Organizing Institutions

Department of Biophysics and Cell Biology,
Medical and Health Science Center,
University of Debrecen

Doctoral School of Molecular Cell Biology and Immunobiology,
University of Debrecen
Sponsor Institutions

Department of Biophysics and Cell Biology,  
Medical and Health Science Center,  
University of Debrecen

Doctoral School of Molecular Cell Biology and Immunobiology,  
University of Debrecen

UNIVERSITY OF DEBRECEN SYMPOSIUM

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FEJLESZTÉSI TERV

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Welcome to the 23rd Wilhelm Bernhard Workshop

Dear Colleagues,

We are pleased to welcome you in Debrecen, Hungary for the 23rd Wilhelm Bernhard Workshop on the Cell Nucleus.

The current meeting, a sequel in the long history of the Workshop, will hopefully live up to its traditions: in its informal and friendly atmosphere, high scientific standards and at the same time its openness to novel perspectives and to serendipitous observations. Interest in the workshop has not waned in the wake of the highly successful and memorable Riga meeting in 2011, as demonstrated by the number of registered participants. We can just hope that the legacy of the current conference will be comparable, helping the perpetuation of this highly enjoyable series of scientific events, to be realized in the forthcoming workshops.

Their history starts in 1968 when Dr. Wilhelm Bernhard, a leading Swiss scientist of the times, who gained international fame for his pioneering works on the ultrastructure of the nucleus, also widely adored for his affectionate and emphatic personality and his intellectual aura of a true Renaissance Man, founded this series of Workshops. (See http://wbworkshops.com/wb.html for details.)

Belonging a worthy tradition is a great honour and also responsibility. We couldn't have endeavoured contributing to it by organizing the current meeting without a lot of help. We got continuous support from members of the International Committee, especially from the organizers of previous workshops. We could count on the support of the University, the Doctoral School of Molecular, Cell and Immune Biology, and the Department of Biophysics and Cell Biology, each giving not only the institutional background but direct financial support as well. Their support, as well as funds obtained from the Visegrad Fund (http://visegradfund.org/) and EMBO (http://www.embo.org/) made it possible to support the attendance of many young scientists and invited speakers.

The honor of organizing the 23rd Wilhelm Bernhard Workshop is also perceived as an acknowledgement of the efforts, enthusiasm and the often so warmly related personality of Professor Egon Hidvégi, the organizer of two past workshops in this country.

An international meeting is a chance for the scientific community it is organized by to get more attention than usual. The University of Debrecen, its Medical and Health Science Center, and particularly its groups involved in research on the cell nucleus, chromatin and related subjects, will enjoy now the privilege of becoming known to so many fellow scientists around the world. We are very much thankful for this opportunity.

Such a meeting is also an occasion and an obligation to show one's country to the world. We will try to show you its faces we deem the most beautiful and unique. In advance, also in line with Dr. Bernhard's legacy, please find a few relevant lines by a worthy representative of Hungarian culture through the links provided on the webpage of the conference.

Once again, we welcome you all in Debrecen, hoping that you will find the meeting both useful and enjoyable:

Gabor Szabo
(acting President of the International Committee)
**International Committee**

Marco Biggiogera (Italy)
Stan Fakan (Switzerland)
Frances Victoria Fuller-Pace (United Kingdom)
Ronald Hancock (Canada)
Peter Hemmerich (Germany)
Daniele Hernandez-Verdun (France)
Egon Hidvégi (Hungary)
Pavel Hozák (Czech Republic)
Jun Janigasawa (Japan)
Susana Moreno Díaz de la Espina (Spain)
Thoru Pederson (USA)
Marion Schmidt-Zachman (Germany)
Peter Shaw (United Kingdom)
Nikolajs Sjakste (Latvia)
Karel Smetana (Czech Republic)
Gábor Szabó (Hungary)
Robert Tanguay (Canada)
Mark Thiry (Belgium)
Roel van Driel (The Netherlands)
Anna von Mikecz (Germany)
Piotr Widłak (Poland)
Lars Wieslander (Sweden)
Olga Zatsepina (Russia)

**Local Organizing Committee**

*Prof. Gábor Szabó*
Lóránt Székvölgyi, Ph.D.
Gábor Szalóki, M.Sc.
Éva Hegedüs, Ph.D.
László Imre, M.Sc.
György Fenyőfalvi, M.D.
András Szántó, M.Sc.
Péter Nánási, M.D.
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General Schedule

Aug. 19.
14.00 Arron  
14.00-18.00 Registration, mounting of posters  
18.00-18.15 Conference opening  
18.15-18.25 Introductory remarks about Wilhelm Bernhard (Klaus Scherrer, Paris, France)  
18.25-19.15 Wilhelm Bernhard Medal Lecture 2013  
19.15-19.50 Session A/1: Nuclear architecture  
19.50-22.30 Welcome reception, poster viewing in the Hall of the Life Sciences Building

Aug. 20.
08.30-11.55 Session A/2: Nuclear architecture  
12.00-13.30 Lunch, poster viewing  
13.30-15.10 Session A/3: Nuclear architecture  
15.25-17.40 Session B/1: Chromatin structure and dynamics  
17.40-19.10 Dinner  
Evening Free program, cultural and touristic events of the Debrecen Flower Carnival 2013

Aug. 21.
08.30-12.15 Session B/2: Chromatin structure and dynamics  
12.30-14.15 Lunch  
13.15-14.15 Meeting of the International Committee  
14.30-16.30 Sightseeing in Debrecen (visit to the Reformed College of Debrecen, viewing Munkácsy’s Christ Trilogy in the Déri museum)  
17.00-23.00 Excursion to Paripa Csárda

Aug. 22.
08.30-12.15 Session C: Transcription, posttranscriptional processes  
12.30-14.00 Lunch, poster viewing  
14.00-18.10 Session D: DNA replication, recombination, repair  
18.45-19.45 Dinner  
20.30-22.00 Ferenc Snétberger concert

Aug. 23.
08.30-12.35 Session E: Pathological aspects, therapeutical targets  
12.35-14.00 Lunch, poster viewing  
14.00-17.35 Session F: Novel methodical approaches  
17.35-17.45 Conference closing, concluding remarks  
18.30- Banquet dinner, farewell party in the Assembly Hall of the old university building

Aug. 24.
Departure
Wilhelm Bernhard

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 ergastoblasm 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 Etrangères 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 Darracq (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.

In a philosophical essay entitled "Spiritualité et Neant dans la Science", completed a month before his death, Dr. Bernhard began with two quotations that expressed his themes: "Science sans conscience n’est que ruine de l’ame" (F. Rabelais, 1532)
"The only wisdom we can hope to acquire is the wisdom of humility. Humility is endless" (T.S. Eliot, "Four Quartets").

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 and 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 Ustron, Poland
2011 Riga, Latvia

The Wilhelm Bernhard Medal
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
2011 Klaus Scherrer
2013 Tom Misteli

Tom Misteli
Tom Misteli is an internationally renowned cell biologist who pioneered the use of imaging approaches to study genomes and gene expression in living cells. He is currently a senior investigator and associate director at NCI’s Center for Cancer Research, where he heads the cell biology of genomes group. He obtained his Ph.D. from the University of London and did postdoctoral training at Cold Spring Harbor Laboratory. His laboratory is interested in uncovering fundamental principles of spatial genome organization and applying this knowledge to the development of novel diagnostic and therapeutic strategies for cancer and aging.
Detailed Program

Aug. 19.

14.00-18.00  Registration, mounting of posters
18.00-18.15  Conference opening
18.15-18.25  Introductory remarks about Wilhelm Bernhard (Klaus Scherrer, Paris, France)
              Tom Misteli (Bethesda, USA)

A. Nuclear architecture

              regulation.
              Yegor S. Vassetzky (Paris, France)

19.50-22.30  Welcome reception, poster viewing

Aug. 20.

A. Nuclear architecture

This session is offered to the memory of Ilya Zbarsky, one of the pioneers of research on nuclear architecture.

Chairman: Anna von Mikecz

08.30-09.05  A2. From cell populations to single molecule analysis of chromosome folding.
              (Plenary lecture)
              Peter Fraser (Cambridge, UK)

09.05-09.30  A3. New models of the nucleus and chromosomes.
              Ronald Hancock (Québec, Canada)

09.30-09.55  A4. An interaction between telomeres and the nucleoskeleton affects chromosome structure.
              Steven Kosak (Chicago, USA)

09.55-10.20  A5. Involvement of nuclear phospholipids in nuclear structure and functions.
              Pavel Hozák (Prague, Czech Republic)

10.20-10.35  Break
Chairman: Ronald Hancock

10.35-11.00  
A6. Regulation of aging and metabolism by the *C. elegans* nuclear lamina.  
Yosef Gruenbaum (Jerusalem, Israel)

11.00-11.25  
A7. The role of eukaryotic genome spatial organization in regulation of transcription.  
Sergey Razin (Moscow, Russia)

11.25-11.50  
A8. Large scale chromatin organization: the case of PcG bodies.  
Ivan Raska (Prague, Czech Republic)

11.50-11.55  
Reminiscences to Prof. Zbarsky  
Yegor S. Vassetzky (Paris, France)

12.00-13.30 Lunch, poster viewing

Chairman: Beáta G. Vértessy

13.30-13.55  
Vasily V. Kuvichkin (Moskow, Russia)

13.55-14.20  
A10. Putative plant homologs of lamins in plants.  
Susana Moreno Díaz de la Espina (Madrid, Spain)

14.20-14.45  
Masahiko Harata (Sendai, Japan)

14.45-15.10  
Péter Vilmos (Szeged, Hungary)

15.10-15.25 Break

**B. Chromatin structure and dynamics**

Chairman: Susana Moreno Díaz de la Espina

15.25-16.00  
B1. Chromatin replication and epigenome maintenance. (EMBO Young Investigator lecture)  
Anja Groth (Copenhagen, Denmark)

16.00-16.25  
B2. Reconstitution of the human nuclear proteome after cell division through NLS modulation.  
Beáta G. Vértessy (Budapest, Hungary)
16.25-16.50
B3. Human inactive X chromosome is compacted through a Polycomb-independent SMACHD1-HBX1 pathway governed by XIST RNA. Chikashi Obuse (Hokkaido, Japan)

16.50-17.15
B4. Lodestar loads the cohesin complex onto the chromosomes in Drosophila. János Szabad (Szeged, Hungary)

17.15-17.40

17.40-19.10 Dinner

Evening Free program, cultural and touristic events of the Debrecen Flower Carnival 2013

Aug. 21.
Chairman: Lóránt Székvölgyi

08.30-09.05
B6. Nuclear architecture studied in space and time: current state and perspectives. (Plenary lecture)
Thomas Cremer (München, Germany)

09.05-09.30
B7. Barr body architecture and development studied with 3D super resolution fluorescence microscopy: X-inactivation is characterized by the collapse of a functional nuclear compartment present in active chromosome territories. Marion Cremer (München, Germany)

09.30-09.55
B8. Pronounced co-localization of immunoglobulin genes and their enhancers in transcription factories at the nuclear periphery in plasma cells. William Garrard (Dallas, USA)

09.55-10.20
B9. Nuclear topology of H3 histones revealed by structured illumination imaging. Christian Schöfer (Vienna, Austria)

10.20-10.35 Break
Chairman: Jürgen Bode

10.35-11.00
B10. Regulation of transcription at PML nuclear bodies. Peter Hemmerich (Jena, Germany)

11.00-11.25
B11. Cell differentiation is dependent on Caspase-medlated genome alterations. Lynn Megeney (Ottawa, Canada)
11.25-11.50  
**B12.** Single-strand discontinuities and R-loops mark higher-order chromatin domains.  
Gábor Szabó (Debrecen, Hungary)

11.50-12.15  
**B13.** Mouse nuclear MYOSIN I knock-out shows interchangeability and redundancy of myosin isoforms in the cell nucleus.  
Tomas Venit (Prague, Czech Republic)

12.30-14.15  
Lunch

13.15-14.15  
Meeting of the International Committee

14.30-16.30  
Sightseeing in Debrecen (visit to the Reformed College of Debrecen, viewing Munkácsy’s Christ Trilogy in the Déri museum)

17.00-23.00  
Excursion to Paripa Csárda

**Aug. 22.**

**C. Transcription, posttranscriptional processes**

Chairman: Pavel Hozák

08.30-09.05  
**C1.** Bimodal regulation of RNA polymerase II transcription kinetics by histone acetylation in single living cells. (Plenary lecture)  
Hiroshi Kimura (Osaka, Japan)

09.05-09.30  
**C2.** Temporal and spatial characteristics of the assembly of the exon junction core complex and associated proteins on Balbani ring gene pre-mRNPs/mRNPs \textit{in vivo}.  
Lars Wieslander (Stockholm, Sweden)

09.30-09.55  
**C3.** Genome-wide approaches allow dissecting signal-specific transcriptional regulation.  
László Nagy (Debrecen, Hungary)

09.55-10.20  
**C4.** Encode data and pervasive transcription - the question about the size of primary transcripts.  
Klaus Scherrer (Paris, France)

10.20-10.45  
**C5.** Contribution of histone variant H2A.Z isoforms to transcriptional activation in hyper-acetylated chromatin.  
Masayuki Kusakabe (Sendai, Japan)

10.45-11.00  
Break
Chairman: Lars Wieslander

11.00-11.25
Piotr Widlak (Gliwice, Poland)

11.25-11.50
Joanna Rzeszowska-Wolny (Gliwice, Poland)

11.50-12.15
C8. The role of transcription factors in control of ovarian functions.
Alexander Sirotkin (Nitra, Slovakia)

12.15-14.00 Lunch, poster viewing

D. DNA replication, recombination, repair

Chairman: Piotr Widlak

14.00-14.35
D1. Replication damage induced by topoisomerase- and poly(ADPribose)polymerase-induced protein-DNA complexes. (Plenary lecture)
Yves Pommier (Bethesda, USA)

14.35-15.00
D2. Topoisomerase I-DNA cleavage complexes impact on CpG island promoters.
Giovanni Capranico (Bologna, Italy)

15.00-15.25
Jorge B. Schwartzman (Madrid, Spain)

15.25-15.50
D4. Dual effect of heat shock on DNA replication and genome integrity.
Artem K. Velichko (Moscow, Russia)

15.50-16.05 Break

Chairman: Nikolajs Sjakste

16.05-16.30
Emmanuelle Fabre (Paris, France)

16.30-16.55
D6. Intrinsic homology-sensing and assembling property of chromatin.
Takashi Ohyama (Tokyo, Japan)

16.55-17.20
D7. Antagonistic DNA unwinding mechanisms of RecQ helicases.
Ralf Seidel (Munster, Germany)

17.20-17.45
D8. Heterochromatin Protein 1beta - a key factor in DNA repair and replication.
Jurek Dobrucki (Krakow, Poland)
Lóránt Székvölgyi (Debrecen, Hungary)

18.10-19.45  Dinner

20.30-22.00  Ferenc Snétberger concert

Aug. 23.

E. Pathological aspects, therapeutical targets
Chairman: Marion Schmidt-Zachman

08.30-09.05  E1.  Enhancing DNA damage foci persistence to promote cancer cell senescence: targeting chromatin and metabolism. (Plenary lecture)
Stephen J. Kron (Chicago IL, USA)

09.05-09.30  E2.  Reversible senescence - restescence - is a unique response of breast epithelial cells to prolonged cell cycle arrest.
Dean Jackson (Manchester, UK)

09.30-09.55  E3.  The LINC complex in nuclear function.
Elisabeth McNally (Chicago, USA)

09.55-10.20  E4.  The role of amyloid in nuclear function and dysfunction.
Anna von Mikecz (Duesseldorf, Germany)

10.20-10.30  Break

Chairman: Peter Hemmerich

10.30-10.55  E5.  Cell Cycle Control of genomic signaling.
Richard G. Pestell (Philadelphia, USA)

Frederick A. Dick (London, Canada)

11.20-11.45  E7.  Reversible polyploidy and parasexual phenomena in tumour cells induced by DNA and spindle damage.
Jekaterina Erenpreisa (Riga, Latvia)

11.45-12.10  E8.  Nucleolar demethylases NO52 and NO66: important players in the development of hematological malignancies.
Marion Schmidt-Zachman (Heidelberg, Germany)
12.10-12.35
E9. The role of proteinase inhibitors in the nucleus.
Natasha Kopitar-Jerala (Ljubljana, Slovenia)

12.35-14.00
Lunch, poster viewing

F. Novel methodical approaches
Chairman: Yosef Gruenbaum

14.00-14.35
F1. Changes to cellular water and ion content by nucleolar stress: investigation by a cryo-correlative nano imaging approach. (Plenary lecture)
Dominique Ploton (Reims, France)

14.35-15.00
Andrea Scharf (Duesseldorf, Germany)

15.00-15.25
F3. Liganded RXR display highly dynamic behavior governed principally by co-activator binding as revealed by single cell imaging.
György Vámosi (Debrecen, Hungary)

15.25-15.40
F4. Fluidigm products in single-cell gene expression analysis and in quantitative RT-PCR.
Radoslav Silar (BioTech-Europe, Czech Republic)

15.40-15.55 Break

Chairman: György Vámosi

15.55-16.20
F5. Specific inhibitors of nuclear transport; potential anti-virals.
David Jans (Clayton, Australia)

16.20-16.45
Jürgen Bode (Hannover, Germany)

16.45-17.10
F7. Antimutagenic and repair-stimulating derivative of 1,4-dihydropiridine AV-153 intercalates in DNA in a single strand break site between two pyrimidines.
Nikolajs Sjakste (Riga, Latvia)

17.10-17.35
F8. ChIP efficiency is changed by fixation-induced poly(ADP)ribosylation.
Sascha Beneke (Zurich, Switzerland)

17.35-17.45 Conference closing, concluding remarks
18.30- Banquet dinner, farewell party

*Aug. 24.*

Departure
Lecture abstracts
W1. NUCLEAR ARCHITECTURE AND DISEASE

Tom Misteli
National Cancer Institute, NIH, Bethesda, MD 20892, USA

In higher organisms, genomes are housed and function in the cell nucleus. While we have learnt a great deal in recent years about the sequence of genomes and the machinery that reads genome information, insights into how genomes function in the context of the architectural framework of the cell nucleus in a living cell are only now emerging. Several key concepts such as the existence of nuclear architectural proteins, the presence of distinct nuclear compartments, the non-random organization of genomes, and the dynamic nature of nuclear architecture are now recognized as driving genome function. Importantly, aberrations in nuclear architecture are now known to lead to diseases ranging from cancer to pre-mature aging. The elucidation of the cell biological properties of the genome will be essential to a full understanding of how genomes function both in health and disease.
Chromosomes are confined to chromosomal territories in the nuclear space. Translocations that lead to exchanges of chromosome arms may disrupt this cellular order and lead to relocalization of genes within the nucleus.

We have studied the localization of cyclin D1 (CCND1) and c-myc gene regions within the nuclei in normal human lymphocytes and the mantle cell lymphoma and Burkitt lymphoma cells and cell lines. We have shown that both CCND1 and c-myc genes were relocalized form the chromosomal periphery into the central perinucleolar region following translocation. We have also shown the presence of nucleolin-binding sites in the vicinity of the CCND1 gene and show that they upregulate transcription from the CCND1 promoter. We also show intensive binding of nucleolin to the CCND1 gene promoter after the translocation and also show that the chromatin organization of the translocated regions changes after the translocation and that these changes span over very large regions. The same pattern is observed in the Burkitt lymphoma, where c-myc is upregulated by nucleolin.

We propose a novel mechanism of carcinogenesis brought about by chromosomal translocations where the relocalization of chromosomal territories within the nuclear space leads to misregulation of translocated genes.

Keywords: Mantle cell lymphoma; Burkitt lymphoma; nuclear organization; epigenetics
A2. FROM CELL POPULATIONS TO SINGLE MOLECULE ANALYSIS OF CHROMOSOME FOLDING

Takashi Nagano1, Yaniv Lubling2, Tim Stevens3, Eitan Yaffe2, Stefan Schoenfelder1, Wendy Dean4, Ernest Laue3, Amos Tanay2, Peter Fraser1

1Nuclear Dynamics Programme, The Babraham Institute, Cambridge, UK; 2Department of Computer Science and Applied Mathematics, Weizmann Institute, Isreal; 3Department of Biochemistry, University of Cambridge, UK; 4Epigenetics Programme, The Babraham Institute, Cambridge, UK

The spatial organization of chromatin and chromosomes in the nucleus has been tightly linked to control of genome functions such as transcription, silencing, replication and repair. Analytical techniques to study higher-order chromatin and chromosome structure and nuclear organization genome-wide (3C, 4C, 5C and HiC) have revealed important aspects of long-range intra- and inter-chromosomal contacts, some of which have functional consequences. These methods all begin with millions of cells and can be used to infer average or probabilistic genome conformations. However, single-cell measurements by fluorescence in situ hybridization show that chromosome and genome conformations, though non-random, vary considerably from cell to cell, even in clonal populations of a single tissue type. Because chromatin and genome conformations are dynamic and preferential, rather than static and specific, multi-cell 3C-based methods are poor indicators of actual chromosome and genome conformation or structures. Here we present the results of a new single-cell Hi-C method that we have devised for high-resolution, single-molecule structural analysis of the mouse genome. We present results of computational analyses and 3D structural modeling of the single mouse X chromosome in male Th1 cells, and an exploration of functional genetic and epigenetic landscapes.
A3. NEW MODELS OF THE NUCLEUS AND CHROMOSOMES

Ronald Hancock

Laval University Cancer Research Centre, Québec, Canada

Our understanding of structure and functions in the nucleus and of the structure of chromosomes has been advanced considerably in recent years by concepts from polymer and colloid science and nanoscience, together with powerful methods for simulation of macromolecules and their interactions. In the nucleus chromatin, a giant linear polymer, is confined and crowded together with proteins and RNAs at a global concentration of hundreds of mg/ml [1]. In these conditions, macromolecules and their interactions are strongly influenced by entropic (also termed depletion) forces which are negligible in dilute solutions. Experiments and simulations show that these strong, specific, and subtle forces are responsible for many features of the nucleus and chromosomes. For example:

- loops in chromatin are predicted by simulations to form due to entropic effects on a linear polymer in confined conditions, as well as discrete chromosome territories due to the spontaneous segregation of unentangled, self-avoiding polynucleosome chains [2, 3];
- compartments containing particular macromolecules (eg PML bodies) are predicted by simulations to form as a result of entropic forces [4], as suggested by experiments [5], raising questions if compartments have specific “functions”.
- finding targets and propagation of signals are facilitated by anomalous diffusion in crowded conditions [6].
- metaphase chromosomes may be structured by entropic forces due to crowding by macromolecules in the surrounding cytoplasm. By mimicking this crowding using inert macromolecules, chromosomes can be isolated without the polyamines and cations usually used [7]. They contain irregular chromatin fibres, consistent with the observed absence of 30 nm fibres [8] and with predictions for polynucleosome chains packed by crowding [1].

References:

A4. AN INTERACTION BETWEEN TELOMERES AND THE NUCLEOSKELETON AFFECTS CHROMOSOME STRUCTURE

Ashley Wood\textsuperscript{1}, Jannie Rendtlew Danielsen\textsuperscript{3}, Catherine Lucas\textsuperscript{1}, Shauna Houlihan, David Scalzo\textsuperscript{3}, Takeshi Shimi\textsuperscript{1}, Robert Goldman\textsuperscript{1}, Erica D. Smith\textsuperscript{1}, Michelle Le Beau\textsuperscript{4}, and Steven Kosak\textsuperscript{1}

\textsuperscript{1}Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA; \textsuperscript{2}Department of Neurology, Feinberg School of Medicine, Northwestern University, Chicago, IL 60611, USA. \textsuperscript{3}Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle Washington 98109, USA; \textsuperscript{4}Section of Hematology/Oncology, Department of Medicine and Comprehensive Cancer Research Center, The University of Chicago, Chicago, IL, 60637, USA

The linearity of eukaryotic chromosomes provides unique structural and functional considerations. Telomeres protect the ends of linear genomes, and the gradual loss of these ends is associated with cellular aging. Telomere safeguarding involves the insertion of the 3' overhang - facilitated by telomere repeat binding factor 2 (TRF2) - into telomeric DNA, forming t-loops. We present evidence indicating that t-loops can also form at interstitial telomeric sequences, forming an interstitial t-loop (ITL). We demonstrate by chromatin immunoprecipitation (chIP) that TRF2 association with interstitial telomeric sequences is stabilized by colocalization with the nuclear lamina protein, lamin A/C. Lamins are intermediate filament proteins located beneath the inner nuclear membrane and throughout the nucleoplasm. Mutations in the gene that encodes lamin A/C, \textit{LMNA}, are associated with Hutchinson Gilford Progeria Syndrome (HGPS), a pre-mature organismal aging disorder. We find that lamin A/C molecularly interacts with TRF2, and that these proteins are upregulated at similar times during cell differentiation. Furthermore, reduction in levels of lamin A/C as well as HGPS mutations in \textit{LMNA} lead to reduced ITL and dramatic telomere loss. We suggest that cellular and organismal aging are intertwined through the effects of the interaction between TRF2 and lamin A/C on chromosome structure.
A5. INVOLVEMENT OF NUCLEAR PHOSPHOLIPIDS IN NUCLEAR STRUCTURE AND FUNCTIONS

Yildirim, S., Sobol, M., Filimonenko, V., Castano, E., Filimonenko, A. and Pavel Hozák
Department of Biology of the Cell Nucleus, Institute of Molecular Genetics ASCR, Viteňská 1083, 142 20 Prague 4, Czech Republic

The nucleus is a highly organized cell compartment, where controlled gene expression, DNA replication, and RNA processing occur. These processes are spatially ordered via the nucleoskeleton, which is involved in nuclear compartmentalization and critical for nuclear functioning [1]. In spite of the growing interest and extensive research concerned to the nuclear organization, so far mostly protein complexes have been found as important for spatial nuclear ordering. We describe here novel structures containing phosphatidylinositol 4,5-bisphosphate (PIP2) which seem to contribute as well. Based on scarce literature data relating to PIP2 presence in interchromatin granule clusters and in the nucleolus, we carried out ultrastructural mapping of PIP2-containing structures using pre-embedding immunolabeling and 3D electron tomography, together with molecular biology experiments. Cell cycle, growth, and overall protein synthesis are dependent on RNA polymerase I (Pol I) transcription. Transcriptionally active cells organize rRNA production in discrete locations of the nucleolus that sequester different steps of RNA synthesis and ribosome biogenesis, although how these structures are organized in detail remains unknown. We found that PIP2 is present on the active ribosomal promoter during the transcription, and PIP2 depletion reduces Pol I transcription which can be rescued by the addition of exogenous PIP2. We then identified direct binding of PIP2 to transcription initiation factor UBF which makes a complex with Pol I in the nucleolus. In addition, PIP2 also binds directly to the pre-rRNA processing factor, fibrillarin, in a transcription-dependent manner. PIP2 binding to UBF and fibrillarin causes conformational changes in these proteins that are important for their binding to nucleic acids. Ultrastructural tomography showed that PIP2-containing structures propagate through the nucleolus where they connect individual fibrillar centers (containing enzymes and transcription factors for ribosomal DNA transcription) and the dense fibrillar component (where the transcription and maturation of rRNA takes place). These results indicate that PIP2 mediates the formation of pre-initiation complex via binding to UBF and Pol I, making a structural platform for RNA Pol I transcription, pre-rRNA processing and formation of nucleoli. In addition to PIP2 presence in nucleoli and in interchromatin granule clusters, the PIP2-positive structures stretch into the nucleoplasm where they appear as previously undescribed 70-100 nm roundish “lipid islets”. We mapped the elemental content of these islets using electron energy-loss microscopy. They appear surrounded by chromatin, and carbon mapping showed high density of organic compounds inside the islets indicating that lipids might be the main inner constituents of these structures. To reveal the plausible functions of these PIP2-containing islets, we mapped mutual localization of PIP2 with nuclear proteins involved in transcription, splicing, and higher order chromatin organization using advanced immunogold electron microscopy and super-resolution light microscopy. We show that at the periphery of the islets, PIP2 co-localizes or is located in immediate vicinity with nascent transcripts, pre-lamin A, LAP2α, H3K4me2, and H3K9me2. Direct binding and mobility assays also showed nucleoplasmic interactions between PIP2 and nuclear myosin I (NM1), which is a part of chromatin remodelling complex B-WICH [2] and promotes Pol I and Pol II transcription [3, 4]. Furthermore, the recruitment of lamin A into NM1-bound lipo-protein complex via interactions with PIP2 was demonstrated. We also revealed the association of PIP2 with core histones in pull-down experiments, and showed that the mobility of histone H2B depends on PIP2. Since lamin A is involved in chromatin remodelling via binding to DNA directly or to BAF and core histones, we propose that PIP2 might modulate the state of chromatin by interactions with NM1, core histones and lamin A. Taken together, this data allow us to suggest that PIP2 plays an important role in the organization of chromatin architecture and thus in regulation of gene transcription.

References:

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Hutchinson-Gilford Progeria Syndrome (HGPS) is a premature aging disease caused by several mutations in the LMNA gene. Progerin, a LMNA splice variant causing HGPS, is also expressed at a low level in the normal population and has been casually linked to histone modifications, transcription regulation and increased DNA damage. However, lamin has yet to be linked to any of the major lifespan regulating pathways, thus leaving a gap in our understanding of its role in normal and premature aging. Dietary restriction acts via conserved pathways to enable better cell maintenance and prolongs lifespan and health-span in multiple organisms. Hallmarks of dietary restriction are reduction in body size and fat content. Our data show that in the nematode Caenorhabditis elegans, multiple aspects of dietary restriction are regulated by lamin, including animal length and fat content, in a pathway mediated by ataxin-2 and dependent on S6K and SREBP. Furthermore, we show that lamin regulates expression level of ataxin-2 and that ataxin-2 regulates the shape of the nuclear envelope. These data have direct relevance to our understanding the mechanisms behind both premature and normal aging.
A7. THE ROLE OF EUKARYOTIC GENOME SPATIAL ORGANIZATION IN REGULATION OF TRANSCRIPTION

Sergey V. Razin1,2,3, Ekaterina S. Gushchanskaya1,2,3, Arkadiy K. Golov1,2 Olga V. Iarovaia1,3, Alexey A. Gavrilov1

1Institute of Gene Biology of the Russian Academy of Sciences, 119334 Moscow, Russia; 2Faculty of Biology, M.V. Lomonosov Moscow State University, 119992 Moscow, Russia; 3LIA 1066 French-Russian Joint Cancer Research Laboratory, Villejuif, France – Moscow, Russia

Development of a panel of the so-called C-methods – experimental protocols that allow the study of the 3D organization of the eukaryotic genome – has permitted to make observations resulting in a new concept in molecular genetics. It became evident that the 3D genome organization constitutes a part of epigenetic mechanisms essential for maintaining the identity of differentiated cells. In this respect, the assembly of distant regulatory elements in common activating complexes – active chromatin hubs – appears to be of primary importance. Here we show that one of the principal assumptions behind the C-methods is not correct. All C-methods are based on the “proximity ligation” which is preferential cross-ligation of interacting DNA fragments that remain joined by protein bridges after solubilization from formaldehyde-fixed nuclei. We show that the proximity ligation in the 3C procedure really occurs within non-lysed nuclei inside a cage formed by cross-linked chromatin fibers. This finding allows a new interpretation of the results of 3C analysis. Our data suggest that regulatory elements participating in formation of an active chromatin hub do not necessarily form a common complex stabilized by protein bridges, but rather are recruited to the same nuclear compartment where they retain a certain degree of mobility. This model is further supported by demonstration that treatment of nuclei by agents inducing decompaction of chromatin performed before the fixation with formaldehyde results in a decrease of 3C signal.
A8. LARGE SCALE CHROMATIN ORGANIZATION: THE CASE OF PcG BODIES

Smigova, J.¹, Juda, P.¹, Bartova, E.² and Ivan Raska¹

¹Charles University in Prague, First Faculty of Medicine, Institute of Cellular Biology and Pathology, Czech Republic; ²Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Brno, Czech Republic

The interphase nucleus of eukaryotes is a highly compartmentalized organelle. Its inner content can be categorized into a chromatin compartment and an interchromatin compartment. The interchromatin compartment harbors numerous distinct protein/RNA-based subcompartments that contain little or no DNA. Such nuclear subcompartments offer a mechanism by which to concentrate and spatially segregate nuclear activities. For example, nuclear bodies represent the local accumulations of a set of resident proteins and RNAs in the interchromatin compartment and offer a microenvironment that can improve the efficiency and fidelity of protein-DNA or protein-RNA interactions that are important for gene expression. Specifically, a Polycomb (PcG) body is considered to be the accumulation of PcG proteins, and it has been proposed that the PcG body acts as a gene silencing factory in the interchromatin compartment. However, the structural basis of the PcG body is still under debate. Diverging reports on protein-based versus chromatin-based nature of the PcG body were published. In order to expand the information on the nature of the PcG bodies in U-2 OS cells, we focused on the behaviour of the PcG proteins encompassed in fluorescent PcG bodies under conditions of hyperosmotically induced molecular crowding that allows to differentiate between interchromatin and chromatin compartments. We observed under these conditions that the PcG bodies disappeared, but persisted as nuclear domains characterized by accumulations of DNA. We found that the disappearance of PcG proteins from the original PcG bodies was associated with their hyper-phosphorylation. Importantly, fluorescence microscopy results showed that the changes observed in hyperosmotically treated cells were quickly reversible after re-incubation of cells in normal medium. We conclude that the PcG body in U-2 OS cells is not a nuclear body, but a chromatin domain.

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A9. NEW MODEL OF NUCLEAR PORE COMPLEXES BASED ON LIPIDS-DNA INTERACTIONS

Vasily Vladimirovich Kuvichkin

Institute of Cell Biophysics, Russian Academy Sciences, 142290, Pushchino, Moscow reg, Russia

The lipid-DNA interactions have been studied for more than 40 years. DNA forms complexes with lipids (DLC) both in vitro and in vivo. We studied interactions between DNA and lipids, organized in liposomes in presence of Me$^{2+}$ - ternary complexes (TC): DNA zwitterionic liposomes - Me$^{2+}$ [1]. Membrane vesicles forming the nuclear envelope with pores are analogs of such liposomes.

In accordance with our new nuclear pore model [2] DLC arise in the chromatin areas with three-stranded hybrids: DNA- low molecular weight RNA (lmwRNA) at their interactions with two membrane vesicles (~70 nm in diameter). The melting temperature of DNA/lmwRNA triple helix is considerably lower than in the same sequence of double-stranded DNA. That can specify preferential attachment of triple-stranded hybrids to the membrane vesicles. The triple helix unwinding during fusion of two nuclear membrane vesicles results in pre-pore structure: DNA-lmwRNA hybrid and a single-stranded DNA (ssDNA) located on the outer diameter of fused “big vesicle”. The “big vesicle” during interaction with double nuclear membrane can form nuclear pore channel, fusing two membranes. This process has several stages and involves surface tension of nuclear membranes and “big vesicle”. The ability of their fusion during close contact is basic condition of pore formation. At the same time ssDNA and hybrid DNA/lmwRNA shifts to pore annulus. Interactions nucleoporins with ssDNA and dsDNA /lmw RNA hybrid in pore annuli form the final structure of the pore complex.

Nuclear pores thereby serve as sites of initiation of transcriptions in a cell, because ssDNA is the best site of transcription initiation. The number of pores in a nucleus specifies the set of genes attached to nuclear envelope with DLC formation. Such attachment of any gene to a nuclear envelope results in enhanced level of expression of this and neighboring genes.

Not all “big vesicles” reach nuclear membrane and turn into pores. Some of them far from the nuclear membrane also enhance transcription. The “big vesicles” can form aggregates which are the basis for building “transcription factories”. The “big vesicles” aggregates of different chromosomes allow to observe the “chromosome kissing” effect.

We propose new mechanism of gene expression regulation and consider the reason of widely-distributed diseases (cancer, Alzheimer’s disease etc) as failure of such gene regulation mechanism.

References.

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A10. PUTATIVE ANALOGS OF LAMINS IN PLANTS

Ciska, M.¹, Masuda, K.² and Susana Moreno Díaz de la Espina¹

¹Centro Investigaciones Biológicas (CSIC), Madrid, Spain; ²Graduate School of Agriculture, Hokkaido University, Sapporo, Japan

Although the organization of the metazoan and plant nuclear lamina is similar, plants lack genes for lamins, which are the main components of the metazoan lamina, and also for most of the lamin-binding proteins. Thus, plants have a lamina not based on lamins and the characterization of the proteins organizing this structure in them is one of the hot topics in the field. In amongst other candidates, members of the plant NMCP/LINC family of proteins which localize to the lamina and share the typical tripartite structure but not sequence similarity with lamins [1, 2, 3, 4] are good candidates to play lamin functions in plants.

NMCP genes are highly conserved in plants except for unicellular algae. The bioinformatic analysis of the NMCP family revealed two types of proteins: NMCP1 (two genes in dicots and one in monocots) and NMCP2 (a single gene). All NMCPs have a highly conserved rod domain formed by two coiled coils separated by a linker. They have highly conserved regions at each end of the rod domain and at the positions of the predicted linkers, a similar pattern to that observed in lamins where these domains mediate head to tail association. The tail domain of the protein is less conserved but contains several specific conserved regions: an NLS, a putative actin-binding site, a stretch of acidic amino acids and a conserved motif at the C-terminus of the protein [4]. The endogenous AcNMCP1 has a molecular weight higher than predicted suggesting post-translational modification of the protein. It is an abundant component of the lamina, although it is also present in the internal nucleoskeleton; and its expression is developmentally regulated in the root [4]. The results demonstrate many similarities of NMCP proteins with lamins (structural organization, position of conserved regions, subnuclear distribution, solubility and expression). Additionally the functional analysis of their mutants suggests that they fulfil some function in maintenance of nuclear shape and size as lamins [2, 5, 6]. Further analysis of the protein partners of NMCP/LINCs and of their involvement in lamin-related functions will contribute to the establishment of a functional homology.

References:

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A11. ACTIN FAMILY PROTEINS INVOLVED IN THE FUNCTIONAL ORGANIZATION OF THE NUCLEUS

Masahiko Harata

Graduate School of Agricultural Science, Tohoku University, Sendai, Japan

Genome function is governed by both the organization of DNA into chromatin and the spatial arrangement of chromatin within the nucleus. As candidates for evolutionarily conserved molecules involved in both chromatin and nuclear organization, we have investigated the actin family, consisting of conventional actin and actin-related proteins (Arps). The Arps are evolved from a shared common ancestor with actin and are classified into ten subfamilies from Arp1 to Arp10. Whereas only a limited amount of actin is present in the nucleus, we have previously found that Arp subfamilies 4, 5, 6, 7, 8, and 9 are localized predominantly in the nucleus [1]. The nuclear Arps have been found in chromatin remodeling and modification complexes as functionally essential components, mostly, together with actin. These observations indicate that the actin family contributes to chromatin function through the activity of these complexes. In addition, we recently showed that nuclear Arps are required for the spatial positioning of chromatin in the nucleus both in yeast and vertebrates [2, 3]. These observations indicate that nuclear Arps are involved extensively in the hierarchical organization of the nucleus. Although the mechanisms by which the nuclear Arps contribute to nuclear organization are still unknown, it has been reported that nuclear Arps directly interact with actin in vitro. Therefore, it is attractive to hypothesize that cross-talk between actin family members is involved in nuclear organization. Indeed, the knockdown of the nuclear Arp4 in human cells increases nuclear actin and also the reactivity of F-actin probes in the nucleus. Another feature of the nuclear actin family, namely the possible cooperation of nuclear Arps and the myosin family will be also discussed.

References:


A12. NUCLEAR FUNCTION FOR A CYTOSKELETAL ACTIN BINDING PROTEIN, MOESIN

Kristó, I., Erdélyi, M., Péter Vilmos

Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

The most dynamic component of the cytoskeleton in every eukaryotic cell is the microfilament network of linear polymers of actin subunits. Extensive research in the past decade has significantly broadened our view about the role actin plays in the life of the cell and added at least two novel aspects to actin research. One of these is the discovery of the existence of nuclear actin which became evident only recently. Another new and interesting perspective in actin research is the investigation of the role actin plays in mitotic chromosome segregation: despite long-held skepticism recent works seem to have revealed the existence of the actin-based, so-called spindle matrix.

Members of the actin binding Ezrin-Radixin-Moesin (ERM) protein family of vertebrates are major regulators of actin dynamics in the cytoplasm. In Drosophila melanogaster, Moesin is the sole representative of the ERM protein family. To the present view Moesin, similarly to its vertebrate counterparts, is responsible for the crosslinking of membrane proteins to the cortical actin network. Surprisingly, we found both in cultured cells and Drosophila embryos that beyond its characteristic cytoplasmic distribution Moesin is present in the interphase nucleus and during mitosis it localizes to the mitotic spindle. In prophase Moesin level rapidly increases in the nucleus and the protein co-localizes with the actin network surrounding the mitotic spindles throughout mitosis. Based on the findings that the mitotic localization of Moesin meets all the distinguishing requirements for spindle matrix components and that it exhibits genetic interaction with them, we conclude that Moesin is a new member of the spindle matrix.

With the help of Drosophila polytene nuclei we also investigated whether the nuclear localization of Moesin has any biological significance or the protein is only stored in the interphase nucleus to facilitate nuclear reorganization during the first steps of mitosis. We found that in the interphase nucleus Moesin accumulates at the nuclear envelope and can be detected both in the nucleoplasm and on the chromosomes where it binds actin in a very dynamic manner. We also found that Moesin transportation into the nucleus is an active process, however the single nuclear localization signal predicted so far plays no role in nuclear import. Our results further revealed that Moesin localizes to the euchromatic regions where it is part of the transcription complex, strongly suggesting that Moesin plays role in transcription.

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B1. CHROMATIN REPLICATION AND EPIGENOME MAINTENANCE

Constance Alabert\(^1\), Jimi-Carlo Bukowski-Wills\(^2\), Sung-Bau Lee\(^1\), Georg Kustatscher\(^2\), Kyosuke Nakamura\(^1\), Flavia de Lima Alves\(^2\), Patrice Menard\(^1\), Jakob Mejlvang\(^1\), Juri Rappsilber\(^2,3\) and Anja Groth\(^1\)

\(^1\)Biotech Research and Innovation Centre (BRIC) and Centre for Epigenetics, University of Copenhagen, Ole Maaløes Vej 5, 2200 Copenhagen, Denmark; \(^2\)Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh EH9 3JR, United-Kingdom; \(^3\)Department of Biotechnology, Technische Universität Berlin, 13353 Berlin, Germany.

In dividing cells, faithful duplication of the genome must be accompanied by reproduction of the chromatin landscape on newly synthesized DNA. Our research focuses on how human cells replicate chromatin and ensure transmission of genetic and epigenetic information during cell division.

Recently, we have developed an explorative proteomic approach, Nascent Chromatin Capture (NCC), to profile chromatin replication in human cells. NCC relies on biotin-dUTP labeling of replicating DNA and cross-linking, followed by affinity-purification of tagged chromatin and quantitative proteomics. By comparing nascent newly synthesized chromatin with mature post-replicative chromatin, we provide association dynamics for 3995 proteins. Highly enriched in nascent chromatin, we find the complete human replisome followed by central players in chromatin assembly and cohesion establishment. In contrast, a large set of chromatin proteins including histone H1 show delayed association. By combining NCC enrichment with experimentally derived chromatin probabilities, we also use the data set to identify new replication factors. Together, this provides an extensive resource to understand DNA replication and restoration of chromatin organization. In addition, this proteomic instrument provides opportunity to address transmission of histone marks and replication stress responses. I will discuss our latest progress using NCC technology to understand chromatin replication and epigenome maintenance.
B2. RECONSTITUTION OF THE HUMAN NUCLEAR PROTEOME AFTER CELL DIVISION THROUGH NLS MODULATION

Gergely Róna1, Zsuzsanna Környei2, Mary Marfori3, Máté Borsos1, Máté Neubrandt2, Ildikó Scheer1, Judit Tóth1, Anna Magyar4, Emília Madarász2, Zoltán Bozóky1, Jonathan J. Ellis3, Ahmed M. Mehdi3, Mikael Bodén3, László Buday1, Bostjan Kobe3, Beáta G. Vértessy1,4

1Institute of Enzymology, RCNS, HAS, Budapest, 2Institute of Experimental Medicine, HAS, Budapest, 3School of Chemistry and Molecular Biosciences, Univ. Queensland, Brisbane, 4Dept. Organic Chem. Eötvös Univ. Budapest, 4Dept. Applied Biotech., BME, Budapest

Phosphorylation adjacent to nuclear localization sequences (NLSs) of cargo proteins is known to regulate nucleocytoplasmic transport. We identify that such phosphorylation of dUTPase, essential for genomic integrity, by cyclin dependent kinase 1 (Cdk1) results in dynamic cell-cycle dependent distribution of the protein. Non-phosphorylatable mutants drastically alter protein re-import into the nucleus during the G1 phase. We determine the molecular basis of this regulation by biophysical methods. Our 3D structural data indicate that phosphorylation of other Cdk1 substrates occurring at analogous NLS sites might result in the same regulatory pattern. By a computational screen of the human proteome, we identify numerous proteins possessing Cdk1 consensus sites at this specific NLS position. Creating a cellular assay, we validate these findings for proteins involved in DNA repair, epigenetics, RNA editing/splicing, and other key processes. Our study argues for a dynamically scheduled Cdk1-driven mechanism that regulates the nuclear proteome during the cell cycle.

Highlights
- Cdk1 phosphorylation of dUTPase at the M phase impedes re-import into the nucleus
- 3D structures of importin-NLS complexes reveal the mechanism of this regulation
- In silico/cellular screens show overall proof-of-concept for trafficking control
- General mechanism governs cell cycle-dependent re-shaping of nuclear proteome
B3. HUMAN INACTIVE X CHROMOSOME IS COMPACTED THROUGH A POLYCOMB-INDEPENDENT SMACHD1-HBIX1 PATHWAY GOVERNED BY XIST RNA

Nozawa, R. S.¹, Nagao, K.¹, Shibata, S.¹, Nozaki, N.², Sado, T.³, Kimura, H.⁴ and Chikashi Obuse¹

¹Graduate School of Life Science, Hokkaido University, Sapporo, Japan; ²Mab Institute Inc., Sapporo, Japan; ³Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan; ⁴Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan

In human female cell nuclei, the inactive X chromosome (Xi) forms a compact structure called the Barr body. On human Xi, two repressive epigenetic marks, the trimethylations of histone H3 Lys9 (H3K9me3) and Lys27 (H3K27me3), are distributed as distinct domains, and the XIST RNA preferentially coats the H3K27me3 domains. Here, we show that the Xi compaction requires HBix1, a heterochromatin protein (HP1)-binding protein, and SMCHD1, which harbors the SMC hinge and GHKL-ATPase domains, and is known to be important for X inactivation in mice. HBix1 and SMCHD1 were enriched on both H3K9me3 and H3K27me3 domains of Xi, in an XIST-dependent manner. The localization of HBix1 on the H3K9me3 and XIST/H3K27me3 territories were mediated through interactions with HP1 and SMCHD1, respectively. Furthermore, HBix1 was required for the localization of SMCHD1 to the H3K9me3 domains. The depletion of HBix1 or SMCHD1, but not the polycomb repressive complex 2 (PRC2), resulted in Xi decompaction, in a similar manner to XIST depletion. Thus, the molecular network involving HBix1 and SMCHD1 links the H3K9me3 and H3K27me3/XIST-coated domains to organize the compact Xi structure.

References:

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B4. LODESTAR LODES THE COHESIN COMPLEX ONTO THE CHROMOSOMES IN DROSOPHILA

János Szabad¹, Szilárd Szikora¹, Tamás Szalontai¹ and Imre Gáspár¹, ²

¹Department of Biology, Faculty of Medicine, University of Szeged, Szeged, Hungary; ²European Molecular Biology Laboratories, Heidelberg, Germany

ldsHor-D is one of dominant female-sterile mutations in Drosophila. The initial cleavage divisions are not completed due to abnormal chromosome segregation in embryos of the ldsHor-D-carrying females. During spermatogenesis ldsHor-D (i) induces mostly equational nondisjunction and (ii) renders chromosomes unstable such that they can be lost in the descending embryos leading to the formation of e.g. XX/0, female/male embryos, gynandromorphs. ldsHor-D is of dominant negative nature and originated through a single base pair replacement mutation in the lodestar gene. The encoded A777T-Lodestar mutant protein is “sticky” and is responsible for all the ldsHor-D-related defects. ldsHor-rvP2 is a loss-of-function (null) lodestar allele. The ldsHor-rvP2/--; hemizygous females are sterile: their embryos die due to unusual cleavage divisions and the elimination of the abnormal nuclei. Both ldsHor-D and ldsHor-rvP2 (in hemizygous condition) induce high frequencies of Y chromosome loss in the Drosophila wing primordia. Highlighting and monitoring a number of components in the Drosophila embryos and in the dividing neuroblast cells in the presence and in the absence of the Lodestar protein clearly showed that this protein is required to lode the cohesin complex onto the chromosomes.
B5. NUCLEOSOME-NUCLEOSOME STACKING: A MAJOR ELEMENT OF CHROMATIN STRUCTURE

Nikolay Korolev¹, Abdollah Allahverdi¹, Alexander P. Lyubartsev¹, Yanping Fan, Chun-Fa Liu², Lars Nordenskiöld¹

¹School of Biological Sciences, Nanyang, Technological University, Singapore; ²Department of Materials and Environmental Chemistry, Arrhenius Laboratory, Stockholm University, Stockholm, Sweden

Folding of genomic DNA into compact but dynamic eukaryotic chromatin is driven by attractive interactions between nucleosome core particles (NCPs) and is inherently related to regulation of DNA replication, transcription and repair. However, little is known about molecular structure of the NCP-NCP contacts as well as about the nature and scale of forces involved. Major component of chromatin, DNA, is highly negatively charged polymer and as a consequence chromatin folding is sensitive both to ionic environment and to sequence and modifications of positively charged histone N-termini (tails). Combining experimental and modelling approaches, we study nucleosome-nucleosome interaction in both general polyelectrolyte and specific structural context. Using novel synthetic organic chemistry/molecular biology methods, folding and intermolecular association of the NCPs and chromatin arrays are studied in dependence of ionic conditions and presence of posttranslational modifications in the histone tails. Also, computational modelling is used to investigate NCP-NCP interaction taking into account atomistic details of the nucleosome structure and adequately describing strong electrostatic forces acting in chromatin. Most experimental data is well-described by electrostatic model. However, NCP-NCP close contact (stacking) shows enhanced sensitivity to presence of the H4 histone tail and to acetylation of lysine 16 in this histone (H4K16Ac) which is beyond electrostatics. Surprisingly, we have experimentally found that in addition to the strong effect of H4K16Ac, chromatin displays similar unfolding behaviour in the presence of major cation of cell cytoplasm, K⁺, while very similar Na⁺ ions promote chromatin compaction. Based on our experimental and modelling data as well as on the analysis of the NCP crystal structures, we propose a novel structural model for exceptional influence of H4 K16 acetylation on chromatin compaction. Also we explain molecular mechanism for different effect of K⁺ and Na⁺ on the nucleosome stacking.
B6. NUCLEAR ARCHITECTURE STUDIED IN SPACE AND TIME: CURRENT STATE AND PERSPECTIVES

Thomas Cremer

Biocenter, Ludwig Maximilians University (LMU), Grosshaderner-Street 2, 82152 Martinsried, Germany

Super resolution fluorescence microscopy has narrowed the resolution gap to electron microscopy with the added potential of ultrastructural studies of nuclei in living cells. We have used structured illumination microscopy (SIM) (Markaki et al. 2012) to compare the functional organization of nuclei in a variety of cell types and species. Further, we established a protocol for correlative microscopy for sequential studies of individual cells with live cell microscopy, SIM and transmission electron microscopy (TEM) (Hübner et al. 2013). Correlative microscopy combines the particular strength of each approach and compensates for its specific limitations. New evidence from mouse embryonic stem cells (see lecture of Marion Cremer), cells in bovine pre-implantation embryos and human hematopoietic cells supports the chromosome territory – interchromatin compartment (CT-IC) model (Cremer and Cremer 2010). Active CTs form a threedimensional network of clustered chromatin domains (CDs), characterized by a compact chromatin core enriched in H3K27me3, a marker for transcriptionally silent chromatin, and a less compact periphery, called the perichromatin region (PR) (Rouquette et al. 2009 and 2010). RNA polymerase II and H3K4me3, a histone modification typical for transcriptionally competent chromatin is enriched in the PR, which lines and also invades a network of interchromatin compartment (IC) channels. This channel network harbors nuclear bodies, pervades all CTs and has direct access to the nuclear pores. The IC channel system and the PR form the active nuclear compartment (aNC), whereas the inactive nuclear compartment (iNC) is represented by the compact chromatin interior of CD-clusters.

References:
B7. BARR BODY ARCHITECTURE AND DEVELOPMENT STUDIED WITH 3D SUPER RESOLUTION FLUORESCENCE MICROSCOPY: X-INACTIVATION IS CHARACTERIZED BY THE COLLAPSE OF A FUNCTIONAL NUCLEAR COMPARTMENT PRESENT IN ACTIVE CHROMOSOME TERRITORIES

Marion Cremer, Smeets, D., Markaki, Y., Schmid, V., Schermelleh, L., Crèmer, T.

Ludwig Maximilians University (LMU), Munich, Germany; Department of Biochemistry, University of Oxford, Oxford, UK

X chromosome inactivation in female mammals, driven by Xist-RNA spreading, leads to chromatin compaction of the inactive X (Xi) territory, with Barr body formation as a structural nuclear hallmark. Using 3D-structured illumination microscopy we (I) explored the spatio-temporal process of Barr body formation in early differentiating mouse embryonic stem cells (mESCs) and (II) compared the three-dimensional ultrastructure of Xi with active chromosome territories (CTs) in man and mouse. We demonstrate that all CTs form a network of structurally linked clusters of chromatin domains (CDs) with an estimated size of ∼1Mbp. CDs of active CTs are lined by a layer of low density chromatin, the perichromatin region (PR), which is enriched for H3K4me3 and RNAPII, denoting transcriptionally competent chromatin. This PR network is co-aligned with a contiguous channel system, the interchromatin compartment (IC), which starts at nuclear pores and pervades CTs. PR and IC form together an intricate active nuclear compartment (ANC) where transcription occurs. The Barr body differs from active CTs by a closing up of CD clusters and a partially collapsed ANC, largely lacking H3K4me3 and RNA Pol II. Yet, the rudimentary channels maintain access to nuclear pores and may be essential for the structural integrity of Xi and for providing accessibility of factors required for (sparse) transcription and replication. Xist-RNA foci, associated with the nuclear matrix protein SAF-A localize within the rudimentary ANC suggesting an interaction with silenced genes by blocking the access of transcription machineries to Xi. In early differentiating mESC initial Xist-RNA spreading precedes Barr body formation which occurs together with subsequent RNAP II exclusion. Activation of an inducible autosomal Xist transgene in male mESCs triggers the formation of an ‘autosomal Barr body’ with less compacted chromatin and incomplete RNAP II exclusion demonstrating the requirement of the X-chromosomal context for a typical Barr body.

References:


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B8. PRONOUNCED CO-LOCALIZATION OF IMMUNOGLOBULIN GENES AND THEIR ENHANCERS IN TRANSCRIPTION FACTORIES AT THE NUCLEAR PERIPHERY IN PLASMA CELLS

Sung-Kyun Park, Yougui Xiang and William T. Garrard

Department of Molecular Biology, UT Southwestern Medical Center, Dallas, TX 75390-9148, USA.

A single plasma cell is estimated to secrete its own mass in antibody molecules each day. To uncover some of the molecular secrets behind such remarkably high levels of gene expression, we have investigated the nuclear organization of transcribing immunoglobulin (Ig) genes at various stages of mouse B cell development, utilizing 3D RNA immuno-FISH and ChIP-3C-Seq technologies. After the pro-B cell stage, ∼80% of Igκ and IgH genes are bi-allelically transcribed. As B cells differentiate to become better suited for high-level expression of antibody molecules, their Ig genes become progressively co-localized in transcription factories, which peaks at the plasma cell stage, with ∼48% co-localization between Igκ-IgH, Igκ-IgJ, and IgH-IgJ gene pairs. Remarkably, ∼26% of the plasma cell nuclei exhibit triplet Igκ-IgH-IgJ gene co-localizations in transcription factories, with ∼45% of the transcribing Ig genes located within <0.5µm from the nuclear periphery. In plasmablasts we identified the trans-chromosomal interacting sequences between Ig genes in transcription factories as their enhancer elements. Moreover, plasma cells from Igκ gene E3′ knockout mice exhibit significant reductions in Ig gene co-localizations at transcription factories, nuclear periphery locales, and Ig gene transcript levels. Furthermore, Ig gene transcripts arising from genes co-localized in the nuclear interior are channeled together between dense chromatin regions from their sites of synthesis toward a subset of nuclear pores. We conclude that plasma cells have optimized coordinated transcriptional and post-transcriptional control of Ig genes by co-localizing them in transcription factories often near the nuclear periphery through their transcriptional enhancers.

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B9. NUCLEAR TOPOLOGY OF H3 HISTONES REVEALED BY STRUCTURED ILLUMINATION IMAGING

Snyers, L., Almeder, M., Weipoltshammer, K. and Christian Schöfer
Department for Cell & Developmental Biology, Medical University of Vienna, Vienna, Austria

Nucleosomal histone H3 exists in several variants and has been implicated in genome organization. Most H3 variants are incorporated into nucleosomes in a replication-dependent manner; interestingly however, the histone H3 variant H3.3 has been shown to be incorporated in a transcription-dependent manner [1]. Histone H3.3 can thus be considered as a marker for transcriptionally active chromatin. It has been furthermore suggested, that H3.3 as well as the canonical, replication-dependent histones H3.1 and H3.2 might be preferentially associated with active chromatin, constitutive and facultative heterochromatin, respectively [2]. It was our aim to study the distribution of the mentioned histone H3 variants in the interphase nucleus and during the cell cycle of mammalian cells and to correlate their topological distributions with nuclear compartments, including chromosome territories, nucleoli, and nuclear speckles.

We expressed histone variants marked with fluorescent proteins in normal and malignant cells and correlated the expression pattern with detection of specific DNA sequences using fluorescent in situ hybridization (FISH) or with markers localized by immunofluorescence detection using conventional confocal microscopy and SIM (structured illumination microscopy).

We found that histone H3.3 largely overlapped with markers indicative of transcriptional activity, corroborating the finding that H3.3 is incorporated into chromatin in a transcription-dependent way. In contrast, markers for transcriptionally silent chromatin showed higher colocalization with H3.1 and H3.2 than with H3.3. We then correlated H3.3 distribution with nuclear compartments. It was surprising though to find that ribosomal genes were not or only very weakly labelled with histone H3.3. This interesting finding may indicate a different nucleosomal architecture or nucleosomal dynamics in (active) ribosomal genes than in genes transcribed into mRNA molecules. We next correlated the expression pattern of histone H3 variants with chromosome territories and found that none of the histones is confined to particular regions within the chromosome territories. In particular, H3.3 was found distributed throughout territories demonstrating that transcriptional activity is not confined to particular regions of the territory. Correlation of H3 histones and SC-35, a marker for nuclear speckles (a.k.a. interchromatin granules), showed that most speckles are devoid of H3 histones including H3.3. However, H3.3 appears often accumulated at the periphery of speckles indicating a close spatial vicinity of speckles and transcriptionally active sequences, which however does not extend deep into the speckles. In mitotic chromosomes, we observed a banding pattern after expression of histone H3 variants which was most conspicuous with histone H3.3. These bands were largely exclusive when compared with banding staining that predominately label silent chromatin. It thus suggests that this banding pattern may represent the transcriptome of the previous interphase.

In summary, we could show that the expression patterns of histone H3 variants could be detected in interphase nuclei and mitotic chromosomes. Structured illumination imaging allowed precise localisation of histones in the context of nuclear chromatin which goes far beyond resolution obtained by conventional fluorescent microscopy. Using these techniques we were able to elucidate some structural-functional relationships in the interphase nucleus and found a distinct banding pattern in mitotic chromosomes.

References:
B10. REGULATION OF TRANSCRIPTION AT PML NUCLEAR BODIES

Tobias Ulbricht, Mohammad Alzrigat, Anna von Mikecz, Viktor Steimle, and Peter Hemmerich

Leibniz-Institute for Age Research, Fritz-Lipman-Institute, Jena, Germany

Promyelocytic leukemia (PML) nuclear bodies are macromolecular assemblies in cell nuclei of almost all human tissues. Usually, nuclei of cultured cells contain approximately 5-30 PML bodies, ranging in size from ~0.2 to 1 μm. Despite extensive research over the past 20 years the precise biochemical function of PML bodies is still unknown. Current models predict that a subset of nuclear proteins may be functionally modified or sequestered at PML bodies.

We have analyzed the role of PML bodies in regulation of the MHC class II gene cluster. We showed that PML is required for efficient IFN-γ-induced MHC II gene transcription through protection from proteasomal degradation of class II transactivator (CIITA), the main transcriptional regulator of MHC class II genes. Live cell imaging revealed that PML isoform II specifically forms a stable complex with CIITA at the periphery of PML bodies. Stabilization of key nuclear factors at PML bodies may be a general biochemical mechanism by which these structures are involved in the regulation of gene expression.
B11. CELL DIFFERENTIATION IS DEPENDENT ON CASPASE-MEDIATED GENOME ALTERATIONS. CASPASE ACTIVITY DIRECTS A COMPLEX DNA DAMAGE/REPAIR RESPONSE TO PROMOTE DIFFERENTIATION

Lynn A. Megeney

The Sprott Centre for Stem Cell Research, The Ottawa Hospital Research Institute, The Ottawa Hospital; Depts. of Medicine and Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON.

Cell differentiation is an adaptation common to all multi-cellular organisms. This complex cell fate is characterized by extensive cytoskeletal changes and genome reorganization, changes that are also conserved features of programmed cell death. Interestingly, caspase proteases are required for the differentiation of a wide variety of cell types, yet it remains unclear how these apoptotic proteins could promote such a cell fate decision. Caspase signals often result in the activation of the specific nuclease caspase activated DNase (CAD), suggesting that cell differentiation may be dependent on a CAD mediated modification in chromatin structure. Here we demonstrate that viable muscle cell nuclei are subject to widespread CAD dependent transient DNA strand breaks during early stages of the skeletal muscle differentiation program. Inhibition of caspase 3 or reduction of CAD expression leads to a dramatic loss of strand break formation and a block in the myogenic program. The global nature of the strand break pattern strongly suggests that the cell must utilize a defined DNA repair process to ensure long term survival of the differentiated cell. Indeed, myoblast differentiation appears to be characterized by robust activation of the base excision repair (BER) pathway, a pattern that immediately follows CAD mediated DNA damage. Loss of key BER factors, such as the scaffold protein XRCC1, lead to a dramatic attenuation in differentiated myoblast viability. In addition to the temporal CAD directed DNA break/repair response, we hypothesized that caspase mediated signals may target multiple proteins to induce a chromatin structure that is conducive to differentiation. Interestingly, we have noted that caspase 7 activation also acts to modify the differentiation process through targeted cleavage of the nuclear matrix protein SATB2, liberating a restrictive chromatin milieu. These results demonstrate that the transient activation of effector caspase enzymes results in profound alterations in the DNA/chromatin microenvironment, biochemical modifications that are essential for cell differentiation.
B12. SINGLE-STRAND DISCONTINUITIES AND R-LOOPS MARK HIGHER-ORDER CHROMATIN DOMAINS

Székvölgyi, L., Hegedüs, É., Fenyőfalvi, G., Szántó, A., Nánási, P., Imre, L. and Gábor Szabó
University of Debrecen, Medical and Health Science Center, Dept. Biophysics and Cell Biology, Hungary

Live, nonapoptotic eukaryotic cells, from yeast to human, disassemble into ~ loop-size ds fragments upon protein denaturing treatments. Applying comet assay run in field inversion mode we could analyze DNA fragmentation also at the individual cell level, having concluded that ds fragmentation is related to endogeneous ss breaks and that these nicks may be juxtaposed with RNA/DNA hybrids [1.]. These structures have been identified as R-loops, sensitive to RNA polymerase inhibitors. We have developed a method to label with biotinylated nucleotides the immediate vicinity of nicks, and a reverse South-Western (rSW) blot procedure to detect, separately in the complementary strands [2.], incorporated labeled nucleotides and R-loops using anti-biotin and anti-RNA/DNA hybrid antibodies, respectively. Making use of these procedures, we mapped endogeneous nicks and R-loops in HCHO-fixed S. cerevisiae, by ChIP-chip on tiling arrays, and by rSW within the repetitive rDNA locus. These studies confirmed the co-localization of these two structures, in line with our complementary results on mammalian cells. Having developed a laser scanning cytometry (LSC) based method to measure superhelicity and size of the DNA loops present in the „halos” of salt-extracted mammalian cell nuclei, we could show that the nuclear domain harbouring nicks and the other domain exhibiting superhelicity, are topologically separated, in G1, S as well as in G2 phases of the cell cycle.

References:
[1.] Székvölgyi et al., PNAS 104(38):14964, 2007
[2.] Hegedüs et al., NAR 37(17):e112, 2009

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B13. MOUSE NUCLEAR MYOSIN I KNOCK-OUT SHOWS INTERCHANGEABILITY AND REDUNDANCY OF MYOSIN ISOFORMS IN THE CELL NUCLEUS

Venit, T., Dzijak, R., Rohozkova, J., Kalendova, B. and Hozak, P.

Department of Biology of the Cell Nucleus, Institute of Molecular Genetics, ASCR, v.v.i., Prague, Czech Republic

Nuclear myosin I is a nuclear isoform of the well-known “cytoplasmic" Myosin 1c protein. Located on the 11th chromosome in mice, NM1 results from an alternative start of transcription of the Myo1c gene adding an extra 16 amino acids at the N-terminus [1]. Previous studies revealed its roles in RNA Pol I and RNA Pol II transcription [2, 3], chromatin remodeling [4], and chromosomal movements [5]. It was thought, that nuclear localization signal is localized within first 16 amino acids. However, we discovered that is localized in the middle of the myosin molecule and therefore directs both Myosin 1c isoforms to the nucleus.

In order to trace specific functions of the NM1 isoform, we generated mice lacking the NM1 start codon without affecting the cytoplasmic Myo1c protein. Mutant mice were analyzed in a comprehensive phenotypic screen and strikingly, no obvious phenotype related to previously described functions has been observed. However, we found minor changes in bone mineral density and the number and size of red blood cells in knock-out mice, which are probably related to previously undocumented functions of NM1 in the cytoplasm. Moreover, in Myo1c/NM1 depleted U2OS cells, the level of Pol I transcription was restored by overexpression of shRNA-resistant mouse Myo1c. The ratio between Myo1c and NM1 proteins is similar in the nucleus and deletion of NM1 did not cause any compensatory overexpression of Myo1c protein. Finally, we found that Myo1c is in interaction with Pol II.

We therefore suggest that both isoforms can substitute each other in their nuclear functions.

References:

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C1. BIMODAL REGULATION OF RNA POLYMERASE II TRANSCRIPTION KINETICS BY HISTONE ACETYULATION IN SINGLE LIVING CELLS

Hiroshi Kimura and Timothy J. Stasevich

Graduate School of Frontier Biosciences, Osaka University, Osaka, Japan

In eukaryotes, post-translational histone modifications play an important role in gene regulation. A variety of residue-specific modifications have been linked to RNA polymerase II activity, but it remains unclear if these marks are active regulators of transcription or just passive byproducts. This is because most studies rely on fixation, which limits temporal resolution to five or ten minutes at best. As well, most studies make measurements on cell populations, so correlated factors may not actually be present in the same cell at the same time. Complementary approaches are therefore needed to probe the dynamic interplay of histone modifications and RNA polymerase II with higher temporal resolution in single living cells. A case in point is histone acetylation. This mark has been linked to all stages of the RNA polymerase II transcription cycle, but it remains uncertain if these links represent a single regulatory mechanism (and its downstream consequences), several distinct regulatory mechanisms, or are merely side-effects of transcriptional activity with no potentiating action.

To address these questions, we developed a system to monitor the dynamic interplay of residue-specific histone modifications and RNA polymerase II phosphorylation in single living cells by fluorescence microscopy, increasing temporal resolution to the tens-of-seconds range. Our single-cell analysis using Fab-based live endogenous modification labeling (FabLEM; ref 1, 2) reveals natural fluctuations in histone H3 Lysine 27 acetylation levels can alter downstream transcription kinetics by as much as 50%. This is achieved by two independent mechanisms. First acetylation enhances the search kinetics of transcriptional activators, and second acetylation accelerates the promoter-escape rate of initiated RNA polymerase II. Signatures of the latter can be found genome-wide using ChIP-Seq. We argue this bimodal regulation leads to a robust and potentially tunable transcriptional response.

References:


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C2. TEMPORAL AND SPATIAL CHARACTERISTICS OF THE ASSEMBLY OF THE EXON JUNCTION CORE COMPLEX AND ASSOCIATED PROTEINS ON BALBIANI RING GENE PRE-mRNPs/mRNPs IN VIVO

Petra Björk and Lars Wieslander
Department of Molecular Biosciences, WGI, Stockholm University

The exon junction complex, EJC, associates with the pre-mRNP/mRNP in a splicing dependent but non-sequence specific way at a region approximately 20 nucleotides upstream of exon-exon junctions. eIF4AIII, the Y14-Mago heterodimer and Barentsz (Btz)/MLN51 form the EJC core that serves as a platform for interactions with a multitude of other proteins in the nucleus and in the cytoplasm, thus being important for export, quality control, translational control and localization. The Balbiani ring genes allow identification of their pre-mRNPs during synthesis and their mRNPs during intranuclear transport in situ. We have used these genes to investigate when and where the EJC core components as well as several EJC associated proteins interact with the pre-mRNPs/mRNPs. We are interested in how association with the pre-mRNP/mRNP is related to nuclear processes such as transcription, splicing, intranuclear movement and export through the nuclear pore complex.
C3. GENOME-WIDE APPROACHES ALLOW DISSECTING SIGNAL-SPECIFIC TRANSCRIPTIONAL REGULATION

László Nagy
Department of Biochemistry and Molecular biology, University of Debrecen Hungary

RXR is an obligatory component of heterodimeric nuclear receptors, but might also act as a homodimer to regulate gene expression. RXR signaling is likely to have a major impact in macrophages, but the genomic basis of its ligand-activation is unknown. Genome-wide studies were carried out to map transcriptional changes, nascent RNA production and cistromic interactions. We identified a restricted network of active enhancer among the 5200 RXR bound genomic regions. Active enhancers are characterized by an increase of enhancer RNA, P300 recruitment and PU.1 release. Using these features as filtering criteria 387 liganded-RXR bound enhancers were linked to 226 genes. The distribution of enhancers is symmetrical relative to TSSs and the examined long-range enhancers communicate with promoters via stable or RXR-induced loops. A set of angiogenic genes (Vegfa, Hbegf, Cxcl2, Litaf, Hipk2, Foxo3) has liganded-RXR controlled enhancers and provides the macrophage with a modular, partly hidden vasculogenic program.
C4. ENCODE DATA AND PERVERSIVE TRANSCRIPTION - THE QUESTION ABOUT THE SIZE OF PRIMARY TRANSCRIPTS

Klaus Scherrer

Institute Jacques Monod, Paris, France

The recent publication of the second wave of ENCODE data in 2012 signifies decisive progress to understand gene expression in eukaryotic cells. The fact that most of the genome is transcribed in the life cycle of animal organisms, and of about 60% in differentiated cells, implies that the DNA-genome is carried over to the RNA level and that most of regulation of gene expression is post-transcriptional. Transcription mapping by micro-array techniques has shown its limitations due to a background of 5% at best; this signifies that much of actual consensus about the transcription landscape has to be revised. Primary transcripts are rare, too rare to show up next to abundant RNA in micro-array mapping. However, the recent RNA-Seq methods allow to pick up rare transcripts, with a resolution of up to 1 in 1000. Such analysis shows that regions upstream to genes are transcribed over tens of kb, as well as intronic and downstream areas. It follows that most of Cis-Regulatory Modules (CRMs) of promotor, enhancer and other TF-binding sites are transcribed and, hence, the putative regulatory sequence information might be carried over to RNA. However, the most potent high throughput methods have thus far been unable to decide, whether the primary transcripts span such domains as single molecules or if there is a patchwork of transcripts each with its own transcription start site (TSS). It makes a conceptual difference in terms of regulation if full domain transcripts (FDTs) are produced from LTRs, to be processed by differential splicing into mRNA and, possibly, non-coding RNA fragments, or if the set of exons corresponding to a gene are copied from a proximal promotor, independently of the upstream or downstream transcripts. We have to resort to "old" - but still relevant - data, which may allow to decide in favor of one of these models and bring into focus the issue of FDTs.

At an early time (1970), the breakthrough of transcription-mapping by EM using the Miller/Hamkalo techniques has shown that not only pre-rRNA in nucleoli, but also bona-fide pre-mRNA in tissue culture cells is made forming "christmas trees" which, most often, embrace large DNA domains. Furthermore, in lampbrush chromosomes, all the DNA-loops are transcribed indicating full genome transcription in oogenesis; a notion now extended by the recent ENCODE data to normal interphase cells. Working over 40 years on the avian alpha-globin genes, we were able to show by more and more refined molecular biology and biochemical methods that up to 33 Kb long globin RNA is made. Most important, pulse-labeling showed that there is no RNA made corresponding by size to the exon-intron structure of the 3 individual globin genes: such RNA shows up later on, most likely after initial processing. Unfortunately, it was never technically possible to show direct conversion of such transcripts into globin mRNA. Nevertheless, the notion that the 33 kb FDTs are precursors of the individual globin pre-mRNAs, processed eventually into globin mRNA, seems the most likely model.
C5. CONTRIBUTION OF HISTONE VARIANT H2A.Z ISOFORMS TO TRANSCRIPTIONAL ACTIVATION IN HYPER-ACETYLATED CHROMATIN

Kusakabe, M.1, Matsuda, R.1, Kitamura, H.1, Hori, T.2, Fukagawa, T.2 and Masahiko Harata1

1Graduate School of Agricultural Science, Tohoku University, Sendai, Japan; 2National Institute of Genetics, Mishima, Japan

Exchange of the canonical histones with non-allelic histone variants regulates epigenetic events. H2A.Z is one of the most evolutionarily conserved histone variants. H2A.Z has been implicated in a number of epigenetic events, including transcription and DNA repair. Recently, we demonstrated that vertebrates have two isoforms of H2A.Z, named H2A.Z-1 and H2A.Z-2, and that these variants have both redundant function and specific functions [1]. By using the deacetylase inhibitor Trichostatin A (TSA), we observed that H2A.Z isoforms are evicted from chromatin in a hyper-acetylated chromatin context. To elucidate the function of H2A.Z isoforms in transcriptional regulation further, we performed H2A.Z gene knockouts in DT40 cells, and established H2A.Z-1 single knockout, H2A.Z-2 single knockout, and H2A.Z double knockout (H2A.Z DKO) cell lines. We firstly searched for genes directly regulated by H2A.Z isoforms through microarray transcriptional analysis, and identified JUN and FOS as H2A.Z target genes. Whereas the expression of JUN and FOS genes were significantly activated by TSA in wild-type cells, this activation was completely abolished by the double knockout of H2A.Z isoforms, but not by the single knockouts. These results indicate that H2A.Z isoforms are required for the activation of the genes upon chromatin hyper-acetylation in a redundant manner. Quantitative chromatin immunoprecipitation (qChIP) analysis revealed the deposition of H2A.Z in both the promoter and the gene body of JUN and FOS genes, and also their eviction upon hyper-acetylation of chromatin by TSA. Together with reports indicating the involvement of H2A.Z in gene repression, we propose the possibility that eviction of H2A.Z in hyper-acetylated chromatin is one of the mechanisms by which histone acetylation activates transcription.

Reference:
C6. CO-REGULATION OF THE NF-KAPPAB SIGNALING PATHWAY BY HEAT SHOCK FACTOR 1

Janus, P. and Piotr Widlak

Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology; Gliwice, Poland

NFκB- and HSF1-dependent pathways are essential components of cellular responses to stress, which play the major role in pathogenesis of serious human diseases, including cancer and response to therapeutic treatments. Both of these transcription factors regulate several genes involved in cell proliferation, apoptosis, inflammation and immune response. Here we aimed to identify NFκB-dependent genes which expression is affected by the hyperthermia-induced HSF1.

Activation of the NFκB pathway and expression of NFκB-dependent genes was analyzed in human U-2 osteosarcoma cells stimulated with TNFα cytokine. Cells were either preconditioned with hyperthermia to activate endogenous HSF1 or engineered to express a constitutively active form of HSF1 in the absence of heat shock. The global chromatin binding of HSF1 was analyzed using the Chip-Seq approach in cells exposed to hyperthermia (genes with hypothetical κB or HSE elements were predicted based on in silico analysis).

We found that hyperthermia, but not the presence of constitutively active HSF1, resulted in a general suppression of the NFκB signaling activation. Additionally, both these factors specifically affected expression of several genes, which expression was modulated by TNFα cytokine. There were 323 genes identified responsive to the cytokine stimulation, 113 of them contained the NFκB-binding motif. Both hyperthermia and the presence of constitutively active HSF1 revealed similar antagonistic effect against cytokine stimulation in 54 of NFκB-responsive genes. There were 12 genes among them where actual promoter-binding of HSF1 was associated with suppression of their cytokine-induced stimulation. We concluded that this set represented a group of stress-related genes directly co-regulated by NFκB and HSF1 transcription factors.
B8. REACTIVE OXYGEN SPECIES AND THE REGULATION OF GENE EXPRESSION

Joanna Rzeszowska-Wolny

Biosystems Group, Institute of Automatic Control, Silesian University of Technology, Gliwice, Poland

Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radicals, or singlet oxygen are produced permanently in low quantities during cellular metabolism, but their levels are increased radically by external factors such as ionizing and UV-A radiation and chemical compounds, and also during cell cycle progression. The main producers of ROS in cells are mitochondria, where oxidants are formed predominantly by complex I or III of the cytochrome chain. Another important source of intracellular oxidants are oxidases that catalyze reactions involving molecular oxygen as the electron acceptor; some of which are widely expressed and evolutionarily conserved. Cellular oxidant and antioxidant levels influence the oxidized or reduced states of proteins and create redox-signaling pathways which can activate transcription factors, kinases, and phosphatases. ROS present in cells can also oxidize nucleic acids, and RNA shows an even greater level of oxidation than DNA in cells exposed to different oxidizing factors. The ability of ROS to induce structural changes in RNA suggests that ROS may also play a role in RNA interference where the efficiency of miRNA biogenesis and action depends on complementary interactions between RNA strands. Until now, oxidative modifications of nucleic acids have been considered rather as a byproduct damage which is deleterious for cells and may lead to mutation and pathology. We have studied ROS levels and their changes during the cell cycle and after X-ray exposure in different cells, and correlated them with changes in mRNA and miRNA levels. Our results support the hypothesis that ROS may modulate RNA interference and play a role in regulating gene expression at the level of RNA.

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C8. THE ROLE OF TRANSCRIPTION FACTORS IN CONTROL OF OVARIAN FUNCTIONS

Alexander Sirotkin

Animal Production Research Center Nitra, 951 41 Luzianky and The Constantine the Philosopher University, 949 01 Nitra, Slovakia

We are the first who studied the control and the role of transcription factors in the regulation of reproductive functions. Our presentation represent a short review of original data concerning external (temperature, calories restriction) and internal (hormones, protein kinases, RNA interference) in control of selected transcription factors (p53, NFkB, STAT, CREB), as well as their role in control of basic ovarian cell functions (proliferation, apoptosis, secretory activity, response to hormonal stimulators).

It was shown, that stress (high temperatures, food restriction), hormonal regulators of reproduction (gonadotropins, GH, oxytocin, some growth factors), pharmacological or genomic regulators of protein kinases (protein kinase A, MAP kinase, CDC2 and other kinases), siRNAs and miRNAs control the expression of these transcription factors within porcine, rabbit and human ovarian cells. Furthermore, the transfection-induced overexpression of these transcription factors altered the proliferation (markers of cell cycle PCNA, cyclin B1, MAP kinase, CDC2 kinase/p34), both nuclear (TdT) and cytoplasmic (bax, bcl-2, caspase 3, p53) apoptosis, release of steroid (progesterone, testosterone, estradiol) and peptide (oxytocin, IGF-I) hormones and prostaglandins (F and G) by cultured ovarian granulosa cells, as well as their response to stress and hormonal treatments.

These observation demonstrate (1) the presence of transcription factors in ovarian cells, (2) the hierarchy of upstream regulators of ovarian transcription factors (environmental factors – hormones - protein kinases – miRNA – transcription factors), (3) the involvement of transcription factors in control of basic ovarian cell functions (proliferation, apoptosis, secretory activity and response to hormones) and (4) that transcription factors are mediator of the upstream regulators action on basic ovarian cell functions.
DNA is normally associated with proteins, such as histones, DNA polymerases, helicases and repair enzymes non covalently, which enables its copying, editing and repair. Yet, a subset of chromatin proteins associates with DNA covalently. DNA topoisomerase are the archetypical example of chromatin protein that form covalent DNA-protein complexes by reversibly linking their catalytic tyrosine to the end of the DNA break they generate to change DNA topology (supercoiling, intertwining, decatenation) [1, 2]. The normally transient topoisomerase cleavage complexes can however be trapped when the DNA contains preexisting damage (abasic sites, oxidized bases, nicks), or in the presence of anticancer drugs, which use this effect to kill cancer cells that are defective in DNA repair, checkpoints and survival pathways. The trapping of topoisomerase cleavage complexes gives rise to replication damage, especially in the case of camptothecin-induced topoisomerase I cleavage complexes (Top1cc).

Tyrosyl-DNA-phosphodiesterase 1 (TDP1), a key repair enzyme for Top1cc, hydrolyzes the phosphodiester bond between the DNA 3’-end and the Top1 tyrosyl moiety. Alternative repair pathways for Top1cc involve endonuclease cleavage. However, it is unknown what determines the choice between the TDP1 and endonuclease repair pathways. By generating TDP1 and PARP1 double-knockout chicken DT40 cells, we demonstrate that TDP1 is epistatic to PARP1 for the repair of Top1cc. We also show that the N-terminal domain of TDP1 directly binds the C-terminal domain of PARP1, and that TDP1 is PARylated by PARP1. PARylation stabilizes TDP1 and enhances TDP1’s recruitment to DNA damage sites. TDP1-PARP1 complexes, in turn recruit XRCC1 in camptothecin-treated cells. This work identifies PARP1 as a key component of Top1cc excision repair by TDP1.

Finally, we will review our recent findings demonstrating that PARP inhibitors trap PARP-DNA complexes, which is a key determinant of their anticancer activity in connection with replication damage [3].

References:
DNA Topoisomerase I (Top1) is required at transcribing regions to relax DNA supercoils generated by RNA polymerases, and is inhibited with high specificity by camptothecin, an effective anticancer agent. As CPT-induced Top1-DNA cleavage complexes (Top1ccs) can affect transcription and genome stability, we investigated the global molecular response to Top1ccs at transcriptional levels. We found that Top1ccs trigger an accumulation of antisense RNA polymerase II transcripts specifically at divergent active CpG-island promoters in a replication-independent and Top1-dependent manner. The accumulation of antisense transcripts was observed upstream to TSS (up to 5000 bp) of promoters of intermediate-to-high activity. Even though maximal accumulation of antisense transcripts required ongoing transcription, Top1ccs increased them even in the presence of DRB, a transcription inhibitor, suggesting that CPT triggers a reduction of the degradation of promoter-associated antisense RNAs. Moreover, kinetics investigations showed that Top1ccs and R-loops increased transiently at transcribed regions, leading to a substantial but transient block of RNA polymerases at promoters. The findings establish that a specific transcriptional response to Top1ccs occur at divergent active CpG-island promoters, which may include a transient stabilization of R-loops, and define molecular events of a response pathway leading to transcription-dependent genome instability.
D3. GEOMETRY AND PLASTICITY OF DNA DURING REPLICATION: 
THE BENEFIT OF DNA ENTANGLEMENTS

Jorge Cebrián\textsuperscript{1}, Víctor Martínez\textsuperscript{2}, María José Fernández\textsuperscript{2}, Christian Schaerer\textsuperscript{2}, Pablo Hernández\textsuperscript{2}, Dora B.Krimer\textsuperscript{1}, Jorge B. Schvartzman\textsuperscript{1}

\textsuperscript{1}Centro de Investigaciones Biológicas (CSIC), Ramiro de Maeztu 9, 28040, Madrid, España; 
\textsuperscript{2}Laboratorio de Computación Científica y Aplicada, FP-UNA, San Lorenzo, Paraguay

During replication, DNA molecules undergo topological changes that affect supercoiling, catenation and knotting. To better understand this process and the role of topoisomerases, the enzymes that control DNA topology in vivo, two-dimensional agarose gel electrophoresis (2Dgels) and atomic force microscopy (AFM) were used to examine partially replicated bacterial plasmids containing replication forks stalled at specific sites. The results obtained and computer simulations based on Metropolis Monte Carlo helped us to predict the thermodynamic stability of these molecules and to determine the potential energy that can be stored in the replicated and unreplicated regions.

Altogether, these observations strongly suggest that type II DNA topoisomerases recognize the geometry (sign and handedness) of DNA duplex crossings. This geometry changes continuously during replication and in partially replicated molecules differs also between the replicated and non-replicated regions. In addition, the data obtained revealed that DNA entanglements (supercoils and catenanes) play an active role in preventing the formation of deleterious knots.
Heat shock (HS) is one of the better-studied exogenous stress factors. However, little is known about its effects on DNA integrity and the DNA replication process. In this study, we show that in G1 and G2 cells, HS induces a countable number of double-stranded breaks (DSBs) in the DNA that are marked by γH2AX. In contrast, in S-phase cells, HS does not induce DSBs but instead causes an arrest or deceleration of the progression of the replication forks in a temperature-dependent manner. This response also provoked phosphorylation of H2AX, which appeared at the sites of replication. Moreover, the phosphorylation of H2AX at or close to the replication fork rescued the fork from total collapse. Collectively our data suggest that in an asynchronous cell culture, HS might affect DNA integrity both directly and via arrest of replication fork progression and that the phosphorylation of H2AX has a protective effect on the arrested replication forks in addition to its known DNA damage signaling function.
Chromosomes are non-randomly organized in the yeast nucleus. Subtelomeres in particular are found close to the nuclear periphery according to the genomic size of their chromosome arm, centromere attachment to the SPB and nucleolar volume. Subtelomeres, as well as other chromosomal loci, occupy "gene territories" recapitulated by computational models based on polymer physics.

We have questioned whether genomic organization could affect efficiency of double strand break (DSB) repair. Breaks of both strands of the DNA are the most dangerous insults to the genome’s integrity. DSBs can be repaired by homologous recombination (HR); in this conserved mechanism, a genome-wide homology search finds sequences similar to those near the break, and uses them as a template for DNA synthesis and ligation. How homologous sequences find each other is unknown.

We show that yeast genomic regions whose gene territories overlap recombine more efficiently than sequences located in spatially distant territories. Tethering of telomeres and centromeres reduces the efficiency of recombination between distant genomic loci, lowering the chances of non-allelic recombination.

Our results challenge current models that posit an active scanning of the whole nuclear volume by the broken chromosomal end; they demonstrate that search for homology is a limiting step in HR, and emphasize the importance of nuclear organization in genome maintenance.
The underlying mechanism of homology-sensing or homologous pairing of chromatin or chromosomes in the early prophase of meiosis I is a major mystery in cell biology. In 2007, we presented the first experimental evidence that double-stranded (ds) DNA molecules can distinguish "self" and "non-self" in the presence of physiological concentrations of Mg$^{2+}$ ions, and self-assemble even in a solution of heterogeneous dsDNA species (Inoue et al., 2007). Subsequently, other groups have confirmed this property of DNA (Baldwin et al., 2008; Danilowicz et al., 2009).

Recently, using AFM and a quantitative interaction assay, we obtained the first direct evidence for DNA sequence-dependent self-assembly of nucleosomes, in which selective association occurs between nucleosomes with identical DNA sequences (Nishikawa and Ohyama, 2013). This DNA sequence-dependent self-assembly of nucleosomes, as well as that of dsDNA, can explain the underlying mechanisms of not only meiotic chromosome pairing, but also mitotic chromosome pairing, including polytene chromosome formation in Diptera, transvection and other similar phenomena. We are currently examining whether DNA methylation influences DNA self-assembly and/or nucleosome self-assembly. A preliminary data suggested that self-assembly of nucleosomes is affected by DNA methylation under a certain condition. All these findings are likely to be a clue to solution of the mystery.
D7. ANTAGONISTIC DNA UNWINDING MECHANISMS OF RECQ HELICASES

Ralf Seidel

University of Münster, Münster, Germany

RecQ helicases play important roles in maintaining genome stability during replication and in controlling double-strand break repair by homologous recombination. Despite severe phenotypes that are seen in knockouts of RecQ helicases, little is known about the direct functions of the different RecQ helicases and how their specific activity is regulated. In this study we investigate how two RecQ helicases from Arabidopsis thaliana, RecQ2 and RecQ3, unwind short DNA hairpins using high resolution magnetic tweezers. This technique allows us to follow the unwinding of individual DNA substrates in real-time.

For RecQ2 we observe that after a hairpin is slowly unwound at about 7-9 bp/s, it abruptly reanneals (rezips). These unwinding-rezipping events occur in bursts of many repetitions which are caused by a single RecQ2 complex. We show that the helicase actively senses and even clamps the unwinding fork ahead of it. When it looses contact to the fork, it is mostly in a weakly bound sliding state on single-strand DNA and can be rapidly pushed back by the rezipping hairpin. Strand-switching then allows the helicase to reestablish contacts to the fork and reinitiate a new round of unwinding.

Remarkably, the behavior of RecQ3 is distinct. Hairpin unwinding by this enzyme proceeds only for 10-20 bp followed by equally slow rezipping of the hairpin due to single-strand DNA translocation after strand switching. Highly repetitive unwinding-rewinding events by a single enzyme are as well reinitiated by strand switching. Strikingly, on mechanically unzipped hairpins, RecQ3 stably rewinds rather than unwinds the hairpin in a processive ATP-driven fashion.

Thus, the two enzymes act antagonistically. While RecQ2 is optimized to keep short DNA structures open and thus to potentially provide accessibility for other proteins, RecQ3 is an effective DNA rewinder that may even help DNA annealing against single-strand binding proteins. These two opposing activities are, however, achieved by a very similar mechanism that involves cycles of directional changes due to strand-switching.
D8. HETEROCHROMATIN PROTEIN 1β-A KEY FACTOR IN DNA REPAIR AND REPLICATION


Division of Cell Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland

BACKGROUND. Heterochromatin protein 1 (HP1) is a small non-histone chromosomal protein, originally described as major component of heterochromatin and an epigenetic regulator. However, HP1 is emerging now as a multifunctional protein involved in several key nuclear processes. We have demonstrated that HP1α and HP1β are recruited to sites of oxidative DNA damage [1, 2, 3]; recruitment to other types of damage has also been demonstrated [4].

METHODS. Recruitment and dynamics of HP1β was studied using live cell imaging, fluorescence recovery after photobleaching (FRAP), fluorescence resonance energy transfer detected by lifetime microscopy (FLIM/FRET) and bimolecular fluorescence complementation assay (BIFC). Local DNA damage was induced by low intensity visible light, in the absence of exogenous photosensitizers [5].

RESULTS and DISCUSSION. We demonstrate that HP1β forms a complex with Proliferating Cell Nuclear Antigen (PCNA), a key factor involved in DNA replication and repair. The HP1-PCNA complex is engaged in DNA replication; it is also recruited to ss and ds DNA breaks [6]. FRET-FLIM live cell studies provide evidence of association and close proximity between HP1β and PCNA in the complex. FRAP demonstrates that HP1β-PCNA complexes are highly mobile in nonreplicating nuclei, but when engaged in DNA replication, within DNA replication foci, they are bound and do not exchange with the mobile pool [7]. HP1β, which is associated with PCNA in the regions of DNA repair, is bound and does not exchange with the mobile pool, suggesting that HP1β in association with PCNA may be a component of a DNA repair complex. The role of HP1 in DNA repair and replication remains unknown; we postulate that HP1β may act as a loading platform which recruits various factors involved in DNA repair and replication.

References

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Histone H3 lysine 4 trimethylation marks meiotic recombination hotspots, but how this ubiquitous chromatin modification relates to initiation of Spo11-dependent DNA double-strand breaks (DSBs) has remained elusive. Here we provide evidence for a cause-effect relationship between recombination initiation and histone H3K4me3 during yeast meiosis. We show that in vivo targeting
(i) Set1, the catalytic subunit of the histone H3K4 methyl transferase complex (COMPASS) and
(ii) Spp1, the PHD-domain component of COMPASS
to recombinationally cold regions is able to induce DSB formation. Interestingly, the presence of Spp1 at recombination sites is a prerequisite of hotspot activation independent of Set1 and histone H3K4 methylation. Spp1 physically interacts both with H3K4me3 and Mer2 - a key protein of the chromosomal axis and the “core” DSB complex – that tethers recombination hotspots to chromosomal axial sites, where Spo11-calalyzed DNA cleavage takes place at nucleosome-depleted regions.

Reference:
E1. ENHANCING DNA DAMAGE FOCI PERSISTENCE TO PROMOTE CANCER CELL SENESCENCE: TARGETING CHROMATIN AND METABOLISM


Ludwig Center for Metastasis Research and Depts. of Molecular Genetics and Cell Biology, Chemistry, and Radiation and Cellular Oncology, The University of Chicago, Chicago Illinois, USA

Despite diverse tissues of origin, malignant cells display a shared set of phenotypes that include loss of replicative senescence, activated metabolism and a combination of genetic and epigenetic changes. By contrast, cancer growth rates vary significantly, and are typically far slower than the most proliferative normal tissues such as mucosal epithelia. Rather than target cell proliferation, an attractive alternative would be to restore cancer cell mortality. While blocking telomerase might have some benefit, simply inducing unrepairable DNA damage via chemotherapy or radiotherapy should be sufficient to induce accelerated senescence. A common pathway mediates replicative and accelerated senescence wherein constitutive DNA damage signaling, resulting from the persistence of foci of modified chromatin that form to mark eroded telomeres or DNA damage along the arms, drives an irreversible cell cycle arrest. In examining how malignant cells are able to resist senescence, we observed a rapid resolution of DNA damage foci after irradiation. Histone deacetylase inhibitors, poly(ADP-ribose) polymerase inhibitors and dozens of other agents identified by screening libraries of small molecule drugs not only delayed foci resolution but also drove irradiated cancer cells into senescence.

Toward identifying mechanisms of action, we discovered several active agents targeted glucose uptake or other steps in glycolysis, suggesting that the Warburg effect of aerobic glycolysis might contribute to rapid DNA damage foci resolution. Indeed, blocking any of several steps in glycolysis and glutaminolysis induced foci persistence and restored the senescence response after irradiation. Further chemical genetic studies with small molecule probes implicate the α-ketoglutarate-dependent dioxygenases as key targets of cancer metabolic reprogramming to mediate both rapid foci resolution and resistance to senescence. A simple model suggests that maintaining high histone and/or DNA methylation levels after DNA damage via impairing α-ketoglutarate-dependent demethylase activity is able to promote rapid foci resolution, perhaps independent of completion of DNA repair. This unanticipated effect of cancer metabolic reprogramming and oncometabolites such as (R)-2-hydroxyglutarate may contribute to multiple features of neoplastic cells that enhance malignancy including genomic instability, resistance to genotoxic therapy and, of course, cell immortality.

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**E2. REVERSIBLE SENESCENCE - RESTESCENCE - IS A UNIQUE RESPONSE OF BREAST EPITHELIAL CELLS TO PROLONGED CELL CYCLE ARREST**

Apolinar Maya-Mendoza¹, Charles H. Streuli and Dean A. Jackson

*Faculty of Life Sciences and Wellcome Trust Centre for Cell-Matrix Research, University of Manchester, Oxford Road, Manchester, M13 9PT; ¹Present address: Department of Genome Integrity, Danish Cancer Society Research Centre; Copenhagen Denmark.*

Mammalian cells have limited replication potential, both in vitro and in vivo. As ageing cells approach their replicative lifespan they enter an irreversible senescent state in which no further cell division can occur. During normal proliferation cycles in human cells a major route for inducing senescence is through persistent activation of the DNA damage response, which may result, for example, from telomere attrition or persistent genotoxic stress. Because of the importance of protecting cells from damage, it is not surprising that in eukaryotes a series of complex mechanisms has evolved to counteract the effects of metabolic and exogenous stresses. Damage arising during DNA replication is a major source of mutagenic stress and the extent of damage dictates whether cells undergo transient cell cycle arrest and damage repair, senescence or apoptosis. Importantly, existing dogma defines these alternative fates as distinct choices. Hence, prolonged cell cycle inhibition in fibroblasts induced a DNA damage response and post-mitotic cell death. However, in clear contrast we have found that breast epithelial cells are able to survive prolonged S phase arrest and subsequently re-enter cycle after many days of being in an arrested, senescence-like state. Interestingly, this novel property of breast epithelial cells is neither classic senescence, nor senescence from which variant cells evolve. According we termed ‘restescence’ to describe the recovery of breast epithelia from a senescence-like cell cycle arrest, which is freely reversible. In contrast to profound levels of chromosomal damage seen in fibroblasts, highly efficient S phase arrest in breast epithelia minimised chromosome damage and protected sufficient chromatin-bound replication licensing complexes to support cell cycle re-entry. These observations have profound implications for breast cancer treatment because therapeutic strategies causing restescence may minimise the effectiveness of some drug regimens.
The LINC complex, which links the nucleus to the cytoplasm, includes nesprin and SUN proteins. The LINC complex provides a mechanical connection between the inner nuclear lamina, the inner and outer nuclear membranes and the cytoplasm. Mutations in LMNA, the gene encoding lamins A and C, and nesprins 1 and 2 lead to inherited diseases that affect both heart and muscle. More than 300 mutations have been reported in LMNA and most of these mutations exert their effect through a dominant negative mechanism. LMNA truncating mutations tend to cause more severe cardiac involvement. Missense LMNA mutations that link to heart and muscle disease distribute all along the length of lamins A and C. Lamins A and C are intermediate filament proteins that assemble as head to tail dimers, followed by aggregation into higher order filaments to provide a network of support and structure to the nuclear membrane. Lamins A and C are major components of the nuclear membrane of terminally differentiated cells where the nuclear lamina regulates an array functions ranging from nuclear transport to gene expression and chromatin binding. How mutations in a broadly expressed gene like LMNA cause striated muscle disease remains unknown, and a central question for understanding how the nuclear membrane functions. Lamin A has a rod like central region and an independently folding immunoglobulin-like domain in its carboxy terminal region referred to as a lamin A tail. Since Ig domains are heavily implicated in protein interactions, we conducted a proteomic analysis of proteins that bind to the tail region of lamin A. Proteins from the muscle cell line C2C12 were tested for their binding properties to lamin A tails, and the proteins were assessed by mass spectrometry. We compared the protein binding profile of normal and mutant lamin A tails, using wildtype lamin A tail to two different LMNA mutants. Of the 130 proteins identified using the method, nearly 20% were known lamin A binding proteins. We identified that proteins of diverse nuclear function as lamin A binding proteins including those implicated RNA binding, processing and splicing. These data suggest that LMNA mutations may disrupt an array of nuclear membrane functions in the laminopathies.

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E4. THE ROLE OF AMYLOID IN NUCLEAR FUNCTION AND DYSFUNCTION

Anna von Mikecz

IUF-Leibniz Research Institute for Environmental Medicine at Heinrich-Heine University, Duesseldorf, D-40225 Duesseldorf, Germany.

The occurrence of amyloid-like protein aggregation in age-associated neurodegenerative diseases and normal aging processes represents, on the background of aging societies, a good reason to analyze the composition of nuclear protein aggregates in order to identify molecular pathways. We characterize the endogenous protein aggregation landscape of the cell by unbiased proteomics and identify the nucleus as a major target of stepwise protein fibrillation that peaks with formation of amyloid-like microdomains. The nucleolus, nuclear speckle domains and spliceosomes are defined as amyloidogenic venues and prevalent components of the endogenous aggregome. A notable similarity between transitional steps of protein fibrillation and formation of different spliceosomal complexes in the splicing cascade is partial structural unfolding of protein components which may explain their aggregation propensity and our observation of amyloid-like spliceosomal microenvironments. Aggregome components that specifically occur in advanced protein fibrillation states may indicate a framework of both functional and aberrant protein aggregation pathways in the nucleus and represent promising targets of further investigations.
E5. CELL CYCLE CONTROL OF GENOMIC SIGNALING

Richard G. Pestell1,2

1,2Kimmel Cancer Center; 2Department of Cancer Biology Thomas Jefferson University, Philadelphia, PA 19107

Recent studies have identified novel molecular mechanism coordinating genomic signaling during cell cycle progression. The induction of G1/S phase cell cycle progression in mammalian cells requires the D type cyclins. Expression of the cyclins is tightly coordinated through induction by growth factor, oncogenic, and metabolic cues. The cyclin D1 encodes a protein that serves as the regulatory subunit of the holoenzyme that phosphorylates the retinoblastoma (pRB) protein to induce DNA synthesis, and phosphorylates the NRF1 protein to inhibit mitochondrial metabolism. In addition a DNA bound form of cyclin D1 coordinates gene expression.

Cyclin D1 regulates gene expression by altering the abundance of nuclear transcription factors (TF) recruited in the context of local chromatin. Cyclin D1 knockout tissues show reduced recruitment of TF associated with reduced recruitment of chromatin remodeling proteins, SUV39, HP1a, HDAC1/3 and cointegrators (P300,CBP) [1]. Cyclin D1 reintroduction restores recruitment of chromatin remodeling proteins, with corresponding changes in gene expression [2]. An unbiased genome wide analysis of cyclin D1 binding sites using ChIP-Seq, identified a hierarchy of cyclin D1 associated TF binding sites, both within the coding but also the non-coding genome [3]. TF binding sites include the CTCF, nuclear receptor binding sites, CRE/AP-1 sites and Oct/POU sites. The pathways in which these genes reside govern chromosomal stability (CIN). The induction of cyclin D1 is shown to induce CIN both in vitro and in vivo using mammary gland targeted inducible transgenic mice. Chromosomal aberrations were confirmed by spectral karyotyping and centrosome abnormalities confirmed by immunofluorescence. The induction of CIN occurs independently of the kinase binding function of cyclin D1. Thus the nuclear DNA binding form of cyclin D1 coordinates genomic stability. Interrogation of gene expression from 2,254 breast tumors identified cyclin D1 expression correlating with CIN in luminal B breast cancer. Cyclin D1 expression is induced early in tumorigenesis and may thereby contribute to tumor progression.

References:


E6. HAPLOINSUFFICIENCY OF AN RB-E2F1-CONDENSIN II COMPLEX CAUSES REPLICATION STRESS AND CONTRIBUTES TO MESENCHYMAL CANCERS

Courtney H. Coschi¹³, Charles Ishak¹³, Srikanth Talluri¹³, Alison L. Martens¹³, Ian Welch⁴, and Frederick A. Dick¹²³

¹London Regional Cancer Program; ²Children’s Health Research Institute; ³Department of Biochemistry; and ⁴Veterinary Services, University of Western Ontario, London, Ontario, Canada

Genomic instability is a characteristic of malignant cells, however, evidence for its contribution to tumorigenesis has been enigmatic. In this study we demonstrate that a complex containing the retinoblastoma protein, E2F1, and Condensin II localizes to major satellite repeats at pericentromeres. In its absence, DNA double strand breaks occur at pericentromeric repeats, with H2AX foci evident in mitosis and G1, a phenotype consistent with replication stress. Surprisingly, loss of even one copy of the retinoblastoma gene (RB1) was sufficient to cause DNA breaks and reduce recruitment of Condensin II to pericentromeres. Furthermore, we determined that RB1 mutation status correlated with copy number variation, chromosomal gains and losses, as well as chromothriptic rearrangements, in cancers of mesenchymal origin. Importantly, the magnitude of these chromosomal abnormalities was indistinguishable between RB1⁺⁻ and RB1⁻⁻ tumors. Lastly, using gene-targeted mice we determined that mutation of just one copy of the murine Rb1 gene causes sarcomas and lymphomas with increased chromosome copy number variation. Our study connects replication stress with a number of common chromosomal abnormalities in cancer through a dosage sensitive complex present at pericentromeric repeats.

This work was supported by MOP64253 from the Canadian Institutes of Health Research.
E7. REVERSIBLE POLYPLOIDY AND PARASEXUAL PHENOMENA IN TUMOUR CELLS INDUCED BY DNA AND SPINDLE DAMAGE

Jekaterina Erenpreisa1, Salmina, K.1, Kosmacek, E. A.2,3, Huna, A.1, Raskina, O.4, Belyayev, A.4, Ianzini, F.2, Hausmann, M.5, Cragg, M. S.6, Mackey, M. A.2

1Latvian Biomedical Research&Study Centre, Riga; 2University of Iowa, Iowa City, USA; 3University of Nebraska, Omaha, USA; 4University of Haifa, Israel; 5University of Heidelberg, Germany; 6University of Southampton, UK.

Recent data incline to think that genetic changes occurring during carcinogenesis create permissive environment for forbidden epigenetic programmes. In particular, reversible polyploidy induced in tumour cells by DNA and spindle damage is emerging as a potential mechanism of treatment resistance and has been shown to be associated with reprogramming to an embryonic stem cell-like state [1-3]. This is a protracted process occurring within 7-14 days, leading to the release of para-diploid clonogenic cells, initiating metastatic tumours in vivo. The modes of polyploidisation and depolyploidisation are far from being fully understood. Our observations on various irradiated, etoposide and paclitaxel treated tumour cell lines using FISH, immunofluorescence and live cell imaging analysis, suggest that this is a multi-step process, which includes parasexual phenomena, with features similar to meiosis and primitive kinds of syngamy. It involves segregation of whole genomes, followed by fusions, mitotic slippage and binucleation, true endomitosis – an analog of evolutionary meiotic prophase [4], segregation of sister-cohesed chromosomes, reductional divisions omitting S-phase, autonomic divisions and fusions of subnuclei, aborted multi-polar divisions followed by fusions, sorting and selective diminution of sub-nuclei, while cancer stem cell-like daughter cells appear within the cytoplasm of the polyploid mother with a proportion released as clonogenic descendants [5,6]. These processes are suggested as being adaptive, programmed, and related to evolutionary ontogenetic cycles.

References:

E8. NUCLEOLAR DEMETHYLASES NO52 AND NO66: IMPORTANT PLAYERS IN THE DEVELOPMENT OF HAEMATOLOGICAL MALIGNANCIES?

Gabrysch, E. and Marion Schmidt-Zachmann

German Cancer Research Center, Clinical Cooperation Unit for Molecular Hematology/Oncology, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

Histone lysine methylation has been implicated in both gene activation and repression depending on the distinct lysine (K) residue that is methylated as well as the number of methyl groups covalently attached. Enzymes containing a Jumonji C (JmjC) domain have been identified to act as histone demethylases and are supposed to be important in developmental control, cell-fate decision and disease.

The two nucleolar proteins NO52 and NO66 belong to this class of epigenetic modifiers although their distinct biological functions are still poorly defined. Performing overexpression and knockdown experiments we analyzed the influence of both proteins on e.g. rDNA transcription, rRNA processing, gene transcription, proliferation and cell cycle progression. Moreover, we studied the mechanisms regulating the expression of the two histone demethylases.

Hematological malignancies, in particular the acute myeloid leukemia (AML) are heterogeneous diseases with respect to morphology, immunophenotype and genetic rearrangements. Furthermore, epigenetic dysfunctions seems to have a central role in the pathology of myeloid malignancy. We used real-time PCR to determine the expression pattern of NO52/NO66 in human leukemia cell lines as well as patient material in order to explore the role(s) of these nucleolar proteins as potential prognostic biomarkers in AML. Most interestingly, protein NO66 might play a key role in controlling haematopoietic stem cell renewal or differentiation since it is missing in CD34+/CD38- stem cells but clearly detectable in CD34+/CD38+ progenitor cells.
E9. THE ROLE OF PROTEINASE INHIBITORS IN THE NUCLEUS

Maher, K. 1,2,5, Butinar, M. 1,2, Vasiljeva, O. 1, Turk, B. 1,4, Grigoryev, S. 5 and Natasha Kopitar-Jerala1

1Department of Biochemistry, Molecular and Structural Biology, Jožef Stefan Institute, Ljubljana SI-1000, Slovenia; 2Jožef Stefan International Postgraduate School, Jamova 39, SI-1000 Ljubljana, Slovenia; 3Centre for Integrated Approaches in Chemistry and Biology of Proteins (CIPKeBiP), Jamova 39, SI-1000 Ljubljana, Slovenia; 4Department of Chemistry and Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Cesta v Mestni log 88A, SI-1000 Ljubljana, Slovenia; 5Department of Biochemistry and Molecular Biology, Hershey Medical Center, Pennsylvania State University, College of Medicine, Hershey, PA, USA

Recently several independent studies have shown the role of endolysosomal cysteine cathepsins in the nucleus, particularly in connection with cell cycle progression, differentiation and cancer progression. Cysteine proteinase inhibitor cystatin B/ stefin B (CSTB) gene is located on human chromosome 21. Mutations in CSTB are associated with progressive myoclonus epilepsy Unverricht-Lundborg disease (EPM1) [1,2]. In stefin B deficient mice (Cstb -/-) increased cerebellar granular cell apoptosis, progressive ataxia, myoclonic seizures were detected [3]. The precise mechanism by which the lack of stefin B influence the disease is not clear yet. We have identified an interaction between stefin B and with histones H2A.Z, H2B, and H3 and cathepsin L in the nucleus. Stefin B-deficient mouse embryonic fibroblasts entered S phase earlier than wild type mouse embryonic fibroblasts [4]. In contrast, increased expression of stefin B in the nucleus delayed cell cycle progression. The delay in cell cycle progression was associated with the inhibition of cathepsin L in the nucleus, as judged from the decreased cleavage of the CUX1 transcription factor. Interaction of stefin B with the Met-75 truncated form of cathepsin L in the nucleus was confirmed by fluorescence resonance energy transfer experiments in the living cells. Stefin B could thus play an important role in regulating the proteolytic activity of cathepsin L in the nucleus, protecting substrates such as transcription factors from its proteolytic processing.

References:


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F1. CHANGES TO CELLULAR WATER AND ION CONTENT BY NUCLEOLAR STRESS: INVESTIGATION BY A CRYO-CORRELATIVE NANO IMAGING APPROACH.

Dominique Ploton¹, Nolin, F.², Wortham, L.², Tchelidze, P.³, Lalun, N.¹, Bobichon, H.¹, Michel, J.²

¹CNRS FRE 3481, University of Reims, France; ²LRN, University of Reims, France; ³University of Tbilissi, Georgia

The cell nucleus contains the highest concentration of macromolecules in the cell. Thus, in mammalian cells, mean concentration of macromolecules varies from 65 to 180 mg/ml for whole nuclei and from 75 to 200 mg/ml for interphase chromatin and nucleolus respectively as measured by using different approaches such as chemical assays, interference microscopy or quantitative Scanning and Transmission Electron Microscopy (STEM) (Hancock, 2004). Such high concentrations induce macromolecular crowding which predicts that a small variation in the water content could produce strong variations in the activity of numerous macromolecules. Data dealing with water content and ions concentration in hydrated nuclear compartments are unknown although water and ions are recognized as crucial players in the organization and function of proteins and nucleic acids. The objective of our study was to quantify both water and ions concentration in the main nuclear domains at nanoscale within control and stressed cells. For this, we developed a new correlative light and electron microscopy (CLEM) imaging approach combining observation of the same cryo-ultrathin section first by fluorescence microscopy (localization of GFP in HeLa cell-line expressing H2B-GFP) and then by cryo-STEM (Nolin et al., 2012). As fluorescence and STEM images were perfectly registered, nuclear compartments containing chromatin (identified by their fluorescence) were clearly localized on STEM images. This correlative approach allowed us to perform a targeted simultaneous quantification of water and of elements. Thus, elements (N, P, K, Cl, Mg and S) were simultaneously identified and quantified by EDXS at a resolution of 20 nm and their concentration was calculated for the first time in mmol/L. In control cells, we found that nucleoplasm contained more water than clumps of interphase condensed chromatin and chromatin of mitotic chromosomes (75 %, 65 % and 63 % respectively). In the nucleolus, fibrillar centers contained more water than both DFC/ GC (83 % and 69 % respectively). As a comparison, cytosol and mitochondria contain 72 and 60 % of water respectively. In the nucleus, concentration of elements ranged from 128 to 462 mmol/L for K, from 23 to 85 mmol/L for Cl and from 13 to 55 mmol/L for Mg. All these data were used for each cell compartment to create an “element descriptor” which gives a completely new image of elementary composition integrating water concentration. We then addressed the consequences of a nucleolar stress induced by actinomycin D (50 ng/ml, 3 hrs) in terms of water and ions content in cell compartments. Interestingly, a nucleolar stress induced a 5 to 10 % increase of water content in all cell compartments. As an example, light nucleolar caps and segregated DFC and GC, resulting from rRNA synthesis inhibition, contain 91 % and 85 % of water respectively. Targeted EDXS analysis evidenced a strong decrease of elements in all compartments, but with significant differences from one element to another one. As an example, Cl decreased more strongly than other elements and Mg decreased more in condensed chromatin than in nucleolus or nucleoplasm (Nolin et al., 2013). Finally, we will give preliminary results concerning the status of water and ions during the different steps of apoptosis induced by a high dose of actinomycin D. These data demonstrate that each step of apoptosis is characterized by a unique “element descriptor” depending both on time and cell compartment.

References:

F2. BREAKDOWN OF NUCLEAR QUALITY CONTROL: SINGLE-CELL IMAGING OF NUCLEOLAR PROTEIN AGGREGATION IN A LIVING ORGANISM

Scharf, A., Piechulek, A., and von Mikecz, A.

IUF - Leibniz Research Institute for Environmental Medicine, Duesseldorf, Germany

We feed the well-characterized nematode *Caenorhabditis elegans* with fluorochrome-labelled nanoparticles and image breakdown of quality control in a living organism on a subcellular level. Silica nanoparticles enter cells and nuclei of *C. elegans* and accumulate in worm organs such as pharynx, intestine, spermathecae and vulva. The distribution pattern is distinct and correlated with a decline of respective organ functions such as pharyngeal pumping. The observed phenotypes are reflected on the molecular level by an impairment of protein homeostasis that manifest in the accumulation of insoluble ubiquitinated proteins. Single cell imaging reveals exposition-linked nuclear aggregation of endogenous proteins especially in nucleoli of intestinal cells. Protein aggregation and reduced neural behaviors represent aging phenotypes that are induced prematurely by silica nanoparticles.

Work in the von Mikecz laboratory is supported by the German Science Foundation (DFG) through grants MI 486/7-1 and GRK 1033.
Retinoid X Receptor (RXR) is a key heterodimeric nuclear receptor. Agonist treatment increased the occupancy of RXR genomic binding regions but hardly affected the number of sites as revealed by ChIP-Seq suggesting a static nuclear behaviour. However, transcription is an inherently dynamic process and the receptor’s diffusion properties in single cells on the sub-second scale are not known. We characterized the nuclear dynamics of RXR during activation using live-cell imaging. By applying FRAP and FCS techniques having different temporal resolution, a highly dynamic behaviour was uncovered at different time scales. A two-state model described best the receptor’s mobility. In the unliganded state most RXRs belonged to the fast population, leaving ~15% for the slow, chromatin bound population. Upon agonist treatment, the fraction of the slow population increased to ~45% as a result of an immediate and reversible redistribution. Coactivator binding was indispensable for redistribution and had a major contribution to chromatin association. The two populations were distributed homogenously in the nucleus. Our results support a model in which RXR is very dynamic and follows hit-and-run kinetics upon activation.

References:

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F5. SPECIFIC INHIBITORS OF NUCLEAR TRANSPORT; POTENTIAL ANTI-VIRALS

David A. Jans, Fraser, J. E. and Wagstaff, K. M.
Dept of Biochemistry and Molecular Biology, Monash University, Clayton, VIC, 3152, Australia

Viral disease is one of the greatest burdens of human health, with an urgent need for new anti-viral strategies. Our work examining a number of DNA and RNA viruses indicates that regulated protein movement into and out of the nucleus through the importin (IMP) superfamily of transporters is central to viral infection. This is particularly striking in the case of RNA viruses where although viral replication generally occurs in the cytoplasm, gene products important in virus replication/assembly traffic into the host cell nucleus generally to dampen the host cell anti-viral response. Using a variety of in vitro and in vivo approaches, we have delineated the IMPs and targeting signals responsible for the nuclear import/export of specific viral proteins from DNA tumor viruses such as cytomegalovirus/Herpes Simplex Virus, as well as the RNA viruses Dengue (DENV), Respiratory Syncytial Virus (RSV), and Human Immunodeficiency Virus (HIV)-1. Intriguingly, many of the different viral gene products utilize either IMPβ1 or IMPα/β for nuclear import, and IMPβ-homologue EXP1/CRM1 for nuclear export, implying that agents targeting their cellular nuclear transport proteins could represent broad spectrum anti-viral agents. Our recent results support this idea, with 3 different novel inhibitors of IMPα/β-dependent nuclear import reducing infection by DENV as well as HIV-1, by reducing nuclear import of the non-structural protein 5 and integrase proteins, respectively. Nucleocytoplasmic transport thus appears to be a viable target of great significance in the fight against pathogenic viruses.

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Wagstaff KM et al. (2012) Biochem J 443, 851-6
The advance of Tyr-recombinases Flp and Cre in the early 1990ies opened novel options for the predictable association of replicating nonviral minicircles with open chromatin structures [1] or the predictable insertion of transgenes into compatible target sites of the mammalian host genome [2]. Both efforts were greatly supported by biomathematical (SIDD-) procedures to analyze the DNA strand separation potential of participating sequences localized both at the genomic target site and on the incoming vector [3]. The spectrum of applications could be further expanded by Flp-Recombinase Mediated Cassette Exchange (RMCE) approaches in 1994 [reviewed in 2]. RMCE enables refined tag-and-exchange strategies to replace an existing target structure by a compatible donor cassette devoid of auxiliary vector parts. Such a "gene swap" safely locks the incoming cassette which, however, can be re-mobilized and exchanged at will in case other compatible donors are provided ("serial RMCE"). During the first decade these features considerably enhanced the potential of systematic genome modifications. Based on the reproducible expression capacity of the resulting cell strains and the availability of multiple heterospecific FRT mutants, a comprehensive tool box [4] emerged to serve a wide spectrum of purposes [5]. While the concept guaranteed predictable expression characteristics [6], it approached, but did not reach the production levels of industrial multiple-copy cell lines. Its exceptional potential emerged, however, once it entered the stem cell field. Here RMCE has started to contribute to the reliable generation of induced pluripotent stem (iPS-) cells and their subsequent differentiation to a variety of cell types for diagnostic and therapeutic purposes. An unanticipated property concerns the capacity to enable serial RMCE reactions [7] even after the reprogramming cassette underwent the obligatory silencing process. During this phase of Flp-induced recombination steps chromatin re-opening is associated with the loss of heterochromatic CpG methylation- and H3K9me3-marks. With these properties in mind we have added composite Flp-recognition target sites ("heterospecific FRT-doublets"), to the LTRs of lentiviral vectors. These "twin sites" enhance the safety of iPS re-programming and –differentiation as they enable the subsequent quantitative excision of transgenes, leaving behind a highly accessible,single “FRT-twin” [7]. This composite element represents a unique genomic tag, serving to characterize the host locus in order to identify genomic targets meeting the recent stringent Genomic Safe Harbor criteria. These tagged „GSHs“ lend themselves to multiple dedicated (re-)uses, solely based on the potential of the actual Flp-RMCE repository.


This work was supported by the Excellence Initiative REBIRTH (Regenerative Biology to Reconstructive Therapy), the SFB 738 (Optimization of conventional and innovative transplants), and a ReGene (Regenerative Medicine and Biology) grant by BMFT (Germany)
F7. ANTIMITAGENIC AND REPAIR-STIMULATING DERIVATIVE OF 1,4-DIHYDROPYRIDINE AV-153 INTERCALATES IN DNA IN A SINGLE STRAND BREAK SITE BETWEEN TWO PYRIMIDINES

Buraka, E.1, Yu-Chian Chen, C.2, Nikolajeva, V.4, Grube, M.5, Gavare, M.5, Duburs, G.3 and Nikolajs Sjakste1,3

1 Faculty of Medicine, University of Latvia, Sharlotes Street 1a, Riga LV1001, Latvia; 2Laboratory of Computational and Systems Biology, School of Chinese Medicine China Medical University, Taichung 40402, Taiwan ROC and Department of Bioinformatics, Asia University, Taichung 41354, Taiwan; 3 Latvian Institute of Organic Synthesis, Aizkraukles Street 21, Riga LV1006, Latvia; 4Collection of Bacterial Strains of the University of Latvia, Kronvalda boulevard 4, Riga LV1586, Latvia; 5Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda boulevard 4, Riga LV1586, Latvia

Ability to intercalate between DNA strands with subsequent inhibition of transcription or replication processes determines cytotoxic activity of numerous anticancer drugs. Strikingly intercalating activity was reported also for some flavonoids considered to be antimutagenic agents and genome stabilizers. Aim of this study was to determine mode of interaction with DNA of antimutagenic and DNA repair stimulating dihydropyridine (DHP) AV-153.

DNA-drug interactions were studied by means of UV-VIS spectroscopy. Ability of a drug to compete with ethidium bromide (EBr) intercalation was determined by measuring fluorescence intensity of EBr-DNA complex (λex = 335, λem = 600) in presence of increasing concentrations of the compound. Interaction with bases was studied by means of infrared spectroscopy.

Compound AV-153 a 1,4 dihydropyridine with ethoxycarbonil groups in positions 3 and 5 evidently interacted with DNA, as addition of the sonicated rat liver DNA to AV-153 solutions caused pronounced hyperchromic and bathochromic effects on the spectra. Reciprocally addition of the compound to DNA solutions caused hyperchromic effect in DNA spectra. Similar effects were observed when intact pTZ57R plasmid was used for titration. Induction of single-strand breaks in the plasmid by peroxinitrite did not change much binding affinity of the compound. However the effect was much stronger when sonicated plasmid was taken for titration. The binding mode did not change when ionic strength of the solution was changed from 5mM to, 10mM, 20mM, 50 mM or 150mM of NaCl. Neither was it influenced by changes of the temperature (25°C, 30°C, 37°C or 40°C). The compound manifested interaction with single-stranded oligonucleotide and hairpin forming oligonucleotides, hyperchromic and hypsochromic effects were observed. Interaction of the compound with AT-rich DNA of Staphilococcus aureus or GC-rich of Micrococcus luteus were much weaker compared to interaction with DNA of similar AT and GC content. AV-153 competed with EBr for intercalation sites in DNA: 116 mM of the compound caused a two-fold decrease of the fluorescence intensity. Computer modelling AV-153 and DNA interactions indicated ability of the compound to dock between DNA strands in the site of a single-strand DNA break and between two pyrimidines. Data of infrared spectroscopy confirmed preferable interactions of the compound with T and C bases.

Thus antimutagenic substance AV-153 appears to intercalate between the DNA strands. Ability to stimulate intensity of DNA repair was also reported for AV-153, it was hypothesized that poly(ADP)ribose polymerase is the main target of the drug. Our data suggest existence of alternative ways of stimulation of DNA repair by AV-153, probably AV-153 opens DNA helix and makes damaged sites more accessible to repair enzymes.
Crosslinking of proteins to DNA is essential for chromatin-immunoprecipitation in cases where interaction is low, i.e. transcription factors bound to specific target sites. Afterwards, reaction is stopped by addition of glycine and samples are washed with buffer. We show now that crosslinking by formaldehyde induces DNA damage as detected by phosphorylation of H2AX (gammaH2AX) and by induction of poly(ADP-ribose) (PAR), an early marker of strand breaks, synthesized by poly(ADP-ribose) polymerases (PARPs). Both posttranslational modifications have been shown to act as signaling hubs altering chromatin structure. We tested three different ChIP protocols for their ability to induce PAR and found in all cases massive PARP activation. PAR synthesis could be either specifically blocked by a PARP inhibitor with no impact on appearance of gammaH2AX, or suppressed by increasing formaldehyde concentration, which also abolished phosphorylation of H2AX. Both approaches enhanced ChIP efficiency in a similar way, but interestingly not all cases tested were altered. We could show that only specific combinations of protein factors with their target DNA were affected, i.e. CTCF at the H19 imprinting control region, but not at the BRCA1 promoter. In summary, we show that standard ChIP protocols are flawed by artificial formation of poly(ADP-ribose) and gammaH2AX. Suppression of PARP activity changes ChIP efficiency, revealing specific protein-DNA interactions that are affected by PARylation. By our proposed modifications of current ChIP protocols it will be possible to analyze the natural chromatin composition.
Poster abstracts
List of posters in alphabetical order (according to first author’s name)
P1. THE PARTICIPATION OF WIP1 PHOSPHATASE IN THE RESPONSE OF U-2 OSTEOSARCOMA CELLS TO RADIATION

Agata Abramowicz, Katarzyna Szoltysek, Patryk Janus, Krzysztof Puszynski, Piotr Widlak

1Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland; 2Silesian University of Technology, Gliwice, Poland

WIP1 is a multi-target serine/threonine protein phosphatase encoded by PPM1D gene. Its major targets include MDM2(Ser395), ATM(Ser1981), p53(Ser15), CHK1(Ser345), CHK2(Thr68), γH2AX(Ser139) and NF-κB(Ser536). These proteins are inactivated by a WIP1-mediated dephosphorylation, hence WIP1 putatively participates in regulation of recovery of cell after induction of DNA damage response (DDR). Overexpression of PPM1D gene has been observed in some types of cancer and usually has been associated with poorer prognosis. Here we aimed to characterize the significance of WIP1 in response to radiation in human osteosarcoma U-2 OS cell line.

U-2 OS cells have been characterized by high expression of PPM1D gene. Two functional gene products are detected in these cells: full-length wild-type protein (66.7 kDa) and C-terminus truncated form (about 50 kDa). The expression of WIP1 in U-2 OS cells was inhibited by lentivirus-delivered shRNA (control cells contained an shRNA construct encoding a scrambled sequence). The WIP1 knock-down was verified by Western-blot – amounts of both protein forms were reduced to approximately 25% of their initial levels. The DDR was induced upon exposure to ionizing radiation (IR, 4 or 10 Gy) or UV-C radiation (10 J/m2), which induce ATM-Chk2-p53 or ATR-Chk1-p53 signaling, respectively. The levels of WIP1 and phosphorylated DDR-related proteins (p53Ser15, Chk2Thr68, Chk1Ser345) were analyzed by Western-blot at different time points after irradiation. The stability of WIP1 was assessed following cycloheximide (100 μg/ml) treatment. Expression of selected p53-dependent genes (NOXA, CDKN1A) was assessed by QRT-PCR. The influence of the WIP1 inhibition on the cell cycle profiles was assessed by flow cytometry. The long-term effects of the WIP1 deficiency on radiation sensitivity of cells were analyzed by the clonogenic test.

Both IR and UV-C induced accumulation of WIP1 in control cells, which was the most prominent after irradiation with 10 Gy. In WIP1-depleted cells some accumulation of WIP1 could be detected after exposure to IR. The most clear influence of WIP1 deficiency was observed at the level of phosphorylation of Chk2Thr68 and p53Ser15 (direct targets of WIP1), which was markedly higher as compared to irradiated control cells (with "normal" level of WIP1). However, we did not observe a noticeable effect of WIP1 depletion on the cell cycle profiles or clonogenic abilities of irradiated cells. We concluded that either WIP1 is able to perform its function after appreciable decrease of its amount (e.g. due to high affinity to its targets) or there are alternative WIP1-independent mechanisms of recovery after DDR, capable of compensating WIP1 deficiency.

This work was supported by Polish National Science Center, Grant No N518-287540.
Inflammation, growth retardation, malnutrition and cardiac overload during early childhood play an important role in the developmental programming of adult cardiovascular diseases. The common causes of these maladies in children are infectious gastroenteritis triggering malnutrition and tachyarrhythmia. Our recent follow up studies indicated that neonatal cryptosporidial gastroenteritis was associated with cardiomyocyte abnormalities. Using real-time PCR, immunocytochemistry and image analysis, we demonstrated that 2 and 6 month-old rats who survived cryptosporidiosis at the age of 10-14 days, showed cardiomyocyte hyperpolyploidization and myosin heavy chain expression balance shift toward the slow isoform beta. Unexpectedly, animals who survived the disease 5.5 month ago, showed more prominent changes than the ones who survived cryptosporidiosis 1.5 month ago. This data suggest that cryptosporidiosis is particularly dangerous for weakened or ageing organism. Based on the observation that myosin heavy chain α possesses approximately 5 times lower ATP-ase activity compared to myosin heavy chain β, it is reasonably to suggest that neonatal cryptosporidiosis decreases cardiac contractile ability and thus may trigger developmental programming of adult cardiovascular diseases.

This study was supported by The Ministry of education and science of Russian Federation, project 8306, RFBR №12-04-01199-a, and RAS presidium program. "Molecular function integration mechanisms for physiologic function realization"
**P3. POLYPLOIDY BOOSTS TRANSCRIPTOME OF SOMATIC CELLS WHICH SHARES CERTAIN FEATURES WITH MALIGNANT PHENOTYPE**

Anatskaya, O. V.¹, Erenpreisa, J. A.², Salmina, K.² and Vinogradov, A. E.¹

¹Institute of Cytology RAS, St Petersburg, Russia and ²Latvian Biomedical Research&Study Centre, Riga

Somatic polyploidy is known to accompany developmental programming, congenital abnormalities and dangerous diseases like hypertension, diabetes, atherosclerosis and cancer. Moreover, recently the involvement of reversible endopolyploidy in resistance of tumour cells to DNA damaging therapy has been shown. However, it is still unclear whether polyploidy maintains the gene dosage balance at transcription level or introduces new features in gene regulation activity. To favour the understanding of this question, we performed cross-species genome-wide analysis of ploidy-associated transcriptomic changes in cardiomyocytes and hepatocytes. Using multi-level protein interaction network and biological pathway analysis for the extensive data obtained with next generation sequencing, we found that polyploidy affects most biological processes. Cardiomyocytes and hepatocytes show similar trends in response to genome duplication. In both cell types, polyploidy predominantly increases transcriptome activity and substantial amount of genes became induced by 10-100 folds. These are the anabolic pathways and pathways involved in stress response, oncogenesis and patterning in early embryogenesis (JUN, FOS, RRAS, MET, KIT, HRAS, WNT, MCM and NFKB –families, BMP, Hedgehog and Sox and growth stimulation (IGF, EGF, FGR SMAD, VEGF, NGF, DUSP, PDGFA, MAPK - families). We also found that polyploidy favours mild hypoxia-associated changes (HIF-1α induction and boosting of sugar metabolism). Thus, somatic polyploidy might be an ingenious evolutionary instrument for fast adaptation to stress and new environments allowing trade-offs between high functional demand, stress, and energy depletion. At the same time and possibly just therefore, endopolyploidy shares important epigenetic features with malignant phenotype.

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Anatskaya, O. V., Sidorenko, N. V., Matveev, I. V., Kropotov, A. V., Kharchenko, M. V. and Vinogradov, A. E.

Institute of Cytology RAS, St Petersburg, Russia

Developmental origin of health and diseases theory (DOHaD) postulates that growth retardation and inflammation in children increase cardiovascular pathology risk decades later. Based on medical statistics, suggesting that gastroenteritis is the most common cause of growth retardation and inflammation in children, we investigated the effects of cryptosporidiosis challenged by the worldwide human enteropathogen – Cryptosporidium parvum on neonatal rat cardiomyocyte. Specifically, we decided to find out how neonatal cryptosporidiosis of various severities affects cardiac anatomy and cardiomyocyte remodeling. Using real-time PCR, cytometry, immunohistochemistry, image analysis and interatrial septum visual examination, we revealed that gradual increase in cryptosporidial invasion was associated with ‘all-or-nothing’ changes. At weak parasitic infection, interatrial septum was closed and there were no statistically significant changes in cardiomyocytes. At moderate and severe infections, all changes in cardiac anatomy and cardiomyocytes were statistically significant and demonstrated approximately similar degree. Compared to control, heart were atrophied and elongated, interatrial septum contained a small window (patent foramen ovale), cardiomyocytes lost protein, became thin and elongated and accumulated additional genomes. Also we found HIF-1α mRNA overexpression. Remarkable, the “all-or-nothing” response to gradual stimulus strengthening is an important criterion of developmental programming, since such a response is commonly a consequence of abnormal anatomic structure formation cell differentiation failure. Our results could be interesting for physicians because they indicate that moderate cryptosporidiosis can be dangerous for neonatal heart and can trigger cardiovascular pathology neonatal programming. Also our results for the first time demonstrate the association between gastroenteritis, patent foramen ovale and cardiomyocyte hyperpolyploidization.

This study was supported by The Ministry of education and science of Russian Federation, project 8306, RFBR №12-04-01199-а, and RAS presidium program. “Molecular function integration mechanisms for physiologic function realization”
The data coming from various fields of research indicate that polyploidy shows different traits in various cell types suggesting that its pure effects are still not completely understood. It is not surprising, because preserving gene dosage balance, genome doubling may induce only very subtle effects that are very difficult to filter from the noise. At the same time catching these effects is important since many regulatory molecules, including transcription factors, are synthesized in a number of just several copies. Also it has been recently shown that even tiny perturbations in DNA specificity, affinity, and promoter design can transform individual TFs between distinct roles and alter the signal processing behaviour of multi-input systems. To find out whether genome duplication affects transcriptome activity, we compared the data from multiple databases obtained with cells from evolutionary distant organisms including yeast and mammals. Using gene network and biological pathway analysis, we indicated that polyploidy activates cytoplasmic and mitochondrial ribosome biogenesis and protein synthesis and degradation through ubiquitin-proteasome system. Also we found that polyploidy boosts histone-related chromatin remodelling and main energy metabolic pathways, including sugar and lipid metabolism. Among the depressed pathways there were cell wall maintenance and late mitosis. Thus, our results suggest cell polyploidy exerts similar effects in evolutionary distant organisms and is a powerful instrument of epigenetic transcriptome regulation. A detailed description of ploidy-related transcriptomic changes is critical for our understanding of gene activity epigenetic control in health in disease because it may be helpful in the development of new approaches to disease prevention and therapy.

This study was supported by The Ministry of education and science of Russian Federation, project 8306, RFBR №12-04-01199-a, and RAS presidium program. "Molecular function integration mechanisms for physiologic function realization"
While nuclear protein aggregation is a well established feature of aging processes and neurodegenerative triplet repeat diseases, the underlying mechanisms are insufficiently understood. In an unbiased approach that includes purification and analysis of SDS-insoluble protein aggregates by mass spectrometry spliceosomal components are identified as major constituents of the nuclear aggregome in different steps of protein fibrillation. Consistently, confocal imaging discovers amyloid-like microenvironments in the center of speckle-domains enriched with pre-mRNA splicing factors. A notable similarity between transitional steps of protein fibrillation and formation of different spliceosomal complexes in the splicing cascade is partial structural unfolding of protein components which may explain their aggregation propensity and our observation of amyloid-like spliceosomal microenvironments. Thus, characterization of the endogenous protein aggregation landscape suggests that an intimate network of protein fibrillation, nuclear organization and gene expression may regulate physiological or pathological protein homeostasis.

Reference:

Work in the von Mikecz laboratory is supported by the German Science Foundation (DFG) through grants (MI 486/7-1) and GRK 1033.
P7. HIGHLY ELEVATED LEVEL OF LYSINE 9-ACETYLATED HISTONE H3 AROUND THE TRANSCRIPTIONAL START SITE OF THE MDR1 GENE IN A MULTIDRUG-RESISTANT CELL LINE

Balint, E., Toth, M., Boros, I. M.

BAYGEN Institute, Bay Zoltan Foundation for Applied Research, Szeged, Hungary; Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary; Chromatin Research Group of University of Szeged–Hungarian Academy of Sciences, Department of Biochemistry and Molecular Biology, University of Szeged, Szeged, Hungary

Failure of chemotherapy in breast cancer presents a major problem and is often due to elevated expression of ATP binding cassette (ABC) type transporters, such as multidrug resistance 1 protein (MDR1). It has been shown that MDR1/ABCB1 gene expression is regulated at the chromatin level by DNA methylation and histone acetylation. However, the modified histone residues have not been identified and the role of various histone acetyl transferases (HATs) is not fully understood. By studying a breast carcinoma model cell line and its MDR1 overexpressing derivative here we show that histone 3 lysine 9 (H3K9) acetylation level is a hundred fold elevated in the promoter and first exon of the MDR1 gene in the drug-resistant cell line compared to the drug-sensitive cell line. The acetylation level of the other examined lysine residues (H3K4, H3K14, H4K8, H4K12) is weakly or not at all elevated in the MDR1 locus, although their acetylation is generally increased genome-wide in the drug-resistant cell. Down-regulation of the expression of HATs PCAF and GCN5 by RNAi effectively reduces the expression of MDR1. Unexpectedly, treatment with a p300-selective inhibitor (HAT inhibitor II) further increases MDR1 expression and drug efflux in the drug-resistant cells. Our data suggest that repeated exposure to chemotherapy may result in deregulated histone acetylation genome wide and in the MDR1 promoter.

This work was supported by NKTH (OMFB-00441/2007 Teller Ede Program NAP-BIO 06/1) to Prof. Eva Kondorosi (BAYGEN Institute, Hungary) and by the Hungarian Scientific Research Fund (OTKA 77443) to I.M. Boros.
Nuclear apparatus in ciliates comprises two types of nuclei: a huge vegetative macronucleus and a small generative micronucleus. As all protists' nuclei, they lack lamins, other proteins providing for nuclear architecture. Actin could be a good candidate for this role. Structural function of nuclear actin has been proved in *Xenopus* oocytes. Moreover, actin has also been found to be involved in chromatin remodeling and transcription.

Here we demonstrate actin dynamics in the nuclei of *Paramecium caudatum* and *P. primaurelia* using TRITC-phalloidin treatment of total cells. We compared nuclear actin localization at different stages of cell cycle, cell division, selfing and nuclear degradation after Latrunculin B treatment. Both macro- and micronucleus in *P. caudatum* were TRITC-phalloidin positive, differences registered in actin distribution within each nucleus. In the macronucleus changes mostly involved amplified nucleoli, while in the micronucleus alterations in the achromatin cap were most prominent.

During cell division attenuation of TRITC-phalloidin fluorescence was recorded in both nuclei. Most perceptible decrease of the signal was observed in the micronucleus where actin was lost both in the chromatin containing area and in the achromatin cap. Two types of DNA containing structures were registered after selfing (intraclonal conjugation causing substantial nuclear reorganization): phalloidin positive and phalloidin negative. Macronuclear anlagen, which have low DNA content, were actin rich, whereas DNA rich fragments of the old macronucleus demonstrated weak fluorescence.

Inhibition of actin polymerization by treatment with different concentrations of Latrunculin B caused agglomeration and vacuolization of nucleoli, fragmentation of the macronucleus and its total destruction under high concentrations of Latrunculin B.

Małgorzata Broszkiewicz¹, Adrianna Magalska¹, Błażej Ruszczycki¹, Iwona Czaban¹, Magdalena Ambrożek², Kamil Parobczak¹, Robert Pawlak³, Grzegorz Wilczyński¹.

¹Nencki Institute of Experimental Biology, Warsaw, Poland; ²Medical Center of Postgraduate Education, Warsaw, Poland; ³University of Leicester, Leicester, United Kingdom

PML protein is a crucial element of characteristic structures involved in creating dynamic architecture of the cell nucleus – PML bodies. However, the PML function at the systemic level is far from understanding. It is widely believed that besides its important role in the prenatal neurogenesis, PML is not expressed in the adult brain. To the contrary, our pilot studies reveal that PML protein does exist in the adult mouse brain, and forms nuclear bodylike structures in the neuronal nuclei. Our preliminary anatomical study indicates that the most pronounced expression of PML occurs in cerebral- and cerebellar cortices. Such a pattern suggests the involvement of PML in higher levels of information processing in the brain. Furthermore, a brief seizure evoked by pentylenetetrazole upregulates PML, and evokes a series of changes in the morphology of the nuclear PML aggregates in the cerebral cortical neurons, culminating in their accumulation and dispersion throughout the nucleus, as observed by high-resolution fluorescence microscopy followed by three-dimensional quantification. Quite unexpectedly, however, an electron-microscopic immunogold analysis revealed that PML aggregates do not form morphologically recognizable objects, e.g. the classic nuclear bodies with fibrous capsule. Nevertheless, these studies demonstrated that seizure causes PML aggregates to tightly associate with decondensed chromatin fibrils. This observation suggests that PML function in neurons might be associated with activity-dependent gene expression. The changes in PML expression seem not to be seizure-specific, as they are evoked also by immobilization stress. Taking into consideration our studies, and the literature data, we foresee that PML has an important function(s) in the brain.
P10. STRUCTURAL STUDIES OF NAPS HHA AND H-NS

CAO, W.1, Yalamanchili, H. K.2, Hao, Q.1, Wang, J. J.2 and Zhang, H.1

1Department of Physiology, The University of Hong Kong; 2Department of Biochemistry, The University of Hong Kong

The nucleoid-associated proteins (NAPs) are known to have low molecular weight and the ability to form dimer or oligomer, most of which can bind to DNA for regulation of gene expression [1]. H-NS, well-studied as one of the NAPs, acts as a global transcriptional repressor[2]. It has independent functional N-terminal domain for oligomerization and C-terminal domain for DNA binding, joined by a flexible linker. H-NS contributes to horizontal genes transfer, cell physiology, and environmental factors like temperature or pH would influence H-NS’s ability towards dimerization or oligomerization as well as binding to DNA [3, 4]. The Hha protein is a member of the Hha-YmoA class, which is only found in the members of Enterobacteriaceae as a modulator of expression of virulence factors in Gram-negative bacteria [1, 6, 7]. Hha’s binding to H-NS but not DNA can modulate expression of the hemolysin operon, which is essential for the H-NS-regulated gene expression[8]. This study aims at solving the structure of Hha and its complex with H-NS by X-ray Crystallography. The structure will show the binding details between H-NS and Hha, and reveal how Hha regulates gene expressions through H-NS.

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Cancer is believed to be responsible for an estimated 7.5 million deaths each year, with the majority of mortality cases accounted to highly aggressive forms. High grade cancers are often difficult to detect before metastasis. If caught at early stages of progression, patient prognosis is often good with an estimated 60-80% survival rate over the following 5 year period. Due to this, our group aims to increase understanding of the cellular processes involved in maturation of cancerous phenotypes from low to high grades through investigating interactions between Nuclear envelope (NE) proteins and the nuclear pore complex (NPC).

Our current aims are to investigate whether an interactive relationship between the NE and NPC exists, with key interest in cancer development. Our data currently suggests that select areas of the NE and NPC show physical interactions and functional relationships, stimulating phenotypes similar to those found within the developmental stages of aggressive cancer forms upon cellular manipulation.

Further aims are to investigate whether 3D systems would be a more viable tool for future investigations into cancer migration and metastasis studies. Conventional 2D systems restrict cells to minimal cellular contact within the developing colonies, as well as limit the methods available to analyse migration and metastasis within these systems.
Thymus is the lymphohaematopoetic organ in fish. Both the lymphoid and non lymphoidal heterogeneous cellular elements of the thymus gland of *Aplocheilus panchax* (Order: Cyprinodontidae, Family: Aplocheilidae), which is widely distributed from Pakistan to Indonesia, via Singapore, have been examined under the transmission electron microscope (TEM). The position of the plasma cells in fish thymus is hardly universally established. The plasma cells in the above fish are the antibody producing sites within the thymic micro-environment. These cells are classified on the basis of their chromatin condensation, nuclear membrane thickness and the position of the nucleolus. Some of these features and others help to characterize the early, intermediate and late stage of the developmental sequences of this cell line. In these cells the heterochromatin materials are dispersed and lie close to the inner nuclear membrane. The euchromatin materials are evenly distributed. The appearance of the nuclear granular materials within the plasma cells is apparently indicative of cellular dysfunction and metabolically inactive state. The significance of the results in fish developmental immunobiology and other related fields will be discussed.
P13. Cytometric analysis of differentiation and cell death in human adipocytes

Minh X. Q. Doan¹, Anita K. Sárvári², Pamela Fischer-Posovszky³, Martin Wabitsch³, Zoltán Balajthy², László Fésüs²,⁴, Zsolt Bacsó¹

¹Department of Biophysics and Cell Biology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary; ²Department of Biochemistry and Molecular Biology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary; ³Division of Pediatric Endocrinology and Diabetes, Department of Children and Adolescent Medicine, University Medical Center Ulm, Ulm, Germany; ⁴MTA DE Apoptosis, Genomics and Stem Cell Research Group, University of Debrecen, Debrecen, Hungary

Understanding adipocyte biology and its homeostasis is in the focus of current obesity research. We aimed to introduce a high-content analysis procedure for directly visualizing and quantifying adipogenesis and adip-apoptosis by laser scanning microscopy in a large population of cells. Slide-based image cytometry and image processing algorithms were utilized and optimized for high-throughput analysis of differentiating cells and apoptotic processes in cell culture at high confluence. Both preadipocytes and adipocytes were simultaneously scrutinized for lipid accumulation, texture properties, nuclear condensation, and DNA fragmentation. Adipocyte commitment was found at day 3, while terminal differentiation of adipocytes occurred at day 9 to 14 with characteristic nuclear shrinkage, eccentric nuclei localization, chromatin condensation, and massive lipid deposition. Preadipocytes were shown to be more prone to TNFα induced apoptosis compared to mature adipocytes. By applying Halo assay and measuring fraction of migrated DNA fragments, we detected and quantified directly DNA damage during the adipogenesis in single cell basis. Importantly, spontaneous DNA fragmentation was observed at early stage when adipocyte commitment occurs. This DNA damage was independent from either spontaneous or induced apoptosis, and probably was part of the differentiation program.
QUANTITATIVE IMAGE ANALYSIS OF REPLICATION STRESS IN CELLS EXPOSED TO DNA TARGETING ANTICANCER DRUGS AND OXIDATIVE STRESS


Division of Cell Biophysics, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Krakow, Poland; Nencki Institute of Experimental Biology, Laboratory of Functional and Structural Tissue Imaging, Polish Academy of Sciences, Warsaw; Brander Cancer Research Institute and Department of Pathology, New York Medical College, Valhalla, New York 10595, USA

BACKGROUND. A recently described method [Berniak et al., submitted] of quantitative analysis of spatial (3D) relationship between discrete nuclear events detected by confocal microscopy was applied in analysis of a dependence between sites of DNA damage signaling (γH2AX foci) and DNA replication (EdU incorporation) in cells subjected to treatment with topoisomerase inhibitors camptothecin (CPT), etoposide (ETP), mitoxantrone (MTX), cis-platinum (CS) or hydrogen peroxide (H2O2).

METHODS. Newly synthesized DNA was fluorescently labeled using a precursor EdU (5-ethynyl-2'-deoxyuridine) and a 'click' reaction. γH2AX foci were labeled by immunofluorescence. Images of replication and histone H2AX phosphorylation sites were recorded using confocal microscopy aided with deconvolution. Nearest-neighbor and correlation analyses were performed using an algorithm written specifically for this task.

RESULTS. CPT induces γH2AX foci, likely reporting formation of double-strand DNA breaks (DSBs), almost exclusively at sites of DNA replication. ETP induces γH2AX in S-phase, with a moderate tendency toward replication sites. MTX induces γH2AX foci with no detectable preference for replication foci. Histone H2AX phosphorylation induced by CS is detected 2 and 4 h after exposure to the drug and largely coincides with replication foci. Oxidative stress leads to induction of γH2AX in replicating as well as non-replicating cells; there was only a weak tendency toward damage at sites of DNA replication.

CONCLUSIONS. High degree of colocalization of EdU and γH2AX sites in cells treated with CPT is coherent with the known mechanism of induction of DSBs by DNA topoisomerase I (topo1) inhibitors at sites of collision of moving replication forks with topo1-DNA "cleavable complexes" stabilized by CPT. The moderate or poor correlation of γH2AX foci with replication sites as seen in the case of ETP or MXT (also observed with these drugs in non-replicating G1 and G2 cells), hints at the mechanism of DNA damage unrelated to replication. The increased number of replication foci observed in cells treated with CPT suggests that stalling replicating forks may trigger activation of new DNA replication origins. This agrees with a postulated plasticity of replication origins.
Studies have shown early on that the DNA of interphase chromosomes in the eukaryotic nucleus is anchored periodically to the nuclear matrix, forming a series of large (50-200 kbp) loops. Since the chromosomal DNA between these fixed attachment points is intact (unnicked), each loop corresponds to a topologically constrained, independent superhelical domain. When isolated nuclei are treated with high salt (2 M NaCl), the histones and other soluble nuclear proteins involved in chromatin-packaging are removed and the DNA bulges out from the nucleus forming a round “halo” surrounding the residual nuclear matrix. The halo, comprising intact, negatively supercoiled (i.e. underwound) DNA loops emanating from the nuclear matrix, can be visualized using fluorescent DNA intercalator dyes. The negatively supercoiled DNA loops can be gradually unwound, relaxed then overwound, with increasing concentrations of the intercalators, allowing the measurement of the length and superhelical density of the DNA loops.

We have developed a sensitive method for the measurement of DNA superhelicity and loop length of individual cell nuclei, based on the use of a novel, bright fluorescent intercalator and Laser Scanning Cytometry (LSC). The average halo radii at different intercalator concentrations were calculated from the measured halo areas and were plotted against the dye concentration, thus generating a characteristic ‘winding curve’. Superhelical densities at different intercalator concentrations were determined by single molecule experiments using magnetic tweezers that could precisely quantify the DNA elongation and unwinding caused by the intercalating dye at the same high salt conditions. Using the intercalator concentration versus superhelical density plot as a standard curve, we were able to convert the dye concentration values of the winding curves to true superhelical densities. By LSC, DNA loop size and superhelicity could be distinguished in the different phases of the cell cycle. Using the fluorescence integrals of the images it was also possible to determine the matrix association of the DNA loops at the different intercalator concentrations.

The DNA loops in the halos appeared completely superhelical throughout the cell cycle, though several thousand nicks were visible in the nuclear matrix associated DNA (Szekvolgyi et al., 2007). This suggests that that the halo- and matrix associated DNA represent two distinct chromatin domains that are topologically isolated. Significant changes were observed during the cell cycle in the length and matrix association of the DNA loops of the halo. We have also found differences in the superhelicity and length of the chromatin loops between different cell types and after various treatments, including differentiation induction and replication inhibition. Our current efforts are focused on the determination of the absolute level of superhelicity of the looped DNA after taking nonspecific dye binding also into account.

References:
Szekvolgyi L. et al., 2007 PNAS 104, 14964-14969.
Vinculin (VCL) is an actin-binding protein responsible to sense the mechanical properties of the extracellular environment. It is the main component of the focal adhesions establishing cell-cell and cell-matrix interaction. Our preliminary data locates VCL to both somatic nuclei as well to meiosis-specific structure in spermatocytes nuclei – the synaptonemal complex. This finding shows VCL as a new player in the mammal meiosis. In *C. elegans*, vinculin ortholog is DEB-1 protein which has ~85% amino acid sequence homology. Depletion of DEB-1 resulted into hermaphrodite sterility and "EMO phenotype" with refractive eggs that fail to hatch. However, till present there was no evidence of DEB-1 participation in meiosis.

This model organism is well-established for the study of meiosis because adult hermaphroditic meiotic nuclei progress in the entire gonad, which allows visualizing all meiotic stages. We therefore localized the DEB-1 with a specific antibody in the whole *C. elegans* gonad and within rachis. As the presence of DEB-1 in meiocytes was microscopically not clearly apparent, so we produced DEB-1 RNAi knock-down (*deb1*-) worms. DEB-1 depletion caused disruption of regular arrangement of germ-line nuclei within gonad, enabled pairing of meiotic chromosomes during zygotene and pachytene and thus the formation of synaptonemal complex. Moreover, statistical evaluation of DAPI stained bodies showed aneuploidy, varying the DAPI stained bodies between 1-10 chromosomes in contrast to 6 chromosomes in the wild type mature oocytes.

We continued with ultra-structural visualization of meiotic chromosomes in zygotene and pachytene wild type and *deb-1*-/ worm gonad. Furthermore we detected DEB-1 and synaptonemal proteins (HIM-3, SYP-2) on ultrathin sections, using immuno-gold labeling method. These TEM results allowed us to describe further changes in morphology of meiotic nuclei within WT and *deb1*-/ gonad.

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Cancer is characterized by an uncontrolled growth and spread of abnormal cells. In carcinogenesis many proteins involved in the regulation of cell proliferation and apoptosis, are degraded through ubiquitin-proteasome system. It seems clear that the inhibition of this system alters cancer growth and for this reason there is an increasing interest in proteasome inhibitors.

Bortezomib (BZ) is the first 26S proteasome inhibitor for the treatment of multiple myeloma and mantle cell lymphoma, and its effect induces accumulation and aggregation of misfolded proteins in the endoplasmic reticulum (ER), resulting in activation of the unfolded protein response (UPR). One of the main effect of ER stress transducers (PERK, IRE1, and ATF6) is the phosphorylation of eIF2α that leads to general inhibition of translation and subsequent translational activation of activating transcription factor 4 (ATF4).

ATF4 belongs to a bZip transcription factors and its uORF1 is a positive-acting element that upregulate genes involved in amino acid metabolism and transport in stressed cells. Its overexpression is detected in tumours and it is believed that it associated with drug resistance.

The aim of this preliminary study is to better understand the subcellular localization and the role of ATF4 in HeLa and MCF7 cancer cell line treated with BZ, by immunofluorescence staining and ultrastructural immunocytochemistry. In particular, we are interested in the possible nucleolar location of ATF4 in relation with ribosomal proteins and impairment of ribosomal biogenesis.

Previous data indicate that cells line show different thresholds for BZ-induced ATF4 nucleus or nucleolar localization; its translocation is time-dependent and reversible after 16h even after bortezomib is removed. We found that ATF4 is associated with RNA and when HeLa cells were permeabilized prior to fixation, a subpopulation of ATF4 localized and were firmly attached to a few bright nuclear foci following proteasome inhibition. Moreover, the presence of ATF4 within the granular region seems to be related to the nucleolar activity. In segregated nucleoli with impaired functions, in fact, ATF4 is excluded from this region.

This work was founded by FAR 2010 and 2011.
P18. MAPPING OF NICKS AND R-LOOPS AT THE RIBOSOMAL DNA LOCUS OF THE YEAST SACCHAROMYCES CEREVISIAE

Éva Hegedűs¹, Endre Kókai², Lóránt Székvölgyi¹, Viktor Dombrádi² and Gábor Szabó¹

¹Department of Biophysics and Cell Biology, and ²Department of Medical Chemistry, Medical and Health Science Center, University of Debrecen, 4012 Debrecen, Nagyerdei krt. 98., Hungary

Agarose embedded, deproteinized S. cerevisiae chromatin exposed to S1 nuclease digestion, or upon denaturation, yield loop-sized DNA fragments (~50-150 kbp), due to the presence of preformed single-strand (ss) discontinuities bordering these loop-size chromatin domains as detected using either FIGE or CHEF gel electrophoretic systems.

In order to compare the distribution of ss discontinuities in the different chromosomes, they were separated by CHEF prior to S1 digestion or denaturation, and analyzed in a 2nd dimension. These experiments have lead to the conclusion that the incidence of nicks is similar in each of the chromosomes, what has been confirmed in ChIP-on-chip experiments.

For mapping of nicks within the naturally amplified rDNA units, we have developed a reverse South-Western protocol. The nicks present in the deproteinized chromatin of agarose-embedded S. cerevisiae could be nick-translated by DNA polymerase I using biotinylated nucleotides. Labeling was limited to the immediate surroundings of the nicks using terminator nucleotides. The rDNA units could be separated from the other, non-rDNA sequences by a restriction digestion and gel electrophoresis [1]. After a 2nd restriction digestion the DNA fragments of rDNA were blotted onto nylon membrane and the restriction fragments containing nicks were detected by anti-biotin antibody. This method was also used for the detection of R-loops in rDNA units, but in this case we used the RNA/DNA hybrid specific S9.6 antibody. We have compared the distribution of nicks and R-loops within the rDNA cluster in the case of various mutant yeast cell strains.

Reference:

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P19. URACIL EXCLUSION FROM THE GENOME OF UNDIFFERENTIATED CELLS IS ESSENTIAL FOR DEVELOPMENT IN DROSOPHILA MELANOGASTER

András Horváth¹, László Henn², Villő Muha¹, Júlia Batki¹, Péter Vilmos², Gergely Róna¹, Miklós Erdélyi², Beáta G. Vértessy¹

¹Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences; ²Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences

Accurate and stable DNA synthesis requires the precise regulation of dNTP pool. This regulation involves i) meeting the requirements of cell cycle or DNA repair dependent DNA synthesis, ii) the maintenance of the proper ratio of dNTP components, and iii) the elimination of the modified nucleotides from the pool. Perturbed regulation results in the appearance of DNA lesions compromising genome integrity mostly by base excision (BER) or mismatch repair. Uracil bases are frequent lesions that can occur in DNA. dUTPase has the essential role to prevent dUMP incorporation into DNA, and an uracil DNA glycosylase, UNG, a component of BER is responsible for uracil removal from the genome.

From previous observations we learned that the fruit fly lacks UNG from the three components and possess only a statio-temporal expression of dUTPase. We showed that the uracil content of DNA depends only on dUTPase expression in this organism. I also examined the transcriptional regulatory mechanism that forms dUTPase expression pattern. My results suggest that DRE motifs located in dUTPase promoter may be responsible for a cell cycle dependent expression, but there might be a DRE independent gonad-specific regulation as well. We also showed that compromising dUTPase expression in proliferating cells, such as imaginal precursor tissues in larva or embryonic tissues, results in genomic uracil accumulation, DNA fragmentation, mitotic errors and cell death.
P20 THE RELATIONSHIP BETWEEN SENESCENCE P21CIP1 AND SELF-RENEWAL PROTEIN OCT4 IN THE ETOPOSIDE-TREATED EMBRYONAL CARCINOMA CELLS

Anda Huna\textsuperscript{1}, Kristine Salmina\textsuperscript{1}, Thomas R Jackson\textsuperscript{2}, Pawel Zayakin\textsuperscript{1}, Jekaterina Erenpreisa\textsuperscript{1}

\textsuperscript{1}Latvian Biomedical Research & Studies Centre, Riga, Latvia; \textsuperscript{2}Cancer Sciences Unit, General Hospital, Southampton, SO16 6YD, UK

Cellular senescence is typically considered a terminal cell fate. However, as we recently showed, in tetraploid cancer cells, which possess inherited stemness and are +/-TP53, therapy-induced senescence may be reversible and intimately associated with self-renewal. The cells of human embryonal carcinoma PA1 treated with Etoposide undergo 2-3 day long full G2 arrest, followed by therapy induced senescence and death of the majority of them, however a small proportion of cells recovers clonogenic growth after a week. We found that in the G2-arrest stage most cells simultaneously accumulate in their nuclei opposite regulators – self-renewal master transcription factor OCT4A and inhibitor of cell cycle and mediator of senescence P21CIP1 and they both depend on activation by DNA damage of TP53 (Jackson et al., 2013). Here we report on studies of the character of this dual expression in the time course as detected by flow cytometry and \textit{in situ} fluorescence measurements using fluorescent microscope, video camera, and Image Pro Plus 4.1 software. In non-treated cells, the two-dimensional distribution of the low intensity OCT4A and background p21CIP1 staining measured in individual cells form rather a compact cluster, while in the treated cells the reactivity for both antibodies is strongly enhanced and the variability amplitude increases. Using the hypothesis of ergodicity, we suggest that this pattern reflects the temporal fluctuations of the protein amounts in individual cells characterizing it as bi-potential and metastable. The bi-potential state has the property to keep alert both networks, however preventing for the time being each other from reaching their destination targets. Initially the amounts of the opposite proteins correlate, however in the time course the “swing” between the regulators appears as shown by decreasing correlation between both factors, therefore, representing the process of dissociation between opposite outliers. Removal of TP53 accelerates this dissociation and leads to simultaneous release of premature mitoses and prematurely senescent cells. Further studies on the patterns and significance of this temporal collaboration of self-renewal and senescence networks for cell fates decisions are on route.

Reference:

P21. NUCLEOSOME-DNA AFFINITY IS HIGHLY SENSITIVE TO CERTAIN H3 MODIFICATIONS AND TO SUPERHELICAL TWIST

László Imre¹, Zoltán Simándi², György Fenyőfalvi¹, Lóránt Székvölgyi¹, László Nagy², Hiroshi Kimura³ and Gábor Szabó¹

¹Department of Biophysics and Cell Biology, Medical and Health Science Center, University of Debrecen, ²Department of Biochemistry and Molecular Biology, Medical and Health Science Center, University of Debrecen, ³Graduate School of Frontier Biosciences, Osaka University, Osaka 565-0871, Japan

We have developed a sensitive and high throughput method, applying a laser scanning cytometer (LSC), for the analysis of histone mobility features. Our method offers sensitive means to determine, quantitatively and in a cell-cycle phase specific manner, a major component of global histone mobility: nucleosome-DNA cohesion. After salt or intercalator elicited elution of agarose-embedded isolated nuclei the remaining histone levels are determined by immunofluorescence labeling. Using a panel of modification-specific monoclonal antibodies we have analyzed the elution profiles of H3 histones carrying different epigenetic marks. The elution characteristics of bulk histones were assessed directly in the cell lines expressing GFP-tagged H3.

Significantly steeper profiles (indicating loosely bound histones) could be measured in the case H3K4me3 and H3K27ac, in contrast with inactive marks like H3K27me1, H3K27me2, H3K27me3, H3K9me1, H3K9me2, H3K9me3 and other active marks, including H3K36me3, H3K4me0, H3K4me1, H3K4me2, H3K9ac, H3K14ac. These data suggest that H3K4me3 and H3K27ac modifications, alone or in combination, have a major role in the basic structural organization of chromatin. This effect is likely to prevail in the absence of reader protein binding to the same modifications, in view of the detection protocol applied. Furthermore, the similar effects of salt and intercalators raise the possibility that superhelical twist and/or DNA strand extension may play a role in gene regulation via influencing DNA-nucleosome affinity.

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P22. NEGATIVE REGULATION OF THE NF\(\kappa\B\) SIGNALING PATHWAY BY THE HEAT SHOCK TRANSCRIPTION FACTOR 1

Janus, P.\(^1\), Kalinowska-Herok, M.\(^1\), Szoltysek, K.\(^1\), Jaksik, R.\(^2\), Handschuh, L.\(^3\), Kimmel, M.\(^2\) and Widlak, P.\(^1\)

\(^1\)Maria Sklodowska-Curie Memorial Center and Institute of Oncology; Gliwice, Poland; \(^2\)Silesian University of Technology; Gliwice, Poland; \(^3\)Institute of Bioorganic Chemistry, Polish Academy of Sciences; Poznan; Poland.

NF\(\kappa\B\)- and HSF1-dependent pathways are the major components of cellular response to stress, which play essential role in pathogenesis and therapy of serious human diseases, including cancer. Both transcription factors regulate numerous genes involved in apoptosis, cell proliferation and inflammatory responses. Here we aimed to identify NF\(\kappa\B\)-regulated genes, which expression is affected by HSF1.

Expression of NF\(\kappa\B\)-dependent genes was analyzed in U2-OS human osteosarcoma cells stimulated with TNF\(\alpha\) cytokine, which activates the canonical NF\(\kappa\B\) pathway. Cells were either preconditioned with hyperthermia (HS) to activate endogenous HSF1 or engineered to express a constitutively active form of HSF1 in the absence of heat shock (aHSF1). The expression of TNF\(\alpha\)-induced genes was analyzed by the expression microarrays. Bioinformatics analysis was made for prediction of hypothetical \(\kappa\B\) and HSE motifs in promoter regions of analyzed genes. Actual sites of HSF1 binding to chromatin were detected in cells subjected to HS by chromatin immunoprecipitation assay coupled with DNA sequencing (ChIP-Seq approach).

Stimulation of U2-OS cells with TNF\(\alpha\) resulted in up-regulation of 190 genes and down-regulation of 133 genes. Among such 323 TNF-regulated genes 113 genes contained hypothetical \(\kappa\B\)-binding motifs and could be considered as putatively NF\(\kappa\B\)-dependent. Actual sites of HSF1 binding were detected in 68 out of 323 TNF-regulated genes, including 35 NF\(\kappa\B\)-dependent genes. We have analyzed effects of hyperthermia and the presence of constitutively active aHSF1 upon expression of TNF-regulated genes. The same effect of hyperthermia and aHSF1 was observed for 192 TNF-regulated genes, including 64 NF\(\kappa\B\)-dependent genes, hence we concluded that observed effect was related to hyperthermia-induced HSF1. For the majority of NF\(\kappa\B\)-dependent genes (54 genes), HSF1 and NF\(\kappa\B\) revealed antagonistic effect: HSF1 inhibited genes up-regulated by TNF (39 genes) or stimulated genes down-regulated by TNF (15 genes). Among them there were 16 NF\(\kappa\B\)-dependent genes where actual HSF1 binding in their promoter regions were detected, including 13 genes inhibited by HSF1, which indicated direct effect of HSF1 upon their regulation.

We concluded that HSF1, in addition of canonical activation of HSP genes, could be involved in direct regulation of genes participating in the NF\(\kappa\B\)-dependent pathway. Here we showed evidence that HSF1 is a potential negative regulator of 13 NF\(\kappa\B\)-dependent genes stimulated by TNF\(\alpha\): IL8, CCL2, CD83, FOSB, EGR1, ATF3, SLC12A7, ZFP36, PPP1R15A, GADD45B, RRAD, IFNGR2, EPB41L2.

This work was supported by Polish National Science Center, Grant N401_563740 and Grant DEC-2012/04/A/ST7/00353.
Phosphoinositides are well known intracellular signalling molecules regulating many cellular processes. Besides the well established cytoplasmic signalling pathway, it is suggested that phosphoinositide signalling occurs even in the cell nucleus. Many enzymes involved in metabolism of phosphoinositides such as phosphatidylinositol phosphate kinases, phosphatases and phospholipases localize to the nucleus, nucleolus and nuclear speckles - intranuclear dynamic structures storing pre-mRNA splicing factors. Moreover, PI(4,5)P2, the most studied nuclear phosphoinositide, localizes to nucleoli and nuclear speckles and is required for regulation of expression and nuclear export of specific mRNA. However, only a little is known about other phosphoinositides within the nucleus.

Our aim is to map localization of different phosphoinositides within the cell nucleus. For this purpose we performed immunofluorescence studies. We used not only specific antibodies but also PH and PX domains, protein modules specifically recognising different phosphoinositides. These domains are fused to GST tag and therefore can be detected by anti-GST antibody and fluorophore labelled secondary antibody. To better understand the nature of nuclear protein-lipid complexes, we would like to pull-down phosphoinositide binding partners in the nucleus by phosphoinositide coated agarose beads. Our preliminary data show that PI(3,4)P2 also localizes to the nucleus but not to the nucleolus and interacts with lamin B.

Using the methods mentioned above, we expect to better understand the character of phosphoinositides-protein interaction and the involvement of these complexes in nuclear processes.

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P24. FUNCTIONS OF MYOSIN 1C ISOFORMS IN THE CELL NUCLEUS

Kalendova, A., Yildirim, S. and Hozak, P.

Institute of Molecular Genetics AS CR v.v.i., Department of Biology of the Cell Nucleus, Prague, Czech Republic

Nuclear myosin 1 (NM1) is a molecular motor localized to the cell nucleus. It is able to bind actin by its motor domain, calmodulin by a neck domain and various cargos by the tail domain. In the interphase nucleus, NM1 participates in chromatin remodelling, DNA transcription and ribosome biogenesis. 16 amino acids shorter isoform of NM1 called myosin 1c (Myo1c) is known to bind acidic phospholipids in the cytoplasm, which enables it to associate with membranes and membranous structures. Since membranous structures do not exist inside of the cell nucleus, PIP2 is believed to be retained in lipoprotein complexes. Here we show that both NM1 and Myo1c are able to bind acidic phospholipids in the cell nucleus, mainly phosphatidylinositol-4,5-bisphosphate (PIP2). NM1 and Myo1c therefore seem to be anchored to such lipoprotein complexes through their interaction with PIP2 and other lipids. Releasing of NM1 from these complexes leads to elevation in pre-rRNA synthesis. On the other hand, wild-type NM1 and lipid-binding mutant of NM1 are both able to associate with transcription initiation factor TIF-1A and RNA polymerase I and therefore initiate the transcription. Inhibition of transcription however causes increase in mobility of NM1. These data suggest existence of two different pools of NM1 and Myo1c - molecules bound to lipoprotein complexes and molecules associated with transcription. Active crosstalk most probably exists between these two pools, because releasing of NM1 from PIP2 causes increase in transcription.

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We examined whether chromatin relaxation during the diplotene stage is accompanied by the synthesis and maturation of mRNA. Larch microsporocytes display changes in chromatin morphology during the diplotene stage, alternating between 4 short stages of chromatin contraction and 5 longer diffusion (relaxation) stages. The nuclei exhibit large amounts of the RNA polymerase II, and high levels of snRNPs involved in splicing. The results reveal high transcriptional activity during the 5 periods when chromatin is being decondensed in the microsporocytes. mRNA is intensively synthesized during the diffusion stages. In these stages, we also observed a high level of protein synthesis. The synchronous development and "timely and fast switching on and off" of RNA and protein synthesis allows the tracking of changes that occur during snRNA synthesis and maturation under "natural conditions". In the cytoplasm, a cyclical occurrence of bodies containing small nuclear RNA was observed. An in vivo investigation using molecular beacon probes showed that CsBs play transport role during snRNP biogenesis in the cytoplasm. We observed a correlation between the occurrence of cytoplasmic snRNP bodies (CsBs), the level of snRNP, and the dynamic formation of Cajal bodies.
Recent studies have shown that splicing can play a role in the regulation of gene expression. Intron retention (IR) is an alternative splicing event where the splicing of an intron is skipped, resulting in an otherwise mature transcript harboring an unprocessed sequence. This process has been shown to be triggered by external stimuli, to be specific to developmental phases and tissue type. A recent study showed that the utilization of stored RNA is a driving force in rapid development. Retention and subsequent removal of introns from pre-mRNAs regulate temporal patterns of translation during rapid and posttranscriptionally controlled spermatogenesis of the fern and during meiosis in fission yeast.

In our research we used larch microsporocytes, which accumulate a large amount of mRNA in nucleus during the diplotene stage. Our study also showed that in the diplotene mRNA appeared in Cajal bodies (CBs). Double labeling of newly formed transcripts and poly(A)RNA shows that appearance of poly(A)RNA containing CBs, followed by a period of high transcriptional activity, and precedes the appearances of a large amount of poly(A)RNA in the cytoplasm. We performed single molecule labeling using ELF 97 mRNA in situ hybridization method to check if accumulated within CBs mRNA harboring retained introns.
Heat Shock Factors (HSFs) are transcriptional activators of heat shock genes. Once activated they form trimers and bind specifically to Heat Shock sequence Elements (HSEs) throughout the genome. HSF1 is the primary factor responsible for the response to different forms of cellular stress (e.g. heat shock), while HSF2 becomes activated during development and differentiation (e.g. during spermatogenesis). Both HSF1 and HSF2 cooperate either during stress or under physiological conditions.

Despite the high degree of conservation of the heat shock response, different cells vary in their ability to induce Heat Shock Proteins (HSPs) synthesis and consequently in sensitivity to damaging agents. Interestingly, some types of cells (for example spermatocytes) lack the typical heat shock response and are hypersensitive to hyperthermia. What’s more, activation of HSF1 in spermatocytes initiates apoptosis leading to infertility of males. To elucidate mechanisms assisting heat induced apoptosis we studied how HSF1 and HSF2 cooperate with each other in regulation of Hsp genes expression during the heat shock response in mouse spermatocytes.

Using the Duolink In Situ kit we detected direct interactions (heterotrimers are also possible) between HSF1 and HSF2 in mouse testes. We observed HSF1/HSF2 complexes both in cytoplasm and nuclei. In spermatogonia, complexes were more abundant in cytoplasm than in nuclei, in spermatocytes – inversely, while in spermatids they were equally distributed. Many complexes were located on the boundary between cytoplasm and nucleus. Following heat shock, the amount of HSF1/HSF2 complexes was enlarged. Next, by ChIP-seq (chromatin immunoprecipitation combined with massive parallel sequencing) we studied the binding of HSF1 and HSF2 to DNA in spermatocytes. Analyses revealed that while increased temperatures induced HSF1 binding to many Hsp genes, HSF2 was only bound at control cells and/or at 38°C, then it was completely released from Hsp promoters at 43°C. Additionally, we stated that binding of HSF1 to the Hsps promoters did not correlate with their transcriptional activation following heat shock.

Obtained results suggest that HSF1 and HSF2 could cooperate in regulation of the transcription of some Hsps genes only at physiological temperatures and in some cases at 38°C, since they bind to the same region of the promoter in these conditions. Although HSF1 and HSF2 interactions exist in spermatogenic cells following heat shock, they do not stimulate activation of Hsp genes expression.

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P28. NEW INSIGHTS INTO XENOPUS LAEVIS LAP2 GENE MODEL, EXPRESSED PROTEINS ISOFORMS AND THEIR INVOLVEMENTS IN EARLY MITOSIS PROCESSES

Kozioł, K.¹, Dubińska-Magiera, M.¹, Chmielewska, M.¹, Goldberg, M. W.², Hutchison, C. J.² and Rzepecki, R.¹*

¹Laboratory of Nuclear Proteins, Faculty of Biotechnology, University of Wroclaw, Przybyszewskiego Str. 63/77, 51-148 Wroclaw, Poland; ²Integrative Cell Biology Laboratories, School of Biological and Biomedical Sciences, The University of Durham, South Road, Durham DH1 3LE, UK;

Previous studies have demonstrated in Xenopus laevis three isoforms of LAP2 protein. Two isoforms were found to be expressed in early late oocytes, eggs and embryonic development (omega and gamma). Starting from the tadpole stage single beta isoform is expressed. Based on all available databases including Xenopus laevis genome draft 6.0 and TXGP UTexas Oktoberfest DB and our MS/MS database from eggs and developmental stages we created an integrated model of XLAP2 gene and possible transcripts synthesized during Xenopus development. We found that more then three previously detected isoforms can be detected.

We also analysed the function of XLAP2 beta isoform using XTC tissue culture cells. XLAP2 beta knockdown induces cell growth arrest and apoptosis proceeded by nuclear envelope abnormalities and partial loss of NE, loss of lamin B expression and intranuclear location of F/G repeat nucleoporins.

XLAP2 beta, during early mitosis (up to metaphase), is preferentially enriched in membrane fractions associated with centrosomes and centrosome associated microtubules but not with bulk ER membranes. This association of beta isoform with spindle microtubules is not dependent on TPX2 protein directly. We found that preferential association of XLAP2 beta is lost when polymerization of microtubules is blocked or depolymerization induced.

Since we previously discovered intranuclear fraction of XLAP2 beta associated with chromatin we performed a series of IP experiments followed by MS/MS analyses using anti XLAP2 antibodies to detect proteins present in complexes with “soluble” fraction of XLAP2 beta during interphase and mitosis. Different sets of proteins were discovered in soluble complexes with XLAP2beta. This all indicate that in Xenopus laevis LAP2 proteins are essential for cell viability and play complex and diverse functions.

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*corresponding author
P29. DNA PROPERTIES THAT ARE DISTINCTIVE OF MEIOTIC RECOMBINATION HOTSPOTS

Osamu Miura, Toshihiro Ogake, Takashi Ohyama
Biology, Waseda University, Tokyo, Japan.

Genetic information is also carried in conformation and physical properties of DNA. Understanding such information constitutes one of the frontiers in the era of epigenetics. We are studying this challenging issue with focusing on the meiotic recombination hotspots. These sites do not have any consensus sequence and the mechanism underlying hotspots formation is poorly understood.

Using the genome databases of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Mus musculus*, we examined characteristics of the mechanical properties of the hotspots. When averaged, a common distinctive mechanical property was clearly detected at the center region of the hotspots for each organism. However, very interestingly, the properties themselves were opposite between the yeasts and *M. musculus*: i.e., the center region is very rigid in the former while very flexible in the latter. Recently, we also examined the other structural properties of the hotspots and found a common distinctive property. We will discuss DNA properties that are distinctive of meiotic recombination hotspots.
Most of nucleolar proteins can exchanged freely between the nucleolus and the surrounding nucleoplasm. The mechanisms by which proteins are accumulated within such non-membrane-bound substructures remain unclear. One way of protein retention in the nucleolus is associated with the presence of specific short amino acid sequences – nucleolar localization signals (NoLSs). The mechanism of their action is still unknown. Analysis of published data revealed that all described NoLSs are enriched with positively charged amino acids (~50% of the total amino acid content). One can suggest that the mechanism of interaction with the nucleolar components is electrostatic. To ascertain this assumption, we used chimeric proteins containing EGFP and short tags containing different amounts (from 1 to 19) of positively charged amino acids – lysines or arginines (such tags were referred to as imitative NoLSs). We found that there was dependence between the charge of the imitative NoLSs and the level of EGFP accumulation inside nucleoli. The level of EGFP accumulation was inversely correlated with the speed of EGFP exchange between the nucleolus and the nucleoplasm. We found that the level of accumulation is higher in case of imitative NoLSs in which positively charged amino acids were not separated by uncharged amino acids. Also, we mapped in detail the signals and their structure in two proteins (MAP3K14 and HIV Tat). The presence of positively charged amino acids in close vicinity of the signal (in MAP3K14) leads to the "blurring" the NoLS boundaries. In fact, in this case, the boundaries can be mapped only approximately. If positively charged amino acids were absent in close vicinity of the signal (in HIV Tat), the boundaries of NoLS coincided with that of the cluster of charged amino acids. The replacement of positively-charged amino acids inside signal led to a decrease in the efficiency of accumulation. Thus, these results are in agreement the hypothesis that the accumulation of proteins in the nucleolus by NoLSs can be determined by the electrostatic interaction of positively charged signals with the components of the nucleolus.
P31. CELL-TYPE-SPECIFIC DELAY IN ACTIVATION OF NF-κB SIGNALING PATHWAY DURING THE HEAT SHOCK RESPONSE

Naumowicz A.1,2, Janus P.1, Widlak W.1, Kimmel M.2

1Center for Translational Research and Molecular Biology of Cancer, M. Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Poland; 2Institute of Automatic Control, Faculty of Automatic Control, Electronics and Computer Sciences, Silesian University of Technology, Gliwice, Poland

The NF-κB signaling pathway is an essential component of cellular response to stress as the main regulator of numerous genes important for inflammation, immune response and cell survival. The family of mammalian NF-κB proteins consists of five NF-κB/Rel and four IκB proteins. NF-κB/Rel are subunits of NF-κB transcription factor acting as dimers, while IκB are their specific inhibitors. The NF-κB heterodimers play a physiological role and their activity remains under strict control. The most common is a dimer composed of p50/RelA (p50/p65) proteins. The TNFα-stimulated canonical activation of NF-κB pathway leads to phosphorylation and proteasomal degradation of IκB protein, resulting in nuclear translocation of NF-κB and its binding to DNA in the κB motif.

Previous studies have shown that the NF-κB activation pathway is blocked by heat shock. We investigated activation of NF-κB pathway in the human lung adenocarcinoma A549 and osteosarcoma U-2 OS cell lines by Western blotting. TNFα treatment for 15 minutes led to activation of NF-κB signaling which was monitored by following the phosphorylation and degradation of IκB and phosphorylation/activation of RelA. Activation reached the maximum 15 minutes post TNFα treatment and IκB level was restored after 60 minutes in both cell lines. Dynamic live cell imaging studies with A549 cell line stably transfected with a construct coding the EGFP-RelA fusion protein confirmed that RelA was translocated to nucleus following TNFα stimulation.

Cells were subjected to heat shock in 43°C and allowed to recover for 1 hour. Then, they were treated with TNFα for 15 minutes. In U-2 OS cells we observed inhibition of TNFα-induced degradation of IκB, thus NF-κB was not activated at least up to 90 minutes post treatment. Surprisingly in A549 cell, we observed activation of the NF-κB signaling during the heat shock response, although it was weaker and delayed in time (15-30 min) comparing to the normal conditions. The living cell imaging of A549 cells with EGFP-RelA fusion protein has also shown the differences in the kinetics of RelA translocation from cytoplasm to nucleus during normal conditions or in response to heat shock after cytokine stimulation.
P32. THE HUMAN WBSCR22 IS INVOLVED IN THE BIOGENESIS OF THE 40S RIBOSOMAL SUBUNITS IN MAMMALIAN CELLS

Õunap, K.¹², Kurg, A.¹ and Kurg, R.²

¹Department of Biotechnology, Institute of Molecular and Cell Biology, University of Tartu;
²Institute of Technology, University of Tartu

WBSCR22 protein which is expressed from Williams-Beuren chromosomal region 22 is a nuclear protein which has a predicted S-adenosylmethionine (SAM) binding motif typical of Rossmann-fold methyltransferases. SAM-dependent MTases represent a diverse class of enzymes which act on protein, small molecule, lipid or nucleic acid methylation and therefore mediate numerous cellular processes. Recently it has been shown that WBSCR22 is highly expressed in invasive breast cancer. Ectopic expression of WBSCR22 enhances tumour cell survival in the vasculature and its depletion in tumour cells reduced metastasis formation. WBSCR22 gene is one of the 26-28 genes which are deleted in microdeletion syndrome Williams-Beuren syndrome. WBS patients usually have cardiovascular problems, intellectual disability, connective tissue and endocrine abnormalities and it is unknown how the deletion of most of the genes contributes to the WBS phenotype.

Our study shows that WBSCR22 protein is involved in ribosome biogenesis and it is therefore important for cell growth. WBSCR22 knock-down with siRNA leads to ribosome small subunit (40S) biogenesis defect by reducing free ribosomal 40S subunit level and decreased 40S/60S ratio. WBSCR22 is also involved in processing of 18S rRNA since WBSCR22 knock-down causes the accumulation of 18S-E pre-rRNA in cell nucleus.

The yeast homolog of WBSCR22, Bud23, sharing 47% similarity on amino acid level, is a ribosomal 18S rRNA methyltransferase required for ribosome biogenesis. Bud23 is a non-essential protein but its deletion leads to slow growth phenotype and defects in pre-rRNA processing and ribosome 40S biosynthesis. The human WBSCR22 partially complements the growth and polyribosome profile of bud23 deletion mutant suggesting that the human WBSCR22 is a functional homologue of yeast bud23. Our data suggest that that human WBSCR22 and yeast Bud23 have similar but probably not identical functions in ribosome biosynthesis. Similarities and differences between yeast Bud23 and human WBSCR22 proteins will be discussed.
P33. HOW QUICK ARE THE FIRST STEPS OF THE HUMAN rRNA PROCESSING?

Popov, A., Smirnov, E., Kovacik, L., Skalnikova, M., Higashinakagawa, T., Cmarko, D. and Ivan Raska

Charles University in Prague, First Faculty of Medicine, Institute of Cellular Biology and Pathology, Prague, Czech Republic and International Center for Molecular, Cellular and Immunological Research Tokyo Women’s Medical University, Tokyo, Japan

Processing of nascent ribosomal RNA (rRNA) in mammalian cells includes a series of cleavages of the primary 47S transcript (pre-rRNA) and produces three RNAs: 18S, 28S, and 5.8S. The sequence of the main processing events in human cells has been established, but little is known about the dynamics of the first cleavages. In the present study, we used real-time PCR to measure levels of pre-rRNA after inhibition of transcription with actinomycin D. Thus we could estimate the half-life of rRNA transcripts in two human-derived cell lines, HeLa and LEP (human embryonic fibroblasts), as well as in mouse NIH 3T3 cells. The primary transcripts seemed to be more stable in the human than in the murine cells. Remarkably, in all cases there was more or less pronounced lag phase in the pre-rRNA decay, which apparently corresponds to the preparatory events preceding the first cleavage. Additionally, we followed the dynamics of the decay of the 5'ETS fragment which is degraded only after the formation of 41S rRNA. According to our estimates, the first three (or four) steps of the processing in human cells take 5 to 8 minutes.

Reference:


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By creating a double-stranded break in DNA at a specified location, one can either integrate a desired sequence through homologous recombination (HR) or could achieve knock-out through non-homologous end joining (NHEJ) mediated repair mechanism. By applying a conditional knock-out strategy we wish to study the role of dUTPase in genomic integrity maintenance in human cells.

dUTPase is an essential enzyme that keeps the dUTP/dTTP ratio low in the cellular environment, thus preventing uracil incorporation into the genome. Several chemotherapeutic drugs routinely applied in the clinic (5FU, 5FdU, methotrexate, raltitrexed), interfere with the thymidylate biosynthetic pathway, which dUTPase is also part of. We would like to further clarify how dUTPase modulates the effects of these drugs in this pathway. We are generating a conditional knock-out system which utilizes a ligand inducible Cre recombinase to cut out an exogenous dUTPase source integrated into the AAVS1 locus of HCT116 cells. The exogenous dUTPase source expresses both nuclear and mitochondrial isoforms along with a green fluorescent protein for selection and cassette detection purposes. The ligand inducible Cre recombinase containing cassette is also integrated into the AAVS1 locus of the other chromosome pair. These integration events were done by a single ZFN pair, simultaneously. The appropriate expression from the cassettes was varified before trying the knock-out of the endogenous dUTPase.

With the inducible dUTPase knock-out cell line we will be able to clarify the role of dUTPase in genome integrity maintenance and their involvement in clinical therapy. This system will also give us the possibility to check the distinct relevance of the two dUTPase isoforms (nuclear and mitochondrial) in the above mentioned processes. The knock-out strategy developed could be also applied for other potentially essential genes which could be of wide interest.
Alive tumor cells can get rid of unwanted DNA. Significance and regulation of this process is a matter of much interest. The excessively amplified DNA can be released, while release of Rad51-enriched MN was reported in irradiated HeLa cells was shown by Haaf et al.[1] and for the first time was suggested as being involved in the sorting of damaged DNA through repair. In association with macroautophagy so far two kinds of the chromatin elimination have been described in tumour cells following DNA or spindle damage: (1) The autophagic elimination of the selected (2-5%) micronuclei (MN) [2] and 2) the autophagy-aided degradation and extrusion of large masses of the chromatin (the whole subnuclei) enriched by repair factors in TP53-mutant polyploid tumour cells observed at later terms after DNA damage, first described on irradiated Burkitt’s lymphoma cell lines [3] and recently also in other cell lines treated by paclitaxel and etoposide [4]. While the first process is associated with mitotic slippage and genomically instable tetraploidy, the second participate in sorting of the chromatin before final de-polyploidisation of giant cells induced by damage. We show that the nuclear envelope limited chromatin sheets (ELCS), DNA repair by homologous recombination and even cytoplasmic autophagy may play a role in this process, which is likely aimed to sort out the damaged and excessive DNA. It was suggested that the chromatin autophagy ultimately serves to balance the genomes and decrease the load of aneuploidy of recovered para-diploid descendants of transiently polyploid cells [5]. We admit that degradation and diminution of large chromatin portions before release of clonogenic sub-cells represent part of the developmental programmed process.

References:
Retinoids are morphogens and have been implicated in cell fate commitment of embryonic stem cells (ESCs) to neurons. Their effects are mediated by RAR and RXR nuclear receptors. However, their transcriptional co-factors required for cell and gene-specific signaling and transcription are not known. Here we show that Protein aRginine Methyl Transferase (PRMT) 1 and 8 have key roles in determining retinoid regulated gene expression in a multistage differentiation model of neurons from murine ESCs. PRMT1 impacts the cistrome of P300 and RXR and appears to act as a repressive modulator, providing the cells with a mechanism to selectively reduce the potency of retinoid signals on Hox genes. PRMT8 is a retinoid receptor target gene itself and acts as a cell type specific transcriptional co-activator of retinoid signaling. Lack of either of them leads to reduced nuclear arginine methylation and disarrayed neuronal gene expression. Importantly, PRMT8 regulates a distinct set of genes, including markers of gliomagenesis. PRMT8 is almost entirely absent in human glioblastoma tissues. We propose that PRMT1 and PRMT8 serve as a rheostat of retinoid signaling to determine neuronal cell specification, and might also be relevant in the development of human brain cancer.

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Breast cancer is the most common oncological disease in women. Improvement of treatment efficiency is a major priority. Extensive studies have been devoted to overcome cancer cell resistance. Also new type anti-cancer therapies have been developed and studied. Recently, cancer stem cells are a completely new area of investigation and number of scientists pay attention to develop agents against them. Therefore it is crucial to develop new strategies for cancer stem cell identification.

The aim of the study is to determine cancer cell resistance, cell population heterogeneity and amount of DNA in cell in luminal and triple negative breast cancer using paraffin sections. It is necessary to find new criteria to estimate the efficiency of anticancer therapy.

Material and methods. In two types of breast cancer - triple negative (n=11) and luminal (n=32) were studied approach to identify cancer stem cells using biological markers – CD44 (clone: DF1485, NovocastraTM, Leica Biosystems Newcastle, United Kingdom), CD24 (clone: ML5, BioSite, Finland), Oct3/4 (clone: N1NK, NovocatraTM, Leica Biosystems Newcastle, United Kingdom) and ALDH (ALDH1A1, BioSite, Finland) – which were estimated immunohistochemically in paraffin sections by semi-quantitative method. In addition, both types of cancer histological samples were stained by Feulgen method to measure DNA amount in the cancer cell nuclei. The integrated optical density (IOD) proportional to amount of DNA and mean optical density proportional to concentration of DNA of nucleus were measured using ImagePro Plus 6.0 software. ANS (8-anilino naphthalene-1-sulfonic acid) and ethidium bromide staining of cancer cells and has several positive aspects – this method is simple and fast and allows easier visually evaluate the composition of the cancer cell population. This method could be useful in future for prognostic purposes using a flow cytometer.

Results. Intensive expression of antigen CD44 and ALDH and positive expression of CD24 and Oct3/4 were seen in morphologically different cells in both breast cancer groups. Semi quantitative histological section evaluation showed that CD44 and ALDH antigen expression was stronger in triple negative breast cancer group comparing to the luminal breast cancer group. CD24 and Oct3/4 antigen expression were also stronger in the triple negative breast cancer group. Expression of these antigens was elevated in two different cell types: small (2-5µm) cells and large cells. However, there could be distinguished also large and small cells with phenotype CD44+/CD24-/ALDH+ which characterizes breast cancer cell resistance.

Cell population with a larger nucleus area and lower average optical density dominated in luminal breast cancer samples. In contrast, triple negative breast cancer cell population showed cells with smaller nucleus area and higher mean optical density.

Conclusions. Overexpression of CD44 was observed in morphologically different cell types, among them in small cells, which possibly could be cancer stem cells. The embryonic cell marker Oct3/4 was observed to be dominant in two cell types - large cells and small cells, which indicate that these are poorly differentiated cells. According to these studies we conclude, that the luminal breast cancer cell populations small cells population contain cells with low and high DNA concentration, but large cells exhibit only low DNA concentration. In triple negative breast cancer cell population large and small cells contain high and low concentration of DNA. Consequently, our study suggested that both small and large cells with high concentration of DNA could be responsible for resistance but small cells could be resistant cell population progenitor.

*Corresponding author
P38. TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 AND CHONDROGENESIS

Somogyi, Cs., Matta, Cs., Földvári, Zs., Katona, É., Takács, R., Juhász, T., Zákány, R.,
Department of Anatomy, Histology and Embryology, Medical and Health Science Centre,
University of Debrecen, Hungary

Our major interest is to study in vitro chondrogenesis, focusing on the relation of various extracellular signals, plasma membrane ion channels, intracellular Ca\(^{2+}\)-concentration changes and protein phosphatases in chondrifying high density cell cultures (HDC). Though, chondrocytes are non-excitable cells, they express a large set of ion channels (e.g. purinergic P2X4, glutamate receptor NMDARs, Kv channels) maintaining a dynamic relationship with the acidic and hypoxic extracellular environment in cartilage. Transient Receptor Potential Vanilloid (TRPV) subfamily of non-selective cation channels of plasma membrane has not been studied in a detailed way during cartilage formation, except TRPV4 activity that contributes through the Sox9 pathway in the process of chondrogenesis.

Our experimental model is a primary chondrogenic HDC system, in which chondrogenic cells are isolated from limb buds of 4-day-old chicken embryos. Spontaneous cartilage formation occurs within 6 days in HDCs.

Chicken chondrogenic cells express mRNAs of various TRPV members during the course of cartilage differentiation. We focused on the polymodal thermo- and mechanosensitive TRPV1 membrane receptor and were able to detect several bands, including the one with the expected MW with two different TRPV1 antibodies in Western blots in samples of chicken articular cartilage or samples from chicken HDCs, as well as in chicken brain and dorsal root ganglia, applied as positive controls. Therefore, we also plan to identify the interested protein and its possible splice variants with mass spectrometry. These experiments are in progress. As avian TRPV1 contains a truncated capsaicin-binding region, it is insensitive to capsaicin or related pharmacological modulators. Therefore, we applied heat treatment on chicken HDCs to prove functionality of the channel and monitored temporal pattern of TRPV1 mRNA expression in a 4 hours period. To clarify the precise function of TRPV1 during chondrogenesis we need to perform transient gene silencing in HDCs.

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P39. *IN VIVO INHIBITION OF RNA POLYMERASES AFFECTS THE LEVEL OF CHROMOSOMAL R-LOOPS*

András Szántó, Péter Nánási, Gábor Szabó and Lóránt Székvölgyi

Departments of Biophysics and Cell Biology, Medical and Health Science Center, University of Debrecen, 4012 Debrecen, Nagyerdei krt. 98., Hungary

Structures known as R-loops are comprised of RNA-DNA hybrids that are thought to be rare byproducts of transcriptional processes and/or experimental procedures. However, recent studies have shown that these structures may be abundant in cells and may pose a threat to genome integrity. In our work presented herein, the quantity and subcellular distribution of endogenous R-loops was investigated in Actinomycin D- and alpha-amanitin-treated Jurkat cells compromised in RNAP/II/III activities. R-loops were detected by indirect immunofluorescence using an RNA-DNA hybrid specific monoclonal antibody (S9.6) and their level was quantified by a laser scanning cytometry (LSC) and confocal laser scanning microscopy (CLSM). In parallel with the detection of R-loops, active RNA synthesis was pulse labeled by an ethenyl-uridine (EU)-based “click” chemistry. Our results show that chromosomal R-loops form in a dynamic, transcription-dependent manner with an average half-life of 26 minutes. Both active transcription and R-loops preferentially localize to the nuclear matrix but they do not colocalize (pearson correlation coefficient r=0.2), suggesting that R-loops detected by the S9.6 antibody are not related to RNA-DNA hybrids travelling within the substrate binding pocket of RNA polymerases upon transcription elongation. We hypothesize that chromosomal R-loops comprise trans-acting regulatory RNAs present at ~50 kbp intervals that help establish loop-size chromatin domains.
It is now firmly established that long-lasting synaptic plasticity involves dramatic changes in gene expression occurring under the influence of specific signaling pathways and transcription factors. Numerous studies have shown that DNA and histone epigenetic modifications play key roles in neuronal plasticity. Recent studies in non-neuronal cells, indicated the existence of epigenetic mechanism of yet another class, related to the nuclei structural remodeling and very poorly understood in neurons.

Therefore, we decided to study the ultrastructure of the cell nuclei in the hippocampal dentate gyrus granule neurons upon seizures induced by kainic acid, an analog of glutamate. Under these conditions the granular neurons instead of degradation, undergo an intensive plasticity phenomena. We found that seizures led to rapid and dramatic enlargement and striking reorganization of internal component-structures of interchromatin granule clusters (IGCs) in granular cell’s nucleus. Moreover, unlike IGCs of control animals, the reorganized IGCs contained activated RNA polymerase II CTD phosphoepitopes. These observations may suggest involvement of IGC in activity-dependent transcription events in neurons.

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P41. CELLULAR STRESS INDUCED BY UV-C IRRADIATION INHIBITS NF-kappaB SIGNALLING PATHWAY IN A P53-INDEPENDENT MANNER.

Szotysek, K.¹, Walaszczyk, A.², Abramowicz, A.¹, Janus, P.¹, Kimmel, M.² and Widłak, P.¹

¹Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology; Gliwice, Poland; ²Silesian University of Technology; Gliwice, Poland

Ultraviolet (UV) radiation is a harmful environmental factor, which causes suppression of the immune system, chronic skin damage and cancer. Exposure to UV induces a variety of cell signalling pathways, including signal transduction pathway regulated by p53, NF-κB and MAPK (ERK, JNK and p38). Actual cellular response to UV-induced stress depends on interactions and crosstalk between different pathways. Here we aimed to analyze kinetics of activation of different signalling pathways in UV-treated cells, and to assess the effect of p53 on the NF-κB-dependent pathway.

Human colon carcinoma HCT116 cell line was used as a model in two congenic variants: either containing or lacking (bi-allelic gene knock-out) transcriptionally competent p53. Cells were treated with the UV-C radiation (20 J/m²) and/or incubated with TNFα cytokine (10 ng/ml for 15 minutes) to induce the classical NF-κB pathway; stimulation with the cytokine was performed at different time points after irradiation (from 1 to 24 hours after UV). Expression of genes and proteins representative for different signalling pathways was assessed by QRT-PCR and Western blotting: AKT (AKT/PKB signalling), JUN and ERK1/2 (MAPK signalling), PTEN, PPM1D, p53 and WIP1 (p53 signalling), IL8, BCL3, NFKB1 and IkBα (NF-κB signalling) and HSPA2, HSPB2 and HSPH2 (the heat shock response).

In both cell variants exposed to UV (p53 positive and p53 negative cells) fast activation of AKT/PKB and WIP1 accumulation (phosphorylation level of AKT declined due to WIP1 induction) was observed, as well as accumulation of HSPs characteristic for cellular response to stress. Strong induction and accumulation of p53 was observed in cells with the wild type TP53 gene. Activation of MAPK signalling was similar in both cell variants at the protein level (late phosphorylation of ERK1/2), however, higher expression level of JUN was observed in cells lacking p53.

Strong effect of UV irradiation was observed on the TNFα-induced activation of the NF-κB pathway. Pre-exposure to UV resulted in suppression of the NF-κB activation (inhibition of the IkBα inhibitor degradation and reduction of nuclear NF-κB) and marked down-regulation of NF-κB-dependent genes (e.g. IL8, BCL3, NFKB1). The strongest inhibitory effect was observed when UV irradiation preceded TNFα stimulation for 6 hours. Most notably, similar inhibitory effect of UV was observed in cells with the different p53 status.

We concluded that UV-related stress-response pathways interfered with the cytokine-induced activation of the NF-κB pathways in the p53-independent manner.

This work was supported by the Polish National Science Center, Grant N301-264536 and Grant 2012/05/B/NZ2/189753.
The nucleolus integrates the gene-rich chromatin domains (CD) consisting of eukaryotic rDNA - giant tandems built by hundreds of r-gene repeats clustered into chromosomal segments defined as NORs. The nucleolus arises due to postmitotic reactivation of rRNA synthesis on non-nucleosomal r-chromatin loops emanating from NOR-bearing chromosomal territories (CT). As a derivate of interphase NORs, the nucleolus reassembles in compliance with the functional topography of NOR-cohered heterochromatic chromosomal segments, forming the unit system of nucleolus-associated DNA (naDNA). The latter comprises looped rDNA containing CD folded into structure of FC with non-ribosomal intra- and peri-nucleolar condensed chromatin called Nucleolus Associated Chromatin (NAC). How this NAC integrating non-nucleosomal and nucleosomal naDNA domains responds to r-genes transcriptional arrest not been previously studied in living cells.

To combine the 4D dynamics of FC together with juxtaposed DFC (FC/DFC complex) and NAC we used confocal microscopy (CM) and electron microscopy (EM) to study the redistribution of GFP-tagged UBF and fibrillarin under the selective block of RNA polymerase I by low dose (0.05µg/ml) of Actinomycin D (AmD) during 8 hours. We found that the treatment with AmD leads to the gradual coalescence and fusion of individual FCs on one side and DFCs on another side followed by their displacement to the nucleolar periphery to form caps. Interestingly, 4D studies demonstrated that segregation and capping of nucleolar components occurred in all nucleoli of the same cell and that nucleoli never fused together, but maintained their relative 3D position in the nucleus. Thus, each nucleolus may represent separate compartment emerging due to the spatial interplay between r-chromatin loop(s) and NAC belonging to distinct NOR-bearing CT that include ribosomal and non-ribosomal genomic regions.

To investigate in more details how condensation of NAC system is linked to the spatial redistribution of FC we developed a correlative study as follows. At the first step, the reorganization of NAC induced by AmD treatment was studied by 4D CM in chosen HeLa cells stably expressing H2B-GFP. At different time points (for example 1h) the same cells were fixed, immunolabeled for UBF, imaged for simultaneous localization of chromatin and UBF and finally processed for EM. By using a precise positioning and orientation system, we were able to find exactly the same cells and image them by EM. By merging images of corresponding areas identified on EM and fluorescent images we identified the intra-nucleolar H2B-GFP label to the filamentous meshwork of Intra-nucleolar Condensed Chromatin (ICC) that links FC to Perinucleolar Condensed Chromatin (PCC) shell. In the course of treatment we observed the gradual condensation of filamentous structure of ICC into coarse clumps and their shift from nucleolar interior towards the PCC. During movement several ICC clumps approach each other and fuse just before being coalesced with PCC shell.

We conclude that fusion of individual FCs on the one hand and DFC zones on the other one is largely involved into the mechanisms of nucleolar segregation and capping. Presumably, concerted condensation of NAC constituents induces forces that pull FC/DFC complex to the PCC shell.
Heat Shock Transcription Factor 1 (HSF1) is a main regulator of the heat (stress) response. It activates heat shock genes, which encode heat shock proteins (HSPs). HSPs accumulation enables cells to survive in suboptimal conditions. Beyond the classical induction of HSPs, HSF1 binds to a broad array of non-HSP genes. This property of HSF1 seems to be important in processes associated with tumorigenesis.

We aimed to study the role of HSF1 in tumor growth. We have constructed a model of mouse B16F10 melanoma cells with an overexpression of constitutively active, human HSF1, with deletion of the regulatory domain (amino acids 221-315; aHSF1). Simultaneously we have constructed cells with down-regulated HSF1 expression using shRNAs specific for 3'UTR or coding sequences of mouse HSF1. The expression of aHSF1 in stably transfected cells led to activation of several inducible Hsp genes (Hsph1, Hspb1, Hspa1), what was confirmed on mRNA and protein level. Transfection with HSF1 shRNA constructs was connected with a decreased level (up to 50-70%) of HSF1 protein and inhibition of the expression of inducible Hsp genes. We found that expression of constitutively active HSF1 as well as reduced HSF1 expression did not affect proliferation of B16F10 melanoma cells. However cells with expression of constitutively active HSF1 were more able to efficiently proliferate in the soft agar and gave rise to larger colonies than control cells. They also migrated more effectively in the transwell migration assay. Moreover, the number of metastases was increased in the lungs after the injection of B16F10 cells with expression of constitutively active HSF1 into the tail vein of C57BL6 mice. Down-regulation of HSF1 expression did not change the ability of cells to grow in soft agar and migrate, but its potential to form lung metastasis in vivo was reduced. Because of aHSF1 enhanced cell migration, we decided to determine the expression profile of genes associated with cell motility using a specific RT^2PCR array. We found that transcription of several genes involved in focal adhesion (Vcl, Cav1, Capn1) was decreased in cells expressing aHSF1, but was not changed in cells with down-regulated expression of HSF1.

We conclude that HSF1 activation might support cell motility and enhance tumor metastasis via influence on the cytoskeleton of a cell, and its connections to the extracellular matrix.

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Human papillomaviruses (HPVs) are among the most common sexually transmitted viral pathogens. While the majority of HPV infections are benign and cleared by the host immune system, a small percentage leads to a persistent infection, which is a significant risk factor for the development of cancer. HPV is the aetiological factor for cervical and other anogenital cancers and is also associated with head and neck cancers. It is estimated that HPVs cause nearly half of all virus-related cancers in females (reviewed in [1]).

The papillomavirus genome is an approximately 8000 bp double-stranded DNA molecule that is comprised of three functional regions: the upstream regulatory region (URR); the early coding region and the late coding region. The URR contains DNA sequences that are responsible for viral replication and transcription control, and it is believed to form the basis of the high tissue tropism for cutaneous or mucosal epithelium that papillomaviruses exhibit. Although several cellular transcription factors are known to bind to this sequence [2-4], no single epithelial specific protein has been identified so far.

In order to identify novel cellular transcription factors that regulate papillomavirus transcription, we have performed a SILAC based DNA pull-down assay using HPV type 11 URR sequence as bait. Human papillomavirus transcription factor E2 was identified in our assay along with several previously characterized cellular transcription factors that include members of the NF-I family [5], Sp1 [6], and components of the transcription factor AP-1 [7, 8]. In addition to the previously known regulators, cellular zinc finger transcription factors ZEB1 and ZEB2 were found to bind to the viral URR sequence. ZEB proteins are important regulators of cell differentiation and their overexpression contributes to malignant cancer progression through the induction of epithelial to mesenchymal transition (reviewed in [9]). Papillomavirus oncogenes E6 and E7 induce ZEB family members [10], which in turn regulate papillomavirus early promoter activity, adding another layer to this complex host-pathogen interaction network.

References:
P45. INVOLVEMENT OF THE NUCLEAR ACTIN-RELATED PROTEIN ARP4 IN THE SUBCELLULAR LOCALIZATION AND POLYMERIZATION OF ACTIN

Yamazaki, S.1, Sogawa, K.2, Tokunaga, M.2, De Lanerolle, P.3 and Harata, M.1

1 Graduate School of Agricultural Science, Tohoku University, Sendai, Japan; 2 Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Tokyo, Japan; 3 Department of Physiology & Biophysics, University of Illinois at Chicago, Chicago, USA

Actin is an integral component of the cytoskeleton which plays a crucial role in a variety of cell functions, including cell migration, adhesion, polarity and shape change in the cytoplasm. The actin family consists of conventional actin and actin-related proteins (Arps). In the cytoplasm, the Arp 2/3 complex regulates actin filament branching and elongation. In contrast, the members of the Arp subfamilies 4-9 accumulate in the nucleus [1], whereas the amount of actin and the other Arps in the nucleus remains low. Recent in vitro analyses revealed that Arp4 binds to the actin monomer (G-actin) and inhibits the formation of the actin filament (F-actin) by forming a heterodimer with actin [2]. Interestingly, formation of nuclear F-actin plays an essential role in gene reprogramming and in the reactivation of the pluripotent gene OCT4 [3]. In contrast, the presence of both G-actin and Arp4 is often observed in a large variety of chromatin remodeling and histone modification complexes [4]. These facts raise the possibility that dynamic interaction between actin and Arp4 in the nucleus contributes to epigenetic regulation. Here, we demonstrate that knockdown (KD) of Arp4 by RNA interference increase nuclear actin in HeLa cells. Moreover, the reactivity of probes towards F-actin in the nucleus was increased by Arp4-KD. These phenotypes of Arp4-KD cells were rescued by the ectopic expression of Arp4 which is resistant to RNA interference. Although Arp4 is an essential component of multiple chromatin remodeling complexes, its KD did not affect the expression of exportin6 and importin9, which contribute to export and import of nuclear actin, respectively. Therefore, we propose that the direct interaction of Arp4 with actin is involved in the subcellular localization and polymerization of actin in the nucleus.

References:
Recently it was shown that at least 5.5% of noncoding conserved elements unique to mammals have evolved from mobile elements and preferably located near the genes involved in the development and regulation of transcription (Lowe et al. 2007). Sequence conservation, widespread evolutionary distribution, and the presence of functional elements such as promoters and transcription factor binding sites in a sequences of satellite DNA, led to the assumption that in addition to participation in centromere shaping, they may also act as cis-regulatory elements of gene expression (Pezer et al. 2011).

Satellite DNA is rapidly evolving portion of eukaryotic genome which evolution moves by the stochastic process of molecular drive (Ugarkovic, 2005). Alpha-satellite DNA is the only currently known centromeric sequence of human DNA, forming kinetochore de novo. Use of alpha-satellite probes as markers of chromosomal rearrangements and detection sites, which are responsible for the development of malignant pathologies in humans, causes further studing of satellites, their conservation and divergence.

Among the factors attracting alpha-satellite DNA, the following should be noted:
- the location in centromeric chromatin of all human chromosomes, that is responsible for mitotic and meiotic homologous chromosomes segregation,
- association with other tandem DNA, such as gamma-satellite whose function is not clarified and location has shown only a few pairs of human chromosomes, but these formed clusters flanked by alpha-satellite DNA,
- proximity to the coding sequences and genes.

Using fluorescence in situ hybridization and immunofluorescence FISH on chromosomes and DNA fibers we detect the satellite repeats which coexist with the genes associated with tumor progression.

To fulfill the potential regulatory function, elements of satellite DNA is predicted to be preferentially located in euchromatin portion of genomes in the vicinity of genes. Designs by sequencing the entire genome allow to determine the presence and distribution of tandem DNA repeats in euchromatin. Analysis of satellite DNA elements scattered in euchromatin and its comparison with homologous elements shown in heterochromatin also may shed light on the lineage of the original DNA and their subsequent evolution.

Transcriptional potential of satellite DNA, as well as their distribution, proximity to protein-coding genes provides strong support for these studies and notes that they, along with transposons are able to be a rich source for the assembly of gene regulatory systems.

Encoding proto-oncogenes and tumor suppressors that we know today are the main molecular centers of both normal and malignant cells. Therefore, the search of ncRNA with both tumor suppressor or driver contains additional mode to identify important biologically active ncRNAs in a huge and largely noncharacterized noncoding transcriptom.

References:
Pezer Z., J. Brajkovi, I. Feliciello, and D. Ugarkovic, Transcription of satellite DNAs in Insects. Prog. Mol. Subcell. Biol
Ugarkovic D., Functional elements residing within satellite DNAs. EMBO Rep.

This work was supported by National Academy of Sciences of Ukraine funding.
P47. PROTEASOMAL GENE CLUSTER OF CHROMOSOME 14: POSSIBLE ASSOCIATION WITH MORBIDITY IN GENERAL

Sjakste, T.¹, Paramonova, N.¹, Sugoka, O.¹, Trapina, O.¹, Dmitrijevs, I.¹, Kalnina, J.², Rumba-Rozenfelde, I.², Sjakste, N.²

¹Institute of Biology of the University of Latvia, Miera 3, Salaspils, LV2169, Latvia; ²Faculty of Medicine, University of Latvia, Sharlotes Street 1a, Riga LV1001, Latvia

Complex and dynamic ubiquitin proteasome system (UPS) active both in the nucleus and cytoplasm is represented by unique in place and time organization of multiple subunits for realization of any physiological process and in response to additional internal or external stimuli. The UPS deregulation implicated in several human pathologies can depend on the structural variations in the genes encoding the proteasome subunits. Spectrum and level of the loci variability and their impact in phenotype realization could be different in males and females. The poster will present results of several studies.

We have analysed the PSMA6, PSMC6 and PSMA3 genotype – sex interactions in human populations and in the association with juvenile idiopathic arthritis (JIA) and bronchial asthma (BA) in Latvian children. Sex specificity was found to be ethnos specific. Several single- and/or multiple loci genotypes and haplotypes were identified as sex specific JIA and BA risk factors in Latvians. Revealed genetic modules potentially have a diagnostic value in medical practice with reference on the sex- and ethnic specificity.

Further we have analyzed variation of the polyA tract locating in the PSMA6 promoter (rs113987343) on the association with juvenile idiopathic arthritis (JIA) and obesity (OB) in Latvian children. The rs113987343 polymorphism was analysed in 174 JIA and 96 OB patients versus 191 healthy individuals. Rare PSMA6 c.-632C allele (rs71640264) disrupts poly(A) tract suggesting the compound PSMA6 c.-655Aₙ[A>C]Aₙ MS structure. In our study we observed variation only in the 5’ part of this motif and suggest the precise locus structure to be c.-655Aₙ[A>C]A₂ for Latvian population. No linkage between the c.-632C allele and repeat number was revealed in Latvians. The heterozygous genotypes A₁₈/₁₉, A₁₉/₂₀, and A₂₀/₂₁ were most frequent in all cohorts; other genotypes appear to be rare. The genotype A₂₁/₂₂ found only in control group (about 5%) appears to be JIA and OB protective (P > 0.05).

Finally the rs2348071 SNP of the PSMA3 gene (c.543+138G>A) has been genotyped in 281 multiple sclerosis (MS) patients (201 women) being diagnosed on relapsing – remitting (188 subjects) or secondary progressive (93 subjects) course of the disease, versus 191 control subjects (117 women) without inflammatory and any autoimmune disorders. In controls the rs2348071 locus showed allele and genotype presentation similar to other Europeans with MAF about 30% and genotype GG being most frequent (53%). In both female and male cohorts of MS patients the minor allele A was observed slightly more frequent than in controls (P < 0.05), frequency of both GG and AA homozygotes was decreased (about 30% and 5% respectively) in favour of heterozygote GA genotypes (from 61% to 71% in patients of relapsing – remitting and secondary progressive course of disease respectively) that was significantly higher than in controls (P < 0.0001; OR = 3.539 [95% CI 2.409 – 5.198] according to co-dominant model). The PSMA3 c.543+138G>A nucleotide substitution potentially could affect binding of several transcription factors, sequence similarity to splicing signals and other modulators of gene expression and finally influence 20S proteasome functionality. Thus the rs2348071 heterozygous genotype appears to be the MS risk factor in Latvian population.

Association of the polymorphisms of genes of the proteasomal gene cluster of Chromosome 14 with several diseases indicate its possible association with morbidity in general.

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<td>Agata</td>
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<td>Katerina</td>
<td><a href="mailto:apnon@ukr.net">apnon@ukr.net</a></td>
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<td>Olga</td>
<td><a href="mailto:olga.anatskaya@gmail.com">olga.anatskaya@gmail.com</a></td>
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<td>Florian</td>
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<td>Zsolt</td>
<td><a href="mailto:bacso@med.unideb.hu">bacso@med.unideb.hu</a></td>
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<td>Eva</td>
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<td>Sascha</td>
<td><a href="mailto:sascha.beneke@vetpharm.uzh.ch">sascha.beneke@vetpharm.uzh.ch</a></td>
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<td><a href="mailto:Bode.Juergen@MH-Hannover.de">Bode.Juergen@MH-Hannover.de</a></td>
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<td>Małgorzata</td>
<td><a href="mailto:m.broszkiewicz@nencki.gov.pl">m.broszkiewicz@nencki.gov.pl</a></td>
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<td>Wei</td>
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<td>James</td>
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<td>Dusan</td>
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<td>Marion</td>
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<tr>
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<td>Bence</td>
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<tr>
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<td>Subrata Kumar</td>
<td><a href="mailto:skdvu@yahoo.co.in">skdvu@yahoo.co.in</a></td>
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<td>Fred</td>
<td><a href="mailto:fdick@uwo.ca">fdick@uwo.ca</a></td>
</tr>
<tr>
<td>20</td>
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<td>Quang Minh</td>
<td><a href="mailto:vivavn@gmail.com">vivavn@gmail.com</a></td>
</tr>
<tr>
<td>21</td>
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<td>Jurek</td>
<td><a href="mailto:jerzy.dobricki@uj.edu.pl">jerzy.dobricki@uj.edu.pl</a></td>
</tr>
<tr>
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<td>Miklós</td>
<td><a href="mailto:erdelyim@brc.hu">erdelyim@brc.hu</a></td>
</tr>
<tr>
<td>23</td>
<td>Erenpreisa</td>
<td>Jekaterina</td>
<td><a href="mailto:katrina@biomed.lu.lv">katrina@biomed.lu.lv</a></td>
</tr>
<tr>
<td>24</td>
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<td>Emanuelle</td>
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</tr>
<tr>
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<td>Fenyőfalvi</td>
<td>György</td>
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<tr>
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<td>Jana</td>
<td><a href="mailto:fukalova@img.cas.cz">fukalova@img.cas.cz</a></td>
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<tr>
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<td>Valentina</td>
<td><a href="mailto:valentina.galimberti01@ateneopv.it">valentina.galimberti01@ateneopv.it</a></td>
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<td>William</td>
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<td>Masahiko</td>
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<tr>
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<td>Zoltán</td>
<td><a href="mailto:simandi@med.unideb.hu">simandi@med.unideb.hu</a></td>
</tr>
<tr>
<td>86</td>
<td>Simsone</td>
<td>Zane</td>
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</tr>
<tr>
<td>87</td>
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<td><a href="mailto:sirotkin@cvzv.sk">sirotkin@cvzv.sk</a></td>
</tr>
<tr>
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<td>Nikolaj</td>
<td><a href="mailto:nikolajs.sjakste@lu.lv">nikolajs.sjakste@lu.lv</a></td>
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<tr>
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<td><a href="mailto:as@chem.pg.gda.pl">as@chem.pg.gda.pl</a></td>
</tr>
<tr>
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<td>Evgeny</td>
<td><a href="mailto:evgeny.smirnov@lf1.cuni.cz">evgeny.smirnov@lf1.cuni.cz</a></td>
</tr>
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<td>Dariusz</td>
<td><a href="mailto:darsmol@umk.pl">darsmol@umk.pl</a></td>
</tr>
<tr>
<td>92</td>
<td>Szabó</td>
<td>Gábor</td>
<td><a href="mailto:szabog@med.unideb.hu">szabog@med.unideb.hu</a></td>
</tr>
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<td>Gábor</td>
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<td><a href="mailto:szantoandras@upcmail.hu">szantoandras@upcmail.hu</a></td>
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<td><a href="mailto:kszoltysik@io.gliwice.pl">kszoltysik@io.gliwice.pl</a></td>
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<td>Vámosi</td>
<td>György</td>
<td><a href="mailto:vamosig@med.unideb.hu">vamosig@med.unideb.hu</a></td>
</tr>
<tr>
<td>102</td>
<td>Vassetzky</td>
<td>Yegor</td>
<td><a href="mailto:vassetzky@igr.fr">vassetzky@igr.fr</a></td>
</tr>
<tr>
<td>103</td>
<td>Velichko</td>
<td>Artem</td>
<td><a href="mailto:velichkoak@gmail.com">velichkoak@gmail.com</a></td>
</tr>
<tr>
<td>104</td>
<td>Venit</td>
<td>Tomas</td>
<td><a href="mailto:tomas.venit@img.cas.cz">tomas.venit@img.cas.cz</a></td>
</tr>
<tr>
<td>105</td>
<td>Vértessy</td>
<td>Beáta</td>
<td><a href="mailto:vertessy@enzim.hu">vertessy@enzim.hu</a></td>
</tr>
<tr>
<td>106</td>
<td>Vilmos</td>
<td>Péter</td>
<td><a href="mailto:vilmosp@brc.hu">vilmosp@brc.hu</a></td>
</tr>
<tr>
<td>107</td>
<td>von Mikecz</td>
<td>Anna</td>
<td><a href="mailto:mikecz@tec-source.de">mikecz@tec-source.de</a></td>
</tr>
<tr>
<td>108</td>
<td>Vosa</td>
<td>Liisi</td>
<td><a href="mailto:liisi.vosa@ut.ee">liisi.vosa@ut.ee</a></td>
</tr>
<tr>
<td>109</td>
<td>Vydra</td>
<td>Natalia</td>
<td><a href="mailto:nvydra@yahoo.co.uk">nvydra@yahoo.co.uk</a></td>
</tr>
<tr>
<td>110</td>
<td>Walaszczyk</td>
<td>Anna</td>
<td><a href="mailto:awalaszczyk@io.gliwice.pl">awalaszczyk@io.gliwice.pl</a></td>
</tr>
<tr>
<td>111</td>
<td>Widlak</td>
<td>Piotr</td>
<td><a href="mailto:widlak@io.gliwice.pl">widlak@io.gliwice.pl</a></td>
</tr>
<tr>
<td>112</td>
<td>Widlak</td>
<td>Wieslawa</td>
<td><a href="mailto:wwidlak@io.gliwice.pl">wwidlak@io.gliwice.pl</a></td>
</tr>
<tr>
<td>113</td>
<td>Wieslander</td>
<td>Lars</td>
<td><a href="mailto:Lars.Wieslander@moltbio.su.se">Lars.Wieslander@moltbio.su.se</a></td>
</tr>
<tr>
<td>114</td>
<td>Yamazaki</td>
<td>Shota</td>
<td><a href="mailto:s.yamazaki@s.tohoku.ac.jp">s.yamazaki@s.tohoku.ac.jp</a></td>
</tr>
<tr>
<td>115</td>
<td>Zimina</td>
<td>Olga</td>
<td><a href="mailto:o.g.alkhimova@imbg.org.ua">o.g.alkhimova@imbg.org.ua</a></td>
</tr>
</tbody>
</table>
The abstract book was edited by:
Gábor Szabó
Lóránt Székvölgyi
Éva Hegedűs
Gábor Szalóki

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