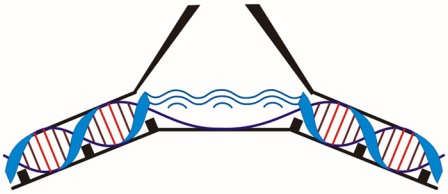

Russian - Swiss
Workshop

Regulation of genome stability by DNA replication and repair

Saint - Petersburg

7th - 9th July 2010

Russian Academy of Sciences
Institute of Chemical Biology & Fundamental Medicine, Novosibirsk
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Russian-Swiss Workshop on

Regulation of genome stability by DNA replication and repair

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PROGRAMME AND SCHEDULE

Day 1: Wednesday 7th July

16:00-17:00 **Registration of participants**

17:00-17:30 **Opening ceremony**

Professor Ulrich Hubscher
RAS Academician Sergey G. Inge-Vechtomov
RAS Academician Dmitry G. Knorre
RAS Corresponding Member Olga Lavrik

1. Decision points in DNA damage response

Chairpersons: U. Hubscher, Y. Pavlov

17:30-18:00	Philip Hanawalt	Lesion sensing and decision points in the DNA damage response
18:00-18:30	Sergey Inge-Vechtomov	Primary lesions of genetic material and their processing: yeast experience
18:30-19:00	Boris Zhivotovsky	DNA damage-mediated cell death

19:00 **Welcome reception**

Day 2: Thursday 8th July

2. DNA damage and its consequences

Chairpersons: G. Dianov, O. Lavrik

10:00-10:30	Ulrich Hübscher	Oxygen as a friend and enemy: how to combat the mutational potential of 8-oxo-guanine
10:30-11:00	Youri Pavlov	Myths and mysteries of the DNA replication
11:00-11:30	Polina Shcherbakova	Mutator variants of replicative DNA polymerases, genome instability and cancer

Coffee break

12:00-12:30	Massimo Lopes	Structural insights into genome instability associated to DNA replication stress
12:30-13:00	Leonid Gening	Activity of DNA polymerase iota in normal and malignant human cells
13:00-13:30	Ekaterina Belousova	Translesion synthesis across oxidative DNA lesions catalyzed by DNA polymerases β and λ

Lunch

3. DNA repair mechanisms and their regulation

Chairpersons: H. Naegeli, B. Zhivotovsky

15:00-15:30	Grigory Dianov	Molecular mechanisms balancing the levels of DNA repair enzymes to the cellular environment
15:30-16:00	Dmitry Zharkov	Diffusional movement of DNA repair proteins along DNA

16:00-16:30	Svetlana Khodyreva	Poly(ADP-ribose) polymerase 1 is a key regulator of damage processing in base excision repair

Coffee break

16:50-17:20	Alex Sartori	CtIP controls EXO1-mediated DNA end resection in human cells
17:20-17:50	Inna Lavrik	Towards understanding life/death decisions in death receptor-induced apoptosis
17:50-18:10	Ekaterina Ilina	Ku antigen interacts with DNA containing abasic sites

18:10-19:30 **Poster session**

Day 3: Friday 9th July

4. Nucleotide excision DNA repair

Chairmen: P. Hanawalt, D. Zharkov

10:00-10:30	Hanspeter Naegeli	Bipartite DNA damage recognition in nucleotide excision repair
10:30-11:00	Jean-Marc Egly	Nucleotide Excision Repair factors are involved in transcription in absence of genotoxic attack
11:00-11:30	Nadejda Rechkunova Olga Lavrik	Cooperative interaction of NER factors on damage DNA

Coffee break

5. Chromatin modifications during DNA repair and cell signaling

Chairmen: J. Lingner, M. Stucki

12:00-12:30	Vincent Dion	Dynamics of double-strand breaks: implications for repair
12:30-13:00	Vladimir Korolev	Chromatin and DNA damage repair

Lunch

14:30-15:00	Manuel Stucki	The role of MDC1 in the mammalian DNA damage response
15:00-15:30	Olga Iarovaia	Mechanism of chromosome rearrangements in treatment related leukemia
15:30-16:00	Joachim Lingner	Regulation of telomerase at chromosome ends by shelterin and terra

16:00-17:00 **General discussion and closing remarks**
(U. Hubscher, O. Lavrik)

17:00-19:00 **Free time**

19:00-23:00 **Banquet**

23:30-02:00 **Trip on the boat**

LECTURES

LESION SENSING AND DECISION POINTS IN THE DNA DAMAGE RESPONSE

Philip C. Hanawalt, Boris P. Belotserkovskii and Graciela Spivak

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Replication and maintenance of the genome are absolute requirements for life. Proliferating cells must duplicate their DNA with astonishing precision in the face of a barrage of endogenous and environmental genotoxic threats. The various modes for processing DNA damage compete with each other to recognize lesions, and each step in a repair pathway may generate an intermediate that is susceptible to intervention by enzymes from other pathways. The result may depend upon which protein encounters the lesion first.

Translocating RNA polymerase (RNAP) sensitively detects damage as it performs one-dimensional scanning of the template DNA during transcription, thus to initiate transcription-coupled repair (TCR). Victims of the genetic diseases, Cockayne syndrome (CS) and UV-sensitive syndrome ($UV^{S}S$), presenting a deficiency in TCR, are characterized by sunlight sensitivity but no cancers. CS patients additionally exhibit developmental and neurological problems as well as segmental progeria. We have postulated that the inefficient processing of oxidative DNA damage may partially account for the CS phenotype. (For recent review see Hanawalt and Spivak, *Nature Reviews Mol. Cell Biology* (2008)

We still do not know the precise signals that can trigger TCR. Some types of damage may occur preferentially in non-canonical DNA and could stabilize those structures. That damage might then become refractory to repair. Futile cycles of TCR in naturally occurring non-canonical DNA structures might contribute to genomic instability. To test these hypotheses we study transcription *in vitro* with purified mammalian RNAP II and T7 RNAP on defined DNA templates containing site-specific lesions and/or non-canonical DNA structures. Z-DNA, H-DNA triplexes and G4-quadruplex-DNA partially arrest transcription. T7 RNAP is also arrested by homopolymer guanine-rich sequences (Gn/Cn) when the cytosine is in the transcribed DNA strand. These sequences produce significant transcription blockage in an orientation-, length- and supercoiling-dependent manner. Based upon the effects of various sequence modifications, solution conditions and substitution of inosine or deazaguanosine for guanosine, we conclude that the blockage is due to formation of unusually stable RNA/DNA hybrids, and that these structures are likely responsible for the transcription-dependent replication blockage observed in G-rich sequences *in vivo* (Belotserkovskii et al., submitted for publication).

PRIMARY LESIONS OF GENETIC MATERIAL AND THEIR PROCESSING: YEAST EXPERIENCE

Inge-Vechtomov S.[1,2], Stepchenkova E.[2], Kochenova O.[1] and Shumega A.[1]

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Each mutation (or recombination) event is preceded by a primary lesion of genetic material. The primary lesions may be processed either into mutation or predominantly may be erased by repair. Unfortunately, the primary lesions usually avoid genetic analyses because of lack of an appropriate experimental model to score their phenotypic expression. Here we present a system, which can fill that gap in the yeast *Saccharomyces cerevisiae*. The system was named the alfa test. It utilizes some features of the cell type determination process controlled by the *MAT* idiomorph. Usually haploid heterothallic strains of *S.cerevisiae* belong either to "a" or to "α" mating type. MAT_a cells efficiently mate MAT_α cells. "Illegitimate α X α mating becomes possible when a MAT_α cell changes its cell type to MAT_a as a result of disturbance of the *MAT* expression. The events responsible for rare "illegitimate" α X α mating (about 1 X 10^{-6} spontaneously) may be studied in selective system. In the system cells with primary lesios have clear phenotype – ability to form hybrids with MAT_α cells. The majority of illegitimate hybrids (more than 50%) selected both spontaneously and induced with several agents preserve their original – α mating type. Only minority of the hybrids do not expressed any mating type as it is typical for regular hybrids heterozygous for MAT_a/MAT_α. We completed illegitimate mating with illegitimate cytoduction experiments, when recipient cell gets only cytoplasm, but no nuclei from its mating partner. In these experiments the majority of the cytoductants also expressed original – α mating type. These cytoductants originated through phenotypic expression of primary lesions as "a" and afterwards were repaired off. The rest of cytoductants distributed among the classes: conventional a-mating type (about 15%), recessive a*(0,5%), nm – non-maters (1,5%), of which the first class arose through cassette transposition - recombination type event, and the two other classes – present real mutations within MAT_α.

Using several exogenous and endogenous mutagens (methylation agents, base analogs, UV radiation and mutations in genes controlling genome stability) we determined ratio of primary lesions and hereditary changes of genetic material caused by these factors. Mutations in genes of some TLS DNA-polymerases unusually demonstrate high rate of illegitimate mating because of elevated frequency of both primary lesions and mutations. We discuss effects of *rev3*, *rev7* and *rad30* mutations. This data along with their theoretical value may have a practical outcome for genetic toxicology, in making the alfa-test more sensitive.

DNA DAMAGE-MEDIATED CELL DEATH

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DNA damage-induced apoptotic pathway includes caspase-2, which is activated within the PIDDosome complex, and causes cytochrome *c* release and caspase activation. Hence, PIDDosome-mediated caspase-2 activation might be an important link between DNA damage and the engagement of the mitochondria-mediated apoptotic pathway. In addition to PIDDosome, caspase-2 is able to use the CD95 DISC as a platform and the recruitment of caspase-8 to this complex is required for activation of both enzymes. Investigation of the contribution of p53 and caspase-2 to apoptosis and mitotic catastrophe (MC) induced by DNA damage in carcinoma cells revealed that both functional p53 and caspase-2 are required for the apoptotic response, which was preceded by translocation of caspase-2 to the cytoplasm. In the absence of functional p53, DNA damage resulted in caspase-2-independent MC followed by necrosis. We found that the final mode of cell death triggered by DNA damage in cancer cells is determined by the profile of proteins involved in the regulation of the cell cycle.

OXYGEN AS A FRIEND AND ENEMY: HOW TO COMBAT THE MUTATIONAL POTENTIAL OF 8-OXO-GUANINE

Barbara van Loon[1#], Enni Markkanen[1#], Elena Ferrari[1#], Jason L. Parsons[2], Grigory Dianov[2] and Ulrich Hübscher[1]

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The maintenance of genetic stability is of crucial importance for any form of life. Oxidative stress causes damage to the highly reactive DNA bases, such as guanine. Due to its prevalence and mutagenic potential 7,8-dihydro-8-oxo-2'deoxyguanine (8-oxo-G) is recognized as one of the most important oxidative DNA lesions (1). Majority of DNA polymerases (pols) bypass 8-oxo-G in an inaccurate manner, by preferentially incorporating ′wrong′ adenine (A) opposite 8-oxo-G, subsequently inducing GC→TA transversion mutations that can give rise to cancer. We were able to show that DNA pol λ in the presence of the auxiliary factors proliferating cell nuclear antigen (PCNA) and replication protein A (RP-A) ensures 1200-fold more efficient incorporation of C opposite 8-oxo-G than A (2,3). Furthermore, we recently provided evidence for the existence of the accurate 8-oxo-G repair pathway, coordinated by MutY glycosylase homologue (MUTYH) and DNA pol λ (4). DNA pol λ is phosphorylated in S phase and thereby protected from degradation via ubiquitination (5). However, nothing is known about the possible mechanism of regulation of DNA pol λ protein levels upon oxidative damage. We will present the identification of E3 ubiquitin ligase involved in the ubiquitination DNA pol λ and its effect on DNA pol λ stability.

1. van Loon, B., Markkanen, E. and Hübscher, U.: Oxygen as a friend and enemy: How to combat the mutational potential of 8-oxo-guanine *DNA repair,* in press, 2010

2. Maga, G., Villani, G., Crespan, E., Wimmer, U., Ferrari, E., Bertocci, B. & Hubscher, U. (2007). 8-oxo-guanine bypass by human DNA polymerases in the presence of auxiliary proteins. *Nature* **447**, 606-8.

3. Maga, G., Crespan, E., Wimmer, U., van Loon, B., Amoroso, A., Mondello, C., Belgiovine, C.,Ferrari,E., Locatelli, G., Villani, G., Hübscher, U.: Replication Protein A and Proliferating Cell Nuclear Antigen coordinate DNA polymerase selection in 8-oxo-G repair *Proc. Natl. Acad. Sci. USA*, **105**, 20689-20694, 2008

4. van Loon, B. & Hubscher, U. (2009). An 8-oxo-guanine repair pathway coordinated by MUTYH glycosylase and DNA polymerase λ. *Proc Natl Acad Sci U S A* **106**, 18201-6.

5. Wimmer, U., Ferrari, E., Hunziker, P. & Hubscher, U. (2008). Control of DNA polymerase lambda stability by phosphorylation and ubiquitination during the cell cycle. *EMBO Rep* **9**, 1027-33.

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MYTHS AND MYSTERIES OF THE DNA REPLICATION

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The asymmetric nature of DNA poses topological problems for replication of the anti-parallel strands by a fork moving in one direction. The replication of the two strands is inherently different. An additional complication is the collision of active transcription apparatus with the replication fork. Recent advances in the genetic, biochemical and structural aspects of DNA replication in eukaryotes greatly improved our understanding of the underlying mechanisms. These novel data include deciphering crystal structures of parts of human and yeast DNA polymerase (Pol) delta, finding an unusual two-Pol tandem in the catalytic subunit of Pol epsilon, finding the iron-sulfur domain in the second primase subunit of Pol alpha, the discovery of new roles of Pol accessory subunits in replication and recombination, and getting new insights into specialized Pols. Based on the data from our laboratory and from the literature, we discuss the latest opinions about the organization and mechanism of the replication fork. Is the lagging strand discontinuous and the leading strand continuous? What are the specific roles of various Pols at the replication fork? Are mutation signatures of Pols sufficient to understand their function? How does the quality of the dNTP pool affect replication? What Pols are required for DNA repair, lesion bypass and genetic recombination? How the accessory Pol subunits contribute to the Pol functions and replication fidelity?

MUTATOR VARIANTS OF REPLICATIVE DNA POLYMERASES, GENOME INSTABILITY AND CANCER

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Accurate DNA synthesis by the replicative DNA polymerases □, □ and □ is critical for maintaining the genome stability and avoiding mutations that can lead to disease. Amino acid changes in the replicative enzymes can reduce their fidelity, resulting in increased incorporation of non-complementary nucleotides and a mutator phenotype. We used a yeast model system to determine if variants of replicative DNA polymerases naturally occurring in human cells can cause increased mutagenesis. We observed that the R696W substitution in the conserved region III of Polδ (analog of the R689W change in the human cancer cell line DLD-1) caused an unprecedented elevation of the spontaneous mutation rate that was incompatible with life in haploid and homozygous diploid yeast. We showed that the catastrophic level of mutagenesis resulted from low-fidelity DNA synthesis by Polδ-R696W. Heterozygotes survive, and the mutation rate depends on the relative expression level of wild-type versus mutant alleles. The similarities between the mutational spectra of the yeast strains producing Polδ-R696W and DLD-1 cells suggest that the altered Polδ could be responsible for a significant proportion of spontaneous mutations in this cancer cell line. The results further suggest that the highly error-prone Polδ-R689W could contribute to cancer initiation and/or progression in humans. We propose a model wherein the accumulation of mutations in oncogenes and tumor suppressor genes in cells heterozygous for the variant Pol δ allele is accelerated by transient changes in gene expression leading to a temporary excess of Polδ-R689W.

STRUCTURAL INSIGHTS INTO GENOME INSTABILITY ASSOCIATED TO DNA REPLICATION STRESS

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DNA replication represents a crucial, but potentially dangerous process that cells have to complete in order to undergo cellular division. Recently, uncharacterized "DNA replication" stress has been linked to DNA damage checkpoint activation upon oncogene expression and in early pre-cancerous lesions, which contributes to prevent full malignant transformation. Remarkably, most of the common DNA damaging cancer chemotherapeutics also interfere with the mechanism of DNA replication, but little structural and genetic information is available on the mechanisms underlying their cytotoxicity.

We aim to gain structural and genetic information about the pathological transitions leading to genome instability under different conditions of DNA replication stress, combining classical cellular and molecular biology assays with single molecule approaches on replication intermediates (DNA fiber analysis, electron microscopy). Our current investigations on oncogene-induced DNA replication stress suggest that replication forks experience elongation problems early after oncogene expression, whereas double strand breaks (DSB) become evident, both physically and through checkpoint activation, only later. Similarly, our observations on a specific class of cancer chemotherapeutics (Topoisomerase I inhibitors) suggest that distinct alterations at replication forks - possibly resulting from accumulation of topological stress - precede DSB, challenging the current model of action of these drugs (replication fork run-off at DNA nicks). These observations have been instrumental to design and perform electron microscopy experiments, by which we are currently addressing which structural abnormalities at DNA replication intermediates precede and possibly determine the final cellular responses to different types of DNA replication stress.

ACTIVITY OF DNA POLYMERASE IOTA IN NORMAL AND MALIGNANT HUMAN CELLS

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An enhanced level of mutagenesis partly caused by disturbed balance of error-prone DNA polymerases activities is one of the key indications of malignant transformation of cells. To estimate the possible role of low-fidelity DNA polymerase iota (Polι) in increasing mutation frequency in mammalian cells, we studied the activity of this enzyme in cell extracts of different mouse organs and of human ocular (melanoma) and eyelid (basal cell carcinoma) tumor tissues. As cofactors in DNA polymerase reactions, we used both Mg^{2+} ions, considered to be the main activators of DNA replication *in vivo*, and Mn^{2+} ions that are the most efficient divalent cations in activating homogenous Polι preparations *in vitro*. In the presence of Mg^{2+} ions, Polι was active only in mouse testis and brain cell extracts, whereas in the presence of Mn^{2+} the activity of Polι was detected in all studied normal mouse organs. In cell extracts of both studied malignant tumors (melanoma and basal cell carcinoma), Polι was active in the presence of both Mg^{2+} and Mn^{2+} ions. In both cases, Mn^{2+} ions were more efficient activators of Polι activity than Mg^{2+}, but to different degrees. In the extracts of basal cell carcinoma in the presence of Mn^{2+} ions, the Polι activity was 2.5-fold higher than in control cells (benign tumors of the same eyelid region). In melanoma cell extracts supplemented with Mg^{2+} or Mn^{2+} ions, the Polι activity was close to that in cell extracts of surrounding normal tissues or in extracts of post-traumatically removed normal eye. In the reactions activated by the majority of cell extracts from normal mice and human tissues, DNA synthesis beyond G incorporated by Polι opposite T template was discontinued. A similar effect called T stop was described for pure preparations of Polι. A distinctive feature of malignant tissue transformation in both melanoma and basal cell cancer was a characteristic change in DNA synthesis when being activated by Mn^{2+} ions it acquires the ability to overcome T stop. A similar feature among cell extracts of different normal mouse organs was only characteristic of testis cell extracts. Our data suggests that the synthesis after T stop was performed by other DNA polymerases of malignant and testis cell extracts rather than by Polι. This similarity between testis and tumor might be explained by a cell division block that occurs in all normal cells but not in testis or malignant cells.

TRANSLESION SYNTHESIS ACROSS OXIDATIVE DNA LESIONS CATALYZED BY DNA POLYMERASES β AND λ

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The main strategy used by pro- and eukaryotic cells for replication of damaged DNA is translesion synthesis (TLS). We investigated TLS activity of the human X-family DNA polymerases β and λ on DNA duplexes containing different lesions resulted from the oxidative stress – abasic site, oxoguanine and thymine glycol. We determined kinetic parameters of dNTP incorporation opposite different DNA damages by DNA polymerases activity in the presence of Mg(II) or Mn(II) ions. Additionally, we investigated the influence of hPCNA and hRPA on TLS-reaction. Further, to discriminate the surrounding proteins that could potentially act during TLS in the cell we applied the photoaffinity labeling approach for modification of Bovine Testis (BT) and HeLa (RC) extract proteins. We found a limited number of modification products among the general pool of proteins. It was confirmed: (i) by Western blotting that the RC 75-80 kDa crosslinking product is the covalent adduct of DNA to pol λ; (ii) by immunoprecipitation with human antibodies that the BT 105 kDa crosslinking product is PARP1.

On the basis of experimental results, DNA polymerases β and λ can be proposed as a "good" candidates for participation in TLS process across AP-site, 8-oxoG and thymine glycole during genome DNA replication on the leading and lagging strands. Moreover, DNA polymerases β and λ can be a components of TLS machine not only during the first stage of the process (i.e. incorporation of dNMP opposite damage) but on the stage of processing of uncomplementary 3'-end of primer.

This work was supported by a grant from the RFBR (No. 09-04-00899-a), by RMES (contract No. 02.740.11.0079), and program of Russian Academy of Science on Molecular and cellular biology.

MOLECULAR MECHANISMS BALANCING THE LEVELS OF DNA REPAIR ENZYMES TO THE CELLULAR ENVIRONMENT.

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DNA lesions occur due to the chemical instability of DNA or are induced by DNA damaging agents. The majority of non-bulky DNA lesions, including base damage, sites of base loss and single strand breaks, are repaired by proteins involved in the Base Excision Repair (BER) pathway. The level of endogenous DNA lesions depends on cellular metabolism and exogenous mutagens and although this level may vary, it is not clear how the levels of BER enzymes are controlled in response to the changing environment. We have investigated the cellular mechanism regulating the levels of BER enzymes and found that the stability of BER enzymes in mammalian cells is linked to and controlled by the level of DNA lesions. We demonstrated that the stability of BER enzymes increases after formation of a repair complex on damaged DNA. We also found that the proteins which are not involved in a repair complex are polyubiquitylated by the E3 ubiquitin ligases Mule and CHIP and subsequently rapidly degraded by the proteasome. In contrast, we demonstrated that deubiquitylation of DNA repair proteins is able to inhibit their degradation by the proteasome and thus elevate protein levels in response to DNA damage. By fractionating human cell extracts we identified the cytoplasmic ubiquitin-specific protease USP47 as the major enzyme involved in BER proteins deubiquitylation. I will discuss a novel dynamic model for the regulation of the steady state levels of BER enzymes.

DIFFUSIONAL MOVEMENT OF DNA REPAIR PROTEINS ALONG DNA

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Proteins that move along DNA may use two fundamentally different movement mechanisms: directed movement with coupled ATP hydrolysis and random one-dimensional diffusion driven by Brownian fluctuations. We have developed a new approach to quantitatively analyze the latter mechanism and used it to study the process of lesion search by several DNA repair enzymes: *Escherichia coli* and human uracil DNA glycosylases, 8-oxoguanine-DNA glycosylases, and AP endonucleases. All these enzymes were able to move along DNA by one-dimensional diffusion over distances up to 40 base pairs. The average travel distance was significantly influenced by ionic strength, Mg^{2+} ions, and competing DNA binding molecules but was barely affected by crowding agents. We have computationally modeled the process of retraction and reinsertion of the amino acids that Fpg DNA glycosylase intercalates into DNA and obtained mutants of this enzyme that affect the travel distance by changing this wedge. An analytical model has been developed that describes the one-dimensional random walk of proteins along DNA in terms of probabilities of the enzyme to move or dissociate at each step.

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POLY(ADP-RIBOSE) POLYMERASE 1 IS A KEY REGULATOR OF DAMAGE PROCESSING IN BASE EXCISION REPAIR

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Poly(ADP-ribose) polymerase 1 (PARP1), functioning as DNA nick-sensor, interacts with base excision repair (BER) DNA intermediates containing single-strand breaks. Bound to DNA breaks, PARP1 catalyzes synthesis of poly(ADP-ribose) covalently attached to some nuclear proteins and itself. Autopoly(ADP-ribosyl)ation of PARP1 facilitates its dissociation from DNA breaks and is considered as a factor regulating DNA repair. PARP1 was identified among the BER proteins cross-linked to the photoreactive branch-point BER DNA intermediate along with apurinic/apyrimidinic endonuclease 1 (APE1), DNA polymerase β (Pol β) and flap endonuclease 1 (FEN1). By functional assays in reconstituted systems, as well as in cell extracts, we demonstrated that PARP1 and its poly(ADP-ribosyl)ation is involved in regulation of activity of the base excision repair enzymes – APE1 (3'-5' exonuclease activity), Pol β, FEN1. PARP1 and its poly(ADP-ribosyl)ation was shown to more efficiently influence DNA synthesis in long patch BER. PARP1's ability to interact with intact AP sites and AP sites processed by APE1 via covalent via Schiff base intermediate was demonstrated in cell extracts and with pure PARP1. The identity of PARP1 as the target for cross-linking to AP sites in cell extracts was proved by MALDI-TOF-MS analysis. PARP1 does not cleave AP sites, but instead forms a stable intermediate with this lesion. Thus, in addition to well-known role of PARP1 as nick-sensor, we demonstrated its interaction with DNA intermediate of the BER process at the stage preceding incision of sugar-phosphate backbone of DNA. Interaction of PARP1 with AP sites, along with the previously detected interaction with DNA breaks, demonstrates PARP1's role as a key sensor of lesions appeared in the BER process. PARP1 is able to interact with AP sites cleaved by APE1 and interaction of PARP1 with this intermediate stimulates synthesis of poly(ADP-ribose) polymer.

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CtIP CONTROLS EXO1-MEDIATED DNA END RESECTION IN HUMAN CELLS

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Cells have evolved two distinct pathways to repair DSBs: non-homologous end-joining (NHEJ) and homologous recombination (HR). Accurate repair of DNA double-strand breaks (DSBs) by HR requires extensive 5'→3' resection of DNA ends to generate 3'- ssDNA tails followed by strand invasion of a homologous template. Investigation of the molecular mechanisms of DNA end resection has recently gained much attention and a two-step model has been postulated: CtIP has been found to cooperate with the MRN complex in the initial phase of DSB resection, most likely by endonucleolytic removal of (modified) nucleotides from the 5'-end ("end-trimming"). In a second step, these intermediates are further resected by two alternative pathways involving EXO1 or BLM, allowing efficient RAD51 nucleoprotein filament formation.

We now show that CtIP, through its ability to physically and functionally interact with EXO1, coordinates DNA end resection in human cells.

TOWARDS UNDERSTANDING LIFE/DEATH DECISIONS IN DEATH RECEPTOR-INDUCED APOPTOSIS

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Apoptosis is a process common to all multicellular organisms. Apoptosis can be triggered by a number of factors including DNA damage, growth factor withdrawal and stimulation of death receptors. These stimuli can also lead to the induction of non-apoptotic pathways. In this study we tried to understand life/death decisions in the cell investigating death receptor signalling and applying systems biology approach. CD95 (APO-1/Fas) is a member of the death receptor (DR) family, a subfamily of the TNF-R (tumor necrosis factor receptor) super family. Engagement of CD95 leads to the induction of the apoptotic and non-apoptotic signaling pathways. Here we addressed the question of life/death decision at CD95 considering the cross-talk between CD95-induced apoptosis and NF-κB activation. Using HeLa-CD95 cells (HeLa cells stably overexpressing CD95) we have generated experimental data on the induction of apoptosis and NF-κB signaling pathway. The data generated were used for building the systems biology model of the CD95 signaling. Using this model and our experimental data we have shown that DED proteins of the DISC: procaspase-8 and c-FLIP regulate both pathways in a dynamic way. Furthermore, our model has allowed to provide the new insights on how life/death decisions are made in a cell.

BIPARTITE DNA DAMAGE RECOGNITION IN NUCLEOTIDE EXCISION REPAIR

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Nucleotide excision repair is a key cellular defense reaction that promotes genome stability by processing helix-distorting DNA adducts arising from UV radiation, chemical carcinogens, reactive metabolic byproducts as well as oxygen radicals. The initial recognition of these widely different DNA lesions is accomplished by a bipartite discrimination process based on the sequential interplay of at least two distinct strategies to sense damaged DNA. Xeroderma pigmentosum group C (XPC) protein conducts the primary survey of the genome, thus triggering a global-genome repair pathway for the removal of all aforementioned lesions. This general repair initiator achieves its unique substrate versatility by avoiding direct contacts with damaged bases and using, instead, a dynamic sensor interface that detects non-hydrogen-bonded bases on the undamaged side of the DNA double helix. Once associated with target sites, XPC protein serves as a docking station to launch the XPD helicase which, as part of the TFIIH complex, carries out a directional DNA scanning process. Recent studies with an archael XPD homolog demonstrate that the collision with a single UV-induced cyclobutane pyrimidine dimer (CPD) inhibits its DNA helicase but, surprisingly, stimulates the accompanying ATPase activity. Protection assays with restriction enzymes and a CPD-specific endonuclease demonstrate that the XPD helicase remains firmly bound to a lesion situated in the strand along which the enzyme moves with 5'–3' polarity. Instead, the helicase enzyme readily dissociates from the damaged substrate after running into a CPD located in the complementary strand. These results indicate that a second more rigorous lesion verification step is carried out by the XPD subunit, which determines the precise location of chemically altered residues and demarcates lesion sites in a strand-specific manner. Future research will be devoted to the question of how this bipartite DNA damage recognition mechanism takes place within the physiologic chromatin context of living cells.

NUCLEOTIDE EXCISION REPAIR FACTORS ARE INVOLVED IN TRANSCRIPTION IN ABSENCE OF GENOTOXIC ATTACK

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Upon gene activation, we found that RNA polymerase II transcription machinery assembles sequentially with the Nucleotide Excision Repair (NER) factors at the promoter. This recruitment occurs in absence of exogenous genotoxic attack, is sensitive to transcription inhibitors, and depends on XPC a DNA damage sensing factor. The presence of these repair proteins at the promoter of activated genes is necessary in order to achieve optimal DNA demethylation and histone post-translational modifications (H3K4/H3K9 methylation, H3K9/14 acetylation) and thus efficient RNA synthesis. Deficiencies in some NER factors impede the recruitment of others and affect nuclear receptor transactivation.

Our data suggest that there is a functional difference between the presence of the NER factors at the promoters (which requires XPC) and the NER factors at the distal regions of the gene (which requires CSB). While the latter may be a repair function, the former is a function with respect to transcription unveiled in the current study. Preliminary data suggest a role for XPG and XPF/ERCC1 the two endonucleases involved in NER as well as in other DNA repair pathways.

COOPERATIVE INTERACTION OF NER FACTORS ON DAMAGED DNA

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The nucleotide excision repair (NER) is one of the major repair systems to remove a wide range of helix distorting lesions from DNA, including those formed by UV light, various environmental mutagens and certain chemotherapeutic agents. Defects in NER are associated with several human autosomal hereditary diseases. The coordination of the assembly of the NER complexes and the sequential individual reactions is achieved through multiple DNA-protein and protein-protein interactions. We have studied two key stages of global genome repair (GGR), namely, damage recognition and assembly of preincision complex. The interaction of key protein factors of the NER process, XPC-HR23B, XPA, and RPA, with DNA structures that mimic NER intermediates has been analyzed. Using DNA duplexes containing photoreactive 5I-dUMP residues in the certain positions either in damaged or in undamaged strands and fluorescein group linked to uridine residue as the lesion, direct evidence of preferential contacts of XPC-HR23B, the damage sensing factor of GGR, with undamaged strand was provided. The photocrosslinking positioning of XPC-HR23B on damaged DNA is in accordance with the X-ray data for Rad4. XPA shows two maximums of crosslinking intensities located on the 5'-side from a lesion. RPA mainly localized on undamaged strand of damaged DNA duplex and damaged bubble-DNA structure. When these proteins were added simultaneously we detected triple complex formation, different modification levels and better protection from nuclease digestion that could reflect some conformational changes during the complex formation. These results show for the first time the direct evidence for the localization of XPA in the 5'-side of the lesion and suggest the key role of XPA orientation combined with RPA binding to undamaged strand for positioning the NER preincision complex. The findings support the mechanism of loading of the structure specific endonuclease ERCC1-XPF by XPA on the 5'-side from the lesion before damaged strand incision. This scenario is in agreement with reported 5'-DNA strand incision by ERCC1-XPF prior to the 3'-DNA strand incision by XPG. Together XPA and RPA would play a structural role and ensure a proper three-dimensional structure of the DNA intermediate for excision in addition of being involved in the DNA damage strand recognition.

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DYNAMICS OF DOUBLE-STRAND BREAKS: IMPLICATIONS FOR REPAIR

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A single double-strand break (DSB) can kill a cell. Consequently, cells have evolved a complex system of repair and signalling pathways that trigger both DSB repair and checkpoint arrest. Despite significant progress in elucidating the roles of specific repair proteins, the organization of the process within the nuclear space is poorly understood. Recently, using budding yeast as a model system, we have shown that irreparable breaks are recruited to the nuclear periphery whereas physiologically relevant spontaneous breaks occurring during S phase assume an internal nuclear location. We have now better characterized the signals and proteins that determine which types of damage will be recruited to the nuclear pore and which will remain internal. We also examine how chromatin movement is regulated during repair. Quantitative fluorescence microscopy is used to analyse DSB location and movement in living cells. We find that spontaneous breaks marked by functional fusions of YFP with Mre11, Rad52, Rad51, or Rad54 are internal, providing strong evidence that repair of DSBs via homologous recombination is concentrated in the nuclear interior. In addition, repair foci formed by factors recruited early in the repair process move as much as an intact chromatin locus, although the movement eventually drops such that repair foci are highly constrained. Both Rad51 and Rad54 appear to be required for internal localization and for constrained movement of Rad52-YFP foci. Rad52-YFP foci induced in G1 – through inhibition of checkpoint kinases via caffeine treatment – also undergo a drop in movement, suggesting that the immobilization is not due to strand invasion of the sister chromatid. Since the loss of chromatin movement during DSB repair is neither a by-product of repair nor determined by checkpoint kinases, we are now examining the possibility that it reflects chromatin remodelling around DSBs.

CHROMATIN AND DNA DAMAGE REPAIR

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In eukaryotic cells the inheritance both accurate DNA sequence and her chromatin organization is key position for the genome stability. Different DNA lesions induced by endo- and exogenous factors creation the problem for this stability. For all comprehension as cell can accomplish this task it is integrated the knownledge about nature these lesions, its detection and repair into chromatin surroundings. Numerous types of DNA lesions and repair pathways and complex chromatin organization complicate this comprehension. Resent progress in each from these spheres helps to clear on molecular and cellular levels significance of these processes interaction. In this report I shall view modern conceptions about regulation of repair processes on chromatin level. I shall view types of histone modifications and its influence repair regulation and the role of remodeling complexes in different branches of repair processes.

THE ROLE OF MDC1 IN THE MAMMALIAN DNA DAMAGE RESPONSE

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Higher order chromatin structure is important for recognition and repair of DNA damage. Many DNA damage response proteins accumulate in large chromatin domains flanking sites of DNA double-strand breaks (DSB). The histone variant H2AX that is phosphorylated by ATM in response to ionizing radiation and the mediator/adaptor protein MDC1 that recognizes phosphorylated H2AX, facilitate this process. Loss of H2AX or MDC1 causes genome instability and is associated with DSB repair and cell cycle checkpoint defects.

MDC1 also interacts the MRE11/RAD50/NBS1 (MRN) complex and its presence is essential for the efficient accumulation of the MRN complex in chromatin regions flanking DSBs. We recently described the mechanism by which MDC1 interacts with the MRN complex and mediates its recruitment to chromatin regions flanking DSBs. We identified a region in MDC1 that is essential for the focal accumulation of the MRN complex at sites of DNA damage. This region contains multiple conserved acidic sequence motifs that are constitutively phosphorylated *in vivo*. We showed that these motifs are efficiently phosphorylated by caseine kinase 2 (CK2) *in vitro* and directly interact with the N-terminal forkhead-associated (FHA) domain of NBS1 in a phosphorylation-dependent manner. Mutation of these conserved motifs in MDC1 or depletion of CK2 by siRNA disrupts the interaction between MDC1 and NBS1 and abrogates accumulation of the MRN complex at sites of DNA double-strand breaks *in vivo*. Thus, our data reveal the mechanism by which MDC1 physically couples the MRN complex to damaged chromatin.

MDC1 also contains an FHA domain at its N-terminus. This FHA domain is thought to be highly relevant for MDC1's physiological role in the DNA damage response. However, no *bona fide* interaction partner for the FHA has so far been discovered. I will present novel data indicating that the FHA domain is implicated in a novel mechanism of DNA damage-induced MDC1 dimerization.

MECHANISM OF CHROMOSOME REARRENGEMENTS IN TREATMENT RELATED LEUKAEMIAS

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Chromosome rearrangements are believed to cause secondary leukaemias which constitute frequent complications of antitumor chemotherapy with DNA topoisomerase II - specific drugs. Here we show that inhibition of DNA topoisomerase II in cultured cells stimulates association of components of the non-homologous end joining system with a known breakpoint cluster region of the human *AML1* gene, suggesting that errors of DNA repair during non-homologous end joining may be a cause of translocations in cells treated with DNA topoisomerase II poisons. Most of secondary leukaemias are associated with translocations involving certain genes. The translocation between *AML1* and *ETO* genes t(8;21)(q22;q22) is frequently observed in secondary leukaemias. *AML1* is a transcription factor and a critical regulator of hematopoietic cell development. In translocation the part of the *AML1* gene is fused with nearly the entire *ETO* gene at 8q22, resulting in an in-frame *AML1–ETO* fusion. Leukaemias are thought to originate because of altered transcriptional regulation of normal *AML1* target genes by in-frame *AML1–ETO* fusion protein. However, for nonhomologous chromosomes to join, they must break at about the same time while either located near each other in the interphase nucleus or be brought together by some mechanism. Chromosome 21 bearing the *AML1* gene is located relatively close to the nuclear center, while chromosome 8 bearing the *ETO* gene occupies a more peripheral nuclear position. Thus there should be a mechanism which brings these two chromosomes or at least the domains of these chromosomes which participate in the translocation event to the same nuclear region. We show that treatment of cells with VP-16 (etoposide), an inhibitor of DNA topoisomerase II widely used in anticancer chemotherapy, causes the *ETO* gene repositioning in the nuclear space which allows *AML1* and *ETO* genes to be localized in the same nuclear layer. Inhibitor studies demonstrate that such an effect is likely to be connected with the formation of stalled cleavable complexes on DNA. Finally, inhibition of *ETO* gene repositioning by 2,3-butanedione monoxime (BDM) suggests that this process depends on nuclear myosin.

REGULATION OF TELOMERASE AT CHROMOSOME ENDS BY SHELTERIN AND TERRA

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Telomerase counteracts telomere shortening at chromosome ends. Telomerase repression and telomere shortening regulates lifespan of cells suppressing tumorigenesis. Recruitment of human telomerase to chromosome ends is thought to be a pivotal step in the activation of telomerase, but the molecular basis for telomerase recruitment is unknown. We have assayed telomerase recruitment via fluorescence in situ hybridization (FISH; Terns-lab) and chromatin immunoprecipitation (ChIP; Lingner-lab). We identified TPP1 and TIN2, components of the telomeric shelterin complex as key telomerase recruitment factors. We have determined that the OB fold of TPP1 is required for the recruitment function of telomerase. Telomerase association with telomeres does not require POT1, which anchors TPP1 to the single-stranded DNA overhang of the telomere, suggesting that TIN2-anchored TPP1 (associated with double-stranded regions of the telomere) plays a major role in the recruitment.

Telomeres are transcribed into TERRA, a large noncoding RNA of unknown function, which forms an integral part of telomeric heterochromatin. TERRA molecules resemble in sequence the telomeric DNA substrate as they contain 5'-UUAGGG-3' repeats which are complementary to the template sequence of telomerase RNA. We demonstrate that endogenous TERRA is bound to human telomerase in cell extracts. Using in vitro reconstituted telomerase and synthetic TERRA molecules we demonstrate that TERRA binds to the RNA template sequence of telomerase. In addition TERRA binds to the telomerase reverse transcriptase (TERT) protein subunit independently of hTR. In vitro studies demonstrate TERRA acts as a potent competitive inhibitor for telomeric DNA in addition to exerting an uncompetitive mode of inhibition. Our data identify TERRA as a telomerase ligand and natural direct inhibitor of human telomerase. Telomerase regulation by the telomere substrate may be mediated via its transcription.

POSTER SESSION

NORMALIZATION OF RECOMBINATION ACTIVITY OF RecA PROTEIN IN *ESCHERICHIA COLI* CELLS

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Recombinational repair, protecting cells from damage of its DNA, must be constrained in its activity that is carried out by the structure-functional limitations of the activity of the main recombination enzyme – RecA protein.

We have previously shown that a single amino acid substitution [D112R] in protomer interface leads to a 50-fold increase of frequency of recombination exchanges per DNA unit length (FRE) compared with wild-type RecA protein. When cultured in liquid growth medium of independent populations of *Escherichia coli* cells, carrying the plasmid with *recAD112R* gene, there was observed a gradual normalization of the recombination level. Analysis of 16 independent populations showed, that the FRE decrease may occur due to changes that appeared as on the plasmid, carrying the *recAD112R* gene, as well as in the chromosome. Sequencing of plasmids that provide the greatest FRE decrease, revealed a deletion of a region size of 633 nucleotide pairs, containing a promoter of the *recA* gene, which led to a decrease in the level of its expression. FRE decrease, induced by the changes in the chromosome, was provided by a point mutation in *pcnB* gene, which is coding the poly(A)-polymerase I, controlling the number of copies of plasmids in bacterial cells.

Correlation between the recombination activity of the RecA protein, expressed in cells, and their biological motility is discovered. Strains with increased FRE value possessed higher expression levels of the genes responsible for the molecular motility, as well as the ability to move along the surface of semisolid agar with higher speed compared with the *E. coli* strains, showing the FRE value at the wild type level.

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EXOGENOUS INJECTION OF NICOTINAMIDEADENINEDINUCLEOTIDE CHANGES THE LEVELS OF HISTONE H2AX PHOSPHORYLATION/DEPHOSPHORYLATION IN MOUSE HEART CELLS AFTER IONIZING RADIATION

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Among all types of DNA damage, double-strand breaks (DSBs) are the most dangerous lesions and could lead to apoptosis or carcinogenesis. One of the earliest responses to DNA DSBs is phosphorylation of 139-serine in core variant histone H2AX (γH2AX) in megabase chromatin domains around DSB sites. The number of γH2AX foci correlates with the number of DSBs during DSBs repair. It is well known that poly-ADP-ribose polymerase (PARP) is activated after DNA damage and it ribosilates chromatin proteins around the damage sites. It was suggested in 1986 by Carson et al. that an intimate relationship exists between DNA single-strand breaks, nicotinamideadeninedinucleotide (NAD) metabolism, and cell viability in quiescent human lymphocytes. An increase in DNA strand break formation in lymphocytes, or a block in DNA repair, accelerates poly-ADP-ribose formation and may induce lethal NAD and ATP depletion, thus cause programmed removal of lymphocytes (and perhaps of other cells) with damaged DNA. We suggest that a relationship could exist between DSBs repair efficiency and NAD cycling in conditions of NAD excess in myocardium cells and cardiomyocytes of C57Bl/6 mice.

Here, using immunochemistry technique and Western blotting we studied the influence of exogenous NAD on the level of phosphorylation/dephosphorylation of histone H2AX in myocardium cells and cardiomyocytes of C57Bl/6 mice after ionizing radiation (IR). It is known, that cardiomyocytes are terminally differentiated and do not proliferate. It is known that γH2AX could concentrate in replication centers of prolifirating cells, and in terminally differentiated cells γH2AX is formed only at DSB sites. Using Western blotting method we found, that injection of exogenous NAD in the dose of 3,8 mg/kg immediately after IR significantly increase the level of γH2AX in myocardium cells 20 min after IR in the dose of 3 Gy in comparison to irradiated control mice. 1 hour after IR the level of γH2AX decreased to the level that is lower than in control mice. Immunohistochemistry (PAP method) confirmed our data on the influence of NAD on γH2AX formation and elimination after IR. It is evident, that exogenous injection of NAD changes the kinetics of phosphorylation/dephosphorylation of H2AX histone and possibly influences DSBs repair in myocardium cells and cardiomyocytes of C57Bl/6 mice after IR.

7,8-DIHYDRO-8-OXO-2'-DEOXYADENOSINE-5'-TRIPHOSPHATE: SYNTHESIS AND USE BY DNA POLYMERASES

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7,8-Dihydro-8-oxoadenine (8-oxo-Ade) is a major product of oxidative damage of adenine, which can be induced by ionizing radiation, environmental oxidizers, or metabolically generated reactive oxygen species. The steady-state levels of 8-oxoAde in eukaryotic DNA is at the same order as the levels of other ubiquitous oxidative purine lesions, such as 7,8-dihydro-8-oxoguanine or formamidopyrimidines. Increased amounts of 8-oxoAde are found in human tumors and degenerating neural tissue. Two major routes of accumulation of 8-oxoAde in DNA can be envisioned. First, Ade bases in DNA may be directly oxidized, in which case an 8-oxoAde pair with thymine inevitably appears. Second, Ade in dATP (and possibly some dATP precursor) may be oxidized, and the resulting 7,8-dhydro-8-oxo-2'-deoxyadenosine-5'-triphosphate (8-oxo-dATP) can in principle be used by DNA polymerases during replicative or repair DNA synthesis. Human and *Escherichia coli* cells possess enzymes that specifically hydrolyze 8-oxo-dATP, underscoring the biological importance of this damaged dNTP. However, virtually no information is available on the utilization of 8-oxo-dATP by DNA polymerases.

We report an efficient method of synthesis of 8-oxo-dATP and a study of its use by several DNA polymerases of bacterial and human origin. 8-oxo-dATP was an inefficient substrate for exonuclease-deficient Klenow fragment of *E. coli* DNA polymerase I and was primarily misincorporated opposite guanine in the template. Human DNA polymerases α and λ did not utilize 8-oxo-dATP as a substrate, whereas DNA polymerase β incorporated it opposite thymine in an error-free manner.

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KU ANTIGEN INTERACTS WITH DNA CONTAINING ABASIC SITES

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One of the most abundant lesions in DNA is abasic (AP) sites arising spontaneously or as intermediates in base excision repair. Residues of deoxyribose in AP sites are in equilibrium between cyclic furanose and acyclic aldehyde forms. The presence of aldehyde function determines the ability of AP site to form Schiff base intermediate with primary amino groups of proteins. This intermediate can be stabilized by $NaBH_4$ treatment and, therefore, AP DNA can be used as an approach in searching proteins, which interact with AP sites. In HeLa cell extract, a predominant product with an apparent molecular mass of 95 kDa was observed. Analogous covalent adducts of proteins with AP DNA were revealed in the extracts derived from human fibroblast, HL-60, K562, and several melanoma cell lines unlike bovine testis nuclear extract. The cross-linked protein was identified as the p80 subunit (Ku80) of Ku antigen (Ku) by immunoprecipitation and MALDI-TOF-MS analysis. Ku is an abundant DNA end binding protein in human cells. Ku is the DNA binding component of DNA-dependent protein kinase (DNA-PK). AP DNAs of different structure were used to study peculiarities of Ku interaction with DNA. Considering the extreme selectivity of AP site-containing DNA probes, we decided to examine the use of this approach for measuring levels of Ku in different cell extracts. We found that the amount of Ku80 estimated by dot-ELISA and AP DNA cross-linking were comparable. Cross-linking using AP DNA allows revealing truncated variants of Ku80 polypeptide (Ku80v). The ability of Ku80v/Ku70 heterodimer to interact with DNA-PK catalytic subunit (DNA-PKcs) is greatly reduced and resulted in increased sensitivity to some DNA damage agents as a consequence of reduced DNA repair. Thus, the ability of Ku80 to form cross-link with baseless deoxyribose can be used as an efficient and easy assay to test the content of Ku antigen in cell extracts. This approach, unlike western blot or estimation of the Ku content based on mRNA levels, reveals forms of Ku that are active in DNA binding, including those, which have aberrations in Ku80, but retain ability to bind DNA. In addition this test is less sensitive to the reaction conditions than the electrophoretic mobility shift assay.

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XPA AND RPA PROTEINS ARE INVOLVED IN THE FORMATION OF CORRECT TOPOGRAPHY OF NER PREINCISION COMPLEX ON DAMAGED DNA

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Nucleotide excision repair (NER) is the major repair pathway to eliminate bulky DNA adducts, such as pyrimidine dimers, arising upon UV irradiation, and chemical adducts, arising upon exposure to carcinogens and some chemotherapeutic drugs. The recognition of damaged sites followed by the preincision complex formation is crucial for successful repair. The interaction of the key NER factors XPA and RPA with DNA duplexes and bubble-DNA structures imitating damaged DNA was investigated using photoaffinity labeling technique, footprinting and electrophoretic mobility shift assay. Using DNA duplexes containing photoreactive nucleotide residues in the certain positions either in damaged or in undamaged strands, direct evidence for preferential RPA contacts with the 5'-side of undamaged strand was provided, however efficient crosslink with damaged strand was also detected when 5I-dUMP was localized to the 5'-side from a DNA damage. The same position yielded maximum level for the XPA modification; in addition two contacts of this protein with undamaged strand were detected. Our crosslinking results demonstrate for the first time that XPA localized nearby ss/dsDNA junction that is positioned 5' to a lesion. In this position XPA would initiate the assembly of the DNA preincision complex by recruiting ERCC1/XPF endonuclease. The assembly would be followed by the incision in the damaged ssDNA towards the 3' direction. The data obtained allow to suggest XPA and RPA structural role in formation of proper three-dimensional structure of the DNA intermediate for excision stage. The model of interaction of proteins within the preincision NER complex is proposed.

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INTERACTION OF PARP2 WITH DNA STRUCTURES MIMICKING DNA REPAIR INTERMEDIATES

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Poly(ADP-ribosyl)ation, one of the posttranslational protein modifications significant for genomic stability and cell survival in response to DNA damage, is mostly catalyzed by PARP1. It was the first protein described to synthesize PAR in response to mitogenic stimuli or genotoxic stress. PARPs now constitute a large family of 17 proteins, in which PARP1 and PARP2 are so far the sole enzymes whose catalytic activity is immediately stimulated by DNA strand-breaks. Whereas the role of PARP1 in the response to DNA damage has been widely illustrated, the contribution of another DNA-dependent PARP, PARP2, has not been studied so far and the most part of published data are based on the experiments *in vivo*. Only limited data on requirement of PARP2 for efficient base excision repair, homologous recombination, DNA stability during mitosis and germ cell differentiation are available.

In present work, few DNA-structures (nick-, gap2-, gap10-, gap20-, flap3-, flap9-, over5-, over3-, hp-, twj-, fwj-DNAs) mimicking intermediates of different DNA metabolizing processes were used to identify the structure, which PARP2 could be addressed to. To determine the contribution of PARP1 and PARP2 to the total poly(ADP-ribose) synthesis, the activation of PARP1 and PARP2, when present separately or together, by each of DNAs was estimated. The K_d values of PARP2-DNA complexes were also measured. As expected, the activity of PARP2 was much lower than PARP1's activity. Surprising, that for PARP2 no correlation between activation efficiency and K_d values was found. PARP2 was activated the most effectively in the presence of over5- and gap2-DNAs but it displayed higher affinity to gap20- and flap9-DNAs. Taken together, these data allow to count efficiency of poly(ADP-ribose) synthesis by PARP2 as V_{max}/K_d and to conclude that over5- and gap20-DNAs are the most effective activators for PARP2. The interactions of PARP2 with few BER enzymes (polβ, XRCC1 and FEN1) were also studied. PARP2 inhibits activity of polβ, but inhibitory effect was reduced in the presence of XRCC1. Unfortunately, we failed to reveal complexes of PARP2 with any of these BER proteins by cross-linking with glutaraldehyde that testifies to existence of transient PARP2-protein-DNA complexes (if at all) or inappropriate positioning of acceptor amino acids.

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INTERACTION OF TOPOISOMERASE I WITH DNAS LACKING TOP1 CLEAVAGE SITES

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DNA topoisomerases are involved in multiple cellular processes including replication, transcription, and recombination. These enzymes are required for a solution of the topological problems arising in the processes of DNA metabolism and influence on their activity. Topoisomerase I (Top1) performs cleavage and religation of DNA strand within specific cleavable site via the formation of DNA-protein covalent intermediate between the catalytic tyrosine residue of the enzyme and 3'-phosphate of the cleaved strand. In the present work the interactions of human Top1 with DNA structures imitating base excision repair intermediates were studied in details. Using specific antibodies against Top1 and PARP1 proteins, modified products of these proteins in bovine testis nuclear extract were identified. The same modified products were detected in cellular extract from human myeloid leukemia and melanoma cell lines, as well as from mouse embryonic fibroblasts. In the extracts prepared from apoptotic human pancreatic carcinoma cells treated with etoposide, topoisomerase II inhibitor, the main protein modified by nicked DNA duplex was attributed to the adduct of DNA with Top1 truncated (apoptotic) fragment. The data obtained allow to suggest, that revealed interaction of Top1 with damaged DNA might be one of the signal mechanisms triggering programmed cell death.

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PHOTOAFFINITY MODIFICATION AND IDENTIFICATION OF NER COMPETENT HeLa EXTRACT PROTEINS INTERACTING WITH HARDLY REPAIRABLE NER_SUBSTRATE ANALOG - 137 bp Fap-dC-DNA

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The 137-bp photoactive double stranded DNA, imitating NER substrate, was created and used as DNA probe for affinity modification. DNA probe bear photoactive 4-azido-2, 5- difluoro-3-pyridin-6-yl (Fap-) group, introduced via linker fragment at cytidine base. The modified dCMP located in specified position of the molecule and contains 3'-[32]-P label. NER competent cell extract proteins (NEP) selectivity and efficiently interact with Fap-containing DNA. Mild UV-irradiation (313 nM, 5 min, 15 J/cm2) of reaction mixture containing the duplex and extract proteins resulted in effective and selective DNA–protein crosslinking. About one quarter of the total amount of radioactive DNA probe was included in restricted number of covalent protein–nucleic acid adducts. Proteins modification pattern produced with extended DNA probe was distinctly affected by ratio of cell extract proteins and DNA concentrations in reaction mixture for crosslinking. Extract proteins concentration decrease resulted in widening of set of effectively photocrosslinked polypeptides and changes distribution of crosslinked DNA probe. The modification pattern of NEP with using 137bp Fap-dC-DNA was drastically influenced by NEP/DNA concentrations ratio. The decrease of the ratio NEP/DNA leads to increase of amount of protein crosslinked in the extract. Comparison of modification patterns observed for recombinant and NE proteins allowed designate several targets of affinity modification. Polypeptide ~70 kDa detected in wide range of NEP concentrations can be attributed as p70 of RPA. Stably present in modification patterns 115-120 kDa crosslinked protein by means functional test was identified as PARP1. Thus PARP1 generally accepted as nick sensor can specifically interact with NER substrate, continuous DNA, bearing arylazide lesion. The 130-140 kDa protein efficiently crosslinked at low NEP concentration presumably was DNA-binding subunit of XPC factor.

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DOMAINS AND LINKERS: HETERONUCLEAR NMR CHARACTERIZATION OF THE STRUCTURE AND DYNAMICS IN MODULAR PROTEINS

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Human genomics and structural biology data indicate that the majority of human proteins are structurally modular with the average of three independently folding domains per polypeptide chain. Until recently the flexible inter-domain linker polypeptides were often not reported in the crystal structures of the respective proteins. This limited our understanding of the essential roles the dynamic linker polypeptides have in protein function. The crystal structural investigations of the DNA repair glycosylases where as much as 30% of the residues can belong to the dynamic parts of the polypeptide chain exemplifies this limitation. With the advent of modern heteronuclear solution NMR methods, the characterization of the domain three-dimensional structure can be preformed hand-in-hand with the determination of the backbone dynamics of the flexible inter-domain linkers. Here we present a heteronuclear (^{1}H, ^{15}N, ^{13}C) solution NMR structure/dynamics study of D6-HP, a modular fragment of actin-regulating protein villin in which two structured domains, D6 and HP, are connected with a flexible 40-residue linker. The structural properties of the domains and the dynamics of the impressively long linker will be presented in the context of the calcium-controlled functional role(s) of the complete protein.

KINETICS OF HISTONE H2AX DEPHOSPHORYLATION AT THE SITES OF DOUBLE-STRAND DNA BREAKS INDUCED BY IONIZING RADIATION IN CULTIVATED MAMMALIAN CELLS

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Histone H2AX is rapidly phosphorylated at serine 139 in mega base chromatin domains surrounding double-strand DNA breaks (DSBs) induced by ionizing radiation (IR) in mammalian cells and forms large nuclear γ-H2AX foci. After reaching the maximum the level of γ-H2AX is decreased gradually with time and dynamics of γ-H2AX elimination mimics dynamics of DSB rejoining. Two different molecular mechanisms of γ-H2AX dephosphorylation were suggested: direct in situ dephosphorylation by phosphatases and replacement of γ-H2AX with unphosphorylated H2AX by chromatin-remodeling complexes. We have found earlier using FRAP assay that kinetics of GFP-H2AX recovery after photo bleaching was equally slow in non irradiated cells and 1 hour after 10 Gy, and the rate of exchange was comparable with kinetics of γ-H2AX foci elimination after IR thus indicating that dephosphorylation could occur by histone exchange. To observe the kinetics of histone exchange after induction of DSBs located more densely in limited area of the nucleus we used micro irradiation of living H2AX-GFP expressing Chinese hamster cells by 405 nm laser. In agreement with our previous results H2AX-GFP mobility in the irradiated areas was found to be as slow as after the action of IR. To estimate the role of phosphatases on γ-H2AX dephosphorylation *in situ* we treated human embryonic fibroblasts (HEF) with forskolin, the activator of adenylate cyclase which also activates protein phosphatase PP2A. It was detected by immunoblotting and by analysis of the overall γ-H2AX-associated fluorescence in HEF nuclei that pre-treatment with forskolin decreased H2AX phosphorylation following IR. The average area occupied by γ-H2AX foci in the nuclei one, three or five hours after IR was decreased in comparison with untreated cells, but the average numbers of γ-H2AX foci did not change, i.e. forskolin did not affect the kinetics of γ-H2AX foci formation and elimination. Forskolin pre-treatment did not change cell survival and the induction of chromosome aberrations after IR indicating that it did not affect the efficiency of repair. Expression of chromatin-remodelling complex Tip60 containing cAMP response elements serving as binding sites for transcription factor CREB was not changed after forskolin pre-treatment, but we cannot exclude that this protein could be activated by posttranslational modifications induced by forskolin. The decrease of spread of γ-H2AX phosphorylation at individual DSB could be due not only to direct activation of phosphatase PP2A by forskolin, but also to stimulation of POLB gene containing cAMP response elements in promoter and involved in base excision repair that could occur in IR-damaged chromatin. Our observations suggest that further experiments are needed to elucidate the possible input of dephosphorylation in situ in the mechanism of γ-H2AX elimination after IR in mammalian cells.

Fap-dC INCLUDED IN EXTENSIVE LINEAR dsDNA DEMONSTRATES PROPERTIES OF NONREPAIRABLE NER SUBSTRATE

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Synthetic analogs of damaged DNA are efficient tools for studying nucleotide excision repair. A wide variety of purine nucleobases adducted with aminofluorenes, benz-a-pyrene diol epoxide (BP-) and cis-diamine-dichloro-Pt have been used to synthesize artificial NER substrates. The examples of the pyrimidine-lesions used to design artificial DNA structures are T-T-dimers and the thymine monoadduct of hydroxymethyl-trimethylpsoralene. We recently suggested dCMP and dUMP in which the role of damages is played by photoactive 4-azido-2, 5- difluoro-3-pyridin-6-yl group (Fap) as well as fluoresceine (Flu), anthracene or pyrene residues joined to the nitrous base using flexible linker fragments. The 50-60 bp duplexes bearing these bulky damages are used for electrophoretic mobility shift experiments, measuring of quantitative characteristic of equilibrium binding of NER proteins/damaged DNA and affinity modification. Circular Fap-dPy-DNAs have been used for Fap-dC- and Fap-dU excision primary evaluation. The universal model DNA which are eligible for different experiments with reconstituted NER system are extended (≥120bp) linear DNA.

The advanced enzymatic approach of artificial DNA structures synthesis allows create NER substrates analogs of any nucleotide sequence and of various architecture easily. This method for synthesis of linear DNA substrates is versatile: wide variety damage-containing substrates can be designed and prepared by changing of modified dNTP. The model DNA137 bp containing Fap-dC, Flu-dU and BP-dG have been synthesized and tested as excision reaction substrates in NER competent HeLa extract. BP-dG-DNA was used as a reference substrate to test NER-competent extract excision activity and to evaluate concurrent properties of Flu-dU-DNA and Fap-dC-DNA. The substrate properties of model DNA can be described as BP-dG-DNA> Flu-dU-DNA>>Fap-dC-DNA.

The data of the present work in combination with previously obtained results allow ascribe the linear Fap-dC-DNA as nonrepairable NER substrate analog, which is able to compete with effective DNA substrates for binding responsible for damage recognition/excision proteins.

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