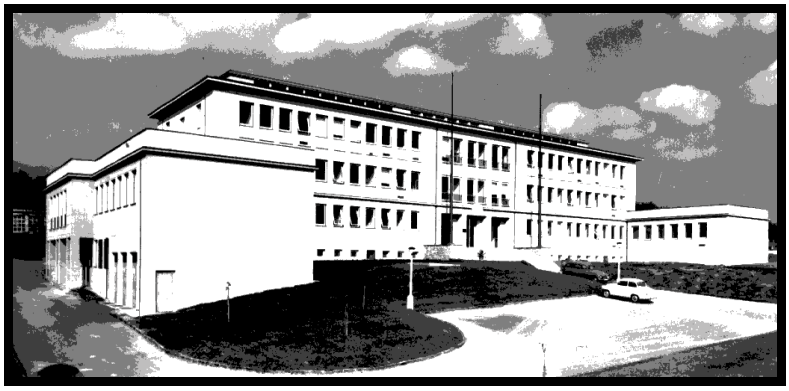
ACADEMY OF SCIENCES OF THE CZECH REPUBLIC

INSTITUTE OF BIOPHYSICS



RESEARCH REPORT 1999

BRNO 2000

CONTENTS

Authorized by the Director of the Institute of Biophysics on 4 February 2000

I. INTRODUCTION

The most important organizational and scientific activity of the Institute in 1999 was the establishment of the Methodological and Educational Center of Molecular Biophysics. In this respect a wide internal discussion was going on.

Its goal was to select progressive and advanced topics, which are being solved at the Institute, to establish new links between research teams and intensify their cooperation. Another significant accomplishment was the establishment of the national ICCBnet node (at the IBP) as a constitutive part of the International Center for Cooperation in Bioinformatics network.

In compliance with the #111/1998 Coll. of Laws concerning universities and the General Cooperation Agreement between the Academy of Sciences, CR and the Masaryk University, Brno, the Institute of Biophysics signed up the Doctoral Study Cooperation Agreement with the Faculty of Science, MU Brno. The Institute of Biophysics and the Faculty of Science presented to the Accreditation Board of the Ministry of Education, Youth and Sports of the Czech Republic a joint application for an extension of Doctoral Study Program Accreditation in Biology, including Molecular and Cell Biology, Genetics, Physiology and Evolutionary Biology of Animals, Immunology as well as accreditation in Physics Doctoral Study involving Biophysics; these studies are realized at the Faculty of Science of the Masaryk University, Brno. Based upon the consent of the Accreditation Board, the Ministry of Education granted to the Institute of Biophysics of the Academy of Sciences the Accreditation to participate in the Doctoral Study Programs specified above.

New Scientific Council was elected in January 1999. Its members are: V. Brabec, J. Fajkus, M. Hofer, J. Hofmanová, S. Kozubek and E. Paleček – internal members, J. Doškař (Faculty of Sci., MU), Z. Šimek (University of Technology Brno), J. Totušek (Faculty of Medicine, MU) – external members. V. Brabec was elected a chairman of the Scientific Council.

Next we present the researchers of our Institute whose achievements were appreciated by various institutions:

J. Kypr has been ranked among 2000 significant researchers of the 20th century for his contribution to the study of nucleic acids, proteins, genes and genomes by the International Biographical Centre (Cambridge, GB).

V. Vetterl was awarded by the Silver Medal of Palacký University, Olomouc.

The team of the Laboratory of Analysis of Biologically Important Molecular Complexes (J. Fajkus, J. Fulnečková, M. Horáková, K. Poláčková and K. Říha) was awarded by the *"Talent 98"* Certificate of Merit of the Ministry of Education, Youth and Sports of the Czech Republic.

K. Neplechová was granted by the award of the Institute of Biophysics AS CR for outstanding scientific results for her paper titled *DNA Interactions of New*

Antitumor Aminophosphine Platinum(II) Complexes published in Molecular Pharmacology.

The Institute of Biophysics participated in organization of the *"Days of Science"* as it did in 1998. The Open Door Day was arranged for on 22nd October and 151 visitors, mainly students of grammar schools, some colleges and universities came to see laboratories.

The laboratory equipment was improved by the acquisition of the ultra-sensitive ITC calorimeter and the URZ ^{60}Co new cobalt source of the Chisostat irradiator.

Abbreviations

AS - Academy of Sciences of the Czech Republic, CR - Czech Republic, IBP - Institute of Biophysics, MU - Masaryk University, GA AS CR - Grant Agency of the Academy of Sciences of the Czech Republic, GA CR - Grant Agency of the Czech Republic, IGA - Internal Grant Agency, MH - Ministry of Health, ME - Ministry of Education, Youth and Sports, MEA - Ministry of Environmental Affairs, JINR - Joint Institute of Nuclear Research, MI - Ministry of Industry

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31 December 1999

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Mgr. Jan Zouhar*
Mgr. Jitka Žlůvová

*internal students

II. SCIENTIFIC ACTIVITIES

In 1999 the research programme of the Institute of Biophysics included 71 projects in total.

Scientific projects supported by the GA of the AS CR:

- 10 individual projects; 3 supplementary projects;
- 3 projects of the Advanced Research Developmental Programme;
- 3 projects of the Supporting Program of Instrumental Equipment in Progressive Research Areas;

Scientific projects supported by the GA CR:

- 16 individual projects, (the Institute was the recipient of 14 grants and the co-recipient of 2 grants);
- 3 complex projects, (the Institute was the recipient of 1 grant and the co-recipient of 2 grants);
- 10 postdoctoral projects;

12 projects were supported by grant agencies of Ministries and other organizations;

11 projects were supported by foreign grant agencies.

This chapter presents an overview of projects and results of which the Institute was the grant recipient and the most significant results of other projects. The projects are grouped into five research programmes.

(1) Electrochemical methods of studies in nucleic acids and proteins

(2) Structure, function and evolution of genomes

(3) Changes in the genomes' structure induced by physical and chemical factors

(4) Relationship between the structure and function of proteins as studied by methods of protein engineering

(5) Control mechanisms of proliferation, differentiation and apoptosis in cell populations

PROGRAMME 1

Electrochemical methods in studies of nucleic acids and proteins

Project 7109 / GA AS CR A4004702

Adsorption of bases, nucleosides, nucleotides and polynucleotides on electrodes

Principal Investigator Vladimír Vetterl

Co-Investigators: Viktor Dražan, Libor Hanák, Naďa Špačková, Jiří Šponer, Luděk Strašák

Project 0808 / GA ME CR

Modernization of practical exercises in experimental method of biophysics

Principal Investigator Vladimír Vetterl

Co-Investigators: Vratislav Kapička, Viktor Dražan, Stanislav Hasoň

Adsorption of cytidine at Au (111) and Hg electrode

Adsorption of cytidine at the surface of a static mercury drop electrode, graphite electrode covered by a mercury film and a single crystal Au (111) electrode was studied by measurement of impedance of electrode double layer, cyclic voltammetry and current – time curves. The dependence of the cytidine adsorption on pH, temperature and ionic environment and the kinetic of cytidine surface layer formation was studied. We have found that the two-dimensional (2-D) condensation of cytidine at the Hg electrode surface can take place in a wide range of pH values similarly as it was observed with cytosine earlier. The tendency to 2-D condensation depends on the ionic environment. The condensed layer of cytosine which is formed at alkalic pH value is more compact and/or less polar (it has a lower value of the differential capacity) than the layer formed at acidic pH value (it has a higher value of the differential capacity). In a more concentrated cytidine solutions another region of 2-D condensation appears on the positively charged Hg surface (around -0.1 V) which might correspond to the condensation of cytidine molecules oriented with their negatively charged riboses towards the mercury electrode surface. 2-D condensation of cytidine was observed at the graphite electrode covered with a mercury film as well. The nucleation process was about ten times faster than at the mercury drop electrode. With Au(111) single crystal electrode an anomalous dependence of 2-D condensation on temperature was observed – the tendency to condensation was increased at higher temperatures.

Adsorption of thymine at Au(111) electrode

The measurement of cyclic voltammograms (CV) of thymine using Au(111) electrode at different scan rates and temperatures has shown that 2-D condensation of adsorbed thymine molecules takes place similarly as it was observed at the Hg surface. The first scan of CV after immersion of the Au electrode into the thymine solution differs from the subsequent CV scans. The results show that at the switching potentials a reconstruction of the electrode surface takes place - a diminution of the atomic flat domains and the arrangements of Au atoms inside the domains. These changes can be detected by scanning tunneling microscopy.

Adsorption of coumarin at Au (111) and Hg electrode

The dependence of CV of coumarin on its concentration, scan number and temperature using Au(111) electrode has shown that two different domains exist on the electrode surface arising from the heat and electrochemical reconstruction of the surface. The adsorption of coumarin at the Hg surface was followed by electrochemical impedance spectroscopy. Coumarin was adsorbed in a broad potential range from +0.1 V up to – 1.85 V. There were three different adsorption regions and reduction at –1.5 V. At higher coumarin concentrations the 2-D condensation of adsorbed molecules took place. Impedance of the electrode double layer depended on frequency in the region of tensammetric peaks. In the potential region of adsorption the frequency dependence of the impedance in the complex plain was a straight line which represented the series combination of ohmic resistance and capacity. In the potential region of reduction there appeared two peaks on the potential dependence of impedance and two semicircles on the frequency dependence of impedance in the complex plane. The reduction of coumarin thus apparently proceeded in two steps.

Structure and dynamics of unusual DNA structures

We continued studies on the structure and dynamics of unusual DNA forms. Their existence is expected in telomeres of eukaryotic chromosomes (four-stranded i-DNA and G-DNA) or in bacteriophage and parvovirus genomes (double-stranded DNA with a zipper motif). Biological functions of these structures are intensively investigated; some of them could be potentially used in medicine as inhibitors of HIV integrase.

The project was aimed at using computational methods of molecular dynamics (program package AMBER 5.0) to analyze some effects not solved experimentally, e.g. the influence of a nucleotide sequence and the presence of modified nucleic acid bases on the structure, dynamics and stability of the molecule. Attention was paid also to the influence of hydration and ion interactions, important for the behavior of the whole system.

All simulations were done using a robust Particle Mesh Ewald method (PME) for a correct treatment of long-range electrostatic interactions. PME is a powerful method to study the molecular dynamics with explicitly represented solvent and is very stable in the nanosecond scale.

An extensive study of structure and dynamics of parallel and antiparallel guanine quadruplexes was finished. We could demonstrate that the structure was stable only if monovalent cations were presented in the ion channel, while the structure without cations in the channel was disrupted during several nanoseconds. The simulations also demonstrated a role of flexible thymine loops on stability of the system or on the ion transport from/to ion channel.

Structure and stability of adenine zipper motifs were studied. These structures were stable on the nanosecond scale. Specific hydration, interaction with ions and deformations of sugar/phosphate backbone were analyzed in detail. These results are now prepared for publication.

Project 9123 / GA AS CR A4004901

Analysis of the interactions of mutagens, carcinogens and anticancer drugs with biopolymers by means of electrochemical and biochemical methods

Principal Investigator František Jelen

Co-Investigators: Emil Paleček, Miroslav Fojta, Miroslav Tomschik

Technician: Irena Postbieglová

Project 9302 / GA CR 301/99/0692

Structural aspects of interactions of checkpoint proteins with DNA in cancer

Principal Investigator Emil Paleček

Co-Investigators: Petr Pečinka, Jiří Bůžek, Václav Brázda, Jan Paleček

Project 8102 / GA AS CR A5004803

Interactions of supercoiled DNA with tumor-suppressor protein p53

Principal Investigator Emil Paleček

Co-Investigators: Petr Pečinka, Hana Černocká, Václav Brázda, Marie Brázdová

Technicians: Ivana Salajková, Danuše Fridrichová, Ludmila Římánková

Project 7302 / GA CR 204/97/K084

Electrodes modified with nucleic acids and proteins. New tools in biochemical and biomedical research

Principal Investigator Emil Paleček

Project 8318 / GA CR 204/98/P091

Electrochemical biosensors for the detection of DNA damaging agents

Principal Investigator Miroslav Fojta

Project 8108 / GA AS CR A4004801

Electrodes as regulators of the cleavage of immobilized DNA by redox-modulated chemical nucleases

Principal Investigator Miroslav Fojta

Co-Investigators: Petr Pečinka, Hana Černocká, Miroslav Tomschik, Luděk Havran, Václav Brázda, Marie Brázdová, Tatiana Kubičárová, Jan Paleček, Jiří Bůžek

Technicians: Danuše Fridrichová, Ivana Salajková, Ludmila Římánková, Irena Postbieglová

Project 441 / IGA MH NC/5343-3

Interactions of tumour suppressor protein p53 with damaged DNA and with lesions induced by anti-cancer drugs

Principal Investigator Miroslav Fojta

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, DNA and its mimic - peptide nucleic acid (PNA) with mercury and carbon electrodes to obtain results potentially useful in research and development of biosensors for the detection of DNA hybridization and DNA damage. 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 metallothioneins as it appears that obtained results may be of use in the research of other metalloproteins, especially of the protein p53, in addition to the application of these methods for analysis of metallothioneins.

In the field II the research was oriented to the questions of influences of some metal ions and oxidation agents on tumor suppressor protein p53 and its interaction with linear and supercoiled DNA, and also on utilization monoclonal antibodies in the research of this protein and DNA structure and interactions.

Reduction and oxidation of peptide nucleic acid and DNA at mercury electrodes

Peptide nucleic acid (PNA) is a DNA mimic that bind strongly and specifically to complementary DNA or RNA oligomers, but in contrast to DNA its backbone does not carry any electric charge. We used voltammetry in cyclic and square-wave modes to study reduction and oxidation signals of single stranded PNA and DNA decamers and pentadecamers with the same base sequences at mercury and carbon electrodes. The signals produced by the DNA oligomers at the hanging mercury drop electrode (HMDE) i.e. the cathodic peak CA (due to reduction of cytosine and adenine) and the anodic peak G (due to oxidation of the guanine reduction product) corresponded to those observed earlier with single stranded chromosomal and plasmid DNAs. PNA yielded similar peaks whose potentials were, however, more negative as compared to DNA oligomers in all oligomers studied. The above peaks were studied by means of adsorptive stripping voltammetry in dependence on accumulation time, oligomer concentration and on some parameters of the electrochemical measurements. Similarities and differences between DNA and PNA were observed. It was concluded the difference in the signals of PNA and DNA are primarily due to different adsorption properties of these compounds. Relations between the guanine content and base sequences on one hand and the electrochemical responses on the other hand were found. At accumulation time (tA) 1 min peak G of PNA gave a linear calibration up to about 500 ng/ml; at tA = 5 min the detection limit of PNA was below 5 ng/ml. Constant current derivative chronopotentiometric stripping analysis (CPSA) at a pyrolytic graphite electrode produced two well-separated oxidation peaks of guanine and adenine residues in contrast to the poorly developed signals obtained by linear sweep (LS) and square wave (SW) voltammetries. These signals were greatly improved as a result of application of a suitable baseline correction method. Using the polynomic method for LSV and moving average baseline correction for SWV the DNA detection limits were comparable to those of CPSA at carbon electrodes as well to those obtained with peak G measurements at the mercury electrodes.

Cleavage of supercoiled DNA by deoxyribonuclease I in solution and at the electrode surface

Cleavage of supercoiled DNA by deoxyribonuclease I (DNase I) in solution and at the surface of the mercury electrode was studied by means of AC voltammetry. This technique produces peak 3 which is produced only by DNAs containing free ends (such as linear double-stranded and single-stranded DNAs and open circular DNAs) but not by covalently closed circular (ccc) DNAs. Formation of a single interruption of the sugar-phosphate backbone in the ccc supercoiled (sc) DNA results in formation of peak 3. Peak I is produced by both ccc DNA molecules as well as by DNAs containing free ends; changes in height of this peak occur due to DNA cleavage. We show that the kinetics of the cleavage of DNA in solution and at the electrode surface substantially differ suggesting restricted accessibility of the surface-confined DNA for the interaction with the enzyme. Cleavage of the immobilized DNA is remarkably influenced by the potential of the electrode surface. At positively charged surface the enzymatic reaction is inhibited in its initial stage while moderately negative charges stimulate the cleavage of the immobilized DNA by DNase I.

Real/time monitoring of enzymatic cleavage of nucleic acids using a quartz crystal microbalance

The use of crystal microbalance (QCM) for monitoring in situ the enzymatic cleavage of surface-confined nucleic acids by nucleases is described. Such real-time monitoring of mass changes associated with the enzymatic digestion indicates that the activity and specificity of nucleases is preserved at the gold surface, and can be used for manipulating surface-confined DNAs and RNAs. These observations indicate great promise for using QCM for elucidating the interactions of nucleic acids with enzymes, and for enhancing the power of hybridization biosensors.

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 (M range of concentrations of CdMT, therefore the same as on HMDE. After deposition of CdMT on composite paste electrode applying potential when complex is reduced, three oxidation peaks can be observed, one of uncomplexed Cd2+ and two of different S-Cd(II) complex formation, in region -200 mV and + 150 mV. There are not complications arising from interactions of electrode products or MT decomposition products with material of the electrode, as encountered when HMDE is used. These results were obtained in cooperation with J. Heyrovský Inst. Phys. Chem., Acad. Sci. CR, Prague.

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

Metallothionein 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. These results were obtained in cooperation with J. Heyrovský Inst. Phys. Chem., Acad. Sci. CR, Prague.

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

Wild type human full length (f.l.) tumor suppressor p53 protein binds preferentially to supercoiled (sc) DNA in vitro both in the presence and absence of the p53 consensus sequence (p53CON). This binding produces a ladder of retarded bands on the agarose gel. Bands revealed by immunoblotting with antibody DO-1 corresponded to the ethidium stained retarded bands. 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. Compared to the intrinsic zinc strongly bound to Cys 176, Cys 238, Cys 242 and His 179 in the p53 core domain, binding of additional Zn2+ to p53 was much weaker as shown by an easy removal of the latter ions by low concentrations of EDTA. Oxidation of the protein with diamide resulted in a decrease of the number of the retarded bands. Under the same conditions, no binding of oxidized p53 to p53CON in a linear DNA fragment was observed. 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. Other divalent cations tested (cadmium, cobalt, nickel) exhibited no such effect. 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. Binding of C-terminal anti-p53 antibody also protected bacterially expressed protein against irreversible loss of activity due to diamide oxidation. Binding the human p53 core domain (segment 94-312) to scDNA greatly differed from that observed with the full-length p53. The core domain did not posses the ability to bind strongly to many sites in scDNA regardless of the presence or absence of p53CON suggesting involvement of some other domain (probably C-terminal) in binding of the full-length p53 to scDNA. Supershift experiments using antibodies against p53 N- or C-terminus suggested that in oxidized p53, scDNA binding through the C-terminus gained importance. These results were obtained in cooperation with Memorial Masaryk Institute of Oncology in Brno.

Monoclonal antibody against DNA adducts with osmium structural probes

Osmium tetroxide complexes with nitrogen ligands (Os,L) have been widely used as probes of the DNA structure. A monoclonal antibody OsBP7H8 against DNA adducts with Os,L was produced in mice. OsBP7H8 does not bind to proteins

or total yeast RNA modified with Os,2,2'-bipyridine (bipy) nor to the unmodified nucleic acids and proteins. The antibody recognizes DNA modified with Os,bipy (DNA-Os,bipy) or with OsO4,1,10-phenanthroline (DNA-Os,phen) but it does not cross-react with oxidized DNA and with DNA adducts of osmium tetroxide complexes with other ligands (such as pyridine, TEMED and bathophenanthroline disulfonic acid). The affinity of OsBP7H8 to DNA-Os,phen is about five-fold higher as compared to DNA-Os,bipy. The antibody can be thus applied either for recognition of single-stranded and distorted regions in DNA (after DNA modification with Os,bipy) or for detection of both single-stranded and double-stranded DNAs (after DNA modification with Os,phen). A new simplified procedure for the dot-blot analysis is proposed, not requiring the purification of DNA-osmium adduct prior to its application to the membrane.

PROGRAMME 2

Structure, function and evolution of genomes

Project 8104 / GA AS CR A5004802

Biophysical analysis of selected regions of the human genome

Principal Investigator Jaroslav Kypr

Co-Investigators: David Häring, Radim Kittner, Martin Výkruta

Technician: Petr Vacula

Using the Pearson correlation coefficient, we analyzed correlations between the nucleotide distributions along the genomes of man, mouse, drosophila, *Arabidopsis, Saccharomyces, Escherichia coli,* retroviruses, herpesviruses and human viruses. The analysis included uninterrupted sequenced genomic regions longer than 10 kb which were deposited in the EMBL database, release 51, June 1997. The length limit was 8 kb in *Arabidopsis.* In total, we analyzed over 1,800 nucleotide sequences having over 50,000,000 nucleotides in length.

The strongest correlations were found between the genomic distributions of cytosine and guanine, in particular in drosophila. These correlations were weaker, but still strong in the human and E. coli genomes. The correlations of guanine with cytosine do not originate from isochores because the isochores were not observed to occur in the drosophila and E. coli genomes. The distribution of adenine correlated with the distribution of thymine in all genomes except for Saccharomyces where adenine anticorrelated with thymine. Still stronger anticorrelations were almost generally observed between the distributions of adenine and cytosine, and between the distributions of guanine and thymine. These anticorrelations presumably originate from unrepaired pairing of guanine with adenine. Hence it seems that besides the complementary adenine/thymine and cytosine/guanine couples, the pairs of adenine/cytosine and guanine/thymine exist which compensate each other in the genomes. Our studies demonstrate that this compensation belongs among the strongest and most universal factors that determine the global nucleotide distributions in genomes.

Further we studied flanking regions of dinucleotide microsatellites in the human, mouse and drosophila genomes. This study revealed that microsatellites were linked to the bulk of genomic DNA through connectors that were frequently much longer than the microsatellites themselves. Nucleotide sequences of these connectors were obviously non-random and rich in adenine and thymine. They also were frequently a continuation of the microsatellite in which the number of mutations increased with the distance from the microsatellite centre. In spite of the mutations, however, the microsatellite motif repetition was still evident very far from the point where the strict repetition of the microsatellite motif was first perturbed. Only the microsatellites composed of the alternating sequences of cytosine and guanine were not inserted into the genomic DNA through the

connectors described above. Simultaneously, their occurrence is very rare in genomes, which may cohere with the absence of the connector regions.

The analyses provided the following: (i) Microsatellite definition in the sense of a strict repetition of its basic short motif does not reflect the true microsatellite nature. The microsatellites are mostly surrounded in genomes by long regions that are evidently their integral parts. (ii) Owing to the fact given in point (i) above, the microsatellites constitute a much larger part of the genomes than it has ever been thought. Extrapolation of our studies suggests that certainly a billion, but perhaps even two billions of nucleotides constitute microsatellites in the human genome if the microsatellites are taken as including their surroundings, which is their integral part. (iii) Polymerases synthesise DNA de novo and the synthesised DNA is a microsatellite in all known cases. This property leads to a hypothesis in connection with the above that microsatellite expansions significantly contributed to the creation of the first eukaryotic genomes.

Project 8304 / GA CR 206/98/0626

Interstrand crosslinks induced in the genetic material by ultraviolet light

Principal Investigator: Jaroslav Kypr

Co-Investigators: Radim Kittner, Jiří Kovanda, Karel Nejedlý

Technician: Jitka Koštiálová

Taking advantage of the previous experience, we chose 31 pUC19, pBR322, ColE1, SV40, fiX174 and M13mp19 restriction fragments of suitable length and oligonucleotide composition. The fragments were irradiated with UV light and the fraction of irradiated molecules of DNA was determined, using the denaturing alkaline gel electrophoresis, that contained interstrand covalent bonds. This experiment was repeated with each fragment until the standard deviation of the average value was below an acceptable level. This way we got relatively very precise data suitable for quatitative analysis. The data were used for studies of how the amount of the interstrand photoproducts depends on the oligonucleotide composition of the irradiated restriction fragments of DNA.

Seven of the possible ten dinucleotide steps existing in DNA showed very high correlation coefficients in the dependences of the amount of the interstrand photoproducts on the dinucleotide composition of the irradiated fragments. This confirmed that the data were precise enough for the quantitative analysis. Formation of the interstrand photoproducts was found to be mostly promoted by the (TA).(TA) and (AT).(AT) dinucleotides while the promotive effect of the (AA).(TT) dinucleotide was much smaller. In line with the expectation, the three dinucleotides composed of only C and G suppressed crosslinking. It was, however, surprising that the (GA).(TC) dinucleotide suppressed the crosslink formation still more. This finding contradicts the notion that the crosslink formation is determined by the local thermostability of the double helix of DNA.

Dependences on the trinucleotide compositions confirmed that the thermostability is not the factor deciding about the formation of crosslinks. This phenomenon was found to be promoted most by trinucleotides none of which was only composed of A and T. For example, (ATA).(TAT) promoted the crosslink formation three times less than (GTA).(TAC). Neither was the highest suppression exhibited by trinucleotides composed of only C and G, which should be observed if the double helix thermostability were the decisive factor. The data obtained with tetranucleotides cannot be reproduced with data obtained with dinucleotides or trinucleotides, which shows that the tetranucleotide level still significantly contributes about where the crosslinks are generated. It is noteworthy that (CATG).(CATG) belongs among the tetranucleotides that strongly suppress crosslinking while (CTAG).(CTAG) is the tetranucleotide exhibiting the most promotive effect among all tetranucleotides. These results obviously demonstrate that the UV light-induced crosslink formation between the complementary strands of DNA is an effect governed by DNA structure and not by DNA thermodynamic properties.

The data described above were further used to construct a computer program predicting the probability of UV light-induced crosslink formation along the long genomic sequences of nucleotides. Application of this program to the six viral and plasmid genomes used in the experimental part of this project permitted to identify loci of preferred crosslink formation as well the loci where the crosslinks almost do not occur. We prepared the fragments containing these loci, irradiated them with UV light and determined the fraction of fragments containing the interstrand crosslink. These experimental data were compared with the prediction which was found to be 82% successful. This result demonstrates that the approach we used was correct. The software will now be used to identify the preferred sites of the UV light-induced crosslinks between the complementary strands of DNA in the human genome.

Project 7107 / GA AS CR A4004701

Role of cytosine in the conformational polymorphism of DNA

Principal Investigator Michaela Vorlíčková

Co-Investigators: Jana Chládková, Markéta Přibylová, Jaroslav Kypr

Technician: Marcela Tůmová

Duplex of d(CCCCGGGG), and other selfcomplementary DNA fragments starting with a block of cytosines on the 5´end, yield CD spectra which indicate base pair stacking characteristic for the A-type structure. Yet, d(CCCCGGGG) undergoes a cooperative trifluoroethanol-induced transition into structure A indicating that some important property, characteristic of B-DNA, is retained in its aqueous duplex. NMR spectroscopy has shown that this property is puckering of the deoxyribose rings. The above-mentioned facts inspire a notion of an unprecedented double helix of DNA in which A-like base stacking is combined

with B-type sugar puckering. In order to find out whether this combination is possible, we used molecular dynamics to simulate the duplex of d(CCCCGGGG). Remarkably, simulations, completely unrestrained by the experimental data, provided a very stable double helix of DNA exhibiting just the intermediate B/A features described above. The double helix contained well-stacked guanines but almost unstacked cytosines. This generated a hole in the double helix centre, which is a property characteristic of A-DNA, but absent in B-DNA. The base pairs stacked tightly at the ends but stacking was loose in the duplex centre. The minor groove was narrow at the double helix ends but wide at the central CpG step where the Watson-Crick base pairs were buckled in opposite directions. The results were obtained in cooperation with the Laboratory of Biomolecular Structure and Dynamics of the Faculty of Science, Masaryk University.

The major control element of the c-myc oncogene is characterized by an unusual DNA strand asymmetry: one strand is a nearly perfect purine, mostly guanine, tract, and the other is a complementary pyrimidine tract. We have studied conformational properties of four fragments of the pyrimidine, cytosine-rich, strand having 16, 22, 27, and 33 nucleotides in length. By means of CD spectroscopy and polyacrylamide gel electrophoresis we have found that all of the fragments formed intercalated cytosine tetraplex even at slightly acidic pH (6.5-7) values. The 16-mer adopted the least thermostable bimolecular tetraplex which appeared as well as melted with a long-lasting kinetics. The other fragments folded into intramolecular tetraplexes appearing and melting with a fast kinetics. Their thermostability increased in the order 27-mer, 22-mer, and 33-mer. The changes in CD spectra accompanying tetraplex formation provided the number of the hemiprotonated cytosine pairs formed during this process. Based on these data we have suggested molecular models of the particular tetraplexes. This result was obtained in cooperation with Chalmers University, Göteborg.

Project 8307 / GA CR 204/98/1027

Conformational polymorphism and expansion of the $(CNG)_n$ and related microsatellite DNA sequences

Principal Investigator Michaela Vorlíčková

Co-Investigators: Iva Kejnovská, Martin Kratochvíl, Iva Hrabcová, Jaroslav Kypr

Using CD spectroscopy and polyacrylamide gel electrophoresis we have found that dodecamer $(CGA)_4$ can adopt several distinct conformations. In the vicinity of neutral pH it forms a parallel-stranded homoduplex containing C^+.C, G.G and A.A base pairs. The GpA dinucleotide is responsible for the stability of this homoduplex, as $(CAG)_4$ forms foldback under the same solvent conditions. Cytosines in the $(CAG)_4$ dodecamer do not accept protons, not even at pH values lower than 4, when the foldback denatures. At slightly alkalic pH values and very low ionic strengths, $(CGA)_4$ also forms a foldback which, however, is transformed

in a bimolecular antiparallel homoduplex at increasing salt concentrations. The transition of $(CGA)_4$ between antiparallel- and parallel-stranded homoduplexes has a long-lasting kinetics. The antiparallel-stranded duplex contains G.C pairs, while adenines occurring in the opposite strands between CpG steps promote transition of the $(CGA)_4$ duplex into a left-handed Z-DNA. The transition has a long-lasting kinetics and takes place under the same conditions as the B-Z transition of $(CG)_4$, but its cooperativity is lower. Thus, the conformational polymorphism of $(CGA)_4$ includes parallel-stranded, antiparallel-stranded, right-handed and left-handed homoduplexes. In contrast, $(CAG)_4$ invariably adopts only a single conformation, i.e. the stable foldback, under all of the examined solution conditions.

Project 6321 / GA CR 301/99/0045

Telomere dynamics in selected types of solid malignant diseases

Principal Investigator Jiří Fajkus

Co-Investigators: Kateřina Krejčí, Eva Sýkorová, Aleš Kovařík

Technicians: Emilie Koudelková, Libuše Jedličková

The aim of the project is to design methods for diagnostics in oncology, based on determination of the length and structure of telomeres and on evaluation of activity and expression of telomerase in malignant tissues. In this respect, protocols for detection of telomerase activity and expression of its RNA and catalytic subunits have been optimized. Important conclusion has been made, that the expression of the telomerase catalytic subunit and the telomerase activity could be used for diagnostic purposes, while the RNA subunit expression seems not to be specific for tumor tissues, and, therefore, it is not suitable as a diagnostic marker.

To increase simplicity and accuracy of the protocol, in situ versions of telomere and telomerase analyses are being established in collaboration with the Danish Cancer Institute.

Project VS97032, ME CR – Program of promoting university research

Analysis of biologically important molecular complexes

Principal Investigator Jiří Fajkus

Co-Investigators: Jana Fulnečková, Mirka Horáková, Marie Skleničková, Lenka Fajkusová, Lumír Krejčí, Kateřina Krejčí, Eva Sýkorová, Lenka Skřišovská

Assistants: Gabriela Kalábová, Klára Poláčková

Technicians: Libuše Jedličková, Emilie Koudelková

Analyses of telomere/subtelomere junctions of plant chromosomes aim to the following outcomes: a) mapping of telomere associated sequences *at Silene latifolia* chromosomes; b) Determination of DNA sequence of regions associated with telomeres of the X-chromosome sorted from the *S. latifolia* genome (in collaboration with laboratory of Dr. Vyskot, this Institute); study of the specific features of the chromatin structure of telomere-associated sequences in *S. latifolia*. During the last year, new subtelomeric sequences, as well as the respective chromatine region, have been characterized.

At the same time, mapping and sequence characterization of subtelomeres in *Nicotiana tomentosiformis* and *N. tabacum* has been finished.

Studies of mechanisms of plant telomerase regulation revealed inhibition of the activity by proteins extracted from telomerase-negative plant tissues. These proteins form complexes with a single-stranded G-overhang of telomeres. The inhibition is due to a competitive binding of proteins to a substrate oligonucleotide, not a direct interaction with telomerase. Affinity of the inhibitory proteins to the G-strand is higher than that of telomerase, which enables these proteins to substitute telomerase from its complex with a substrate. These interactions are sequence-specific (not species-specific) and resistant to high salt concentration. In the case of complex formed by proteins extracted from *Nicotiana tabacum,* its molecular weight 40 kDa was determined. Formation of complexes showing telomerase inhibition may be involved in developmental and cell-cycle control of plant telomerase.

Part of scientific activities was focused on the study of de novo methylation of the Pa-promoter in bcr-abl gene, telomerase activity and disease progression in CML patients. A new perspective for our laboratory is connected with studies on yeast recombination factors.

A new assay has been designed to screen for mutations which disrupt specific interactions in recombinosome, a nucleoprotein complex of recombination factors of the RAD52 epistasis group (includes e.g., RAD51, RAD52, RAD54 and RAD55). Selection led to mutants with disrupted interaction to one of the RAD51-associated proteins, while other interactions remained intact.

Furthermore, suppressor mutations, restoring protein interactions in the recombinosome, were also isolated.

Project 8308 / GA CR 204/98/0191

The relationship between the methylation status, structure and function of tobacco 5S rDNA locus

Principal Investigator Roman Matyášek

Co-Investigators: Jaroslav Fulneček, Aleš Kovařík, Jiří Fajkus, Miloslava Fojtová, Blažena Koukalová, Milan Bezděk

Technicians: Emilie Koudelková, Libuše Jedličková, Danuše Fridrichová

Project 9324 / GA CR 204/99/D001

Studies on mechanisms of DNA methylation and demethylation in higher plants

Principal Investigator Jaroslav Fulneček

Guarantor: Roman Matyášek

1. Characterization of 5S rDNA in genomes of the genus Nicotiana

The long scale organization of 5S rDNA loci in some *Nicotiana* species was characterized using the *in situ* hybridization, PFGE and Southern blot hybridization. Two 5S DNA specific hybridization probes were used: (a) probe derived from nontranscribed spacer specific for T-genomic component (b) probe specific for transcribed part of tobacco 5S rDNA unit. We could demonstrate that all 5S rDNA families in species *N. digluta, N. glutinosa, N. kawakamii, N. otophora, N. setchelii, N. tabacum, N. tomentosa, N. tomentosiformis* (subtribus *Tabacum)* contained T-specific sequences (even though individual families have different sizes of monomeric units). In addition, *N. tabacum* genome contained S-genomic component. The species *N. rustica* (subtribe *Rustica*), *N. longiflora* and *N. sylvestris* (subtribe *Petunioides*) did not contain sequences homological with T-specific probe. It appears that the probe is specific for the subtribe *Tabacum* and thus it can be used for phylogenetic studies.

1C *N. tabacum* genome contains 900 – 1300 copies of short monomeric units (431 bp) of the S-type and 900 – 1700 copies of long monomeric units (646 bp) of the T-type; 1C *N. tomentosiformis* genome contain 400 units of the T-type*; N. sylvestris*, 400 units of the S-type and *N. otophora*, 1400 units of the T-type. In *N. tabacum* the copy number of both families was the same in roots and leaves. The high level of copy number polymorphisms can be used for fingerprinting

of tobacco cultivars. It appears that the size of monomeric units of S and T 5S rDNA families are in the relation 2 : 3 in the *N. tabacum* genome, and thus it is possible that dimer of the longer unit and trimer of the shorter unit have similar nucleosomal structure.

S and T families of 5S rDNA in *N. tabacum* can be distinguished by *in situ* hybridization; both loci show partial decondensation at interfase nuclei indicating transcriptional activity.

The T-family is present on the long arm of the chromosome 8 in all species of the section *Tomentosae*; otherwise, the chromosome 8 does not contain any other known repetitive sequences (rDNA, HRS60, GRS, GRD, NTRS). In *N. kawakamii* and *N. otophora* there is an additional T-locus on the short arm of the chromosome 12, and in *N. setchelii* there are still two additional T-loci on chromosomes 5 and 7 (in the chromosome 7 with GRS loci).

Based on these studies we could conclude, that over 5 million years of evolution of the composite *N. tabacum* genome, 5S-rDNA loci evolved mainly due to the alteration of the copy number of 5S rDNA monomeric unit.

Analysis of the methylation status of some cytosines in non-transcribed part of 5S rDNA of *N. tabacum* has been performed using methylation sensitive restriction endonucleases. Both families of 5S rDNA sequences in *N. tabacum* display a high level of methylation at cytosines in symmetric CG and CNG sequences. Preliminary results show, that germination of *the N. tabacum* seeds is accompanied with partial hypomethylation of some cytosine residues.

2. Nucleotide sequences of 5S rDNAs and their genomic methylation status in other plant species

Presently we prepare primers derived from 5S rDNA loci of *A. thaliana*, *S. cereale* and *T. aestivum* designed for amplification of transcribed 5S rDNA. Cloned PCR products are sequenced and the sequences will be used for comparative analysis of the structure and methylation status of 5S rDNA genes, using bisulphite genomic sequencing.

Project 8321 / GA CR 521/98/0045

Factors involved in regulation of DNA methylation in plants

Principal Investigator Aleš Kovařík

Co-Investigators: Blažena Koukalová, Roman Matyášek

Technicians: Emilie Koudelková, Libuše Jedličková

Grant Co-Recipient: Institute of Organic Chemistry and Biochemistry AS CR, Prague

Principal Investigator Antonín Holý

Cadmium-induced apoptosis in plant cells

The work explored the influence of cadmium on a suspension cell culture of *Nicotiana tabacum* (TBY-2) by examining cell morphology, viability and DNA integrity. Changes of these parameters were strikingly dependent on concentration of cadmium in the culture medium: a concentration of 50 - 100 μmol/l $CdSO_4$ induced apoptotic changes including DNA fragmentation into oligonucleosomal units, while 1 mmol/l Cd^{2+} showed strong cytotoxicity, but no fragmentation of DNA. Low cadmium concentrations (below 10 μmol/l) effected neither cell viability nor DNA integrity.

A detailed kinetic study showed a significant delay in the onset of apoptosis after the application of high concentration of cadmium. From days 0 - 3 after the application of 50 μmol/l $CdSO_4$, the morphology of the cells, their viability and growth were indistinguishable between control and treated cells, whereas the "domain" DNA fragmentation into 50 - 200 kb fragments was observed at the DNA level. Then, in days 4 - 7, commenced a characteristic and rapid decrease in cell viability, accompanied with distinct changes in cell morphology and chromatin fragmentation to oligonucleosomes. The results suggest that chronic exposure of plant cells to cadmium induces frequent double strand breaks in DNA. The programmed cell death may be triggered when the threshold of DNA damage is reached.

Development of a new computerized method for counting of cells

We have developed a simple method for estimation of viable cell counts in plant suspension cultures. The cells are stained with fluorescein diacetate (FDA) and 20 μl aliquots of diluted cell suspension are spotted onto a nylon membrane. After drying membranes are scanned on a Phosporimager and the fluorescence is measured for each spot. The relationship between cell counts and fluorescence intensity appeared to be fairly linear ranging from as little as 250 up to 10 000 cells/spot. The duration of the test is about 15 min. Because of the simplicity the test may be particularly useful where routine counting of large number of specimens is required.

Project 9311 / GA CR 521/99/0696

Kinetics of DNA methylation in embryogenesis and seed germination

Principal Investigator Boris Vyskot

Co-Investigators: Jiří Široký, Karel Říha, Bohuslav Janoušek, Eduard Kejnovský, Jitka Žlůvová, Jaromíra Hodurková, Vladimíra Hykelová

Technician: Nikol Kumanová

In this project we have characterized individual stages of embryogenesis, seed germination, and seedling development in the model dioecious plant *Silene latifolia*. In order to follow DNA methylation kinetics during development, an immunostaining technique on plant tissue cryosections has been mastered. Comparative levels of global DNA methylation were studied in individual stages of embryo and plant development using a mouse monoclonal antibody (kindly provided by Dr. M. Ruffini-Castiglione, University of Pisa) followed by an indirect immunodetection. During a very early embryo development (till the heart embryo) no differences in the intensities of immunolabelling were found. However, in the following stages a high level of DNA methylation was detected in terminal regions of cotyledons and shoot and root meristems. During seed germination, a rapid and large demethylation occurred in endosperm nuclei which was obviously connected with their metabolic activation. A similar gross DNA demethylation was detected in hypocotyl and radicula nuclei (except the quiescent centre of root meristem). The demethylation of cotyledons occurred later before their cell divisions. The strongest signals of DNA methylation were found in the quiescent centre of shoot apical meristem for the whole period of plant vegetative growth.

In collaboration with Dr. D. E. Shippen (Texas A&M University, College Station) and Dr. J. Fajkus (this Institute) we have investigated the architecture of telomeres in the dicot plants *Silene latifolia* and *Arabidopsis thaliana* using the PENT (primer extension/nick translation) assay. We have shown that G-overhangs longer than 30 nucleotides are a common feature of plant telomeres and these structures persist throughout the cell cycle. However, only half of the telomeres in *S. latifolia* seedlings possess long G-overhangs. The remaining fraction of telomeres either carry no overhangs or overhangs less than 12 nucleotides in length. G-overhangs were also detected in *Silene* seeds and leaves, tissues that lack telomerase activity. The fraction of telomeres with detectable G-overhangs decreased from 50% in seeds to 35% in leaves, which could reflect the relative timing of telomerase inactivation in these tissues. Taken together, the data suggest 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.

We have continued our collaboration with Prof. I. Negrutiu (Ecole Normale Supérieure de Lyon) on structure and function of *Silene latifolia* sex chromosomes. Asexual mutants, cumulating two developmental defects that

characterize the sexual dimorphism in this species, were produced by gamma ray irradiation of pollen and screening in the M1 generation. The mutants harbour a novel type of mutation affecting an early function in sporogenous cell differentiation within the anther (*stamen promoting function*). Using both molecular and karyological analyses these mutants were shown to result from interstitial deletions on the Y chromosome. We have presented evidence that such deletions tentatively cover the central domain on the (p)-arm of the Y chromosome (Y2 region). By comparing stamen development in female and asexual flowers we have shown that they share the same block in anther development. We conclude that the *stamen promoting function* gene(s) is Y-linked and responsible for the male sexual dimorphism in the third floral whorl in *S. latifolia*.

Project 9511 / GA AS CR A5004901

Nuclear structure and histone acetylation in plant cells

Principal Investigator Boris Vyskot

Co-Investigators: Jiří Široký, Jaromíra Hodurková, Jitka Žlůvová, Martina Lengerová

Technician: Nikol Kumanová

Recent studies show that histone acetylation is a wide-spread chromatin modification connected with potential transcriptional activity. Only very few data demonstrating the role of histone acetylation in regulation of gene expression in plants are available. To follow overall changes in bulk chromatin we study dynamics of histone H4 acetylation during the process of seed germination and plantlet development when rapid and gross changes in metabolic activities occur. This research is realized in collaboration with Prof. B. M. Turner (University of Birmingham Medical School) who also kindly provided specific antisera against H4 histones acetylated at different positions of N-terminal lysines. Plant histone extracts have been studied using AUT gels and western blotting. Our analyses performed on the model species *Silene latifolia* indicate that in quiescent seeds H4 nucleosomal histones are maintained in the monoacetylated isoform. A rapid increase in H4 acetylation was observed during seed germination and early plantlet growth and development. The maximum level of H4 acetylation (including the presence of pentaacetylated isoform) was detected relatively late, at the 5^{th} day after imbibition, which probably reflects the beginning of large-scale transcription. A recent technique of immunoprecipitation enabling to separate acetylated and non-acetylated fractions of chromatin has been mastered. This will allow us to study a correlation between histone acetylation and gene expression on the molecular level.

Chromatin structure was also analyzed in root meristematic nuclei blocked at the G2 stage of cell cycle using staining and immunocytological techniques. The results showed that heterochromatic foci were clustered at nuclear periphery,

while euchromatin was found in central regions of nuclei. The immunostaining confirmed a modified character of the heterochromatin: it was DNA hypermethylated and H4 histone underacetylated. The heterochromatin foci disappeared after both Trichostatin A (an inhibitor of histone deacetylases) and 5-azacytidine (an inhibitor of DNA methyltransferases) treatment. An immunohistotechnique has been also developed to follow changes in histone H4 acetylation patterns during plant development.

Recent immunocytological and molecular data show that heterochromatic nuclear regions, both constitutive and facultative, are differently modified (cytosine hypermethylation and histone hypoacetylation) and late replicating, as compared to euchromatin. Intrusive and/or additive (supernumerary) DNA sequences are often functionally silenced which is accompanied by their heterochromatinisation. We have presented a number of karyological studies on autotetraploid female cells of *Silene latifolia*. Immunofluorescence analyses do not indicate any global differences in the DNA methylation, histone H4 acetylation, and chromosome replication patterns which could arise as a consequence of the whole chromosome set duplication of the original diploid genome. Similarly, a number of silver-positive nucleoli roughly correlates to the ploidy level. The early replication and H4 hyperacetylation have been detected at all subterminal chromosome regions which indicates, together with cDNA *in situ* hybridization patterns, the localization of gene-rich regions. The DNA methylation and chromosome replication patterns, but not the histone H4 acetylation, show differences among the four X chromosomes present: one to three X chromosomes were observed as hypermethylated and/or late replicating. Taken together, our data demonstrate that there is no overall silencing of the additional two sets of autosomes in the tetraploid cells, but the X chromosomes could be subject to an irregular dosage compensation.

Facultative and constitutive heterochromatin properties are also studied on another dioecious model plant, *Rumex acetosa*. Two Y chromosomes, present only in males, do not play an active role in sex determination, but they are necessary for male fertility. These sex chromosomes are heterochromatic, harbour many accumulated DNA repeats, and form chromocenters in interphase nuclei. However, our analyses did not reveal any differences in DNA methylation labeling intensity between the Y chromosomes and autosomes in male cells. Similarly, the immunolabelling analysis showed a similar DNA methylation pattern between the two X chromosomes in female cells which indicate the absence of dosage compensation phenomenon in this plant species. In collaboration with Dr. C. C. Ainsworth (University of London) we localize sex-specific DNA clones using the FISH technique. The verified Y-specific probes will be used to confirm the character of sex-bodies in male interphase nuclei.

Project 6325 / GA CR 521/96/K117

New methods for effective studying and mapping of crop plants

Principal Investigator Jaroslav Doležel, Institute of Experimental Botany AS CR, Olomouc

Grant Co-Recipient: Institute of Biophysics AS CR, Brno

Investigator: Jiří Široký

Co-Investigators: Boris Vyskot, Eduard Kejnovský, Martina Lengerová, Helena Dušková-Horáková, Vladimíra Hykelová

This project is focused on the study of evolution of sex chromosomes in some dioecious plant species in genus *Silene*. We continued in karyological analysis of mitotic chromosomes of various *Silene* species to obtain the data needed for morphological comparison of chromosomes, especially sex chromosomes. Using *in situ* hybridization we localized rDNA genes, namely 25S- rDNA and 5S-rDNA genes, in *S. latifolia*, *S. vulgaris*, *S. pendula*, and *S. chalcedonica*. In *S. latifolia*, *S. vulgaris*, and *S. pendula*, 25S-rDNA genes were localized on 5 or 6 pairs of chromosomes while in *S. chalcedonica* only on two chromosomal pairs. Genes for 5S-rDNA were found on only one chromosomal pair in *S. chalcedonica* in contrast to two pairs in other species tested. In addition, *S. chalcedonica* exhibited significantly higher nuclear DNA contents as was shown by flow-cytometry.

We have started experiments focused on localization and more detailed characterization of single copy and low copy sequences linked to sex chromosomes of *Silene latifolia*. We used DNA sequences as follows. First, *MROS3* gene, specifically expressed in male reproductive organs, located on the X chromosome with a homologue (pseudogene) on the Y chromosome (collaboration with the University of Tokyo). Second, *SlY1* gene, the first discovered functional gene located on a plant Y chromosome, and its X-linked homologue, *SlX1* (collaboration with Ecole Normale Supérieure de Lyon). Third, a group of short DNA sequences exhibiting a sex-specific polymorphism (a collaboration with the University of North Carolina).

These sex-specific DNA sequences and genes were used for *in situ* hybridization (FISH) on mitotic chromosomes to show their chromosomal localization. To improve the sensitivity of FISH experiments we both introduce new techniques which should enable detection of low copy sequences as well as techniques based on *in situ* amplification (PRINS). In parallel, we prepared by flow sorting pure X and Y chromosomes. These sorted chromosomes are used for PCR amplification of the sequences and genes mentioned above to show their specific localization on sex-chromosomes.

Using inverse PCR we analyze the regions flanking these sex-specific sequences and genes. It will be helpful in determining the copy number of these genes as well as in searching for X and Y homology. In addition, amplification of flanking

sequences will be used in preparation of longer probes that are more convenient for FISH experiments. We also started with experiments studying chromosomal distribution of retroelements in various species of genus *Silene*, especially focused on expected accumulation of retroelements in non-recombining parts of sex chromosomes.

Project 8317 / GA CR 521/98/P061

Study of chromatin changes during the plant microsporogenesis and microgametogenesis

Principal Investigator Bohuslav Janoušek

Guarantor: Boris Vyskot

This research is focused on the roles of DNA methylation and histone acetylation in the course of pollen differentiation. The vegetative pollen nucleus is, contrary to generative and sperm nuclei, characterized by a relaxed chromatin structure and its transcripts are utilized during pollen tube germination. Using antisera specific for acetylated isoforms of histone H4 we demonstrated that during pollen maturation in *Lilium longiflorum* histones H4 in the vegetative nucleus are largely deacetylated at N-terminal lysines 5 and 8. However, during the subsequent pollen tube germination the vegetative nucleus becomes H4 hyperacetylated. Now we have correlated these data with DNA methylation immunoanalyses. After the first asymmetric pollen mitosis the vegetative nucleus is hypermethylated in comparison with both the microspore and the generative nucleus. These data show that there is a correlation between histone H4 underacetylation and DNA hypermethylation in the vegetative nucleus. This phenomenon obviously reflects the fact that the vegetative pollen cell becomes quiescent after pollen maturation and H4 underacetylation and DNA hypermethylation could present mechanisms to ensure its long-term genome inactivation. This hypothesis has been supported by similar data obtained by DNA methylation immunostudies on tricellular pollen in *Silene latifolia*.

Gagea lutea belongs to rare plant species which display a sperm dimorphism. Its two sperm cells regularly differ in size and number of nucleoli. However, our immunoanalyses did not show any differences in their H4 histone acetylation pattern. In *G. lutea*, the vegetative and generative nuclei are H4 acetylated in a similar extent which indicates that the vegetative nucleus inactivation does not occur as in *L. longiflorum*. This result could be explained by the fact that in *G. lutea* the pollen tube growth and development is a very rapid process.

The role of DNA methylation in regulation of gene expression is further studied on 5' non-transcribed regions of genes expressed during microgametogenesis (*MROS*, male reproductive organ specific genes, isolated by Dr. S. Matsunaga, University of Tokyo). The *MROS1* gene is specifically expressed in mature pollen grains. We have started to study its 5' non-transcribed region and found that

it consists of two parts, variable and conservative. The conservative region is CG dinucleotide rich and will be used to study cytosine methylation using genomic sequencing.

PROGRAMME 3

Changes in the genome structure induced by physical and chemical factors

Project 7103 / GA AS CR A5004702

Effects of geometric isomerism in antitumour dinuclear platinum complexes on binding properties and conformational alterations in DNA

Principal Investigator Viktor Brabec

Co-Investigators: Zdeňka Balcarová, Ctirad Hofr, Eva Janovská, Jana Kašpárková, Hana Loskotová, Kamila Neplechová, Olga Nováková, Oldřich Vrána, Renata Žaludová

Technician: Milada Kořínková

The major focus of this project was the DNA binding profile of a novel trinuclear platinum phase I clinical agent (BBR3464). The structure of BBR3464 is best described as two *trans*-$[PtCl(NH_3)_2]$ units linked by a tetra-amine $[trans\text{-}Pt(NH_3)_2\{H_2N(CH_2)_6NH_2\}_2]^{2+}$ unit. The 4+ charge of BBR3464, the presence of at least two Pt coordination units capable of binding to DNA and the consequences of such DNA binding are remarkable departures from the cisplatin structural paradigm. The chemical and biological features argue that the drug should be considered the first clinical representative of an entirely new structural class of DNA-modifying anticancer agents. The high charge on BBR3464 facilitates rapid binding to DNA with a $t_{1/2}$ of ~40 min, significantly faster than the neutral cisplatin. The melting temperature of DNA adducted by BBR3464 increased at low ionic strength but decreased in high salt for the same r_b. This unusual behavior is in contrast to that of cisplatin. BBR3464 produces an unwinding angle of 14^o in negatively supercoiled pSP73 plasmid DNA, indicative of bifunctional DNA binding. Quantitation of interstrand DNA-DNA cross-linking in plasmid pSP73 DNA linearized by *Eco*RI indicated approximately 20% of the DNA to be interstrand cross-linked. While this is significantly higher than the value for cisplatin, it is, interestingly, lower than that for dinuclear platinum compounds such as $[\{trans\text{-}PtCl(NH_3)_2\}_2H_2N(CH_2)_6NH_2]^{2+}$ (BBR3005) where interstrand cross-linking efficiency may be as high as 70-90%. Either the presence of charge in the linker backbone or the increased distance between platinating moieties may contribute to this relatively decreased ability of BBR3464 to induce DNA interstrand cross-linking. Fluorescence experiments with ethidium bromide were consistent with the formation of long-range delocalized lesions on DNA produced by BBR3464. The sequence preference for BBR3464 on plasmid DNA was determined to the exact base pair by assaying extension of the polynucleotide by $Vent_R(exo^+)$ DNA polymerase. Strong sequence preference for single dG or d(GG) sites was suggested. The presence of relatively few blocks on DNA in comparison to either cisplatin or BBR3005 was indicative of high sequence selectivity. Finally,

immunochemical analysis confirmed the unique nature of the DNA adducts formed by BBR3464. This analysis showed that antibodies raised to cisplatin-adducted DNA did not recognize DNA modified by BBR3464. In contrast, DNA modified by BBR3464 inhibited the binding of antibodies raised to transplatin-adducted DNA. Thus, the bifunctional binding of BBR3464 contains few similarities to that of cisplatin but may have a subset of adducts recognized as similar to the transplatinum species. In summary, the results point to a unique profile of DNA binding for BBR3464, strengthening the original hypothesis that modification of DNA binding in manners distinct from that of cisplatin will also lead to a distinct and unique profile of antitumor activity.

Project 8113 / GA AS CR A7004805

Interactions of DNA with antitumor platinum drugs of the second generation

Principal Investigator Oldřich Vrána

Co-Investigators: Ctirad Hofr, Vladimír Kleinwächter, Jaroslav Malina, Kamila Neplechová, Renata Žaludová

Mechanistic studies of a novel class of aminophosphine platinum(II) complexes as potential anticancer agents were performed. These new agents, which have demonstrated activity against murine and human tumor cells including those resistant to cisplatin are *cis*-[$PtCl_2(Me_2N(CH_2)_3PPh_2$-*P*)$_2$], **1** and *cis*-[$PtCl(C_6H_{11}NH(CH_2)_2PPh_2$-*N*,*P*)($C_6H_{11}NH(CH_2)_2PPh_2$-*P*)], **2**. We studied modifications of natural and synthetic DNAs in cell-free media by **1** and **2** by various biomedical and biophysical methods and compared the results with those obtained when DNA was modified by cisplatin. The results indicated that **1** and **2** coordinated to DNA faster than cisplatin. Bifunctional **1** formed DNA adducts coordinating to single adenine or guanine residues or by forming cross-links between these residues. In comparison with cisplatin, **1** formed the adducts more frequently at adenine residues and also formed less bidentate lesions. The monofunctional **2** only formed DNA monodentate adducts at guanine residues. In addition, **1** terminated DNA synthesis *in vitro* more efficiently than cisplatin while **2** blocked DNA synthesis only slightly. DNA unwinding studies, measurements of circular dichroism spectra, immunochemical analysis and studies of the B-Z transition in DNA revealed conformational alterations induced by the adducts of **1**, which were distinctly different from those induced by cisplatin. Complex **2** had little influence on DNA conformation. It is suggested that the activity profile of aminophosphine platinum(II) complexes, which is different from that of cisplatin and related analogues, might be associated with the specific DNA binding properties of this new class of platinum(II) compounds.

We also continued in the studies of DNA reactions with antitumor cisplatin in the presence of sulfur-containing amino acids and oligopeptides. The first studies included the measurement of the reaction kinetics of several platinum compounds {cisplatin, carboplatin [*cis*-diammine-1,1-cyclobutanedicarboxylatoplatinum(II)] and monofunctional [Pt(dien)Cl]Cl [chlorodiethylenetriamineplatinum(II)

chloride]} with L-methionine (Met) with the aid of high-pressure-liquid chromatography (HPLC). The intermediate product of these reactions was isolated which contained Pt and Met in the ratio of 1:1 and its reaction with DNA (calf-thymus and plasmid pSP73) was also investigated. The complexes cisplatin-Met and [Pt(dien)Cl]Cl-Met reacted with DNA, but the reaction rate was lower than that of nonconjugated cisplatin or [Pt(dien)Cl]Cl. In addition, the hypothesis suggested by other authors was verified according to which the reaction of carboplatin with Met leads to the opening of its chelate ring and consequently to enhancement of the reaction rate of carboplatin with DNA. However, this hypothesis was not verified. The reaction rate of the carboplatin-Met complex with DNA was considerably lower than in the case of nonconjugated carboplatin (after 12 days less than 10 % of the complex was bound). The sites at which the complex cisplatin-Met binds to plasmid DNA were also determined by replication mapping assay using PCR. We have found that preferential binding sites of the complex were guanine residues similarly as in the case of the binding of nonconjugated cisplatin, but the distribution and number of binding sites were distinctly different.

Project 6303 / GA CR 305/99/0695

Affection of DNA conformation by metal-based anticancer drugs. Relations to the development of new cytostatics

Principal Investigator Viktor Brabec

Co-Investigators: Jana Kašpárková, Hana Loskotová, Jaroslav Malina, Kamila Neplechová, Olga Nováková

Technician: Milada Kořínková

Project 8315 / GA CR 307/97/P029

Molecular mechanism underlying antitumor activity of bis(platinum) complexes

Principal Investigator Jana Kašpárková

Guarantor: Viktor Brabec

Project 8319 / GA CR 204/97/P028

Biophysical analysis of the effect of antitumor ruthenium complexes on DNA

Principal Investigator Olga Nováková

Guarantor: Viktor Brabec

Project 8323 / GA CR 301/98/P231

Reaction of DNA with platinum complexes containing aminophosphine ligands. Relation to the development of new platinum drugs

Principal Investigator Kamila Neplechová

Guarantor: Viktor Brabec

Modifications of natural DNA in a cell-free medium by cisplatin tethered to AT specific, minor groove binder distamycin, were studied by various methods of biochemical analysis or molecular biophysics. These methods included: binding studies by means of differential pulse polarography and flameless atomic absorption spectrophotometry, mapping of DNA adducts by means of transcription assay, use of ethidium bromide as a fluorescent probe of DNA adducts of platinum, measurements of DNA unwinding by gel electrophoresis, measurements of CD spectra, interstrand cross-linking assay employing gel electrophoresis under denaturing conditions, measurements of melting curves with the aid of absorption spectrophotometry and use of terbium ion as a fluorescent probe of distorted base pairs in DNA. The results indicated that attachment of distamycin to cisplatin changed several features of DNA binding mode of the parent platinum drug. Major differences consisted in different conformational alterations in DNA and in a considerably higher efficiency of the conjugated drug to form in DNA interstrand cross-links. Cisplatin tethered to distamycin, however, coordinated to DNA with similar base sequence preferences as the untargeted platinum drug. The results pointed to a unique profile of DNA binding for cisplatin-distamycin conjugates, suggesting that tethering cisplatin to minor groove oligopeptide binders might also lead to an altered profile of biological activity.

Project 413 / Howard Hughes Medical Institute, USA, HHMI 75195-540201

DNA interactions of platinum-group metals: relations to their antitumor activity

Principal Investigator Viktor Brabec

Project 069/P 981n Ministero Degli Affari Esteri, Italy

Nuovi farmaci antitumorali di platino

Principal Investigator Viktor Brabec

Project National Institutes of Health (NIH, USA) 1R01CA78754-01

Mechanistic studies on new platinum clinical agents

Principal Investigator Viktor Brabec

Project 436 / National Science Foundation (NSF, USA) a ME CR, ME 152

DNA interactions of polynuclear platinum

Principal Investigator Viktor Brabec

Investigations within these foreign projects were mainly focused on studies of modifications of natural DNA and synthetic oligodeoxyribonucleotide duplexes in a cell-free medium by analogues of antitumor cisplatin containing enantiomeric amine ligands, such as *cis*-[$PtCl_2$(RR-DAB)] and *cis*-[$PtCl_2$(SS-DAB)] (DAB = 2,3-diaminobutane). These modifications were studied by various methods of molecular biophysics and biophysical chemistry. These methods included: DNA binding studies by pulse polarography and atomic absorption spectrophotometry, mapping of DNA adducts using transcription assay, interstrand cross-linking assay employing gel electrophoresis under denaturing conditions, differential scanning calorimetry, chemical probing, bending and unwinding studies of the duplexes containing single, site-specific cross-link. The major differences resulting from the modification of DNA by the two enantiomers consisted in the

thermodynamical destabilization and conformational distortions induced in DNA by the 1,2-d(GpG) intrastrand cross-link. It has been suggested that these differences are associated with a different biological activity of the two enantiomers observed previously. In addition, the results of this study were also consistent with the view that formation of hydrogen bonds between the carbonyl oxygen of the guanine residues and the ”quasi equatorial” hydrogen of the *cis* amine in the 1,2-d(GpG) intrastrand cross-link plays an important role in determining the character of the distortion induced in DNA by this lesion.

In addition, we performed studies to reveal further details of DNA binding mode of bifunctional trinuclear platinum compound BBR3464 and its relationship to the remarkable potency and antitumor activity of this new drug. By choosing an appropriate sequence where stop sites occurred in replication mapping experiments using DNA polymerase and PCR technique:

5’-T’$_{23}$ G’$_{24}$ A’$_{25}$ A’$_{26}$ T’$_{27}$ T’$_{28}$ C’$_{29}$ G’$_{30}$ A’$_{31}$ G’$_{32}$ C’$_{33}$ T’$_{34}$ C’$_{35}$ G’$_{36}$ G’$_{37}$ T’$_{38}$ A’$_{39}$

3’-A$_{23}$ C$_{24}$ T$_{25}$ T$_{26}$ A$_{27}$ A$_{28}$ G$_{29}$ C$_{30}$ T$_{31}$ C$_{32}$ G$_{33}$ A$_{34}$ G$_{35}$ C$_{36}$ C$_{37}$ A$_{38}$ T$_{39}$

molecular modeling on 1,4-interstrand (G’$_{30}$ to G$_{33}$) and 1,5-intrastrand (G$_{33}$ to G$_{29}$) cross-links was carried out. These analyses confirmed the similarity in energy between the two forms of cross-link. The similarity in energies was attributed to strain being present in different sections of each model. The model with the linker group within the groove exhibits strain in the BBR3464 molecule, while the model with the linker outside the major groove exhibits DNA strain in the region of platinum binding. These initial modeling calculations indicated that long-range intrastrand and interstrand cross-links were almost equally favored. Whether this is due to chain length of BBR3464 being so flexible or the charge along the backbone is yet to be determined.

Project 423 / D8/0009/97

European Cooperation in the Field of Scientific and Technical Research, project COST D8.10, Chemistry of metals in medicine

Metal recognition of DNA and drug design

Coordinator Peter Sadler, Department of Chemistry, University of Edinburgh, United Kingdom

Investigators in the countries of EC:

J.C. Chottard (France), J. Reedijk (The Netherlands), J.-M. Malinge (France), G. Natile (Italy), G. Lowe (United Kingdom)

Principal Investigator v CR Viktor Brabec

Project 433 / D8/0017/97

European Cooperation in the Field of Scientific and Technical Research, COST D8.50, Chemistry of metals in medicine

The development of ruthenium antitumor compounds

Coordinator Enzo Alessio, Department of Chemistry, University of Trieste, Italy

Investigators in the countries of EC:

M. Coluccia (Italy), B. Keppler (Germany), L. Messori (Italy), G. Natile (Italy), J. Reedijk (The Netherlands), P. Sadler (United Kingdom)

Principal Investigator v CR Viktor Brabec

Project 403 / D1/0012/92

European Cooperation in the Field of Scientific and Technical Research, COST D8.40, Chemistry of metals in medicine

Platinum-linked nucleotides analogs as viruses inhibitors

Coordinator Giovanni Natile, Department of Pharmaceutical Chemistry, University of Bari, Italy

Investigators in the countries of EC:

P. Sadler (United Kingdom), P. Bukovec (Slovenia), J. Kobe (Slovenia), E. De Clercq (Belgium)

Principal Investigator v CR Vladimír Kleinwächter/Oldřich Vrána

Within the studies planned for the Cost projects in the Czech Republic some initial results of the studies described above for projects GA CR (reg. no. 305/99/0695) and GA AS CR (reg. no. A5004702) were obtained.

Project 7305 / GA CR 202/97/0874

The structure of interphase nucleus and its changes after irradiation

Principal Investigator Stanislav Kozubek

Co-Investigator: Václav Kroha

Investigators: Emilie Lukášová, Eva Bártová, Alena Cafourková, Pavla Jirsová, Michal Kozubek, Magdalena Skalníková

Technicians: Vladimíra Fučíková, Hana Křivánková

We have continued our studies on the interphase chromatin structure using the densely ionizing radiation (in collaboration with Institute of Nuclear Physics AS CR). The methodological work describing the hardware and software used in our cytometers was published. Studies of the topography of 9/22 chromosomes, ABL/BCR genes and the induction of exchange aberrations in the nuclei of G_0-lymphocytes were completed and also published. The results of these studies show, that the origin of chromosomal aberrations can be deduced from the known organization of chromosomes in the cell nucleus. Two other papers, describing the topographic changes of genes and centromeres in different phases of the cell cycle and during cell differentiation were accepted for publication. It follows from these studies that changes of the chromatin structure are associated with changes of gene expression. For example, during the myeloid differentiation, the condensation of the chromosome 8 and the change of its localization in the cell nucleus take place simultaneously with changes of the expression of the c-myc gene localized in this chromosome. Some progress in the study of chromatin structure during the process of DNA repair was achieved. It seems, that the process of DNA repair is accompanied by chromatin decondensation and the termination of this process leads to the initial state of chromatin condensation. The decrease of the mutual distances between homologous genetic loci observed during the chromatin decondensation corresponds to the radial displacement of the genetic loci, and is not related with the mutual interaction of homologous chromosomes in the recombination reparation. The knowledge accumulated from our studies of the topography of genes, centromeres and chromosomes in the nuclei of different cells enable as to make some generalization. For example, it is evident that the localization of genetic regions in cell nuclei can be characterized by their specific distances from the nuclear centre, however, their angle distributions are essentially random. If there are more than two copies of a gene or a chromosome in the cell nucleus they all have the same topographic characteristics as compared with the loci in the diploid nucleus. The same genes, centromeres or chromosomes have very similar topography in different cells of human blood. These results are prepared for the next publications.

Project 8322 / GA CR 202/98/P253

Changes in the structure of interphase nuclei of human leukemic cell lines after treatment with differentiating agents and after gamma-irradiation

Principal Investigator Eva Bártová

Guarantor: Stanislav Kozubek

In the frame of this postdoctoral grant we investigate the nuclear topography of centromeric heterochromatin and the topography of some chromosomes and genes such as *c-myc*, *abl* and *bcr*. Chromatin structure is detected in the experiments concerning myeloid differentiation and after gamma irradiation of leukemic cells. In the case of radiobiological experiments the positioning of centromeric heterochromatin of chromosomes 1, 8, 9, 12, 17, 18 and X was determined. The results were presented at the conferences (Fluorescence Microscopy and Fluorescence Probes; XXII Days of Radiation Hygiene).

The topography of the *c-myc*, *abl*, *bcr* genes was studied during myeloid differentiation of leukemic cells. The reason was that these genes are important in the development of chronic myelogenous leukemia (CML). The appearance of the *bcr/abl* oncoprotein is fundamental phenomenon in the pathogenesis of CML, however, it is known that chimeric oncoprotein *bcr/abl* requires *c-myc* gene for its activity. The nuclear topographic characteristics of the mentioned genes were summarized and the manuscript was accepted for publication. The nuclear organization of the *c-myc* gene, its relationship to the nuclear topography of centromeric region of chromosome 8 and whole chromosome 8 location in comparison with chromosome 8 condensation are described. These nuclear parameters are associated with the changes in the *c-myc* gene expression.

Project 415 / IGA MH CR, NM15-3

Use of the interphase FISH technique for screening of individuals with high risk of haemoblastic malignancies

Principal Investigator Stanislav Kozubek

Co-Investigators: Josef Drbal, Jan Trnka

Investigators: Emilie Lukášová, Eva Bártová, Michal Kozubek, Alena Cafourková, Pavla Jirsová

Topographic characteristics of specific chromosomes and genes that are involved in translocations typical of different types of non-Hodgkin lymphoma (NHL) were studied at individuals at which some of these diseases were detected immunohistochemically. The topological parameters of specific genetic loci were studied in lymphocytes isolated from the peripheral blood and in bone marrow cells. In two individuals with mantle cell lymphoma, the topological parameters of

genes BCl 1 (11q21) and IgH (14q32) were compared not only between lymphocytes from the peripheral blood and bone marrow cells but also with cells isolated from the lymphatic nodes, that were affected by the translocation between these genes. Topography of genes and chromosomes is followed in different phases of the cell cycle of bone marrow cells and lymphatic nodes and in G_0 phase of T lymphocytes. The infiltration of malignant cells from the lymphoma (containing the characteristic genetic change) into peripheral blood and bone marrow cells is also evaluated. The presence of genetic translocations is determined in metaphase spreads.

The biological material was obtained from the Masaryk Oncological Institute: bone marrow cells and peripheral blood from six individuals with follicular lymphomas, with typical translocation t(14;18), five individuals with diffuse large cell lymphomas, with characteristic t(14;18) and also other changes such as t(3;14) and trisomy 12, two individuals with mantle cell lymphomas characterized by translocation t(11;14), one individual with T-cells chronic lymphocytic leukemia, one individual with B-cell chronic lymphocytic leukemia, with characteristic trisomy 12 and the deletion of 11q, and four individuals with non identified NHL.

The evaluation of genetic changes is performed by means of FISH using the chemically modified DNA probes specific for the assumed genetic change (translocation). If the assumed translocation is not detected, the FISH is repeated with other combination of probes. The DNA probes for some translocation break-points (IgH, BCL1, BCL2) are prepared after amplification of the specific cosmide and PAC clones and DNA isolation from these clones.

So far, no genetic changes were found in bone marrow cells and in lymphocytes isolated from the peripheral blood of nine analyzed patients. The detection of the characteristic genetic translocation in these cells can be found only in the case of their infiltration by malignant cells from the affected node. It follows from our results that the peripheral blood and bone marrow cells of these individuals were not infiltrated by malignant cells. The same conclusion was obtained from the immunohistological analysis. The topography of specific chromosomes and genes is studied in both cell types of all mentioned individuals. The parameters obtained are compared with these parameters in B and T lymphocytes of healthy individuals. The results are evaluated and they will be known at the end of the next year. They should show whether there are some differences in the topography of critical loci between healthy and affected individuals that could predict the risk of specific malignancy.

Project VS97031, ME CR

The use of image analysis in the study of mechanisms of the induction, in diagnostics and for the prevention of deleterious human diseases

Principal Investigator Stanislav Kozubek

Co-Investigator: Emilie Lukášová

Co-Investigators: Michal Kozubek, Eva Bártová, Magdalena Skalníková, Adriana Jergová, Andrea Marečková, Alena Cafourková, Pavla Jirsová

Both the hardware and the software of the high resolution confocal cytometer (HRCM II) was finished and the whole system was activated. HRCM II consists of the automated microscope ZEISS Axiovert 100, confocal unit CARV (Atto corp., USA) and CCD camera MicroMax (Princeton Instruments). The whole system is driven by personal computer equipped with two Intel Pentium II/350 MHz processors. This equipment makes it possible to automate the acquisition in confocal mode and the combination of the both confocal and the conventional modes. The advantages of both modes remain preserved. Conventional mode enables fast image acquisition and the determination of the positions of small (point) light sources (e.g. visualized genes, centromeres etc.) but it cannot analyze objects with a stained volume because the images are blurred by the non-confocal beams. Objects with the stained volume usually provide substantially more light and therefore the confocal mode does not prolong the acquisition time.

The laboratories were equipped with a new furniture and also with modern instrumentation for molecular and cell biology in Faculty of Informatics, Masaryk University Brno. The software was optimized for the acquisition and the image analysis, evaluation of the chromatic aberrations and resolution of the whole system. The driving software has been adapted for supercomputer of the Faculty of Informatics.

Many biological experiments have been performed in our laboratory, a part of the results was published in impacted journals and the other part will be published in a short time. In addition to the analysis of the topographic characteristics in human haemopoetic cells, the first experiments with tissues were also done. These experiments showed that FISH together with the image analysis were applicable in looking after the characteristics of the health and tumor tissues. The goal of our future research is finding the acceptable parameters that would characterize the tumor and could be used for the diagnostics.

Project 409 / JINR Dubna

Detection of stable aberrations in human lymphocytes irradiated with heavy ions

Principal Investigator Stanislav Kozubek

Co-Investigator: Evgenij A. Krasavin

Investigators: Emilie Lukášová, Eva Bártová, Pavla Jirsová, Alena Cafourková, (CR), Raisa D. Govorun, Michail Repin (Russia)

Technicians: Vladimíra Fučíková, Hana Křivánková

The results can be divided in two parts according to the type of radiation used for irradiation of human lymphocytes. Chromosome aberrations induced by different doses of protons with the energy of 1 GeV were evaluated in the first mitosis after irradiation of lymphocytes by the classical cytogenetic analysis in the whole genome and by FISH in chromosomes 1 and 2. The results were compared with the frequencies of aberrations induced in human lymphocytes with gamma rays (0.66 MeV; dose rate 3.6 Gy/min). It follows from this comparison, that there is no difference in the total number of aberrations induced by the same doses of both radiation types in the whole genome and in chromosomes 1 and 2. The results show also that the fraction of aberrations induced in chromosomes 1 and 2 with both types of radiation represents about 35 % of aberrations induced in the whole genome. Translocations were the most frequent type of aberrations induced by both radiation types. It follows from these results that the damages induced in the genome by the particles of very high energy does not differ from those induced by the sparsely ionizing radiation of gamma rays.

Exchange aberrations were detected between 9 chromosomes in human lymphocytes irradiated with He ions of mean energy of 30 MeV using the repeated FISH the fifth day after irradiation. Two different chemically modified DNA probes specific for two different chromosomes were used in each hybridization. The positions of individual mitoses on microscope slide were recorded after the first hybridization and used in all successive experiments for automatic scanning of images. The comparison of images from 5 successive hybridisations allowed us to identify the exchanges among the selected chromosomes. The frequency of aberrant mitoses was about 30 times lower the fifth day after irradiation than in the first mitosis indicating that during this period of time, the cells that were not damaged and those that were damaged only slightly were divided preferentially. 70 % to 80 % of aberrant cells contained only one damage in identified chromosomes. The translocations were the most frequent but there were also fragments and numerical aberrations (trisomies). The exchange aberrations that were found most frequently in the first mitoses were detected also in aberrant cells the fifth day after irradiation. The results show that the individual exchanges induced by the densely ionizing radiation can be stable in dividing cells.

Project 9322 / GA CR 202/99/0959

Employment of multiple-beam optical traps for controlled manipulation and rotation of microobjects

Principal Investigator Miroslav Liška, Technical University, Brno

Co-Investigators: Pavel Zemánek, Emilie Lukášová

Investigators: Zdeněk Harna, Jiří Kršek, Miloš Jákl, Alexandr Jonáš, Libor Šrámek, Josef Lazar, Ondřej Číp, Pavel Pokorný, Stanislav Kozubek, Eva Bártová

A system which enables creation of several optical traps by splitting the laser beam in two parts and by using an optical deflector in one of these parts was developed. This system is combined with an UV pulse laser so that a complex apparatus for optical trapping and cutting is obtained. Movable lenses ensure independent 3D positioning of a beam focus of the trapping and cutting beam without power losses at the back objective aperture. The pulse length is short enough to prevent the heat to spread to the surroundings. This unique tool is convenient for the operations inside the living cells without their injury. The trapping and cutting beam was used for the fusion of human promyelocytic HL 60 cells and for optical rotation of these cells.

PROGRAMME 4

Relationships between the structure and function of proteins as studied by methods of protein engineering

Project 6323 / GA CR 206/96/K188

Cytokinins and auxins in regulation of plant development

Principal Investigator Ivana Macháčková, Institute of Experimental Botany AS CR, Prague 6

Grant Co-Recipient: Institute of Biophysics AS CR

Co-Investigator: Břetislav Brzobohatý

Investigators: Hana Bubeníčková, Jan Hejátko, Jan Nejedlík, Alena Reková

Project 420 / ME CR, programme KONTAKT, NSF, ES040

A phytohormone specific β-glucosidase as a tool to investigate the subcellular compartmentation of phytohormone conjugation

Principal Investigator Břetislav Brzobohatý

Project National Science Foundation, USA, grant INT-9600462

U.S. - Czech Plant Research on Subcellular Compartmentation of Phytohormone Conjugation

Coordinator Natasha V. Raikhel, Michigan State University, East Lansing, USA

Principal Investigator v CR Břetislav Brzobohatý

The goal of our research is to understand the subcellular compartmentation in regulation of phytohormone activity by reversible conjugation. We explored the feasibility of re-direction of cytokinin-O-glucoside specific β-glucosidase Zm-p60.1 into selected subcellular compartments, and evaluated the ability of the re-directed enzyme to release active cytokinins from their inactive forms, cytokinin-O-glucosides.

Information in *Zm-p60.1* cDNA, specifying subcellular targeting, was modified to achieve re-direction of individual Zm-p60.1 derivatives from plastids/chloroplasts into (i) vacuole (*Zm-p60.vl*, *Zm-p60.vc*), and (ii) secretion into extracellular space (*Zm-p60.ex*). Individual recombinant cDNAs were placed under control of a strong constitutive promoter, CaMV35S, and used in constructing transgenic tobacco plants.

Experimental work was focused on the confirmation of successful re-direction of Zm-p60.1 derivatives in transgenic tobacco plants. Extensive pulse-chase experiments revealed that the proteins were properly translocated into the tobacco secretory system and that the signal sequence used to direct them into the ER was removed by an ER signal peptidase. In addition, the barley lectine CTPP in Zm-p60.vl became glycosylated and subsequently removed from the preprotein, indicating passage through the ER and Golgi apparatus and redirection from the default secretory pathway into the vacuole. Accumulation of processed Zm-p60.vl and Zm-p60.vc in protoplasts indicated their retention in the vacuole. Zm-p60.ex accumulated in the incubation medium which indicated its secretion. The subcellular localization in transgenic tobacco plants of the native Zm-p60.1 and its derivatives with altered targeting information, were examined by indirect fluorescent confocal microscopy and by electron microscopic immunocytochemistry. Together with the pulse-chase labeling experiments, the results of indirect fluorescent confocal microscopy and electron microscopic immunocytochemistry proved that targeting of Zm-p60.vl and Zm-p60.vc to the vacuole, and Zm-p60.ex to the extracellular matrix was achieved. These experiments represent first successful re-direction of a plastid enzyme into the vacuole, and extracellular matrix.

Project 430 / European Commission, DG XII, programme INCO-COPERNICUS, grant PL966135

The role of phytohormones in control of plant development and architecture

Coordinator Klaus Palme, Max-Delbrück-Laboratorium in der Max-Planck-Gesellschaft, Köln, Germany

Grant Co-Recipient: Institute of Biophysics AS CR

Principal Investigator Břetislav Brzobohatý

Investigator: Jan Hejátko

We isolated the knock-out mutant of *Arabidopsis thaliana* with an insertion of the maize transposon En-1 in the exon 5 of the *CKI-1* gene. The absence of the *CKI-1* function allowed to study the biological role of the putative cytokinin receptor CKI-1. 25% of the progeny derived from self-pollination of the mutant plant were expected to be homozygous for *En-1::cki1* allele, assuming the Mendelian inheritance. Since the mutation was expected to be recessive, 25% of the progeny were to exhibit a phenotypic alteration. However, no visible phenotype alteration

was detected in any of the vegetative stages. Therefore, Southern blot analysis was performed to ascertain the presence of *En-1::cki1* allele in the genome. The molecular analysis revealed that none of the 36 tested plants carried *En-1::cki1* allele in the homozygous state. Thus, *En-1::cki1* allele might be lethal for one of the gametes. Presently we perform an extensive crossing experiment to test this eventuality. In this respect, the microscopic analysis will be applied to study gamete development in the mutant.

As the first step in a biophysical characterization of CKI1 protein, a member of a two-component histidine kinase family, CKI1 putative extracellular domain was expressed in an *E. coli* expression system. The extracellular domain expected to bind cytokinins, shares the lowest degree of homology with other two-component histidine kinases. Thus, the extracellular domain represents the smallest part of the protein that might allow us to study interactions between CKI1 and individual cytokinins. Simultaneously, it is the best region to be used for raising specific anti-CKI1 antibodies. Sequence coding for the extracellular domain was fused to a sequence coding for a His-tag, and expressed in *E. coli*. The recombinant protein accumulated almost exclusively in inclusion bodies preventing direct examination of cytokinin binding activity. The recombinant protein was purified by IMAC chromatography under denaturing conditions and used for raising specific anti-CKI1 antibodies in rabbits. To obtain a functional form of the extracellular domain, renaturation of the inclusion bodies was initiated.

Project 9116 / GA AS CR A7004902

Involvement of the chromosomal protein HMG1 in DNA end joining by DNA ligases

Principal Investigator Michal Štros

Co-Investigator: Anna Kolíbalová

Technician: Božena Krönerová

In the course of the study of the ability of HMG1 to bend DNA by circularisation of short DNA fragments by T4 DNA ligase we have found that HMG1 could significantly stimulate DNA ligation. We have shown that the „HMG box“ domain B of HMG1 is responsible for the ability of the protein to enhance DNA ligation by T4 DNA ligase. In our further experiments we have studied the effect of amino acid sequences flanking the domain B on its ability to stimulate DNA ligation. By comparison of a number of domain B polypeptides of different lengths we have shown that the C-terminal flanking sequence of the domain B is necessary for an efficient stimulation of DNA ligation by T4 DNA ligase. Using site-directed mutagenesis we have identified a number of conserved amino acid residues (mainly basic or hydrophobic), the mutation of which significantly diminished the ability of the HMG box domain to stimulate DNA end joining. Pull-down assay, electron and scanning force microscopy revealed that HMG1 could promote (non-covalent) association of DNA molecules via their ends. These

findings have led us to propose that HMG1 stimulates DNA end joining by functionally and evolutionarily different DNA ligases (T4 DNA ligase, human DNA ligase IV) by virtue of its ability to "bridge" ends of two DNA molecules while leaving them accessible for ligation.

Project 9316 / GA CR 301/99/0691

Involvement of chromosomal proteins HMG1 and HMG2 in transcription. Identification of novel cellular binding partners for HMG1 and 2

Principal Investigator Michal Štros

Co-Investigator: Anna Kolíbalová

Technician: Božena Krönerová

Octamer transcription factors and tumor-suppressor protein p53 were isolated from *E. coli* or lysates of baculovirus-infected insect cells to study the influence of HMG1 on their binding to DNA. PCR (polymerase chain reaction) and site-directed mutagenesis were used to construct the individual mutants of the HMG1 domain B. The presence of the mutations was verified by DNA sequencing.

In our preliminary experiments we have found that HMG1 could enhance binding of tumor-suppressor protein p53 or its DNA-binding core domain (p53CD) on DNA. The subtle differences in stimulation of p53 binding on DNA depend on the DNA sequence and are likely related to the bendability of the DNA.

PROGRAMME 5

Mechanisms of the control of proliferation, differentiation and apoptosis in cell populations

Project 9306 / GA CR 524/99/0694

Polyunsaturated fatty acids and cytokines - their role in maintenance of homeostasis at the cell population level

Principal Investigator Alois Kozubík

Co-Investigators: Jiřina Hofmanová, Jiří Pacherník, Jan Vondráček, Jaromíra Netíková, Jiřina Holá, Martina Kovaříková, Alena Vaculová, Robert Janík

The goal of the project is to obtain a more detailed information about interactions between cytokines TGF-β1 and TNFα and intracellular signaling molecules involved in regulation of cell proliferation, differentiation and apoptosis in human leukemia cell line HL-60. Attention was focused on understanding of cooperation of these molecules with polyunsaturated fatty acids (PUFAs) and their products - eicosanoids. The project was resolved in two levels:

I. The character and significance of interactions of the listed molecules was described. An extensive data set of "dose response" characteristics reflecting cytokinetic parameters after treatment of cells with individual compounds was obtained. Following mathematical evaluation, these data will be utilized for "optimization" of the model suitable for studies of combined effects.

II. Studies on mechanisms underlying these effects were started. 1) We demonstrated that *i*, combined treatment with TGF-β_1 and all-*trans* retinoic acid (ATRA) is accompanied with enhancement of differentiation and shift towards monocytic lineage (ATRA is known as inducer of granulocytic differentiation of HL-60 cells), as supported by increased CD11b/CD14 cell surface antigen expression and oxygen radical production in comparison to effects of ATRA alone; *ii*, ATRA treatment alone lead to spontaneous apoptosis while treatment of cells with ATRA and TGF-β1 results to the significantly decreased number of apoptotic cells (and thus increased viability) in comparison with ATRA alone; *iii*, inhibition of apoptotic and cytotoxic effects of ATRA is independent of Bcl-2 protein expression (a major regulator of programmed cell death which is down-regulated during ATRA-induced differentiation). These results documented an important role of TGF-β1 in regulation of both differentiation and programmed cell death induced by ATRA in myeloid cells. 2) The activity of jun-N-terminal kinase (JNK) on sub- and confluent human immortalized keratinocytes HaCaT after treatment with various inducers was investigated. It is known that the responses of sub- and confluent cell populations to extracellular stimuli are different. This can be caused by contact inhibition or by changes of activity of the key signal molecules such as JNK that may be activated by various extracellular

stimuli (cytokines, mitogens, etc.). We demonstrated that cell confluency can inhibit the induction of the JNK activation detected as JNK/c-Jun phosphorylation and JNK nuclear translocation.

Another part of the study was focused on methodology. The conditions for gene transfection in HL-60 cells using electroporation were optimized, since in hemopoietic cells only very low yields have been reached so far (~1%). Using plasmids phGFP–S65T and CMV–βgal and post–pulse separation of live cells by gradient centrifugation we found that optimal conditions for HL-60 cells electroporation range from 275 to 325 volts for voltage, 750–900 μF for capacitance and 10–20 μg. Using these conditions, we achieved 11–14 percent of yield of positive transfected cells.

Project 8314 / GA CR 525/98/1266

Biochemical and cellular markers of toxic and carcinogenic effects of xenobiotics

Principal Investigator Miroslav Machala, VÚVL, Brno

Co-Investigator: Jiřina Hofmanová

Co-Investigators: Alois Kozubík, Jan Vondráček, Jaromíra Netíková, Jiřina Holá, Kateřina Minksová, Karel Souček

On three mammalian cell lines Hepa-1 (mouse hepatoma cells), HL-60 (human myeloid leukemia cells) and HaCaT (human keratinocytes) modulations of selected biochemical markers (enzyme activities of cytochromes - CYP1A and CYP4A and oxidative products of arachidonic acid) after exposure to the model xenobiotics, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and clofibrate, were studied. After treatment with TCDD the induction of CYP1A activity was observed. After treatment with clofibrate, changes in cytokinetics were observed, especially potentiation of differentiation in myeloid HL-60 cells.

Transgenic cell line H4IIEGudLuc was used for detection of dioxin-like activity, cell line MVLN for detection of estrogen activity and mouse hepatoma cell line Hepa-1 was used to detect parameters of oxidative stress like production of reactive oxygen species, decrease in glutathione concentration and lipid peroxidation. TCDD, 17beta-estradiol and redox-cycling quinones such as menadione and 2,3-dimethoxynaphtoquinone were used as model toxicants. We detected estrogenic and dioxin-like activities in several oxygenated polycyclic aromatic hydrocarbons.

The above mentioned approaches were used for detection of dioxin and estrogen-likes activities in the samples from atmosphere and stable dust in animal farms. In several samples, estrogenic activity was detected.

Project 8320 / 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 Jan Vondráček

Guarantor: Alois Kozubík

We investigated, whether DMSO-induced differentiation can increase sensitivity of HL-60 cells to apoptic signals mediated by Fas- and TNF receptors (TNFRI and II). The study was based on fact that Fas and TNFR I have similar structure and they share some elements of signaling pathways. The results were also compared with results from cells treated with another differentiation inducer, *all-trans* retinoic acid (ATRA). Under standard conditions (10% fetal bovine serum), HL-60 cells show only low sensitivity to Fas-mediated signaling. However, a significant up-regulation of Fas expression was detected in DMSO-treated cells. In serum-free conditions, almost 40% of the cells pre-treated with DMSO underwent apoptosis after 16h incubation. However, the observed up-regulation was probably not solely responsible for this effect. Increased apoptosis was also observed in DMSO-pre-treated cells incubated with TNF-α where no upregulation of expression of either TNFR I or II was observed after treatment with DMSO. HL-60 cells were sensitive to apoptosis induction by TNF-α also when grown under standard conditions. Moreover, DMSO potentiated action of TNF-α, while ATRA almost completely abrogated apoptosis induced by this cytokine. Since a number of authors have shown that Bcl-2 may modulate sensitivity of some cell types to Fas-induced apoptosis, we studied expression of this protein during differentiation of HL-60 cells. However, its down-regulation was more pronounced in ATRA-treated cells, where no increase in apoptosis was observed. The observed effects of both death receptor agonists represent an interesting model that could provide insight into mechanisms that make myeloid leukemia cells resistant to apoptotic stimuli during differentiation.

Project 417 / IGA MH CR 4095-3

Metabolic and regulating effects of lipids in artificial nutrition

Principal Investigator Zdeněk Zadák, Faculty Hospital KU, Hradec Králové

Co-Investigator: Jiřina Hofmanová

Co-Investigators: Alois Kozubík, Martina Kovaříková, Alena Vaculová

Technician: Eva Koukalová

In the cell line HT-29, derived from human colon adenocarcinoma, we studied: (1) dependence of cytokinetic parameters on quantity and type of n-3 and n-6

polyunsaturated fatty acids (PUFAs), (2) interaction of cytokine TNFα with arachidonic acid (AA) metabolism and the consequences of these interactions on behavior of cells in different stage of differentiation induced by sodium butyrate. The effects of n-6 PUFAs (linoleic and AA) and n-3 PUFAs (alpha-linolenic and eicosapentaenoic) on cell cycle, proliferation, viability and apoptosis were time- and concentration-dependent. High PUFA concentrations (100 μM) decreased cell proliferation and viability and transiently blocked the cells in S-phase. Similar effects were observed after longer treatment (96-120 hours) with higher concentrations of TNFα (15-30 ng/ml). After combined treatment of cells with AA and TNFα we observed additional (1) increase of number of cells in S-phase, and (2) increase of the number of apoptotic cells (decrease of cell viability). Specific inhibitors of AA conversion, indomethacin (inhibitor of cyclooxygenases) and nordihydroguiaretic acid (inhibitor of lipoxygenases), decreased the proliferation of both non-differentiated and differentiated cells. On the other hand eicosatetraynoic acid (competitive inhibitor of AA metabolism) inhibited the proliferation of non-differentiated but not differentiated cells. After combined treatment of cells with TNFα and inhibitors mentioned above the effects on cell proliferation were mostly additive and did not depend on the level of differentiation.

Project 9312 / GA CR 306/99/0027

Enhancement of G-CSF action by adenosine signaling: Testing of its potential clinical use in murine models

Principal Investigator Michal Hofer

Co-Investigators: Jiřina Holá, Jaromíra Netíková, Iva Pipalová, Milan Pospíšil, Antonín Vacek, Lenka Weiterová-Juchelková

Reassuming our previous experiments, we assessed the frequency of micronucleated polychromatic erythrocytes (MNPCEs) in the mouse bone marrow. The influence of dipyridamole (DP) and/or adenosine monophosphate (AMP) was tested in unirradiated animals and those irradiated with a sublethal (6.5 Gy) dose of gamma-rays.

In nonirradiated mice, the application of the drugs led to slight but significant elevation of the frequency of MNPCEs. On the contrary, the frequency of MNPCEs induced by irradiation was significantly reduced by preceding administration of DP or AMP and, in particular, of their combination.

The changes observed in the bone marrow cells may be explained by a supposed mechanism of the action of DP and AMP, mainly by the elevation of extracellular adenosine and activation of adenosine cell receptors with following cardiovascular effects evoking hypoxia in tissues.

Project 410 / MPO CR PZ-Z2/25/97

Radioprotective and chemoprotective effects of the immunomodulators adamantylamide dipeptide (AdDP)

Principal Investigator Michal Hofer

Co-Investigators: Iva Pipalová, Antonín Vacek, Lenka Weiterová-Juchelková

Technician: Věra Reichmannová

Administration of the synthetic immunostimulatory compound adamantylamide dipeptide (AdDP) was found to induce stimulation of haematopoiesis in mice exposed to sublethal doses of ionizing radiation and to increase survival of experimental animals irradiated with a lethal dose. These results suggest that it is possible to extend the spectrum of clinical indications for the administration of AdDP to the conditions of haematopoietic suppression, especially in oncological practice.

Project 7326 / GA CR 308/97/1141

The role of oxygen metabolites and nitric oxide in the development of intestinal reperfusion injury

Principal Investigator Antonín Lojek

Co-Investigators: Milan Číž, Hana Čížová-Slavíková

Technician: Lenka Vystrčilová

The influence of ischemia/reperfusion (I/R) on the formation of reactive oxygen and nitrogen metabolites was studied using the laboratory rat small intestine ischemia/reperfusion model. Arteria mesenterica superior was occluded for a period of 45 minutes. Blood samples were collected 2 and 4 hours after releasing the clamp. In plasma samples, the Griess reaction was utilized to observe a significant increase in the nitrite ion concentration, which is an indirect proof of nitric oxide synthase (NOS) activation after I/R. Further experiments, based on continual chemiluminescence (CL) analysis, served to confirm increased metabolic activity of neutrophils in the whole blood. To elucidate NOS contribution to the overall CL activity, 1 mM L-NAME (N-nitro-L-arginine methyl ester) known as an NOS inhibitor, and 2.5 mM L-arginine (a substrate for NOS), were gradually added to the reaction mixture. While L-NAME significantly decreased the CL activity, a subsequent addition of L-arginine resulted in its parial restoration. These results confirm a significant role of NOS in increasing oxidant production after I/R. Oxidative stress was manifested by increased plasma lipid peroxide levels. Further experiments were therefore designed to ascertain a possible applicability of L-NAME in reducing oxidative

stress *in vivo*. A similar objective was pursued when applying substances with antioxidant effects (dimethylthiourea) and iron chelators (deferoxamine). The experiments are continued.

Project 8301 / GA CR 524/98/0190

The role of endogenous antioxidants in the regulation of post-ischemic oxidative stress

Principal Investigator Milan Číž

Co-Investigators: Antonín Lojek, Hana Čížová-Slavíková, Lukáš Kubala

Technician: Blanka Panáková

The Wistar rat small intestine ischemia/reperfusion model was also employed in advancing studies on the oxidative stress. The results have confirmed increased total antioxidant capacity (TRAP) in serum as a consequence of ischemia/reperfusion (2-4 hours). Among the individual low-molecular antioxidants studied, only uric acid produced changes comparable with TRAP (correlation coefficient r = 0.93). After a slight decrease (2 hours of reperfusion), the total SH-groups returned to the control values (4 hours of reperfusion). These changes were induced almost exclusively by changes in protein SH-groups while the contribution of non-protein SH-groups was negligible. Ischemia/reperfusion of the small intestine also brought about reduced levels of serum albumin and ceruloplasmin. Out of enzymatic antioxidants, we measured superoxide dismutase activity where a mild increase was observed after both 2- and 4-hour reperfusion. Increased TRAP did not prevent lipid peroxidation in the intestinal mucosa.

Having ascertained previously that allopurinol (xanthin oxidase inhibitor) fed to rats in drinking water at a dose of 8.73 mg/day for a period of one week had no significant effect on changes induced by the ischemia/reperfusion of the intestine, allopurinol was applied to rats intraperinoneally 30 minutes prior to operation at a dose of 50 mg/1 kg of body weight. Thus applied allopurinol eliminated above all the ischemia/reperfusion induced changes in leukocyte numbers and metabolic activity, in the serum total antioxidant capacity and uric acid concentration, and in lipid peroxidation of the intestinal mucosa. On the one hand, *in vitro* experiments proved that allopurinol has no direct trapping effects concerning the peroxyl radical, while on the other hand, it does influence the superoxide radical production through the xanthin/xanthin oxidase system.

Project 437 / IGA MH CR 4796-3

Reactive oxygen species in relation to hemodialysis and kidney transplantation

Principal Investigator Vladimír Soška, Faculty Hospital U sv. Anny, Brno

Co-Investigators: Milan Číž, Antonín Lojek, Hana Čížová-Slavíková, Lukáš Kubala

We ascertained the modified expression of the CD11b/CD18 and CD62-L antigens on neutrophils during hemodialysis. Compared with blood samples taken from patients before hemodialysis, the blood samples obtained after hemodialysis showed increased CD11b/CD18 expression while that of CD62-L was reduced. Results obtained hitherto from monitoring phagocyte chemiluminescence (CL) have provided evidence for a reduction in their metabolic activity after hemodialysis. This trend was observed while using various activators - starch, fMLP and opsonized zymosan particles. Further analyses confirmed a significant reduction in the total antioxidative capacity in the plasma of patients after hemodialysis, manifested by an elevated malondialdehyde concentration in the plasma samples, documenting an increased lipid peroxidation. The results of analyses of samples collected from patients after kidney transplantation could not be evaluated as yet due to the non-homogeneity of the group.

Project 9327 / GA CR 524/99/D022

The influence of different time of ischemia and reperfusion upon the development of reperfusion injury of intestine

Principal Investigator Hana Čížová-Slavíková

Guarantor: Milan Číž

October 1999 set off the first investigation stage of a postdoctoral grant devoted to the time-related development of ischemia/reperfusion injury of the small intestine of the laboratory rat. We concentrated our attention to the changes of neutrophil metabolic activity, total antioxidant capacity of the plasma, and the relative contribution of individual antioxidant components to the intestinal damage. To refine the methodology, a new spectrophotometrical technique of detecting ascorbic acid concentration in the serum has been introduced. Lipid peroxidation in blood serum and in the intestine is evaluated as a measure of oxidative stress induced by ischemia/reperfusion. In agreement with the time schedule for the project, this initial stage will be completed and assessed in June 2000.

Laboratory of computer and information services (CIS)

Responsible Person Josef Jursa

Technician: Martin Varga

Standard services of the laboratory:

- Operation, servicing and development of the IBP local area network (LAN)
  - Operation of the connection of the IBP LAN to Brno Academic Computer Network (BACN) and to the Internet
  - Cary on e-mail server
  - Cary on www server of the IBP (http://www.ibp.cz) including data updating
  - Current maintenance and development of computer technique (hardware and software), utilized by all projects at the IBP (servers, graphic workstations and simple PCs with Internet access); hardware is operating under UNIX, MS Windows NT, MS Windows95 a MS DOS systems.
  - Consulting and guidance services for individual projects (an expert assistance in problems with computer technique and computer network).
- Operation and servicing of a ICCBnet (International Center for Cooperation in Bioinformatics network) national node of the Czech Republic - http://ICCBnet.ibp.cz
- Mirroring of the Protein Database (PDB) accessible through the Internet
- Sequence databases and accompanying software - Wisconsin GCG package - accessible to 150 users from Academy of Sciences and universities in the Czech Republic
- Operation and servicing of a library server used by Academy of Sciences in Brno region

In the beginning of the 1999 there was installed the BSD/OS operating system on a new hardware of the IBP server – two processor PC with a disk array.

Terminal server was installed to make the IBP LAN accessible to users by telephone lines, which enables distant working.

The information system on the IBP www server (http://www.ibp.cz) was substantially extended.

Project 435 / ME CR 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 Josef Jursa

In the 1999 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). Database server SGI Origin200 was extended (processor, disk space and more powerful UPS were added) and databases were installed – structural Protein Database (PDB) and sequence databases with software for data processing – Wisconsin GCG package. PDB is accessible to the users in Central Europe through the Internet and GCG package is utilized by 150 academic users (Academy of Sciences and universities) in the Czech Republic. The project also established a redundant connection of the IBP to the Brno Academic Computer Network to ensure uninterrupted accessibility of the database server.

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Termination of DNA synthesis at the adducts of platinum antitumor compounds

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Interaction of PNA and DNA oligomers with charged surfaces

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Europien School of Haematology – Seventh European Conference on Apoptosis, Ein Gedi, Israel, 14. – 17. 11. 1999

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Vorlíčková, M.:
Cirkulární dichroismus – princip a použití při studiu struktury biopolymerů

Přednáška pro posluchače kurzu Biofyzikální chemie, PřF MU, Brno, 2. 11. 1999

Vorlíčková, M.:
CD spektroskopie

Přednáška pro posluchače kurzu Biofyzikální chemie, PřF MU, Brno, 29. 11. 1999

Vrána, O., Brabec, V.:
DNA interstrand cross-linking by antitumor cisplatin depends on nucleotide sequence, supercoiling and temperature

5thInternational Symposium on Applied Bioinorganic Chemistry, Corfu, Greece, 13. – 17. 4. 1999

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Roles of DNA methylation in plant development

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DNA methylation and histone acetylation studies in Silene latifolia

Graduate School of Enegineering, Osaka University, Osaka, Japan, 2. 11. 1999

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Highly selective DNA hybridization biosensors based on PNA probes
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Untraditional pharmacotherapeutic approaches to prevention and therapy of acute radiation disease
XXII. Days of Radiation Hygiene, Jáchymov, 16. – 18. 11. 1999
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III. Pracovní setkání biochemiků a molekulárních biologů, PřF MU Brno, 3. 2. 1999, poster

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Turánek, J., Záluská, D., Vacek, A., Hofer, M., Ledvina, M., Ježek, J.:
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Pollen sterility coincides with the ectopic maize glucosidase expression and amylogenesis defect
8th days of Plant Physiology, Olomouc, 7. - 10. 7. 1998
In: Book of Abstracts, nestr.

E. Overview of publication activities in 1999

1. Full-length papers published in 1999	53
supplementary papers due to RR 1998	6
2. Short communications published in 1999	1
supplementary comm. due to RR 1998	3
3. Chapters in monographs	4
4. Monographs, textbook	1
5. Scientific lectures	
presented in the CR	67
presented abroad	70
6. Abstracts presented	
conferences in the CR	36
conferences abroad	44
supplementary items due to 1998	
conferences in the CR	2
conferences abroad	2
7. Articles popularizing science	7
8. Citations in the Science Citation Index	
October - December 1998	168
January - September 1999	412

IV. INTERNATIONAL CONTACTS

As in the previous years, international contacts in 1999 were realized in connection with research projects supported by various grant agencies both from the Czech Republic and from abroad, or on the basis of competitions organized by the Academy of Sciences of the Czech Republic (hereafter the Academy of Sciences or AS CR) or as invitations, etc. The contacts were either active (study stays of scientists of the Institute of Biophysics and their participation in conferences abroad) or passive (stays of foreign scientists in the Institute of Biophysics).

In most cases the travel expenses have been covered by particular research grants and hence the full responsibility for the success and scientific contribution of the stays abroad has been taken by the particular investigators.

Only those scientists, who succeeded in a competition for travel grants in 1999, obtained a partial support for stays abroad from central funds of the Academy of Sciences. They received a part of a reciprocal quota allocated to a particular agreement of the Academy of Sciences with a foreign partner institution.

An overview of international contacts in 1999 is given in the following Tables:

Foreign guests

Country	Central funds of AS CR	Grants	Other sources (visitor's own expenses)
Austria			1
Finland		1	
France		1	
Germany		1	1
Great Britain			1
Hungary		1	
Israel	2		1
Japan			1
Russia			1
Slovakia		1	6
Serbia		1	
Turkey			1
USA		4	2
Uzbekistan			1
Total	**2**	**10**	**16**

Travels of scientists abroad

Country	Central funds of AS CR	Grants	Other sources (invitation, etc.)
Austria		2	1
Belgium	1	1	1
Chile		2	
Egypt			1
Finland	1		1
France		2	2
Germany		6	12
Great Britain	1	4	6
Greece		2	
Hungary		3	1
India		2	
Ireland		2	
Israel	1	1	3
Italy		4	
Japan			4
Poland		5	
Portugal		1	
Russia			1
Slovakia	2	7	5
Spain		1	
Sweden	1		
Switzerland		1	
Turkey			2
USA		5	6
Total	**7**	**49**	**46**

A. Overview of international cooperation and list of foreign grants

The joint research based on direct agreements with foreign laboratories or on grants received from abroad continued as given 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*

ISRAEL

Weizmann Institute of Science, Rehovot
J. Fajkus
Analysis of the structure of plant chromosome termini

SWITZERLAND

University of Zürich, Institute of Pharmacology and Toxicology, Zürich
V. Brabec
Nucleotide excision repair of DNA adducts of antitumor platinum drugs

2. Foreign grants

GERMANY

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

European Space Agency
S. Kozubek (1999)
Topical ream in biotechnology: Biotechnologies for environmental monitoring

GREECE

Laboratory of Physical Chemistry, Faculty of Sciences, University of Thessaloniki, Thessaloniki
V. Vetterl (1996 - 2000)
Programme KONTAKT, Ministry of Education of CR, EM 022
Interactions of biopolymers and *their components at interfaces*

ITALY

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

JAPAN

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

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/CZ Grant yielded under the agreement between NSF and ME CR, ME 152
V. Brabec (1998 - 2000)
DNA interactions of polynuclear platinum

National Science Foundation, Grant INT-9600462
MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing
B. Brzobohatý (1996-1999)
U.S.-Czech plant research on subcellular compartmentation of phytohormone conjugation
In CR: Programme KONTAKT, Ministry of Education of CR, EM 040
B. Brzobohatý (1996-1999)
A phytohormone specific β-glucosidase as a tool to investigate the subcellular compartmentation of phytohormone conjugation

OTHER FUNDING

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 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. D8/0012/97 (OC D8.40)
O. Vrána (1997 - 2001)
Platinum-linked nucleotides analogues as viruses inhibitors

INCO COPERNICUS PL 966135
B. Brzobohatý (1997 - 2000*)*
The role of phytohormones in control of plant development and *architecture*

Joint Institute of Nuclear Research Dubna, No. 409, Russia
S. Kozubek (1999 - 2000)
The structure of cell nuclei and *its relation to genetic changes induced by densely ionizing radiation*

3. Foreign scholarships and other support

E. Benková - a scholarship sustaining 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 two-year study stay at the Haartman Institute, University of Helsinki, Finland

V. Dražan - the Erasmus-Socrates scholarship supporting a ten-months study stay at the Institut für Chemie – Physikalische und Theoretische Chemie, Freie Universität Berlin, Germany

J. Hejátko – a six-months study stay at the Max-Planck-Institut für Züchtungs-forschung, Köln am Rhein, Germany

V. Hykelová – a doctoral scholarship for a study stay at Ecole Normale Supèrieure de Lyon of the French Ministry of Education, France

J. Malina – a scholarship for a six-months study stay at the Department of Chemistry, Virginia Commonwealth University, Richmond, USA

R. Matyášek - a scholarship granted by the Royal Society for a long-term study stay at the Queen Mary and Westfield College Laboratory, University of London, Great Britain

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

K. Říha – the NATO scholarship for one-year study stay at the Department of Biochemistry and Biophysics, Texas A. and M. University, USA

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

B. Stays abroad

1. Stays within the framework of agreements of the Academy of Sciences

BELGIUM

A. Kovařík accomplished his study stay at the Laboratorium voor Genetica, Universiteit Gent. The stay was focused upon the study of relation between DNA methylation and gene expression in transgenic organisms. The results proved connection between methylation of asymmetrical positions of cytosins and transcriptional activity of the reporter gene of three transgenic lines of *N. tabacum* having different levels of expression.

FINLAND

A. Lojek continued in his cooperation with the Department of Biochemistry, University of Turku and studied the formation of reactive metabolites of oxygen and nitrogen activated by phagocytes.

GREAT BRITAIN

M. Lexa was involved in the isolation of insertion mutants in defined genes of *Arabidopsis thaliana* to be used in the analysis of developmental processes. This work was done at the Department of Plant Sciences, University of Oxford.

ISRAEL

V. Vetterl continued in the investigation of surface layers, stimulating biological membranes, at the Weizmann Institute of Science, Rehovot. He was engaged in the measurement of the influence of alkyl chain upon the impedance of electrode double layer and the effect of ion channels on the conductance of the double-layer.

SLOVAKIA

J. Hofmanová and *A. Kozubík* continued in their cooperation with the Department of Molecular and Cell Biology of the Faculty of Science, UPJŠ and studied mechanisms of non-genotoxic carcinogenesis.

SWEDEN

E. Paleček visited the Lundberg Laboratory, University of Gothenburg to exchange the constructs of the p53 protein and to establish cooperation in constructing the DNA biosensors.

2. Participation at conferences and workshops

AUSTRIA

K. Krejčí participated in the 2nd European Cytogenetics Conference, Wien, 3. – 6. 7. 1999

A. Lojek participated in the Seventh Vienna Shock Forum, Wien, 13. – 16. 11. 1999

BELGIUM

H. Loskotová attended the EU-ESF Practical Training Course – Chemistry of Metals in Biological Systems, Louvain-la-Neuve, 14. – 24. 5. 1999

CHILE

H. Slavíková took part in the ICRO-UNESCO International Training Course and in the International Symposium on the Plant Polyphenols Antioxidants in the Biology and Pathology of Free Radicals, Santiago de Chile, 19. – 30. 7. 1999

FRANCE

J. Kašpárková and *H. Loskotová* participated in the 5th FGIPS Meeting in Inorganic Chemistry, Toulouse, 26. – 31. 10. 1999

GERMANY

M. Brázdová attended the EMBO Course on Protein Purification and Characterization, Berlin, 19. – 26. 9. 1999

M. Číž took part in the SFRR (Europe) Summer Meeting: Antioxidants, Adaptation, Aging, Dresden, 2. – 5. 7. 1999

C. Hofr participated in the 2nd International Conference on Application of Biocalorimetry, Halle, 28. – 31. 3. 1999

S. Kozubek took part in the ESACP Congress, Heidelberg, 10. – 11. 4. 1999 and in the seminar of researchers participating in the ESA Topical Team in Biotechnology, Köln am Rhein, 28. – 30. 4. 1999

K. Minksová and *J. Vondráček* participated in the 9th Annual Meeting of SETAC-Europe, Leipzig, 25. – 29. 5. 1999

GREAT BRITAIN

V. Brabec and *J. Kašpárková* participated in the BIOMED and COST D8 Programme St. Anne´College conferences, University of Oxford, 27. – 28. 3. 1999 and the 8th International Symposium on PLATINUM and other metal coordination compounds in CANCER CHEMOTHERAPY, Oxford, 28. – 31. 3. 1999

R. Matyášek participated in the Annual Conference of the Royal Society, London, 15. – 17. 6. 1999

GREECE

V. Brabec and *O. Vrána* participated in the 5th International Symposium on Applied Bioorganic Chemistry, Korfu, 13. – 17. 4. 1999

HUNGARY

V. Vetterl participated in the 5th International Microsymposium on Electrochemical Impedance Analysis, Balatonfoeldvar, 31. 5. – 3. 6. 1999

INDIA

V. Brabec and *O. Nováková* participated in the International Symposium on Recent Trends in Biomedical Research, Mumbai, 27. – 29. 10. 1999

IRELAND

M. Hofer participated in the Eleventh International Congress on Radiation Research, 18. – 23. 7. 1999

ISRAEL

J. Fulneček and *J. Jursa* participated in training of administrators of National Nodes of the International Center for Cooperation in Bioinformatics Network, Rehovot, 11. – 20. 7. 1999

E. Paleček participated in the Novel Approaches in Biosensors and Rapid Diagnostic Assays, Eliat, 10. – 14. 10. 1999

J. Vondráček participated in the 7th European Conference on Apoptosis, Ein Gedi, 14. – 17. 11. 1999

ITALY

M. Fojta and *E. Paleček* took part in the 'p53: Twenty Years On' Workshop, Trieste, 20. – 22. 5. 1999

JAPAN

V. Brabec participated in the 29th Symposium on Nucleic Acids Chemistry, Maebashi, 10. – 12. 11. 1999

POLAND

J. Fulneček, J. Jursa and *M. Varga* participated in training of servicing staff of national nodes of the International Center for Cooperation in Bioinformatics Network, Warsaw, 13. – 19. 9. 1999

RUSSIA

V. Brabec participated in the conferences of grant holders supported by the HHMI (USA) "1999 Meeting of International Scholars“, Moscow, 22. – 25. 6. 1999

SLOVAKIA

E. Jagelská participated in the conference Štruktúra a stabilita proteínov SSP´99, Košice, 8. – 11. 9. 1999

K. Minksová and *J. Vondráček* participated in the XX. Xenobiochemické sympózium, Smolenice, 20. – 21. 5. 1999

J. Paleček participated in the 20th International Specialized Symposium on Yeasts, Smolenice, 23. – 28. 5. 1999

V. Vetterl participated in the XXII. dny lékařské biofyziky, Košice, 25. – 30. 5. 1999

TURKEY

F. Jelen and *V. Vetterl* participated in the 20th Biomedical Science and Technology Symposium, Izmir, 6. – 8. 10. 1999

USA

M. Fojta and *E. Paleček* participated in the 11th Conversation in the Discipline Biomolecular Stereodynamics, Albany, 15. – 19. 6. 1999 and the NERM ´99: North-East Regional Meeting of the American Chemical Society, Postdam, 22. – 24. 6. 1999

J. Kašpárková participated in the 90th Annual Meeting of American Association for Cancer Research, Philadelphia, 10. – 16. 4. 1999

J. Šponer participated in the 11th Conversation in the Discipline Biomolecular Stereodynamics, Albany, 15. – 19. 6. 1999

M. Štros participated in the Keystone Meeting - DNA Replication, Recombination and DNA Repair, Taos, 15. 2. – 2. 3. 1999

3. Invited study stays or study stays within the framework of joint projects

GERMANY

M. Brázdová, M. Fojta and *J. Paleček* attended a seminar at the Max-Planck-Institut für biophysikalische Chemie, Göttingen (8. – 10. 4. 1999) within the framework of a cooperation

F. Jelen (5. – 12. 11. 199) and *E. Paleček* (8. – 23. 4. and 29. 10. – 12. 11. 1999) spent their study stay focused upon working on joint grant at the same place

M. Štros worked at the Max-Planck-Institut für Biophysikalische Chemie, Göttingen, Germany (7. – 20. 3. and 2. 9. – 13. 10. 1999)

GREAT BRITAIN

B. Brzobohatý spent his study stay at the Department of Plant Sciences, University of Oxford (12. 10. – 9. 12. 1999)

J. Fulneček (11. – 21. 10. 1999), *A. Kovařík* (20. 9. – 2. 10. 1999) and *R. Matyášek* (22. 10. – 8. 11. 1999) worked at the A. R. Leitch Laboratory, School of Biological Sciences, Queen Mary and Westfield College, University of London

J. Hodurková spent her study stay at the Department of Anatomy, The Medical School, Birmingham (3. 10. – 2. 11. 1999)

HUNGARY

M. Číž and *A. Lojek* visited the cooperating J. Hamar Laboratory at the National Institute of Traumatology, Budapest (24. – 27. 3. 1999)

ITALY

V. Brabec visited the partner Laboratory of Inorganic and Bioorganic Chemistry, Department of Chemistry, University of Florence (2. – 12. 2. 1999) and the Department of the Pharmaceutical Chemistry, University of Bari (8. – 22. 7. 1999) within the COST program

JAPAN

V. Brabec spent one study months at the National Institute for Advanced Interdisciplinary Research, Tsukuba (14. 10. – 18. 11. 1999)

J. Fajkus and *B. Vyskot* visited the partner Laboratory of Molecular Genetics and Breeding, Institute of Molecular and Cellular Biosciences, University of Tokyo and the Department of Integrated Biosciences, Graduate School of Frontier Sciences, University of Tokyo (22. 10. – 5. 11. 1999) within the frame of joint project

PORTUGAL

V. Vetterl worked at the Coimbra University to prepare research programs for students participating in the Erasmus-Socrates Project (27. 4. – 12. 5. 1999)

SLOVAKIA

C. Hofr and *O. Vrána* visited the Department of Biochemistry of the P. J. Šafárik University and the Institute of Experimental Physics, SAS in Košice with an intention to sign a cooperation concerning the study of stability and conformation changes of biological macromolecules (25. – 27. 10. 1999)

SPAIN

V. Vetterl visited the Faculty of Pharmacology, Seville University and the Department of Physical Chemistry, University of Alicante, where he discussed a possibility of student exchange and their education in Molecular Biophysics and Physics of Biopolymers (28. 1. – 19. 2. 1999)

SWITZERLAND

J. Kašpárková completed the study stay at the Department of Pharmacology and Toxicology, University of Zürich (1. – 5. 10. 1999)

USA

B. Brzobohatý worked at the Plant Research Laboratory, Michigan State University, East Lansing (27. 7. – 24. 9. 1999)

All realized study stays have had a significant impact to the progress of projects and their results are altogether presented in the Chapter II of this Report.

C. Cooperation with international governmental and non-governmental organizations

V. Kleinwächter - the chairman of the Czech Committee for Biophysics (IUPAB) - resigned from the post on 1. 6. 1999. *J. Šlotová* is the secretary and *E. Paleček* is the member of this Committee, *V. Brabec* and *S. Kozubek* were coopted to the Committee on 3. 11. 1999 and *S. Kozubek* was elected a vice chairman

J. Šlotová is a representative of the Czech Republic in the ICSU. She took part in the meeting of Expert Commission for preparation of materials for the General Assembly of the ICSU in 1999 in Cairo, Egypt (26. 9. – 1. 10. 1999)

V. Brabec is a representative of the CR in the Managing Board of the European Program of Scientific and Technological COST D8 and a member of the Evaluation Committee of the 5 Frame Programme of the EU in Brussels, Belgium

J. Fajkus was nominated for an external expert for the evaluation of the 5 Frame Programme projects of the EU in Brussels, Belgium

S. Kozubek is a member of the Programme Advisory Committee, Joint Institute for Nuclear Research, Dubna, Russia. Next he worked as a member of the European Commission Directorate General XII for the evaluation of the INCO COPERNICUS Projects, Section Radiation Protection in Brussels, Belgium

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

D. International conferences organized by the Institute of Biophysics

Institute of Biophysics – The Laboratory of Analysis of Biologically Important Molecular Complexes in cooperation with the Faculty of Science, MU Brno organized on 17. – 22. 5. 1999 a course of lectures named the „Genetic Codes“, which was lead by professor. E. N. Trifonov from the Weizmann Institute of Science, Rehovot, Israel. The course was a part of the doctoral study of Molecular and Cell Biology and it was completed by an exam.

V. DOCTORAL STUDIES, PEDAGOGICAL AND OTHER ACTIVITIES

A. Postgraduate studies

Before the Committee for Doctoral Dissertations V. Vetterl successfully defended his work in the field of biophysics, *Influence of ion environment upon intermolecular interaction of nucleic acids and their compounds,* and B. Vyskot successfully defended his work *Study of epigenetic processes with higher plants.*

Advanced education of students was based either on internal or external research studies or on postgraduate studies.

(a) Research studies

M. Fojtová finished her internal research study.

Number of research students (31 December 1999):

internal research students:	4
external research students:	5

Seven scientists were active as research students' advisors, other two acted as advisors - specialists.

(b) Postgraduate studies (PGS)

In 1999 the Institute of Biophysics participated in postgraduate education of students at universities, mainly at the Faculty of Science of the Masaryk University in Brno. In total, 45 students worked towards the doctor degree in the Institute. 19 of them were external postgraduate students and 26 of them were internal students.

Mgr. V. Dražan, Mgr. D. Häring, Mgr. K. Krejčí and Mgr. H. Loskotová finished their studies by passing the Rigorous Examinations and defending Doctor Theses, which originated at the Institute.

Numbers of postgraduate students are given in the following table.

Total number of students	External	Internal	Year of study
15	7	8	I.
5	2	3	II.
12	2	10	III.
5	2	3	IV.
2	2	0	V.
1	1	0	VI.
5	3	2	accomplishing study

PGS are carried out in the following items of specialization (a number of students is given in parentheses):

biophysics (10); 2 students accomplished their Doctor Theses
medical biophysics (1)
molecular biology (14)); 2 students accomplished their Doctor Theses
genetics (4)
animal physiology (6)
plant physiology (1)
immunology (1)
environmental chemistry (1)
biochemistry (2); 1 student accomplished his Doctor Thesis
botany (2)
informatics (3)

Thirteen scientists of the Institute acted as students' advisors.

Based upon the positive standpoint of the Accreditation Board of 24 November 1999 the Ministry of Education, Youth and Sports conferred on the Institute of Biophysics the Accreditation extending Doctoral Study Programs in Biology to Molecular and Cell Biology, Genetics, Physiology and Evolutionary Biology of Animals and Immunology and Accreditation extending DSP in Physics with Biophysics.

The Scientific Council of the Institute of Biophysics approved membership of scientists of the Institute in the particular Branch Councils, teachers of doctoral programs and students' advisors.

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

BC for Biophysics:

M. Bezděk, V. Brabec, E. Paleček, J. Šlotová, V. Vetterl, M. Vorlíčková

BC for Molecular and Cell Biology:

J. Fajkus, B. Koukalová, J. Kypr, E. Paleček, V. Vetterl

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

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

BC for Genetics:
M. Bezděk, E. Paleček, B. Vyskot

BC for Environmental Chemistry and Ecotoxicology:
A. Kozubík

Scientists of the Institute of Biophysics are members of PGS Branch Councils at other faculties:

Faculty of Medicine, Masaryk University in Brno
BC for 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, V. Kleinwächter, E. Paleček

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. Cooperation with universities

Pedagogical activities

The process of continuing cooperation with the Faculty of Science of the Masaryk University in Brno and other universities consisted in the following activities:

Faculty of Science, Masaryk University, Brno:

Lectures and practical exercises were carried out by 16 scientists of the Institute (including 2 professors and 3 assistant professors). 16 scientists acted as advisors for 32 students working on their Diploma Theses.

The following scientists were members of examining committees for State Final Examinations:
E. Paleček - a field of Molecular Biology
J. Hofmanová, A. Kozubík - a field of Animal Physiology

J. Hofmanová and A. Kozubík were members of the examining committee for Rigorous Examinations in the field of Animal Physiology and Developmental Biology.

V. Kleinwächter and V. Vetterl were members of the examining committee for Admission and Rigorous Examinations in field of Biophysics.

E. Paleček was a member of the Scientific Council of the Faculty of Science.

Other universities:

Faculty of Medicine, Masaryk University, Brno - V. Vetterl is a member of the Scientific Council

Faculty of Science, Palacký University, Olomouc - V. Kleinwächter and J. Šlotová are members of the Scientific Council and together with V. Vetterl are members of the examining committee for State Final Examinations, field of Biophysics. V. Vetterl is a member of the Scientific Council at Palacký University.

Faculty of Medicine, Palacký University, Olomouc - V. Vetterl gave lectures and led practical exercises in medical biophysics for foreign students

Faculty of Science UK, Prague - B. Vyskot is a member of the State Doctoral Examinations in the field of Biology, specialization Molecular Biology of Plants

An overview of lecture cycles and practical exercises given by scientists of the Institute of Biophysics

A. Faculty of Science, Masaryk University, Brno

Summer term 1998/1999

Field	Course	Lecturer
Biophysics	Introduction into biophysics II	V. Vetterl, V. Kleinwächter
	Radiation biophysics	S. Kozubek
	Molecular biophysics	V. Vetterl
	Molecular biophysics of mutagens, cancerogenes and cytostatics	V. Brabec
	Biophysical properties and computer analysis of nucleic acids, proteins, genes and genomes	J. Kypr
	Electrical and magnetic properties of molecules	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	A. Kozubík
Plant Physiology	Developmental genetics	B. Vyskot
Animal Physiology, Ecotoxicology	Advanced methods in biological research	A. Kozubík, J. Hofmanová
	Applied physiology	A. Kozubík, J. Hofmanová
	Genotoxicity	J. Hofmanová
	Health Risks	A. Kozubík

Winter term 1999/2000

Field	Course	Lecturer
Biophysics	Introduction into biophysics I	V. Vetterl, V. Kleinwächter
	Molecular biophysics	V. Vetterl, V. Kleinwächter
	Experimental methods in biophysics	V. Vetterl
	Bioelectrochemistry	V. Vetterl, F. Jelen
Molecular Biology and Genetics	Chemistry of nucleic acids	E. Paleček, M. Fojta
	Molecular biology and yeast genetics	J. Paleček
Animal Physiology, Ecotoxicology	Physiology of cell systems	A. Kozubík, J. Hofmanová, A. Lojek
	Special immunological methods	M. Číž

B. Faculty of Medicine, Palacký University, Olomouc

Two-term course for foreign students

	Medical biophysics	V. Vetterl

Cooperation in research

The Institute participated in 3 cooperative research projects carried out together with universities in 1999. The projects were granted by the GA CR. Two of these grants had the scientists of the IBP as principal investigators.

The Institute of Biophysics continued in cooperation with the Department of Physical Electronics of the Faculty of Science, MU Brno in the Joint Laboratory of Biophysics.

Within the programme of the ME CR named *Support of Research in the Universities* scientific and educational work continued in the Joint Laboratory of Molecular Physiology of Plants at the Faculty of Science, MU (*B. Brzobohatý*, project #VS96096), and in the Laboratory of Analysis of Significant Molecular Complexes in the Institute of Biophysics (*J. Fajkus*, project #VS97032) and in the Laboratory of High-Resolution Cytometry at the Faculty of Informatics, MU (*S. Kozubek*, project #VS97031).

Within the programme of the ME CR named *Development of the Universities* was realized the *Innovation of teaching experimental methods of biophysics* project (investigator *V. Vetterl*) in cooperation of the Faculty of Science, MU and Institute of Biophysics.

Within the programme RECETOX (*Regional Research Center for Atmospheric Chemistry and Effects of Atmospheric Pollutants*) the cooperation 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 programme.

The Institute of Biophysics (J. Hofmanová and A. Kozubík) was emboded in newly created University Oncological Center (UOC). A. Kozubík is a member of the UOC coordination committee – he is in charge of coordinating of experimental groups of the Center of Experimental Oncology (CEO).

C. Applied research

A. Kovařík cooperated with the II. Internal Clinic of Faculty Hospital in Brno in the „Detection of mycotic infections in clinical material“. By an appropriate selection of oligonucleotid primers sensitivity enhancement of the PCR method was achieved. By using purified DNA *Candida albicans* as a template for PCR amplification a sensitivity for finding of 1 pg of DNA was achieved that corresponds to 1-5 cells of infectious agent.

A. Lojek and *M. Číž* continued in their cooperation with the Center of Cardio-vascular and Transplant Surgery in Brno to solve 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* cooperated as well with the Pilsen Prazdroj a.s. brewery in the field of testing oxidative and antioxidative properties of beer in relation to its durability, sensational qualities and oxidative and antioxidative parameters of blood.

J. Fajkus continued in his cooperation with the Institute of Children Health in Brno and with the Faculty Hospital and Clinic, Brno within the project of the GA CR and the ME CR.

Continuing cooperation went on with medical institutions within the frame of three joint projects supported by the GA MH CR. The results are presented in the Chapter I of this Report.

D. Membership in scientific institutions

M. Bezděk is a member of the Czech Committee for Transgenic Plants and of the General Assembly of the AS CR (for the period 1994 - 1998). Since 1995 he is a member of the Council of the Programme titled *Support of Research in the Universities at the ME CR*.

V. Brabec is a 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 GA AS CR. He is a member of the #3 Branch Council *Medical Sciences* and a member of the #301 Sub-Branch Committee *Molecular Biology, Genetics and Experimental Oncology* of the GA CR and a member of the #7 Branch Council *Medical Sciences* of the GA AS CR.

M. Fojta is a member of the #204 Sub-Branch Committee *Molecular and Cellular Biology* of the GA CR.

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

V. Kleinwächter is a member of the # 4 Branch Council *Chemical Sciences* of the GA AS CR.

S. Kozubek was elected a member of the General Assembly of the AS CR for the period 1998 - 2002.

A. Kozubík is a member of the #5 Branch Council for *Agricultural Sciences* and of the #524 Sub-Branch Committee *Physiology and Pathology of Animals* of the GA CR.

J. Kypr is a member of the Scientific Council of the Academy of Sciences.

E. Lukášová is a member of the #202 Sub-Branch Committee for *Physics* of the GA CR.

E. Paleček is a member of the #5 Branch Council *Molecular and Cellular Biology* of the GA 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 CR for the *Research and Development,* a member of the permanent working group (for biology) of the Accreditation Committee of the Government of CR for the Universities and a member of the ME CR Committee for *Evaluating Research Intentions and Results* of institutions for granting institutional support to the research and development in science.

J. Široký is a member of the #5 Branch Council *Agricultural Science* and of the #521 Sub-Branch Committee *„Plant Production, Genetics and Breeding“* of the GA CR.

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

M. Štros is a member of the #4 Branch Council for *Chemical Sciences* of the GA AS CR.

M. Vorlíčková was a member of the General Assembly of the AS CR for the period 1994 - 1998.

V. Vetterl was nominated by the Board of the Fund of University Development for a member of the item F3 *Innovation of Biomedicine Programs*.

O. Vrána is a member of the #5 Branch Council *Molecular and Cellular Biology* of GA AS CR.

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

Scientists of the Institute of Biophysics are members of Boards for Doctor Degrees in Biophysics, Biochemistry and Immunology (*E. Paleček*), and Boards for "Candidate" Degrees in Biophysics (*E. Paleček* - a chairman, *M. Bezděk*, *A. Vacek* - members), Biochemistry (*J. Kypr*) and Normal and Pathological Physiology (*M. Pospíšil*).

V. Brabec is a member of the Slovak Board for Doctor Degrees in Molecular Biology.

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

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

V. Vetterl - *Český časopis pro fysiku*

E. Membership in scientific societies

International scientific organizations and societies

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

V. Brabec - a member of the International Society of Electrochemistry

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

M. Číž - a member of the Society for Free Radical Research

J. Fajkus - a member of the American Association for Microbiology

M. Hofer - a member of the Council of the European Society for Radiation Biology

J. Hofmanová - a member of the New York Academy of Sciences, the European Tissue Culture Society and the Society for Leukocyte Biology (USA)

V. Kleinwächter - a member of the IUPAB Commission for Radiation and Environmental Biophysics

S. Kozubek - a member of the Programme Advisory Committees in the Joint Institute of Nuclear Research, Dubna, Russia, and of the European Society for Radiation Biology

A. Kozubík - a member of the New York Academy of Sciences, the European Tissue Culture Society and the Society for Leukocyte Biology (USA)

K. Krejčí – a member of the European Cytogenetics Association and the Danish Center for Gerontology

A. Lojek - a member of the New York Academy of Sciences, Society for Free Radical Research and the European Shock Society

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

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

J. Šlotová - a representative of the Czech Republic in ICSU, a member of the General Committee of ICSU and a member of the European Society for Radiation Biology

M. Štros - a member of the New York Academy of Sciences

V. Vetterl - a member of the Bioelectrochemical Society

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

A. Vacek - a member of the International Astronautical Academy

National organizations and committees

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

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

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

M. Číž - a member of the Czech Immunological Society and a member of the Czech Society for Biochemistry and Molecular Biology

H. Čížová - a member of the Czech Society for Biochemistry and Molecular Biology

M. Fojtová - a member of the Society of Experimental Plant Biology

M. Hofer - a board member of the Czech Radiobiological Society at the Czech J. Ev. Purkyně Medical Society

J. Hofmanová - a member of the Society for Tissue Cultivation at the Czech Oncological Society and of the Czech Radiobiological Society at the Czech J. Ev. Purkyně Medical Society, a member of the University Oncologic Center

V. Kleinwächter - a board member of the branch Chemical Physics and Biophysics of the Union of Czech and Slovak Mathematicians and Physicists, a chairman of the Czech Ostomy Association and a board member of the Brno Branch of the Czech Society for Biochemistry and Molecular Biology

A. Kolíbalová - a member of the Czech Society for Biochemistry and Molecular Biology, a member of the Czechoslovak Biological Society, and a member of the Biotechnological Society

B. Koukalová - a member of the Mendel Genetic Society

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

S. Kozubek - a board member of the Czech Radiobiological Society at the Czech J. Ev. Purkyně Medical Society, a member of the National Committee for the Exploitation and Research of Cosmic Space and a vice-chairman of the Czech Committee for Biophysics (IUPAB)

A. Kozubík - a member of the Society for Tissue Cultivation at the Czech Oncological Society and a member of the Czech Radiobiological Society at the Czech J. Ev. Purkyně Medical Society

A. Lojek - a member of the Czech Immunological Society

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

I. Pipalová - a member of the Council of the Society for Laboratory Animal Science

J. Šlotová - a scientific secretary of the Czech Committee for Biophysics (IUPAB)

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

V. Vetterl - a board member of the branch Chemical Physics and Biophysics of the Union of Czech and Slovak Mathematicians and Physicists

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

O. Vrána - a scientific secretary of the Biophysical Section of the Czechoslovak Biological Society

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