INSTITUTE OF BIOPHYSICS OF THE CZECH ACADEMY OF SCIENCES, V. V. I.

REPORT 2012–2014

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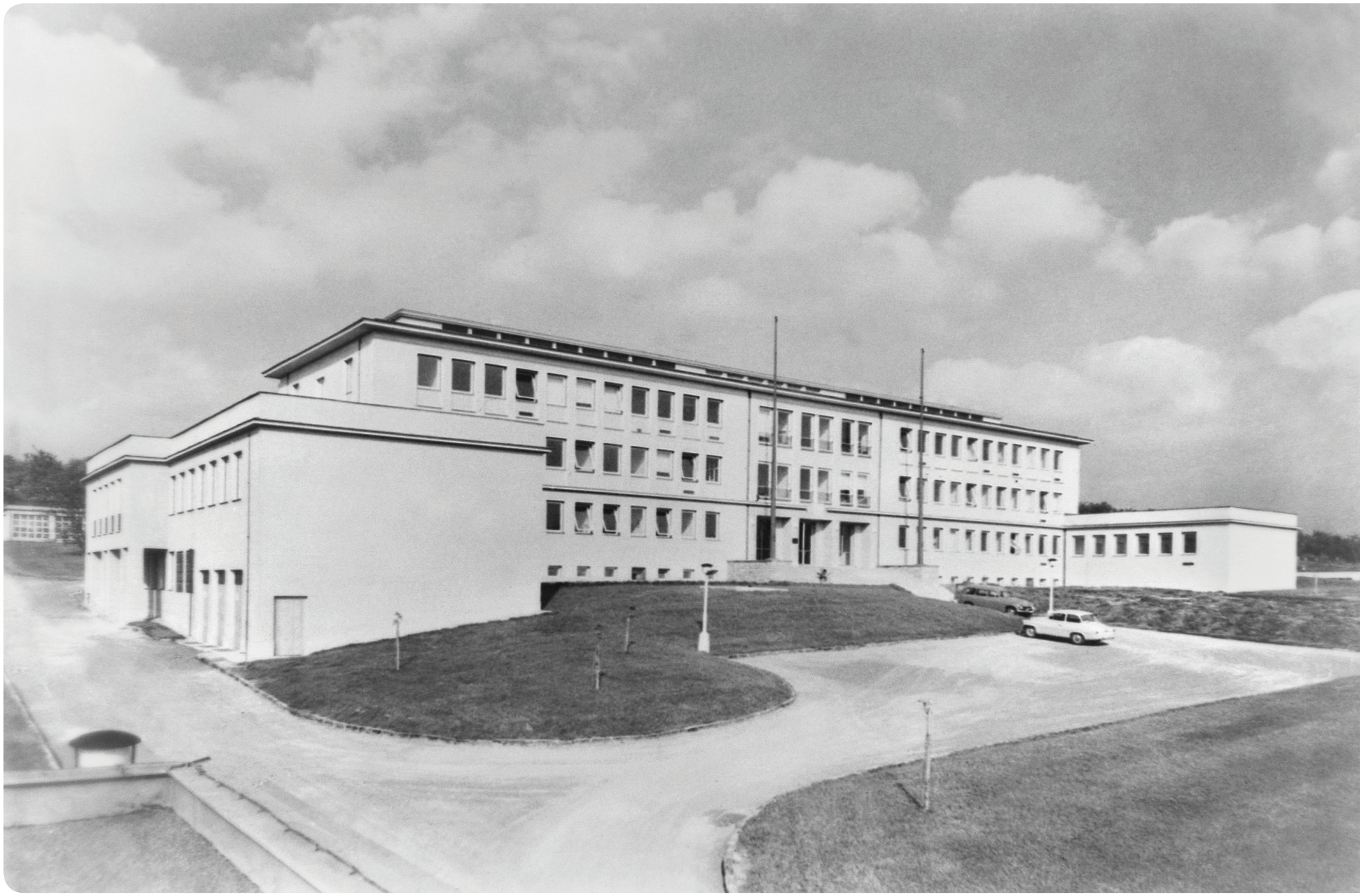
CONTENT

INTRODUCTION

The mission of the Institute of Biophysics, Czech Academy of Sciences (IBP) is both basic and oriented research of the structure, function and dynamics of biological systems (biomolecules, cell components, cells and cell populations) using a broad spectrum of biophysical methods. IBP has a long tradition of first-rate research and competitive environment.
In various evaluations within the CR or CAS, the Institute is being ranked among the best, regardless of the character of these evaluations. For the period between 2010 and 2014, the IBP has 742 impacted publications and 6,647 citations on the WoS (data from 8 April 2015), which constitutes a much larger ratio of publications and quotations per worker than any other biological institutes of CAS, which focus on similar topics. The individual departments of the institute focus on different research fields, but from the point of view of effectiveness, they are quite balanced. The prominent position of the institute within the CAS and CR is corroborated by prestigious awards, which our workers were awarded (for example, Prof. J. Šponer was awarded the Academic Award in 2014, Prof. E. Paleček received the Česká hlava award in the same year). The average age of workers of the institute is relatively low, in all departments, there are a number of young people at various degrees of their scientific career. The ratio of men and women is positive as well.
Since 2005, the institute has an evaluation system, which is being performed annually and the results for the whole period are presented on the Internet. In accordance with the results of the evaluation, funding of individual departments is adjusted every year, or, by a decision of the Council, fundamental changes in the organizational structure of the institute are made (establishment and disestablishment of departments). In the past 10 years, such decisions were made in quite a few cases. As a result of the evaluation, some teams grew in numbers significantly. On the contrary, during the stated period, in 5 cases, independent teams were dissolved.
IBP significantly contributes to further education of the most educated part of the population of the CR. 60-70 doctoral students are constantly trained at the IBP; almost every single one of these students (excluding exceptions) reach the point of defence of their theses. All departments of the institute take part on the training; in some departments, this constitutes more than 10 semester lectures per year.
The equipment at the IBP is the best since the establishment of the institute. It was purchased in cooperation with CEITEC, ICRC and also with CAS. The total value of the equipment purchased for IBP from these sources constitutes approximately CZK 100,000,000 in the last 10 years. We consider these investments appropriate, we have trained experts for all the equipment and we guarantee a full use of these investments. We also presume the central laboratories of IBP to be used in an appropriate extent by other workers of the region as well. We believe that this amazing equipment, which fits the needs of our workers perfectly, will lead to further increase of quality of research of our institute while keeping the expenses at the smallest level possible.



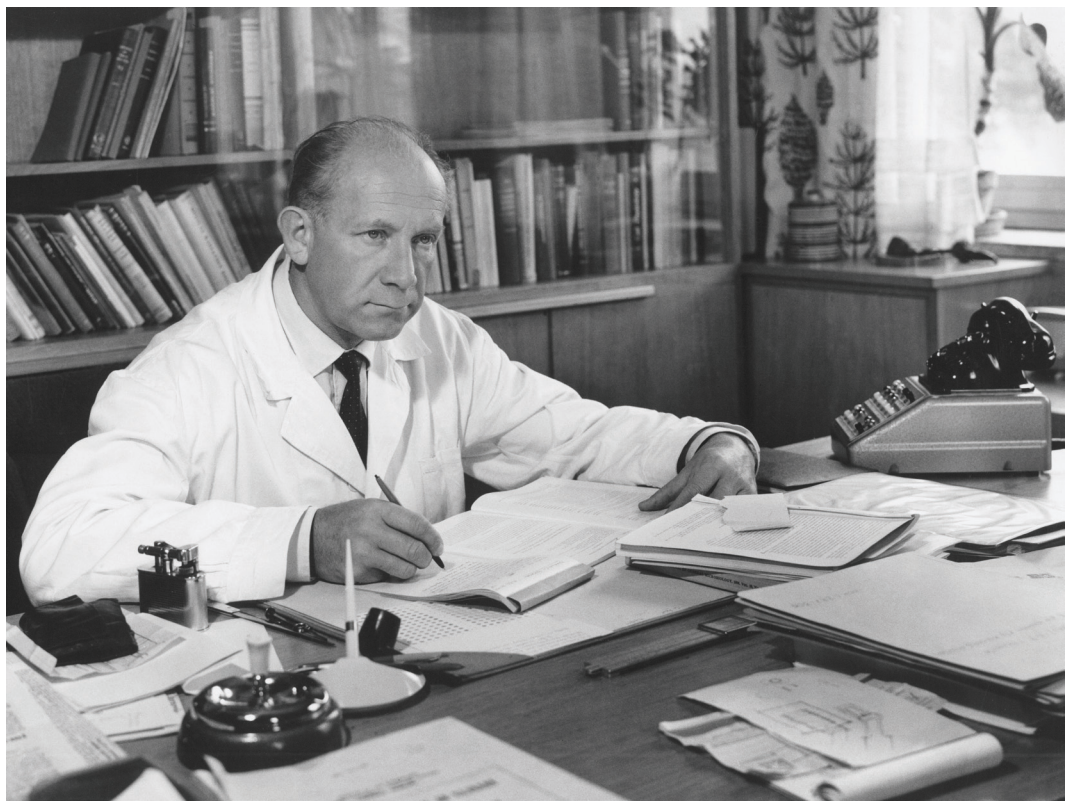
View of the Institute of Biophysics AS CR shortly after its establishment

BRIEF HISTORY

Research aimed at investigation of physical properties of biological systems has a long tradition in Brno. The history of biophysics in the former Czechoslovak Republic is closely connected with two men, who were active at the Masaryk University in the early thirties, Professors Ferdinand Herčík and Vilém Laufberger. Whereas Prof. Laufberger moved to Charles University in Prague in 1935, Ferdinand Herčík remained in Brno.
Prof. Herčík´s scientific interests, which ranked from studies of effects of ionising radiation on living organisms to molecular biophysics, prompted him to initiate actions directed at establishing a specialised laboratory devoted to research in biophysics.
On 1 February 1954, he established the Laboratory of Biophysics as a part of the former Czechoslovak Academy of Sciences. On 1 January 1955, the Laboratory was transformed into the Institute of Biophysics and Prof. Ferdinand Herčík was named director of the Institute. In the initial stage, the Institute was predominantly equipped for radiobiological research.
Because of its experience and scientific results, the Institute was included into the principal task of the State Research Programme, "Biophysical research of the living matter" until 1990, and became a centre for postgraduate education in biophysics and participated also in pre-graduate education in cooperation with Masaryk University .
In 1966, after death of Prof. Ferdinand Herčík, the former scientific secretary Dr. Zdeněk Karpfel was elected a director of the Institute. Under his leadership, the research aims gradually changed from radiobiology to cell and molecular biophysics. In this period, the Institute grew and in 1989, it consisted of 97 research fellows.

At the beginning of 1990, Dr. Milan Bezděk was elected a director of the Institute of Biophysics. The new scientific management respected the traditional research activities. The research concerned three thematic areas: molecular biophysics, biophysics of complex systems, biophysics of the effects of external factors.
In 1997, Dr. Bezděk was elected a member of the Scientific Council of the Academy and resigned from the position of the director. In the same year, Dr. Jana Šlotová has been elected a new director.
In 1999, Ministry of Education of the Czech Republic granted to the Institute of Biophysics the accreditation to participate in the Doctoral Study Programmes in cooperation with Faculty of Science of the Masaryk University in biophysics, molecular and cellular biology, genetics, physiology and evolutionary biology of animals and immunology.
The management of the Institute of Biophysics paid attention to the systematic renewal of the infrastructure (computers and networking as well as scientific instrumentation) and establishing creative conditions for the research.
In 2005, Assoc. Prof. Stanislav Kozubek has been elected a new director and substantial changes have been made in the Institute of Biophysics. The internal evaluation of the scientific performance was introduced and 2006 was the first year, in which the evaluation was carried out and applied.
Since 2007, the Institute of Biophysics changed its legal statute to public research organization. As a consequence, the Council of the Institute of Biophysics has been newly elected and Assoc. Prof. Stanislav Kozubek has been newly selected and appointed the Director of the Institute of Biophysics (by the President of the Czech Academy of Sciences).

BRIEF HISTORY

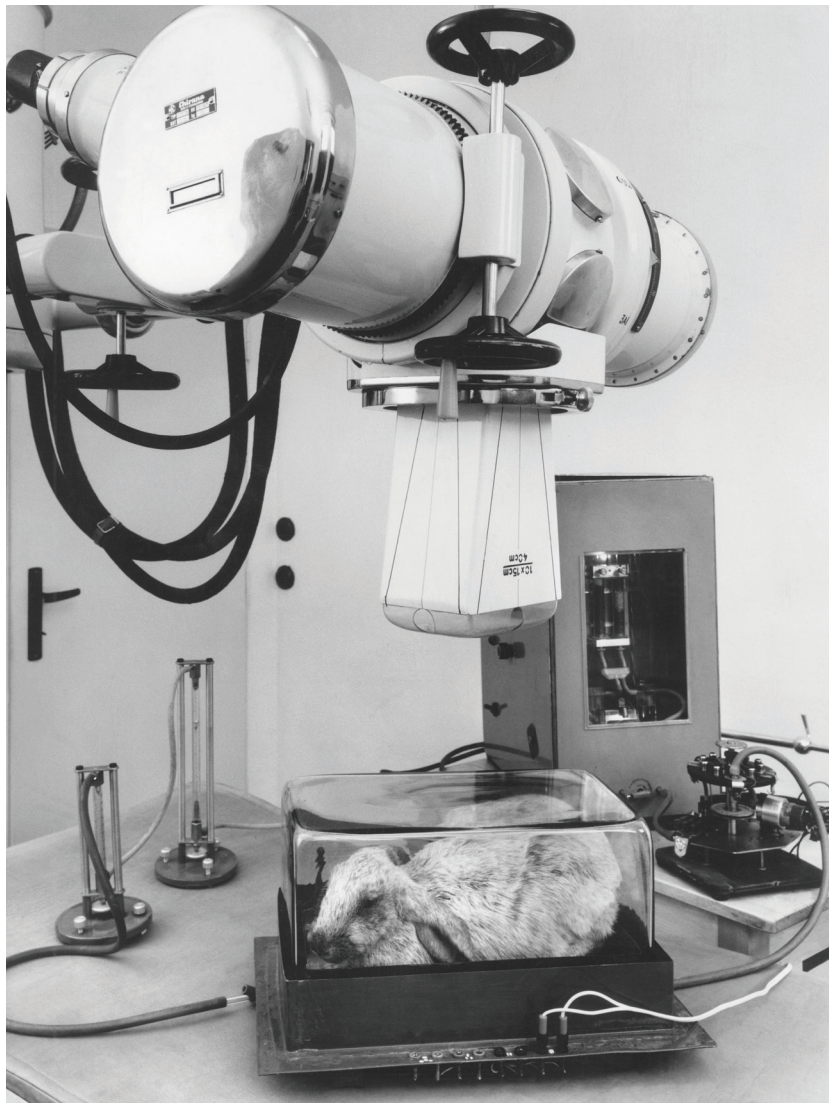


Ferdinand Herčík, founder and the first director of the Institute

Zdeněk Karpfel, director of the Institute (1966–1990)
Jana Šlotová, director of the Institute (1997–2005)

Visit of the first Czech cosmonaut Vladimír Remek at the Institute on the occasion of the evaluation of the Intercosmos programme. From the left: Oldřich Pelčák, Antonín Vacek, Vladimír Remek and Miloš Klimek

Milan Bezděk (in the middle) in a discussion with Jana Šlotová and Helena Ilnerová (president of the Czech Academy of Sciences, on the left)



Historical views of laboratories of the Institute

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Viktor Brabec

DEPARTMENT OF CELL BIOLOGY AND RADIOBIOLOGY
Martin Falk

DEPARTMENT OF BIOPHYSICAL CHEMISTRY AND MOLECULAR ONCOLOGY
Miroslav Fojta

DEPARTMENT OF MOLECULAR EPIGENETICS
Aleš Kovařík

DEPARTMENT OF MOLECULAR CYTOLOGY AND CYTOMETRY
Stanislav Kozubek

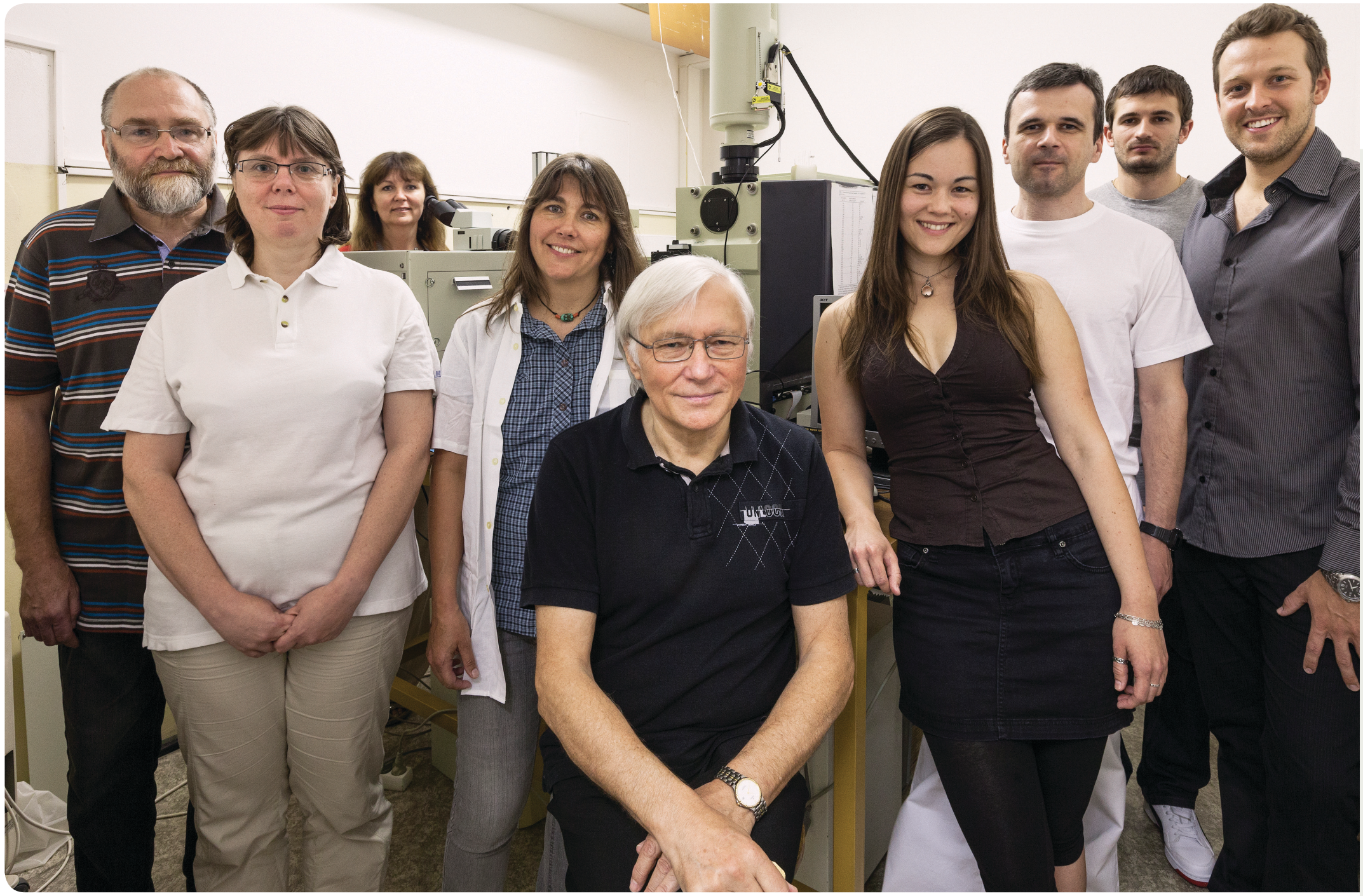
DEPARTMENT OF CYTOKINETICS
Alois Kozubík

DEPARTMENT OF FREE RADICAL PATHOPHYSIOLOGY
Antonín Lojek

DEPARTMENT OF STRUCTURE AND DYNAMICS OF NUCLEIC ACIDS
Jiří Šponer

DEPARTMENT OF CD SPECTROSCOPY OF NUCLEIC ACIDS
Michaela Vorlíčková

DEPARTMENT OF PLANT DEVELOPMENTAL GENETICS
Boris Vyskot



SCIENTISTS Viktor Brabec, Jana Kašpárková, Hana Kostrhunová, Jaroslav Malina, Olga Nováková, Marie Vojtíšková, Oldřich Vrána
TECHNICAL ASSISTANT Milada Kořínková
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DEPARTMENT OF MOLECULAR BIOPHYSICS AND PHARMACOLOGY

THE DEPARTMENT IS INVOLVED IN STUDIES OF MOLECULAR AND CELLULAR MECHANISMS OF ANTITUMOR AND ANTIMICROBIAL EFFECTS OF NEW METAL-BASED COMPLEXES IN RELATION TO DESIGN OF NEW ANTICANCER DRUGS AND ANTIBIOTICS

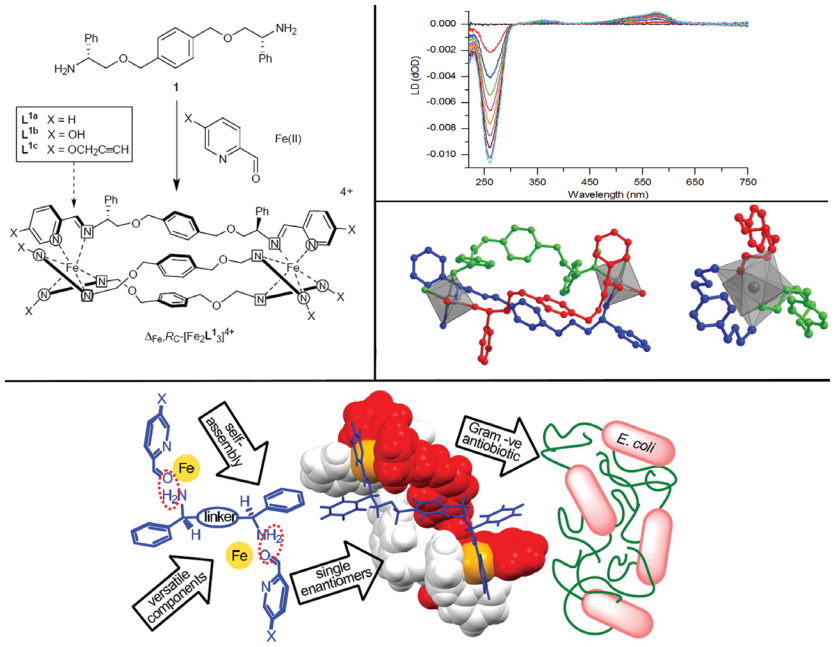
In this Department, research is focused on interactions of metal-based compounds, for example those containing iridium, osmium, platinum and ruthenium, with DNA and other biomacromolecules. These compounds exhibit marked antitumor or antimicrobial effects derived from their capability to bind to DNA. Determination of biophysical properties of DNA damaged by these compounds, also including their combination with physical and epigenetic factors and understanding cellular responses to these damages, makes it possible to infer efficiency of these compounds in treatment of cancer and bacterial diseases.

The concept of this research has been extended by inclusion of processes connected with recognition and repair of damaged DNA, which provides the results of greater biological significance and relevance to clinical practice. The focus of this work is to understand the molecular and cellular mechanisms of action of metallodrugs and at rational design and development of novel agents of relevance for anticancer and antimicrobial strategies and of interest to the pharmaceutical industry. This biophysical research is, therefore, of high importance to society and it can be expected that this subject matter will continue to develop in the near future.

SELECTED RESULTS

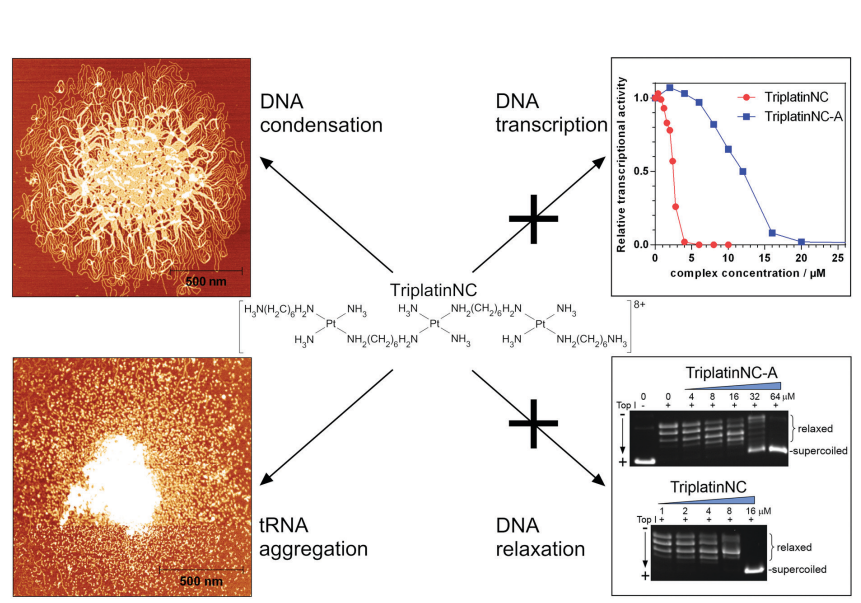
OPTICALLY PURE, WATER-STABLE METALLOHELICES WITH ANTICANCER AND ANTIBIOTIC ACTIVITY

Biophysical properties of the metallohelices—chiral assemblies of two or more metal atoms linked by short or relatively rigid multidentate organic ligands—can be regarded as non-peptide mimetics of α-helices, because they are of comparable size and have shown some relevant biological activity. We have characterized some interactions of these helicates with biomacromolecules analogous to the interactions of peptides with molecules of biological significance. The results indicate that these chiral metallohelices can be used in medicine, for example as antitumor drugs and antibiotics. This study was performed in collaboration with researchers of the University of Warwick in England.



SUBSTITUTION-INERT TRINUCLEAR PLATINUM COMPLEXES EFFICIENTLY CONDENSE/AGGREGATE NUCLEIC ACIDS AND INHIBIT ENZYMATIC ACTIVITY

The trinuclear platinum complexes (TriplatinNC and TriplatinNC-A) are biologically active agents that bind to DNA through noncovalent (hydrogen bonding, electrostatic) interactions. We have shown that these Pt complexes condense DNA and small transfer RNA molecules with an unprecedented potency. The results indicate that the mechanisms for the biological activity of these trinuclear platinum complexes are associated with their ability to condense/aggregate nucleic acids with consequent inhibitory effects on crucial enzymatic activities. Additionally, these trinuclear platinum complexes may also have potential for gene delivery, because the transfection of DNA in gene therapy depends largely on the possibility of achieving its condensation by simple artificial molecules.



SELECTED OUTPUTS

Brabec, V.; Howson, S. E.; Kaner, R. A.; Lord, R. M.; Malina, J.; Phillips, R. M.; Abdallah, Q. M. A.; McGowan, P. C.; Rodger, A.; Scott, P. Metallohelices with activity against cisplatin-resistant cancer cells; does the mechanism involve DNA binding? Chem. Sci. 2013, 4, 4407–4416

Howson, S. E.; Bolhuis, A.; Brabec, V.; Clarkson, G. J.; Malina, J.; Rodger, A.; Scott, P. Optically pure, water-stable metallo-helical 'flexicate' assemblies with antibiotic activity. Nature Chemistry 2012, 4, 31–36

SELECTED OUTPUTS

Malina, J.; Farrell, N. P.; Brabec, V. Substitution-inert trinuclear platinum complexes efficiently condense/aggregate nucleic acids and inhibit enzymatic activity. Angew. Chem. Int. Ed. 2014, 53, 12812–12816

Malina, J.; Farrell, N. P.; Brabec, V. DNA condensing effects and sequence selectivity of DNA binding of antitumor noncovalent polynuclear platinum complexes. Inorg. Chem. 2014, 53, 1662–1671

SELECTED OUTPUTS

Pracharova, J.; Zerzankova, L.; Stepankova, J.; Novakova, O.; Farrer, N. J.; Sadler, P. J.; Brabec, V.; Kasparkova, J. Interactions of DNA with a new platinum(IV) azide dipyridine complex activated by UVA and visible light: Relationship to toxicity in tumor cells. Chem. Res. Toxicol. 2012, 25, 1099–1111

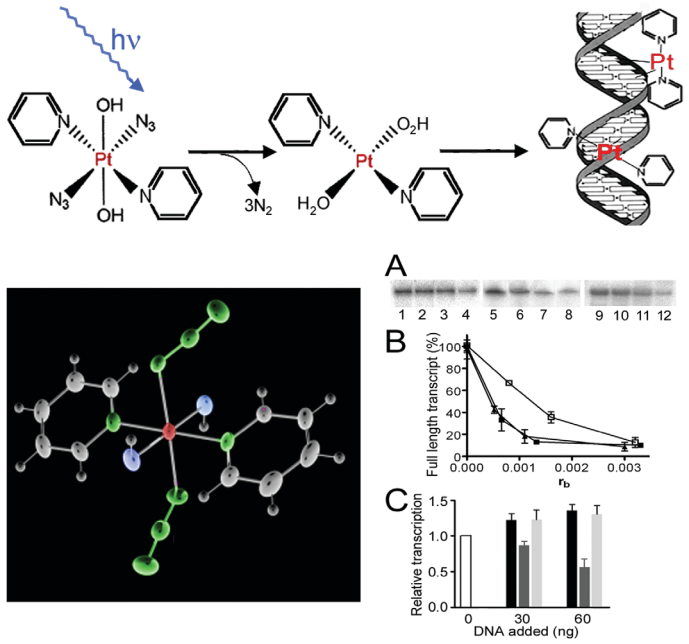
SELECTED OUTPUTS

Malina, J.; Natile, G.; Brabec, V. Spontaneous translocation of antitumor oxaliplatin, its enantiomeric analogue, and cisplatin from one strand to another in double-helical DNA. Chem. Eur. J. 2013, 19, 11984–11991

Malina, J.; Kasparkova, J.; Farrell, N. P.; Brabec, V. Walking of antitumor bifunctional trinuclear PtII complex on double-helical DNA. Nucleic Acids Res. 2011, 39, 720–728

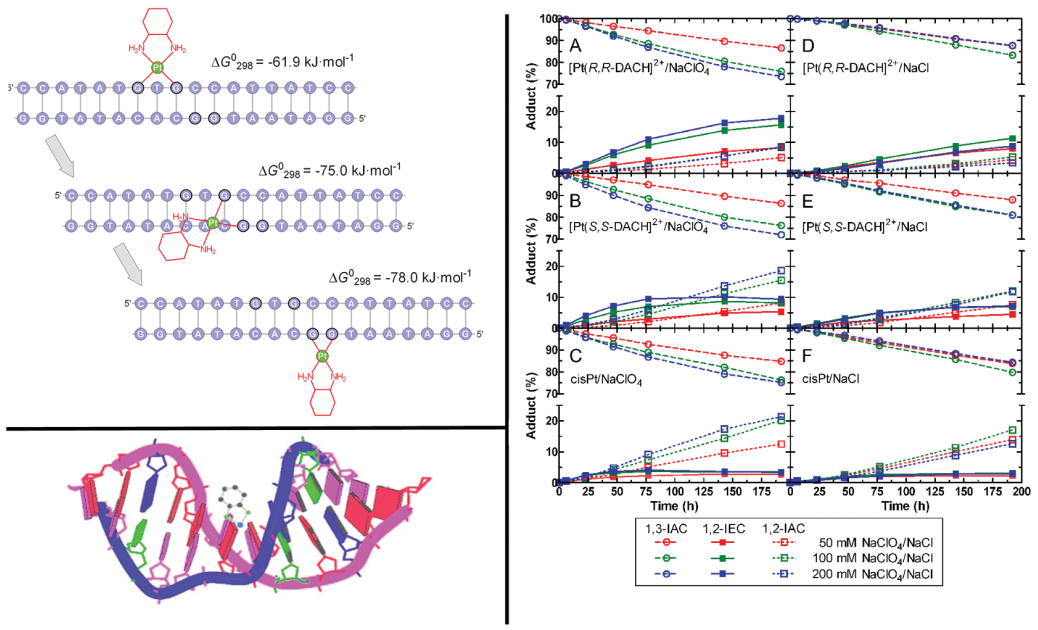
MECHANISMS OF ANTITUMOR EFFECTS OF NEW PHOTOACTIVATABLE PLATINUM(IV) COMPLEXES

Cisplatin (*cis*-$[PtCl_2(NH_3)_2]$) is a widely used anti-tumor agent that acts primarily by adducting DNA to form intrastrand cross-links. Toxicity to the patient limits the use of this highly effective drug, indicating the need for a more selective alternative. Design of photoactivatable Pt(IV) diazido complexes, such as *trans,trans,trans*-$[Pt(N_3)_2(OH)_2(pyridine)_2]$ (1), which upon exposure to ultraviolet or visible light is converted to a highly reactive Pt(II) analog, is one approach. Our results show that photoactivated 1 disrupts conformation of DNA in cancer cells more profoundly than conventional cisplatin. However, due to its lack of toxicity in the absence of light, 1 might be a safer antitumor agent for clinical use.



WALKING OF ANTITUMOR PLATINUM COMPLEXES ON DOUBLE-HELICAL DNA

Platinum complexes, such as cisplatin, oxaliplatin and trinuclear complex BBR3464 belong among efficient anticancer agents. DNA binding and the consequences to structure and function are the mechanistic paradigm, by which these metallodrugs exert their antitumor activity. We have shown, using oligonucleotide duplexes containing single, site-specific cross-links of cisplatin, oxaliplatin and BBR3464 and gel electrophoresis that, under physiological conditions, the coordination bonds between platinum and N7 of G residues involved in the cross-links can be spontaneously cleaved. This cleavage may lead to the linkage isomerization reactions between these metallodrugs and double-helical DNA. Differential scanning calorimetry of duplexes containing single, site-specific cross-links of cisplatin, oxaliplatin and BBR3464 reveals that one of the driving forces that leads to the lability of DNA cross-links of these metallodrugs is the difference between the thermodynamic destabilization induced by the cross-link and by the adduct into which it could isomerize. The rearrangements may proceed in this manner: cross-links, originally formed in one strand of DNA, can spontaneously translocate from one DNA strand to its complementary counterpart, which can evoke walking of the platinum complex on DNA molecule.



SCIENTISTS Martin Falk, Jiří Fajkus, Michal Hofer, Michal Štros, Emilie Lukášová, Miloslava Fojtová, Jana Fulnečková, Eva Sýkorová, Zuzana Hoferová, Denisa Komůrková
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PhD. STUDENTS *running* Ladislav Dokládal, Petr Fajkus, Martin Kučírek, Soodabeh Abbasi Sani, Jian Bagherpoor Alireza, Iva Falková, Lucie Ježková

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DEPARTMENT OF CELL BIOLOGY AND RADIOBIOLOGY

IN OUR DEPARTMENT, WE INVESTIGATE A BROAD SCALE OF QUESTIONS ASSOCIATED WITH A COMPLEX RESPONSE OF CELLS AFTER THEIR EXPOSURE OF DIFFERENT KINDS OF IONIZING RADIATIONS; WE ALSO EXPLORE THE PRINCIPLES OF CHROMATIN ORGANIZATION IN NUCLEI OF EUKARYOTIC CELLS AND THE RELATIONSHIP BETWEEN THIS ORGANIZATION AND CHROMATIN FUNCTION UPON PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS.

Laboratory of Chromatin Function, Damage, and Repair investigates the effects of different kinds of ionizing radiations (gamma rays, accelerated protons and heavy ions) on normal and tumor cells, especially the character of DNA damage and mechanisms of DNA repair. The research group tries to reveal what roles do the chromatin structure, epigenetic modifications, and nuclear architecture play in the cell response to irradiation, genome instability, and carcinogenesis. The researchers extensively explore how to make the best use of current radiotherapy and how to improve it further; nowadays, they mostly study radiosensitizing effect of metal nanoparticles (nanomedicine).

Laboratory of Experimental Hematology studies effects of ionizing radiation on hematopoiesis and, in general, also possibilities of radioprotection. The experiments are carried out at levels from individual cells to model organisms (mice).
Laboratory of DNA-Molecular Complexes focuses its research on the structure and evolution of telomeres and their importance for the stability of chromosomes and plant speciation. Solved questions concern the nucleoprotein composition of telomeres and telomerases, interactions between telomere components, structure-function relationships between telomerase subdomains, and alternative strategies of telomere maintenance.

The role of epigenetic mechanisms in regulation of expression of genes and stabilization of telomeres and genomes is also an important topic.
Laboratory of Analysis of Chromosomal Proteins focuses on the interactions between different chromatin components that are important in the context of the DNA repair, genome stability, and effects of medical drugs (that bind to chromatin). The interactions of HMGB (HMG, High-Mobility Group) proteins with various DNA structures, nucleosomes, and biologically important proteins, like histones, tumor suppressors (p53/p73), topoisomerases, and telomerases are the main object of analyses.

SELECTED RESULTS

HETEROCHROMATIN REMAINS INCOMPLETELY DIFFERENTIATED IN NEUTROPHILS OF AML LEUKEMIA PATIENTS, REGARDLESS OF THE TREATMENT WITH THE DISEASE REMISSION–THIS PREVENTS FUNCTIONING OF AML NEUTROPHILS IN THE IMMUNE RESPONSE

Upon an immune activation, neutrophils fire chromatin nets to immobilize infectious agents. We have discovered that this ability is lost in a variable extent in AML neutrophils that show features of incomplete chromatin differentiation. In addition, we have revealed that the repair of DNA double strand breaks is down-regulated in normal neutrophils but not immature neutrophils from AML patients. The protein HP1 is incompletely replaced with the serpin MNEI in heterochromatin of AML neutrophils; hence, we suggest that the immunodetection of HP1 is a gentle method to sensitively monitor AML neutrophil maturation and functionality in clinical practice.

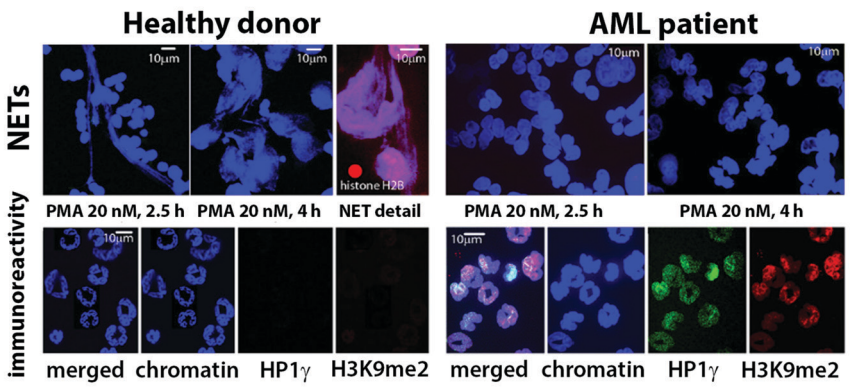

FIGURE 1. HP1γ and histone H3 dimethylated at lysine 9 (H3K9me2) are immunodetected in nuclei of AML neutrophils but absent in nuclei of healthy donors; this indicates incomplete differentiation of neutrophils from AML patients (lower line). Because of improper chromatin structure, AML neutrophils cannot fire chromatin nets when activated with PMA (upper line).

SELECTED OUTPUTS

Lukášová, E.; Kořistek, Z.; Klabusay, M.; Ondřej, V.; Grigoryev, S.; Bačíková, A.; Řezáčová, M.; Falk, M.; Vávrová, J.; Kohútová, V.; Kozubek, S. Granulocyte maturation determines ability to release chromatin NETs and loss of DNA damage response; these properties are absent in immature AML granulocytes. Biochim. Biophys. Acta. 2013, 1833, 767–779

BRCA1 ALTERNATIVE SPLICING VARIANTS (BRCA1-ASVs) CAN NEGATIVELY INFLUENCE THE REPAIR OF DNA DOUBLE STRAND BREAKS (DSBs)

Alternative splicing of pre-mRNAs is an important post-transcriptional regulatory mechanism. Its deregulation in tumor cells may lead to production of irregular ASVs. During the screening of high-risk breast cancer families, we ascertained numerous BRCA1-ASVs with an unknown clinical significance. Consequently, we have demonstrated that the ASVs studied can negatively influence repair of DNA double strand breaks, which represent the most serious damage of the genome.

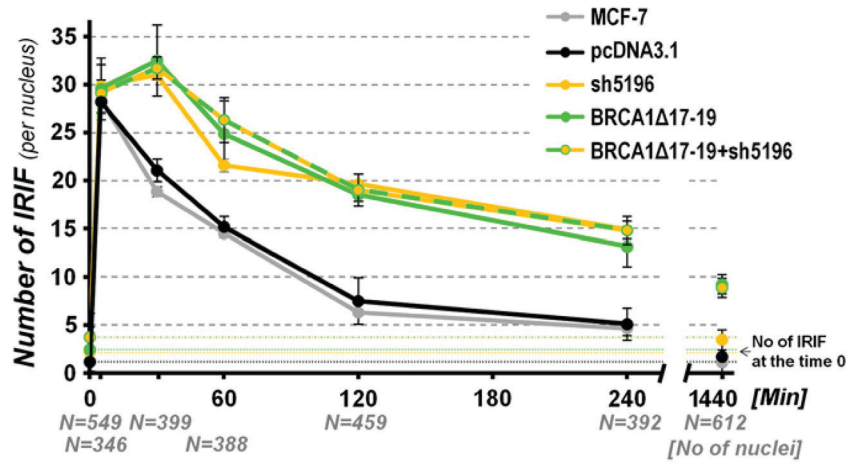

FIGURE 2. A negative effect on DSB repair kinetics of BRCA1 ASVs with deleted exons 17 – 19 (i.e. a part of BRCT domain) in MCF7 cells.). x-axis: time post irradiation [min]; y-axis: number of DSBs (IRIFs); curves: *grey* – control MCF7s (wtBRCA1), *black* – MCF7s transfected with empty pcDNA3.1 vector, *orange* – MCF7s with sh5196-mediated inhibition of wtBRCA1 expression, *green* – MCF7s expressing BRCA1Δ17-19 ASV together with wtBRCA1, *orange-green* – MCF7s with expressed BRCA1Δ17-19 ASV and inhibited wtBRCA1. Irradiation: ^{60}Co γ-rays, 1.5 Gy.

SELECTED OUTPUTS

Sevcik, J.; Falk, M.; Kleiblova, P.; Lhota, F.; Stefancikova, L.; Janatova, M.; Weiterova, L.; Lukasova, E.; Kozubek, S.; Pohlreich, P.; Kleibl, Z. The BRCA1 alternative splicing variant Δ14-15 with an in-frame deletion of part of the regulatory serine-containing domain (SCD) impairs the DNA repair capacity in MCF-7 cells. Cell Signal. 2012, 24, 1023–1030

Sevcik, J.; Falk, M.; Macurek, L.; Kleiblova, P.; Lhota, F.; Hojny, J.; Stefancikova, L.; Janatova, M.; Bartek, J.; Stribrna, J.; Hodny, Z.; Jezkova, L.; Pohlreich, P.; Kleibl Z. Expression of human BRCA1Δ17-19 alternative splicing variant with a truncated BRCT domain in MCF-7 cells results in impaired assembly of DNA repair complexes and aberrant DNA damage response. Cell Signal. 2013, 25, 1186–1193

SELECTED OUTPUTS

Falk, M.; Hausmann, M.; Lukášová, E.; Biswas, A.; Hildenbrand, G.; Davídková, M.; Krasavin, E.; Kleibl, Z.; Falková, I.; Ježková, L.; Štefančíková, L.; Ševčík, J.; Hofer, M.; Bačíková, A.; Matula, P.; Boreyko, A.; Vachelová, J.; Michaelidisová, A.; Kozubek, S. Determining Omics spatiotemporal dimensions using exciting new nanoscopy techniques to assess complex cell responses to DNA damage: part B – structuromics. Crit. Rev. Eukaryot. Gene Expr. 2014, 24, 225–247

MECHANISMS OF HOW CHROMOSOMAL TRANSLOCATIONS ARE FORMED DEPEND BOTH ON THE QUALITY OF IONIZING RADIATION (USED TO INDUCE DNA DAMAGE) AND CHROMATIN STRUCTURE

Because of localized energy deposition, high-LET (linear energy transfer) radiations directly induce multiple DSBs (primary DSB clusters) that represent substrates for chromosomal translocations. On the other hand, low-LET radiations create isolated DSBs, which can occasionally cluster secondarily (secondary clusters) due to repair processes.

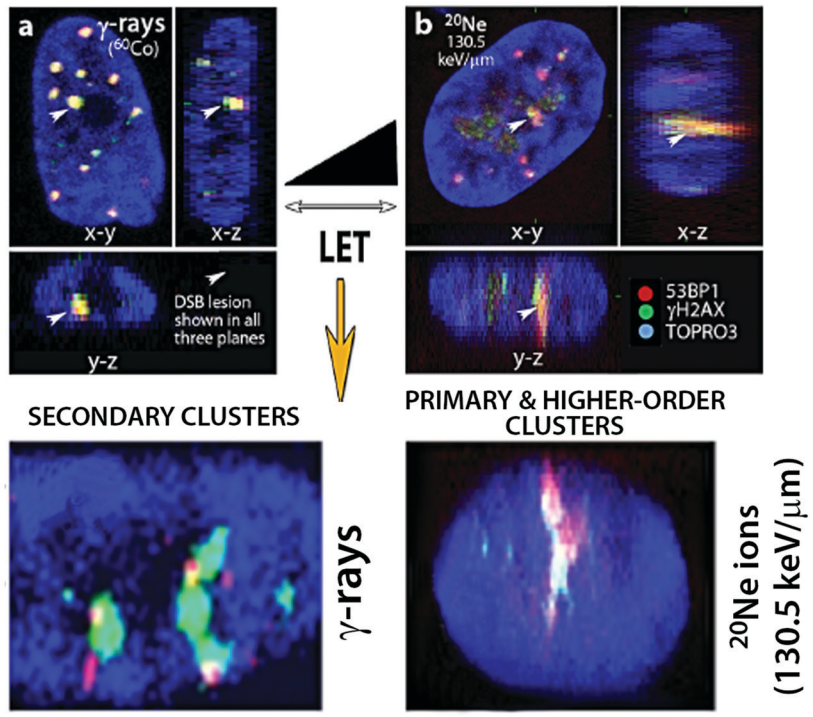

FIGURE 3. Primary and secondary DSB clusters formed upon irradiating cells with accelerated ^{20}Ne ions (130.5 keV/μm) and γ-rays (^{60}Co), respectively. *Red + green*: 53BP1 and γH2AX, respectively (DSB markers), *blue*: chromatin.

SELECTED OUTPUTS

Polanská, E., Dobšáková, Z., Dvořáčková, M., Fajkus, J., Štros, M. HMGB1 gene knockout in mouse embryonic fibroblasts results in reduced telomerase activity and telomere dysfunction. Chromosoma. 2012, 121, 419–431

HMGB1 AND HMGB2 PROTEINS INFLUENCE TELOMERASE ACTIVITY AND TELOMERE FUNCTION

Telomere sequences at chromosome ends are elongated by the telomerase, an enzyme consisting of catalytic and RNA subunits (TERT, TR). We have discovered that a functional relationship exists between HMGB1 and telomeres: knockout of HMGB1 in mouse embryonic cells causes (I) a significant decrease of telomerase activity, (II) chromosomal abnormalities, (III) accumulation of γH2AX foci in telomeres, and (IV) telomere shortening. Interestingly, even though HMGB2 has similar functions in cells like HMGB1, the knockout of HMGB2 has the opposite effect on the telomerase activity. Hence, the telomerase activity also seems to be regulated by different expression of HMGB1 and HMGB2 proteins. Our findings can contribute to elucidation of the roles of these proteins in maintaining chromosome stability and cancer development.

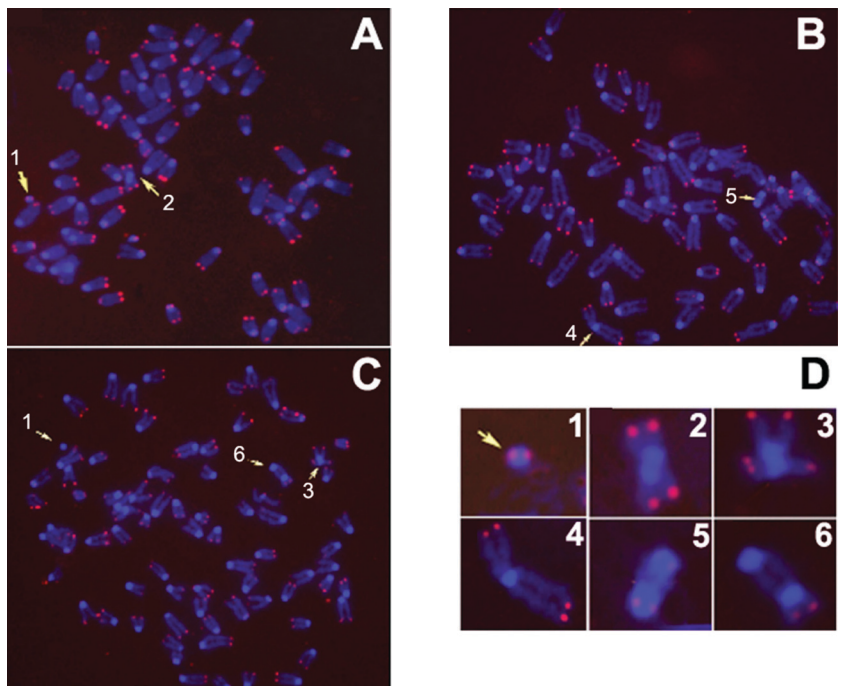

FIGURE 4. HMGB1-/- MEFs show increased chromosome instability. FISH with a Cy3-labeled telomeric PNA probe (red) in HMGB1-/- MEFs (panels A-C); chromosomes are stained with DAPI (blue). The arrows indicate chromosomal aberrations which are shown in detail in the lower part: (1) minichromosomes; (2–4) Robertsonian-like fusions (the most common, 12/57 cases); (5–6) end-to-end fusions. A total of 57 metaphases were analyzed.

SCIENTISTS Miroslav Fojta, Emil Paleček, Václav Brázda, Eva Brázdová-Jagelská, Marie Brázdová, Hana Černocká, Vlastimil Dorčák, Lukáš Fojt, Stanislav Hasoň, Luděk Havran, Veronika Ostatná, Zdeněk Pechan, Hana Pivoňková, Mojmír Trefulka, Radim Vespalec
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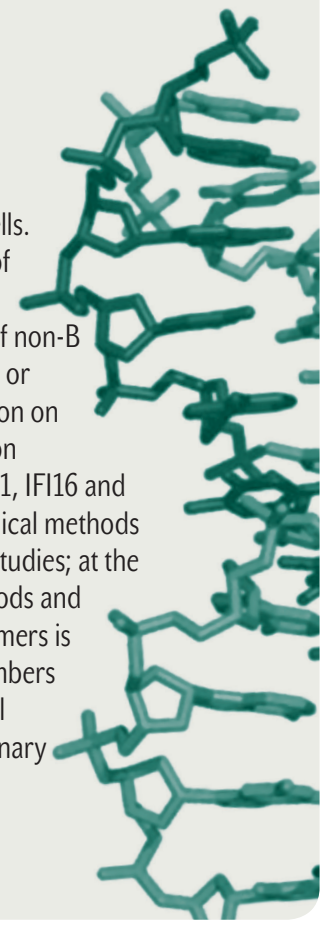
DEPARTMENT OF BIOPHYSICAL CHEMISTRY AND MOLECULAR ONCOLOGY

THE DEPARTMENT OF BIOPHYSICAL CHEMISTRY AND MOLECULAR ONCOLOGY (DBCMO) STUDIES THE STRUCTURE OF NUCLEIC ACIDS, PROTEINS, CARBOHYDRATES AND OTHER BIOLOGICALLY IMPORTANT MOLECULES IN SOLUTIONS AND AT SURFACES AND DEVELOPS NOVEL ELECTROCHEMICAL BIOSENSORS AND BIOASSAYS.

In the field of electrochemical analysis of nucleic acids, the DBCMO holds the world primacy because of the discovery of DNA electroactivity, made by Emil Paleček at the end of the 1950's. Since then, the DBCMO has dealt with the study of nucleic acids on electrodes and in solutions which resulted in laying foundations of the contemporary nucleic acids electrochemistry. As evidenced by number of publications, including invited reviews, this research is extraordinarily fruitful. The DCBMO contributes to the progress in various areas significantly, including systematic studies of electrochemical detection of DNA damage, DNA hybridization and DNA-protein interactions. Various techniques of DNA labelling have been established at the DBCMO to improve its electrochemical analysis.

Protein electrochemistry has been studied during last two decades. The research is directed especially towards the study of proteins which do not contain "non-protein" redox centres and exhibit electrochemical activity because of catalysis of hydrogen evolution. This approach is unique in the context of world science and it opens new possibilities for the studies of protein structure and interactions. Using electrocatalytic "peak H" in combination with constant current chronopotentiometric stripping (CPS) on mercury or solid amalgam electrodes, it is possible to detect protein denaturation, aggregation as well as small changes of protein structure and protein-DNA interactions.
The DBCMO is engaged in systematic studies of proteins important in cancer, particularly their interactions with DNA *in vitro* and in cells. Our efforts are focused on the effects of DNA nucleotide sequence, structure (DNA superhelicity and/or formation of non-B structures such as cruciforms, triplexes or quadruplexes) and chemical modification on DNA binding by p53-family transcription factors or other proteins(such as BRCA1, IFI16 and others). Newly developed electrochemical methods mentioned above are applied in these studies; at the same time, development of novel methods and their applications in analysis of biopolymers is supported by skills of the DBCMO members in biochemical and molecular biological methods, creating a strong interdisciplinary background of the department.

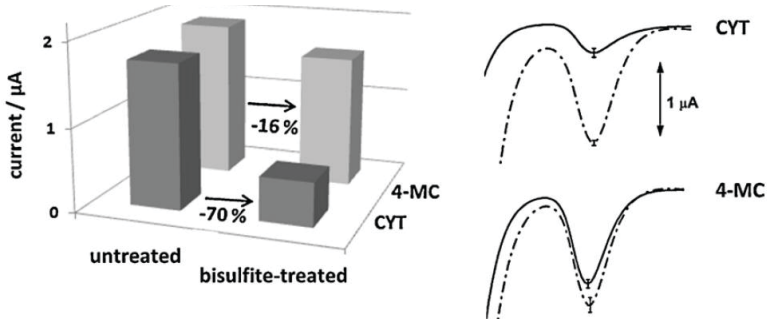


SELECTED RESULTS

ELECTROCHEMISTRY OF NUCLEIC ACIDS: LABEL-FREE ELECTROCHEMICAL ANALYSIS OF DNA METHYLATION

Methylation of cytosine is an epigenetic modification of DNA involved in mechanisms of chromatin structure regulation, gene expression control and, consequently, cell differentiation. Because of its importance, different methods, which frequently use selective deamination of non-methylated cytosine with bisulfite, are developed for the detection of the 5-methylcytosine (5-mC) in DNA. Using 5-mC reduction signal at mercury or solid amalgam electrodes, we have designed a simple label-free technique for the determination of cytosine methylation level in bisulfite-treated DNA. Cytosine, which is deaminated to electrochemically "silent" uracil, does not contribute to the voltammetric signal obtained after the bisulfite treatment.

This result represents another of a series of applications of nucleic acids electrochemistry in the DBCMO. The field was founded almost 60 years ago in Brno by E. Paleček and in 2012, it was reviewed thoroughly in an invited *Chemical Reviews* paper [see the chapter "Selected papers"]. The review has recently received enough citation to be placed in the top 1% of its academic field based on threshold for the field and publication year by the Web of Science.

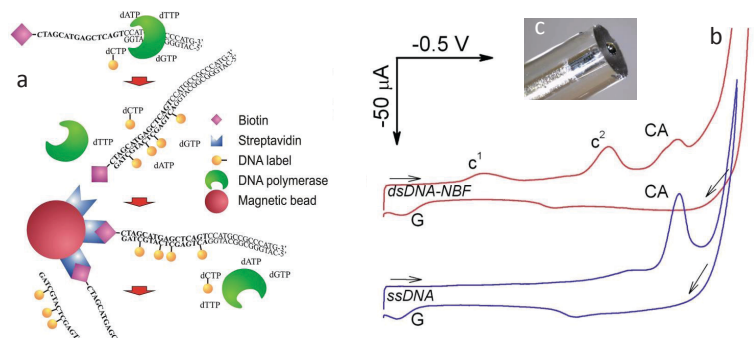


Comparison of voltammetric responses of non-methylated CYT (containing 10 cytosines) and methylated MC (4 of the 10 cytosines methylated) oligonucleotides. (A) Column graph showing CA peak heights of CYT and MC prior (left) and after (right) bisulfite treatment. (B) Voltammetric responses corresponding to the column graph measured prior to (dashed dot) or after (solid) bisulfite treatment (the residual reduction signal after the bisulfite treatment of CYT comes from adenine residues present in both oligonucleotides).

LABELLING OF BIOPOLYMERS WITH ELECTROACTIVE SPECIES FOR BIOSENSORS & BIOASSAYS

We have contributed to the development of techniques of biopolymers labelling with various electroactive groups in order to improve electrochemical analysis of the biopolymers and to extend application potential of electrochemical methods in experimental biology and biomedicine. One of these techniques is based on chemical modification of the biopolymers with oxo-osmium complexes with bidentate nitrogenous ligands. Osmium tetroxide reagents have been used for the modification of thymine residues in DNA with applications in e.g., studies of protein-DNA binding. Complexes of six-valent osmium have been applied for modification of sugar moieties, such as the 3′-terminal ribose in RNAs and for introduction of electroactive tags into various carbohydrates including glycan components of glycoproteins.

The other approach is based on enzymatic incorporation of labelled nucleotides using DNA polymerases and modified deoxynucleoside triphosphates (dNTPs) as substrates. In a close collaboration with the group of Prof. M. Hocek, (IOCB ASCR, v.v.i., Prague) we have extended the palette of electrochemically oxidizable or reducible DNA labels applicable to multipotential redox coding of nucleobases and nucleotide sequences, and developed electrochemical methods for their analysis.



General scheme of labelled DNA preparation, using the polymerase-based techniques (a), examples of cyclic voltammograms (b) of unmodified DNA (blue) and DNA labelled with nitrobenzofurazane (red), and a photo of a solid amalgam electrode on which the voltammograms were recorded (c) and.

SELECTED OUTPUTS

Hocek, M; Fojta, M; Nucleobase modification as redox DNA labelling for electrochemical detection. Chem Soc Rev 2011, 40, 5802–5814

Balintova, J; Plucnara, M; Vidlakova, P; Pohl, R; Havran L; Fojta, M; Hocek, M; Benzofurazane as a New Redox Label for Electrochemical Detection of DNA: Towards Multipotential Redox Coding of DNA Bases. Chem-Eur J 2013, 19, 12720–12731

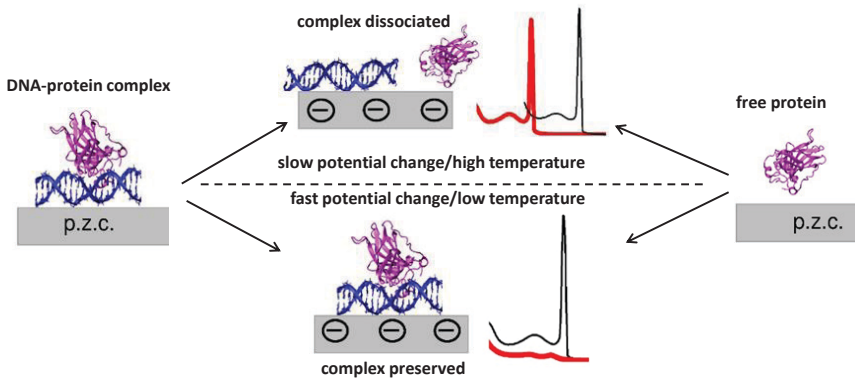
SELECTED OUTPUTS

Palecek, E; Bartosik, M; Electrochemistry of Nucleic Acids. Chem Rev 2012, 112, 3427–3481

Palecek, E; Tkac, J; Bartosik, M; Bertok, T; Ostatna, V; Palecek, J; Electrochemistry of Nonconjugated Proteins and Glycoproteins. Toward Sensors for Biomedicine and Glycomics. Chem Rev 2015, 115, 2045–2108

ELECTROCHEMISTRY OF PROTEINS: SENSITIVE METHODS FOR PROTEIN STRUCTURE AND INTERACTIONS

We have developed a method, based on combination of electrocatalytic activity of some amino acid residues and constant current chronopotentiometric stripping (CPS), allowing analysis of practically any protein. We have shown that proteins are not denatured when adsorbed to bare metal electrodes at potentials close to zero charge, but they can denature at negatively charged electrodes. Such denaturation can be avoided in CPS at high current densities. Using CPS with thiol-modified Hg electrodes, we have observed excellent correlation with structure and stability of the tumor suppressor protein p53. Recently, we have used this method for the analysis of DNA-protein interactions. The sequence-specific binding of p53 core domain (CD) to DNA resulted in a striking decrease in the electrocatalytic reduction signal of free p53. This decrease is related to changes in the accessibility of the electroactive amino acid residues in the p53CD-DNA complex. By adjusting current density and temperature, weaker non-specific binding can be either eliminated or distinguished from the sequence-specific binding. The high resolving power of this method is based on the disintegration of the p53-DNA complex by the electric field effects at a negatively charged surface and fine adjustment of the millisecond time intervals for which the complex is exposed to these effects.

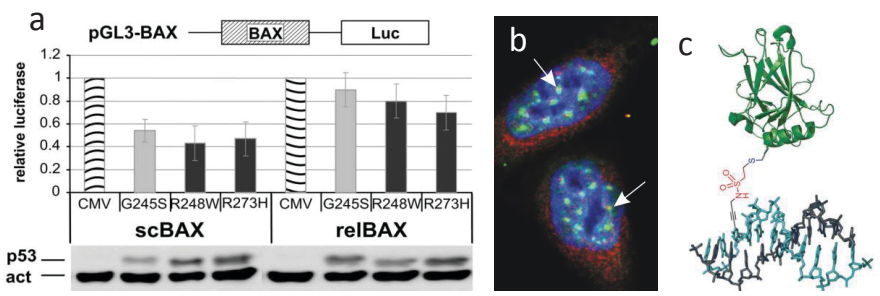


Scheme of the detection of p53 core domain interactions with DNA using CPS at thiol-modified mercury electrode (p.z.c.: potential of zero charge).

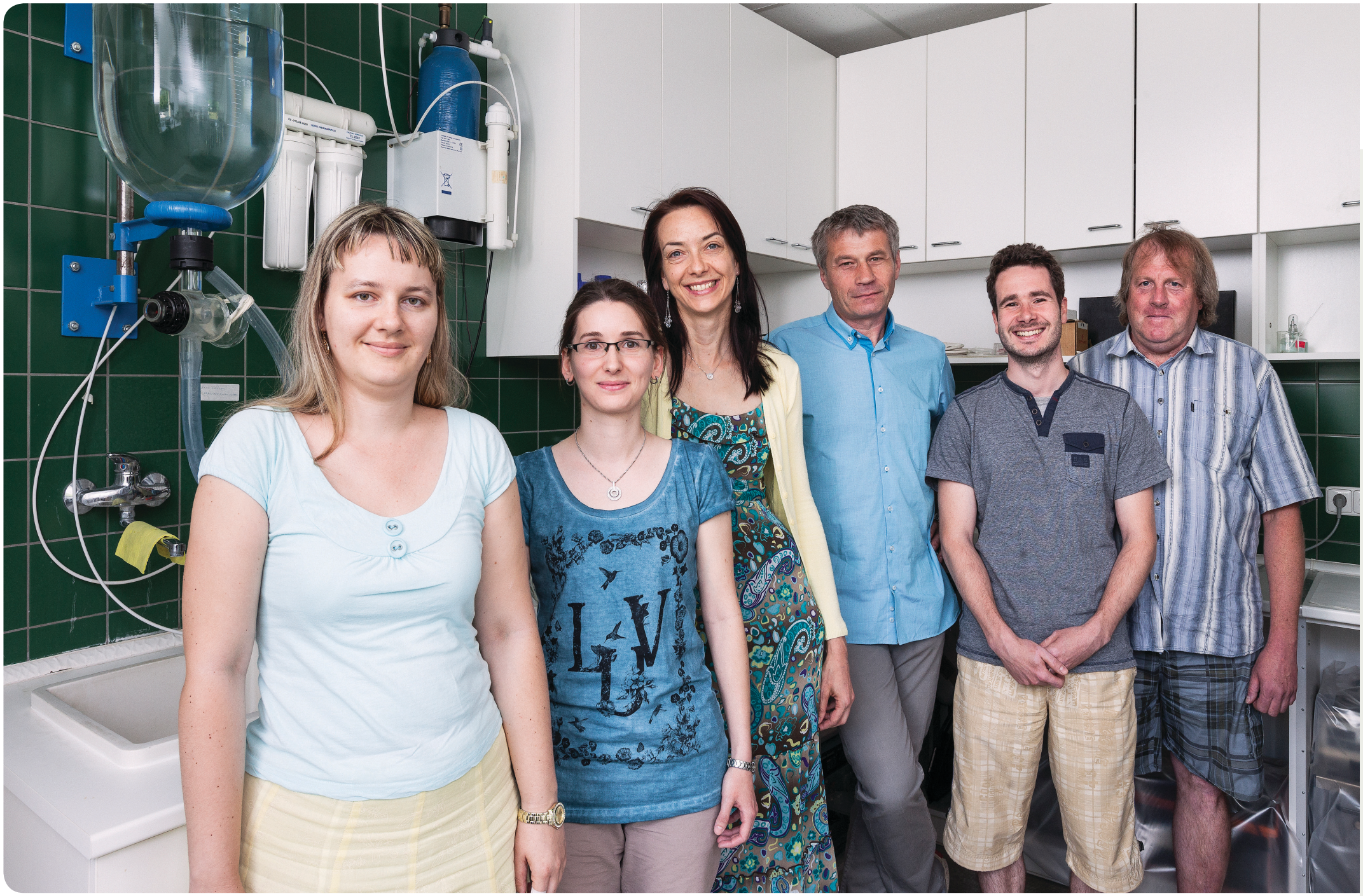
STUDIES OF INTERACTIONS OF PROTEINS IMPORTANT IN CANCER WITH DNA IN VITRO AND IN CELLS

In this area we contributed by systematic studies of the interactions of the p53-family transcription factors and other proteins with DNA in various topological states and/or adopting diverse alternative (non-B) structures. We have shown that cancer-related mutant p53 proteins (mutp53) retain the ability to bind preferentially supercoiled DNA (previously reported for the wild-type p53, wtp53) and that the DNA superhelicity modulates interactions of both wtp53 and mutp53 with DNA not only *in vitro*, but also in cell lines (as evidenced by chromatin immunoprecipitation and reporter expression assays involving various natural promoters such as BAX or MSP). Preferential binding to supercoiled DNA and to cruciform DNA structures was also observed for the gamma-interferon-inducible protein IFI-16 or 14-3-3 protein. These observations extend the insight into the mechanisms of regulation of crucial cellular processes including transcription, cell cycle and defence against malignant transformation.

For the protein-DNA binding studies we have developed and applied new analytical techniques based on electrochemistry and/or chemically modified DNAs. In collaboration with M. Hocek group (IOCB ASCR Prague), novel reactive DNA probes for covalent crosslinking of DNA-protein complexes were introduced and successfully applied for the p53 protein-DNA bioconjugation.



Evidence of the effect of DNA superhelicity on the BAX promoter repression in cells using luciferase reporter assay (a), colocalization of 14-3-3 protein with cruciform DNA in cell nuclei (b) and scheme of covalent conjugate of a reactive DNA probe with p53 core domain (c).



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DEPARTMENT OF MOLECULAR EPIGENETICS

WE FOCUS ON THE ROLE OF EPIGENETIC MECHANISMS IN ORGANISATION OF DNA IN CELL NUCLEUS, EPIGENETIC REGULATION OF TRANSGENE EXPRESSION AND EVOLUTION.

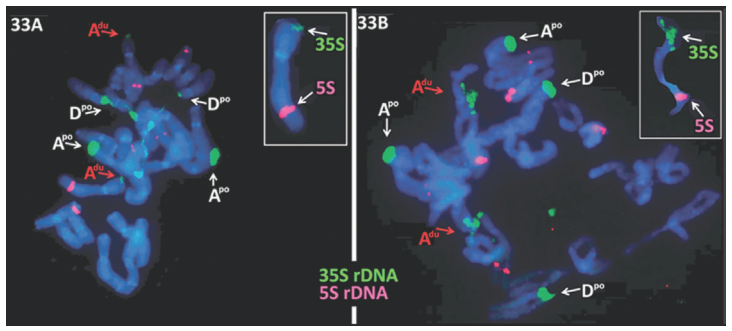
The term epigenetics came into general use in the early 1940s, when British embryologist Conrad Waddington used it to describe the interactions between genes and gene products, which determine developmental pathways and give rise to an organism's phenotype (observable characteristics). Since then, information revealed by epigenetics studies has revolutionized the fields of genetics and developmental biology. Specifically, researchers have uncovered a range of possible chemical modifications to deoxyribonucleic acid (DNA) and to proteins called histones that associate tightly with DNA in the nucleus. These modifications can determine when or even if a given gene is expressed in a cell or organism. In our research, we focus on the role of epigenetic mechanisms in organisation of DNA in cell nucleus, epigenetic regulation of transgene expression and evolution.

We address the important questions: How do small RNAs, nucleosome modification and remodelling mechanisms cooperate in epigenetic regulation of the non-coding and coding information in DNA and how does this control several important cell physiology functions? What is the relationship between nuclear architecture, chromatin arrangement and chromatin modifications in the determination of epigenetic states? What are the effects of external signals on epigenetic states and reprogramming? How do epigenetic mechanisms mediate gene environment interactions? How do the epigenetic mechanisms influence stability of transgene expression, which is the key factor in application of transgene technology in biotechnology and medicine. What is the role of epigenetic factors in harmonising gene expression after the "genomic shock" in hybrids and allopolyploid species?

SELECTED RESULTS

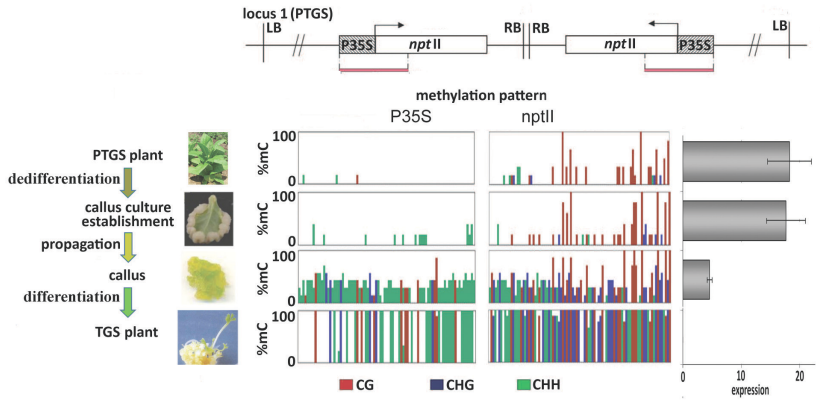
MOLECULAR MECHANISMS OF GENE DOSAGE CONTROL IN INTERSPECIFIC HYBRIDS AND ALLOPOLYPLOIDS

Polyploidy, in which the entire chromosome complement multiplies (3x, 4x), is widespread in plants. When polyploidy occurs after interspecific hybridisation, the process is called allopolyploidy. In allopolyploids, one manifestation of gene dosage control is nucleolar dominance, an epigenetic phenomenon in which the ribosomal rRNA genes (rDNA) of one progenitor are repressed. To study the relationship between nucleolar dominance and rRNA gene dosage, we have studied allotetraploid *Tragopogon mirus*, recently formed from the diploid parents *T. dubius* (Du-genome donor) and *T. porrifolius* (Po). We have used molecular, cytogenetic and genomic approaches to analyze rRNA gene activity in two sibling plants with widely different rRNA gene dosages. Example of cytogenetic analysis of ribosomal rRNA genes in plants 33A and 33B by fluorescence in situ hybridisation (FISH) is shown in Figure below. Plant 33B had ~ 400 rRNA genes in Du-genome, which is typical for *T. mirus* with expression dominance of Du-genes in all organs. Its sister plant 33A harboured a homozygous deletion at chromosome A^{du} that reduced the number of Du-genes to about 70 copies resulting in biparental rDNA expression in root but not in leaf where Du-rDNA dominance was maintained. We hypothesize that active, decondensed rDNA units (A^{du}) are most likely to be deleted via recombination. The silenced homeologs could be used to ameliorate mutational damage and contribute to evolutionary success of polyploids.



THE INFLUENCE OF CELL DEDIFFERENTIATION ON EPIGENETIC STATE OF PLANT TRANSGENES

Understanding processes leading to cell dedifferentiation and differentiation is in the centre of interest of both plant and animal physiologists. Here, we have studied the influence of cell dedifferentiation in *Nicotiana tabacum* (tobacco) plants, also known as callusogenesis (left margin of the Figure), on epigenetic modifications of transgenes. In transgene locus 1, the residing reporter gene nptII was silenced at the posttranscriptional (PTGS) level. Transcriptionally silenced (TGS) epialleles were generated in a callus culture (see the right part of Figure for the RNA analysis). PTGS to TGS conversion in callus was accompanied by spreading of cytosine methylation from the transcribed region into the promoter (central part of the Figure shows distribution of methylated cytosine residues along the sequence). We show that cell culture resulted in blurring of the parental epigenetic expression and methylation patterns at the silencing locus associated with increased epiallelic diversity. Regenerated plants showed high interindividual but low intraindividual epigenetic variability, indicating that the callus induced epiallelic variants were transmitted to plants and became fixed. We hypothesize that epigenetic changes associated with dedifferentiation may influence setting of epigenetic states of endogenous genes. These observations should be considered in application of stem cells in human medicine.



SELECTED OUTPUTS

Dobešová, E; Malinská, H; Matyášek, R; Leitch, AR; Soltis, DE; Soltis, PS; Kovařík A; Silenced rRNA genes are activated and substitute for partially eliminated active homeologs in the recently formed allotetraploid, Tragopogon mirus (Asteraceae). Heredity 2015, 114, 356–365

Matyášek, R; Tate, JA; Lim, YK; Šrubařová, H; Koh, J; Leitch, AR; Soltis, DE; Soltis, PS; Kovařík, A; Concerted evolution of rDNA in recently formed Tragopogon allotetraploids is typically associated with an inverse correlation between gene copy number and expression. Genetics 2007, 176, 2509–2519

SELECTED OUTPUTS

Křížová, K; Depicker, A; Kovařík, A; Epigenetic switches of tobacco transgenes associate with transient redistribution of histone marks in callus culture. Epigenetics 2013, 8, 666–676

Křížová, K; Fojtová, M; Depicker, A; Kovařík, A; Cell culture-induced gradual and frequent epigenetic reprogramming of invertedly repeated tobacco transgene epialleles. Plant Physiol 2009, 149, 1493–1504

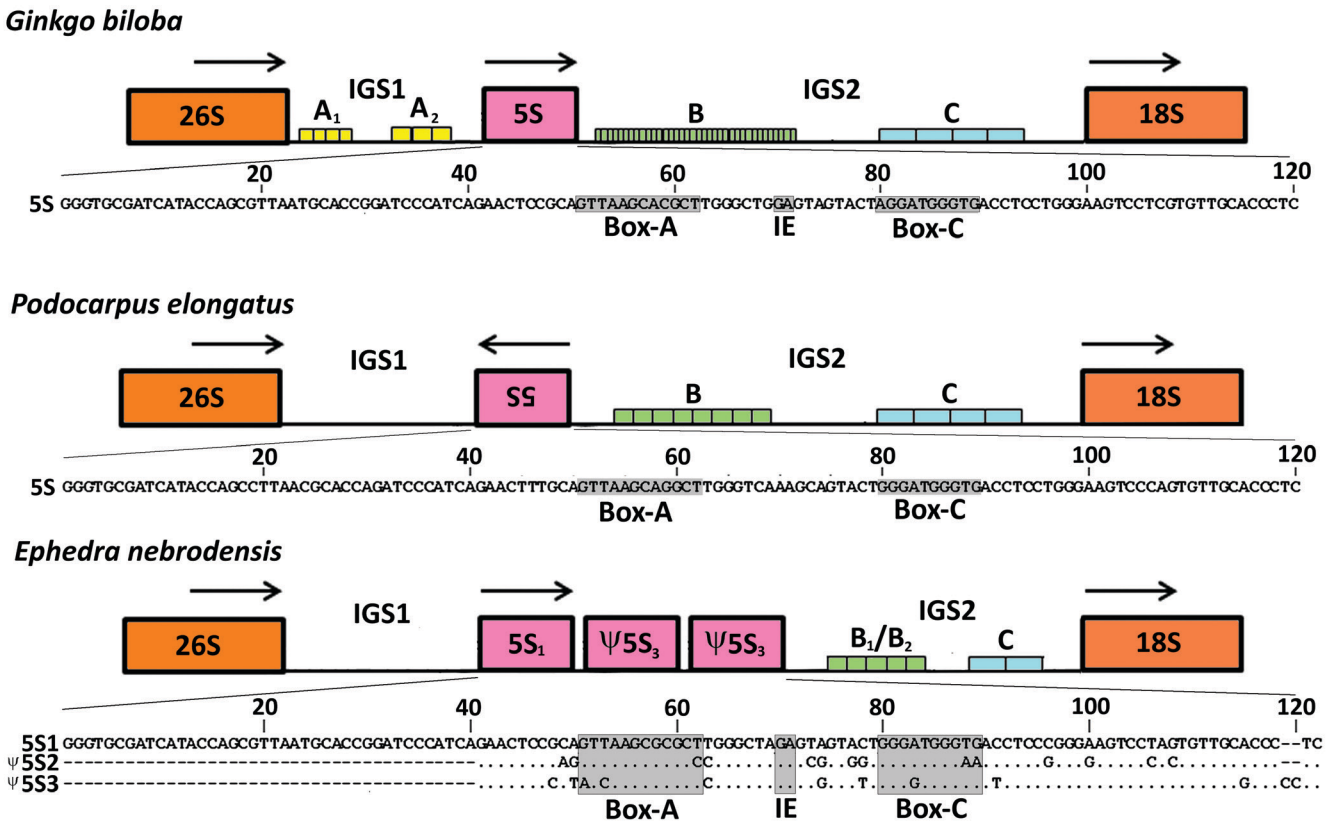
SELECTED OUTPUTS

Garcia, S; Kovařík, A; Dancing together and separate again: gymnosperms exhibit frequent changes of fundamental 5S and 35S rRNA gene (rDNA) organization. Heredity 2013, 111, 23–33

Garcia, S; Panero, JL; Široký, J; Kovařík, A; Repeated reunions and splits feature the highly dynamic evolution of 5S and 35S ribosomal RNA genes (rDNA) in the Asteraceae family. BMC Plant Biol 2010, 10, 176

ORGANISATION OF 5S AND 35S rRNA GENES IN PLANTS

Eukaryotic ribosomes are composed of 5S, 5.8S, 18S and 26S rRNAs plus about 70 proteins. While the 18S, 5.8S and 26S genes are always co-transcribed at 35S rDNA loci, the 5S rRNA is usually transcribed from separate loci (S-arrangement). However, linked 35S-5S rDNA (L- arrangement) also occurs in several phylogenetic groups including plants. Here, we have studied chromosomal and genomic organisation of rDNA in several gymnosperm species. We have observed the L-arrangement in some *Gnetales* and *Coniferales* species, and in Ginkgo (right part of Figure, pink colour). *Cycadales* exhibit separate organization of rDNA (black colour). Linked 5S rRNA genes occurred as single-copy insertion or as short tandem embedded in the 26S–18S rDNA intergenic spacer (left part of the Figure). The 5S transcript may be encoded by the same (*Ginkgo, Ephedra*) or opposite (*Podocarpus*) DNA strand as the 18S–5.8S–26S genes. In addition, pseudogenised 5S copies were found in *Ephedra*. Both L- and S-type units have been largely homogenised across the genomes. Phylogenetic relationships based on the comparison of 5S coding sequences suggest that the 5S genes independently inserted IGS at least three times in the course of gymnosperm evolution. Frequent transpositions and rearrangements of basic units may indicate relatively relaxed selection pressures imposed on genomic organisation of 5S genes in plants.



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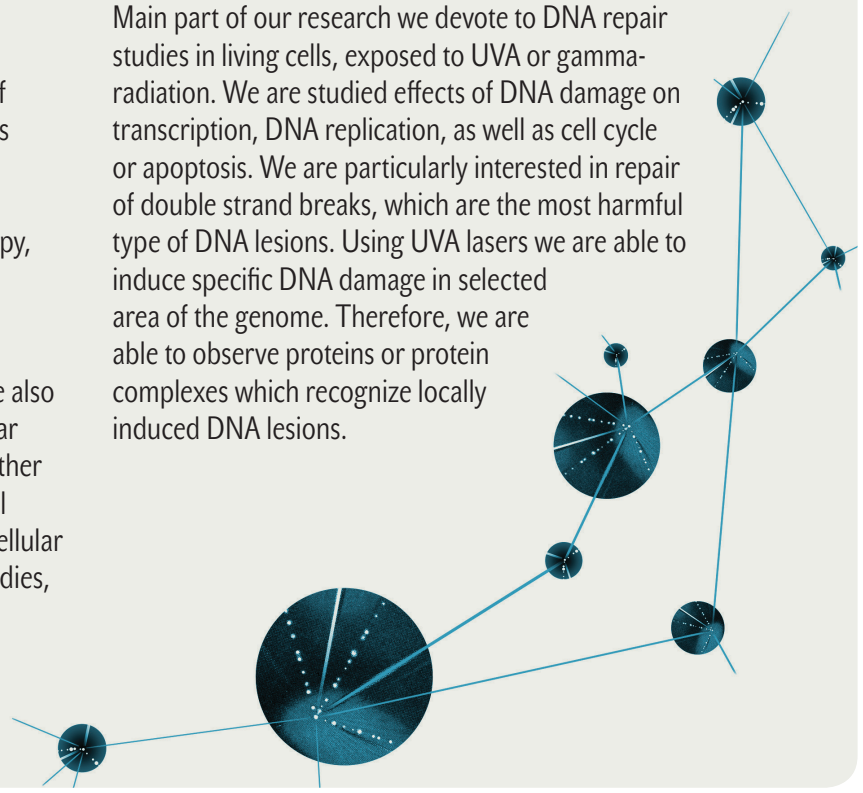
DEPARTMENT OF MOLECULAR CYTOLOGY AND CYTOMETRY

WE ARE FOCUSED ON THE NUCLEAR ARCHITECTURE WHICH REGULATES TRANSCRIPTION OR DNA REPAIR PROCESSES. ANOTHER FIELD OF OUR RESEARCH IS THE STUDY OF EPIGENETIC EVENTS IN THE CELL NUCLEUS, WE ESPECIALLY AIM AT POST-TRANSLATION MODIFICATIONS OF HISTONES.

Team members of the Department of Molecular Cytology and Cytometry are interested in the studies of structures and processes in the cell nucleus. We are focused on the nuclear architecture which regulates transcription or DNA repair processes. Besides, we studied nuclear arrangement of chromosomal territories (CTs) in various cell types and transmission of chromosome positioning from mother to daughter cells.
Another field of our research is the study of epigenetic events in the cell nucleus, we especially aim at post-translation modifications of histones (PTMs). We study regulatory role of PTMs of histones in ribosomal genes and we are interested in understanding how PTMs of histones influence chromatin architecture, gene expression and DNA repair. Our research is focused on histone code changes after the cell treatment by inhibitors of enzymes that mediate histone signature, including methylation or acetylation.
We investigate nuclear processes by the use of techniques of molecular biology or proteomics to obtain a detailed picture of the molecular events occurring in the cell nucleus.
In addition, high resolution confocal microscopy, combined with GFP technology, enables us to study processes in living cells; for example, during the cell cycle and cell differentiation.
Using fluorescence protein technology, we are also able to monitor changes in trajectory of nuclear protein bodies and their interactions with another regions of the cell nucleus. Moreover, confocal microscopy in combination with HeLa-Fucci cellular model also enables us to provide cell cycle studies, when various phases of the cell cycle could be distinguished in this cell type.

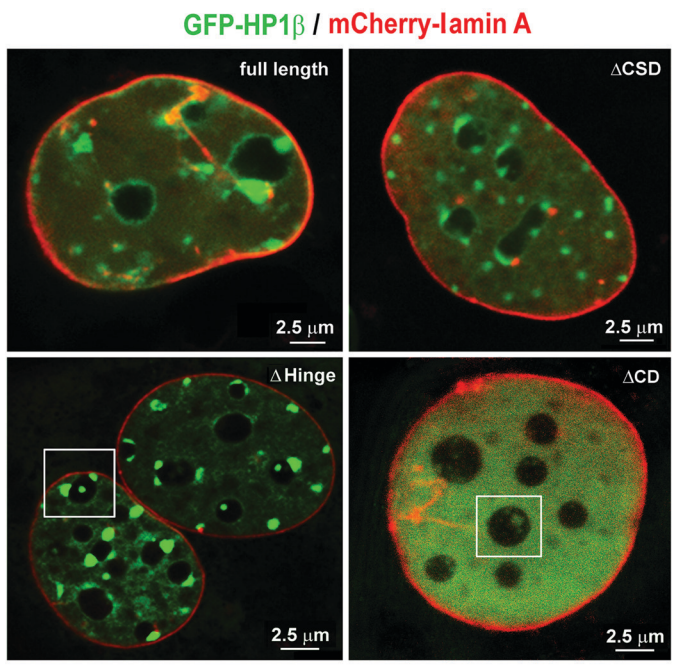
Main part of our research we devote to DNA repair studies in living cells, exposed to UVA or gamma-radiation. We are studied effects of DNA damage on transcription, DNA replication, as well as cell cycle or apoptosis. We are particularly interested in repair of double strand breaks, which are the most harmful type of DNA lesions. Using UVA lasers we are able to induce specific DNA damage in selected area of the genome. Therefore, we are able to observe proteins or protein complexes which recognize locally induced DNA lesions.



SELECTED RESULTS

THE ROLE OF A-TYPE LAMINS IN DNA REPAIR PROCESSES AFTER UVA RADIATION-INDUCED DNA DAMAGE

The optimal repair of DNA lesions is fundamental for physiological processes. In the study Sehnalová et al. (2014) we investigated the recruitment of HP1β, 53BP1 and BMI1 proteins to ultraviolet (UVA)-induced DNA lesions. We found that UVA irradiation of nuclear lamina abolished A-type lamins. However, irradiation did not affect the recruitment of HP1β, 53BP1 and BMI1 to DNA lesions. We also analysed the function of full-length HP1β and HP1β with deleted CD, CSD or hinge domains (Fig. 1) at UV-induced DNA lesions. Fluorescence intensity of GFP-HP1β and GFP-BMI1 at UVA-induced DNA lesions were unaffected by deficiency in A-type lamins, whereas those parameters of mCherry-53BP1 were changed. We conclude that only the 53BP1 status at DNA lesions is probably affected by A-type lamin deficiency.

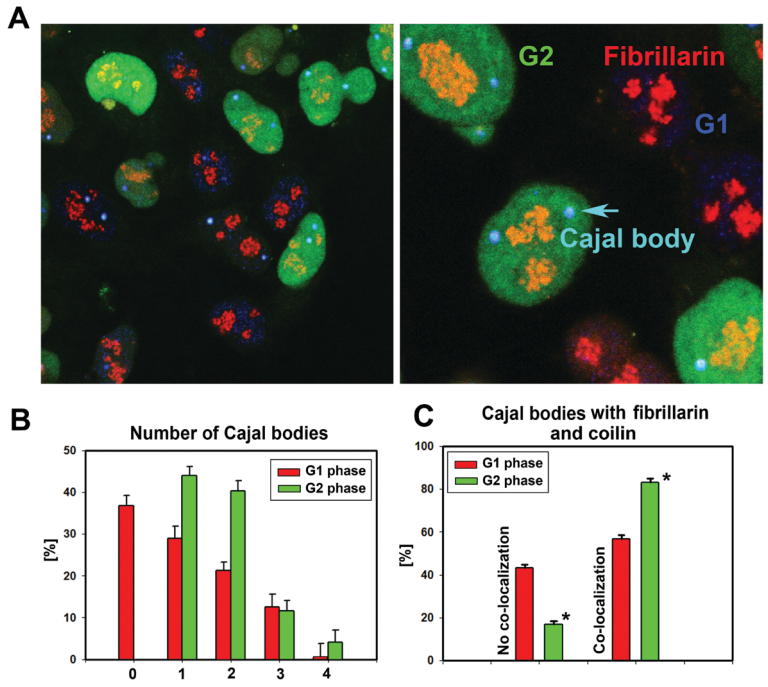


SELECTED OUTPUTS

Sehnalová, P; Legartová, S; Cmarko, D; Kozubek, S; Bártová, E; Recruitment of HP1β to UVA-induced DNA lesions is independent of radiation-induced changes in A-type lamins. Biol Cell 2014, 106, 151–165

NUCLEAR MORPHOLOGY OF CAJAL BODIES IS DEPENDENT ON THE CELL CYCLE

Cajal bodies (CBs) (Fig. 2A) are important nuclear structures containing proteins that preferentially regulate RNA-related metabolism. We investigated the cell-type specific nuclear distribution of Cajal bodies and the level of coilin (a main protein of CBs) in non-irradiated and irradiated human tumour cell lines or embryonic stem (ES) cells. The number of Cajal bodies per nucleus was cell cycle-dependent (Fig. 2B), with higher numbers occurring in G2 phase (green) compared with those in G1 (red) phase of the cell cycle. We also found that the number of Cajal bodies, containing both coilin and fibrillarin, increased in G2 phase. These experiments pointed out the cell cycle phases a very important to study from the view of protein mobility and protein foci trajectory at various types of DNA lesions.



SELECTED OUTPUTS

Foltánková, V; Matula, P; Sorokin, D; Kozubek, S; Bártová, E; Hybrid detectors improved time-lapse confocal microscopy of PML and 53BP1 nuclear body colocalization in DNA lesions. Microsc Microanal 2013, 19, 360–369

SELECTED OUTPUTS

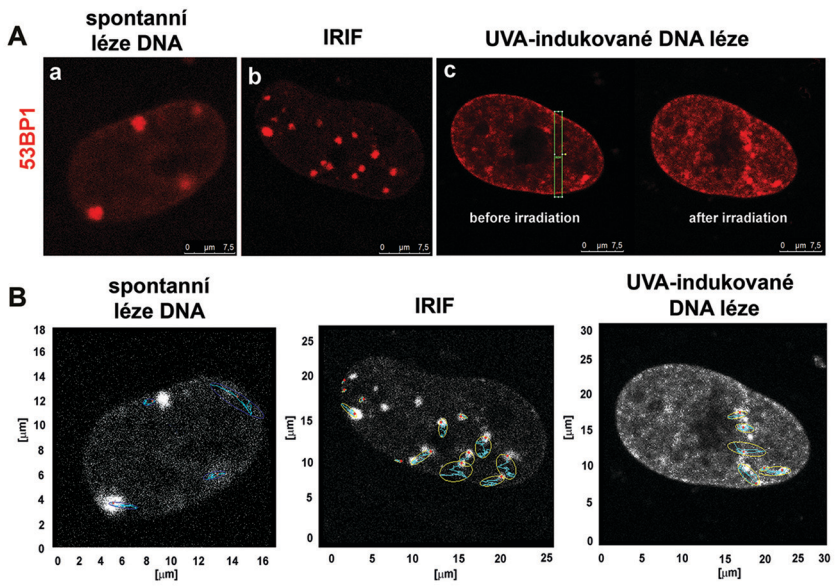
Bártová, E; Foltánková, V; Legartová, S; Sehnalová, P; Sorokin, DV; Suchánková, J; Kozubek, S; Coilin is rapidly recruited to UVA-induced DNA lesions and γ-radiation affects localized movement of Cajal bodies. Epigenetics Chromatin 2014, 5, 460–468

SELECTED OUTPUTS

Stixová, L; Sehnalová, P; Legartová, S; Suchánková, J; Hrušková, T; Kozubek, S; Sorokin, DV; Matula, P; Raška, I; Kovařík, A; Fulneček, J; Bártová, E; HP1β-dependent recruitment of UBF1 to irradiated chromatin occurs simultaneously with CPDs. Epigenetics Chromatin 2014, 7, 39

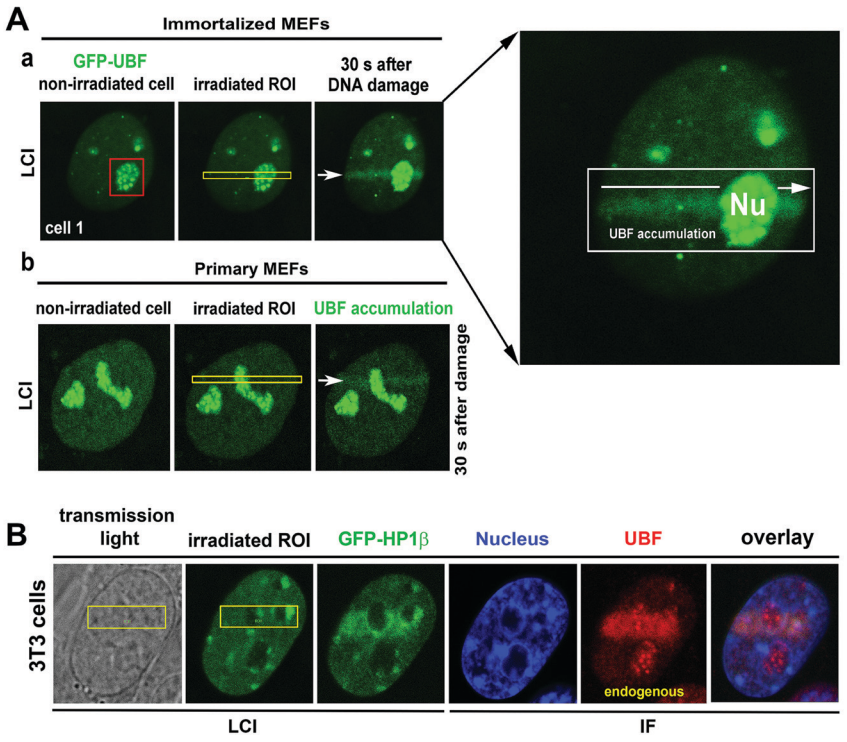
HYBRID DETECTORS USED FOR ADVANCED ANALYSIS OF IMAGES ACQUIRED BY CONFOCAL MICROSCOPY

We used hybrid detectors (HyDs) to monitor the trajectories and interactions of promyelocytic leukemia nuclear bodies (shown by Foltánková et al., 2013) and mCherry-53BP1-positive DNA lesions (Fig. 3A). In comparison with photomultiplier detectors, which are used for standard analysis by confocal laser scanning microscopes, HyDs significantly eliminated photobleaching of GFP and mCherry fluorochromes during image acquisition. Using this technique we found that trajectories of 53BP1-positive nuclear bodies were changed after γ-irradiation (Fig. 3B). We showed that hybrid detectors (HyDs) represent a useful tool for live-cell imaging because of their sensitivity. These detectors can be used with lower laser intensities to illuminate the sample, which reduces both photodamage and phototoxicity. Therefore, HyDs are useful for time-lapse confocal microscopy. Importantly, HyDs significantly eliminated photobleaching of GFP and mCherry fluorochromes during image acquisition, and they permitted us to perform real-time image acquisition for a longer time period relative to the time allowed when using photomultipliers. We also combined HyD-based confocal microscopy with a tailored advanced image analysis called single particle tracking analysis (Fig. 3B).



RECRUITMENT OF GFP-UBF1, HP1β, γH2AX, AND 53BP1 TO UVA-INDUCED DNA LESIONS

We analyzed the DNA-damage response after ultraviolet A (UVA) and γ-irradiation of mouse embryonic fibroblasts and focused on upstream binding factor 1 (UBF1), a key protein in the regulation of ribosomal gene transcription. Cells transiently expressing GFP-UBF1 were microirradiated using a 355-nm UVA laser (Fig. 4A). Recombinant GFP-UBF1 (green) in iMEFs (Fig. 4Aa) and primary MEFs (Fig. 4Ab) was monitored after local microirradiation by a 355-nm UVA laser. Endogenous UBF1/2 (red) was analyzed by immunofluorescence (Fig. 4B) after local UVA microirradiation of ROIs (yellow) in 3T3 cells stably expressing GFP-HP1β (green). Irradiated cells were fixed in 4% formaldehyde and stained with appropriate antibodies. Live cell images are labelled as (LCI) and immunofluorescence as (IF).



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DEPARTMENT OF CYTOKINETICS

MOLECULAR MECHANISMS INVOLVED IN THE CONTROL OF CELL PROLIFERATION, CELL SURVIVAL/DEATH, OR ALTERATIONS OF CELL PHENOTYPE AND CELL-TO-CELL COMMUNICATION, WHICH PLAY A ROLE IN THE MULTISTEP DEVELOPMENT OF HUMAN TUMORS AND WHICH CAN BE EXPLOITED IN TUMOR THERAPY, ARE THE PRIMARY FOCUS OF THE RESEARCH CARRIED OUT IN THE DEPARTMENT OF CYTOKINETICS.

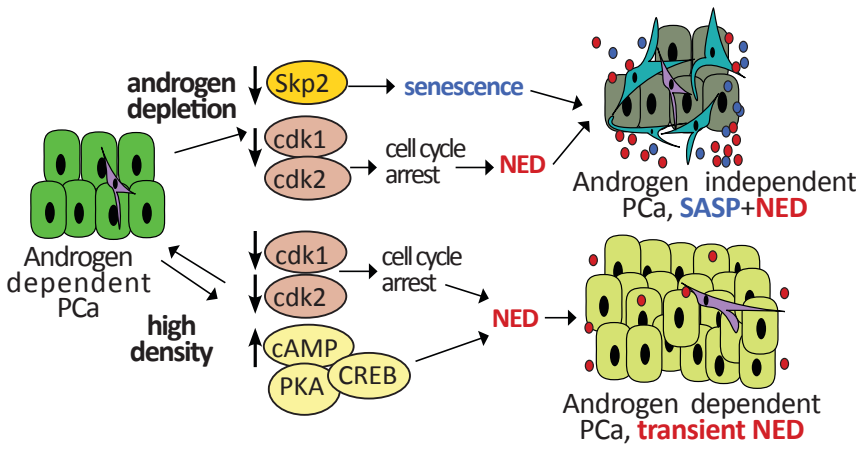
In our work, we presently focus on the molecular mechanisms, which regulate the cytokinetics (or, dynamics of both normal and transformed cell populations), including: i) physiological protein regulators involved in developmental, microenvironmental and oncogenic signaling, such as tumor necrosis factor and transforming growth factor-beta cytokine families, fibroblast growth factors, Wnt pathway signaling or PAS proteins (aryl hydrocarbon receptor); and ii) specific lipid molecules, which constitute both structural and signaling cellular components. Using modern and innovative *in vitro* cell culture techniques, now also coupled with state-of-the-art *in vivo* models, our goal is to contribute to our understanding of cancer and to bring novel results that could be exploited both in cancer prevention and its therapy.

We explore the interactions of these physiological regulatory pathways with specific bioactive food components (such as polyunsaturated fatty acids) or therapeutic drugs, which can either reduce tumor incidence or which could be employed in cancer therapy. The disruption of both inter- and intracellular signaling pathways, including those involved in oncogenic and developmental signaling, is also considered a major target of environmental toxicants. Given the multistep nature of tumor development, we therefore study the potential role of carcinogenic and/or endocrine-disrupting environmental chemicals during oncogenic disease development, with particular focus on tumor initiation and promotion. Once the malignant disease progresses into its final stages, in order to develop future effective anti-tumor therapies, it is also essential to understand the signaling pathways within the context of plasticity of cancer cells, tumor heterogeneity and therapy resistance. Therefore, we also use our expertise in cancer cell biology to cooperate with molecular pathologists and clinicians. This is directed to the very important goal of being able to confirm the results of our studies in clinically relevant models and material.

SELECTED RESULTS

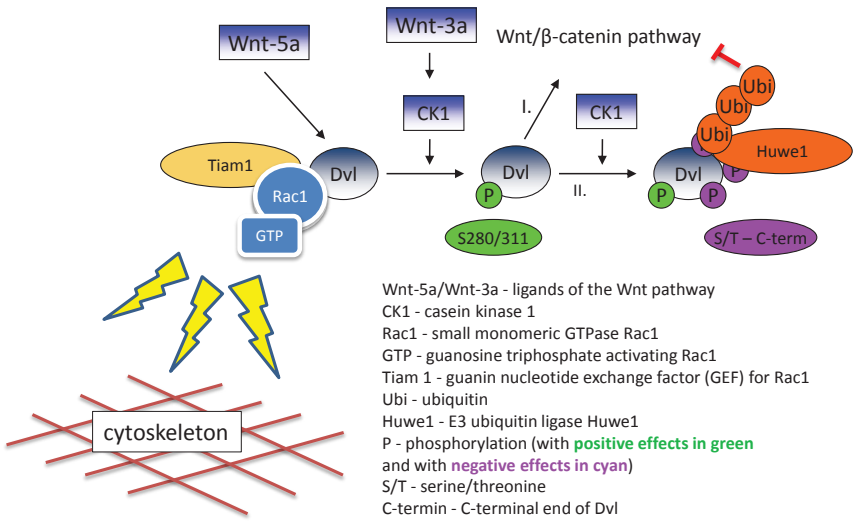
HETEROGENEITY AND PLASTICITY OF CANCER CELLS

Tumor heterogeneity and the plasticity of cancer cells present challenges for effective clinical diagnosis and therapy. Such challenges are epitomized by neuroendocrine (NE) transdifferentiation (NED) and the emergence of NE-like cancer cells in prostate cancer (PCa).
Interestingly, androgen deprivation therapy, a widely used treatment for advanced prostate cancer, induces both the emergence of NE-like prostate cancer cells and the senescence-associated secretory phenotype in prostate cancer epithelial cells. The induction of the senescence-associated secretory phenotype by androgen depletion is closely connected to the regulation of the cell cycle machinery through the down-regulation of S-phase kinase-associated protein 2, whereas the emergence of neuroendocrine-like cancer cells (through the process of NED) is under separate control. Moreover, our results imply a new relationship between high cell density-induced cell cycle attenuation and promotion of NED and suggest that high cell density is a trigger of intracellular signaling that can mediate reversible NED in prostate cancer cells. This may help to understand the role of tumor tissue environment and its plasticity.



MOLECULAR MECHANISMS OF WNT SIGNALING

Morphogenetic proteins from the Wnt family are crucial regulators of embryonal development and homeostasis in the adult organism. Wnt pathway deregulation often leads to the tumor formation and is implicated in the pathogenesis of many other diseases. Wnts bind membrane receptors from the Frizzled family, which transduce signal to phosphoprotein Dishevelled (DVL). At the level of DVL, signal is analyzed and depending on the ligand/coreceptor/cell, it is further transduced downstream via one of at least four signaling pathways. Molecular mechanisms, which direct signal at the level of Dishevelled are unknown. Our lab focuses on understanding of the crucial events between the receptor, Dishevelled and downstream pathway components. We are trying to apply our findings directly to clinically relevant problems e.g. pathogenesis of cancer or leukemia.



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Bernatik, O; Sedova, K; Schille, C; Sri Ganji, R; Cervenka, I; Trantirek, L; Schambony, A; Zdrahal, Z; Bryja, V; Functional Analysis of Dishevelled-3 Phosphorylation Identifies Distinct Mechanisms Driven by Casein Kinase 1ε and Frizzled5. Journal of Biol. Chemistry 2014, 289: 23520–23533

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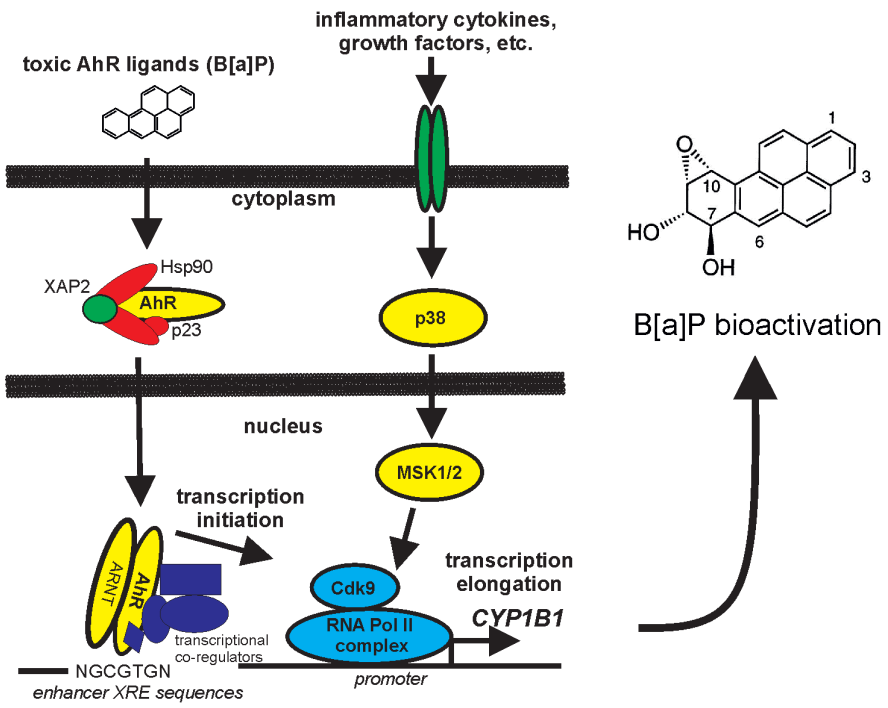
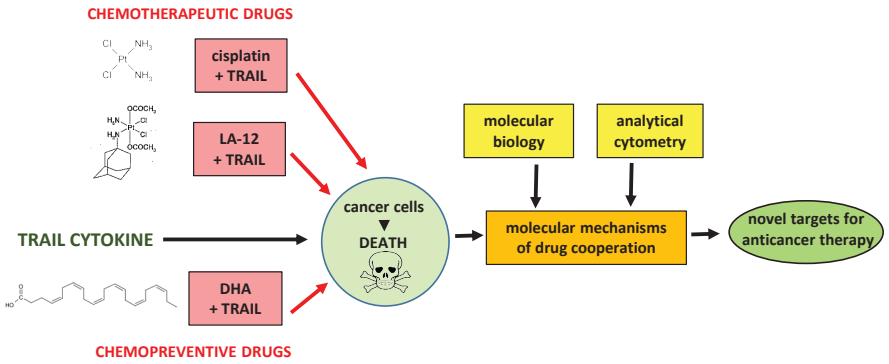
THE ROLE OF INFLAMMATORY MEDIATORS IN BIOACTIVATION OF ENVIRONMENTAL CARCINOGENS

Long-term deregulated inflammation represents one of the key factors contributing to cancer etiology. The analysis of genotoxicity and metabolism of benzo[a]pyrene (B[a]P), a highly carcinogenic polycyclic aromatic hydrocarbon, has revealed that inflammatory conditions may significantly accelerate B[a] P metabolism and formation of ultimate mutagenic B[a]P metabolite, via cytochrome P450 1B1 (CYP1B1)-catalyzed metabolism. CYP1B1 is an enzyme with a unique tumor-specific expression pattern, which bioactivates a wide range of carcinogenic compounds. We described a novel mechanism of the regulation of CYP1B1 expression via the p38/MSK1 kinase cascade, which leads to an increased CYP1B1 gene transcriptional elongation. Our results show that inflammatory reaction may alter metabolism of carcinogens and thus contribute to their tumor-initiating effects via a cell-specific modulation of CYP1 expression.



PLATINUM-BASED DRUGS AND TRAIL IN CANCER THERAPY

Common cancer cell resistance to currently available chemotherapy is a major obstacle in successful treatment, and evokes a need for application of agents (or their combinations) with higher effectiveness and novel mechanism of action. Recently, we have shown the unique ability of recently introduced platinum (IV) complex LA-12 over conventionally used cisplatin to enhance killing effects of tumor necrosis factor-related apoptosis inducing ligand (TRAIL) in cancer cells, especially at the level of mitochondrial apoptotic pathway. We have also reported on a higher cytotoxicity and favorable properties of LA-12 compared to „classical" platinum (II) complexes to effectively modulate cancer cell cycle and death regulatory mechanisms.



DIETARY FATTY ACIDS IN MODULATION OF CANCER CELL DIFFERENTIATION/DEATH

We have discovered an important association between cellular lipid alterations and distinct differentiation/apoptotic response of human colon epithelial cells with various tumorigenic potential induced by dietary fatty acids (short-chain fatty acid butyrate and essential polyunsaturated docosahexaenoic acid – DHA, ω-3 type). Our work also highlighted a promising potential of DHA when combined with clinically important cytokine TRAIL for selective elimination of colon cancer cells via targeting apoptotic signaling pathways at the level of mitochondria and sphingolipid metabolism.

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DEPARTMENT OF FREE RADICAL PATHOPHYSIOLOGY

DEPARTMENT OF FREE RADICAL PATHOPHYSIOLOGY FOCUSES ON THE ROLE OF REACTIVE METABOLITES AND DIFFERENT SIGNALING MOLECULES IN PATHOLOGICAL PROCESSES RELATED TO IMMUNE AND VASCULAR DISORDERS.

We focus on the study of mechanisms leading to generation of reactive oxygen and nitrogen metabolites by phagocytes and its possible modulation using substances derived from physiological mediators (such as serotonin and its analogues, nitro-fatty acids, and methylarginines), drugs (such as anti-histamines), and dietary supplements (such as polysaccharides and polyphenols, isolated from plants). Evaluation of new molecular mechanisms underlying pathological processes in cardiovascular system is another important objective of our department. We focus on the inflammation-derived disturbances of physiological functions, as well as on the damage to vascular endothelium and heart tissue.
In order to study the ischemia-induced damage of cardiac muscle, we employ models of fetal and neonatal hearts, combined with embryonic stem cells differentiated to functional cardiomyocytes. These experiments allow us to study the pathological mechanisms induced by hypoxia. Moreover, we can evaluate the promotion of the regeneration processes, which are based on differentiation of progenitor cells in cardiac tissue. Combining our interest in phagocytes, we are clarifying the importance of abundant enzyme of neutrophil granulocytes, myeloperoxidase, in the development of cardiovascular pathological processes.
Additionally, our attention focuses on the protective role of nitro-fatty acids in several models of cardiovascular disorders (e.g. atherosclerosis, pulmonary hypertension, and atrial fibrosis).

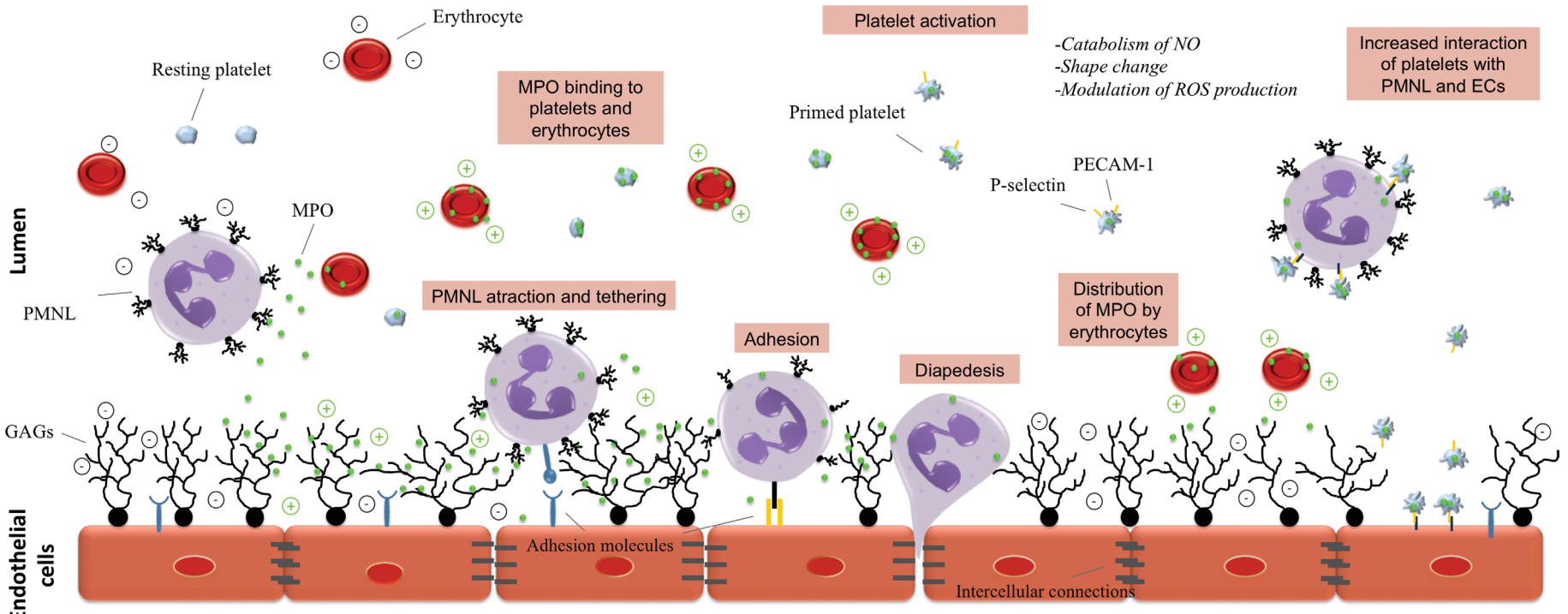
Notably, these endogenously produced signaling mediators represent the potential therapeutics for treatment of chronic and acute inflammation, linked to pathology of cardiovascular diseases.
Other important projects are aimed at the role of L-arginine and its endogenously produced derivatives, methylarginines, on development of vascular dysfunction associated with impaired bioavailability of nitric oxide. Our recent findings confirmed a new molecular mechanism of asymmetric dimethyl arginine action in endothelial and smooth muscle cells. We have discovered that this methylarginine, identified as one of the potential therapeutic targets in treatment of cardiovascular diseases, represents a possible casual link in the progression of pulmonary hypertension.

SELECTED RESULTS

THE ROLE OF MPO IN REGULATION OF ENDOTHELIAL AND IMMUNE FUNCTIONS

Myeloperoxidase (MPO) is an abundant hemoprotein, expressed by neutrophil granulocytes. The release of MPO is a hallmark of vascular inflammation and contributes to the pathogenesis of vascular inflammatory processes. We show that upon degranulation from neutrophils, MPO binds to the surface of endothelial cells in an electrostatic-dependent manner and undergoes transcytotic migration to the underlying extracellular matrix. Interestingly, MPO also binds to the surface of blood cells including thrombocytes and erythrocytes.

Interaction of thrombocytes with MPO induces their partial activation. Furthermore, we have shown that MPO binds to erythrocytes, which correlates with the clinical conditions of patients with cardiovascular diseases. MPO, which is bound to erythrocytes, affects the function of blood vessels *in vivo* adversely. The presented importance of MPO in the development of blood vessel inflammation and endothelial dysfunction opens pharmacological interventions focused on MPO.



Proposed mechanism of MPO action in vasculature. Collectively, the data reveal that MPO binds to ECM proteins on the basis of electrostatic interactions, and MPO chlorinating and oxidizing activity is potentiated upon association with these proteins.

SELECTED OUTPUTS

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SELECTED OUTPUTS

Lojek, A; Denev, P; Ciz, M; Vasicek, O; Kratchanova, M; The effects of biologically active substances in medicinal plants on the metabolic activity of neutrophils. Phytochem Rev 2014, 13, 499–510

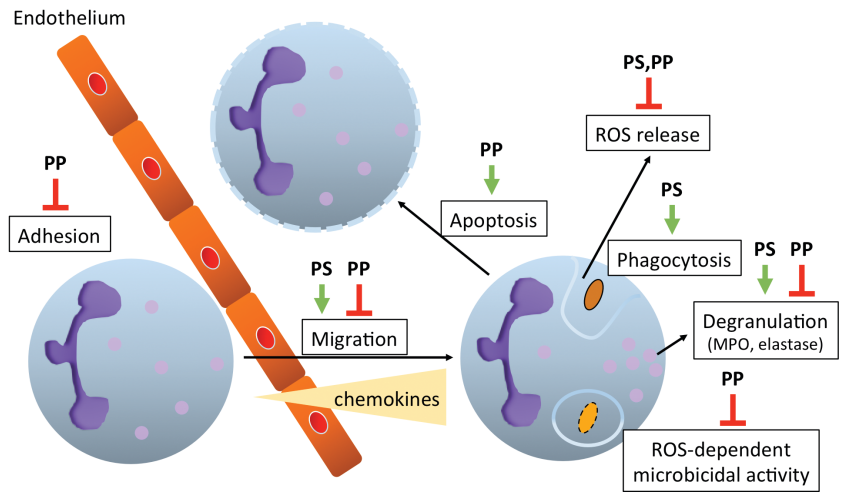
SELECTED OUTPUTS

Klinke, A; Möller, A; Pekarova, M; Ravekes, T; Friedrichs, K; Berlin, M; Scheu, KM; Kubala, L; Kolarova, H; Ambrozova, G; Schermuly, RT; Woodcock, SR; Freeman, BA; Rosenkranz, S; Baldus, S; Rudolph, V ;Rudolph, TK; Protective effects of 10-nitro-oleic acid in a hypoxia-induced murine model of pulmonary hypertension. Am J Respir Cell Mol Biol 2014, 51, 155–162

MODULATION OF NEUTROPHIL OXIDATIVE BURST

Platelet mediators like serotonin and histamine can have a protective function against neutrophil-derived oxidative stress and oxidative damages. The increase in local concentrations of serotonin and histamine significantly reduced the formation of reactive oxygen species by neutrophils. We have discovered that the inhibitory effect of histamine was caused rather by the binding of histamine to H2R than to H4R. Our results also confirmed the fact that serotonin is a very strong antioxidant while histamine is not.

We have also studied the anti-inflammatory properties of medicinal plants and small fruits, which are a rich source of polysaccharides and polyphenols, and which are therefore suitable raw materials for the production of functional foods. Especially the extracts from berries almost completely blocked the formation of neutrophil-derived oxidants induced using receptor binding activators. On the other hand, the effect of extracts on neutrophils activated with receptor by-passing stimuli was much milder, indicating that these extracts interfere with the signaling cascade of phagocyte activation upstream to the protein kinase C activation.

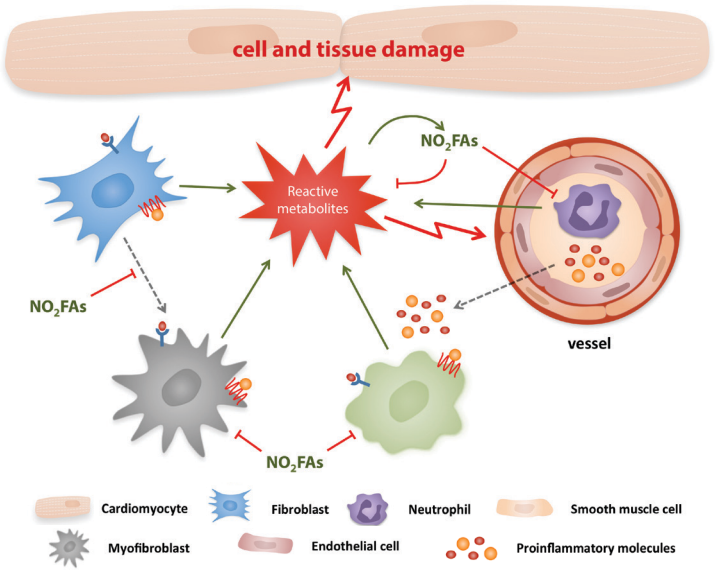


Summary of the stimulatory (↓) and inhibitory (⊥) effects of polyphenols (PP) and polysaccharides (PS) on neutrophils.

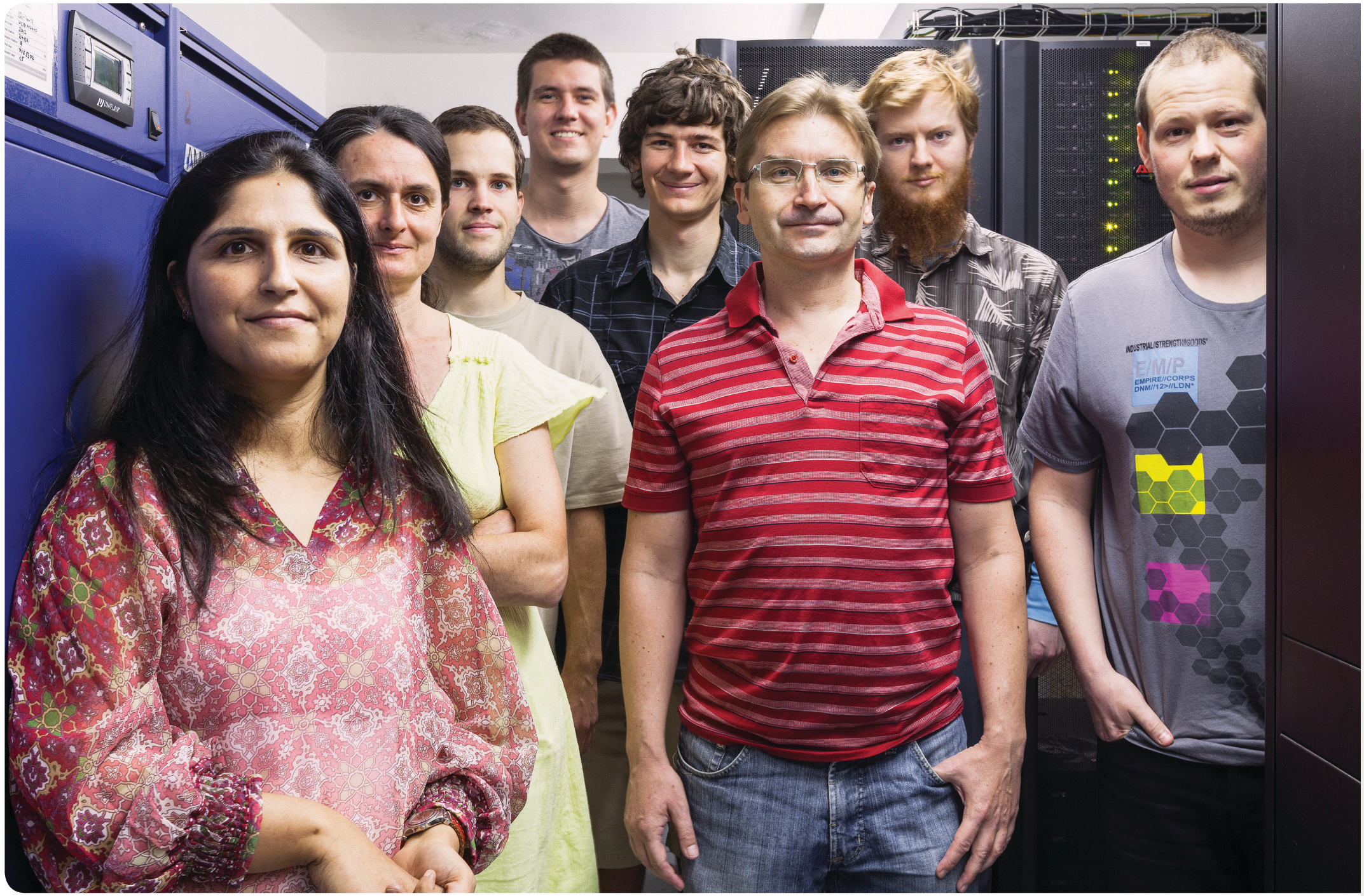
THE CARDIOPROTECTIVE EFFECTS OF NITRO-FATTY ACIDS

Heart failure is associated with enhanced activation of macrophages, neutrophils, and fibroblasts, as well as with production of oxidants and pro-inflammatory mediators. The oxidative milieu generated during inflammation consists of a broad spectrum of oxidizing, nitrosating, and nitrating species and their products, which are destroying the heart tissue. Nitrated unsaturated fatty acids (NO_2FAs), endogenous products of oxidant-induced nitration reactions, represent novel signaling mediators leading to secondary changes in protein function via electrophilic-based modifications.

Our studies have shown that NO_2FAs are present in the vasculature at nanomolar to low micromolar concentrations, enough to exert biological actions including inhibition of neutrophil, macrophage, and fibroblast activation, pro-inflammatory cytokine secretion, and vascular smooth muscle cell proliferation. In light of the above–described cell signaling roles of NO_2FAs, it may be expected that there is a "threshold level" over which their physiological activities switch from signaling to pharmacological actions. Therefore, NO_2FAs are suggested as highly promising compounds for treatment of various cardiovascular and inflammatory disorders.



Proposed mechanism of nitro-fatty acids (NO_2FAs) action in cardiovascular system.



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EXTERNAL CO-WORKERS Pavel Banáš, Michal Otyepka, Naděžda Špačková, Tomáš Dršata, Petr Jurečka, Martin Ferus
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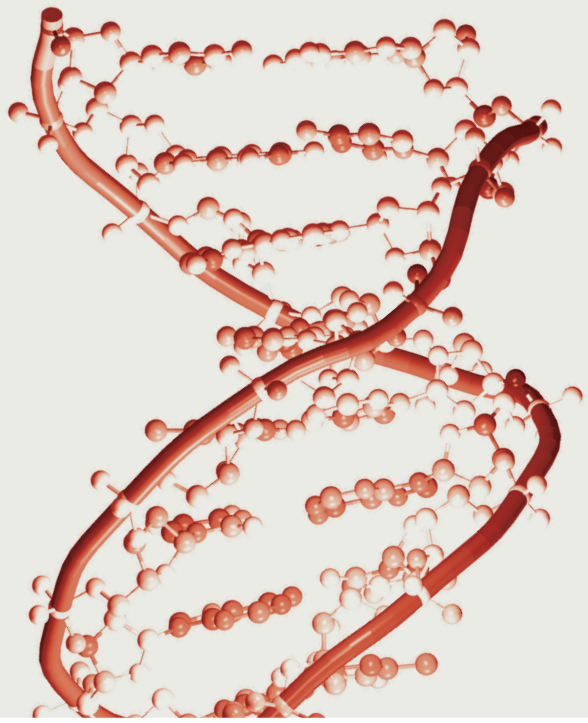
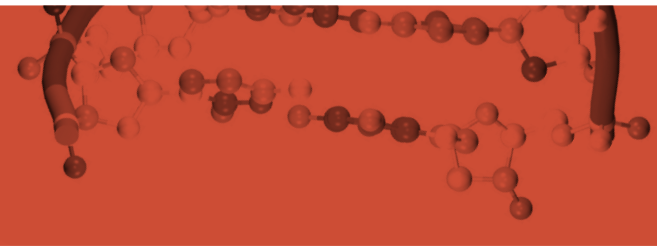
DEPARTMENT OF STRUCTURE AND DYNAMICS OF NUCLEIC ACIDS

AT THE DEPARTMENT OF STRUCTURE AND DYNAMICS OF NUCLEIC ACIDS, WE USE STATE OF THE ART COMPUTATIONAL TECHNIQUES TO STUDY BIOLOGICAL AND CHEMICAL PROCESSES OF THE NUCLEIC ACIDS. OUR RESEARCHERS WORK WITH CONTEMPORARY RNA AND DNA MOLECULES AND THEY ALSO EXAMINE THEIR EVOLUTIONARY DEVELOPMENT AND THE ORIGIN OF LIFE.

We use three principal theoretical research techniques. **Quantum chemical (QM) calculations** are state of the art physical-chemistry methods, which provide accurate and physically complete description of smaller molecular systems. The technique reveals direct structure–energy relations that cannot be obtained by any other technique. Such calculations play indispensable role in reference evaluation of nature and magnitude of all kinds of molecular energy contributions that shape up nucleic acids, such as base stacking, base pairing, backbone conformational preferences, etc. QM calculations enable us to study chemical reactions at the level of changes in electronic structure. **Classical explicit solvent molecular dynamics (MD)** simulations characterize structural dynamics of nucleic acids and their complexes with proteins on scale of hundreds of thousands of atoms. The currently available simulations can be extended to a microsecond time scale. With this technique, biomolecules are modeled at the atomistic level of descriptions, using classical potential functions, which are also known as empirical force fields. Approximations inherent to the force fields represent the main limitation of this approach. **Structural bioinformatics** aims, among other things, to provide classification of molecular interactions in nucleic acids based on structural and sequence data. This technique enables our researchers to obtain useful information out of large amounts of raw data.

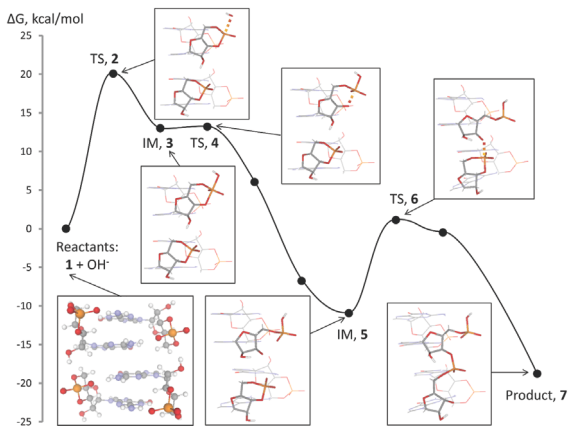


SELECTED RESULTS



ORIGIN OF LIFE THEORY

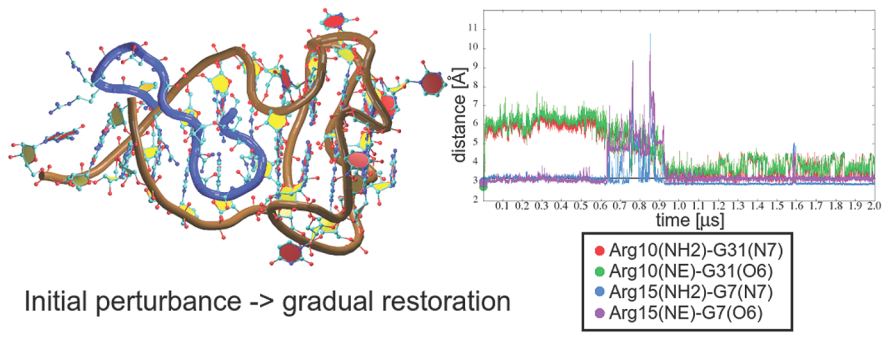
Another research topic of our group concerns the origin of the first genetic molecules. We address this problem at various levels. Our main goal is to reconstruct the origin of the terrestrial life on the basis of formamide, a simple organic precursor. We collaborate with leading experimental laboratories. In the Ferus et al. PNAS, 2014 paper we propose a model for the formation of nucleobases in a prebiotically relevant process. We suggest that extraterrestrial impacts could synthesize the building blocks of the first life-giving molecules. We have simulated the high-energy synthesis of nucleobases from formamide during the impact of an extraterrestrial body. Based on GC-MS, high-resolution FT-IR spectroscopic results and theoretical calculations, we present a comprehensive mechanistic model, which accounts for all steps, which take place in the studied impact chemistry. The work has been highlighted by the PNAS editorial board as being of exceptional significance and acquired a world-wide attention. In the paper Sponer et al. J. Phys. Chem. B 2015, we address the polymerization of prebiotic precursors leading to the first RNA sequences. We present a ring-opening polymerization scenario, which is so far the only known way to selectively generate 3′,5′-linked oligomers in a prebiotically relevant reaction. In a related study (Stadlbauer et al. Chem. Eur. J., 2015) we suggest a model, how these simple, ancient oligonucleotides could acquire their catalytic function, i.e. the property, which made evolution possible.



A pillared architecture formed upon self-assembling of 3′,5′-cyclic GMPs enables a ring-opening-polymerization selectively leading to 3′,5′-linked oligonucleotides. Results reported in Sponer et al. J. Phys. Chem. B 2015.

MOLECULAR SIMULATIONS OF PROTEIN/RNA COMPLEXES.

One of our key research subjects in molecular dynamics simulations are the protein/RNA complexes. In living biological organisms, the RNA molecules always interact with proteins, from the moment of their creation to the moment of their degradation. While the simulations of isolated RNA molecules provided a lot of significant information over the years, the inclusion of proteins is a necessary step to further our studies of the RNA molecules onto the next level. In the paper *Krepl et al. JCTC, 2015*, we have analyzed over 30 μs of unrestrained molecular dynamics simulations of six diverse protein-RNA complexes in explicit solvent. The simulations have shown variable behavior, ranging from systems which were essentially stable to systems with progressive deviations from the experimental structure. For some systems, μs-scale simulations were necessary to achieve stabilization after initial sizable structural perturbations. The simulations were affected by numerous factors, including properties of the starting structures, force field imbalances and real molecular flexibility. These factors, and thus the simulation behavior, differ from system to system. We have analyzed the effects of the properties of the starting structures, uncertainties caused by simulation sampling and limitations of the force field. Our study is the most extensive one that has ever been done on protein/RNA complexes and can serve as a benchmark for further simulation studies.



Initial perturbance -> gradual restoration

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Krepl M; Havrila M; Stadlbauer P; Banas P; Otyepka M; Pasulka J; Stefl R; Sponer J; Can We Execute Stable Microsecond-Scale Atomistic Simulations of Protein-RNA Complexes? J. Chem. Theory Comput. 2015, 11, 1220–1243

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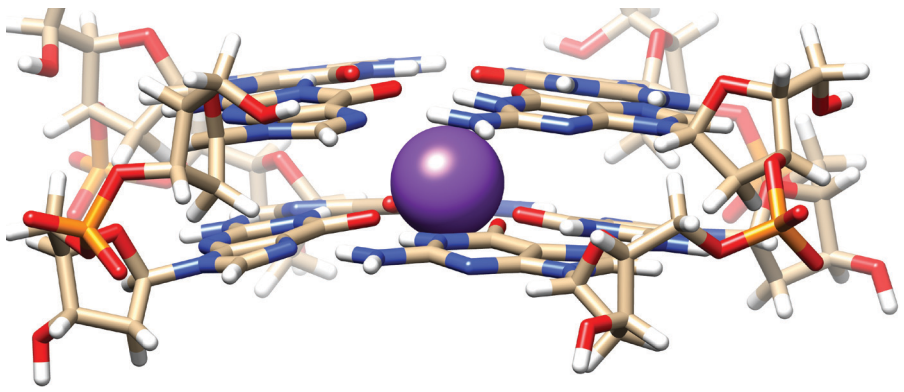
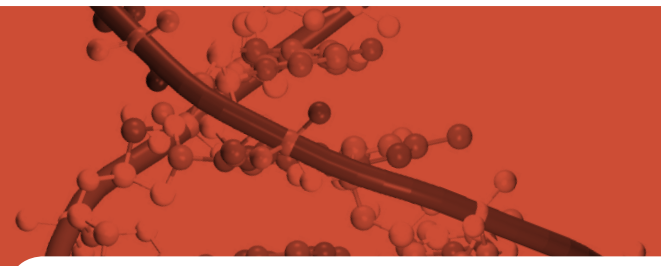
Stadlbauer P; Krepl M; Cheatham T E, III; Koca J; Sponer J; Structural dynamics of possible late-stage intermediates in folding of quadruplex DNA studied by molecular simulations. Nucleic Acids Res. 2013, 41, 7128−7143

LARGE-SCALE QUANTUM CHEMICAL CALCULATIONS OF NUCLEIC ACIDS.

Atomistic molecular dynamics (MD) simulation using classical force field has been the most common computational method to study nucleic acids. However, its accuracy is limited by the approximate nature of the empirical force fields. Modern electronic structure (quantum chemical, QM) computations offer an inherently more accurate description of a studied system as compared to force fields. However, the applicability of QM methods to nucleic acids is limited by the size of the systems that can be handled, difficulties in inclusion of solvent, and essentially lack of any sampling. In our recent studies, we have pioneered applications of large-scale QM computations to essentially complete nucleic acids building blocks, which we consider a new methodological advance, substantially complementing the MD simulations using classical force fields. For the first time, we have applied such computations to a system that represents a sufficiently complete DNA fragment. Specifically, seven distinct folds of the cation-stabilized two quartet guanine quadruplex stem (Sponer et al, JACS 2013). Our computations revealed surprisingly large differences between the MM and QM descriptions, leading to a major revision in the predicted energy order of different G-DNA stem arrangements. We have also discussed the limitations of such computations in detail. Large-scale QM computations on nucleic acids represent one of our main research goals in the near future. Further, we use QM calculations in studies of RNA catalysis (ribozymes) and testing and tuning of the simulation force fields.

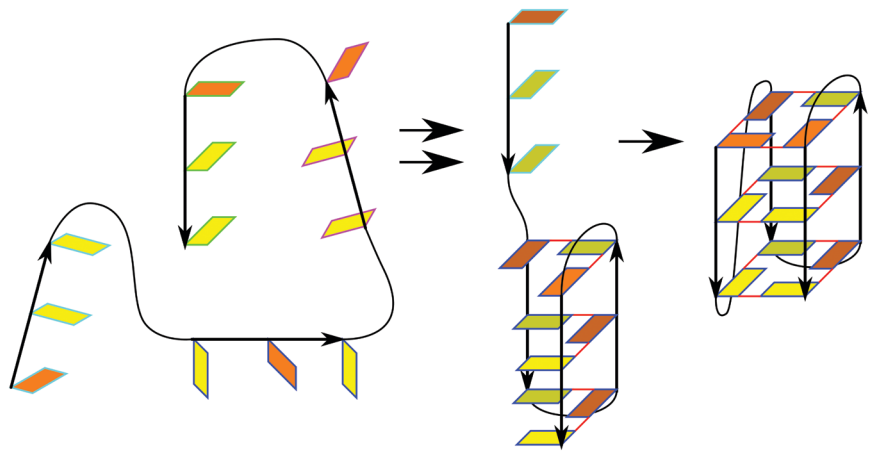


Cation-stabilized two-quartet quanine quadruplex stem.



COMPUTATIONAL STUDIES ON GUANINE QUADRUPLEXES FOLDING

Guanine-rich oligonucleotides can fold into four-stranded structures called G-quadruplexes (GQ). GQs became hot topic molecules in the last decade, because they are prevalent in human genome, particularly in gene-regulatory sites and in telomeres, which makes them interesting therapeutic targets. Our department utilizes molecular dynamics (MD) simulations to study stability and folding pathways of various GQs. With the current computational power, we are capable of running MD simulations up to 10 μs, at least 10-times longer than average nucleic acid simulations. Our recent studies have shown differences in formation mechanisms of all-anti parallel stranded GQs and of GQs with antiparallel strands, containing mixture of syn and anti nucleotides (Stadlbauer et al., NAR, 2013). Simulations of triplex intermediates have shown loop-type combinations, viable in folding, and also possible interconversion between lateral and diagonal type of loop (Stadlbauer et al., Biochimie, 2014). We demonstrate that the GQ folding is a complex multi-pathway process and that contemporary GQ folding mechanisms, derived from available experimental data, are oversimplified.



SCIENTISTS Michaela Vorlíčková, Iva Kejnovská, Daniel Renčiuk, Jaroslav Kypr, Karel Nejedlý
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DEPARTMENT OF CD SPECTROSCOPY OF NUCLEIC ACIDS

THE LABORATORY DEALS WITH STRUCTURAL PROPERTIES OF DNA IN SELECTED GENOMIC REGIONS IMPORTANT FROM THE POINT OF THEIR FUNCTION OR PATHOLOGY

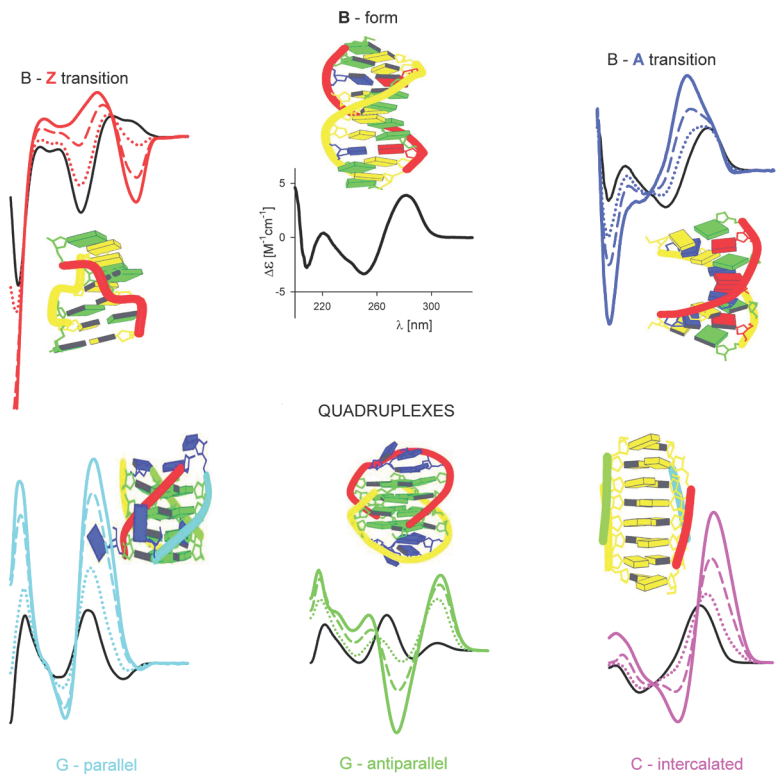
The basic arrangement of DNA is a right-handed Watson-Crick B-type double helix. Depending on sequence, DNA can adopt many other structures, including the right-handed A-type double helix typical of RNA, the left-handed Z-DNA, various kinds of hairpins and slipped structures, parallel-stranded double helices, various kinds of triplexes, guanine quadruplexes, intercalated cytosine tetraplexes, and other less well-characterized structures. Transition of a DNA region into an anomalous conformation makes it recognizable by selected proteins, while the protein-DNA interaction is the base for the control of biological processes. The main methodology of our laboratory is a circular dichroism (CD) spectroscopy.
The spectroscopic method is extremely sensitive to DNA conformation, mainly to its structural switches, and provides spectra characteristic of particular DNA arrangements.

Our laboratory contributed to the knowledge of all the above-mentioned DNA structures and in the last decade, our effort has been mainly focused on tetra-stranded structures—quadruplexes. Quadruplexes frequently occur in gene promoters and control their function. Telomeres are another important place of their formation. Telomere quadruplexes check genome stability, influence aging, and they are also associated with cancer prevention, and thus have become targets for the design of anticancer agents. We have contributed to the view of an extensive conformational polymorphism of the human telomere DNA. Transitions among its various quadruplex structures may be associated with different telomere functions. Reprogramming of differentiated cells into pluripotent stem cells is another 'hot topic', in which our laboratory is currently interested. In this process, the quadruplexes play an important role again.

SELECTED RESULTS

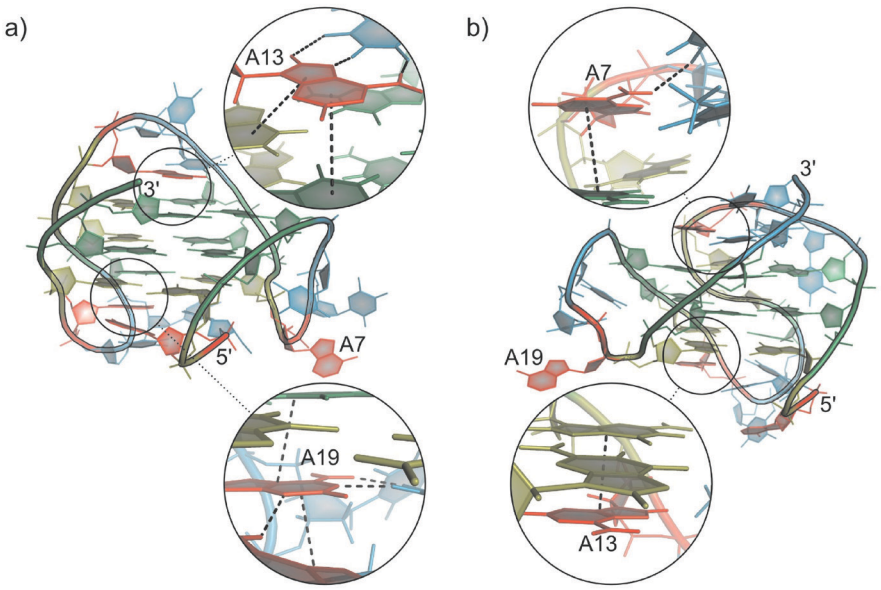
CD SPECTROSCOPY IS A USEFUL METHOD FOR STUDYING NUCLEIC ACID STRUCTURES AND INTERACTIONS

It provides characteristic spectra of particular DNA arrangements and sensitively reflects their changes. The figure shows some examples of DNA structures and of their CD spectra: Right-handed B-(black) and A-DNA (blue) duplexes, left-handed Z-DNA duplex (red), guanine parallel (cyan) and antiparallel (green) quadruplexes, and cytosine intercalated tetra-stranded structure (pink).



LOSS OF LOOP ADENINES ALTERS HUMAN TELOMERE QUADRUPLEX FOLDING

Cellular DNA is constantly subjected to exogenous and endogenous damaging events while abasic (ap) lesions are the most frequent lesions occurring in DNA. Using CD and NMR spectroscopies, we have discovered that a loss of adenine in the first (ap7), second (ap13), or third (ap19) loop of the human telomere DNA quadruplex does not hinder its formation, but changes its structure. The ap7 and ap19 sequences form so-called hybrid-1 (a) and hybrid-2 (b) quadruplexes, respectively, with ap site located in a propeller-like loop. In these structures, adenine A7 (a) and adenine A19 (b) are situated out of the quadruplex core, so that their loss has a minimal negative effect on the quadruplex stability. In view of important functions of the telomeres in aging and carcinogenesis, the change in their quadruplex structure may have serious biological consequences.



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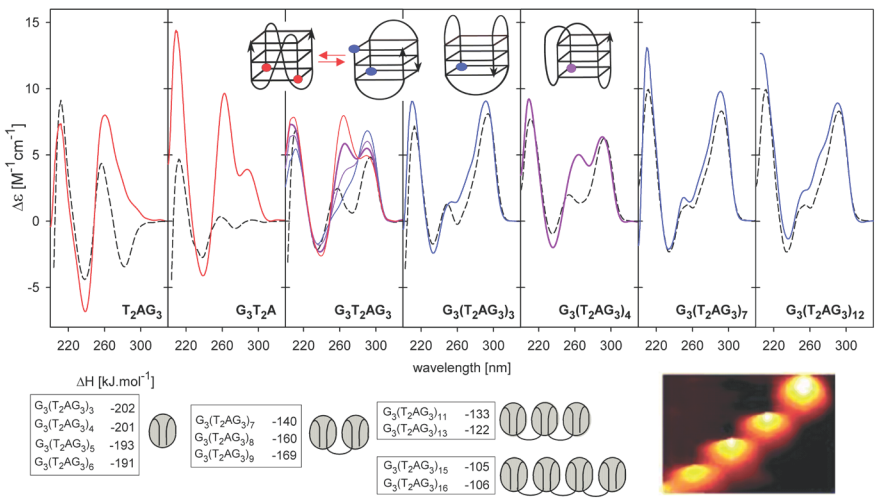
quadruplexes at high DNA concentrations. Biopolymers 2014, 101, 428–438

ARRANGEMENT OF LONG HUMAN TELOMERE DNA MOLECULES

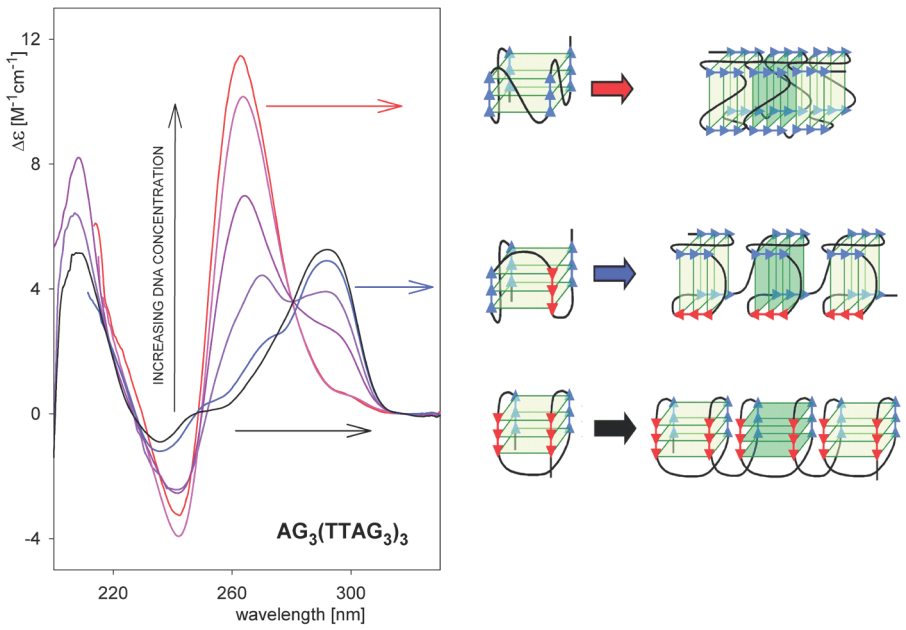
Telomeres play an important role in cellular aging and cancer. Telomeric DNA forms quadruplexes, which are pivotal elements for maintaining telomere integrity and controlling cancer cell proliferation. CD spectroscopy has shown (upper panel) that the structure of the human telomere DNA sequences formed by repeated $(G_3TTA)_n$ blocks depends on the number (n) of the repeats. CD spectra with dominating 260 nm band drawn in red and the spectra drawn in blue (maximum at 295 nm) correspond to the prevailing parallel and antiparallel quadruplexes, respectively. The spectra of G_3TTAG_3 reflect the temperature-induced isomerization between the two quadruplex structures. No analogous isomerization took place with $G_3(TTAG_3)_4$ (violet). The sequence adopted a single quadruplex structure, which we have named a (3+1) quadruplex[1]. The existence of the (3+1) quadruplex has been proven in 2006 by NMR[2].
On the basis of sharp decreases in enthalpy values with sequences containing an integral multiple of four G_3 blocks (bottom panel), which indicate division of the original unit into more parts, we have suggested that long telomere molecules are formed by a set of small quadruplexes like beads on a string. Four years later the beads have been visualized (bottom right) by AFM[3].



[1]M.V. et al.: NAR 2005, 33: 5851, [2]Luu, K.N. et al.: JACS 2006, 128: 9963, [3]Xu et al.: Angew. Chem. 2009, 48: 7833

STRUCTURE OF THE HUMAN TELOMERE DNA QUADRUPLEX IS POLYMORPHOUS

The arrangement of the telomeric quadruplex in physiologically relevant K^+ solutions has not yet been unambiguously determined. Distinct quadruplex structures were observed using various methods. We have explained the cause of distinct results reported by different laboratories obtained by crystallography, NMR, optical spectroscopies and other methods. The reason for this is the dependence of the quadruplex arrangement on DNA concentration, which is by orders distinct in the individual methodological approaches.
We have shown that the arrangement of the telomeric DNA depends on DNA concentration: at low concentrations, used with spectroscopic measurements, the core quadruplex structure $G_3(TTAG_3)_3$, as well as long molecules composed of several (G_3TTA) blocks, forms an antiparallel quadruplex. Under concentrations used with NMR measurements, the DNA forms so called (3+1) quadruplex reported by NMR, and at the highest DNA concentrations, a parallel quadruplex observed by X-ray in crystal is formed. We have confirmed this conclusion in cooperation with our colleagues from the Institute of Physics in Prague by Raman spectroscopy.



SCIENTISTS Boris Vyskot, Eduard Kejnovský, Jiří Široký, Bohuslav Janoušek, Roman Hobza, Jitka Žlůvová
POSTDOCS Zdeněk Kubát, Radim Čegan, José Luis Rodriguez Lorenzo
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PhD. STUDENTS *running* Vojtěch Hudzieczek, Veronika Šlancarová, Viktor Tokan, Wojciech Jesionek, Roman Gogela, Ivan Vogel, Viera Kováčová, Pavlína Šteflová, Tereza Králová *theses defended in 2012–2014* Jiří Baloun, Hana Blavet, Eva Nevrtalová

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DEPARTMENT OF PLANT DEVELOPMENTAL GENETICS

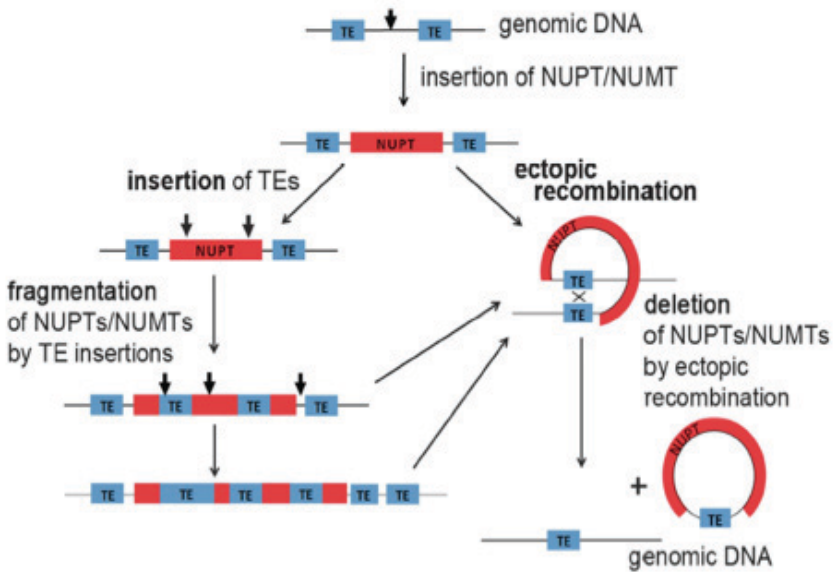
THE DEPARTMENT FOCUSES ON STUDIES OF DEVELOPMENTAL PROCESSES, WHICH PLAY THE KEY ROLE IN PLANT REPRODUCTION.

We work on model dioecious species that evolved heteromorphic sex chromosomes. These chromosomes are structurally analyzed and sequenced in order to reveal specific evolutionary processes. By regulation of function of corresponding genes, basic reproduction processes will be controlled. Special attention is paid to development of original methods for modification of plant genome. The most important achievements include genetic mapping of model dioecious plants, discovery of phylogenetic relationships in sex evolution, and transposon mobility.
The existence and evolution of sex chromosomes is a basic question in evolutionary biology. While sex chromosomes are ancient in majority of animal species, plant sex chromosomes have evolved relatively recently, making dioecious plants good models for the study of early steps of sex chromosome evolution. *Silene latifolia* (syn. *Melandrium album* or white campion) is a dioecious plant possessing heteromorphic sex chromosomes, X and Y. It formally resembles the mammalian type of sex determination, since the gender is controlled by the dominant Y chromosome-linked genes, present only in male individuals. Recent data on *S. latifolia* show that both the X and Y chromosomes harbor active genes, but that they are slightly divergent due to the genetic degeneration of alleles in the non-recombining region of the Y. Our laboratory focuses mainly on isolation and characterization of DNA sequences from *S. latifolia* sex chromosomes.
We have constructed X- and Y-specific libraries using DNA template obtained from laser microdissected chromosomes. New sex-chromosome linked sequences are used for FISH mapping of *S. latifolia* and related *Silene* species in order to search for the sex chromosome homologues.
We have recently improved FISH strategy for differentiating the sex chromosomes by chromosome painting. This approach represents a quick tool to compare organization of plant genomes.
We have also generated new sex linked markers by constructing and screening a sample bacterial artificial chromosome (BAC) library to look for appropriate FISH probes. We also study the roles of repetitive DNA sequences in the evolution of sex chromosomes. We follow accumulation of promiscuous DNA and tandem repeats on the Y chromosome. Our data indicate a new type of retrotransposons carrying tandem repeats that reveal a unique mechanism of tandem repeat amplification in the genomes. The results of our current research show that divergence of the sex chromosomes of *S. latifolia* is already in process and degeneration of the Y chromosome by accumulation of specific sequences has begun.

SELECTED RESULTS

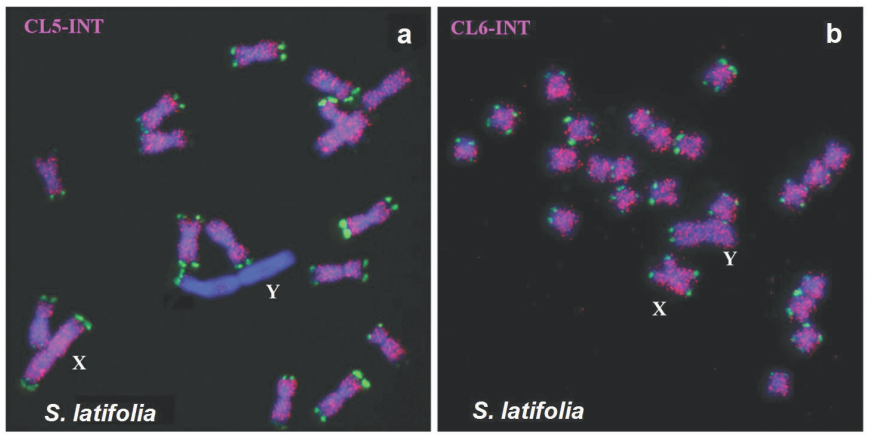
DYNAMICS OF DNA REPEATS IN THE PLANT NUCLEAR GENOME

Sex chromosomes are an ideal system to study the effects of suppressed recombination on repetitive DNA dynamics. We have studied chromosomal distribution of microsatellites using FISH and NGS data analysis and discovered that microsatellites have expanded on the evolutionary young Y chromosomes of sorrel *(Rumex acetosa)* and white campion *(Silene latifolia)* while, on the other hand, the evolutionary old Y chromosomes of human did not show such expansion of microsatellites and TEs dominated here. The high abundance of microsatellites in the neighborhood of transposable elements (TEs) suggested that microsatellites are probably targets for TE insertions. On the basis of these data, we have built a model proposing that microsatellites expand on evolutionary young Y chromosomes and that they are replaced by transposable elements in evolutionary older Y chromosomes.



SEX-SPECIFIC SILENCING OF RETROELEMENTS IN DIOECIOUS PLANTS BY EPIGENETIC MECHANISMS

Retrotransposons are mobile elements related to retroviruses, but they do not form infectious particles and are amplified only within the genomes. Some retrotransposons are present in many thousands of copies. We studied the question why some retrotransposons differ in their presence in the sex chromosomes of the dioecious plant *Silene latifolia*. We performed a deep comparative study of three closely related retroelements Ogre, one of them being absent on the chromosome Y. We have discovered that this phenomenon is caused by different epigenetic regulation of these elements. The family of Ogre elements missing in the Y chromosome is largely silenced in male plants, while in females it can be multiplied and integrated into the autosomes and the Y chromosome.



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SELECTED OUTPUTS
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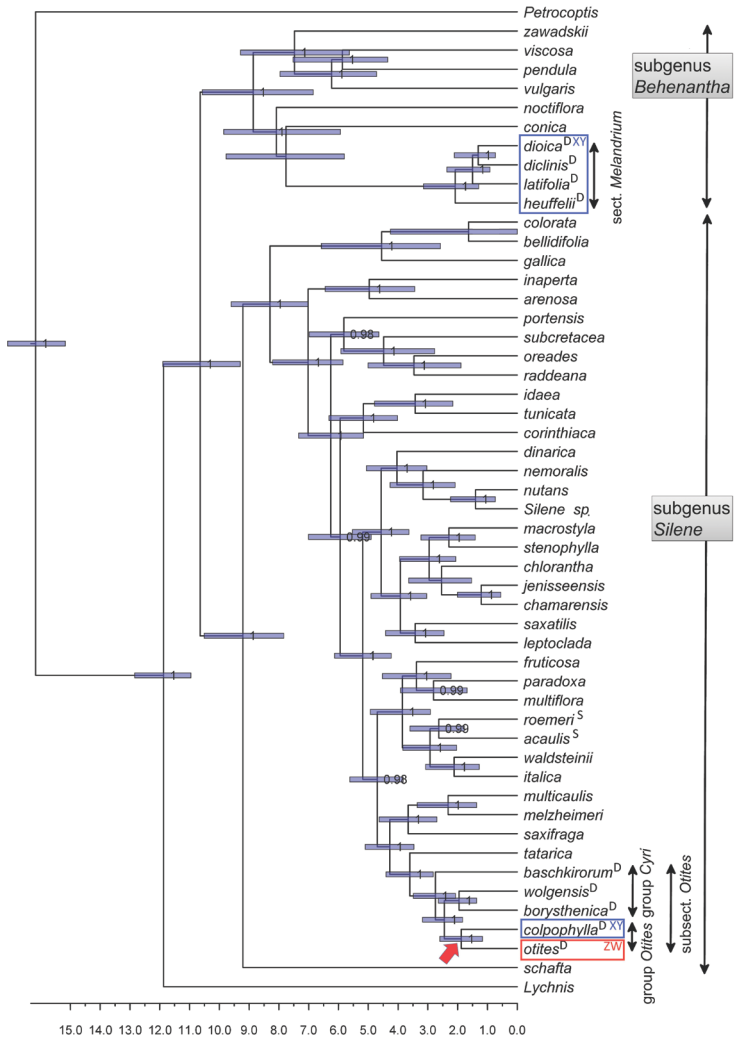
SIMPLIFIED WORKFLOW TO DETECT SEX LINKED MARKERS USING COMBINATION OF CLASSICAL GENETIC SEGREGATION ANALYSIS WITH RNA-SEQ DATA

In dioecious sorrel *(Rumex)* species, two different sex-chromosomal systems and sex-determining mechanisms have been described: XX/XY with an active Y chromosome *(e.g., R. acetosella)* and XX/XY_1Y_2 with sex determination based on the X/A ratio *(e.g., R. acetosa)*. The role of the X/A ratio in the sex determination of *R. acetosa* resembles the sex determining system of *Drosophila*, where the primary genetic sex determining signal is provided by the ratio of X-linked genes to autosomal genes. Recent studies on *R. acetosa* show massive accumulation of organellar DNA and speci-fic microsatellites on both Y chromosomes. Only limited information about expressed markers (genes) linked to sex chromosomes is available in *R. acetosa* so far. We have developed a pipeline for detection of sex linked genes based on nucleotide polymorphism analysis. In our approach, cross of preferably distant populations is used for tracking of nucleotide polymorphisms. Our pipeline presents a simple and freely accessible software tool for identification of sex chromosome linked genes in species without existing reference genome. On the basis of combination of genetic crosses and RNA-Seq data we designed high-throughput and cost-efficient approach for a broad community of scientists focused on sex chromosome structure and evolution. Individual sex linked expressed markers are subsequently selected for BAC library screening. Our data suggest that both X and Y chromosomes contain many active genes in *R. acetosa*. Subsequent structural and functional analysis of X and Y chromosome linked regions shed the light on evolutionary pathways forming plant sex chromosomes.

EVOLUTION OF SEX DETERMINATION SYSTEMS

In many plant species, the development of male and female organs is restricted to separate individuals: males and females. Usually, only one of the sexes produces two types of gametes. Presence of both types of heterogamety in closely related species is rare. We have tested possible switch in heterogamety in two closely related species of the plant genus *Silene: S. colpophylla* and *S. otites* (the most recent common ancestor is marked by a red arrow). On the basis of the phylogenetic analysis and genetic mapping of molecular markers, we have proven that this switch took place approximately two million years ago. The occurrence of XY and ZW within one group of closely related species is a unique finding in plants.



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Slancarova, V; Zdanska, J; Janousek, B; Talianova, M; Zschach, C; Zluvova, J; Siroky, J; Kovacova,
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systems with heterogametic males and females in Silene. EVOLUTION, 2013, 67: 3669–3677

Soukupova, M; Nevrtalova, E; Čížková, J; Vogel, I; Cegan, R; Hobza, R; Vyskot, B; The
X-chromosome is necessary for somatic development in the dioecious Silene latifolia:
cytogenetic and molecular evidence and sequencing of a haploid genome.
CYTOGENETIC AND GENOME RESEARCH, 2014, 143: 96–103

Steflova, P; Tokan, V; Vogel, I; Lexa, M; Macas, J; Novak, P; Hobza, R; Vyskot, B; Kejnovsky, E;
Contrasting patterns of transposable element and satellite distribution on sex chromosomes in
the dioecious plant Rumex acetosa. GENOME BIOLOGY AND EVOLUTION, 2013, 5: 769–782

PROJECTS

DEPARTMENT OF MOLECULAR BIOPHYSICS AND PHARMACOLOGY

GAP205/11/0856; GA CR; Thermodynamics of DNA damaged by physical and chemical agents; 2011–2015; Mgr. Jaroslav Malina, Ph.D.

GAP301/10/0598; GA CR; Metallodrugs based on osmium, platinum and ruthenium complexes. From mechanistic studies to novel, more efficient chemotherapy of cancer; 2010–2014; Prof. RNDr. Viktor Brabec, DrSc.

GD301/09/H004; GA CR; Molecular and structural biology of selected antitumor drugs.From mechanistic studies to chemotherapy of tumors; 2009–2012; Prof. RNDr. Viktor Brabec, DrSc.

LD14019; MEYS; Molecular and cellular pharmacology of new conjugated organometalic compound. Relations to development of anticancer drugs.; 2014–2016; Prof. RNDr. Jana Kašpárková, PhD.

LH13096; MEYS; Targeting DNA interactions with platinum anticancer drugs with damaged-DNA binding-proteins; 2013–2015; Prof. RNDr. Viktor Brabec, DrSc.

LH14317; MEYS; Mechanisms of antitumor effects of non-traditional platinum; 2014–2016; Prof. RNDr. Viktor Brabec, DrSc.

M200041201; AS CR; Non-traditional antibiotic and anticancer metallodrugs. Mechanistic studies, molecular and cellular pharmacology; 2012–2015; Prof. RNDr. Viktor Brabec, DrSc.

GA13-08273S; GA CR; Biophysical analysis of DNA condensation. Effect of new organometallic compounds of biological significance.; 2013–2016; Mgr. Olga Nováková, Dr.

CM1105; COST-EU; Functional metal complexes that bind to biomolecules; 2012–2016; Prof. RNDr. Viktor Brabec, DrSc.

GPP303/11/P047; GA CR; Molecular and cellular pharmacology of novel class of antitumor iridium complexes. Implications for innovations in cancer chemotherapy; 2011–2013; Mgr. Anna Kisová, Ph.D.

GA14-21053S; GA CR; Mechanistic studies on dual targeting of DNA and histone deacetylase with bifunctional inhibitors; 2014–2016; Prof. RNDr. Viktor Brabec, DrSc.

FP7-IDEAS-ERC 247450; Bioinorganic chemistry for the design of new medicines; 2010–2015; Prof. RNDr. Viktor Brabec, DrSc. (Co-Principal Investigator).

DEPARTMENT OF CELL BIOLOGY AND RADIOBIOLOGY

GAP301/10/0590; GA CR; Involvement of nuclear HMGB proteins in sensitizing of human cells to anticancer drugs inhibiting DNA topoisomerases; 2010–2012; RNDr. Michal Štros, DSc.

GAP303/11/0128; GA CR; Role of adenosine A3 receptor signaling in regulation of hematopoiesis: knowledge obtained from adenosine A3 receptor knock-out mice; 2011–2014; Doc. MUDr. Michal Hofer, CSc.

GAP305/12/2475; GA CR; HMGB proteins: involvement in biology of telomeres and human embryonic stem cells; 2012–2015; RNDr. Michal Štros, DSc.

GA13-06943S; GA CR; Structural and functional components of plant telomeres; 2013–2017; Mgr. Eva Sýkorová, CSc.

GA305/08/0158; GA CR; Activation of adenosine receptors combined with cyclooxygenase inhibition in modulation of radiation-induced myelosuppression; 2008–2012; MUDr. Michal Hofer, CSc.

GA521/09/1912; GA CR; Telomeres of algae; 2009–2012; Doc. RNDr. Jiří Fajkus, CSc.

LD12039; MEYS; The influence of higher-order chromatin structure on the mechanism of DNA double-strand break (DSB) repair and formation of chromosomal translocations in cells irradiated with gamma rays and medically relevant ion beams; 2012–2014; RNDr. Martin Falk, PhD.

GP13-10948P; GA CR; Alternative telomeres with uknown sequence in plants; 2013–2015; Mgr. Vratislav Peška, Ph.D.

GP13-11563P; GA CR; Analysis of replication and DNA damage response in plants deficient in accurate chromatin assembly; 2013–2015; Mgr. Martina Dvořáčková, Ph.D.

IAA500040801; AV0; Telomeres and telomerase: transition from molecular to structural biology approach.; 2008–2012; Mgr. Eva Sýkorová, CSc.

OVBIOFYZ20101; MO0; RECEPTOR - An agonist of adenosine A3 receptors in therapy of acute radiation diseas; 2010–2012; Doc. MUDr. Michal Hofer, CSc.

MP1002; COST-EU; Nanoscale insights into Ion Beam Cancer Therapy (Nano-IBCT); 2012–2014; RNDr. Martin Falk, PhD.

Grant of Plenipotentiary of the Czech Republic in JINR Dubna; Chromatin structure modification and DNA repair pathways inhibition as tools to therapeutically increase or decrease cell survival upon the action of ionizing radiation of different quality; 2012, 2013, 2014, and continues; RNDr. Martin Falk, PhD.

The 3+3 Grant for Cooperation with JINR Dubna; Effect of RBE on DNA damage and complex cellular response to ionizing radiation – New insights into the mechanisms of formation of chromosomal translocations; 2012, 2013, 2014, and continues; RNDr. Martin Falk, PhD.

DEPARTMENT OF BIOPHYSICAL CHEMISTRY AND MOLECULAR ONCOLOGY

GAP205/10/2378; GA CR; Induced organization of biomolecules at interfaces by interacting with electrically charged surfaces as a tool for medical diagnostics; 2010–2014; Mgr. Stanislav Hasoň, Ph.D.

GAP206/11/1638; GA CR; Novel electrochemical sensors and sensing techniques for the analysis of nucleic acids structure and interactions; 2011–2015; Doc. RNDr. Miroslav Fojta, CSc.

GAP206/12/2378; GA CR; Influence of conformation and chemical modification on the behavior of oligonucleotides at electrodes: towards the development of DNA sensors; 2012–2014; Mgr. Luděk Havran, Dr.

GAP301/10/1211; GA CR; Transcriptional activities of wild-type and mutant p53, decision between cell proliferation, cell cycle arrest and apoptosis; 2010–2012; Mgr. Václav Brázda, Ph.D.

GAP301/10/2370; GA CR; The role of p53 DNA structure-selective binding in brain cancer; 2010–2012; Mgr. Marie Brázdová, Ph.D.

GAP301/11/2076; GA CR; DNA binding, stress-induced expression and transactivation activity of p73 protein isoforms; 2011–2015; Mgr. Hana Pivoňková, Ph.D.

GA13-36108S; GA CR; Interactions of p53 family proteins with biologically significant DNA quadruplexes; 2013–2015; Mgr. Marie Brázdová, Ph.D.

GPP206/11/P739; GA CR; Sequence-specific electrochemical sensing of PCR-amplified genomic DNA fragments; 2011–2015; Mgr. Petra Horáková, Ph.D.

GPP301/10/P548; GA CR; Reaction of Six-valent and Eight-valent Osmium Complexes with Biomacromolecules and Their Application in Biomedicine; 2010–2012; Ing. Mojmír Trefulka, Ph.D.

IAA400040901; AV0; DNA labeling with redox markers for electrochemical sensing. Applications in analysis of nucleotide sequences and molecular diagnostics; 2009–2013; Doc. RNDr. Miroslav Fojta, CSc.

GBP206/12/G151; GA CR; Center of novel approaches to bioanalysis and molecular diagnostics; 2012–2018; Doc. RNDr. Miroslav Fojta, CSc.

ME09038; MEYS; Interactions of proteins and nucleic acids with surfaces. New tools for biomedicine; 2009–2012; prof. RNDr. Emil Paleček, DrSc.

7AMB12AR028; MEYS; Spectroscopic and electrochemical characterization of photo-oxidized proteins; 2012-2013; RNDr. Veronika Ostatná, Ph.D.

EE2.3.09.0046; MEYS; Modern biophysical methods:advanced education and training in experimental biology; 2009–2012; Doc. RNDr. Miroslav Fojta, CSc.

EE2.3.30.0019; MEYS; Development of modern trends in experimental biology: importance of biomolecular interactions for functions of cellular structures; 2012–2015; Doc. RNDr. Miroslav Fojta, CSc.

GA13-00956S; GA CR; Emerging roles for the proteins of Anterior Gradient family in cancer development; 2013–2017; RNDr. Veronika Ostatná, Ph.D. (Co-Principal Investigator).

GAP301/11/2055; GA CR; New methods of analysis of proteins and their glycosylation in cancer - combination of electrochemistry, microfludic biosensors and mass spectrometry.; 2011–2014; Doc. RNDr. Miroslav Fojta, CSc. (Co-Principal Investigator).

GA203/09/0317; GA CR; Construction of novel functional nucleic acids for applications in chemical biology, catalysis and self assembly.; 2009–2013; Doc. RNDr. Miroslav Fojta, CSc. (Co-Principal Investigator).

DEPARTMENT OF MOLECULAR EPIGENETICS

GAP501/10/0208; GA CR; Genome reunions in plants: from DNA to chromosomes and reverse; 2010–2012; RNDr. Aleš Kovařík, CSc.

GA13-10057S; GA CR; Structure, expression and evolution of ribosomal RNA genes in plant genomes: new approaches to old questions; 2013–2016; RNDr. Aleš Kovařík, CSc.

GA14-34632S; GA CR; Genome wide range mapping of rDNA structure and epigenetic modfications in plant hybrids using third generation single molecule real-time sequencing; 2014–2016; RNDr. Roman Matyášek, CSc.

GA206/09/1751; GA CR; The impact of genomic shock associated with interspecific hybridization and polyploidization on evolution of rDNA loci in young invasive weeds.; 2009–2013; RNDr. Roman Matyášek, CSc.

GPP501/11/P667; GA CR; Reprogramming of epigenetic state of transgenic and endogenic loci during dedifferentiation and plant regeneration.; 2011–2013; Mgr. Ing. Kateřina Křížová, Ph.D.

MEB021114; MEYS; The structure and expression of ribosomal RNA genes in invasive Spartina anglica allopolyploid species that formed recently in Europe; 2011–2012; RNDr. Aleš Kovařík, CSc.

DEPARTMENT OF MOLECULAR CYTOLOGY AND CYTOMETRY

EE2.3.30.0030; MEYS; Development of human resources in the field of cell biology; 2012–2015; Doc. RNDr. Stanislav Kozubek, DrSc.

GAP302/10/1022; GA CR; Chromatin dynamics during DNA repair; 2010–2014; Doc. RNDr. Stanislav Kozubek, DrSc.

GA13-07822S; GA CR; The role of epigenetic processes in DNA repair; 2013–2016; Doc. RNDr. Eva Bártová, Ph.D.

TA03010598; TA0; Modified nucleosides, nucleotides and oligonucleotides and their application in biomedicine; 2013–2016; RNDr. Karel Koberna, CSc.

7F14369; MEYS; NuArch: Nuclear Architecture in the regulation of autophagy, DNA repair and gene expression; 2014–2017; Doc RNDr. Eva Bártová, Ph.D.

LD11020; MEYS; Epigenetics normal and tumor cells.; 2011–2013; Doc. RNDr. Eva Bártová, Ph.D.

GBP302/12/G157; GA CR; Dynamics and Organization of Chromosomes in the Cell Cycle and during Differentiation under Normal and Pathological Conditions; 2012–2018; Doc. RNDr. Stanislav Kozubek, DrSc.

TD09/05; COST-EU; Epigenetics from Bench to Bedside; 2010–2013; Doc . RNDr. Eva Bártová, Ph.D.

PIRSES-GA-2010-269156-LCS ; Marie Curie project-EU; Study on protein dynamics in living cells after DNA injury; 2011–2014; Doc RNDr. Eva Bártová, Ph.D.

PROJECTS

DEPARTMENT OF CYTOKINETICS

GAP301/11/1730; GA CR; Changes of lipid metabolism and composition during colorectal carcinogenesis - the effects of butyrate and polyunsaturated fatty acids; 2011–2013; Prof. RNDr. Jiřina Hofmanová, CSc.

GAP503/11/1227; GA CR; Interactions of inflammatory mediators and Ah receptor signaling in toxic effects of polycyclic aromatic hydrocarbons; 2011–2013; Doc. RNDr. Jan Vondráček, Ph.D.

GA13-07711S; GA CR; The role of Hippo signaling pathway and organic toxicants in alterations of liver cell function and xenobiotic metabolism; 2013–2016; Doc. RNDr. Jan Vondráček, Ph.D.

GA13-09766S; GA CR; Lipid nutritional factors as modulators of xenobiotic metabolism and toxicity in colon epithelial cells; 2013–2017; Prof. RNDr. Alois Kozubík, CSc.

GD204/09/H058; GA CR; Intercellular signalling in development and disease; 2009–2012; Mgr. Vítězslav Bryja, Ph.D.

GPP301/12/P407; GA CR; The role of MDM2 in epithelial-to-mesenchymal transition: implication for cancer progression; 2012–2014; Mgr. Eva Slabáková, Ph.D.

NT11201; MZ0; New mechanisms of platinum-based drug action as a tool for anticancer therapeutic strategies; 2010–2014; Prof.. RNDr. Alois Kozubík, CSc.

PIPPMS M 200041203; ASCR; The role of epithelial to mesenchymal transition in plasticity of normal and cancer stem cells; 2012–2015; Mgr. Karel Souček, Ph.D.

GP13-31488P; GA CR; Influence of Dishevelled posttranslational modifications on Wnt signaling pathways; 2013–2015; Mgr. Ondřej Bernatík, PhD.

GD303/09/H048; GA CR; Molecular mechanisms of selected pathological processes in the cell; 2009–2012; Prof. RNDr. Jiřina Hofmanová, CSc. (Co-Principal Investigator).

CZ.1.07/2.4.00/31.0245; MEYS; OrganoNET – partnership for education and research in the field of visualisation of tissues and organs; 2012–2014; Mgr. Karel Souček, Ph.D. (Co-Principal Investigator).

NT13573; MZ0; Role of S-phase kinase-associated protein 2 in biology of prostate cancer stem cells and tumor progression; 2012–2015; Mgr. Karel Souček, Ph.D. (Co-Principal Investigator).

DEPARTMENT OF FREE RADICAL PATHOPHYSIOLOGY

GA524/08/1753; GA CR; The influence of L-arginine and its analogues on the generation of reactive oxygen and nitrogen species by professional phagocytes; 2008–2012; Doc. RNDr. Antonín Lojek, CSc.

GCP305/12/J038; GA CR; Importance of the interaction of myeloperoxidase with the endothelial glycocalyx for leukocyte recruitment and vascular fuction; 2012–2014; Mgr. Lukáš Kubala, Ph.D.

GP13-40824P; GA CR; New Role for Nitrated Fatty Acid in Modulation of Phagocyte-derived Inflammatory Responses; 2013–2015; Mgr. Gabriela Ambrožová, Ph.D.

GP13-40882P; GA CR; Role of L-arginine, methylarginines and their metabolism in regulation of endothelial homeostasis; 2013–2014; Mgr. Michaela Pekarová, Ph.D.

LD11010; MEYS; The effects of histamine receptor H4R antagonists on the production of reactive oxygen and nitrogen metabolites by phagocytes; 2011–2013; Doc. RNDr. Antonín Lojek, CSc.

LD11015; MEYS; Role of hypoxia and intracellular redox status disbalance in a regulation of cell selfrenewal and differentiation; 2011–2013; Mgr. Lukáš Kubala, PhD.

LD14030; MEYS; Molecular mechanisms of serotoninergic system interactions with cells of immune system; 2014–2016; RNDr. Milan Číž, Ph.D.

AS CR – Programme "The Support of international cooperation projects" The importance of nitrated fatty acids as endogenous cardioprotective mediators; 2012–2015; Mgr. Michaela Pekarová, Ph.D.

BM1404; COST – EU; European Network of Investigators Triggering Exploratory Research on Myeloid Regulatory Cells (Mye-EUNITER); 2014–2018; Mgr. Michaela Pekarová, Ph.D.

CM1201; COST – EU; Biomimetic Radical Chemistry; 2012–2016; RNDr. Milan Číž, Ph.D.

BM0806; COST – EU; Recent advances in histamine receptor H4R research; 2009–2013; Doc. RNDr. Antonín Lojek, CSc.

TD0901; COST – EU; Hypoxia sensing, signalling and adaptation; 2009–2013; Mgr. Lukáš Kubala, PhD.

GA13-29358S; GA CR; Materials for integration of animal cells with organic electronics: towards future bioelectronic devices; 2013–2016; Mgr. Jan Víteček, Ph.D. (Co-Principal Investigator).

DEPARTMENT OF STRUCTURE AND DYNAMICS OF NUCLEIC ACIDS

GAP208/10/2302; GA CR; Theoretical and Experimental Studies Related to the Prebiotic Chemistry of Nucleic Acids; 2010–2013; Judit E. Šponer, Ph.D.

GAP208/11/1822; GA CR; Structure and dynamics of DNA. Advanced computational studies.; 2011–2015; Prof. RNDr. Jiří Šponer, DrSc.

GAP208/12/1878; GA CR; Catalytic strategies of RNA and RNP enzymes studied by multi-scale computational approach; 2012–2016; Prof. RNDr. Jiří Šponer, DrSc.

GA14-12010S; GA CR; Theoretical and experimental studies on the origin of life; 2014–2016; Judit E. Šponer, Ph.D.

GA203/09/1476; GA CR; Structural dynamics, molecular interactions and function of key RNA motifs; 2009–2012; Prof. RNDr. Jiří Šponer, DrSc.

Computer equipment is kindly provided by the project "CEITEC-Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund.

GBP305/12/G034; GA CR; Centre for RNA Biology; 2012–2018; Prof. RNDr. Jiří Šponer, DrSc. (Co-Principal Investigator).

GD203/09/H046; GA CR; Biochemistry on the crossroad from in silico to in vitro; 2009–2012; Prof. RNDr. Jiří Šponer, DrSc. (Co-Principal Investigator).

DEPARTMENT OF CD SPECTROSCOPY OF NUCLEIC ACIDS

GAP205/12/0466; GA CR; DNA quadruplexes in the human genome associated with diseases and aging; 2012–2016; Prof. RNDr. Michaela Vorlíčková, DrSc.

GP14-33947P; GA CR; Regulation of the embryonic stem cell pluripotency factor Oct4 via guanine quadruplex in its promoter; 2014–2016; Mgr. Daniel Renčiuk, Ph.D.

IAA500040903; AV0; Biophysics and bioinformatics of genomic DNA fragments very rich in guanine and adenine; 2009–2013; RNDr. Jaroslav Kypr, CSc.

Sciex fellowship, No. 11.137, Atomic structure of biologically important DNA fragments, Univerzity of Zurich, Switzerland, 05–11/2012

GA13-28310S; GA CR; Evolutionary conserved structural features of centromeric and telomeric DNA; 2013–2016; Prof. RNDr. Michaela Vorlíčková, DrSc. (Co-Principal Investigator).

GA202/09/0193; GA CR; Formation and dynamics of nucleic acid motifs involved in regulation of gene expression; 2009–2013; Prof. RNDr. Michaela Vorlíčková, DrSc. (Co-Principal Investigator).

DEPARTMENT OF PLANT DEVELOPMENTAL GENETICS

LH14002; MEYS; Functional Genomics of Dioecious Plants; 2014–2016; prof. RNDr. Boris Vyskot, DrSc.

GAP305/10/0930; GA CR; Sex chromosomes and dynamics of transposable elements; 2010–2014; Doc. RNDr. Eduard Kejnovský, CSc.

GAP501/10/0102; GA CR; Comparative Analysis of Plant Sex Chromosomes; 2010–2014; Prof. RNDr. Boris Vyskot, DrSc.

GAP501/12/2220; GA CR; Sex chromosome evolution - chromosome-specific genomics in genus Silene; 2012–2016; RNDr. Roman Hobza, Ph.D.

GA13-06264S; GA CR; Sex determination and sexual dimorphism in plants; 2013–2016; RNDr. Bohuslav Janoušek, Ph.D.

GA522/09/0083; GA CR; Isolation of Sex Linked Genes to Study Evolution of Sex Chromosomes in Plants; 2009–2013; RNDr. Roman Hobza, Ph.D.

GBP501/12/G090; GA CR; Evolution and Function of Complex Plant Genomes; 2012–2018; prof. RNDr. Boris Vyskot, DrSc.

GD204/09/H002; GA CR; Plant Developmental Biology and Genetics; 2009–2012; Prof. RNDr. Boris Vyskot, DrSc.

GPP501/10/P483; GA CR; Retrotransposon colonizing only recombining part of genome of dioecious plants; 2010–2012; Mgr. Zdeněk Kubát, Ph.D.

GP13-34962P; GA CR; Silene dioica as a model species for heavy metal tolerance; 2013–2015; Ing. Radim Čegan, Ph.D.

CZ.1.07/2.4.00/17.0008; MEYS; InterDoc – Internationalization of Doctor Studies in the Area of Botany and Plant Physiology; 2011–2014; Prof. RNDr. Boris Vyskot, DrSc. (Co-Principal Investigator).

COOPERATION WITH UNIVERSITIES

Teaching and education is a very important mission of the Institute. All departments are involved in bachelor's and master's degree programmes, the special attention is paid to doctoral studies programmes. Every year, researchers provide 72 cycles of semester lectures or seminars at Masaryk University or other Czech and Slovak universities. They supervise about 40 diploma students and 60 doctoral students every year. The institute collaborates with universities also in research, and students regularly participate in IBP research activities. This collaboration is also advantageous because the laboratory equipment and expertise are used in both organizations.

Members of IBP are involved in the following courses

MASARYK UNIVERSITY, BRNO

Milan Číž, Lukáš Kubala, Antonín Lojek: Free radicals in animal physiology

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

Jiří Fajkus, Martina Dvořáčková:
Seminar of the Department of functional genomics and proteomics

Jiří Fajkus, Miloslava Fojtová, Eva Sýkorová: Analysis of chromatin structure

Jiří Fajkus, Miloslava Fojtová: Applied genomics and proteomics

Jiří Fajkus, Miloslava Fojtová: Methods in genomics and proteomics

Miroslav Fojta, Emil Paleček, Miloslava Fojtová and Michaela Vorlíčková:
Chemical properties, structure and interactions of nucleic acids
(Bi7015 in Czech, at the Faculty of Science)

Miroslav Fojta, Michaela Vorlíčková, Miloslava Fojtová and Daniel Renčiuk:
Chemical properties, structure and interactions of nucleic acids
(S1001 in English, doctoral programme at CEITEC MU)

Miroslav Fojta, Václav Brázda, Marie Brázdová, Hana Pivoňková:
Chemistry of nucleic acids – laboratory practice
(Bi7016 in Czech, at the Faculty of Science)

Miroslav Fojta, Michaela Vorlíčková, Miloslava Fojtová and Iva Kejnovská:
Chemical properties, structure and interactions of nucleic acids – practical course
(S1002 in English, doctoral programme at CEITEC MU)

Miloslava Fojtová: Seminar in genomics and proteomics branch (I-IV)

Jiřina Hofmanová, Alois Kozubík: Health risks

Jiřina Hofmanová, Alois Kozubík: Genotoxicity and carcinogenesis

Jiřina Hofmanová, Alois Kozubík: Special methods of animal physiology

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

Alena Hyršlová Vaculová: Mechanisms of cell death, function, methods

Alena Hyršlová Vaculová: Molecular biology and genetics

Alena Hyršlová Vaculová: Scientific work methodics

František Jelen: Bioelectrochemistry 1, 2

Eduard Kejnovský, Roman Hobza: Evolutionary genomics

Stanislav Kozubek, Eva Bártová: Molecular physiology of the genome

Stanislav Kozubek, Martin Falk: Radiation biophysics

Alois Kozubík: Introduction to the study of general biology

Alois Kozubík, Jiřina Hofmanová: Physiology of cell systems

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

Lukáš Kubala, Antonín Lojek, Milan Číž: Special physiology of blood

Antonín Lojek, Milan Číž, Lukáš Kubala: Immunology

Antonín Lojek, Milan Číž, Lukáš Kubala: Photobiology

Olga Nováková: Selected themes of application biophysics

Karel Souček: Journal club – Cancer biology I, II

Karel Souček, Eva Slabáková, Alena Hyršlová Vaculová, Eva Bártová:
Analytical cytometry

Karel Souček, Zuzana Pernicová, Šárka Šimečková, Jan Remšík:
Analytical cytometry – practical course

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

Jiří Šponer: Structure and dynamics of nucleic acids

Jiří Šponer: Introduction to biophysics

Jan Vondráček: Applied chemistry and biochemistry

Jan Vondráček: Physiology of pharmaceuticals and toxic compounds

Oldřich Vrána: Biophysics

Oldřich Vrána: Vibration spectroscopy of biopolymers

Oldřich Vrána: Spectroscopic study of biopolymers

Oldřich Vrána, Olga Nováková, Jana Kašpárková, Jaroslav Malina, Michaela Vorlíčková: Experimental methods of biophysics

Boris Vyskot: Developmental genetics

Boris Vyskot: English seminar for PhD. students (spring, autumn)

PALACKÝ UNIVERSITY OLOMOUC

Viktor Brabec: Biophysics of nucleic acids

Viktor Brabec: Seminar in biophysics

Viktor Brabec: Structure, properties, and function of nucleic acids

Viktor Brabec: Physical properties of nucleid acids

Viktor Brabec, Jana Kašpárková: Experimental methods of biophysics

Jana Kašpárková: Molecular biophysics of mutagens, cancerogens and cytostatics

Jana Kašpárková: Molecular biophysics

Jiří Šponer: Structure and dynamics of nucleic acids

Jan Vondráček: Molecular toxikology

Jan Vondráček: Environmental toxikology

Boris Vyskot: Developmental biology

Boris Vyskot: Epigenetics

MENDEL UNIVERSITY OF AGRICULTURE AND FORESTRY IN BRNO

Roman Hobza: Genetic engineering

Roman Hobza: Genetic engineering, practical course

Vojtěch Hudzieczek: Plant Developmental Genetics, practical course

Boris Vyskot: Plant Developmental Genetics

UNIVERSITY OF VETERINARY AND PHARMACEUTICAL SCIENCES BRNO

Marie Brázdová: Biochemistry (Lectures in Czech)

Marie Brázdová: Biochemistry (Lectures in English)

Eduard Kejnovský, Roman Hobza: Evolutionary biology

CHARLES UNIVERSITY IN PRAGUE

Jiří Šponer: Structure and dynamics of nucleic acids

COMENIUS UNIVERSITY IN BRATISLAVA

Veronika Ostatná: Medical biophysics

UNIVERSITY OF SOUTH BOHEMIA IN ČESKÉ BUDĚJOVICE

Roman Hobza, Eduard Kejnovský: Evolutionary genomics

UNIVERSITY OF OSTRAVA

Václav Brázda: Basics of genetic engineering

Vojtěch Hudzieczek, Boris Vyskot: Developmental biology

BRNO UNIVERSITY OF TECHNOLOGY

Miroslav Fojta: Molecular biology

Aleš Kovařík: Basic bioinformatics

HONORS AND AWARDS

2012

Martin Bartošík: Award of the Minister of Education, Youth and Sports for excellent students and graduates in study programmes (Minister of Education, Youth and Sports)

Vítězslav Bryja: Ministry of Education, Youth and Sports award for outstanding research, experimental development and innovation for the year 2012
(Minister of Education, Youth and Sports)

Hana Pivoňková: The Otto Wichterle Award (President of the Czech Academy of Sciences)

2013

Miroslav Fojta: Heyrovky-Ilkovic-Nernst Lecture (German Chemical Society, Czech Chemical Society, Slovak Chemical Society)

Jiřina Hofmanová: Award for outstanding achievement
(The 18th World Congress on Advances in Oncology and 16th International Symposium on Molecular Medicine, Hersonissos, Crete, Greece, 10.–12. 10. 2013)

Vojtěch Hudzieczek: Award for the best presentation (The International PhD Student Conference on Experimental Plant Biology (Pavol Jozef Safarik University in Kosice)

2014

Michaela Pekarová: The Otto Wichterle Award (President of the Czech Academy of Sciences)

Emil Paleček: The Czech Head Award (Government of the Czech Republic)

Emil Paleček: the Silver Commemorative Medal of the Czech Senate (Senate of the Parliament of the Czech Republic)

Jiří Šponer: Academic Award–Praemium Academiae (President of the Czech Academy of Sciences)

INTERNATIONAL CONFERENCES AND WORKSHOPS ORGANIZED OR CO-ORGANIZED BY THE INSTITUTE

2012

45th Heyrovsky Discussion - Electrochemistry of Biopolymers and Bioactive Compounds

International Conference of PhD Students in the field of experimental plant biology

Advanced Confocal Microscopy and Living Cell Studies

International Conference for Polyploidy, Hybridisation and Biodiversity

Metallodrugs I: Design and mechanism of action

Functional Genomics and Proteomics for Sustainable Agriculture

12th International Workshop on Radiation Damage to DNA

2013

Metallodrugs II: Design and mechanism of action

V4 International Conference Analytical Cytometry VII

2014

Metallodrugs III: From DNA interactions to chemotherapy of cancer

47th Heyrovsky Discussion on Electrochemistry of Organic and Bioactive Compounds

Methods in Plant Science 6

Confocal microscope Leica TSC SP5-X

RESEARCH INFRASTRUCTURES

LABORATORY OF CELLULAR BIOPHYSICS (LCB)

The Laboratory has been established in 2009 with the aim to overcome underfunding in the area of cell analyses and links technology of confocal microscopy and flow cytometry and sorting.

CONFOCAL MICROSCOPY

SPINNING DISC CONFOCAL MICROSCOPY (SDCM)

Confocal microscopy system based on Leica DMXA epi-fluorescence microscope with motorized stage and Piezzo z-control, MicroMax CCD high-resolution camera, rotating spinning disc, powerful Ar-Kr laser (2,5W) with AOTF has been available at the IBP for approximately 15 years. Recent upgrades involve replacement of computer control with new Acquiarium software and 4 laser diodes instead of the laser. SDCM provides images of quality comparable to images made with CLSM, scanning is faster and light damage to cells as well as photobleaching can be reduced.

CONFOCAL LASER SCANNING MICROSCOPY (CLSM) WITH WHITE LASER AND UV-LASERS

The laboratory is equipped with confocal microscope Leica TSC SP5-X with white light laser (470-670 nm in 1 nm increments); powerful argon laser (488 nm) and UVA lasers (355 nm and 405 nm). Chamber for cell cultivation under microscope is used for living cell studies. The following are the main microscopy-related techniques: 3D-image analysis of confocal sections, co-localization analysis (protein foci co-localization), de-convolution, tile scanning and reconstruction of larger biological objects (e.g. mouse brain sections), FRAP, FRET, UVA-micro-irradiation (induction of DNA lesions by 355-nm or 405-nm UVA lasers), time-lapse confocal microscopy, single particle tracking analysis etc.

FLOW CYTOMETRY

BD FACSVERSE FLOW CYTOMETRY

BD FACSVerse system is equipped with three lasers (405nm, 488nm and 640nm) with separated optical paths. The fluidics system uses vacuum, excitation of a sample takes place in the steel cuvette, which improves optical parameters. The instrument can measure FSC, SSC and 8 fluorescence parameters. This system serves the users from IBP on a daily basis.

FACSARIA II SORP FLOW CYTOMETRY

This flow cytometer and 4-way cell sorter is equipped with five lasers (355nm, 405nm, 488nm, 561nm and 639nm) with separated optical paths and fixed optics. It uses the principle of hydrodynamic focusing in the environment of high pressure. The instrument can measure FSC, SSC and 18 fluorescence parameters at the same time and also aseptically sort particles up to 4 tubes or multiple-well plate (up to 384-wells), these can be cooled or heated at preset temperature by the connected water bath. The system serves as shared instrument to many departments but also to the cooperating partners (e.g. Masaryk University, Veterinary Research Institute, etc.).

ATTUNE® ACOUSTIC FOCUSING CYTOMETER

Attune Acoustic Focusing Flow Cytometer is the first cytometer that uses ultrasonic waves (over 2 MHz), rather than hydrodynamic forces, to position cells into a single, focused line along the central axis of a capillary. This system is equipped with two lasers and four detectors and is routinely used for analyses of in vivo samples and for educational activities.

FACSAria II Sorp Flow cytometry

RESEARCH INFRASTRUCTURES

CEITEC–IBP CENTRAL FACILITIES

Three departments have developed close contacts with the Central European Institute of Technology (CEITEC), particularly with the Structural Biology programme at CEITEC Masaryk University (biopolymer research) and Advanced Nanotechnologies and Microtechnologies programme at CEITEC Brno University of Technology (biosensing). To support these collaborations, joint laboratories IBP-CEITEC have been established in IBP building. The laboratories are equipped with instrumentation which, being owned by CEITEC, is available for IBP researchers. This instrumentation involves powerful computer clusters to pursue state-of-the-art computational modelling of biopolymers structure and dynamics, inductively coupled plasma mass spectrometry (ICP-MS) for elemental analysis (particularly determination of metals in modified biopolymers and other biological samples), CD spectrometer for nucleic acids structure studies, isothermal titration microcalorimeter and ELISA system for interaction studies, and a multimode electrochemical analyser. In addition, collaboration and personal connection exists between IBP and CEITEC. J. Fajkus serves as a co-ordinator of the research programme Genomics and proteomics of plant systems in CEITEC. Collaboration based on common projects makes it possible to use the CEITEC infrastructure such as Core Facilities Genomics, CF Proteomics, Plant Cultivation Facilities – greenhouses and phytotrones, advanced microscopy instrumentation, NMR, Crystallography, Cryoelectron microscopy etc. as internal users.

FNUSA–ICRC–IBP CENTRAL FACILITIES

Because of the cooperation between IBP and FNUSA-ICRC, several state-of-the-art instruments are placed at IBP AS CR and they are available to the research teams from IBP and FNUSA. They include nitric oxide analyser (Sievers NOA-280i, Analytix), electron paramagnetic resonance spectrometer (MiniScope MS 500, Magnettech), image microflow system (Cellix/Zeiss), automated liquid handling station (EpMotion 5075, Eppendorf), high-performance, medium- to high-throughput PCR platform (96- or 384-well plates, LightCycler 480, Roche), multi-species optical and X-Ray imaging system (IVIS Lumina XR, Perkin Elmer). The nitric oxide analyser and electron paramagnetic resonance spectrometer are routinely used for determination of nitric oxide production and various reactive oxygen species formation in different kind of biological samples. The image microflow system consists of the inverted fluorescent microscope (Zeiss Observer Z1) and from a set of pumps from Cellix that allow to perform performance of experiments under flow conditions in specialised microfluidic chips. This system is used to study modulation of adhesion of leukocytes to model surfaces and to study physiology and pathological functions of endothelial cells under flow conditions mimicking the real conditions in the body. Automated liquid handling station is used for wide spectrum of applications, including medium throughput cell based assay, RT-PCR reaction setup, ELISA, etc. PCR platform is frequently used by many users, mostly for analyses of gene expression. The optical and X-Ray imaging system is installed in a brand new small animal facility at IBP CAS. This system integrates multiple modalities including photographic, bioluminescent, fluorescent imaging, X-Ray, radioisotopic/ Cerenkov and high speed real-time imaging in small animals - rodents.


PURELAB Option
RESERVOIR
ELGA
ELGA

RESEARCH INFRASTRUCTURES

ANIMAL FACILITY

At the beginning of 2015, a new serum pathogen free (spf) small animal facility became operative and substituted for the previous conventional facility. This new spf animal facility will provide IBP with high-quality small experimental animals (mice and rats), which meet the highest requirements for the absence of microbial pathogens negatively influencing experimental results. The clean space of the facility is equipped with its own air-handling system fitted out with powerful antimicrobial filters. The facility can house up to 4,000 mice and rats, including immunodeficient and genetically modified ones, which could not be bred in IBP in the past. A breeding unit with individually ventilated cages is assigned to the most fragile animals. Thus, investigators from IBP are newly able to perform a wide spectrum of animal studies including sophisticated experimentation on immunodeficient and rare small animal strains.

OTHER MODERN EQUIPMENT TO BOOST RESEARCH

In 2011 IBP has purchased Typhoon FLA 9000, a versatile laser scanner for biomolecular imaging applications, including quantitative and sensitive measurements of radioisotopic labels such as phosphorus, chemifluorescent Western blots, and multiplex fluorescence as well as digitization of colorimetric assays. Typhoon FLA 9000 delivers (i) Versatility: image radioisotope-, multifluorescent-, chemifluorescent-, and colorimetric-labeled samples (ii) High resolution and quantitation: a pixel resolution of up to 10 µm and a linear signal response over five orders of magnitude provides precise quantitation in gels, blots, tissue sections, and arrays.
The scanner has quickly become one of the busiest devices at IBP, it is currently being used by more than 60% of its laboratories. The average day scanning time (net) is estimated to be 2–3 hours. Most methods involve radioactivity scanning of P[32]-labelled samples, but non-radioactive fluorescence samples are also being analyzed. The outputs from experiments have been published in scientific journals. Only in the Laboratory of Molecular Epigenetics, there have been seven papers published since the installation of the equipment. The scanner is also used by the researchers from the Brno academic community.

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CENTER OF INFORMATION TECHNOLOGIES (CIT)

Standard services of the Center include maintenance of local area network (LAN), connection from IBP LAN to Brno Academic Computer Network and to the Internet, exchange and IP telephony, IBP e-mail server, including antivirus and antispam systems, IBP web server including data update, fileserver and secure data storage including data backups, VMware server cluster.
CIT also takes care of development and maintenance of computer hardware and software jointly used by all laboratories (servers and PCs with connected scientific instruments) running under UNIX, MS Windows 7/8. CIT also provides consulting services for individual scientists.
Main attention of CIT is devoted to the security issues. Security patches were installed in time and antivirus databases were regularly updated. All e-mails are monitored at the server by a virus scanner together with special software, designed to detect and defang dangerous elements inside e-mail messages (dangerous attachments are renamed, so that they cannot be run automatically on PC). In addition, e-mails are scanned by an antispam system.

LIBRARY

A part of CIT takes care of online access to scientific journals over Internet and manages subscriptions to scientific informational resources. Library manages information exchange among libraries and interlibrary borrowing services, takes care of printed versions of journals and books in IBP and arranges access of users to them. Library also offers reprographic services to IBP scientists.

CONTACT Published by: Institute of Biophysics, CAS
ADDRESS Královopolská 135, 61265 Brno
E-MAIL ibp@ibp.cz
WEB www.ibp.cz
EDITORS Alois Kozubík, Antonín Lojek
LAYOUT AND DESIGN Linda Kriegerbecková
PHOTOGRAPHY Michal Hladík
SCIENTIFIC DOCUMENTS Research Departments
PRINTED BY POINT CR, s.r.o.
September 2015

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