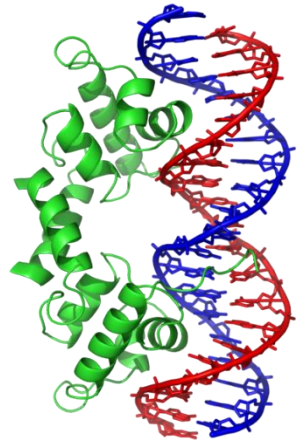
Institute of Chemical Biology & Fundamental Medicine SB RAS

The First Meeting in the Frame of French-Siberian Centre of Research and Education

“Nucleic Acid - Protein Interactions for Life Sciences”



May 13-15, 2013

Akademgorodok, Novosibirsk, Russia

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PROGRAMME AND SCHEDULE

DAY 1: Monday, May 13

11:00 – 11:30 **Registration**

11:30 – 12:00 **Opening ceremony**

Valentine VLASSOV, Academician RAS
Nikolay LYAKHOV, Academician RAS
Patrick CURMI, APILIFE Coordinator
Alain KROL, LIA Coordinator
Olga LAVRIK, APILIFE Coordinator, RAS Corresponding Member

12:00-13:15 Session 1.
Chairpersons: Marc Mirande and Andre Dietrich

12:00-12:25 *The tRNA3Lys packaging complex involves association of human mitochondrial LysRS with the polyprotein GagPol from HIV-1: a new pharmacologic target ?*
J. Dias, L. Kobbi, A. Nail, A. Szklarz, M. Comisso and **Marc MIRANDE*** (Centre de Recherche de Gif)

12:25-12:50 *Manipulating Mitochondrial Genetics*
D. Mileshina, F. Weber-Lotfi, P. Boesch, N. Ibrahim, M. Koulintchenko, G. D'Souza, V. Saxena, V. Tarasenko, Y. Konstantinov, R. Lightowlers and **Andre DIETRICH*** (Institut de Biologie Moleculaire des Plantes)

12:50-13:15 *Mitochondrial DNA Import Mechanisms*
Frederique WEBER-LOTFI*, N. Ibrahim, M. Koulintchenko, D. Mileshina, Y. Konstantinov, R. Lightowlers and A. Dietrich
*(Institut de Biologie Moleculaire des Plantes)

13:15-13:40 *Specificity of DNA Import into Isolated Mitochondria of Plants and Mammalians*
Milana KOULINTCHENKO*, N. Ibrahim, E. S. Klimenko, Yu. M. Konstantinov, F. Lotfi, A. Dietrich
*(Siberian Institute of Plant Physiology and Biochemistry)

13:40-15:00 Lunch

15:00-16:15 Session 2.
Chairpersons: Olga Lavrik and Patrick Curmi

15:00-15:25 *Influence of multifunctional protein YB-1 on the enzymes involved in the repair of AP-sites in DNA*
Pavel PESTRYAKOV*, E. Fomina, S. Khodyreva, D. Zharkov, L. Ovchinnikov, P. Curmi and O. Lavrik
*(ICBFM SB RAS)

15:25-15:50 *Impact of the YB-1 Protein on Recognition and Repair of DNA Damages*
Dmitry KRETOV*, L.P. Ovchinnikov, O. Lavrik, V. Joshi and P.A. Curmi
*(Université d'Evry val d'Essonne)

15:50-16:15 *Biomolecular Interactions on Surface Investigated at High Resolution by AFM*
Loïc HAMON*, P.A. Curmi, V. Joshi, M. Sukhanova, O. Lavrik and D. Pastre
*(Université d'Evry val d'Essonne)

16:15-16:40 Coffee Break // Poster Session

16:40-17:55 Session 3.
Chairpersons: Olga Fedorova and Jean-Christophe Amé

16:40-17:05 *Active DNA Demethylation and Epigenetic Reprogramming: Insight into Mechanism of Recognition of 5-Hydroxymethyluracil by Methyl-Binding Domain Protein 4*
Inga GRIN*, S. Moréra, A. Vigouroux, S. Couvé, V. Henriot, M.Saparbaev and A.A. Ishchenko
*(Institute de Cancérologie Gustave Roussy)

17:05-17:30 *The Mechanism of DNA Lesion Search by Human 8-Oxoguanine DNA Glycosylase*
Nikita A. KUZNETSOV*, A.A. Kuznetsova, A.A. Ishchenko, M.K. Saparbaev and O.S. Fedorova
*(ICBFM SB RAS)

17:30-17:55 *The Kinetic Mechanism of Human Apurinic/Apyrimidinic Endonuclease 1 in NIR Pathway*
Nadejda TIMOFEYEVA*, V.V. Koval, A.A. Ishchenko, M. Saparbaev and O. Fedorova
*(ICBFM SB RAS)

18:30 **Welcome reception (Restaurant "Vkusnyi centre")**

DAY 2: Tuesday, May 14

10:00-11:15 Session 4.
Chairpersons: Valérie Schreiber and Svetlana Khodyreva

10:00-10:25 *PARG Facilitates Cellular Recovery From Prolonged Replication Stress*
G. Illuzzi, E. Fouquerel, J.-C. Amé, F. Dantzer and **Valérie SCHREIBER*** (Université de Strasbourg)

10:25-10:50 *Interactions of HMGB1 and HMGB2 Proteins with Apurinic/Apyrimidinic Sites in DNA*
Ekaterina ILINA*, M. Kutuzov, D. Modestov, S. Agdauletova, O. Lavrik and Khodyreva S.N. *(ICBFM SB RAS)

10:50-11:15 *Comparative Analysis of Interaction of Human and Yeast DNA Damage Recognition Complexes with Damaged DNA in Nucleotide Excision Repair*
Nadejda RECHKUNOVA*, Y. Krasikova, E. Maltseva, P. Pestryakov, I. Petruseva and O. Lavrik *(ICBFM SB RAS)

11:15-11:45 Coffee Break // Poster Session

11:45-13:00 Session 5.
Chairpersons: Elena Rykova and Loïc Hamon

11:45-12:10 *Interaction of Ddc1 and RPA with Single-Strand/Double-Strand DNA Junctions In Cell Free Extracts of Saccharomyces Cerevisiae*
Maria SUKHANOVA*, C. D'Herin, P.A van der Kemp., S. Boiteux and O.I. Lavrik *(ICBFM SB RAS)

12:10-12:35 *Circulating DNA for Cancer Diagnostics: Concentration, Epigenetic Characteristics, Association with Blood Complexes and Cells*
Elena RYKOVA*, E.S. Morozkin, E.M. Loseva, A.A. Ponomaryova, A.M. Kurilshikov, I.V. Morozov, I.S. Zaporozhchenko, K.Y. Kapitskaya, T.L. Azhikina, L.O. Bryzgalov, E.V. Antontseva, T.I. Merkulova, N.V. Cherdyntseva, V.V. Vlassov, P.P. Laktionov *(ICBFM SB RAS)

12:35-12:55 *Intracellular Targets of Immunoinhibiting Extracellular DNA*
Anna CHEREPANOVA*, T. Duzhak, V. Vlassov, P. Laktionov *(ICBFM SB RAS)

13:00-14:30 Lunch

14:30–15:45 Session of LIA 1.
Chairpersons: Olga Dontsova and Eric Westhof

14:30– 14:55 *Recoding UGA as Selenocysteine: Idiosyncratic and Shared Factors for mRNP Assembly and Translation*
Alain KROL (University of Strasbourg)

14:55– 15:20 *Eukaryote-Specific Features of mRNA Path in the Human Ribosome*
Dmitry GRAIFER*, Yu.S. Khairulina, D.E. Sharifulin, A.G. Ven'yaminova, G.G. Karpova *(ICBFM SB RAS)

15:20– 15:45 *Lys53 of Ribosomal Protein L36al is Close to the CCA End of a tRNA at the P/E Hybrid Site on the Human Ribosome*
Konstantin BULYGIN*, S. Baouz, A. Woisard, C. Hountondji, G. Karpova *(ICBFM SB RAS)

16:00- 22:30 Free time, cultural programme

DAY 3: Wednesday, May 15

10:00– 11:15 Session of LIA 2.
Chairpersons: Galina Karpova and Alain Krol

10:00– 10:25 *"Unusual" RNA Base Pairs in Recognition and Decoding*
Eric WESTHOF* (University of Strasbourg)

10:25– 10:50 *New features of telomerase RNA processing*
M. Rubtsova, A. Malyavko, D. Vasilkova, M.Zvereva, E. Smekalova, V. Prassolov, E. Westhoff and **Olga DONTSOVA***
*(Department of Chemistry and Belozersky Institute Lomonosov Moscow State University)

10:50– 11:15 *New players in recognition of AP sites: Implication in DNA repair*
Svetlana KHODYREVA*, E. Ilina, M. Kutuzov, M. Sukhanova, J.-C. Amé, V. Schreiber and O. Lavrik *(ICBFM SB RAS)

11:15-11:45 Coffee Break // Poster Session

11:45– 13:00 Session of LIA 3.
Chairpersons: Marina Zenkova and Dmitry Graifer

11:45-12:10 *Identification of recombinant products of non-enzymatic reaction of RNA cleavage/ligation*
Marina ZENKOVA*, Ya. Staroselec, E. Brenner, V. Vlassov
*(ICBFM SB RAS)

12:10-12:35 *Nucleic acid based anticancer therapeutics*

T. Kabilova, **Elena CHERNOLOVSKAYA*,** M. Zenkova and V. Vlassov *(ICBFM SB RAS)

12:35-13:00 *Identification of molecular targets of RNase A in antitumor therapy*
O. Patutina**, Nadejda MIRONOVA*,** E. Brenner, A. Kurilshikov, V. Vlassov, M. Zenkova *(ICBFM SB RAS)

13:00-14:30 Lunch

14:30-14:55 *Ubiquitin musical chairs: Juggling multiple molecular signals between shuttles and receptors*
Michael GLICKMAN (Bio-Rad)

14:55-15:20 *SPR: applications in biomolecular interaction analysis*
Anna SYCHEVA (Bio-Rad)

15:20-16:00 CONCLUDING REMARKS

Patrick CURMI, Olga LAVRIK, Alain KROL, Valentin VLASSOV

19:00 **Gala Dinner (Restaurant "House of Scientists")**

LECTURES

THE tRNA3Lys PACKAGING COMPLEX INVOLVES ASSOCIATION OF HUMAN MITOCHONDRIAL LysRS WITH THE POLYPROTEIN GagPol FROM HIV-1: A NEW PHARMACOLOGIC TARGET?

José Dias, Lydia Kobbi, Aurélien Nail, Anne Szklarz, Martine Comisso, and Marc Mirande

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Cytosolic and mitochondrial LysRS are encoded by alternative splicing of a single gene and can only be distinguished according to their very N-terminal sequences. Beyond its role in translation, mitochondrial LysRS (mLysRS) is also hijacked from the host cell following HIV-1 infection to carry the primer tRNA3Lys into the virions1. Using monospecific antibodies, we previously showed that only mLysRS is taken up in viral particles along with tRNA3Lys, the primer for reverse transcription of the HIV-1 genome. Maturation of the precursor of mLysRS upon its mitochondrial targeting is a prerequisite to form a complex with tRNA2. We screened all the viral proteins to identify the partners of LysRS responsible for the formation of the tRNALys packaging complex. We showed that mLysRS associates with the Pol domain of GagPol. This interaction is highly specific, as assessed by the KD value of about 3 nM between mLysRS and Pol. More specifically, the transframe (TF) and integrase (IN) domain proteins of Pol interact with the catalytic domain of LysRS3. A model of the assembly of the mLysRS:tRNA3Lys:GagPol packaging complex is proposed, which is also consistent with the release of its different components after maturation of GagPol into the virions. To identify the protein-interaction sites between LysRS and GagPol, we introduced a non-natural photoactivable amino acid, pBpa, at different positions of LysRS. The first results of this approach will be presented. These data open new perspectives for the search of a new class of inhibitors of the HIV-1 development cycle that would block the packaging of tRNA3Lys into viral particles.

1Kaminska et al. J. Virol. (2007) 81: 68-73.

2Dias et al. Biochemistry (2012) 51, 909-916.

3Kobbi et al. J. Mol. Biol. (2011) 410, 875-886.

Daria Mileshina[1,2], Frederique Weber-Lotfi[1], Pierre Boesch[1,3], Noha Ibrahim[1,3], Milana Koulintchenko[1,2,3], Gerard D'Souza[4], Vaibhav Saxena[4], Vladislav Tarasenko[1,2], Yuri Konstantinov[2], Robert Lightowlers[3] and Andre Dietrich[1,*]

[1]Institut de Biologie Moleculaire des Plantes, CNRS/UdS, Strasbourg, France, [2]Institute of Plant Physiology and Biochemistry, RAS, Irkutsk, Russia, [3]Medical School, University of Newcastle, UK, [4]Massachusetts College of Pharmacy and Health Sciences, Boston MA, USA

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Mitochondrial genome expression is essential for organelle functional efficiency and inter-compartment cross-talk. Manipulation of mitochondrial genetics is thus of interest for a range of fundamental investigations and is appealing to treat neurodegenerative diseases caused by organelle DNA mutations. In plants, mitochondrial genetics underlies key breeding tools. Given the importance of these issues, transforming mitochondria has been a long standing goal that was unfortunately reached only in a couple of unicellular organisms. Contrasting with the failure to transform the organelles in whole cells, we established that isolated plant and mammalian mitochondria can functionally import DNA [1, 2]. The process is sensitive to a number of effectors and can accommodate large size linear DNA [3]. Remarkably, the imported DNA functionally joins the organelle genetic system. Marker sequences under the control of a mitochondrial promoter are expressed *in organello* [1, 2]. Imported DNA carrying oxidative lesions is repaired [4,5]. Constructs carrying fragments of mitochondrial DNA undergo homologous recombination with the resident DNA [6]. On that basis, we develop nanocarrier-mediated cell uptake and mitochondrial targeting of functional gene constructs. DNA incorporation into mammalian cells and expression of a marker gene placed under the control of the mitochondrial heavy-strand promoter were obtained, potentially opening novel prospects for mitochondrial transfection.

[1] Koulintchenko M, Konstantinov Y, Dietrich A (2003) EMBO J. 22:1245-1254

[2] Koulintchenko M, Temperley RJ, Mason PA, Dietrich A, Lightowlers RN (2006) Hum. Mol. Genet. 15:143-154

[3] Ibrahim N, Handa H, Cosset A, Koulintchenko M, Konstantinov Y, Lightowlers RN, Dietrich A, Weber-Lotfi F (2011) Pharm. Res. 28:2871–2882

[4] Boesch P, Ibrahim N, Paulus F, Cosset A, Tarasenko V, Dietrich A (2009) Nucleic Acids Res. 37:5690-5700

[5] Boesch P, Ibrahim N, Dietrich A, Lightowlers RN (2010) Nucleic Acids Res. 38:1478-1488

[6] Mileshina D, Koulintchenko M, Konstantinov Y, Dietrich A (2011) Nucleic Acids Res. 39:e115

Frederique Weber-Lotfi[1,*], Noha Ibrahim[1], Milana Koulintchenko[1,2], Daria Mileshina[1,2], Yuri Konstantinov[2], Robert Lightowlers[3] and Andre Dietrich[1]

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We previously demonstrated that isolated plant, mammalian and *Saccharomyces cerevisiae* mitochondria are able to import double-stranded DNA through an active mechanism [1-3]. The imported DNA can be transcribed, repaired or integrated into the genome *in organello* [4-6]. The voltage-dependent anion channel seems to be involved in DNA translocation through the mitochondrial outer membrane. For the inner membrane, inhibition studies of the uptake using specific effectors pointed to an involvement of the adenine nucleotide translocator in plants, but the challenge of understanding which channel(s) can be recruited or hijacked by double-stranded DNA molecules remains mostly open. In the present studies, we used both biochemical approaches and *S. cerevisiae* genetic tools to identify the still elusive inner membrane proteins participating in mitochondrial DNA import. Strikingly, among the candidates from the inner membrane carrier family selected on the basis of biochemical data with plant organelles, only the two minor forms of the adenine nucleotide translocator turned out to be required for optimal DNA translocation into isolated yeast mitochondria. Conversely, we highlighted a putative contribution of Mdm33, a protein that controls mitochondrial morphology in *S. cerevisiae*. Taken together, our data suggest that there are significant variations in the mitochondrial DNA import mechanism between different organisms and that even in a given organism multiple pathways might operate.

[1] Koulintchenko M, Konstantinov Y, Dietrich A (2003) EMBO J. 22:1245-1254

[2] Koulintchenko M, Temperley RJ, Mason PA, Dietrich A, Lightowlers RN (2006) Hum. Mol. Genet. 15:143-154

[3] Weber-Lotfi F, Ibrahim N, Boesch P, Cosset A, Konstantinov Y, Lightowlers RN, Dietrich A (2009) Biochim. Biophys. Acta 1787:320-327

[4] Boesch P, Ibrahim N, Paulus F, Cosset A, Tarasenko V, Dietrich A (2009) Nucleic Acids Res. 37:5690-5700

[5] Boesch P, Ibrahim N, Dietrich A, Lightowlers RN (2010) Nucleic Acids Res. 38:1478-1488

[6] Mileshina D, Koulintchenko M, Konstantinov Y, Dietrich A (2011) Nucleic Acids Res. 39:e115

SPECIFICITY OF DNA IMPORT INTO ISOLATED MITOCHONDRIA OF PLANTS AND MAMMALIANS

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It is well known that the horizontal gene transfer (HGT) is one of the substantial factors mainly in evolution of bacteria. Recent studies indicate that plant mitochondria are unusually active in HGT relative to all other organelles of multicellular eukaryotes. In addition to a large and complex main mitochondrial genome plant mitochondria contain small circular and linear DNAs regarded as extrachromosomal replicons or plasmids. Linear mitochondrial plasmids are present in many fungi and in some plants, but they seem to be absent from most animal cells. They usually have a common structural feature, called an invertron that is characterized by the presence of terminal inverted repeats (TIRs) and proteins covalently attached to their 5' termini. The Brassica 11.6-kb plasmid, one of the linear mitochondrial plasmids in plants, shows a non-maternal inheritance, in contrast to mitochondrial genomes. The origin of these plasmids is unknown, but indirect evidence indicates the possibility of horizontal transfer from fungal mitochondria. Those particularities allow to assume that plant mitochondria might possess a mechanism of a natural competence to take up foreign DNA, resembling that of the process in bacterial cells. To study DNA import into plant mitochondria and into human mitochondria we used the 11.6 kb linear plasmid from rapeseed (Brassica napus L.). This plasmid is characterized by the terminal inverted repeats present at each end of the molecule. It was shown that (1) the efficiency of the import of large DNA molecules into plant mitochondria depends on the sequence; (2) the specificity of DNA import is mediated by the presence of certain elements in their sequence, namely TIRs at the 5' and 3' end of the molecules. Conversely, efficiency of DNA import into mammalian mitochondria depended neither on the DNA sequence, nor on its size. For DNA molecules with "medium" sizes, between 4-7 kb, it was established that import into plant mitochondria barely has any sequence specificity and does not clearly depend on the DNA size. The structural features of linear plasmids present in mitochondria of many plant species could be a convenient tool to investigate the mechanisms of DNA transfer through mitochondrial membranes and serve as mitochondrial replicative vectors.

The work was financially supported by a grant from the Russian Fund for Basic Research 12-04-01400.

INFLUENCE OF MULTIFUNCTIONAL PROTEIN YB-1 ON THE ENZYMES INVOLVED IN THE REPAIR OF AP-SITES IN DNA

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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) pathway. Multifunctional protein YB-1 is known to participate in cellular response to genotoxic stress and was shown to interact with several components of BER – DNA glycosylases NTH1, NEIL2, DNA polymerase beta and DNA ligase III. Here we investigate the influence of YB-1 on one of the major BER enzymes, responsible for AP site cleavage, AP endonuclease APE1, and on bifunctional DNA glycosylase NEIL1, participating in APE1 independent pathway of AP site repair, as well on the enzyme exhibiting dRP-lyase activity during BER – DNA polymerase beta.

YB-1 was shown to inhibit the cleavage of AP site located in single-stranded DNA and in unpaired "bubble" DNA structures by both APE1 and NEIL1. "Bell-shape" stimulation of APE1 activity by moderate amounts of YB-1 was observed on double-stranded DNA and DNA containing AP-site in combination either with mispaired bases or oxidative lesion (5-fluorouracyl) in +1 position of the opposite DNA strand. Excess YB-1 was shown to have inhibitory effect on APE1. NEIL1 was stimulated by YB-1 on the same substrates over the wide concentration range. At the same time NEIL1-mediated cleavage of the 5-foU containing strand was suppressed by YB-1, when this lesion was located clustered to AP-site. Apart from that, YB-1 was shown to modulate dRP-lyase activity of DNA polymerase beta. These data indicate that YB-1 can modulate the repair of DNA, containing clustered lesions, both by stimulating AP-site repair and also serve as coordinator of short and long patch pathways of the repair. On the other hand, YB-1 may have a function in avoiding a possible generation of doublestrand breaks and frameshift mutations, arising from the cleavage of single-stranded portion of DNA substrate already used by different DNA-processing pathway.

This work was supported by RFBR grants no 11-04-00559, 12-04-00178, 12-04-33162

IMPACT OF THE YB-1 PROTEIN ON RECOGNITION AND REPAIR OF DNA DAMAGES

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YB-1 is a multifunctional DNA-, RNA-binding protein, composed of three main domains: A N-terminal alanine-proline rich domain, an evolutionarily conserved cold-shock domain, and a large C-terminal domain. 3D structure was determined just for CSD, and, in contrast, AP and C-terminal parts of YB-1, according to theoretical predictions, should be unstructured. In the cell cytoplasm, YB-1 is involved in many critical processes like the regulation of translation, mRNA stabilization and due to its ability to interact with actin and tubulin, in mRNA localization. It has also been determined that YB-1 can perform some functions in the nucleus, like regulation of transcription, splicing of RNA and perhaps repair of DNA lesions. In this studying we tried to investigate which role can YB-1 play in DNA repair process.

Many authors reported that treatment of cells with different DNA-damaging drugs induce the translocation of YB-1 into the nucleus. However these reports are contradictory regarding drugs and conditions that can trigger such effect. We thus decided to first tackle this question using a series of drugs, each of them having a specific DNA damaging mechanism. As a marker of DNA-damage we used antibody anti-γH2AX, which label double-stranded breaks. Unexpectedly, we found that among the drugs tested, actinomycin D only may induce some cytoplasmic-nuclear redistribution of YB-1, however co-localization with γH2AX was not detected.

In addition, it was previously proposed that nuclear translocation of YB-1 resulted from its specific cleavage by 20S proteasome which produces a truncated form of YB-1. We thus produced eukaryotic vector coding for the corresponding short YB-1 and found that such truncated YB-1 mostly localize to the nucleus. We also observed that this part of YB-1 has a particular affinity to the nucleolus region and co-localizes with UBF, a transcription factor of RNA-polymerase I. Finally, the treatment of cells, which overexpressed the truncated form of YB-1, with actinomycin D induced the dissociation of nuclear speckles and also provoked translocation of short YB-1 into the cytoplasm. Together this data indicate that a short form of YB-1 can be detected in nucleolus where it may play a specific role linked to transcription or nucleic acid damage protection.

BIOMOLECULAR INTERACTIONS ON SURFACE INVESTIGATED AT HIGH RESOLUTION BY AFM

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Atomic Force Microscopy is a powerful technique for studying interactions between biomolecules at the nanometric scale. Before imaging, biomolecules like DNA or nucleoprotein complexes should be adsorbed and immobilized on a substrate. The interaction between the biomolecules and the substrate should preserve the structure and reactivity of interacting biomolecules, even if the adsorption leads to geometrical rearrangements from 3D in solution to 2D on the surface. A good compromise lies in a weak adsorption allowing biomolecule diffusion on surface and thus unaltered interactions with their partners.

Even though numerous substrates have been used up to now, we believe, that mica surface, the first that has been used decade ago, is still the best combining atomic flatness and weak electrostatic interactions between the substrate and the biomolecules.

In the last 10 years, we have focused our attention first on the mechanism of the DNA adsorption on mica by multivalent ions and the reactivity of adsorbed biomolecules toward their partners. The results after these fundamental investigations enable us to reveal by AFM some major structural changes on nucleoprotein filaments or to image site-specific DNA protein complexes. For example, we detect at the single molecule level, the interaction of proteins implicated in DNA repair like PARP-1 and PARP-2 with specific site (AP sites) on long DNA fragments. Other parts of our activity concern the imaging by AFM of RNA / protein complexes or the influence of chemicals on microtubule stability.

ACTIVE DNA DEMETHYLATION AND EPIGENETIC REPROGRAMMING: INSIGHT INTO MECHANISM OF RECOGNITION OF 5-HYDROXYMETHYLURACIL BY METHYL-BINDING DOMAIN PROTEIN 4

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Changes in DNA methylation patterns are generally associated with epigenetic changes in human cancers. Many human cancers commonly demonstrate global DNA hypomethylation concomitant with specific hypermethylation of tumor-suppressor genes. Active DNA demethylation in mammals occurs via hydroxylation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) by the Ten-eleven translocation family of proteins (TETs). 5hmC residues in DNA can be further oxidized by TETs to 5-carboxylcytosines and/or deaminated by the AID/APOBEC family proteins to 5-hydroxymethyluracil (5hmU). Excision and replacement of these intermediates is initiated by DNA glycosylases such as thymine-DNA glycosylase (TDG), methyl-binding domain protein 4 (MBD4) and single-strand specific monofunctional uracil-DNA glycosylase 1 (SMUG1) in the base excision repair pathway (BER). These findings suggest a new unexpected role of the mismatch-specific thymine/uracil DNA glycosylases in the control of epigenetic information via removal of oxidation/deamination products of 5mC.

Here, we report detailed biochemical and structural characterization of human MBD4 which contains mismatch-specific TDG activity. Full-length as well as catalytic domain (residues 426–580) of human MBD4 (MBD4cat) can remove 5hmU when opposite to G with good efficiency. Here, we also report six crystal structures of human MBD4cat: an unliganded form and five binary complexes with duplex DNA containing a T•G, 5hmU•G or AP•G (apurinic/apyrimidinic) mismatch at the target base pair. These structures reveal that MBD4cat uses a base flipping mechanism to specifically recognize thymine and 5hmU. The recognition mechanism of flipped-out 5hmU bases in MBD4cat active site supports the potential role of MBD4, together with TDG, in maintenance of genome stability and active DNA demethylation in mammals.

THE MECHANISM OF DNA LESION SEARCH BY HUMAN 8-OXOGUANINE DNA GLYCOSYLASE

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Extensive structural studies of human DNA glycosylase hOGG1 have revealed the substantial conformational changes of enzyme molecule; however, at present there is little information about the time scale of rearrangements of protein structure as well as dynamic behavior of individual amino acids. Here we examined the role of certain catalytically important amino acids in hOGG1 enzymatic pathway and described their involvement in the step-by-step mechanism of oxidative DNA lesion recognition. The conformational dynamics of hOGG1 wild-type and mutants Y203W, Y203A, H270W, F45W, F319W and K249Q and DNA-substrates were investigated by the fluorescence stopped-flow method.

The analysis of kinetic data obtained in this study significantly improves understanding of the step-by-step molecular mechanism of hOGG1 lesion recognition process. Our data showed that the function of Tyr-203 residue is not only to conserve of the kinked state of DNA duplex but to serve as "lesion-sensor needle" in the discrimination between normal and damaged bases. Also we demonstrate that Lys-249 plays an important role in the early step of the damaged nucleotide binding and flipping out process. The suggestion that Asp-268 residue is responsible for hydrolysis of N-glycosidic bond, whereas Lys-249 is a key amino acid in sugar-phosphate bond cleavage (β-elimination reaction) was confirmed. The role of His-270 in the oxoG flipping out process was identified.

This study was made possible by grants from the Program of the Russian Academy of Sciences "Molecular & Cell Biology" (6.11), RFBR (13-04-00013 and 12-04-31066) and Russian Ministry of Education and Science (SS-64.2012.4, SP-4012.2013.4, 8092, 8473 and 14.B37.21.0195), the Grant from Russian Government to support leading scientists (No. 11.G34.31.0045) to N.A.K., A.A.K. and O.S.F, as well as the grants [ANR Blanc 2010 Project ANR- 09-GENO-000, PICS N5479, RB 2013] to M.K.S. and [#2012 00029161] to A.A.I.

THE KINETIC MECHANISM OF HUMAN APURINIC/APYRIMIDINIC ENDONUCLEASE 1 IN NIR PATHWAY

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The major human apurinic/apyrimidinic endonuclease 1 (APE1) is a key enzyme in the base excision repair (BER) and nucleotide incision repair (NIR) pathways. BER is initiated by DNA glycosylases, excising the damaged and/or mispaired bases to produce apurinic/apyrimidinic sites (AP sites). AP sites are generated also through the spontaneous loss of bases (mainly purines). During BER pathway DNA in human cells is hydrolytically nicked 5' to the AP site by APE1. Repair of certain base lesions can be initiated directly by the AP endonucleases alone in NIR, by-passing the DNA glycosylase step. During this process, an AP endonuclease introduces a nick 5' to the damaged deoxynucleotide, generating a 3'-hydroxyl terminus and a 5'-phosphate terminus. Thus, the NIR pathway avoids the formation of potentially toxic AP-intermediates. Using a stopped-flow fluorescence method we analyzed the conformational dynamics and kinetic mechanism of wild-type APE1 and its mutants APE1K98A and NΔ61APE1. DNA substrates used in this study contained AP site, tetrahydrofuran, 5,6-dihydrouridine (DHU) or α-2'-deoxyadenosine. Our data suggest that APE1 can pre-exist in two conformations and that the conformational selection and induced fit occur during the enzyme action. The enzyme release from the complex with the nicked DNA product limits the overall NIR process and determines its rate in the steady-state conditions. The comparison of the kinetic constants of mutants to those of wtAPE1 provides us with a view of the roles of lysine-98 and of REF1 domain. We have shown that during both BER and NIR pathways Lys98 is important in the 5'-phosphodiester bond hydrolysis of DNA substrate. This amino acid substitution influences the catalysis in NIR more extensively, than in BER pathway. The REF1 is required for the 5'-phosphodiester bond hydrolysis in NIR, but not in BER pathway. Our data reveal that APE1 uses the same active site to catalyze the cleavage of DHU- and AP-substrates. The protein is probably able to form different conformations in the region of the active site, which are responsible for the incision of such structurally unrelated lesions as AP site and DHU.

This study was made possible by grants from the RAS Program "Molecular & Cell Biology" [6.11], RFBR [13-04-00013] and RMES [SS-64.2012.4, 8092, 8473, 11.G34.31.0045] to N.A.T., V.V.K. and O.S.F, as well as the grants [ANR Blanc 2010 Project ANR- 09-GENO-000, PICS N5479, RB 2013] to M.K.S. and [#2012 00029161] to A.A.I.

PARG FACILITATES CELLULAR RECOVERY FROM PROLONGED REPLICATION STRESS

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Poly(ADP-ribosyl)ation is a post-translational modification of proteins involved in a wide number of biological processes including DNA repair, transcription and cell death. The poly(ADP-ribose) cellular level is regulated by the poly(ADP-ribose) polymerases (PARPs) and the degrading enzyme poly(ADP-ribose) glycohydrolase (PARG), influencing the cell fate decision between life and death in response to DNA damage. Using shRNA to prevent the expression of all PARG isoforms in a cellular model (shPARG), we have shown that the absence of PARG increased radiosensitivity and affected the repair of radioinduced single (SSB) and double (DSB) strand breaks (1). We have also demonstrated a functional link between PARG and the repair/replication factor PCNA (2): binding to PCNA contributes to PARG recruitment to laser induced DNA damage sites and to replication foci. The latter observation prompted us to investigate the contribution of PARG in DNA replication. shPARG cells showed increased sensitivity to hydroxyurea (HU), a drug triggering replication arrest, or replication fork collapse and DSB after long treatment. shPARG cells treated with long but not short HU treatment showed defect in S-phase restart, strong PAR synthesis, increased □H2AX but decreased RPA hyper-phosphorylation, supporting a complex role for PARG in the processing of collapsed fork and associated DSB that is under investigation.

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Project funded by CNRS, Université de Strasbourg, LABEX Medalis, Ligue contre le Cancer (Equipe Labellisée), EDF.

INTERACTIONS OF HMGB1 AND HMGB2 PROTEINS WITH APURINIC/APYRIMIDINIC SITES IN DNA

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High-mobility group box 1 protein (HMGB1), abundant multi-functional non-histone chromatin protein, has been shown previously to interact with apurinic/apyrimidinic (AP) sites via covalent intermediate, Schiff base [Prasad et al., 2007], but the functional significance of this interaction has not been investigated. The closest homolog of HMGB1 is HMGB2. In this work we made a comparative analysis of the interaction of HMGB1 and HMGB2 proteins with AP sites, single or in clustered damages. The clustered damages in DNA were represented by AP site in one DNA strand and AP site (or its analog) in the complementary strand, with the distance between AP sites being within 1.5 turns of DNA helix. Recognition and cleavage of the AP sites by both HMGB-proteins depend on the presence and location of additional AP site. In general, HMGB-proteins more efficiently interact with AP sites in clusters, especially when the distance between the AP sites is less than one turn of DNA helix. Both HMGB-proteins were shown to inhibit the activity of key enzymes of base excision repair – AP endonuclease 1 and DNA polymerase β. HMGB2 was found to interact more efficiently with DNA compared to HMGB1. A comparative analysis of profiles of the protein cross-linking to different AP DNA in HeLa cell extract and purified HMGB1 allowed to attribute cross-links of extract proteins to HMGB1, suggesting a possibility of interactions of HMGB1 with AP DNA in the presence of other DNA-binding proteins. Peptide mass mapping (based on MALDI-TOF-MS analysis) of cell extract protein(s) cross-linked to AP DNA revealed both HMGB1 and HMGB2 proteins.

This work was supported by RFBR grant 12-04-31664 and by RAS program “Molecular and Cellular Biology”.

COMPARATIVE ANALYSIS OF INTERACTION OF HUMAN AND YEAST DNA DAMAGE RECOGNITION COMPLEXES WITH DAMAGED DNA IN NUCLEOTIDE EXCISION REPAIR

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The human XPC-RAD23B complex and its yeast ortholog, Rad4-Rad23, are the primary initiators of global genome nucleotide excision repair (GG-NER). The interaction of these proteins with damaged DNA was analyzed using model DNA duplexes containing a single fluorescein-substituted dUMP analog as a lesion. An electrophoretic mobility shift assay revealed similarity between human and yeast proteins in DNA binding. Quantitative analyses of XPC/Rad4 binding to the model DNA structures were performed by fluorescent depolarization measurements. XPC-RAD23B and Rad4-Rad23 proteins demonstrate approximately equal binding affinity to the damaged DNA duplex. Both proteins bind to the damaged 15 nt bubble-DNA structure mimicking the intermediate of preincision stage in GG-NER or the "transcription bubble" with the highest affinity that is reduced in the following order: damaged bubble>undamaged bubble>damaged duplex>undamaged duplex. The affinity of XPC/Rad4 for various DNAs was shown to correlate with DNA structure abnormalities. Using photoreactive DNA containing 5-iodo-dUMP in defined positions, XPC/Rad4 location on damaged DNA was shown. Under conditions of equimolar binding to DNA both proteins exhibited the highest level of crosslinks to 5I-dUMP located exactly opposite the damaged nucleotide. The positioning of the XPC and Rad4 proteins on damaged DNA by photocrosslinking footprinting is consistent with X-ray analyses of the Rad4-DNA crystal complex. The identity of the XPC and Rad4 location illustrates the common principles of structure organization of DNA damage-scanning proteins from different eukaryotes.

This work was supported by RFBR (Nos. 12-04-31323, 12-04-33162, and 13-04-00538); the Russian Ministry of Education and Science (contract 14.B37.21.0188); the Program of the Presidium of RAS 'Molecular and Cellular Biology'.

INTERACTION OF DDC1 AND RPA WITH SINGLE-STRAND/DOUBLE-STRAND DNA JUNCTIONS IN CELL FREE EXTRACTS OF SACCHAROMYCES CEREVISIAE

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DNA lesions and structural alterations induce binding of repair and checkpoint proteins to site of DNA damage to initiate DNA damage response. Genetic and biochemical studies in S. cerevisiae have shown that Ddc1-Mec3-Rad17 checkpoint clamp and RPA act at the early steps of the checkpoint response, recognizing DNA repair/replication intermediates containing single-stranded (ss) regions and single to double-stranded (ds) DNA junctions. However structural requirements for interaction of the proteins with DNA at the molecular level have remained obscure. In this study, we have prepared photoreactive partial DNA duplexes containing 3′-recessed end (5′-ss/dsDNA) or 5′-recessed end (3′-ss/dsDNA) and used it to label proteins that interact with these DNA substrates in cell extracts from wild-type or checkpoint mutant cells. The resulting photocrosslinked proteins were analyzed by peptide mass fingerprinting. We found that Ddc1 was photocrosslinked by the 5'-ss/dsDNA substrates independently of the other components of checkpoint clamp. We did not observe any detectable crosslinking of subunits of Ddc1-Mec3-Rad17 clamp with 3'-ss/dsDNA substrates in cell extract. We found that p70 subunit of RPA (RPA p70) is predominant crosslinking product with 3'-ss/dsDNA substrates. Thus, the Ddc1 and RPA may have preference for binding of certain DNA structures and recognize different types of DNA damage. Extracts deleted for Ddc1 (ddc1) did not display labeling of full-length RPA p70 with neither 5'-ss/dsDNA nor 3'-ss/dsDNA substrates. We found that RPA p70 undergoes a proteolytic cleavage in ddc1 extract. The cleavage of RPA p70 is suppressed by specific proteasome inhibitor MG 132, indicating involvement of proteasome in the degradation. Addition of purified Ddc1 or C-terminal fragments of Ddc1 to ddc1 extract slightly inhibits of RPA p70 cleavage. Thus the high levels of proteolytic activity observed in ddc1 extract can be partially suppressed by Ddc1. The results reveal a novel and potentially important biochemical property of Ddc1 in preventing proteolytic degradation of the DNA binding protein, such as RPA.

The work was supported by RFBR (grants № 11-04-00559, 12-04-92601, 12-04-33162); Program of RAS "Molecular and Cell Biology".

CIRCULATING DNA FOR CANCER DIAGNOSTICS: CONCENTRATION, EPIGENETIC CHARACTERISTICS, ASSOCIATION WITH BLOOD COMPLEXES AND CELLS

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Circulating nucleic acids (cirNA) were found in the blood as cell-free or being absorbed at cell surface. Cell-free nucleic acids (cfNA) are more fragmented as compared to cell-surface bound nucleic acids (csbNA) and are integrated into complexes with proteins or membrane-bearing particles. Whether csbNA are packed into specific structures is not clear, however certain cell-surface proteins were shown to bind "naked" NA and mediate their transport into nuclear cells. Naturally occurring complexes of cfNA with biopolymers along with exosomes, microparticles can also be absorbed at cell surface and transported into cells. Ratio of cfNA to csbNA in the blood depends from the state of a donor: main part of cirNA from healthy subjects are bound with cells, whereas cancer patients have a different distribution of cfNA and csbNA depending from tumor type. CirDNA epigenomic studies provide promising data for development of non-invasive cancer biomarkers based on bisulfite–converted DNA or methyl-CpG binding protein–enriched DNA. CsbDNA fraction from cancer patients contains aberrantly methylated DNA originated from cancer cells and along with cfDNA represents the valuable source of material for cancer diagnostics. As far as apoptosis is the main source of cirDNA, comparative study using massive parallel sequencing of cell-free apoptotic DNA was made which demonstrates overrepresentation of coding DNA sequences in apoptotic DNA as compared with genome DNA. Functional analysis of the overrepresented peaks was made using data from UCSC Genome Bioinformatics Site which revealed that apoptotic DNA is enriched with hypermethylated CpG-rich regions and regions containing DNase I hypersensitive sites.

The research has been carried out with support of the grants from RFBR № 11-04-12105-offi-m-2011, SB RAS Program in collaboration with other scientific organizations № 65.

INTRACELLULAR TARGETS OF IMMUNOINHIBITING EXTRACELLULAR DNA

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Extracellular DNA (exDNA) bound to the cell surface (csbDNA) via DNA–protein interactions, inhibit poly(I:C)-induced production of proinflammatory interleukins by human umbilical vein endothelial cells (HUVEC) [1]. In this study cellular targets of immunoinhibiting csbDNA have been identified using ODNs containing the nucleotide motifs frequently found in csbDNA and displaying the same immunoinhibiting effect.

Complexes of [32P]-labeled ODNs (ss- and ds-ODNs) with biopolymers of membrane–cytosolic extracts and living HUVEC, including the composition and the affinity, have been studied by electromobility shift assay (EMSA). ODN-binding proteins were identified by MALDI-TOF mass spectrometry after the isolation of the complexes containing biotinylated ODNs followed with PAGE.

Both ss- and ds-ODNs form strong ODN–protein complexes with similar electrophoretic mobility after incubation with the membrane–cytosolic extracts of HUVEC either when added extracellularly or lipofected into cells. Cross-competition experiments and trypsin treatment demonstrate the involvement of the same proteins in the binding of ss- and ds-ODNs. ODN-binding proteins were identified as DNA-binding components of DNA-dependent protein kinase (DNA-PK), namely, Ku70 and Ku80 proteins and confirmed by supershift assay with anti-Ku70 monoclonal antibodies. High affinity for DNA, diverse cellular localizations and functions of Ku70/80 suggests its possible involvement in the csbDNA binding both on the cell surface and inside the cell, penetration of exDNA into the cell and cellular compartments and mediation of cirDNA biological effects. Whether Ku is directly involved in dsRNA signaling or have auxiliary functions, mediating ODN localization or polyIC-proteins interactions, remains to be investigated.

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RECODING UGA AS SELENOCYSTEINE: IDIOSYNCRATIC AND SHARED FACTORS FOR mRNP ASSEMBLY AND TRANSLATION

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About forty years ago, the readout of the genetic code was found more flexible than previously envisioned. As a matter of fact, certain circumtances make that stop codons switch from nonsense to sense, as examplified by UGA that can encode the selenium-containing amino acid selenocysteine (Sec) found in selenoproteins. These proteins, carrying Sec in the active site, can fulfill as varied functions as defence against reactive oxygen species, sperm and thyroid hormone maturation, muscle biogenesis, etc... Reading selenocysteine instead of stop at the UGA codon is a complex event that requires both dedicated RNAs and proteins but also general factors. Mutations in some of these components or in the selenoproteins themselves may lead to embryonic lethality or various pathologies, attesting the importance of selenoproteins in health and disease. At the molecular level, the central question is to understand how assembly of a selenoprotein mRNA-protein complex occurs in an orderly and temporal fashion so that approaching ribosomes are cleared at the UGA Sec codon. Recent data will be presented, providing novel insight into selenoprotein mRNA interaction with the ribosome (in collaboration with the group of Prof. Galina Karpova, Institute of Chemical Biology and Fundamental Medicine, SB RAS, Novosibirsk).

EUKARYOTE-SPECIFIC FEATURES OF mRNA PATH IN THE HUMAN RIBOSOME

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Protein synthesis is conducted by ribosomes, very complicated cellular ribonucleoprotein machineries translating genetic information incoming as mRNA. Specific interactions of ribosomal ligands (tRNAs, mRNA, etc.) with the ribosome underlie the work of the ribosomal machinery, and knowledge of the structural basis of these interactions is of principal importance. These interactions in prokaryotes are known at the atomic level due to X-ray crystallography that has not yet been applied to ribosomes from higher eukaryotes. We determined fine structure of the mRNA binding channel of the human ribosome at the level of ribosomal protein peptides neighboring mRNA using a set of labeled mRNA analogues bearing cross-linker at designed locations and an original methodology for mapping of cross-links on ribosomal proteins (rps). We found that major part of the protein environment of mRNA in the ribosome corresponds to peptides that have no homology in bacterial ribosomal proteins. So, mRNA region 5' of the codons interacting with tRNAs neighbors mainly an eukaryote-specific peptide of rpS26e [1], and eukaryote/archaea-specific decapeptide of rpS15e is located at the ribosomal decoding site [2]. Several hypotheses concerning roles of these peptides in translation are discussed. We discovered also a peculiarity of the translation process in eukaryotes that principally differs it from the analogous process in bacteria. It turned out that human ribosomes, in contrast to bacterial ones, require the participation of 2'-OH of mRNA for accommodation of the codon-anticodon duplex to the ribosomal P site, which could be essential for start codon selection during translation initiation.

This study was supported by RFBR grant # 11-04-00597 to G.K. and by the Russian Academy of Sciences Presidium program "Molecular and cell biology" (grant to G.K.).

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Lys53 OF RIBOSOMAL PROTEIN L36AL IS CLOSE TO THE CCA END OF A tRNA AT THE P/E HYBRID SITE ON THE HUMAN RIBOSOME

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Using a set of periodate-oxidized tRNAs (2', 3'-dialdehyde derivatives of tRNA), we have revealed that the CCA end of the P-site-bound tRNA can cross-linked to ribosomal protein RPL36AL of the large subunit of the human ribosome [1]. This protein belongs to the L44e protein family present in all eukaryotic and archaeal ribosomes, and it is known that its homolog, RPL44, is located at the archaeal ribosomal E site.

We have shown that:

1) the cross-link of RPL36AL to the CCA end of tRNA is specific for a tRNA at the P/E hybrid site, as a tRNA in all other tRNA positions of pre-translocational ribosomes could not be cross-linked to this ribosomal protein;

2) the cross-link is formed most efficiently with C74 and C75 of the P/E-tRNA than with the ultimate A of the tRNA;

3) Lys53 of protein RPL36AL cross-links to these tRNA nucleotide residues;

4) Lys53 is located close to a conserved 49GGQ51 motif that is present in the proteins of the L44e family in eukaryotic 80S ribosomes and that is identical to the universally conserved motif of class 1 release factors implicated in promoting peptidyl-tRNA hydrolysis.

Possible implication of RPL36AL in the hydrolysis of peptidyl-tRNA during transpeptidation is discussed.

This work was supported by grants from the Russian Foundation for Basic Research (11-04-00597-a) to G.K.

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"UNUSUAL" RNA BASE PAIRS IN RECOGNITION AND DECODING

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RNA molecules are characterized by the formation of hydrogen-bonded pairs between the bases along the polymer. All base-base interactions present in nucleic acids, involving at least two "standard" H-bonds, can be classified in twelve families where each family is a 4x4 matrix of the usual bases. The common Watson-Crick pairs belong to one of these families and the other eleven families gather the non-Watson-Crick pairs. The Watson-Crick pairs form the secondary structure and all the other families are critical for the tertiary structure. In several of those twelve families, the 4x4 matrix is partially filled because only some base-base oppositions are able to lead to the formation of two "standard" H-bonds with proper geometry and distances. Some of the missing base-base oppositions are, however, observed with "unusual" features in an increasing number of crystallographically defined crystal structures. Such "unusual" base pairs involve protonation of one of the base, bifurcated H-bonds, water-mediated base pairing, and especially tautomeric forms of the bases. The origins of those observations can be ascribed partly to the free energy of crystal formation and packing together with the law of mass action in the absence of ligand competition. But, most importantly, the observations stress the dominant role of active site tightness in fidelity: the ribosome binds most favorably base pairs with Watson-Crick geometry and selects accordingly the correct codon-anticodon complex. Various environmental (ions, antibiotics) or structural factors (rRNA or tRNA mutations) can contribute positively or negatively to the overall free energy of selective binding leading to permissive or hyper-accurate ribosomes.

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NEW FEATURES OF TELOMERASE RNA PROCESSING

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Telomerase, a ribonucleoprotein, is responsible for the maintenance of eukaryotic genome integrity by replicating the ends of chromosomes. The core enzyme comprises the protein TERT, conserved among the species, and an RNA subunit (TER) that, in contrast, displays large variations in size and structure.

We identified the RNA telomerase component from thermotolerant yeast H. polymorpha and describe its structural features. Application of site directed mutagenesis and 3' RACE allowed us to determine that HpTER is processed by first splicing event.

Human telomerase RNA is processed from primary RNA polymerase II transcript. To investigate the details of hTR 3'-end processing we created stable cell lines where hTR gene with its 3'-end flanking region is followed by a region coding for IRES and GFP. Additionally, a similar construct coding for hTERT and tdTomato proteins was used to analyze the influence of hTERT overexpression on efficiency of hTR 3'-end processing. As a result we could suggest endonuclease cleavage of long hTR transcript as a primary event in hTR processing. The region of the transcript downstream from the cleavage site appears to be relatively stable and could play a role in telomere length maintenance.

NEW PLAYERS IN RECOGNITION OF AP SITES: IMPLICATION IN DNA REPAIR

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The apuririnic/apyrimidinic (AP) sites are considered to be common lesions in genomic DNA, arising at a frequency of 10,000 to 50,000 lesions per mammalian cell per day. If unrepaired, AP sites present mutagenic and cytotoxic consequences to the cell. Attempted repair of bistranded AP sites (i.e. situated in both DNA chains) can result in formation of double-strand breaks – the most deleterious DNA lesion. One can suggest an existence of proteins that are able to specifically interact with AP sites and regulate their processing.

Along with intact AP site, the products of AP site cleavage via β-elimination and hydrolysis – 4-hydroxypenten-2-al at the 3' end (3'-PUA) and 5'-dRP, respectively, are also able to form Schiff base intermediates with primary amino groups of proteins. The Schiff base can be reduced by NaBH4 treatment that results in formation of an irreversible covalent bond between protein and DNA.

DNA containing AP sites (isolated or in clustered DNA damages) were used to trap in mammalian cell extracts proteins interacting with AP sites. Cross-linked proteins were identified by peptide mass mapping (based on MALDI-TOF-MS analysis) and immunochemical approaches. Among proteins reactive to AP sites: high-mobility group box 1 and 2, abundant multi-functional non-histone chromatin proteins, poly(ADPP-ribose) polymerase 1, Ku80 subunit of Ku antigen and AP- endonuclease 1 were identified. Interaction of some proteins reactive to AP sites with DNA containing isolated and bistranded AP sites and their influence on AP site processing by AP enonuclease1 were studied in reconstituted systems.

This work was supported by RFBR grant 13-04-01426, by RAS program on Molecular and Cellular Biology and by the Ministry of Education and Science of Russia, project 14.B37.21.0188.

IDENTIFICATION OF RECOMBINANT PRODUCTS OF NON-ENZYMATIC REACTION OF RNA CLEAVAGE/LIGATION

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Studies of the last decades provided evidences that "there was once an RNA world" – time period, when RNA polymers served as "carriers" of the genetic information and also provided some catalytic activities necessary for its replication. Regarding to the hypothesis, the primitive RNA molecules have to be synthesized of the RNA monomers, then at the posterior stages, the RNA molecules had to be replicated via template-directed ligation reactions. One important obstacle of this pathway is an emergence of the novel sequences including catalytic ribonucleic acids, as far as not all sequences could be replicated via template-directed polymerization.

We assumed that novel RNA molecules could be formed in the recombination process consisting of two consecutive cleavage and ligation reactions. Both reactions can proceed in RNA molecules due to the presence of 2'-hydroxyl group in ribose and its ability to form 2', 3'-cyclic phosphate. RNA fragments with 5'-hydroxyl group and 2', 3'-cyclic phosphate, formed as a result of RNA cleavage, could cross-interact with each other forming new phosphodiester linkages and yielding new RNA molecules.

Here we describe results of our studies on the consecutive cleavage/ligation reaction of short (about hundred nucleotides long) fragments of viral RNA, proceeding in the presence of magnesium ions. Cleavage/ligation of the RNA was shown to occurre even in the absence of metal ions and its efficiency demonstrated an escalating pH – yield profile within pH interval from 6.0 to 8.8, which suggests base catalysis of the reaction proceeding via increasing of nucleophilic properties of the attacking 5'-hydroxyl group. We show, that these consecutive cleavage/ligation reaction of the fragments of viral RNAs results in the formation of the wide diversity of new products of RNA recombination. We found that efficient RNA ligation occurs mostly in internal loops, internal bulges and in stem structures, turned into stem-loops upon ligation.

This work was supported by RAS programs "Molecular and cellular biology" and "Fundamental sciences to medicine 2012-24", Scientific school № 2972.2012.4.

NUCLEIC ACID BASED ANTICANCER THERAPEUTICS

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Short double-stranded RNAs containing specific sequences are able to activate the mammalian innate immune system. The immunostimulatory activity of dsRNA can be applied to antiviral and antitumor therapy. Here we identified a set of 19-bp RNA duplexes with 3 nt overhangs in the 3'-ends that display immunostimulating activity (here and after ImmunoStimulating RNA or isRNA) and studied their sequence/activity relationships. It was found that the introduction of substitutuions in the middle part of isRNA sequence (10 – 16 positions counting from the 5'-end of strand 1) does not alter the antiproliferative activity; while substitutuions in the 3'-end region of isRNA substantially reduce it. isRNAs efficiently inhibit the proliferation of human oral epidermoid carcinoma cells (IC50 values varied from 10 to 100 nM). Our data demonstrated that antiproliferative effects of isRNAs are related to cell growth arrest, rather than the induction of apoptosis. These isRNAs strongly stimulate the synthesis of interferon-α (IFN-α), and to a lesser extent the synthesis of tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) in adherent peripheral blood mononuclear cells (PBMC). An intravenous injection of isRNA/Lipofectamine complexes into C57BL mice increases IFN-α and IL-6 levels in the blood serum up to 15-fold and 3-fold, respectively, compared to the control mice. The results of *in vivo* experiments show that single intravenous injection of isRNA complexed with transfection reagent efficiently increases the level of IFN-α, and to a lesser degree the level of pro-inflammatory cytokine IL-6 in mice blood serum. We found that isRNA reduces the metastases area in the liver, kidneys and heart of CBA/LacSto mice with hepatocarcinoma G-29 and cause a slight, but reliable inhibition of the primary tumor growth. The results of experiments with C57BL mice with implanted melanoma B16 show that isRNA efficiently inhibits tumor growth and metastasis spreading in the lungs. The results obtained clearly demonstrate the pronounced immunostimulatory and antiproliferative properties of the isRNAs under study. Hence, these short double stranded RNAs can be considered as potential agents for the therapy of oncological and viral diseases.

The work was financially supported by RAS programs Molecular and Cell Biology (no. 22-1) and Science to Medicine (no. 44), the Ministry of Science and Education of RF (no. 14.740.11.1058), RFBR (no.11-04-01017-a) and SB RAS (no.85).

IDENTIFICATION OF MOLECULAR TARGETS OF RNASE A IN ANTITUMOR THERAPY

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In recent years, ribonucleases are regarded as perspective anticancer drugs. Novel data, suggesting an important role of miRNAs in mediating tumor growth and invasion, have provided researchers with a new area to search for possible molecular targets of cytotoxic ribonucleases. Previously, antitumor and antimetastatic properties of pancreatic ribonuclease RNase A have been verified (Patutina O.A. et al., Biochimie 2011). It was found that observed tumoricidal activity of RNase A was accompanied by a reduction in pathologically elevated levels of extracellular RNAs and an increase in ribonuclease activity of blood plasma of tumor-bearing animals. In the present study, by high-throughput SOLiD sequencing technology we performed an analysis of genome-wide profiles of miRNAs in tumor and serum of mice after treatment with RNase A. Sequencing data revealed that RNase A therapy resulted in an apparent alteration in the levels of 215 serum and tumor-derived miRNAs. Analysis of miRNA expression profile showed that the pool of miRNA with most significantly changed expression level contained a considerable number of ascertained tumor-associated miRNAs, such as miRNAs from let7 family, miR-21, miR-10b, miR-145, miR-451a, miR-29b1, miR-17 and others. RNase-mediated downregulation of miR-10b, miR-145a, let7g and miR-451a was validated by qRT-PCR. The drop of miRNA expression was attended by an upregulation of tumor and metastasis suppressor genes Pten, Timp3, CD82 and Brms1. The obtained data give the evidence that antitumor and antimetastatic effects of RNase A is associated with alteration in miRNA profiles in tumor tissue and blood serum of animals promoting the strengthening of adhesive properties of tumor cells and impeding an ability to metastasize.

This work was supported by RAS programs “Molecular and cellular biology” and “Fundamental sciences to medicine 2012-24”, by RFBR grant 11-04-01012, Scientific school № 2972.2012.4 and Fellowship of the President of Russian Federation for young scientists 2307.2012.4.

SPR: APPLICATIONS IN BIOMOLECULAR INTERACTION ANALYSIS

Anna Sycheva

Surface plasmon resonance technique is widely used for qualitative and quantitative analysis of biomolecular interactions. The method allows determination of kinetic (rate constants) and thermodynamic (equilibrium dissociation constant) parameters.

I used Bio-Rad Proteon biosensor to study a number of protein-protein and protein-nucleic acid interactions. Here is an overview of this research.

Protein-protein interactions

In partnership with a group from the Institute of Bioorganic Chemistry we determined the variant of TRAIL protein with the highest potential to induce apoptosis in cancer cells. We analyzed interaction of five TRAIL variants with death receptors, decoy receptors and osteoprotegerin, a soluble TRAIL-binding protein. The best-performing variant showed a very weak affinity for decoy receptors and osteoprotegerin while retained an almost wild-type affinity for one of the death receptors. This variant was then proved to be active in cancer cell culture experiments as well[1].

Protein-RNA interactions

In collaboration with a group from the Institute of Protein Research we extensively studied binding of prokaryotic ribosomal protein L1 to messenger and ribosomal RNA. It was previously shown that L1 recognizes the same motif in both RNA molecules. However, SPR analysis demonstrated that the affinity of L1 for mRNA was 3 to 5 orders of magnitude lower than the affinity for rRNA. These observations were key to determine the structural elements responsible for the stability of protein-RNA complexes[2].

In another series of experiments point mutations were introduced into the RNA-binding domain of L1 protein. The threonine residue in the triad Thr-Met-Gly which is crucial for RNA recognition was substituted by phenylalanine, valine and alanine. Although all these amino acid substitutions resulted in the appearance of bulges on the protein-RNA contact surface, protein variants retained the ability to specifically bind to rRNA. Kinetic analysis showed that these changes in the surface relief had little effect on protein-RNA recognition but significantly reduced the life time of protein-RNA complexes[3].

Protein-DNA interactions

In collaboration with the Russian Institute of Physico-Chemical Medicine we used SPR technique to study the interaction of histone-like HU protein from Acholeplasma laidlawii with DNA duplexes. Apart from kinetic and equilibrium parameters determination we addressed the question of binding cooperativity. Hill coefficient calculation indicated that binding sites on the DNA were independent[4].

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POSTER SESSION

IMPROVING THE QUALITY OF THE DNA TEMPLATE BY REPAIR ENZYMES FOR PCR AMPLIFICATION OF DEGRADED DNA

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Despite the fact that DNA in living organisms is used as the main carrier of genetic information, the chemical stability of this molecule is limited. In living organisms, such DNA damage can lead eventually to the emergence of mutations, tumors, and contribute to aging. During the life of the organism, the repair systems resist the accumulation of damage in DNA, but with the death of the organism, these processes cease working, and the accumulation of DNA damage becomes irreversible. The accumulation of damage in DNA can be a problem when it is necessary to analyze its sequence. For example, the efficiency of PCR is sharply reduced if the template is subjected to oxidation or apurinization. This is particularly actual in studies of "ancient DNA and DNA in forensic practice: the samples for expertise are often subjected to adverse physical and chemical factors which arise prior to their collection and during DNA extraction from them. The accumulation of damage in postmortem human DNA obstructs PCR greatly, in some cases making the amplification of DNA from the samples impossible. We are developing a system in which repair enzymes are used to improve the quality of degraded DNA matrices before PCR. In most cases, lesions in postmortem DNA are located opposite undamaged second strand and, therefore, can be correctly repaired. We are creating a kit that includes several major DNA glycosylase, AP endonuclease, DNA polymerase and DNA ligase, the combined effect of which leads to repairing much of the damage in the test DNA prior to its use as a template for PCR. We also propose to include translesion thermostable DNA polymerase in the PCR kit to effectively overcome the residual damage in the amplification process. It is assumed that the primary use of our system will be found in the analysis of forensic samples, food samples and "ancient DNA".

This work was supported by the U.M.N.I.K. program (№06У/02-10).

STOPPED-FLOW KINETICS OF AP-SITE CLEAVAGE BY WILD TYPE APN1 FROM SACCHAROMYCES CEREVISIAE AND ITS H83A MUTANT

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Cellular DNA is continually attacked by endogenous and exogenous factors. This attack leads to formation of various DNA damages, with abundant apurinic/apyrimidinic (AP) sites arising. AP-sites are very mutagenic and genotoxic DNA damages and they are repaired mainly by AP-endonucleases in a base excision repair pathway. AP-endonuclease 1 from Saccharomyces cerevisiae (Apn1) is known to possess the major apurinic/apyrimidinic activity incising phosphodiester backbone 5′ to the AP-site. Up to date the spatial structure of Apn1 and its complex with DNA is not reported, so kinetic features of the interactions of Apn1 and its mutant form with DNA and conformational dynamics of the reactants would shed light on the role of key aminoacid residues involved in recognition and catalysis.

In the present study the conformational dynamics of DNA substrates during AP-endonuclease catalytic cycles were investigated. Measurements were carried out by 2-aminopurine (2-aPu) and pyrrolocytosine (PyrC) fluorescence, which were incorporated in different strands of DNA to detect conformational changes of both strands. Pre-steady-state kinetics of the repair process was studied by stopped-flow method and rate constants of each elementary step were calculated by global non-linear regression fitting.

The analysis of experimental data has resulted in the conclusion that both S. cerevisiae Apn1 and Apn1 H83A cleaves AP-sites in DNA during multi-stage process. Conformational changes of DNA substrate were shown to take place in the course of specific complex formation. When interacting with the enzymes, both DNA strands are involved in the recognition process. The initial conformation of double-stranded nucleic acid is of great importance for the formation of the proper enzyme-substrate complex. The location of 2-aPu relative to the damage was shown to have no influence on substrate cleavage by Apn1; but in the case of Apn1 H83A location of 2-aPu is significant.

The work is supported by grants from the Program of the Russian Academy of Sciences “Molecular & Cell Biology” (6.11), the Russian Foundation for Basic Research (RFBR 13-04-00013 and 11-04-01377), the Russian Ministry of Education and Science (SS-64.2012.4, 8092, 8473, 14.B37.21.0195), and the Grant from Russian Government to support leading scientists (11.G34.31.0045).

MECHANISTIC FEATURES OF ESCHERICHIA COLI AND HUMAN 8-OXOGUANINE-DNA GLYCOSYLASES IN THE EXCISION OF DAMAGED BASES

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DNA glycosylases play the opening act in a highly conserved process for excision of damaged bases from DNA called the base excision repair pathway (BER). DNA glycosylases attend to a wide variety of lesions arising from both endogenous and exogenous factors. The types of damage include alkylation, oxidation, and hydrolysis. A major DNA oxidation product is 8-oxoguanine (8-oxoG), a base with a high mutagenic potential. In bacteria, this lesion is repaired by formamidopyrimidine-DNA glycosylase (Fpg), while in the case of humans this function belongs to 8-oxoguanine-DNA glycosylase (OGG1). We have attempted a comprehensive characterization of 8-oxoG recognition by DNA glycosylases. First, we have obtained thermodynamic parameters for melting of DNA duplexes containing 8-oxoG in all possible nucleotide contexts. The energy of stacking interactions of 8-oxoG was in strict dependence on 8-oxoG nucleotide environment, which may affect the recognition of damage and the efficiency of eversion of 8-oxoG from DNA helix by glycosylases. Next, we established how the flexibility of DNA context affects damage recognition by these enzymes [Kirpota et al., 2011]. Then, we have found that DNA containing 8-oxoG next to a single strand break provides a good substrate for Fpg, as soon as all structural phosphate residues are maintained. Using site-directed mutagenesis, we have addressed the functions of many previously unstudied amino acid residuess that were predicted to be important for Fpg activity by molecular dynamics simulation and phylogenetic analysis. Of note, many substitutions abolished the excision of 8-oxoG but did not affect the cleavage efficiency of abasic substrates. Finally, we investigated the contribution of separated structural domains of Fpg to specific enzyme-substrate interaction. Surprisingly, despite the absence of the catalytic domain, C-terminal domain of Fpg possessed a low residual ability to recognize and cleave abasic substrates. Our study sheds light on mechanism details of Fpg and OGG1 activity, with the ultimate goal of understanding how binding energy can be spent by these enzymes for catalysis.

This work was supported by RAS Presidium (Molecular and Cellular Biology Program), RFBR (11-04-00807, 12-04-33231).

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ENZYMES OF DNA REPAIR AS TARGETS FOR ANTITUMOR PREPARATIONS

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One of the most important task of medicinal chemistry is creation of new drugs, including antineoplastic, based on synthetic transformations of natural biologically active nontoxic compounds. Among vegetable metabolites triterpene acids (glycyrrhetinic and betulonic acid), polyphenoles (usnic acid) and some other are of great interest.

Our team has devised synthesis scheme for broad range of natural biologically active compounds derivatives as well as methods for determination of inhibitory characteristic in reactions catalyzed by key BER (base excision repair) enzymes. A repertoire of selective inhibitors of individual BER enzymes has been revealed as well as multipurpose BER inhibitors which suppress several enzymes at once.

Among usnic acid derivatives tested several aromatic compounds have been revealed which inhibit PARP1 in millimolar range and don't influence other BER enzymes. Piperazine derivatives of betulonic acid – quaternary amines – have influenced all enzymes tested (PARP1, DNA polymerase β and APE1) in millimolar range. Among glycyrretinic acid derivatives tested mild selective inhibitor of APE1 and class of compounds - DNA polymerase β inhibitors (with ketonic group in 11 position) were found. Varacin analogs with acyclic substituent in the side chain allowed to obtain DNA polymerase β and APE1 inhibitors working in millimolar range.

Obtained data allow to suggest transformation ways for more active compounds synthesis.

This work was supported by the Program of Russian Academy of Sciences «Molecular and Cellular Biology» and RFBR 12-04-33162, 12-04-92601

EFFECT OF Mg2+ IONS ON STRUCTURE OF THE HUMAN RIBOSOMAL PROTEIN S13 BINDING SITE IN THE CENTRAL DOMAIN OF THE 18S rRNA

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It is known that Mg2+ ions at high concentrations stabilize the structure of the 16S rRNA in a conformation favorable for binding to the ribosomal proteins in the course of the eubacterial 30S ribosomal subunits assembly in vitro. Effect of Mg2+ on the formation of the 18S rRNA structure at the 40S subunit assembly remains poorly explored. Here, we show that the sequentional increase of the Mg2+ concentration from 0.5 mM to 20 mM leads to a significant decrease of the affinity of recombinant human ribosomal protein S13 (rpS13e) to a RNA transcript corresponding to the central domain fragment of the 18S rRNA (18SCD). The regions near the rpS13e binding site in 18SCD (including the nucleotides of helices H20 and H22), whose availabilities to hydroxyl radicals were dependent on the Mg2+ concentration, were determined. It was found that increase of the concentrations of Mg2+ results in the enhanced accessibilities of nucleotides G933-C937 and C1006-A1009 in helix H22 and reduces those of nucleotides A1023, A1024, and A1028-S1026 in the helix H20. Comparison of the results obtained with the crystallographic data on the structure of the central domain of 18S rRNA in the 40S ribosomal subunit led to conclusion that increase of Mg2+ concentrations results in the reorientation of helices H20 and H24 relatively helices H22 and N23 to form a structure, in which these helices are positioned similarly to their positioning in 40S subunits. Hence, saturation of the central domain of 18S rRNA with coordinated Mg2+ ions leads to changes in its structure similar to those as the rpS13e binding does, preventing this way binding of rpS13e to the isolated rRNA.

This work was supported by grants from the Russian Foundation for Basic Research (11-04-00672-a) to A.M. and by the Russian Academy of Sciences Presidium program ‘‘Molecular and cell biology’’ to G.K.

PRE-STEADY-STATE ANALYSIS OF HUMAN AP ENDONUCLEASE 1 EFFECT ON DNA GLYCOSYLASES TURNOVER

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Exposure of the cellular DNA to reactive oxygen species (ROS) generated either by the normal metabolism of the cell or by chemical and physical exogenous agents, is at the origin of lesions that can have genotoxic or mutagenic consequences. Such damage is handled by the highly conserved base excision repair (BER) pathway, initiated by a damage-specific DNA glycosylase. BER is a multi-step process and can be reconstituted with a limited number of proteins. The importance of the coordination of the various steps in the BER pathway is suggested by the finding of multiple interactions between the proteins of the pathway.

DNA glycosylases begin removal of base lesion in DNA by cleaving N-glycosidic bond between the damaged base and the ribose sugar leaving an abasic (AP) site. The AP site is processed by AP endonuclease 1 (APE1), which cleaves the DNA backbone on the 5'-side of the AP site. It has been shown that human APE1 stimulates the activity of hOGG1 as well as several other human or murine DNA glycosylases. The mechanism of such stimulation, however, remains controversial.

In this study, we analyzed the rate of the displacement of human DNA glycosylases (AAG, hOGG1, NEIL1, TDG core, MBD4 core, hUNG2) by APE1 using oligonucleotide substrates with tetrahydrofuran AP analogue (F) or specific DNA base damage placed in different positions. The F is a non-cleavable for DNA glycosylases but is cleavable by APE1. The direct measurement of pre-steady-state binding and cleavage of 2-aminopurine labeled F-substrates complexed with DNA glycosylases demonstrated different rates of DNA transfer from one enzyme to another. Using DNA substrates contained specific DNA base damage the rate of the base excision and DNA glycosylase turnover were calculated.

This study was made possible by grants from the Program of the Russian Academy of Sciences “Molecular & Cell Biology” (6.11), RFBR (13-04-00013 and 12-04-31066) and Russian Ministry of Education and Science (SS-64.2012.4, SP-4012.2013.4, 8092, 8473 and 14.B37.21.0195), the Grant from Russian Government to support leading scientists (No. 11.G34.31.0045)

TYROSYL-DNA PHOSPHODIESTERASE 1 – A NEW PLAYER IN BASE EXCISION REPAIR

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Tyrosyl-DNA phosphodiesterase 1 (Tdp1) catalyzes the hydrolysis of the phosphodiester linkage between the DNA 3′ phosphate and a tyrosine residue as well as a variety of other DNA 3′ damaged termini. Recently we determined that human Tdp1 interacts with AP sites and catalyzes the AP-site-cleavage reaction to generate breaks with the 3′- and 5′-phosphate termini. The removal of the 3′-phosphate is performed by polynucleotide kinase phosphatase (PNKP). PNKP and Polβ play key roles in this repair pathway and the activity of both enzymes are stimulated by XRCC1. The data suggest a role of Tdp1 in the new AP-endonuclease independent BER pathway in mammals. Tdp1 is more efficient in the cleavage of AP site in single-strand or bubble DNA structures. AP site that is opposite to bulky DNA lesion is hydrolyzed by Tdp1 faster than single AP site located in dsDNA. Tdp1 is also able to cleave synthetic analogs of AP-site – tetrahydrofuran and non-nucleotide units (decandiol and diethylenglycol) in contrast to enzymes possessing AP-lyase activity (e.g. Neil1 and EndoIII). Tdp1 mutants (SCAN1 and H263A) can bind the AP-site-containing DNA but do not reveal endonuclease activity. This new activity of Tdp1 can contribute to repair of AP sites particularly in DNA structures containing ssDNA region or AP site in the context of cluster-type lesions.

This work was supported by RFBR (Nos. 12-04-00337, 12-04-33162); the Program of the Presidium of RAS ‘Molecular and Cellular Biology’.

DAMAGE PROCESSING BY HUMAN 8-OXOGUANINE-DNA GLYCOSYLASE MUTANTS WITH THE OCCLUDED ACTIVE SITE: STOPPED-FLOW STUDY

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8-Oxoguanine-DNA glycosylase hOgg1 is one of the most important human DNA repair enzyme that removes 8-oxoguanine, a pre-mutagenic oxidative purine base lesion, from DNA. In order to understand the mechanisms of substrate recognition and processing by DNA glycosylases we have chosen to replace Cys253 with leucine or isoleucine. Amino acid Cys-253 of hOgg1 molecule participates in 8-oxoguanine coordination after its extrusion from DNA helix to the base-binding pocket. The hypothesis was that the added steric bulk and inability to form the Cys253(thiolate)–Lys249 dipole would exclude 8-oxoguanine from the pocket and disable its excision but still allow the enzyme to act on AP sites. The stopped-flow kinetic study in a combination with fluorescent detection was used in order to reveal conformational dynamics during protein-DNA interaction. Changes in fluorescence signals indicate sequential conformational transitions in macromolecules during the catalytic cycle. DNA substrates contained damaged bases or abasic sites. It was shown that although base-binding pocket occlusion distorts the active site of hOgg1 and greatly decreases the catalytic proficiency of the enzyme, it does not fully prevent both 8-xoguanine and AP-site damages sampling and excision.

For both mutant forms, a notable increase in the nicked product formation rates was observed in a presence of 8-bromoguanine (BrGua) during interaction with both AP- and oxoGua-substrates. Of note, the β-elimination rate didn't depend on the order of mixing. This may indicate that even if BrGua binds to the occluded OGG1 active site in the absence of DNA, this binding is not tight enough to completely prevent subsequent entrance and co-ordination of oxoGua-substrate.

Results obtained for mutant forms C253I and C253L of hOgg1 glycosylase resonate well with the concept of active site plasticity of enzymes. They indicate that the active site is flexible enough to compensate partially distortions caused by inappropriate amino acid residues.

The work is supported by grants from the Program of the RAS "Molecular & Cell Biology" (6.11), the RFBR (13-04-00013 and 11-04-01377), the Russian Ministry of Education and Science (SS-64.2012.4, 8092, 8473, 14.B37.21.0195), and the Grant from Russian Government to support leading scientists (11.G34.31.0045).

DNA-PROTEIN INTERACTIONS IN NER AND THEIR REGULATION

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The coordination of the assembly of the NER complexes and the sequential individual reactions is achieved through multiple DNA-protein and protein-protein interactions. The interaction of key protein factors of the NER process, XPC-RAD23B and XPA, with DNA structures mimicking NER intermediates has been analyzed. Using DNA containing photoreactive 5I-dUMP residues in the certain positions either in damaged or in undamaged strands and fluorescein group linked to uridine residue as a lesion, the topography of XPC-RAD23B-DNA and XPA-DNA complexes was determined. Obtained results fill the gap between biochemical results for XPC-RAD23B and X-ray structure for yeast ortholog Rad4-Rad23 and confirm the assumption that XPA may function as a link protein in the NER complex. Influence of substitutions of lysine in DNA binding cleft of XPA by glutamate (XPA K141E, K179E, and K141/179E; recombinant plasmids were kindly provided by Hanspeter Naegeli, University of Zürich) on the interaction with various DNA structures was analyzed. Both K179E and K141E mutations result in moderate decrease in DNA binding affinity and do not influence on the protein positioning on partially open DNA duplex. Tandem mutation K141/179E dramatically reduced XPA affinity to DNA. The results allow suggesting the key role of XPA orientation combined with RPA binding to undamaged strand for positioning the NER preincision complex.

Poly(ADP-ribose)polymerase-1 (PARP-1) is one of the candidate to participate in NER regulation. PARP-1 is rapidly activated in response to DNA damage using NAD+ as a substrate to form poly(ADP-ribose) (PAR) subunits. Using EMSA we revealed the influence of PARP1 on binding of XPC-RAD23B and XPA to model DNA-structures modulated by NAD+.

This work was supported by RFBR (grants no. 12-04-31323, 12-04-33162, 13-04-00538).

INSIGHTS INTO THE REPAIR OF OXIDATIVE DNA DAMAGE BY GLYCOSYLASES FROM MOLECULAR DYNAMICS SIMULATIONS

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It is well known that DNA is highly prone to damage induced by various physical and chemical agents. Base oxidation is a prevailing damage type arising on exposure to reactive oxygen species, which leads to mutations, cell death due to stalled replication, and regulatory dysfunction, ultimately resulting in aging, carcinogenesis and many pathological processes. One of the primary pathways for dealing with such damage is base excision repair, initiated by damage-specific enzymes, DNA N-glycosylases.

The most common damaged DNA base, 8-oxoguanine (OG), is highly mutagenic because it can form a stable Hoogsten pair with adenine. A system to prevent such mutations, known as GO-system, is possessed by both prokaryotes and eukaryotes. The GO-system comprises three enzymes, one of which, Fpg DNA glycosylase, excises OG from OG:C pairs in bacteria. Eukaryotes, including humans, possess a functional analogue of Fpg, OGG1. The X-ray structures of both these glycosylases are known.

Molecular dynamics (MD) simulation nowadays is an essential part of biochemical research. We combine a classic MD simulation with a newly developed efficient trajectory analysis tool, MDTRA, to analyze the specificity of Fpg for the base opposite to the lesion. A number of structural features have been identified making cytosine rather than adenine a preferred opposite base. Furthermore, DNA distortion by adenine opposite OG in the complex with Fpg is found to be long ranged, and even the positioning of an everted OG in the active site depends on the type of an opposite base.

In order to understand the importance of interactions within a base-binding pocket for OGG1 glycosylase, the dynamics of two models with a bulky substituent inside it (mutants C253I and C253L) was investigated. According to a known glycosylase reaction mechanism, the key parameters of catalytically important amino acids (Lys249 and Asp268) were checked. Analysis of populations of catalytically favorable conformations revealed the fact that the mutant forms should expose a reduced glycosylase activity, though not forfeiting it at all. Indeed, further pre-steady-state and steady-state kinetic experiments verified this inference.

This work was supported by RFBR (11-04-00807-a) and RAS Presidium (Mol Cell Biology 6.12).

IDENTIFICATION OF RIBOSOMAL PROTEINS PEPTIDES NEIGHBORING mRNA REGION 5' OF THE CODONS INTERACTING WITH tRNAS ON THE HUMAN RIBOSOME

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One of key ribosomal ligands is mRNA bearing the genetic information. During translation, the ribosome binds a 30-50 nucleotides long mRNA fragment including codons interacting with tRNAs. Site-directed cross-linking studies on human ribosomes with application of different mRNA analogs that bore a 4-azido-2,3,5,6-tetrafluorobenzoyl cross-linker at an U residue in specific location showed that ribosomal protein (rp) S5e neighbors mRNA nucleotide in position -3 with respect to the first nucleotide of the P site codon, and rpS26e interacts mRNA 5 'of codons bound to tRNAs. Here, we determined rpS26e and rpS5 peptides neighboring mRNA on the human 80S ribosome with the use of an original methodology based on the application of various proteolytic agents for selective cleavage of the cross-linked protein with subsequent SDS-PAGE separation of the labeled modified peptides and their identification. The rpS5e site cross-linked to the mRNA nucleotide in position -3 was mapped to the fragment 89-152. This fragment belongs to the conserved part of the protein; in the bacterial counterpart of rpS5e, rpS7p, this fragment contains amino acid residue R79 that interacts with mRNA according to X-ray data on bacterial ribosomes. This indicates the conserved nature of the immediate protein environment of the E site mRNA codon on the small ribosomal subunit. As for rpS26, it turned out that photoactivatable group of mRNA analogs at the nucleotides in positions -4, -6 and -9 cross-linked to the same dodecapeptide in positions 60-71 in the central part of the rpS26e [1]. An analysis of protein sequences of the rpS26e family revealed that motif YxxPKxYxK within the mentioned fragment is conserved in eukaryotes but not in archaea. A comparison of the crystal structure of the 40S subunit with available cryo-EM images of the 40S subunit complexed with eIF3 showed that the location of rpS26e on the 40S subunit overlaps with the eIF3 binding site. We suggest that motif YxxPKxYxK interacts with eIF3, which is involved in recruitment of the mRNA to the 40S subunit and has no counterparts in bacteria and archaea.

The work was supported by the Presidium of Russian Academy of Sciences (Program on Molecular and Cell Biology, grant to G.K.) and by the Russian Foundation for Basic Research (11-04-00597 to G. K.).

[1] Sharifulin D. E., Graifer D. M., Karpova G. G. (2012) Nucleic Acids Res. 40, 3056-3065.

PROTEOMIC ANALYSIS OF EXOSOMES FROM BLOOD OF HEALTHY DONORS AND BREAST CANCER PATIENTS

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Microvesicles release by neoplastic cells are the most popular targets of current cancer research, due to their potential involvement in cancer propagation and carrying a large number of tumor distinctive molecules. In particular, experimental evidence demonstrate that in blood of mammary carcinoma patients tumor specific miRNA&mRNA (let-7, miRNA-195, miRNA-103) are carried by membrane-wrapped vesicles (exosomes) and thus, exosomes circulating in blood can gain the benefits for search of breast cancer biomarkers.

Blood from healthy women and breast cancer patients were fractionated into plasma and cellular fractions (Tamkovich, 2005, Clin. Chem.), cell-surface-bound exosomes were eluted from cell surface with PBS/EDTA and trypsin solutions and were filtered through 0.22μm micropore filters. Plasma and cell fraction were clarified by centrifugation at 1000 g, exosomes were pelleted by ultracentrifuged at 100 000 g for 90 min at 4°C, resuspended in PBS and stored at -80°C. Protein concentration was determined using the NanoOrange kit. Exosomes were characterized by TEM as 40-80 nm membrane – wrapped particles and spot-analysis confirming well-established exosome markers (CD-63, CD-81, CD-24, CD-9). SDS-PAGE analysis shows a distinct silver staining set of proteins from 8 to 250 kDa. Protein bands were cut out from PAG and followed with in-gel trypsin digestion, peptides extraction and identification by MALDI TOF/TOF and maXis 4G. Set of proteins previously found in exosomes including mitochondrial methyl-transferase-like protein 17, Actin, cytoplasmic, Serum albumin, Estradiol 17-beta-dehydrogenase, ERS protein, KIAA 0378, Nuclear autoantigen sp-100, Nineins (initial and isoforms CRA-d, CRA-h, 2) were identified in pilot experiments.

It was found that exosomes are circulating not only in plasma but in at least comparable amount at the surface of blood cells. Comparison of proteins and contents of cell-free and cell surface bound nucleic acids in healthy and illness patients will demonstrate usefulness of proteome or nucleotide markers for diagnostics, prognostics and monitoring of breast cancer.

The present work was supported by grant from RFBR (N 12-04-31365).

Сборник материалов конференции

“Nucleic Acid - Protein Interactions for Life Sciences”

(Meeting in the frame of French-Siberia Centre of Research and Education)

May 13-15, 2013

Akademgorodok, Novosibirsk, Russia

Издание сборника осуществлено при поддержке:

РФФИ (грант № 13-04-06037_Г)

Подготовка оригинал-макета, редактирование, дизайн

Р.О. Анарбаев

Ответственный за выпуск

Н. А. Лебедева

Издательство ИХБФМ СО РАН

Российская Федерация, 630090, Новосибирск, пр. Лаврентьева, 8

www.niboch.nsc.ru

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