Organizing Institutions

Maria Skłodowska-Curie Memorial
Cancer Center and Institute of Oncology
Gliwice Branch
Welcome to  
the Wilhelm Bernhard Workshop 2009!

Dear Friends,

We are very pleased to welcome you in Ustroń for this 21st International Workshop on the Cell Nucleus. We are quite sure that this Workshop, once more, will represent a moment of fruitful and stimulating discussion between scientists from all over the world, meeting in the spirit that Wilhelm Bernhard left 31 years ago.

Have a good time in Ustroń and welcome again!

Piotr Widłak  
Mieczysław Chorąży
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Stan Fakan, Switzerland
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General schedule:

31st August (Monday)
12.00 - 18.00  Arrival, registration
18.30  Opening Ceremony - The Wilhelm Bernhard Medal Lecture 2009
          Concert and Welcome Party

1st September (Tuesday)
Morning Session: Structure and Dynamics
Afternoon Session: Chromosomes and Chromatin
Outdoor dinner on Czantoria mountain

2nd September (Wednesday)
Morning Session: The Nucleolus and Ribosome Biogenesis
A trip to Szczyrk, Żywiec, Koniaków and Wisła in the afternoon/evening

3rd September (Thursday)
Morning and Early Afternoon Session: The Nucleus in Pathological Conditions
Poster Session (poster are presented since 1.09.2009)
Afternoon Session: DNA Damage and Repair
Farewell party

4th September (Friday)
9.00 – 12.00  Departure

The Opening Ceremony will be held in Ustoń City Culture Center Prażakówka
(Daszyńskiego str. 28)
All sessions will be held in Spa Hotel Diament (Zdrojowa str. 3)
Wilhelm Bernhard
1920-1978

Wilhelm Bernhard was born November 8, 1920, in the village of Worb, Switzerland. As a youth he pursued his earliest scientific interest, astronomy, grinding his own telescope mirror to scan the sky. After completing his studies at Berne and Geneva for the M.D. degree in 1946, he served briefly in the Swiss Army as physician for ski-borne troops, scaling the heights with his patrol. In 1947 he went to Paris, France, for training in pathology where he met Professor Charles Oberling, early propounder of the viral theory of cancer. They discovered in each other a mutual boundless curiosity, zest for life, and love of history and the arts. Professor Oberling invited Dr. Bernhard to head the new laboratory of electron microscopy in Villejuif, one of the first established in France.

Some of the earliest descriptions of the ultrastructure of cell organelles (nucleolus, endoplasmic reticulum, Golgi apparatus, lysosomes, centrioles, microtubules) emanated from this laboratory, where students and guests from many countries gathered to learn and to collaborate. But during the early years, Dr. Bernhard's primary interest was to study the neoplastic cell and the viruses known to induce certain animal tumors, with the ultimate goal of determining whether viruses could be causally linked to human cancer. Thus, between 1953 and 1966 he and his colleagues demonstrated the structure and intracellular development, first of the Rous sarcoma virus and then, successively, the viruses associated with the Shope fibroma, mouse mammary tumor, Murray-Beg avian endothelioma, avian erythroblastosis and myeloblastosis, murine leukemia, the polyoma, and SV40 viruses and adenoviruses. It was he who proposed the now established classification of types A, B, and C murine oncogenic viruses. It was he who wrote, after years of comparative studies, especially of human leukemias, that there is no specific ultrastructural change in neoplastic cells.

Dr. Bernhard's first task at Villejuif in 1947 had been to develop procedures for obtaining sections of cells thin enough for high resolution electron microscopy. With the first premethacrylate waxes that he tested after shivering through long hours of sectioning in a cold room, he was not only able to obtain ultrathin sections but also to stain adjacent sections and demonstrate that the newly discovered granular endoplasmic reticulum was indeed the basophilic ergastoplasm of Garnier and Bouin. From that time, he eagerly sought new cytochemical techniques that would extend even further the information that could be gained from the electron microscope. With his colleagues, he pioneered in the development and utilization of electron microscope autoradiography, water-miscible embedding resins, enzymic digestion of specific components of cells, cryoultramicrotomy, immunocytochemistry with peroxidase as marker, concanavalin A labeling of cell membranes, a specific stain for DNA, and a selective stain for ribonucleoprotein.

At a time when most electron microscopists focused primarily on the myriads of structures in the cytoplasm, Dr. Bernhard attempted to decipher the ultrastructure of the nucleus. The perichromatin fibrils and their role in RNA synthesis, the phenomenon of nucleolar segregation, the existence of different types of perichromatin granules, and the formation of abnormal nuclear bodies were all described in his laboratory, using cultured cells treated with various drugs and hormones and processed by cytochemical procedures. At the time of his death, he and his colleagues were pursuing nonnucleolar transcription units and the organization of DNA in interphase nuclei.

Dr. Bernhard's influence was felt well beyond his laboratory. He was a charter member of the Société Française de Microscopie Électronique and the European Cell Biology Organization. He founded and ran the highly successful European Nucleolar Workshop (now Wilhelm Bernhard Workshop on the Cell Nucleus). He was the driving force behind the annual Franco-Russian cell biology conferences, and represented the Ministère des Affaires Étrangères in scientific missions in South America, India, Japan, and South Korea. A strong advocate of the thesis that science should have no national boundaries, he visited and invited to his laboratory scientists from all parts of the world.
His influence was not limited to the world of biology. Among his friends were sculptors, poets, philosophers, and painters. He was an illuminating guide to concerts, exhibitions, and architectural gems of Paris, the antique shops of the flea market, and the churches and chateaux of the Loire region. He transformed a large quarry behind the laboratory into an extraordinary rose garden. He liked Mozart, Proust, and…. American Westerns. All who knew him were touched by his charm and elegance, and his taste for beauty and adventure.

Dr. Bernhard rose through the ranks of the Centre Nationale de Recherches Scientifique from Attaché (1948 to 1953), Chargé de Recherches (1953 to 1956), Maître de Recherches (1956 to 1961), to Directeur de Recherches (1961). Throughout this period he was Chef du Laboratoire de Microscopie Électronique at the Institut de Recherches Scientifiques sur le Cancer at Villejuif. He was named Chevalier de l'Ordre Nationale du Mérite (1973), held honorary membership in the Royal Microscopical Society, the Spanish Society of Pathology, and the Académie Leopoldina, Halle (Germany), and was awarded the title Doctor Honoris Causa by the Université de Bruxelles (1969) and by the Université de Bale (1969). He was awarded the Prix Louise Darraq (Lauréat de l'Académie des Sciences, 1957); Prix du Lauréat du Concours de la Ligue Nationale Suisse Contre le Cancer, 1960; Grand Prix Scientifique de la Ville de Paris, 1964; Prix Paul Ehrlich (with R. Dulbecco), Frankfurt, 1967; Hartmann Muller Memorial Lecturer, Zurich, 1968; Ricketts Award, University of Chicago, 1972; Schleiden Medal, Académie Leopoldina, Halle, 1976. On December 4, 1978, he received posthumously the Prix Lacassagne of the Ligue Nationale Française Contre le Cancer.

On October 9, 1978, Dr. Wilhelm Bernhard died suddenly in Buenos Aires, on his way to a scientific meeting in Mendosa. Thus ended the career of an adventurous pioneer in electron microscopy applied to cancer research.

Elizabeth H. Leduc, Etienne de Harven, Cancer Research 39:2811 (1979)

The Wilhelm Bernhard International Workshops on the Cell Nucleus:

1969 Liblice, Czechoslovakia
1971 Lake Balaton, Hungary
1973 Abisko, Sweden
1975 Varna, Bulgaria
1977 Salamanca, Spain
1979 Weimar, Germany
1981 Safed, Israel
1983 Banyuls-sur-Mer, France
1985 Krakow, Poland
1987 Stevensbeek, The Netherlands
1989 Suzdal, USSR
1991 Les Diablerets, Switzerland
1993 Balatonaliga, Hungary
1995 Spa, Belgium
1998 Lac-Delage, Canada
1999 Prague, Czech Republic
2001 Arcachon, France
2003 Pavia, Italy
2005 Münsterschwarzach Abbey, Germany
2007 St Andrews, Scotland
2009 Ustroń, Poland
The Wilhelm Bernhard Medal
Honorary Lecture

1987  Oscar Miller
1989  Harris Busch
1991  Werner W. Franke
1993  Masami Muramatsu
1995  Karel Smetana
1999  Thoru Pederson
2001  Eliza Izaurralde
2003  Stan Fakan
2005  Bertil Daneholt
2007  Joseph G. Gall
2009  William T. Garrard

William T. Garrard
Born in 1942, Seattle, USA. Received B.Sc. (microbiology) from University of Washington, Seattle (1966) and Ph.D. (microbiology) from University of California, Los Angeles (1971). Professor of biochemistry at The University of Texas Southwestern Medical Center, Dallas, since 1974.

Professional interests include: apoptosis, chromosome structure, control of gene expression, molecular immunology, DNA methylation, developmental molecular biology, chromosome replication, organization of DNA sequences, yeast molecular genetics, functional genetics.
**21st Wilhelm Bernhard Nuclear Workshop**  
**Ustroń, 31.08.2009 – 4.09.2009**  

*Scientific program*

### 31.08.2009

**18.30**  
Opening Ceremony

**The Wilhelm Bernhard Lecture**

**William Garrard** (Dallas): Activation and silencing of the immunoglobulin kappa gene locus through DNA looping and nuclear repositioning

**Artistic Performance of the “Czantoria” Ensemble**

*Welcome Party*

### 1.09.2009

**9.00 – 13.00**  
Session *Structure and Dynamics*  
*(Susana Moreno Diaz de la Espina and Ronald Hancock – chairpersons)*

**Roel van Driel** (Amsterdam): How multi-protein complexes assemble and function on chromatin (25’)

**Paola Vagnarelli** (Edinburgh): Repo-Man and Condensin regulate chromosome structure and dynamics in mitosis (25’)

**Andrea Scharf** (Dusseldorf): Proteasomal proteolysis and transcription (20’)

**Jana Fukalova** (Prague): Actin and actin-binding proteins in the nucleus (20’)

**Sukriye Yildrim** (Prague): Nuclear myosin I binds to phosphatidylinositol-4,5-biphosphate in the cell nucleus (20’)

*Coffee break (20’)*

**Karsten Rippe** (Heidelberg): Dynamic organization, interaction and function of PML nuclear bodies in telomerase independent lengthening of telomeres (25’)

**Peter Hemmerich** (Jena): PML regulates MHC class II transcription at nuclear bodies by stabilization of CIITA, the transcriptional master regulator at MHC class II gene promoters (25’)

**Dariusz Jan Smoliński** (Toruń): mRNA, a new component of Cajal bodies (20’)

**Jan-Peter Siebrasse** (Bonn): Nuclear trafficking of single native mRNP particles in living cells (20’)

**Toru Higashinakagawa** (Tokyo): Functional knockdown analysis of Medaka Polycomb group gene *OLEED* (20’)

13.00 – 14.00 Lunch

14.00 – 18.00 Session Chromosomes and Chromatin

(William Garrard and Peter Shaw – chairpersons)

Hilmar Strickfaden (Martinsried): Changes of chromosome arrangement in cycling cells: Theodor Boveri’s model revisited (25’)

Garry Morgan (Nottingham): Gene expression in the amphibian oocyte – lampbrush chromosome structure and function (25’)

Peter Shaw (Norwich): Meiotic homologue pairing and the Ph1 locus (20’)

Takashi Ohyama (Tokyo): How genomic DNA is functionally folded in a nucleus (20’)

Gabor Szabo (Debrecen): Chromatin loops emanating from R-loops (20’)

Coffee break (20’)

Fabienne Hans (Grenoble): Molecular distinctions between Aurora A and B: a single residue change transforms Aurora A into correctly localized and functional Aurora B (25’)

Maria Malanga (Naples): Poly(ADP-ribose): a molecular switch of DNA Topoisomerase I functions (25’)

Krzysztof Staroń (Warszawa): Linker domain regulates subnuclear distribution of human topoisomerase I (20’)

Ivan Raska (Prague): A new complex epigenetic marker: the replication-coupled, cell cycle-dependent, dual modification within the histone H4 tail (20’)

Vladimir Vorobyev (St. Petersburg): Structural targets of the non-histone chromatin protein HMGB1 (20’)

19.00 Outdoor Dinner on Czantoria Mountain

2.09.2009

9.00 – 13.00 Session The Nucleolus and Ribosome Biogenesis

(Marco Biggiogera and Marc Thiry – chairpersons)

Marion Schmidt-Zachmann (Heidelberg): Functional characterization of nucleolar proteins and their roles in haematological malignancies (25’)

Sui Huang (Chicago): The PNC is associated with DNA and novel RNA-protein complexes (25’)

Francoise Lamaye (Liege): Reptile cell cultures for nucleolar studies (20’)

Karel Smetana (Prague): Computer assisted image nucleolar and cytoplasmic RNA density measurements of differentiating cells represented by human erythroid and granulocytic precursors (20’)

Victoria Barygina (Moscow): The mobile fraction of fibrillarin can serve as an indicator of rDNA transcription (20’)

Coffee break (20’)

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Lasse Lindahl & Janice Zengel (Baltimore): Mechanism of correlation of ribosome assembly and cycle control (25’)

Coralie Carron (Toulouse): A link between pre-rRNA processing and reformation of the nucleolus at the end of mitosis (25’)

Nicolas Rolland (Nancy): Study on the assembly of U3 snoRNP, an RNP essential for ribosomal RNA maturation (20’)

Belinda Jane Westman (Dundee): Wrestling with SUMO, the nucleolus and the C/D box snoRNA-associated protein, NOP5/NOP58

Olga Koroleva (Reading): Hypoxia-induced relocation of eIF4A-III to nucleolus and splicing speckles (20’)

13.00 – 14.00 Lunch

14.00 Trip to Szczyrk, Żywiec (the Habsburg’s Brewery), Koniaków and Wisła (dinner at Kubalonka)

3.09.2009

9.00 – 10.50 Session The Nucleus in Pathological Conditions (Joanna Rzeszowska and Karel Smetana – chairpersons)

Eric Schirmer (Edinburgh): Extensive tissue variation in the nuclear envelope proteome and its implications for genome organization and the pathology of laminopathies (25’)

Anna von Mikecz (Dusseldorf): Nuclear protein aggregation: the interface between function and pathology (25’)

Frances Fuller-Pace (Dundee): Transcriptional co-activation of the tumour suppressor p53 by the RNA helicase p68: modulation of p53 function in response to DNA damage (20’)

Wiesława Widlak (Gliwice): Mechanism of the HSF1-regulated apoptosis of spermatogenic cells (20’)

Jekatrina Erenpreisa (Riga): The role of meiotic cohesion REC8 in the genome reduction of gamma-irradiation-induced endopolyploid tumour cells (20’)

Coffee break (20’)

11.10 – 13.00 Poster Session

14.00 – 15.00 Session The Nucleus in Pathological Conditions (continuation)

Marek Rusin (Gliwice): The response of nuclear structures to treatment with resveratrol, a chemopreventive agent (20’)

Piotr Widlak (Gliwice): DNA Fragmentation Factor – the major player in apoptotic breakdown of the cell nucleus (20’)

Mykola Czekan (Gliwice): Karyometric analysis of cell nuclei in papillary thyroid cancer and their correlates in gene expression profile (20’)

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15.00 – 18.00  Session **DNA Damage and Repair**

*(Marion Schmidt-Zachmann and Piotr Widlak – chairpersons)*

**Adriaan B. Houtsmuller** (Rotterdam): Molecular mechanisms of gene transcription and DNA repair in living cells (25’)

**Francois-Michel Boisvert** (Dundee): A quantitative proteomic analysis of changes in subcellular proteome localization induced by DNA damage (20’)

*Coffee break (20’)*

**Sergey Shaposhnikov** (Oslo): Comet assay combined with FISH for looking at DNA damage and repair in relation to organization of the nucleus (25’)

**Jerzy Dobrucki** (Kraków): Heterochromatin protein 1 in DNA damage response - recruitment or dissociation from repair sites? (20’)

**Nikolajs Sjakste** (Riga): The tightly bound to DNA proteins: an old problem revisited by novel approaches (20’)

**Roman Jaksik** (Gliwice): The elements of the systemic cellular response to stress; the regulation of transcript levels in cells exposed to ionizing radiation (20’)

**Ronald Hancock** (Quebec): Double-strand breakage of the DNA of a minichromosome in human cells by γ-radiation and its repair (20’)

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19.00  **Farewell Party**
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ACTIVATION AND SILENCING OF THE IMMUNOGLOBULIN KAPPA GENE LOCUS THROUGH DNA LOOPING AND NUCLEAR REPOSITIONING

William T. Garrard

Department of Molecular Biology UT Southwestern Dallas, Texas USA

We have investigated the higher-order chromatin structure and composition of the mouse immunoglobulin (Ig) kappa locus as a function of activation and silencing during B cell development. LPS-induced transcription of the mouse Igκ gene in B lymphocytes utilizes three distal enhancers (Ei, E3', and Ed) and requires the transcription factor NF-κB, whose family members include RelA and c-Rel. To determine step-wise many of the requirements and events that lead to the establishment of the fully active state of the Igκ locus, we have utilized chromosome conformation capture (3C) and ChIP-3C technology along with inducible biological systems, chemical inhibitors, dominant negative and knock-down or knockout of NF-κB family members. We find that prior to transcriptional induction, rearranged V gene promoters are complexed with either the enhancer Ei or E3' (but not with both nor with Ed), forming the “poised” conformation, but that pair-wise interactions between the three enhancers occur only after LPS-activation and the transient and essential recruitment of RelA. ChIP-3C experiments reveal that these enhancer complexes are in close proximity to RNA polymerase II, providing evidence that transcription factories are the molecular ties for the bases of these DNA loops. These processes are actin-filament-dependent but independent of new protein synthesis, transcription or c-Rel.

During B cell differentiation one of the two Igκ alleles becomes silenced (allelic exclusion). We have previously identified a recombination silencer termed Sis, which is responsible for targeting germline Igκ miniloci carried on yeast artificial chromosomes (YACs) to pericentromeric heterochromatin [Immunity 24, 405 (2006)]. Sis possesses bound Ikaros, a repressor protein that is thought to be responsible for such targeting because it co-localizes with pericentromeric heterochromatin. We now have generated mice with a deletion of Sis in the endogenous locus. 3D FISH reveals that Sis is indeed responsible for targeting the native locus to pericentromeric heterochromatin. Sis-/- mice exhibit normal levels of gene rearrangement and allelic exclusion but exhibit a markedly skewed Igκ repertoire because of striking alterations in the V genes used for gene rearrangement. Because we have found that Sis also possesses bound CTCF, a protein responsible for creating chromosomal loops by bridging together distal cis-acting sequences, we hypothesize that Sis-/- mice have markedly altered V gene looping patterns in pre-B cells undergoing gene rearrangement. Interestingly, older Sis-/- mice exhibit enlarged spleens, glomerular damage and increased serum autoantibodies. We suggest that these autoimmunity phenotypes may relate to the skewed antibody repertoire.

Research supported by NIH and the Robert A. Welch Foundation. Important contributors to these results include Drs. Zhe Liu, Yougui Xiang, Piotr Widlak and Jane Skok.
To understand how multi-protein complexes assemble and function on chromatin, we have combined in vivo analysis of the mammalian nucleotide excision DNA repair machinery with computational modeling. We found that the components of the DNA repair machinery exchange rapidly between repair sites and the nucleoplasm, whereas their net accumulation at damaged DNA evolved on a much slower timescale. A predictive Kinetic model constrained by these data shows how the assembly of multi-protein complexes is orchestrated by progressive enzymatic modifications of the DNA substrate, leaving considerable freedom for the binding mode of individual proteins. We demonstrate that the faithful recognition of DNA lesions is time-consuming, while repair complexes form rapidly through random and rapidly reversible assembly. Systems analysis reveals a fundamental conflict between specificity and efficiency of chromatin-associated protein machineries and shows how a trade-off is negotiated through reversibility of protein binding.
Chromosome structure and dynamics are extremely important during mitosis. During mitotic exit, chromosomes undergo a radical de-condensation in an orderly and timely manner in order to give rise to a functional G1 nucleus. We have shown that condensin is essential for the maintenance of a compact chromosome structure during anaphase chromosome segregation but it is dispensable for their hyper-condensation in telophase. We have also identified the Repo-Man/PP1 complex as a crucial regulator of chromosome architecture during mitosis. This complex re-localises to the segregating chromatids at anaphase onset and inactivates an as-yet unidentified substrate, which cooperates with condensin in prometaphase for the compaction of mitotic chromosomes.

We have recently demonstrated that a cdc2/cyclinB phosphorylation site on Repo-Man is crucial for Repo-Man/PP1 complex localisation and activity during mitosis.

In order to identify the chromosomal substrates of the Repo-Man PP1 complex, we have conducted pull-down experiments of tagged Repo-Man followed by mass-spectrometry from cells where mitotic exit has been driven by inhibiting CDK1. Our preliminary results suggest that Repo-Man is able to interact with chromatin and this interaction is sufficient to trigger deposition of early nuclear envelope components independent of mitotic exit.

Key words: mitotic chromosomes, condensin, protein phosphatase, anaphase
TRANSCRIPTION is the first step in gene expression and hundreds of proteins must interact with a small region of the DNA strand to successfully transcribe one gene. It is essential for the cell to coordinate and regulate these proteins: all players must be at the right time in the right place to obtain correctly transcribed nascent RNAs. One helpful nanomachine in this complex regulation process is the proteasome. Many components of the transcription machinery are degraded by the proteasome, e.g. the RNA pol II or transcription activators\(^1,2\), but the spatial structure-function relationship between the proteasomal proteolysis and transcription is unknown.

Nuclear proteasomes are active in distinct domains: proteins in the nucleus are degraded in proteolytic foci or centers\(^3\). A chromatin profiling shows that active proteasomal proteolysis is not localized in heterochromatic regions of the nucleus, but is clearly visible in the euchromatin which hosts nuclear processes such as DNA methylation, chromatin remodelling and the transcription. To resolve the spatial structure-function relationship of the active proteasomal proteolysis and transcription we use a dynamic localization-approach: (1) Active proteasomal proteolysis is analyzed in relation to global transcription in modulated cell systems and local transcription in single cells. (2) The mobility of proteolytic foci enables us to investigate the dynamic interaction of both processes. Thus, the dynamic contribution of proteasome-dependent proteolysis to global and local transcription will be discussed.

References:

Key words: proteasomal proteolysis, regulation of gene expression
ACTIN AND ACTIN-BINDING BINDING IN THE NUCLEUS

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Nuclear actin plays an important role in such processes as chromatin remodeling, transcriptional regulation, RNA processing, and nuclear export. Recent research has demonstrated that actin in the nucleus exists in dynamic equilibrium between monomeric and polymeric forms. Numerous actin-binding proteins (ABPs) regulate the dynamics of actin filaments in the cytoplasm. The presence of some ABPs in the nucleus have been reported but little is known about their nuclear functions.

Spectrin was first characterized as a cytoskeletal protein underlying the plasma membrane of the erythrocyte and linking actin filaments to integral membrane proteins. Recent studies has provided evidence that spectrin is present in the nucleus of mammalian cells. Nonerythroid αspectrin (αSpII) is part of nuclear protein complex involved in DNA repair. Spectrin βIV (βSpIVΣ5) associates with the nuclear matrix and PML nuclear bodies and spectrin βII interacts with nuclear proteins. Member of spectrin protein family and focal adhesion and stress fiber-associated proteins, α-actinin 1 and 4 were identified as class IIa histone deacetylases-interacting proteins. In addition a novel splice variant of α-actinin 4 which is predominantly localized in the nucleus was isolated. Filamin A was the first actin filament cross-linking protein identified in non-muscle cells. Human filamin A (hsFLNa) was shown to interact with BRCA2 in the nucleus. C-terminal 100kDa fragment of FLNa colocalized with androgen receptor to the nucleus where it regulates androgen receptor activity.

We addressed the issue of nuclear localization and possible nuclear functions of several ABPs. As a first step, we performed a study on their nuclear localization by means of fluorescent and electron microscopy using cell models with different levels metabolic activity. Fluorescent detection of ABPs was done on intact cells and on cells from which most of chromatin has been removed to reveal possible attachment to underlying nuclear structures. We analyzed as well the ultrastructural distribution of ABPs in various nuclear compartments of HeLa cells and resting human lymphocytes. The areas of significant immunogold labeling were mapped using previously developed spatial statistical algorithm. We confirmed nuclear presence of several ABPs also on Western blots of HeLa cells nuclear extracts. In order to define possible binding partners, co-immunoprecipitations of ABPs with the key proteins of important nuclear processes, such as transcription, replication, splicing and cell cycle regulation, were performed. The involvement of ABPs in nuclear processes will be discussed.

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Key words: actin, actin-binding proteins, nucleus
NUCLEAR MYOSIN I BINDS TO PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE IN THE CELL NUCLEUS

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FRAP (Fluorescence Recovery After Photobleaching) and FCS (Fluorescence Correlation Spectroscopy) are two complementary methods to measure protein mobility. The mobility of proteins allows one to describe their interactions with other molecules. We used these methods to measure the mobility of our protein of interest, nuclear myosin I (NM1), in the cell nucleus. NM1 is a single-headed motor protein which takes part in transcription by RNA polymerase I and II. It was shown that NM1 binds phosphatidylinositol-4,5-bisphosphate (PIP2) by its tail domain and a subpopulation of NM1 is tethered to plasma membrane via PIP2. After depletion of PIP2 by specific phosphatase, NM1 is released from the plasma membrane to the cytoplasm. We used FRAP and FCS to determine whether NM1 binds to PIP2 also in the nucleus. When PIP2 binding was abolished by single-point mutation in the tail domain, we observed that NM1 became more mobile, while block of transcription or actin depolymerization had no apparent affect on the mobility of NM1 in the nucleus. These results suggest that NM1 binds via PIP2 to some larger immobile structures in the nucleoplasm. Further investigations are needed to identify the binding partners and to elucidate the functional significance of NM1 interactions with PIP2 in the nucleus.

This study was supported by the Grant Agency of Czech Republic (Reg. No. 204/07/1592 and Reg. No. 204/05H023), by the Ministry of Education, Youth and Sports of the Czech Republic (Reg. No. LC06063), and by the institutional grant AV0Z50520514.

Key words: FCS, FRAP, NM1, PIP2
DYNAMIC ORGANIZATION, INTERACTION AND FUNCTION OF PML NUCLEAR BODIES IN TELOMERASE INDEPENDENT LENGTHENING OF TELOMERES

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Telomerase-negative tumor cells maintain their telomeres via an alternative lengthening of telomeres (ALT) mechanism. This process involves the association of telomeres with promyelocytic leukemia nuclear bodies (PML-NBs) into complexes that are termed APBs (for ALT associated PML bodies). Recently, we have presented results on the mobility of telomeres and PML-NBs as well as their interaction in human U2OS osteosarcoma cells, in which the ALT pathway is active (1). An U2OS cell line was investigated that had lac operator repeats stably integrated adjacent to the telomeres of chromosomes 6q, 11p and 12q. Via autofluorescent LacI bound to the lacO arrays the telomere/PML-NB mobility in correlation with telomere repeat length was evaluated. The results suggested that the shortening of telomeres results in an increased mobility that facilitates the formation of APBs. With this system we have further investigated the role of PML-NBs in the ALT pathway. Results will be presented that provide new insight into the assembly process of PML-NBs and APBs, their structure and composition and the mechanism of alternative telomere lengthening (2).

References:
PML REGULATES MHC CLASS II TRANSCRIPTION AT NUCLEAR BODIES BY STABILIZATION OF CIITA, THE TRANSCRIPTIONAL MASTER REGULATOR AT MHC CLASS II GENE PROMOTERS

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The eukaryotic nucleus is highly compartmentalized with respect to both, structure and function. Chromosome territories are arranged non-randomly and a gene's position within the nucleus can impact on its transcriptional activity. Promyelocytic leukemia nuclear bodies (PML NBs) are subnuclear protein assemblies involved in many nuclear activities, including DNA repair and transcriptional control. The precise biochemical functions of PML NBs in these processes however remain unknown. Current models discuss PML NBs as sites of storage and/or posttranslational modification of specific nuclear proteins, or as assembly sites for nuclear protein complexes. PML NBs transiently accumulate a selected set of transcription-associated factors and they also spatially associate with transcriptionally active genomic regions. Here we demonstrate that IFNγ induces a nonrandom association between PML NBs and the major histocompatibility class II gene cluster in the nucleus of various cell lines. We also show that a subset of MHC class II specific transcription factors colocalize with PML NBs. Most importantly, PML depletion by RNAi leads to destabilization of CIITA, the master transcription factor of MHC class II genes. This in turn results in downregulation of IFNγ-induced MHC class II mRNA expression. Our observations strongly suggest that PML NBs are directly involved in the regulation of MHC class II transcription levels by association with the respective genomic region and by PML-mediated stabilization of CIITA.
**mRNA, A NEW COMPONENT OF CAJAL BODIES**

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In larch microsporocytes, spherical structures are present in which poly(A) RNA accumulates. There were one to several bodies per cell and they were often present in the vicinity of the nucleolus. Transcripts were observed rarely after a prolonged time of incubation with BrU (over 2h). No nascent transcripts were observed within them. Splicing factors of the SR family, were also not observed therefore distinguish them from speckles. The absence of the above-mentioned elements within bodies containing poly(A) RNA disqualifies them as sites of synthesis and preliminary stages of primary transcript maturation. However, they contained abundant elements of the splicing machinery commonly occurring in Cajal bodies (CB), i.e. Sm proteins or small nuclear RNA (snRNA). The molecular composition as well as the characteristic ultrastructure of bodies containing poly(A) RNA prove that these were Cajal bodies. The question arises whether the observed poly(A) RNA in CB are mRNA’s or other polyadenylated RNA’s, i.e. non-coding “mRNA-like” transcripts. There were examined several probes complementary to known mRNAs from *Larix sp.* Double labeling to U2 snRNA (marker of CB) and mRNAs of (1) Sm proteins (SmE, SmD1 and SmD2), (2) Pol II RNA RPB2 subunit and (3) some house keeping gene’s mRNAs like catalase, ATPase, α-tubulin or peroxidase, showed that there are present in CBs. The localization of mRNA using electron microscopy indicated that a specific accumulation of this RNA class occurs in oval Cajal bodies built of coiled fibrils. This is the first report on the presence of mRNA within Cajal bodies.

The investigations performed here indicate that in the analyzed microsporocytes, the level of poly(A) RNA is high. The discrepancy of the results obtained here with data from the literature could came from lower level of poly(A) in somatic cells than generative cells like larch microsporocytes. Thus, it cannot be excluded that its detection was easier than in somatic cells. It is unknown if mRNA accumulation in CBs represents typical developmental strategy of cells during microsporogenesis. The lack of data on this subject may be due to the fact that the localization of mRNA during microsporogenesis in other plants has not been performed to date, and investigations on Cajal bodies in these cells are still fragmentary. The role of mRNA-rich CBs in RNA metabolism in larch microsporocyte development will be discussed.

*Key words: nuclear bodies, coiled body, pre-mRNA, snRNP, poly(A) RNA, meiotic prophase I*
NUCLEAR TRAFFICKING OF SINGLE NATIVE MRNP PARTICLES IN LIVING CELLS

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Over the past years several studies addressed the mobility and intracellular trafficking of mRNP particles and a plethora of molecular details has been disclosed by now. However, one of the major obstacle of this endeavor is the visualization of mRNA molecules without interfering with their native behavior. In a recent study we analyzed the intranuclear mobility of Balbiani ring (BR) mRNP particles in living salivary glands of Chironomus tentans (1). For that purpose we microinjected fluorescence labeled DNA- or RNA-oligonucleotides complementary to the highly repetitive portion of the target mRNA. Meanwhile we established a new protein based labeling approach by using recombinant hrp36, the C. tentans homologue of hnRNP A1, to overcome the loading of the mRNA with an artificial probe. The trajectories of the resulting native, fluorescent mRNPs can be followed using a selective focal plane illumination microscope, which was recently set up in our lab (2). Since hrp36 protein accompany the mRNA from the transcription site to the cytosolic ribosomes the nuclear trafficking, including the NPC passage, can directly be monitored in living cells with high time resolution. This approach further allows to explore directly the influence of mRNP-interacting proteins on the mobility and movement of the mRNP particles.

References:
FUNCTIONAL KNOCKDOWN ANALYSIS OF MEDAKA POLYCOMB GROUP GENE, OLEED

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Polycomb group (PcG) refers to a family of genes that are well conserved in a wide variety of organisms. Initially, they were supposed to function in the maintenance of epigenetic marks across cell divisions, termed “cellular memory”. A recent growing body of evidence, however, suggests their participation in pleiotropic developmental processes, making it hard simply to interpret their function as cellular memory keeper. Prompted by these contexts, we simply inhibited the expression of Polycomb homologs in medaka by hypomorphic knockdown with morpholino antisense oligos and deduced their possible functions from the resulting phenotypes. Here, we discuss the function of one of medaka Polycomb homologs, oleed, an ortholog for Drosophila esc and mouse eed. Upon knockdown of oleed, cyclopia phenotype was generated. Knockdown of another medaka PcG, olezh2, an ortholog for Drosophila E(z), also gave rise to cyclopia. Treatment of the embryo with trichostatin A, which inhibits the activity of HDAC, a transient component of ESC-E(Z) complex, similarly produced cyclopia phenotype. These and other lines of evidence suggested the participation of ESC-E(Z) complex as a whole is functioning downstream of hedgehog signaling pathway. The involvement of trimethylation of at lysine 27 of histone H3 was also suggested. More recently, we found knockdown of oleed led to another phenotype, the reversal of left-right asymmetry of internal organs, such as heart looping, position of liver and gallbladder. The status of Kupffer’s vesicle epithelial cilia, the rotation of which generates leftward fluid flow and induces side-specific gene expression, was disorganized in knockdown embryos. To sum up all the data thus far available, we discuss the role of OLEED in left-right patterning through maintenance of transcriptional status of Noto.

Key words: Polycomb, medaka, knockdown, histone methylation, organ asymmetry
In 1909 Theodor Boveri (1862-1915) published a seminal study on embryos of the horse roundworm Parascaris equorum during the first two cell cycles. His observations led him to several conclusions: (1) Chromosomes occupy distinct chromosome territories (CTs) in the cell nucleus. (2) CT order is stably maintained during interphase. (3) Changes of chromosome neighbourhoods occur during mitosis, in particular during prometaphase, when chromosomes become attached to the spindle and move toward the metaphase plate. (4) The rather symmetrical movements of both sets of chromatids during anaphase lead to rather symmetrical arrangements of CTs in the two daughter nuclei. As a consequence of profound changes of chromosome order during prometaphase neighbourhood arrangements of CTs can differ greatly from one cell cycle to the next.

To test Boveri’s claims with a state-of-the-art experimental design, we performed life-cell experiments with cultured human cells that stably express H4-photoactivatable-GFP. We activated GFP fluorescence in selected areas of interphase and early prophase nuclei, as well as in prometaphase, metaphase, anaphase and telophase by laser microirradiation (440 nm). Using time-lapse, spinning disc confocal microscopy, we traced labelled chromatin through the remaining part of mitosis and analyzed the distribution of labelled chromatin in the daughter nuclei. Our results are in clear concordance with the predictions derived from Boveri’s one hundred year old studies.

Key words: mitosis, nuclear architecture, chromosomal neighbourhoods, higher order genome architecture
Amphibian oocytes display nuclear structures in extraordinary morphological detail. In particular, their chromatin is uniquely organized as lampbrush chromosomes (LBCs). This type of organization is characterized by highly-extended chromatin loops upon which transcription occurs at much greater rates than in somatic nuclei, and this means that individual active transcription units (TUs) are resolvable using the light microscope. We are studying the structure and function of these TUs with respect to the co-transcriptional recruitment of RNA-binding proteins. It has been well established that components of the major spliceosome are targeted to the nascent RNP arrays of each TU and a similar localization pattern has been shown for a number of hnRNP proteins. Most of the splicing factors and hnRNPs examined so far associate along the entire length of almost all TUs, raising questions as to the nature and specificity of the mechanisms governing their recruitment to nascent transcripts. One possible clue is provided by the presence of some of these proteins also in two types of oocyte nuclear body, Cajal bodies (CBs) and speckles/interchromatin granule clusters (IGCs). Many components of the transcriptional apparatus are also present at high concentration in CBs, and RNA polymerase subunits and transcription factors have likewise been shown to be specifically targeted to CBs. It has been suggested that oocyte CBs are sites at which the transcriptional machinery and spliceosomes co-assemble into unitary particles prior to their transport to all loops to initiate transcription. To examine further the nature of co-transcriptional recruitment we sought to examine the behaviour of proteins that might be expected to be involved in the splicing of only a subset of genes. One such was RBM6 (RNA Binding Motif), a putative alternative splicing factor involved in regulating apoptosis, which nonetheless showed a general targeting to LBC TUs and IGCs. Interestingly, expression of exogenous, full-length RBM6 in oocytes caused changes in loop morphology and the assembly of novel structures on the surface of most IGCs. However, another alternative splicing factor and minor hnRNP, CELF1, was distributed to only a small number of transcription units, often in a localized pattern in which the protein was first detectable a considerable distance beyond the transcription start site. This pattern suggests that CELF1 recruitment is not dependent on pre-loading events occurring during the initiation of pol II transcription. The implications that the patterns of recruitment to LBC TUs exhibited by CELF1 and other transcript-binding proteins have for the structure and function of active genes will be considered.

*Key words: nascent RNP; RBM6; CELF1; Cajal body; IGC*
MEIOTIC HOLOGUE PAIRING AND THE PH1 LOCUS

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During meiosis, the homologous chromosomes must recognize each other and move together before intimate pairing and recombination take place. The mechanism of homologue recognition is not understood. It is likely to involve at least two levels of searching: first a colocalization of specific chromatin regions, such as telomeres (the telomere bouquet); second a specific sequence comparison mechanism, which in many organisms has been shown to involve double strand breaks, single strand resecting and invasion. In hexaploid wheat, Ph1 is a major locus that controls the specificity of homologue pairing. In Ph1 deletion mutants, there is a significant level of pairing between homoeologues – equivalent chromosomes from different constituent genomes – whereas in the presence of Ph1 no such incorrect pairing is seen. We have shown that in the Ph1 mutant the telomere bouquet is formed and sub-telomeric regions pair correctly, but this correct pairing must be subsequently over-ridden by incorrect pairing arising elsewhere in the genome as illegitimate pairing is seen at metaphase I. One source of incorrect pairing is likely to be at the centromeres, which associate into 7 foci in wheat at the same time as the telomere bouquet forms. In the presence of Ph1 these resolve correctly, or disperse completely if no true homologues are present as in a wheat rye hybrid, but in the Ph1 mutant a proportion of incorrect associations remain; we propose this is one factor in the observed illegitimate pairing at metaphase I. Recent mapping of Ph1 has suggested that genes showing homology to yeast Ime2 and mouse cdk2 are responsible for these phenotypic effects (Al-Kaff et al., Ann Bot 2008, 101:863-872). cdk2 is involved in both the meiotic and the mitotic cycles at S-phase, and is responsible for phosphorylating histone H1 and other proteins. Ime2 is a meiotic non-cyclin-dependent protein kinase, and among other activities it regulates the expression of Hop1 and other meiotic genes. In support of the proposed role of Ph1 as an Ime2 homologue, in the Ph1 mutant, ASY1, a homologue of Hop1, is deregulated. Furthermore, we have shown that at the time of the telomere cluster, there is a change in chromosome conformation, which may be a result of specific phosphorylation of chromatin proteins by the Ph1 kinase. In the wild type this conformational change occurs synchronously in the homologues, whereas in the Ph1 mutant, the conformational change is unsynchronized between homologues. Furthermore, we have preliminary evidence that the telomere clustering, and chromosome conformation change, occurs at the stage of pre-meiotic replication, again in agreement with the role of cdk2 in the pairing phenotype.

Key words: cyclin dependent kinase; wheat; chromatin; chromosome; meiosis


HOW GENOMIC DNA IS FUNCTIONALLY FOLDED IN A NUCLEUS

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Despite remarkable progresses in genome science, we are still far from a clear understanding of how genes are functionally wrapped up in chromatin, how chromatin fibers are packaged into a nucleus without entanglement, and how these chromatin structures are faithfully inherited across cell divisions. Here, we present three topics that suggest clues for understanding these issues.

At first, we point out the biological importance of left-handedly curved DNA structures. We found that the curved DNA structures that mimic left-handed supercoils can activate transcription in chromatin context. This effect was detected in various assay systems heretofore employed. Our results can explain why transcriptional regulatory regions often contain curved DNA structures.

The second topic concerns the importance of the mechanical properties of DNA. We have heretofore "believed" that many eukaryotic class II gene promoters contain some core element, such as TATA box, Inr (initiator sequence), DPE (downstream promoter element), or G/C-rich sequence. However, the "core-less" promoters account for more than 50% of the human promoters in the EPD (eukaryotic promoter database). Here, we report that all human promoters have a common mechanical property that can drive transcription. Furthermore, we show the “DNA-flexibility map” of whole human genome and discuss how mechanical properties of DNA are implicated in the functional folding of genomic DNA.

Finally, we discuss self-assembly of DNA. Some proteins are known to have a property of self-assembly, which is an important mechanism to construct supramolecular architectures for cellular functions. While, no report has been presented for self-assembly of double-stranded (ds) DNA molecules. Recently, we found the phenomenon of self-assembly of dsDNA molecules, which occurs in aqueous solutions containing physiological concentrations of Mg²⁺ ions. We show that DNA molecules preferentially interact with the molecules with an identical sequence and length even in a solution composed of heterogeneous DNA species. The attractive force causing DNA self-assembly may function in various biological processes, such as folding of repetitive DNA, recombination between homologous sequences, and synopsis in meiosis.

Key words: chromatin, curved DNA, DNA flexibility, DNA self-assembly, gene expression
We have shown (Szekvolgyi et al., PNAS, 104/38:14964–14969, 2007) that the genomic DNA of resting and proliferating mammalian cells and yeast spheroplasts harbor ss breaks, primarily nicks, positioned at loop-size intervals that could be efficiently labelled in situ by DNA polymerase I holoenzyme, but not by Klenow fragment and terminal transferase unless after ribonucleolytic treatments. The RNA molecules involved comprise R-loops detected by an RNA/DNA hybrid specific antibody. Quantitative microscopic analysis of nicks and R-loops over a population of cell nuclei demonstrates that the R-loops are indeed juxtaposed with the nicks. Data obtained by immuno-FISH were in line with this result: the breakpoint cluster region (bcr) of the Mixed Lineage Leukemia (MLL) gene previously shown to be a nick-prone region, colocalized with the nicks. R–loops and biotin-dUTP-tagged ss breaks also appear to be enriched within the MLL bcr in ChIP samples obtained with either the anti-R-loop or anti-biotin antibodies. Comparing the global distribution of the ss breaks in two distinct yeast species, based on the number of chromatin particles yielded by the nuclei upon denaturation as determined by quantitative image analysis, the ss breaks appear to separate ~30 kb regions on either strand. Mapping by indirect end labeling in S. cerevisiae chromosome I as well as in XII within the rDNA cluster, performed on denaturing gels using single-stranded probes, suggest that a fraction of the nicks detected are site-specific. These data, taken together, allow envision a model with ≥50 kb chromatin loops emanating from their anchorage distinguished by nick-accumulation and the presence of RNA/DNA-hybrids.

This work has been supported by the Hungarian Scientific Research Fund (OTKA) [T72762 to G. Sz.].

Key words: R-loops, chromatin, nicks, yeast, MLL
MOLECULAR DISTINCTIONS BETWEEN AURORA A AND B: A SINGLE RESIDUE CHANGE TRANSFORMS AURORA A INTO CORRECTLY LOCALIZED AND FUNCTIONAL AURORA B

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Aurora A and Aurora B, paralogue mitotic kinases, share highly similar primary sequence. Both are important to mitotic progression, but their localizations and functions are distinct. We have combined shRNA suppression with overexpression of Aurora mutants to address the cause of the distinction between Aurora A and Aurora B. Aurora A residue G198, mutated to Asparagine to mimic the aligned N142 of Aurora B, causes Aurora A to bind the Aurora B binding partner INCENP, but not the Aurora A binding partner TPX2. The mutant Aurora A rescues Aurora B mitotic function. We conclude that binding to INCENP is alone critical to the distinct function of Aurora B. Although G198 of Aurora A is required for TPX2 binding, N142G Aurora B retains INCENP binding and Aurora B function. Thus, while a single residue change transforms Aurora A, the reciprocal mutation of Aurora B does not create Aurora A function. An Aurora A-Δ120 N-terminal truncation construct reinforces Aurora A similarity to Aurora B, as it does not associate with centrosomes, but instead associates with kinetochores.

Key words: Aurora kinases, passenger protein, shRNA rescue, mitosis
Human DNA topoisomerase I plays a dual role in the regulation of gene expression by controlling DNA topology on one side, and pre-mRNA splicing, on the other. In fact, besides relaxing supercoiled DNA, topoisomerase I exhibits a kinase activity targeted at the serine-arginine (SR)-rich family of splicing factors (SR-proteins). The two activities are mutually exclusive; under conditions of substrate availabilities for both DNA relaxation and protein phosphorylation, the latter prevails.

Poly(ADP-ribose) (PAR), a polymer of NAD\(^+\)-derived ADP-ribose units, is a multifaceted regulator of topoisomerase I functions. First PAR influences individual catalytic steps of the DNA relaxation reaction differentially: it inhibits DNA cleavage while it stimulates the religation activity of the enzyme. Such effects persist even in the presence of drugs (camptothecin, topotecan) that stabilize the catalytic intermediate topoisomerase I-cleaved DNA covalent complex (cleavage complex). Second, topoisomerase I-dependent phosphorylation of ASF/SF2, a prototypical SR-protein, is suppressed by PAR. Finally, in the presence of both DNA and ASF/SF2, PAR acts as a modulator of the relative expression levels of the DNA cleavage and protein kinase activities and is able to induce topoisomerase I functional switch in a dose-dependent manner.

PAR specific binding at multiple target sites both in topoisomerase I and ASF/SF2 is a plausible underlying mechanism; different binding affinities and relative concentrations of interacting partners are key determinants of the functional outcome. Such a regulatory mechanism might operate in cells and impact on genome stability (through control of cleavage complex formation and dissolution) and gene expression (by setting a balance between DNA relaxation and phosphotransferase activities).

**Key words:** PAR, topoisomerase I, noncovalent interactions, SR-proteins
LINKER DOMAIN REGULATES SUBNUCLEAR DISTRIBUTION OF HUMAN TOPOISOMERASE I


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The response to the chemotherapy drug camptothecin (CPT) is translocation of its target protein topoisomerase I (topoI) from the nucleolus to nucleoplasm. The molecular mechanism backing this process seems to include several elements that depend on different regions of the protein. Here, we asked which regions of topoI are responsible for its subnuclear localization and its movement to nucleoplasm upon CPT treatment. To address this, subnuclear distribution of fluorescently tagged fragments of topoI was studied in HeLa cells untreated or treated with CPT. TopoI is a single polypeptide composed of 4 domains: N-terminal, core, linker and C-terminal. The fragments containing not more than the N-terminal and core domains were initially localized in the nucleolus but moved to nucleoplasm, mainly to the nuclear speckles, in the CPT treated cells. This was because of two sequences that flank the N-terminal domain. However, the fragment containing the N-terminal, core and the linker domains did not move to nucleoplasm upon CPT treatment but instead remained concentrated in the nucleolar caps. An increase of the linker flexibility in the complete topoI, resulted from a point mutation A653P, prevented it from translocation to nucleoplasm in the presence of CPT. Based on the above we suggest that the linker domain is a key factor that regulates subnuclear distribution of topoI. Binding of CPT and/or inhibition of rRNA transcription induces changes in linker flexibility, what results in the enzyme redistribution.

Key words: topoisomerase I, camptothecin, translocation
A NEW COMPLEX EPIGENETIC MARKER: THE REPLICATION-COUPLED, CELL CYCLE DEPENDENT, DUAL MODIFICATION WITHIN THE HISTONE H4 TAIL

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We shall describe an evidence for the reversible, cold-dependent detection of the epitope (referred to as epiC), recognized by a monoclonal anti-actin antibody in diploid human fibroblast cell nuclei and mitotic chromosomes. With a help of the detailed 3D confocal and cross-correlation analysis along with biochemical and immunochemical experiments, we shall show that the epiC positivity appears on the newly replicated chromatin domains with some delay (of about 1 h) with respect to their DNA replication and then persists on the newly replicated chromatin until next early G1 phase, during which it disappears. However, while this epiC positivity is detected in the early replicated chromatin domains, it is not found in the mid/late replicating domains either during S phase, or at any other cell cycle phases. The unique spatio-temporal pattern of epiC positivity suggests that some replication-coupled modulation of early replicated chromatin domains, which could be involved in transfer/maintenance of epigenetic information on transcriptionally competent part of genome, is detected. Indeed, in contrast to the actin epitope, epiC consists of different amino acid sequence within histone H4 tail carrying two posttranslational modifications and is a new complex epigenetic histone marker that might represent a global marker related to the timing decision point. The relevance of epiC marker in the transfer/maintenance of epigenetic information on the transcriptionally competent part of the genome will be dealt with.

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Key words: chromatin, cell cycle, replication, histone H4 dual modification
Background: HMGB1 is an abundant chromatin protein which lives a double life: as an architectural transcription factor and as an extracellular signal. Here we show how its DNA-binding properties can function to chaperone its own antibodies onto chromatin and use this property as a tool to characterize its preferential binding sites on chromatin.

Methods: We employed a ChIP (chromatin immunoprecipitation) protocol with formaldehyde-crosslinked chromatin from 15-day chicken embryo erythrocytes after sonication of nuclei to yield chromatin fragments containing 200-600 bp of DNA. Separately, soluble chromatin was obtained by micrococcal nuclease (MNase) digestion of nuclei followed by sucrose gradient fractionation to give oligonucleosomes (trimer to hexamer), to which HMGB1 was not covalently attached. Two ChIPs were performed: with anti-HMGB1 antiserum and with anti-HMGB1 antiserum pre-incubated with HMGB1 protein. The DNA sequence content of the antibody-Bound (immunoprecipitated) fractions at several genes/loci was determined by real-time PCR.

Results: The capacity of HMGB1 to carry anti-HMGB1 antibodies to the chromatin is shown from slot blots. Pure HMGB1, DNA, the chromatin after crosslinking/sonication, and oligonucleosomes were applied to membranes which were developed using affinity-purified anti-HMGB1 antibodies and the anti-HMGB1 antibodies pre-incubated with excess HMGB1 protein. Comparing development with anti-HMGB1 antibodies alone with development using antibodies pre-incubated with HMGB1 showed a significant decrease of the chemiluminescent signal from HMGB1 with pre-incubated antibodies but an increase for the chromatin, oligonucleosomes and even the pure DNA. This approach assumes that antibody-recognition epitope on HMGB1 does not coincide with its DNA binding site, e.i. binding of HMGB1 to DNA does not displace the antibody.

ChIP experiments using HMGB1 antiserum with crosslinked/sonicated chromatin indicate that HMGB1 is about equally distributed over all tested sites, whether on inactive genes, on heterochromatin, or at transcriptionally active sites. In contrast, when MNase-derived oligonucleosomes were used for the ChIP, HMGB1 was located preferentially at inactive and heterochromatin sites, as compared to the active points. When the HMGB1 antiserum was pre-incubated with HMGB1, an even greater preference for inactive/heterochromatin sites was observed, particularly with the oligonucleosomes.

Conclusion: The ChIP data show that HMGB1 preferentially chooses compact heterochromatin-like structures as targets when the protein is freely mobile (in vitro oligonucleosomes), though this is not the case with the in vivo crosslinked chromatin. When HMGB1 protein is added to its antibodies it has the expected blocking effect on the binding to free HMGB1 but because HMGB1 is a DNA binding protein there is apparent recognition of free DNA and chromatin in consequence of HMGB1 carrying antibodies to the DNA. Thus antibodies to a non-specific DNA-binding antigen can be ‘chaperoned in’ to any DNA-containing target, with misleading results.

Key words: chromatin, non-histone chromatin protein HMGB1, chromatin immunoprecipitation (ChIP)
FUNCTIONAL CHARACTERIZATION OF NUCLEOLAR PROTEINS AND THEIR ROLES IN HAEMATOLOGICAL MALIGNANCIES

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The nucleolus is a complex nuclear substructure in which key steps of ribosome biogenesis take place. However, it is well established that numerous non-ribosomal functions can be linked to the nucleolar compartment, e.g. control of the cell cycle, nuclear export processes and the sequestration of regulatory molecules. The nucleolus has received increasing interest of pathologists and hematologists, because a general and important difference between cancer and normal cells is hyperactivity and pleomorphism of the nucleoli. Nucleoli of cancer cells often show great variations of size, shape, fine structure, and cytochemical composition, probably as the result of increased ribosome biosynthesis as well as changes in the mechanisms controlling cell proliferation.

Over the years we have reported on the identification of several constitutive nucleolar proteins (NO38/NPM1, NO29/NPM3, NOH61, NO66, NO52), which seem to function in different steps during ribosome biogenesis. One major aim of our studies is the analysis of the precise biological roles of these nucleolar components in regulating rDNA transcription, rRNA processing and/or maturation of pre-ribosomes by RNA interference (RNAi) as well as the determination of their interaction partners. The identification of distinct topogenic protein complexes should shed some light on the complex process of ribosome biosynthesis in higher eukaryotic cells.

Acute myeloid leukemia (AML) is a heterogeneous disease with respect to morphology, immunophenotype and genetic rearrangements. Recently, frame shift mutations of the NPM1 gene were identified as a very frequent abnormality in adult patients with de novo AML, predominantly in cases with a normal karyotype. These NPM1 mutations have gained tremendous attention in recent times, because of their prognostic significance.

We have started to examine the roles of the nucleolar proteins NO52, NO66 (putative histone demethylases) as well as NPM3 in the development of hematological malignancies with a specific emphasis on AML.

Key words: nucleolus, ribosome biogenesis, acute myeloid leukemia (AML)
The perinucleolar compartment (PNC), a multi-component nuclear structure, is associated with malignant transformation both in cultured cells and tumor tissues in vivo. A high PNC prevalence generally associates with poor prognosis for several cancer models and PNC prevalence closely correlates with the metastatic capacity of human prostate cancer cells in mouse metastasis models. To understand the functional association between PNCs and malignancy, we are investigating the molecular complexes that associated with the PNC. Previous findings have shown that the structural integrity of PNC is dependent upon pol III transcription and the PNC is enriched with RNAs transcribed by RNA polymerase III and RNA binding proteins that are primarily indicated in pol II transcription (ie. PTB, CUGBP). Using RNA pull-down and immunoprecipitation approaches, we found that some of the PNC associated RNAs are in the same complex with PTB and CUGBP. These associations are independent of pol II transcription and appears to derive from the newly synthesized pol III RNA since a pol III transcription inhibition disassemble PNCs without significantly affect the distribution of the mature functional RNPs, such as MRP in the nucleolus. Furthermore, we found that PNCs are associated with DNA locus/loci through chemical-biology analyses as well as in CDK mutant that undergoes endoreplications. Together, our findings support a working model, in which, PNC forms through nucleation of novel RNA-protein complexes onto yet to be identified DNA loci. Such a nucleation may play a role in post-transcriptional regulation of a subset of pol III RNAs or may play a role in expression regulation of the associated loci. Studies are underway to investigate these possibilities.

THE PNC IS ASSOCIATED WITH DNA AND NOVEL RNA-PROTEIN COMPLEXES

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REPTILE CELL CULTURES FOR NUCLEOLAR STUDIES

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Recently, an exhaustive statement of literature about the fine structure of the nucleolus in vertebrate cells (Thiry and Lafontaine, 2005, TCB 15:194-199) has revealed that in anamniotes the nucleolus consisted of two major compartments (a fibrillar zone surrounding by a granular zone) whereas in amniotes it was composed of three main compartments (the fibrillar center, the dense fibrillar component and the granular component). Although the precise structure of the nucleolus was well-known in mammals and birds, it had only been described in two lizard species.

During the two last years, we have investigated the ultrastructural organization of the nucleolus in different species belonging to four groups of reptiles (lizard, snake, turtle and crocodile). Our results clearly indicated that both nucleolar types were present in reptiles: a bipartite nucleolus in turtles and in snakes and a tripartite nucleolus in lizards and in crocodiles, suggesting that the tripartite nucleolus should be appeared in some reptiles. It is also interesting to note that all the tissues from a same species had the same type of nucleolus, although the importance and the repartition of those components can vary from a tissue to another one. Curiously, in bipartite nucleoli, we also showed that the fibrillar zone was not homogeneous but contained densely-contrasted, fibrillar cordons. Both, DNA and AgNOR proteins were preferentially detected in these cordons. In tripartite nucleoli, AgNOR proteins were observed in the fibrillar centres and in the dense fibrillar component but DNA was only significantly detected in the fibrillar centres.

To better understand the functional organization of the nucleolus in reptiles, and in particular in bipartite nucleolus, we developed in vitro cell cultures from tissues (liver and spleen) of an aquatic turtle Trachemys scripta scripta. A lung epithelial cell culture from a lizard Gekko gecko was used as control of tripartite nucleolus. Under these in vitro conditions, the nucleolar compartmentalization was identical to that observed in tissue samples. When ribosomal RNA synthesis was preferentially inhibited by a treatment with actinomycin D, we observed in tripartite nucleoli, a classical segregation of three main nucleolar components. By contrast, in bipartite nucleoli, we showed that the electron-dense cordons of the fibrillar zone were concentrated in the centre of segregated fibrillar zone.

These results suggest that both distinct fibrillar components in the tripartite nucleolus would come from a redistribution of components present in the fibrillar zone of the bipartite nucleolus.
Since quantitative information on the nucleolar and cytoplasmic RNA concentration in differentiating and maturing cells is very limited, the present study was undertaken to provide additional data on in differentiating cells of the erythroid and granulocytic lineage in situ. The satisfactory number of erythroid and granulocytic precursors in bone marrow of patients suffering from the chronic phase of chronic myeloid leukaemia without a large increase in the granulocytic to erythroid ratio facilitated computer assisted image RNA density measurements in both nucleoli and cytoplasm at the single cell level.

As expected, the measurements indicated a significant decrease of the nucleolar and cytoplasmic RNA concentration only in advanced stages of differentiation. In the erythroid lineage, the resulting ratio of the nucleolar to cytoplasmic RNA density was very stable and did not change during differentiation, being similar in the early and advanced stages of erythroblastic development. The marked similarity of the nucleolar to cytoplasmic RNA density ratio indicated a “symmetric decrease” of the RNA concentration in nucleoli and cytoplasm during the erythroblastic differentiation. This phenomenon might be related not only to the nucleolar RNA transcription but also to its transport and cytoplasmic consumption. However, no such symmetry of the nucleolar and cytoplasmic RNA decreasing concentration during differentiation cells has been noted in the granulocytic lineage. In late differentiation stages the reduction of the cytoplasmic RNA density was larger than that of the nucleolus. Thus, as reported previously on induced apoptotic cells, RNA in nucleoli of differentiating and maturing cells of the granulocytic lineage appeared to be somehow “frozen”. Complementary experiments with cytostatic treatment of granulocytic progenitors in vitro inducing apoptosis or signs of differentiation onset also exhibited a similar phenomenon.

In summary, disregarding the possible interpretation, the presented results suggest differences in the differentiation and maturation process between two studied cell lineages in human bone marrow, which were detected by computer assisted RNA image densitometry at the single cell level. It should be also mentioned that other approaches to study differentiating human erythroid and granulocytic precursors in bone marrow biopsies would be very difficult or impossible.

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Key words: nucleolar and cytoplasmic RNA density, differentiating cells
THE MOBILE FRACTION OF FIBRILLARIN CAN SERVE AS AN INDICATOR OF rDNA TRANSCRIPTION

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Fibrillarin (321 aa in human) is a mobile nucleolar protein that is involved in methylation and processing of pre-rRNA. Fibrillarin serves as an autoantigen in human scleroderma diseases and is targeted by autoantibodies raised in genetically susceptible mice by regular administrations of sublethal doses of HgCl₂.

Human fibrillarin contains two cystein residuals (Cys99 and Cys268), which are thought to be responsible for the protein binding to Hg(II). In the present study we tested the idea that binding of Hg(II) to SH-groups of Cys99 and Cys268 modifies fibrillarin properties in living cells. To reach the goal, we used plasmids encoding human fibrillarin fused to EGFP, where the both cysteins were substituted by the serine residuals (Ser99/Ser268). The plasmids were used for transient transfection of HeLa cells, and the mobility of the wild-type and mutant fibrillarin fusions were analyzed by FRAP and a confocal laser scanning microscope.

In nucleoli of control HeLa cells, fibrillarin was located in foci that were coincident with endogenous fibrillarin revealed with an anti-fibrillarin antibody as well as with BrUTP-positive sites - the markers of on-going rRNA synthesis. In nucleoli, a mobile fraction of fibrillarin was 59±5%. HeLa cells exposure to 75 µM HgCl₂ for 1.5-2 h induced migration of endogenous fibrillarin and fibrillarin-EGFP from nucleoli to discrete foci in the nucleoplasm, caused the arrest of rRNA synthesis and diminished the mobile fraction of the nucleolar fibrillarin to 48±4%. Unexpectedly, the location and mobility of mutant fibrillarin-EGFP were similar to those of wild-type fibrillarin both in control and in mercury treated cells. These observations showed that Cys99 and Cys268 per se do not play a crucial role in fibrillarin interaction with mercury.

To test the idea that diminution of the fibrillarin mobile fractions in HgCl₂-treated cells was due to inhibition of rDNA transcription, we measured the mobile fraction of fibrillarin-EGFP following a well-known inhibitor of ribosome synthesis, actinomycin D. In other experiments HeLa cells were incubated with DRB (5,6-Dichlorobenzimidazole 1-β-D-ribofuranoside), which does not affect the transcription, but inhibits pre-rRNA maturation. After treatment with actinomycin D the mobile fraction of fibrillarin decreased to 37±5%, whereas following DRB it remains at the control level - 60±8%. All together our results show that the mobile fraction of fibrillarin can serve an indicator of rDNA transcription in living cells.

Key words: fibrillarin, mobile fraction, HgCl₂, actinomycin D, DRB
MECHANISMS OF CORRELATION OF RIBOSOME ASSEMBLY AND CELL CYCLE CONTROL

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It has long been known that ribosome biogenesis is coordinated with growth conditions and cell growth rate. It is important to understand the mechanisms underlying this correlation, because human cancers and other diseases involve loss of the normal cell cycle control and ensuing inappropriate expansion of cell populations. However, the mechanistic linking of ribosome biogenesis and cell growth is only partially understood. It is known that some proteins have dual roles in ribosome biogenesis and control of the cell cycle (e.g. yeast Nop7 alias Yph1). To gain a deeper insight, we have systematically repressed the synthesis of individual ribosomal proteins in Saccharomyces cerevisiae and observed the effects on cell cycle progression using flow cytometry. We find that the cell cycle is arrested or delayed in different ways depending on which ribosomal protein is no longer synthesized, suggesting that the effect of blockage of ribosome synthesis is not simply the consequence of reduced protein synthesis capacity. We are also isolating and analyzing temperature-sensitive mutants with alterations mapping in ribosomal protein genes that affect the cell cycle.

As the first step in pursuing a mechanistic understanding of the basis for defects in cell cycle control, we are currently characterizing the effects of repressed synthesis or mutational change of selected ribosomal proteins using confocal microscopy. Our thinking is focused on possible dual roles of individual proteins and controlled sequestration of specific cell cycle and budding factors in nucleolar complexes that also contain intermediates in ribosomal biogenesis.
A LINK BETWEEN pre-RRNA PROCESSING AND REFORMATION OF THE NUCLEOLUS AT THE END OF MITOSIS

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Our systematic analysis of the role of ribosomal proteins involved in the small ribosomal subunit (see poster entitled “Overview of the role of the ribosomal proteins in maturation and nuclear export of mammalian 40S ribosomal subunits” by O’Donohue et al.) showed that human cells depleted of a sub-class of ribosomal proteins displayed small nucleoplasmic bodies. Further examination of these phenotypes by video-microscopy identified them as residual pre-nucleolar bodies (PNBs), which persisted for increased lengths of time. Indeed, in control cells, PNBs form in telophase and contain nucleolar components such as pre-ribosomal factors (e.g. fibrillarin, B23) and pre-rRNAs, whose maturation had not been completed before cell division. These pre-rRNAs and factors regroup in the PNBs at the end of mitosis. As assessed in live control cells expressing B23-GFP, PNBs are incorporated into the reforming nucleoli and disappear progressively in G1. In contrast, PNBs were still observed several hours post-mitosis in the nucleoplasm of upon depletion of a subset of ribosomal proteins. Northern blots and FISH experiments using a series of probes to the pre-rRNA transcribed spacers showed that the pre-rRNAs found in residual PNBs corresponded to intermediates whose maturation is specifically blocked by depletion of the ribosomal proteins. A similar phenotype was observed upon depletion of Bystin/ENP1, a pre-ribosomal factor required for the maturation of the 40S particles. Our current working model is that recruitment of PNBs components at the end of mitosis is linked in part to correct processing of the pre-rRNAs that they contain. Our preliminary experiments indicate that this phenotype of residual PNBs correlates with G1 phase arrest of cells defective in ribosome biogenesis. The presence of these residual PNBs could participate to the stress signal that provokes cell cycle arrest.

Key words: nucleolus, mitosis, pre-nucleolar bodies, pre-rRNA processing
STUDY ON THE ASSEMBLY OF U3 snoRNP, AN RNP ESSENTIAL FOR RIBOSOMAL RNA MATURATION

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SnoRNPs with C/D boxes, in particular U3 snoRNP, play a major role in rRNA biogenesis. U3 snoRNA contains two conserved pairs of boxes C'/D and B/C that are needed to bind the stably associated proteins. Binding of protein Snu13p/15.5 kD to each of the conserved motifs is a prerequisite for recruitment of the 4 other U3 snoRNP proteins, namely: Nop1p, Nop56p and Nop58p on the C'/D motif and Rrp9p on the B/C motif. Rrp9p is a U3 specific protein which is essential for yeast growth and involved in the assembly of the maturosome on the pre-rRNA. We previously identified the RNA sequence and structure requirements for efficient association of Rrp9p on the B/C motif by expression of variant U3 snoRNAs in yeast followed by immunoselection assays. In order to study the amino acid determinants for Snu13p-Rrp9p interaction, we developed an in vitro reconstitution assay based on the association of protein Rrp9p contained in a Snu13p depleted extract on a preformed U3 snoRNA/recombinant Snu13p complex. Protein Rrp9p shows homology with proteins containing WD-40 domains. We used the known 3D structure of the beta subunit of a human G protein that contains 7 WD-40 motifs and 3D structure homology modeling methods to propose a 3D structure model for protein Rrp9p. Based on the 7 WD-structure that we established for Rrp9p and on the known Snu13p 3D structure, we identified putative interacting regions between these two proteins and tested the hypotheses by site-directed mutagenesis. The data obtained will be presented.

In parallel, we identified the C'/D determinants needed for association of proteins Nop1p, Nop56p and Nop58p to U3 snoRNA by production of variant U3 snoRNAs in cellulo. The data also revealed that very low amounts of U3 snoRNA are sufficient to ensure yeast growth and that stable U3 snoRNPs lacking Nop58p protein can be detected in cellulo. When applying the in vitro reconstitution system described above, we failed to assemble the Nop1p, Nop56p and Nop58p proteins on a preformed C'/D RNA/recombinant Snu13p complex. This observation is in agreement with our previous finding of the involvement of numerous cellular factors in assembly of C/D box RNP (Rsa1p protein, R2TP complex, HSP90 chaperone). Finally, co-expression assays in E. coli revealed a possible interaction between Rrp9p and Rsa1p. Implication of this observation for U3 snoRNP assembly will be discussed.

Future studies will concern the search for Rrp9p protein partners which are involved in maturosome assembly.

Key words: rRNA biogenesis, U3 snoRNP assembly, Rrp9p, 3D structure homology modeling
The ability of eukaryotic cells to exploit a variety of different post-translational modifications to proteins is vital for proper functioning. One such modification is the conjugation of target proteins to members of the SUMO family of proteins (SUMO 1–4 in humans). SUMOylation, in general, effects the intra- or inter-molecular interactions of the modified protein and can change its stability, localisation or activity. SUMOylation is of particular importance for nuclear processes and it is clear that different nuclear functions are affected depending on the location of SUMOylation. For example, SUMOylation at the nuclear pore of RanGAP1 affects nucleocytoplasmic shuttling, whereas SUMOylation of transcription factors (such as p300) in the nucleoplasm can result in transcriptional repression.

There are to date only handful of examples of nucleolar proteins that are modified by SUMOylation. It has been reported that SUMOylation influences the nucleolar localisation of proteins and more recently, that deSUMOylation of nucleophosmin is necessary for rRNA processing. However, it is likely that the importance of SUMOylation in the nucleolus is not yet fully characterised. Thus, we sought to identify novel nucleolar SUMOylated targets by exploiting the highly advanced technique known as SILAC (stable incorporation of labelled amino acids in cell culture) in combination with mass spectroscopy. Interestingly, although these targets possess a range of different functions, several are involved in either snoRNA and/or rRNA biogenesis, such as the C/D box snoRNP protein, Nop5/Nop58. Subsequent investigations have focussed on this protein, and constitute the first evidences for a role of SUMOylation in the function of C/D box snoRNPs.

Both in vitro and in vivo assays have confirmed that Nop58 is a substrate for SUMOylation and mutational analyses have revealed the sites of SUMO modification. Interestingly, the highly related protein Nop56 is not a good substrate for SUMOylation. This difference may explain how these two similar proteins have evolved to possess distinct functions and we are now investigating the role of SUMOylation in the function of Nop58. Fluorescence microscopy reveals that non-SUMOylatable Nop58 mutants are able to localise correctly to Cajal bodies and the nucleolus, and consistent with this observation, it appears that mutants are able to interact with other core C/D box proteins. However, detailed examinations of these protein complexes by immunoprecipitations (IP) experiments combined with SILAC-MS suggest that SUMOylation of Nop58 affects its interactions with nucleolar C/D-box-snoRNP accessory proteins. Furthermore, non-SUMOylatable Nop58-GFP exhibits a lower affinity for several different snoRNAs as tested by IPs/QPCR. We are now investigating the importance of Nop58 SUMOylation for the proper formation of fully functional C/D box snoRNPs, by examining the size and molecular composition of complexes that contain non-SUMOylatable Nop58, and the ability of these complexes to mediate correct rRNA processing and promote snoRNA stability.

Key words: SUMO, nucleolus, Nop5/Nop58, snoRNA, SILAC
HYPOXIA-INDUCED RELOCATION OF eIF4A-III TO NUCLEOLUS AND SPlicing SPECKLES

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We identified the Arabidopsis orthologue of the mammalian DEAD box helicase, eIF4A-III, the putative anchor protein of Exon Junction Complex (EJC) on mRNA. The EJC links the different aspects of mRNA biogenesis such as transcription, splicing, export, surveillance and translation. The EJC is integrally involved in mRNA biogenesis, is deposited on mRNAs as a result of pre-mRNA splicing, determines the export of mRNPs via the nuclear pore complex and is involved in mRNA surveillance and nonsense-mediated mRNA decay. The EJC contains more than 20 different proteins of which eIF4A-III, Y14, Mago and MLN51 form the tetrameric core of the EJC which acts as the RNA-binding platform anchoring other EJC components to the spliced mRNA. The parallel elucidation of the function of this nuclear protein complex and its position with respect to nuclear architecture is required to fully understand mRNA biogenesis and how the cell responds to the many physiological processes and growth and environmental stimuli which they encounter.

Our work examines dynamic behaviour of Arabidopsis eIF4A-III, showing its relocation from nucleoplasm to the nucleolus and speckles during hypoxia or treatment by respiratory inhibitor, and its physical interaction and co-localization with other members of the EJC. Arabidopsis eIF4A-III interacts with an orthologue of the core EJC component, ALY/Ref, and co-localises with other EJC components such as Mago, Y14 and RNPS1, suggesting a similar function in EJC assembly to animal eIF4A-III. A GFP-eIF4A-III fusion protein showed localisation to several sub-nuclear domains: to the nucleoplasm during normal growth, and to the nucleolus and splicing speckles in response to hypoxia. The presence and differential dynamic properties of eIF4A-III in these different regions and compartments of the nucleus may reflect different stages of EJC assembly and interactions with mRNA targets. Treatment with the respiratory inhibitor sodium azide produced an identical response to the hypoxia stress. Treatment with the proteasome inhibitor, MG132, led to accumulation of GFP-eIF4A-III mainly in the nucleolus suggesting that transition of eIF4A-III between sub-nuclear domains and/or accumulation in nuclear speckles is controlled by proteolysis-labile factors. As revealed by FRAP analysis, the nucleoplasmic fraction was highly mobile, while the speckles were the least mobile fractions and nucleolar fraction had an intermediate mobility. Sequestration of eIF4A-III into nuclear pools with different mobility is likely to reflect the transcriptional and mRNA processing state of the cell. Our work may provide an insight into early stages of direct oxygen sensing, which precedes (and most likely controls) any changes in translational activity.

Reference:

Key words: eIF4A-III, nucleus, nucleolus, splicing speckles, hypoxia
EXTENSIVE TISSUE VARIATION IN THE NUCLEAR ENVELOPE PROTEOME AND ITS IMPLICATIONS FOR GENOME ORGANIZATION AND THE PATHOLOGY OF LAMINOPATHIES

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Interest in the nuclear envelope has grown recently because of its association with a variety of inherited diseases called laminopathies that range from several muscular dystrophies to lipodystrophy, cardiomyopathy, neuropathy, dermopathy, bone diseases and the progeroid aging syndromes. Each of these diseases preferentially affects a different tissue, yet the nuclear envelope proteins mutated are expressed in nearly all tissues. This conundrum could be explained if tissue specific partners of these proteins work in tissue-specific functional complexes that are specifically disrupted in the tissues expressing pathology. This predicts that there would be tissue variation in the nuclear envelope proteome. We have directly tested this, finding that 60% of the protein complement of the nuclear envelope varies between liver, muscle, and leukocyte tissues. Moreover, some of the tissue-specific proteins identified vary in differentiation paradigms that could reflect functions in the pathologies of laminopathies. Data from several laboratories indicates that chromatin organization is altered in several laminopathies and our proteomic datasets include several proteins involved in chromatin modifications, gene regulation and genome organization. The leukocyte datasets in particular should have identified several proteins involved in tethering chromatin to the nuclear periphery as lymphocytes have an especially dense concentration of peripheral heterochromatin. Indeed we find that two novel lymphocyte-specific nuclear envelope transmembrane proteins can recruit a specific chromosome locus to the nuclear periphery when overexpressed. Further study of these interactions will include testing whether they are disrupted in patients with nuclear envelope diseases.

Key words: nuclear envelope, proteome, laminopathy, genome organization
NUCLEAR PROTEIN AGGREGATION: THE INTERFACE BETWEEN FUNCTION AND PATHOLOGY

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The nucleus represents a cellular control unit that regulates all events concerning the storage and processing of DNA and RNA. It is organized by highly crowded, dynamic assemblies of proteins and nucleic acids in molecular machines, ribonucleoprotein complexes, clusters of ongoing nuclear processes, nuclear bodies, and chromatin. While the genomes of man and other species have been sequenced, it is largely unknown how the dynamics of nuclear macromolecules are regulated in order to maintain the function of the nucleus throughout interphase. Recent work suggests direct engagement of a nuclear ubiquitin-proteasome system (nUPS) in the first steps of gene expression. Proteasomal activity is localized in subnuclear compartments such as speckles, PML nuclear bodies and distinct proteolytic foci in euchromatic regions. A tuned balance of ubiquitination and proteasome-dependent protein degradation of nuclear proteins in distinct nucleoplasmic proteolysis factories is instrumental in nuclear function and, when deregulated, leads to abnormal protein aggregation in the cell nucleus. Thus, we introduce the idea that aberrant nuclear protein aggregates represent congestion of nuclear function that, in conditions of proteasomal deficiency, may be involved in the development of protein aggregation diseases such as polyglutamine disorders or other age-related degenerative conditions.
The DEAD box RNA helicase p68 is a growth- and developmentally-regulated protein that has been shown to be aberrantly expressed in a range of cancers and to act as a potent co-activator of several highly regulated transcription factors, including the critical tumour suppressor p53. p53 is induced and activated in response to several stresses, including DNA damage, and induces expression of a host of downstream target genes involved in growth arrest, apoptosis and DNA repair. In a previous study, we demonstrated that suppression of p68 by RNAi results in inhibition of p53 target gene expression following DNA damage suggesting that p68 plays an important role in the p53 DNA damage response.

Using siRNA-mediated p68 knockdown we have shown that p68 is critical for the induction of the key growth arrest gene p21 and for the induction of cell cycle arrest in cell line models. In contrast p68 knockdown appears to have little effect on expression of the apoptosis-associated gene PUMA, or induction of apoptosis in cells. These data are consistent with the idea that p68 is important in determining the outcome of inducing p53 (i.e. cell death vs cell survival) in cancers.

I shall discuss possible mechanisms by which p68 may modulate p53 function to influence the choice between growth arrest and apoptosis in response to DNA damage. I shall also discuss our recent findings from a study of p68 expression in breast cancer.
MECHANISM OF THE HSF1-REGULATED APOPTOSIS OF SPERMATOGENIC CELLS

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Apoptosis of male germ cells can be induced by different types of environmental insults, including the heat shock. Spermatocytes, the most stress-sensitive male germ cells, do not respond to the heat shock in a “classical” way. Such classical heat shock response involves activation of HSF1 that results in expression and accumulation of inducible heat shock proteins (HSPs), which reveal several cell-survival and anti-apoptotic functions. We have previously found that expression of constitutively active HSF1 in spermatocytes of transgenic mice does not lead to activation of inducible Hsp70 genes, but instead induces caspase-dependent apoptosis that causes male infertility. Additionally, activation of HSF1 down-regulates Hsp genes constitutively expressed in testes: spermatocyte-specific Hspa2 and spermatid-specific Hspa1l. It is paradoxical because HSF1 is the archetypal activator of Hsp genes. More detailed study revealed that down-regulation of Hspa2 gene occurs prior to the onset of HSF1-induced apoptosis, and during later developmental stages germ cells undergoing HSF1-induced apoptosis are essentially lacking the Hspa2 protein. The finding indicates a functional relationship between down-regulation of the Hspa2 and apoptotic degeneration of a seminiferous epithelium in response to activation of HSF1. The cis-acting DNA sequences responding to such repression localize in the immediate promoter region of the Hspa2 gene, but none of them is directly associated with HSF1. This suggests that the spermatocyte-specific activity of HSF1 could misdirect a network of transcription factors required for proper regulation of Hspa2 and possibly other genes involved in apoptosis.

Here we aimed to identify genes differentially regulated by HSF1 in somatic and spermatogenic cells. We have performed the genome-wide transcriptional analysis in control and heat-shocked mice cells, either isolated hepatocytes or spermatocytes. The Affymetrix GeneChip system was used for such analysis and expression of selected genes was validated by RT-PCR. Genes that are differently expressed in mouse spermatocytes and hepatocytes subjected to the heat shock have been identified. Examples of genes up-regulated by the heat shock in hepatocytes but down-regulated in spermatocytes include actual or putative transcription factors (e.g., Jun, Fos, Klf6, Atf3, Nfkbiz), as well as genes involved in proliferation and apoptosis (e.g., Egr1 and Egr2, Bag3, Phlda1). Among a few genes specifically up-regulated in spermatocytes were apoptosis-related genes Dap1, Pyhin1, Dido1 and Mdm1. The next step of the study was the chromatin immunoprecipitation assay combined with DNA microarray (the ChIP on chip method) that enabled direct identification of HSF1 transcriptional targets. This approach would allow disclosing genes where the heat shock-dependent regulation is either directly or indirectly regulated by the HSF1 binding, and elucidating the molecular background for differential regulatory properties of HSF1 in somatic and male germ cells.
THE ROLE OF MEIOTIC COHESIN REC8 IN THE GENOME REDUCTION OF GAMMA-IRRADIATION-INDUCED ENDOPOLYPLOID TUMOUR CELLS

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Escape from mitotic catastrophe and generation of endopolyploid tumour cells (ETCs) represents a potential survival strategy of tumour cells in response to genotoxic treatments. ETCs that resume the mitotic cell cycle have reduced ploidy and are often resistant to these treatments. In search for a mechanism for genome reduction, we previously observed that ETCs express meiotic proteins among which REC8 (a meiotic cohesin component) is of particular interest, since it favours reductional cell division in meiosis. The two prerequisites are known necessary for reductional division in meiosis: homologous chromosomes must first be paired and cross-linked by recombination and secondly, sister chromatids of each homolog must be cohesed to allow for homolog disjoining during the first meiotic division, with both of these processes requiring REC8. The central feature of the chromosome reduction in meiosis is omission of the S-phase between two meiotic divisions.

In the present investigation, we induced endopolyploidy in p53-dysfunctional human tumour cell lines (Namalwa, WI-L2-NS, HeLa) by gamma irradiation, and analysed the subcellular localisation of REC8 in the resulting ETCs. We observed by RT-PCR and Western blot that REC8 is constitutively expressed in these tumour cells, along with Sgo1 and Sgo2, and that REC8 becomes modified after irradiation. REC8 localised to paired sister centromeres in ETCs, the former co-segregating to opposite poles. We also found that ETCs display gH2AX foci of DNA double strand breaks repair colocalised with meiotic recombinase DMC1. By BrdU labelling, we found that a subset of ETC miss S-phase during a time frame longer than their normal cell cycle and progress to mitosis. Altogether, our observations indicate that radiation-induced ETCs express features of meiotic cell divisions and that these may facilitate chromosome segregation and genome reduction in ETCs.

Reference:

Key words: REC8, tumour cell, irradiation, endopolyploidy, genome reduction
THE RESPONSE OF NUCLEAR STRUCTURES TO THE TREATMENT WITH RESVERATROL - A CHEMOPREVENTIVE AGENT

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Phytochemical - resveratrol decreases cancer risk and improves the health of laboratory animals. The molecular mechanisms of beneficial effects of resveratrol are not well understood. We examined how resveratrol influenced the growth of two human cancer cell lines of different origin: osteosarcoma (U-2 OS) and lung adenocarcinoma (A549), and how it modulated the expression, as well as the localization of key proteins, including WRN and BRCA1, involved in DNA repair and the cell cycle regulation. Resveratrol-induced growth arrest was associated with signs of stress-induced senescence, with transient upregulation of BRCA1 in A549 cells and permanent upregulation of this protein in U-2 OS cells. Interestingly, in A549 cells, the downregulation of BRCA1 after prolonged resveratrol treatment was associated with overexpression of p21(WAF1) protein. Neither downregulation of BRCA1, nor upregulation of p21 was observed in U-2 OS cells. The BRCA1 bodies formed after the treatment frequently co-localized with WRN helicase and TRF1 telomeric protein. Moreover, in U-2 OS cells, the resveratrol treatment was associated with the signs of telomeric instability: the formation of micronuclei filled with telomeric DNA and the appearance of extrachromosomal telomeric repeats. Resveratrol also activated DNA damage signaling system, what was manifested as the phosphorylation of histone H2AX at serine 139 and of p53 at serines 15 and 37. Our data indicate that resveratrol interferes with DNA metabolism and arrests the cell cycle progression, however, apparently it engages different mechanisms to slow down the growth of A549 cells and of U-2 OS cells.
DNA FRAGMENTATION FACTOR – THE MAJOR PLAYER IN APOPTOTIC BREAKDOWN OF CELL NUCLEUS

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Apoptosis, or programmed cell death, is a fundamental process essential for both development and maintenance of tissue homeostasis, which allows to eliminate excessive, unnecessary or damaged cells from multicellular organisms. Cells undergoing apoptosis show specific biochemical and morphological features. One of the hallmarks of the terminal stages of apoptosis is nuclear breakdown. First, large fragments of about 50 kb and more are excised from chromosomes, which reflects domain structure of the chromatin. This large scale DNA fragmentation is followed by more advanced internucleosomal cleavage termed “DNA laddering”. Although several enzymes have been implicated in such DNA cleavage reactions, caspase-activated DNase (CAD), also termed DNA fragmentation factor (DFF), is the major endonuclease responsible for internucleosomal DNA cleavage during apoptosis. In non-apoptotic cells, DFF exists in nucleus as a heterodimer, composed of CAD/DFF40 latent nuclease subunit, and ICAD/DFF45 chaperone and inhibitor subunit. Apoptotic activation of caspases (mostly caspase-3) results in the cleavage of the inhibitor and release of the nuclease, which form active homo-oligomers. The nuclease can be further stimulated or inhibited by other regulatory factors, including abundant chromatin proteins histone H1, HMGB1 or topoisomerase II. Noteworthy, double-stranded DNA is the exclusive substrate of DFF, while other nucleic acids could inhibit its activity. DNA breakdown is usually temporally and functionally correlated with chromatin condensation, characteristic morphological hallmark of cells undergoing apoptosis. Although apoptotic cell death can occur without significant DNA degradation, this phenomenon apparently facilitates the engulfment of resulting apoptotic cell corpses by phagocytes and eliminates the transforming potential of any damaged or mutated DNA. Importantly, defects in apoptotic DNA fragmentation could be associated with a number of disease, including cancer and autoimmunity.
KARYOMETRIC ANALYSIS OF CELL NUCLEI IN PAPILLARY THYROID CANCER AND THEIR CORRELATES IN GENE EXPRESSION PROFILE

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Background: Diagnostic criteria of papillary thyroid carcinoma (PTC) are defined by the presence of characteristic cell nuclei features, “optically clear” distribution of chromatin, irregular nuclear profile, pseudoinclusions and grooves. We hypothesize that these features are driven by specific molecular mechanisms and assess whether karyometric parameters of PTC nuclei do correlate with gene expression profile assessed by microarray analysis.

Material and methods: Post-operative thyroid frozen samples and respective paraffin blocks from 41 patients with PTC were analyzed. Images were obtained by light microscopy with a semiautomatic computer image analyzer. One hundred thyroid nuclei were assessed in each case. Based on pre-processed nuclear images (noise elimination by median filter and distribution of grey levels into 3 classes based on internal gray levels segmentation) we calculated parameters directly related to nuclear size, shape and chromatin distribution. For chromatin pattern we applied texture analysis with co-occurrence matrix. For each karyometric parameter, mean value and its variance were assessed and correlated with gene expression patterns by permutation-based test. Global significance was calculated for relation of each parameter to gene expression profile (evaluated by Affymetrix HG-U133A).

Results: We found out that the variability of nuclear size is the most significantly associated with PTC gene expression profile in PTC. Nucleus area variance was positively correlated with expression of 54 genes and showed inverse correlation to 95 genes. Ten genes (HDAC1, KIAA0329, ARHGEF17, CASC3, PEX11A, ERCC3, MUC1, ITGA1, SELENBP1, SLC2A1) significantly correlated with nuclear area coefficient of variation (NACV) were selected for validation by QPCR. Validation of obtained correlation results was performed on independent set of 36 PTC. Expression of five genes (PEX11A, ITGA4, SELENBP1, HDAC1 and ARHGEF17) significantly correlate with NACV.

Conclusions: Among karyometric parameters investigated, it is anisotropy of PTC nuclei which significantly correlate with its gene expression profile. Functional importance of this fact requires further studies.

Key words: quantitative microscopy, image analysis, karyometry, gene expression profile
The in vivo reaction kinetics of vital processes inside the eukaryote nucleus such as gene transcription regulation are poorly understood. We fluorescently tag androgen receptors (AR) and coregulators with GFP and color variants to elucidate their mode of action in living cells using confocal time-lapse imaging and quantitative fluorescence assays such as fluorescence recovery after photobleaching (FRAP) and fluorescence resonance energy transfer (FRET). In addition, for optimal interpretation of the complex and massive amount of experimental results we develop and apply computer modeling methodology to simulate both FRAP and FRET assays as well as the behavior of proteins inside living cells.

The picture emerging mainly from FRAP experiments on GFP-AR is one of freely mobile receptors that find their targets, gene promoters, by free diffusion and random collision. In addition, their interaction with protein-DNA complexes appears to be much more dynamic than previously anticipated. Combined FRAP and FRET experiments using double-tagged YFP-AR-CFP suggest that conformational changes of the androgen receptor after promoter binding facilitate binding of coregulators.
A major challenge in cell biology is to identify the subcellular distribution of proteins within cells, and to characterize how protein localization changes under different cell growth conditions and in response to stress and other external signals. Protein localization is usually determined either by microscopy, or using cell fractionation combined with protein blotting techniques. Both these approaches are intrinsically low throughput and limited to the analysis of known components. Here we use mass spectrometry based proteomics to provide an unbiased, quantitative and high throughput approach for measuring the subcellular distribution of the proteome, termed ‘spatial proteomics’. This method also allows a direct proteome-wide comparison of changes in protein localization in response to a wide range of physiological and experimental perturbations.

The spatial proteomics method analyses a whole cell extract created by recombining differentially labelled subcellular fractions derived from cells in which proteins have been mass labelled with heavy isotopes. This approach was used to measure the relative distribution between cytoplasm, nucleus and nucleolus of over 2,000 proteins in HCT116 cells. The ability of spatial proteomics to characterize dynamic changes in protein localization at a systems level was demonstrated by analyzing the redistribution of the proteome elicited during the cellular response to DNA damage following treatment of HCT116 cells with etoposide. These spatial proteomics data provided unexpected new evidence showing that DNA damage alters the properties of the MCM complex. The MCM proteins, which act as a replicative helicase during DNA synthesis, are required for processive DNA replication and are a target of S-phase checkpoints. Interestingly, we also found other known DNA repair proteins clustered with a similar redistribution profile to the MCM and RPA complex replication proteins, which suggests a possible mechanism linking DNA replication and DNA repair. This demonstrates how spatial proteomics can be used to not only look at individual protein responses under different conditions, but also how different groups of proteins can be analyzed to have a better understanding of underlying functional interactions.

Spatial proteomics represents a ‘second generation’ approach to MS-based proteomics that not only identifies proteins but also provides an unbiased and quantitative measurement of protein properties; in this case the annotation of subcellular proteome localization under different conditions. While we illustrate this using cells exposed to etoposide, it could just as well be applied to assess other changes in the localization of proteins, for example following gene knock-outs, metabolic perturbations, after activation of signalling pathways, after viral infection or following any other altered conditions in cells.
The DNA in eukaryotic cells is organized into loop domains that represent basic structural and functional units of chromatin packaging. The comet assay, a sensitive method for monitoring DNA damage and repair, involves electrophoresis of nucleoids of supercoiled DNA attached to the nuclear matrix. Breaks in the DNA relax the supercoiling and allow DNA loops to expand, and on electrophoresis to move towards the anode, giving the appearance of a comet tail. The % of DNA in the tail reflects the break frequency. We have used fluorescent in situ hybridisation (FISH) to investigate the structure of the chromatin within comet preparations and to study specific DNA sequences within comets. Using large-insert genomic probes and human Cot-I DNA we found that, under neutral electrophoresis conditions, the probed sequence of DNA is seen as a linear array, consistent with extension from a fixed point on the nuclear core or matrix. After alkaline electrophoresis, the appearance of the fluorescent probes suggests that linear DNA has coalesced into a granular form. Another direction that we are taking in FISH-comet technology is detection of DNA sequences with ‘padlock probes’ (circularisable oligonucleotide probes). We have applied probes that hybridise to Alu repetitive elements and to mitochondrial DNA. During the sequence of stages in the comet assay, mitochondrial DNA progressively disperses into the surrounding agarose gel, showing no tendency to remain with nuclear DNA in the comets. In contrast, Alu probes remain associated with both tail and head DNA and can be used as an alternative way of staining comets, allowing us to visualise either single or both single and double stranded DNA in comets. To study specific gene sequences within comets, we have made a list of repair genes of interest to us and, as a starting point, have designed padlock probes to target three of these genes: the 8-oxoguanine-DNA glycosylase-1 (OGG1) gene, the xeroderma pigmentosum group D (XPD) gene, and the hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene. Our initial experiments resulted in strong signals with no unspecific non-comet-bound background. These results look promising, suggesting further experiments with gene specific probes.

Key words: comet assay, fluorescent in situ hybridisation, DNA damage and repair, DNA loops
HETEROCHROMATIN PROTEIN 1 IN DNA DAMAGE RESPONSE - RECRUITMENT OR DISSOCIATION FROM REPAIR SITES?

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We studied recruitment of DNA repair proteins to damage sites in live cells, by microscopy approaches, using a new method of inflicting local, sublethal damage in nuclei of live cells. Oxidative damage, which was inflicted by exciting DNA-intercalated ethidium with focused green light, triggered recruitment of base excision repair enzymes. Surprisingly, an epigenetic regulator, heterochromatin protein 1 (HP1) (Zarebski et al., 2009) was recruited to damage as well. HP1 is a constitutive component of heterochromatin, and plays an important role in transcriptional repression and regulation of euchromatic genes, however it was not known to be required for repair of oxidative damage. The finding of HP1 recruitment is particularly puzzling, since in another study HP1 was shown to dissociate from chromatin as a result of DNA damage (Ayoub et al. 2008). Technical aspects of live cell imaging that may explain these contradictory results will be discussed.

References:
THE TIGHTLY BOUND TO DNA PROTEINS: OLD PROBLEM REVISITED BY NOVEL APPROACHES

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The tightly bound to DNA proteins (TBP) is a protein group that remains attached to DNA after its deproteinization. Functional significance of this protein group was never completely understood, despite numerous promising results. Many published data appear to be doubtful nowadays as applied methods now seem to be insufficiently potent. Our research consortium has focused efforts on characterization of the TBP spectrum and TBP-DNA interactions using several novel approaches. TBP spectrum in plant and animal tissues was studied by combination of 2D electrophoresis and mass-spectrometry, distribution of TBPs along several genes was studied using hybridization with the oligonucleotide DNA arrays, distribution of TBPs along barley chromosomes was studied by means of PCR method with primers for barley molecular markers, sequences binding TBPs were cloned and sequenced. DNA was fractionated in TBP-free (F) and TBP-enriched fractions (R) by means of separation on nitrocellulose, alternatively TBP-DNA complexes were obtained by DNase digestion.

R fraction isolated from chicken erythrocytes gave a strong hybridization signal in position corresponding to 60 Kb from the 5′end of the alpha globin gene domain. The probe derived from the HD3 cell TBP-bound DNA weakly hybridized to this oligonucleotide. Thus cessation of transcription of the domain should be followed by F – R transition of this site. F and R DNA isolated from HD3 cells induced to synthesize globin, apoptotic HD3 cells and liver cells manifested specific hybridization patterns with the array. In the barley Amy32b gene transition from watery ripe to the milky ripeness stage of kernel was followed by decrease of TBP binding along the whole gene. Expression of the Bmy1 gene coupled to ripening was followed by release of the exon III and intron III sequences from complexes with TBPs.

Cloning and sequencing of TBP-enriched DNA from barley shoots yielded a high proportion of CT-motif sequences and a specific GC-sequence. Computational analysis of the motifs revealed their similarity with transposable elements. Barley TBPs are homologous to several replication proteins, proteins involved in regulation of transcription and RNA processing, transposon binding proteins, ubiquitin-ligase and phosphatase. TBP spectrum in rat organs was tissue specific, drastic differences were revealed between liver and hepatoma TBPs.

Marker analysis revealed changes in association of chromosome 1H and 7H sites with TBPs between first leaf and coleoptile and at two stages of barley shoot development. Tight DNA-protein complexes of the nuclear matrix and those detected by NPC-chromatography were revealed as also involved in tissue- and development-dependent transitions, however, in sites different from TBP-DNA interactions.

We can conclude that formation of the tight DNA-protein complexes depends on cell lineage, transcription and development stage. Probably, TBPs are involved in regulation of gene activity. TBP form a specific group of nuclear proteins different from the nuclear matrix proteins.
Exposure to stressing factors such as ionizing radiation induces a plethora of changes in the cell, including oxidative damage to most of the cellular macromolecules and changes in many processes on the transcript and protein levels. Microarray assays show that a few minutes after irradiation of human melanoma Me45 cells, the levels of some transcripts change significantly suggesting that processes of stabilization or destabilization of mRNA may play a role in these early changes. The nucleotide composition and the structure of the non-coding regions of mRNA, which are known to interact with microRNAs (miRNAs) and proteins, may be of importance in this effect. Using NucleoSeq, a new bioinformatic tool which we have developed, we extracted from the EMBL database and analyzed the nucleotide sequences of over 12000 transcripts which showed different behavior after irradiation. Significant differences in nucleotide composition were observed between mRNAs showing greater than 20% down- or up-regulation 15 min after irradiation, and these differences disappeared stepwisely over the next 12-24 h. Up-regulated transcripts showed longer 3’- untranslated regions (UTRs) and a higher content of A and T in both coding and 3’ sequences, which were highly correlated with the isochore location of these genes. We also discovered significant differences in the number of potential miRNA-binding sites between transcripts up- or down-regulated after irradiation, and a higher miRNA sequence content is correlated with increased stability of transcripts suggesting that the miRNA silencing mechanism may be blocked by irradiation.

This work was supported from the grant PBZ-MNiI-2/1/2005.
INDUCTION AND REPAIR OF DNA DOUBLE-STRAND BREAKS (DSBs) CAUSED BY IONIZING-RADIATION IN THE 170 kb EPSTEIN-BARR VIRUS MINICHROMOSOME
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One of the most important lesions produced in mammalian cells by ionizing radiation (IR) are DNA double-strand breaks (DSBs). This kind of DNA damage is believed to play the major role in radiation-induced lethality and formation of chromosome deletions, and is therefore crucial in the response of cells to radiotherapy. However, in spite of many years of research the precise nature and mechanisms of the origin of chromosome breaks still remain elusive. A major focus of our research is therefore aimed towards understanding these processes and developing new models which will allow us to look more precisely into the nature of induction and repair of DSBs.

As a model we use the naturally-occurring 172 kb-long Epstein-Barr virus minichromosome, which is stably maintained at ~50 copies in the Raji (Burkitt’s lymphoma) cell line. We have characterized the migration in pulse-field gel electrophoresis (PFGE) of all the different topological forms of the episome DNA: supercoiled (FI), relaxed circular (FII), linear (FIII) and nicked circular, which show a different migration pattern than those of small plasmid DNAs. Based on these data, we demonstrated that ionizing radiation produces several single-strand breaks (SSBs) and, surprisingly, only one double-strand break (DSB), or a cluster of very close DSBs, in the whole minichromosome DNA. Precise detection and quantitation using in-gel hybridisation and FISH on spread DNA molecules shows no significantly hypersensitive sites for DSB formation, and suggests the existence of a novel protection mechanism in which after the induction of a first DSB, minichromosome DNA become less sensitive for the induction of further ones.

We have also found that by incubation of irradiated cells at 37°C it is easy to explore in vivo the kinetics, stages and enzymes required for repair of DSBs. Our experiments indicate that like cellular DNA, DSBs in the EBV minichromosome are mainly repaired by the nonhomologous end-joining (NHEJ) pathway, during which the majority of linear molecules are converted to the supercoiled form without any detectable intermediate products. No misjoining of fragment ends, which would result in linear DNA oligomers, is detected. There is no preferential repair in the highly transcribed regions vs. non transcribed ones, and the repair is performed with the same efficiency along the entire episomal DNA. By the same approach and using new and potent specific inhibitors we were able to explore the participation of different factors like PARP, topoisomerases I and II, histone deacetylases and DNA polymerases, in DSB repair processes. Catalytic inhibitors of topoisomerase 2 (ICRF-193) or of both topoisomerases 1 and 2 (F 11782) have no effect on the repair of minichromosome DNA, resolving a controversy in the literature about the requirement for topoisomerases for repair of DSBs.

Key words: minichromosom, γ-radiation, double-strand breaks, DSB repair
Poster abstracts

List of posters in alphabetical order (according to first author’s name)
1) **POLYCOMB GROUP GENE OLEED PARTICIPATES IN LEFT-RIGHT PATTERNING THROUGH REGULATION OF CILIogenesis IN MEDAKA**

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Polycomb group (PcG) genes play a pivotal role in epigenetic regulation. The products of PcG genes form multimeric protein complexes, namely PRC1 and PRC2, and regulate chromatin structure of numerous target genes. The function of PcG, however, still remains poorly understood especially in developmental context. Here, we report that PcG gene **oleed**, a main component of PRC2, is involved in left-right axis determination in medaka, *Oryzias latipes*. The precise left-right patterning is essential for the proper development and function of most organs. In humans, abnormality in the left-right axis due to disturbance of the ciliary function results in serious genetic disorders, such as Kartagener syndrome. Hypomorphic knockdown of **oleed** using morpholino antisense oligos preferentially caused left-right reversal of the position of liver, gallbladder and heart looping. In mammal and fish, left-right axis is initially determined by cilium-generating leftward fluid flow. An asymmetric signal is transmitted to the left side of the lateral plate mesoderm, thereby activating left side-specific gene expression that leads to the asymmetric organogenesis. Expression pattern of **Spaw**, the first left-side marker gene in fish, was randomized when function of **oleed** was inhibited. The Kupffer’s vesicle (KV) epithelium was ciliated, but their length, distribution, and motility were abnormal. Additionally, we found that **Noto**, which is required for the node formation and ciliogenesis in mouse, was markedly downregulated upon knockdown of **oleed**. The data showed that **oleed** was required for maintenance but not for the initiation of **Noto** expression. Based on these results, we propose that **oleed** governs ciliogenesis in the Kupffer’s vesicle, a key organ for left-right patterning, through regulation of **Noto**. This is the first evidence that PcG genes are involved in ciliogenesis and subsequent left-right patterning.

Key words: Polycomb group genes, chromatin, left-right patterning, genetic diseases

2) **THE INFLUENCE OF PDT ON THE MEWO CELL LINE IN VITRO**

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Melanoma is the most severe of all skin neoplasms as it may grow rapidly and metastasize. The application of photodynamic therapy (PDT) opens new perspectives in treatment of this tumor. PDT is an effective local cancer treatment that induces cytotoxicity through intercellular generation of reactive oxygen species. The disintegration of cellular structures and modulation of genetic information induced by PDT direct cancer cells to a death pathway. This combined treatment is based on the specific photosensitizer accumulation in the tumour tissue, followed by irradiation with visible light. The hydrophobic photosensitizer tends to localize in the plasma and subcellular membranes, making these structures especially sensitive to the photooxidative damage. The photochemical interactions of the photosensitizer, light and molecular oxygen produce singlet oxygen and other forms of active oxygen. The oxidative stress is a factor which initiate the cell death in PDT.

The aim of this study was to assess in vitro photodynamic therapy which induces oxidative stress in the human melanoma MEWO cell line. We examined the cells viability and the photosensitizer localization. The Photofrin® (Ph®) was used for the photodynamic therapy in vitro as a photosensitizer. The cells were incubated for 18 h with 20 µg/ml of Ph II in DMEM (medium). Than they were irradiated with the light intensity of 10 mW/cm² using a lamp with polarized light and red filter (632.8 nm). The cells viability was determined by MTT assay as mitochondria metabolic function. The localization of (Ph®) was examined under the
confocal microscopy. Mito-Tracker Green was used as molecule marker to assign the location of mitochondrias.

Viability studies have shown, that there are significant differences between samples after PDT and samples without irradiation. In samples without irradiation viability was much higher. After 24 hours incubation with 20 µg/ml concentration of Ph® and with irradiation survived about 20 % of cells. In the same combination, but without irradiation survived above 80% of cells. More over we demonstrated that Ph® accumulates mainly in the mitochondrial membranes. This leads to disturbances of mitochondrial transmembrane potential and finally with high chance to apoptptic cell death.

Key words: melanoma, oxidative stress, photodynamic therapy, apoptosis

3) CHARACTERIZATION OF GSTP1 UPSTREAM REGION AND IDENTIFYING TRANSCRIPTION FACTOR BINDING SITES

Chwieduk A., Slonchak A., Rzeszowska-Wolny J.

Glutathione S-transferases are a superfamily of enzymes responsible for interaction of a large variety of toxic, electrophilic compounds and organic peroxides. One of the most important GST isoenzymes is glutathione S-transferase Pi (GSTP1) which performs important functions in the detoxification of endogenous and exogenous xenobiotic compounds and in protection against oxidative stress. High levels of GSTP1 expression have been associated with many human cancers. In the clinic, GSTP1 is involved in resistance against antineoplastic drugs, and therefore overexpression is a bad prognostic for patient survival.

The promoter region of the human GSTP1 gene contains a polymorphic short tandem repeat (STR) locus consisting of (ATAAA) repeat units showing length and sequence variation. We have examined the structure of the upstream sequence of the GSTP1 gene containing ATAAA repeats. The function of this AT-rich region is still unknown. In the present study, a polymerase chain reaction and sequencing were used to investigate whether the repeat region differed in size.

GSTP1 is transcriptionally silenced by promoter hypermethylation in several human tumors. Methylation-specific PCR was used to analyze the GSTP1 promoter methylation status in cultured cancer cells. We report that the GSTP1 promoter was hypermethylated in MCF7 breast cancer cells and partly methylated in BeWo (human choriocarcinoma), Me45 (human melanoma) and HCT116 (colorectal carcinoma) cells. The loss of glutathione S-transferase P1 expression in MCF7 cells resulted from hypermethylation of CpG sites in the GSTP1 promoter region.

To investigate how the GSTP1 gene is transcriptionally regulated, truncated promoter fragments were obtained by PCR and cloned into the pGL3basic vector upstream of the luciferase gene. This experiment provided evidence that the GSTP1 gene is positively regulated by a NF-kB element and negatively regulated by a NFkB-like element and CRE in all cell types tested.

EMSA (Electrophoretic Mobility Shift Assays) analysis showed that ARE, NF-kB and CRE sites of the human GSTP1 promoter interact specifically in vitro with transcription factors from BeWo, Hbl-100 (mammary epithelial cancer cell line) and Me-45 cells.

This work was supported by a grant from the Ministry of Science and Education (Poland), number N40115732/3043.
4) USE OF HALOGENATED PRECURSORS TO DEFINE A TRANSCRIPTION TIME WINDOW (TTW) AFTER TREATMENT WITH HYPOMETABOLIZING MOLECULES

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Hibernation is an adaptation developed by some species to survive during winter, when food is scarce and temperature low. During lethargy, these animals drastically modify their metabolism to live in extreme conditions and, throughout hibernation, all metabolic activity is decreased.

DADLE, an opioid-like peptide, has been signalled as a possible trigger for natural hibernation, and synthetic enkephalins were therefore considered interesting for inducing a hypometabolic state.

DADLE is capable to mimic the Hibernation Induction Trigger action and can induce an hibernation-like state in HeLa cells (Vecchio et al., 2006). Moreover, its injection in summer active animals can induce lethargy. Further experiments showed that this molecule is also capable of determining a torpor state in rats (Biggiogera et al., 2006). Data on DALE, another member of the enkephalin family used to study hypometabolism, are still scarce in literature.

The aim of this work is to understand how DADLE and DALE act on transcription by using a new two-step pulse chase methods (Vecchio et al., 2008, Jaunin et al., 2000). We have tried to pinpoint a transcription time window (TTW) by the incorporation of two RNA precursors: iodouridine (I-Ur) and chlorouridine (Cl-Ur). By this method we attempted to determine whether DADLE and DALE treatments affect transcription duration.

I-Ur and Cl-Ur were given to HeLa cells for 15 min each with an interval of 15 min between the treatments. After embedding, the labelling of the perichromatin fibrils (PF) was evaluated at electron microscopy on control, peptide-treated cells and after recovery.

We observed that the area occupied by PF decreased after DADLE or DALE treatment, while the number of double labelled PF increased when compared to control cells.

Finally, the ratio between double labelling density and the area occupied by PF indicate that the permanence in the nucleus of newly synthesized RNA is shorter in control cells than after DADLE or DALE treatment. Since we can find a double labelled fibril only if its transcription time is longer than 15 min, it seems that the two peptides can slow down the transcription rate and RNA export. However, their effect is reversible and transcription resumes after 48 h up to the control levels.

The method we propose seems to be a valuable technique to compare the effect of different treatments on transcription.

Key words: transcription, hypometabolic state, opioids, electron microscopy, immunocytochemistry

5) APPROACHING MAF1 DOMAINS INTERACTION IN REPRESSION OF RNA POLYMERASE III TRANSCRIPTION

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During life yeast cell encounters several different situations that demand a rapid adaptation of cellular metabolism to changing life conditions. One of the first steps of the events leading cell to handle with an unexpected situation occurs by inhibition of the transcription dependent to RNA polymerase III (Pol III). The Maf1 protein was identified in our laboratory as a negative regulator of the Pol III apparatus in Saccharomyces cerevisiae yeast cells. Maf1 plays a central role in coupling different signal transduction pathways
to Pol III transcriptional machinery. Secretory pathway defects, nutrient limitation, DNA damage, low nutrients availability, oxidative stress and chemical treatment with different drugs influence repression of the Pol III transcription according to the Maf1 activity. Maf1 is a phosphoprotein that in favorable growth conditions is phosphorylated and its presence is visible in cytoplasm. Different stress conditions, change to non-fermentable carbon source and approaching stationary phase cause dephosphorylation of Maf1 and its relocation to the nucleus. Dephosphorylated Maf1 interacts directly with N-terminal region of the largest subunit of Pol III leading to repression of transcription. Nevertheless the exact sequence of events appearing during action of the Maf1 remains not clear. The Maf1 protein is highly conserved through evolution, suggesting that the regulatory mechanisms involving Maf1 may be similar from yeast to man.

Our approach concerns a link between structure and activity of Maf1. The family of eukaryotic Maf1 share highly conserved aminoacid sequence with easily recognizable two regions called A and BC domain. The function of each of the domains is unknown. With the yeast 2H system we were able to prove their physical interaction and define the minimal region of A domain involved. This interaction is supported by the screen for suppressor mutants of a single point mutation in A domain. We have found Maf1 mutants carrying additional mutations localized in the BC domain that suppress phenotype of the single point mutation in A domain. Using yeast 2H system we analyzed also a direct influence of the single point A domain mutation and identified suppressor BC domain mutations on interaction between both of Maf1 domains. Cellular functionality of mutated Maf1 proteins were investigated by Immunofluorescence and Western/Northern hybridization. The results of our researches will be widely presented on the poster.

Key words: RNA Pol III, Maf1p, transcription

6) ARABIDOPSIS LINKER HISTONE VARIANTS HAVE DIFFERENT AFFINITY TO CHROMATIN IN VIVO

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The central role of core histones in chromatin structural transitions that directly affect gene expression is now widely acknowledged. In contrast, little is still known about H1 linker histones role in these processes, despite numerous data from in vitro studies suggesting H1’s critical function in determining regularity of higher order chromatin structures. Recently, a significant down-regulation by RNAi of genes encoding all three H1 variants in Arabidopsis thaliana has been shown to affect precise regulation of gene expression that was correlated with changes in specific DNA methylation pattern in many chromosome regions (Wierzbički & Jerzmanowski, 2005). In both higher plants and animals numerous non-allelic H1 variants co-occur in the same cells. The wider biological meaning of this variability is unknown. Arabidopsis, in addition to two somatic H1 variants (H1-1 and H1-2) has a characteristic evolutionary conserved plant H1 variant designated H1-3, the expression of which is strongly up-regulated by drought stress and ABA treatment.

We determined the tissue localization of H1-3 in Arabidopsis using transgenic plants expressing the H1-3:GFP fusion protein. The application of FRAP (Fluorescent Recovery After Photobleaching) technique showed that H1-3 has dramatically lower affinity to chromatin than the remaining two somatic variants H1-1 and H1-2. These data are consistent with the concept that drought-induced H1-3 variant in Arabidopsis may modulate chromatin structure for adaptation of transcription profile to stress conditions.
7) IDENTIFICATION OF NOVEL (HYPOTHETICAL) PROTEIN PARTNERS OF THE MAJOR APOPTOTIC NUCLEASE DFF; YEAST TWO-HYBRID BASED STUDY

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The major apoptotic nuclease, DNA fragmentation factor (DFF), also termed Caspase-activated DNase (CAD), is primarily responsible for internucleosomal DNA cleavage during the terminal stages of apoptosis. In non-apoptotic cells, DFF exists in the nucleus as a heterodimer, composed of a 45 kD chaperone and inhibitor subunit (DFF45/ICAD) and a 40 kD latent nuclease subunit DFF40/CAD. Activation of the nuclease depends on caspase-3-mediated cleavage of DFF45/ICAD inhibitor and formation of DFF40/CAD homooligomers. Caspase-activated DFF40/CAD homo-oligomers can further interact with additional activators or inhibitors; however, only few of them had been identified so far.

Here we used a yeast-two and -three hybrid system in aim to identify novel proteins that potentially interact with DFF40/CAD. *S. cerevisiae* AH105 strain was transformed with human DFF40 (cloned into pGBT9 vector) and then mated with *S. cerevisiae* Y187 strain carrying human brain embryo or HeLa cell cDNA libraries. Alternatively, to identify proteins potentially interacting with the DFF heterodimer, *S. cerevisiae* AH105 strain was transformed with both human DFF40 and DFF45 (cloned into bi-cistronic pBridge vector); an approach called a yeast-three hybrid system. In addition, *S. cerevisiae* AH105 strain was transformed with human DFF45 to search for possible partners of this protein alone.

The screening revealed DFF45 as the only partner of DFF40 when expressed separately. However, screening of HeLa cDNA library revealed that DNA sequences present in chromosome 10 (ESTs CV366200.1 and DB313950) potentially encode for protein that interact with the DFF heterodimer. In contrast, the screening revealed several proteins that potentially interact with DFF45, including GFAP, FHL1, FBXO28, FOSL1, PGK1 and PCNT.

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8) SWITCHING OF MUTUALLY EXCLUSIVE ENZYMATIC ACTIVITIES CONTROLLED BY PEPTIDE LINKER: THE CASE OF HUMAN TOPOISOMERASE I

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SF2/ASF acts in spliceosomes influencing the determination of splicing sites. The physiological function of SF2/ASF is directed by phosphorylation carried out by several kinases: SRPK1, Clk/Sty, PRP4 and topoisomerase I (topoI). In case of topoI, which is both the kinase and DNA topoisomerase, mutual effects of interaction between topoI and SF2/ASF are observed: topoI phosphorylates SF2/ASF, whereas SF2/ASF inhibits DNA relaxation activity of topoI.

SF2/ASF protein belongs to the group of the SR proteins that are equipped with an arginine-serine-rich (RS) domain and one or two RRM (RNA recognition motif) domains. In the latter case both domains are separated by the linkers of different length and property. The linker of SF2/ASF is relatively long (29 amino acid residues) and contains a flexible glycine tract (9 glycine residues). In this work we analyzed how features of the linker influence ability of SF2/ASF to: (i) be phosphorylated by topoI and (ii) inhibit DNA nicking activity of topoI, which is a key step in its relaxation activity.

We found that the length and flexibility of the linkers, determined by the RMSD analysis, did not influence phosphorylation of SF2/ASF by topoI for linkers longer than 9
residues. However, SF2/ASF without the linker region was a comparatively poor substrate for topol acting as a kinase. It was in contrast to phosphorylation carried out by Clk/Sty kinase that was independent of the presence of the linker. We also found that the length of the linker is an important determinant of the ability of SF2/ASF to inhibit DNA nicking activity of topol: the shorter was the linker, the lower inhibition of DNA nicking was observed.

We further identified two binding sites for SF2/ASF on topol and two binding sites for topol on SF2/ASF. The competition experiments for the kinase activity of topol in the presence of topol fragments containing one or both binding sites for SF2/ASF suggest that the length of the linker determines ability of SF2/ASF to interact with both binding sites on topol and, therefore, influences not only phosphorylation of SF2/ASF but also its inhibitory action on DNA nicking activity of topol.

9) FUNCTION OF NUCLEAR MYOSIN I
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Nuclear myosin I (NM1) is a 120 kDa molecular motor localized in the cell nucleus. It consists of a single head or motor domain which binds actin and has ATPase activity, neck domain which provides a site for calmodulin binding and regulation of motor activity by calcium, and tail domain which binds cargo through phosphatidylinositol-4,5-biphosphate (PIP2). NM1 was shown to be involved in chromatin remodeling, repositioning of transcriptionally activated regions in the nucleoplasm and also in transcription with RNA polymerase I. Here we tested whether NM1 also takes part in transcription with RNA polymerase II and III and try to resolve the mechanism of NM1 involvement in transcription by mutational analysis of NM1. We observe a significant decrease in transcription elongation after NM1 immunodepletion by all three RNA polymerases in two different in vitro transcription systems. Furthermore, NM1 co-purifies with the promoter region during the transcription reactions. The NM1 immunodepleted transcription systems allows us to test whether add-back of wild-type NM1 or mutants with disabled actin binding, motor function, and PIP2 binding would restore normal transcription. For this we are currently expressing NM1 in insect cells. We also found Arp6 and Vigilin to interact with NM1 by immunoprecipitation. Arp6 and Vigilin are required for gene silencing in heterochromatin and Arp6 a part of chromatin remodeling complexes essential for this process which might imply a role for NM1 in heterochromatin transition.

Key words: NM1, transcription, RNA polymerase

10) INTERFERENCE BETWEEN THE HEAT SHOCK RESPONSE AND NFκB-DEPENDENT SIGNALING PATHWAY
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NFκB is a family of transcription factors that regulate numerous genes important for pathogen- or cytokine-induced inflammation, immune response and cell proliferation. NFκB also activates several genes that promote cell survival, which contributes to aggressive tumor growth and resistance to chemotherapy and radiation in cancer treatment. HSF1 is the primary transcription factor responsible for cellular response to different forms of stress (e.g., a heat shock), which upon stress-induced activation binds regulatory DNA elements, termed heat
shock elements (HSE), present in promoters of heat shock proteins (HSPs) genes, and activates their expression. In general, HSPs function as molecular chaperones in regulation of cellular homeostasis and promoting survival. HSPs over-expression is frequently found in many types of cancer, and is usually associated with poor prognosis. On the other hand, however, hyperthermia is an adjuvant treatment used to sensitize cancer cells to radio- and chemotherapy, possibly affecting pathways that promote cell survival.

Here we aimed to address possible mechanisms by which hyperthermia and HSF1-dependent signaling interfered with NFκB-dependent pathways. The U2OS osteosarcoma human cell line was used as an experimental model. The heat shock response was induced by mean of hyperthermia (incubation at 43°C for one hour). Alternatively, cells were transfected with mutated constitutively active HSF1 with deletions in regulatory domain (HSF1ΔRD) to activate HSF1-dependent signaling in the absence of the heat shock. Cells were incubated with TNFα cytokine to activate the NFκB pathway, and then expression of NFκB-regulated genes was assessed by RT-PCR. The activation of the NFκB signaling pathway was monitored by mean of degradation of IκBα inhibitor and appearance of active DNA-binding NFκB forms in nuclear extracts.

We have observed that TNFα-induced activation of NFκB was inhibited in cells subjected to hyperthermia, and four hours recovery in physiological temperature was necessary to allow full activation of NFκB. On the other hand, NFκB remained to be fully activatable by TNFα treatment in cells containing constitutively active mutated HSF1 at normal temperature. Interestingly, however, expression of several TNFα-activated and NFκB-dependent genes, including genes encoding TNFα and IL-6, was down-regulated in the presence of active HSF1. Our findings clearly indicates functional interference among hyperthermia, HSF1- and NFκB-dependent signaling pathways.

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11) 3D STRUCTURE OF 10 NM CHROMATIN FIBER DEDUCED FROM DNA FLEXIBILITY

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DNA is highly compacted in a nucleus. In humans, genomic DNA is folded in length by as much as 200,000-fold. Despite remarkable progress in genome science, we are still far from a clear understanding of how genes are functionally folded in a nucleus or in chromatin. Polymer models have been used to describe chromatin in the interphase nucleus. However, the models heretofore presented failed to consistently explain chromatin structures at different stages of compaction. This seems to be caused by ignoring physical properties of DNA. The challenge of the present study is to construct a model depicting 3D architecture of 10 nm chromatin fiber, the fundamental structure of chromatin, based on the physical properties of DNA.

S. cerevisiae has an advantage in the modeling, for positioning of nucleosomes over the genome has been estimated by Segal et al. (Nature 442, 772-778, 2006). Thus, this organism was subjected to the modeling. We regarded DNA as a chain of 3 bp segments, which greatly differs from previous procedures, assumed that any linker DNA is folded as a self-avoiding worm-like chain, and drew the path of linker DNAs. The chain angle θ, which is the angle between neighboring segments, was determined by persistence length of given DNA, which was calculated from its flexibility determined, in turn, by flexibility parameter sets of Brukner et al. (EMBO J. 14, 1812-1818, 1995) and Packer et al. (J. Mol. Biol. 295, 85-103, 2000). In order to know the accuracy of the modelling, we subjected the resulting models of interphase
chromatin to the analyses of chromatin size and spatial distance between genes. Each chromosome was packaged in a cube with a side 1 µm in the model, being consistent with the size of yeast nucleus. Furthermore, spatial distances between several sets of genes agreed well with those determined experimentally by other groups, suggesting that our modeling protocol and the resulting models themselves were highly reliable. The protocol, evaluation and application of this new modeling will be discussed.

Key words: genomic DNA, chromatin, polymer model, 10 nm chromatin fiber

12) NUCLEOLAR PROTEIN MYBBP1A IS A TRANSDUCER OF NUCLEOLAR STRESS

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It has been proposed that some sort of stress sensor monitors nucleolar structure and function and regulates p53 levels. The impairment of nucleolar function stabilizes and activates p53. However, the mechanisms underlying this regulation are still unclear. Here, we show that nucleolar disruption induces acetylation and accumulation of p53 without its phosphorylation. We screened nucleolar proteins which are involved in acetylation and stabilization of p53 using siRNA library and identified Myb-binding protein 1a (MYBBP1A). Nucleolar disruption led to translocation of MYBBP1A from nucleolus to nucleoplasm. MYBBP1A then binds to p53 and facilitates the complex formation among p53, MYBBP1A, and p300 to induce p53 acetylation. Also, MYBBP1A inhibited binding of p53 to HDM2 and stabilized p53. MYBBP1A depletion significantly abrogated p53 activation and apoptosis induced by nucleolar disruption. These results demonstrate that MYBBP1A plays a key role in transducing nucleolar stress signal.

Key words: MYBBP1a, nucleolus, p53, nucleolar stress

13) INFLUENCE OF IONIZING RADIATION ON GLYCOSYLATION PATTERN IN HUMAN K562 AND ME45 CANCER CELLS

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Glycosylation is a ubiquitous protein modification and is involved in many physiological and pathological processes. Alterations of carbohydrate determinants were found associated with cancer progression and metastasis. Lectins are oligomeric proteins with saccharide-binding sites that can recognize and bind particular glycoconjugates.

The aim of this work was to follow the changes of the level of some glycoconjugates in cells exposed to ionizing radiation. The FITC-labeled lectins PHA-E, ConA, LCA, PNA, and UEA that bind carbohydrate conjugates known to accompany the enhanced metastasis of human cancers were chosen for this study. Human cultured K562 lymphoblastoid and Me45 melanoma cells were exposed to 4 Gy of ionizing radiation and allowed to recover in standard culture conditions. Samples of control and irradiated cells were collected at different time points after exposure, incubated with a lectin, and the average level of bound lectin per cell was calculated on the basis of fluorescence assessed for 100 cells.
Exposure to ionizing radiation had a profound effect on the level of all sites recognized by the lectins, and irradiation had different effects on glycosylation in both cell lines. In K562 cells the levels of sites recognized by all except PHA-E increased in the first 3 hours after irradiation, and the amount recognized by ConA and LCA decreased below the control level during the next 48 hours. The level recognized by PHA-E and UEA showed an increase during the next 96 h in K562 cells. Me45 cells responded differently, showing directions of change opposite to K562 cells at short times after irradiation (mainly a decrease) for all except sites recognized by the lectin LCA. The levels of mRNAs coding for the glycosyltransferases MGAT3, MGAT2, ALG3, C1GALT, FUT4 that potentially could participate in changes of glycosylation patterns was also followed.

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14) DNA DAMAGE AND APOPTOSIS INDUCED BY EXPOSURE TO IONIZING RADIATION OR TO FACTORS RELEASED BY IRRADIATED CELLS IN COLORECTAL CANCER CELLS WITH DIFFERENT TP53 STATUS

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Aim: Cells exposed to ionizing radiation release factors which can induce DNA damage, chromosomal instability, apoptosis and changes in proliferation rate of neighboring untreated cells, phenomena known as bystander effects. The purpose of this work was to compare the changes in DNA damage and cellular responses induced by direct irradiation and bystander effects in cells which does or does not contain p53 protein.

Methods: HCT116 colorectal cancer cells with wild-type or knocked-out p53 protein were irradiated with 2 Gy of γ-rays. After 1 hour of incubation, medium was collected and applied to non-irradiated p53+/+ and p53-/- HCT116 cells (bystanders) that were further incubated in standard conditions for different times. DNA methylation and 8-oxo-dG levels were assessed by HPLC and apoptotic and mitotic cell fractions by microscopic methods.

Results: P53 status does not significantly influence the level of 8-oxo-dG in untreated control cells. Irradiated p53-/- but not wild-type cells showed a significant increase in level of oxidative damage at 3 hours after irradiation, and in bystander cells the highest level of oxidative damage was observed during 1 hour of growth in irradiated cells conditioned medium (ICM). The status of P53 seemed to influence the level of uracil in DNA, which was elevated in p53-/- cells in comparison to wild-type cells, and the level of cytosine methylation which was higher in cells with wild type p53. Both irradiation and growth in ICM caused an increase in apoptosis and both p53 +/- and p53-/- directly irradiated cells showed the increase in apoptotic cell frequency earlier than bystander cells. TP53-/- cells showed higher level of apoptosis after both treatments. The NFκB inhibitor, parthenolide (1 μM 3 hours before irradiation) similarly influenced the level of apoptosis in cells differing in p53 status and highly enhanced the apoptosis level in bystander cells.

Conclusion: p53 status markedly affects DNA modifications after irradiation and in the bystander effect. NFκB probably takes some part in bystander signaling in HCT116 cells.
15) IMPROVING HETEROCHROMATIN REMODELLING OF CLONED EMBRYOS: A KEY TO CLONING EFFICIENCY?


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The organization of centromeric and pericentric heterochromatin in 1-cell fertilized mouse embryos is completely different from the organization observed in somatic cells. This unique organization is also observed in 60% of embryos obtained by nuclear transfer using embryonic stem cell (ESNT). Here, we show that remodelling into a zygotic-like organization also occurs after somatic cell nuclear transfer (SCNT), supporting the hypothesis that reorganization of constitutive heterochromatin can occur regardless of the source and differentiation state of the starting material. However, remodelling efficiency in SCNT embryos appears lower than in ESNT embryos and is accompanied by a lower developmental potential. Interestingly, treatment of SCNT embryos with the deacetylase inhibitor trichostatin A (TSA) improves nuclear remodelling at the first cell stage as well as development to term, but not to blastocyst. Together, the results suggest that proper organization of constitutive heterochromatin in early embryos has long-term effects, especially in cloning procedures.

16) INTERACTIONS OF THE SWC4 PROTEIN OF CHROMATIN REMODELING COMPLEXES IN SACCHAROMYCES CEREVISIAE

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The Swc4 protein, encoded by an essential gene, is shared by two chromatin-remodeling complexes in Saccharomyces cerevisiae cells: NuA4 (Nucleosome Acetyltransferase of H4) and SWR1. The SWR1 complex catalyzes ATP-dependent exchange of the nucleosomal histone H2A for H2A.Z (Htz1p). The activity of NuA4 is responsible mainly for acetylation of the H4 histone but also for the acetylation of H2A and H2A.Z. We have previously isolated a collection of swc4 mutants and showed that the essential function of the Swc4 protein is retained in its N-terminal part, within the first 269 amino acids of the 476 amino acids long protein. We have also showed that the truncated swc4 mutant does not interfere with the activity of NuA4 in vivo.

Further, in a yeast two-hybrid screen that we performed using Swc4p as bait, we isolated Vps36p as a Swc4p-binding partner. Vps36p is a component of the ESCRT-II (Endosomal Sorting Complex Required for Transport) complex in yeast. The human ortholog of Vps36p - EAP45 - is known to perform also a nuclear function: as a component of the RNA polymerase II complex. The physiological consequences of the swc4, vps36Δ and swc4vps36Δ mutations suggest that Vps36p might be a bifunctional protein.

Key words: Saccharomyces cerevisiae; chromatin-remodeling complexes; essential genes; Endosomal Sorting Complex Required for Transport (ESCRT)
17) FUNCTIONAL ANALYSIS OF A NUCLEOLAR PROTEIN, NUCLEOMETHYLIN IN MOUSE LIVER

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In eukaryotic cells, ribosome biosynthesis is the most energy-consuming process, and it adapts to changes in intracellular energy status. A central process in this adaptation is the regulation of transcription of rRNA genes. However, the mechanism that links energy status and rRNA transcription is largely unknown. We identified a novel protein complex, eNoSC, which senses intracellular energy status and epigenetically controls the rDNA locus in order to change the ratio between the numbers of active and silent gene clusters. eNoSC contains a novel nucleolar protein, Nucleomethylin (NML), which has a methyltransferase-like domain and binds histone H3 dimethylated at Lys9 at the rDNA locus, along with the NAD+-dependent deacetylase SIRT1 and histone methyltransferase SUV39H. Our results also indicate that eNoSC promotes restoration of energy balance by limiting rRNA gene transcription, thus protecting cells from energy deprivation-dependent apoptosis. (Cell 2008)

In this study, to address the function of NML in vivo, we have generated ubiquitously NML-expressed transgenic mice (NML-TG), using β-actin promoter. We found that NML-TG mice exhibited delayed liver regeneration and accumulation of triglyceride in hepatocytes at 48 hours after partial hepatectomy. To understand the molecular mechanism responsible for this response, we examined gene expression profile using DNA microarray and metabolic profile using CE-TOFMS. These tests reveal the changes of gene expression associated with metabolisms and cell cycle, and the metabolites change of glycolysis. These results suggest that NML has regulated hepatocyte proliferation and hepatocyte energy metabolism after partial hepatectomy. We are currently investigating about NML target genes in regenerating liver.

Key words: nucleolus, NML, liver regeneration, metabolism, proliferation

18) ULTRASTRUCTURAL ANALYSIS OF ORGANIZATION SPICING IN THE RETICULAR AND CHROMOCENTRIC NUCLEUS OF PLANT

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Sequences of non-coding pre-mRNA are removed by the multistages splicing. Several differences were confirmed between kingdoms of plants and animals, despite of the high conservatism of this process. Plants introns usually do not contain polypyrimidine tract and are shorter. The quantity and diversity among SR-proteins and snRNP is larger with plants, than with animals. Few studies concerning the architecture of the plant nucleus do not explain ultimately, if those differences among plants and animals have their reflection at the ultrastructural organization of splicing.

The location of snRNA, SR-proteins, PANA antygen and RNA polimerase II was studied, to know nuclear domains involved to process of splicing in the reticulate (Allium cepa) and chromocentric (Lupinus luteus) cell nucleus of plants.

The detection of the PANA antigen (marker of interchromatin granules clusters) and SR-proteins demonstrated, that in both types of the plants the interchromatin granules with the
diameter of 20-25 nm, filling considerable volume interchromatin area of cellular nucleus, they are the equivalents of the interchromatin granules clusters, observed in the animal cells. These granules do not create clusters, as is observed in animal cells, but they significant fill up the interchromatin area, particularly in the chromocentric nucleus. The some of gold grains occurred also on the periphery of condense chromatin. However detection of snRNA shown the various location in two types of the cellular nucleus. The largest pool of snRNA was connected to chromatin, particularly with the periphery of *Allium*. The signal in interchromatin areas, except Cajal bodies, was observed rarely. In *Lupinus* cells, snRNA, similarly as SR-proteins and PANA antigen, occur in the interchromatin area, mainly in perichromatin fibrils but also in interchromatin granules. It’s suggest that the location of spliceosomes components in plants can be connected with the architecture of the cellular nucleus.

The partial colocalization of snRNA and SR-proteins in reticular nucleus did not let qualify the place of the occurrence of splicing in this type of cellular nucleus. Because splicing is the contrantranscriptional process that is why there was carried out the additional location of RNA polimerase II. The various forms of the phosphorylated polimarase II RNA in the cellular nucleus of *Allium* became visible in the interchromatin area, and the considerable pool was also connected with periphery of chromatin.

The colocalization of snRNA, SR-proteins and RNA polimerase II on the periphery of the chromatin suggests, that similarly as with animals, the perichromatin area can be essential domain in the spatial organization of splicing in the reticular type cell nucleus of plants.

19) OVERVIEW OF THE ROLE OF THE RIBOSOMATIC PROTEINS IN MATURATION AND NUCLEAR EXPORT OF MAMMALIAN 40S RIBOSOMATIC SUBUNITS

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Although essential for cell growth, ribosome production remains partially understood in mammals where specific cleavages and additional steps are undergone. The function of the 33 proteins of the small ribosomal subunit (RPS) and their involvement in maturation and transport of the pre-40S particles has been addressed through a systematic knock-down with siRNAs in human HeLa cells. Depletion of each RPS impaired 40S subunit production, except in the case of RPS25. Simultaneous knock-down showed that RPS27 and RPS27L are two isoforms with redundant functions in humans. RPSs were classified according to their requirement in pre-rRNA processing. Fifteen of these proteins ("initiation" RPS; i-RPS) are strictly necessary for initiating the processing of the pre-rRNA in the 18S rRNA maturation pathway, i.e. for processing of the 5'-ETS. A more heterogeneous group was composed of "progression" RPS proteins (p-RPS), which are required at various stages in the processing of the 18S rRNA. A subset of p-RPSs is required for efficient nuclear export of the pre-40S particles, among which RPS15 and RPS17 appear to have a prominent role. Absence of other p-RPSs blocks final processing of the 18S-E RNA in the cytoplasm. Unlike all the other cleavage steps, the early cleavage site in the 5'-ETS, which is found in vertebrates but not in yeast, is rather insensitive to depletion of RPS proteins. This work provides the first in vivo functional map of the involvement of the RPS proteins in the biogenesis of the 40S particles in mammals and shows a pivotal role of ribosomal protein assembly in initiation of pre-rRNA maturation.

*Key words: pre-rRNA processing, human ribosomal proteins, systematic knock-down*
20) NUCLEAR ACTIN BINDING PROTEINS OF THE PLANT NUCLEUS: SPECTRIN-LIKE PROTEINS

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We have revealed the presence of actin in the plant nucleus associated with transcription foci and also with the nucleoskeleton (NSK) (1). Nuclear actin is modulated by different actin binding proteins (ABP). Plants lack orthologues of the main structural nuclear ABPs of animal cells, such as lamins LAPs and nesprins. Up to date, the only plant nuclear ABPs detected are NMI (1), profilin and ADF. We have investigated the presence of a class of structural nuclear ABPs in the plant nucleus, the spectrin-like proteins. Spectrin is a family of acidic α-helical high molecular weight multifunctional ABPs. All the proteins of the spectrin superfamily contain arrays of spectrin repeats involved in self-association, interaction with other proteins, targeting, etc. Flanking the repeats they have an N-terminal calponin homology (CH) domain for actin binding and a C-terminal region with different functional domains. Several members of the spectrin protein superfamily with different functions have been identified in the mammalian nucleus, such as α spectrin II and β spectrin II, β spectrin IV Σ5, nesprins, etc. (2). α Spectrin II is involved in DNA repair. The truncated 72-kDa-β-spectrin IV Σ5 associates with PML bodies and the nuclear matrix. Nesprins associate with lamins, emerin, and actin, and probably play a role in nuclear organization and integrity.

Although no clear orthologues of spectrin were found in the Arabidopsis genome (3), spectrin-like proteins have been detected in several plant tissues and species. Spectrin-like antigens have been also reported in meristematic nuclei of carrot, pea and onion (4, 5). In this study we characterize the nuclear spectrin-like proteins in Allium cepa, investigate their actin-binding ability, and their distribution in the different nuclear compartments including the NSK. For that we used an antibody recognizing α and β chains of chicken spectrin in western blots, 2-D electrophoresis, immunoprecipitation, and confocal immunofluorescence and immunogold labellings. We demonstrate the existence of spectrin-like proteins with different phosphorylation isoforms in onion nuclei that co-immunoprecipitate and partly co-localize with actin. They show diverse distributions in the asynchronous meristematic nuclear population: nuclear periphery, intranuclear tracks and in dots, and also in the NSK. Our results suggest that spectrin-like proteins are structural components of the plant nucleus that could act as ABPs.

References:

Key words: plants, nucleus, nucleoskeleton, ABPs, spectrin

21) IN CAENORHABDITIS ELEGANS NANOPARTICLE-BIO-INTERACTIONS BECOME TRANSPARENT: SILICLE-NANOPARTICLES INDUCE REPRODUCTIVE SENESCENCE

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While expectations and applications of nanotechnologies grow exponentially, little is known about interactions of engineered nanoparticles with multicellular organisms. Here we propose the transparent roundworm Caenorhabditis elegans as a simple but anatomically and biologically well defined animal model that allows for whole organism analyses of nanoparticle-bio-interactions. Microscopic techniques showed that fluorescently labelled
nanoparticles are efficiently taken up by the worms during feeding, and translocate to primary organs such as epithelial cells of the intestine, as well as secondary organs belonging to the reproductive tract. The life span of nanoparticle-fed Caenorhabditis elegans remained unchanged, whereas a reduction of progeny production was observed in silica-nanoparticle exposed worms versus untreated controls. This reduction was accompanied by a significant increase of the ‘bag of worms’ phenotype that is characterized by failed egg-laying and usually occurs in aged wild type worms. Experimental exclusion of developmental defects suggests that silica-nanoparticles induce an age-related degeneration of reproductive organs, and thus set a research platform for both, detailed elucidation of molecular mechanisms and high throughput screening of different nanomaterials by analyses of progeny production.

Key words: nanotoxicology, aging, Caenorhabditis elegans, microscopy

22) INVESTIGATION OF THE ROLE OF PML NBs IN MOUSE POLYOMAVIRUS INFECTION

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Previously, we observed the association of Promyelocytic Leukaemia Nuclear Bodies (PML NBs) with Mouse Polyomavirus (MPyV) large tumorigenic antigen (LT) clusters in the nuclei of infected cells. We further studied the involvement of PML NBs components in this association and determined whether LT itself is responsible for the association with PML NBs. We found out that in the cells transiently expressing LT antigen, LT clusters are not formed and replication of MPyV genome is essential for LT clusters formation and targeting to the vicinity of PML NBs. FISH analysis of the cells fixed at different times post infection showed that viral DNA -PML NBs association appears as fast as 10 hours post infection and increases with progression of infection. Simultaneously, MPyV infection leads to the increasing of the size of PML NBs.

Also, we examined whether the polyomavirus profits by association with PML NBs or PML NBs are parts of defence mechanism against virus. We found that MPyV early and late gene transcription as well as genome replication were increased in PML negative cells. Moreover, we demonstrated that in the cells with strong association between PML NBs and MPyV DNA the late transcription of MPyV genome was delayed.

By now, we excluded two structural proteins of PML NB SP100 and DAXX as possible agents responsible for interaction between MPyV DNA and PML NBs. However, the role of these proteins in the repression of the MPyV expression is under investigation.

Altogether, our data suggest a defence role of PML NBs against MPyV. Nevertheless, PML NBs may also contribute to the viral genome maintenance based on the similar function towards to the cellular DNA.

Key words: polyomavirus, large T, PML, Daxx, Sp100

23) ROLE OF MEIOTIC COHESIN REC8 IN COORDINATION OF CHROMOSOMAL AND CENTROSOMAL CYCLES FOR SELF-RENEWAL OF TUMOUR CELLS

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The evidence has been accumulated that a proportion of endopolyploid cells which are induced in p53-dysfunctional tumours have the potential to reduce the genome to para-diploidy
and give rise to repopulating growth of para-diploid cells with increased resistance to anti-cancer therapy (Sundaram et al., 2004; Puig et al., 2008). While studying by immunofluorescence the role of meiotic cohesin REC8 in somatic reduction of lymphoid tumour cells in vitro, we observed in many early endopolyploid tumour cell (ETC) metaphases that REC8 formed a ring-like structure in addition to its centromeric localisation. Such structures have previously been observed with NuMA, a nuclear microtubule-binding protein that binds to the separating centrosomes during early metaphase and until the onset of anaphase where it contributes to the organization and stabilization of microtubules in astral spindle poles. Co-staining disclosed the almost perfect co-localisation of both REC8 and NuMa to these metaphase and anaphase spindle poles. NuMA protein is known to interact with the mitotic analogue of REC8 – cohesin SCC1. Interestingly that in turn SCC1 was found to be required for the \textit{in vitro} assembly of astral poles, indispensable for division of HeLa cells (Gregson et al., 2001). In addition, we found that REC8 is colocalised at centrosomes and astral poles of ETC with the protein of the embryonic stem- and germ-line OCT4_B isoform. This cytoplasmic isoform was found both in early embryogenesis and somatic tumour cells but its function remains hitherto unknown. Our observation allow to suggest that REC8/OCT4_B complex activated in the induced by DNA damage ETC may coordinate their chromosome and centrosome cycle as an essential feature of self-renewal of cancer stem cells resistant to anti-cancer therapy.

\textit{Key words:} endopolyploid tumor cells, REC8, NuMa, astral poles, self-renewal

24) THE ROLE OF THE SEQUENCE THAT ATTACH CHROMOSOMES TO THE NUCLEAR ENVELOPES IN OVERCOMING OF POSITION EFFECTS

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It is now postulated that independent tissue-specific expression of eukaryotic genes is in close connection with the intranuclear organization of chromosomes. Thus the DNA sequences participated in attaching of chromosomes to the nuclear envelopes are of great interest.

We have a sequence of this type isolated from murine nuclear envelopes (EnvM4) which is thought to be involved in the process of bordering of structural and/or functional chromosomal domains. We found that it possess polypourine/polypirymidine tracks that are present in a great amount of genomes (from bacteria and plants to human) with up to 100% homology. This may represent the general nucleotide motif attaching DNA to the nuclear (cellular) envelopes shared among species.

To examine the influence character of EnvM4 on the expression of reporter genes the P-transformation method of Drosophila melanogaster was used. We injected into Drosophila embryos with inactivated yellow and white genes reporter which contained these genes and regulatory elements flanked with EnvM4 and managed to achieve 10 lines. Each of them showed high level of expression of reporters that indicates the overcoming of position effects. To investigate the role of EnvM4 sequences in this process we deleted white gene enhancer in vivo and saw decreasing of the expression level of white gene to basic one, but not the yellow gene. This means that the sequences flanking the genes do not impact the gene expression itself but participate in maintenance of defined expression level. As far as EnvM4 fragments are connected with the nuclear envelopes they possibly can establish an independent domain of gene and help to eliminate any influence of surrounding sequences.

\textit{This work was supported by Russian Academy of Sciences (Program "Biological diversity", subprogram "Gene pools and genetic diversity", section "Development genetics".}

\textit{Key words:} nuclear organization, gene expression, position effects
25) QUANTIFICATION AND POSITIONING OF SUBNUCLEAR STRUCTURES IN SENESCENT CELLS

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The process of cellular senescence was first described by Hayflick and Moorhead. They observed that normal human fibroblasts were able to enter a state of irreversible growth arrest after serial cultivation in vitro (1).

In the nucleus of senescent cells, the chromatin undergoes dramatic changes through the formation of areas of facultative heterochromatin called senescence-associated heterochromatin foci (SAHFs). SAHFs are composed of modifications and associated proteins characteristic of transcriptionally silent heterochromatin, such as heterochromatin protein 1 gamma (HP1γ). When cells become senescent, HP1γ is phosphorylated on serine 93, a modification required for its binding to SAHFs (2).

Here, we observed the formation of SAHFs by quantifying the number of HP1γ-Foci during the cumulative population doublings of human fibroblasts (WI-38). In growing cells the mean value of HP1γ-Foci per cell nucleus was nine whereas this value increases in senescent cells up to 23 HP1γ-Foci per cell nucleus. These results underline the observation that the close structure and function relationship in the nucleus changes during senescence. The quantification of HP1γ-Foci provides a useful tool to monitor these changes and the senescent state of serial cultivated human fibroblasts.

We currently develop methods for positioning of nuclear substructures in correlation with SAHFs. Major emphasis is laid on analysis of proteasomes and proteasomal proteolysis (3) in senescent nuclei.

Key words: cellular senescence, nucleus, HP1γ-foci, nuclear substructures

References:

26) EFFECTS OF AN OXIDATIVE STRESS ON HUMAN K562 AND ME45 CANCER CELLS

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Cultured cells exposed to oxidizing factors like H2O2 or ionizing radiation respond with an immediate increase in DNA strand breaks which gradually disappear after about 180 min. Both H2O2 and ionizing radiation are known to induce oxidative damage to DNA, and 8-hydroxyguanine (8-OH-dG) is the best studied example. The oxidative DNA modifications are mainly repaired by base excision repair and less frequently by nucleotide excision repair, and both systems use endonucleases that incise DNA to remove damaged fragments on one strand and thus induce some DNA strand breaks which can be detected experimentally.

The main purpose of this study was to compare these responses in two human cancer cell lines, leukemic K562 and melanoma Me45 cells, and to study the relationship between DNA strand breaks and the level of oxidative damage in DNA to understand how much of the observed strand breaks originate from DNA repair.
K562 and Me45 cells were exposed for 5 min to 100 µM H₂O₂ added to the culture medium, and after washing suspended in fresh medium and incubated in standard conditions. The levels of DNA strand breaks and 8-hydroxyguanine (8-OH-dG) were measured by comet assays and high pressure liquid chromatography with electrochemical detection (HPLC-EC), respectively, in samples collected after different time intervals.

The dynamics of single strand break repair differed significantly between K562 and Me45 cells, with melanoma cells showing a monotonic decrease whereas in K562 cells some increase in strand breaks was seen about 1 hour after the exposure to H₂O₂.

The level of 8-OH-dG was measured at different times after H₂O₂ or ionizing radiation exposure in the DNA of K562 cells. In the first minutes after both treatments the level of 8-OH-dG in DNA dropped below the level of that in control cells, and then gradually increased. These results suggest that exposure to both H₂O₂ and ionizing radiation very rapidly induce DNA repair systems that efficiently remove damaged bases from DNA, even those which are present in control cells. The increase in both strand breaks and 8-OH-dG observed after 1 hour in K562 cells could result from induction of new reactive oxygen or nitrogen species in these cells, a phenomenon known in other cells from the literature.

This work was supported by a grant from the Silesian University of Technology nr BK-209/RAu1/2008, t.3.

27) FEATURES EXTRACTION BASED ON PARTIAL LEAST SQUARES METHOD FOR CLASSIFICATION IN DNA MICROARRAY DATA

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Recent studies suggest that gene expression profiles may represent a promising alternative for clinical cancer classification. Molecular-based approaches have opened the possibility of investigating the activity of thousands of genes simultaneously and can be used to find genes involved in neoplasia. A big problem in applying microarrays in classification problem is dimension of this data (Nguyen et al., 2002) because the high dimensionality of gene expression microarray data set hurts generalization performance of classifiers. Most statistical methodology for classification does not work well when there are more variables than samples. The key aspect is to build a good tool for classification that uses small number of genes. In this paper we describe multiclass features extraction since these are more difficult than binary ones (Zhang et al., 2004). The gene selection for classifier is a very important problem. Over the past few years many algorithms was proposed to resolve this problem. However, most of the studies are designed to binary dimension reduction problems and only a few involve multiclass cases. The optimal selection of most significant genes for multiclass analysis is still an open problem. We propose a gene selection method based on Multivariate Partial Least Squares (MPLS) (Höskuldsson et al., 1988). Then we compare the results with Recursive Feature Elimination (RFE) method (Guyon et al., 2002). The standard way to use PLS algorithm is only for dimension reduction and not for selecting significant genes. We used this method for searching best genes for classification. The new idea is to use PLS not only as multiclass approach, but to construct more binary selections that use one versus rest (OvR) and one versus one (OvO) methods. In this article we sort the obtained weight vector w from MPLS algorithm and choose only the corresponding genes with the highest weights in vector w. In order to classify DNA microarray samples the support vector machines (SVM) technique is used. The code is written in Matlab. For error estimation we use balanced bootstrap 632+ (variance-reducing bootstrap 632+). To show the performance of ours system we use accuracy results for multiclass problems. For tests we used Affymetrix HGU-133 plus2 microarrays. The data set consisted of gene expression profiles for 143 microarrays of colon tissue. The specimens included three classes: tumor, normal and polyps arrays. Our results show that the
new PLS-based method to select significant genes has very high accuracy rate and can be used for differentiate microarrays data. In every tests PLS used as standard multiclass approaches was worser then combination of two-class PLS approach. Implemented method Bootstrap Based Feature Ranking BBFR (Fujarewicz et al., 2005) can help to find new genes that take part in neoplasia.

This work was supported by a grant from the Silesian University of Technology nr BK-209/RAu1/2008, t.3.

28) FUNCTIONAL INTERACTIONS BETWEEN SIGNALING PATHWAYS THAT DEPEND ON P53 AND NFκB TRANSCRIPTION FACTORS IN CELLS EXPOSED TO GENOTOXIC STRESS

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Signaling pathways that depend on p53 or NFκB transcription factors are essential components of cellular responses to stress. In general, p53 is involved in either activation of cell cycle arrest or induction of apoptosis, while NFκB exerts mostly anti-apoptotic functions; both regulatory pathways apparently interfere with each other yet molecular details of such interactions remains to be elucidated.

Here we aimed to analyze effects of NFκB pathway on activation of p53-dependent genes, and effects of p53 pathway on activation of NFκB-dependent genes. Colon carcinoma HCT116 cell line was used, in two congenic variants either containing or lacking transcriptionaly competent p53. Cells were incubated with TNFa cytokine to induce NFκB, and or treated with ultraviolet radiation to induce p53 pathway; both factors were used in several different combinations. Activation of NFκB and p53 pathways was monitored by Western-blotting. The level of expression of selected genes was assessed by semi-quantitative RT-PCR or quantitative real-time Q-RT-PCR. We have observed that time of activation of several NFκB-dependnt genes was delayed in p53-deficient cells.

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29) NUCLEO-CYTOPLASMIC SHUTTLING OF HUMAN COHESINS SA1 AND SA2 EXPRESSED IN YEAST SACCHAROMYCES CEREVISIAE

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The cohesin complex ensures accurate separation of sister chromatids into two daughter cells. Several models for the cohesin complex have been proposed, but the one-ring embrace model is currently the most popular. The complex comprises four core protein subunits, essential for cell viability, that are conserved from yeast to vertebrates. In Saccharomyces cerevisiae mitotic cells, the cohesin complex consists of Scc1/Mcd1, Smc1, Smc3, and Irr1/Scc3. In human mitotic cells, the cohesin complex is composed of Rad21, Smc1, Smc3, and two Irr1 orthologues, SA1 and SA2. Each complex can contain only one of the two SA proteins and it is unknown whether the composition of the complex is stable or whether SA1 and SA2 are exchangeable.

We initially expressed human SA1 and SA2 proteins in yeast mutants to check whether they can replace Irr1p. Although neither of the SA proteins substituted for Irr1p function, we found that SA1p expressed in yeast has a nuclear localization, while the majority of SA2p is present in the cytoplasm. Analysis of the amino acid sequence of SA2 allowed us to identify putative nuclear export signals (NES) characteristic for Crm1p karyopherin – dependent
export. Using yeast mutants defective in the CRM1 gene or sensitive to Leptomycin B (Crm1p inhibitor) we found that SA2 is exported from the nucleus in a Crm1p-dependent manner. We assume that such export can be an element of SA1-SA2 exchange in the cohesin complex. At present, the putative NES-es are tested to identify those which are indeed functional.

Moreover, although SA proteins can not replace Irr1p we found that their presence in yeast cells provoked phenotypic changes, which indicates that some functions are interchangeable between yeast and human cohesins.

Key words: chromosome, segregation, nucleus, protein, transport

30) LOCALIZATION OF Nopp140 WITHIN MAMMALLIAN CELLS DURING INTERPHASE AND MITOSIS

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Nopp140 is a nonribosomal, nucleolar protein. Unlike most other nucleolar proteins, Nopp140 does not carry RNA-binding motifs or glycine/arginine-rich stretches, as deduced from the known primary sequences of different species. On the contrary, its amino- and carboxy-termini are separated by a long, central domain, consisting of ten repeats of acidic serine clusters alternating with lysine-, alanine-, and proline-rich basic stretches. Most serine residues of the acidic repeats are phosphorylated by casein kinase 2, which makes Nopp140 one of the most highly phosphorylated proteins in the cell with ~80 phosphates per molecule. Nopp140 has also been detected in the dense fibrillar component of nucleoli and in Cajal bodies. Despite these biochemical characteristics and these locations, the biological functions of Nopp140 are still unclear.

In order to shed light on the cellular function of Nopp140, we re-investigated the precise location of this protein within different mammalian cells during the cell cycle by immunofluorescence confocal microscopy coupled to three-dimensional (3D) image reconstructions and immunogold electron microscopy.

During interphase, 3D image reconstructions of confocal sections revealed that nucleolar labelling appeared as several tiny spheres organized in necklaces. Moreover, after an immunogold labelling procedure, gold particles were detected not only over the dense fibrillar component but also over the fibrillar centers of nucleoli in untreated and actinomycin D-treated cells. Labelling was also consistently present in Cajal bodies. After pulse-chase experiments with BrUTP, colocalization was more prominent after a 10-15 min chase than after a 5 min chase.

During mitosis, confocal analysis indicated that Nopp140 organization was lost. The protein dispersed between and around the chromosomes in prophase. From prometaphase to telophase, it was also detected in numerous cytoplasmic nucleolus-derived foci. During telophase, it reappeared in the reforming nucleoli of daughter nuclei.

Altogether, these results strongly suggest that Nopp140 could be a component implicated in the early steps of pre-rRNA processing.
31) LOCALIZATION OF TRANSGENES ACTIVATED BY AN ARTIFICIAL CURVED DNA

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Unusual DNA structures, such as curved DNAs, poly(dA·dT) sequences, and Z-DNA-forming sequences frequently occur in eukaryotic genomes. Many reports have indicated that they play an essential role in the formation, stability and positioning of nucleosomes, and consequently in DNA packaging in nuclei. Among these structures, curved DNAs have been proved to have an activity to facilitate transcription. They can organize local chromatin infrastructure to be appropriate for transcription initiation. Thus, curved DNAs seem to be a promising tool in “chromatin engineering” for efficient expression of transgenes.

The spatial organization of genome in a nucleus is known to influence gene expression. Therefore, the next issue to be addressed, to develop curved DNA-based chromatin engineering, is the nuclear localization of the transgenes activated by curved DNA structures. On this view, we performed the imaging analysis using the lac operator/lac repressor-GFP system. We constructed a 10 kbp plasmid including 64 direct repeats of lac operator followed by a reporter array. This array contained 180 bp artificial curved DNA segment (T20), the herpes simplex virus thymidine kinase promoter and the luciferase reporter gene. Mouse embryonic stem (ES) cells and HeLa cells were transfected with this plasmid and LacI-GFP expression vector, and several cell lines were established. Also established were control cell lines each of which deleted T20 from the transgene locus. Fluorescence microscopy observation of the ES cell lines showed that the reporter was located at nuclear periphery in both T20-containing and T20-less lines. The same was true for the T20-less HeLa control cell lines. However, the T20-containing reporter moved to internal region of the HeLa nucleus. These results suggest that the effect of DNA curvature on reporter localization in the nucleus differs between cells. The underlying mechanism will be discussed.

Key words: bent DNA, gene expression, chromatin, nucleus, live cell imaging

32) DAMAGE INDUCED IN ENDOTHELIAL CELLS AND CARDIOMYOCYTES UPON EXPOSURE TO IONIZING RADIATION

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Radiation-induced damage of cardiovascular system is one of reported side effects of radiotherapy. Heart failure related to radiotherapy most possibly involves long-term effects of damage of cardiac microcirculation. Here we aimed to analyze radiosensitivity of cardiac endothelial cells.

Experimental model. 3 types of endothelial cells were used: primary cells isolated from hearts of C57BL/6J mice, H5V cells (isolated from mouse embryo heart) and b.END3 cells (isolated from mouse brain). In addition, primary cardiomyocytes were isolated from the same mice strain. Cells were irradiated in vitro with 2 Gy dose of ionizing radiation. Radiosensitivity of cells was measured using γH2A.X staining, induction of apoptosis and clonogenic survival. We have analyzed effect of either direct irradiation or so called bystander effect (in different combination of cell types). In addition, permeability of EC monolayer was analyze to determine possible effect of irradiation upon inter-cellular interaction. Additionally, heart sections from animals irradiated in vivo were analyzed by electron microscopy to reveal possible radiation-induced changes in cell ultrastructure.
We observed that in vitro radiosensitivity of tested endothelial cells was similar to radiosensitivity of other cell types. However, endothelial cells were resistant to radiation-induced bystander effect. Exposure to ionizing radiation (even at doses up to 16 Gy) did not result in acute permeability of EC monolayer. We also observed changed morphology of mitochondria of cardiomyocytes from mice irradiated in vivo.

This work was supported by the FP7/EURATOM Grant CARDIORISK.

33) HIGHER-ORDER NUCLEAR STRUCTURE IN EPILEPTOGENESIS

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Even though the molecular mechanisms of gene-expression in neurons are quite broadly described in the literature, little is known about the relationship between these processes and the architecture of the neuronal cell nucleus. For example, it is firmly established that waves of gene-expression occurring after neuronal stimulation, comprise plenty of genes playing important roles in cognitive and epileptic phenomena. However, it has never been examined whether these bursts of transcriptional activity involve any regulation at the level of higher-order nuclear structure, including chromosomal domains. Accordingly, we have performed studies on the structure of neuronal nucleus in epileptic animals. Using electron microscopy, we found the appearance of large interchromatin granule clusters (IGCs) in epileptic nuclei. We confirmed this observation by immunofluorescence-confocal analysis of IGC marker, a spliceosome assembly factor SC-35. Interestingly, there was also aggregation of spots immunoreactive for phosphorylated and acetylated Histone H3, a marker of transcriptionally active chromatin. The findings are consistent with the results of bioinformatic analysis of transcription profiling upon epileptogenesis (published databases), showing tendency for coordinated expression of positional gene-clusters along chromosomes. Taken together, our results suggest that upon epileptogenesis there is prominent reorganization of neuronal nucleus, putatively involving clustering of highly expressed genes in the form of molecular factories, where transcription, splicing, and (possibly) export of pre-mRNA are orchestrated. More generally, the phenomenon could occur not only upon epileptogenesis, but also in plasticity related to learning and memory formation which could be a topic to follow up in the future.

Key words: epileptogenesis, interchromatin granules, confocal microscopy

34) LIVE CELL IMAGING OF HETEROCHROMATIN PROTEIN 1 INVOLVEMENT IN DNA DAMAGE RESPONSE - THE IMPORTANCE OF RECRUITING IN EARNEST

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Involvement of various factors in DNA damage response can be studied in situ, in live cells, using various new optical microscopy techniques. Recruitment of repair enzymes tagged with fluorescent proteins to damaged chromatin can be visualized by live cell fluorescence microscopy. In order to visualize recruitment of repair factors, damage is inflicted in a small area of the nucleus. Subsequently changes of local concentration of a fluorescently labelled repair protein are studied. We have shown that an epigenetic regulator, heterochromatin
protein 1, is recruited to sites of oxidative chromatin damage\textsuperscript{2,3} which was inflicted by interaction of a beam of focused green light with a DNA-intercalated ethidium. In another report dissociation but no recruitment was detected\textsuperscript{4}. We demonstrate that the experimental conditions are crucial in studies of recruitment of repair factors to DNA damage. If the local damage is too severe, i.e. the concentration of a photosensitizer, or a dose of light are too high, recruitment of HP1 does not occur. The level of local HP1 accumulation is dependent on the conditions of the assay. Thus, the conditions of experiments aimed at detecting recruitment of repair factors to DNA damage must be carefully optimized to avoid a false negative result.

References:

Key words: DNA damage response, heterochromatin protein 1, HP1, recruitment
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In 2008, “Czantoria”, the foremost artistic ensemble from Ustroń, celebrated its 20th anniversary. On the occasion of celebrating the 700th anniversary of the City of Ustroń, during the “What makes the people of Ustroń sing inside?” gala concert in 2005, “Czantoria” was bestowed the official title of Ustroń City Honorary Ensemble. During the past twenty years “Czantoria” gave over 600 concerts. Currently it has 52 singers. The repertoire is varied, from popular folk songs, especially from the Cieszyn region, through popular songs, as well as patriotic and religious music, notably carols. The concerts have been invariably bringing to the audience moments of deep feelings, joy and artistic emotion. “Czantoria” has been performing regularly for spa and resort visitors home and abroad. It went on tours to Germany, Italy, Bulgaria, Hungary, Lithuania, Slovakia and Czech Republic. The artistic success of “Czantoria” undoubtedly goes back to immense efforts of late Marian Zyla, the founder and first artistic director of the Ensemble, as well as to its present Bandmaster and Musical Director, Władysław Wilczak. Past years have witnessed dozens of singers performing for the Ensemble (including late Bolesław Iskrzycki, Rafał Winter, Emil Fobr, Ernestyna Kisiala, Dorota Gerlic, Władysław Hładki and Henryk Jankowski). Such rotation does not make things necessarily easy for musical directors to keep up the performance level but, what is encouraging, “Czantoria” enjoys a constant inflow of talented young singers bringing with them an invigorating breath of youth and energy. “Czantoria” ensemble has had the privilege of working with top artists such as Ewa Kornas-Biegas and Magdalena Chudzieczek-Cieslar (sopranos) or with somewhat younger generation of Musical Academy graduates such as Katarzyna Siwiec, Joanna Szczęśniowska and Izabela Zwias (all of them conductors). In its Jubilee Year “Czantoria” is musically in top shape; thanks to the effort of the gifted conductor, high artistic level and rich repertoire are the trademarks of the Ensemble. The audience reception of the Ensemble is usually spontaneous and quite often ends up in joint feast singing. The Ensemble is proud to have an eight-volume Chronicle, kept from the very beginning by Jan Albrewczyński. Entries witness excellent reviews and reception of “Czantoria” everywhere. The Executive Board of the “Czantoria” Association for the Promotion of Folk Culture expresses its deep gratitude to the Ustroń Mayor’s Office for financial support and harmonious cooperation on behalf of Cieszyn Land, our beloved Little Motherland.
"Czantoria" Ensemble

Program of Concert

August 31, 2009

"Ustroński" Polonaise

Hej Groniczki [hey, grohnitschki] – Hey, Mountain Ridges
Beskidzie, Beskidzie [beskeedgae] – Beskid Mountains
Tam koło Cieszyna [tahm kowo tschescheena] – There, By Cieszyn

Górals [gooral] – Highlander
Dziewczko z Poręby [dzevheitschko s poremby] – Hey, Girl From Poremba
Dysc [dystz] – Rainfall

Szumi jawor [shoomi yavor] – Maple Rustle
Śpiewam ja śpiewam [spyevam ya spyevam] – I Am Singing, I Am Singing
Sarna [sarna] – Roe Deer
Orawa [orawah] – Orava

Umrzyła Gorolka [oomziwa goorolka] – Last Breath of Highlander Woman
Sobótkowe Śpiewki [sobootkove spyevkee] – Bonfire Songs
Płyniesz Olzo [puynesch olzou] – Olza River
Pod Twoim okienkiem [pohd tweem okyenkem] – Under Your Window
Wiązanka „Kajżeś była Hanuliczko” [kayzesh bewa hanoolitschko] – ”Where Have You Been, Hanuliczka” Medley

Wiązanka „Pije Kuba” [peeye cooba] – ”Jake Is Drinking” Medley
Karolinka [caroleenkah] – Little Carolyn
Wiązanka „Szła dziewczka” [schwa dzevetschka] – ”Young Girl Walking” Medley
Ustroń city and countryside

Ustroń owes its charm to the surrounding mountain tops of Wielka Czantoria (995 m), Mała Czantoria (866 m), Lipowski Groń (745 m) and Równica (884 m), covered with beautiful beech and spruce trees.

History

Though it was mentioned for the first time in 1305, Ustroń received town rights in 1956. The name "Ustroń" was written on a map of Poland dating from 1558, made by Wacław Grodecki for the Polish king, Sigismund II Augustus. From the second half of the XVI century the town was a manorial land, and from the first half of XVII century it was owned by the Habsburgs. The first records of tourists come from the 1650s - when people would come here not only to appreciate a favourable climate and a beautiful setting but also for the whey treatment, applied in case of anaemia and digestive track ailments.

Ustroń was mainly a pastoral and agricultural settlement till the discovery of an iron ore in the XVIII century. This resulted in founding the steelworks in 1772, and later in constructing the ironworks, a foundry and a rolling mill. Metallurgy as well as ideal climatic conditions and picturesque countryside were key factors for the development of Ustroń as a health resort. Szmelcerze (smelters; people working the great furnaces), discovered that water heated up by slag revealed soothing properties, especially in the treatment of rheumatic pains. When the medical research confirmed this discovery, archduke Albert ordered to build a guest house with baths called "Hotel Kuracyjny". The plaque on the building says: "Archduke Albert constructed the baths for people to heal their illnesses. 1804."

In the second half of XIX century a spring rich in ferrous water and layers of therapeutic mud were discovered, which added to Ustroń's popularity as a health resort.

By decree of National Government in Opawa in 1882 Ustroń was recognized as an Austrian health resort. In 1901 a first baths house was built managed by a company called "First Austrian – Silesian Mud Baths in Ustroń". Ustroń received town rights on 28, December 1956 and was given health resort rights in 1967. Five years later WRN in Katowice gave the town a status of a health resort.

Things to see

There are only a few examples of the town's old traditional buildings. Constructed in 1769, the wooden St Anna's Church in Ustroń Nierodzim, is one of the most precious, and a reconstructed wooden building from the second half of the XVIII century is also worth seeing. Another example of interesting architecture is the Roman Catholic Church of St Clement from 1788. In front of it you can see two statues by the XVIII century sculptor Waclaw Donay: one of them represents St Joseph with the Child, the other Jan Nepomucen. The Protestant Church dedicated to the Apostle Jacob, was constructed in 1835. The church, built in classical style, has a large nave with adjoining aisles. The altar, designed by the Austrian architect Repreuet, stands in the semicircular chancel. While walking through the streets of the town, make a visit to Ironworks and Smithy Museum housed in the former headquarters of the ironworks. The exhibition presents the history of the ironworks in Ustroń. You can see miniature models of various machines as well as some iron objects. The rooms on the ground floor are used for temporary exhibitions. They also house Ustroń Modern Art Gallery. An interesting tourist attraction is Sobieski's Oak Tree from 1683. The legend has it that the tree was planted by the local people to commemorate King Sobieski's victory at Vienna. Taking a red-coloured trail from Zawodzie to Równica you get to the XVIII century stone altar which was used by the persecuted Lutherans as a place of religious worship.

Tourism and sport

An unusual setting amidst the picturesque slopes of Czantoria and Równica, well-marked walks, mountain hikes and sport facilities provide excellent conditions for climbing, skiing, cycling and other summer and winter sports. There are numerous signposted trails for walking, hiking and cycling. One of the main attractions is the chair lift to the top of Czantoria and Summer Toboggan-Run also from Czantoria. The nearby clearing of Stoklosica commands fine panoramic views over Ustroń and the Beskid Śląski region; whereas from the top of Czantoria, on a sunny day, you can admire the Tatra Mountains and Mała Fatra. From the top of Czantoria you can cross the border to the Czech Republic. The top of Równica is accessible on foot and by car. Apart from the wonderful views, tourists can enjoy a homely atmosphere of the hostel and a rustic charm of the old huts.

The outdoor dinner and bonfire at Czantoria Mountain (Tuesday night) and the trip to Szczyrk, Żywiec, Koniaków and Wisła (Wednesday afternoon and night)
Ustroń City Map

Katowice

- St Anna's Church
- Manor House
- Protestant Church of Apostle Jacob
- Roman Catholic Church of St Clement
- Ironworks and Smithy Museum
- "Prazakowka" restaurant
- Railway Station "Zdroj"