40th Anniversary of the Institute of Protein Research
Russian Academy of Sciences

INTERNATIONAL CONFERENCE ON

"PROTEIN BIOSYNTHESIS, STRUCTURE AND FUNCTION"

June 9 – 13, 2007
Pushchino, Russia

The Organizing Committee warmly thanks for the support:

Russian Academy of Sciences,

Russian Foundation for Basic Research,

Institute of Protein Research, RAS

PROGRAM

SATURDAY, 9 JUNE

OPENING CEREMONY

12:00 Opening lecture by **A. S. Spirin**

13:00 Greetings

14:00 Get-together reception

SUNDAY, 10 JUNE

10.00 ***Morning session***

PROTEIN BIOSYNTHESIS AND REGULATION

Chairman: L. P. Ovchinnikov

N. E. Shirokikh and A. S. Spirin. Localization of the ribosomal initiation 48S complex on eukaryotic mRNAs by the method of primer extension inhibition with the use of fluorescent label (15 min).

V. A. Shirokov, G. S. Kopeina, K. V. Gromova, Zh. A. Afonina, V. D. Vasiliev, A. S. Spirin. Step-wise formation of polyribosomes and circular translation of polysomal mRNA in a long-term eukaryotic cell-free system

O. M. Alekhina, K. S. Vassilenko, A. S. Spirin. Translation of non-capped mRNAs in a eukaryotic cell free system: acceleration of initiation rate in the course of polysome formation (15 min).

N. L. Korneeva, R. E. Rhoads. Alteration of eIF4F activities affects mRNA selection for translation (15 min).

O. Thiébeauld, H-S. Park, A. Geldreich, T. Hohn and L. A. Ryabova. The role of host factors in TAV-activated reinitiation of translation (20 min).

11:30-11:45 *Coffee break*

Chairperson: L. A. Ryabova

A. A. Selyutina, A. V. Sorokin, V. M. Evdokimova, L. P. Ovchinnikov. Cleavage of YB-1 by the 20S proteasome: a possible mechanism of cleavage, effect of YB-1 truncation on its functions and intracellular distribution (15 min).

D. N. Lyabin, I. A. Eliseeva, O. V. Skabkina and L. P. Ovchinnikov. Regulation of YB-1 mRNA translation (15 min).

E. S. Bochkareva, A. A. Herskovits, J. Adler, L. Bahari and E. Bibi. *In vivo* dissecting the functional roles of the E. coli SRP and its receptor, FtsY in membrane protein biogenesis (20 min).

A. G. Ryazanov. Elongation factor-2 kinase: from translational control to longevity (20 min).

13:30-15:00 *Lunch*

15.00 ***Evening session***

STRUCTURE OF THE RIBOSOME AND OF PROTEIN-SYNTHESIZING MACHINERY COMPONENTS

Chairman: S. V. Nikonov

G. Yusupova, L. Jenner, B. Rees, D. Moras and M. Yusupov. Messenger RNA structure on the ribosome in different functional states (20 min).

S. V. Tishchenko, E. Yu. Nikonova, O. S. Kostareva, A. D. Nikulin, V. G. Klyashtorny, S. A. Volchkov, O. S. Nikonov, N. L. Davydova, N. A. Nevskaya, S. V. Nikonov, M. B. Garber, W. Piendl. Crystallization and structural investigation of regulatory complexes between ribosomal protein L1 and specific mRNA fragments (15 min).

G. M. Gongadze, A. V. Korobeinikova, A. P. Korepanov, M. V. Bazhenova, A. V. Sarskikh, M. B. Garber. Properties of the bacterial CTC family proteins associated with the ribosome (15 min).

A. P. Korepanov, M. G. Bubunenko, M. B. Garber. Genetic analysis of the assembly of the 30S ribosomal subunit platform in *Escherichia coli* (15 min).

16:20-16:35 *Coffee break*

Chairperson: M. B. Garber

E. A. Stolboushkina, O. S. Nikonov, A. D. Nikulin, S. V. Nikonov, M. B. Garber, U. Bläsi. Crystallization of the heterotrimeric archaeal translation initiation factor aIF2 (10 min).

O. S. Nikonov, E. A. Stolboushkina, A. D. Nikulin, D. Hasenöhrl, U. Bläsi, D. J. Manstein, R. V. Fedorov, M. B. Garber and S. V. Nikonov. New insights into the interactions of the translation initiation factor 2 from Archaea with guanine nucleotides and initiator tRNA (15 min).

S. Ch. Agalarov, A. A Kalinichenko, A. Kommer, A. S. Spirin. Ribosomal protein S1 induces a conformational change of the 30S ribosomal subunit (15 min).

A. V. Zhigailov, E. S. Laletina, N. S. Polimbetova, D. M. Graifer, B. K. Iskakov. Study of plant 18S rRNA 3' domain accessibility in the composition of wheat germ 40S ribosomal subunit (15 min).

S. V. Nikonov, S. A. Volchkov, N. A. Nevskaya, M. B. Garber. Interactions of ribosomal proteins with ribosomal and messenger RNAs (20 min).

18:00-20:00 POSTER SESSION

20:00-23:00 EVENING CLUB (socializing)

_____ *MONDAY, 11 JUNE* _____

10.00 ***Morning session***

BIOCHEMISTRY OF NUCLEIC ACIDS

Chairperson: O. I. Lavrik

A. B. Chetverin, H. V. Chetverina. Molecular colonies (20 min).

H. V. Chetverina, M. V. Falaleeva, T. R. Samatov, A. V. Kravchenko, Yu. A. Zabolotneva, A. B. Chetverin. Diagnostic potential of the molecular colony technique (15 min).

V. I. Ugarov, A. B. Chetverin. Functional circularity of Qβ replicase templates (15 min).

M. V. Falaleeva, H. V. Chetverina, V. I. Ugarov, E. A. Uzlova, A. B. Chetverin. Polynucleotide phosphorylase from *Thermus thermophilus* as a tool for studies on RNA recombination (10 min).

11:10-11:25 *Coffee break*

Chairman: A. B. Chetverin

O. I. Lavrik. Coordination of DNA repair machines studied by affinity labeling technique combined with functional assay (20 min).

A. V. Oleinikov, S. Francis, V. A. Malkov, E. Rossnagle, T. K. Mutabingwa, M. Fried, P. E. Duffy. Malaria parasite tricks and challenges in development of anti-malarial vaccine (20 min).

O. V. Denisova, A. V. Chernov, T. Y. Koledachkina, N. I. Matvienko. A new tag-based approach to high-throughput analysis of CCWGG methylation (10 min).

N. V. Zyrina, L. A. Zheleznaya, E. V. Dvoretsky, V. D. Vasiliev, A. Chernov and N. I. Matvienko. Template independent DNA synthesis by Bst DNA polymerase in the presence of site-specific DNA nickases (10 min).

V. N. Ksenzenko, A. I. Krutilina, A. S. Glukhov, N. V. Akulenko, S. O. Garbuzinskiy, O. V. Galzitskaya, A. V. Kaliman,. L. A. Shaloyko. Site-specific endonucleases encoded by T5-like bacteriophages: biochemical properties and genetic role (15 min).

13:30-15:00 *Lunch*

_____ *MONDAY, 11 JUNE* _____

15.00 ***Evening session***

STRUCTURE AND FUNCTION OF PROTEINS

Chairman: S. A. Potekhin

A. A. Vazina, N. F. Lanina, V. N. Korneev, I. P. Dolbnya, W. Bras. The principles of nanostructural organization of the multidomain muscle protein titin (20 min).

V. V. Rogov, K. Schmöe, N. Yu. Rogova, F. Löhr, F. Bernhard, V. Dötsch. Structural analysis of the Rcs signalling pathway in ENTERO bacteria (20 min).

Y. V. Sergeev, J. F. Hejtmancik and P. T. Wingfield. Energetics of domain-domain interactions and entropy driven association of β-crystallins (20 min).

16:10-16:25 *Coffee break*

Chairman: V. V. Rogov

S. Ryazantsev, E. Neufeld, Z. H. Zhou and L. Rome. Electron microscopy as a critical component of nanoscience research (20 min).

K. Severinov, T. Kazakov, E. Semenova, A. Kazakov, M. Gelfand. Structure, function and evolution of post-translationally modified microcins (20 min).

D. E. Agafonov and R. Lohrmann Protein composition of human snRNPs and spliceosomal complexes revealed by two-dimensional electrophoresis (15 min).

Yu. V. Mitin, A. Yu. Khrushchev, L. V. Klimenko, I. A. Kashparov. Non-linear antimicrobial peptides (10 min).

L. V. Gushchina, A. G. Gabdulkhakov, V. V. Filimonov. The structure of the chimeric protein imitating SH3-peptide interaction (10 min).

17:45-18:00 *Coffee break*

Chairman: V. V. Filimonov

A. Koglin, C. Klammt, N. Trbovic, D. Schwarz, B. Schneider, B. Schäfer, S. Sobhanifar, F. Löhr, F. Bernhard, V. Dötsch. Structural investigation of the membrane proteins TehA and YfiK (20 min).

F. Bernhard, D. Schwarz, F. Junge, C. Klammt, B. Schäfer, V. Dötsch. Cell-free expression of polytopic integral membrane proteins in preparative scales (20 min).

J-S. Li, M. Ikeguchi, Y. Matsumura, M. Shinjo and H. Kihara. α-helix-rich intermediate in the folding pathway (20 min.)

20:00-23:00 EVENING CLUB (SOCIALIZING)

TUESDAY, 12 JUNE

10.00 ***Morning session***

CELL BIOLOGY

Chairman: O. V. Fedorov

O. Denisenko. hnRNPs and epigenetic control of gene expression (20 min).

A. S. Kostyukova. Formation of tropomodulin/tropomyosin complex at the pointed end of the actin filament (20 min).

E. S. Nadezhdina, P. A. Ivanov, A. Yu. Lomakin. Microtubules in stress granule dynamics (15 min).

A. A. Minin, O. E. Nekrasova. Role of intermediate filaments in mitochondria distribution (15 min).

E. S. Nadezhdina, A. V. Burakov, O. V. Kovalenko, O. N. Zhapparova, I. B.Brodsky, L. A. Zinovkina, E. S. Potekhina, N. A. Shanina, V. I. Rodionov. Dynein, dynactin and protein kinase LOSK in microtubule array organization (15 min).

11:30-11:45 *Coffee break*

INVESTIGATION OF PROTEINS IN SOLUTION

Chairperson: O. V. Galzitskaya

G. V. Semisotnov. Denaturation and renaturation of globular proteins: scientific and biotechnological aspects (20 min).

V. V. Filimonov. Equilibrium folding intermediates and their association (20 min).

B. S. Melnik, S. R. Evdokimov, V. V. Marchenkov, N. V.Kotova, G. V. Semisotnov. The analysis of Multi-stage denaturation and renaturation kinetics of monomeric globular proteins: carbonic anhydrase B (15 min).

E. I. Tiktopulo, V. D. Vasiliev, N. G. Koretskaya, S. A. Potekhin. Structural transformation of cry 3A δ-endotoxin and its mutant form (C14) depending on pH of the medium and ethanol (15 min).

13:30-15:00 *Lunch*

_____ *TUESDAY, 12 JUNE* _____

15.00 ***Evening session***

INVESTIGATION OF PROTEINS IN SOLUTION

Chairman: G. V. Semisotnov

V. E. Bychkova. Conformational changes occurring in proteins in the presence of membranes and their relation to protein functioning (20 min).

V. A. Balobanov, N. S. Katina, N. B. Ilyina, I. A. Kashparov, E. N. Samatova, V. E. Bychkova. Phase diagrams of apomyoglobin in coordinates of pH, temperature and urea concentration (10 min).

V. M. Tischenko, S. D. Knight, A. V. Zavialov, S. MacIntyre, E. I. Tiktopulo. Subunit destabilization and release from *Yersinia pestis* F1 subunit-chaperone complex (15 min).

S. A. Potekhin, A. F. Bunkin, S. M. Pershin, A. A. Nurmatov, R. S. Khusainova. Selective interaction of biomacromolecules with spin isomers of water molecules (15 min).

16:10-16:25 *Coffee break*

THE THEORY OF PROTEIN STRUCTURE. BIOINFORMATICS.

Chairman: A. V. Finkelshtein

A.V. Efimov. Novel structural motifs and structural trees of proteins (20 min).

A. V. Kajava. Parallel superpleated β-structure as a common fold for amyloid and prion fibrils (20 min).

B. Reva, Y. Antipin and C. Sander. Specificity of protein function encoded in conservation patterns (20 min).

N. S. Bogatyreva, N. V. Dovidchenko, D. N. Ivankov, A. V. Finkelstein. Strict computation of electrostatic interactions in a corpuscular medium (15 min).

17:30-17:45 *Coffee break*

Chairman: A. V. Efimov

O. V. Galzitskaya, S. O. Garbuzynskiy, D. N. Ivankov, A. V. Glyakina, M. Yu. Lobanov, A. V. Finkelstein. Prediction of folding nuclei in globular proteins (15 min).

Yu. N. Chirgadze, R. V. Polozov, V.S. Sivozhelezov, E. I. Zheltukhin. Structural principles of complex formation of transcription factors with B-DNA: transcription factors with three α-helices (15 min).

M. S. Gelfand. Evolution of bacterial regulatory systems (20 min).

A. V. Finkelstein, G. V. Semisotnov, V. E. Bychkova. Protein folding problem: theory and experiment. In the memory of Oleg B. Ptitsyn (30 min).

CLOSING CEREMONY

_____*WEDNSDAY, 13 JUNE*_____

12:00 ***PICNIC***

CONFERENCE ABSTRACTS

LOCALIZATION OF THE RIBOSOMAL INITIATION 48S COMPLEX ON EUKARYOTIC mRNAS BY THE METHOD OF PRIMER EXTENSION INHIBITION WITH THE USE OF FLUORESCENT LABEL

N. E. Shirokikh, A. S. Spirin
Institute of Protein Research, RAS

(1) Eukaryotic cellular mRNAs are naturally capped and polyadenylated. According to the scanning model originally proposed by M. Kozak, the so-called native 40S ribosomal subunit (40S:eIF3:eIF2:eIF1:eIF1A complex) first binds to the cap-binding protein complex (eIF4E:eIF4G:eIF4A) at the 5'-end of mRNA, and then successively scans the 5'-untranslated region (leader sequence) of mRNA until it encounters the initiation codon. The process of scanning is unidirectional (5'→3') and catalyzed by an ATP-dependent RNA helicase (eIF4A). On the other hand, there are special cases of cap-independent initiation of translation of viral non-capped mRNAs when the 40S ribosomal subunit binds directly and specifically to a well-structured RNA module called “internal ribosome entry site” (IRES), thus omitting the steps of 5'-end binding and leader sequence scanning. At the same time, some uncapped leader sequences devoid of defined IRES structures have been shown to strongly enhance the initiation of translation of eukaryotic mRNAs in cell-free systems. One of them is oligo(A) leader typical of highly expressible pox virus RNAs.

(2) The study of the assembly of translation initiation complexes on different mRNAs is possible with the primer extension inhibition technique (toe-printing method). Here we applied the toe-printing method for studies of 48S initiation complex using fluorescently labeled primers with subsequent analysis of reverse transcription products by capillary electrophoresis. This improved technique was shown to have several advantages, being more precise and more quantitative as compared with the original version.

(3) We used the improved toe-printing technique to identify the set of initiation factors required for the formation of the 48S initiation complex on luciferase mRNA with oligo(A) leader, as compared with a classical eukaryotic mRNA, capped beta-globin mRNA, with its own leader. It was found that the formation of the initiation 48S complex on Luc-mRNA with oligo(A) leader manifested its independence of eukaryotic initiation factors eIF4F and eIF4A, in contrast to the beta-globin mRNA. The 48S complex could be well formed on mRNA with oligo(A) leader in the absence of the eIF4 group factors also when competitive amounts of beta-globin mRNA were added to the reaction mixture.

(4) Our findings suggest that eukaryotic mechanisms of translation initiation may be more diverse than usually thought. In any case it seems likely that efficient initiation of translation of eukaryotic mRNAs can be realized without caps, structured IRESes, and unidirectional ATP-dependent scanning. Similarly to prokaryotic initiation, affinity and direct binding of native small ribosomal subunits to some internal polynucleotide sequences, their diffusional sliding (“phaseless wondering”) along polyribonucleotide chain and specific fixation at the initiation codon may be characteristic of the eukaryotic initiation process as well.

STEP-WISE FORMATION OF POLYRIBOSOMES AND CIRCULAR TRANSLATION OF POLYSOMAL MRNA IN A LONG-TERM EUKARYOTIC CELL-FREE SYSTEM

V. A. Shirokov, G. S. Kopeina, K. V. Gromova, Zh. A. Afonina, V. D. Vasiliev, A. S. Spirin
Institute of Protein Research, RAS

The time course of polysome formation was studied in a long-term wheat germ cell-free translation system using sedimentation and electron microscopy techniques. The polysomes were formed on uncapped firefly luciferase mRNA with special enhancing 5' and 3' untranslated regions (UTRs). The formation of fully loaded polysomes was found to be a long process that required many rounds of translation and proceeded via several phases starting from short linear polysomes, their subsequent folding into short double-row clusters, and then gradual elongation of the clusters into heavy-loaded double-row strings containing up to 40 ribosomes. The formation of the double-rows with interaction between antiparallel 5' and 3' halves of a polysome was considered to be topologically equivalent to circularization of polysomes. Only a slow exchange with free ribosomes and free mRNA was observed in the double-row type polysomes, this suggesting that polysomal ribosomes reinitiate translation within the circularized polysomes.

TRANSLATION OF NON-CAPPED MRNAS IN A EUKARYOTIC CELL FREE SYSTEM: ACCELERATION OF INITIATION RATE IN THE COURSE OF POLYSOME FORMATION

O. M. Alekhina, K. S. Vassilenko, A. S. Spirin
Institute of Protein Research, RAS

Translation initiation is a crucial phase in protein synthesis. It is at this stage that translational control is realized both in prokaryotic and eukaryotic cells. Cell-free translation systems serve as an important tool for studying the molecular mechanisms of translation initiation. Real time monitoring of the translation of non-capped luciferase mRNA in a wheat germ cell-free system has been performed by continuous in situ measurement of the luminescence increase in the translation mixture. The phenomenon of acceleration of translation has been discovered. It has been shown that the acceleration is accompanied by the loading of translating polysomes with additional ribosomes, and thus is caused mainly by a rise in the initiation rate, rather than the stimulation of elongation or the involvement of additional mRNA molecules in translation. The acceleration requires a sufficient concentration of mRNA and depends on the sequence of the 5' untranslated region (UTR). It can be abolished by an addition of the excess cap analogue (m7GpppGm). As the acceleration does not depend on the preliminary translation of other mRNAs in the same extract, the conclusion has been made that the effect is not due to activation of the ribosome population or other components of the system during translation, but rather it is the consequence of intra-polysomal events. The acceleration observed is discussed in terms of the model of two overlapping initiation pathways in eukaryotic polysomes: translation of non-capped mRNAs starts with eIF4F-independent initiation at 5' UTR, and after the formation of sufficiently loaded polysomes, they rearrange in such a way that the mechanism of re-initiation of terminating ribosomes switches on. The eIF4F-mediated circularization of polysomes may be considered as a possible event that leads to the re-initiation switch and the resultant acceleration effect.

ALTERATION OF EIF4F ACTIVITIES AFFECTS MRNA SELECTION FOR TRANSLATION

N. L. Korneeva, R. E. Rhoads
Louisiana State University Health Sciences Center in Shreveport, USA

The rate of translation initiation in eukaryotic cells depends on both features of the mRNA itself and also the level and activity of the eIF4F complex, which consists of eIF4G, eIF4E and eIF4A. The recruitment of capped mRNA to initiation complexes by eIF4F involves several discrete steps, including cap-recognition by eIF4E, mRNA binding by eIF4G, ATP-dependent unwinding of mRNA secondary structure by the helicase eIF4A, binding to the 40S ribosomal subunit through eIF3, and recognition of the 3'-terminal poly(A) tract PABP. Rather than a static scaffold, eIF4G serves as a dynamic motor facilitating the stepwise interaction of initiation factors, ribosomal subunits, and mRNA for assembly of the initiation complex. We and others have obtained data consistent with a model whereby binding of initiation factors and mRNA to eIF4G causes conformational changes within eIF4G domains that lead to altered eIF4F cap-recognition, RNA-binding, or RNA-unwinding. Modification of eIF4E and other members of eIF4F complex can affect the *spectrum* of mRNAs translated. Both *trans*-acting and *cis*-acting factors can reduce the efficiency of cap-dependent translation. *Trans*-acting factors include the limiting amount of eIF4F in cells under normal conditions. *Cis*-acting factors include a highly structured 5'-UTR of mRNA, the presence of upstream AUGs, and poor sequence context for the initiating AUG, all of which are found in the 5'-UTRs of mRNAs for many low-abundance protein. mRNAs with these properties encode a disproportionate share of proteins involved in cell growth and cell cycle progression, *e.g.*, proto-oncoproteins, growth factors, growth factor receptors, and cyclins. Translation of these mRNAs requires high levels and activity of the eIF4F complex. The Mnk kinase binds eIF4G and phosphorylates both eIF4E and eIF4G in eIF4F. Using Mnk inhibitors, we have shown a direct, positive correlation between Mnk kinase activity and the rate of *in vitro* translation of capped-mRNA with a highly structured 5'-UTR. These effects were dependent on eIF4E and did not occur with either uncapped or unstructured mRNA. Modification of Mnk activity *in vivo* caused suppression of a malignant cell phenotype as well as reduced translation of cancer-related mRNAs. Based on these data, we hypothesize that eIF4E phosphorylation status affects two *coupled* activities of eIF4F, cap-recognition and secondary structure unwinding, during initiation step of translation, rather than just cap-recognition as suggested by earlier studies. Our model postulates cooperative roles for eIF4E and eIF4A in the modulation of eIF4F activity during ribosome loading onto capped mRNAs containing highly structured 5'-UTRs.

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THE ROLE OF HOST FACTORS IN TAV-ACTIVATED REINITIATION OF TRANSLATION

O. Thiébeauld[1], H-S. Park[2], A. Geldreich[1], T. Hohn[2], L. A. Ryabova[1]
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The cauliflower mosaic virus (CaMV) reinitiation factor, transactivator: viroplasmin (TAV) is required to activate translation of viral and artificial polycistronic mRNAs in plants, where the eukaryotic cell environment normally precludes the occurrence of reinitiation. To accomplish its function, TAV participates in multiple interactions with host factors including ribosomal proteins and eukaryotic translation initiation factors (eIFs). TAV is proposed to interact with eIF3 and the 60S ribosomal subunit via multiple contacts mediated by at least three ribosomal proteins: L13, L18 and L24. TAV either keeps eIFs bound to the translating ribosome or helps to reacquire them *de novo* during or after termination of translation of the first ORF. Indeed, TAV might prevent loss of eIF3 from the translating ribosome after the first initiation event. Here we studied the function of a novel TAV-interacting protein, TAIP, in host cells and in the TAV-mediated transactivation process. In addition to interactions with TAV, TAIP binds the 60S ribosomal subunit via at least one ribosomal protein, L7. Although TAIP is detected on 60S and 80S ribosomes isolated from wheat germ extract, recombinant purified TAIP can be loaded on the 60S ribosomal subunit, but not on the 80S ribosome. Full-length TAIP and TAV are detected in the polyribosomal fractions isolated from CaMV-infected turnip plants but not from healthy plants. Transiently overexpressed TAIP increases TAV-mediated transactivation of polycistronic translation in plant protoplasts, suggesting that TAIP is required for reinitiation. The cellular function of TAIP might be to promote the 60S subunit joining step during translation initiation since recombinant TAIP increases the 80S complex formation assembled at CrPV IRES RNA. We suggest that TAIP is exploited by TAV to allow 60S joining at the reinitiation step of polycistronic translation.

CLEAVAGE OF YB-1 BY THE 20S PROTEASOME: POSSIBLE MECHANISM OF CLEAVAGE, EFFECT OF YB-1 TRUNCATION ON ITS FUNCTIONS AND INTRACELLULAR LOCATION

A. A. Selyutina[1], A. V. Sorokin[1], V. M. Evdokimova[1,2], L. P. Ovchinnikov[1]
[1]Institute of Protein Research, RAS, [2]Department of Pediatrics, British Columbia Research Institute for Children's and Women's Health, and the University of British Columbia

YB-1 is a DNA/RNA-binding nucleocytoplasmic shuttling protein whose regulatory effect on many DNA- and mRNA-dependent events is determined by its location in the cell. We found that *in vitro* YB-1 undergoes a specific proteolytic cleavage by the 20S proteasome, which splits off a 105-amino-acid long C-terminal fragment by breaking the bond between E^{219} and G^{220}. This cleavage appears to be ubiquitin- and ATP-independent, and is abolished by the association of YB-1 with messenger RNA. The inhibitory analysis has shown that YB-1 cleavage was performed mostly by post-acidic (caspase-like) activity of the 20S proteasome. Supporting evidence also came from mutated E-219-R YB-1 that was almost completely insensitive to cleavage. Truncated YB-1 has the same affinity for RNA/DNA as its full-length form, but truncation slightly reduced YB-1 ability to inhibit cap-dependent translation in rabbit reticulocyte lysate. We found that the same limited proteolysis of YB-1 occurs *in vivo* and is triggered by DNA-damaging drugs in NIH3T3 cells, as well as in cancer cells from patients with tumors of different origin (breast, lung and ovarian cancer). Drug treatment of NIH3T3 cells resulted in accumulation of truncated YB-1 in nuclear punctuate structures resembling nuclear speckles, i.e., zones of accumulation of transcriptional and splicing factors. YB-1 truncation and accumulation in the nucleus correlates with appearance of cell resistance against the used drug. We believe that inhibition of caspase-like activity of the proteasome can reduce generation of truncated YB-1 and its transfer into the cell nucleus, and that such proteasome inhibitors can be used to prevent multidrug resistance of cancer cells.

This work was supported in part by grant from the Russian Foundation for Basic Research (07-04-00403-a) and grants from the Russian Academy of Sciences (Programs on "Molecular and Cellular Biology" and "Basic Sciences to Medicine").

REGULATION OF YB-1 MRNA TRANSLATION

D. N. Lyabin, I. A. Eliseeva, O. V. Skabkina, L. P. Ovchinnikov
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YB-1 is a multifunctional DNA/RNA-binding protein, a member of the protein family with the evolutionary ancient cold-shock domain. YB-1 is involved in practically all events of storing, transfer, and expression of genetic information and its amount in the cell is strictly regulated. We have shown that in its 3' UTR, YB-1 mRNA contains a ~80 nt regulatory sequence which specifically interacts with two major mRNP proteins, YB-1 and PABP (poly(A)-binding protein). As shown by mapping, specific binding sites of YB-1 and PABP overlap, and these two proteins compete with each other for binding to the regulatory sequence. In a cell-free translation system YB-1 suppresses its own synthesis at concentrations insufficient for inhibition of translation of other mRNAs. This suppression is accompanied by YB-1 mRNA accumulation in the form of free mRNPs, which indicates that the inhibition occurs at the stage of initiation, either at the step of 40S ribosomal subunit binding to mRNA or at the previous steps of mRNA interactions with translation initiation factors. PABP restores translation of YB-1 mRNA that has been inhibited by YB-1, and stimulates translation of even poly(A)$^-$ YB-1 mRNA. PABP sequestration by exogenous poly(A) resulted in accumulation of poly(A)$^-$ YB-1 mRNA in the form of free mRNPs. Thus, together, YB-1 and PABP may maintain YB-1 concentration, as well as the YB-1/PABP ratio, at the level optimal for translation of all other cellular poly(A)$^+$ mRNAs.

Attachment of the poly(A) tail to YB-1 mRNA strongly inhibits its translation with no effect on its stability, which may be a result of the presence of a specific PABP-binding site within 3' UTR. Indeed, partial deletion of the specific PABP-binding site abolished inhibition of YB-1 mRNA translation caused by polyadenylation. This unusual regulation of YB-1 mRNA translation by the poly(A) tail could provide selective translation of YB-1 mRNA under conditions of total mRNA deadenylation. In this case transition of the majority of mRNAs into the pool of free non-translated mRNPs will be accompanied by increasing synthesis of YB-1 required to accomplish packaging of mRNAs released from polysomes and their storing in the masked form.

Supported in part by the Russian Academy of Sciences (Programs on "Molecular and Cellular Biology" and "Basic Sciences to Medicine").

IN VIVO DISSECTING THE FUNCTIONAL ROLES OF THE *E. COLI* SRP AND ITS RECEPTOR, FtsY IN MEMBRANE PROTEIN BIOGENESIS

E. S. Bochkareva, A. A. Herskovits, J. Adler, L. Bahari, E. Bibi
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The bacterial version of the mammalian signal recognition particle (SRP) machinery plays an important role in membrane protein biogenesis. The *Escherichia coli* SRP system consists of 4.5S SRP-RNA and two proteins, Ffh and FtsY, which are homologues of the eukaryotic SRP54 and the SRP-receptor α-subunit, respectively. In order to dissect *in vivo* the functional roles of SRP and FtsY at different steps of the pathway, we analyzed the consequences of genetic depletion of Ffh and FtsY in *E. coli* cells. We found that in Ffh-depleted cells the amount of polytopic membrane proteins remains unchanged, they localize to the membrane, but their proper insertion and folding are disrupted. In contrast, the amount of the same membrane proteins is considerably reduced in cells depleted of FtsY. In addition, the amount of membrane-bound ribosomes decreased markedly in cells deprived of FtsY, whereas binding of ribosomes to the membrane is not interrupted in Ffh depleted cells.

We consider these data in frame of a model suggesting that during the process of membrane protein targeting and biosynthesis, ribosomes encounter two membrane binding sites: a docking site and a SecYEG translocon-connected site. Proper membrane protein insertion and folding require both companions, SRP and FtsY and take place near the translocon. On the other hand, binding of ribosomes to the docking site requires FtsY and can occur in the absence of SRP. Our recent results have shown that SRP is able to dissociate ribosomes from this site. Thus, our results highlight the central role of FtsY in ribosome targeting to the membrane and the possible role of SRP during the transfer of ribosomes translating membrane proteins from their docking site to the translocon.

ELONGATION FACTOR-2 KINASE: FROM TRANSLATIONAL CONTROL TO LONGEVITY

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Elongation factor 2 kinase (eEF2 kinase) was discovered in the Institute of Protein Research in the laboratory of A.S. Spirin (1). It was identified as calcium-dependent protein kinase that phosphorylates and inactivates eEF2 and therefore can regulate the global rate of protein synthesis at the elongation stage. Cloning and sequencing of eEF2 kinase led to the discovery of a new class of protein kinases, named alpha-kinases, whose catalytic domains display no sequence homology to conventional eukaryotic protein kinases (2). Several mammalian alpha-kinases have been identified including channel-kinases TRPM6 and TRPM7, representing a novel type of signaling molecules that contain a protein kinase domain fused to an ion channel (3). These channel-kinases play a key role in the regulation of calcium and magnesium homeostasis in vertebrates (4).

To investigate the physiological role of eEF2 kinase and other alpha-kinases we generated knockout mice with the disruption of genes of various alpha-kinases. Our studies of eEF2 kinase knockout mice revealed that eEF2 kinase plays important role in the modulation of stress resistance and longevity. We found that inactivation of eEF2 kinase confers resistance to the lethal effect of gamma-radiation, protects mice from hair graying caused by radiation and protects cells in the intestine from the radiation-induced cell death. We also found that fibroblasts and kidney epithelial cells derived from eEF2 kinase knockout mice are resistant to cell death induced by various cytotoxic agents. Analysis of long-term survival of mice revealed that knockout of eEF2 kinase results in a significant increase in maximal lifespan. Our recent studies suggest the molecular mechanism by which eEF2 kinase through alteration in protein synthesis elongation rate can control cell survival, tissue stress resistance and ageing.

1. Ryazanov, A.G. et al. (1988) *Nature*, 334: 170-173
2. Ryazanov, A.G. et al. (1997) *Proc. Natl. Acad. Sci.*, 94: 4884-4889.
3. Drennan, D. and Ryazanov, A.G. (2004) *Prog. Biophys. Mol. Biol.*, 85: 1-32.
4. Schmitz, C. et al. (2003) *Cell*, 114: 191-200.

MESSENGER RNA STRUCTURE ON THE RIBOSOME IN DIFFERENT FUNCTIONAL STATES

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Recent crystal structures of 70S ribosome containing functional ligands provided information about the general organization of the ribosome and its functional centres. Very little is known about the molecular basis of the interaction of mRNA with the ribosome at different states of the translation. We have compared X-ray structures of ribosome complexes modelling translation initiation, post-initiation and elongation states (Yusupova et al., 2006). In the initiation and post-initiation complexes, the presence of the Shine-Dalgarno (SD) duplex causes strong anchoring of the 5'-end of mRNA on the platform of the 30S subunit, where numerous interactions between mRNA and the ribosome take place. Conversely, the 5'-end of the "elongator" mRNA lacking SD interactions is flexible, suggesting a different exit path for mRNA during elongation. The post-initiation ribosome complex reveals that after initiation of translation, while SD interaction is still present, mRNA moves in the 3'-5' direction with simultaneous clockwise rotation and lengthening of the SD duplex, bringing it in contact with ribosomal protein S2. The E-codon nucleotides in this state are found in the classical A-helical orientation favourable for forming base-pairs with the E-tRNA anticodon. In the post-initiation ribosome complex, the tRNA in the A site is stable and its electron density including the CCA-end is fully visible. During translation the mRNA moves into the ribosome "entry" site surrounded by ribosome proteins S3, S4 and S5. Two additional ribosome complexes containing linear or hairpin structures at the 3'-end of mRNA have been determined.

Yusupova G, Jenner L, Rees B, Moras D, Yusupov M (2006) Structural basis for messenger RNA movement on the ribosome. Nature, 444: 391-394.

CRYSTALLIZATION AND STRUCTURAL INVESTIGATION OF REGULATORY COMPLEXES BETWEEN RIBOSOMAL PROTEIN L1 AND SPECIFIC mRNA FRAGMENTS

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A series of works on the structures of complexes between ribosomal protein L1 and its targets on 23S rRNA and mRNA has been carried out by our group during last years. The structures of the ribosomal L1 protuberance and three regulatory L1-mRNA complexes have been determined and analyzed. In contrast to 23S rRNA binding, L1 binds mRNA only through its domain I. We conclude that domain II of the protein does not contribute to the RNA recognition, but by being involved in 23S rRNA binding, renders the stability of the ribosomal complex higher than the stability of the regulatory complex. This difference fits the requirements of feed-back regulation of translation based on direct competition between two binding sites. To confirm that domain I of L1 recognizes targets for the protein on rRNA and mRNA, truncated L1 containing only domain I have been prepared and crystallized. Its structure is practically identical to that within the intact L1 protein. Stable complexes of the domain with fragments of 23S rRNA and mRNA for L1 have been crystallized. The structure of this domain in complex with mRNA has been solved and compared with the L1-mRNA complex structure. RNA-protein interfaces in the two structures are identical. This is a structural confirmation of the crucial role of the domain I in RNA recognition.

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PROPERTIES OF THE BACTERIAL CTC FAMILY PROTEINS ASSOCIATED WITH THE RIBOSOME

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The CTC family consists of proteins homologous to the product of the gene *ctc* (catabolite controlled) of *Bacillus subtilis*. Genes of the CTC family proteins were found in Bacteria only. These proteins have a domain homologous to *Escherichia coli* 5S rRNA-binding one-domain ribosomal protein L25. Analysis of the structures of two complexes of 5S rRNA with proteins of this family, *E. coli* L25 and *Thermus thermophilus* TL5, revealed that amino acid residues interacting with RNA can be divided into two groups. The first group consists of non-conserved residues. Strongly conserved residues of the second group form intermolecular hydrogen bonds shielded from the solvent. We introduced point mutations into the RNA-binding site of proteins TL5 and L25. It turned out that replacements of residues of the first group do not influence the stability of the RNA-protein complex, whereas replacements of residues of the second group lead to destabilization or disruption of the complex. These data allow us to conclude that this group of strongly conserved residues forms an RNA-recognition module on the surface of the proteins. However, analysis of 300 CTC proteins reveals a few naturally occurring replacements for the second group of amino acid residues among representatives of Bacilli and Cyanobacteria, which have at the same time some changes in the loop E region of 5S rRNA (the binding site for proteins TL5 and L25). We have shown that CTC proteins with such mutations can form stable RNA-protein complexes with corresponding 5S rRNAs. This is an example of co-evolution of the structures of two interacting macromolecules. We have also shown that *E. coli* cells lacking the gene for L25 protein are viable, but grow slower than the parental strain. The ribosomes from L25-defective and parental cells translate *in vitro* at the same rate either poly(U) or natural mRNA. The only difference observed was that the mutant ribosomes synthesized natural polypeptides with less productivity, compared to wild type ribosomes both *in vivo* and *in vitro*. Thus we suggest that although the CTC protein is not essential for the bacterial cell viability, it is required for efficient functioning of the bacterial translation apparatus.

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GENETIC ANALYSIS OF ASSEMBLY OF THE PLATFORM OF THE 30S *ESCHERICHIA COLI* RIBOSOME

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Assembly of ribosomal subunits from isolated rRNAs and r-proteins is a well-coordinated stepwise process, which has been dissected thoroughly *in vitro*. According to an existing *in vitro* model, a set of proteins (the so-called primary-binding) must first bind to naked rRNA. Such a binding is required for assembly of a set of "secondary-binding" proteins into the forming ribosome, *etc*. Thus, ribosomal proteins are believed to perform structural function during ribosome assembly. We focused our study on the role of the ribosomal proteins of the platform of the 30S subunit in viability of *E. coli* cells and translation. Using the modern "recombineering" technique we have performed knockouts of *E. coli* chromosomal genes *rpsF*, *rpsK*, *rpsO*, *rpsR* and *rpsU* encoding for ribosomal proteins S6, S11, S15, S18 and S21, correspondingly. Our data show that r-proteins S6 and S15 are not required, while the rest of the proteins are essential for *E. coli* viability. Our findings are consistent with the previous *in vitro* data, where essential by our approach proteins were shown to be important for ribosome assembly/functioning. At the same time, S15-deficient strain is viable. It is striking, since the S15 protein is the well known "primary binding" r-protein. Its incorporation in the 30S ribosome *in vitro* is shown to be required for assembly of S6, S18, S11 and S21. The S15-deficient strain is compromised for growth at normal conditions. Besides, the strain is cold sensitive. ΔS15 ribosomes contain all the proteins except for S15. Ribosomes from the ΔS15 strain are completely dissociated into subunits in the standard *in vitro* conditions where control ribosomes remain associated. Another interesting result is that the S18 protein may be incorporated into the ribosome in the absence of the S6 protein, though these proteins are thought to stimulate assembly of each other into the 30S subunit *in vitro*. Our findings suggest that assembly of 30S ribosomes *in vivo* may differ from the proposed *in vitro* model.

CRYSTALLIZATION OF THE HETEROTRIMERIC ARCHAEAL TRANSLATION INITIATION FACTOR aIF2

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The structure of a key component of translation initiation in Eukarya and Archaea, initiation factor a/eIF2 (heterotrimeric protein - α,β,γ), is of great interest. At present the structures of its isolated subunits and intersubunit dimers (αγ and βγ) are determined. We plan to determine the structure of the full heterotrimeric protein and its functional complex with GTP and Met-tRNA$_i^{Met}$. Determination of these structures should contribute significantly to the understanding of the molecular mechanism of translation initiation.

We have cloned the genes for all the three subunits of the hyperthermophilic archaeon S*ulfolobus solfataricus* IF2 (Ss-aIF2), obtained overproducing strains and purified the subunits and the full heterotrimeric protein on a preparative scale. The largest γ subunit of Ss-aIF2 has been crystallized in the nucleotide-free and nucleotide-bound forms. The size of the largest crystals was about 450x100x100 µm. The crystals of nucleotide-free Ss-aIF2γ and nucleotide-bound Ss-aIF2γ belong to the space groups $P3_121$ and $P3_1$, and the structures were determined at 2.9 Å and 2.65 Å resolution, correspondingly. The structures have been solved and analysed. A new model for initiator tRNA binding to the Ss-aIF2αγ has been developed.

Recently, we have crystallized the full heterotrimeric initiation factor Ss-aIF2αβγ. Large monocrystals with dimensions about 450x250x50 µm diffract X-rays to 2.85 Å resolution. Structural studies of the Ss-aIF2αβγ are being carried out now.

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NEW INSIGHTS INTO INTERACTIONS OF THE TRANSLATION INITIATION FACTOR 2 FROM ARCHAEA WITH THE NUCLEOTIDES AND INITIATOR tRNA

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Heterotrimeric translation initiation factor a/eIF2 delivers charged initiator tRNA to the small ribosomal subunit. In this work, we have determined the structures of aIF2γ from the archaeon *Sulfolobus solfataricus* in the nucleotide-free and GDP-bound forms. Comparison of the free, GDP and Gpp(NH)p-Mg^{2+} forms of aIF2γ reveals a sequence of conformational changes upon GDP and GTP binding. Our results show that the affinity of GDP to the G-domain of the γ subunit is higher than that of Gpp(NH)p. At variance with the other report we describe an alternative position for Met-$tRNA_i^{Met}$ on the αγ-dimer, which is based on the location of a pyrophosphate molecule in domain II of the γ-subunit. In the model reported here, the acceptor stem of the tRNAi is approximately perpendicular to that of tRNA in the ternary complex EF-Tu-Gpp(NH)p-tRNA. According to our analysis, the elbow and T-stem of Met-$tRNA_i^{Met}$ in this position should make extensive contact with the α-subunit of aIF2. Thus, this model is in good agreement with experimental data, which show that the α-subunit of aIF2 is necessary for the stable interaction of aIF2γ with Met-$tRNA_i^{Met}$.

RIBOSOMAL PROTEIN S1 INDUCES A CONFORMATIONAL CHANGE OF THE 30S RIBOSOMAL SUBUNIT

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A comparative study of the 30S ribosomal subunit in the complex with protein S1 and the subunit depleted of this protein has been carried out by the hot tritium bombardment method. Differences in exposure of some ribosomal proteins within the 30S subunit depleted of S1 and within the 30S-S1 complex were found. It was concluded that protein S1 binds in the region of the neck of the 30S ribosomal subunit inducing a conformational change of its structure.

STUDY OF PLANT 18S rRNA 3' DOMAIN ACCESSIBILITY IN THE COMPOSITION OF WHEAT GERM 40S RIBOSOMAL SUBUNIT

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Some plant viral mRNA 5'- and 3'-untranslated regions (5'-, 3'-UTRs) with known translation enhancer ability show partial complementarity to the 3' domain of plant 18S rRNA. Such complementarity might serve as a means to bind 40S ribosomal subunits and may explain in part the translation enhancer property of these UTRs. In support to this, artificial insertion into mRNA 5'-UTR of a short sequence with complementarity to 3' domain of 18S rRNA has enhanced translation of reporter mRNA in wheat germ (WG) cell-free system.

In order to demonstrate that 3' domain of 18S rRNA is indeed accessible in the composition of WG 40S and can really be involved in complementary interaction with mRNA we used the UV cross-linking approach. For this we designed oligodeoxyribonucleotide (oligoDNA "N2") that was complementary to plant 18S rRNA from 1639 to 1651 nucleotide (nt.) and a control oligoDNA ("N5"), which did not possess such a complementarity. About 95% of ^{32}P-labeled "N2" in concentration of 6 x 10^{-6}M did bind to WG 40S, whereas only 11% of "N5" could bind in the same concentration.
Further we synthesized 4-[*N*-(2-chloroethyl)-*N*-methylamino]benzylmethyl-5'-phosphoramidite derivatives of oligoDNA "N2" (denoted as "RN2") and used it in cross-linking experiments. It was shown that both 18S rRNA and many ribosomal proteins were modified during the alkylation reaction and that these modifications were site-specific.

Treatment with RNase H has revealed that "RN2" binds to 40S subunit via base pairing in the expected region of 18S rRNA. After cleavage of cross-linked 18S rRNA with RNase H, two expected fragments were detected: the first - unlabelled one of approximately 1640 nucleotides, and the second – radio-labeled fragment of 170 nucleotides.

These data suggest that 3' domain of plant 18S rRNA is exposed in 40S ribosomal subunit and is accessible for base pairing with other RNAs.

INTERACTIONS OF RIBOSOMAL PROTEINS WITH RIBOSOMAL AND MESSENGER RNAs

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Specific interactions between ribosomal proteins and ribosomal and messenger RNAs are responsible for the conformation of the ribosomal subunits and feedback regulation of protein synthesis at the translation level. In the most cases specific contacts are formed by complementary surfaces containing the butt-end of a β-sheet and adjacent loops of the protein and the distorted shallow groove of RNA. The contact regions can be divided into two parts: internal and external ones. The internal structurally invariant part is rather used for the RNA-protein recognition whereas the external labile part - for an additional stabilization of the complex. Polar atoms of amino acid residues and nucleotides of the internal parts form intermolecular conserved hydrogen bonds inaccessible to the solvent. The hydrogen-bonding capacity of these atoms is almost without exception fully saturated. Due to a relief of contacting surfaces these hydrogen bonds have different directions and probably determine the specificity of interactions.

MOLECULAR COLONIES

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Molecular colony technique (MCT) was invented by us as a tool for the detection of airborne RNA molecules that invade Qβ replicase reactions producing the impression of a *de novo* (template-free) RNA synthesis (Chetverin *et al*., 1991, *J. Mol. Biol*. 222, 3-9). As far as those experiments demonstrated the ability of MCT to detect, enumerate and clone single nucleic acid molecules, a number of potential applications of this method became immediately evident (Chetverin & Chetverina, 1995, *Russian Federation Patent* 2,048,522; 1997, *U.S. Patent* 5,616,478). The first application of MCT has been the studies on chemical reactions between single RNA molecules generating replicable recombinant sequences, which were detected and analyzed after being amplified by Qβ replicase in the form of RNA colonies (Qβ-MCT). In particular, those studies resulted in the discovery of spontaneous rearrangements in RNA sequences that occur at a rate of 10^{-9} h^{-1} per internucleotide bond. The polymerase chain reaction version of MCT (PCR-MCT), also called “polony technology”, which allows DNA and RNA sequences to be amplified in the form of DNA colonies, has been used for a cell-free gene cloning, including screening *in situ*, and for an extremely sensitive and reliable diagnostics in a digital format. Now, owing to efforts of several laboratories in Russia and in the United States, MCT has become an elaborated technology with unique research capabilities and great biotechnological potentials.

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DIAGNOSTIC POTENTIAL OF THE MOLECULAR COLONY TECHNIQUE

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Carrying out polymerase chain reaction (PCR) in a thin layer of polyacrylamide gel generates a 2D pattern of DNA colonies, each individual colony comprising numerous copies of one starting template molecule. This molecular colony technique (MCT) allows single molecules DNA or RNA targets to be detected and quantified in a digital manner, by counting the number of colonies, without the need for measuring signal intensities. Also, by spatially separating the colonies of amplified molecules, MCT eliminates any competition between different targets in multiplex assays and substantially reduces the interference from a nonspecific synthesis caused by mishybridization of primers with one another and with non-target nucleic acids. We explored the diagnostic potential of MCT by assaying DNA and RNA targets in the whole blood and in bone marrow. To this end, a complete diagnostic procedure was developed, including long-term storage of clinical samples under conditions that ensure full preservation of the DNA and RNA integrity, isolation of nucleic acids by methods that provide for a 100% yield of either DNA or RNA or both of them and remove any inhibitors of reverse transcription and PCR, efficient reverse transcription and in-gel PCR amplification of analyzed molecular targets, and detection of DNA colonies by hybridization with target-specific fluorescent probes. The results show that MCT assay can reliably detect 1 DNA target molecule and 2 RNA molecules in 100-µl aliquots of the whole blood, which is the highest sensitivity ever achieved in molecular diagnostics. Applicability of this assay for diagnosing viral infections and for monitoring the minimal residual disease in cancer patients is demonstrated.

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FUNCTIONAL CIRCULARITY OF QB REPLICASE TEMPLATES

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Qβ replicase (RNA-directed RNA polymerase of bacteriophage Qβ) exponentially amplifies certain RNAs (RQ RNAs) *in vitro*. Although Qβ replicase can initiate and elongate on a variety of RNAs, only some of them are recognized as legitimate templates. GTP-dependent initiation on a legitimate template drives the enzyme to a “closed” conformation capable of elongation in the presence of aurintricarboxylic acid (ATA), a powerful inhibitor of RNA-protein interactions. The structural basis for the discrimination between a legitimate template (an RQ RNA) and an illegitimate template (e.g., the 5' fragment of an RQ RNA) is unknown. RQ RNA could potentially form a hairpin immediately adjacent to a “stalk” formed by the complementary 5'-GGG… and …CCC-3' ends. To explore the role of putative interactions between the complementary RNA termini in the legitimate initiation, we studied effects of the 5' fragment on initiation on the 3' fragment, and also effects of single and double G to A substitutions within the 5'-GGG… sequence on initiation on both the RQ135 RNA and its 3' fragment. Legitimate initiation was monitored by the synthesis of the complementary template copy in a two-step process, in which incubation in the presence of GTP and Mg^{2+} (initiation) was followed by incubation in the presence of all four NTPs and ATA (elongation). We found that the 5' fragment decreases 10 to 30-fold the GTP requirement of initiation on the 3' fragment, which becomes close to the GTP requirement of initiation on the full-length template (RQ135 RNA). On the contrary, mutations within the 5'-GGG… sequence increase the GTP requirements of initiation on both the full-length template and its 3' fragment. The results suggest that the 5' terminus of RQ135 RNA participates in the legitimate initiation and support the idea of a functional circularity of legitimate Qβ replicase templates.

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POLYNUCLEOTIDE PHOSPHORYLASE FROM *THERMUS THERMOPHILUS* AS A TOOL FOR STUDIES ON RNA RECOMBINATION

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Reverse transcription followed by polymerase chain reaction (RT-PCR) is widely used to monitoring recombinations among RNA molecules both *in vitro* and *in vivo*. However, such an assay is heavily prone to artifacts due to the ability of reverse transcriptases of switching between RNA templates at a high frequency. In this work, the artifactual recombination during reverse transcription was essentially eliminated by selective degradation of the secondary templates, to which reverse transcriptase is switching, with polynucleotide phosphorylase (PNPase) from *T. thermophilus*, a 3'→5' exoribonuclease that catalyses processive phosphorolysis of RNA into nucleoside-5'-diphosphates.

The Tth PNPase gene was PCR-amplified using *T. thermophilus* chromosomal DNA as a template, ligated into a plasmid, and expressed in *E. coli* cells. The PNPase was isolated and deprived of any endonuclease activity. The purified enzyme was found to destroy unprotected RNA at 65°C, when the RNA secondary structure is melted. However, RNA remains intact under the same conditions if its 3' terminus is protected by hybridization with a complementary oligodeoxyribonucleotide or by phosphorylation of its 3'-OH group. If a mixture of protected and unprotected RNAs is treated with Tth PNPase, then only unprotected RNA is degraded. Selective degradation of the secondary templates in a mixture of recombining RNAs allows the artifactual recombination during RT-PCR to be decreased by at least 3 orders of magnitude. This approach can be used for monitoring RNA recombination between any RNA species in complex RNA mixtures, including RNA populations of living cells.

We also found that an oligodeoxyribonucleotide hybridized to an internal segment of RNA protects from Tth PNPase both that segment and the upstream sequence. A closer examination of the degradation products revealed that a 9-nt downstream portion of the RNA is also protected. It follows that Tth PNPase can be used for a precise 3'-terminal trimming of RNA stands to any desired length.

This work was supported by the RAS program "Molecular and Cellular Biology".

COORDINATION OF DNA REPAIR MACHINES STUDIED BY AFFINITY LABELING TECHNIQUE COMBINED TO FUNCTIONAL ASSAY

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DNA is continuously damaged by oxygen radicals, alkylating agents, X-rays and environmental mutagens. The outcome of DNA damage is contributing to aging and cancer. Base excision DNA repair (BER) represents a multiprotein system catalyzing complex transformation of damaged DNA. This process requires a finely tuned action of numerous enzymes and protein factors. DNA – protein intermediates that appear in the course of this process are too complicated and very dynamic to be studied by X-ray analysis or other instrumental techniques. Photoaffinity labeling was intensively applied to study assembly of repair machines. The main idea of our approach consists in introduction of photoreactive moieties into DNA in the course of DNA transactions by DNA polymerases. A wide range of photoreactive analogs of dNTPs has been synthesized and used to design photoreactive intermediates of BER either in systems reconstituted of purified proteins or in mammalian nuclear or cellular extracts. Photoreactive branch point intermediates of BER were formed in mammalian cellular extracts to identify the composition of protein ensembles interacting with damaged DNA. The main target proteins covalently linked to branch point BER intermediate were identified by the immunoprecipitation assay and by MALDI-MS as poly(ADP-ribose) polymerase1 (PARP1), flap endonuclease1 (FEN1), DNA polymerase β (Polβ) , apurinic/apyrimidinic endonuclease1 (APE1) and the high mobility group box 1 protein (HMGB1). Crosslinking experiments combined with the functional assay show that PARP1 and APE1 can discriminate DNA intermediates of short-patch and long-patch BER pathways to regulate the process. It was discovered that the switch of BER pathways is mediated by mutual action of APE1, Polβ and PARP1.Chemically reactive DNA structures containing apurinic/apyrimidinic (AP) sites were used for proteomic analysis of protein targets in cellular extracts. PARP1, XRCC1 and Ku70/80 were found to interact with the AP-site containing DNA structures. Therefore affinity labeling combined with the functional assay is a powerful tool to explore proteomic ensembles of DNA repair and to identify the functional composition of these machines.

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MALARIA PARASITE TRICKS AND CHALLENGES IN DEVELOPMENT OF ANTI-MALARIAL VACCINE

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Severe malaria syndromes caused by *Plasmodium falciparum* kill about 2 million people worldwide each year including 200,000 pregnant women in Africa. Attempts to make anti-malarial vaccine over last 60 years have been unsuccessful. The reasons are complex parasite-host interactions, including expression of variable proteins on the surface of infected red blood cells and parasite sequestration, which will be discussed. Nevertheless, immunity against malaria in endemic areas develops over the lifetime, and it takes only a few episodes in childhood to develop protection against severe malaria (severe anemia, respiratory distress, and cerebral malaria). Protection against pregnancy malaria (PM), a special case of malaria in semi-immune adults, also develops after 2 - 3 pregnancies and serves as an excellent model for development of anti-severe malaria vaccine.

We studied the differential parasite gene expression and development of protective immunity in PM using high throughput approaches, including oligonucleotide and protein arrays, and clinical and laboratory parasite isolates. We found that transcription of six genes was substantially increased in parasites collected from pregnant women and each gene encoded a protein with a putative export sequence and/or transmembrane domain. These genes include *var2csa*, a member of the variant PfEMP1 gene family, as well as five conserved genes of unknown function.

We expressed all domains of VAR2CSA and VAR1CSA (6 and 7 domains per protein, respectively; each domain of 30-50 kDa is crosslinked by 6-12 disulfide bonds) using a novel approach that allows folding, rapid purification, immobilization and quantification of target antigen in array format. Both proteins were previously implicated in PM. We found that the reactivity to all VAR2CSA domains was higher with sera from multigravid females than primigravid females or males. Conversely, the reactivity to VAR1CSA was higher with sera from males. Seroreactivity was strongly influenced by antigenic variation of VAR2CSA domains, attributed mostly to the flexible loops. One of the conserved genes expressed by placental parasites, *PFD1140w*, was expressed using continuous cell-free system. Its seroreactivity was highest in multigravid females, similarly to VAR2CSA.

These results strengthen VAR2CSA as a vaccine candidate for pregnancy malaria, highlight a possible role for the conserved genes as pregnancy malaria vaccine targets, and suggest that a suite of genes is involved in the genesis of a well-defined clinical phenotype of *P. falciparum*.

A NEW Tag-BASED APPROACH FOR HIGH-THROUGHPUT ANALYSIS OF CCWGG METHYLATION

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DNA methylation is an important mechanism of gene regulation in many eukaryotic organisms. Non-CpG methylation occurring in the context of CNG sequences is found in plants at a large number of genomic loci. However, little information is available so far about non-CpG methylation in mammals. Efficient methods that would allow detection of scarcely localized methylated sites in small quantities of DNA are required to elucidate the biological role of non-CpG methylation both in plants and animals. We report a novel approach that would allow to detect localization of sites of CCWGG methylation (W is A or T), a particular case of CNG methylation, in genomic DNAs of bacteria and eukaryotes. This technique is based on digestion of DNAs with methylation-sensitive restriction endonucleases *Eco*RII-C and *Ajn*I. We used bacteriophage and plasmid DNAs either methylated or unmethylated at CCWGG sites to test specificity and efficiency of the tag-based approach. We used a modified enzyme *Eco*RII-C, which, unlike its wild type prototype, can completely digest genomic DNA at unmethylated CCWGG sites. 5'-protruding strands were filled in using Klenow enzyme 3'→5' exo$^-$ in the presence of desoxynucleotides mixture (dCTP, dATP, dTTP) and ddGTP. At the next step, DNA fragments were digested with *Ajn*I endonuclease at remaining methylated CCWGG sites. Purified products were ligated with biotinilated oligonucleotide duplexes Tag-f/Tag-r and loaded on streptavidin coated paramagnetic beads. For excision of DNA tags we used *Bsp*D6II type IIS endonuclease which cleaves DNA strands 16/14 nucleotides apart from its recognition site 5'-CTGAAG-3'/5'-GTTCAG-3' presented within the Tag-f/Tag-r duplex. After the cleavage with *Bsp*D6II, DNA ends were ligated with linker duplexes represented by a set of oligonucleotides which are complementary to all possible combinations of 2-nucleotide overhangs. Utilization of PCR amplification of tag sequences potentially increases the sensitivity of the approach, which makes it suitable for developmental studies of methylation in a reasonably small number of cells. PCR products were digested with *Xba*I endonuclease and ligated into arrays of tags. Ligation products were cloned into pUC19 cloning vector at *Xba*I site. This allows high throughput sequencing of tags, identification of flanking regions and their exact positions in the genome. The tag sequences can be use to identify exact location of methylated CCWGG site in the genome.

We conclude, that the tag-based approach provides a highly efficient tool for selective identification of methylated CCWGG sites in a variety of genomes. Notably, knowing the genomic sequence is not a required prerequisite for using this method.

TEMPLATE INDEPENDENT DNA SYNTHESIS BY Bst DNA POLYMERASE IN THE PRESENCE OF SITE-SPECIFIC DNA NICKASES

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Initiation of DNA synthesis in a typical replication mode requires a template DNA strand and a primer. Ogata and Miura found that highly purified DNA polymerases from thermophilic bacteria were able to synthesize DNA without template and primer DNA. The double-stranded DNA product synthesized comprises numerous short repeating sequences resembling satellite DNA in eukaryotes. These sequences exhibit a quasipalindromic structure with a repeating unit (motif). This phenomenon was called 'creative' or *ab initio* DNA synthesis. Liang *et al*. showed that 'creative' synthesis of DNA can be enhanced by the addition of a thermostable restriction endonuclease for which there is a recognition site within the highly repetitive palindromic motif sequence.

We found that highly efficient DNA synthesis without template and primer DNAs occurs when site-specific DNA nickases N.*Bsp*D6I (recognition sequence: 5'…GAGTCN$_4$↓…3'), N.Alw I (recognition sequence: 5'…GGAGTCN$_4$↓…3'), N.*Bbv*C IA (recognition sequence: 5'…GC↓TGAGG…3'), and N.*Bsm* I (recognition sequence: 3'…CTTAC↓GN…5') were added to a reaction mixture containing deoxynucleoside triphosphates and the large fragment of *Bst* DNA polymerase. In the presence of the N.*Bsp*D6I DNA nickase over a period of 2 h, virtually all the deoxynucleoside triphosphates become incorporated into DNA. Inactivation of the N.*Bsp*D6I nickase by heating inhibits DNA synthesis. Optimal N.*Bsp*D6I activity is required to achieve high yields of synthesized DNA. Electron microscopy data revealed that the majority of DNA molecules have a branched structure. In the presence of N.*Alw* I, N.*Bbv*C IA, and N.*Bsm* I in a reaction mixture the template-independent DNA synthesis displayed the resembling kinetics. Cloning and sequencing of the fragments synthesized demonstrated that the DNA product mainly consists of non-palindromic tandem repeats containing nickases recognition sites and insertions of random nucleotides.

We also discuss a possible mechanism that addresses template-independent DNA synthesis stimulated by the N.*Bsp*D6I nickase. Linear DNA fragments encompassing uniformly oriented motifs are cut by the N.*Bsp*D6I nickase on one strand of DNA. The *Bst* DNA polymerase extends this strand using a 3'-terminus exposed by the nick and displaces the parent strand. After elongation the *Bst* DNA polymerase adds random 3' NN overhangs due to its intrinsic nucleotidyl transferase activity (unpublished observation). During subsequent rounds, more displaced DNA strands carrying random 3' NN overhangs accumulate in the reaction mixture. It is plausible that the newly synthesized strand has a 3' overhang of N' N' complementary to the 3'-terminus of one of the previously displaced strands. The large fragment of the *Bst* DNA polymerase can mediate joining of the complementary ends and carry out DNA synthesis. Importantly, this mechanism is supported by our data showing short insertions of random nucleotides in the boundary regions between blocks of uniformly oriented motifs. Recurring rounds of strand displacement lead to the appearance of single-stranded molecules with motifs arranged in the reverse orientation. The 3'-termini of these DNA strands may form hairpin structures and prime DNA synthesis. Nicking and displacement of such strands would create molecules with larger blocks of motifs. As the number of these molecules increases, the probability of intermolecular hybridization increases and branched molecules appear.

SITE-SPECIFIC ENDONUCLEASES ENCODED BY T5-LIKE BACTERIOPHAGES: BIOCHEMICAL PROPERTIES AND GENETIC ROLE

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Eleven nicking and one DNA double strand cleaving endonucleases encoded by three T5-like bacteriophages were identified. Two of them, F-TfII and F-TfIII, were expressed in *Escherichia coli* cells and purified nearly to homogeneity. One Zn^{2+} ion was found to be bound with each F-TfII and F-TfIII molecule. The activity of both enzymes is stimulated by a wide range of divalent metal ions and reaches optimal levels at extreme conditions (pH 10.0; 50°C – 55°C). F-TfII, F-TfIII as well as homologous to them F-TfIIV were shown to be able to initiate homing. DNA footprinting, modification-interference assays, mutational analysis of binding sites and computer modeling of enzyme-DNA complexes provide evidences that highly homologous F-TfII – F-TfIIV endonucleases recognize their DNA substrates in a different mode. The evolution of recognition properties of these endonucleases will be discussed.

THE PRINCIPLES OF NANOSTRUCTURAL ORGANIZATION OF THE MULTIDOMAIN MUSCLE PROTEIN TITIN

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The molecular mechanism of the high elasticity of titin should be considered within the framework of the physics of structural transitions in crystalline polymers. Experimental data on stretching of titin fibre coincide with the concept of orientational melting: the melting temperature of crystallites (β-barrel) oriented along the force vector is higher than that of the ones square with it. Schematic models for structural dynamics of titin fibre during stretching were suggested.

A nanoscale morphological model of a titin molecule consisting of an array of rigid domains of Ig- and FN3-types covalently-connected by conformationally variable short loops of a polypeptide chain is proposed. The main peculiarity of our model is the presence of non-equivalent positions of domains due to three possible types of orientational distortions sensitive to the environment, namely splay, twist and bend of the conformationally variable loops. The line group of symmetry of the structure can be determined as SM with axial translation $\tau\infty$.

The steady-state stress-strain curves for titin fibre show an elastic behavior similar to that of crystalline polymers, the stress-strain relation of titin fibre clearly displays three stages. The stretching of titin fibre produces considerable changes in the X-ray diffraction patterns: the SAXS reflections disappear, but the WAXS ring reflections at spacings of 0.98 nm and 0.46 nm persist.

In an individual titin macromolecule, a novel double-phase state of configuration of the single polypeptide chain was revealed experimentally by X-ray diffraction and mechanical technique. The highly ordered crystallites (β-barrels) become more rigid and co-exist with a fully melted, unfolded polypeptide chain. The nanophase-separated morphologies of the titin molecule with characteristic lengths on the nanometer scale can develop only during stretching.

The functional heterogeneity of a titin strand determined by the amino acid sequences of homologous domains and loops is adjusted by a local change of the force vector inside the sarcomere at a given time. Under the influence of external forces the structure of any domain can become either rigid or flexible, depending on its orientation in the titin strand. Nanophase separation of structure can develop in any covalently-connected multidomain macromolecule. Today for the analyses of X-ray diffraction patterns of nanophase separated biological systems it is prospective to use the conceptions developed for description of structurally inhomogeneous silicate nanoparticles consisting of inter-penetrating fragments with different symmetry. Regularly oriented fragments with different symmetry, for which the requirements of classical crystallography become violated, were called “centaurs”.

The hyper elasticity of titin is discussed using the theory of nanostructural symmetry of rod-like morphology of biological materials. Nanoparticles in which numbers of “surface” and “volume” atoms are comparable, can be assembled from a rather scanty set of special atomic groups (generating clusters or domains) into geometrical structural complexes in which the coherent boundaries of clusters are secured. Nanoparticles are presumed to be associated with the presence of numerous determined states as intermediate phases, which can be described by non-crystallographic groups involving an infinite point group. In the terms of the concept of nanostructural self-organization the origin of the functional heterogeneity along the titin strand becomes interpretable.

Thus, the size of a nanoparticle can be considered as a physicochemical factor, which determines specific properties of nanostructure, and the assembly laws are defined by the generating clusters themselves and topological properties of space or physical fields.

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STRUCTURAL ANALYSIS OF THE Rcs SIGNALLING PATHWAY IN ENTEROBACTERIA

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Bacteria as well as lower eukaryotes use phosphorylation cascades in order to respond to changing environmental conditions. A key mechanism of signalling pathways is the communication of membrane integrated sensor kinases with cytoplasmatic effector proteins by complex reversible phosphorylation reactions involving multistep phosphorelay systems.

The Rcs regulatory network is a global signalling system that controls a variety of operons involved in capsule synthesis, virulence, motility or cell division. The membrane bound sensor unit is formed by a heterodimer of the hybrid kinases RcsC and RcsD while the cytoplasmatic effectors RcsA and RcsB form a heterodimer upon DNA-binding. The arrangement of enzymatic domains with histidine kinase, phospho-receiver, phospho-transfer and DNA-binding activities is characteristic of the Rcs system and essential for the modulation of signal transfer. We present the structural evaluation of two central functional domains (RcsC phosphoreceiver – RcsC-PR, and RcsD histidine phosphotransfer – RcsD-HPt domains) of this signalling system by heteronuclear high resolution NMR spectroscopy. We further describe the so far unique structural fold of newly identified domains integrated in the RcsC and RcsD sensor kinases as well as in kinases of other bacterial signalling systems. Phospho-transfer mechanisms and phosphorylation-dependent cascade of protein-protein interactions has been analysed by multiple approaches. We have mapped the interaction of RcsC-PR with RcsD-HPt and characterized the conformational effects of Mg^{2+} and the phosphorylation mimetic BeF_3^- on RcsC-PR.

ENERGETICS OF DOMAIN-DOMAIN INTERACTIONS AND ENTROPY DRIVEN ASSOCIATION OF β-CRYSTALLINS

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β-Crystallins are major protein constituents of the mammalian lens, where their stability and association into higher order complexes are critical for lens clarity and refraction. They undergo modification as the lens ages, including cleavage of their terminal extensions. The energetics of βA3- and βB2-crystallin association was studied using site-directed mutagenesis and analytical ultracentrifugation. Recombinant (r) murine wild type βA3- and βB2-crystallins were modified by removal of either the N-terminal extension of βA3 (rβA3Ntr) or βB2 (rβB2Ntr), or both the N- and C-terminal extensions of βB2 (rβB2NCtr). The proteins were expressed in Sf9 insect cells or *E.coli* and purified by gel-filtration and ion-exchange chromatography. All β-crystallins studied demonstrated fast reversible monomer-dimer equilibria over the temperature range studied (5 ^{o}C to 35 ^{o}C) with a tendency to form tighter dimers at higher temperatures. The N-terminal deletion of rβA3 (rβA3Ntr) significantly increases the enthalpy (+10.9 kcal/mol) and entropy (+40.7 cal/deg mol) of binding relative to unmodified protein. Removal of both N- and C- terminal extensions of rβB2 also increases these parameters but to a lesser degree. Deletion of the βB2-crystallin N-terminal extension alone (rβB2Ntr) gave almost no change relative to rβB2. The resultant net negative changes in the binding energy suggest that βA3-and βB2-crystallin association is entropically driven. The thermodynamic consequences of the loss of βA3-crystallin terminal extensions by *in vivo* proteolytic processing could increase their tendency to associate and so promote the formation of higher order associates in the aging and cataractous lens.

ELECTRON MICROSCOPY AS A CRITICAL COMPONENT OF NANOSCIENCE RESEARCH

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The field of NanoScience is concerned with the study of matter on the nanoscale (10^{-9}m), a scale where the fundamental biological, chemical and physical processes take place. The key and revolutionary aspect of nanoscience is that we are now able to manipulate matter on the nanoscale and can create or modify it by design. Modern electron microscopy (EM) is used for structural analysis and visualization in the nano-world. Historically, nanoscience has been associated with "nanostructures" of inorganic matter: fullerenes, nanotubes, quantum dots, etc. Nevertheless, there is a large area where extremely sophisticated processes take place at the nanoscale level - in biological matter. Indeed, nano-machinery is involved in major biological processes such as transcription, translation, and protein biosynthesis, as well as virus host interactions. In 2002, the State of California established the California NanoSystems Institute (CNSI). The Electron Imaging Center for Nanomachines (EICN) is a key core facility of this institution, and will provide access to modern electron microscopes and cutting edge EM techniques. It will be equipped with state-of-the-art instruments – a 200kV TF20, a 300kV Titan STEM, and a Titan Kryos electron microscope from FEI (The Netherlands), as well as SEM, a PACT-2 (Leica, Austria) high-pressure freezer and other equipment for sample preparation, cryo-electron microscopy (cryo-EM) and tomography, and 3D reconstruction and visualization. Structural analysis of biological "nanomachines" and their localization/interaction in cells are a significant part of the research that will be featured at EICN.

A number of individual proteins, macromolecular complexes, DNA/RNA-protein complexes, and viruses have been studied using modern EM approaches including cryo-EM and 3D reconstruction. Structural information obtained by EM is used to alternate/create new functions/properties of the "nano-objects." For instance, vault particles (Rome, UCLA) are modified in such a manner that they may be used as a therapeutic delivery vehicle, targeting particular cells, etc. Modified proteins, DNA etc. may function as structural blocks of artificial "nano-machinery" including "nano-computers," "nano-sensors" and other "nano"-devices.

STRUCTURE, FUNCTION, AND EVOLUTION OF POST-TRANSLATIONALLY MODIFIED MICROCINS

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Microcins are a class of small (<10 kDa) ribosomally-synthesized antibiotics produced by *Enterobacteriaceae* and active against closely related bacterial species. While some microcins are active as unmodified peptides, others are heavily modified by dedicated maturation enzymes. Microcins from the latter, post-translationally modified group target essential molecular machines inside the cells. Our recent structural and functional data about two distinct post-translationally modified microcins: microcin J (McJ), which inhibits bacterial transcription by targeting DNA-dependent RNA polymerase, and microcin C (McC), which inhibits translation by targeting an aminoacyl-tRNA synthetase, will be discussed. While both McJ and McC are produced by *E. coli*, inferences based on sequence and structural similarities with peptides encoded and/or produced by phylogenetically diverse bacteria will be presented to put microcins into a larger perspective.

PROTEIN COMPOSITION OF HUMAN snRNPs AND SPLICEOSOMAL COMPLEXES REVEALED BY TWO-DIMENSIONAL ELECTROPHORESIS

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Pre-mRNA splicing is catalyzed by the spliceosome, which consists of U1, U2, U4/U6 and U5 snRNPs and a number of protein factors. Spliceosome assembly proceeds via several intermediate stages in a stepwise manner accompanied by recruitment of different proteins and snRNPs. The most stable intermediates (named spliceosomal complexes E, A, B and C) can be isolated by affinity purification under different stringency. Mass spectrometry of human spliceosomal complexes purified under native conditions revealed a high number of spliceosome associated proteins that taken together results in hypothetical particles with at least a doubled total molecular mass as compared to the mass visualized by other physical-chemical methods. This implies that proteins are presented in very different amounts that is quite far from the stoichiometric ratio and calls for a more quantitative assessment of the spliceosome protein composition. Here we report about two-dimensional electrophoresis that could suit for such a purpose in a similar way as one employed for separation of ribosomal proteins. The advantages of the suggested approach are discussed.

NON-LINEAR ANTIMICROBIAL PEPTIDES

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Tetrapeptides Arg-Leu-Ala-Arg and Lys-Leu-Ala-Arg are repeated in the primary structure of a series of natural antimicrobial peptides. This allows concluding that these tetrapeptides are the main structural elements of antimicrobial peptides.

The presented work is dedicated to the synthesis and study of non-linear peptides containing this kind of tetrapeptides: [Lys-ε-(Lys-Leu-Ala-Lys)]n n= 1,2,3,4. The synthesis of these peptides was carried out using activated pentafluorophenyl esters.

Antimicrobial activity of the synthesized peptides depends on the n-value and reached 15 μM at n=4 (*E.coli*), which is close to activities of several natural antimicrobial peptides.

The obtained non-linear peptides have no fixed secondary structure.

THE STRUCTURE OF THE CHIMERIC PROTEIN IMITATING SH3-PEPTIDE INTERACTION

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Domains that recognize proline-rich sequences are supposed to be of key importance as they mediate transient protein–protein interactions in signal transduction within eukaryotic cells. We have designed a chimeric protein (WT-C-II-A) by extending the chain of spectrin SH3 domain with a proline-rich decapeptide PPPVPPYSAG trough a twelve-residue linker. This oligopeptide should supposedly stick to the conservative binding site, while its linkage should increase the affinity. Nevertheless, DSC and fluorescence data did not prove that the binding occurred in solution.

The crystals of the chimera were grown at pH 4.5, 2 M ammonium sulfate, and protein concentration of 7.5 mg/ml. The crystals are referred to P32 space group with elementary unit parameters of a=b=36.390, c=112.17, α=β=90.00°, γ=120.00° and allow structure determination at 1.8 Å resolution. A set of diffraction data from one crystal was collected on the DESY synchrotron in Hamburg and processed by the CCP4 program package.

The phase problem has been solved by the molecular replacement method. Most likely the asymmetric unit contains from 2 to 4 molecules, with Matthews's coefficient varying from 4.29 to 2.14 and the solvent content from 71% to 42%. The published structure of α-spectrin SH3 domain (1.8 Å resolution; PDB code 1SHG) was used as the initial approximation. The final model has been refined to the R-factor of 22.6% and R_{free} of 25.9% at 1.85 E, 67 water molecules and 2 acetate molecules were localized within the free part of the elementary unit. It was found that two SH3 globules are packed "face-to-face" making a triple complex with one central ligand fragment (PPPVPPY), which adopts orientations either I or II with respect to the binding sites. The localizations of both linkers and the rest of the ligand are not defined.

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CELL-FREE EXPRESSION OF POLYTOPIC INTEGRAL MEMBRANE PROTEINS IN PREPARATIVE SCALES

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The preparation of sufficient amounts of functionally folded membrane proteins is usually the first and major bottleneck in structural and functional approaches. Cell-free expression techniques have been emerged in recent times as a powerful perspective for fast and highly efficient production systems as they eliminate most intrinsic problems of membrane protein production in conventional *in vivo* systems. The direct synthesis of membrane proteins into defined artificial hydrophobic environments offers completely new possibilities of membrane protein production. In addition, the open nature of cell-free reactions and the unrestricted access allows intensive optimizations of expression conditions and protocols can be individualized according to specific requirements of particular target proteins.

We demonstrate the high level cell-free production of a variety of polytopic membrane proteins of prokaryotic as well as of eukaryotic origin and belonging to a comprehensive selection of different families. Membrane proteins involved in transport, efflux, signalling, metabolism or biosynthesis can be produced by cell-free expression in mg amounts in a single ml of reaction mixture. The throughput optimization of membrane protein expression was established as an integrated process with a robotic platform and its efficiency is demonstrated with the expression of representative subsets of the *E. coli* inner membrane proteome. The quality of synthesized membrane proteins was assessed by structural and specific functional assays and a number of conditions could be identified that can have strong impacts on the functional folding of synthesized proteins. The high quality of the isolated samples is demonstrated by single particle analysis and by complex formation with their natural peptide ligands. By systematic engineering of the human endothelin B receptor, we could identify and co-localize the interface for homo-dimer formation and the binding site for the natural peptide ligand endothelin-1 to an area of 40 amino acids centred on the first transmembrane helix. Cell-free systems are thus promising tools for the fast isolation and characterization of so far highly difficult membrane proteins and future developments like liposome-based expression protocols will even further extend their applications.

A STABLE α-HELIX-RICH INTERMEDIATE IS FORMED BY A SINGLE MUTATION OF THE β-SHEET PROTEIN, src SH3, AT pH3

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Recently, we have found a transient intermediate on the folding pathway of src SH3. Intending to investigate the structure of the transient intermediate, we tested a mutant of src SH3, named A45G, using circular dichroism, fluorescence and X-ray solution scattering, and incidentally found that it forms a stable α-helix-rich intermediate (I_{eq}) (different from the native β-sheet-based secondary structure) at pH 3.0, but contains only β-sheets at pH6.0, whereas wild-type SH3 forms only β-sheets at both pH 3.0 and 6.0. The intermediate I_{eq} shows a circular dichroism measured at θ_{222}=-10,300 deg*cm^{2}*dmol^{-1}, indicating a 31 % α-helix proportion, as estimated by the CONTIN program. X-ray scattering gave the radius of gyration for I_{eq} as 19.1 Å at pH 3.0 and 15.4 Å at pH 6.0, and Kratky plots showed a clear peak at pH 3.0, 4.0 and 6.0, indicating that I_{eq} too is compact. In these parameters, I_{eq} closely resembles the kinetically obtained intermediate I_{kin}, which we found on the folding pathway of wild-type SH3 at pH 3.0 (radius of gyration 18.7 Å and θ_{222}= -8,700 deg*cm^{2}*dmol^{-1}, indicating a 26% α-helix proportion (Li *et al*, Biochemistry, in press)). Refolding experiments with A45G were done at pH 6.0 by stopped-flow apparatus monitored by circular dichroism, and compared to kinetic experiments with wild-type SH3 at pH 6.0. The result showed an α-helix-rich intermediate at the same dichroism amplitude, but 9 times slower in formation-rate. A pH-jump experiment from pH 3.0 to pH 5.9 on A45G was also performed. This showed no bursts, and the rate of conformation-change was almost as fast as the refolding rate of A45G at pH 6.0. These kinetic experiment data would be consistent with I_{eq} being nearly identical to the I_{kin}, which appeared on the folding pathways of both wild-type SH3 and A45G.

hnRNPs AND EPIGENETIC CONTROL OF GENE EXPRESSION

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Epigenetics has been defined as heritable changes in gene expression states that are not associated with alterations in DNA sequence. Instead, DNA and histone modifications are believed to serve the role of stable persistent marks maintaining the gene expression state. However, despite the common belief, the notion that epigenetic changes are not linked to alterations in DNA sequence has no experimental support. We used budding yeast as a model system to address this issue. In *Saccharomyces cerevisiae*, cryptic mating locus *HMR* is an established example of epigenetic control of gene expression. This locus is located near the telomere on the right arm of chromosome III, it contains a1 and a2 genes flanked by E and I silencers. In the wild type cells, *HMR* is well silenced but in *sir1*Δ background, cells split into two fractions, with *HMR* in either ON (~99%) or OFF (~1%) transcription state. Eventually cells switch *HMR* to the opposite expression state in a poorly characterized process called the *epigenetic switch*. We found that cells selected in the *HMR* OFF state always have the nearest telomere much longer than unselected cells indicating that the telomere length governs the transcription state at *HMR*. In cells lacking hnRNP K like protein Hek2, cells enriched in the *HMR* OFF state have normal size of the proximal telomere but instead they have ~30% more copies of rRNA genes than unselected cells. As the yeast genome contains an array of 150 copies of rRNA genes, extra 50 copies represent ~4% of the yeast genome. We further explored this phenomenon to show that there is causal link between the rRNA copy number and *HMR* expression. Our data show that epigenetic control of gene expression in yeast is governed by alterations in DNA, and implicate hnRNPs in these processes.

FORMATION OF TROPOMODULIN/TROPOMYOSIN COMPLEX AT THE POINTED END OF THE ACTIN FILAMENT

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Tropomodulin is a family of proteins that cap the pointed, slow-growing end of actin filaments and require tropomyosin for optimal function. Tropomodulin is an elongated molecule; its C-terminal half is composed of alternating alpha helices and beta strands, forming a LRR (leucine-rich repeat) domain, while the N-terminal half is mostly disordered. The disordered part of Tropomodulin contains three of four known binding sites, two tropomyosin-binding sites, residues 1-38 and 109-144, and a tropomyosin-dependent actin-capping site, residues 48-92. It was shown that one tropomodulin molecule simultaneously binds two tropomyosin molecules in a cooperative manner. The affinities of tropomodulin as well as leiomodin, the larger homolog of tropomodulin, for different isoforms of tropomyosin were determined and compared. It was shown that the binding of tropomyosin to tropomodulin and leiomodin is isoform-specific and differs almost 100-fold for different tropomyosin isoforms. A proposed model for the binding of tropomodulin to actin and tropomyosin at the pointed end of the filament shows how the tropomodulin-tropomyosin accentuates the asymmetry of the pointed end and suggests how subtle differences among tropomyosin isoforms may modulate actin filament dynamics.

MICROTUBULES IN STRESS GRANULE DYNAMICS

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Stress granules are micron size RNP-structures, which are formed in the cytoplasm under stress conditions. They consist of translation initiation complex components such as eIF3, mRNA, polyA-binding protein (PABP), and some specific stress-activated proteins, e.g. TIA-1. Stress granules exist in dynamic equilibrium with polysomes. Cycloheximide induces dissociation of stress granules while puromicin increases them. Previously we showed that the arsenate-induced stress granule formation is dependent on microtubules. Stress granules did not assemble in nocodazole-treated cells. Probably, stress granule components moved along microtubules reflecting the movement of some components of the translational machinery. We decided to study whether stress granules could move along microtubules, and whether their disassemby also depends on microtubules.

We performed a time-lapse videomicroscopy study of living HeLa cells expressing EGFP-PABP and EGFP-TIA-1. Fluorescent stress granules were formed under arsenate treatment, and these granules moved around in the cytoplasm. The movement of stress granules was mostly chaotic though sometimes rather rapid. We measured the velocity of stress granule movement at three second intervals and found that it was independent of the stress granule size. At least 35% granules moved with the velocity more than 0.2 μm/s (threshold rate for active transport), and sometimes they demonstrated displacement with the rate up to 1.0 μm/s. After disruption of microtubules with nocodazole, less than 5% granules exceeded the threshold velocity. This observation confirmed that the stress granules movement was driven by microtubule-dependent transport.

We found that cycloheximide treatment induced dissociation of stress granules: about 70-80% of HeLa cells treated with arsenate had stress granules, and only 20% cells contained such granules when cycloheximide followed arsenate. If microtubules were disrupted with nocodazole, cycloheximide did not influence the number of cells with stress granules. Disruption of actin filaments with latrunculin B did not affect either the stress granule formation under arsenate or their dissociation under cycloheximide.

We used FRAP analysis to study stress granule component mobility. After photobleaching of individual EGFP-PABP-containing granules with argon laser, we observed a rather slow recovery of fluorescence. The half-time of recovery was 450-500 s. If EGFP-PABP was photobleached in the cytoplasm, the fluorescence recovery was more rapid, in a few seconds. After nocodazole treatment of cells, the mobility of EGFP-PABP in the cytoplasm did not change though the half-time of recovery of photobleached stress granules increased two-three times. These experiments confirmed our assumption that stress granule components move along microtubules.

We developed a method of isolation of stress granule-enriched preparation. The fraction of stress granules was isolated from EGFP-TIA-1-expressing arsenate-treated cells and contained stress granules visible by fluorescence microscopy. To obtain the granules, cells were lysed with 40x passing through a hypodermic syringe needle, the cell debris was sedimented at 500 g, stress granules were sedimented at 4000 g, resuspended, treated with Triton X-100 and resedimented. In comparison with the same cytopasm preparation isolated from control cells, the preparation of stress granules was enriched with EGFP-TIA-1, eIF3, PABP, and also with microtubule motor protein dynein. Dynein was also found in stress granules by immunofluorescence staining. Interestingly, stress granules did not contain dynein co-factor dynactin, which is thought to connect dynein with membrane organelles. Stress granules probably contained kinesin I, and lacked kinesin II. Thus, stress granule components might move along microtubules driven by common microtubule motor proteins kinesin I and dynein. We also found that focal adhesion protein paxillin partially migrates to stress granules under arsenate treatment. This cytoskeletal protein was shown previously to bind to PABP, and it might be a linker between translation machinery components and microtubule proteins.

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ROLE OF INTERMEDIATE FILAMENTS IN MITOCHONDRIA DISTRIBUTION

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Proper distribution of mitochondria in cells is important for their function and is achieved by the interaction of these organelles with the cytoskeleton. Mitochondria move to long distances using microtubules as tracks and to shorter distances along actin filaments. Besides they can anchor to certain structures of the actin cytoskeleton but the mechanisms of these interactions are largely unknown. Recent data have implicated intermediate filaments (IF) in cross-linking of different cytoskeleton structures and organelles in cells. In order to investigate their possible role in mitochondria anchoring, we analyzed the movements of these organelles in cells isolated from vimentin-null mice devoid of IF. Our data show that mitochondria motility in cells lacking IF is much higher than in wild type cells that contained IF or cells with restored IF. Interestingly, the motility of mitochondria in vimentin-null cells was insensitive to disruption of fibrillar actin (F-actin) in contrast to IF-containing cells. These data indicate that IF are involved in the interaction of mitochondria with F-actin.

We have found earlier that activation of protein kinase C (PKC) with phorbol ester leads to enhanced mitochondria motility while inhibition of this enzyme causes their stoppage. In this study we show that activation of PKC increases movements of mitochondria even in cells with disrupted F-actin that points to the target of PKC other than F-actin. As soon as activation of PKC does not affect mitochondria motility in vimentin-null cells we conclude that this kinase controls mitochondria interaction with IF.

So, we demonstrate that IF are responsible for mitochondria distribution and PKC controls their interaction.

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DYNEIN, DYNACTIN AND PROTEIN KINASE LOSK IN MICROTUBULE ARRAY ORGANIZATION

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Interphase microtubules are organized into a radial array with the centrosome in the center. Cytoplasmic dynein is known to be involved in the establishment of radial microtubule (MT) arrays. In interphase cells, anti-dynein reagents such as CC1 fragment of dynactin subunit p150Glued induce loss of radial MT organization, suggesting that dynein is involved in MT nucleation, retention of MTs at the centrosome, orin the structural integrity of the centrosome itself. To distinguish between these possibilities, we examined the effect of microinjection of anti-dynein reagents (CC1 fragment, 74.1 antibody, recombinant dynamitin) on MT distribution and centrosome protein composition in cultured fibroblasts. We found that while the levels of major centrosomal proteins involved in MT nucleation or anchoring did not change significantly in the injected cells, anti-dynein reagents indeed induced rapid loss of radial MT organization. MT nucleation rates at the centrosome measured with EB-1 protein comets also remained unaffected by anti-dynein reagents; leaving the only remaining possibility that dynein is required for MT stabilization at the centrosome. To further examine the mechanism of dynein-dependent MT stabilization, we tested the effects of dynein-blocking reagents in cells pre-treated with MT-stabilizing drug taxol. We found that taxol treatment protected radial MT organization from disruption by anti-dynein reagents. Our results support a model for the organization of a radial MT array that involves dynein-dependent stabilization of MT minus ends from disassembly.

Radial microtubule organization is a subject of cellular regulation probably driven by protein phosphorylation. Few protein kinases which regulate the interphase microtubule array were described before. Ste20-like protein kinase LOSK (SLK) was identified by us as a microtubule and centrosome associated protein. While expressed in cells, LOSK/SLK N-terminal catalyticaly active domain (LOSK-ΔT) had no obvious effect on microtubule organization. However, either LOSK/SLK activity inhibition by over-expression of dominant-negative mutant LOSK^{K63R}-ΔT or LOSK/SLK depletion by RNAi led to a disordered microtubule arrangement. Microtubule disorganization was prominent in Vero, CV-1, and CHO-K1 cells and unclear in HeLa and PEK cells. The effect was the result neither of microtubule stabilization nor of centrosome disruption. In cell with suppressed LOSK activity the centrosome lost its ability to anchor to cap microtubules, and the level of centrosomal dynactin was reduced. However, the centrosome ability to nucleate microtubules was not affected. Vero cells with over-expressed LOSK^{K63R}-ΔT had unaltered Golgi though were unable to polarize Golgi at the wound edge. Thus, protein kinase LOSK/SLK is required for microtubule organization in certain cell types, and its effect is probably mediated by dynactin.

We showed also that in aged Vero cytoplasts (17 hours after enucleation) the microtubule system lost the radial organization and became chaotic. To clear up the reasons for that, we studied the centrosome activity and its position in the cytoplasts, and the distribution of dynein-dynactin components in the cell. It was found that the centrosome in aged cytoplasts with a chaotic microtubule system was still active and was located in the central region of the cytoplasm, while after total disruption of microtubules it moved to the cell periphery. The microtubules in such cytoplasts were not stabilized, but they lost their ability to interact with the cortex. Moreover, the cortex of aged cytoplasts did not contain dynactin, in contrast to new-made cytoplasts. Therefore, we concluded that cytoplasm-dispersed motors were involved into the centrosome positioning in the center of the cell and that the microtubule-cortex interaction influenced the radial structure of microtubule system directly, via regulation of the microtubule steady-state dynamics.

We found that large dynactin subunit p150Glued had two isoforms. The shorter 1B isoform had a deletion of 20 a.a. in its second microtubule-binding domain. We established that the longer 1A isoform was expressed in nervous cells, and the shorter isoform in non-nervous ones. When expressed in fibroblast-like cells, 1A isoform was distributed along microtubules, and 1B isoform bound to microtubule plus ends. Probably, this difference in the distribution of dynactin isoform reflects the difference in microtubule-dependent transport organization in neurons and fibroblast-like cells.

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DENATURATION AND RENATURATION OF GLOBULAR PROTEINS: SCIENTIFIC AND BIOTECHNOLOGICAL ASPECTS

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The report represents some experimental results of globular proteins denaturation and renaturation studies. Scientific aspects of these studies concern to the clarification of the structure and stability of the intermediate states, rates of protein refolding, as well as the influence on protein refolding of protein ligands, intramolecular cross-linking, nonspecific intermolecular association and chaperones. The multi-stage sequential mechanism of protein unfolding and refolding has been demonstrated using carbonic anhydrase as an example. Biotechnological aspects of protein denaturation and renaturation studies include the recommendations on reactivation of recombinant proteins from inclusion bodies and prevention of newly synthesized proteins aggregation. The usage of denatured proteins for purification and analysis of cellular chaperones has been demonstrated using GroEL chaperonin as an example. These studies were supported in part by the RAS Program "Molecular and Cellular Biology", HFSPO, RFBR and by the Howard Hughes Medical Institute Awards.

EQUILIBRIUM FOLDING INTERMEDIATES AND THEIR ASSOCIATION

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The existence of equilibrium intermediates on folding pathways of small globular proteins is widely accepted since the concept of “molten globule” has been introduced into the protein folding area. This concept, however, became rather vague as a result of a biased application of its formal criteria, such as “residual” secondary structure or seemingly non-cooperative unfolding, to diverse chain conformations. Some of them include small well-structured cores exhibiting wide unfolding transitions due to the low heat effect, as, for example, in the case of eukine lysozyme. Others form small cooperatively unfolding clusters through equilibrium association of short β-structured segments. These oligomeric complexes might be more stable than the native conformation at elevated temperatures or low pH, as it occurs with Cro repressor and its modifications. Rather frequently the ability to form “molten globule” oligomers increases as a result of point mutations destabilizing the native structure with respect to these intermediate states. The properties of partially folded states revealed in our structural and thermodynamic studies will be discussed in this report.

THE ANALYSIS OF MULTI-STAGE DENATURATION AND RENATURATION KINETICS OF MONOMERIC GLOBULAR PROTEINS: CARBONIC ANHYDRASE B

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Until last time the most experimental studies of protein refolding have been focused on small globular proteins, which fold in a two-state manner. There are a lot of approaches to analyze the experimental data obtained for these proteins to study the transition state structure that determines the protein folding rate. However, the applicability of developed approaches is greatly restricted or even unrealizable (in many cases) for large proteins, whose refolding goes through an accumulation of intermediate states.

This work evolves the previously developed approach to analyze protein refolding kinetics with the burst phase that cannot be measured in experiment [Baryshnikova et al., 2005, Prot. Sci.14 (10), 2658-2667] for proteins, whose refolding goes through more than one kinetic intermediate state. The refolding/unfolding kinetics and earliest intermediate population of carbonic anhydrase B are measured in a wide range of urea concentrations. On the base of these data, we calculated the rate constants of refolding/unfolding elementary stages as well as free energies of intermediates and transition states. Thus, we show that our approach makes possible to analyze the multi-stage unfolding/refolding kinetics of proteins to obtain the free energy landscape and to study the transition states structure.

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STRUCTURAL TRANSFORMATION OF Cry 3A δ-ENDOTOXIN AND ITS MUTANT FORM DEPENDING ON pH OF THE MEDIUM AND ETHANOL

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Protein biological functions are often realized by means of molecule dimerization, oligomerization or fibrillation. To characterize in more detail the conformational state of the toxin, which the protein acquires in conditions modeling the near-membrane environment, it has been studied at different pH and in the presence of ethanol. It is known that conditions bringing the protein to a corresponding conformation are low pH and dielectric constant as well as a temperature rise.

Protein Cry 3A δ-endotoxin is synthesized as a protoxin that as a result of proteolysis forms a toxin resistant to subsequent proteolysis. We have analyzed alcohol solutions of the toxin and its mutant form (W218-C) at pH 2.0, 2.5 and 3.0. On electron micrographs the preparations studied in alcohol-free solutions are seen as a field uniformly occupied by molecules of about 60 Å. The melting of such solutions yields one heat absorption peak. If the protein solution contains ethyl alcohol, the temperature dependence of the heat capacity is of a complex nature. According to electron microscopy data, in such conditions Cry 3A δ-endotoxin and its mutant forms C14 are a trimer. In the micrographs the whole field is occupied only by geometrical trimers, the angle of the molecule-triangle being directed outwards and they being connected through their flat side arranged at an angle of 120 degrees relative to each other. The heat absorption curve has two peaks. Each peak has a quite high degree of reversibility. Diagrams of possible toxin transitions with the formation of fibrillar structures have been drawn using the experimental microcalorimetry, electron microscopy and near and far CD data obtained at different temperatures.

$$\text{pH 2.0:} \quad F \leftrightarrow U \qquad (1)$$

$$\text{pH 2.5:} \quad N(\tau) \rightarrow I(\tau) \rightarrow F \leftrightarrow U \qquad (2),$$

where N is the native state, I is the intermediate state, F is the fibrillar state, and U is the denatured state.

At pH 2.0 the two first states (N and I) are absent. Molecules turn into thread-like or fibrillar structures because of the reduction of the energetic barrier. The transition from the F state into the N one occurs already at room temperature. The rise of pH to 2.5-3.0 renders an enhancement of the energetic barrier of molecule transition into an oligomeric thermostable structure. Under such conditions an oligomeric structure is formed only if the temperature increases.

CONFORMATIONAL CHANGES OCCURRING IN PROTEINS IN THE PRESENCE OF MEMBRANES AND THEIR RELATION TO PROTEIN FUNCTIONING

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The conformational behavior of water-soluble proteins functioning in the presence of membranes is not completely understood so far. By different experimental techniques it was shown that a conformational state of some proteins undergoes transition from the native to intermediate state in the presence of artificial phospholipid membranes. This intermediate state has a fluctuating tertiary structure and a pronounced secondary structure; it is rather compact and similar to the molten globule state of these proteins in aqueous medium. This is the state in which proteins realize their functions.

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PHASE DIAGRAMS OF APOMYOGLOBIN IN COORDINATES OF pH, TEMPERATURE AND UREA CONCENTRATION

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To estimate properties of different conformational states of apomyoglobin over a wide range of experimental conditions, the phase diagrams were constructed. For this purpose, pH-, temperature- and urea-induced denaturation/renaturation of the protein was investigated. Conformational changes of apomyoglobin were studied by tryptophan fluorescence and circular dichroism. Stability and population of the native, intermediate and unfolded states were calculated from the obtained experimental data. For construction of a three-dimensional phase diagram, the middles of all transitions were plotted in coordinates of pH, temperature and urea concentration. The maximum of the native state stability was observed at pH 6.2 and at 300K in the absence of denaturant. The native state showed a similar heat melting at all pH above 5.0, while its stability against urea increased with pH. Below pH 5.0, the native state stability and temperature melting parameters decreased in a similar way. The maximal stability of the intermediate state was observed at pH 6.2 and at 330K in the absence of urea, and it decreased with lowering pH. Cold and heat denaturations of the intermediate state displayed different pH-dependences.

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SUBUNIT DESTABILIZATION AND RELEASE FROM YERSINIA PESTIS F1 SUBUNIT-CHAPERONE COMPLEX

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Periplasmic chaperones from the chaperone/usher pathway of Gram negative bacteria assist in folding and transport of protein subunits of filamentous adhesins, via a process that is physically unlinked to the bacterial inner membrane and hence a source of cellular energy. Previously, we showed that the periplasmic chaperone, Caf1M, from Yersinia pestis traps an activated conformation of the Caf1 subunit, preserving folding energy and a potential energy source to drive subsequent steps of assembly. In this study, we elucidate the mechanism of chaperone-induced destabilization of subunits by mutagenesis of the Caf1M and Caf1 donor strand binding motifs. Calorimetry measurements together with high-resolution structural studies show that insertion of the excessively large donor residues of the chaperone in pockets 1-4 as well as too small donor residues in pocket 5 of the acceptor groove distorts the subunit hydrophobic core, increasing the free energy of the subunit fold by at least 32-35 kJ mol-1. A rigid two-donor strand platform produces the largest and energetically most costly distortions in pockets 1-3. Chaperone-to-subunit donor residue substitutions in positions 126 and 128 partially relieve distortions, increasing the Caf1M-Caf1 complex stability. (The distorted “open” structure of the chaperone-bound subunit is a consequence of higher affinity of the chaperone to a pre-folded rather than folded state of the subunit.) During fiber growth, chaperone donor strands are substituted by the donor strand of an incoming subunit. The donor strand exchange is initiated in pocket 5 and followed by displacement of large donor residues of the chaperone by smaller donor residues of the subunit, proceeding through a series of cooperative steps each involving local closure of the acceptor cleft.

SELECTIVE INTERACTION OF BIOMACROMOLECULES WITH SPIN IZOMERS OF WATER MOLECULES

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Recently the effects of the spin state of water molecules on its absorption on lyophilized DNA, lysozyme and some inorganic sorbents were studied. It was shown that the absorption rates of ortho and para water from vapor differ noticeably. A clear distinction in the sorbtion kinetics is determined by the difference in quantum statistics for spin isomers, which in its turn can give rise to remarkable differences in physical-chemical properties of orto and para water.

Four-photon spectroscopy of H2O molecule rotational transitions in biological macromolecules (protein, DNA) aqueous solutions shows a considerable growth (more than 10 times) of water molecule rotational lines intensity. This reflects the increasing concentration of quasi-free H2O molecules. Obviously, the macromolecules are able to influence the concentration of spin isomers at their nearest surroundings only. Probably, macromolecules affect the H-bond network in the solvate shells and it leads to the growth of free H2O effective concentration.

The lines intensity of H2O ortho/para isomers in protein/DNA solutions increases disproportionately. The rotational lines of ortho isomers are considerably stronger than those of the para one in the presence of native macromolecules. Such selectivity decreases subsequently for solution of denatured DNA. Thus, spin-isomers properties are different with respect to hydration of the macromolecule surface and depend on the macromolecule conformation.

These results demonstrate the possibility of water spin-isomers to affect the structure and stability of biological macromolecules and open a new opportunity in biology and medicine.

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NOVEL STRUCTURAL MOTIFS AND STRUCTURAL TREES OF PROTEINS

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Novel structural trees for two superfamilies, (α+β)-proteins containing ψ-motifs and β-proteins containing φ-motifs, have been constructed and analyzed. Novel structural motifs having unique overall folds and a unique handedness are taken as root structures of the trees. One of them can be represented as a combination of the right-handed βαβ-unit and the so-called ψ-motif, and the other called the φ-motif is formed by three β-strands folded so that together they resemble the Greek letter φ(phi). The larger protein structures of each superfamily are obtained by a stepwise addition of α-helices and/or β-strands to the corresponding root motif taking into account a restricted set of rules inferred from known principles of the protein structure. Applications of the constructed structural trees for protein structure comparison, protein classification and protein folding are discussed.

PARALLEL SUPERPLEATED BETA-STRUCTURE AS A COMMON FOLD FOR AMYLOID AND PRION FIBRILS

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In several serious neurodegenerative diseases, including Alzheimer's disease, Huntington's disease and transmissible prion diseases, proteins that are normally soluble and innocuous polymerize into amyloid fibrils that correlate with the disease state. Amyloids also accumulate in other pathogenic conditions such as amylin fibrils in type 2 diabetes. Despite much effort, no high resolution structure has yet been determined for the amyloid fibrils. Recently, we proposed a new fold called the parallel superpleated beta-structure as the structural basis for several kinds of amyloid fibrils (1, 2). In this fold, beta-strands of an amyloidogenic domain zig-zag in a planar serpentine arrangement. Serpentines are stacked axially, in register, generating an array of parallel beta-sheets, with a small left-hand twist. The interior of the filament is stabilized not only by packing of apolar side-chains but also by stacked polar/uncharged residues which are predicted to form H-bonded ladders, similar to those found in beta-helical proteins. The parallel superpleated beta-structure model emerged after a detailed analysis of the experimental data on yeast Ure2p prion filaments. We also suggested that amyloid fibrils of human amylin, glutamine expansions in huntingtin, the core of yeast Sup35 protein, alpha-synuclein have such a fold. During the last 2 years, a series of experimental works have confirmed the occurrence of the parallel superpleated beta-structural fold in several amyloid fibrils (3). This finding can have wide applications in the identification of amyloidogenic protein sequences and the discovery of therapeutic agents for the prevention and treatment of amyloid diseases.

(1) Kajava, A.V., Baxa, U., Wickner, R.B., Steven, A.C. A model for Ure2p prion filaments and other amyloids: The parallel superpleated beta-structure. (2004) *Proc Natl Acad Sci U S A* 101, 7885

(2) Kajava, A.V., Aebi, U., Steven, A.C. The parallel superpleated Beta-structure as a model for amyloid fibrils of human amylin. (2005) *J. Mol.Biol*. 348:247

(3) Kajava A.V., Squire J.M., Parry D.A. Beta-structures in fibrous proteins. (2006) *Adv Protein Chem*. 73:1.

SPECIFICITY OF PROTEIN FUNCTION ENCODED IN CONSERVATION PATTERNS

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Background. Genome projects are generating a rapidly increasing number of protein sequences, but our knowledge of functional details lags behind. Fortunately, functional constraints in evolution have created information-rich conservation patterns in protein families. If one can decode these patterns, one can derive detailed functional hypotheses. Here, we focus on decoding the patterns of specificity residues. Such residues are conserved in each protein subfamily, but differ between functionally diverse subfamilies.

Results. We present a new algorithm to solve the combinatorial complex problem of identifying specificity residues and, simultaneously, the corresponding optimal division into subfamilies. In our approach, called combinatorial entropy optimization (CEO), we optimize a conservation contrast function over different assignments of proteins to subfamilies. We validate the method by comparing sets of predicted specificity residues with sets of experimentally known functional residues, such as interaction residues observed in three-dimensional macromolecular complexes, and get good agreement between prediction and observation.

Conclusion. The method, at http://proteinkeys.org, takes a multiple sequence alignment as input and returns subfamilies and a set of specificity residues. The computed subfamilies may be used, e.g., to assign a likely function to new protein sequences or to choose maximally informative targets for structural genomics projects. The computed specificity residues may be used to design highly specific mutation experiments that test function with minimal side effects; to build sharper and more informative evolutionary trees that more accurately reflect functional relatedness; to predict interactions with proteins; and, to estimate the functional consequences of genetic variation.

STRICT COMPUTATION OF ELECTROSTATIC INTERACTIONS IN A CORPUSCULAR MEDIUM

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Prediction of ligand binding or protein structure requires very accurate force field potentials: even small errors in potentials can make some “wrong” structure (from zillions possible) more stable than the single “correct” one. Despite huge efforts to optimize them, currently used force field potentials are still not able to bring an approximate, homology-based model of protein structure closer to its native conformation. One of the main difficulties is connected with computation of electrostatic interactions in a non-uniform corpuscular medium. In this work we present a strict algorithm for this computation in a medium, where each atom (or molecule, depending on the desired approximation) is considered as a three-dimensional harmonic oscillator.

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PREDICTION OF FOLDING NUCLEI IN GLOBULAR PROTEINS

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Our theoretical approaches for prediction of folding/unfolding nuclei in three-dimensional protein structures are based on a search for free energy saddle points on networks of protein folding/unfolding pathways. Under some approximations, this search is performed rapidly by the dynamic programming method or by the method of kinetic equations and results in prediction of Φ values, which can be compared with those found experimentally. We compare the theoretically obtained and experimental Φ values (which characterize involvement of residues in folding nuclei) for 17 proteins, where Φ values are now known for many residues. We show that the model provides good Φ value predictions for proteins whose structures have been determined by X-ray analysis (the average correlation coefficients for two methods are 0.65 and 0.62), with a more limited success for proteins whose structures have been determined by NMR techniques only (the average correlation coefficients for two methods are 0.34 and 0.29). The transition state free energies computed from the same model correlate well with logarithms of experimentally measured folding rates at mid-transition (the correlation coefficient is -0.73).

The developed approach was applied to amyloidogenic proteins. The regions important for their "normal" folding into their native structures were predicted and compared with experimentally found regions, which are important for the amyloid formation (the so-called amyloidogenic regions). We revealed that amyloidogenic regions are typically incorporated into the native structure early during its formation (not later than at the rate-limiting step of a "normal" folding process).

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STRUCTURAL PRINCIPLES OF COMPLEX FORMATION OF TRANSCRIPTION FACTORS WITH B-DNA: TRANSCRIPTION FACTORS WITH THREE α-HELICES

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Complexes of five transcription factors with similar structures, containing three α-helices, were considered. Two stages of complex formation were studied for protein interactions with the major groove region of B-DNA: the docking stage at the distances of 5.0-6.0 Å and the stage of atomic contacts at the distances of 2.5-3.9 Å. ***At the docking stage*** the recognition features were examined by electrostatic potentials, which determine mutual orientation of the molecules. The recognition area of protein displays a clear positive potential. Oppositely, the molecule of B-DNA as a whole displays a weak or strong negative potential depending on the degree of ionization of phosphate groups. Positive potentials of definite atomic groups of nucleotide bases were not displayed since these groups are dislocated at the bottom of the DNA major groove. We can conclude that at the first stage the protein and B-DNA could easily form a preliminary low-resolution complex. However, this complex is non-specific, the protein molecule is not fixed, and it could slide along the long axis of B-DNA. ***At the atomic contacts stage*** the protein recognizing α-helices forms contacts by means of polar side groups, which are disposed at the helix surface in positions 0, 4, 5, 7 and 9, where 0 is the position of the first contacting residue. The contacts of the polar amino acids in positions 0, 4, 5 are essentially with bases, while the ones in positions 7 and 9 with the phosphate groups. The DNA recognizing area in the major groove region covers a part of the promoter sequence TAAT. Summing up the results at the two stages of recognition, we can conclude that the final specific nucleotide binding at the stage of atomic contacts becomes possible as a result of compensation of a very strong screening electrostatic effect of the phosphate backbone of B-DNA.

EVOLUTION OF BACTERIAL REGULATORY SYSTEMS

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Computer analysis of conserved DNA sites and RNA structures in upstream gene regions is a powerful technique of comparative genomics used for prediction of regulatory interactions and for functional annotation of genes. Sometimes this can be done at a surprising level of detail. For example, starting with a new conserved motif upstream of ribonucleotide reductase genes in a diverse set of bacteria, we have identified the responsible transcription factor, found other genes regulated by this factor, demonstrated the functional link to the regulation of replication, and finally, predicted the mechanism of regulation (repression by cooperative binding to tandem sites overlapping with promoters). Analysis of regulatory sites in conjunction with other comparative genomic techniques may lead to non-trivial functional predictions, such as the changes in the protein composition of bacterial ribosomes under conditions of zinc starvation. Both these predictions were subsequently validated in experiment.

Current availability of hundreds of bacterial genomes allows one to go deeper and to reconstruct the history of events that have shaped the regulatory systems observed in extant bacteria. One such case is the evolution of transcription regulation of the iron homeostasis in alpha-proteobacteria. It includes changes in transcription factor specificity, complete loss of transcription factors and emergence of new ones, as well as re-wiring of regulatory cascades. Another example is the T-boxes, RNA structures regulating amino acid metabolism in Firmicutes (low-GC Gram-positive bacteria): they have been duplicated (sometimes at a very fast rate), changed their specificity, and formed unusual tandem regulatory structures.

PROTEIN FOLDING PROBLEM: THEORY AND EXPERIMENT. IN THE MEMORY OF OLEG B. PTITSYN

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We give an overview of development of the protein folding problem, and show that a mystery of this phenomenon (colloquially known as "the Levinthal paradox") is now, at last, understood in its basics due to combined efforts in theoretical and experimental studies. The main attention is paid to the works on protein folding in vitro, which revealed a nucleation mechanism of folding and explained the role of the folding intermediates.

Our work was supported in part by the RAS Program “Molecular and Cellular Biology”, RFBR, ISSEP, HFSPO, CRDF, INTAS, and by the Howard Hughes Medical Institute Awards.

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POSTER 1

LEADER SEQUENCE OF TOBACCO MOSAIC VIRUS RNA POSSESSES COOPERATIVELY MELTED, COMPACT CONFORMATION

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The 5'-untranslated region (5' UTR) of RNA of tobacco mosaic virus (TMV), called the omega sequence, is known as an mRNA leader promoting very efficient initiation of translation. The central part of the sequence consists of many CAA repeats, which were reported to be mainly responsible for the enhancing activity of the omega leader. In this work we synthesized the polyribonucleotides containing either the natural omega sequence or the regular (CAA)n sequence, and studied them using UV spectrophotometry and analytical ultracentrifugation methods. It was demonstrated that the polyribonucleotides manifest significant hypochromicity, cooperative melting of its structure upon heating, high melting temperature and the sedimentation coefficient typical of compactly folded RNA of this size. Thus, the omega leader and (CAA)n sequence seem to be well structured elements of mRNA.

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POSTER 2

MYOGLOBIN AND MITOCHONDRIA: HOW DOES "OXYGEN DEPOT" WORK?

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The new mechanism of oxymyoglobin deoxygenation in cells is proposed, involving its direct interaction with the mitochondrial membrane. As a result, myoglobin affinity to oxygen markedly decreases, which corresponds to a shift of the dissociation curve to higher partial oxygen pressure. Up to now, two general mechanisms of myoglobin functioning have been generally accepted, "oxygen depot" and "facilitated diffusion". Both are simply physical, not implying any interaction with mitochondria or other cell structures and metabolites, as well as alterations in myoglobin affinity to oxygen. Kinetics of deoxygenation of sperm whale oxymyoglobin has been studied spectrophotometrically in the presence of respiring rat liver and heart mitochondria (with succinate) under conditions of direct contact between them and separation by a film permeable for only low-molecular weight compounds. It has been shown that, under physiological conditions, dissociation of oxygen from oxymyoglobin is possible only at its direct contact to mitochondria. The deoxygenation rate does not depend on the protein concentration (zero order on $[MbO_2]$, as against the first order reaction in the absence of mitochondria) and completely coincides with the rate of oxygen consumption by mitochondria according to the polarographic data. The dependence of the deoxygenation rate on the protein and mitochondria concentration, and also on the overall charge of myoglobin has been studied, using horse (pI 7,1) and sperm whale (pI 8,3) proteins, their zinc complexes (pI > 8,3), and the myoglobin derivative carboxymethylated at histidines (pI 5,2). The results indicate the importance of electostatic interaction of myoglobin with the mitochondrial membrane. No deoxygenation is observed, if oxymyoglobin is separated from mitochondria, though oxygen concentration in the protein solution is equal to zero. Thus, myoglobin functions as a usual oxygen transporter, that is, like hemoglobin, binds oxygen with high affinity and dissociates it with lower one.

POSTER 3

BACTERIAL sm-LIKE PROTEIN Hfq: STRUCTURE AND INTERACTION WITH RNA

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Hfq is a small (8-11 kDa) thermostable bacterial protein that was first identified as a host factor required for plus-strand replication of the Qβ RNA bacteriophage. In bacterial cells Hfq appears to function as a global regulator of gene expression. Synthesis of more than 30 proteins is affected in an *Escherichia coli* hfq-mutant including the synthesis of the σS subunit of RNA polymerase and, consequently all σS-dependent genes. Hfq stimulates rpoS translation by mediating the binding of small RNAs (sRNAs) DsrA and RprA to their complementary target sequence in the 5´ untranslated region of rpoS mRNA, thereby allowing ribosome binding.

In crystal as well as in solution, Hfq forms a symmetric doughnut–shaped homohexamer. Comparative analysis of known structures of this protein family shows that the fragment of the Sm-fold, responsible for oligomerization, is strongly structurally conserved. Three conserved hydrogen bonds between β-strands of adjacent molecules hold together the monomers. Two hydrogen bonds provided by highly conserved His57 additionally stabilized the hexamer formation. Mutant forms of protein Hfq from *P. aeruginosa* with substitutions of His57 by alanine, threonine or asparagine have been isolated and purified. The stability of the Hfq mutant forms was characterized by various physical and biochemical methods including X-ray crystallography.

A large amount of RprA, DsrA and OxyS small regulatory RNAs as well as two different rpoS mRNA fragments have been obtained. Interactions of all these RNAs with Hfq and the small regulatory RNA with rpoS mRNA fragments were studied. All small regulatory RNAs as well as the rpoS mRNA fragments form complexes with the Hfq hexamer at a 1:1 molar ratio, whereas high-molecular complexes with 1:2 molar ratios are formed with an increasing concentration of the protein. Protein Hfq forms triple complexes with all RNA-rpoS mRNA binary complexes obtained by us. The protein also binds to OxyS RNA and forms a triple complex with an equimolar mixture of OxyS RNA and rpoS mRNA. This fact seems to confirm the presence of two different RNA binding sites for OxyS RNA and rpoS mRNA on protein Hfq. A wide variety of crystallization conditions of Hfq in complex with various regulatory RNA and RNA fragments have been scanned without success. Rather high lability of the complexes is the most likely reason of the failure. At present a new approach to solve the crystallization problem is proposed.

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POSTER 4

NEW POSSIBILITIES OF ANALYTICAL ULTRACENTRIFUGATION FOR ANALYSIS OF HYDRODYNAMIC PROPERTIES OF PROTEINS

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The new generation of Beckman analytical ultracentrifuges (Optima XL) with digital scanning and the development of new approaches for solution of the main equation of sedimentation analysis (the Lamm equation) has opened new possibilities for the use of this method. One of the best programs for an analysis of sedimentation data SEDFIT was developed by P. Schuck. Here the possibilities of this program for resolution of native proteins of 10-70 kDa and their mixtures are demonstrated. The optimal conditions for the best resolution were found. The results of our investigation show that such parameters as molecular mass, sedimentation coefficient, diffusion coefficient can be obtained from one sedimentation experiment. It was shown that for proteins with M>30 kDa the deviation of the values of experimental molecular mass from those calculated from the amino acid composition is 1-2%. For proteins with M~15kDa the deviation is in the range of 5-7%. It was demonstrated that program SEDFIT can resolve the components in a mixture differing twice in molecular mass. The unique experiments for the resolution of three components in solution are described. The obtained results open new principal possibilities for studying the complex formation by biological macromolecules using the analytical ultracentrifugation method.

POSTER 5

PROTEOLYSIS OF THE RIBOSOMAL PROTEIN S1 FROM *ESCHERICHIA COLI* AND *THERMUS THERMOPHILUS* LEADS TO THE FORMATION OF TWO DIFFERENT FRAGMENTS

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As a result of limited trypsin proteolysis of S1 ribosome protein from *Thermus thermophilus* (molecular mass 60 kDa) 71 amino acid residues are spit off from the C-end of a molecule, and 25 amino acid residues are split off from the N-end. The stable high-molecule mass fragment has molecular mass of 49 kDa, retains RNA-binding properties and is capable of interacting with the 30S ribosome subunit, whereas the fragment derived from *Escherichia coli* has molecular mass of 41.3 kDa and retains RNA-binding properties only. Thus, despite the high homology of *E. coli* и *T. thermophilus* proteins, the proteolysis leads to the formation of two different fragments that may evidence of essential differences between their structures.

POSTER 6

INFLUENCE OF POINT MUTATIONS IN THE NF-κB P50 SUBUNIT DNA-BINDING LOOP ON THE PROTEIN STABILITY AND ITS COMPLEX FORMATION WITH DNA

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It is known that eukaryotic transcription factor NF-κB takes part in the activation of many cellular oncogenes and viral replication. The key moment of these processes is the interaction of NF-κB with the κB-site of recognition in chromosomal or viral DNA. Therefore the search for NF-κB selective inhibitors is one of the main goals of pharmacotherapy. To date a number of NF-κB inhibitors on the basis of antisense oligonucleotides, aptamers and inhibitor protein IκB have been proposed. In the present work we suggest a novel approach based on the use of mutant forms of the NF-κB p50 subunit responsible for specific DNA-binding, which are unable to bind with DNA.

The purpose of the work was to study the influence of point amino acid substitutions in the human NF-κB p50 subunit, participating in the interaction with DNA minor groove (R57, R59, C62 and K147), on the protein stability and its binding with the κB-site in DNA. By site-directed mutagenesis amino acid residue R57 was replaced by alanine (protein R57A), R59 by glutamic acid and alanine (proteins R59E and R59A, respectively), C62 and K147 by tryptophan (proteins C62W and K147W, respectively).

By gel-shift it was found that mutant protein R59A does not interact with the DNA duplex containing the κB-site, whereas the dimeric state and protein stability are not changed in comparison with the wild type p50 subunit as shown by sedimentation analysis and SAXS. Mutant forms C62W and R57A reveal the same properties, however, they can form a complex with DNA at an excess of the protein (protein:DNA=4:1). The protein R59E binds to the specific DNA duplex with a low affinity as against the wild type of protein but it is more stable and interacts with DNA as a tetramer. The specificity and efficiency of DNA binding for mutant protein K147W are similar to those for the wild type, but it has an increased tendency to associate.

On the basis of our results we can conclude that in perspective the best inhibitors for the regulation of NF-κB-dependent transcription in cells may be obtained on the basis of mutant proteins R59E and R59A.

POSTER 7

UNSTRUCTURED REGIONS IN ELONGATION FACTORS EF1A FROM THREE OVERKINGDOME OF THE LIVING WORLD

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A new characteristic for classification of the Living World based on the ability of amino acid sequences to form unstructured regions that appear as loops in their 3D structure is described. Our approach is in principle different from RNA and protein phylogenies that are based on the alignment of amino acid sequences from different organisms. Introduction of new structural-functional characteristic in itself is of undoubted interest because megataxonomy and microphylogeny lack features that may resolve evolutionary relation between different groups of organisms though apparent abundance of such characteristics is present. We used the program *Fold/Unfold* to search for unstructured regions in the elongation factors EF1A. The reliability of loop prediction was checked against five factors whose structure is known from X-ray analysis. In addition to two cross-bridges between three structural domains in the elongation factors, the program predicts extra loops. Not counting the effector loop that is inherent to all factors, there are six. Three (A, B and C) of the six different loops are revealed in the first domain, one loop (D) in the second, and two loops (E and F) in the third domain of the factor, all six of which are never found in the same factor. Signatures of elongation factors for each Overkingdom of the Living World have been found for several dozen typical representatives from each Overkingdom. These signatures lead to the variation of the number of loops and their localization within the factor domains. The obtained data lead us to believe that the approach based on the prediction of unstructured protein loops – up to six – must have higher resolution than the method based on the indels (insertion + deletion), the number of which equals one for the same elongation factors. In our analysis, the specificity of sequences is important, in addition to the existence of loops. Since the total number of loops predicted in the factors increases with the complexity of an organism, we propose the following about the role of the loops in evolution: holding to the principle of “thrifty inventiveness”, Nature operates with different universal inserts (loops) adapting their number and location among the factor domains as well as their amino acid composition so that the protein will perform special functions: one in protozoa and several in higher organisms.

This work was supported by the Russian Foundation for Basic Research.

POSTER 8

PREDICTION OF PROTEIN FOLDING RATES

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Search and study of the general principles that govern kinetics and thermodynamics of protein folding generate a new insight into the factors controlling this process. Based on the known experimental data and using theoretical modeling of protein folding process, we demonstrate that it is possible to predict protein folding rate from its spatial structure and even from its sequence.

The folding rates obtained from the dynamic programming method, method of kinetic equations and Monte Carlo simulations correlate reasonably well with logarithms of experimentally measured folding rates at mid-transition.

It has been shown that chain length is the main determinant of the folding rate for proteins with three-state folding kinetics. The logarithms of folding rates of such proteins in water strongly anti-correlate with their chain length (the correlation coefficient being -0.80). At the same time, chain length has no correlation with the folding rate (the correlation coefficient is -0.07 only) for two-state folding proteins, which are rather similar in their sizes.

Our analysis demonstrates that α/β proteins have both the greatest number of contacts and the slowest folding rates in comparison with proteins from the other structural classes. Because α/β proteins are also known to be the oldest proteins, it can be suggested that proteins have evolved to pack more quickly and into looser structures.

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POSTER 9

A STABLE α-HELIX-RICH INTERMEDIATE IS FORMED BY A SINGLE MUTATION OF THE β-SHEET PROTEIN, src SH3, AT pH3

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Recently, we have found a transient intermediate on the folding pathway of src SH3. Intending to investigate the structure of the transient intermediate, we tested a mutant of src SH3, named A45G, using circular dichroism, fluorescence and X-ray solution scattering, and incidentally found that it forms a stable α-helix-rich intermediate (I_{eq}) (different from the native β-sheet-based secondary structure) at pH 3.0, but contains only β-sheets at pH6.0, whereas wild-type SH3 forms only β-sheets at both pH 3.0 and 6.0. The intermediate I_{eq} shows a circular dichroism measured at θ_{222}=-10,300 deg*cm^{2}*dmol^{-1}, indicating a 31 % α-helix proportion, as estimated by the CONTIN program. X-ray scattering gave the radius of gyration for I_{eq} as 19.1 Å at pH 3.0 and 15.4 Å at pH 6.0, and Kratky plots showed a clear peak at pH 3.0, 4.0 and 6.0, indicating that I_{eq} too is compact. In these parameters, I_{eq} closely resembles the kinetically obtained intermediate I_{kin}, which we found on the folding pathway of wild-type SH3 at pH 3.0 (radius of gyration 18.7 Å and θ_{222}= -8,700 deg*cm^{2}*dmol^{-1}, indicating a 26% α-helix proportion (Li *et al*, Biochemistry, in press)). Refolding experiments with A45G were done at pH 6.0 by stopped-flow apparatus monitored by circular dichroism, and compared to kinetic experiments with wild-type SH3 at pH 6.0. The result showed an α-helix-rich intermediate at the same dichroism amplitude, but 9 times slower in formation-rate. A pH-jump experiment from pH 3.0 to pH 5.9 on A45G was also performed. This showed no bursts, and the rate of conformation-change was almost as fast as the refolding rate of A45G at pH 6.0. These kinetic experiment data would be consistent with I_{eq} being nearly identical to the I_{kin}, which appeared on the folding pathways of both wild-type SH3 and A45G.

POSTER 10

AN ALPHA-HELICAL BURST IN THE src SH3 FOLDING PATHWAY

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Src SH3 is a small all-β-sheet protein composed of a single domain. We studied the folding behavior of src SH3 at various conditions by circular dichroism (CD), fluorescence and X-ray solution scattering methods. On the src SH3 folding pathway, an α-helix-rich intermediate appeared not only at subzero temperatures but also above 0□. The fraction of α-helix in the kinetically observed intermediate is c.a. 26% of the native value based on kinetic CD experiment. X-ray solution scattering revealed the intermediate was compact, but not fully packed. The analysis of CD implies that the amplitude of the burst phase is proportional to the helical fraction calculated according to the helix-coil transition theory. This strongly suggests that the initial folding core is formed by the collapse of much less stably-existing α helices.

1. Li et al. Biochemistry, in press.

POSTER 11

REDOX FUNCTION AND PEROXIDASE ACTIVITY OF CYTOCHROME c IN MITOCHONDRIA ARE REGULATED BY CARDIOLIPIN

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Redox properties of cytochrome c (cyt *c*) are critical to its normal electron transport functions in mitochondria as well as to its participation in apoptotic signaling via the peroxidase oxidation of two anionic phospholipids, CL and PS. Peroxidation of CL contributes to the permeabilization of mitochondria membranes, and subsequent cyt *c* release into the cytosol. However, the process that triggers the change of cyt *c* function from the electron shuttle to a peroxidase is unknown. The previous work established that upon interaction with CL, cyt *c* loses its tertiary structure with a consequent increase of its peroxidase activity. During apoptosis, it has been found that the fraction of CL-bound cyt *c* increased markedly. We have hypothesized that the binding of CL to cyt *c* acts as a switch that turns off its electron transport function and turns on its peroxidase catalytic function. The redox behavior of cyt *c* bound to CL-containing membranes was studied using direct voltohmmetry measurements of cyt *c* adsorbed on alkanethiol monolayers and equilibrium redox titrations in the presence and absence of CL. The effects of CL binding on the reduction potential of cyt *c* in the presence of purified Complex III, as well as on the regulation of electron transport activity in the electron transport chain in mitochondria, were examined by EPR and optical absorbance. The data show that the binding of cyt *c* to CL causes a significant (~350-400 mV) negative shift of the redox potential of cyt *c* and, as a result, inhibition of cyt *c* reduction in mitochondria and interruption of mitochondrial electron transport. These findings suggest that CL acts as a switch and regulates cyt *c*'s mitochondrial redox functions.

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POSTER 12

HETEROGENITY OF THE MOLTEN GLOBULE STATE OF APOMYOGLOBIN

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Urea- and pH-induced equilibrium and kinetic folding and unfolding of a dozen of mutated apomyoglobins revealed a difference in behavior of proteins with substitution of hydrophobic conserved non-functional (i.e. uninvolved in heme binding) residues belonging to A, G, H helices. It is shown that in transition from the intermediate to native state the apomyoglobin folding nucleus is formed mostly by non-conserved amino acids from B, C, and E helices. However, conserved residues belonging to A, G and H helices form more contacts in the intermediate state than those from other helices.

This work was supported in part by the RAS Programs of “Molecular & Cellular Biology”, by an INTAS grant, and by the Howard Hughes Medical Institute Awards.

POSTER 13

pH-INDUCED DENATURATION OF MUTANT FORMS OF APOMYOGLOBIN

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The amino acid sequences of globin family contain six conserved non-functional (i.e. uninvolved in heme binding) sites on A, G and H helices. These residues can be important at the stage of the native state formation, as well as at the stage of formation of the intermediate state. Here, pH-induced denaturation of mutant proteins, where amino acids in these sites had been substituted by alanine, was studied. The contribution of substituted residues to stability of the both protein states was estimated. For comparison, seven non-conserved residues with a similar number of contacts were also investigated. It was shown that the effect of the substitutions in conserved non-functional sites on stability of both the native and intermediate states was stronger than that in non-conserved sites. However, due to the absence of the rigid tertiary structure in the intermediate state, all introduced substitutions affect the stability of the intermediate state much weaker than that of the native state. The portion of contacts in the intermediate state was estimated for both sets of mutant proteins. It appeared to be larger for conserved residues, emphasizing their role in the formation of nonspecific hydrophobic interactions upon the intermediate state formation.

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POSTER 14

LIGAND-DEPENDENT ASSEMBLY OF GroEL CHAPERONIN OLIGOMERIC PARTICLE

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The refolding of oligomeric GroEL (hsp60) and GroES (hsp10) chaperones has been studied by electrophoresis, fluorescence, limited pulse-proteolysis and light scattering. It was shown that GroES adopts native oligomeric structure in itself. In contrast, the formation of GroEL oligomeric structure is ligand-dependent and occurs only in the presence of Mg-ADP or Mg-ATP. Both the rate of the formation and the yield of GroEL oligomeric structure are dependent not only on protein concentration but on the concentration of Mg-ADP or Mg-ATP and ionic strength of the solution. Moreover, at low concentrations of GroEL monomers for their effective specific assembly except Mg ions and the adenyl nucleotides the presence of GroES co-chaperonin is necessary.

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POSTER 15

UNIQUE TRAITS OF *HALOARCULA MARISMORTUI* FLAGELLAR FILAMENTS

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Both Bacteria and Archaea use rotation of spiral flagellar filaments for motility in liquid media. A bacterial flagellar filament consists of only one type of protein (flagellin) arranged into 11 longitudinal rows and each row consists of subunits in only one (R or L) conformational state. For most of known Archaea, their flagella are composed of several types of protein subunits named “flagellins” as well. All archaeal flagellins have remarkable homology with each other and no homology with bacterial flagellins.

The external part of flagella of extreme halophilic archaea *H. salinarum* consists of five flagellins (A1, A2, B1, B2, and B3), which are coded by five different genes located tandemly in two loci. Earlier we have demonstrated that two different A-flagellins (A1 and A2) of *H. salinarum* are necessary and sufficient to form a longitudinal and spiral filament. The cells of mutant strains with only one A flagellin had straight flagella. Thus, the multicomponent nature of flagella from *H. salinarum* may be explained first by the requirement of two A flagellins for the formation of a flagellar filament with a spiral shape and, secondly, by the possible participation of some B flagellins in the final stage of flagellar assembly.

In the presented work we investigate the protein composition of flagellar filaments of other halophilic archaea *Haloarcula marismortui.* Formerly, this microorganism was considered as motionless, but recently after its full genome sequencing, the motility-related genes were identified. In contrast to *H. salinarum*, until now a laboratory culture of *H.marismortui* has not exhibited evident motility suggesting that the expression of the motility genes may be restricted to specific environmental conditions. Three flagellin genes *(flaA1, A*2 and *B*) are present in the *H. marismortui* genome. All the chemotaxis and flagellar genes (with the exception of *flaA*1 and *flaA*2, which are located on Chromosome II and pNG100 plasmid accordingly) are encoded on major Chromosome I. The probable *H. marismortui flaA1* gene protein product cannot be unambiguously named as a full-value third flagellin. It consists of only 84 a.a. and lost both the leader peptide (necessary for flagellin assembly) and the relatively conservative C-terminal flagellin part.

In this study we have succeeded in finding conditions favorable for *H. marismortui* motility and have purified and studied the flagellar filaments of the archaea. The results of electron microscopy showed that *H. marismortui* cells had bunches of several spiral flagella. The *H. marismortui* flagellar filaments are approximately 1.5 times thicker (18 nm) than other characterized archaeal filaments (11—12 nm). We have found that spiral *H. marismortui* flagellar filaments are composed of only one flagellin (FlaB). We supposethat the structural organization of archaeal flagellar filaments is more diverse than it was accepted until now and the filaments of some archaea (among these are *H. marismortui*) are close to the “bacterial type” of organization when spirality of supramolecular structure is provided by two stable conformational states of a single type of the same molecule (in contrast to two different flagellin molecule types in archaea *H. salinarum*).

We have discovered that *H. marismortui* cells demonstrate a phenomenon resembling the well-known flagellin phase variation in Enterobacteria: synthesis of the FlaB flagellin is terminated depending on external conditions, at this only FlaA2 flagellin is detected in produced flagellar filaments.

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