic and MALDI–TOF mass spectrometric study
Site-specific chemical modification is a useful technology in characterisation of proteins, but the number of chemical probes of the protein structure reacting with proteins under mild conditions in aqueous solutions is rather limited. Here we studied the reaction of osmium tetroxide, 2,2′-bipyridine (Os,bipy) with several peptides using capillary zone electrophoresis (CZE) and matrix-assisted laser desorption-ionisation–time-of-flight mass spectrometry (MALDI–TOF MS). Both techniques showed formation of a stable complex of Os,bipy with tryptophan residues. In CZE peaks with different migration times and UV-Vis spectra were observed. MALDI–TOF MS showed the formation of a product with characteristic isotopic pattern corresponding to the presence of osmium atom. Oxidation of cysteine and methionine side chains to cysteic acid and methionine sulfone by Os,bipy was detected by CZE and confirmed by MALDI–TOF and post-source decay (PSD) mass spectra. PSD showed specific shifts of molecular weights of the peptides and their fragments after the derivatisation. We believe that Os,bipy may become a useful agent in the characterisation of proteins.

Electrochemical determination of lead and glutathione in a plant cell culture
Differential pulse anodic stripping voltammetry (DPASV) as the tool for analysis of lead in the plant cell culture was established. For the cultivation procedure, lead in Pb(II)-ethylenediaminetetraacetic acid (Pb-EDTA) chelate has been used. The detection limit of lead was found at 500 pM in phosphate buffer (pH 5.5), and 100 nM in prepared cells intracellular extract (20 pg Pb(II)/mg cells). For determination of cysteine-rich peptides, voltammetry in differential mode (DPV) in cobalt(Ill)-containing ammonia buffer (Brdicka reaction) was used. In this short communication, we present suitable voltammetric techniques for the physiological study of lead and thiols in plant cell culture.

Cyclic voltammetric study of the redox system of glutathione using the disulfide bond reductant tris(2-carboxyethyl)phosphine

Glutathione in the reduced (GSH) and oxidized (GSSG) forms was studied by cyclic voltammetry. Tris(2-carboxyethyl)phosphine (TCEP) as the disulfide bond reductant and/or hydrogen peroxide as the sulfhydryl group oxidant were used. Cyclic voltammetry measurements, following the redox state of glutathione, were performed on a hanging mercury drop electrode (HMDE) in borate buffer (pH 9.2). It was shown that in aqueous solutions TCEP was able to reduce disulfide groups smoothly and quantitatively. The TCEP response at -0.25 V vs. Ag/AgCl/3 M KCl did not disturb the signals of the thiol/disulfide redox couple. The origin of cathodic and anodic signals of GSH (at -0.44 and -0.37 V) and GSSG (at -0.69 and -0.40 V) glutathione forms is discussed. It was shown that the application of TCEP to the conservation of sulfhydryl groups in peptides and proteins can be useful instrument for the study of peptides and proteins redox behavior.

In the field II our attention was further focused on the structure and interations of the tumor suppressor protein p53. We succesfully continued investigations of the supercoil-selective DNA binding of p53 and the studies of regulation of the protein DNA-binding activities at the level of its posttranslational modifications. In collaboration with the Laboratory of Molecular Biophysics and Pharmacology, studies of p53 binding to DNA modified with novel platinum cytostatics have been carried out. After granting the FP6 EU integrated project (No. 502983), intensive research aimed to applications of electrochemical techniques and chemical probes in analysis of mutant p53 proteins was started.

Investigations of the supercoil-selective DNA binding of wild type p53 suggest a novel mechanism for controlling p53 function

The tumor suppressor protein, p53, selectively binds to supercoiled (sc) DNA lacking the specific p53 consensus binding sequence (p53CON). Using p53 deletion mutants, we have previously shown that the p53 C-terminal DNA-binding site (CTDBS) is critical for this binding. Here we studied supercoil-selective binding of bacterially expressed full-length p53 using modulation of activity of the p53 DNA-binding domains by oxidation of cysteine residues (to preclude binding within the p53 core domain) and/or by antibodies mapping to epitopes at the protein C-terminus (to block binding within the CTDBS). In the absence of antibody, reduced p53 preferentially bound scDNA lacking p53CON in the presence of 3-kb linear plasmid DNAs or 20-mer oligonucleotides, both

containing and lacking the p53CON. Blocking the CTDBS with antibody caused reduced p53 to bind equally to sc and linear or relaxed circular DNA lacking p53CON, but with a high preference for the p53CON. The same immune complex of oxidized p53 failed to bind DNA, while oxidized p53 in the absence of antibody restored selective scDNA binding. Antibodies mapping outside the CTDBS did not prevent p53 SCS binding. These data indicate that the CTDBS is primarily responsible for p53 SCS binding. In the absence of the SCS binding, p53 binds sc or linear (relaxed) DNA via the p53 core domain and exhibits strong sequence-specific binding. These data support a hypothesis that alterations to DNA topology may be a component of the complex cellular regulatory mechanisms that control the switch between latent and active p53 following cellular stress.

Activation of the DNA-binding ability of latent p53 protein by protein kinase C is abolished by protein kinase CK2

Tumor suppressor protein p53 is one of the most important regulators of cell proliferation and differentiation and of programmed cell death, triggering growth arrest and/or apoptosis in response to different cellular stress signals. The sequence-specific DNA-binding function of p53 protein can be activated by several different stimuli that modulate the C-terminal domain of this protein. The predominant mechanism of activation of p53 sequence-specific DNA binding is phosphorylation at specific sites. For example, phosphorylation of p53 by PKC (protein kinase C) occurs in undamaged cells, resulting in masking of the epitope recognized by monoclonal antibody PAb421, and presumably promotes steady-state levels of p53 activity in cycling cells. In contrast, phosphorylation by cdk2 (cyclin-dependent kinase 2)/cyclin A and by the protein kinase CK2 are both enhanced in DNA-damaged cells. We determined whether one mechanism to account for this mutually exclusive phosphorylation may be that each phosphorylation event prevents modification by the other kinase. We used nonradioactive electrophoretic mobility shift assays to show that C-terminal phosphorylation of p53 protein by cdk2/cyclin A on Ser(315) or by PKC on Ser(378) can efficiently stimulate p53 binding to DNA *in vitro,* as well as binding of the monoclonal antibody Bp53-10, which recognizes residues 371-380 in the C-terminus of p53. Phosphorylation of p53 by CK2 on Ser(392) induces its DNA-binding activity to a much lower extent than phosphorylation by cdk2/cyclin A or PKC. In addition, phosphorylation by CK2 strongly inhibits PKC-induced activation of p53 DNA binding, while the activation of p53 by cdk2/cyclin A is not affected by CK2. The presence of CK2-mediated

phosphorylation promotes PKC binding to its docking site within the p53 oligomerization domain, but decreases phosphorylation by PKC, suggesting that competition between CK2 and PKC does not rely on the inhibition of PKC-p53 complex formation. These results indicate the crucial role of p53 C-terminal phosphorylation in the regulation of its DNA-binding activity, but also suggest that antagonistic relationships exist between different stress signalling pathways.

GRANTS:

GA AS CR A4004402
Electrochemical detectors of DNA hybridization and their applications in DNA diagnostics
Principal investigator: M. Fojta, 2004 - 2007

GA AS CR A4004110
Binding of tumor suppressor protein p53 to DNA. The influence of DNA superhelicity and posttranslational modifications of the protein
Principal investigator: E. Paleček, 2001 - 2004

GA AS CR A1163201
Application of adsorptive transfer and elimination techniques in oligo-nucleotides and nucleic acids research
Principal investigator: L. Trnková, Fac. Sci. MU Brno, co-investigator: F. Jelen, 2002 - 2004

GA AS CR B5004203
Regulation of transcription factors binding to consensus sequences in superhelical DNA. Influence of phosphorylation of tumor supressor proteins p53, p73 and SMAD4 to their binding activity
Principal investigator: V. Brázda, 2002 - 2004

GA AS CR KJB4004302
Application of chemical structural probes and electroanalytical methods in DNA damage detection. Development of DNA sensors
Principal investigator: L. Havran, 2003 - 2005

GA AS CR IBS5004355
Possibilities of electrochemical methods in genomics.
Basis for development of DNA sensors.
Principal investigator: E. Paleček, 2003 - 2005

GA AS CR S5004009
Untraditional therapeutic approaches in oncology
Principal investigator: A. Kozubík, co-investigator: E. Paleček, 2000 - 2004

GA AS CR S5004107
Applications of biophysical methods in biotechnological and clinical praxis
Principal investigator: V. Vetterl, co-investigator: E. Paleček, 2001 - 2005

GA AS CR K4055109
Physics, chemistry and informatics for biological, ecological and medical applications
Principal investigator: K. Ulbrich, IMCH AS CR Prague, co-investigator: E. Paleček, 2001 - 2004

GA CR 203/04/1325
New approaches in development of electrochemical sensors for DNA damage
Principal investigator: M. Fojta, 2004 - 2006

GA CR 301/04/P025
Influence of DNA superhelicity on sequence specific and structure selective binding of the p53 protein
Principal investigator: V. Brázda, 2004 - 2006

GA CR 301/02/0831
Interaction of protein p53 and its homologues with DNA and their role in malignant tranformation
Principal investigator: B. Vojtěšek, MOÚ Brno, co-investigator: E. Paleček, 2002 - 2004

GA CR 204/02/0734
Interactions of tumor suppressor proteins with DNA. Roles of DNA structure and protein modifications
Principal investigator: M. Fojta, 2002 - 2004

GA CR 203/02/0422
New trends in electrochemistry of nucleic acids and their applications in environmental chemistry
Principal investigator: F. Jelen, 2002 - 2004

GA CR 204/03/0566
Electrochemistry in protein analysis and in detection of DNA hybridization
Principal investigator: E. Paleček, 2003 - 2005

IGA MH CR NC/7574 - 3
Recognition of DNA damage by tumor suppressor proteins. Effects of anti-cancer drugs
Principal investigator: M. Fojta, 2003 - 2005

Ministry of Education, Youth and Sports 1K04119
Interactions of mutant p53 proteins with genomic DNA *in vitro* and *in vivo*
Principal investigator: M. Fojta, key person: M. Brázdová, September 2004 - August 2007

Ministry of Industry and Trade 1H-PK/42
Research and development of a new-type electrochemical biosensor for the detection of nucleotide sequences and genotoxic agents in the environment
Project leader: M. Fojta, guarantor: E. Paleček

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

LABORATORY OF BIOMACROMOLECULE PHYSICS(LBP)

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BACHELOR:	DANA OHLÍDALOVÁ

The effect of methylation on the adsorption and two-dimensional (2-D) condensation of cytosine adsorbed at the mercury electrode was studied. 2-D condensation of 5-methylcytosine molecules adsorbed at the mercury surface and resulting in the formation of a compact layer and capacitance pit on C-E curves starts to occur at much lower bulk concentrations of 5-methylcytosine than it was observed with cytosine and is faster. The capacitance pit of 5-methylcytosine was observed at two different potential regions, around –0.5 V and –1.2 V. The dependence of the C-E curves on pH has shown that the capacitance pit appears in the range between pH 4 and pH 5.8, i.e. at the pH values close to the pK of 5-methylcytosine (pK = 4.6) similarly as it was observed with cytosine. The existence of the two separate potential regions of 2-D condensation of 5-methylcytosine can be explained by different orientations of adsorbed 5-methylcytosine molecules in the two potential regions. Maximum observed on C-E curves at the potential around -0.8 V is obviously tensammetric peak resulting from the reorientation of adsorbed molecules. Electrochemical impedance spectroscopy has proved the tensammetric character of this peak.

A study of adsorption of nucleic acids on pencil graphite electrode modified by a mercury layer of a different thickness using electrochemical and optical methods

The adsorption kinetics of the single stranded polyA (in 0.3 M NaCl + 50 mM Na2HPO4 , pH 8.3) on the pencil graphite electrode modified by a mercury layer (Hgm-PenE) of different thickness were measured. The rate constants of adsorption were measured from the plots of the surface coverage against the time of adsorption for polyA. The plots were fitted by Langmuir model and Diffusion controlled Langmuir model.

The surface of the bare pencil graphite electrode (PenE) is characterised in diffractive optical element based sensor (DOE) images by a lower value of intensity and poorly developed peaks, which means a rougher surface. The DOE images of the pencil graphite electrode modified by a mercury layer (Hgm-PenE) of different thickness are characterised by a higher value of intensity and sharper peaks, which means a smoother surfaces. The optical roughness Ra of the thin Hgm-PenE surfaces is higher compared with the thick Hgm-PenE surfaces. The surface of the polyA modified PenE is rougher compared with the polyA modified Hgm-PenE surfaces. The optical roughness Ra of the PenE surface after adsorption of polyA was increased. After adsorption of the polyA on the thin Hgm-PenE surface the value of the Ra was slightly decreased. In the case of the polyA adsorption on the thick Hgm-PenE surface the higher increases of the Ra-value was detected.

Effect of electromagnetic fields on biological objects

The effect of extremely low frequency (ELF) magnetic fields (f = 50 Hz, amplitude of magnetic field induction B_m = 10 mT) and highfrequency magnetic fields in GSM band used in mobile communication (f = 900 Mz, irradiation power 0.5 W.cm $^{-2}$) on bacteria *(Eschechirichia coli, Paracoccus denitrificans)*, yeast *(Saccharomyces cerevisiae)*, plant *(Arabidopsis thaliana)* and leukemic cells was studied.

1. The effect of ELF magnetic fields on *E. coli* was studied in our laboratory and in the Department of Medical Physics, Faculty of Medicine, University of P.J. Šafařík, Košice (J. Sabó). In both laboratories it was found that the ELF magnetic field causes the decrease of number of colony forming unit (CFU) in bacterial culture. Bacteria were exposed 4.5h since its inoculation. The decrease was larger with the longer exposure time and with higher magnetic field induction.

2. *P. denitrificans* were exposed to ELF magnetic field and the growth curves were measured after 24 hours. The decrease of the number of bacteria was observed after magn The denitrificatial activity of P.denitrificans was measured electrochemically using pentile graphite or pyrolytic graphite electrodes. Bacteria were adsorbed on the surface of the electrode and exposed to ELF magnetic field and high frequency electromagnetic field 900 MHz for 24 hours. The biological activity of bacteria was determined from the reduction peak of a voltamogram. The exposure to ELF field resulted in the decrease of reduction peak It means that ELF field has demaged bacteria. No effect was observed after 900 MHz field exposure.
3. Effect of ELF magnetic filed on yeasts was studied in cooperation with Faculty of Medicine, Masaryk University, Brno. Pilot studies showed changes in the growth curves of exposed yeasts and the dependenc on the time of exposure.
4. In cooperation with LMCC we have estimated the effects of ELF magnetic field on the cytoskelet of human leukaemia cells. The cytoskelet was damaged after long-time (4 days) magnetic exposure, however, no effect was observed after short-time (2 hours) exposure.
5. In cooperation with LPDG, the effect of ELF magnetic field on the DNA of *A. thaliana* was studied using the commet-assay technique. No chromosome damage after a short-time exposure (1 hour) was observed.

Dosimetric study – electromagnetic fields in the public transportation

We have started the dosimetric study in order to determine the ELF magnetic field exposure of people in public transportion in Brno, Ostrava and Prague. We have measured magnetic field induction in different types of wagons and in different passanger places. Until now no values exceeding the health limit were found.

GRANTS:

GA AS CR A4004404
Interaction of biopolymers with ligands and detection of their conformation changes at interfaces by electrochemical and optical methods
Principal investigator: V. Vetterl, 2004 - 2006

GA AS CR S5004107
Application of biophysical methods in biotechnological and clinical praxis
Principal investigator: V. Vetterl, 2001 - 2005

GA AS CR KJB4004305
Chemically modified solid electrodes in electrochemical analysis of nucleic acids and their components
Principal investigator: S. Hasoň, 2003 – 2005

GA AS CR K4055109
Physics, chemistry and informatics for biological, ecological and medical applications
Principal investigator: K. Ulbrich, IMCH AS CR Prague, co-investigator: V. Vetterl, 2001 - 2004

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

LABORATORY OF STRUCTURE AND DYNAMICS OF NUCLEIC ACIDS (LSDNA)

HEAD:	DOC. RNDR. JIŘÍ ŠPONER, DRSC.
SCIENTISTS:	MGR. NAĎA ŠPAČKOVÁ, PH.D.
	JUDIT E. ŠPONER, PH.D.

We have continued with intense computational studies of various aspects of structure, dynamics and molecular interactions of DNA and RNA, utilizing state-of-the-art molecular dynamics (MD) and quantum chemical methods.

MD simulations were utilized to study RNA-protein complex between *E. coli* Loop E – Helix IV (LE-HeIV) rRNA and ribosomal L25 protein. The simulations reveal a very good overall qualitative agreement between the experimental and simulated structures which is quite promising considering further studies of RNA-protein interactions. The major groove of LE is a prominent rRNA cation binding site. In absence of divalent cations, LE massively interacts with monovalent cations via inner-shell binding. The HeIV region shows clear bi-stability of its major groove explaining the observed difference between HeIV X-ray and NMR structures. In agreement with the experimental data, the simulations suggest that α1-helix of L25 is the least stable part of the protein. The rRNA-protein complex is, besides direct solute – solute contacts, mediated by a number of highly specific hydration sites with long-residing water molecules, some of them bound even permanently in the simulation. Long-residency hydration sites thus represent important elements of the three-dimensional structure of rRNA.

Ribosomal RNA K-turn motifs are asymmetric internal loops characterized by a sharp bend in the phosphodiester backbone resulting in "V" shaped structures, recurrently observed in ribosomes and showing a high degree of sequence conservation. We have carried out extended explicit solvent molecular dynamics simulations of selected K-turns, in order to investigate their intrinsic structural and dynamical properties. The simulations reveal an unprecedented dynamical flexibility of the K-turns around their X-ray geometries. The K-turns sample, on the nanosecond timescale, different conformational substates. The overall behavior of the simulations suggests that the sampled geometries are essentially isoenergetic and separated by minimal energy barriers. The nanosecond dynamics of isolated K-turns can be qualitatively considered as motion of two

rigid helix stems controlled by a very flexible internal loop which then leads to substantial hinge-like motions between the two stems. We suggest that K-turns are well suited to act as flexible structural elements of ribosomal RNA. They can mediate large-scale motions or they can allow a smooth assembling of the other parts of the ribosome.

Computational analysis of $d(GGGGTTTTGGGG)_2$ guanine quadruplexes containing either lateral or diagonal four-thymidine loops was carried out using a wide range of state-of-the-art computational techniques. The study provides for the first time, within the approximations of the applied force field, a qualitatively complete analysis of the available loop conformational space.

Binding modes of hydrated Zn^{2+} and Mg^{2+} cations to the N7 and O6 positions of guanine have been characterized by state-of-the-art ab initio model calculations. We analyzed how differences in the electronic structure of the cationic complexes translate to differences in the biological function of the two studied metals. The thermodynamic driving force of the metal binding process was estimated on the basis of interaction energies and total electronic energies. The computed results unambiguously reveal that the N7 position of guanine exhibits a greater propensity to bind Zn^{2+} than Mg^{2+} while both cations have a similar affinity to bind to O6. Contrary to the intuitive expectations, however, the computed data do not suggest any superiority of the N7 inner shell binding mode for Zn^{2+} compared to the O6 binding. For Mg^{2+} the O6 inner shell binding mode is favored over the N7 one.

Hydrogen-bonded nucleic acids base pairs substantially contribute to the structure and stability of nucleic acids. We performed a new quantum-chemical reference study of base pairing for a wide range of base pairs with binding energies spanning from -5 to -47 kcal/mol. The molecular structures were obtained using the RI-MP2 (resolution of identity MP2) method with extended cc-pVTZ basis set of atomic orbitals. The interaction energies were calculated using the Complete Basis Set (CBS) extrapolation at the RI-MP2 level. For some base pairs, Coupled-Cluster corrections with inclusion of noniterative triple contributions (CCSD(T)) were derived. The calculations were compared with selected medium quality methods. Very good performance of nonpolarizable Cornell et al. force field is confirmed and this indirectly supports the view that H-bonded base pairs are primarily stabilized by electrostatic interactions.

GRANTS:

MEdYS CR LN00A016
Program „Research Centres“, Biomoolecular Centre
Head: J. Šponer
Principal investigator, J. Koča, Fac. Sci. MU Brno, co-investigator: J. Šponer, 2000 - 2004

GR067507 Senior Wellcome Trust International Research Fellowship
Principal investigator: J. Šponer, 2003 - 2007

PROGRAM II

BIOPHYSICS OF NUCLEIC ACIDS COMPLEXES

LABORATORY OF MOLECULAR BIOPHYSICS AND PHARMACOLOGY (LMBP)

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	MGR. JANA KAŠPÁRKOVÁ, PH.D.
	MGR. VICTORIA MARINI, PH.D.
	MGR. OLGA NOVÁKOVÁ, DR.
	MGR. VIKTOR VÍGLASKÝ, PH.D.
	RNDR. MARIE VOJTÍŠKOVÁ, CSC.
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	ING. MILADA KOŘÍNKOVÁ
	EMILIE MORNSTEINOVÁ
GRADUATE STUDENTS:	MGR. VENDULA BURSOVÁ
	MGR. PETROS CHRISTOFIS
	ING. PAVLA HERINGOVÁ
	MGR. KATEŘINA CHVÁLOVÁ
	MGR. VLASTIMIL MAŠEK
	ING. JIŘÍ NAVRÁTIL
	MGR. RADIM PROKOP
	MGR. FRANCISCO JAVIER RAMOS
	ING. HANA RYBNÍČKOVÁ
	MGR. KRISTÝNA STEHLÍKOVÁ
	RNDR. JANA ZAHORECOVÁ
BACHELORS:	BC. KATEŘINA AMBROŽOVÁ
	BC. VLADAN BERNARD
	BC. JAN BREZOVSKÝ
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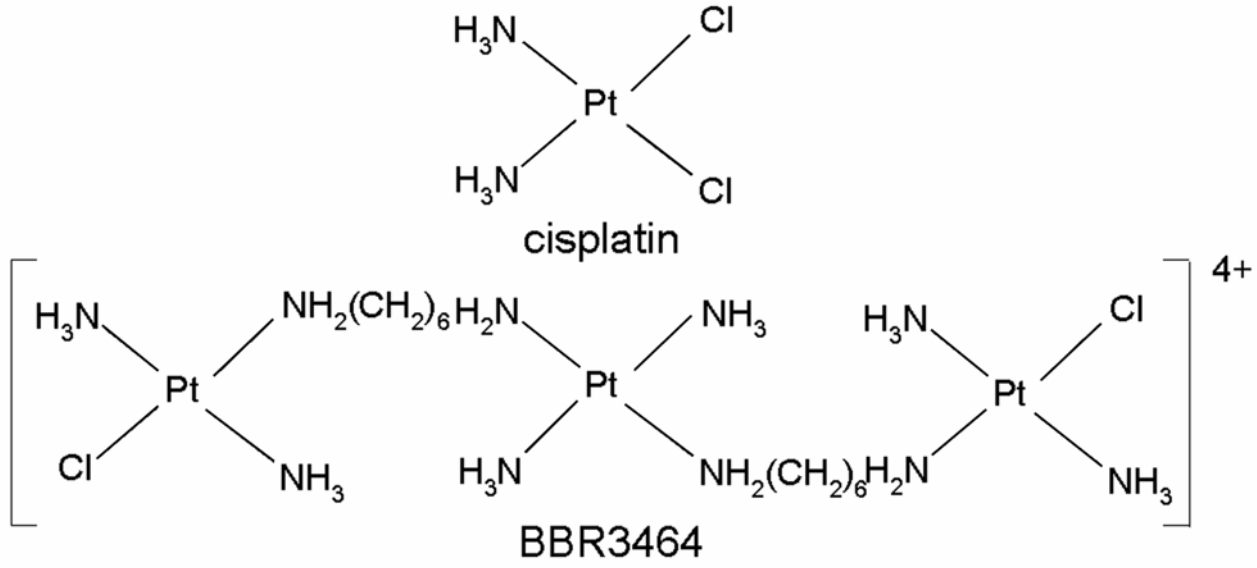
	IVA SEDLÁŘOVÁ
	MARTINA SLÁMOVÁ
	MAGDALENA ZÁVODNÁ

Differential recognition by the tumor suppressor protein p53 of DNA modified by the novel antitumor trinuclear platinum drug BBR3464 and cisplatin

Multinuclear platinum complexes represent a new class of anticancer drugs that contain two reactive platinum centers linked by a variable-length alkanediamine chain and are characterized by different DNA binding profile with respect to that of their mononuclear counterparts. The trinuclear platinum agent [{*trans*-$PtCl(NH_3)_2\}_2\mu$-*trans*-$Pt(NH_3)_2\{H_2N(CH_2)_6NH_2\}_2]^{4+}$ (BBR3464), the first example of this class to enter clinical trials, is more potent than conventional mononuclear cisplatin [*cis*-diamminedichloroplatinum(II)]. BBR3464 retains significant activity in human tumor cell

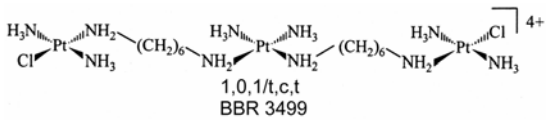


lines and xenografts that are refractory or poorly responsive to cisplatin, and displays a high activity in human tumor cell lines that are characterized by both wildtype and mutant p53 gene. In contrast, on average, cells with mutant p53 are more resistant to the effect of cisplatin. It has been hypothesized that the sensitivity or resistance of tumor cells to cisplatin might be also associated with cell cycle control and repair processes that involve p53. DNA is a major pharmacological target of platinum compounds and DNA binding activity of the p53 protein is crucial for its tumor suppressor function. This study, using gel-mobility-shift assays, was undertaken to examine the interactions of active and latent p53 protein with DNA fragments and oligodeoxyribonucleotide duplexes modified by BBR3464 in a cell free medium and to compare these results with

those describing the interactions of these proteins with DNA modified by cisplatin. The results indicate that structurally different DNA adducts of BBR3464 and cisplatin exhibit a different efficiency to affect the binding affinity of the modified DNA to p53 protein. It has been suggested that different structural perturbations induced in DNA by the adducts of BBR3464 and cisplatin produce a differential response to p53 protein activation and recognition and that a 'molecular approach' to control of downstream effects such as protein recognition and pathways of apoptosis induction may consist in design of structurally unique DNA adducts as cell signals.

Effect of the geometry of the central coordination sphere in antitumor trinuclear platinum complexes on DNA binding

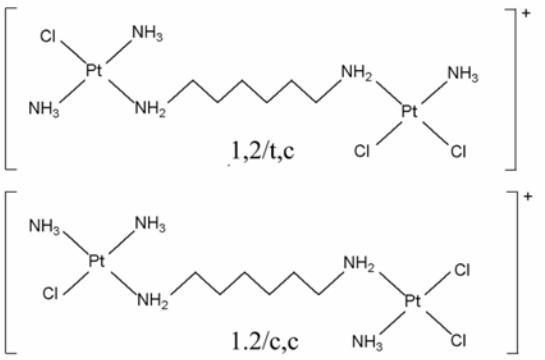
The geometry of the coordination spheres in antitumor trinuclear platinum compounds affects their potency. For example, the central cis unit of [{*trans*-$PtCl(NH_3)_2\}_2\mu$-*cis*-$Pt(NH_3)_2\{H_2N(CH_2)_6NH_2\}_2]^{4+}$ (1,0,1/t,c,t, BBR3499) results in substantially reduced cytotoxicity. It has been shown that the interactions of polynuclear platinum drugs with target



DNA are distinct from the mononuclear-based cisplatin family. In the present work the DNA binding of 1,0,1/t,c,t in cell-free media was examined by the methods of molecular biophysics and compared to the binding of 1,0,1/t,t,t. The binding of 1,0,1/t,c,t is slower and less sequence specific. 1,0,1/t,c,t also forms on DNA long-range delocalized intrastrand and interstrand cross-links similarly as 1,0,1/t,t,t, although the frequency of interstrand adducts is markedly enhanced. Importantly, the adducts of 1,0,1/t,c,t distort DNA conformation and are repaired by cell-free extracts considerably more than the adducts of 1,0,1/t,t,t. It has been suggested that the unique properties of long-range interstrand cross-links of bifunctional trinuclear platinum complexes and resulting conformational alterations in DNA have critical consequences for their antitumor effects.

Trifunctional dinuclear platinum complexes as DNA-protein cross-linking agents

DNA-protein cross-linking agents may have a host of possible applications, including identification of contact sites, isolation of weakly bound proteins within a multiprotein complex, and representing an attractive target for chemotherapeutic intervention. For the latter application, it is therefore desirable to explore the potential selectivity of such interactions in terms of both DNA lesions and protein target. The trifunctional dinuclear platinum compounds 1,2/c,c [{*cis*-$PtCl(NH_3)_2$}μ-$H_2N(CH_2)_6NH_2${*cis*-$PtCl_2(NH_3)$}] and 1,2/t,c, [{*trans*-$PtCl(NH_3)_2$}μ-$H_2N(CH_2)_6NH_2${*cis*-$PtCl_2(NH_3)$}] contain a monofunctional platinum coordination sphere linked to a *cis*-$[PtCl_2(amine)_2]$ moiety. The compounds have been

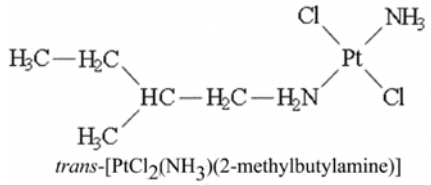


examined for their DNA binding and ability to induce covalent ternary DNA-protein cross-links. Comparison was made with representative bifunctional dinuclear platinum compounds [{$PtCl(NH_3)_2$ μ-$H_2N(CH_2)_nNH_2]^{2+}$. DNA modified by the trifunctional compounds is able to bind and cross-link *Bam*HI, a sequence-specific DNA-binding protein that recognizes the palindromic sequence GGATCC and also very efficiently binds and cross-links SP1, a sequence-specific Zn finger protein that induces a bend in the DNA upon binding. Two representative nonsequence-specific DNA-binding proteins, the Klenow fragment from DNA polymerase I and Klenow exonuclease minus (which has been mutated to remove the 3′-5′ proofreading domain), both bind modified DNA and effectively cross-link to the DNA. Data from circular dichroism, inhibition of ethidium bromide fluorescence, interstrand cross-

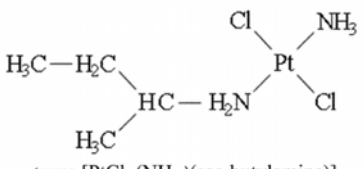
linking and unwinding assays are all consistent with (Pt,Pt) interstrand cross-links as the dominant lesion of trifunctional compounds and the most likely structure to form the ternary DNA-protein cross-links. In vitro transcription of RNA is inhibited by the platinum compounds and indicate G residues as primary binding sites. Binding to calf thymus DNA as assessed by differential pulse polarography is rapid and essentially quantitative. An increase in melting temperature of CT DNA adducted by the platinum compounds is observed at low salt concentrations but at high salt, modification results in a decrease of *t*m. In summary, the trifunctional agents may find use as protein-targeting drugs and as probes for conformational effects on DNA-protein interactions.

DNA interactions of new antitumor platinum complexes with trans geometry activated by a 2-metylbutylamine or sec-butylamine ligand

Cisplatin [*cis*-diamminedichloroplatinum(II)] is a potent anticancer drug for the treatment of testicular and other germ-cell tumors, but its clinical use is limited by the diminished activity against a number of cancers, the acquired resistance developed by many tumors and severe side effects. Therefore, the search continues for an improved platinum antitumor agent and in this search the clinical inactivity of trans isomer of cisplatin (transplatin) was considered up to recently a paradigm for the classical structure-pharmacological activity relationships of platinum drugs. However, to this end several new analogues of transplatin which exhibit a different spectrum of cytostatic activity including activity in tumor cells resistant to cisplatin have been identified. The global modification of mammalian and plasmid DNAs by novel platinum compounds, *trans*-[$PtCl_2(NH_3)(Am)$], where Am = 2-methylbutylamine or sec-butylamine was investigated in cell-free media using various biochemical and biophysical



trans-[$PtCl_2(NH_3)$(2-methylbutylamine)]



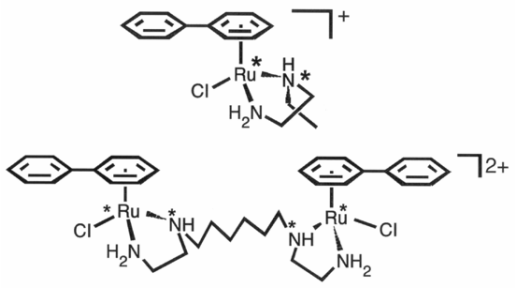
trans-[$PtCl_2(NH_3)$(sec-butylamine)]

methods. These modifications were analyzed in the context of the activity of these new compounds in several tumor cell lines including those resistant to

antitumor cisplatin. The results showed that the replacement of one amine group by 2-methylbutylamine or sec-butylamine ligand in clinically ineffective transplatin resulted in a radical enhancement of its activity in tumor cell lines so that they are more cytotoxic than cisplatin and exhibited significant antitumor activity including activity in cisplatin-resistant tumor cells. Importantly, this replacement also markedly altered DNA binding mode of transplatin and reduced the efficiency of repair systems to remove the adducts of the new analogues from DNA. The results support the view that one strategy to activate trans geometry in bifunctional platinum(II) compounds including circumvention of resistance to cisplatin may consist in a chemical modification of the ineffective transplatin which results in an increased efficiency to form DNA interstrand cross-links.

Induced-fit recognition of DNA by organometallic complexes with dynamic stereogenic centers

Organometallic chemistry offers novel concepts in structural diversity and molecular recognition that can be used in drug design. Here, we consider DNA recognition by η^6-arene Ru(II) anticancer complexes by an induced-fit mechanism. The stereochemistry of the dinuclear complex $[((\eta^6$-biphenyl)RuCl(en))$_2(CH_2)_6]^{2+}$ (en = ethylenediamine) was elucidated by studies of the half unit $[(\eta^6$-biphenyl)RuCl(Et-en)$]^+$ (where Et-en is $Et(H)NCH_2CH_2NH_2$). The structures of the separated *R**Ru*R**N and *S**Ru*R**N diastereomers



of $[(\eta^6$-biphenyl)RuCl(Et-en)$]^+$ were determined by x-ray crystallography; their slow interconversion in water ($t_{1/2}$ = 2 h, 298 K, pH 6.2) was observed by NMR spectroscopy. For $[(\eta^6$-biphenyl)RuCl(Et-en)$]^+$ and $[((\eta^6$-biphenyl)RuCl(en))$_2(CH_2)_6]^{2+}$ the *R**Ru*R**N configurations are more stable than *S**Ru*R**N (73:27). X-ray and NMR studies showed that reactions of $[(\eta^6$-biphenyl)RuCl(Et-en)$]^+$ and $[((\eta^6$-biphenyl)RuCl(en))$_2(CH_2)_6]^{2+}$ with 9-

ethylguanine gave rise selectively to *S**Ru*R**N diastereomers. Dynamic chiral recognition of guanine can lead to high diastereoselectivity of DNA binding. The dinuclear complex $[((\eta^6\text{-biphenyl})\text{RuCl(en)})_2(CH_2)_6]^{2+}$ induced a large unwinding (31°) of plasmid DNA, twice that of mononuclear $[(\eta^6\text{-biphenyl})\text{RuCl(Et-en)}]^+$ (14°), and effectively inhibited DNA-directed RNA synthesis *in vitro*. This dinuclear complex gave rise to interstrand cross-links on a 213-bp plasmid fragment with efficiency similar to bifunctional cisplatin, and to 1,3-GG interstrand and 1,2-GG and 1,3-GTG intrastrand cross-links on site-specifically ruthenated 20-mers. Complex $[((\eta^6\text{-biphenyl})\text{RuCl(en)})_2(CH_2)_6]^{2+}$ blocked intercalation of ethidium considerably more than mononuclear $[(\eta^6\text{-biphenyl})\text{RuCl(Et-en)}]^+$. The concept of induced-fit recognition of DNA by organometallic complexes containing dynamic stereogenic centers via dynamic epimerization, intercalation, and cross-linking may be useful in the design of anticancer drugs.

GRANTS:

GA AS CR A5004101
Structure, recognition and biochemistry of DNA modified by antitumor platinum drugs
Principal investigator: V. Brabec, 2001 - 2005

GA AS CR KJB5004301
Molecular mechanisms underlying anticancer effects of a new drug BBR3464
Principal investigator: J. Kašpárková, 2003 - 2005

GA AS CR K4055109
Physics, chemistry and informatics for biological, ecological and medical applications
Principal investigator: K. Ulbrich, IMCH AS CR Prague, co-investigator: V. Brabec, 2001 - 2004

GA AS CR K4055109
Physics, chemistry and informatics for biological, ecological and medical applications
Principal investigator: K. Ulbrich, ÚMCH AV ČR Prague, co-investigator: V. Brabec, 2001 - 2004

GA CR 305/02/1552A
Platinated oligonucleotides for selective modulation of gene expression, relations to antisense strategy and development of new drugs
Principal investigator: V. Brabec, 2002 - 2004

GA CR 202/01/D110
Microcalorimetric analysis of thermodynamic stability of DNA affected by anticancer platinum complexes
Principal investigator: C. Hofr, 2001 - 2004

GA CR 204/03/H016
Structural biophysics of macromolecules
Principal investigator: V. Brabec, 2003 - 2007

IGA MEdYS CR 1K03010
A study of thermodynamic stability of DNA modified by new antitumor platinum complexes
Principal investigator: V. Brabec, 2003 - 2005

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

LABORATORY OF DNA MOLECULAR COMPLEXES (LADMC)

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	IVA ŠANTRŮČKOVÁ

Laboratory is focused on structure-function relationships in complexes of proteins and nucleic acids which participate in metabolism of genetic material. Within this focus, a specific attention is paid to analysis of eukaryotic chromosome ends (telomeres), to the study of their synthesis by means of telomerase or so called alternative mechanisms, and to the isolation and characterisation of telomere-binding proteins. While this research in plants is of basic science nature, in human cells and clinical samples the research is targeted to the field of molecular diagnostics.

Our research in 2004 advanced our understanding of evolution of plant telomeres in the plant order of Asparagales. Our previous results showed that a distinct clade included plant species where the ancestral, *Arabidopsis*-type of telomeric repeats [TTTAGGG]n had been partially, or fully, replaced by the human-type telomeric sequence [TTAGGG]n. Telomerases of these species synthesise human repeats with a high error rate *in vitro*. Recently we further characterized the structure of telomeres in these plants by analyzing the overall arrangement of major and minor variants of telomeric repeats using fluorescence *in situ* hybridization on extended DNA fibres of *Ornithogalum* plants (Fig. 1). Whilst the telomeric array is predominantly composed of the human variant of the repeat, the ancestral, *Arabidopsis* type of telomeric repeats

was ubiquitously observed at one of the ends and/or at intercalary positions. Another variant of the repeat typical of *Tetrahymena* [TTGGGG]n

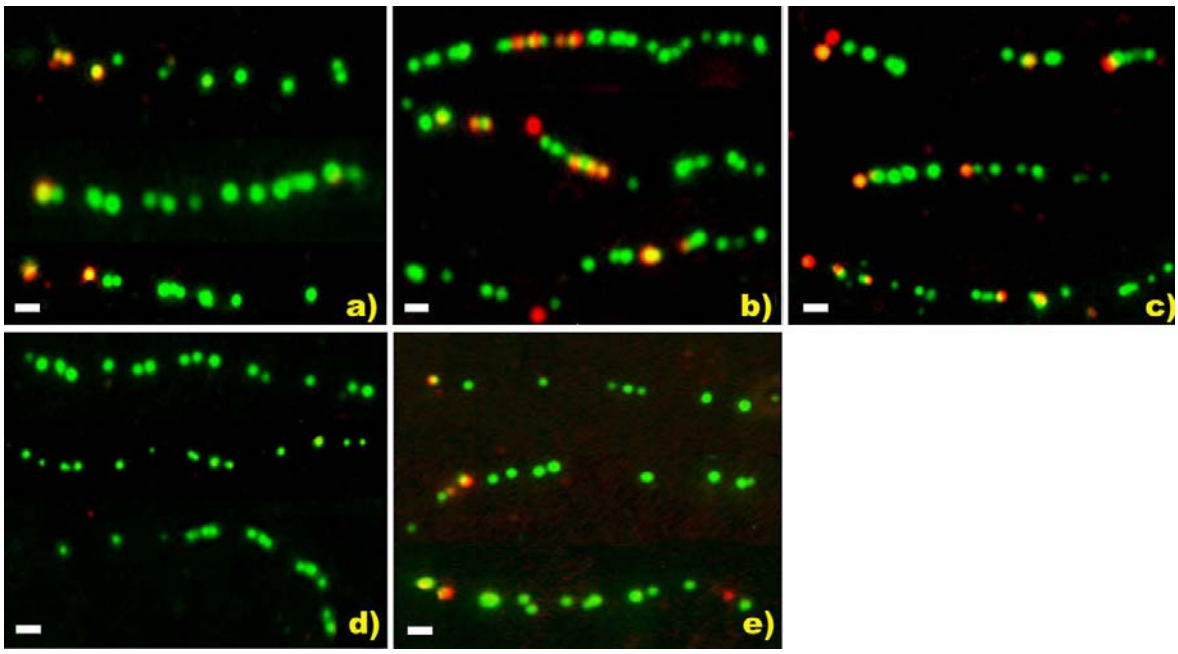

Fig. 1. Co-localization between major (human-type) and variant repeats in telomeres of Ornithogalum virens. Telomeric localisation of human-, Arabidopsis- and Tetrahymena-type repeats have been demonstrated previously (Sykorova et al., 2003). A detailed analysis of their mutual arrangement on extended DNA fibers shows that the ancient (Arabidopsis-type) telomeric repeat (Cy3-labelled, red, a-c) is ubiquitously present in telomeric arrays of human-type-specific signals (FITC-labelled, green). Three types of localization of Arabidopsis-type repeats can been observed: a) at one of the ends of the telomeric fibre; b) intercalary position; c) both terminal and intercalary position. Tetrahymena-type repeats (Cy3-labelled, red, d-e) are mostly absent (d) in telomeric arrays of human-type-specific signals (FITC-labelled, green). In about 20% of fibers, Tetrahymena-type repeats localise to terminal or subterminal parts of the arrays (e). Scale bar corresponds to 1μm.

was observed interspersed in about 20% of telomeric fibres. Micrococcal nuclease digestions indicated that Asparagales plants with a human-type of

telomere have telomeric DNA organised into nucleosomes. However, unexpectedly, the periodicity of the nucleosomes is not significantly shorter than bulk chromatin as is typical of telomeric chromatin. Using electrophoretic mobility shift assays we detected in Asparagales plants with a human-type of telomere a 40 kDa protein that forms complexes with both *Arabidopsis*- and human-type of G-rich telomeric strand. However the protein shows a higher affinity to the ancestral *Arabidopsis*-type of sequence. Two further proteins were found, a 25 kDa protein that binds specifically to the ancestral sequence and a 15 kDa protein that binds to the human-type of telomeric repeat. Our results thus provide first glimpse on the rise of the complex organisation of the telomere repeats in Asparagales and on events leading to the stabilisation the new telomere at the point of mutation.

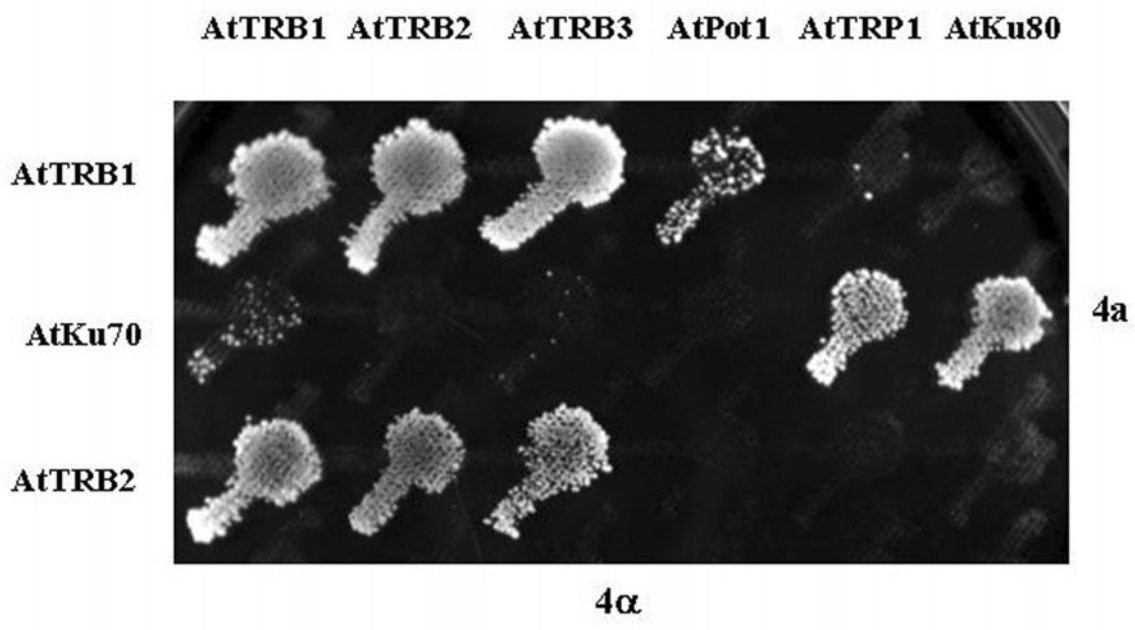

Fig. 2. Two-hybrid assay for interactions among A. thaliana putative telomere-binding proteins. 4a (horizontal rows) and 4α (vertical rows) denote different mating types of the yeast strain (PJ69-4a and PJ69-4α, respectively). Previously identified interactions of AtKu70×AtKu80 and AtTRB2×AtTRB3 are used as positive controls.

Another study of telomere-binding proteins has been performed in a more common model organism, *Arabidopsis thaliana*. Although a number of candidate telomere-binding proteins have been identified by homology searches to plant genome databases and tested for their affinity to telomeric DNA sequences *in vitro*, there is minimal data relevant to their telomeric function. To address this problem we made a collection of cDNAs of putative telomere binding proteins of *A. thaliana* to analyze their protein-protein interactions with the yeast two-hybrid system (Fig.2). Our results show that one myb-like protein, AtTRP1, interacts specifically with AtKu70, the latter protein having a previously described role in plant telomere metabolism. In analogy to the interaction between human Ku70 and TRF2 proteins, our results suggest AtTRP1 is a likely homologue of TRF2. The AtTRP1 domain responsible for AtKu70 interaction occurs between amino acid sequence positions 80-269. Another protein AtTRB1, a member of the single myb histone (Smh) family, shows self-interaction and interactions to the Smh family proteins AtTRB2 and AtTRB3. Protein AtTRB1 also interacts with AtPot1, the *Arabidopsis* homolog of oligonucleotide-binding (OB)-fold-containing proteins which bind G-rich telomeric DNA. In humans, the TRF1-complex recruits hPot1 to telomeres by protein-protein interactions where it is involved in telomere length regulation. Possibly AtTRB1 has a similar role in recruiting AtPot1.

In a medical part of our research, the study of using telomerase as a diagnostic and a predictive factor in colorectal cancer was followed by a similar study in multiple myeloma (MM). Telomerase activity was detected in CD138+ fractions of bone marrow from MM patients obtained by immunomagnetic separation and also in the remaining CD138- fraction. Samples were taken from newly diagnosed MM patients, from patients in maximum response to treatment and in relaps. Our recently developed dual-colour real-time TRAP technique has been applied for telomerase activity measurement. From 40 samples, 12 showed increased telomerase activity in CD138+ fraction, 21 a similar activity in both fractions and in the remaining 7 samples the activity in CD138- fraction was higher than that in the CD138+ fraction. These results show that the activity of telomerase is not restricted to CD138+ fraction of bone marrow of MM patients. From 5 samples coming from maximum treatment response stage, 3 had no telomerase activity and 2 had only weak activity. On the other hand, a wide range of activities (from zero to high) were detected in 6 samples coming from the relaps stage suggesting involvement of telomerase-independent alternative telomere lengthening in some MM cases. This is supported by results of *in situ*

analysis of telomeres in bone marrow cells of MM patients which show frequent losses or amplifications of individual telomeric signals.

The last part of our application-oriented research is focused on utilization of structural variability of subtelomeres for genotyping of potato varieties. Subtelomeric regions reveal a relatively high degree of polymorphism due to the increased frequency of recombination events in these chromosome loci. In a search for molecular markers applicable for genotyping of potato varieties, we focused on two possible sources of polymorphism occurring in this region. i) arrangement of blocks of subtelomeric chromatin; ii) structure of telomere-subtelomere boundary. The analysis of internal arrangement of subtelomeric sequences have shown several types of cultivar-specific spectra of PCR products arising from variant orientation of the sequence units of the ST3-subtelomeric sequence, or from different lengths of regions linking the individual sequence units. Further, the telomere-subtelomere boundary sequences have been amplified using telomeric and ST3-specific primers and the products obtained have been cloned. Sequence analysis of the clones resulted in characterisation of a novel telomere-associated sequence (FIN2). Primers derived from this sequence have then been used alone or in combination with telomeric or ST3-specific primers to generate cultivar-specific spectra of PCR products. The described combinations of sequence-specific primers may be used for fast, cheap and reproducible PCR-genotyping of selected potato varieties.

GRANTS:

GA AS CR S5004010
Development of novel diagnostic techniques for oncology
Principal investigator: S. Kozubek, co-investigator: J. Fajkus, 2000 - 2004

GA AS CR K5011112
Molecular and cellular basis of severe disorders
Principal investigator: J. Kuneš, IP AS CR Prague, co-investigator: J. Fajkus, 2001 - 2004

GA CR 204/02/0027
Molecular analysis of the structure and function of typical and alternative telomeres in plants
Principal investigator: J. Fajkus, 2002 - 2004

IGA MH CR NC7043-3
Telomerase activity as a new prognostic factor in multiple myeloma
Principal investigator. R. Hájek, LF MU Brno, co-investigator: J. Fajkus, 2002 - 2004

NAZV1164
Characterization of potato genotypes by DNA fingerprinting method
Principal investigator: H. Polzerová, Výzkumný ústav bramborářský Havlíčkův Brod, co-investigator: J. Fajkus, 2001 - 2004

IGF16/01 (University hospital Brno)
Research of prognostic factors in multiple myeloma,
Principal investigator: R. Hájek, LF MU Brno, co-investigator: J. Fajkus, 2003 - 2005

143100008 IGA MEdYS CR (Research project of MU)
Genomes and their function
Principal investigator: J. Relichová, co-investigator: J. Fajkus, 1999-2004

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

LABORATORY OF ANALYSIS OF CHROMOSOMAL PROTEINS (LACP)

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GRADUATE STUDENTS:	MGR. EVA MUSELÍKOVÁ-POLANSKÁ
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Comparison of HMGB1 protein binding with HMGB1 having mutated all three intercalating residues (previously reported to be involved in recognition and binding to distorted DNA) to DNA minicircles and hemicatenated DNA loops (hcDNA) revealed that the extreme affinity of HMGB1 for hcDNA ($K_d < 1$ pM) is to be explained by binding of the protein to the hemicatenane. Similar conclusion was made upon binding of tumor suppressor protein p53 to hcDNA [Štros etal. (2004) *Biochemistry 43*, 7215-7225]. We have also discovered that HMGB1 is able to induce an altered DNA comformation of DNA minicircles depending on the linking number of the topoisomers. The latter finding is evidenced by anomalous increased migration of DNA-HMGB1 complexes in non-denaturing polyacrylamide gels, and *not* their retardation despite higher mass of these complexes relative to the protein-free DNA.

We have also demonstrated that HMGB1 can stimulate intermolecular DNA associations resulting in enhanced ligation of short or long DNA fragments. This finding helped us to understand some aspects of the stimulatory effect of HMGB1 on activity of a number of biologically important nuclear enzymes.

GRANTS:

GA AS CR K4055109
Physics, chemistry and informatics for biological, ecological and medical applications
Principal investigator: K. Ulbrich, IMCH AS CR Prague, co-investigator: M. Štros, 2001 - 2004

GA CR 301/02/0952
Tumor supressor proteins of p53 family and chromosomal protein HMGB1. A study of a functional consequence
Principal investigator: M. Štros, 2002 - 2004

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

PROGRAM III

BIOPHYSICS AND BIOINFORMATICS OF GENOMES

LABORATORY OF CD SPECTROSCOPY OF NUCLEIC ACIDS (LSNA)

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	MGR. PETRA ŠKOLÁKOVÁ

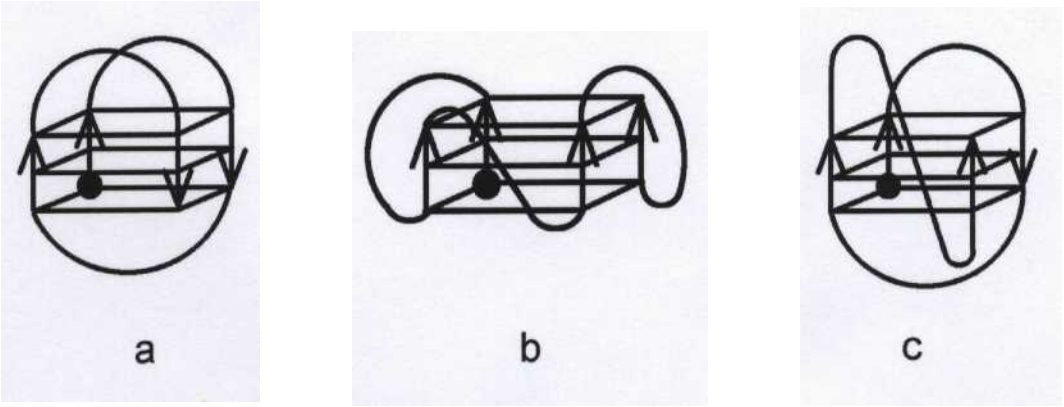
The topology of tetraplex arrangements that are formed by human telomere $(TTAG_3)_n$ sequences differs depending on the number of $TTAG_3$ repeats. It has been shown recently that ATG_3TTAG_3T and $AG_3(TTAG_3)_3$ fragments form, in the crystal, bimolecular and intramolecular tetraplexes respectively, with the TTA loops positioned on the exterior of the tetraplex core in a propeller-like arrangement (figure). In this way all the four tetraplex chains remain oriented in the same direction (parallel-stranded tetraplexes). We have found that (ATG_3TTAG_3T) as well as G_3TTAG_3 fragments form two types of bimolecular associates migrating with a distinct mobility in the gel. Their CD spectra correspond to the simultaneous presence of both parallel-stranded (ps) and antiparallel-stranded (aps – two parallel and two antiparallel chains) tetraplexes, while their populations change in dependence on solvent conditions and the sample history. At low temperatures, for example, the aps tetraplex prevails which, with a very slow kinetics, isomerizes into a ps tetraplex at 37 ^{o}C. However, the DNA fragments with four G3 blocks, i.e. $AG_3(TTAG_3)_3$ as well as $G_3(TTAG_3)_3$, form, in contrast to crystal, exclusively intramolecular antiparallel tetraplexes in solution (figure). Surprisingly, extension of the motif by one repeat preserves the tetraplex of $G_3(TTAG_3)_4$ still as intramolecular, but its CD spectrum gives evidence of an increase in the population of parallel strands. It thus means that the redundant repeat adopted a propeller-like loop observed in the crystal (figure), so that the $G_3(TTAG_3)_4$ tetraplex consists of three mutually parallel chains and one antiparallel chain. Such formation has been observed by NMR with $G_3T_4G_4$. The ps and aps chain populations remain unchanged with a further increasing repeat number, e.g. with $G_3(TTAG_3)_5$. However, this fragment forms, apart from intramolecular, also bimolecular tetraplexes. The fragment of $G_3(TTAG_3)_7$ with eight G_3 blocks forms only an intramolecular antiparallel tetraplex again. The finding of the ps tetraplexes with propeller-like loops and their detection in solution threw light on our long-lasting problem of how

to explain the fact that bimolecular and intramolecular tetraplexes can provide CD spectra characteristic of ps tetraplexes.

The tetraplex of the $G_3(TTAG_3)_3$ fragment, i.e. of the shortest repeat that folds intramolecularly, is the most stable of all $G_3(TTAG_3)_n$ tetraplexes, including that of $G_3(TTAG_3)_7$. It is characterized by an extremely fast electrophoretic migration indicating its compact arrangement. This anomaly disappears with increasing motif length. A twofold long motif migrates already regularly, just correspondingly to its length.

In contrast to G-rich strands, complementary C-rich strands form a single type of i-tetraplex arrangement. Its molecularity only changes in dependence on fragment length: C_3TAAC_3 forms a bimolecular tetraplex, $(C_3TAA)_3C_3$ and longer fragments form intramolecular tetraplexes. The redundant C3TTA in the case of $(C_3TAA)_4C_3$ remains free, in the same frequency on the 3′- or 5′- end of the molecule. The fragment $(C_3TAA)_5C_3$ forms only one type of intramolecular tetraplex, probably with C_3TAA overhangs on both ends. In addition, it also forms bimolecular tetraplexes.

G-rich and C-rich complementary human telomere DNA fragments associate to form classical heteroduplexes even under conditions when G-rich or C-rich strands adopt tetraplexes. However, the heteroduplexes dissociate into the particular tetraplexes under conditions of a simultaneous stability of guanine and cytosine tetraplexes, i.e. in the presence of K^+ ions and acidic pH. The dissociation takes place in more acidic pH values with increasing motif length and simultaneously the ability of mutual interaction of the particular tetraplexes decreases. An exception in this respect is represented by the duplex $G_3(TTAG_3)_3.(C_3TAA)_3C_3$. It dissociates into tetraplexes more easily than shorter fragments, and the tetraplexes are even more reluctant to mutual interaction than those of the longer fragments. We have recently found that dissociation of the $G_3(TTAG_3)_3.(C_3TAA)_3C_3$ heteroduplex takes place nearly under physiological conditions (10 mM K^+ and pH 6). Our results show that the $G_3(TTAG_3)_3$ sequence represents the optimum length for tetraplex appearance. Once formed, a compact tetraplex does not interact with the complementary strand, which may be the essence of the gradual telomere shortening in the consequential syntheses.



Tetraplex arrangement of the sequence (a) $G_3(TTAG_3)_3$ in solution, (b) $AG_3(TTAG_3)_3$ in crystal and (c) $G_3(TTAG_3)_4$ in solution.

GRANTS:

GA AS CR A4004201
DNA tetraplexes and their occurrence in the human genome
Principal investigator: M. Vorlíčková, 2002 - 2006

GA AS CR K5052113
Structure, expression and interaction of genomes
Principal investigator: V. Pačes, IMG AS CR Prague, co-investigator: M. Vorlíčková, 2001 - 2004

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

LABORATORY OF DNA BIOPHYSICS AND GENOME BIOINFORMATICS (LDBGB)

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TECHNICAL ASSISTANT:	SOŇA KAŠKOVÁ
GRADUATE STUDENT:	MGR. IVANA VASILENKOVÁ

The Laboratory explores principles of primary, secondary and tertiary structures of genomic molecules of DNA, restriction and PCR fragments and synthetic oligonucleotides using spectroscopic, electrophoretic, photochemical, PCR and computer methods. The aim is to find out how the genomic molecules of DNA arose, how they function and how they undergo changes.

Research continued of a method to monitor and explore conformational isomerism of the genomic molecules of DNA. For this purpose, we irradiated the pUC19, pBR322 and phiX174 molecules of DNA with various doses of UV light and cleaved the irradiated molecules with more than 20 type II restrictases. In general, the irradiation hindered the cleavage the more the higher the dose of irradiation was. In line with previous studies, the (A+T) and bipyrimidine content in the restriction site belonged among the factors that on average increased resistance of the UV irradiated DNA with respect to the restrictase cleavage. However we also observed a significant influence of the UV irradiation on (G+C)-rich sites containing low bipyrimidine contents. In addition, nucleotide sequences flanking the restriction sites influenced in some cases (e.g. HindIII), but did not influence in other cases (e.g. SalI) the protective effect of UV irradiation. Neoschizomer couples SmaI and AvaI, or SacI and Ecl136II cleaved the UV irradiated DNA similarly. Our results demonstrate that the intrastrand thymine dimers in the restriction site certainly are not the only photoproduct blocking the restrictases. UV irradiation of A-form DNA made DNA less resistant to restrictase cleavage than UV irradiation of B-form DNA, which is in accord with the known higher resistance of the A-form towards damage caused by UV light. In some cases, DNA transition into the A-form entirely protected it

from UV light-induced damage that is recognized by restrictases. The present study further demonstrates that creation of partial digests used in genomic studies, can be extended to some restrictases rich in (G+C) and containing few bipyrimidine dinucleotides. In this work, we generated extensive quantitative data that we will further use to monitor and explore the B-A conformational isomerizations in genomic molecules of DNA.

The method has so far been used to detect and study the B-A transtion in linearized DNA of pUC19. The B-A transition was induced by the most common B-A transition inducer, i.e. ethanol, and the transition was detected by the most common method, i.e. the CD spectroscopy. The B-A transition induction is not trivial in such a long molecule as the pUC19 DNA is because it tends to precipitate in the presence of ethanol. We carefully got rid the DNA from proteins and worked with as low concentrations of DNA and ions as possible. This way we managed to induce the B-A transition even in pUC19 DNA as documented by CD spectroscopy. Then we irradiated this DNA with an appropriate dose of UV light, the irradiated DNA was transferred into a restrictase buffer and cleaved with the corresponding restrictase. This protocol was used with 17 restrictases that cleaved the pUC19 DNA at various sites. Then we plotted the amount of uncleaved, i.e. UV damaged DNA as a function of the ethanol concentration at which the DNA was irradiated. These dependences generally showed an S-shaped course characteristic for the cooperative B-A transition. Using the curves we determined the transition beginning and midpoint for each restrictase. These data map the B-A transition course along the whole polylinker of pUC19 DNA and, further, at six evenly distributed sites along the plasmid. The transition midpoints fall into the B-A transition region determined by CD spectroscopy. The midpoint values negatively correlate with the (G+C) content in the corresponding restriction site and its neighborhood. This method complements previous methods to study the B-A transition in DNA. It makes mapping posssible of the B-A transition course along PCR or restriction fragments of genomic DNA even 50 kilobases long because such fragments can be separated in agarose gels by a normal electrophoresis. This method will make studies possible of the molecular biology meaning of the B-A transition in genomic molecules of DNA.

Development continued of a method to simulate spatial shapes of genomic molecules of DNA. We extracted cartesian coordinates of the phosphorus atoms from the NDB database of oligonucleotide crystal structures. Using the coordinates, we calculated geometrical parameters for each nucleotide, dinucleotide and trinucleotide that suffice to build the oligonucleotide molecular structures in the phosphorus atom representation. We developed a software for this purpose to construct the phosphorus atom models for each of the 144 oligonucleotide crystal structures whose coordinates were used in the model development. 61 of the 144 structures were B-DNA, 83 A-DNA.

The B-DNA and A-DNA structures were treated separately. We compared the models with the original experimentally determined molecular structures. The RMSD of the model and experimental structures was 1.1 Å for the A-DNA structures and 3.4 Å for the B-DNA structures.
In this part of the project, the most interesting finding was that the separation of the consecutive phosphorus atoms in the DNA backbone was significantly larger with the purine nucleotides than the pyrimidine nucleotides thongh the primary structure is the same in both cases. This means that the sugar-phosphate backbone is regular in DNA even as a consequence of the alternation of the purine and pyrimidine nucleeotides. This irregularity is extremely expressed in molecules such as poly(dA)·poly(dT) or poly(dG)·poly(dC) where substantially different helices of the purine strand and the pyrimidine strand are bound to generate a double helix that is substantially different from the classical B-DNA. This unusual double helix is called a heteronomous DNA.
The approach described above makes it possible to extrapolate the experimental data, i.e. the oligonucleotide crystal structures, to much longer DNA molecules that do not crystallize properly for the diffraction analysis. It is essential in this approach that each DNA nucleotide is only represented by a single, i.e. phosphorus atom in this representation because this significantly shortens the calculations. This is a key property to simulate DNA molecules of the human genome because each of them contains tens to hundreds millions of nucleotides.
We started simulations of genomic DNA with viral genomes. For this purpose we wrote the software GENOMESHAPE. This software calculates, within several seconds, cartesian coordinates of all phosphorus atoms of e.g. the EB virus DNA whose length is about 170 kbp. Its simulated spatial structure contains an evident three dimensional motif reflecting the known repetitive primary structure contained within this genome. Then we simulated the whole genome of yeast consisting of 16 molecules of DNA whose lengths fall within 0.2-1.5 Mbp. Our current software and hardware makes simulation of even this genome easily possible. The simulations show that the molecules of DNA of this eukaryotic organism consist of compact domains separated by more or less straight connectors. We think that genomic molecules of DNA are bent at many places and globally compacted even in the absence of chromosomal proteins. Finally we performed simulations of the DNA molecules of the human chromosomes 21 and 22 whose lengths are around 30Mbp. We found more or less separated compact domains in these huge molecules and distinctly separated telomere domains. One results indicate that the above approach can be used to simulate the whole human genome. Now we will develop a further module of GENOMESHAPE that will automatically identify characteristic geometrical shapes in the human molecules of DNA.

GRANTS:

GA AS CR A1004201
Biophysical properties of (guanin+cytosine) and (adenin+thymine) regions in the DNA molecules of human chromosomes
Principal investigator: J. Kypr, 2002 - 2006

GA AS CR A1004301
Conformational transitions in plasmid DNA
Principal investigator: K. Nejedlý, 2003 - 2005

GA AS CR K5052113
Genome structure, expression and interactions
Principal investigator: V. Pačes, IMG AS CR Prague, co-investigator: J. Kypr, 2001 - 2004

IGA MH CR NM7634-3/2003
A method development for prediction of pathological expansion of trinucleotide repeats in the human genome
Principal Investigator: J. Kypr, 2003 - 2005

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

LABORATORY OF MOLECULAR EPIGENETICS (LME)

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	RNDR. BLAŽENA KOUKALOVÁ, CSC.
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	MGR. JANA BEDŘICHOVÁ
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UNDERGRADUATE STUDENT:	KATEŘINA KŘÍŽOVÁ

Tragopogon mirus and *T. miscellus* are allopolyploid (2n=24) plant species that arose relatively recently on the American continent by interspecific hybridization of parental diploid plants *T. dubius, T. pratensis* and *T. porrifolius*. The diploid species did not occur in pre-columbian era and were introduced to America from Euroasia during colonization. In contrast to most other models of plant allopolyploids both *T. mirus* (*T. dubius* x *T. porrifolius*) a *T. miscellus* (*T. dubius* x *T. pratensis*) have a well-defined origin and appear to be an excellent system for genetic studies.

Evolution of ribosomal RNA genes in recently formed Tragopogon allopolyploids

Genes coding for ribosomal RNA (rDNA) are actively transcribed sequences composed of tandemly arranged units. Our recent studies showed gene conversion of parental rDNAs in several *Nicotiana* allopolyploids (Kovarik et al. 1996, Lim et al. 2000, Matyasek et al. 2003) suggesting that rDNA constitutes a dynamic part of plant genomes. We have proposed that rDNA units from different subgenomes tend to interact in the plant nucleus due to their high transcription and chromatin decondensation. In our current work we have analyzed rDNA in two allotetraploid species *T. miscellus* and *T. mirus*. Since there is evidence for their recent and recurrent orgin we have analyzed five different populations of *T. mirus* and three populations of *T. miscellus*. These populations arose separately at geographically distinct places in the north-western part of the USA. About 5-10 plants from each population were analyzed for the rDNA types. In total we have analyzed about 70 DNAs by Southern blot hybridization, PCR, single stranded conformation polymorphisms. The rDNA loci on chromosomes were

visualized by fluorescence in situ hybridization (FISH). We found that the parental rDNA units were present in all allotetraploid plants analyzed. However, the copy number of parental units was not balanced and the ratios were typically skewed away from *T. dubius*. In some individuals the content of *T. dubius* units was less than 5% of total rDNA. However, in one population of *T. mirus* from Palouse (Wyoming, USA) the general trend appeared to be reversed - *T. dubius* units dominated over those of *T. porrifolius* origin. Thus concerted evolution may occur bidirectionally within a single species. Herbarium specimens of plants collected in early fifties appeared to be a convenient control to living plants experiments. We have extracted DNA from dried leaves from herbarium specimens. Using PCR we showed that plants close to date of their origin had the parental rDNAs in nearly balanced ratios (1:1). In this way we have provided perhaps one of the best evidence for concerted evolution of rDNA in allopolyploid species. The FISH study of rDNA chromosomal localization showed that units elimination was probably accompanied by their replacement with units from the partner genome. Tandemly arranged ribosomal rRNA genes are more rapidly evolving than the non transcribed satellite sequences in *Tragopogon* allotetraploids (see below).

Satellite repeats evolution in recently formed Tragopogon allopolyploids

To better characterize diploid and tetraoploid genomes of *Tragopogon* we have isolated and cloned two highly repetitive sequences TGP7 and TPRMBO from *T. porrifolius* and *T. pratensis*, respectively. The TGP7 satellite is based on a 532 bp monomeric unit; the TPRMBO satellite has a 160 bp monomeric unit. Cytogenetic analysis (FISH) showed that TGP7 is a subtelomeric repeat while the TPRMBO is a centromeric repeat. The number of FISH signals on chromosomes in allotetraploids corresponded to that of parental diploid species suggesting that number of loci and copy number have not been dramatically changed by allopolyploidy. Moreover the size of genome in allotetraploids (20-24 pg/nucleus) was about the double of that of diploids (10-12 pg/nucleus). Thus contrast to other allopolyploid species including *Triticum*, *Brassica* and *Nicotiana* there is no evidence for rapid evolution of satellite sequences or chromosomal instability in *Tragopogon* allotetraploids of recent and recurrent origin.

GRANTS:

GA AS CR S5004010
Development of new diagnostic tools in oncology
Principal investigator: S. Kozubek, co-investigator: A. Kovařík, 2000 - 2004

GA AS CR K5052113
Structure, expression and interaction of genomes
Principal investigator: V. Pačes, IMG AS CR Prague, co-investigator: A. Kovařík, 2001 - 2004

GA CR 204/03/P104
The structure and expression of ribosomal RNA genes in allotetraploid and their parental genomes
Principal investigator: J. Fulneček, 2003 - 2005

GA CR 521/04/0775
Epigenetic regulation of gene expression in transgenic and endogenous loci of higher plants
Principal investigator: A. Kovařík, 2004 - 2006

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

PROGRAM IV

MOLECULAR CYTOLOGY AND CYTOGENETICS

LABORATORY OF MOLECULAR CYTOLOGY AND CYTOMETRY (LMCC)

HEAD:	DOC. RNDR. STANISLAV KOZUBEK, DRSC.
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	RNDR. EVA BÁRTOVÁ, PH.D.
	MGR. PAVLA JIRSOVÁ, PH.D.
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	HANA KŘIVÁNKOVÁ
GRADUATE STUDENTS:	MGR. EVA FALTÝSKOVÁ
	MGR. ANDREA HARNIČÁROVÁ
	MGR. GABRIELA GALIOVÁ
BACHELOR:	BARBORA GABRIELOVÁ

A new investment, laser Innova 70 Spectrum (Coherent) with acusto-optical tuning filter (AOTF, Brimrose) for fast and computer controlled selection of the laser line, were installed in our laboratory. The Innova laser together with unique confocal unit CSU-10 (Yokogawa) provides fast and fully automated acquisition of high-quality images. The LEICA DMRXA microscope, CSU-10 confocal unit, CoolSnap CCD camera and Innova laser are controlled by a powerful computer.

Ultrafast-high-resolution image acquisition software

The development of software for fast acquisition of 4D (3D + time) images started as an integral part of the EU project "3D Genome". The goal of this development is optimization of all steps of image acquisition when the microscope components run in parallel whenever it is possible. Recently, we have achieved 20 high-resolution images/s in 2D space. The next step will be the acquisition of a stack of single-colour images with the rate of 20 sections per second. The goal of the development will be a complete 3D stack of 2-3 colour images stored to computer memory within a one second interval; 4D images will be generated with the mentioned time interval or faster for smaller number of stack sections.

Tracking software

Further we are developing software for automated tracking of cell structures in time. This software can analyze a large number of images with such reliability that there is practically no need for user corrections. The efficiency of the software is demonstrated in Fig. 1, where the results of analysis of 1000 images of living cell nucleus, stored to computer memory in 1 min, are presented. The computer program performs registration of 4D images, shows the 3D images as maximum x-y, x-z and y-z projections and enables the user to page through the images in time. 3D image analysis is supplemented with tracking objects in time, numbering of the objects and drawing their trajectories.

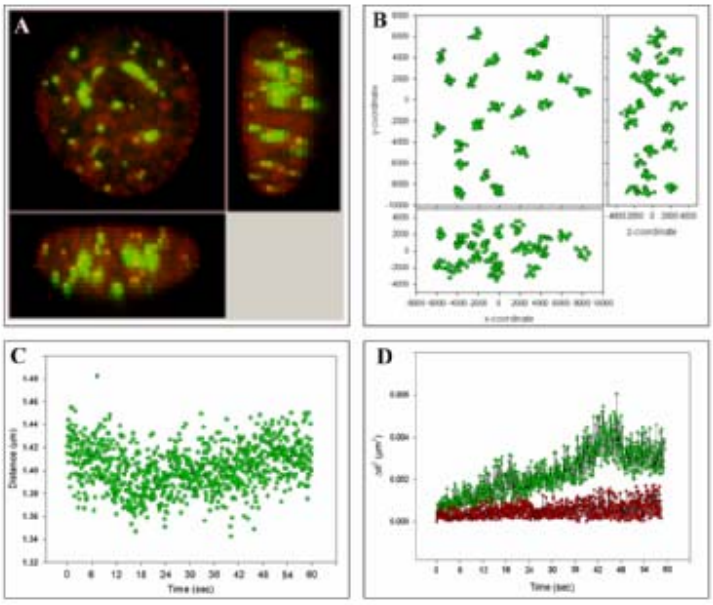

Fig. 1. Analysis of HP1 foci in living cells. A) visualization of chromatin in the cell nucleus (red, H2B-RFP) with HP1 foci (green, HP1-GFP); x-y, x-z and y-z projections are shown as maximum images (all structures are visible through the whole object); B) positions of foci found by computer analysis (circles) at different time intervals form clusters, which shows relatively slow movement of the foci; C) illustrative plot of distances between two foci against time for short intervals (60 ms); D) plot of the mean value of differences in distances squared (Δd^2) with standard deviations against time for living cells (green) and fixed cells (red). The increasing part of the plot could be interpreted as Brownian diffusion. For approx. 40 s the plot riched plateau, which indicates constriction of the diffusion at the distance of about 0.06 μm.

Gene silencing in living cells

Experiments were performed using MCF-7 human mammary carcinoma cells, transfected by H2B-RFP and HP1-GFP constructs (Fig. 1). We visualized cell nuclei (H2B-RFP) together with HP1 foci (HP1-GFP) as well as HP1 foci together with transgenes stained by Cy3. The results of tracking these objects showed that transgenes are directed to the nearest HP1 focus and move in some restricted compartments inside the cell nuclei. The motion of transgenes is not random Brownian motion but represents a combination of diffusion and directional motion (Fig. 2). On the other hand, HP1 foci are very stable in time with very small coefficient of diffusion and substantially restricted random movement. The positions of HP1 foci are changing mostly due to common directional deformation of a large parts of the cell nucleus. Transgene expression is silenced by HP1 foci and, consequently, after several days after transfection, practically all transgenes are localized in the foci and the HP1 structures terminate their illumination (but can be visualized after cell fixation immunochemically).

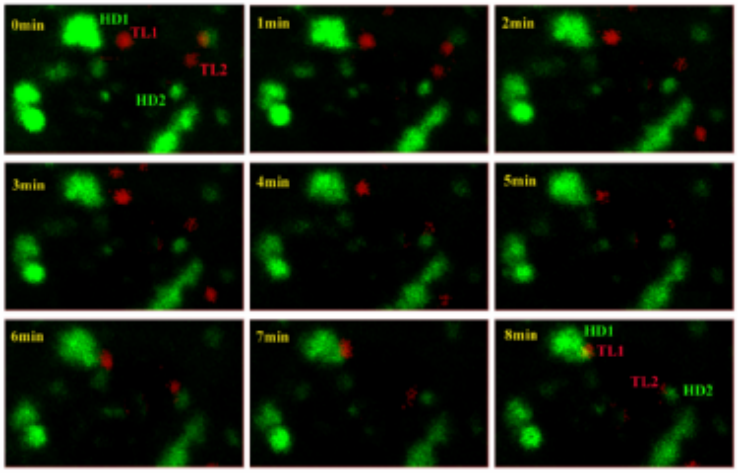

Fig. 2. Directional movements of two transgene loci TL1 and TL2 to the relevant HP1 foci HD1 and HD2.

Methylation of H3K9 histone as a marker of epigenetic mutation preceding genesis of malignant diseases

H3K9 histone methylation is usually associated with transcriptionally repressed chromatin. The methyl moiety shields the negative charge of DNA molecule which lowers electrostatic repulsion and allows thus stronger chromatin compaction. It is well known that the histone H3 methylated in K9 position binds HP1 protein (Heterochromatin protein 1); bound HP1 protein then undergoes dimerisation which is important for creation of compact chromatin structure characterized by lowered gene expression. Colocalization of HP1 protein with intensively stained chromatin subdomains (heterochromatin) was experimentally demonstrated for many cell types, which has proven the above described hypotheses. It could be expected that the importance of HP1 protein will increase during differentiation associated with total decrease of gene expression and formation of intensively stained chromatin regions inside the cell nucleus (e.g. during the myeloid or monocytic differentiation). Progressively stronger methylation of the H3K9 can be therefore presupposed with differentiation process. Surprisingly, our experiments revealed that H3 histones are not methylated and HP1 protein is absent in terminally differentiated cells (granulocytes); mono-, di- and trimethylation of H3K9 as well as the presence of all three HP1 isoforms (alpha, beta, gamma) was analyzed using immunohistochemistry and confirmed by Western blotting. Using the anti-CENPA antibody, it was shown that chromatin regions intensively stained by TOPRO contains centromeres and that the absence of epitopes mentioned above is not an artifact resulting from insufficient antibody penetrance into the compact chromatin (results from Western blotting). Understandably, the question about the origin of such a heterochromatin, formed by alternative mechanisms and invoked in terminally differentiated cells, arises in our minds. What are its properties from the point of view of epigenetic regulation of gene expression? Is this heterochromatin included in gene silencing similarly to that methylated in H3K9? All these questions are the subject of our further investigation. Without any answer to outlined questions we tried to apply our findings in clinical practice.

Owing to the important role of granulocytes in leukemogenesis, we have determined the presence of HP1 protein as well as the methylation status of H3K9 in blood cells of patients suffering by aggressive or chronic type of myeloid leukemia (AML and CML). It was shown that granulocytes of AML and CML patients contain methylated histone H3 (mainly dimethylation was

studied) and even the presence of HP1 was observed in the case of AML patients. Due to this reason we have studied also the degree of methylation during the patient treatment. The results obtained show that methylation is preserved even in case of apparently patients after Gleevec application (in the absence of the malignant clone). This result is extremely interesting since it means that (1) patients after Gleevec therapy are not completely cured despite the "eradication" of cells carrying genetic aberration and (2) genetic aberration probably arises already on the background of higher granulocytes methylation, which is not detected in normal cells. These observations lead to the hypothesis that the primary cause of malignant diseases is not the genetic aberration itself but – at least in some cases – epigenetic changes of chromatin structure. We can speculate that genetic instability occurs as a consequence of epigenetic changes, which consequently results in development of genetic aberrations, probably of (partially) specific kind. Only some cells (e.g. those carrying the BCR/ABL translocation) from the whole mutated population are enforced in further transformation. Why is the HP1-heterochromatin of differentiated cells the marker of their malignancy is another from many arising questions. Again, we can speculate about the relation between the heterochromatin appearance and gene expression. HP1-heterochromatin, more flexible due to highly dynamic binding of HP1 to H3K9, is probably more appropriate for regulation of gene expression in dividing cells, whereas "alternative" heterochromatin play a part in hereditary silencing of genes. In any case, deserved intensive research activities have to be dedicated to this problem of very high importance.

Studies of experimentally induced changes in histone H3 methylation

The aim of our further experiments was the study of the total dimethylation H3K9 during induced differentiation, apoptosis and after chromatin modifications stimulated by the treatment with histone hyperacetylating and DNA demethylating agents. In additional experiments, the H3K4 dimethylation, H3K9 acetylation and the level of the HP1 protein (HP1 beta) was determined in the interphase nuclei of selected cell types. During megakaryocytic differentiation of leukemic K562 cells, we observed a reduced dimethylation and acetylation of H3K9 while opposite effect was revealed during enterocytic cell maturation. Pro-apoptotic agent etoposide, characterized by an immense clinical applications, did not change the level of H3K9 dimethylation, however, the H3K9 acetylation was reduced. The hyperacetylating drug, trichostatin A (TSA), increased the level of H3K9 acetylation and simultaneously reduced the level of H3K9 dimethylation. Further inhibitor of histone deacetylase (HDAC),

sodium butyrate (NaBt), increased both H3K9 dimethylation and acetylation. The observed changes of H3K9 dimethylation correlated with the levels of heterochromatic protein HP1. Moreover, nuclear arrangement of HP1 was related to the changes in the level of H3K9 dimethylation determined by western blots. The HP1 protein was condensed into well-separated foci after NaBt treatment while TSA stimulated dissociation of the regions studied. Experimentally induced total DNA hypomethylation did not lead to the expected decrease in the level of H3K9 dimethylation. H3K9 dimethylation in the processes studied represents a dynamic epigenetic mark, while H3K4 dimethylation is a relatively stable chromatin modification that undergoes only slight modifications.

Genes dedicated for particular function are localized on chromosomes in clusters

Recently discovered clustering of highly expressed genes represented a strong impuls for further investigations. In our experiments, performed in collaboration with Faculty of Informatics, Masaryk University, time changes of the gene expression were investigated for 19,000 genes in 3 different pathways of cell differentiation. Using advanced mathematical methods, the genes were divided into groups according to their behaviour (time dependence of expression). We found that (1) individual groups form on chromosomes localized clusters and (2) projection of a group corresponding to one particular differentiation type to another the obtained distribution is again strongly non-random with a prevalence of one or several target groups. It seems that individual cellular functions are provided by groups of genes localized on chromosomes in clusters, which allows common regulation of gene expression. More complex functions are combined from simpler by joining the clusters together under common regulation.

Clusters of highly expressed genes are localized in the central regions of the cell nucleus

In the frame of the EU project “3D Genome”, the nuclear localization of one chromosome 11 region with increased gene expression (RIDGE) and one anti-RIDGE were studied. We found that RIDGEs are localized substantially more centrally in the cell nuclei of human fibroblasts and lymphocytes as compared with anti-RIDGEs. It confirms earlier conclusions on radial arrangement of chromatin in cell nuclei. These results are in agreement with findings of R. van Driel (Amsterodam) and T. Cremer (Munchen) groups. At present the project

continues by studies of individual loci inside chromosome 11 RIDGE and anti-RIDGE regions.

GRANTS:

GA AS CR S5004010
Development of new diagnostic techniques for oncology
Principal investigator: S. Kozubek, 2000 - 2004

GA AS CR A1065203
The use of multiple optical tweezers to controlled manipulation and rotation of microobjects
Principal investigator: P. Zemánek, ISI AS CR Brno, co-investigator:
E. Lukášová, 2002 - 2006

GA AS CR A5004306
Human genome structure
Principal investigator: S. Kozubek, 2003 - 2008

GA AS CR K5052113
Structure, expression and interaction of genome
Principal investigator: V. Pačes, IMG AS CR Prague, co-investigator:
S. Kozubek, 2001 - 2004

GA CR 202/02/0804
Chronic myeloid leukemia radiation risk estimation based on BCR -ABL distance in hematopoietic cells
Principal investigator: E. Lukášová, 2002 - 2004

GA CR 202/04/0907
High-resolution cytometry of living cells
Principal investigator: S. Kozubek, 2004 - 2006

IGA MH CR NC 6987-3
Epigenetic control of gene expression in malignant diseases
Principal investigator: S. Kozubek, 2002 - 2004

IGA MH CR 1A8241-3
New possibilities of diagnostics of leukemia using DNA-microarrays
Principal investigator: S. Kozubek, co-investigators: I. Koutná, FI MU Brno, J. Mayer, IHK FN Brno, 2004 - 2006

IGA MEdYS CR ME 565
Structure and function of interphase chromosomes in normal and malignant cells
Principal investigator: S. Kozubek, 2002 - 2004

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

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	BC. MARTINA HRUBÁ
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	MICHAELA MARKOVÁ
	LUKÁŠ BLAHA
	HANA KUBEKOVÁ
	DANA PAULÍČKOVÁ
	MARTINA TALIANOVÁ
	JIŘÍ ŽÁK
	VÁCLAV DIOPAN
TECHNICAL ASSISTANT:	MARTINA KAŠÍKOVÁ

Silene latifolia is a key plant model to study sex determination and sex chromosome evolution. Current studies have been based on genetic mapping of the sequences linked to sex chromosomes with analysis of their characters and relative positions on the X and Y chromosomes. Until recently, very few DNA sequences have been physically mapped to the sex chromosomes of *S. latifolia*. Here we present multicolor FISH analysis of *S. latifolia* chromosomes based on the presence and intensity of FISH signals on individual chromosomes. We have generated new markers by constructing and screening a sample BAC library for appropriate FISH probes. Five newly isolated BAC clones yielded discrete

signals on chromosomes. Two clones were specific for one autosome pair and three clones hybridized preferentially to the sex chromosomes. We present the FISH hybridization patterns of these five BAC inserts together with previously described repetitive sequences (X-43.1, 25S rDNA, and 5S rDNA), and use them to analyze the *S. latifolia* karyotype. The autosomes of S. latifolia are difficult to distinguish based on their relative arm lengths. Using one BAC insert and the three repetitive sequences, we have constructed a standard FISH karyotype that can be used to distinguish all autosome pairs (Fig. 1). We also analyze the hybridization patterns of these sequences on the sex chromosomes and we discuss the utility of the presented karyotype mapping strategy to study sex chromosome evolution and Y chromosome degeneration.

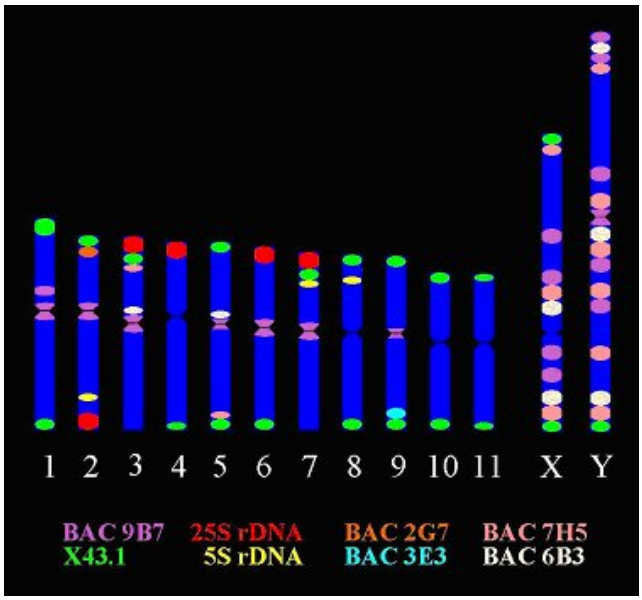

Fig. 1. Idiogram of S. latifolia chromosomes with location of FISH signals. Autosome pairs are ordered according their relative length. All autosome pairs possess specific FISH patterns and therefore can be easily distinguished. DNA probes (BACs and repeats) are indicated in corresponding colors.

We also present an improved FISH strategy for differentiating the sex chromosomes of the dioecious model plant, *Silene latifolia*. Fixed mitotic protoplasts were dropped on a polyethylene naphthalate membrane, the X or Y chromosomes were isolated using nitrogen laser beam microdissection, catapulted by laser pressure, and amplified by DOP-PCR. A modified FAST-

FISH protocol based on a short hybridization time combined with a low concentration of probe was used. The success of this approach is demonstrated by the differential labeling of the X and Y chromosomes and it could represent a quick method for comparing organization of plant genomes (Fig. 2).

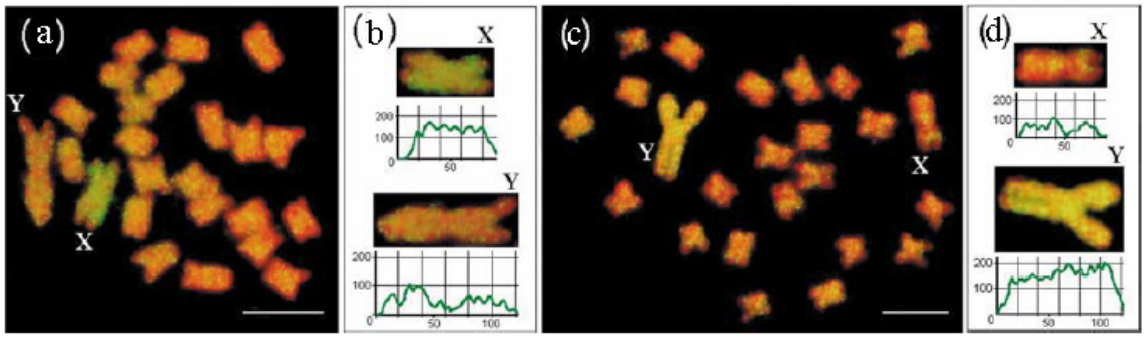

Fig. 2. FAST-FISH patterns on male metaphase chromosomes of S. latifolia. (a,b) The preferential labeling of the X chromosome when the DOP-X probe was used. (c,d) The clearly distinct Y chromosome labeling in the case when the DOP-Y was applied.

The Mre11/Rad50/Nbs1 complex is involved in many aspects of chromosome metabolism. Aberrant function of the complex is associated with defects in the DNA checkpoint, double-strand break repair, meiosis, and telomere maintenance. Here, we report the consequences of Mre11 dysfunction for the stability of mitotic and meiotic chromosomes in *Arabidopsis thaliana* (Fig. 3). Although plants homozygous for a T-DNA insertion in a conserved region of the MRE11 gene are viable, they exhibit growth defects and are infertile. Analysis of mitotic chromosomes prepared from the mutant plants revealed abundant dicentric chromosomes and chromosomal fragments. Fluorescence in situ hybridization showed that anaphase bridges are often formed by homologous chromosome arms. The frequency of chromosome fusions was not reduced in mre11 ku70 double mutants, suggesting that plants possess DNA end-joining activities independent of the Ku70/80 and Mre11 complexes. Cytogenetic examination of pollen mother cells revealed massive chromosome fragmentation and the absence of synapsis in the initial stages of meiosis. The fragmentation was substantially suppressed in mre11 spo11-1 double mutants,

indicating that Mre11 is required for repair but not for the induction of Spo11-dependent meiotic DNA breaks in *Arabidopsis*.

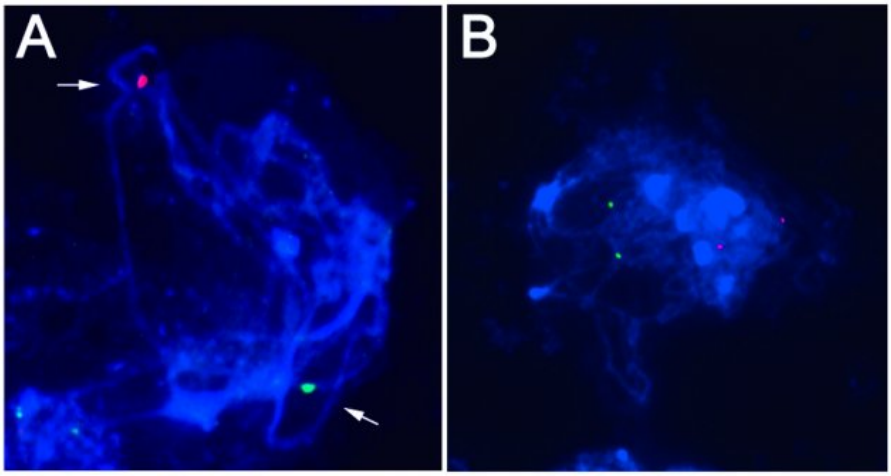

Fig. 3. Meiotic spreads from PMCs of wild-type (A) and mre11-3 (B) plants corresponding to the pachytene stage were hybridized with BACs F6F3 (chromosome 1, terminal, red) and F1N10 (chromosome 4, intercalary, green). The paired signals indicated by arrows in the wild-type pachytene indicate synapsis of homologous chromosomes.

GRANTS:

GA AS CR A6004304
Epigenetic consequences of telomere dysfunction
Principal investigator: B. Vyskot, 2003 - 2006

GA AS CR K5052113
Structure, expression and interaction of genome
Principal investigator: V. Pačes, IMG AS CR Prague, co-investigator:
B. Vyskot, 2001 - 2004

GA CR 204/02/0417
Structure and function of sex chromosomes in the model dioecious plant, *Silene latifolia*
Principal investigator: B. Vyskot, 2002 - 2004

GA CR 522/02/1485
Histological and functional analysis of the Y-chromosome linked gene complexes in *Silene latifolia*
Principal investigator: B. Janoušek, (2002 - 2004)

GA CR 521/02/0427
Construction of *Silene latifolia* BAC library and its application
Principal investigator: E. Kejnovský, 2002 - 2004

GA CR 522/03/0354
Cytogenetic study of nuclear chromatin in plants
Principal investigator: J. Široký, 2003 - 2005

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

LABORATORY OF MOLECULAR ANALYSIS OF PLANT DEVELOPMENT (LMAPD)

HEAD:	DOC. RNDR. BŘETISLAV BRZOBOHATÝ, CSC.
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	MGR. JAN ZOUHAR, DR.
	RNDR. JAN HEJÁTKO, PH.D.
GRADUATE STUDENTS:	MGR. ALENA REKOVÁ
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	MGR. JAKUB HORÁK
	RNDR. JAN HEJÁTKO
	MGR. PAVEL MAZURA
	MGR. MARKÉTA ŠÁMALOVÁ
	MGR. PETRA BORKOVCOVÁ
	MGR. HANA BUBENÍČKOVÁ
	MGR. MICHAL SLANÝ
	MGR. PŘEMYSL SOUČEK
	MGR. HANA RYŠAVÁ
DIPLOMA STUDENT:	PAVEL MAZURA, BC.

Biological function of a putative cytokinin receptor CKI1

Previously, we have found that a two-component sensor histidine kinase CKI1, originally implicated in cytokinin perception, is required for completion of megagametogenesis in *Arabidopsis*. However, *CKI1* expression analysis suggests CKI1 function in sporophytic tissues, too. As the mutant *cki1* alleles cannot be obtained in the homozygous state, we have employed posttranscriptional gene silencing to elucidate the potential role of CKI1 in sporophytic tissues. Using RT-PCR, transgenic lines displaying a reduction in *CKI1* mRNA levels were identified. Surprisingly, the vegetative development is not markedly altered in the transgenic lines. However, the lines are semi sterile. Confocal laser scanning microscopy revealed that the reduction in *CKI1* mRNA levels resulted in dramatic defects in early megagametogenesis. The pleiotropic nature of the defects included defects in mitosis, formation and positioning of the vacuole, and embryo sac stability. Taken together with the

analysis of megagametophyte development in *cki1* mutants and *CKI1* expression in ovule sporophytic tissues prior to meiosis, CKI1 might be involved in sporophyte-megagametophyte communication even before the onset of meiosis. This communication might be critical for proper mitosis, vacuole formation and embryo sac stability.

Functional architecture of an active site in a maize β-glucosidase Zm-p60.1

The project aims to deepen our understanding of the molecular determination of aglycone specificity in β-glucosidases. Previously, we have found at least two distinct modes of molecular determination of preferential affinity for aromatic aglycones in two β-glucosidase sub-families. In the first sub-family represented by a maize β-glucosidase Zm-p60.1, the preference for substrates possessing an aromatic aglycone is thought to be determined by a cluster of aromatic amino acid residues F193-F200-F461-W373. In an attempt to analyze the relationships of the two modes of aglycone binding, we have substituted individually amino acid residues in the cluster by corresponding amino acid residues (F193A, F200K, F461L, W373K) found in the active center of a representative of the second subfamily, a β-glucosidase of *B. napus*. A novel production and purification method was developed for the mutant enzymes. Enzyme kinetic analysis using the chromogenic substrate p-nitrophenyl-β-D-glucosidase indicates a role for F193 in preventing solute entry into the Zm-p60.1 aglycone binding site. Opening the aglycone binding site to solute apparently results in higher affinity of the enzyme for the substrate in its ground state and, thereby, reduced turnover number due to increased activation energy. Further, stacking interactions between a polar aromatic aglycone and W373 might be substituted by an interaction of a positively charged lysine residue (W373K) with a partial negative charge of the aglycone. The aliphatic amino acid residue appears functionally equivalent to the aromatic residue in position 461 (F461L). Substitution F200K results in decreased affinity of the enzyme for the substrate and decreased turnover number.

GRANTS:

GA AS CR K5052113
Structure, expression and interaction of genome
Principal investigator: V. Pačes, IMG AS CR Prague, co-investigator: B. Brzobohatý, 2001 - 2004

GA CR 203/02/0865
Functional architecture of an active site in a maize β-glucosidase
Principal investigator: B. Brzobohatý, 2002 - 2004

IGA MEdYS CR, LN00A081, 2000 - 2004
Signalling pathways in plants
Principal investigator: B. Brzobohatý, 2002 - 2004

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

PROGRAM V

KINETCS OF CELL POPULATIONS

LABORATORY OF CYTOKINETICS (LC)

HEAD:	DOC. RNDR. ALOIS KOZUBÍK, CSC.
SCIENTISTS:	DOC. RNDR. JIŘINA HOFMANOVÁ, CSC.
	RNDR. JAN VONDRÁČEK, PH.D.
	MGR. MARTINA KOVAŘÍKOVÁ, PH.D.
	MGR. KAREL SOUČEK, PH.D.
	MGR. ALENA VACULOVÁ, PH.D.
	MGR. KATEŘINA CHRAMOSTOVÁ, PH.D.
	MGR. JIŘINA PROCHÁZKOVÁ, PH.D.
RESEARCH FELLOWS:	MGR. JAROMÍRA NETÍKOVÁ
	BC. MARTINA URBÁNKOVÁ
GRADUATED STUDENTS:	MGR. JIŘÍ ŠTIKA
	MGR. JIŘINA ZATLOUKALOVÁ
	ING. VIKTOR HORVÁTH
UNDERGRADUATE STUDENTS:	MGR. LENKA STIXOVÁ
	RNDR. LENKA ŠVIHÁLKOVÁ-ŠINDLEROVÁ
	RNDR. ZDENĚK ANDRYSÍK
	ING. VIKTOR HORVÁTH
	LENKA UMANNOVÁ
	HANA NOVOTNÁ
	JIŘÍ SUCHÝ
	LENKA KOČÍ
	EVA LINCOVÁ
	ZUZANA KOUBKOVÁ
	OLGA BLANÁŘOVÁ

In 2004, the studies were focused on the understanding of the role of lipidic membrane components, especially polyunsaturated fatty acids (PUFAs). The research was related to the effects of environmental factors on cell signaling. The interaction with specific endogenous regulators of proliferation, differentiation and apoptosis in cancer as well as non-cancerous cell populations was investigated.

The effects of fatty acids and endogenous apoptotic regulators of TNF family on colon epithelial cells

Polyunsaturated fatty acids (PUFAs) of n-6 (arachidonic acid, AA) or n-3 (docosahexaenoic acid, DHA) type were found to modulate proliferation and/or cell death induced by sodium butyrate or apoptotic inducers from TNF family (TNF-alpha, anti-Fas, TRAIL) in the human colon adenocarcinoma cell line HT-29. These effects were associated particularly with changes in cell cycle, cell membrane phospholipid composition and oxidative metabolism after PUFA treatment.

The apoptotic response to TNF-alpha and anti-Fas antibody (CH-11) was increased by pretreatment of HT-29 cells with AA or DHA. The higher amount of floating cells, subG_0/G_1 population and apoptotic cells was detected in pretreated cells. These effects were further potentiated by protein synthesis inhibitor cycloheximide. The effects of CH-11 were associated with activation of caspase-8, -9, and -3, cleavage of poly(ADP-ribose)polymerase (PARP), and decreased mitochondrial membrane potential, but these parameters were not significantly changed in PUFA pretreated cells.

Modulation of HT-29 cell apoptosis at both molecular (pro-caspase-3, -8, Bid, PARP cleavage) and cellular (cell viability and adhesion) levels was demonstrated also after cooperative action of DHA and TRAIL.

The studies of interaction of sodium butyrate (NaBt) and TNF-alpha during differentiation of colon epithelial HT-29 and FHC cells were focused on the role of transcription factors NF-κB and PPAR-$\tilde{\gamma}$ Protein expression (Western blotting) of both factors and their activities were detected using stable transfectants prepared by transfection of both cell lines with reporter constructs for transcription factors studied. While PPAR-γ expression was not changed in HT-29 cells after both 4 and 24 hours, its activity was enhanced already after 4 hour NaBt treatment and particularly after the combined treatment with TNF-alpha. NF-κB expression was increased by NaBt and its activity was higher after TNF-alpha treatment. On the other hand, both expression and activity of NF-κB were decreased after combined NaBt and TNF-alpha treatment. In FHC cells, PPAR-γ expression was not changed, while NF-κB expression was suppressed either by TNF-alpha or its combination with NaBt.

The effects of cytostatics

Anti-tumor potency of platinum(IV) complexes with increasing hydrophobicity of their ligands was compared. Cytotoxic potential of the new platinum(IV) complex, coded as LA-12 [(OC-6-43)-bis (acetato)(1adamantylamine)

amminedichlo- roplatinum(IV)], was compared with the series of complexes of the general formula (OC-6-43)-bis(acetato) (alkylamine)amminedichloro-platinum(IV). Alkylamine ligands with increasing hydrophobicity were: isopropylamine, cyclohexylamine, 1-adamantylamine and 3,5-dimethyl-1-adamantylamine. The platinum(IV) complexes were coded as LA-4, LA-2 (known as JM-216), LA-12 and LA-15, respectively. Cytotoxicity was tested on the panel of cancer cell lines using the microplate tetrazolium (MTT) assay and the results were verified by microscopy. HPLC was used to measure hydrophobicity, stability of complexes in various buffers and velocity constants for their reactivity with glutathione. Platinum(IV) complexes with bulky hydrophobic ligands (LA-12 and LA-15) demonstrated about one order higher velocity constant for pseudo-first-order reaction with glutathione in comparison to cisplatin, LA-4 and LA-2, whose velocity constants were close to those measured for cisplatin and related platinum(II) complexes. Cytotoxicities of LA-12 and LA-15 towards cisplatin-resistant epithelial carcinoma A2780/cisR were superior to cisplatin, LA-4 and LA-2 in both 24- and 72-h continuous exposure MTT tests. Rapid induction of apoptosis in the treated cancer cell lines and no cisplatin cross-resistance were found for LA-12, which is a potential candidate for clinical testing.

The results achieved in ecotoxicology

Using model epithelial cell line WB-F344, isolated from adult rat liver, we studied effects of selected groups of toxic compounds that are either present at high levels in the environment or are being used for mechanistic studies of toxicants. We found that genotoxic polycyclic aromatic hydrocarbon (PAH) dibenzo(a,l)pyrene, which is an efficient mutagen and carcinogen, induced activation of MAP kinases ERK1/2 and p38, and stimulated phosphorylation of p53 tumor suppressor at serine 15. Using specific inhibitors of MAP kinases and of p53 transcriptional activity, we found that MAP kinase activation and p53 phosphorylation are closely associated with induction of programmed cell death. A similar type of effect was observed also when using model tissue-specific derivatives of 7-*H*-dibezo(c,g)carbazole. It was found that the ability of these compounds to stimulate cell proliferation and to activate aryl hydrocarbon receptor (AhR) are closely related. The effect at cellular levels depends on the balance between induction of apoptosis and cell proliferation.

Polychlorinated biphenyls (PCBs) are an important group of environmental contaminants. A number of PCBs are carcinogenic in vivo. Using model compound from the group of dioxin-like PCBs (PCB126, PCB105), non-dioxin-

like PCBs (PCB47, PCB153) and hydroxylated PCB metabolites (4-OH-PCB187, 4'-OH-PCB79), we found that the AhR-activating compounds can release WB-F344 cells from contact inhibition. The stimulation of cell proliferation corresponded with ability to induce CYP1A1 mRNA expression, a model AhR target. Induction of cell proliferation was associated with an increased expression of cyclin A and D2, as well as with increased cyclin-dependent kinase 2 activity. These results shown that the tumor promoting effects of dioxin-like PCBs or their metabolites in liver could be related to an AhR-mediated induction of cell proliferation.

With an aim to better describe the role of AhR in these effects, we studied impact of model AhR ligands, flavonoids, which act as either agonists or antagonists of AhR. We found that both agonists and antagonists induce similar effects, i.e. induction of cell cycle progression and cell proliferation, although the antagonists are only weak inducers of AhR-dependent genes.

Our results contribute to a general understanding of regulatory mechanisms in epithelial cells that might affect carcinogenesis. These findings might help to better describe the mechanisms of carcinogenic effects of PAHs. These problems are further studied in collaboration with a Johannes Gutenberg-University, Mainz, Germany, aiming to describe the role of AhR and cell cycle regulatory proteins in effects of polyaromatic compounds on liver epithelial cells.

The results achieved in area of applied research

Practical applications of the research of PUFA effects on cancerous and non-cancerous cell populations should lead towards development and optimalization of parenteral lipid emulsions. In cooperation with Infusia Hořátev a. s., it was demonstrated that

1) supplementation of cells *in vitro* with lipid emulsions caused changes of fatty acid content in cell lipids and in cell oxidative metabolism, 2) the response of human cell lines derived from normal fetal colon (FHC) or colon adenocarcinoma (HT-29) was differently affected. The concentration of emulsions which did not affected HT-29 cells increased percentage of floating and subG_0/G_1 FHC cells probably due to their higher reactive oxygen species production and lipid peroxidation.

GRANTS:

GA AS CR B6004407
Interactions of genotoxic and nongenotoxic effects of polycyclic aromatic hydrocarbons in regulation of cell proliferation
Principal investigator: J. Vondráček, 2004 - 2006

GA AS CR S5004009
Alternative therapeutic strategies in oncology
Principal investigator: A. Kozubík, 2000 - 2004

GA AS CR K5011112
Molecular and cellular basis of severe disorders
Principal investigator: J. Kuneš, IPH AS CR Prague, co-investigator:
A. Kozubík, 2001 - 2004

GA CR 524/04/0895
Mechanisms of cell death induced by dietary lipid components and endogenous apoptotic regulators in colon epithelial cells
Principal investigator: J. Hofmanová, 2004 - 2006

GA CR 525/03/1527
Chemical identification and *in vitro* screening of toxicity of aromatic contaminants in agricultural production environment
Principal investigator: M. Machala, VÚVeL Brno, co-investigator:
J. Vondráček, 2003 - 2005

GA CR 524/03/0766
Modulation of proliferation, differentiation and apoptosis of hemopoietic cells – interactions of cytokines, drugs and lipid nutrition compounds
Principal investigator: A. Kozubík, 2003 - 2005

GA CR 524/02/P051
The interaction of cytokine TNF-α with butyrate during differentiation and apoptosis of colon epithelial cells
Principal investigator: M. Kovaříková, 2002 - 2004, supervisor: J. Hofmanová

MPO CR - Program TANDEM No. FT-TA/015
The development of lipid emulsions for parenteral nutrition with specific pharmacological effects
Principal investigator: J. Mikeska, Infusia, a.s. Hořátev, co-investigator: A. Kozubík

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

LABORATORY OF FREE RADICAL PATHOPHYSIOLOGY (LFRP)

HEAD:	RNDR. ANTONÍN LOJEK, CSC.
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	RNDR. HANA ČÍŽOVÁ, PH.D.
RESEARCH FELLOW:	MVDR. IVANA PAPEŽÍKOVÁ
	MGR MARTINA PAVELKOVÁ
TECHNICAL ASSISTANTS:	BLANKA PANÁKOVÁ
	LENKA VYSTRČILOVÁ
GRADUATE STUDENTS:	MGR. DANIELA KOMRSKOVÁ
	MGR. LUCIE GALLOVÁ
	MGR. VERONIKA HÁJKOVÁ
	MGR. JANA FRANKOVÁ
UNDERGRADUATE STUDENTS:	JANA KRÁLOVÁ
	ANETA MORAVCOVÁ
	LUCIE GOJOVÁ
	JANA HAZDROVÁ
	MICHAELA MAŇASOVÁ

Hyaluronic acid (HA), due its unique biophysical properties and physiological compatibility, significantly contributes to wound healing. HA is used as scaffold for healing wound where the normal healing process is impaired. Unfortunately the turnover of HA in the mammalian body is surprisingly high due to hyaluronidase digestive activity. In skin, which contains 50% of total body hyaluronan, the half-life of HA is about one day. The aim of this study was to determine and compare the response of human leukocytes to 1.52 MDa HA and HA of the same batch where a resistance to hyaluronidase digestion was reached by treatment of HA with *N*-Ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide (HA-DR). Neither HA nor HA-DR influenced metabolic activity of resting blood phagocytes. In contrast, the oxidative burst of phorbol myristate acetate-activated phagocytes was significantly decreased after co-incubation with HA but not HA-DR. Expression of CD11b on PMNL as well as on monocytes increased for both HA and HA-DR treated samples in a dose dependent manner. The expression of CD25 on lymphocytes was also increased similarly after HA and HA-DR treatment. Therefore our results show a slight immunostimulatory

effect of the compounds tested. It can be concluded that chemical modification of HA did not change significantly its biological potential.
The aim of this study was to clarify the effect of carvedilol on the production of reactive oxygen (ROS) and reactive nitrogen species (RNS) by leukocytes. Phagocytes from rat peripheral blood were used as a model for ROS production; RAW 264.7 cell line (murine macrophages) was used for the study of inducible nitric oxide synthase (iNOS) expression and nitric oxide production. The production of ROS by resting phagocytes and phagocytes activated with different kinds of stimuli was measured using luminol-enhanced chemiluminescence (CL). Carvedilol (0.1 μM – 100 μM) dose-dependently inhibited both the spontaneous and the activated CL of phagocytes, although the effect of carvedilol was also dependent on the type of activator. This suggests that carvedilol, besides its antioxidative properties, affects the metabolic pathway of NADPH oxidase activation. As observed in the second part of the study, carvedilol in 100 μM concentration completely inhibited the production of nitrites by RAW 264.7 cells as determined by Griess reaction. Carvedilol at a concentration of 10 μM had only a slight inhibitory effect and no changes were observed at lower concentrations of carvedilol. This result correlated with the result of Western-blot analysis, where the total inhibition of the expression of inducible iNOS after the co-incubation with 100 μM carvedilol was detected.

The effect of serotonin in a concentration range of 10^{-2} M - 10^{-7} M on various parameters of oxidative burst of phagocytes was studied using various luminol-enhanced chemiluminescence (CL) methods. Serotonin inhibited the CL response of opsonized zymosan-activated neutrophils in human whole blood in a dose dependent manner. Since luminol-enhanced CL of phagocytes is widely considered to be dependent on a reaction of the myeloperoxidase (MPO) system, the effect of serotonin on the activity of MPO was studied in further experiments. Serotonin exerted a dose dependent inhibition of the MPO activity: 99% inhibition (10^{-3} M serotonin), 95% inhibition (10^{-4} M serotonin), 27% inhibition (10^{-5} M serotonin) and 17% inhibition (10^{-6} M serotonin). The lowest concentration of serotonin (10^{-7} M) did not inhibit the activity of MPO at all. Antioxidant properties of serotonin were further studied in various chemical systems producing individual ROS. Only the two highest concentrations of serotonin showed a peroxyl radical-scavenging effect: the total peroxyl-radical antioxidative parameter (TRAP) values were 4959 mmol/l and 410 mmol/l at concentrations of 10^{-3} M and 10^{-4} M, respectively. As for the other ROS, serotonin exerted the strongest antioxidant potential against hydroxyl radical,

lower antioxidant potential against superoxide anion, and the weakest antioxidant potential against hydrogen peroxide. It can be concluded that serotonin could affect the oxidative burst of phagocytes. Both decreasing the generation of ROS and direct scavenging of ROS were responsible for its inhibitory effects.
The effects of four wine polyphenols (catechin, epicatechin, quercetin and resveratrol) in concentrations of 10, 25, 50 and 100μmol/l on different parameters of oxidative metabolism of phagocytes were investigated. It was found that all four polyphenols exerted high antioxidative capacity against peroxyl radical (TRAP method) and decreased the chemiluminescence of murine macrophages RAW 264.7. The long-term effects of polyphenols were studied in RAW 264.7 cells pre-incubated with polyphenols for 1h and stimulated with lipopolysacharide for 24h. It was found that only quercetin diminished the production of macrophage-derived reactive oxygen metabolites. Moreover, quercetin and resveratrol decreased release of nitric oxide (Griess reaction, spectrophotometry) in the dose-dependent manner. In the case of quercetin, the decrease in nitric oxide production corresponded with a decrease in iNOS expression (Western blotting).

GRANTS:

GA AS CR B6004204
Antioxidative properties of flavonoids with respect to the oxidative burst of phagocytes and cooperation between phagocytes and endothelial cells
Principal investigator: M. Číž, 2002 - 2004

GA AS CR K5011112
Molecular and cellular basis of severe disorders
Principal investigator: J. Kuneš, IPH AS CR Prague, co-investigator: A. Lojek, 2001 - 2004

GA CR 305/04/0896
Effects of carvedilol on metabolic activity of neutrophils and monocytes
Principal investigator: A. Lojek, 2004 - 2006

GA CR 524/04/0897
The role of serotonin in mutual interactions of platelets and professional phagocytes
Principal investigator: M. Číž, 2004 - 2006

GA CR 524/02/0395
The influence of hyaluronic acid on the functions and interactions of leukocytes and epithelial cells under physiological and inflammatory conditions
Principal investigator: A. Lojek, 2002 - 2004

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

LABORATORY OF EXPERIMENTAL HEMATOLOGY (LEH)

HEAD:	MUDR. MICHAL HOFER, CSC.
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	MUDR. ANTONÍN VACEK, CSC.
	MGR. LENKA WEITEROVÁ, PH.D.
	RNDR. ZUZANA HOFEROVÁ, CSC.
RESEARCH FELLOWS:	RNDR. JIŘINA HOLÁ
	MGR. DENISA ŠKAPOVÁ-ŠTREITOVÁ
TECHNICAL ASSISTANT:	KVĚTA LÁNÍKOVÁ

In 2004, the research was aimed especially at the study of mechanisms by which an inhibition of proliferation of hematopoietic progenitor cells can be obtained employing pharmacological activation of adenosine membrane receptors. It was found out that these effects can be achieved by the administration of N^6-cyclopentyladenosine (CPA), an adenosine receptor agonist specific for the A_1 receptor subtype. In experiments in mice, the ability of 5-fluorouracil (5-FU), a cytotoxic drug killing preferentially cells in the cycle, was methodically exploited. CPA administered before an injection of 5-FU protected by means of suppression of proliferation progenitor cells for granulocytes and macrophages (GM-CFC) and increased, thus, their numbers at the evaluation in the time interval following 5-FU injection. However, if CPA was given after an injection of 5-FU, the inhibition of proliferation of GM-CFC became evident as a decrease of their numbers in the subsequent period and in an aggravation of the hematopoietic suppression produced by 5-FU. These findings represent the first evidence of the possibility of employing activation of adenosine receptors for inhibition of cell proliferation in the hematopoietic system.

In this year, experiments *in vitro* were commenced which are targeted at testing the ability of adenosine to potentiate the stimulating action of selected cytokines and hematopoietic growth factors on cell proliferation in the cultures of normal mouse bone marrow cells. The hitherto obtained findings give evidence for mutually potentiating action of adenosine and interleukin-3 on the production of GM-CFC. The results of these and following experiments will serve for finding drug combinations suitable for evaluation of their ability to support hematopoietic processes in experiments *in vivo* or if need be, in clinical practice.

GRANTS:

GA AS CR K5011112
Molecular and cellular basis of severe disorders
Principal investigator: J. Kuneš, IPH AS CR Prague, co-investigator: M. Hofer, 2001 - 2004

GA CR 305/02/0423
The effects of adenosine analogs on hematopoiesis
Principal investigator: M. Hofer, co-investigator: J. Vácha, LF MU Brno, 2002 - 2004

AVOZ5004920 (Institutional Research Plan)
Biophysical properties of living systems and their changes under the influence of environmental factors
Principal investigator: J. Šlotová, 1999 - 2004

RESEARCH AND TECHNOLOGICAL CENTRES

BIOMOLECULAR CENTRE

COORDINATOR:	MASARYK UNIVERSITY BRNO
PARTICIPANT:	INSTITUTE OF BIOPHYSICS AS CR BRNO
HEAD IN THE IBP AS CR:	DOC. RNDR. JIŘÍ ŠPONER, DRSC.

Using molecular-dynamic and thermodynamic methods the analysis of kinetics of intermediary states in the forming of guanine quadruplex (G-DNA) was performed. In this manner explicit inclusion of ions in the channel of the quadruplex was possible. As a result a model allowing quantitative reconstruction of the way of formation of the G-DNA stem could be obtained.

We also performed extensive simulations of the „kissing“ dimmer DIS of the initiation sequence of the virus HIV-1 and the H3 DID retrovirus of the Moloney murine leukemia. We found that kissing complexes are stabilized by the structural pocket, mainly occupied by divalent cations.

SIGNALLING PATHWAYS IN PLANTS

COORDINATOR: INSTITUTE OF EXPERIMENTAL BOTANY AS CR, PRAGUE

PARTICIPANT: INSTITUTE OF BIOPHYSICS AS CR BRNO

HEAD IN THE IBP AS CR: DOC. RNDR. BŘETISLAV BZOBOHATÝ, CSC.

We isolated and characterized insertion mutations in the gene coding the response regulator AAR21 in *Arabidipsis thaliana*.

CENTRE OF INFORMATION TECHNOLOGIES (CIT)

HEAD:	RNDR. JOSEF JURSA, CSC.
TECHNICIAN:	LUKÁŠ POSÁDKA

Standard services of the laboratory:

- Operation, servicing and development of the IBP local area network (LAN)
- Operation of the connection of the IBP LAN to Brno Academic Computer Network (BACN) and to the Internet
- Cary on the e-mail server
- Cary on the www server of the IBP (http://www.ibp.cz) inclusive data updating
- Running maintenance and development of computer technique (hardware and software), utilized by all projects solved at the IBP (servers, graphic workstations and simple PCs with Internet access), which is working under UNIX, MS Windows NT/2000/XP and MS Windows 95/98/ME operating systems.
- Consulting and guidance services for individual projects. (Expert help - in a limited amount - with solving problems connected with computer technique and computer network.)

Operation and servicing of a ICCBnet (International Center for Cooperation in Bioinformatics network) national node of the Czech Republic - http://ICCBnet.ibp.cz:

- Mirroring of the Protein Database (PDB) accessible through the Internet
- Sequence databases and accompanying software - Wisconsin GCG package - accessible to users from Academy of Sciences and universities in the Czech Republic
- Operation and servicing of a library server used by Academy of Sciences in Brno region

In the 2004 there was connected CISCO 2600 router to the LAN, which enables to route telephone calls through Internet provider CESNET. This way, researchers at the IBP can communicate with colleagues in similarly equipped

laboratories without any payments to telephone operators. Phoning this way, all other long distance calls are much cheaper.

Main attention of CIT was devoted to security. Accent was done to install security patches in time and regularly updating antivirus databases. All e-mail transfer is monitored at the server by two independent virus scanners and consequently is scanned with software designed to detect and defang dangerous elements inside an e-mail messages. E.g. dangerous attachments are renamed, so that they cannot be run automatically on PC without user knowledge. In addition e-mail is scanned by a system for limitation of un-requested e-mail (spam).

III. PUBLISHED REPORTS

A. PAPERS PUBLISHED IN JOURNALS AND MONOGRAPHS

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C. PAPERS PRESENTED AT CONFERENCES AND IN SCIENTIFIC SOCIETIES

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Electrochemistry of nucleic acids and proteins
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In: Abstracts, p. 25

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Electrochemistry of nucleic acids and proteins. New possibilities in development of biosensors for genomics and proteomics (invited lecture)
55th Annual Meeting of the International society of Electrochemistry, Thessaloniki, Greece, 19. – 24. 9. 2004
In: Book of Abstracts I, nestr.

Paleček, E., Billová, S., Fojta, M., Havran, L., Jelen, F., Masařík, M.:
Electrochemistry of nucleic acids and proteins. Applications in biomedicine and DNA sensing (invited lecture)
II. Uluslararasi Katilimli Moleküler Tani Ve Uygulamalari Sempozyumu (II. Symposium on Molecular Diagnosis and Application), Izmir, Turkey, 10. – 14. 5. 2004
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The effect of carvedilol on the superoxide generation in and myeloperoxidase release from activated human neutrophils in vitro
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Recognition of DNA modified with platinum complexes by protein p53
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In: Poster Abstracts, p. 99a

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AhR- and ER-mediated activities in human blood samples collected from PCB-contamined and background region in Slovakia

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Inhibition of GJIC by new hydrophobic platinum(IV) complex LA-12 through activation of MEK-1/2

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Procházková, J., Kozubík, A., Souček, K., Hofmanová, J.:

Modifications of arachidonic acid metabolism influencing the activity of NF-κB induced by TNFα in leukaemic cell line HL-60

Cytokinematics 2004 – The 8th International Symposium on Microscopy of Live Cells in the Post Genomics Era, Hradec Králové, 5. – 7. 9. 2004

In: Final Programme & Abstracts, p. 31

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In: Program. Sborník Abstrakt, p. 37

Ptáček, J., Neplechová, K., Sýkorová, E., Novotná, M., Fajkus, J.:

Polymorphism in subterminal chromosome regions applied for genotyping of potato varieties

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In: Book of Abstracts, p. 146

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The interaction of antitumor platinum compounds with DNA in the presence of sulfur-containing compounds

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Telomer-vazebné proteiny A. thaliana *ze skupiny SMH1* (Single Myb Histone 1)

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Evolution of a rapidly spreading satelite homologous to intergenic 26-18S rDNA spacer in natural and synthetic Nicotina species

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In: Atherosclerosis, 5, 2004, p. 156

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DNA sequence context modulates recognition of cisplatin-modified DNA by XPA and RPA repair proteins
Metal Compounds in the Treatment of Cancer, Bari, Italy, 19. – 22. 2. 2004
In: Book of Abstracts, p. 18

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Recognition of DNA modified by antitumor cisplatin by RPA and XPA proteins
4th DNA Repair Workshop, Smolenice, Slovakia, 2. – 5. 5. 2004
In: Book of Abstracts, p. 47

Stixová, L., Souček, K., Procházková, J., Hofmanová, J., Kozubík, A.:
Modulation of cytokinetics in differentiating leukemia cells
Cytokinematics 2004 – The 8th International Symposium on Microscopy of Live Cells in the Post Genomics Era, Hradec Králové, 5. – 7. 9. 2004
In: Final Programme & Abstracts, p. 33

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Effect of the low-frequency magnetic fields on the living organisms
Biological Effects of EMFs – 3rd International Workshop, Kos, Greece, 4. – 8. 10. 2004
In: Proceedings Vol. II, p. 944-949

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XXVII. Dny lékařské biofyziky, Hradec Králové, 19. – 21. 5. 2004
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Loss of Arabidopsis-type telomeres in the evolution of Solanaceae
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In: Book of Abstracts, p. 63

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Three distinctive types of telomere in the plant group Asparagales
Plant & Animal Genome XII, San Diego, California (U.S.A.), 10. – 14. 1. 2004
In: Final Abstracts Guide, p. 92

Sýkorová, E., Lim, K. Y., Kunická, Z., Chase, M. W., Bennet, M. D., Leitch, A. R., Fajkus, J.:
Evolutionary switchpoints in plant telomeres
EMBO Workshop / 58th Harden Conference – Telomeres and Genome Stability, Cambridge, U.K., 3. – 7. 4. 2004
In: Programme and Abstracts, p. 28

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Species divergence in Asparagales is associated with the evolution of novel types of telomeres
15th International Chromosome Conference, West London, U.K., 5. – 10. 9. 2004
In: Chromosome Res., 12 (Suppl. 1), 2004, p. 165

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Evolution of plant telomeres – step back or forward?
3rd European Workshop on: Role of Telomeres and Telomerase in Cancer and Aging, Ladenburg, Germany, 21. – 23. 11. 2004
In: Abstracts, nestr.

Sýkorová, E., Neplechová, K., Skleničková, M., Lim, K. Y., Chase, M. W., Leitch, A. R., Fajkus, J.:

How do Alliaceae maintain their telomeres? – revisited

15th International Chromosome Conference, West London, U.K., 5. – 10. 9. 2004

In: Chromosome Res., 12 (Suppl. 1), 2004, p. 59-60

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Chromatin structure of Asparagales telomeres – old story with a new end?

EMBO Workshop / 58th Harden Conference – Telomeres and Genome Stability, Cambridge, U.K., 3. – 7. 4. 2004

In: Programme and Abstracts, p. 17

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Arachidonic acid release and formation of reactive oxygen species induced by methylated anthracene derivatives in rat liver epithelial cells

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Mre11-deficient Arabidopsis exhibit aberrant meiosis and chromosomal instability in somatic cells

15th International Chromosome Conference, London, U.K., 5. – 10. 9. 2004

In: Chromosome Res., 12 (Suppl. 1), 2004, p. 82

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Molecular interactions in nucleic acids. How to relate the gas phase trends with condensed phase data and nucleic acids

Molecules of Biological Interest in the Gas Phase, Exeter, U. K., 13. – 18. 4. 2004

In: Book of Abstracts, p. I. 27

Šponer, J.:

Structure, dynamics and molecular interactions of nucleic acids

International Senior Research Fellows Meeting, Bangalore, India, 24. – 26. 3. 2004

In: Programme and Abstracts, p. 47-48

Trnková, L., Topinková, J., Jelen, F., Postbieglová, I.:
Elimination voltammetry of oligonucleotides
55th Annual Meeting of the International society of Electrochemistry, Thessaloniki, Greece, 19. – 24. 9. 2004
In: Book of Abstracts I, nestr.

Vacek, A., Hofer, M.:
Naše zkušenosti s využitím TF k léčbě poruch krvetvorby
IMMODIN 2004, Prostějov, 8. – 9. 10. 2004

Vacek, J., Trnková, L., Jelen, F., Kizek, R.:
Electrochemical determination of cysteine-containing biomolecules by means of catalytic reactions on a mercury electrode
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In: Book of Abstracts I, nestr.

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Ethanol acts as a potent agent sensitizing the colon cancer cells to the death ligand-induced apoptosis
Mechanisms of Cell Death – 5th Symposium of the International Cell Death Society, Maynooth, Ireland, 25. – 28. 6. 2004
In: Programme and Abstract Book, p. 69

Vařecha, M., Amrichová, J., Ondřej, V., Lukášová, E., Kozubek, S., Matula, Pa., Matula, Pe., Kozubek, M.:
In vivo imaging of cell interior using automated high-resolution cytometry
Biophysics of the Genome, Brno, 12. – 13. 10. 2004
In: Biophysics of the Genome: Proceedings of the Workshop Held at Brno, p. 76-82

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Adsorpce nukleových kyselin na povrchu elektrod
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Fakulta matematiky, fyziky a informatiky, Univerzita Komenského, Bratislava, Slovensko, 16. 11. 2004

Vojtíšková, M.:

Reparační mechanismy lidského genomu

Lékařská fakulta MU, Brno, 10. 11. 2004

Vojtíšková, M., Sedlářová, I., Stehlíková, K., Brabec, V.:

Exprese rekombinantního XPA proteinu pomocí bezbuněčného RTS systému

VIII. Pracovní setkání biochemiků a molekulárních biologů, Brno, 3. – 4. 2. 2004

In: Sborník příspěvků, p. 76

Vondráček, J., Bryja, V., Chramostová, K., Krčmář, P., Hampl, A., Kozubík, A., Machala, M..

Disruption of contact inhibition in rat liver epithelial cells by dioxin-like PCBs – a potential tumor-promoting mechanism?

PCB Workshop, Illinois, Illinois (USA), 13. – 15. 6. 2004

In: Book of Abstracts, nestr.

Vondráček, J., Chramostová, K., Bryja, V., Krčmář, P., Andrysík, Z., Faust, D., Dietrich, C., Hampl, A., Kozubík, A., Machala, M.:

Disturption of contact inhibition and deregulation of cell cycle induced by both persistent and non-persistent AhR ligands in rat liver epithelial cells

SETAC Europe – 14th Annual Meeting, Praha, 18. – 22. 4. 2004

In: Abstracts, p. 187-188

Vondráček, J., Chramostová, K., Bryja, V., Šindlerová, L., Andrysík, Z., Krčmář, P., Hampl, A., Kozubík, A., Machala, A.:

Deregulation of cell cycle control in rat liver epithelial cells by aryl hydrocarbon receptor ligands

XIX. Biochemický sjezd, Olomouc, 31. 8. – 3. 9. 2004

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Disruption of contact inhibition in rat liver epithelial cells by various types of AhR ligands
Dioxin 2004 –24th International Symposium on Halogenated Environmental Organic Pollutants and POPs, Berlin, Germany, 6. – 10. 9. 2004
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Vondráček, J., Chramostová, K., Švihálková-Šindlerová, L., Andrysík, Z., Kozubík, A., Pěnčíková, K., Krčmář, P., Gábelová, A., Machala, M.:
Interactions of genotoxic and nongenotoxic effects of PAHs
Central and Eastern European Environmental Health Conference, Praha, 24. – 27. 10. 2004
In: Book of Abstracts, p. 37

Vyskot, B..
Epigenetika
X. ročník kurzu pro učitele středních škol, Brno, 3. – 5. 2. 2004

Vyskot, B.:
Silene latifolia: Structure and function of sex chromosomes
Silene-Microbotryum Meeting, University of Virginia, Virginia (U.S.A.), 28. - 29. 8. 2004
In: Program, nestr.

Weiterová, L, Hofer, M., Pospíšil, M., Znojil, V.:
Regulatory role of adenosine signalling in heamatopoiesis: mobilization and in vitro studies
9th Congress of the European Hematology Association, Geneva, Switzerland, 10. – 13. 6. 2004
In: Hematol. J., 5 (Suppl. 2), 2004, 194

Zahorecová, J.:
Transcription mapping of DNA adducts of metal-based drugs
COST D20 – Joint Working Group Meeting 0001-00 and 0009-01: DNA and Protein interactions of platinum – and non-platinum anticancer agents, Vienna, Austria, 5. – 6. 11. 2004

Zahorecová, J., Nováková, O., Van der Schilden, K., Brabec, V.:

DNA binding properties of a new series of polynuclear ruthenium polypyridyl complexes

Metal Compounds in the Treatment of Cancer and Viral Diseases (COST D20 Conference), Garmisch – Partenkirchen, Germany, 27. - 28. 8. 2004

In: Book of Abstracts, p. P-57

D. Supplementary reports due to the Research Report 2003

Ježek, J., Palša, S., Lukášová, E., Kozubek S., Jákl, P., Šerý, M., Jonáš, A., Liška, M., Zemánek, P.:

Employment of laser induced fusion of living cells for the study of spatial structure of chromatin

In: Proceedings of the 13th Polish-Czech-Slovak Optical Conference on Wave and Quantum Aspects of Contemporary Optics (Proc. SPIE 5259). Washington, SPIE-The International Society for Optical Engineering 2003, p. 336-340

E. OVERVIEW OF PUBLICATION ACTIVITIES IN 2004

1. Full-length papers	86
- supplementary, due to 2003	4
2. Short communications	2
3. Popularizing articles	2
4. Lectures - presented in the CR	6
- presented abroad	3
5. Lectures presented at conferences:	
National	21
International in the CR	27
International abroad	94
6. Abstracts of conferences:	
National	21
International in CR	15
International abroad	88
- supplementary, due to 2003	1
7. Lectures published in full extenso	12

IV. INTERNATIONAL CONTACTS

As in previous years, international contacts were established in connection with research projects, supported by various grant agencies both from the Czech Republic and from abroad, on the basis of competitions organized by the Academy of Sciences of the Czech Republic (hereafter the Academy of Sciences CR or AS CR) or at the invitation of foreign institutions, etc.

An overview of international contacts in 2004 is provided in tables as follows:

Travels of scientists abroad

Country	*AS CR competition*	*Grants*	*Other sources*
Australia		1	
Austria		10	2
Belgium	1	6	3
Brasilia		1	
Bulgaria	1		
Canada		1	
China		1	
Croatia		1	
Estonia		2	
Finland	1	1	
France		9	3
Germany		22	4
Great Britain		7	5
Greece		7	1
Hungary		1	2
Israel	1	1	
Italy		6	
Japan			1
Luxemburg		3	
Mexico		3	
New Zealand		1	
Norway		3	
Poland		5	

Russia		1	
Scotland		3	
Slovak Republic	2	17	8
Spain		1	
Switzerland		3	
The Netherlands		4	1
Turkey		2	1
USA		3	9
Total	6	130	40

Foreign guests

Country	*AS CR competition*	*Grants*	*Other*
Bulgaria	1		
France	2	1	
Great Britain		1	
Hungary	1	1	
Israel		1	
Italy		1	
Slovak Republic	2		1
Spain		1	
Switzerland		1	2
Turkey		1	
USA			1
Total	6	8	4

A. Overview of international co-operations of the Institute of Biophysics and foreign grants in 2004

Joint research based on direct agreements with foreign laboratories and projects which received grants from abroad continued as shown below.

1. Direct agreements with foreign laboratories

BULGARIA
Institute of Oceanology, BAS, Varna
A. Lojek - Oxidant/antioxidant properties of marine biota - indication for coastal marine environment and human health assessment

FINLAND
University of Turku, Department of Biochemistry, Turku
A. Lojek - Role of phagocytes in the oxidative injury of animal cells and tissues

GERMANY
Max Planck Institut für biophysikalische Chemie, Göttingen
E. Paleček - Aggregation of the alfa-synucleine using electrochemical methods

GREAT BRITAIN
The University Court of the University of Edinburgh
V. Brabec - Collaboration and Material Transfer Agreement

Queen Mary and Westfield College, University of London
A. Kovařík - Research in the field of plant genetics and epigenetics

ITALY
European Institute of Oncology, Milano
S. Kozubek - Mechanisms of the origin of promyelotic leukemia

SLOVAK REPUBLIC
Institute of Experimental Physics, Slovak Academy of Sciences, Košice
A. Kozubík - Investigation of biological membranes a their models, structure and stability of nucleic acids and proteins, their interaction with drugs

Institute of Biological and Ecological Sciences, Faculty of Natural Sciences, P. J. Šafárik University, Košice
A. Kozubík - Regulation, proliferation, differentiation and apoptosis in tumor cell populations *in vitro* and *in vivo*

USA
Virginia Commonwealth University, Richmond
V. Brabec - Mechanistic studies on new platinum clinical agents

2. Foreign Grants

ARGENTINA
Grupo de Innovación Tecnologica en la Universidad de Buonos Aires
L. Novotný (IFCH JH AV CR Prague), E. Paleček (2002 - 2004) - Interaction of peptides, proteins and DNA with electrodes and new electrochemical methods for biochemistry and molecular genetics

FRANCE
CNRS/AS CR Collaboration, Institute Jacques Monod, Paris
M. Štros (2003 - 2004) – Alternative structures of the DNA and the function of eukaryotic genomes

GERMANY
DAAD/AS CR Collaboration, Institut für Toxicologie, Johannes Guttenberg Universität, Mainz
J. Vondráček (2004 – 2005) - Disturbances in the contact inhibition by polyaromatic compounds

GREAT BRITAIN
The Wellcome Trust, 0673646/Z/03/Z
V. Brabec (2004 - 2007) - Platinum and ruthenium complexes. From DNA damage to cancer chemotherapy

The Leverhulme trust F/07476/G
J. Fajkus (2001 - 2004) - Loss and gain of typical telomere repeats in a major radiation of monocots

Senior Wellcome Trust International Research Fellowship for Biomedical Research in Central Europe, GR067507MF
J. Šponer (2003 - 2007)

GREECE
KONTAKT Program, MEdYS CR, STC CR/Greece, ME 685
Laboratory of Physical Chemistry, Department of Chemistry, Aristotle University, Thessalonica
KONTAKT Program, MEdYS CR, STC CR/Greece, RC-3-24

Laboratory of Physical Chemistry, Department of Chemistry, Aristotle University, Thessalonica
V. Vetterl (2003 - 2005) Develoment of methods and construction of sensors for the detection of DNA-drug interactions

ITALY
KONTAKT Program, MEdYS CR, STC CR/Italy, No. 16
Universita Cattolica del Sacro Cuore, Roma
M. Číž (2002 - 2004) - Chemiluminescent determination of the role of neutrophils in the development of oxidative stress-induced injury

JAPAN
KONTAKT Program, MEdYS CR, STC CR/Japan, ME 565
Department of Life Science, Faculty of Bioresources, Mie University, Mie
S. Kozubek (2002 - 2006) - Structure and function of interphase chromosomes in normal and malignant cells

RUSSIA
Committee for collaboration with Joint Institute for Nuclear Research, Dubna
S. Kozubek (2002 - 2004) - Estimation of the risk of chronic myeloid leukemia from low levels of radiation

SLOVAK REPUBLIC
KONTAKT Program, MEdYS CR, STC CR/SR
Institute of Experimental Oncology, Slovak Academy of Sciences, Bratislava
V. Brabec (2004 - 2005) - Platinum complexes: From DNA damage to cancer chemotherapy

KONTAKT Program, MEdYS CR, STC CR/SR
Unifersity of P.J. Šafárik, Košice
A. Kozubík (2004 - 2005) - The role of phospholipid metabolism in photocytotoxic effects of hypericin

KONTAKT Program, MEdYS CR, STC CR/SR
Laboratory of biophysics
V. Vetterl (2004 - 2006) - Study of DNA hybridization

USA
Howard Hughes Medical Institute (HHMI), INTNL 55000313
J. Kašpárková (2001 - 2005) - Basis for new structure - pharmacological relationship of platinum antitumor drugs

National Institute of Health (NIH), 5R01CA708754-07
V. Brabec (2004 - 2007) - Mechanistic studies on new platinum clinical agents

Other fundings

COST OC D20.001
V. Brabec (2001 - 2005) - Biochemistry, structural and cell biology of anticancer platinum drugs of second generation
COST OC D21.001
V. Brabec (2002 - 2005) - Characterisation of metalloproteins, key molecules in biological processes
COST OC D20.003
V. Brabec (2002 - 2005) - Intracellular and extracellular target sites for anti-cancer activity and toxiciry of ruthenium complexes
COST OC D21.005
E. Paleček (2002 - 2005) - Characterisation of metalloproteins important in cancer and their interaction with DNA
COST OC D20.004
O. Nováková (2003 - 2005) - Strategy of non-covalent recognition of DNA for design and synthesis of new metal-based drugs

Multilateral Collaboration

EU Research Training Network - HPRN-CT-2002-00145
Co-ordinator: M. Hannon, UK; Participant: *V. Brabec* (2002 - 2004) - Structural effects arising from major groove DNA recognition by metallo-supramolecular cylinders

EU6RP, LSGH-CT-2003-503441

Co-ordinator: R. van Driel, NL; Participant: *S. Kozubek* (2004 - 2006)

3D Genome structure and function

EU6RP, proposal No. 502983

Co-ordinator: K. Wiman, Sweden; Participant: *E. Paleček* (2004 - 2008)

Mutant p53 as a target for improved cancer treatment

B. Co-operations with international governmental and non-governmental organizations

J. Šlotová is a representative of the CR in the ICSU
S. Kozubek is a chairman of the Czech Committee for Biophysics, *V. Brabec, E. Paleček, J. Šlotová* and *V. Vetterl* are members of this Committee
V. Brabec is a representative of the CR in the Managing Board of the European Program of Scientific and Technological Research, COST D20 and D21, and is a member of the panel of European Commission for the evaluation of Marie Curie projects
J. Fajkus is an expert for evaluation of the projects of 5FWP EC „Quality of Life“ in Brussels, Belgium
A. Kovařík is a member of the Commission for Academical Degrees of the University of Gent, Belgium
S. Kozubek is a member of the Programme Advisory Committee, Joint Institute for Nuclear Research, Dubna, Russia
E. Paleček is a consulting member of NATO for DNA biosensors, and a member of the Gregor Mendel Trust; as a trustee since 2002

C. International conferences organized by Institute of Biophysics

Structural and Cellular Biology of Non-Classical Antitumor Platinum Compounds, Bari (Italy), 19. - 22. 2. 2004

Third workshop on Biophysics of the Genome, Brno, 12. - 13. 10. 2004

V. DOCTORAL STUDIES, CO-OPERATION WITH UNIVERSITIES AND OTHER ACTIVITIES

A. Postgraduate studies

In 2004, the Institute of Biophysics successfully continued to participate in postgraduate education (doctoral studies - DSP) at universities, mainly at the Faculty of Science of Masaryk University in Brno. In total, seventy two students worked towards a doctor's degree at the IBP. Twenty one of them were external or combined postgraduate students, fifty one of them were internal students and 7 students interrupted their studies.

Total number of students	*External/ /combined*	*Internal*	*Year*
18	2	16	I.
11	2	9	II.
14	2	12	III.
14	4	10	IV.
6	5	1	V.
2	1	1	VI.
2	2	0	VII.
5	3	2	absolvents

DSP students belong to fields of specialization as follows:

biophysics (20); from them 4 students their study interrupted/finished
molecular biology (25); from them 2 students accomplished their Doctor's Theses and 3 students their study interrupted/finished
genetics (8); 2 students accomplished their Doctor's Theses
animal physiology (8)
immunology (4)
environmental chemistry (1)
botany (1)
anatomy and physiology of plants (2)
microbiology (1); this student accomplished her Doctor's Theses
medical biology (1)
20 scientists of the IBP were appointed as PGS student advisors

Doctoral Theses - undertaken at the IBP and defended in 2004:

S. Billová: Interaction of the protein p53 with DNA and new methods of the analysis of proteins

M. Falk: Hypotheses on the relation between the higher level structure of chromatin and expression of genes

J. Hejátko: Identification of CKI1, a putative sensor histidine kinase, as a key regulatory component of the megagametogenesis in *Arabidopsis thaliana*

R. Hobza: The structure and evolution of plants sex chromosomes

K. Skalická: Dynamics of plant allopolyploid genomes - analysis of natural and synthetic hybrids

The following scientists of the IBP are members of Branch Councils (BC) for postgradual studies at the Faculty of Science of Masaryk University in Brno:

BC for DSP Physics: *V. Brabec* (garant for DSP biophysics)
BC for Biophysics: *V. Brabec* (chairman), *S. Kozubek, J. Šlotová, V. Vetterl (members)*

BC for DSP Biology: *B. Vyskot*
BC for Molecular and Cell Biology: *J. Fajkus, J. Kypr, E. Paleček, V. Vetterl*
BC for Physiology and Developmental Biology of Animals: *J. Hofmanová, A. Kozubík*
BC for Immunology: *M. Číž, A. Lojek*
BC for Genetics: *B. Vyskot*

BC for DSP Chemistry:
BC for Environmental Chemistry and Ecotoxicology: *J. Hofmanová, A. Kozubík*

BC for DSP Biochemistry: *J. Šponer*
BC for Biochemistry: *J. Šponer*

In addition to this, IBP scientists are members of these of Branch Councils at other faculties:

Faculty of Medicine, Masaryk University in Brno:
BC for Biophysics: *V. Vetterl*
BC for Molecular Biology: *V. Vetterl*

Faculty of Medicine, Palacký University in Olomouc:
BC for Medical Biophysics: *V. Vetterl*

Faculty of Science, Palacký University in Olomouc:
BC for Physical and Analytical Chemistry: *E. Paleček, V. Vetterl, O. Vrána*
BC for Botany: *B. Vyskot*
BC for Biophysics: *V. Brabec, S. Kozubek, J. Šlotová, V. Vetterl*

Faculty of Science, Charles University in Prague:
BC for Anatomy and Physiology of Plants: *B. Vyskot*

Faculty of Mathematics and Physics, Charles University in Prague:
BC for Molecular and Biological Structures: *V. Brabec*

B. Co-operation with Universities

Masaryk University in Brno:
S. Kozubek is a member of the Scientific Council

Faculty of Science, Masaryk University in Brno:
J. Šlotová is a member of the Scientific Council

Faculty of Medicine, Masaryk University in Brno:
J. Šlotová is a member of the Scientific Council
J. Fajkus is a member of the board of the biology section and a member of the commission for attestations, section of biology
A. Kozubík is a member of the commission for attestations, section of biology

Palacký University in Olomouc:
B. Vyskot is a member of the Scientific Council

Faculty of Sciences, Palacký University in Olomouc:
V. Brabec is a member of the Scientific Council

Faculty of Sciences, P.J. Šafárik University, Košice, Slovak Republic:
A. Kozubík is a member of the Scientific Council

In 2004 there was completed habilitation of *B. Brzobohatý, J. Kašpárková, A. Lojek*, *M. Fojta* (at Faculty of Sciences, Masaryk University in Brno) and all of them were nominated as assistant profesors.

C. Membership in scientific institutions

V. Brabec is a member of the Slovak board for doctor's degrees in molecular biology

M. Bezděk is a member of the Czech Committee for Transgenic Plants

M. Hofer is a member of the Sub-branch Committee 305 "Physiology, pharmacology, toxicology" of the Grant Agency CR and is a member of the Branch Council for Theoretical Medical Fields and Pharmacy at the J. E. Purkyně Military Medical Academy in Hradec Králové

J. Jursa is a member of the South Moravian Regional Committee for Computer Technology and a member of the Council for Computer Technology of the AS CR

S. Kozubek was elected a member of the General Assembly of the AS CR for the period 2003 – 2005, a member of the Director Advisory Board of the State Office for Nuclear Safety and is a Co-ordinator of the Consorcium for DNA microarrays

A. Kozubík is a member of the Scientific Council of the Masaryk Oncological Institute, Brno, a member of the Co-ordination Committee of the University Oncological Centre, Brno and a member of the Scientific Council of the program RECETOX at the Faculty of Science, MU Brno

J. Kypr is a member of the Sub-branch Committee 301 "Molecular Biology, Genetics and Experimental Oncology" of the Grant Agency CR

A. Lojek is a member of the Branch Committee 5 " Agricultural Sciences" and the chairman of the Sub-branch Committee 524 "Physiology and Pathology of Animals" of the Grant Agency CR

E. Paleček is a member of the Scientific Council of the AS CR, is a member of the Supervisory Committee of the GA AS CR, a founding member of the Learned Society of the Czech Republic, a member of the Bioethical Committee at the Council of the Government of the CR for research and development, a member of the permanent working group (for biology and ecology) of the Accreditation Committee of the Government of CR for the Universities and a member of the Ministry of Education, Youth and Sport CR Committee for evaluating research plans and results of institutions for granting institutional support to research and development in science

J. Šlotová is a vice-chairman of the Council for International Affairs of the AS CR and a member of the General Assembly of the AS CR

M. Štros is a member of the Sub-branch Committee 204 "Cellular and Molecular Biology"

V. Vetterl is a member of the Branch Council 1 "Mathematical and Physical Sciences and Informatics" of the Grant Agency AS CR

M. Vojtíšková is a member of the Council for qualification degrees in Genetics of the Ministry of health of the CR

B. Vyskot is a member of the Accreditation Committee of the Government of the CR for the Universities and chairman of its working group for biology and ecology, a member of the Learned Society of the Czech Republic and a member of board for doctor's degree (DSc.) in molecular biology, genetics and biology of plants

The following scientists were members of editorial boards of scientific journals:

V. Brabec - Bioorganic Chemistry and Applications
E. Paleček - General Physiology and Biophysics, Bioelectrochemistry and Bioenergetics and Talanta (Guest-editor)
J. Šponer - Journal of Biomolecular Structure and Dynamics (Senior Editor)
V. Vetterl - Czech Journal for Physics
B. Vyskot - Biologia plantarum (Academia, Kluwer) - Co-editor

D. Membership in scientific societies

International scientific organizations and societies:

V. Brabec - member of the Biophysical Society USA, of the Society of Biological Inorganic Chemistry and of the American Society for Biochemistry and Molecular Biology
V. Brázda - member of The Biochemical Society
B. Brzobohatý - member of the Federation of European Societies of Plant Physiology, of the Society for Experimental Biology, of the International Plant Growth Substances Association and of the American Society of Plant Biologists
M. Číž - member of the Society for Free Radical Research
H. Čížová - member of The Oxygen Society
J. Fajkus - member of the American Association for Microbiology
J. Fulneček - member of the DNA Methylation Society
E. Frimlová - member of the Federation of European Societies of Plant Physiology
J. Hejátko - member of the American Society of Plant Biologists
M. Hofer - member of the Council of European Society for Radiation Biology
J. Hofmanová - member of the European Tissue Culture Society, of the International Society for Analytical Cytology and of the International Society for Predictive Oncology

J. Kašpárková - member of the Society of Biological Inorganic Chemistry and of the American Association for the Advancement of Science
S. Kozubek - member of the European Society for Radiation Biology
A. Kozubík - member of the European Tissue Culture Society, of the Society for Leukocyte Biology (USA), of the International Society for Analytical Cytology and of the International Society for Predictive Oncology
A. Lojek - member of the Society for Free Radical Research
E. Paleček - member of the Bioelectrochemical Society
M. Pospíšil - member of the International Astronautical Academy and of the European Society for Radiation Biology
M. Štros - member of the American Society for Biochemistry and Molecular Biology
V. Vetterl - member of the Bioelectrochemical Society and of the International Society of Electrochemistry
M. Vorlíčková - member of the Biophysical Society USA
A. Vacek - member of the International Astronautical Academy
J. Zouhar - member of the American Society of Plant Biologists

National scientific organizations and committees:

M. Bezděk - member of the of the Czech Society for Biochemistry and Molecular Biology and of the Mendel Genetic Society
V. Brabec - member of the Czech Committee for Biophysics (IUPAB)
B. Brzobohatý - member of the Czech Society for Biochemistry and Molecular Biology and of the Society for Experimental Plant Biology
M. Číž - member of the Czech Society for Biochemistry and Molecular Biology
H. Čížová - member of the Czech Society for Biochemistry and Molecular Biology
J. Fajkus – board member of the Mendel Genetic Society
M. Fojtová - member of the Society of Experimental Plant Biology
E. Frimlová - member of the Society of Experimental Plant Biology
M. Hofer - board member of the Czech Radiobiological Society at the Czech JEP Medical Society
J. Hofmanová - member of the of the Society for Tissue Cultivation at the Czech Oncological Society, of the Czech Radiobiological Society at the Czech JEP Medical Society and founding member of the Czech Society for Analytical Cytometry
B. Koukalová - member of the Mendel Genetic Society and of the Czech Biological Society

A. Kovařík - member of the Society of Experimental Plant Biology and of the Mendel Genetic Society

S. Kozubek - chairman of the Czech Committee for Biophysics (IUPAB), board member of the Czech Radiobiological Society at the Czech JEP Medical Society, member of the National Committee for the Exploitation and Research of Cosmic Space and a member of the Advisory Board of the State Office for Nuclear Safety

A. Kozubík - member of the Society for Tissue Cultivation at the Czech Oncological society, of the Czech Radiobiological Society at the Czech JEP Medical Society and founding member of the Czech Society for Analytical Cytometry

L. Kubala - member of the Czech Society for Biochemistry and Molecular Biology

A. Lojek - member of the Czech Immunological Society

R. Matyášek - member of the Mendel Genetic Society

E. Paleček - member of the Czech Committee for Biophysics (IUPAB)

J. Šlotová - member of the Czech Committee for Biophysics (IUPAB)

M. Štros - member of the Czech Society for Biochemistry and Molecular Biology

V. Vetterl - board member of the of the Chemical Physics and Biophysics Branch of the Union of Czech and Slovak Mathematicians and Physicists and member of the Czech Committee for Biophysics (IUPAB)

M. Vorlíčková - member of the Czech Society for Biochemistry and Molecular Biology

O. Vrána - chairman of the Biophysical Section of the Czech Biological Society

J. Vondráček - member of the Czech Immunological Society and of the Czech Society for Biochemistry and Molecular Biology

B. Vyskot – board member of the Plant Biotechnology Section of the Czech Biotechnological Society