Saint Petersburg State University, Russia

Scientific Center of Russian Academy of Sciences, Russia

University of Nebraska Medical Center, U.S.A.

Genome Instability, Evolution and Human Diseases

Saint Petersburg

June 14-17, 2013

Organizers

SAINT PETERSBURG STATE UNIVERSITY

EPPLEY CANCER CENTER

Saint Petersburg scientific center RAS

Partners

Sponsors

Лабораторная служба Хеликс

ХИМЭКСПЕРТ

The conference is organized in the framework of the General Agreement for Cooperation between SPbSU and UNMC. We acknowledge SPbSU for the research supporting grant 1.44.716.2013 and UNMC for the financial support. The Conference is additionally funded by RFBR grant #13-04-06019, NIGMS/NIEHS grant #1R13ES022918-01. We also greatly acknowledge the financial support from sponsors.

Organizing committee:

Sergei Inge-Vechtomov, St. Petersburg State University, Russia
Youri Pavlov, University of Nebraska Medical Center, U.S.A.
Polina Shcherbakova, University of Nebraska Medical Center, U.S.A.

Elena Stepchenkova, St. Petersburg State University, Russia
Edward Ramspott, University of Nebraska Medical Center, U.S.A.
Ludmila Japaridze, Scientific Center of Russian Academy of Sciences, Russia
Alsu Saifitdinova, St. Petersburg State University, Russia
Aleksandr Rubel, St. Petersburg State University, Russia
Anna Atsapkina, St. Petersburg State University, Russia
Tatiana Davydova, St. Petersburg State University, Russia
Charlotte Stalnaker, University of Nebraska Medical Center, U.S.A.
Krista Brown, University of Nebraska Medical Center, U.S.A.
Anna Zhuk, St. Petersburg State University, Russia

Arranged by: Alexey Domoratsky

Contributors to artistic images of the design: Olga Kochenova, Anna Zhuk

Scientific Program

WELCOME ADDRESS AND INTRODUCTORY COMMENTS

Alexei Zavarzin (St. Petersburg University, Russia)

Kenneth Cowan (Eppley Institute for Research in Cancer, University of Nebraska Medical Center, U.S.A.)

Sergei Inge-Vechtomov (St. Petersburg University and Russian Academy of Sciences, Russia)

KEYNOTE LECTURE

Lawrence Loeb (University of Washington, USA) *"Human cancers express a mutator phenotype: origin, consequences and targeting"*

DNA REPLICATION MECHANISMS AND FORK DYNAMICS

Luca Pellegrini (University of Cambridge, U.K.) *"Mechanism for priming DNA synthesis in eukaryotic replication"*

Tahir Tahirov (Eppley Institute for Research in Cancer, U.S.A.) *"Structure and function of human B family DNA polymerases"*

Erik Johansson (Umea University, Sweden) *"Enzymatic properties of yeast DNA polymerase epsilon"*

Hiroyuki Araki (National Institute of Genetics, Japan) *"Establishment of replication forks at origins"*

Helmut Pospiech (University of Oulu, Finland, and Leibniz Institute for Age Research - Fritz Lipmann Institute, Germany) *"Distinctive activities of human replicative DNA polymerases during S phase – DNA polymerase epsilon, but not DNA polymerases alpha/delta are associated with lamins throughout S phase"*

Amir Aharoni (Ben-Gurion University, Israel) *"Tight regulation of PCNA-mediated DNA replication in S. cerevisiae"*

Luis Blanco (Centro de Biologia Molecular Severo Ochoa, Spain) *"PrimPol, an archaic DNA primase/DNA polymerase in human cells"*

Poster session #1

CELLULAR RESPONSES TO REPLICATION IMPEDIMENTS

Peter Burgers (Washington University School of Medicine, USA) *"How do replication forks signal stress?"*

Helle Ulrich (Institute of Molecular Biology gGmbH, Germany) *"Mechanism of ubiquitin-dependent DNA damage bypass"*

Haruo Ohmori (Gakushuin University, Japan) *"REV7 versus MAD2"*

Alena Makarova (Washington University School of Medicine, U.S.A.) *"DNA polymerase ζ and Rev1-mediated mutagenesis in yeast"*

Ekaterina Beloussova (Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, Russia) *"Multiply damaged sites repair: participation of BER and translesion DNA synthesis"*

SHAPING THE GENOME BY MUTATION AND RECOMBINATION (PART I)

Svend Petersen-Mahrt (IFOM-Fondazione Istituto FIRC di Oncologia Molecolare, Italy) *"DNA instability is enhancing genome dynamics in immunity and epigenetics"*

Youri Pavlov (Eppley Institute for Research in Cancer, USA) *"Genome-wide mutation avalanches in diploid yeast cells"*

Andrei Chabes (Umea University, Sweden) *"dNTPs and maintenance of genome stability"*

Roel Schaaper (NIEHS, USA) *"DNA precursors and DNA replication fidelity"*

Nicholas Burgis (Eastern Washington University, U.S.A.) *"Removal of noncanonical purines from nucleoside triphosphate pools"*

Bradley Preston (University of Washington, USA) *"Genetic instability and cancer: Walking the mutator high wire"*

Iwona Fijalkowska (Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Poland) *"Contribution of non-catalytic components of replisome to the final fidelity of replication"*

Poster session #2

SHAPING THE GENOME BY MUTATION AND RECOMBINATION (PART II)

KEYNOTE LECTURE

Michael Lynch (Indiana University, USA) *"Evolution of the mutation rate"*

Anna Malkova (Indiana University-Purdue University Indianapolis, U.S.A.) *"Molecular mechanisms of genetic instabilities resulting from Break-Induced Replication"*

Martin Kupiec (Tel Aviv University, Israel) *"Effect of nuclear architecture on the efficiency of double-strand break repair"*

Andrei Kuzminov (University of Illinois at Urbana-Champaign, U.S.A.) *"Ultraviolet-induced chromosomal fragmentation: pathways, mechanisms, and contribution to lethality"*

DIVERSE PATHWAYS IN HEREDITY AND DISEASE

Sergei Mirkin (Tufts University, U.S.A.) *"Genome weak links"*

Polina Shcherbakova (Eppley Institute for Research in Cancer, USA) *"Mutagenic replication in DNA damage response and human cancer"*

Richard Wood (University of Texas MD Anderson Cancer Center, USA) *"Mammalian DNA polymerase ζ and cancer"*

Roland Lill (Philipps-Universitat Marburg, Germany) *"The role of mitochondria and iron-sulfur protein biogenesis in genome stability and DNA metabolism"*

Shay Ben-Aroya (Bar-Ilan University, Israel) *"Characterization of CIA2, a conserved gene with a role in maintaining the stability of chromosomes"*

Yury Chernoff (Georgia Institute of Technology, U.S.A.) *"Heritable variability at the protein level"*

Abstracts are the responsibility of the authors.
These abstracts should not be cited in bibliographies. Material herein should be treated as personal communications and should be cited as such only with the consent of the author.

Abstracts of poster presentations

Elizaveta S. Ageeva
The Khakas State University of N.F. Katanov, Russia

Ethno-population features of CYP2C19 +681G>A at patients with ulcer disease at the aboriginal and non-aboriginal inhabitants of Khakassia.

The purpose of the present research is the detection of features of polymorphism of a gene of CYP2C19 +681G>A at patients with infection H. pylori among aboriginal and non-aboriginal inhabitants of Khakassia. We conducted a survey of the patients with ulcer disease (UD) (the Khakas people – 50 and the Caucasoid people – 50). The group of control was made by healthy donors of 30 the Khakas people and 30 Caucasoid people. The polymorphism genes determined by a method of the PDRF-analysis. Allele G forms a site for a restriction enzyme Sma I. The association of polymorphisms with UD analysed by means of criterion of the relation of chances (OR - odd ratio). The research showed that genotype AA of +681 genes of CYP2C19 isn't found at the Khakas people with UD. In group of healthy donors - 5,8 %. Heterozygotes of GA of +681 genes of CYP2C19 was founded at 10,7 % of sick UD and at 27,5 % of healthy donors (p ct expediency of appointment and efficiency of eradication therapy.

*presenting author is underlined

Daniel Dovrat, Yearit Fridman, Lyad Zamir and Amir Aharoni
Ben-Gurion University, Department of Life Sciences, Israel

Tight regulation of PCNA-mediated DNA replication in *S. cerevisiae*.

DNA replication is a fundamental process that is essential for cell division and the transmission of genetic information. Proliferating cell nuclear antigen (PCNA) mediates DNA replication and repair in eukaryotes by recruiting many proteins to the DNA template. PCNA must tightly regulate the binding and dissociation events of different DNA modifying enzymes to allow for accurate progression of DNA replication and repair. Despite large number of studies, little is known regarding how PCNA regulates the binding of different partners during DNA replication. Here, we used protein engineering, biochemical and genetic approaches to generate and characterize novel PCNA mutants with enhanced affinity for several key partners. We found that increases in PCNA-partner interaction affinities led to severe in vivo phenotypic defects. Surprisingly, such defects are much more severe than those induced by complete abolishment of the respective interactions. These mutants revealed the susceptibility of DNA replication for minor alterations in PCNA-partner interactions highlighting the importance of tight regulation of these interactions for PCNA mediated DNA replication (1). In addition, to characterize the switching of partners on PCNA during Okazaki fragment maturation, we have generated and characterized heterotrimeric forms of PCNA in which one or two subunits of PCNA are inactive due to mutations. These partially active heterotrimers of PCNA were characterized in vitro and revealed that switching of partners on PCNA during Okazaki fragments maturation can take place only on one active PCNA monomer. These results directly show that the classical “tool-belt” model in which PCNA simultaneously binds two-three partners is not essential for Okazaki fragment maturation and suggest a dynamic model for switching of partners on PCNA during complex processes. Finally, we

examined the evolution of PCNA-partner interaction network using gene replacements in *S. cerevisiae*. We discovered that PCNA-partner interactions tightly coevolved in fungal species leading to specific modes of recognition. We found that fungal PCNA-partner interaction networks diverged into two distinct groups as a result of such coevolution and that hybrid networks of these groups are functionally noncompatible in *S. cerevisiae* (2). Our results indicate that the coevolution of interaction networks can form functional barriers between fungal species and thus can promote and fix speciation.

(1) Fridman Y. et al. (2010) PLoS Biology. 8 (10) e100
(2) Zamir L. et al. (2012) Proc. Natl. Acad Sci. USA. 109(7):E406-14

Daniil Andreychuk and Andrei Chabes
Medical Biochemistry and Biophysics Umeå University, Sweden

Effect of imbalanced dNTP pools on mitochondrial DNA stability

Imbalanced dNTPs reduce nuclear genome stability in a manner that is highly dependent on the nature and degree of the imbalance. Altering the ratio of the four dNTPs also dramatically affects telomere length homeostasis. Using recently created yeast strains with different and defined dNTP pool imbalances, we investigate how these imbalances affect stability of the mitochondrial genome. Preliminary results will be presented.

Yulia Andreychuk and Andrei Chabes
Medical Biochemistry and Biophysics Umeå University, Sweden

Regulation of Ixr1 transcription factor by DNA damage

Regulation of Ixr1 transcription factor by DNA damage S. cerevisiae Ixr1 is a high mobility group (HMG) transcription factor first identified by its ability to bind DNA modified by the anticancer drug cisplatin. Deletion of IXR1 increases resistance of yeast cells to cisplatin. We have recently demonstrated that Ixr1 is involved in regulation of dNTP pools and the ribonucleotide reductase RNR1 gene after DNA damage. In this project, we investigate how Ixr1 is regulated by DNA damage, the role of Ixr1 in organization of chromatin and identify additional genes regulated by Ixr1. Preliminary results will be reported.

Andrey G. Baranovskiy, Nigar D. Babayeva, Tahir H. Tahirov
Eppley Institute for Research in Cancer and Allied Diseases University of Nebraska Medical Center, USA

Crystallization of B-family human DNA Polymerases.

In our presentation we will summarize our progress with structural studies of human B family polymerases.

Olesya B. Belopolskaya
Vavilov Institute of General Genetics Russian Academy of Sciences, Russia

DNA polymorphism in children with malignant brain tumors

Pediatric brain tumor incidence is now rising in the majority of highly developed countries, especially among children younger than 5. The role of environmental factors in childhood carcinogenesis, in general and in the CNS tumor development risk, is under investigation. This study presents the results of research on DNA polymorphism in children with malignant brain tumors (303 patients, age 8.73 ± 0.24; 464 controls, age 27.49 ± 0.43). Genotyping was performed using an allele-specific tetraprimer reaction for the genes of the first (CYP1A1 (3 sites)) and second phases of xenobiotic detoxication (GSTM1, GSTT1). CYP1A1 rs2606345 and rs4646903 were associated with brain cancer risk (recessive model, P = 0.016, OR = 1.60, 95%CI 1.09-2.34; dominant model, P = 0.046, OR = 1.44, 95%CI 1.01-2.07, respectively). GSTM1 deletion was also associated with brain cancer (P = 0.0001, OR = 1.80, 95%CI 1.34-2.42). Given the multinational and multi-age nature of the cases we have identified the most numerous homogeneous subset - Slavic boys ≤ 12 years, the average age of pubertal onset in boys, and Slavic controls. A comparison of distributions between cases (Slavic boys ≤ 12 years) and controls revealed significant effects of GSTM1 deletion (P = 1 x 10-4, OR = 2.50, 955 CI 1.57 - 4.0) and this association remained significant in the whole male sample (P = 1.4 x 10-5, OR = 2.42, 95% CI 1.63-3.60). On average, girls begin puberty at age 10 and we have next identified female subset - Slavic girls ≤ 10 years and Slavic controls. Increased genotype-specific risk was associated with CYP1A1 rs2606345 in the whole female sample (dominant model, P = 0.0063, OR = 1.91, 95% CI 1.19 - 3.06). Subgroup analyses performed on sex revealed different estimated associations in male and female groups. Noteworthy, that the same direction of these association effects was

observed in the both sex groups, though non-significant for CYP1A1 rs2606345 in male sample and for GSTM1 deletion in female sample. The obtained results, if confirmed by independent studies, can be useful for identifying the genetic risk factors involved in the formation of malignant tumors in children.

Ekaterina A. Belousova[1], Inna A. Vasil'eva[1], Nina A. Moor[1], Timofey S. Zatsepin[2], Tatiana S. Oretskaya[2], Olga I. Lavrik[1]

[1]*Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia*

[2]*Chemistry Department and A.N. Belozersky Institute of Physico-Chemical Biology, Moscow, Russia*

Multiple damaged sites repair: participation of BER and TLS proteins

The lesions in genome DNA can be created, for example, under ionizing radiation and various chemical oxidants as a single damage or as a part of multiple damaged sites within 1–2 helical turns (clustered lesions or MDSs). Such MDSs consist of two or more closely spaced lesions, and repair of these sites can be dependent on the types of clustered lesions. One of the major lesions forming in the cell as a product of the oxidation of the thymine methyl group is 5-formyluracil (5-foU). It is known that 5-foU residue in DNA have to miscode with all four nucleobases, generating primarily transversion or transition mutations. In addition, 5-foU could be spontaneously hydrolyzed from the DNA under physiological conditions and revealed to form an apurinic/apyrimidinic site (AP-site). Both 5-foU and AP-site are generally repaired by base excision repair system (BER), but it have been proposed that 5-foU can be removed from DNA sequence by nucleotide repair system (NER) [Kino K., Shimizu Y., Sugasawa K., Sugiyama H., and HanaokaF., Biochemistry, 2004, 43, 2682-2687]. So if

the cluster contains 5-foU and AP-site simultaneously, the order of the repair mechanisms could be found (BER or NER). In any case, a DNA polymerase which will be used for DNA synthesis during repair process has to catalyze DNA synthesis across the lesion. During the BER process DNA polymerases beta and lambda can be proposed for this goal [Belousova E. A., Lavrik O. I., Molecular Biology, 2010, 44, 839–855]. Here, we investigated the activity of DNA polymerases beta and lambda during the repair process on clustered damaged DNA containing 5-foU and AP-site in the range of a few nucleobases.

This work was supported by a grant from the RFBR (№12-04-00178-a, 11-04-00559-a), and the program of the Russian Academy of Science "Molecular and cellular biology".

Shay Ben-Aroya
Mina and Everard Goodman Faculty of Life Sciences Bar-Ilan University, Israel

Characterization of CIA2, a conserved gene with a role in maintaining the stability of chromosomes

Characterization of CIA2, a conserved gene with a role in maintaining the stability of chromosomes Lilach Emuna, Liron Goor, Marina Volpe and SHAY BEN-AROYA The Mina and Everard Goodman, Faculty of Life Sciences Bar-Ilan University A significant number of eukaryotic genes and regulatory pathways have evolved to ensure that cells replicate and segregate their genomes with high fidelity and at the right time. Problems associated with the failure to maintain such tight restrictions on growth can lead to genome instability. In the vast majority of malignancies, this instability, termed Chromosomal Instability (CIN), appears to involve gain and/or loss of whole chromosomes or large

segments of chromosomes, leading to aneuploidy. Like other phenotypes characteristic of cancer, it is possible that mutations in genes that control chromosome stability are responsible for CIN. However, so far, only a handful of genes thought to be important for this phenotype have been identified. To expand the known spectrum of genes that play a role in maintenance of genome stability, the collections of temperature sensitive (Ts) yeast mutants were screened for a CIN phenotype. Among others, this list also contains several conserved genes with unknown function. One such gene is CIA2. The goal of this project is to gain a mechanistic insight into the function of CIA2 with the emphasis on its involvement in maintaining genome integrity. Using several high-throughput approaches such as Synthetic Genetic Array (SGA), Protein-fragment Complementation Assay (PCA), and Synthetic Dosage Lethality (SDL) we were able to draw a comprehensive physical and genetic interaction maps. These approaches reveal roles for CIA2 at the final steps of Iron sulfur cluster (ISC) biogenesis, and suggest that mutated CIA2 stimulates nuclear genome instability by inhibiting the production of ISC-containing protein(s), which are required for maintenance of nuclear genome integrity. In order to identify such potential target proteins, we performed a SDL screen. The results reveal that Cia2 modify Rad3 with ISC, and that this modification is important for its function in nucleotide excision repair (NER). Another potential target is Ipl1, the yeast ortholog of the human protein AuroraB kinase, a key regulator of mitosis. We show that the human ortholog of Cia2, hCia2, localizes with components of the mitotic pathway such as tubulines at spindle pole body, and the midbody matrix surrounding the compacted midzone microtubules during cytokinesis. These results may suggest that Cia2 modify ISC-containing mitotic protein(s) at their site of action.

Sara García-Gómez[1], Aurelio Reyes[2], Julie Bianchi[3], María Martinez[1], Sean G. Rudd[3], Sandra Chocrón[1], Silvana Mourón[4], Juan Méndez[4], Ian J. Holt[2], Aidan J. Doherty[3] and Luis Blanco[1]

[1]*Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, Spain.*

[2]*MRC Mitochondrial Biology Unit, Wellcome Trust/MRC, Cambridge, UK*

[3]*Genome Damage and Stability Centre, Univ. Sussex, Brighton, UK.*

[4]*Centro Nacional de Investigaciones Oncológicas, Madrid, Spain.*

PrimPol, an archaic DNA primase/DNA polymerase in human cells

We describe a second primase in human cells which has the fascinating ability to start DNA chains with dNTPs, unlike regular primases that use exclusively NTPs. This selectivity for priming with dNTPs (a hallmark of archeal primases) provides this human enzyme with the ability to synthesize de novo relatively long DNA chains (up to 4 kb); hence, its is a new DNA polymerase, as well as a primase, and we have accordingly named it PrimPol. A detailed analysis of the primase requirements demonstrated the large preference to form a dimer in which a deoxynucleotide occupies the 3´-position, whereas both a ribonucleotide or a deoxynucleotide are valid substrates at the 5´ (primer) position. PrimPol polymerase activity allows template-directed extension of pre-existing RNA or DNA primers, preferentially using dNTPs. Other features of human PrimPol, are: 1) preferential activation by manganese ions; 2) distributivity during primer extension; 3) lack of dRP lyase activity; 4) no proofreading 3´-5´ exonuclease. Remarkably, PrimPol is able to polymerize past some of the most common oxidative lesions as abasic sites and 8oxoG lesions, and also across a CPD lesion. Interestingly, a Zn-finger present at the C-terminus of PrimPol is required for specific priming at certain sequences (or lesions?). Conversely, the Zn finger is fully dispensable (even detrimental) for extending a pre-existing primer, suggesting that mobilization of the Zn-finger could avoid its interference during further polymerization, thus

optimizing the potential of PrimPol also as a DNA polymerase. Based on its peculiar enzymatic properties, PrimPol could facilitate replication fork progression by acting as a translesion polymerase, or as a specific primase capable to reinitiate downstream of lesions that pause or block DNA elongation by replicative polymerases, particularly at the leading strand. Human PrimPol is located both at the nuclear and mitochondrial compartments, where its downregulation affected both mtDNA maintenance and nuclear DNA replication, generating genome instability. We have generated a PrimPol knock out (KO) mouse model that resulted viable and fertile, but tending to show a shorter lifespan. Accordingly, we observed that both KO adult mouse primary fibroblasts or KO mouse embryonic fibroblasts (MEFs) have a deficient respiratory function, and a large aneuploidy in the nucleus. PrimPol, a "self-sufficient" DNA polymerase with the capacity to restart DNA synthesis when needed, i.e. to bypass damaged templates, probably evolved as one of the most antique solutions to facilitate replication of small genomes threatened by environmental damage.

Nicholas E Burgis, Richard P. Cunningham, and Peter C. Dedon, Bo Pang, Jose L. McFaline, Min Dong, Koli Taghizadeh, Matthew R. Sullivan, C. Eric Elmquist, Amanda Hill, Anthony Gall, Ashley C Moore, Martin K Aune, Stephen Heid, Ayaka Mori
Eastern Washington University

Removal of noncanonical purines from nucleoside triphosphate pools

Inosine triphosphate (ITP) pyrophosphohydrolase, or ITPase, is a ubiquitous, ancient enzyme that protects cells by preventing noncanonical purines from accumulating in (deoxy) nucleoside triphosphate ((d)NTP) pools. ITPase catalyzes the hydrolysis of the acidic anhydride bond between

the alpha and beta phosphates in ITP, and other noncanonical nucleoside triphosphates, producing the corresponding nucleoside monophosphate and pyrophosphate. This activity prevents noncanonical (d)NTPs from integrating into nucleic acids or interfering with other cellular processes. Quantification of (deoxy)inosine in nucleic acids isolated from Escherichia coli and *Saccharomyces cerevisiae* cells with defined purine metabolism mutations revealed large increases of (deoxy)inosine in both DNA (600-fold) and RNA. We observed maximum effects for cells unable to convert IMP to XMP or AMP (IMP dehydrogenase, guaB; Adenylosuccinate synthetase, purA and ADE12), and unable to remove (d)ITP from the nucleotide pool (ITPase, rdgB and HAM1). Our observations suggest that disturbances in purine metabolism, caused by known genetic polymorphisms, could increase the burden of mutagenic nucleobases in DNA and interfere with gene expression and RNA function, a situation possibly exacerbated by the nitrosative stress of concurrent inflammation. Our results also suggest a mechanistic basis for the pathophysiology of human inborn errors of purine nucleotide metabolism. In mammals, ITPase is encoded by the ITPA gene. Itpa homozygous null knock-out mice die before weaning and have gross cardiac abnormalities. Human ITPA polymorphism is linked to adverse reactions to the immunosuppressive prodrugs azathioprine and 6-mercaptopurine and protection against ribavirin-induced hemolytic anemia. Bioactivation of these drugs result in the formation of noncanonical nucleoside triphosphates. Human ITPase enzymes engineered to modulate nucleobase specificity may be valuable tools for studying the role of ITPase in heart development and drug metabolism or the development of gain-of-function mutants or inhibitory molecules. Based on x-ray crystallography and amino acid sequence data, a panel of putative human ITPase nucleobase specificity mutants has been generated. We targeted eight highly conserved amino acid positions within the ITPase sequence that correspond to amino acids predicted to directly interact with the nucleobase or help organize the nucleobase binding pocket.

The ability of the mutants to protect against exogenous and endogenous noncanonical purines was tested with two E. coli complementation assays. Nucleobase specificity of the mutants was investigated with an in vitro biochemical assay using ITP, GTP and ATP as substrates. This methodology allowed us to identify gain-of-function mutants and categorize the eight positions according to their ability to protect against noncanonical purines.

Andrei Chabes
Medical Biochemistry and Biophysics Umeå University, Sweden

dNTPs and maintenance of genome stability

Imbalanced dNTP pools in mice We have recently created a collection of yeast strains with various dNTP pool imbalances by introducing mutations in the allosteric specificity site of ribonucleotide reductase, a key enzyme in dNTP biosynthesis. The S-phase checkpoint was activated by the depletion of one or several dNTPs. In contrast, when none of the dNTPs was limiting for DNA replication, even extreme and mutagenic dNTP pool imbalances did not activate the S-phase checkpoint and did not interfere with the cell cycle progression. We are in the process of creating several mouse strains with mutations in ribonucleotide reductase that will hopefully result in different dNTP pool imbalances. If successful, preliminary results on viability, genome stability and aging will be presented.

Andrei Yu Chernenkov, Dmitriy Fedorov, Anna Spirina, Tatiana Evstyukhina, Vyacheslav Peshekhonov, Vladimir Korolev
Petersburg Nuclear Physics Institute, Russia.

The Interactions Between the Yeast *Saccharomyces cerevisiae* Hsm3 Protein and Chromatin Remodeling Factors.

We established the role of yeast *Saccharomyces cerevisiae* Hsm3 protein as one of the key players in homologous recombination and postreplicative repair pathways. The damaged Hsm3p causes the D-loop destabilization and prevents its processing. The same time it's known that the Hsm3p has chaperone functions and binds with the subunits of the proteasome complex. In addition it was shown that Hsm3 protein is coprecipitated with the members of HAT-B/NuB4 histone acetyltransferase complex – Hat1p, Hat2p and Hif1p. We have genetically proved that the Hsm3p interacts with the subunits mentioned above and is able to effect on the deoxyribonucleotide pool. Also we studied the interactions between the Hsm3 protein and one of the nucleosome assembly and checkpoint factors – Asf1p.

Zoulfia Darieva
Faculty of Life Sciences Manchester University, UK

Role of Pkc1 in coordinating chromatin modifications in response to DNA damage.

Role of Pkc1 in coordinating chromatin modifications in response to DNA damage. Pkc1 , the only member of the protein kinase C family in budding yeast, is important for the cell wall biogenesis, proliferation and stress responses. Recent studies implicate Pkc1 in a variety of nuclear

functions such as SPB duplication, transcriptional regulation of the G2/M transition, mitotic recombination and DNA repair. However, how does Pkc1 exert its effects at the molecular level is largely unknown. Mutants defective in Pkc1 are sensitive to DNA damage. We demonstrate that one of the nuclear targets of Pkc1 is the histone acetyltransferase Rtt109 required for modification of H3 K9, K27 and K56.These acetylation events play a key role in replication-coupled and repair-coupled nucleosome assembly . Pkc1 mutants are defective in H3K56 and H3 K9 acetylation, and exhibit an increased frequency of spontaneous chromosome breaks. They also demonstrate a phenotype Rtt109 cells andΔsimilar to H3K56ac mutants in the presence of DNA damage drugs. An Rtt109 mutant lacking Pkc1 – phosphorylation site mimics the Rtt109 phenotype suggestinga direct link between Pkc1,Δ Rtt109 and histone modification changes. Collectively, these studies place Pkc1 as a key integrator of DNA damage responses through mediating epigenetic changes and ultimately changing gene expression programmes and other chromatin-associated processes.

Milena Denkiewicz, Katarzyna Suski-Grabowski, Urszula Wrońska, Iwona J. Fijałkowska

Laboratory of Mutagenesis and DNA Repair, Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Poland

GINS complex – a new player involved in the maintenance of genomic stability *in S. cerevisiae*

Described in 2003 in eukaryotes, the GINS complex (an acronym for go-ichi-ni-san, for Japanese 5-1-2-3, after the four subunits of the complex - Sld5, Psf1, Psf2 and Psf3) was identified as a novel factor essential for both the initiation and elongation of replication. All four GINS subunits are essential for cell viability. GINS is very well conserved and its homologues

are found from the archea to human cells. Multiple interactions of GINS with major replication proteins position this complex at the heart of the eukaryotic replication machinery, therefore the GINS complex is a good candidate for playing an important function in the maintenance of genomic integrity. Recently, we have investigated the possible influence of the GINS complex on the final fidelity of DNA replication in the yeast *S. cerevisiae*. In our laboratory, several new temperature-sensitive mutants in PSF1 gene encoding one subunit of GINS complex were isolated. We observed that temperature-sensitive alleles of PSF1 gene cause a mutator phenotype by increasing level of spontaneous mutations. Furthermore, we demonstrated that a significant part of the PSF1-dependent errors was a subject to the mismatch repair system. Therefore, we concluded that they are replication errors. Interestingly, the two psf1 mutants exhibit other typical for DNA replication mutants phenotypes, like defective S-phase progression and dumb-bell cell morphology. Moreover, we established that one of the mutants exhibits only weak interaction with Dpb2, a noncatalytic subunit of Pol epsilon. The level of mutagenesis was investigated in several strains possessing psf1 alleles and impaired catalytic subunits of DNA polymerases. The obtained data add new implications to our knowledge about the functional interactions of GINS subunits with Pol epsilon subunits and its role in genetic stability.

Ruchika Sharma and Rao N Desirazu
Indian Institute of Science, India

Interplay of UvrD Helicase with MutL and MutS in Haemophilus influenzae, and its modulation by ATP.

UvrD helicase is a vital component of DNA mismatch repair (MMR) pathway, wherein it initiates the removal of misincorporated base in the

newly-synthesized strand by unwinding it and making it available for exonuclease action. During MMR pathway, UvrD works in concert with multiple proteins and interaction with other proteins modulates its activity and specificity. This investigation demonstrates the functional and physical interaction of Haemophilus influenzae UvrD (HiUvrD) with homologous MutL (HiMutL) and MutS (HiMutS). The helicase activity of HiUvrD was stimulated by cognate HiMutL, which was further enhanced in the presence of HiMutS in a mismatch-dependent manner. ATP-binding by HiMutL and noticeably, ATP-hydrolysis by HiMutS was found to be essential for stimulation of HiUvrD. HiUvrD was found to directly associate with HiMutL, and remarkably, the affinity between the two proteins was substantially enhanced in presence of ATP or its analogs. The ATPase activity of HiUvrD was non-specifically attenuated by single-stranded DNA binding (SSB) protein whereas the helicase activity was enhanced at low SSB concentration. However, DNA unwinding by HiUvrD was inhibited at higher concentrations of SSB, which could be overcome by increasing HiUvrD concentration as well as in presence of HiMutL and HiMutS. Importantly, HiMutS and HiMutL confer competitive advantage to HiUvrD to overcome barriers like SSB that block its helicase function. Taken together, our results emphasize the ATP-dependent modulation of HiUvrD by HiMutL and HiMutS, which could influence its functions during DNA mismatch repair pathway.

D.V.Fedorov, S.V.Kovaltsova , T.A.Evstuhina, V.T.Peshekhonov,
A.Yu.Chernenkov, V.G.Korolev
Petersburg Nuclear Physics Institute, Russia.

Characterization of yeast hsm6 mutants

Previously we reported about the collection of *yeast Saccharomyces cerevisiae* mutants characterized for the increased spontaneous mutagenesis

mutation rate. In this study we examined the HSM6 gene. Mutation hsm6-1 increased the UV-induced mutagenesis and decreased the UV-induced mitotic crossover in the centromere – ADE2 gene region. The HSM6 gene was mapped on the left arm of the chromosome II in the region where the PSY4 gene is localized. The epistatic analysis shown that the hsm6-1 mutation is an allele of PSY4 gene. The sequencing of hsm6-1 mutant allele brought out the frameshift mutation Lys218Glu that caused the stop-codon appearance in the next position. The interactions between the hsm6-1 and rad52 mutations are epistatic. Summarizing all data we propose that the PSY4 gene plays a key role in the regulation of cell withdrawal from the checkpoint caused by the DNA damage.

Rais A. Ganai, Matthew Hogg and Erik Johansson
Dept. of medical biochemistry and biophysics Umeå University, Sweden

Enzymatic properties of yeast DNA polymerase epsilon

Enzymatic properties of yeast DNA polymerase epsilon DNA polymerase epsilon is a multi-subunit B family polymerase that is involved in leading strand DNA replication in eukaryotes. DNA polymerase epsilon in yeast consists of four subunits, Pol2, Dpb2, Dpb3, and Dpb4. Pol2 can be further divided into two domains, the N-terminal catalytic domain and a C-terminal subunit binding domain. The catalytic domain of Pol2 contains both the polymerase site and a 3'–5' exonuclease site that is responsible for proofreading the newly built DNA. The objective of this study is to explore the enzymatic mechanisms by which polymerase epsilon builds new DNA efficiently and with high fidelity.

Roza Goncharova
Institute of Genetics and Cytology National Academy of Sciences of Belarus, Republic of Belarus

Genome integrity and diagnostics of genome instability in human lymphocytes in the risk groups.

Genome integrity and stability underlie viability and resistance of any organism to the negative environmental factors. Genome instability (GI) is due to dysfunctions of various processes involved in maintaining and replicating the genome. GI is characterized by increased cellular susceptibility to mutagenic effects and hereditability of this acquired property. Taking into account possible contribution of induced GI to morbidity, the study of this phenomenon in populations seems to be a new urgent task. The aim of our investigation was assessment of genomic reaction norms in the healthy population of Belarus and detection of some features of GI in human blood lymphocytes in the different risk groups. As a new approach, the levels of endogenous DNA damage, H2O2-induced DNA damage, kinetics and efficiency of DNA repair for 3 h incubation of treated lymphocytes in vitro were determined using the comet assay. This approach was used for GI diagnostics in the groups of children suspected of chromosome instability and microdeletion syndromes [Savina et al., 2011; Savina et al., 2012 a]. Due to this approach, some distinctive features of GI were found in anemia Fanconi and Nijmegen breakage syndrome patients, as well as abnormal cellular response to DNA damage was revealed in Williams syndrome patients. In the latter case, GI was closely associated with a 7q11.23 microdeletion. It was interesting to examine genome integrity of blood lymphocyte in a sizeable group of people exposed to occupational hazards. In this study [Savina et al., 2012 b], against a background of non-genotoxicity of occupational surroundings, individuals with increased cellular sensitivity to DNA damage were found. Their

proportion was higher among elderly and chronically diseased persons indicating the association of GI with morbidity. The results obtained demonstrated expediency and necessity of the individual approach to IG diagnostics that suggested determination of the genomic reaction norms in isolated blood lymphocytes. Based on the results in healthy population (n=172), the reference intervals were determined for all the parameters studied [Savina, Kuzhir, 2012], which can be used for comparison with individual data to estimate genome stability/instability in lymphocytes. Thus, among patients with bladder cancer 50% of the group showed increased sensitivity of lymphocytes to oxidative stress as compared to 14% in the control group. The results as a whole demonstrate contribution of GI in cancers and other hereditary and non-hereditary diseases as well as the efficacy of proposed technology for revealing GI in both the risk groups and individuals.

Ewa Grabowska, Aleksandra Respondek, Małgorzata Jaszczur, Piotr Jonczyk and Iwona J. Fijalkowska

Laboratory of Mutagenesis and DNA Repair, Institute of Biochemistry and Biophysics PAS, Poland

Mutation in central part of Psf1p subunit of GINS complex has impact on DNA replication fidelity.

With regard to the fidelity of DNA replication the main focus has been concentrated on DNA polymerases for years. However, it is a relevant question concerning the contribution of the other proteins that comprise the replisome to the final fidelity of DNA replication. One of such element is GINS complex, heterotetramer composed of Psf1p, Psf2p, Psf3p and Sld5p. GINS complex is essential for the processes of initiation and elongation of DNA replication. In order to investigate a possible role for the GINS

complex in maintaining the fidelity of DNA replication, we characterized strains carrying psf1-1 (kindly provided by H. Araki) temperature-sensitive alleles of the PSF1 gene. We have found that the psf1-1 mutation decreased the affinity of Psf1p for the Psf3p subunit as measured by two-hybrid analysis. Interestingly, Psf1p interacts with Dpb2p – the noncatalytic subunit of major replicase DNA polymerase epsilon. Moreover, we established that mutant form of Psf1p (psf1-100) exhibits only weak interaction with Dpb2, a noncatalytic subunit of Pol epsilon. To get more information whether proper functioning of a GINS complex influence the fidelity of DNA replication and the fidelity of polymerase epsilon we analyzed spectrum of mutagenesis in several yeast strains possessing mutated psf1 alleles and/or pol2-4 allele (lack of proofreading of Pol ε) and other control strains. Isolated mutant forms of Psf1p increased the mutation rate of base substitutions and frameshifts. Our results indicate that GINS complex is an important element in controlling the level of spontaneous mutagenesis and genetic stability in yeast cells.

Peter Hällman and Erik Johansson
Dept of medical biochemistry and biophysics Umeå University, Sweden

Studies of the Pol epsilon accessory subunits, Dpb3 and Dbp4.

F.Ingel, L.Krivtsova, N.Yurtseva, N.Antipanova, T.Legostaeva,
B.Antsiferov, S.Ivanov
FSBO "A.N.Sysin Research Institute for Human Ecology and Environmental Hygiene" of Ministry of Health, Russia

Modification of children's genomic instability is realized trough imbalance of autonomous system, induced by factors of their microsocial environment.

The genomic instability means a series of mechanisms related to transformation of stable genome of normal cell in an unstable characteristic for genome of tumor cells (Suzuki K. et al, 2004; Smith M.et al, 2003). Previously, we have shown that stress as a nonspecific response of the body to the external action of any nature is a factor modifying the level of genomic instability adults and children (Ingel F.I. et al, 1993-2009). Subsequently, the validity of these findings has been proven – in particular - in several studies that investigated the mechanisms by which the immune response influences to DNA instability (Reiche EM, Nunes SO, Morimoto HK.; 2004). The complex of methods of socio-psychological analysis of family, included into the cytogenetic study, revealed the influence of several ethical and emotional factors (in particular - the quality of parental satisfaction with their family life, etc) to the level of children's genomic instability. Further investigations of the effect of microsocial environment of family's and school' to genomic instability of preschoolers of middle and older age and primary schoolers showed the involvement of mechanisms of regulation of autonomic balance of children's body into processes that - for the end - are realized as changes in genomic instability (as increasing of the levels of genetic damage, aneuploidy and changes in proliferation of peripheral blood lymphocytes, as well as their mitotic activity and apoptosis). As summarizing the results of the studies it must be concluded that one of the physiological mechanisms responsible for increasing of children's genomic instability and susceptibility of genome to

environmental mutagens (and, consequently, for their health effects in in adulthood, binding with a wide range of diseases, including, in particular, immunodeficiency states (Cerbone M et al, 2012; Isoda T et al, 2012. .), neuropsychiatric diseases including schizophrenia and autism. (Smith CL, Bolton A, Nguyen G., 2010) and cancer (de Miranda NF, Björkman A, Pan-Hammarström Q, 2011) is connected with misbalance of autonomic nervous system.

Ivanova E.A., Vafina G.H., Ivanov R.S., Karpova L.M.
Institute of Biology Ufa Science Centre Russian Academy of Science, Russia

Genetic variation at reorganization proteome of cell nuclei of plants in the environment.

Flower plants have the same epigenetic mechanisms as many animal cells. Plants and animals have more fundamental similarities than the appearance. In plants, there are many genes that are equivalent to those of the animals. They also have high developed epigenetic system. It is known that about 98% of the genome is not involved in coding proteins. This is associated with the regulation of gene expression by epigenetic mechanisms. These mechanisms could be influenced in the natural circadian cycles. In the environment the plants have to adapt or perish. Many of the plant response are related with the changes in the programs of development of the cells. One mechanism may be the modification of proteins by limited proteolysis. Our knowledge about which specific protein modifications are set to certain positions in the genome, and how these modifications are the part of the molecular of memory are fragmented. It was confirmed that the bases of vernalization are the epigenetic processes on the level modifying chromatin. After vernalization of the parent plant, the mature seeds have of changing of program development. The plant received some form of

molecular memory that persists even after cell division. But this memory can be erased in the next generation of somatic cells, if not two factors: the duration of the cold period and the increase in the light of the day. Changes in DNA and associated proteins, must meet the following criteria of epigenetics. 1. Two organisms are genetically identical, but phenotypically – distinct. 2. Organism continues to be under the influenced the event, although it happened many years ago. It is important to know what changes of the chromatin are in the base of various epigenetic phenomena, and what the role of epigenetic enzymes is in reorganization of chromatin. In the cell nucleus there are proteins which are rich by arginine. The arginine is the amino acid which has resonance properties. Its N-terminal groups are actively involved in processes of interchromosomal modification. However, the biochemical and biophysical mechanisms of this process are not enough studied. Arginine-riched histones by the amino acid sequences are the evolutionarily stable the proteins. This indicated about their important role in the preserving and realization of genetic information in eukaryotes. The aim of this work was to find the dynamics of localization of the Arg-X protease-sensitivities sites in histone and non-histone blocks as the possible zones that affect the conformational changes of the total interphase chromatin during the reprogramming spring varieties to the winter varieties and again to the spring varieties. Isolation of cell nuclei, their supramolecular structures: nucleoplasm, chromatin, nuclear matrix, as well as histones and non-histones with definition in them the Arg-X protease-sensitivities sites was done with use patents developed by E.A. Ivanova, G.H. Vafina.

Erik Johansson
Dept medical biochemistry and biophysics Umeå University, Sweden

Structural and functional studies of DNA polymerase epsilon

DNA polymerase epsilon is a multi-subunit B family polymerase that is involved in leading strand DNA replication in eukaryotes. DNA polymerase epsilon in yeast consists of four subunits, Pol2, Dpb2, Dpb3, and Dpb4. Pol2 can be further divided into two domains, the N-terminal catalytic domain and a C-terminal subunit binding domain. The catalytic domain of Pol2 contains both the polymerase site and a 3'–5' exonuclease site that is responsible for proofreading the newly built DNA. To further understand the influence of the different domains and subunits during the process of DNA synthesis, we have studied the enzymatic properties of DNA polymerase epsilon in single-turnover pre-steady state experiments. Our primary focus has been to elucidate if the non-catalytic subunits influence the chemical steps, processivity or loading of DNA polymerase epsilon onto the primer-template. We have also studied the transition of the primer-end between the exonuclease and polymerase site. Our findings will be discussed in the context of earlier described DNA polymerases.

Piotr Jonczyk[1], Katarzyna Maslowska[1,2], Roel Schaaper[2], Iwona J. Fijalkowska[1],

[1]Institute of Biochemistry and Biophysics PAS, Poland, [2]National Institute of Environmental Health Sciences, USA

Control of the E. coli error-prone SOS response by intracellular dNTP pools

Critical elements of cellular responses to DNA damage are inducible systems, such as the well-characterized E. coli SOS system. The SOS system entails the induction of multiple factors serving to promote DNA repair, including error-prone factors, such as DNA polymerase Pol V, responsible, through translesion synthesis, for improved survival at the cost of elevated mutagenesis. Of critical importance for DNA synthesis are the cellular dNTPs, as efficiency of lesion bypass is dependent on dNTP levels, while misinsertion rate is governed by the ratio of correct and incorrect dNTPs. In addition, nucleotide levels are important for induction and maintenance of the SOS system through the nucleotide requirements of the RecA protein, which serves as the activator of the system. To address the role of dNTP levels in SOS mutagenesis, we have investigated the interaction between the SOS mutator effect mediated by the recA730 mutation (constitutive SOS induction) and alterations of dNTP pools occurring in strains affected in nucleotide metabolism. Remarkably, we observed a near complete suppression of the SOS mutator effect when recA730 was combined with ndk (nucleoside diphosphate kinase) or dcd (dCTP deaminase) defects. Measurements of β-gal expression from the umuDC promoter showed that loss of mutator activity resulted from failure to express the umuDC operon encoding Pol V. This issue was further explored by microarray studies that allowed analysis of the complete spectrum of SOS inducible genes (SOS regulon). Remarkably, in the presence of the ndk or dcd deficiencies a shut-down was observed of the entire SOS system. The results reveal that the cellular dNTP levels play a

critical role in induction of the SOS response. The effect is mediated most likely through the direct role of the dNTPs in the activation of RecA protein. This insight is supported by the measured dNTP pool changes in the respective strains.

V.N.Kipen, S.B.Melnov
International Sakharov Environmental University, Minsk, Belarus

Frequency of 1100delc in gene CHEK2 in population of mammary gland cancer patients from Chernobyl affected area (Belarus)

Currently it is considered that approximately up to 5% of breast cancer cases have hereditary nature (Imianitov E.N. et. al. 2007; Semiglazov V.F. et. al. 2008). It is accepted that the main share of breast cancers are caused by unfavorable "genetic passport", i.e. combination of predisposing low penetrating and relatively often – mid penetrating alleles with moderate and at the same time clinically significant effect to risk of breast cancer. The last one includes CHEK21100delC, NBSl657del5, BRIP1, PALB2. As it is mentioned, all known high- and mid- penetrating genes associated with cancer play an important role in maintaining genome integrity. If we summarize the effect of all known genetic determinants of breast cancer to total risk, so according to polygenic model 8 confirmed low penetrating predisposing genes provide above 5% of genetic risk; and high penetrating genes provide 20-25%, at that mid penetrating genes give less than 3% to the total risk. CHEK2 gene is located in long arm of chromosome 22, and it codes kynase which plays an important role in reaction pathways to DNA damage (Falck J. et. al, 2001.) This role includes coordination of cellular cycle with mechanisms of DNA reparation, with survival or apoptosis of cells. Defects in CHEK2 lead to development of sporadic and hereditary tumors evidencing that CHEK2 is a suppressor of

tumor growth. Mutation of CHEK2 1100delC causes formation of defect protein which could serve as kynase. The study demonstrated that changed protein was either not expressed or its expression is much decreased (Dong et al., 2003). Since 2002 when CHEK2 1100delC was accepted as low penetrating allele predisposing to breast cancer (Meijers-Heijboer et.al. 2002; Vahteristo et.al., 2002), this mutation has been thoroughly studied. We have already examined group of patient (from Chernobyl affected area) with clinically verified diagnosis of breast cancer with respect 1100delC mutation in gene CHEK2, and individuals from the control group. The samples were standardized by age criterion (average age of the main group was 52,26±2,69 years, and control one: 50,98±4,21 years). All the subjects of both groups at the time of examination were urban residents of Minsk region, Belarus. In the main group 1100delC mutation was found in two patients (3,9±2,7%), in the control group no mutation was recorded.

Olga V. Kochenova, Shcherbakova P.V.
Eppley Institute for Research in Cancer and Allied Diseases University of Nebraska Medical Center, USA

DNA polymerase ζ synthesizes long stretches of DNA during lesion bypass in vivo.

DNA polymerase ζ (Polζ) is implicated in a variety of processes in the cell such as translesion synthesis (TLS), homologous recombination, replication of undamaged DNA in response to an impediment of replication fork, etc. The main function of Polζ in these events is to extend a distorted primer terminus which causes stalling of replisome. Polζ-dependent synthesis is highly mutagenic and characterized by increased frequency of GC→CG transversions and complex mutations. Thus, the level of Polζ-dependent mutagenesis is believed to be directly proportional to the length

of the DNA stretches synthesized by Polζ. However, this remains poorly studied. We attempted to determine how many nucleotides Polζ incorporates during bypass of abasic site (AP site) and UV lesions in yeast. Double-stranded substrate with analog of AP site (tetrahydrofurane) was introduced into the apn1Δapn2Δ yeast strain. Mutagenic A or C incorporation opposite AP site allowed us to specifically select for TLS products. We show that TLS across from AP site during replication of the plasmid occurs in ~1% of the transformed cells. The sequencing of 80 products of AP site bypass detected a 3-fold increase in mutagenesis downstream the lesion associated with TLS. Polζ-specific signature (GC→CG transversions) is found within 1.2 Kb region from the lesion site, but not in the sequenced control substrates. Since the length of DNA stretches synthesized by Polζ may vary during bypass of different DNA lesions we attempted to determine how many nucleotides Polζ incorporates after mutagenic TLS across from UV lesion on the chromosome. To address this question, we developed a new reversion assay (the ura3-G764A allele). Revertants of ura3-G764A mutation were selected after 60 J/m2 of UVB-light irradiation. Reversion of ura3-G764A occurred with a frequency of 2.4x10-9 at this dose. We show that reversion to Ura+ phenotype is completely dependent on Polζ and is abolished in Polζ-deficient strain. The sequencing of 100 revertants reveals that Polζ-dependent bypass of UV lesions at G465A site in the URA3 gene is associated with error-prone DNA synthesis downstream the lesion within 1Kb region. We argue that Polζ is efficient in synthesizing long stretches of DNA during TLS in vivo, which may indicate that Polζ alone is responsible for filling the gap left behind the re-priming of replication downstream the lesion. The factors that may affect the length of Polζ-dependent synthesis are to be determined.

Koltovaya N., Balasoiu M., Angel L., Del Giudice L.
JINR Laboratory of Radiation Biology Joint Institute for Nuclear Research, Russia

SANS and HRTEM study of iron and tellurite nanoparticles in mitochondria.

The effects of iron and potassium tellurite on the growth and survival of rho+ and rho0, yfh1Δ and tellurite resistant Saccharomyces cerevisiae strains, and the dymanic of accumulation of metals by cells were investigated. Transmission electron microscopy and small angle neutron scattering data on cells grown in the presence of metals are presented. CDK1 and mitochondrial DNA stability in budding yeast Koltovaya N The inheritance of mitochondria is cell regulated. We obtained the genetic data that the CDC28 kinase, the central regulator of cell cycle progression, affects mtDNA stability and inheritance of mitochondria. CDC28 participate in checkpoint activation and is a target of checkpoints. Recently it was found that a Rad53-dependent checkpoint of mtDNA quantity exists. It is very important to identify mtDNA checkpoint proteins because changes in mtDNA content have been linked to multiple forms of cancer, and DNA damage checkpoint proteins are commonly altered in human tumors. It is possible that changes in tumor cell metabolism, such as mitochondrial respiration decrease and aerobic glycolysis increase, can arise from mtDNA checkpoint dysregulation. So we first identify the new mitochondrial aspect of the central kinase CDK1.

Neta Agmon[1], Batia Liefshitz[1], Christophe Zimmer[2] , Emmanuelle Fabre[3] and Martin Kupiec[1]

[1]Department of Molecular Microbiology and Biotechnology, Tel Aviv University, Israel,

[2]Groupe Imagerie et Modélisation, Département Biologie Cellulaire et Infection, Institut Pasteur, France,

[3]Groupe Régulation Spatiale des Génomes, Département Génomes et Génétique, Institut Pasteur, France.

Effect of nuclear architecture on the efficiency of double strand break repair

Effect of nuclear architecture on the efficiency of double-strand break repair The most dangerous insults to the genome's integrity are those that break both strands of the DNA double helix. These double strand breaks (DSBs) can be repaired by homologous recombination (HR); in this conserved mechanism the broken ends are converted into single-stranded ends and covered by the Rad51 strand-exchange protein. A global genomic "homology search" then finds sequences similar to those near the break and uses them as a template for DNA synthesis and end ligation, resulting in a repaired chromosome. Chromosomes are spatially constrained and occupy restricted territories within the nucleus. Taking advantage of recent data on yeast nuclear organization, we show here that genomic regions whose nuclear 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 and thus reduces the chances of non-allelic recombination. Our results challenge current models that posit an active scanning of the whole nuclear volume only by the broken chromosomal end, and emphasize the important role of nuclear organization in genome maintenance.

Sharik R. Khan and Andrei Kuzminov
Department of Microbiology, University of Illinois at Urbana-Champaign, USA

Ultraviolet-induced chromosomal fragmentation: pathways, mechanisms, and contribution to lethality

Ultraviolet (UV) irradiation is the most common DNA-damaging condition for surface organisms, and it can be lethal in sufficient doses, but the mechanisms of UV-induced lethality are still unclear. UV induces a well-defined one-strand DNA lesion, called pyrimidine dimer, which blocks progress of individual replisomes, but it does not stop replication forks and is repairable by excision. Yet, ultraviolet irradiation starts killing cells of any type at densities of lesions that are completely repairable, and this killing is associated with formation of irreparable double-strand breaks in chromosomal DNA. To understand how these double-strand breaks are induced by UV, we follow their formation in repair-proficient Escherichia coli cells, as well as in mutants, deficient for the RecBCD enzyme, responsible for linear DNA degradation and repair in E. coli. I will present evidence for two pathways of UV-induced chromosomal fragmentation (early and late), suggesting distinct mechanisms. Both pathways operate only in cells that are actively replicating their DNA, suggesting that replication forks are the sites of chromosomal fragmentation. The early fragmentation coincides with inhibition of DNA replication and depends on excision repair, but is independent of recombinational repair functions, including Holliday junction resolution. The mechanism of the early fragmentation is, therefore, compatible with the replication fork collapse model. The late fragmentation coincides with restoration of DNA replication, is independent of excision, but completely depends on recombinational repair functions. The mechanism of the late fragmentation is, therefore, compatible with the replication fork reversal with subsequent

breakage model. Both fragmentation mechanisms, since they happen at replication forks, should lead to completely repairable chromosomal lesions. Moreover, the number of breaks itself should be limited by the number of replication forks. Yet, fragmentation is increased with UV doses, indicating formation of irreparable double-strand DNA breaks. Contribution of the two known fragmentation mechanisms to UV-caused lethality, as well as possible mechanisms for irreparable chromosomal lesions, will be considered.

Artem G. Lada, Igor B. Rogozin, Youri I Pavlov
Eppley Institute for Research in Cancer and Allied Diseases University of Nebraska Medical Center, USA

Genome-wide kataegis induced by AID/APOBEC deaminase in yeast revealed by next-generation sequencing.

Editing deaminases of AID/APOBEC superfamily perform a variety of essential functions in different organisms. APOBEC1 edits mRNA. AID and APOBEC3G are involved in adaptive and innate immunity, respectively. PmCDA1 is involved in immune response in lamprey. Astonishingly, such a variety of functions are performed using the same catalytic mechanism - deamination of cytosine to uracil in the single-stranded RNA or DNA. Deamination in DNA also is a potential threat for the genome stability that ultimately can cause cancer. The mechanisms of targeting of deaminases to their physiological substrates are unknown. Similarly, little is known about the mechanisms of genome protection from mutagenic effects of deaminases. We found that expression of PmCDA1 in yeast leads to increase of mutation frequency in both haploid and diploid strains, as measured at the CAN1 reporter gene. In order to better understand the mechanisms of deaminase-induced mutagenesis, we

employed Illumina next-generation sequencing technology. We have sequenced genomes of diploid uracil-DNA-glycosylase deficient yeast can1 mutant clones induced by the expression of PmCDA1. Each genome contained thousands of predominantly heterozygous mutations. All mutations were C to T or G to A transitions, as expected from the cytosine deamination. Mutational context was typical for the PmCDA1. Most of mutations were distributed more or less randomly across the genome. However, some of the substitutions were found in clusters. Similar clusters have been recently found in breast cancers. We propose that the clusters may result from processive deaminase action, where enzyme binds to the DNA and move along it, catalyzing multiple deaminations. Such clusters were observed in the very strong "hot" regions in the genome. Mutations in these sites were found in different yeast clones. Obviously, these regions of the genome are highly accessible to deaminases. Most likely, the borders of these regions are determined by the local scarcity of factors protecting the genome from deamination. The parameters of action of deaminases on genomic DNA are evolutionary conserved and studies of the mechanism of appearance of the clusters is in yeast model will help understanding of origin of certain human cancers.

Igor N Lebedev, Sazhenova E.A., Lepshin M.V.
Laboratory of Cytogenetics, Institute of Medical Genetics, Russia

Multilocus methylation defects of embryo imprintome in human miscarriages.

Genomic imprinting is an epigenetic phenomenon resulting in monoallelic expression of imprinted genes in a parent of origin-dependent manner. It is well known that this balanced expression is strongly required for normal embryo development in mammals. But little information is available for understanding the role of imprinting defects for abnormal human embryo development and early pregnancy losses, excluding cases of hydatidiform moles. Indeed several studies had no success to search for uniparental disomy in spontaneous abortions with normal karyotype. Previously, we hypothesized that expected pathogenetic effects of imprinting abnormalities in human embryo development can be visualized at the epigenetic level. In our studies hypomethylation of KCNQ1OT1 and PLAGL1 imprinted genes was found in 9.5 and 10.3% of spontaneous abortions, respectively. Moreover some embryos had hypomethylation of maternal alleles in both genes, which was associated with recurrent pregnancy loss in their mothers. Current DNA methylation microarray technologies provide an excellent possibility to analyze epigenetic status of multiple imprinted genes (or imprintome) simultaneously. Using GoldenGate Methylation Cancer Panel I (Illumina) DNA methylation index was estimated for promoter regions of 51 human imprinted genes in placental tissues of 13 first trimester spontaneous abortions with normal karyotype. For the first time, multiple epimutations were observed. Hypomethylation or hypermethylation of promoter regions from 4 to 12 imprinted genes per each embryo was found. Comparative analysis of trophectoderm and epiblast derivates has revealed that 78% of epimutations were confined by single tissue type indicating a high incidence of

postzygotic origin of DNA methylation defects. Our data provide first evidence that aberrations of imprinting maintenance in somatic cells after the wave of epigenetic genome reprogramming can be a significant factor for disturbances of human embryo development. Moreover, it was shown that the total incidence of multilocus methylation defects on maternal and paternal alleles of imprinted genes, which lead to suppression of embryo development, was significantly higher than the rate of epimutations, which can promote embryo growth. This fact supports at the epigenetic level the "sex conflict" hypothesis, which explains the appearance of monoallelic imprinted genes expression in the evolution of mammals. Our studies were supported by grants № P303 and P806 of Federal Program "Scientific and educational personnel of innovative Russia» for 2009-2013 years and grant № 08-04-01344 of Russian Foundation for Basic Research. Dear colleagues, Let me introduce my self. My name is Igor Lebedev. My position is a Head of Laboratory of Cytogenetics of Institute of Medical Genetics, Tomsk, Russia. The main field of scientific researches in our laboratory is cytogenetics and epigenetics of human prenatal development. The focus of studies is related to analysis of lethal chromosomal abnormalities, chromosomal mosaicism, and CNV in spontaneous abortions by using molecular cytogenetic technologies, such as FISH, CGH, array-CGH. In the area of epigenetics our interests are related to genomic imprinting, X-chromosome inactivation, genome-wide analysis of DNA methylation. The presented work is a summary of our studies of the phenomenon of genomic imprinting during abnormal embryo development. The main take home message from these studies is that abnormalities of imprinted genes result from multiple epigenetic changes in their regulatory regions in somatic embryo cells. This thesis supports the common idea of a high level of embryo genome instability at early stages of development, that confirmed also by our studies of germline and somatic STR mutations, chromosomal mosaicism, as well as current data from preimplantation genetic diagnosis. These studies have a significant impact to understanding

the key regulatory mechanisms of normal embryo development and pregnancy losses.

Alena Makarova and Peter Burgers
Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110, USA

DNA polymerase ζ- and Rev1-mediated mutagenesis in yeast.

Polζ plays a key role in mutagenesis and DNA translesion synthesis in eukaryotes. Previously, a two subunit Rev3-Rev7 complex had been identified as the minimal assembly required for catalytic activity *in vitro*. We demonstrate that *S. cerevisiae* Polζ binds the Pol31 and Pol32 subunits of Polδ *in vivo* forming a four subunit Polζ complex ($Pol\zeta_4$). A [4Fe-4S] cluster in Rev3 is essential for the formation of $Pol\zeta_4$ and for damage-induced mutagenesis. The Pol32 subunit is essential for complex formation, providing an explanation for the long-standing observation that *pol32*□ strains are defective for mutagenesis. Purified Pol ζ_4 complex demonstrated enhanced catalytic and PCNA-dependent DNA polymerase activity on damaged and non-damaged DNA compared to two subunit Polζ ($Pol\zeta_2$). Deletion of the C-terminal PCNA-interaction motif in Pol32 attenuates PCNA-dependent TLS *in vitro* and mutagenesis *in vivo*. Furthermore, a mutant form of PCNA, encoded by the *pol30-113* allele of PCNA, fails to stimulate $Pol\zeta_4$ activity, providing an explanation for the defective mutagenesis phenotype of this allele. A stable $Pol\zeta_4$ complex can be identified in all phases of the cell cycle suggesting that the complex is not regulated at the level of protein interactions between Rev3-Rev7 and Pol31-Pol32. We propose that formation of four subunit complex is indispensable for the *in vivo* function of Polζ.

Karolina Makiela-Dzbenska, Garbacz M, Araki H, Flis K, Jonczyk P and Fijalkowska IJ

Institution of Biochemistry and Biophysics PAS, Poland

Contribution of Dpb2p, the non-catalytic subunit of DNA polymerase epsilon, to the fidelity of DNA replication in *Saccharomyces cereviasiae*.

DNA polymerase epsilon is composed of four subunits: Pol2p (polymerase and 3'→5' exonuclease activity), Dpb2p, Dpb3p and Dpb4p. The biological role of Pol2p was established. However, the function of other three subunits is still investigated. To find out whether non-catalytic, essential Dpb2p influences the fidelity of Pol epsilon and to verify the participation of Pol zeta in spontaneous mutagenesis in dpb2 mutant strain we determined the spectrum of mutagenesis in CAN1 locus for eight different isogenic strains. In parallel, we isolated Pol epsilon holoenzymes from wild type strain and dpb2-100 mutant strain and compared biochemical activities of both holoenzymes. In the analyzed spectra of mutagenesis four strains were bearing dpb2-100 allele (dpb2-100, dpb2-100 rev3, dpb2-100 pol2-4 and dpb2-100 pol2-4 rev3) and the remaining four strains (WT, rev3, pol2-4 and pol2-4 rev3) have been used as the appropriate controls for particular comparisons. We estimated the rates and percentage of each individual class or type of mutation in analyzed strains. Comparison of spectrum of mutations generated by mutated Pol epsilon dpb2-100 HE in strain deficient in Pol zeta (rev3) demonstrated that the presence of mutant Dpb2p facilitates more frequent participation of error-prone DNA polymerase zeta in DNA replication. Strongly elevated level (multiplicative effect) of each type of mutations observed in spectrum of pol2-4 dpb2 double mutant showed that Pol epsilon, with mutated Dpb2p, generates enhanced number of errors which are subject to 3' → 5' proofreading. In samples of Pol epsilon holoenzymes purified from dpb2-100 mutant strain we observed about 85% deficiency of the Dpb2p subunit.

In vitro analysis revealed that both enzymes bind to dsDNA with similar affinity, perform DNA synthesis with similar processivity and degrade dsDNA with similar efficiency. Interestingly, we demonstrated that the fidelity of DNA replication in dpb2-100 strain negatively correlates with Dpb2p level in vivo. Presented data suggest that impaired Dpb2p subunit, not only facilitates participation of error-prone Pol zeta in DNA replication but also influences the fidelity of Pol epsilon.

Mikhailov V.M.[1], Neuschtadt E.l.[2], Skripkina N.S.[2], Mitrofanova L.B.[3], Alpatov M.B.[3], Deev R.V.[4], Serikov V.B.[5], Kaminskaya E.V.[1], Popov V.B.[1], Grinchuk T.M.[1], Nikolsky N.N.[1]

[1]*Institute of Cytology RAS,*

[2]*City Pathological-Anatomical Bureau,*

[3]*Almazov's Federal Centre for Cardiology and Endocrinology, Saint Petersburg,*

[4]*Stem Cells Institute, Moscow, Russia,*

[5]*CHORI, Oakland, CA, USA*

Tumors of GFP-expressed C57BL/6 mice

The therapeutic use of genetically modified cells is limited by insertional mutagenesis which is followed by tumors. It was calculated that vector insertion site induces the zone of DNA instability with size up to 10 KB. Effects of insertions are studied well using vectors produced on the base of retro- and adenoviruses on the cell populations of adult organisms. The question arises about how much the genome of transgenic animals which are produced at the zygote stage is destabilized. These are results of histological study of tumors developed from the cells of GFP expressed C57BL/6 mice (C57BL/6-GFP(+) mice). Initial transgenic C57BL/6 mice were produced by method of Okabe (Jackson Laboratory, Okabe et al.

1997). During 5 years C57BL/6 GFP(+) mice were cross breeding with C57BL/6 mice. There were 14 generations. The last one contained 4 mice with subcutaneous tumors, which slides were studied. All tumors were histologically classified as moderately differentiated adenocarcinoma. At the same time bone marrow of C57BL/6-GFP(+) mice were used to produce mesenchymal stem cells (MSC-GFP(+). Transplantation of MSC GFP(+) cells of 43-45 passages to mdx mice were followed by formation of tumors in 100%. Transplantation of MSC GFP(+) cells of 15 passages to mdx mice didn't lead to tumors. Transplantation of the same cells to C57BL/6 mice did not generate tumors too. Histologically tumors were classified as sarcomas. Parts of tumors consisted from differentiated cells such as chondrocytes, adipocytes, nerve cells, and bone bulks with hematopoiesis (Mikhailov et al., 2010). MSC-GFP(+) cells had changed characteristic of transformed cells in their karyotypes even during the early passages of cultivation. Chromosome number varied from near diploid to near tetraploid, in rare cases reaching number 160. Marker and randomly rearranged chromosomes were observed too (Grinchuk et al., 2008; Popov et al., 2009). Spontaneous tumors in transgenic mice C57BL/6-GFP(+) in vivo had revealed epithelial origin view (adenoma). MSC-GFP(+) cells of 43-45 passages had formed in the mdx mice connective tissue tumors. We consider that such prominent difference between tumor types of different origin are not accidental. C57BL/6 mice are known to have very little level of spontaneous tumor occurrence (Medvedev, 1966). We can suggest that genome destabilization of transgenic C57BL/6-GFP(+) mice is reason of tumors formation during mice breeding. Destabilization in MCK-GFP(+) cells genome in vitro is more intense because of the MSC-GFP(+) next instability in cell culture and lack of immune control.

Alena V Makarova, Artem Ignatov, Nataliya Miropolskaya and Andrey Kulbachinskiy

Institute of Molecular Genetics (Russian Academy of Sciences), Laboratory of Molecular Genetics of Microorganisms, Moscow

Single amino acids substitutions in the fingers domain increase the fidelity of human DNA polymerase iota

Human DNA polymerase iota (Pol i) is a Y-family DNA polymerase that participates in translesion DNA synthesis and demonstrates very low fidelity on undamaged DNA. The solved structures of Pol i catalytic core in the complexes with different dNTPs and DNA templates revealed a unique organization of the Pol i active site and proposed the utilization of noncanonical interactions during base-pairing. To better understand the structure-function relationships in the active site of Pol i we investigated the role of ten key active site residues in nucleotide incorporation by introducing single amino acids substitutions in full length human Pol i protein. None of investigated amino acid substitutions was found to significantly increase the efficiency of incorrect nucleotide incorporation. However, we demonstrated that mutations of residues Y39 and Q59 within the fingers domain dramatically increase the fidelity of Pol i on undamaged DNA, particularly opposite templates purines. We also showed that mutation of Y39 residue contacting with a sugar of the incoming nucleotide increases the rate of ribonucleotide incorporation by Pol i suggesting the role of Y39 in the mechanism of discrimination between ribo- and deoxynucleotides. The project was supported by the Russian Foundation for Basic Research.

Y. Nikonorov, G. Benkovskaya and R. Somova
Institute of Biochemistry and Genetics Russian Academy of Science, Russia

Whether the role of intensive activity of transposones is always negative?

The evident toxic effect of intensive transposones reproduction allowed supposing their participation in ageing processes. Some of investigators consider manifestation of transposone activity in the species genome the adaptive trait. In our experiments with the strains of Musca domestica selected for increased and reduced life span (Sh28 and L2, accordingly) we investigated possible participation of transposone Hermes in development of these signs. The least variations of Hermes copy number observed in pupal DNA from all the strains, including the origin strain S. In larval DNA copy number exceeded the obtained value. Evaluating the copy number of transposone DNA in adults muscle tissues we revealed the increase of copy number in the selected strains against to pupal stage and the increase of copy number in the muscle tissues of adults from strain Sh28 as compared to the strain S and to the strain L2. It is possible that in the genome of flies from strain L2 some copies of transposone localized in the regulatory regions of genes which not necessarily must expressed for normal vital functions of house flies in the laboratory conditions and the most part of resident copies keep their previous localization. It would lead to decrease the whole metabolism level and accordingly to reduce the volume of nascent toxic metabolites, prolonging the life span of adults. In the somatic cells of flies from strain Sh28 increased transposition activity probably provoked by appearance of breaks and breaches in DNA molecule. The consequences of such excess activity can manifest as decrease of life span. But it is very possible that in some individuals from this strain positive effect manifests caused by intense transposone activity since new copies of transposone inserting into the breaks of genomic DNA thereby “repairing” these injuries and prolonging the life of damaged cells. Thus, as

a result of selection we got two strains of M. domestica differ significantly by the life span. During over than 30 generations of inbreeding in the strains remained the increased activity of Hermes manifests as the self-excited generation of individual variability of transposone copy number in the set of generations. We presume to propose that under the stress situations the positive effect can be observed concerned with maintenance of viability of organism in spite of risk of acquisition of unfavorable mutations by the germ line cells due to the increase of transposone activity.

Acknowledgments. Investigations funded by RFBR 12-04-01450-a and 11-04-97005-r_povolzhye_a.

Tomo Hanafusa and Haruo Ohmori

Institute for Virus Research, Kyoto University (Present address, Gakushuin University , Japan

Interactions between Rev7/Mad2B or Mad2 and Rev3 in various organisms

Interactions between Rev7/Mad2B or Mad2 and Rev3 in various organisms We reported that the human Rev7(hRev7=hMad2B or hMad2L2) binds to hRev3, recognizing the 1877-ILKPLMSPP-1885 sequence in hRev3 and that hMad2 also binds to the same sequence (Genes Cells, 15:281-296, 2010). We examined whether Rev7 and Mad2 in other organisms, such as budding and fission yeasts, bind to an identical sequence in the same organisms.

Isoz I, Persson U, Volkov K, Johansson E.
Dept of medical biochemistry and biophysics Umeå University, Sweden

The C-terminus of Dpb2 is required for interaction with Pol2 and for cell viability.

DNA polymerase ε (Pol ε) participates in the synthesis of the leading strand during DNA replication in *Saccharomyces cerevisiae*. Pol ε comprises four subunits: the catalytic subunit, Pol2, and three accessory subunits, Dpb2, Dpb3 and Dpb4. DPB2 is an essential gene with unclear function. A genetic screen was performed in *S. cerevisiae* to isolate lethal mutations in DPB2. The dpb2-200 allele carried two mutations within the last 13 codons of the open reading frame, one of which resulted in a six amino acid truncation. This truncated Dpb2 subunit was co-expressed with Pol2, Dpb3 and Dpb4 in *S. cerevisiae*, but this Dpb2 variant did not co-purify with the other Pol ε subunits. This resulted in the purification of a Pol2/Dpb3/Dpb4 complex that possessed high specific activity and high processivity and holoenzyme assays with PCNA, RFC and RPA on a single-primed circular template did not reveal any defects in replication efficiency. In conclusion, the lack of Dpb2 did not appear to have a negative effect on Pol ε activity. Thus, the C-terminal motif of Dpb2 that we have identified may instead be required for Dpb2 to fulfill an essential structural role at the replication origin or at the replication fork.

P.Pestryakov, L.P. Ovchinnikov and O.I. Lavrik
Institute of Chemical Biology and Fundamental Medicine, SB RAS, Russia
Institute of Protein Research, RAS, Pushchino, Russia

Interaction of multifunctional protein YB-1 with AP-sites and its influence on AP endonuclease 1, tyrosyl-DNA phosphodiesterase 1 and DNA glycosylase NEIL1

Apurinic/apyrimidinic sites (AP-sites) which represent one of the most abundantly generated DNA lesions in the cell are generally repaired by base excision repair (BER) system. The main pathway of BER involves the activity of AP endonuclease 1 (APE1), whereas backup pathways can utilize the enzymatic activities of bifunctional DNA-glycosylases/AP-lyases, e.g. NEIL1, and tyrosyl-DNA phosphodiesterase 1 (Tdp1). Multifunctional protein YB-1 is one of the eukaryal proteins having high affinity towards damaged DNA; moreover, it interacts with several components of DNA repair pathways and is transcriptionally activated in response to genotoxic stress. Here we show that YB-1 directly interacts with AP-sites in DNA and has higher affinity toward DNA containing AP-site and additional oxidatively-generated or bulky lesion in opposite DNA strand. It was found that YB-1 affects the in vitro activities of AP-site cleaving enzymes of both major and backup pathways of BER, namely APE1, NEIL1 and Tdp1 during the repair of AP-sites present in DNA alone or in combination with the lesion of another type. We show that, presence of roughly stoichiometric amounts of YB-1 stimulates APE1 by affecting Km (i.e. enzyme affinity to the substrate), but not Vmax (i.e. catalytic stage) of the enzymatic reaction, whereas excess of YB-1 inhibits AP-site cleavage. YB-1 stimulates AP-lyase activity of NEIL1 on DNA, containing AP-site alone or in context of clustered lesions, and inhibits NEIL1-mediated cleavage of the opposite DNA strand, containing 5-formyluridine. It was also found, that both YB-1 and replication/repair-associated protein

RPA effectively inhibit APE1, NEIL1 and Tdp1 on substrates, when AP-site was located in single-stranded DNA. Such behavior is presumably a consequence of YB-1 and RPA competition with enzymes for the DNA substrate. Taken together our data may indicate that YB-1 can modulate the repair of AP-sites in DNA both by positively stimulating APE1 during the major BER pathway, stimulating AP-lyase and inhibiting N-glycosylase activities of NEIL1 during backup pathway of BER and avoiding the possible generation of double-strand breaks, arising from the cleavage of single-stranded portion of DNA substrate already used by different DNA-processing pathway. This work was supported by Russian Academy of Sciences (Molecular and Cellular biology program), grants from RFBR (12-04-33162, 12-04-00178, 11-04-00559).

Helmut Pospiech, Markku Vaara Anna K. Rytkönen, Harri Itkonen, Tomi Hillukkala, Zhe Liu, Daniel Schaarschmidt, Tamar Nethanel, Gabriel Kaufmann, Raija Sormunen, Esa Läärä, Amal Rahmeh, Marietta Y. W. T. Lee, Heinz-Peter Nasheuer, Juhani E. Syväoja

Department of Biochemistry University of Oulu, Finland

Leibniz Institute for Age Research - Fritz Lipmann Institute, Germany

Distinctive activities of human replicative DNA polymerases during S phase – DNA polymerase epsilon, but not DNA polymerases alpha/delta are associated with lamins throughout S phase.

DNA polymerases (Pols) α, δ, and ε replicate the bulk of chromosomal DNA in eukaryotic cells, Pol ε being the main leading strand and Pol δ the lagging strand DNA polymerase. To get more insight into the contributions of human replicative Pols to DNA synthesis during S-phase, they were studied by multiple techniques. Pol δ was three to four times more intensely UV cross-linked to nascent DNA in late compared with

early S phase, whereas the cross-linking of Pols α and ε remained constant. Moreover, Pol δ neutralizing antibodies inhibited replicative DNA synthesis most efficiently in late S-phase nuclei, whereas antibodies against Pol ε were most potent in early S phase. By applying chromatin immunoprecipitation (ChIP), we found that at G(1)/S arrest, all three DNA polymerases were enriched with DNA containing the early firing lamin B2 origin of replication and, 2 h after release from the block, with DNA containing the origin at the upstream promoter region of the MCM4 gene. Pol α, δ, and ε were released from these origins upon firing. Reciprocal ChIP of the three DNA polymerases revealed that at G1/S arrest and early in S phase, Pol α, δ, and ε were associated with the same nucleoprotein complexes, whereas in late S phase Pol ε and Pol α/δ were largely associated with distinct complexes. At G1/S arrest, the replicative DNA polymerases were associated with lamins, but in late S phase only Pol ε, not Pol α/δ, remained associated with lamins. Consistently, Pol ε, but not Pol δ, was found in nuclear matrix fraction throughout the cell cycle. Ultrastructural localization of the pols by immuno-electron microscopy revealed Pol ε to localize predominantly to ring-shaped clusters at electron-dense regions of the nucleus, whereas Pol δ was mainly dispersed on fibrous structures. Taken together, although all three replicative Pols were associated with representive early and late origins of replication, Pol ε and Pol α/δ seem to pursue their functions at least in part independently in late S phase, either by physical uncoupling of lagging strand maturation from the fork progression, or by recruitment of Pol δ, but not Pol ε, to post-replicative processes such as translesion synthesis or post-replicative repair.

Aleksandr A. Rubel[1,2], Tatiana A. Ryzhova[1], Kiril S. Antonetc[1], Alexey P. Galkin[1,2]

[1]Department of Genetics St. Petersburg State University, Russia

[2]St. Petersburg Branch of N.I. Vavilov Institute of General Genetics of the RAS, Russia

Analysis of interaction of mammalian amyloid in yeast based system.

Alzheimer disease is associated with accumulation amyloid beta peptide (Aβ) which in soluble oligomeric isoform leads to synaptic dysfunction and neuronal death. Recently, it was shown that cell surface Prion Protein (PrP^C) acts as a receptor for Aβ oligomers. The two short regions of PrP^C containing residues 23-27 and 95-110 are important for effective binding of PrP^C to Aβ oligomers and probably to other β-sheet conformers. PrP^{Sc} oligomers, but not PrP^C, also efficiently bind Aβ monomers and accelerate their aggregation. To identify the PrP sequences that are essential for interaction between PrP polymers and Aβ peptide we have used yeast based system. We have shown that PrP(28-89) and PrP(90-109) fragments are critical for binding of proteinase-resistant PrP polymers with Aβ peptide.

This work is supported by The Ministry of Eucation and Sience of Russian Federation; The Russian Federation President Grant for Young PhDs (MK-1152.2013.4); Research grant from St. Petersburg State University.

Valentina V. Ignatova[1], Aleksandr A. Rubel[1,2], Alsu F. Saifitdinova[1]
[1]*Saint Petersburg State University, Russia,*
[2]*St-Petersburg Branch of Vavilov Institute of General Genetics, Russia*

Changes in transcribed non-coding regions of the genome as a precondition for evolution.

Recent studies have shown that the non-coding sequences specify vast and hidden layer of regulatory information including determination of species specificity. Changes of the promoter transcribed region structures have an impact on the evolution of living organisms. Untranslated regions of mRNAs are involved in many regulatory pathways that control mRNA localization, stability and translation efficiency. We have found modifications for yeast promoters, which apart from affection transcription properties can increase efficiency of translation of mRNA.

This work is supported by The Ministry of Eucation and Sience of Russian Federation; The Russian Federation President Grant for Young PhDs (MK-1152.2013.4); Research grant from St. Petersburg State University.

Sushma Sharma and Andrei Chabes
Medical Biochemistry and Biophysics, Umeå University, Sweden

Regulation of dNTP metabolism in SOD1 mutants

Regulation of dNTP metabolism in SOD1 mutants The Sod1 protein is a cytosolic copper-zinc superoxide dismutase in Saccharomyces cerevisiae, which catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide and is important in antioxidant defense. It has previously been reported that the loss of Sod1 in yeast leads to oxygen-dependent

sensitivity to DNA-damaging agents and that the induction of the ribonucleotide reductase (RNR) proteins following DNA damage is reduced in the sod1Δ. In this study we investigate whether dNTP pools are affected in sod1 mutants both before and after DNA damage, and how changes in dNTP pools contribute to genome instability in sod1 mutants. Preliminary results will be reported.

A.A.Shiriaeva[1], E.I.Stepchenkova[1, 2], S.G. Inge-Vechtomov[1, 2]
[1]*Saint Petersburg State University, Russia,*
[2]*St-Petersburg Branch of Vavilov Institute of General Genetics, Russia*

Impact of homologous recombination into genome stability estimated by the "α-test" in yeast *Saccharomyces cerevisiae.*

Double-strand breaks are important source of genome instability. They can be induced by ionizing radiation or replication fork collapse. Double-strand breaks may be generated naturally in initial steps of programmed processes, such as mating-type switching in yeast *Saccharomyces cerevisiae* or V(D)J recombination in immune system. Homologous recombination plays an essential role in double-strand break repair. This process is mutagenic, it can lead to loss of heterozygosity, genetic rearrangements and multiple mutations in regions surrounding the break. Rad52 is one of the key players during homologous recombination. In this study we examined effects of the RAD52 gene knockout on genome stability using the α-test. The α-test is based on genetic system, controlling mating type in yeast. S. cerevisiae haploid cells may have a or α mating type. Hybridization is possible only between two haploid cells which have opposite mating types a and α. Mating type is determined by two alleles of the MAT locus: MATa and MATα. This locus is located on the right arm of chromosome III. Chromosome III also contains HMRa and HMRα cassettes

with silent genetic information for the a and α mating type. Different genetic events, such as loss of chromosome III, loss of the chromosome arm, recombination between the MAT locus and the cassette HMRa, conversion from HMRa to the MAT, point mutations and temporary changes (DNA lesions) in the MATα locus, may lead to mating type switching α → a. It is possible to register those events in selective conditions. For that two strains of the same mating time should be plated on media for hybrid selection. If expression of the MATα locus is disturbed by mutation or damage, the cell changes its mating type α → a and can mate with α-type cell. It looks like illegitimate hybridization α x α. The frequency of illegitimate hybridization increases after exposure to DNA-damaging agents. If both arms of the chromosome III are marked, we can analyze the phenotypes of illegitimate hybrids and estimate frequencies of chromosome III, loss of the chromosome arm, recombination between the MAT locus and the cassette HMRa, conversion from HMRa to the MAT, point mutations and temporary changes. In this research we revealed that substantial part of spontaneous damages and lesions induced by UV or camptothecin is repaired by homologous recombination. This process is error-prone and accompanied by chromosome rearrangements, loss of entire chromosome or its arm.

Shulenina L.V., Raeva N.F., Mikhailov V.F.
Burnazian Federal Medical Center of the Federal Medical Biological Agency, Russia

The content of miRNA and the development of breast tumors in irradiated rats.

The "non-targeted effects" of ionizing radiation including bystander effects and genomic instability are unique in that no classic mutagenic event occurs in the cell The cells respond to stress induced by ionizing radiation exposure through complex processes by activating many pathways ranging from DNA damage processing, signal transduction, altered gene expression, cell-cycle arrest, and genomic instability to cell death . The current data suggests that the exposure to radiation provokes cellular responses controlled in part by gene expression networks. miRNAs regulate gene expression and have been shown to control multiple intracellular processes involved in the response to cellular stress. In our study, by using of ɣ-radiation-induced rat breast tumor model, we explored the possibility that microRNA may be involved in mechanisms of tumorogenesis. Here we investigated a families of miRNAs regulating tumor suppressor/oncogene network, e.a.miR34, miR21 and let-7. The qPCR RT technologies has been used as a powerful tool to recognize miRNA in normal and tumor samples. The content of mature microRNAs in bone marrow and breast tumor cells were measured 75 days, 315 and 550 days after 2,5 Gy-acute irradiation of animals. It was shown that the content of miR34 in the bone marrow cells is reliably reduced in the period before the formation of tumors (75 days after irradiation). Is revealed the group of animals with high level of miR21 in these cells. These changes may be markers of effectivity control of genome stability. Probably, the consequence of radiation- induced instability of genome is formation of tumors in distant period after the irradiation (200 - 550 days) with high miR21 and let 7 levels.

Hollie M. Siebler, Artem Lada, Andrey Baranovskiy, Yinbo Zhang, Tahir Tahirov, Youri Pavlov

Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, USA

Critical Role of C-Terminal Domain of DNA Polymerase Zeta (ζ) in Induced Mutagenesis.

B-family Pol ζ is responsible for 50% of spontaneous and nearly all induced mutations. It participates in error-prone lesion bypass. It is renowned for its ability to extend a mismatched primer terminus after insertion of the initial base across from a lesion, fixating the mutation into the newly synthesized DNA strand. Deletion of Rev3, encoding the catalytic subunit of Pol ζ, results in embryonic lethality in mice (Rev3-/-) and ablation of induced mutagenesis in yeast (rev3). Knocking down REV3 can decrease development of cancer cell resistance to cisplatin. Understanding recruitment of Pol ζ and its binding partners is important for understanding its role in genomic instability and cancer through mutagenesis. Much remains unknown about Pol ζ. Because of its inaccuracy, access to undamaged DNA is precisely regulated. We study the regulation and recruitment of Pol ζ to sites of damaged DNA. We and others found that the C-terminus of REV3, specifically the Fe-S cluster, is critical for UV mutagenesis in yeast. Disrupting this site leads to a complete defect of UV mutagenesis. The presence of this Fe-S cluster opens new possibilities for regulation of polymerase switches at the fork. Pol ζ has been found as a four-subunit complex, bound to two subunits of Pol δ (POL31/32). We proposed that the polymerase switch was regulated by an exchange of POL31/32 between Pols δ and ζ (JBC, 2012, 287:17281). Intriguingly, deletion of the entire C-terminus (Rev3ΔC) has a less severe effect than disruption of the Fe-S cluster (Rev3Fe-S). At low doses, Rev3ΔC behaves like wild type while at higher doses induced mutagenesis drops to 24% of

normal levels. We have shown that this residual mutagenesis is dependent on the catalytic activity of REV3. Rev3ΔC is expressed at similar levels to wild type. UV mutagenesis in Rev3ΔC requires REV1, Ub-PCNA, and POL32. We propose that wild type Pol ζ interacts with PCNA via POL31/32 and REV1, Rev3ΔC interacts only via REV1, and Rev3Fe-S is incapable of either interaction. Therefore, at low doses interaction through REV1 allows for full function of Pol ζ but at higher doses it needs POL31/32. Another possibility is that mutating the Fe-S cluster causes a conformational change in the catalytic site, rendering it inactive. Rev3ΔC prevents important interactions, but the catalytic site is unaffected.

N.A. Skryabin[1], I.N. Lebedev[1], N.V. Cherdyntseva[2]
[1]*Institute of Medical Genetics, Tomsk, Russia*
[2]*Institute of Oncology, Tomsk, Russia.*

Dynamics of epigenetic and chromosomal instability in breast cancer

The questions regarding the role of genetic, chromosomal and epigenetic abnormalities in the induction of genomic instability in malignant tumors as well as in the discovering of cancer hallmarks are in the focus of attention. Our study was aimed to examine the dynamics of epigenetic and chromosomal aberrations in the samples of breast cancer with different degrees of malignancy. Biological material for the study was obtained from 28 women with breast cancer, including tissues with benign proliferative processes (n = 7), malignant tumors (n = 40) and regional lymph nodes with metastases (n = 6). DNA methylation profiling was done using a GoldenGate Cancer Panel I microarray (Illumina), which covered 1505 CpG-sites within 807 genes. Molecular cytogenetic study was performed by high-resolution conventional comparative genomic hybridization (HR-CGH). Cluster analysis of DNA methylation microarray

data have revealed a group of samples with a high affinity for the methylation profile in which the presence of samples with benign proliferative processes and metastases was significantly higher than in the rest groups of samples.

Elena I. Stepchenkova[1, 2], Youri I. Pavlov[3]
[1]St. Petersburg Branch of Institute of General Genetics, RAS, Russia
[2]St. Petersburg State University, Russia
[3]University of Nebraska Medical Center, USA

Role of purine nucleotide biosynthesis pathway in control of mutagenesis level in yeast *Saccharomyces cerevisiae*.

Rate of spontaneous mutagenesis is controlled on several levels: sanitization of the cellular pools of nucleic acid precursors, DNA replication and postreplicative repair. All spontaneous mutations arising during replication or repair are made by DNA polymerases whose fidelity is highly dependent on quality and availability of DNA precursors – deoxyribonucleoside triphosphates of adenine, guanine, cytosine and thymine. Contamination or disturbance of nucleotide pool may be mutagenic and cause genome instability. One possible mechanism of mutation induction is incorporation of noncanonical nucleotides into DNA during replicative or repair DNA synthesis when nucleotide pool is contaminated. Our data suggest that another mechanism may take place: if dNTP pool is disturbed low fidelity Pols may be attracted into replication fork more often. We have shown that in yeast strains caring ade mutations rate of spontaneous mutagenesis is several times higher than in wild type strain. It may be seen only in media with low concentration of adenine when cells are starving for this compound. Our data show that increase of spontaneous mutagenesis in ade mutants is caused by Polζ activity.

Mutation ade12 stands out the group of the ade mutations. The ade12 mutants demonstrate even higher level of Polζ-dependent mutagenesis and have multiple pleotropic effects. The ADE12 gene controls first step of AMP biosynthesis from IMP. Mutants ade12 accumulate IMP, potentially mutagenic compound. It is possible that in ade12 mutants both mechanisms are realized: incorporation of noncanonical nucleotides and attraction of low fidelity polymerase for replication. This idea is supported by our data about extreme sensitivity of the ade12 mutant to mutagenic base analog HAP (6-hydroxylaminopurine). The balances between those two mechanisms and their details have to be studied yet. Genetic system of purine biosynthesis de novo and base analog HAP are very useful tools for research in this area.

Oksana Kidgotko, Maria Kustova, Vassilina Sokolova, Mikhail Bass and Vadim B. Vasilyev

Department of Molecular Genetics, Institute of Experimental Medicine, Russia

Modeling mitochondrial diseases: evidence of mtDNA paternal inheritance in several sequential generations.

Inheritance of mutant mitochondrial DNA (mtDNA) and its distribution among tissues and organs during prenatal development is very important for understanding the mechanisms of pathologies that develop secondary to the disruption of energy metabolism and are often termed as 'OXPHOS' (oxidative phosphorylation) diseases. The list of such maladies contains some 120-200 nosologies, this inaccuracy being partly justified by noticeable variety of syndromes, which results from the diversity of alterations in mtDNA. To clarify the genetic background and pathogenesis of OXPHOS diseases one needs understanding the regularities of mutant mtDNA inheritance and distribution among tissues/organs. Modeling

OXPHOS diseases in animals might be of great help. In previous studies we obtained laboratory mice developing from zygotes into which human mitochondria had been injected. It was shown that human mtDNA is transmitted by transmitochondrial (TM) mice along the maternal lineage down to the third and even the fourth generation. Human mtDNA in amounts used never caused alterations of energy metabolism, but was a reliable marker in the studies where distribution of mitochondrial genome among organs was traced. Using previously obtained TM mice we studied the possibility of transmission of foreign (human) mtDNA by male mice to their progeny. A number of lineages founded by male descendants of a TM female were analyzed and paternal mtDNA inheritance was registered more than once, especially in a lineage where human mtDNA was transmitted by males at least in four sequential generations. The pattern of paternally transmitted foreign mtDNA distribution among mouse organs partially coincided with that evidenced in our experiments with maternal inheritance of mtDNA, though some differences were observed. Our results confirm the data of other authors indicating the possibility of mtDNA paternal inheritance. However, to our knowledge, this is the first case demonstrating mtDNA transmission along the paternal lineage for more than two generations. Persistence of paternal mtDNA in several sequential generations of experimental animals allows suggesting that purifying mechanisms aimed at elimination of paternally inherited mtDNA species are not as strict as has been postulated. Our experimental data may be useful for better understanding 'mitochondrial genetics' and especially the peculiarities of inheritance of OXPHOS diseases, which is of high importance for correct genetic counseling.

Anna E. Zawada, Krzysztof Flis, Piotr Jonczyk, Iwona J Fijalkowska
Laboratory of Mutagenesis and DNA Repair, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Poland

Influence of two human POLE2 counterparts on different DPB2 yeast phenotypes.

The multi-subunit DNA polymerase epsilon holoenzyme of *Saccharomyces cerevisiae* consists of four subunits: Pol2p, Dpb2p, Dpb3p and Dpd4p. Mutants in dpb2 allele previously isolated in our laboratory are mutators and show temperature-sensitive phenotype as well as altered protein-protein interactions between Pol2p and mutated forms of Dpb2p. Interestingly, alterations in POLE2 (human ortholog of DPB2 gene) were identified in 5 out of the 16 cases of human colorectal cancer and in 2 out of 24 cases of human breast cancer. DNA replication machinery is structurally and functionally conserved. This provides an opportunity to evaluate the consequences of DNA polymerase epsilon variants found in human cells, such as the causative relationship between them and cancer in humans, by studying analogous mutations in yeast. Moreover, yeast is a genetically tractable model to study the effects of those variants on viability, growth and mutagenesis. Having this in mind, we are planning to introduce into yeast cells two synthetic counterparts of wild type and mutated version of POLE2 gene (optDpE2 and optDpE2*) which encode p59 – the human ortholog of the yeast Dpb2p protein. We have changed their native DNA sequence taking yeast codon usage into consideration. Consequently, both constructs should be translated in yeast cells more efficiently than native POLE2 DNA sequence. Amino acid sequence of optDpE2 is intact, being identical to human protein, while optDpE2* has one amino acid change that corresponds to one of the reported mutations identified in 2 out of 16 cases of human colorectal cancer. We have fused POLE2 gene to LacZ gene in order to check protein expression from chosen vectors and obtained a

positive result. We have also observed a strong protein-protein interaction between human p59 and C-end of yeast Pol2p in the yeast two-hybrid system. Currently, we have been conducting the following experiments:
- complementation of the lethal phenotype of a non-functional yeast DPB2 gene with POLE2;
- suppression of temperature-sensitive phenotype of dpb2 mutant alleles integrated into chromosome;
- suppression of mutator phenotypes of dpb2 mutant alleles by POLE2 allele.

L.B.Zelena, S.I.Voychuk, E.N.Gromozova
Zabolotny Institute of Microbiology and Virology, National Academy of Sciences, Ukraine

Saccharomyces cerevisiae genome instability under different stress factors.

Organisms, yeast in particular, are affected by the variety of physical, chemical and biological stresses. They adapt to live in such environmental conditions by establishing responses to maintain cell survival and growth. The consequences of these events are associated with changes in cellular physiological processes, activation defense mechanisms, changes in gene expression and others. In our research genome variability occurring in *Saccharomyces cerevisiae* under influence of different stress factors was evaluated using PCR with primers to short nucleotide repeats. The level of genome polymorphism observed in yeast cells after treatments with 0.3 M H2O2, 1 M sorbitol and electromagnetic irradiation (40.68MHz, 30 Wt, 60 min) was analyzed and the values were compared between three treatments. Amplification with primer to tetranucleotide repeat did not show any differences in amplicon patterns obtained with yeast DNA after treatments used in the study, although there were about 700-bp and 1300-bp PCR-

fragments that distinguished control and treated samples. As a result of PCR with primer to pentanucleotide repeat amplicon sets specific for every treatment were obtained. The level of polymorphic bands was the highest in yeast samples that were incubated with H2O2, the lowest – exposed to electromagnetic irradiation. Thus, changes in repeated sequences of yeast genome revealed in the present study can indicate DNA rearrangements occurring under different type of stresses.

Petrova A.V., Zhouravleva G.A.
Saint Petersburg State University, Russia

Chromosomes instability in the yeast strains mutant for the essential gene SUP45

In eukaryotes, two protein factors, eRF1 and eRF3, are essential for the translation termination. Factor eRF1 recognizes all three stop codons and releases a newly synthesized peptide. Factor eRF3 stimulates termination activity of eRF1 in the GTP-dependent manner. In yeast *S. cerevisiae*, eRF1 and eRF3 are encoded by essential genes, SUP45 and SUP35, respectively. Deletion of the C-terminal domains of eRF1 or eRF3 leads to cell death. Despite this, we have previously isolated the non-lethal nonsense mutations in SUP45 and SUP35 genes, which reduced the amount of eRF1 or eRF3. In this work, using strains of different origin, we show that sup45 mutations that reduce the efficiency of translation termination result in spontaneous loss of heterozygosity in diploid strains. Using polymerase chain reaction we have shown that diploids bearing sup45 mutations contain only one copy of MAT locus. Direct analysis of chromosomes by pulsed-field gel electrophoresis (CHEF) demonstrated rearrangement of chromosomes II and III in most of diploid strains with sup45 mutations in different genetic background. The chromosome

rearrangements were further investigated by “alfa-test” which utilizes illegitimate hybridization to detect different type of events leading to mating type switch. Using this approach we demonstrated that the frequency of spontaneous illegitimate hybridization in the presence of sup45-102 mutation increases in 4-8 times compared with WT strain accompanied by 25% increase in the chromosome III loss. These results propose that decrease in the fidelity of the translation termination leads to different cellular defects.

Anna Zhuk, Elena Stepchenkova, Sergey Inge-Vechtomov
Saint Petersburg State University, Russia,
Saint Petersburg Branch of Vavilov Institute of General Genetics, Russia

Studying the initial stages of mutagenesis: phenotypic expression of primary DNA lesions and their further processing using the alpha-test

Primary lesions are structural changes in genomic DNA caused by various endogenous and exogenous factors. The major forms of DNA lesions include: base modifications, ultraviolet-induced photoproducts and thymidine dimers, single- and double-strand breaks, abasic sites, oxidative damage, DNA-protein cross-links and others. Primary lesions of genetic material are the underlying cause of gene and genome mutations that lead to changes of phenotypic features and increase the risk of cancer, inherited diseases and diseases related to aging. The genetic consequences of DNA damage such as stable inheritable genetic changes (gene and genomic mutations) are well established in carcinogenesis. However, little is known about efficiency of primarily DNA lesion expression, length of their life in cell or ratio between correct and error-prone processing of primary lesions. A small number of genetic studies show that the primary DNA lesions may be expressed phenotypically as nonheritable changes. The question we are

trying to answer in our research is: could primary DNA lesions affect the phenotype of cells before they are repaired? To examine this possibility, we identify the types of primary DNA lesions that are able to alter the cellular phenotype and their further processing during repair in Saccharomyces cerevisiae, using a method called the alpha-test. The alpha-test utilizes genetic system controlling mating types in heterothallic yeast *Saccharomyces cerevisiae*. In the alpha-test primary lesions induce disturbance of MATα locus expression, leading to temporally or permanent mating type switch α →a in yeast cells. The frequency of mating type switching reflects phenotypic expression of primary DNA lesions. Thus, the alpha-test allows to score the primary lesions that are processed into stable inheritable genetic changes through inaccurate repair and distinguish them from phenotypic expression of primary lesions that were correctly eliminated by repair. To identify the nature of primary lesions that are able to affect phenotype of cells we determined the spectra of inherited and temporary changes of genetic material in the alpha-test induced by different mutagens, which cause specific DNA damage in combination with defects of repair systems. Our results indicate that primary DNA lesions such as single- and double-strand breaks, base modifications, and unpaired nucleotides are able to change the cellular phenotype of yeast cells (as temporary switching of the mating type α → a) before they are either fixed as heritable genetic changes or eliminated by repair.

Andrei Zinovyev, Inna Kuperstein, Emmanuel Barillot, and Wolf-Dietrich Heyer

Institut Curie (France), INSERM U900 "Cancer Genome" Department, France

Synthetic lethality within Homologous Recombination DNA repair pathway.

Synthetic-lethality describes the observation that the combination of two viable mutants results in cell death as a double mutant. Systematic analysis of synthetic lethality constitutes a critical tool for systems biology to decipher molecular pathways. The most accepted mechanistic explanation of synthetic lethality is that the two genes function in two parallel, mutually compensatory pathways, known as between-pathway synthetic lethality. However, recent genome-wide analyses in yeast identified a significant number of negative within-pathway genetic interactions. The molecular mechanisms leading to within-pathway synthetic lethality are not fully understood, but include accumulation of partial defects leading to degradation of an essential pathway, internal redundancy within an essential pathway, or negative interactions within a complex with an essential function. Here, we propose a novel mechanism leading to within-pathway synthetic lethality involving two genes functioning in a single non-essential pathway. This type of synthetic lethality termed within-reversible-pathway synthetic lethality involves reversible pathway steps, catalyzed by different enzymes in the forward and backward directions, and kinetic trapping of a potentially toxic intermediate. Within-reversible-pathway synthetic lethality has been documented in several combinations of gene defects in recombinational DNA repair providing experimental validation of the concept. Mathematical modeling recapitulates the possibility of kinetic trapping and revealed the potential contributions of synthetic, dosage-lethal interactions in such a

genetic system as well as the possibility of within-pathway positive masking interactions. Analysis of yeast gene interaction and pathway data suggests broad applicability of this novel concept. The observation that defects in two genes which function in a single non-essential pathway can lead to synthetic lethality significantly extends the canonical interpretation of synthetic-lethal or synthetic-sick interactions with direct implications to reconstruct molecular pathways and improve cancer therapy.

Медицинская

Биотехнологическая компания

«Медико-генетический центр «Жизнь»

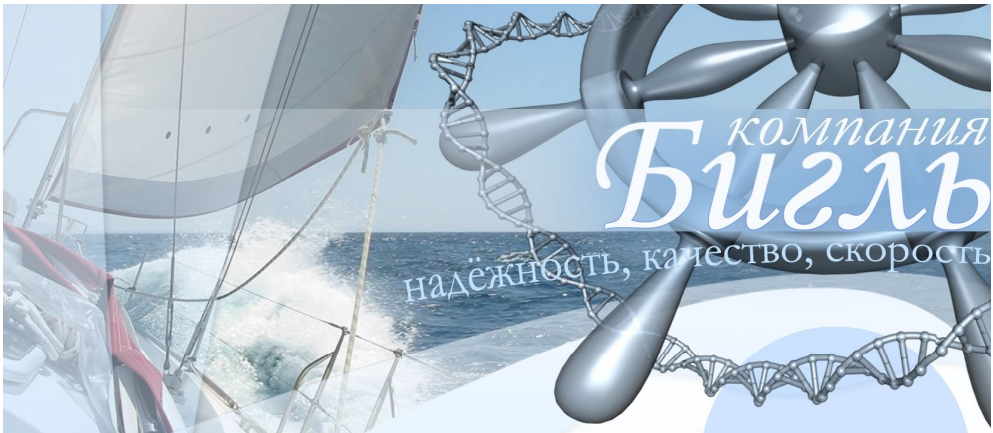
- Разработка молекулярно-генетических тест-систем,
- Молекулярно-генетические исследования «под заказ»,
- Подбор праймеров под любые гены «заказчика»,
- Молекулярно-генетические исследования и диагностика (более 150 генов, 250 SNP),
- Полногеномное секвенирование NGS,
- Научные исследования (генетический паспорт, гены «предрасположенности», спортивная генетика, генетика старения).

Общество с ограниченной ответственностью "БиоГлот"

197341, г. Санкт-Петербург, Коломяжский пр. д.28, к.2, пом. 31-Н, Лит А

тел./факс (812) 6432858, моб (812) 9476538, +7(921)7567809

www.lifemedical.ru, www.sportgenetic.ru



компания
Бигль
надёжность, качество, скорость

синтез олигонуклеотидов

олигонуклеотиды
зонды для real-time ПЦР
секвенирование

наши цены

16 руб/шаг

- гарантированное количество – не менее 2 OE
- срок выполнения заказа - до 5 дней
- бесплатная доставка по России!

дополнительные услуги

- синтез длинных олигонуклеотидов (>50 оснований) – 18 руб/шаг
- дизайн праймеров для Ваших целей
- проверка качества работы синтезированных олигонуклеотидов на Вашей матрице
- оптимизация условий их работы
- синтез генов

контакты

http://www.biobeagle.com

order@biobeagle.com

тел./факс. +7 (812) 366 38 19

мы можем синтезировать больше трёх букв!

Отпечатано копировально-множительным участком отдела обслуживания учебного процесса физического факультета СПбГУ.
Приказ № 571/1 от 14.05.03.
Подписано в печать 06.06.13 с оригинал-макета заказчика.
Ф-т 30х42/4, Усл. печ. л. 4. Тираж 100 экз., Заказ №1706.
198504, СПб, Ст. Петергоф, ул. Ульяновская, д. 3, тел. 929-43-00.