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RIBOSOMES

MOLECULAR STRUCTURE, ROLE IN BIOLOGICAL FUNCTIONS AND IMPLICATIONS FOR GENETIC DISEASES

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Preface

Ribosomes are essential protein-RNA complexes that synthesize proteins according to the gene library of the cell in all living organisms. In this book, the authors present current research in the study of the molecular structure, biological functions and implications for genetic disease of ribosomes. Topics include ribosomal alterations and immune-related illness; the role of ribosomes in mediating hypoxia response and tolerance in eukaryotes; the potential biomedical use of ribosome-inactivating proteins; coarse-graining the nano-machine ribosome to elucidate its functional dynamics; and structural aspects of ribosomal ligands interactions providing the work of the human translational machinery.

Chapter 1 - The chapter is devoted to the review of structural principles of molecular processes providing functioning of the protein synthesizing system in higher organisms. Site-directed cross-linking and related data on structural elements of the human ribosome that form binding sites of mRNA, acceptor terminus of tRNA and hepatitis C virus IRES, and on peptides of the translation termination factor eRF1 participating in the organization of the stop codon recognition site are considered. Comparison of the structural information gained with translational system from higher organisms with the respective information on bacterial and lower eukaryotic systems is given. New insights into molecular mechanisms of translation based on the discussed data are presented.

Chapter 2 - Specific ribosome-directed stressors have the capacity to damage 28S ribosomal RNA and interfere with ribosomal functions during translation and cellular homeostasis. This can provoke so-called ribosomal stress responses that are closely associated with various disease processes including immunosuppressive or hypersensitivity-linked dysfunctions in humans and domestic animals. Since the primary toxic actions of most ribosomal stress-inducing agents generally inhibit global protein synthesis, highly dividing cells, including leukocytes and epithelial cells, are the most susceptible targets. Here, responses of immune cells and systems to acute and chronic exposure to ribosome-inactivating stress in various experimental models are discussed. This review will specifically focus on modulation of immune suppression and hypersensitivity diseases caused by ribosomal dysfunction. Evidence-based approaches characterizing ribosome-inactivating stressor and their implication as critical etiological factors of immune-associated diseases are also presented.

Chapter 3 - Ribosome biogenesis and protein synthesis are highly energy-consuming processes and therefore, key control points for cellular growth and division under various conditions of stress. Hypoxia is a state of poor oxygen supply that is experienced by diverse

organisms under numerous physiological and disease conditions. Hypoxia greatly reduces cellular energy production and hence, organisms must develop effective strategies to cope with this low oxygen state. One common strategy that eukaryotes, ranging from yeast to mammals, employ to adapt under hypoxia is the conservation of cellular energy via suppression of metabolism and protein synthesis. These biological changes occur as a result of coordinated cellular responses orchestrated by various signal transducers and regulators, such as HIFs in higher eukaryotes and Mga2 and Hap1 in yeast. Studies in various eukaryotes have indicated that ribosomes have an essential role in mediating responses to hypoxia. In yeast the expression of various ribosomal and related proteins are altered under hypoxic conditions. Interestingly, it appears that deletion of certain genes involved in ribosome biogenesis, including *NSR1, BUD21, RAI1* or *RPL20A*, minimizes such alterations and enhances the survival of yeast under hypoxic conditions. Such deletions also diminish the effect of key transcriptional regulators, like Swi2 and Swi3, whose targets are altered under hypoxia in the wild-type strain. Similarly, in *C.elegans* reduced activity of arginyl-transfer RNA (t-RNA) synthetase confers hypoxia tolerance. Mammals acquire hypoxia tolerance via global reduction in RNA translation and ribosome biogenesis. In this chapter, the authors will review the role of ribosomes in conferring tolerance to hypoxia and discuss future directions to elucidate the molecular mechanisms underlying hypoxia tolerance.

Chapter 4 - Analyses suggest that protein coding genes occur within ribosomal RNA genes, enabling to code for additional proteins without increasing genome size. Stop codons can be translated by antisense antitermination (suppressor) tRNAs with anticodons matching stop codons, hence overlap coding might not be limited to stopless 'open reading frames'. Alignment analyses detect several putative protein coding genes within mitochondrial rRNAs and the mitochondrial D-loop region that include stops. These putative overlapping genes are probably functional because corresponding RNA abundances in GenBank's EST database increase with the length of the putative overlap coding region (but not for regions not involved in overlap coding), and predicted protein secondary structures resemble those formed by their presumed homologues from GenBank. In addition, numbers of putative overlap coding genes increase with predicted capacities of antisense tRNAs to match stop codons when comparing mitochondrial genomes from each primate and *Drosophila* species. In human mitochondrial genomes, single nucleotide polymorphisms are less frequent in putative rRNA-encoded overlapping protein coding genes than in the rest of each 12S, 16S rRNAs and the D-loop, as one would expect if the coding function of these regions cause excess purifying selection against polymorphisms. However, further analyses based on polymorphism frequencies and proportions of pathogenic mutations among these yield partially inconclusive results, probably because of weaknesses intrinsic to indirect estimation of purifying selection as indirect evidence for gene function. Previous analyses showed that overlapping protein coding genes in frameshifted and antisense sequences of regular mitochondrial protein coding genes coevolve with capacities of antisense tRNAs to translate stop codons, reassigning stop codons to code for amino acids: UAR are reassigned to glutamine in primates, tryptophane in turtles, and serine in *Drosophila*; AGR stops are reassigned to arginine in primates and glycine in the olive ridley (*Lepidochelys*) and lysine in other turtles. Analyses of mitochondrial rRNAs converge with analyses of protein coding genes and strengthen the hypothesis that mitochondrial genomes code according to a stopless genetic code for previously undetected, cryptic overlapping protein coding genes. It is

probable that pathogenicity of at least some polymorphisms is due to the previously unsuspected function of rRNA and D-loop regions as protein coding regions.

Chapter 5 - Temperature up-shift or down-shift is one of the major environmental stresses encountered by microorganisms. Especially, one of the main consequences of temperature down-shift on cells is conformational changes of secondary structures of RNAs. These cold-shock induced structural changes of RNAs have significant effect on the global gene expressions, i) by improper termination of transcription, ii) inefficient RNA degradation, iii) translation of alternatively-structured mRNAs, and iv) biogenesis of ribosome and processing of rRNA. Several cold-shock proteins are produced to counteract these effects and thus allow cold acclimatization of the cell. This chapter describes how specific cold shock proteins modulate the ribosome biogenesis and eventually translation, to ensure effective protein production at low temperature.

Chapter 6 - Ribosome is crucial for protein synthesis in cells. Ribosome-inactivation proteins (RIPs) are a large family of unique enzymes that inhibit protein synthesis by site-specifically and irreversibly modifying the ribosomal RNA. According to their different enzymatic activity, RIPs can be classified into ribonuclease RIPs and N-glycosidase RIPs. RIPs have drawn the attention of many researchers due to their potential use as antivirus and anticancer reagents and as other biological and biomedical tools. In this chapter, the progress in the study of RIPs and the problems encountered in biological and biomedical research are discussed.

Chapter 7 - Coarse-grained computational techniques can provide information on conformational dynamics of the supramolecule ribosome, for which full-atom approaches become computationally demanding. Elastic network models and coarse-grained molecular dynamics simulations are the most commonly used methods for simple descriptions of the biomolecular structure. Usually, an amino acid or a nucleotide residue is represented by a single node, and simplified potential energy functions are used for interacting nodes. These approaches have been successful in reproducing the micro-to-milliseconds dynamics information, such as the experimentally observed ratchet-like rotation of the ribosomal subunits. Furthermore, they have provided insights on the structure-dynamics-function relationship of the ribosomal complex. This review summarizes various coarse-graining approaches on the nano-machine and recent studies that focus on its different functional sites.

Chapter 8 - In living cells and organisms, proteins are often made through decoding genetic information stored as sequences of nucleotides. The central machinery in the process is ribosome. Every protein is translated from the 5' end to the 3' end of an mRNA. Starting from the translation initiation site, every three consecutive nucleotides are considered as a codon and read as a single amino acid. Cellular proteins, which are made from 61 sense codons of a total 64 codons, typically comprise 20 natural amino acids. However, there are unusual cases existing in nature. Under certain circumstances, nature can use codons that are typically nonsense to code natural as well as unusual amino acids (*e.g.* selenocysteine and pyrrolysine). In addition, ribosomal frameshifting is also not uncommon, in which case one or two nucleotides in a coding sequence are skipped to make proteins based on a shifted frame. In the past decade or so, researchers have invented procedures in laboratories to biosynthesize proteins containing unusual amino acids. Such exploration has made a big impact. This chapter summarizes methods used in nature and in laboratories to incorporate unusual amino acids into proteins. In particular, recent studies, which re-engineered ribosome and other

related translational factors, are emphasized. Therapeutic applications of this technology are also discussed.

Chapter 9 - Bowen-Conradi syndrome (BCS), a lethal autosomal recessive disorder which affects infants in the Hutterite population, is caused by a point mutation in *EMG1*, a highly conserved gene necessary for biogenesis of the small subunit of the ribosome. Research on EMG1 function has centered on yeast models, due to their genetic tractability for examining a cell-essential protein. The effects of the BCS-causing mutation on human EMG1 protein function are therefore unknown. The authors previously showed that EMG1 protein levels in BCS patient fibroblasts were severely reduced compared to control cells, and the authors therefore went on to examine EMG1 stability. Using pulse-chase metabolic labeling in cells transiently transfected with wild type or mutant EMG1, the authors found that the mutant protein rapidly associated with a detergent-insoluble fraction. Inhibition of the proteasome resulted in the accumulation of a higher-molecular weight band likely corresponding to ubiquitinated EMG1, suggesting that EMG1 is normally degraded by this pathway. Using immunofluorescence in fibroblasts, the authors found that both wild type and mutated EMG1 were localized to the nucleolus, and that proteasome inhibition resulted in the accumulation of EMG1 in nucleoplasmic foci which may correspond to sites of proteasome-mediated degradation. These data suggest that the mutated EMG1 protein is unstable, leading to a reduced level of EMG1 in the cell. Although the mutated protein localizes normally, the reduction in its level is likely to impact downstream pathways that significantly alter cell function in BCS patients.

Contents

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Chapter 1

Structural Aspects of Ribosomal Ligands Interactions Providing the Work of the Human Translational Machinery

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Abstract

The chapter is devoted to the review of structural principles of molecular processes providing functioning of the protein synthesizing system in higher organisms. Site-directed cross-linking and related data on structural elements of the human ribosome that form binding sites of mRNA, acceptor terminus of tRNA and hepatitis C virus IRES, and on peptides of the translation termination factor eRF1 participating in the organization of the stop codon recognition site are considered. Comparison of the structural information gained with translational system from higher organisms with the respective information on bacterial and lower eukaryotic systems is given. New insights into molecular mechanisms of translation based on the discussed data are presented.

Introduction

One of the key events of the cell life is protein synthesis that is conducted on ribosomes, very complicated ribonucleoproteins, whose function is translation of genetic information incoming as sequences of trinucleotides-codons of mRNAs into the polypeptide chains of proteins. Understanding molecular mechanisms of translation is the problem of basic

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importance, especially with human ribosomes, because the nature of many diseases relates to disturbances in the functioning and regulation of the protein-synthesizing system.

The work of ribosomes has features common to all organisms. In the course of translation, the ribosome binds various specific ligands, which provides the sequence of events leading to the polypeptide chain synthesis. The main ligands of the ribosome are mRNAs bearing the genetic information to be translated, and transfer RNAs (tRNAs) carrying amino acid (aa) residues attached to the 3'-terminal riboses via complex ester bonds; each tRNA is specific to one particular aa. The mRNA binding site of the ribosome is located on the small subunit, and three tRNA binding sites (A, P and E sites) are composed of both small and large subunits. Aminoacyl (A) site is designed for binding of incoming aminoacyl-tRNA (aa-tRNA) whose anticodon recognizes mRNA codon bound at this site, i.e., in the A site selection of aa-tRNA cognate to the mRNA codon located at this site takes place. This process is called decoding. The function of the peptidyl (P) site is binding of tRNA carrying nascent peptide chain, and the role of the exit (E) site is binding of discharged tRNA before it leaves the ribosome. When the A site is occupied with aa-tRNA cognate to the mRNA codon present there, peptide bond between the peptidyl residue of the peptidyl-tRNA at the P site and aa residue of the aa-tRNA is immediately formed. Peptidyl transferase center (PTC) located at the large ribosomal subunit is responsible for the catalysis of peptide bond formation (transpeptidation). As the result, the peptidyl moiety elongated by one aa becomes attached to the A site tRNA, and the P site tRNA gets discharged. After the transpeptidation, the translocation takes place that includes the movement of the peptidyl-tRNA from the A to the P site, and of the discharged tRNA from the P to the E site with the accompanying movement of mRNA codons bound to the respective tRNAs. After completion of this process the ribosome becomes ready to the next peptide elongation cycle. The first elongation cycle is preceded by initiation of translation that is completed by formation of the ternary complex of the ribosome with mRNA and initiator tRNA aminoacylated with methionine (in bacteria, with formylmethionine), in which the mRNA start codon (usually, AUG) and Met-$tRNA_i^{Met}$ are bound at the P site. Termination of protein synthesis takes place when one of three triplets, UAA, UAG or UGA, binds at the A site. These triplets do not encode any aa and serve as stop signals (stop, or nonsense codons). The work of the ribosome is assisted with specialized proteins called translation factors. The factors operating in the elongation are functionally similar in all kingdoms of life; one of them (in prokaryotes, EF-Tu, and in eukaryotes, eEF-1) in complex with GTP delivers aa-tRNA to the A site and another (in prokaryotes, EF-G, in eukaryotes, eEF-2) promotes translocation in a GTP-dependent manner. Functional resemblance is also observed with factors taking part in translation termination in all organisms. So, stop codons are always recognized by class 1 termination factors, or polypeptide chain release factors (RF1/RF2 in bacteria and eRF1 in eukaryotes), that trigger hydrolysis of the complex ester bond in the peptidyl-tRNA, enabling the synthesized polypeptide to leave the ribosome; termination involves also class 2 termination factors that are ribosome-activated GTPases (RF3 in bacteria and eRF3 in eukaryotes).

On the other hand, the functioning of ribosomes from different kingdoms of life has a number of distinctions, which are the most pronounced between bacteria and eukaryotes. These distinctions concern mainly initiation and termination of translation. So, initiation in eukaryotes is much more complicated than in bacteria and involves more steps and larger set of initiation factors; moreover, the ways by which the mRNA start codon is placed at the P site in bacterial and eukaryotic ribosomes are principally different (for review, see Rodnina &

Wintermeyer, 2009; Voigts-Hoffmann et al., 2012; Aitken & Lorsch, 2012). In bacteria, mRNAs bind directly to small ribosomal subunits so that the AUG codon occurs at the P site, which is assisted by the complementary interactions between so called Shine–Dalgarno (SD) sequences, which are generally present in the mRNAs upstream of the start codon, and the anti-SD sequences near the 3'-terminus of the small subunit rRNA. Eukaryotic mRNAs lack SD sequences; they bind the small ribosomal subunit (that is already bound with initiator Met-tRNA) near the 5'-terminal 7-methylguanosine ("cap"), and then the subunit scans along the mRNA until the start codon AUG is recognized. The majority of eukaryotic initiation factors, including all ones responsible for the cap recognition and scanning, have no bacterial counterparts. The alternative pathway of translation initiation concerns genomic RNAs (gRNAs) of viruses, which have specific structured fragments upstream of the start codon, so called Internal Ribosomal Entry Site (IRES) that provide prokaryotic-like mode of initiation of their translation, namely, direct placement of the start codon at the P site region without scanning (e.g., see Hellen & Sarnow, 2001). Such IRES is contained in the gRNA of hepatitis C virus (HCV), one of the most dangerous human pathogens; this IRES is the object of numerous studies. As for the differences in translation termination in bacteria and eukaryotes, they concern, at first, the number of termination factors involved and the mechanisms of termination. Termination in bacteria is performed with two class 1 release factors, RF1 and RF2, which recognize UAG/UAA and UAA/UGA stop codons, respectively. In contrast, in eukaryotes all three stop codons are recognized with the same factor eRF1. Besides, in bacteria and eukaryotes roles of class 2 release factors and GTP hydrolysis in termination are different, as well as are mechanisms of GDP/GTP exchange on the RF3 and eRF3 (e.g., see Rodnina & Wintermeyer, 2009; Korostelev, 2011). Evidently, specific features of the work of eukaryotic translational machinery are based on molecular interactions that do not occur in bacteria.

The translational machinery became a subject of extensive studies utilizing various approaches in two last decades of XX century (reviewed in Rheinberger, 2004), but a breakthrough took place at the boundary between the XX and XXI centuries when the structures of bacterial ribosomal subunits had been deciphered at the atomic level by X-ray crystallography (Ban et al., 2000; Wimberly et al., 2000; Schluenzen et al., 2000). The Nobel Prize in Chemistry has been awarded for this work in 2009. Data on the structures of bacterial ribosomes and their various complexes with ribosomal ligands (tRNAs, mRNAs, etc.) obtained by means of X-ray crystallography made it possible to understand how the ligands interact with the ribosome and why the interactions are specific.

Eukaryotic ribosomes have a pronounced structural similarity with bacterial ones, but they are larger and more complex. So, eukaryotic rRNAs are longer than prokaryotic, and eukaryotic ribosomal subunits in addition to the complete set of ribosomal proteins homologous to their bacterial counterparts contain a number of other ribosomal proteins (about one-third of the total number). Ribosomes from eukaryotes still remain much less studied than ribosomes from bacteria. So, cryo-electron microscopy (cryo-EM) images of eukaryotic ribosomes reported (e.g., see Chandramouli et al., 2008; Taylor et al., 2009; Armache et al., 2010) do not provide direct information on the fine structures of the keystone functional sites of the ribosome, namely, of the mRNA binding center including the decoding site and of the peptidyl transferase center, since particular mRNA codons as well as tRNA acceptor ends were not yet visualized by this method. Very recently reported cryo-EM study of the mammalian ribosomal termination complex carried out at 17-18 Å resolution (Taylor et

al., 2012) presented a mechanistic insight into the coordination between eRF3-dependent GTP hydrolysis and subsequent peptide release by eRF1 but did not provide information on amino acid fragments of eRF1 participating in the organization of stop codon recognition site. As for X-ray crystallography, the first recently reported studies concern only mRNA- and tRNA-free ribosomes from lower eukaryotes, namely, yeast (Ben-Shem et al., 2010; 2011) and *Tetrahymena thermophila* (Rabl et al., 2011; Klinge et al., 2011). Numerous data on the structural and functional organization of the human translational system have been accumulated with the use of a site directed cross-linking. This approach is based on the application of tRNA and mRNA analogs containing chemically reactive (generally, photoactivatable) groups in the selected locations. These ligands being bound within a model ribosomal complex form cross-links with neighboring components of the translational machinery, whose identification enables making conclusions regarding the structure of the ligand binding site.

This chapter is a review on site-directed cross-linking and other related data on the structures of the mRNA binding and the peptidyl transferase centers of the human ribosome and of the stop codon recognition site on the human eRF1, and on the structure of the HCV IRES binding site on the small ribosomal subunit. New insights into molecular mechanisms of translation in higher organisms based on the reviewed data are discussed.

Tools and Model Complexes

The set of tools for structural and functional studies of the human translational system included reactive derivatives of ribosomal ligands, namely, mRNA analogs, tRNA analogs and HCV IRES derivatives. Two types of mRNA analogs were used, namely, derivatives of relatively short (6 to 12 nt long) oligoribonucleotides bearing a *p*-azidotetrafluorobenzoyl (ATB) group attached to the base of the selected nucleotide (Demeshkina et al., 2000; 2003a & b; Graifer et al., 2004; Molotkov et al., 2005; 2006), or to the 3'- or the 5'-terminal phosphate (Bulygin et al., 1997; 2002; 2011) (Figure 1), and 40-50 nt long synthetic mRNAs carrying 4-thiouridine (s^4U) residues at designed positions (Chavatte et al., 2001; Bulygin et al., 2005; 2009).

HCV IRES derivatives bore either ATB groups at selected locations (Laletina et al., 2006; Babaylova et al., 2009) (Figure 2) or s^4U residues randomly substituted uridines over the whole molecule (Otto et al., 2002). tRNA analogs were used that contained s^4U either attached to the 3'-terminus of tRNA as an additional nucleotide (Bulygin et al., 2008) or introduced instead of the 3'-terminal A76 or the penultimate C75 (Bulygin et al., 2012). Chemical groups introduced into the ribosomal ligands can be activated with mild UV-light to form active intermediates capable of cross-linking to both RNA and proteins. In addition to photoactivatable tRNA analogs, tRNA derivatives with periodate oxidated 3' ribose (2',3'-dialdehyde derivatives of tRNA) were applied (Baouz et al., 2009; Hountondji et al., 2012). The latter derivatives were capable of cross-linking only to ribosomal proteins.

In cross-linking studies, model complexes were assembled by direct binding of the ligands to the ribosome without translation factors. In these complexes tRNA was bound at the P site (to which its affinity is higher than to the other ribosomal sites (see Watanabe, 1972; Lill et al., 1986; Graifer et al., 1992)) where it interacted with the cognate mRNA

codon (Figure 3). To obtain complexes where, in addition to the P site, either the A or the E site was occupied with tRNA molecule too, tRNA cognate to the codon present at the respective site was bound to the ternary complex of 80S ribosomes with the mRNA analog and tRNA at the P site. By analogy, termination complexes were formed by binding of eRF1 to the ternary complexes of 80S ribosomes with tRNA at the P site and mRNA analogs, where the A site was programmed with a stop codon (Chavatte et al., 2001; 2002; Bulygin et al., 2002; 2003; 2010; 2011) (Figure 3, complex 5). In the related studies carried out with rabbit ribosomes, s4U-containing mRNA analogs were applied for site-directed cross-linking in 48S and 80S initiation complexes assembled with the use of the appropriate initiation factors (Pisarev et al., 2006; 2008). The cross-linking of HCV IRES derivatives was studied in their binary complexes with 40S ribosomal subunits, which are easily formed without any initiation factors (Pestova et al., 1998; Hellen & Sarnow, 2001). In addition to cross-linking experiments, hydroxyl radical footprinting studies were carried out with the use of tRNA analogs deprived of the 3'-terminal trinucleotide CCA; in these studies, conclusions on the 28S rRNA nucleotides participating in binding of tRNA CCA termini were made based on the comparison between the data with 28S rRNA isolated from the ribosomal complexes containing full size $tRNA^{Asp}$ and the results obtained with $tRNA^{Asp}$ lacking in the CCA end (Bulygin et al., 2012).

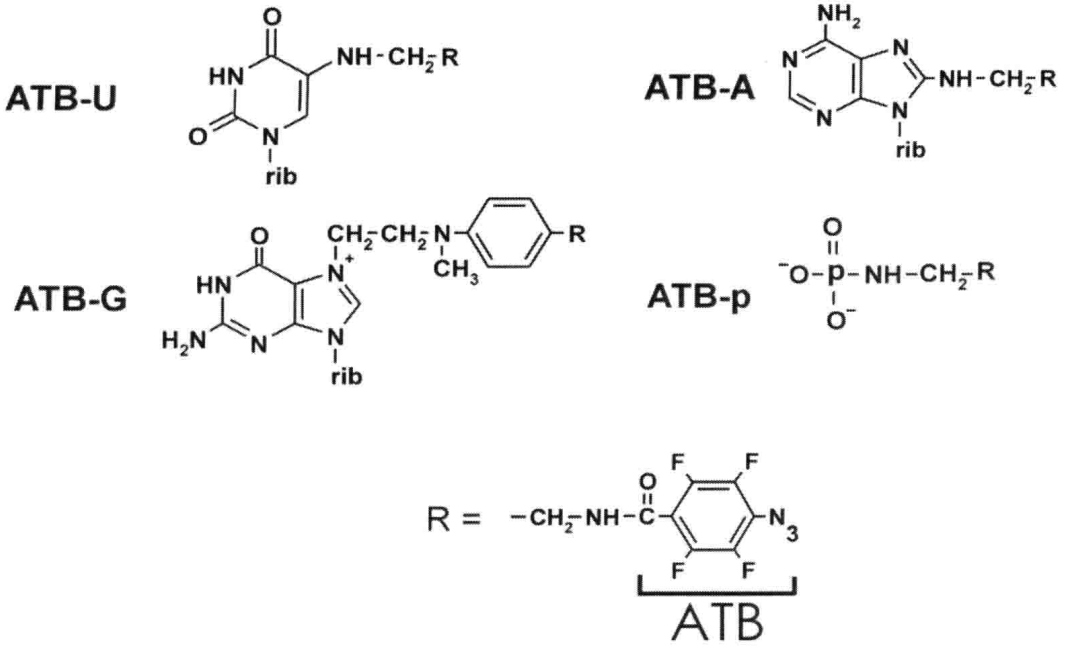

Figure 1. Structures of ATB-modified nucleosides and terminal phosphate in mRNA analogs.

mRNA Binding Center

18S rRNA Nucleotides and Ribosomal Proteins Neighboring mRNA

First systematic studies of the mRNA binding center of the human 80S ribosome were carried out with the application of a large set of short ATB-derivatized mRNA analogs

(Demeshkina et al., 2000; 2003a & b; 2005; Bulygin et al., 1997; 2002; 2003; Graifer et al., 2004; Molotkov et al., 2005; 2006) (Figure 4).

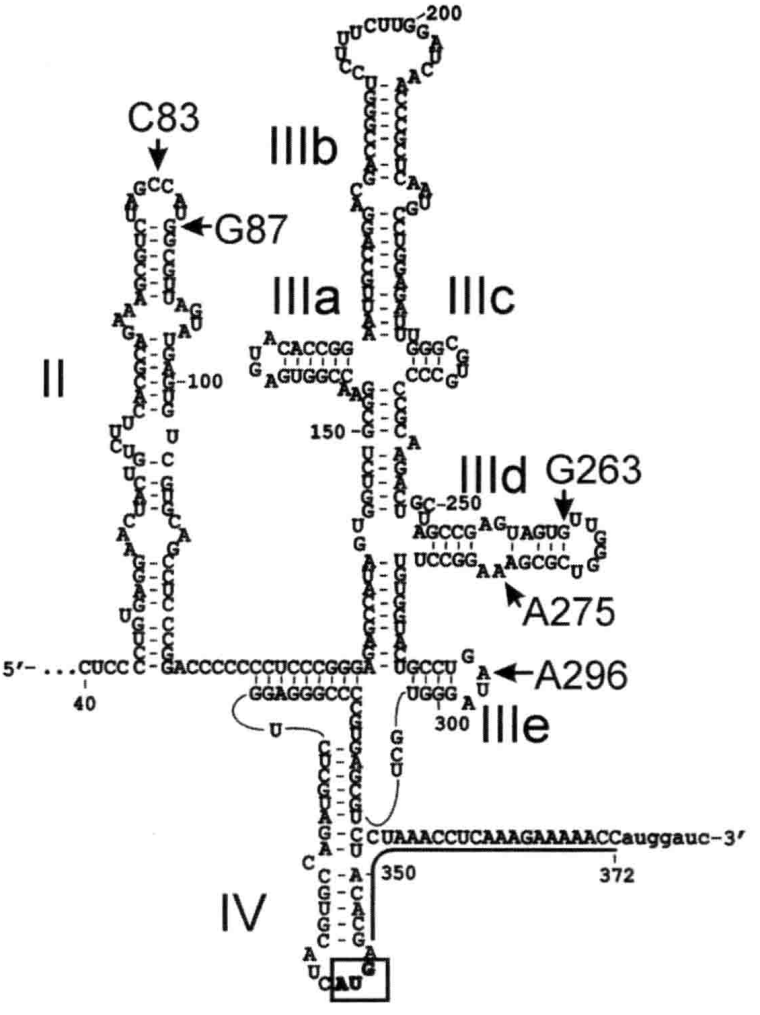

Figure 2. Secondary structure of the RNA transcript corresponding to HCV IRES according to Zhao & Wimmer, 2001. Domains and subdomains are indicated, start AUG codon is boxed, a part of the open reading frame (ORF) is underlined; nucleotides, which present in the transcript but are not the HCV IRES constituents, are given in lowercase letters.

These studies provided detailed information on the molecular environment of mRNA nucleotides in positions from -9 to +12 with respect to the first nucleotide of the P site codon on the 80S ribosome on the level of 18S rRNA nucleotides and identities of ribosomal proteins. Analysis of the data obtained made it possible to find for the first time universal features of the mRNA binding site in all kingdoms and to identify peculiarities specific to eukaryotic (or even to mammalian) ribosomes (Graifer et al., 2004; Molotkov et al., 2005; 2006). All cross-linked 18S rRNA nucleotides fell into the conserved core of the small subunit rRNA secondary structure, and positions of the cross-linked 18S nucleotides almost completely coincided with those of 16S rRNA nucleotides that interact or neighbor the mRNA nucleotides in the respective positions (Ogle et al., 2001; Yusupova et al., 2001; Selmer et al., 2006). These results experimentally confirmed the widely accepted idea on the

conserved rRNA "core" of the ribosome. Data on mutual positioning of mRNA and 18S rRNA in the human ribosome obtained then with s^4U-containing relatively long mRNA analogs (Bulygin et al., 2005; 2009) were in a good agreement with the above mentioned results. In some cases, 18S rRNA nucleotides cross-linked to s^4U residues and to ATB-derivatized nucleotides at the same mRNA positions did not exactly coincide, but all these nucleotides were located very close (within 10-15 Å) to each other on the available 40S subunit spatial structures (Ben-Shem et al., 2011; Rabl et al., 2011). Notably, the cross-linking data showed that the molecular environment of the A site codon practically does not depend on the codon type (sense or stop codon) and on the presence of termination factor eRF1 (Bulygin et al., 2003). Data on 18S rRNA nucleotides contacting mRNA in rabbit 48S and 80S initiation complexes assembled with the use of a set of the appropriate initiation factors and mRNAs containing s^4U at positions from -26 to +11 (relatively to the first nucleotide of the AUG codon) (Pisarev et al., 2006; 2008) turned out to be very similar to those discussed above. All this shows that the conserved core of mammalian 18S rRNA accommodates mRNA to the ribosome very similarly during all steps of translation.

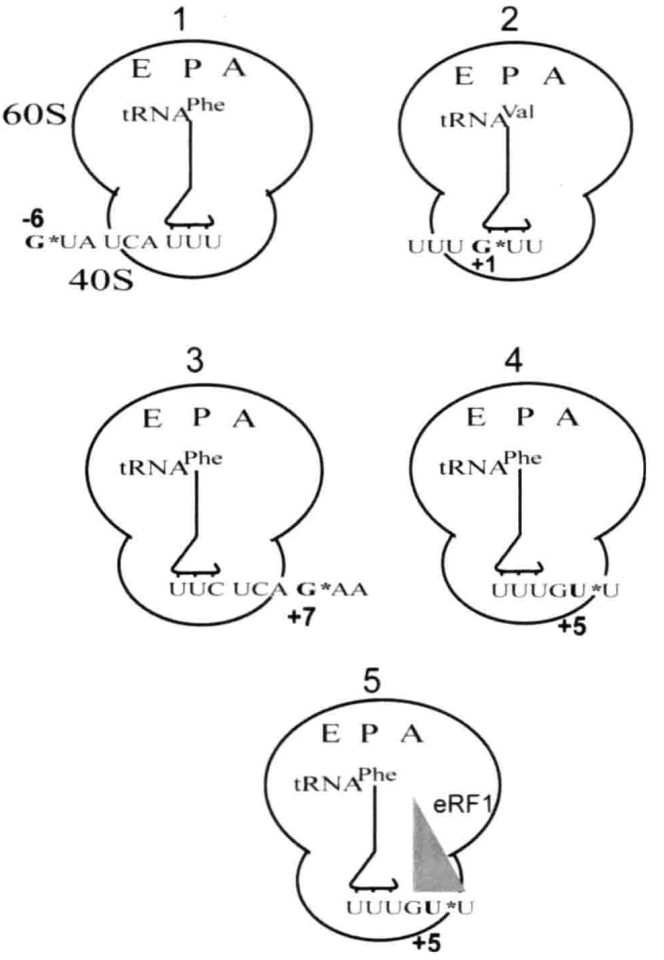

Figure 3. Examples of model 80S ribosomal complexes with ATB-modified mRNA analogs. A, P, and E are the tRNA binding sites. The derivatized nucleotides of mRNA analogs are in bold and marked with asterisks; their positions with respect to the first nucleotide of the P site codon are indicated.