ACADEMY OF SCIENCES OF THE CZECH REPUBLIC

INSTITUTE OF BIOPHYSICS

RESEARCH REPORT
2000

Brno 2001

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CONTENTS

I. INTRODUCTION

In 2000, the periodical evaluation of the research plan and results of the Institute of Biophysics of the AS CR for 1995 - 1999 was accomplished. According to document No. 281 of April 22, 1998, the evaluation should review the usefulness of the institution's support in scientific research. The preparation of the required documents was based on five topics – programmes that include research activities of the Institute. The documents contained the most important scientific results achieved, success rate of grants, editorial activities within the assessed period, educational activities, domestic and foreign co-operation and, last but not least, the use of state financial funds was assessed. The Evaluation Committee of the AS CR nominated two domestic reviewers (Prof. Dr. A. Kotyk, DrSc., Prof. Dr. R. Petrásek, CSc.) and three foreign experts (Prof. Dr. M. Hausmann, Prof. Dr. T. Jovin – both from Germany, Prof. Dr. G. Natile – Italy). The evaluation was accomplished on October 24, 2000 in the Institute of Biophysics, with representatives of the Evaluation Committee AS CR present (Prof. Dr. O. Nečas, DrSc., Prof. Dr. S. Zadražil, DrSc.). In summary, this Committee declared that the scientific results of the Institute contributed to the progressive recognition of important biophysical characteristics of living systems. As a result of the evaluation, the Institute of Biophysics was identified as the most important Czech institution in the field of biophysics.

The establishment of the joint laboratory, the Laboratory of Functional Genomics and Proteomics, was an important step in the integration of the scientific-educational co-operation between the Institute of Biophysics and the Faculty of Science MU. A Contract of Co-operation has been agreed on for this purpose.

Two projects under the AS CR "Programme for Research and Development," with the IBP as principal investigator, backed the concentration of scientific capacities on the complex solution of scientific topics which are most important to the Institute. In addition, two Institute laboratories were integrated into the "Research Centres" Programme of the Ministry of Education, Youth and Sports of the CR.

The Scientific Council of the Institute included the following members: *V. Brabec* (chairman), *J. Fajkus, M. Hofer, J. Hofmanová, S. Kozubek* and *E. Paleček* – internal members; *J. Doškař* (Faculty of Science MU), *Z. Šimek* (VUT, i.e. technical university, Brno), *J. Totušek* (Faculty of Medicine MU) – external members.

On the occasion of the Days of Science, the Open Door Day was held on October 20, 2000. 86 grammar school students, 8 university students, 6 scientists from other institutions - in total 101 visitors - visited the Institute laboratories. During 2000, other visitors, both laymen and scientists, also visited the Institute.

Furthermore, we present the Institute researchers whose achievements were appreciated by various institutions:

E. Paleček was awarded by the AS CR Prize for his work, "Biophysical Research into the Structure and Interactions of DNA", and by a Mendel Medal from the College of Liberal Arts and Sciences, which was awarded him by the University of Villanova, Pennsylvania, USA.

Based on the proposal of the rector of MU, *J. Fajkus* was awarded the "Prize of the Ministry of Education, Youth and Sports of the CR for Research" for discovery of the plant telomerases and for contribution to research into telomeres.

V. Brázda was awarded the "League against Cancer" prize for young scientists for the best set of publications on oncology in 1999.

H. Loskotová was awarded the Institute of Biophysics AS CR Prize for her work "Conformational Analysis of Site-specific DNA Cross-links of Cisplatin-distamycin Conjugates", published in the Biochemistry.

The Scientific Council of P.J. Šafařík University in Košice, Slovak Republic, awarded *M. Pospíšil* with an honourable doctor's degree.

The chairman of the AS CR praised *M. Jelínková* and *A. Eliáš* for their long-standing work for the Institute and for the Academy of Sciences of the CR.

In 2000, a new operational and accommodation building has been built. In addition to this, the experimental animal facility has been renovated and accredited and other reconstruction works in the main building (canteen, kitchen and corridor floors) continued.

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II. SCIENTIFIC ACTIVITIES

Individual Laboratories, grouped into five Programmes, undertake the research done by the Institute of Biophysics:

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.

II. Biophysics of Nucleic Acid Complexes

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

Laboratory of Analysis of DNA Molecular Complexes - LADMC
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
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, CSc.

IV. Molecular Cytology and Cytogenetics

Laboratory of Molecular Cytology and Cytometry - LMCC
RNDr. Stanislav Kozubek, DrSc.

Laboratory of Plant Development Genetics - LPDG
doc. RNDr. Boris Vyskot, DrSc.

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

V. Kinetics of Cell Populations

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

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

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

*** * ***

Laboratory of Computers and Information Systems - LCIS
RNDr. Josef Jursa, CSc.

The scientific projects were supported by grants from various grant agencies as follows:

Grant Agency of the Academy of Sciences of the Czech Republic

- 10 standard grants, 2 additional integration grants, 2 additional postgraduate grants
- 2 grants under the Programme for Support of Research and Development
- 3 grants under the Project for Development of Scientific Research in Key Science Areas
- 3 grants under the Programme for Support of Device Equipment for Progressive Science Branches

Grant Agency of the Czech Republic

- 17 individual grants; 14 of these had IBP scientists as principal investigators, whilst for the remaining grants they were partial investigators
- 3 complex grants; 1 of these had IBP scientists as principal investigators, whilst for the remaining 2 grants they were partial investigators
- 15 postgraduate grants

Grant Agencies of Ministries of the Czech Republic

- Ministry of Health of the CR:
  - 7 grants; 5 of these grants had IBP scientists as principal investigators, whilst for the other 2 grants they were partial investigators
- Ministry of Industry and Trade of the CR:
  - 1 grant where the IBP scientists were partial investigators
- Ministry of Education, Youth and Sports of the CR:
  - "Research Centres" Programme - 2 grants where the IBP scientists were partial investigators
  - "Development of Universities" Programme - 2 grants where the IBP scientists were partial investigators
  - 4 grants under the "COST" Programme
  - 4 grants under the "KONTAKT" Programme
  - 1 grant under the "INFRA 2" Programme

Foreign Grant Agencies

10 grants

PROGRAM I

BIOPHYSICAL CHEMISTRY OF MACROMOLECULES

LABORATORY OF BIOMACROMOLECULE PHYSICS (LBP)

HEAD:	PROF. RNDR. VLADIMÍR VETTERL, DRSC.
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GRADUATE STUDENTS:	MGR. NAĎA ŠPAČKOVÁ MGR. LUDĚK STRAŠÁK MGR. STANISLAV HASOŇ MGR. JAKUB DVOŘÁK

In the year 2000 we have continued to study the physical principles of the interactions of nucleic acids and model compounds with the electrode surface and exploitation of this knowledge in the applications of nanotechnology by developing biosensors, in biotechnology and in clinical praxis. We have studied the effect of the quality of the electrode surface on the mechanism of formation and/or dissolution of self-assembled layers of biomolecules and on the adsorption/desorption processes. The surface of film electrodes (glassy carbon electrode (GC) and pyrolytic carbon electrode (PC) covered by a mercury film) was studied by several independent methods in order to determine the area and morphology of the surface:

I. Electroanalysis with the aid of cyclic voltammetry
II. Microscopy
III. Profilometry

The graphite electrodes were provided with the mercury film by galvanization in $Hg(NO_3)_2$.

Ad I) The active area of the electrode surface S_A was determined amperometricaly using the redox system $Cd^{2+} \Leftrightarrow Cd^0$. It was found that $S_A(GC) = (0.014 \pm 0.0008)$ cm^2, $S_A(PC) = (0.205 \pm 0.002)$ cm^2 .

Ad II) From the macroscopic point of view the pictures of the surface of GC and PC electrodes show that GC electrode has a more glassy, harder and less wrinkled structure in comparison with the PC electrode (see Fig.). From this observation it can be supposed that at the GC surface there will be smaller islands of mercury due to a lower surface tension between the mercury and the electrode surface. At the PC surface between the microparticles of mercury there are larger forces and therefore they can make a more compact layers.

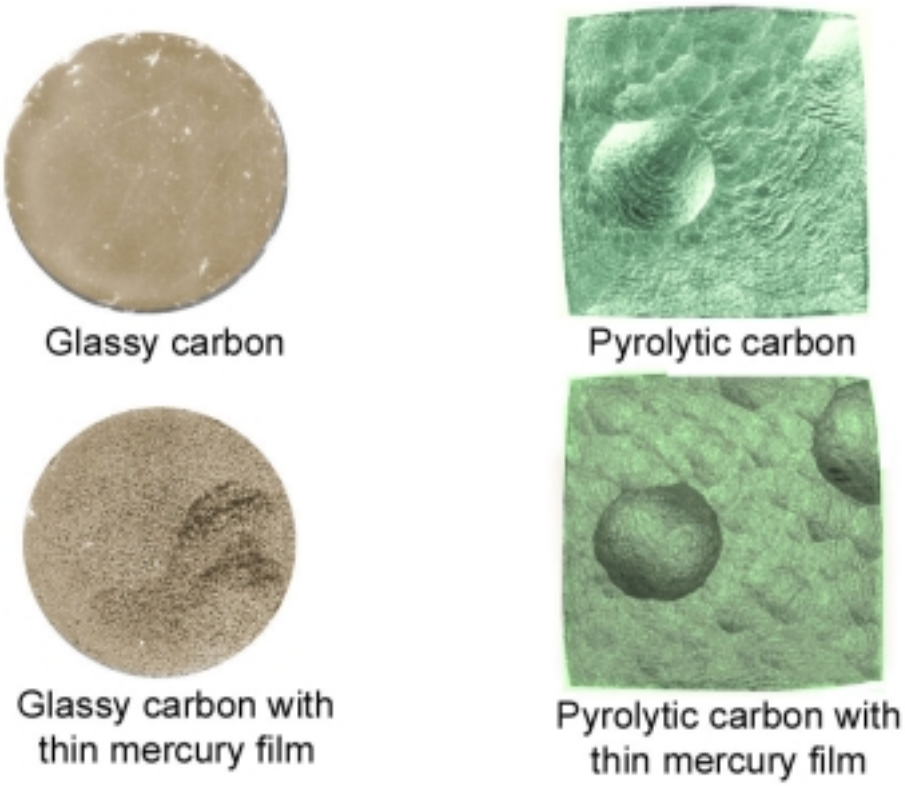
Ad III) The measurement of the roughness of PC and GC electrodes, both covered and uncovered with Hg film, was performed. The roughness of the PC electrode surface was higher and more asymmetric than the roughness of the GC surface.

The kinetics of formation and dissolution of the self-assembled 2D condensed layers of cytidine

The GC and PC electrodes were used to study the effect of the geometry and roughness of the electrode surface on formation and dissolution of the self-assembled 2D condensed layers of cytidine. The results were compared with the experiments performed at the hanging mercury drop electrode (HMDE). The measurement of the time dependence of the d.c. current (I-t curves) revealed the mechanism taking place at the beginning of the transformation of one surface state in the another. The time dependence of the differential capacitance (C-t curves) has brought information on structure changes (secondary transitions) in the cytidine layers taking place at longer time intervals.

The impedance and admittance measurements were used for the study of DNA and PNA adsorption. Native, denatured and damaged calf thymus DNA was studied by admittance measurements using hanging mercury drop electrode. We used adsorptive stripping technique with application of forward (negative-going) and reverse (positive-going) potential scan.

The effect of alternating current voltage amplitude, frequency, adsorption potential and adsorption time on the height of the signal was studied. Native double-stranded (ds) DNA produced peak 3 and inflexion 2 on the forward but not on the reverse curves. Irradiation of ds DNA with relatively small doses of γ-radiation (up to 80 Gy) resulted in an appearance of peak 3 and 2 on reverse voltammetric curves. Similar peaks were observed when ds DNA was sonicated. These results suggest that in damaged regions of ds DNA bases are accessible for the interaction with the electrode producing peak 3 without passing through the potential region U around -1.2 V, where ds DNA is supposed to be slowly opened.



(A) The picture demonstrates that the mercury surface film at the glassy carbon electrode is not continuous, and that mercury forms not evenly distributed microscopic droplets at the electrode surface.

(B) Light microscopy revealed that the electrode of the pyrolytic carbon is completely cowered by mercury. It filled small corrugations and roughness at the electrode surface; at the same time, bigger topological formations were conserved.

We have continued in the simulation of unusual forms of DNA by molecular dynamics methods. Modern computation methods of molecular modeling yield an unique view of the structure and dynamics of biomolecules on the atomic level and conveniently complement experimental techniques (X-ray, NMR) by solving tasks not accessible experimentally. The aim of the calculations was the study of interactions of nucleic acid bases with metal ions inclusive bivalent Pt in order to clear up the effect of metals on the tautomeric equilibrium of bases, their basicity and acidity, base pairing and vertical interactions. The calculation of base pair and vertical interactions in water were performed using the continuous solvent model. These techniques enabled us to correlate calculations with the results of electrochemical measurements and to find out which properties of bases and their complexes are important for their condensation at the electrode surface.

We have obtained experimental and theoretical data concerning forces which maintain the conformation stability of biomacromolecules, and forces which take place in interactions of biomacromolecules with ligands, in particular with mutagens, carcinogens and compounds which have a relation to the regulation of cell growth and to the tumor diseases like the protein p53.

GRANTS:

GA AS CR A4004002
Structure and interactions of nucleic acids and polypeptides at metal surfaces
Principal investigator: V. Vetterl, 2000 – 2002

GA CR 204/97/K084
Electrodes modified with nucleic acids and proteins. New tools in biochemical and biomedical research
Principal investigator: E. Paleček, principal co-investigators: O. Dračka, Fac. Sci. MU Brno, L. Novotný, IPCH J.H. AS CR Prague, B. Vojtěšek, MOÚ Brno, 1997 – 2002

GA CR 203/00/P081
Adsorption of nucleic acid bases and their derivatives at electrodes
Principal investigator: V. Dražan, 2000 – 2002

LABORATORY OF BIOPHYSICAL CHEMISTRY AND MOLECULAR ONCOLOGY (LBCMO)

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In the past year our work was concentrated mainly to two research fields:

Field I. *Properties of nucleic acids and proteins at surfaces and possibilities of their using in DNA biodetectors.*

Field II. *Structure and interaction of DNA and proteins in oncological research especially with respect to the protein p53.*

In the field I the investigations were focused to studies of the interaction of proteins and DNA with mercury and carbon electrodes to obtain results potentially useful in research and development of biosensors for the detection of DNA interactions (including DNA hybridization and DNA interactions with drugs) and DNA damage. Novel approaches involving new electrode types (mercury film, Ag/Hg, compressible HMDE) and new detection techniques (admittance measurements) were introduced in this research field. The work in this field possessed contact points with the field II, mainly in the investigations where electrochemical methods were oriented to the studies of proteins and peptides (especially metallothioneins) as it appears that obtained results may be of use in the research of other metalloproteins such as protein p53. Nevertheless, applications of novel electroanalytical approaches in analysis of metallothioneins appeared also possible.

1. Voltammetric and chronopotentiometric measurements with nucleic acid-modified mercury film on a glassy carbon electrode

Mercury film plated on a glassy carbon electrode was used for the analysis of nucleic acids. Using voltammetry in cyclic, square-wave and alternating current modes and constant current chronopotentiometry redox as well as tensammetric signals of DNA, RNA and synthetic polynucleotides were obtained. Behavior of nucleic acids at the mercury film electrode (MFE) was compared with that observed previously at HMDE. Nucleic acid-modified MFE appeared suitable for various applications, including a sensor for the detection of DNA damage. Using AC voltammetry at the MFE, it was possible to detect both single- and double-strand breaks in DNA.

2. Electrode potential-modulated cleavage of surface-confined DNA by hydroxyl radicals detected by an electrochemical biosensor

Formation of sb in covalently closed supercoiled (sc) DNA can be detected using an electrochemical biosensor based on scDNA-modified mercury electrode. By controlling the potential of the electrode, this technique can be employed in studies of redox reactions involved in formation of DNA strand breaks, and to detect species involved in these reactions. ScDNA anchored at HMDE was cleaved by catalytic amounts of iron/EDTA ions in the absence of chemical reductants when appropriate electrode potential (sufficiently negative to reduce $[Fe(EDTA)]^-$ to $[Fe(EDTA)]^{2-}$) was applied. The process required oxygen or hydrogen peroxide. In the absence of transition metal ions, significant DNA damage was observed at potentials sufficiently negative for reduction of dioxygen at the mercury electrode.

3. Adsorptive transfer stripping AC voltammetry of DNA complexes with intercalators

Using alternating current adsorptive transfer stripping voltammetry at hanging mercury drop electrode (HMDE), conformational changes of DNA due to binding of DNA intercalators were studied. Untwisting of DNA by the intercalators in solution resulted in an altered DNA adsorption. After medium exchange and intercalator removal, the surface-confined DNA probably adopted a restrained structure characterized by an enhancement of DNA peak 2 and decreased intensity of DNA peak 3. Upon introduction of DNA single-strand breaks, the specific behavior of DNA-intercalator complexes was eliminated.

4. Adsorptive stripping voltammetry of denatured DNA on Hg/Ag electrode

The voltammetric behavior of DNA on a hemispherical Hg/Ag electrode (MSE) was compared with that on a classical hanging mercury drop electrode (HMDE). The considerably lower capacity values of MSE's in the positive polarization region were probably due to the higher adsorptivity of chlorides. In the presence of DNA two capacitance peaks were obtained at both electrodes. The application of the more user-friendly and sturdy construction of Hg/Ag electrode required an appropriate electrochemical pretreatment of the electrode/solution interface.

5. Distinction between native and denatured DNA by means of compression

Native and denatured DNA show different behavior on a compression mercury drop electrode in voltammetric and chronoamperometric measurements. The effects of both forms of adsorbed DNA on cyclic voltammetry of Cd^{2+} ions were followed in course of the growth, compression and repeated growth of the mercury electrode. The measurements have indicated different compression-expansion properties of the two DNA. Different behavior was also observed on compression I-*t* curves after DNA accumulation on slowly growing or stationary mercury drop electrode.

6. Adsorptive stripping analysis of DNA with admittance detection

Native, denatured and damaged calf thymus DNA was studied by adsorptive stripping admittance measurements using hanging mercury drop electrode with application of forward (negative-going) and reverse (positive-going) potential scan. Native double-stranded DNA

produced peak 3 and inflexion 2 on the forward but not on the reverse curves. Damage to ds resulted in an appearance of peak 3 and 2 on reverse voltammetric curves.

7. The "presodium" catalysis of electroreduction of hydrogen ions ion mercury electrodes by metallothionein. An investigation by constant current derivative stripping chronopotentiometry

Metallothionein (MT) yields on mercury electrodes a "presodium" catalysis of the evolution of hydrogen, which is, as a peak-shaped signal, particularly well measurable in chronopotentiometry, and which is, unlike presodium catalytic effects of other biopolymers, strongly dependent on the presence of cobalt ions in the solution. The behavior of the "presodium" derivative chronopotentiometric peak under various experimental conditions was tested.

8. Constant current chronopotentiometric stripping analysis of Cd-metallothionein on carbon and mercury electrodes. Comparison with voltammetry

CPSA on hanging mercury electrode, using positive or negative stripping current revealed similar behavior of cadmium metallothionein as observed with voltammetry, but higher sensitivity has been achieved. CPSA on renewable composite paste electrode increased sensitivity by more than order of magnitude comparing to voltammetry and allows the study in μM range of concentrations of CdMT, therefore the same as on HMDE. Three oxidation peaks can be observed, one of uncomplexed Cd^{2+} and two of different S-Cd(II) complex formation.

In the field II the research was oriented to the questions of p53 interaction with linear and supercoiled DNA, namely the roles of the individual protein domains in p53 supercoil-selective (scs) p53 DNA binding, effects of environmental conditions (oxidation agents, metal ions) on the p53 scs, sequence-specific and non-specific DNA binding and use of monoclonal antibodies for modulation of DNA-binding activity of p53.

1. Binding of p53 and its core domain to supecoiled DNA

We investigated the binding of human full length p53 and its isolated core domain (p53CD) to negatively supercoiled (sc) DNA. Significant differences were observed; p53CD produced a relatively small and continuous retardation of scDNA in contrast to the ladder of distinct bands formed by p53 in agarose gels. Competition between scDNAs and their linearized (lin) forms showed a preference for scDNAs by both p53 and p53CD, but the ratios characterizing the distribution of the protein between sc and lin pBluescript DNAs were substantially higher for p53 (sc/lin > 60) than for p53CD (sc/lin ~ 4). Strong binding of p53 to scDNA not containing the p53 consensus sequence may represent a new p53 binding mode, which we tentatively denote supercoil-selective (SCS) binding. Targets of this binding may include: (a) DNA segments defined both by the nucleotide sequence and local topology and/or (b) strand crossings and/or bending.

2. Effects of oxidation agents and metal ions on binding of p53 to supercoiled DNA

Wild type human full length (f.l.) tumor suppressor p53 protein binds preferentially to supercoiled (sc) DNA yielding a ladder of retarded bands on the agarose gel. The intensity and the number of bands of p53-scDNA complex were decreased by physiological concentrations of unchelated zinc ions. Nickel and cobalt ions inhibited binding of p53 to scDNA and to p53CON in linear DNA fragments less efficiently than zinc. Oxidation of the protein with diamide resulted in a decrease of the number of the retarded bands. In agreement with the literature oxidation of f.l. p53 with diamide was irreversible and was not reverted by an excess of DTT. We showed that in the presence of 0.1 mM zinc ions, oxidation of p53 became reversible. We suggested that the irreversibility of p53 oxidation was due, at least in part, to the removal of intrinsic zinc from its position in the DNA binding domain (after oxidation of the three cysteines to which the zinc ion is coordinated in the reduced protein) accompanied by a change in the p53

conformation. Supershift/competition experiments using antibodies against p53 N- or C-terminus suggested that oxidized p53 bind scDNA exclusively through its C-terminus.

3. Specific modulation of p53 binding to consensus sequence within supercoiled DNA by monoclonal antibodies

Monoclonal antibodies (MAbs) were used to study the binding of wild-type human p53 protein to the consensus sequence (p53CON) in a 474 bp DNA fragment and to supercoiled (sc) DNAs with and without p53CON. Supershifting of p53-DNA complexes by MAbs in agarose gels was used to study activation of p53 for sequence specific binding to scDNA. C-terminal specific Bp53-10.1 activated the sequence-specific binding of p53 to p53CON within the pPGM1 scDNA but did not influence binding of p53 to pBluescript scDNA (not containing p53CON). If p53 was incubated with DO-1 prior to addition of Bp53-10.1, no activation of p53 was observed but a portion of the pPGM1 DNA dissociated from the sequence specific immune complex. This is the first demonstration of DNA dissociation from the p53 immune complex due to binding of the N-terminal antibody. Our results suggest that an antibody harbored at the N-terminus can efficiently modulate the p53 sequence-specific binding within scDNA upon attachment of Bp53-10.1 to the p53 C-terminus.

4. Precise characterization of monoclonal antibodies to C-terminal region of p53 protein using PEPSCAN ELISA technique and new non-radioactive gel shift assay

The DNA-binding activity is cryptic but can be modulated through the C-terminal region of the p53 protein by several different stimuli, including phosphorylation by casein kinase II (CKII), protein kinase C (PKC) or binding of the C-terminal monoclonal antibody PAb421. Monoclonal antibodies to the C-terminal region of p53 protein are able to activate the latent form of p53 and induce binding to DNA. To characterize such antibodies, we used a combination of the PEPSCAN ELISA procedure and a newly developed non-radioactive gel shift assay. Monoclonal antibodies from the Bp53 series displayed higher affinities for the human, rat and mouse p53 proteins than did the conventional antibody PAb421. In addition, these antibodies were able to activate the sequence-specific DNA binding functions in latent forms of p53 protein and, in contrast to PAb421, they were able to recognize both PKC phosphorylated and PKC non-phosphorylated forms of p53 protein.

GRANTS:

GA CR 301/99/0692
Structural aspects of interactions of checkpoint proteins with DNA in cancer
Principal investigator: E. Paleček, 1999 - 2001

GA CR 204/97/K084
Electrodes modified with nucleic acids and proteins. New tools in biochemical and biomedical research
Principal investigator: E. Paleček, principal co-investigators: O. Dračka, Fac. Sci. MU Brno, L. Novotný, IPCH J.H. AS CR Prague, B. Vojtěšek, MOÚ Brno, 1997 – 2002

GA CR 204/98/P091
Electrochemical biosensors for the detection of DNA damaging agents
Principal investigator: M. Fojta, 1998 - 2000

GA CR 301/00/D001
Binding of human and mouse tumor suppressor protein P53 to linear and supercoiled DNAs
Principal investigator: V. Brázda, 2000 - 2003

GA CR 301/99/D078
Role of the p53 domains and the oligomerization state of the protein in its molecular interactions
Principal investigator: J. Paleček, 1999 - 2001

GA CR 204/00/D049
Influence of chemical modification of DNA and synthetic oligonucleotides on their electrochemical behavior
Principal investigator: L. Havran, 2000 - 2003

GA AS CR A5004803
Interactions of supercoiled DNA with tumor-suppressor protein p53
Principal investigator: E. Paleček, 1998-2000

GA AS CR A4004801
Electrodes as regulators of the cleavage of immobilized DNA by redox-modulated chemical nucleases
Principal investigator: M. Fojta, 1998 - 2000

GA AS CR A4004901
Analysis of the interactions of mutagens, carcinogens and anti-cancer drugs with biopolymers by means of electrochemical and biochemical methods
Principal investigator: F. Jelen, 1999 - 2001

GA AS CR IAC4004003
Adsorptive properties of biopolymers and their components on electrode surfaces
Principal investigator: V. Dražan, guarantor: F. Jelen, 2000

IGA MH CR NC5343-3/1999
Interactions of tumor suppressor protein P53 with damaged DNA and with lesions induced by anti-cancer drugs
Principal investigator: M. Fojta, 1999 - 2001

PROGRAM II

BIOPHYSICS OF NUCLEIC ACIDS COMPLEXES

LABORATORY OF MOLECULAR BIOPHYSICS AND PHARMACOLOGY (LMBP)

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1. Sequence specificity, conformation and recognition by HMG1 protein of major DNA interstrand cross-links of antitumor dinuclear platinum complexes

Interactions of high mobility group (HMG)-domain proteins with DNA modified by cisplatin plays a role in mechanisms underlying its antitumor activity. A structural motif recognized by HMG-domain proteins on cisplatin-modified DNA is a stable, directional bend of the helix axis. In the present work, bending induced in DNA by major adducts of a novel class of antitumor compounds, represented by the formula [{*trans*-[$PtCl(NH_3)_2$]}$H_2N(CH_2)_{2-6}NH_2$]Cl_2, was investigated. The oligodeoxyribonucleotide duplexes containing various site-specific interstrand cross-links of these bifunctional dinuclear platinum drugs were purified and characterized by Maxam-Gilbert footprinting, chemical probing and phasing assay. It was demonstrated that the cross-links of the dinuclear compounds bent the helix much less those of cisplatin. Gel retardation assay revealed very weak recognition of DNA adducts of dinuclear complexes by HMG1 protein. Hence, the mediation of antitumor properties of dinuclear platinum complexes by HMG-domain proteins is unlikely so that polynuclear platinum compounds may represent a novel class of platinum anticancer drugs acting by a different mechanism than cisplatin and its analogues. A further understanding of how polynuclear platinum compounds modify DNA and how these modifications are processed in cells should provide a rational basis for the design of new platinum drugs rather than searching for cisplatin analogues.

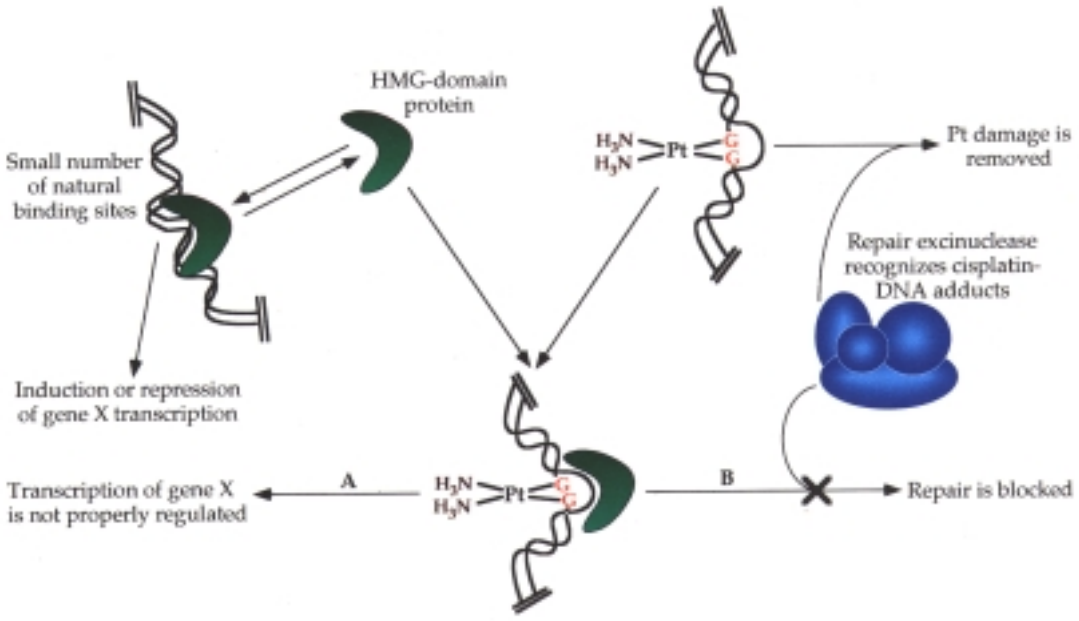

Figure 1. Models for HMG protein involvement in the mechanism of action of cisplatin and its analogues. A) When a cell is exposed to a lethal dose of cisplatin, 10^4-10^5 DNA adducts are formed. If HMG-domain proteins bind with similar affinity to these lesions and to their natural binding sites, the proteins could be titrated away from their transcriptional regulatory function. B) The HMG-domain proteins could block access of the excision repair complex and shield the adducts from repair.

2. Conformational analysis of site-specific DNA cross-links of cisplatin-distamycin conjugates

The requirement for novel platinum antitumor drugs led to the concept of synthesis of novel platinum drugs based on targeting cisplatin to various carrier molecules. We have shown that attachment of DNA minor-groove-binder distamycin to cisplatin changes several features of DNA binding mode of the parent platinum drug. Major differences comprise different conformational changes in DNA and a considerably higher interstrand cross-linking efficiency. The studies performed this year have been directed to the analysis of oligodeoxyribonucleotide duplexes containing single, site-specific adducts of platinum-distamycin conjugates. These uniquely modified duplexes were analyzed by Maxam-Gilbert footprinting, phase-sensitive gel electrophoresis bending assay and chemical probes of DNA conformation. The results have indicated that the attachment of distamycin to cisplatin mainly affects the sites involved in the interstrand cross-links so that these adducts are preferentially formed between complementary guanine and cytosine residues. This interstrand cross-link bends the helix axis by ~35° toward minor groove, unwinds DNA by approximately 95° and distorts DNA symmetrically around the adduct. In addition, CD spectra of restriction fragments modified by the cisplatin-distamycin conjugates have demonstrated that distamycin moiety in the interstrand cross-links of these compounds interacts with DNA. This interaction facilitates the formation of these adducts. Hence, the structural impact of the specific interstrand cross-link detected in this study deserves attention when biological behavior of cisplatin derivatives targeted by oligopeptide DNA minor-groove-binders is evaluated.

3. Steric control of DNA interstrand cross-link sites of trans*-platinum complexes. Specificity can be dictated by planar nonleaving groups*

Recent findings that novel *trans*-dichloroplatinum(II) complexes exhibit antitumor activity violate the classical structure-activity relationships of platinum(II) complexes. These novel "nonclassical" trans-platinum complexes also comprise those containing planar aromatic amines. Our initial studies have shown that these compounds form a considerable amount of DNA interstrand cross-links (up to ~30 %) with a rate markedly higher than clinically ineffective transplatin. The work carried out this year has shown, using Maxam-Gilbert footprinting that *trans*-[$PtCl_2(NH_3)$(quinoline)] and *trans*-[$PtCl_2(NH_3)$(thiazole)], representatives of the group of new antitumor trans-dichloroplatinum complexes containing planar amines, preferentially form DNA interstrand cross-links between guanine residues at the 5'-GC-3' sites. Thus, DNA interstrand cross-linking by *trans*-[$PtCl_2(NH_3)$(quinoline)] and *trans*-[$PtCl_2(NH_3)$(thiazole)] is formally equivalent to that by antitumor cisplatin, but different from clinically ineffective transplatin which preferentially forms these adducts between complementary guanine and cytosine residues. This result shows for the first time that the simple chemical modification of structure of an inactive compound alters its DNA binding site into a DNA adduct of an active drug.

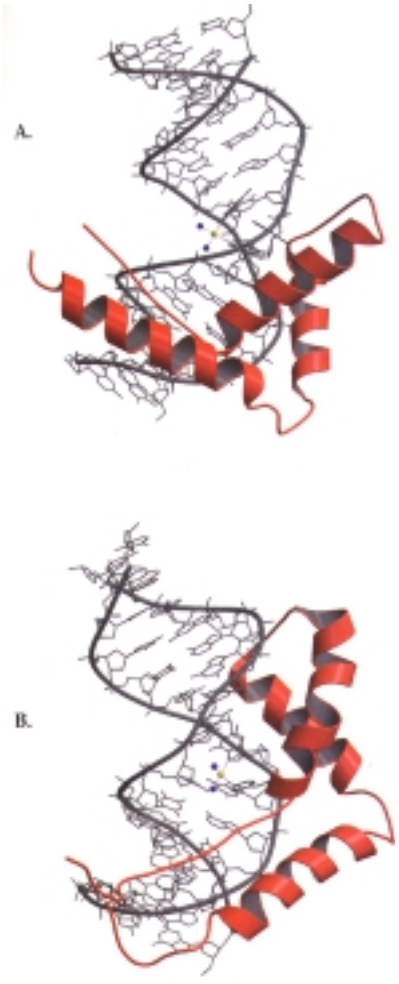

Figure 2. Comparison of binding modes of HMG1 domain A (A) and domain B (B) to DNA modified by antitumor cisplatin.

4. DNA sensor for the determination of antitumor platinum compounds

Recent activity has centered upon the design of biosensors that exploit interactions between the surface-confined DNA and target drugs for their rapid screening. The studies performed this year yielded details of the new strategy to determine platinum or platinum binding to DNA. The strategy is based on a new DNA biosensor capable of monitoring changes in the intrinsic electrooxidation response of the immobilized DNA probe induced by the aqua form of chlorodiethylenetriamineplatinum(II) chloride {$[Pt(dien)(H_2O)]^{2+}$}. A wax impregnated graphite electrode coated by linearized plasmid DNA was employed as a sensitive biosensor for the detection of platinum compounds. The sensor relies on monitoring changes in the intrinsic electrooxidation response of the surface-confined DNA resulting from its interaction with platinum compounds and requires no label or indicator. Short reaction times (2 – 10 min) are sufficient for monitoring submicromolar levels of platinum complexes. It has been suggested that DNA biosensors could be used for quantitating antitumor platinum drugs in various samples including those used when studying mechanisms underlying their antitumor effectiveness. Thus, the resulting DNA biosensor offers a sensitive and portable tool for determining platinum in biological samples or platinum binding to DNA in cell-free media.

GRANTS:

GA CR 305/99/0695
Affection of conformation of DNA by antitumor metal complexes. Relations to the development of new anticancer drugs
Principal investigator: V. Brabec, 1999 - 2001

GA CR 307/97/P029
Molecular mechanism of action of antitumor dinuclear platinum complexes
Principal investigator: J. Kašpárková, 1998 - 2000

GA CR 204/97/P028
Biophysical analysis of the effect of antitumor ruthenium complexes on DNA
Principal investigator: O. Nováková, 1998 - 2000

GA CR 301/98/P231
Reactions of DNA with platinum complexes containing aminophosphine ligands. Relation to the development of new antitumor platinum drugs
Principal investigator: K. Neplechová, 1998 - 2000

GA CR 301/00/0556
Dinuclear metal-based agents as agents cross-linking DNA and proteins
Principal investigator: R. Žaludová, 2000 - 2002

GA CR 305/00/D008
Reactions of antitumor trifunctional dinuclear platinum complexes with biomacromolecules
Principal investigator: H. Kostrhunová, 2000 - 2003

GA AS CR A5004702
Effect of geometric isomerism of antitumor dinuclear platinum complexes on binding mode and conformational alterations of DNA
Principal investigator: V. Brabec, 1997 - 2000

GA AS CR D5004004
Spektropolarimeter JASCO J-720
Principal investigator: V. Brabec, 2000

GA AS CR A7004805
Interactions of DNA with antitumor platinum drugs of the second generation
Principal investigator: O. Vrána, 1998 - 2001

IGA MH CR NL6058-3/2000
Recognition and repair of DNA damaged by platinum antitumor drugs. Relations to the development of new anticancer drugs
Principal investigator: J. Kašpárková, 2000 - 2002

IGA MH CR NL6069-3/2000
Mutagenic effects of antitumor platinum drugs. Relations to the development of new anticancer drugs
Principal investigator: O. Vrána, 2000 - 2002

LABORATORY OF ANALYSIS OF DNA MOLECULAR COMPLEXES (LADMC)

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I. In continuity with previous results of the laboratory in the field of structure and function of plant telomeres, results have been obtained in three main streams:

1. Inhibition of plant telomerase by telomere-binding proteins

Contrary to the situation in human somatic cells, the activity of telomerase in plant cells is precisely regulated in response to changes in cell division rate. To explore this regulatory mechanism, the effect on telomerase activity of protein extracts from nuclei of telomerase-negative tissues was examined. An inhibition of telomerase activity was found which was species-non-specific. This inhibition was due to proteins which form salt-stable, sequence-specific complexes with the G-rich telomeric strand and reduce its accessibility, as shown by gel retardation and by terminal transferase (TdT) extension of G-rich telomeric and non-telomeric (substrate) primers. A 40 kDa polypeptide was detected by SDS-PAGE after cross- linking the complex formed by extracts from tobacco leaf nuclei. Such proteins may be involved in regulation of telomerase activity in plants.

2. *Characterization of repetitive sequences isolated from subtelomeric regions of Nicotiana tomentosiformis chromosomes*

We have isolated and characterized a new repetitive sequence, TAS49, from terminal restriction fragments of *Nicotiana tomentosiformis* genomic DNA by means of a modified vectorette approach. The TAS49 was found directly attached to telomeres of *N. tabacum* and one of its ancestors, *N. tomentosiformis,* and also at inner chromosome locations. No association with telomeres was detected neither in *N. otophora* nor in the second tobacco ancestor, *N. sylvestris*. PCR and Southern hybridization reveal similarities in the arrangement of TAS49 on the chromosomes of 9 species of the genus Nicotiana, implying its occurrence as a subunit of a conserved complex DNA repeat. TAS49 belongs to the family of dispersed repetitive sequences without features of transposons. The copy number of TAS49 varies widely in the genomes of 8 species analyzed being lowest in *N. sylvestris,* with 3300 copies per diploid genome. In *N. tomentosiformis,* TAS49 forms about 0.56% of the diploid genome, corresponding to 17400 copies. TAS49 units are about 460 bp long and show about 90% of mutual homology, but no significant homology to DNA sequences deposited in GenBank and EMBL. Although

genomic clones of TAS49 contain an open reading frame encoding a proline-rich protein similar to plant extensins, no mRNA transcript was detected. TAS49 is extensively methylated at CpG and CpNpG sites and its chromatin forms nucleosomes phased with a 170 +/- 8 bp periodicity.

3. Analysis of architecture of plant telomeres

In yeast, ciliates and mammals, the G-rich strand of the telomere forms a 3' overhang on the chromosome terminus. Here we investigate the architecture of telomeres in the dicot plants *Silene latifolia* and *Arabidopsis thaliana* using the PENT (primer extension/nick translation) assay. We show that both Arabidopsis and Silene telomeres carry G-overhangs longer than 20 - 30 nucleotides. However, in contrast to yeast and ciliate telomeres, only half of the telomeres in Silene seedlings possess detectable G-overhangs. PENT reactions using a variety of primers and reaction conditions revealed that the remaining fraction of Silene telomeres carries either no overhangs or overhangs less than 12 nucleotides in length. G-overhangs were observed in Silene seeds and leaves, tissues that lack telomerase activity. These findings suggest that incomplete DNA replication of the lagging strand, rather than synthesis by telomerase, is the primary mechanism for G-overhang synthesis in plants. Unexpectedly, we found that the fraction of telomeres with detectable G-overhangs decreased from 50% in seedlings to 35% in leaves. The difference may reflect increased susceptibility of the G-overhangs to nuclease attack in adult leaves, an event that could act as a precursor for the catabolic processes accompanying leaf senescence.

II. In the field of development and applications of molecular-diagnostic techniques for oncology, following outcomes have been published:

1. Detailed mapping of methylcytosine positions at the CpG island surrounding the Pa promoter at the bcr-abl locus

Chronic myelogenous leukemia (CML) is associated with a translocation of the protooncogene c-abl from chromosome 9 to chromosome 22, where it fuses to proximal exons of the bcr gene. The expression of the hybrid gene bcr-abl is regulated by the bcr promoter and results in a translation product with high tyrosine kinase activity. In most CML cases, one of two abl promoters (Pa) is nested within the bcr-abl transcription unit, but appears to be usually silent. Recently, de novo methylation of the Pa region and its correlation with disease progression were reported. As these previous studies were limited to the use of methylation-sensitive restriction endonucleases, our aim here was to obtain a complete map of methylcytosines and its variants in CML patients and in model cell lines. To achieve this, bisulfite conversion of cytosines (but not methylcytosines) to uracils in genomic DNA was employed. After modification, the region of interest was PCR-amplified and the products were cloned and sequenced. The results show methylation at a high level and in a homogenous pattern in the BV173 cell line, corresponding to the translocated abl alleles. Variant methylation observed in K562 cells correlates with multiple bcr-abl loci and an intact chromosome 9. Patients that were methylation-positive in restriction analysis showed sporadic and heterogenous occurrence of methylcytosines in bisulfite modification assays. Corresponding results were obtained using a quantitative Southern analysis of the extent of methylation. We conclude that restriction analysis combined with PCR is able to find rare cases of hypermethylation, e.g., for diagnostic purposes, but does not reflect the dominating level of methylation in Ph-positive cells.

2. Telomerase activity and expression and telomere analysis in situ in the course of treatment of childhood leukemias

Childhood leukemia samples of peripheral blood and bone marrow were assayed for telomerase activity and expression on the day of diagnosis and in the course of chemotherapy. A tight correlation between both parameters and clinical response was observed in most patients. A unique case was observed in which telomerase activity was only moderately increased on diagnosis; it gradually increased in the course of therapy, and a subsequent decrease occurred only after application of intensified therapy. This patient did not respond to therapy, his disease

progressed and he finally died during intensified therapy. In another patient, analysis of telomere lengths using quantitative dideoxy-PRINS revealed a single telomere expansion on chromosome 4, suggesting involvement of a telomerase-independent mechanism of telomere elongation.

III. The last research focus of the laboratory is a study of recombination factors. This field has been developed in collaboration with Danish Institute of Agricultural Science, Foulum, Denmark.

1. Molecular dissection of interactions between Rad51 and members of the recombination/repair group

Recombination is important for the repair of DNA damage, chromosome segregation during meiosis, and has been also shown to participate in the regulation of cell proliferation. In the yeast *S. cerevisiae,* recombination requires products of the *RAD52* epistasis group. The Rad51 protein associates with the Rad51, Rad52, Rad54, and Rad55 proteins to form a dynamic complex. We describe a new strategy to screen for mutations which cause specific disruption of interaction between certain protein(s) in the complex leaving other interactions intact. This approach defines distinct protein interaction domains, and protein relationships within the Rad51-complex. Alignment of the mutations onto the constructed 3D model of the Rad51 protein reveal possible partially overlapping interfaces for the Rad51-Rad52 and the Rad51-Rad54 interactions. Rad51-Rad55 and Rad51-Rad51 interactions are affected by the same spectrum of mutations, indicating similarity between the two modes of binding. Finally, a subset of mutations within Rad51 which disrupt interaction with mutant Rad52 protein but activate the interaction with Rad54, suggest that dynamic changes within the Rad51 protein may contribute to an ordered reaction process.

2. Homomeric interaction of the mouse Rad52 protein

The Rad52 protein plays a crucial role in repairing DNA damage and homologous recombination, possibly by virtue of its ability to catalyze annealing of single-stranded DNA. In agreement with recent genetic data, we here present results based on the two-hybrid system, demonstrating that mouse Rad52p is able to form homomeric complexes. A small domain necessary and sufficient for the self-interaction is located in the conserved N-terminus of the protein. These data contribute to the important insights into the architecture of the multi-protein complex involved in recombinational DNA repair.

3. Essential function of the Saccharomyces cerevisiae Sgs1 helicase for the Sgs1-Top3 complex

The *Saccharomyces cerevisiae* gene SGS1 encodes a DNA helicase that shows homology to the *Escherichia coli* protein RecQ and the products of the BLM and WRN genes in humans, which are defective in Bloom's and Werner's syndrome, respectively. Recently, it has been proposed that this helicase is involved in maintaining the integrity of the rDNA and that loss of Sgs1 function leads to accelerated aging. Sgs1 has been isolated on the basis of its genetic interaction with both topoisomerase I and topoisomerase III, as well as in a two-hybrid screen for proteins that interact with the C-terminal portion of topoisomerase II. We have defined the minimal structural elements of Sgs1 required for its interactions with the three topoisomerases, and demonstrate that the complex phenotypes associated with sgs1 mutants are a consequence of a dysfunctional Sgs1-Top3 complex. We also report that the synthetic relationship between mutations in SGS1 and SRS2, which encodes another helicase implicated in recombinational repair, likewise result from a dysfunctional Sgs1-Top3 interaction. Our findings indicate that Sgs1 may act on different DNA structures depending on the activity of topoisomerase I, Srs2 and topoisomerase III.

GRANTS:

GA CR 301/99/0045
Telomere dynamics in selected types of solid malignant tissues
Principal investigator: J. Fajkus, principal co-investigators: R. Vyzula, FN Brno-Bohunice, L. Fajkusová, VÚZD Brno, 1999 - 2001

GA CR 204/00/P012
Study of telomeres in selected malignant diseases using in situ techniques
Principal investigator: K. Krejčí, guarantor: J. Fajkus, 2000

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

ME CR VS97032
Analysis of biologically important molecular complexes
Principal investigator: J. Fajkus, 1997 - 2000

LABORATORY OF ANALYSIS OF CHROMOSOMAL PROTEINS (LACP)

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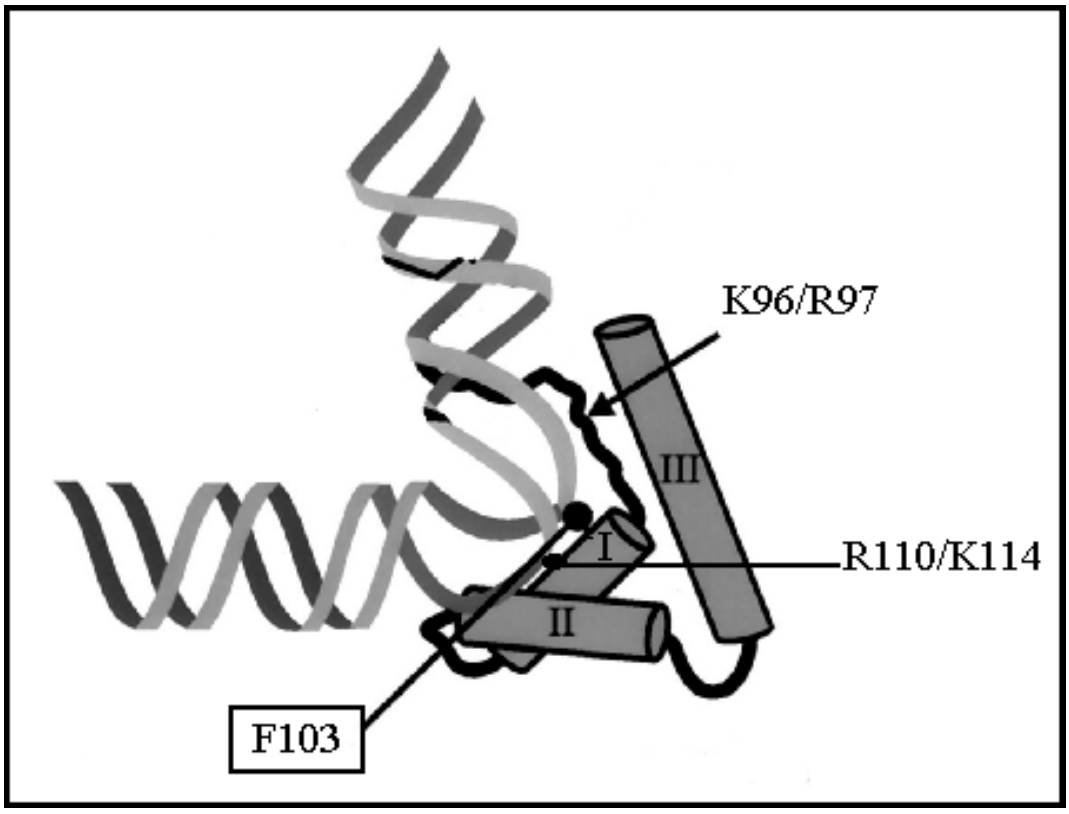
RESEARCH FELLOWS:	ING. ALENA BAČÍKOVÁ RNDR. DAGMAR ZACHOVÁ
GRADUATE STUDENTS:	MGR. EVA MUSELÍKOVÁ MGR. MARTINA MATULOVÁ

We have further studied the involvement of nonhistone protein HMG-1 in ligation of diverse termini of DNA fragments by human DNA ligase I and T4 DNA ligase. We have demonstrated that HMG-1 could enhance cohesive-end and blunt-end DNA ligation by T4 DNA ligase via its B-domain. Pull-down assays, electron and scanning force microscopy revealed that HMG1 can associate two DNA molecules via their ends even in the absence of complementary overhangs. We proposed that HMG-1 protein might be involved in the rejoining of DNA breaks by different DNA ligases due to its ability to bring DNA duplexes and their termini into a close proximity while leaving the ends accessible for ligation.



Mutational analysis of central domain of HMG-1 contributed to understanding of importance of several evolutionarily highly conserved amino acids in binding the protein to four-way DNA junctions (4WJs) and DNA supercoiling. We have found that basic amino acids of the extended N-terminal strand (Lys^{96}/Arg^{97}) and helix I (Arg^{110}/Lys^{114}) of the B domain participate in DNA binding and supercoiling. The putative intercalating hydrophobic Phe^{103} of helix I is important for DNA supercoiling but dispensable for binding to supercoiled DNA and 4WJs. Our results allowed us to propose a model depicting interactions of B domain with closed circular DNA in the course of DNA supercoiling.

GRANTS:

GA CR 301/99/0691
Influence of chromosomal proteins HMG-1 and HMG-2 on transcription
Principal investigator: M. Štros, 1999 - 2001

GA AS CR A7004902
Involvement of chromosomal protein HMG-1 on DNA end-joining by human DNA ligases
Principal investigator: M. Štros, 1999 - 2001

PROGRAM III

BIOPHYSICS AND BIOINFORMATICS OF GENOMES

LABORATORY OF CD SPECTROSCOPY OF NUCLEIC ACIDS (LSNA)

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GRADUATE STUDENT:	MGR. PETR FOJTÍK
UNDERGRADUATE STUDENTS:	MICHAL ZEMÁNEK KLÁRA BEDNÁŘOVÁ

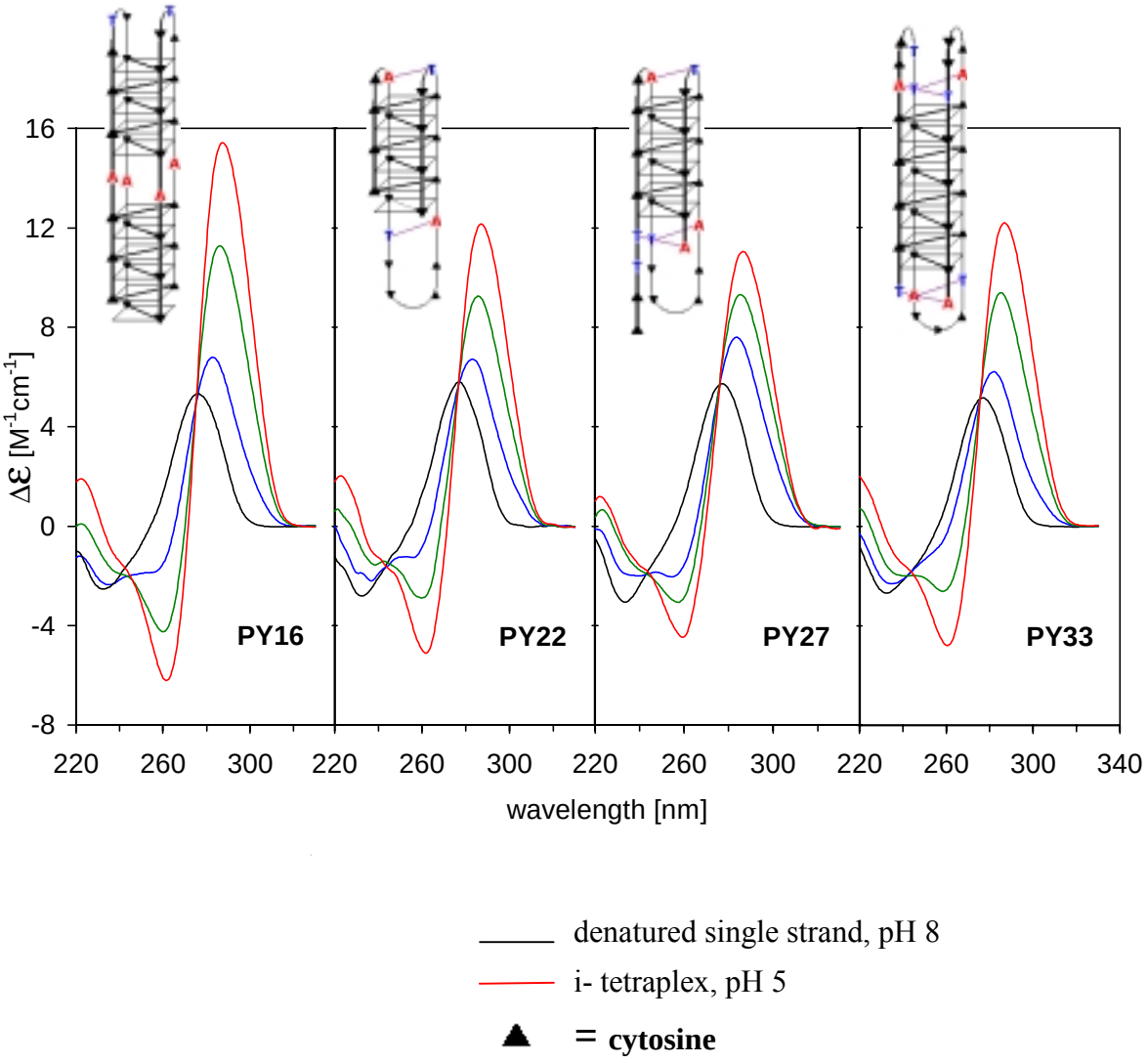
The human gene for cartilage oligomeric matrix protein contains five tandem repeats of the GAC trinucleotide. Its expansion by one repeat causes multiple epiphyseal dysplasia while expansion by two repeats or, remarkably, deletion of one repeat causes pseudoachondroplasia. We used CD spectroscopy, PAGE and UV absorption spectroscopy to compare conformational properties of the DNA strands containing four, five, six and seven repeats of the GAC trinucleotide. The $(GAC)_n$ strands were found to form four distinct ordered conformations depending on the solution conditions. The first was a foldback stable at slightly alkaline pH values and low and medium ionic strength. Increasing salt concentration induced a transition of the foldback into an antiparallel right-handed homoduplex. Both the conformers contained the Watson-Crick G.C pairs while the intervening adenines little contributed to their B-like conformation. The third conformation was a parallel homoduplex stabilized by the hemiprotonated C^+.C pairs and by the GpA steps that also (in contrast to the ApG steps) favored the parallel DNA strand orientation. The parallel homoduplex was stable even at neutral pH. The fourth conformation was the left-handed Z-DNA, which formed easier with $(GAC)_n$ than with $(GC)_n$ of comparable length indicating that the adenines of $(GAC)_n$ promoted the left-handed duplex. This is a new finding that will contribute to a better understanding of the B-Z transition in DNA. Our study shows that stability of the above four conformers strongly depends on the GAC repeat number.

Another trinucleotide whose expansion in the human genome can cause pathological effects, is the CGG trinucleotide. Some literary data show that repeated (CGG)n regions can form a tetraplex, but there is no convincing evidence for that even in „in vitro“ conditions. This problem is especially complicated by a possibility of mixed GCGC tetrad formation. That is why we studied the tetraplex formed by the 11-mer GCGGTTTGCGG, containing the mixed GCGC as shown by NMR spectroscopy. We have found that the 11-mer tetraplex provides a characteristic CD spectrum distinct from the CD spectra of both parallel and antiparallel tetraplexes, formed by guanine tetrads only. In contrast to the guanine tetraplexes, the mixed tetraplex is stabilized by sodium cations and destabilized by potassium cations. We have found conditions stabilizing guanine tetrads with (CGG)n as well as conditions stabilizing intercalated cytosine tetrads with the complementary (CCG)n strands. Under no conditions, however, we observed CD spectra indicating formation of mixed GCGC tetrads by (CGG)n or (CCG)n.

Studies continued on cytosine tetraplexes formed by fragments of various length of the cytosine-rich strand, occurring in the promoter of the human c-myc gene. From the CD spectra and migration in polyacrylamide gels, we determined the number of hemiprotonated cytosine pairs and the number of strands in the cytosine tetraplexes formed by the particular fragments (16, 22, 27 and 33 nucleoside residues in length). On the basis of this data we proposed models of their 3D structures (Figure). This result was obtained in collaboration with Chalmers University, Gothenburg.

GRANT:

GA CR 204/98/1027
Conformational polymorphism and expansion of the $(CNG)_n$ and related microsatellite DNA sequences
Principal investigator: M. Vorlíčková, 1998 - 2000



LABORATORY OF DNA BIOPHYSICS AND GENOME BIOINFORMATICS (LDBGB)

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UNDERGRADUATE STUDENTS:	EVA ZEMANOVÁ RADIM ZEMEK

Both proteins and RNA are mostly composed of invariant structural motifs that are conserved in the evolution. The structural invariants are, for example, used in computer genomics and computer proteomics. With DNA, structural invariants have not yet been considered. This fact motivated us to develop a method suitable to search for structural invariants in DNA.

Our method takes the use of the crystal structures of DNA whose cartesian coordinates are deposited in the NDB database. Each dinucleotide occurs many times in the crystal structures. Comparison of their geometries shows dinucleotide variability. Variability close to zero suggests a structural invariant.

We have extracted cartesian coordinates from the NDB database of 526 neighboring base pairs occurring in 64 crystal structures of B-DNA and of 550 base pairs occurring in 85 crystal structures of A-DNA. All of the pairs included either adenine with thymine or guanine with cytosine. Mispairs and modified bases were excluded. We calculated all distances between the carbon, nitrogen and oxygen atoms of the neighboring base pairs, their average values and standard deviations. The average value of the standard deviations over all interatomic distances in a base pair dimer reflects variability of the dimer in DNA. We found that this variability significantly depended on the base sequence in the dimer.

Surprisingly, the (ApT).(ApT) dimer exhibited the lowest variability in B-DNA. All dimers composed of only GC pairs were more variable. This finding leads to the conclusion that thermostability of the double helix of DNA is not determined by forces that cause its flexibility/rigidity. This accords with our notion about hydrophobic stabilization of the double helix of DNA in which both hydrogen bonding between the complementary bases and base stacking are of a secondary importance. We account for the (ApT).(ApT) step rigidity by cations bound in the minor groove where they fix the mutual position of the thymines whereas the adenines are bound in the major groove through an attractive interaction of their amino groups. In line with experimental studies in solution, our method shows that the (TpA).(TpA) and (CpA).(TpG) steps are the most variable in B-DNA. This correspondence illustrates that the crystal structures can be used to assess important properties of DNA in aqueous solution though the particular crystal structures are qualitatively different from the aqueous DNA.

The sequence of steps according to the variability is approximately opposite in A-DNA compared to B-DNA. The (ApT).(ApT) step is an exception because it belongs among the most invariant steps not only in B-DNA but also in A-DNA. It is remarkable that the steps composed of only GC pairs show similar variability in A-DNA and B-DNA. This fact supports our notion

that the (G+C) blocks made possible that the genetic functions that originally evolved on RNA, could be taken on by DNA.

Studies continued to understand the formation of covalent bonds between the complementary strands of DNA upon UV irradiation. Dependencies of the number of crosslinks on the tetranucleotide composition of the UV irradiated DNA were used to identify the most prone and most resistant regions in six plasmid and viral genomes. We prepared restriction fragments containing these extreme regions and subjected them to an experimental analysis. Results of all experiments were qualitatively in agreement with the predictions. In some cases, the agreement was semiquantitative or even quantitative. The two fragments showing the highest propensity for crosslinking shared the (ATTTTATA).(TATAAAAT) octamer duplex. This octamer is a candidate hotspot of the UV light-induced interstrand crosslinking. Correspondence between prediction and experiment motivated us to develop software predicting crosslinking probability along the whole DNA molecules of the human chromosomes 21 and 22. This map indicated that the human DNA molecules contain regions more than ten times more susceptible to interstrand crosslinking than the plasmid and viral genomes. Taking into account the huge lengths of the human DNA molecules, this finding suggests that even very low doses of UV irradiation generate interstrand crosslinks in the human genome.

Previous findings and software concerning correlation of genomic distributions of nucleotides were now used to analyze the whole *E. coli* genome. We found using cluster analysis that this genome was composed of two kinds of segments that were characteristic by qualitatively distinct correlation. The first kind of segments showed strong correlation of guanine with cytosine and adenine with thymine whereas adenine anticorrelated with cytosine and guanine with thymine. The second kind of segments showed low correlation typical of random sequences. Both kinds of segments were almost equally abundant in the *E. coli* genome and their maximum length was about 70 kb. In contrast, they differed by the (G+C) content because, on average, the highly correlated segments were richer in (A+T) than the segments showing low correlation.

An analogous analysis of the (almost) complete nucleotide sequences of the DNA molecules of the human chromosomes 21 and 22 showed that they were very similar regarding the nucleotide correlation and both also had mosaic structures composed of two qualitatively different kinds of segments. The segments most differed by the correlation of G with C like in *E. coli* but they differed from the *E. coli* segments mostly by the A/C anticorrelation that were strong in both kinds of segments. In addition, the human segments significantly differed by neither the (G+C) content, nor the dinucleotide composition or length.

The most probable explanation of the above observations is a dependence of point mutations on the local (G+C) content and on the repair mechanisms that are specific with particular organisms.

GRANTS:

GA CR 206/98/0626
Interstrand crosslinks induced in the genetic material by ultraviolet light
Principal investigator: J. Kypr, 1998 - 2000

GA CR 204/00/D012
Correlations and variations of nucleotide and short oligonucleotide distributions in the genome of *Caenorhabditis elegans*
Principal investigator: D. Häring, 2000 - 2003

GA AS CR A5004802
Biophysical analysis of selected regions of the human genome
Principal investigator: J. Kypr, 1998 - 2000

GA AS CR C5004003
Conformational variability of dinucleotides in DNA
Principal investigator: S. Neugebauerová, guarantor J. Kypr, 2000

LABORATORY OF MOLECULAR EPIGENETICS (LME)

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TECHNICAL ASSISTANTS:	DANUŠE FRIDRICHOVÁ LIBUŠE JEDLIČKOVÁ EMILIE KOUDELKOVÁ

1. A lack of genetic interaction between parental 5S rDNA clusters in alloploid Nicotiana tabacum

N. tabacum is thought to be an allotetraploid derived from the ancestors of modern *N. sylvestris* (section Alatae, maternal S genome donor) and *N. tomentosiformis* (section *Tomentosae*, paternal T genome donor). Two families of 5S rDNA are reported to be present in *N. tabacum* having basic units of 431 bp, 646 bp respectively. We investigated whether two families of 5SrRNA genes in the genome of *Nicotiana tabacum* have their origin in the diploid progenitors. Ancestral diploid species *N. sylvestris* and *N. tomentosiformis* harbour units of 430 bp and 650 bp, respectively. DNA sequencing revealed that the length polymorphism of basic units are caused by differences in size of intergenic spacer sequences. The spacer sequence of the shorter tobacco unit showed high sequence homology (>95%) to the spacer of *N. sylvestris*; the longer spacer was highly homologous (>95%) to that of *N. tomentosiformis*. The longer spacer sequence differed from the shorter spacer by multiple insertions of GC-rich DNA. The *in situ* hybridization on metaphase tobacco chromosomes confirmed the presence of short family on S-chromosome and long family on T-chromosome. Thus *N. tabacum* appears to have two homeologous clusters of 5S rDNA inherited from both ancestors. The 5S rRNA genes do not appear to have undergone gene conversion as is seen for the 18S-5.8S-26S rDNA units (see below). The sequence diversity of parental gene families across the species was low and comparable to that of within species suggesting absence of changes in the nucleotide composition of both spacer and transcribed sequences. The long–range organization of 5S rRNA gene clusters mapped by PFGE revealed restriction polymorphisms possibly reflecting observed copy number heterogeneity between different species and even between different tobacco varieties. *In cis* but not *in trans* concerted evolution is thus demonstrated for 5S repeats in allotetraploid tobacco. A decrease in the copy number is often associated with an increase in copy in the second family. DNA methylation analysis with a number of methylation sensitive restriction endonucleases, including specific for individual families, showed that both families of 5S rDNA sequences in tobacco are uniformly highly methylated. Both gene families show decondensation pattern at interphase indicating that both ancestral clusters are potentially active.

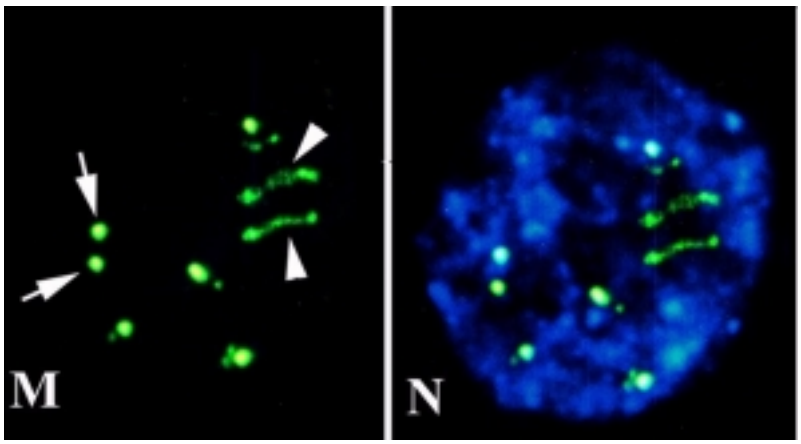
2. Gene conversion of parental 18S-5.8S-25S rDNA in alloploid Nicotiana tabacum

In higher plants the genes coding for 18S-5.8S-25S rRNA and 5S RNA are located in separated loci. We examined the structure, intranuclear distribution and activity of 18S-5.8S-25S ribosomal DNA (rDNA) in *Nicotiana sylvestris* (2n=2x=24) and *N. tomentosiformis* (2n=2x=24) and compared these with patterns in *N. tabacum* (tobacco, 2n=4x=48). *Nicotiana sylvestris* has three rDNA loci, one locus each on chromosomes 10, 11, and 12. In root tip meristematic interphase cells, the site on chromosome 12 remains condensed and inactive, whilst the sites on chromosome 10 and 11 show activity at the proximal end of the locus only. *Nicotiana tomentosiformis* has one major locus on chromosome 3 showing activity and a minor, inactive

locus on chromosome 11. In *N. tabacum* cv. 095-55, there are four rDNA loci on T3, S10, S11/t and S12 (S11/t carries a small T genome translocation). The locus on S12 remains condensed and inactive in root tip meristematic cells while the others show activity, including decondensation at interphase and secondary constrictions at metaphase. Gene conversion in *N. tabacum* is consistent with previous works. However, a PCR experiment using primers specific for the S genome rDNA intergenic sequences (IGS) showed that rDNA gene conversion has not gone to completion in *N. tabacum.* Using restriction enzyme analysis we demonstrated that about 8% of the rDNA units remain of the *N. sylvestris*-type. The minority of S-derived units displayed significantly increased level of cytosine methylation compared to the T-derived units. The data indicate that active, undermethylated genes are of the *N. tomentosiformis*-type. The *N. sylvestris*-type units presumable localize to the inactive loci (i.e. on chromosome S12). Nevertheless, some S-chromosomes also show expression of 18S-5.8S-25S rRNA genes indicating that rDNA units at these loci are of *N. tomentosiformis* origin. The results suggest that methylation and/or DNA condensation has reduced or prevented gene conversion from occurring at inactive genes at rDNA loci. Alternatively, active undermethylated units may be vulnerable to gene conversion, perhaps because they are decondensed and located in close proximity within the nucleolus at interphase.

3. Inheritance of epigenetic states

Reestablishment of cytosine methylation status after drug-induced hypomethylation has been studied in tobacco HRS60 and NTRS repeats in callus culture. The degree of hypomethylation at CG (HpaII) and CNG (MspI) sites was modulated by variable concentration of a hypo-methylation drug, dihydroxypropyladenine (DHPA), an inhibitor of cellular SAH-hydrolase. The drug has been previously shown to preferentially inhibit methylation at CNG and nonsymmetrical motifs. At low concentration (5 μM) the drug induced specific hypomethylation of CNG motifs. Passage of cells onto the drug-free medium resulted in almost complete (>90%) remethylation of cytosines at CNG only after 2-3 cell divisions. At high concentration of the drug (25-100 μM) some CG sites were hypomethylated in the HRS60 sequences and remethylation of these sites did not occur even after 15-16 cell divisions. Thus fundamental differences exist in mechanism of methylation of CG and CNG sites. The results suggest that de novo methylation of CNG and nonsymmetrical motifs is fast on sequences with preexisting CG methylation but slow or non-existing on methylation-free templates.



Panel M: *In situ* hybridization of tobacco interphase nucleus using 18S-5.8S-25S rDNA probe labeled with FITC. The three highly condensed loci are clearly distinguished from one largely decondensed locus. The variable degree of chromatin condensation could correspond to different epigenetic states of rDNA. Panel N: same nucleus contrasted with DAPI staining.

GRANTS:

GA CR 521/98/0045
Factors involved in regulation of DNA methylation in plants
Principal investigator: A. Kovařík, principal co-investigator: A. Holý, IOCHB AS CR Prague, 1998 - 2000

GA CR 204/98/0191
Relation between DNA methylation, structure and expression of 5S rDNA loci in tobacco
Principal investigator: R. Matyášek, 1998 - 2000

GA CR 204/99/D001
Mechanisms of DNA methylation and demethylation in higher plants
Principal investigator: J. Fulneček, 1999 - 2001

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

PROGRAM IV

MOLECULAR CYTOLOGY AND CYTOGENETICS

LABORATORY OF MOLECULAR CYTOLOGY AND CYTOMETRY (LMCC)

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UNDERGRADUATE STUDENT:	KATARÍNA ROSENBERGOVÁ-BUCHNÍČKOVÁ

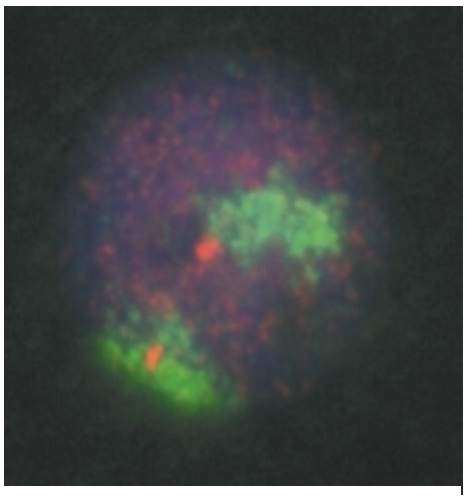


Fig. 1. c-MYC genes (red) in chromosome territories (green) in the nucleus of HL-60 cell (blue). The genes were visualised using PRINS technique; chromosome territories were visualised by FISH.

The development of a basic software for confocal 3D cytometry was finished and initial experiments were performed using this software. The improvement of the hardware consisted in the automation of a combined confocal and non-confocal mode of image acquisition, saving the advantages of both approaches; with the newly developed software the superposition of both images was made possible. The approaches of the visualization of genetic structures were improved, particularly, the PRINS technique for individual genes was optimized.

Chromosome 8 territories and c-MYC genes are visualized in the nucleus of HL-60 cell using FISH and PRINS techniques (Fig. 1).

An extensive series of experiments using 5 types of cells derived from human blood and also cells derived from human colon was finished. In these experiments the topographic characteristics of several genes (ABL, BCR, c-MYC, IGH), centromeres (1, 8, 9, 18) and chromosomes (HSA 8, 9, 14, 18, 22) were investigated. We demonstrated that genetic loci (genes, centromeres, chromosomes) were localized in spherical layers (shells) and a distance from the centre of the nucleus to a locus was characteristic for the given locus and was independent of the cell type. The positioning of the loci within the layers was random. This conclusion was achieved by measuring the locus– centre –locus angles for a large number of nuclei in 3D space and comparing the results with theoretical expectation obtained with a sinus function (see Fig. 2).

We could further demonstrate that some chromosomes displayed a pronounced polar organization (e.g. HSA 9), whereas in some other chromosomes (e.g. HSA 8) such a polar organization could not be detected. The sequence of spherical layers for individual genetic structures as well as the polarity of chromosomes are conserved to some extent in the cell cycle, during differentiation and in the repair induced by radiation. After the addition of the third copy of a chromosome to the cell nucleus, its genetic elements will be localized in the same spherical layers as 2 original homologues in the normal diploid nucleus.

The degree of expression of genes of a given chromosome depends on its localization in the nucleus. We found that chromosome domains were, in average, the nearer to the centre of the nucleus, the higher was their content of R-bands (i.e. apparently expressed chromosome regions). The actual position of a domain in the particular cell nucleus can be variable; however, one of the

domains is always nearer to the centre of nucleus and its genes are probably expressed to the higher extent.

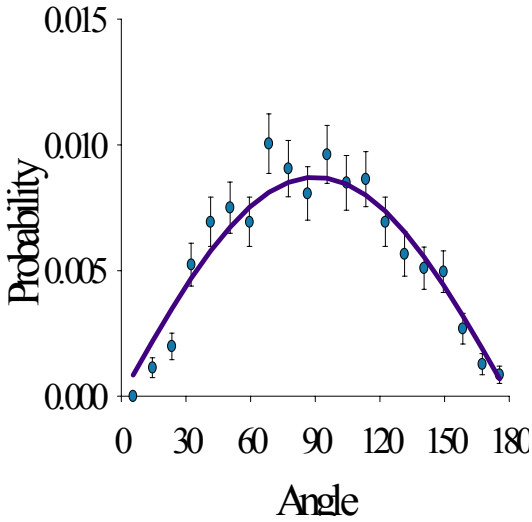


Fig. 2. The distribution of centromere centre of nucleus-centromere angels in 3D space for HL-60 cell nuclei has been determined. Experimental distribution has been calculated from 800 measurements and corresponds quite well to theoretical line. Confocal cytometry developed in collaboration with Faculty of Informatics MU was used.

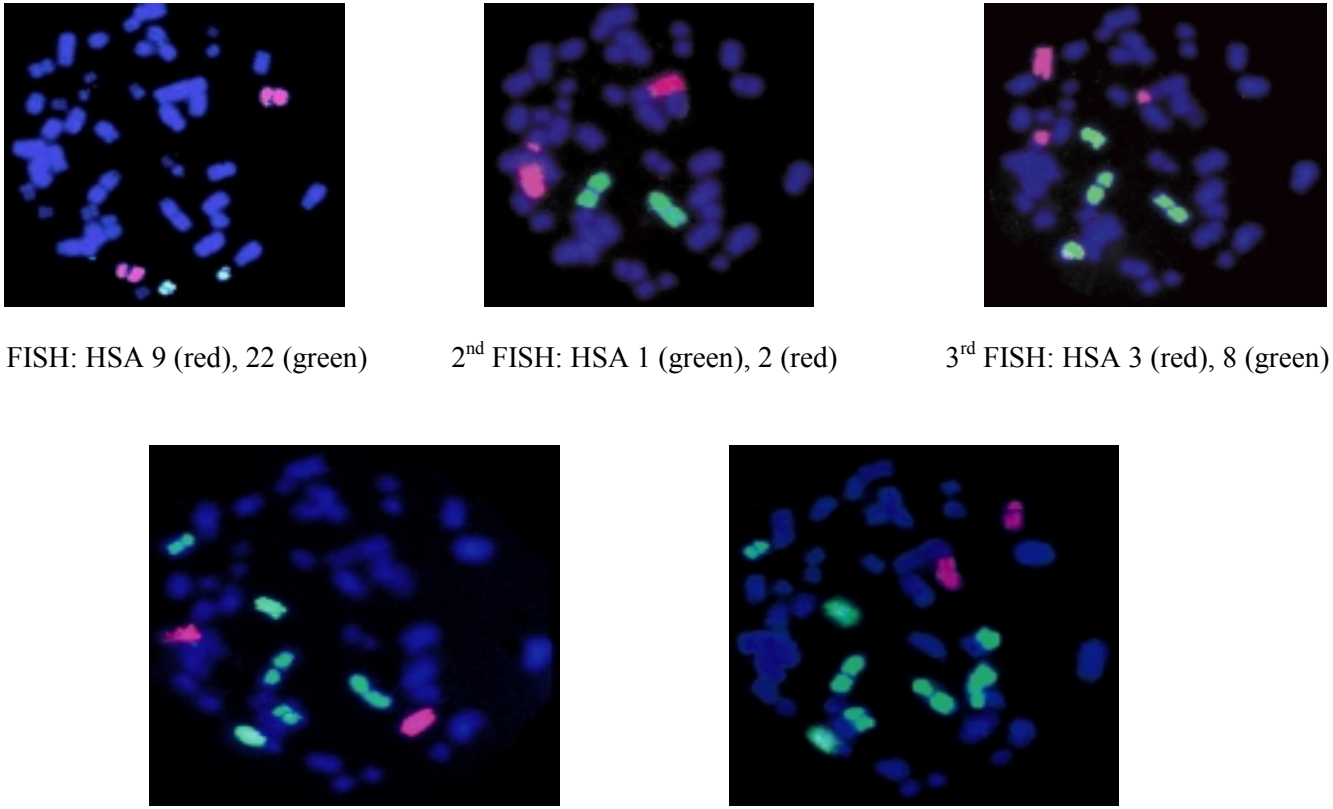
Differentiation brings about changes in the higher-order chromatin structure. For example, myeloid differentiation leads to the condensation of a large proportion of chromatin and its shift to the nuclear periphery. Hereditary down-regulation of some genes is probably also related to changes of their position – the genes are shifted to the periphery of the cell nucleus. We observed similar kinetics of the condensation of a domain of the 8th chromosome and the down regulation of the c-MYC gene suggesting a causal relation between the two processes. The c-MYC gene and the domain of the chromosome 8 are visualized in the HL-60 cell nucleus in Fig. 1. The c-MYC gene that is expressed in these cells, is localized on the periphery of the chromosome domain

Ionizing radiation induces exchange chromosome aberrations in cells. The frequency of aberrations is, for the given chromosome pair, determined by the mutual positioning of chromosomes in the cell nucleus (Fig. 3). The exchanges originate in those chromosome pairs that are near to each other. We could observe tethering between c-MYC and IGH loci in our previous experiments.

In further studies we could demonstrate such a bond between ABL and BCR genes in Go lymphocytes using both 2D and 3D analysis. These our results were confirmed by Nikiforova et al. (Science 290, 138-141, 2000) who studied RET and H4 genes in thyroid cells and found that the substantially increased incidence of thyroid malignant tumors after Chernobyl accident was related to the tethering of the mentioned genes.

Spatial neighborhood of two genetic loci increases the probability of their interaction after irradiation. This phenomenon is pronounced for densely ionizing radiation where the exchange aberrations are induced between those chromosome pairs that are localized near to each other. Exchange aberrations for the same chromosome pairs (e.g. 9 and 22 or 14 and 18) can be found with frequent occurrence also in haemoblastoses. This phenomenon can be observed, to some extent, also in the case of gamma-radiation, where the formation of aberrations takes longer time and more chromosome pairs may interact.

Irradiation of cells causes changes of the higher-order structure of chromatin. According to literary data homologous genetic structures come to the proximity in consequence of the recombinational repair using the second copy of chromosome as a template. In our experiments, we also observed the motion of homologous genetic structures near to each other; we showed, however, that this shift was a consequence of radial motion in the direction to the nuclear centre (probably due to decondensation of chromatin during the enzymatic repair). The spherical layers of genetic structures were, therefore, shifted to the centre of cell nucleus, however, the distribution of the loci inside the layers remained random (Fig. 4). These facts allowed us to reject the above-mentioned mechanism involving the recombinational repair.



1st FISH: HSA 9 (red), 22 (green)

2nd FISH: HSA 1 (green), 2 (red)

3rd FISH: HSA 3 (red), 8 (green)

4th FISH: HSA 4 (red), 11 (green)

5th FISH: HSA 7 (green), 10 (red)

Fig. 3. Repeated hybridisation of the same mitosis of human lymphocyte that was irradiated by γ-radiation (3 Gy). Dicentric aberration can be seen between HSA 2 and 3 (2nd and 3rd FISH); translocation between HSA 7 and unidentified chromosome (5th FISH).

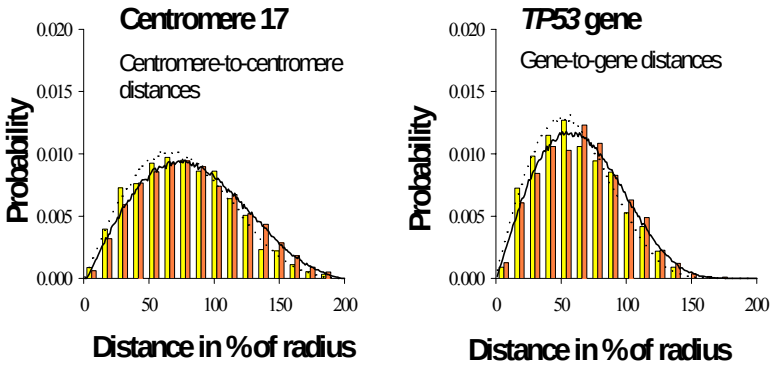


Fig. 4. The distance distributions between two genetic loci (centromeres 17 and TP53 genes) in nuclei of ML-1 cells in control (red) and after irradiation with the dose of 5 Gy (yellow). Experimental distributions correspond to the theoretical one that was calculated on the assumption of random positioning of the loci in spherical layers.

GRANTS:

GA CR 202/98/P253
Changes of the structure of interphase nuclei of human leukemic cell lines after the influence of differentiating agents and radiation
Principal investigator: E. Bártová, guarantor: S. Kozubek, 1998 - 2001

GA CR 202/99/P008
Image analysis in the study of the structure of interphase cell nucleus
Principal investigator: M. Kozubek, FI MU Brno, guarantor: E. Lukášová, 1999 - 2001

GA CR 202/99/0959
The use of multiple optical tweezers to controlled manipulation and rotation of microobjects
Principal investigator: M. Liška, VUT Brno, principal co-investigator: E. Lukášová, 1999 - 2002

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

ME VS 97 031
The use of image analysis for the study of the mechanisms of cancerogenesis, in diagnostics and for prevention of deleterious human diseases
Principal investigator: S. Kozubek, 1997 - 2000

MH NM/15-3
The use of interphase FISH for the screening of individuals with increased risk of malignant haemoblastoses
Principal investigator: S. Kozubek, 1998 - 2000

MH NC 5955
The topography of specific genetic loci in normal and malignant cell nuclei and its use for the diagnostics and treatment of solid tumors
Principal investigator: E. Lukášová, 2000 - 2002

LABORATORY OF PLANT DEVELOPMENTAL GENETICS (LPDG)

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GRADUATE STUDENTS:	MGR. JITKA ŽLŮVOVÁ MGR. JAROMÍRA HODURKOVÁ MGR. VLADIMÍRA HYKELOVÁ MGR. MARTINA LENGEROVÁ MGR. ROMAN HOBZA
UNDERGRADUATE STUDENTS:	MARTINA LENGEROVÁ PŘEMYSL SOUČEK

The results obtained can be summarized into two basic sections: (I) DNA methylation and histone acetylation as control mechanisms of epigenetic processes, and (II) structure, function, and evolution of plant sex chromosomes.

(I) DNA methylation and histone acetylation as control mechanisms of epigenetic processes:

1. DNA methylation patterns during seed germination

We have studied global changes in DNA methylation during seed germination and shoot apical meristem development in *Silene latifolia* using an indirect immunohistochemical approach. The data show that a rapid decrease in global DNA methylation during seed germination occurs first in endosperm tissue and subsequently in hypocotyls. Using bromodeoxyuridine pulses, we demonstrate that these demethylation events occurred before cell division had begun. In the early postgermination period, a decrease in DNA methylation was detected in cotyledons, also before cell division was observed. Taken together, these results indicate that DNA demethylation takes place in a non-replicative way, probably by the action of DNA demethylases. The central zone of the shoot apical meristem remains highly methylated during the whole period of vegetative growth and in this region, only a low cell division activity was found. However, upon the transition of the shoot apical meristem to the floral bud, the meristem both decreased its high methylation status and its cells started to divide. These data indicate that the central zone of the shoot apical meristem represent a relatively quiescent germ-line which is activated upon flowering to form spores and gametes.

2. DNA methylation dynamics in pollen development

In our pollen studies, we have concentrated on the analysis of the DNA methylation status of the 5´ upstream region of the *MROS1* gene (*M*ale *R*eproductive *O*rgan *S*pecific; collaboration with S. Matsunaga, University of Tokyo) which is expressed in pollen grains of *Silene latifolia*. Genomic sequencing analysis of cloned PCR products obtained from the modified pollen and leaf DNA showed a similarity both in 5-mC content and in the location of methylated sites. About 25% cytosines were methylated both in the pollen and leaf DNA. It was possible to distinguish a hypermethylated region located 150 bp upstream from the transcription start. In this 50bp sized region, approximately 66% of cytosines were methylated. In the rest of studied sequence including both non-transcribed part (100bp) and transcribed region (110bp), only 3% of cytosines were methylated. A uniformity of clones obtained from pollen DNA suggests that

the region studied is not involved in the prominent global changes of the DNA methylation of the vegetative nucleus which we observed previously using an immunohistochemical approach.

3. Role of DNA methylation in sex expression

We have analyzed six DNA sequences (DD3, 7, 14, 26, 44, and 51) prepared by RT-PCR analysis (RT-PCR differential display) using RNA isolated from male floral buds of *Silene latifolia* (collaboration with Sarah R. Grant, University of North Carolina). These sequences can represent sex determining loci which correspond with a male specific expression during phase of plant development when stamen primordia appear. We have designed primers and amplified these DD sequences using male and female DNA genomic samples. Both male and female DNA enabled to amplify the same single products, only the DD3 primers provided different products between males and females. To localize the DD sequences more precisely on chromosomes we have separated the autosomes and the X chromosomes by sorting and performed PCR analyses.

4. Histone acetylation during seed germination

The pattern of histone H4 acetylation during seed germination and early plant development of *Silene latifolia* was studied by western blotting. The data showed that histone H4 in the quiescent seed is stored preferentially in its non-acetylated and monoacetylated isoforms. The incorporation of [^{14}C]-uridine into germinating seeds and seedlings was measured to estimate intensity of RNA synthesis. A clear positive correlation between the level of H4 acetylation and transcription was found. Hyperacetylated histone H4 isoforms were detected in the stages of early plantlet development, when an extensive RNA synthesis occurred. To demonstrate the possibility of chromatin immunoprecipitation technique (ChIP) to study an acetylation status of individual genes, we perform this assay with affinity purified antibody against H4-AcLys16 in seed and seedling samples during different developmental stages. DNA, isolated from acetylated and non-acetylated chromatin fractions, is hybridized with a model 25S rDNA probe. We conclude that histone H4 hyperacetylation seems to be connected with transcriptional activation during seed germination and early development.

5. Origin of endosperm facultative heterochromatin

We provide a cytometric evidence for the events in the course of which three whole genomes in the pentaploid endosperm nuclei of *Gagea lutea* become heterochromatinised. In this plant, the embryo sac is tetrasporic: three chalazal spindles fuse and two triploid nuclei are formed. The nuclei in the chalazal part show a stronger condensation compared with the micropylar ones. The pycnosis of the triploid polar nucleus is maintained during endosperm proliferation, while the micropylar polar nucleus and the sperm nucleus remain euchromatic. The origin of the heterochromatic masses in the endosperm is evident from the distribution of heterochromatic chromosomes in the first endosperm mitosis and the following interphase. DNA content measurements confirm a 3:2 relationship of heterochromatic and euchromatic chromosome sets, which is usually maintained up to the cellularised endosperm. This research was realized in collaboration with J. Greilhuber (University of Vienna).

(II) Structure, function, and evolution of plant sex chromosomes:

1. Structure of chromosome ends

We have investigated the architecture of telomeres in *Silene latifolia* using the PENT (primer extension/nick translation) assay. We show that *Silene* telomeres carry G-overhangs longer than 20-30 nucleotides. However, only half of the telomeres in *Silene* seedlings possess detectable G-overhangs. PENT reactions revealed that the remaining fraction of *Silene* telomeres either carry no overhangs or overhangs less than 12 nucleotides in length. G-overhangs were also observed in *Silene* tissues that lack telomerase activity. Unexpectedly, we found that the fraction of telomeres with detectable G-overhangs decreased from 50% in seedlings to 35% in leaves. The difference could reflect increased susceptibility of the G-overhangs to nuclease attack in

adult leaves which could act as a precursor for the anabolic processes accompanying leaf senescence. Taken together, the data argue that incomplete DNA replication of the lagging strand, rather than synthesis by telomerase or C-strand specific nuclease digestion, is the primary mechanism for G-overhang synthesis in plants. This research was realized in collaboration with D. E. Shippen (Texas A&M University).

2. Structure and evolution of Silene genome

We localized a group of *MROS* (*M*ale *R*eproductive *O*rgan *S*pecific) genes, first known genes specifically expressed in male reproductive organs of *S. latifolia*. To localize these genes we used PCR on flow-sorted chromosomes (in collaboration with J. Doležel, Institute of Experimental Botany, Olomouc). We found that *MROS1*, *2* and *4* genes are only autosomal while *MROS3* gene has both autosomal and X-linked copies. At least two X-linked *MROS3* genes are tandemly arranged. Database search revealed that *MROS3* gene homologues are present in *Arabidopsis* genome, also in tandem arrangement. In order to map the structure and evolution of *S. latifolia* sex chromosomes, a BAC (bacterial artificial chromosome) library of *S. latifolia* male is constructed (collaboration with S. Grant, University of North Carolina).

3. Genetic mapping of Silene sex chromosomes

The first three genes localized on the Y chromosome (*SlY1*, *SlY3*, and *SlY4*), possessing their homologues on the X chromosome (*SlX1*, *SlX3*, and *SlX4*, respectively) represent unique molecular markers to study evolution of the sex chromosomes within the genus *Silene*. The aim of this research is to utilize these genes for mapping on the sex chromosomes of *S. latifolia*, *S. dioica*, and *S. diclinis*, as well as on the chromosomes of related hermaphrodite species *S. noctiflora* and *S. conica*, to reveal processes responsible for the origin of the sex chromosomes (especially translocations and inversions). We have measured frequency of crossing-over between the *SlX1* and *SlX2* genes using PCR with corresponding primers: the first data show that the frequency is very high (about 40%) which indicates a long distance between these genes. This research is run in collaboration with I. Negrutiu (Ecole Normale Supérieure de Lyon) and D. Charlesworth (University of Edinburgh).

4. Cytogenetic markers for Silene sex chromosomes

Using the double target *in situ* hybridization with DNA probes labeled with biotin and digoxygenin the precise localizations of 5S rRNA and 25S rRNA genes on the *Silene* spp. chromosomes were determined. In all species tested chromosomes bearing both clusters of rDNA loci were detected in addition to chromosomes possessing a signal of only 5S rDNA or 25S rDNA. In *S. latifolia* and *S. pendula* there are two pairs of chromosomes having both types of signals contrary to *S. vulgaris* and *S. chalcedonica* where the both signals were observed only on one pair of chromosomes. In *S. chalcedonica*, 5S rDNA and 25S rDNA loci are co-localized on the shorter arm of metacentric chromosome No. 5. The method of bicolor FISH thus enabled a more precise karyotyping and unambiguous assignment of chromosome pairs. These rDNA probes will be used as cytogenetic markers to identify individual chromosomes when hybridized with promising BAC probes.

5. Structure of Rumex sex chromosomes

Functional features of *Rumex acetosa* sex chromatin in metaphase and interphase nuclei were analyzed using immunocytochemical (antibodies raised against 5-methylcytosine or N-terminal acetylated histones H4) and FISH (25S rDNA and Y-specific repetitive probes) techniques. Our results have not revealed any global differences in DNA methylation labeling between the heterochromatic Y chromosomes and the X chromosome or the autosomes. The only prominent hypermethylated domains corresponded to inactive nucleolar organizing regions. Comparative staining analyses of female and male interphases revealed two heterochromatic, male-specific peripheral bodies. A double immunolabelling technique have demonstrated that these bodies represent persistent sex chromosomes Y_1 and Y_2 (using the Y-specific DNA probe) and are

largely underacetylated in histone H4 (using the polyclonal antiserum against H4 acetylated at N-terminal lysine 5). We conclude that plant constitutive heterochromatin (represented by the sorrel Y-chromosomes) is depleted in nucleosomal histone acetylation, similarly as we had previously observed in facultative heterochromatin.

Localization of *MROS* (male reproductive organ specific) genes using PCR on sorted chromosomes: (a) a gene located on the autosomes, (b) a gene linked both to the autosomes and to the X chromosome.

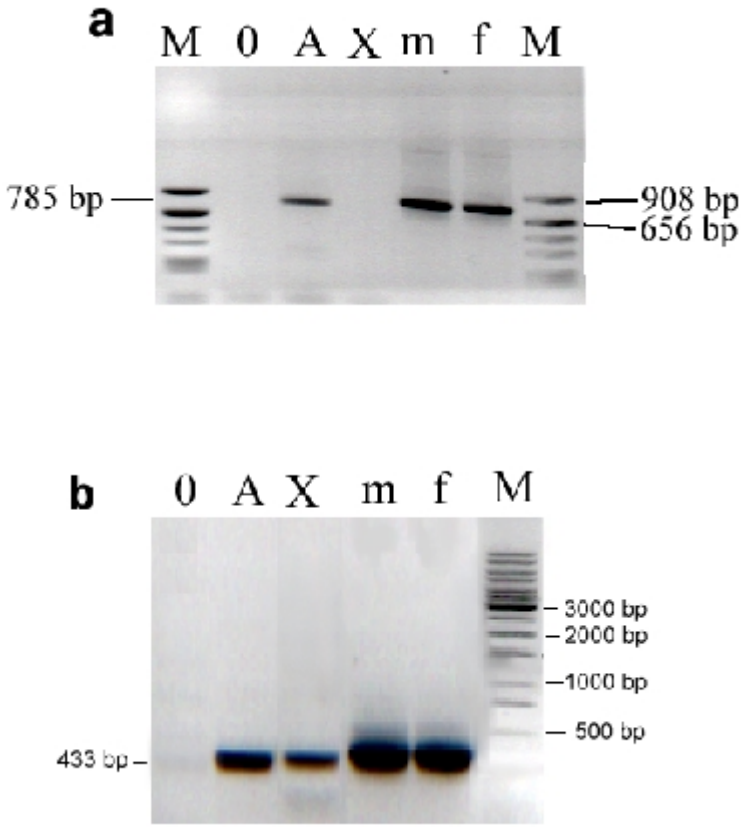

Fig. 1. Agarose electrophoretic separation of PCR products obtained using (a) *MROS2* gene specific primers, and (b) *MROS3* gene specific primers. Negative control without DNA template (0), 1,000 autosomes (A), 100 X chromosomes (X), male genomic DNA (m), and female genomic DNA (f). M – length markers (1 kb ladder plus pBR322/AluI).

GRANTS:

GA AS CR A5004901
Nuclear structure and histone acetylation in plant cells
Principal investigator: B. Vyskot, 1999 - 2001

GA AS CR D5004005
Confocal laser scanner microscope
Principal investigator: B. Vyskot, 2000

GA CR 521/99/0696
Kinetics of DNA methylation in embryogenesis and seed germination
Principal investigator: B. Vyskot, 1999 - 2001

GA CR 521/96/K117
New methods for effective studying and mapping of crop plants
Principal investigator: J. Doležel, IEB AS CR Olomouc, principal co-investigator: J. Široký, 1996 - 2001

GA CR 521/98/P061
Study of chromatin changes during plant microsporogenesis and microgametogenesis
Principal investigator: B. Janoušek, guarantor: B. Vyskot, 1998 - 2000

LABORATORY OF MOLECULAR ANALYSIS OF PLANT DEVELOPMENT (LMAPD)

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1. Biological function of a putative cytokinin receptor CKI1

CKI1 was discovered and assigned a putative cytokinin receptor based on biological effects caused by CKI1 overproduction in *Arabidopsis transgenic* plants. However, no data on CKI1 function under normal conditions has been published. Here, biological function of CKI1 is investigated by functional genomics and molecular biophysics approaches.

A knock-out mutant in *CKI-1* gene was isolated in an *Arabidopsis thaliana* population mutagenized with the maize transposon En-1. The mutant carries an insertion of the transposon in an exon 5 of the *CKI-1* gene. The mutation resulted in partial block in gamethophyte development.

En1 excision events from the cki1::En1 allele were analyzed to prove unequivocally the causal relationship between cki1::En1 allele and the described mutant phenotype. Screening for normal silique development in population of about 250 individual Arabidopsis plants heterozygous for the cki1::En1 allele resulted in identification of a revertant, fully fertile, plant in which the En1 excision can be traced back by presence of an in-frame footprint that apparently does not interfere with a normal biological function of CKI1. In complementary screening for an En1 excision event that preserves the mutant phenotype we have identified a plant in which En1 excised from the cki1::En1 allele while the mutant phenotype was retained. However, molecular analysis of the excision locus proved presence of a footprint that causes loss of function in CKI1 gene. These two results represent a genetic prove of the causal relationship between the cki1::En1 allele and the mutant phenotype. The results confirm a crucial role of CKI1 in plant reproductive development.

2. The role of cytokinin metabolism in plant growth and development

To study specific effects of localized cytokinin overproduction in transgenic plants, a system for tight spatial regulation of ipt expression is being established. A recently described transcription activation system (Moore et al., Proc. Natl. Acad. Sci. USA, 95: 376-381, 1998) is employed. In this system ipt is inserted behind a synthetic promoter (pOp) which is inactive when introduced into wild-type plants but is activated when these plants are crossed with others that express a novel transcription activator protein, LhG4. Transgenic tobacco plants carrying pOp-ipt were generated. More than 80% of pOp-ipt transformants regenerated normally in tissue culture and grew under greenhouse conditions without any detectable phenotype alteration indicating that the pOp promoter is virtually silent in most transformants in the absence of LhG4. Initial evidence for activation of ipt in pOp-ipt transgenic plants was provided by re-transformation of several independent pOp-ipt transgenic plants with a plant transformation vector carrying LhG4 under

control of the CaMV35S promoter. About 70% of the pOp-ipt transgenic plants re-transformed with CaMV35S-LhG4 produced calli that could grow on media not supplemented with cytokinins. Thus, the majority of the pOp-ipt transgenic plants carried at least one activatable pOp-ipt copy. Ipt activation by LhG4 in intact plants will be studied after crosses between pOp-ipt and CaMV35S-LhG4 transgenic plants

3. Cytokinins and auxins in regulation of plant development

We aim to analyze feasibility of regulated cytokinin release from cytokinin-O-glucosides in individual subcellular compartments using a maize β-glucosidase, Zm-p60.1, specific for cytokinin-O-glucosides. Zm-p60.1 is a plastid/chloroplast enzyme. Information specifying subcellular targeting in Zm-p60.1 cDNA has been modified to achieve re-direction of individual Zm-p60.1 derivatives from plastids/chloroplasts into (i) the vacuole (Zm-p60.vl, Zm-p60.vc), and (ii) secretion into the extracellular space (Zm-p60.ex). Success of the re-direction strategy has been confirmed in transgenic tobacco expressing the individual derivatives from a constitutive promoter.

To analyze effects of temporal increases in Zm-p60.1 enzyme activity separately in chloroplasts, vacuoles and the extracellular matrix, the individual Zm-p60.1cDNA derivatives were placed under control of a tetracyclin-inducible promoter. The resulting transcriptional fusions were transformed into tobacco. Preliminary analysis of the transformants is in progress.

GRANTS:

GA AS CR A5004001
Transcription activation system for studying the relationship between cytokinin metabolism and action in Arabidopsis and tobacco
Principal investigator: B. Brzobohatý, principal co-investigators: A. Kuderová, Fac. Sci. MU Brno, I. Macháčková, IEB AS CR Prague, 2000 - 2002

GA CR 206/96/K188
Cytokinins and auxins in regulation of plant development
Principal investigator: I. Macháčková, IEB AS CR Prague, principal co-investigator: B. Brzobohatý, 1996 - 2001

ME CR VS96096
Study of molecular basis of regulation processes in plants
Principal investigator: B. Brzobohatý, 1996 - 2000

PROGRAM V

KINETICS OF THE CELL POPULATIONS

LABORATORY OF CYTOKINETICS (LC)

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1. Studies on effects of cytokines

Regulation of cytokinetics (i.e. proliferation, differentiation and apoptosis) in both normal and tumor cell populations is a complex process, where cytokines and cell membrane phospholipid products, especially arachidonic acid (AA) metabolites - eicosanoids, play an important role. We used a number of structurally different compounds inhibiting AA metabolism (see figure) to investigate the role of selected cytokines (TGF-β1 and TNF-α) in cell signalling: eicosatetrayenoic acid (ETYA, competitive inhibitor of AA metabolism), nordihydroguiaretic acid (NDGA, general lipoxygenase inhibitor), MK-886 (5-lipoxygenase-activating protein inhibitor) baicalein (12-lipoxygenase inhibitor), indomethacin (INDO), flurbiprofen, ibuprofen (cyklooxygenase-1 and -2 inhibitors), niflumic acid (cyklooxygenase-2 inhibitor), proadifen and 9-hydroxyellipticine (cytochrome P450 inhibitors). Theses compounds are of potential importance as anti-tumor agents. We employed tumor cell populations of various origin in order to generalize our findings.

We studied the role of Bcl-2 and Bax proteins in regulation of programmed cell death in NB4 cells derived from acute promyelocytic leukemia with translocation t(15;17). We found that TGF-β1 potentiates the all-trans retinoic acid (ATRA)-induced differentiation in the cells. The combined treatment with ATRA plus TGF-β1 led to a significant increase in CD11b surface antigen expression, activity of non-specific esterases and production of oxygen radicals. The effects of TGF-β1 correlated with decreased Bax expression and slowed down decrease of Bcl-2 expression induced by ATRA. These changes could be responsible for the observed inhibition of apoptosis and increased cell viability after the combined treatment with ATRA plus TGF-β1. Thus, our findings suggest that TGF-β1 modulates apoptosis of myeloid leukemia cells by regulation of the Bcl-2 family proteins.

Moreover, we have studied effects of TNF-α and anti-Fas antibody. We tested whether the Fas-mediated apoptosis can be potentiated by DMSO in leukemia cell lines HL-60, U937, ML-1 and Jurkat. DMSO treatment significantly increased expression of Fas following 48 h incubation in

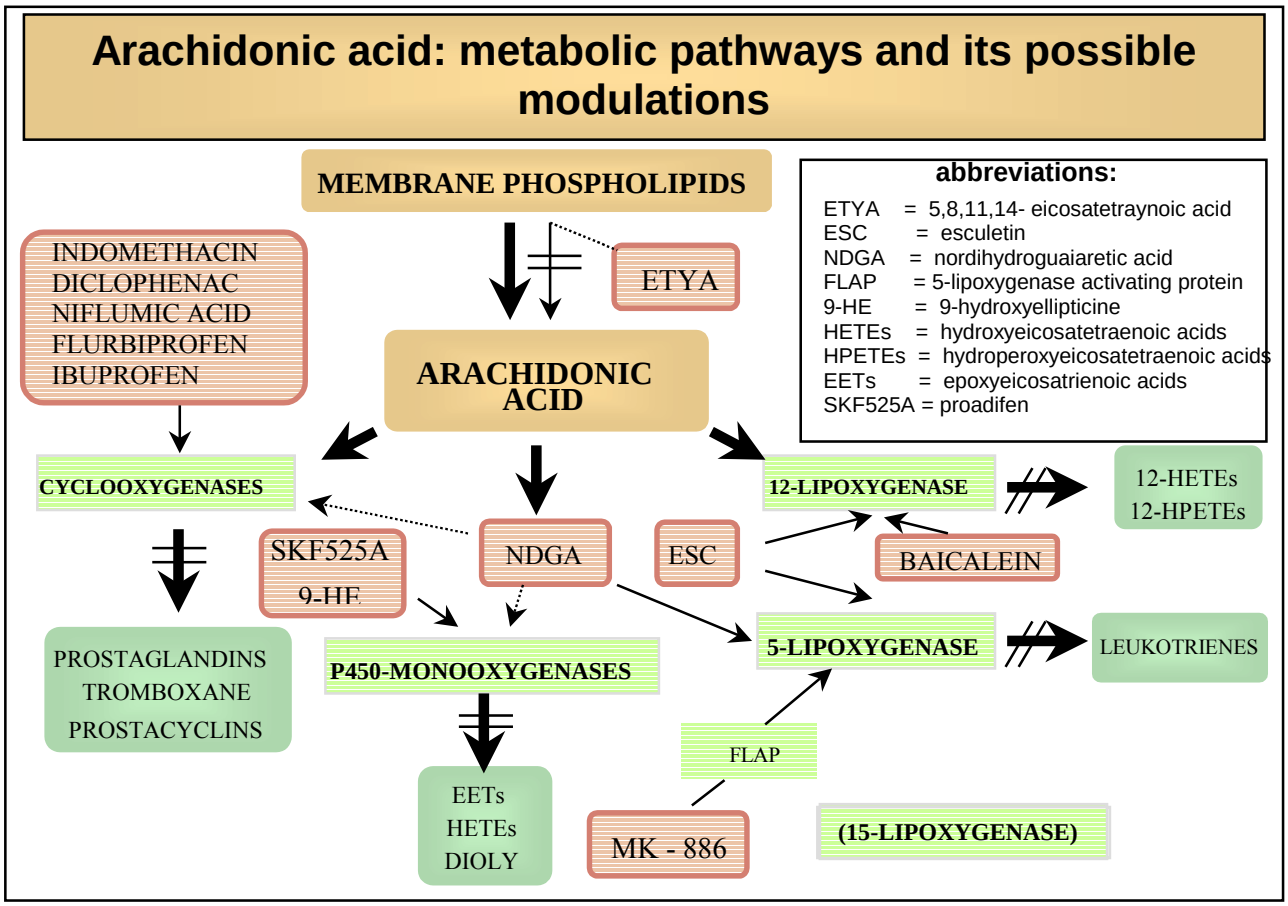
all studied cell lines with exception of ML-1 cells. Application of DMSO significantly increased percentage of apoptotic U937 cells after 40 h incubation with anti-Fas monoclonal antibody. We could show that apoptosis can be potentiated even after preincubation of U937 cells with DMSO, followed by anti-Fas treatment. Furthermore, we have studied effects of DMSO on expression of the Bcl-2 family proteins (Bcl-2, Bax, Mcl-1) and FAP-1 that may affect cell sensitivity towards apoptotic stimuli. We found that DMSO and ATRA may induce FAP-1 expression during the terminal stage of differentiation.

We studied the effects of AA metabolism inhibitors on apoptosis induced by TNF-α. We demonstrated that indomethacin synergistically enhances the effects of TNF-α. Moreover, we investigated effects of peroxisome proliferator-activated receptors (PPAR) ligands on the action of TNF-α. We found that this type of activity is not responsible for the effects of indomethacinand showed that indomethacin can suppress the inhibition of apoptosis induced by ATRA.

To contribute to the understanding of the mechanisms of action of AA metabolism inhibitors in human immortalised keratinocytes HaCaT, we studied effects of structurally unrelated inhibitors on the following parameters: 1) oxidoreductase activity (MTT assay), total amount of proteins and distribution of cell in cell cycle phases in order to assess cell proliferation, 2) PARP protein degradation to determine apoptosis and 3) changes in cell morphology and differentiation assessed as changes in F-actin distribution and expression of cytokeratin and E-cadherin. ETYA, NDGA, esculetin and MK-886 decreased proliferation in HaCaT cells, while cyclooxygenase inhibitors, indomethacin and piroxicam, had no effect. Esculetin and NDGA blocked cells in S-phase, while ETYA and MK-886 in G1-phase of cell cycle. Although we did not observe any changes in expression of cytokeratin and E-cadherin after application of inhibitors, addition of esculetin led to F-actin redistribution, correlating with an increased cell size and adhesivity. These findings suggest multiple biological effects of the compound under study.

Proteins of c-Jun family, AP-1 transcription factor components, play an important role in cell cycle regulation. Therefore, we studied efface of AA metabolism inhibitors on c-Jun expression. We have defined a model of changes in expression of c-Jun family proteins (c-Jun, JunB and JunD) and c-Jun activation (phosphorylation at Ser73) in HaCaT cells during the transition from subconfluent to confluent cell population. To further characterise the model, we investigated cell proliferation, cell cycle and changes in pRb protein expression and phosphorylation. We studied effects of NDGA, esculetin, MK-886 and AA itself on c-Jun expression in subconfluent cells. The increased expression of c-Jun was followed by increased pRb expression and phosphorylation, which correlated with an increased amount of cells in S-phase of cell cycle. We observed strong inducing effects of 50 μM NDGA both on subconfluent and confluent cell populations. Application of either esculetin or MK-886 led to weak increase in c-Jun expression.

We studied changes in cell cycle, proliferation and apoptosis in human adenocarcinoma-derived HT-29 cells after application: 1) selected polyunsaturated fatty acids (n-3 class, docosahexaenoic acid - DHA and n-6 class, AA) and TNF-α, 2) AA metabolism inhibitors (ETYA, NDGA, baicalein, indomethacin and niflumic acid) and their combinations with TNF-α during sodium butyrate (NaBt)-induced differentiation. The findings suggest that AA or DHA plus TNF-α, applied either simultaneously or consecutively, interact to potentiate their anti-proliferative or pro-apoptotic effects on tumor cells in a concentration- and time-dependent manner. Modulation of AA metabolism by specific inhibitors induces changes in cell cycle, reduces proliferation and induces apoptosis in HT-29 cells in an inhibitor- and concentration-dependent manner. While even low concentrations of NDGA had anti-proliferative effects, high concentrations of indomethacin were necessary to induce apoptosis. We found that NaBt (inducing cell cycle block and differentiation) sensitised cells to effects of inhibitors. However, effects combinations with TNF-α were only additive, suggesting that both types of compound act through independent and separated pathways.



2. Studies in environmental toxicology

Our aim was to complement detection systems suitable to gain data for environmental risk assessment. We used four cell lines representing different types of tissues (HL-60, HaCaT, HT-29 a Hepa-1 (murine hepatoma cells)) to follow changes in cytokinetic parameters, proliferation, differentiation, cell cycle and apoptosis, after application of model xenobiotics with nongenotoxic mode of action. Dioxin (TCDD) affected only proliferation of Hepa-1cells, without a clear dependence on concentration. On the other hand, peroxisome proliferator clofibrate affected all basic cytokinetic parameters in all types of cells, although to a different extent. Moreover, we investigated parameters of cytokinetics after combined treatment with either TCDD or clofibrate plus AA metabolism inhibitors. A significant interaction was found for combination of clofibrate with 9-hydroxy-ellipticine (CYP1A1 inhibitor).

Moreover, a number of mutagenic polycyclic aromatic hydrocarbons (PAHs) and their derivatives were identified in various environmental samples. The aim of our study was to determine their dioxin-like and estrogenic activities using *in vitro* reporter gene assays and to compare the findings with dioxin-like activities of 16 PAHs considered to be priority pollutants. We found that a majority of compounds under study interacts with Ah receptor and that this type of activity may contribute to toxic effects of these pollutants.

These findings contribute to understanding of regulatory mechanisms in myeloid and epithelial cells, leading changes in cytokinetics and affecting cancer ethiology. They may be implicated in alternative therapeutic strategies (especially differentiation therapy) for cancer treatment, optimisation of lipid nutrition of colon carcinoma patients and in environmental risk assessment.

GRANTS:

GA CR 524/99/0694
Polyunsaturated fatty acids and cytokines - their role in maintenance of homeostasis at the cell population level
Principal investigator: A. Kozubík, 1999 - 2001

GA CR 525/98/1266
Biochemical and cellular markers of toxic and carcinogenic effects of xenobiotics
Principal investigator: M. Machala, VÚVL Brno, principal co-investigator: J. Hofmanová, 1998 - 2000

GA CR 312/98/P011
The role of metabolism of arachidonic acid in apoptosis induced by TNF and anti-Fas during the differentiation of human leukemic line HL-60 cells
Principal investigator: J. Vondráček, guarantor: A. Kozubík, 1998 - 2000

GA CR 301/00/0563
Modulation of tumor cells defense: Definition and inhibition of survival mechanisms suppressing apoptosis mediated by Fas and employed by tumor cells
Principal investigator: M.A. Sheard, MOÚ Brno, principal co-investigator: J. Vondráček, 2000 - 2002

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

IGA MH CR NC/6171-3
Changes of lipid metabolism and their effects in colorectal carcinoma patients - perspective use in nutritional support
Principal investigator: Z. Zadák, FN UK Hradec Králové, principal co-investigator:
J. Hofmanová, 1998 - 2000

ME 966/2000
Support of study program „Cellular and Molecular Physiology„
Principal investigator: V. Šimek, PřF MU Brno, principal co-investigators: A. Kozubík,
J. Hofmanová, 2000

ME 976/2000
Effects of cytokine TGF in regulation of apoptosis induced by retinoic acid
Principal investigator: K. Souček, principal co-investigators: J. Šmarda, Fac. Sci. MU, Brno,
Z. Andrysík, 2000

LABORATORY OF EXPERIMENTAL HEMATOLOGY (LEH)

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TECHNICAL ASSISTANT:	VĚRA REICHMANNOVÁ
GRADUATE STUDENTS:	MGR. ZUZANA HOFEROVÁ MGR. LENKA WEITEROVÁ

In 2000, studies on the topic of pharmacological stimulation of haematopoiesis damaged by ionizing radiation or cytostatic therapy continued. In the experiments *in vivo* and *in vitro* techniques were combined; radiation source used was ^{60}Co-irradiator Chisostat, a model cytostatic drug was 5-fluorouracil (5-FU).

Following the previously demonstrated radioprotective efficacy of the immunomodulator adamantylamide dipeptide (AdDP) (see Annual Report 1999 of the Institute of Biophyics), mechanisms of haematopoiesis-stimulating effects of this drug, prepared for clinical trials, were studied. It was found that co-stimulating activity of AdDP assesed as stimulation of proliferation of progenitor cells for granulocytes and macrophages (GM-CFC) *in vitro* is dependent on the presence of adherent (stromal) bone marrow cells in cultures. From these observations it can be deduced that AdDP does not support the proliferation of GM-CFC by a direct effect, but indirectly through evoking production of cytokines by stromal cells of the haematopoietic microenvironment.

Studies evaluating modulation of the kinetcs of regeneration of haematopoiesis damaged by 5-FU followed preceding series of experiments in the frame of which synergistic effects of drugs elevating extracellular concentration of adenosine (dipyridamole /DP/ + adenosine mono-phosphate /AMP/) and granulocyte colony-stimulating factor (G-CSF) on granulopoiesis, suppressed by ionizing radiation, were described. In this year interesting observations were made concerning stimulatory effects of the combination of DP + AMP + G-CSF on erythropoiesis damaged by 5-FU. Application of the above mentioned drug combination in a four-day regimen starting after administration of 5-FU, led to a significant elevation of the number of progenitor cells for erythrocytes (BFU-E) in the bone marrow and numbers of erythrocytes in the peripheral blood. From the theoretical point of view as well as from the perspective of a possible practical use it is noteworthy that the effects of the combined therapy by means of DP + AMP + G-CSF are not limited only to granulopoiesis but that they may serve also for modulation of anaemia evoked by cytostatics.

GRANTS:

GA CR 306/99/0027
Enhancement of G-CSF action by adenosine signalling: Testing of its potential clinical use in murine models
Principal investigator: M. Hofer, principal co-investigator: J. Vácha, LF MU Brno, 1999 - 2001

GA MI PZ Z2/25/97
Radioprotective and chemoprotective effects of the immunomodulator adamantylamide dipeptide (AdDP)
Principal investigator: M. Hofer, 1997 - 2001

LABORATORY OF FREE RADICALS PATHOPHYSIOLOGY (LFRP)

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Oxidative and antioxidative properties of N-nitro-L-arginine methyl ester (L-NAME) (which is known as the selective inhibitor of nitric oxide synthase) were studied using the reaction of luminol with 1) superoxide radical generated by the system xanthine oxidase (0.1 U/ml) / hypoxanthine (1 mg/ml), 2) hydrogen peroxide (2 mM), 3) hydroxyl radical generated by the reaction of hydrogen peroxide (2 mM) with ferrous sulfate (1 mM) and 4) peroxyl radical induced by the thermal decomposition of 2,2-azo-bis-2-amidinopropane hydrochloride. CL induced by superoxide, hydrogen peroxide as well as hydroxyl radical was inhibited with L-NAME in a concentration dependent manner. It proves the antioxidant properties of L-NAME against these reactive oxygen species (ROS).

The ROS production and myeloperoxidase activity, which are useful markers of HL-60 myeloid cell maturation, were measured using flow-cytometric, luminometric and spectrophotometric methods in HL-60 cell line differentiated into neutrophil- or monocyte- like cells. Our results obtained using different fluorescent probes, luminophores and activators demonstrate particularities in chemiluminescence and flow-cytometric analyses of HL60 cell-derived extra- and intracellular ROS generation. Another contribution of the study is the improvement of the CL method for detecting myeloperoxidase activity in myeloid cells after their differentiation.

Generation of reactive oxygen species, expression of CD11b/CD18 and CD62L (i.e. surface molecules) on neutrophils and monocytes, the total radical-trapping antioxidative potential of plasma and some other biochemical parameters of plasma were investigated in patients with regular hemodialysis (HD). Blood samples were collected just before and just after the HD procedure. Significant decrease in both spontaneous and activated ROS generation was found after HD. Although the antioxidative potential of plasma was relatively high in patients before HD, these values decreased significantly even below those of healthy controls after HD. The expression of CD11b/CD18 increased both on neutrophils and monocytes, although the expression of CD62L did not change. All observed plasma lipids were found significantly increased after the HD procedure – total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol and triglycerides as well. Apolipoproteins A-I and B were also increased after HD. The increase in plasma lipids and lipoproteins correlated with the increase in albumin concentration. In additional experiments, the influence of two types of HD membrane was tested – non–complement activating polysulfone membrane and complement activating hemophan membrane. Hemophan membrane caused more intensive activation and fault of function of blood phagocytes than polysulfone membrane.

An influence of the duration of ischemia on the production of reactive oxygen metabolites and mobilisation of natural antioxidative mechanisms was studied on a model of

ischemia/reperfusion of rat small intestine. Arteria mesenterica superior was occluded for 15, 30, 45, 60 or 90 minutes and then reperfusion was set. Samples of sera and intestinal tissue were taken at the end of ischemia or after 120 minutes of reperfusion. Ischemia intervals longer than 30 minutes induced increased metabolic activity of circulating neutrophils and serum antioxidative potential. Significant increase in these parameters was also observed after 120 minutes of reperfusion independently on the duration of preceding ischemia. Ascorbic acid and uric acid were the major constituents significantly contributing to the increase in serum antioxidative potential. Contrary to the increased serum antioxidative capacity an increased lipoperoxidation both in serum and intestinal tissue was observed.

In the next set of experiments deferroxamine (iron chelator) and dimethylthiourea (hydroxyl radical scavenger) in the dose of 10 mg/kg of body weight were applied intraperitoneally to rats with induced ischemia/reperfusion of small intestine just before the set of ischemia. Neither deferroxamine nor dimethylthiourea affected the increased metabolic activity of whole blood phagocytes and both of them induced only mild increase in serum total antioxidative capacity. Despite that their protective effect was manifested by decreased lipoperoxidation in intestinal mucosa. Out of antioxidative enzymes, neither SOD nor catalase given intraperitoneally (separately or in combination), prevented both the generation of reactive oxygen species by phagocytes and lipoperoxidation in serum and intestinal mucosa.

GRANTS:

GA CR 524/00/1223
Reactive oxygen and nitrogen metabolites generated by neutrophils under physiological and pathophysiological conditions
Principal investigator: A. Lojek, 2000 - 2002

GA CR 524/98/0190
The role of endogenous antioxidants in the regulation of post-ischemic oxidative stress
Principal investigator: M. Číž, 1998 - 2000

GA CR 524/99/D022
The influence of different time of ischemia and reperfusion upon the development of reperfusion injury of intestine
Principal investigator: H. Čížová, guarantor: M. Číž, 1999 - 2001

IGA MH CR 4796-3
Reactive oxygen species in relation to hemodialysis and kidney transplantation
Principal investigator: V. Soška, FN U sv. Anny Brno, Principal co-investigators: M. Číž, A. Lojek, 1998 - 2000

RESEARCH CENTRE

BIOMOLECULAR CENTRE

COORDINATOR:	MASARYK UNIVERSITY BRNO
PARTICIPANT:	INSTITUTE OF BIOPHYSICS AS CR BRNO
HEAD (IBP AS CR):	RNDR. JIŘÍ ŠPONER, CSC.
GRADUATE STUDENT:	MGR. NAĎA ŠPAČKOVÁ

We have continued in the simulation of unusual forms of DNA by molecular dynamics methods. Modern computation methods of molecular modelling represent respected techniques which yield an unique view on the structure and dynamics of biomolecules on the atomic level and conveniently complement experimental techniques (e.g. X-ray, NMR) in problems which cannot be solved experimentally. The aim of the calculations was the study of interactions of nucleic acid bases with metal ions inclusive platinum in order to clear up the effect of metals on the tautomeric equilibrium of bases, their basicity and acidity, base pairing and vertical interactions. The calculation of base pair and vertical interactions in water was performed using the continuous solvent model. These techniques enable to correlate calculations with results of electrochemical measurements with the aim to find out which properties of bases and their complexes are important for their condensation at the electrode surface.

The experimental and theoretical information was obtained about forces which maintain the conformation stability of biomacromolecules and which participate in their interactions with ligands, in particular with mutagens, carcinogens and compounds having a relation to the regulation of cell growth and to the tumour diseases, like the protein p53.

GRANT:

ME CR LN00A016
Program „Research Centre“ Biomolecular Centre
Coordinator: J. Koča, Fac. Sci. MU Brno, participant: J. Šponer, 2000 - 2004

SIGNALLING PATHWAYS IN PLANTS

COORDINATOR:	INSTITUTE OF EXPERIMENTAL BOTANY AS CR PRAGUE
PARTICIPANT:	INSTITUTE OF BIOPHYSICS AS CR BRNO
HEAD (IBP AS CR):	RNDR. BŘETISLAV BRZOBOHATÝ, CSC.
SCIENTIST:	MGR. JAN ZOUHAR, DR.
GRADUATE STUDENTS:	MGR. JAN HEJÁTKO MGR. PETRA BORKOVCOVÁ MGR. HANA BUBENÍČKOVÁ

Biological function of a putative cytokinin receptor CKI1

CKI1 was discovered and assigned a putative cytokinin receptor, based on biological effects caused by CKI1 overproduction in *Arabidopsis* transgenic plants. However, no data on CKI1 function under normal conditions has been published as yet. Here, biological function of CKI1 is investigated by functional genomics and molecular biophysics approaches.

A knock-out mutant in CKI-1 gene was isolated in an *Arabidopsis thaliana* population mutagenised with the maize transposon En-1. The mutant carries an insertion of the transposon in an exon 5 of the CKI-1 gene. The mutation resulted in a partial block in the gamethophyte development.

En1 excision events from the cki1::En1 allele were analyzed to unequivocally prove the causal relationship between cki1::En1 allele and the described mutant phenotype. Screening for normal silique development in population of about 250 individual *Arabidopsis* plants heterozygous for the cki1::En1 allele resulted in identification of a revertant, fully fertile, plant in which the En1 excision can be traced back by presence of an in-frame footprint that apparently does not interfere with a normal biological function of CKI1. In complementary screening for an En1 excision event that preserves the mutant phenotype we have identified a plant in which En1 excised from the cki1::En1 allele while the mutant phenotype was retained. However, molecular analysis of the excision locus proved the presence of a footprint that causes loss of function in the CKI1 gene. These two results represent a genetic prove of the causal relationship between the cki1::En1 allele and the mutant phenotype. The results confirm a crucial role of CKI1 in plant reproductive development.

GRANT:

ME CR LN00A081
Program „Research Centres“ Signalling pathways in plants
Coordinator: I. Macháčková, IEB AS CR Prague, participant: B. Brzobohatý, 2000 - 2004

LABORATORY OF COMPUTER AND INFORMATION SERVICES (LCIS)

HEAD: RNDR. JOSEF JURSA, CSC.

TECHNICAL ASSISTANT: LUKÁŠ POSÁDKA

Standard services of the laboratory:

- Operation, servicing and development of the IBP local area network (LAN)
  - Operations on the connection of the IBP LAN to the 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) including data updating
  - Current maintenance and development of computer techniques (hardware and software), utilized by projects solved at the IBP (servers, graphic workstations and simple PCs with Internet access), working under UNIX, MS Windows NT/2000, MS Windows 95/98/ME and MS DOS operating systems
  - Consulting and guidance services for individual projects (an expert help with solving computer technique and computer network connected problems)
- Operation and servicing of the 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 the library server used by Academy of Sciences in Brno region

The project Infra-2 LB98210 finished in the 2000. There was established a National Node of the ICCBnet (International Center for Cooperation in Bioinformatics network) in the Czech Republic at the IBP (http://ICCBnet.ibp.cz) and installed a database server with a mirror of the Protein Data Bank (PDB), accessible through Internet. The mirror serves to users in Central and East Europe. The Protein Data Bank collects structural data of proteins, nucleic acids and their complexes. In addition there was installed the GCG Wisconsin Package, a database collection of sequences of genomes, nucleic acids and proteins (GenBank, EMBL, PIR, SWISS-PROT, TrEMBL). The database is provided with about 150 computer programmes to search the database, to work with the data and to utilise them. The Wisconsin Package provides comprehensive software tools for database searching and sequence comparison, fragment assembly, mapping and gene finding, evolutionary analysis, primer selection, protein analysis, and nucleic acid secondary structure prediction. The GCG Package is accessible to academic users (Academy of Sciences and universities) in the Czech Republic.

GRANT:

ME CR Program Infra-2 LB98210
Establishing of a national node of an ICCBnet (International Center for Cooperation in Bioinformatics network) and creating an access to selected international databases for academic users of the Czech Republic
Principal investigator: J. Jursa, 1998 - 2000

III. PUBLISHED REPORTS

References are given in the original language.

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Telomerase activity, expression and telomere in situ analysis in the course of treatment of childhood leukemias

DNA Structure and Interactions – Their Biological Roles and Implications in Biomedicine and Biotechnologies, Brno, 19. - 23. 7. 2000

In: Book of Abstracts, D-14

Malina, J., Natile, G., Brabec, V.:

DNA interactions of antitumor cisplatin analogs containing enantiomeric amine ligands

International Conference on DNA Conformation, Modification and Recognition in Biomedicine, Brno, 2. - 5. 7. 2000

In: Program and Abstracts, P 106

Malina, J., Natile, G., Brabec, V.:

DNA interactions of antitumor cisplatin analogs containing enantiomeric amine ligands

International Symposium Metals in Environmental Madicine Wroclaw, Poland, 18. - 21. 10. 2000

In: Book of Abstracts, p. 61

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Crystallographic study of the maize cytokinin gluco-side-specific ß-glucosidase Zm-p60.1

19th European Crystallographic Meeting, Nancy, France, 25. - 31. 8. 2000

In: Abstracts, p. 246

Mašek, K., Zídek, Z., Ševčík, J., Franková, D., Hofer, M.:

The immunomodulatory effects of trypsin

International Conference on Immunopharmacology in Perspective, Praha 10. - 13. 5. 2000

In: Program and Abstracts, nestr.

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Two families of 5S rDNA in allotetraploid N. tabacum

DNA Structure and Interactions – Their Biological Roles and Implications in Biomedicine and Biotechnologies, Brno, 19. - 23. 7. 2000

In: Book of Abstracts, D-12

Mejstříková, K., Čížová, H., Lojek, A., Číž, M.:
Vliv allopurinolu na I/R poškození tenkého střeva potkana a jeho antioxidační vlastnosti in vitro
XVII. biochemický sjezd, Praha, 7. - 10. 9. 2000

Minksová, K., Vondráček, J., Machala, M., Netíková, J., Holá, J., Hofmanová, J., Kozubík, A.:
Účinky xenobiotik na cytokinetiku modelových buněčných linií
Konference ERA 2000 – Hodnocení rizik pro životní prostředí, Brno, 12. - 15. 3. 2000
In: Program a sborník konference, pp. 184-185, poster

Minksová, K., Vondráček, J., Machala, M., Netíková, J., Holá, J., Hofmanová, J., Kozubík, A.:
Changes of cytokinetic parameters in four cell lines after clofibrate and TCDD treatment
Buňky II/Cells II, České Budějovice, 5. - 6. 9. 2000
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Antitumor efficient platinum complexes with trans-geometry and their reactions with DNA
International Conference on DNA Conformation, Modification and Recognition in Biomedicine, Brno, 2. - 5. 7. 2000
In: Program and Abstracts, P 111

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Steric control of DNA interstrand cross-link sites of antitumor trans-platinum complexes
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In: Abstracts Oral Presentation 1173

Nováková, O., Brabec, V.:
Translesion synthesis by Klenow fragment of Escherichia coli DNA polymerase I past platinum-DNA adducts
International Conference on DNA Conformation, Modification and Recognition in Biomedicine, Brno, 2. - 5. 7. 2000
In: Program and Abstracts, P 113

Nováková, O., Brabec, V.:
Translesion synthesis by Klenow fragment of Escherichia coli DNA polymerase I past platinum-DNA adducts
5th European Biological Inorganic Chemistry Conference, Toulouse, France, 17. - 20. 7. 2000
In: Conference Book, nestr.

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Biological effects of lipoxygenase inhibitors on human keratinocyte line HaCaT
Buňky II/Cells II, České Budějovice, 5. - 6. 9. 2000
In: Cell II. Ed.: J. Berger, p. 80

Paleček, J., Brázda, V., Brázdová, M., Fojta, M., Pospíšilová, S., Vojtěšek, B.,Paleček, E.:
Specific modulation of p53 binding to supercoiled DNA with and without consensus sequence by monoclonal antibodies
10th International p53 Workshop Monterey, California, USA, 5. - 8. 4. 2000
poster

Paleček, E.:

History and new trends

Analytica Conference 2000, München, Germany,11. - 14. 4. 2000

Paleček, E.:

Binding of tumor suppressor protein p53 to supercoiled DNA

NIH, Bethesda, MD, USA, 30. 10. 2000

Paleček, E.:

1. Local DNA structures, PNA and DNA hybridization sensors

2. Interaction of tumor suppressor protein p53 with DNA. Effect of DNA supercoiling

Arizona State University, Phoenix, Arizona, USA, 28. 11. 2000

Paleček, E.:

Binding of tumor suppressor protein p53 to supercoiled DNA

Georgia Tech., Atlanta, Georgia, USA, 30. 11. 2000

Paleček, E.:

Electrochemical analysis of DNA

New Mexico State University, Las Cruces, NM, USA, 17. 11. 2000

Paleček, E.:

Elektrochemie nukleových kyselin a bílkovin. Od polarografie DNA k senzorům pro moderní genomiku

Heyrovského přednáška 2000, Praha, 20. 12. 2000

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Analysis of nucleic acids and proteins with electrodes. Detection of DNA hybridization and DNA damage

51st Annual ISE Meeting, Warsaw, Poland, 3. - 8. 9. 2000

In: Meeting Programme, nestr.

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Preparation of DNA hybridisation probes for the EWS and FLI-1 genes and determination of the basic topographic parameters in nuclei of human T-lymphocytes and Ewing sarcoma cells

Workshop on Higher-Order Structure of Cell Nuclei and Genetic Effects of Radiation, Valtice, 7. - 8. 11. 2000

In: Abstracts, p. 26

Pečinka, P., Jagelská, E., Vojtěšek, B., Paleček, E.:

Effect of temperature on binding of wild type and mutants of p53 to supercoiled DNA

DNA Structure and Interactions – Their Biological Roles and Implications in Biomedicine and Biotechnologies, Brno, 19. - 23. 7. 2000

In: Book of Abstracts, B-12

Qu, Y., Malina, J., Brabec, V., Cox, J., Farrell, N.:

Long-range interstrand cross-links formed by polynuclear platinum complexes

International Conference on DNA Conformation, Modification and Recognition in Biomedicine, Brno, 2. - 5. 7. 2000

In: Program and Abstracts, P 117

Shmakova, N. L., Govorun, R. D., Krasavin, E. A., Kozubek, S., Kutsalo, P. V., Lukashova, E., Repin, M. V., Abou-Zeid, O. A., Fadeeva, T. A.:

Radiobiological research with mammalian cells at JINR accelerators

Workshop on Higher-Order Structure of Cell Nuclei and Genetic Effects of Radiation, Valtice, 7. - 8. 11. 2000

In: Abstracts, p. 28

Skalníková, M., Kozubek, S., Lukášová, E., Bártová, E., Jirsová, P., Gaňová, A., Koutná, I., Matula, Pa., Matula Pe., Paseková, R., Kozubek, M.:

Spatial arrangement of genes, centromeres and chromosomes in human blood cell nuclei

Workshop on Higher-Order Structure of Cell Nuclei and Genetic Effects of Radiation, Valtice, 7. - 8. 11. 2000

In: Abstracts, p. 30

Souček, K., Andrysík, Z., Hofmanová, J., Kozubík, A.:

Transforming growth factor β1 (TGF-β1) affects Bcl-2 and Bax protein levels in myeloid leukaemia cell line NB4 after retinoic acid treatment

Buňky II/Cells II, České Budějovice, 5. - 6. 9. 2000

In: Cell II. Ed.: J. Berger, p. 125

Souček, K., Kubala, L., Lojek, A., Kozubík, A.:

Analysis of reactive oxygen species production and myeloperoxidase activity in chemically induced differentiation of human myeloid leukemia cells

Dresden Chemiluminescence Days, Dresden, Germany, 10. - 13. 5. 2000

In: Abstracts, p. 21

Strašák, L., Hasoň, S., Vetterl, V.:

Frequency dependence of adsorption of native and denatured DNA

International Conference on DNA Conformation, Modification and Recognition in Biomedicine, Brno, 2. - 5. 7. 2000

In: Program and Abstracts, P 144

Strašák, L., Vetterl, V., Šmarda, J.:

Nízkofrekvenční elektrická a magnetická pole a bakterie Escherichia coli: viabilita růstové křivky a oxidoredukční aktivita

XXIII. dny lékařské biofyziky, Darovanský Dvůr, 23. - 26. 5. 2000

In: Sborník abstrakt, p. 62, poster

Strašák, L., Vetterl, V., Šmarda, J.:

Vliv magnetických polí o nízkých frekvencích na bakterie Escherichia coli

Tomáškovy dny 2000, Brno 7. - 9. 6. 2000

In: Sborník souhrnů přednášek, p. 21

Sýkorová, E., Fajkus, J.:

Metody studia struktury chromatinu

2. Metodické dny, Červenohorské sedlo, 2. - 6. 10. 2000

Sýkorová, E., Horáková, M., Mikako, I., Fukui, K., Fajkus, J.:

DNA and chromatin structure of telomere-subtelomere junctions in Silene latifolia

DNA Structure and Interactions – Their Biological Roles and Implications in Biomedicine and Biotechnologies, Brno, 19. - 23. 7. 2000

In: Book of Abstracts, D-13

Šámalová, M.:

Regulovatelné systémy exprese transgenu u rostlin

2. Metodické dny, Červenohorské sedlo, 2. - 6. 10. 2000

Ševčíková, S., Souček, K., Šmarda, J.:

Partial v-myb suppression by Fos and Jun proteins

Buňky II/Cells II, České Budějovice, 5. - 6. 9. 2000

In: Cell II. Ed.: J. Berger, p. 73

Špačková, N., Šponer, J.:

Large scale molecular dynamics simulations of four-stranded DNA assemblies: i-DNA and guanine quadruplex

International Conference on DNA Conformation, Modification and Recognition in Biomedicine, Brno, 2. - 5. 7. 2000

In: Program and Abstracts, P 129

Špačková, N., Berger, I., Šponer, J.:

Simulace čtyřřetězcových struktur nukleových kyselin I-DNA a G-DNA pomocí molekulové dynamiky

IV. Pracovní setkání biochemiků a molekulárních biologů. Biochemie a molekuární biologie na prahu nového tisíciletí, PřF MU, Brno 3. - 9. 2. 2000

In: Sborník příspěvků, p. 90

Špačková, N., Berger, I., Šponer, J.:

Unusual nucleic acid structures: G-DNA, i-DNA and A-zipper. Insights from large-scale molecular dynamics simulations

DNA Structure and Interactions – Their Biological Roles and Implications in Biomedicine and Biotechnologies, Brno, 19. - 23. 7. 2000

In: Book of Abstracts, L4-2

Šponer, J.:

Base stacking and H-bonding: from quantum chemistry to computer simulations

International Conference on DNA Conformation, Modification and Recognition in Biomedicine, Brno, 2. - 5. 7. 2000

In: Program and Abstracts, O 53

Šponer, J., Hobza, P.:

Modern computational studies of interactions of nucleic acid bases, their ingluence on the structure and dynamics of nucleic acids

DNA Structure and Interactions – Their Biological Roles and Implications in Biomedicine and Biotechnologies, Brno, 19. - 23. 7. 2000

In: Book of Abstracts, L4-1

Šponer, J., Hobza, P.:

DNA base amino groups and their role in molecular interactions

DNA Structure and Interactions – Their Biological Roles and Implications in Biomedicine and Biotechnologies, Brno, 19. - 23. 7. 2000

In: Book of Abstracts, C-13

Štika, J., Vondráček, J., Hofmanová, J., Kozubík, A., Šimek, V.:

Role of arachidonic acid metabolites in effects of TNF-α on proliferation, viability, differentiation and apoptosis of human leukaemic cell line HL-60

Buňky II/Cells II, České Budějovice, 5. - 6. 9. 2000

In: Cell II. Ed.: J. Berger, p. 135

Štros, M., Cherny, D., Jovin, T. M.:

HMG1 stimulates DNA end joining by promoting association of DNA molecules via their ends

International Conference on DNA Conformation, Modification and Recognition in Biomedicine, Brno, 2. - 5. 7. 2000

In: Program and Abstracts, O 54

Trnková, L., Kizek, R., Dračka, O.:

Application of elimination methods in electrochemical study of DNA

DNA Structure and Interactions – Their Biological Roles and Implications in Biomedicine and Biotechnologies, Brno, 19. - 23. 7. 2000

In: Book of Abstracts, C-11

Turánek, J., Záluská, D., Vacek, A., Borkovcová, P., Bláha, L., Ledvina, M., Mašek, K.:

Stimulation of nonspecific immunity and haemopoiesis by synthetic analogues of muramyldipeptide incorporated in liposomes

International Conference on Immunopharmacology in Perspective, Praha 10. - 13. 5. 2000

In: Program and Abstracts, nestr.

Vacek, A., Hofer, M., Hoferová, Z., Mašek, K.:

Co-stimulating action of sera of mice administered adamantylamide dipeptide (AdDP) on the proliferation of granulocyte-macrophage progenitor cells (GM-CFC)

International Conference on Immunopharmacology in Perspective, Praha 10. - 13. 5. 2000

In: Program and Abstracts, nestr.

Vaculová, A., Hofmanová, J., Kovaříková, M., Šimek, V., Kozubík, A.:

Účinky vysoce nenasycených mastných kyselin (VNMK) a cytokinu TNFα na buněčný cyklus, proliferaci a buněčnou smrt lidské linie adenokarcinomu kolonu HT-29

XXIV. Brněnské onkologické dny a XIV. Konference SZP s tématikou Postižení gastrointestinálního traktu z multidisciplinárního pohledu, Brno 24. - 26. 5. 2000

In: Abstrakta, p. 189, poster

Vaculová, A., Hofmanová, J., Kovaříková, M., Šimek, V., Kozubík, A.:

The effect of polyunsaturated fatty acids and cytokine TNF-α on proliferation and death of human colon adenocarcinoma HT-29 cell line

Buňky II/Cells II, České Budějovice, 5. - 6. 9. 2000

In: Cell II. Ed.: J. Berger, p. 81

Vetterl, V.:

Adsorption of nucleic acids, polynucleotides and nucleic acid components at mercury electrode

2nd International Conference on Basic Sciences and Advanced Technology, Assiut, Egypt, 5. - 8. 11. 2000

In: Abstract, p. 199

Vetterl, V., Jelen, F., Dražan, V., Strašák, L., Hasoň, S.:

Dielektrické ztráty u nativní a denaturované DNA

II. pracovní setkání fyzikálních chemiků a elektrochemiků. Fyzikální chemie a elektrochemie na konci druhého tisíciletí, PřF MU Brno, 10. 2. 2000

In: Sborník příspěvků, p. 39

Vetterl, V., Jelen, F., Dražan, V., Strašák, L., Hasoň, S.:

Impedance elektrodové dvojvrstvy v roztocích nativní a denaturované DNA

XXIII. dny lékařské biofyziky, Darovanský Dvůr, 23. - 26. 5. 2000

In: Sborník abstrakt, p. 66

Vetterl, V., Jelen, F., Dražan, V., Strašák, L. Hasoň, S.:

Electrochemical impedance spectroscopy of native and denatured DNA

DNA Structure and Interactions – Their Biological Roles and Implications in Biomedicine and Biotechnologies, Brno, 19. - 23. 7. 2000

In: Book of Abstracts, C-9

Vetterl, V., Jelen, F., Dražan, V., Strašák, L., Hasoň, S., Dvořák, J.:

Electrochemical impedance spectroscopy of single-stranded and double-helical polynucleotides

Heyrovský Memorial Symposium on Advances in Polarography and Related Methods, Praha, 30. 8. - 1. 9. 2000

In: Sborník, p. 5

Vetterl, V., Jelen, F., Hasoň, S., Běluša, P.:

Adsorptive stripping analysis of damage DNA with admittance measurements

Heyrovský Memorial Symposium on Advances in Polarography and Related Methods, Praha, 30. 8. - 1. 9. 2000

In: Sborník, p. 44

Vondráček, J., Bláha, L., Machala, M.:

Estrogenní aktivita environmentálních vzorků a vybraných organických kontaminantů

Konference ERA 2000 – Hodnocení rizik pro životní prostředí, Brno, 12. - 15. 3. 2000

In: Program a sborník konference, pp. 194-195 poster

Vondráček, J., Sheard, M. A., Minksová, K., Hofmanová, J., Kozubík, A.:

Modulace apoptózy zprostředkované Fas v buněčných liniích myeloidního původu pomocí dimethylsulfoxidu

XVII. biochemický sjezd, Praha, 7. - 10. 9. 2000

Vondráček, J., Štika, J., Kozubík, A.:

Modulation of TNF-alpha-induced apoptosis in human myeloid HL-60 cells byinhibitors of arachidonic acid metabolism

8th Euroconference on Apoptosis, Davos, Switzerland, 14. - 17. 10. 2000

In: Program and Abstracts, Poster 69

Vorlíčková, M.:

Konformační polymorfie DNA očima CD spektroskopie

Seminář Fyzikálního ústavu UK Praha, 12. 12. 2000

Vrána, O., Brabec, V.:

L-methionine does not increase the rate of reaction of platinum antitumor drugs with DNA

International Conference on DNA Conformation, Modification and Recognition in Biomedicine, Brno, 2. - 5. 7. 2000

In: Program and Abstracts, P 137

Vrána,O., Brabec, V.:

Does L-methionine increase the rate of reaction of platinum antitumor drugs with DNA?

3rd European Biophysics Congress, München, Germany, 9. - 13. 9. 2000

Vrána, O., Brabec,V.:

Does L-methionine increase the rate of reaction of platinum antitumor drugs with DNA?

International Symposium Metals in Environmental Medicine, Wroclaw, Poland, 18. - 21. 10. 2000

In: Book of Abstracts, p. 79

Vyskot, B.:

Epigenetic control of gene expression in plants

Mendel Centenary Congress, 5. GPZ – Tagung, Brno, 7. - 10. 3. 2000

Vyskot, B.:

Epigenetic control of gene expression in plants

Seminar Department of Biology University of North Carolina, Chapel Hill, USA, 5. 6. 2000

Vyskot, B.:

Epigenetic processes in plant development

Special Seminar Department of Biology, Texas A&M University College Station, Texas, USA, 13. 6. 2000

Vyskot, B.:

Epigenetic control of gene expression in plants

Institut für Botanik der Universität Wien, Austria, 9. 10. 2000

Vyskot, B.:

Epigenetic regulation of plant reproductive development

Plant Biotechnology for the New Millennium, Taejon, Korea, 4. 11. 2000

Vyskot, B.:

Epigenetic regulation of plant reproductive development

University of Chinju, Korea, 6. 11. 2000

Vyskot, B.:

Srovnávací vývojová biologie

Seminář: Pokroky v molekulární biologii, ÚMG AV ČR a Centrum PDSB při UK a AV ČR, Praha, 14. 11. 2000

Vyskot, B.:

Epigenetická dědičnost

Seminář: Pokroky v molekulární biologii, ÚMG AV ČR a Centrum PDSB při UK a AV ČR, Praha, 14. 11. 2000

Weiterová, L., Hofer, M., Pospíšil, M., Znojil, V., Vácha, J., Vacek, A., Pipalová, I.:

Mutually potentiating effects of drugs elevating extracellular adenosine and granulocyte colony-stimulating factor on erythropoietic recovery following 5-fluorouracil-induced haematotoxicity in mice

International Symposium Promoted by the Spanish Purine Club, Madrid, Spain, 9. - 13. 7. 2000

Zehnulová, J., Farrell, N., Brabec, V.:

Conformation of DNA site-specific adducts of antitumor trinuclear platinum compounds

International Conference on DNA Conformation, Modification and Recognition in Biomedicine, Brno, 2. - 5. 7. 2000

In: Program and Abstracts, P 140

Zehnulová, J., Farrell, N., Brabec, V.:

DNA intrastrand and interstrand cross-links of novel bifunctional trinuclear platinum antitumor agent

5th European Biological Inorganic Chemistry Conference, Toulouse, France, 17. - 20. 7. 2000

In: Conference Book, nestr.

Žaludová, R., Krestýn, E., Kašpárková, J., Brabec, V.:

Liquid crystalline microphase of DNA modified by platinum complexes

International Conference on DNA Conformation, Modification and Recognition in Biomedicine, Brno, 2. - 5. 7. 2000

In: Program and Abstracts, P 139

Žlůvová, J., Janoušek, B., Vyskot, B.:

DNA methylation changes during plant ontogenesis

IV. Pracovní setkání biochemiků a molekulárních biologů. Biochemie a molekuární biologie na prahu nového tisíciletí, PřF MU, Brno 3. - 9. 2. 2000

In: Sborník příspěvků, p. 44

Žlůvová, J., Vyskot, B.:

DNA methylation dynamics during plant ontogenesis

Němcův cytologický den, Brno 25. 5. 2000

In: Program a sborník abstraktů, p. 42

Žlůvová, J., Vyskot, B.:

Changes in chromatin structure during plant development

DNA Structure and Interactions – Their Biological Roles and Implications in Biomedicine and Biotechnologies, Brno, 19. - 23. 7. 2000

In: Book of Abstracts, L5-15

D. Supplementary reports due to the Research Report 1999

Turánek, J., Záluská, D., Vacek, A., Borkovcová, P., Thurnvaldová, J., Bláha, L., Mašek, K.:
Stimulation of haemopoiesis, nonspecific immunity and protection of mice against radiation injury by adamantyldipeptide incorporated in liposomes
4th International Conference Liposome Advances, Progress in drug and vaccine delivery, London, GB, 13. - 17. 12. 1999
In: Abstracts, 135

E. Overview of publication activities in 2000

1. Full-length papers	62
supplementary papers due to RR 1999	2
2. Short communications	14
3. Chapters in monographs	1
4. Articles popularizing science	4
5. Scientific lectures - presented in the CR	5
- presented abroad	18
6. Abstracts presented	
- conferences in the CR	155
- conferences abroad	36
supplementary abstracts due to RR 1999	1

IV. INTERNATIONAL CONTACTS

As always, 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 2000 is provided in tables as follows:

Foreign guests

Country	AS CR competition	Grants	Other sources
Austria		3	2
Belgium	1	3	1
Egypt	2		
Finland	2		1
France	1	4	2
Germany		13	8
Great Britain		5	2
Greece		1	2
Hungary		4	
Italy		6	
Israel	3		
Korea			1
Netherlands		2	
Norway		1	
Poland		3	
Romania			1
Slovakia	4	7	1
Spain	1	1	
Switzerland		1	
Taiwan	1		
Turkey			1
USA		3	9
Total	15	57	31

Travels of scientists abroad

Country	AS CR agreements	Grants	Other sources	Conferences
Australia				2
Austria		1		5
Belgium				5
Belorussia				1
Bulgaria				2
Canada				3
China				1
Denmark				3
Finland			1	1
France	1		2	12
Germany			2	22
Great Britain		1	2	19
Greece		1		3
Guinea-Bissau				3
Hungary		2		3
Italy				10
Israel		1		6
Japan			1	4
Libya				1
Netherlands				8
Norway				2
Peru				1
Poland			3	10
Portugal				1
Romania				3
Russia		4		27
Slovakia			8	1
Slovenia				3
Spain		2		9
Sweden				8
Switzerland				7
Thailand			4	
Turkey		3		2
Ukraine				10
USA		2	2	24
Total	1	17	25	222

A. OVERVIEW OF INTERNATIONAL CO-OPERATION OF THE INSTITUTE OF BIOPHYSICS AND FOREIGN GRANTS IN 2000

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

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
M. Štros
Interaction of HMG Proteins with DNA and Chromatin

Max-Planck-Institut für Züchtungsforschung, Köln
B. Brzobohatý
Scientific Research in the Field of Plant Molecular Biology

Collaborative Research and Development Agreement between november AG, Erlangen and IBP AS CR Brno
E. Paleček

ISRAEL

Weizmann Institute of Science, Rehovot
J. Fajkus
Analysis of the Structure of Plant Chromosome Termini

JAPAN

Chiba Cancer Center Research Institute, Division of Biochemistry, Chiba
M. Štros
A study on the Effect of Chromosomal Proteins HMG1/2 on the Binding of Proteins of p53 Family to DNA and on their Role as Transcriptional

2. Foreign Grants

FRANCE

CNRS/AS CR Collaboration, Ecole Normale Supérieure de Lyon
B. Vyskot (2000 – 2002)
Molecular Analysis of Sex Chromosomes and Dioecy in Silene latifolia

GERMANY

Volkswagen Stiftung
E. Paleček (1997 - 2000)
Tumor-Suppressor-Protein p53 und Seine Interaktionen mit DNA

Volkswagen Stiftung
M. Kozubek (FM MU), E. Bártová (IBP) (1999 - 2002)
Automated Micro-axialtomography of Tumour-correlated FISH Pattern

GREAT BRITAIN

The Welcome Trust, 062366/Z/00/Z
V. Brabec (2000 - 2003)
DNA Interactions of Platinum Anticancer Drugs. Relation to the Development of New Cytostatics

Royal Society, RS/PDF/BLL – Department of Plant Sciences, University of Oxford
B. Brzobohatý (1997 - 2001)
The Role of Cytokinin Metabolism in Plant Growth and Development

GREECE

Laboratory of Physical Chemistry, Faculty of Sciences, University of Thessaloniki
V. Vetterl (1996 - 2000), KONTAKT Program, Ministry of Education, Youth and Sports of the CR, ES 022
Interactions of Biopolymers and their Components at Interfaces

Institute of Physical Chemistry "Demokritos", Athens
V. Brabec (2000 - 2001), KONTAKT Program, Ministry of Education, Youth and Sports of the CR
Molecular Mechanism of Anticancer Activity of Ruthenium Complexes

ITALY

Ministero degli Affari Esteri, Progetto Rep. Ceca, 069/P981n
V. Brabec (1997 - 2000)
Nuovi farmaci antitumorali di platino

JAPAN

MONBUSHO No. 11694196, Japanese Ministry of Education
J. Fajkus, B. Vyskot (1999 - 2001)
Joint Research on Differentiation and Growth Specificity of Plant Cells

SLOVAKIA

Institute of Experimental Pharmacology, SAS, Bratislava
A. Lojek (2000 - 2001), KONTAKT Program, Ministry of Education, Youth and Sports of the CR, CR/SR co-operation No 67
Influence of Thrombocytes on Oxidative Flare-up of Neutrophils

USA

Howard Hughes Medical Institute, Ann Arbor, Michigan, HHMI 75195-540201
V. Brabec (1995 - 2000)
DNA Interactions of Platinum-group Metals: Relations to Their Antitumor Activity

National Institutes of Health (NIH), 1R01CA78754-01
V. Brabec (1998 - 2002)
Mechanistic Studies on New Platinum Clinical Agents

USA/CR, grant yielded under the agreement between the NSF and Ministry of Education, Youth and Sports of the CR, ME 152
V. Brabec (1998 - 2000)
DNA Interactions of Polynuclear Platinum

OTHER FUNDING

COST D8, Chemistry of Metals in Medicine, Reg. No D1/0012/92 (OC D8.40)
O. Vrána (1997 - 2001)
Platinum-linked Nucleotides Analogs as Virus Inhibitors

COST D8, Chemistry of Metals in Medicine, Reg. No D8/0017/97 (OC D8.50)
V. Brabec (1997 - 2001)
The Development of Ruthenium Antitumor Compounds

COST D8, Chemistry of Metals in Medicine, Reg. No D8/0009/97 (OC D8.10)
V. Brabec (1997 - 2001)
Metal Recognition of DNA and Drug Design

COST D20/003/00, a project involving 11 laboratories from 8 countries
V. Brabec – co-ordinator (2000 - 2004)
Biochemistry, Structural and Cellular Biology of Non-classical Antitumor Platinum Compounds

Joint Institute of Nuclear Research Dubna, No. 409, Russia
S. Kozubek (1999 - 2002)
The Structure of Cell Nuclei and its Relation to Genetic Changes Induced by Densely Ionising Radiation

3. Foreign Scholarships and Grants

E. Benková – a scholarship supporting a long-term postdoctoral study at the Max-Planck-Institut für Züchtungsforschung, Köln am Rhein, Germany

B. Brzobohatý – a scholarship granted by the Royal Society for a study stay at the Department of Plant Sciences, University of Oxford, Great Britain

J. Bůžek – a scholarship for a three-year study stay at the Haartman Institute, University of Helsinki, Finland

V. Dražan – a scholarship for a one-year study stay at the Universität Regensburg, Germany

J. Hejátko – a scholarship for twelve-month study stay at the Max-Planck-Institut für Züchtungsforschung, Köln am Rhein, Germany

V. Hykelová – a scholarship for a ten-month doctoral study stay at the Ecole Normale Supérieure de Lyon, France

J. Mrázek – continued in his study stay at the Department of Mathematics, Stanford University, USA

K. Říha – continued in his study stay at the Department of Biochemistry and Biophysics, Texas A. and M. University, USA

M. Tomschik – a scholarship for a two-year study stay at the NIH Bethesda, USA

B. STAYS ABROAD

1. Stays within the Framework of Agreements of the Academy of Sciences CR

BELGIUM

A. Kovařík attended a one-month study stay at the Laboratorium voor Genetica, Universiteit Gent. He continued in his studies of the relationship between DNA methylation and gene expression in transgenic organisms.

EGYPT

M. Brázdová and *V. Vetterl* participated at the international congress in Assiut (see article 2).

FINLAND

M. Číž continued in his co-operation with the Department of Biochemistry, University of Turku during his 28-day study stay. Amongst other a joint project focusing on the role of oxidative stress in the progress of multiple sclerosis has been prepared.

V. Vetterl visited the Department of Physics, University of Joensuu and the Institute of Materials Chemistry, Tampere University of Technology. He gained an awareness of study methods of rhodopsin and model compounds that use the surface plasmatic resonance and the measurement of surface tension of films created by the Langmuir-Blodget equipment.

FRANCE

J. Žlůvová attended a study stay at the Ecole Normale Supérieure de Lyon within the joint grant. A histological analysis of *Silene latifolia* deletion mutants which prove impaired development of claspers has been pursued.

ISRAEL

J. Fajkus attended a 14-day study stay at the Department of Structural Biology, Weizmann Institute of Science, Rehovot, where he presented the results of his studies related to the chromatin of telomer-associated sequences, and discussed their relation to theoretical predictions performed based on models that have been created at the Israel laboratory.

A. Lojek visited the School of Pharmacy at the Hebrew University of Jerusalem, where he discussed issues related to studies of the creation of oxygen radicals and the protection of animal cells and tissues from their deleterious impact.

V. Vetterl continued in his studies of DNA interactions and model substances with surfaces using methods of surface spectroscopy and the measurement of interface impedance at the Weizmann Institute of Science, Rehovot.

SLOVAK REPUBLIC

M. Číž, H. Čížová, L. Kubala and *A. Lojek* visited the Institute of Experimental Pharmacology of the SAS in Bratislava, where they organized a workshop and established co-operation in the field of monocytes and thrombocytes interactions.

SPAIN

A. Lojek visited the Instituto de Investigaciones Biomedicas de Barcelona; the stay was focused upon the equipment and methodology of the institute and on preparation of the proposal of a joint research project with the partners.

TAIWAN

M. Štros attended a three-week study stay at the Institute of Marine Biotechnology in Keelung. He started a program of co-operation in studies of UBF HMG1 domain function as the RNA polymerasis I transcription factor.

2. Participation in Conferences and Workshops (Based on Invitations or Grants)

EGYPT

M. Brázdová a *V. Vetterl* participated in the Second International Congress on Basic Sciences and Advanced Technology, Assiut, November 5 - 8, 2000

FRANCE

E. Lukášová participated in the 20th International Congress on Analytic Cytology ISAC, Montpellier, May 20 - 25, 2000

O. Nováková and *J. Zehnulová* participated in EUROBIC 5 (5th European Biological Inorganic Chemistry Conference), Toulouse, July 17 - 20, 2000

GERMANY

E. Bártová participated in the 3rd European Biophysics Congress, Munich, September 8 - 13, 2000

V. Dražan and *E. Paleček* participated in the Symposium on Electrochemistry of DNA, Munich, April 11 - 14, 2000

M. Fojta participated in ESEAC 2000: Electroanalysis Conference, Bonn, June 11 - 15, 2000

E. Bártová, C. Hofr, S. Kozubek and *O. Vrána* participated in the 3rd European Biophysics Congress, Munich, September 8 - 13, 2000

L. Kubala and *K. Souček* participated in the 2nd Dresden Chemiluminescence Days, Dresden, May 10 - 13, 2000

N. Špačková participated in the Conference at the Berlin Humboldt School on Structural Biology, Berlin, November 23 - 25, 2000

GREAT BRITAIN

V. Brabec, C. Hofr and *K. Neplechová* participated in the 34th International Conference on Co-ordination Chemistry, Edinburgh, July 8 - 14, 2000

M. Číž and *H. Čížová* participated in the Summer Meeting of the Society for Free Radical Research - Liverpool 2000, Liverpool, July 19 - 22, 2000

GREECE

V. Brabec, J. Kašpárková and *H. Kostrhunová* participated in the 2nd International Conference on Chemical Sciences for Sustainable Development, Halkidiki, June 6 - 11, 2000

HUNGARY

M. Číž, H. Čížová, L. Kubala and *A. Lojek* participated in the 2nd International Workshop on Oxidative Stress in Ischemia/Reperfusion Injury, Sümeg, September 29 - October 10, 2000

ITALY

M. Fojta and *M. Fojtová* participated in the International Conference on Basic and Clinical Aspects of Cell Cycle Control, Siena, May 29 - 31, 2000

KOREA

B. Vyskot participated in the Symposium on Plant Biotechnology for the New Millennium, Chongju, November 3 - 4, 2000

POLAND

J. Malina and *O. Vrána* participated in the Symposium on Metals in Environmental Medicine, Wroclaw, October 18 - 22, 2000

E. Paleček participated in the 51st ISE Meeting, Warsaw, September 6 - 8, 2000

ROMANIA

K. Neplechová participated in the Conference on New Biophysical Methods in Biology and Medicine, Neptun, September 26 - 30, 2000

SPAIN

L. Weiterová participated in the International Symposium Purines 2000: Biochemical, Pharmacological and Clinical Perspectives, Madrid, July 9 - 13, 2000

SWITZERLAND

J. Vondráček participated in the 8th Euroconference on Apoptosis, Davos, October 13 - 19, 2000

USA

V. Brabec participated in the International Conference of Holders of Grants awarded by the Howard Hughes Medical Institute, Chavy Chase, Maryland, June 6, 2000

J. Fajkus participated in the World Health Forum 2000, Dallas, February 20 - 25, 2000

J. Paleček participated in the International p53 Workshop, Monterey, April 4 - 8, 2000

3. Invited Study Stays or Study Stays within Joint Projects, Expert Activities

AUSTRIA

M. Lengerová studied a method of fluorescence *in situ* hybridization using a probe prepared from artificial bacterial chromosomes at the Institute of Botany and Botanical Gardens, Vienna University (October 23 - 26, 2000)

B. Vyskot was invited by the same institute to the workshop to give a synoptic lecture on "Epigenetic control of gene expression in plants" (October 9 - 10, 2000)

BELGIUM

V. Brabec participated in the meeting of the EU COST D20 – Chemistry action (November 23 - 26, 2000), where he defended a new multilateral project and was appointed as its co-ordinator

S. Kozubek participated in two evaluation stages for projects of the 5th EU Framework Program (January 10 - 14 and January 24 - 27, 2000), and in evaluation for projects of the "Quality of Life – 7.1 Cancer Research" EU Program (November 13 - 17, 2000)

FRANCE

V. Vetterl participated as opponent in the defence of a thesis at the Joseph Fourier University in Grenoble (October 8 - 14, 2000)

GERMANY

E. Bártová studied a method of fluorescence *in situ* hybridization on fibre glass and its application in axial tomography at the Institute of Applied Physics, Heidelberg (March 28 - April 2, 2000)

M. Brázdová studied the interaction of the p53 protein and DNA in the Laboratory of Molecular Biology at the Max-Planck-Institut für biophysikalische Chemie, Göttingen (July 25 - August 19, 2000)

B. Brzobohatý participated in the preparation of materials for the final report of the INCO-COPERNICUS PL966135 project at the Max-Planck-Institut für Züchtungsforschung/Max-Delbrück-Laboratorium, Köln am Rhein (May 2 - 13, 2000)

E. Paleček visited the Max-Planck-Institut für biophysikalische Chemie, Göttingen (March 27 - April 16, 2000)

J. Paleček studied the interaction of the p53 protein and DNA particularly under the electron microscope in the Laboratory of Molecular Biology at the Max-Planck-Institut für biophysikalische Chemie, Göttingen (July 27 - August 11, 2000)

ITALY

V. Brabec and *J. Kašpárková* attended two study stays at the Department of Pharmaceutical Chemistry, University of Bari, covered by a joint grant (May 1 - 9 and September 21 - 28, 2000). They visited the Dipartimento di Chimica Inorganica Analitica e Chimica Fisica, University

of Mesina, where they started a program of co-operation on the new EU COST D20 project and gave several lectures for postgraduate students (September 28 - October 5, 2000).

NETHERLANDS

F. Jelen and *V. Vetterl* visited the Laboratory of Electrochemistry, University of Utrecht (June 11 - 18, 2000). They discussed results of measurement of electrode double layer impedance in nucleic acid solutions.

NORWAY

V. Brabec attended a study stay at the Department of Chemistry, University of Bergen, which is also participating in the COST D8 project (February 24 - 27, 2000)

SLOVAK REPUBLIC

J. Fajkus participated as opponent in the defence of habilitation at the University of Komenský in Bratislava (September 28, 2000).

TURKEY

F. Jelen attended a study stay at the Department of Analytical Chemistry, Faculty of Pharmacy, University of Izmir. He studied the interactions of nucleic acids with various substances that create stable adducts with nucleic acids (April 18 - May 18, 2000).

USA

E. Kejnovský worked under a joint grant at the Department of Biology, University of North Carolina, Chapel Hill; he has composed a partial *Silene latifolia* library, which will be used for studies of the evolution of sex chromosomes of the *Silene* species (April 5 - July 6, 2000).

E. Paleček visited several places during his study stay in the USA. He tested a new approach in the DNA and protein analysis at the Department of Chemistry and Biochemistry, NMSU, Las Cruces (four weeks). He lectured on the results achieved in the IBP laboratory at these further places: NIH-NCI Bethesda, Department of Microbiology Arizona State University, Phoenix, School of Chemistry and Biochemistry, Georgia Tech., Atlanta (October 25 - December 4, 2000).

N. Špačková visited the laboratory of the Biomolecular Science Centre at the Bowling State University, Ohio (November 9 - 19, 2000).

B. Vyskot visited the Department of Biology, University of North Carolina, Chapel Hill under a joint grant and became familiar with the construction of genome Silene libraries. Thereafter he studied plant developmental defects at the Department of Biochemistry, Texas A. and M. University (May 31 - June 19, 2000).

All the study stays completed contributed significantly to the carrying out of projects, and their results are mostly given in chapter II of this report.

C. CO-OPERATION WITH INTERNATIONAL GOVERNMENTAL AND NON-GOVERNMENTAL ORGANIZATIONS

S. Kozubek worked as the chairman of the Czech Committee for Biophysics (IUPAB); *V. Brabec, E. Paleček, J. Šlotová* and *V. Vetterl* worked as members of this Committee.

B. Brzobohatý is a member of the Czech Committee for Molecular Biology and Biochemistry.

J. Šlotová is a representative of the CR in the ICSU. She participated in the workshop of national ICSU representatives on the continuing development of life, environmental protection and positive utilization of biotechnology, Vienna, Austria (June 16, 2000).

V. Brabec is a representative of the CR in the Managing Board of the European Program of Scientific and Technological Research, COST D8, and a member of the Evaluation Commission of the 5th EU Framework Program in Brussels, Belgium.

J. Fajkus was nominated as an external expert for project evaluation for the 5th EU Framework Program in Brussels, Belgium.

S. Kozubek is a member of the Programs Advisory Committee, Joint Institute for Nuclear Research, Dubna, Russia. In addition to this, he worked as a member of the European Evaluation Commission for projects of the 5th EU Framework Program and the "Quality of Life" Program in Brussels, Belgium.

M. Pospíšil is a member of the International Astronautical Academy (IAA).

D. INTERNATIONAL CONFERENCES ORGANIZED BY THE INSTITUTE OF BIOPHYSICS

International Conference on DNA Conformation, Modification and Recognition in Biomedicine, Brno, July 2 - 5, 2000

MENDEL-BRNO 2000: Conference on DNA Structure and Interactions. Their Biological Roles and Implications in Biomedicine and Biotechnology (on the occasion of the Centenary Anniversary of the Rediscovery of G. Mendel's Seminal Work), Brno, July 19 - 23, 2000

Higher-Order Structure of Cell Nuclei and Genetic Effects of Radiation, Valtice, November 7 - 8, 2000

V. DOCTORAL STUDIES, TEACHING AND OTHER ACTIVITIES

A. POSTGRADUATE STUDIES

The further education of students took place on the basis of internal or external research studies and on postgraduate studies.

(a) Research studies

The following theses were defended before the Committee for Defending Candidate Theses in the field of biophysics:

M. Fojtová (IBP) / Genetic and Epigenetic Changes of Plant Genomes

L. Fajkusová (VÚZD) / Molecular Diagnostics of Duchenn and Becker Muscular Dystrophy

L. Doležal (Faculty of Medicine, Palacký University, Olomouc) / Contribution to the Optimization of Biophysical and Technical Quality of Ultrasonic Display in Medicine

Number of research students to December 31, 2000:

internal research students: 3
external research students: 2

Six scientists functioned as advisors to research students; other two scientists functioned as specialist advisors.

(b) Postgraduate studies (PGS)

In 2000, the Institute of Biophysics successfully continued to participate in postgraduate education (doctoral studies) at universities, mainly at the Faculty of Science of Masaryk University in Brno. In total, fifty seven students worked towards a doctor's degree at the IBP. Fifteen of them were external postgraduate students and forty-two of them were internal or combined students.

Total number of students	External	Internal/ /combined	Year
21	1	20	I.
11	3	8	II.
5	2	3	III.
7	3	4	IV.
4	2	2	V.
9	4	5	graduates (accomplished studies)

PGS students belong to fields of specialization as follows:

biophysics (12), 3 students accomplished their Doctor's Theses
molecular biology (21), 4 students accomplished their Doctor's Theses
genetics (5)
animal physiology (9), 1 student accomplished her Doctor's Thesis
immunology (3)
environmental chemistry (1)
biochemistry (1), 1 student accomplished his Doctor's Thesis
botany (2)
plant physiology (1)
microbiology (1)
medical biophysics (1)

17 scientists of the IBP were appointed as PGS student advisors.

Doctoral Theses – undertaken at the IBP and defended in 2000:

V. Brázda / Interactions of p53 Protein and Superhelical DNA. Influence of p53 C-end Domain Deletion on the DNA Relation

J. Fulnečková / Nucleoprotein Structure of Plant Telomers

C. Hofr / Biophysical Analysis of the Interactions of DNA and Antitumor Platinum and Ruthenium Complexes

M. Horáková / Structure and Dynamics of Plant Telomers and Subtelomers

M. Kovaříková / The Role of Fatty Acids in the Regulation of Cytokinetics of Tumor Colon Cancer Cells and their Interactions with TNF-α

L. Krejčí / Homologous Recombination and DNA Repair: Beginning at the Break, the Break at the Beginning

J. Malina / Analysis of Interactions with Antitumor Active Ruthenium and Platinum complexes

S. Neugebauerová / Conformation Characteristics of DNA Resulting from Analysis of Interatomic Distances among Adjacent Bases in Oligonucleotide Crystal Structures

The following scientists of the IBP are members of PGS Branch Councils at the Faculty of Science of Masaryk University in Brno:

Branch Council for Biophysics:
M. Bezděk, V. Brabec, E. Paleček, J. Šlotová, V. Vetterl, M. Vorlíčková, F. Jelen

Branch Council for Molecular and Cell Biology:
J. Fajkus, B. Koukalová, J. Kypr, E. Paleček, V. Vetterl

Branch Council for Physiology and Developmental Biology of Animals:
J. Hofmanová, A. Kozubík

Branch Council for Immunology:
M. Číž, J. Hofmanová, A. Kozubík, A. Lojek

Branch Council for Genetics:
M. Bezděk, E. Paleček, B. Vyskot, B. Brzobohatý

Branch Council for Environmental Chemistry and Ecotoxicology:
A. Kozubík

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

Faculty of Medicine, Masaryk University in Brno
BC for Biophysics: *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*

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*

J. E. Purkyně Military Medical Academy in Hradec Králové
BC for Medical Theoretical Specialization and Pharmacy: *M. Hofer*

BC for Biophysics is in charge also at the Palacký University in Olomouc.

B. CO-OPERATION WITH UNIVERSITIES

Teaching Activities and Membership in University Councils

Masaryk University in Brno:

S. Kozubek is a member of the Scientific Council

Faculty of Science, Masaryk University in Brno:

J. Hofmanová a *A. Kozubík* completed their habilitation on March 8, 2000 and were nominated as assistant professors at the Department of Physiology and Developmental Biology of Animals.

B. Vyskot completed his professor degree procedure in the field of Molecular Biology and Genetics with a lecture to the MU Scientific Council on October 17, 2000.

Long-term co-operation with the Faculty of Science continued. Sixteen scientists of the Institute (including two professors and five assistant professors) carried out lectures and practical exercises. Sixteen scientists were appointed as advisors for thirty students working on their Diploma Theses.

The following scientists were members of examination committees for state final examinations:

E. Paleček – the field of Molecular Biology

J. Hofmanová and *A. Kozubík* – the field of Physiology and Developmental Biology of Animals; they were members of examining committees for first doctoral examinations and doctorate degrees in the field of Physiology and Developmental Biology of Animals.

V. Vetterl was a member of the examination committee for admission and first doctoral examinations in the field of Biophysics.

V. Brabec oversees studies for the master's degree in the field of Biophysics at the Faculty of Science at Masaryk University in Brno.

J. Fajkus is a member of the Competition Committee of the Biology Section of the Faculty of Science MU, and a member of the Biology Section Council of the Faculty of Science.

J. Šlotová is a member of the Scientific Council at the Faculty of Science.

Faculty of Medicine, Masaryk University, Brno:

V. Vetterl is a member of the Scientific Council.

<u>Co-operation with other universities:</u>

Palacký University, Olomouc:
V. Vetterl is a member of the Scientific Council.

Faculty of Science, Palacký University, Olomouc:

J. Šlotová is a member of the Scientific Council and together with *V. Vetterl* is a member of the examination committee for state final examinations in the field of Biophysics.

Faculty of Medicine, Palacký University, Olomouc:

V. Vetterl gave lectures and led practical exercises in medical biophysics for foreign students.

Charles University, Prague:

Faculty of Science, Charles University, Prague:

B. Vyskot is a member of a committee for state doctoral examinations in the Biology study program; specialization in Molecular Biology of Plants.

OVERVIEW OF LECTURE CYCLES AND PRACTICAL EXERCISES GIVEN BY SCIENTISTS OF THE INSTITUTE OF BIOPHYSICS

A. Faculty of Science, Masaryk University Brno **Spring term 1999/2000**

Field	*Course*	*Lecturer*
Biophysics		
	Introduction into biophysics II.	V. Vetterl
	Molecular biophysics	V. Vetterl, V. Brabec
	Experimental methods in biophysics	V. Vetterl, J. Kypr, K. Nejedlý
	Biophysical properties and computer analysis of nucleic acids, proteins, genes and genomes	J. Kypr
	Bioelectrochemistry I	F. Jelen, V. Vetterl
Molecular Biology and Genetics		
	Developmental genetics	B. Vyskot
	Biophysical properties and computer analysis of nucleic acids, proteins, genes and genomes	J. Kypr
	Mutagenesis - biological impacts of ionizing radiation	A. Kozubík
	Basis of proteomics	B. Brzobohatý, J. Zouhar
	Structure and functions of eucaryotic chromosomes	J. Fajkus, L. Krejčí
Plant Physiology		
	Developmental genetics	B. Vyskot
Animal Physiology, Ecotoxitology		
	Advanced methods in biological research	A. Kozubík, J. Hofmanová
	Genotoxicity	J. Hofmanová, A. Kozubík
	Health risks	A. Kozubík, J. Hofmanová

Autumn term 1999/2000

Field	*Course*	*Lecturer*
Biophysics		
	Introduction into biophysics I.	V. Vetterl, V. Kleinwächter
	Molecular biophysics	V. Brabec, V. Vetterl, V. Kleinwächter
	Experimental methods in biophysics	V. Vetterl, M. Vorlíčková
	Bioelectrochemistry II.	F. Jelen, V. Vetterl
	Radiation biophysics	S. Kozubek
Molecular Biology and Genetics		
	Chemistry of nucleic acids	E. Paleček, M. Fojta
	Molecular biology and yeast genetics	J. Paleček
	Protein engineering	B. Brzobohatý
	Basis of genomics	B. Brzobohatý
	Molecular aspects of evolution	M. Bezděk
Animal Physiology, Ecotoxicology		
	Physiology of cell systems	A. Kozubík, J. Hofmanová, A. Lojek
	Special immunological methods	M. Číž

B. Faculty of Education (Pae F) MU Brno **Spring term 1999/2000**

Biophysics	S. Kozubek

C. Faculty of Medicine UP Olomouc

Two-term course for foreign students

Medical biophysics	V. Vetterl

D. VUT (Technical University) Brno – Faculty of Chemistry

Two-term course

Chemistry and technology of environmental protection	O. Vrána

Co-operation in research

The institute participated in fifteen projects supported by various grant agencies and carried out in co-operation with universities in 2000. Ten of these grants had scientists of the IBP as principal investigator, five of them as co-partial investigator.

The IBP continued in co-operation with the Department of Physical Electronics of the Faculty of Science, MU Brno in the Joint Laboratory of Biophysics.

The final presentation and defence of results took place successfully for three projects under the Ministry of Education, Youth and Sport CR Program, "Support of Research at Universities" in December 2000:

Project VS96096, *B. Brzobohatý* – Laboratory of Molecular Physiology

Project VS97032, *J. Fajkus* – Laboratory of Analysis of Significant Molecular Complexes

Project VS97031, *S. Kozubek* – Laboratory of High Resolution Cytometry

All these projects will continue as research activities at Masaryk University. A joint laboratory of the Faculty of Science MU Brno and IBP, known as the Laboratory of Functional Genomics and Proteomics, was established based on the Laboratory of Molecular Physiology and on the Laboratory of Analysis of Significant Molecular Complexes at the Faculty of Science MU. *J. Fajkus* was appointed as head of this new joint laboratory. The Laboratory of High Resolution Cytometry is the principal investigator at the Faculty of Informatics.

Within the RECETOX (*Regional Research Centre for Atmospheric Chemistry and Effects of Atmospheric Pollutants*) program, co-operation with the Department of Environmental Protection (Faculty of Science, MU, Brno) has been further enhanced. *A. Kozubík* was a member of the Scientific Council of this program at the Faculty of Science, MU Brno.

IBP representatives *J. Hofmanová* and *A. Kozubík* continued in co-operation with the University Oncological Centre (UOC) in Brno. *A. Kozubík* is a member of the UOC co-ordination committee – he is in charge of co-ordinating experimental groups of the Centre of Experimental Oncology (CEO).

C. APPLIED RESEARCH

E. Paleček has been a member of the Scientific Council of the German biotechnological company *november AG*, Erlangen since 1999. In 2000 this company concluded an agreement with the IBP that included financial support of basic research by the Laboratory of Biophysical Chemistry and Molecular Oncology in the field of the electrochemistry of nucleic acids and biosensors on the basis of electrodes modified by DNA. Another laboratory, Laboratory of Physics of Biomacromolecules, is participatings in this co-operative project as well.

The Laboratory of Physics of Biomacromolecules laboratory continued in co-operation with the Institute of Medical Biology at the Faculty of Medicine, MU Brno (J. Šmarda) in studies of the influence of low-frequency electromagnetic fields on living organisms. They determined that the *Escherichia coli K12 Row* bacteria, in terms of quality, behave in the same way in the homogenous electromagnetic field as in the non-homogenous electromagnetic field, with the same frequency and maximum capacity of magnetic induction. Tracking the dynamics of their growth showed that cells divide in the electromagnetic field, but reproduce much more slowly in comparison with cells in the control culture.

Laboratory of Analysis of Chromosomal Proteins Continued in its co-operation with the B. Vojtěšek Laboratory, Masaryk Oncological Institute, Brno in the field of HMG1/2 proteins influence on interactions of some proteins of the p53 family with DNA.

J. Fajkus, in co-operation with Res. Inst. Child Health and Faculty Hospital Brno, performed the complete dystrophin mRNA sequence analysis in 20 Duchenne muscular dystrophy and Becker muscular dystrophy patients. In 13 cases, deletions in mRNA were detected using reverse transcription-polymerase chain reaction and in another seven cases, point mutations were found using the protein truncation test. Sixteen patients diagnosed with Duchenne muscular dystrophy showed the presence of deletions or of nonsense point mutations. From four patients with the Becker muscular dystrophy phenotype, three cases were associated with deletions conserving the translational frame and one was associated with a nonsense mutation E1110X. In the case of the E1110X mutation, an alternative splicing of dystrophin mRNA (3485-3640del) was detected in this patient which included the E1110X mutation site (nucleotide 3536) and did not change the translation reading frame. Individual nonsense point mutations were characterized by sequence analysis, which showed five novel mutations with respect to those reported in the Cardiff Human Gene Mutation Database http://uwcm.web.cf.ac.uk/uwcm/mg/hgmd0.html and the Leiden muscular dystrophy pages http://www.dmd.nl/.

A. Kovařík co-operated on the "Detection of mycotic infections in clinical material" project at the II. Internal Clinic of the Faculty Hospital in Brno.

A. Lojek and *M. Číž* continued in their co-operation with the Centre of Cardio-vascular and Transplant Surgery in Brno to complete the project for the reduction of the toxicity of reactive oxygen metabolites of patients with myocardial ischemic disease and patients with transplant organs. *A. Lojek* and *M. Číž* also co-operated with the Pilsen Prazdroj a.s. brewery.

A. Kozubík and *J. Hofmanová* started a program of co-operation with the Infusia Hořátev, a.s. company. They became members of expert teams in the field of development and innovation of grease emulsions.

D. MEMBERSHIP IN SCIENTIFIC INSTITUTIONS

M. Bezděk is a member of the Czech Committee for Transgenic Plants. Since 1995 he has been a member of the Council of the *Support of Research at Universities* Program at the Ministry of Education, Youth and Sports CR.

V. Brabec is an elected member of the General Assembly of the AS CR for the period 1998 – 2002 and he was elected a member of the Supervisory Committee of the AS CR General Assembly in 1999. He is a member of the Sub-branch Committee 301 "Molecular Biology, Genetics and Experimental Oncology" of the Grant Agency CR.

M. Fojta is a member of the Sub-branch Committee 204 "Molecular and Cellular Biology" of the Grant Agency CR.

M. Hofer is a member of the Branch Council for Theoretical Medical Fields and Pharmacy at the J. E. Purkyně Military Medical Academy in Hradec Králové.

F. Jelen is a member of the Branch Council 4 "Chemical Sciences" of the Grant Agency AS CR.

J. Jursa is a member of the South Moravian Regional Committee for Computer Technology.

S. Kozubek was elected a member of the General Assembly of the AS CR for the period 1998 – 2002 and a member of the Program Advisory Committee, Joint Institute of Nuclear Research Dubna, Dubna, Russia.

A. Kozubík was chairman of the Branch Council 5 "Agricultural Sciences" and a member of the Sub-branch Committee 524 "Physiology and Pathology of Animals" of the Grant Agency CR. He is also a member of the Scientific Council of the Masaryk Oncological Institute, Brno.

J. Kypr is a member of the Scientific Council of the AS CR, member of the Branch Committee 3 "Medical Sciences" and 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 Sub-branch Committee 524 "Physiology and Pathology of Animals" of the Grant Agency CR.

E. Lukášová is a member of the Sub-branch Committee 202 "Physics" of the Grant Agency CR.

E. Paleček is a member of the Branch Council 5 "Molecular and Cell Biology" of the Grant Agency AS CR, 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 intentions and results of institutions for granting institutional support to research and development in science.

J. Široký is a member of the Branch Council 5 “Agricultural Science“ and of the Sub-branch Committee 521 “Plant Production, Genetics and Breeding“ of the Grant Agency CR.

J. Šlotová is a member of the Council for International Co-operation and a member of the General Assembly of the AS CR.

M. Vorlíčková is a member of the Branch Council 1 “Mathematical and Physical Sciences, Informatics” of the Grant Agency AS CR.

V. Vetterl was nominated by the Board of the Fund of University Development to be a member of the F3 item "Innovation of Biomedicine Programs ".

O. Vrána is a member of the Branch Council 5 "Molecular and Cellular Biology " of the Grant Agency AS CR.

B. Vyskot is a member of the Accreditation Committee of the Government of the CR for universities and chairman of its working group for biology and ecology.

Scientists of the Institute of Biophysics of the AS CR are members of boards for doctor’s degrees in biophysics, biochemistry and immunology (*E. Paleček)* and candidate doctor degrees in biophysics (*E. Paleček* - chairman, *M. Bezděk*, *A. Vacek* - members).

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

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

E. Paleček - *General Physiology and Biophysics* and *Bioelectrochemistry and Bioenergetics*

V. Vetterl - *Český časopis pro fysiku* (*Czech Journal for Physics*).

E. MEMBERSHIP IN SCIENTIFIC SOCIETIES

International Scientific Organizations and Societies

E. Benková – member of the Federation of European Societies of Plant Physiology

V. Brabec – member of the International Society of Electrochemistry

V. Brázda – member of the Biochemical Society

B. Brzobohatý – member of the Federation of European Societies of Plant Physiology and of the Society for Experimental Biology

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 and of the British Royal Society for Ageing

J. Fulneček – member of the DNA Methylation Society

M. Hofer – member of the Council of the 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

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

K. Krejčí – member of the Danish Cancer Society and of the Danish Centre for Gerontology

L. Krejčí – member of the Danish Society for Biochemistry and Molecular Biology

A. Lojek – member of the Society for Free Radical Research

E. Paleček – member of the Bioelectrochemical Society and of the New York Academy of Sciences

M. Pospíšil – member of the International Astronautical Academy and of the European Society for Radiation Biology

J. Šlotová – representative of the Czech Republic in the ICSU

M. Štros – member of the New York Academy of Sciences and of the American Society for Biochemistry and Molecular Biology

A. Vacek – member of the International Astronautical Academy

V. Vetterl – member of the Bioelectrochemical Society

M. Vorlíčková – member of the Biophysical Society USA, Bethesda, Maryland

National Organizations and Committees

M. Bezděk – member of the Czech Society for Biochemistry and Molecular Biology, board member of the Biophysical Section of the Czechoslovak Biological Society and board member of the Mendel Genetic Society

V. Brabec – member of the Czech Committee for Biophysics (IUPAB)

B. Brzobohatý – member of the Czech Committee for Biochemistry and Molecular Biology and member of the Czech Society for Biochemistry and Molecular 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

M. Fojtová – 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 Society for Tissue Cultivation at the Czech Oncological society and member of the Czech Radiobiological Society at the Czech JEP Medical Society

B. Koukalová – member of the Mendel Genetic Society and member of the Czech Biological Society

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

S. Kozubek – board member 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 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 and member of the Czech Radiobiological Society at the Czech JEP Medical Society

A. Lojek – member of the Czech Immunological 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 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 – scientific secretary of the Biophysical Section of the Czechoslovak Biological Society

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