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Similar Features in the Mechanisms of mRNA Translation Initiation in Eukaryotic and Prokaryotic Systems

D. E. Andreev, I. M. Terenin, S. E. Dmitriev, and I. N. Shatsky

Belozersky Institute of Physicochemical Biology, Moscow State University, Moscow, 119992 Russia;
e-mail: shatsky@libro.genebee.msu.su

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Abstract—Similar features in the mechanisms of mRNA translation initiation on prokaryotic and eukaryotic ribosomes are discussed with examples from mRNAs with nonstandard 5'-untranslated regions (5'-UTRs) and mRNAs lacking 5'-UTR (leaderless mRNAs).

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INTRODUCTION

Until recently, an already settled opinion existed that the mechanisms of translation initiation in prokaryotes and eukaryotes are fundamentally different. That viewpoint is well grounded. Only three initiation factors are known for bacteria (IF1, IF2, and IF3), whereas translation initiation in eukaryotes involves nine canonical and a number of auxiliary mRNA-binding proteins [1]. Bacterial and eukaryotic mRNAs have different signals for primary mRNA binding. The majority of prokaryotic mRNAs are polycistronic and are capable of binding small ribosomal subunits in internal regions of their polynucleotide sequences, whereas eukaryotic mRNAs are monocistronic and the primary binding of the 40S ribosomal subunit occurs predominantly at the 5' end. The only exception to this rule is the so-called internal ribosome entry sites (IRESs), discovered in a number of viral and eukaryotic mRNAs [2, 3]. However, unlike the Shine–Dalgarno (SD) signal and the A/U-rich sequences recognized by bacterial S1 [4], the IRESs studied so far are rather long (300–400 nt) and are intricately organized structures with highly specific binding regions for individual components of the eukaryotic translational machinery [2, 3].

The last decade, however, brought new data that bridge the mechanisms underlying translation initiation in eukaryotes and prokaryotes. It was discovered that the eukaryotic initiation factors eIF1, eIF1A, and eIF5B are structural and functional analogs of the bacterial factors IF1, IF2, and IF3 despite the lack of primary structure homology and the difference in their sizes [5–8]. Nonetheless, even the similarity of these initiation factors did not make it easy to suggest parallels between the bacterial and eukaryotic mechanisms of translation initiation, as the binding of mRNA and the search for the AUG initiation codon by eukaryotic 80S ribosomes looks very complex. However, interesting cases were recently reported that favor a considerably higher similarity in the mechanisms of searching for the initiation codon in mRNA by 70S and 80S ribosomes than was believed before. These works were mainly performed in the labs headed by Pestova and Hellen, the team of Boni, and our lab. This review discusses the molecular mechanisms involved in the binding of mRNAs carrying noncanonical initiation regions to the ribosomes of mammals and bacteria. These mechanisms reveal new parallels between the systems of translation initiation in pro- and eukaryotes. However, it is expedient to consider the current concept of the mechanisms of mRNA binding in bacteria (first and foremost, *Escherichia coli*) before describing these unusual observations.

THE MODERN CONCEPT OF MOLECULAR MECHANISMS INVOLVED IN BINDING AND THE SEARCH FOR THE INITIATION CODON IN PRO- AND EUKARYOTES

Recent works suggest that not all researchers involved in molecular biology are having a clear view of what particular signals in prokaryotic mRNA determine the specificity and efficiency of the search for its initiation codon. According to widespread opinion, the SD–antiSD interaction plays a key role in the primary binding of the ribosome and selection of a particular initiation triplet in mRNA. In other words, it is believed that RNA–RNA interactions are characteristic of prokaryotic translation initiation, whereas eukaryotic initiation is presumably based on mRNA–protein interactions. However, the fact that an mRNA-binding component, ribosomal protein S1, an obliga-

tory member of translation initiation in *E. coli* and other Gram-negative bacteria, is located on the 30S ribosomal subunit [9] is completely neglected. The mRNA region that binds S1 is located immediately upstream of the SD sequence. It has no evident consensus. The only thing that is clear is that, enriched in U or A, such a region acts as a powerful enhancer of translation initiation [4]. Most probably, it is S1, rather than the SD sequence, that is responsible for the primary binding of mRNA. Efficient translation initiation requires that the SD sequence does not exceed 5–6 nt. Otherwise, the complex formed is too stable and long-lived, which drastically reduces translation efficiency. Interestingly, S1 counteracts the formation of a very long SD–antiSD duplex [10].

Concepts on the role of the bacterial initiation factors recently have not undergone any serious changes [1]. Bacterial IF3 assists the cognate codon–anticodon interaction between the mRNA initiation triplet and the initiator tRNA by destroying all other codon–anticodon interactions. In eukaryotes, scanning factor eIF1 (see below) is a functional analog of IF3. IF2 binds the initiator tRNA on the surface of the 30S subunit in the presence of GTP and, presumably, stimulates the association of the resulting 30S initiation complex with the 50S ribosomal subunit to form the 70S initiation complex. The functional analog of this factor in eukaryotes is the 40S–60S-joining factor eIF5B. IF1 (eIF1A in eukaryotes) prevents the binding of the initiator tRNA in the A site, thereby stimulating its strict landing in the P site [11]. Note that, unlike eukaryotes, bacteria do not need helicase and ATP hydrolysis to position the mRNA initiator region into the mRNA-binding tunnel of the 30S subunit. This distinction may be explained as follows. First, prokaryotic ribosomes bind to the mRNA initiation regions immediately after they are synthesized by RNA polymerase; therefore, blocking initiation sequences due to complementary pairing with distal mRNA regions is excluded. Translation initiation in eukaryotes takes place on completely synthesized mRNA molecules. Second, the initiation signals in prokaryotic mRNA are, as a rule, located directly upstream of the initiation codon. Finally, as demonstrated by the Noller team [12], the mRNA-binding tunnel of the 30S subunit displays helicase activity 3' of the ribosomal A site. Nonetheless, it is known that stable secondary structures involving the SD sequence and the initiation triplet strongly inhibit translation in bacteria.

Unlike in prokaryotic mRNAs, the main initiator signal in eukaryotic mRNAs is the 5'-terminal cap. It is in this region that all translation initiation events commence in the overwhelming majority of eukaryotic mRNAs (Fig. 1). The cap binds with eIF4E, a component of the multimeric complex eIF4F. The main structural component of this complex is its large subunit eIF4G (170 kDa), which has the binding sites for the cap-binding subunit eIF4E, two molecules of helicase eIF4A, and the poly(A)-binding protein (PABP). The interaction between PABP, which is bound to poly(A) at the mRNA 3' end, and eIF4G essentially stabilizes the association between the overall complex of the factors and the mRNA 5' end; as a consequence of this interaction, the mRNA forms a ring [13]. The mRNA complex with these factors binds to the 40S ribosomal subunit, which already carries the triple complex eIF2–GTP–Met-tRNA$_i$, eIF3, eIF1A, eIF1, and eIF5. This interaction yields the so-called 48S complex. Factor eIF3 is a large multisubunit complex, which is now intensely studied; it serves as a platform for the assembly for a majority of other factors, including eIF4G, a subunit of the cap-binding complex eIF4F. In addition, eIF3 stimulates the binding of the triple complex eIF2–GTP–Met-tRNA$_i$ [1]. The binding of the 43S complex to mRNA–eIF4F–PABP is followed by the scanning of the mRNA 5'-UTR in the 3' direction in search for the closest initiation codon in the optimal nucleotide context …ANNAUGG/A… [14]. The scanning is accompanied by the unfolding of the 5'-UTR secondary structure by helicase eIF4A, which requires ATP hydrolysis. Helicase eIF4A requires the assistance of an additional factor, eIF4B (see [15] and references herein), for melting especially stable hairpins. Scanning factor eIF1 (also bound to eIF3), a complete analog of prokaryotic IF3 [8], is obligatorily involved in the search for the initiation codon. Once the initiation codon is found and the codon–anticodon interaction between it and Met-tRNA$_i$ (48S complex) is established, eIF5 induces hydrolysis of GTP within the triple complex eIF2–GTP–Met-tRNA$_i$, releasing eIF2 from the 40S subunit. The subsequent events have been studied in less detail. It is known that, upon the release of eIF2–GDP, eIF5B attaches the 60S ribosomal subunit to the 48S complex via hydrolysis of another GTP molecule, all initiation factors leave the ribosome, and the resulting 80S initiation complex becomes ready for elongation of the polypeptide chain. Prokaryotic IF2 is an analog of eIF5B [5]. Bacteria lack an analog of the eukaryotic scanning factor eIF2; however, such an analog has been found in archaebacteria, although its function is as of yet vague.

Thus, the binding to mRNA and the selection of the initiation triplet in eukaryotes is based completely on the RNA–protein interactions. The antiSD sequence is absent in the eukaryotic 18S rRNA. Yet some papers still suggest SD–antiSD interactions between the 5'-UTR of individual eukaryotic mRNAs and the 18S rRNA of the 40S subunit. However, experimental confirmations of the involvement of such interactions into the initiation are usually missing. The exclusion of this is the works by the Mauro team ([16] and the references therein). This team reported sufficiently reli-

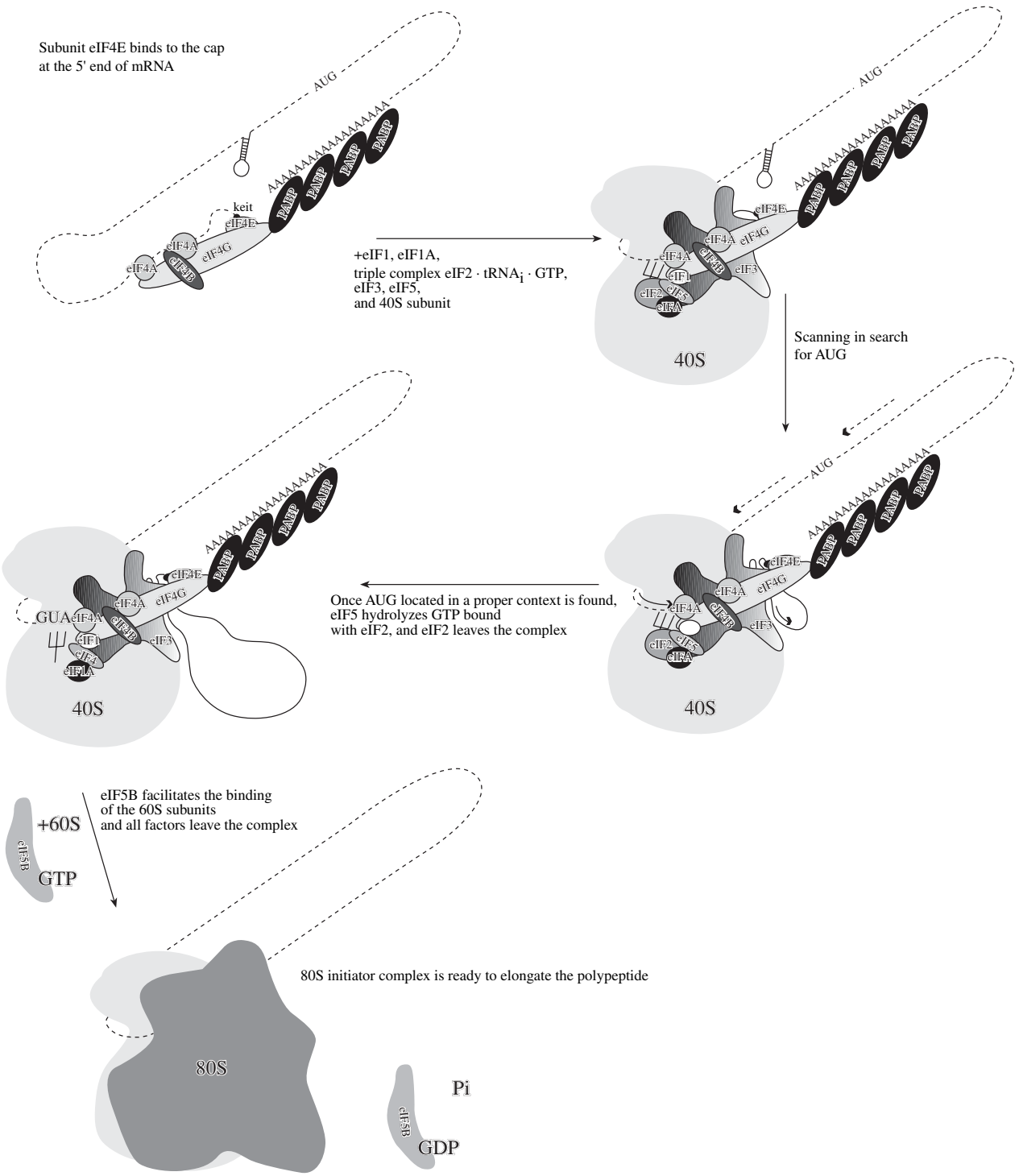

Fig. 1. Scheme of cap-dependent translation initiation in eukaryotes. See text for details.

able experimental data favoring the SD–antiSD interactions between a nonanucleotide element of the 5'-UTR of the mouse *Gtx* homeodomain mRNA and the complementary sequence of hairpin 26 of the mouse 18S rRNA [16]. However, many questions need to be answered before all doubts concerning the existence of SD–antiSD interactions for at least individual mRNAs will be settled. In any case, the most reliable experimental confirmation of such interactions in eukaryotes will be a direct recording of a duplex between mRNA and the 18S rRNA within the 48S initiation complex.

The translation initiation scenario described above is true for the overwhelming majority of eukaryotic mRNAs, but not for all. A number of genomic RNAs in animal RNA viruses lack a cap at the 5' end, while their 5'-UTRs reaching 1000 nt in size display a well-developed secondary structure. Such RNAs follow the above mechanism of internal translation initiation [2, 3], which is typical of bacteria. According to this mechanism, the 40S subunit binds to a specific structure, IRES, within the 5'-UTR, rather than to the mRNA 5' end. It is assumed that IRESs are present in certain cell mRNAs, although this issue is now being actively discussed [17]. Even related viruses may have IRESs with completely distinct structures, and may require essentially different sets of the above canonical initiation factors [2, 3]. The works performed by Pestova–Hellen and colleagues as well as in our own lab made an essential contribution to the research of several viral IRES structures and their requirements to both canonical and auxiliary protein factors. The approach used in these works is an in vitro reconstruction of the initiation complexes from purified components and their analysis by toeprinting [18].

A MECHANISM UNDERLYING THE FORMATION OF THE 48S INITIATION COMPLEX ON THE *Rhopalosiphum padi* VIRUS (RhPV) RNA

The genomic mRNA of RhPV, which infects the bird cherry-oat aphid *Rhopalosiphum padi*, has the 5'-UTR of 579 nt. This RNA is uncapped; its 5' end is presumably covalently bound with a virus protein, which precludes the classic cap-dependent mechanism of translation initiation. Indeed, it was demonstrated that the 5'-UTR of the RhPV RNA carries an IRES [19]. Amazingly, this element is capable of directing translation in insects, wheat, and mammalian cell-free systems with equal efficiency. Most IRESs so far known are highly specific and unrecognizable to the translation components from heterologous systems. The IRES of the RhPV RNA was studied using an original system for the assembly of the 48S preinitiation complex from purified components with subsequent toeprinting [18]. As anticipated, this IRES efficiently formed the 48S initiation complex in the presence of all canonical mammalian initiation factors. However, a withdrawal of eIF4B from the incubation mixture had no effect on the yield of the complex (eIF4B assists eIF4A helicase in melting hairpins in the 5'-UTR during scanning). Moreover, the 48S complex was formed, although with a low yield, even in the absence of all group 4 initiation factors, eIF4A, eIF4B, and eIF4F. Note that the substitution of ATP with its uncleavable analog failed to further decrease the yield of the 48S complex [20].

The ability of the RhPV IRES to assemble the 48S initiation complex in the absence of the group 4 initiation factors and ATP hydrolysis is completely untypical of eukaryotes, except for the IRESs of the hepatitis C virus, several flaviviruses, and the cricket paralysis virus (CrPV), whose mechanism of translation initiation was earlier demonstrated to be considerably closer to the prokaryotic than to the eukaryotic [2].

To localize the IRES within the RhPV 5'-UTR, large deletions (~100 nt each) covering the entire 5'-UTR were obtained. The abilities of the resulting mutant variants of the RhPV RNA to form the 48S complex were tested in the assembly system. It was found that none of the deletions suppressed the activity of the RhPV IRES. Only a deletion of two thirds of the AUG-proximal 5'-UTR sequence (nucleotides 199–599) drastically inhibited the IRES activity. Chemical and enzymatic probing showed that the 5'-terminal third of the 5'-UTR is highly structured, its middle part is low-structured, and base pairing is virtually absent in the U-rich AUG-proximal region of the 5'-UTR (Fig. 2) [20]. Thus, unlike other IRESs so far studied, the RhPV IRES lacks specific binding sites for the translation initiation components, which provides an explanation for its ability to operate in various eukaryotic cell-free systems. The internal binding of the 40S ribosomal subunit to this RNA is nonspecifically directed by the long U-rich single-stranded region.

Although similar experiments have not been performed with a bacterial system, there are good grounds to expect that such IRES is also active in the *E. coli* system, especially because *E. coli* S1 displays a pronounced affinity for U-rich sequences. As was demonstrated by the Boni team, if the 5'-UTR of an eukaryotic mRNA has an appropriate nucleotide sequence (signal) for S1, the SD sequence is unnecessary for the binding of *E. coli* 30S ribosomal subunits [21].

Note that scanning factor eIF1 is absolutely essential for the formation of the 48S complex by the wild-type RhPV IRES and all its active deletion variants [20]. An almost complete analogy with this fact has been reported: the formation of the bacterial 30S initiation complex on mRNA lacking the SD sequence but capable of binding the 30S subunit via S1 depends fully on IF3, a functional analog of eIF1 [21]. Thus, scanning is not an exclusive characteristic of eukaryotic translation initiation. Scanning also occurs when the 30S subunit localizes the initiation codon, although a rather short sequence of the initiation region is presumably scanned in bacterial mRNAs.

SPECIFIC FEATURES OF THE BINDING BETWEEN THE IRES OF THE HEPATITIS C VIRUS RNA AND THE MAMMALIAN 40S RIBOSOMAL SUBUNITS

Surprising specific features were found in the binding of the IRES of the hepatitis C virus (HCV) RNA

1 gataaaagaa cctataatcc cttcgcacac cgcgtcacac cgcgctatat gctgctcatt

61 aggaattacg gctccttttt tgtggataca atctcttgta tacgatatac ttattgttaa

121 tttcattgac ctttacgcaa tcctgcgtaa atgctggtat agggtgtact tcggatttcc

181 gagcctatat tggttttgaa *aggaccttta agtccctact atactacatt gtactagcgt*

241 *aggccacgta ggcccgtaag atattataac tattttatta tattttattc acccccacа*

301 *ttaatcccag ttaaagcttt ataactataa gtaagccgtg ccgaaacgtt aatcggtcgc*

361 *tagttgcgta* ***acaactgtta gtttaatttt ccaaaattta tttttcacaa tttttagtta***

421 ***agattttagc ttgccttaag cagtctttat atcttctgta tattatttta aagtttatag***

481 ***gagcaaagtt cgctttactc gcaatagcta ttttatttat tttaggaata ttatcacctc***

541 ***gtaattattt aattataaca ttagctttat ctatttata*** **AUG**tctacgat gtcttgcacc

Fig. 2. Nucleotide sequence of the 5'-UTR of the *Rhopalosiphum padi* virus (RhPV) RNA. The IRES nucleotides are italicized; the completely unstructured region of the RhPV IRES is in bold.

and related elements of certain pestiviruses (and even some representatives of picornaviruses) with the 40S subunit. With such IRESs the mammalian translation initiation machinery utilizes a specific mode of selecting the initiation codon in the corresponding mRNAs. Since this issue has been elucidated in several reviews [2, 3], we will only briefly consider the unusual features of the HCV IRES-like structures. The nucleotide sequence (about 300 nt) of these elements folds into a unique structure (Fig. 3), which has no analog in mammalian cell mRNAs. To form the 48S initiation complex, such IRESs only need two initiation factors, eIF2 and eIF3. This means that scanning is not required. The unique features of IRESs related to the HCV IRES are to a considerable degree explainable as they are capable of forming stable and specific binary complexes with mammalian 40S subunits at the first initiation stage in the absence of the initiation factors and initiator tRNA. Ribosomal proteins, but not rRNAs, are involved in the binding. Which particular ribosomal protein(s) of the 40S subunit are involved in this primary binding is as of yet unclear; however, the number of candidates already exceeds ten. It is important that plant and yeast 40S subunits fail to bind these IRESs. A model was proposed that the HCV IRES binds directly with the 40S subunit so that the initiation AUG codon is immediately directed to the P site [22]. Consequently, there is no need in eIF1, eIF1A, or the group 4 factors. For the formation of the 48S initiation complex, it remains only to attach the triple complex eIF2–GTP–Met-tRNA$_i$, as observed experimentally [22]. The role of eIF3 is still rather unclear. It is only known that this factor stimulates the binding between eIF2–GTP–Met-tRNA$_i$ and the 40S subunit. Presumably, eIF3 acts at the next stage, when eIF5 and eIF5B facilitate the binding of the 48S initiation complex formed on the HCV IRES to the large ribosomal subunit.

Thus, the HCV IRES acts as both prokaryotic S1 and the SD sequence, positioning the initiation codon of the HCV RNA in the immediate vicinity of the ribosomal P site, although this IRES is considerably larger than a standard translation initiation site in prokaryotic mRNA. In any case, the mechanism of translation initiation at the HCV IRES is absolutely untypical of eukaryotic mRNAs.

IRES-LIKE ELEMENT IN THE mRNAs ENCODING THE RIBOSOMAL PROTEIN S1 IN *E. coli* AND OTHER γ-PROTEOBACTERIA

The previous sections give examples of eukaryotic mRNAs that utilize translation initiation mechanisms that are untypical of eukaryotes and similar to the translation initiation mechanisms of bacteria. The S1 mRNA of *E. coli* and other γ-proteobacteria (*rps*A mRNA) provides a striking example of untypical bacterial translation initiation, which resembles IRES-dependent initiation in eukaryotes. The translation initiation region (TIR) of the *rps*A mRNA is among the most efficient in *E. coli*, despite the absence of the canonical SD sequence. TIR of the *rps*A mRNA is about 100 nt and contains three hairpin structures. It is assumed that these hairpins form a specific spatial structure where individual regions are oriented in a strictly determined manner, which is necessary for an efficient interaction with components of the translational machinery. A disturbance of this specific IRES-

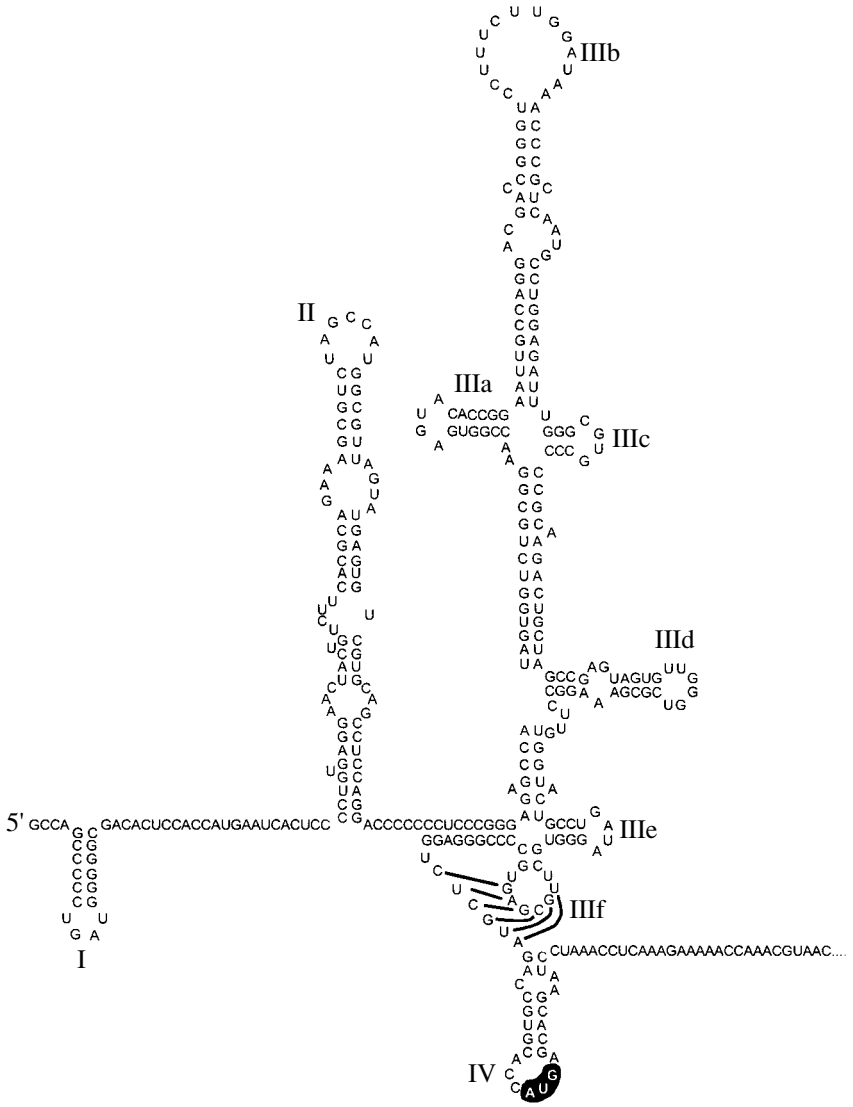

Fig. 3. Structure of the 5'-UTR of the human hepatitis C virus RNA. The domains are designated with Roman numerals. The IRES of the HCV RNA comprises domains II, III, and IV and the initial region of the coding sequence, shown in the figure. Subdomains IIIa, IIId, and IIIe and domain IV play the main role in the factor-free formation of the binary complex between the IRES of the HCV RNA and the 40S ribosomal subunit. The factor eIF3 binds to the region where hairpins IIIa, IIIb, and IIIc are juxtaposed. The precise function of domain II is as of yet unclear.

like structure upon binding with additional S1 molecules considerably decreases the efficiency of translation initiation on the *rps*A mRNA, thereby providing autogenic control of S1 synthesis [23].

INITIATION FACTOR-INDEPENDENT BINDING OF LEADERLESS mRNAs WITH 70S AND 80S RIBOSOMES

Leaderless mRNAs, i.e., the mRNAs that start directly with the initiation codon AUG, are found in all three kingdoms of living organisms, although they have not yet been discovered in mammalian cells. In 1992 it was found in our lab that leaderless mRNA encoding the cI phage λ repressor was capable of directly binding to 70S ribosomes in the absence of the initiation factors [24]. Moreover, IF3 prevented the binding of this mRNA with the 30S subunit. These conclusions were confirmed in experiments with other leaderless mRNAs in other labs, which demonstrated that direct binding to 70S ribosomes is most likely the main mechanism of translation initiation on such mRNAs [25]. Then it was decided to check whether mammalian 80S ribosomes are able to initiate translation in such a manner. A model mRNA in which the *cI* mRNA sequence is followed by *lac*Z was chosen for these experiments. Evidently, such model mRNA has no specific eukaryotic features. The binding of this

mRNA to 40S ribosomal subunits or 80S ribosomes was also examined in a purified system by toeprinting. In the presence of Met-tRNA$_i^{Met}$ the *cIlac*Z mRNA was capable of binding 80S ribosomes, but not 40S subunits, without any eukaryotic translation initiation factors [26]. The mutation of the 5'-terminal AUG codon to GUG completely inhibited such binding. As in the case of *E. coli* 70S ribosomes, even an insignificant extension of the 5' end inhibited the binding considerably, and a longer, even unstructured, leader $(CAA)_{19}$ stopped it completely. Our work of 1992 [24] provided an explanation for this fact. Although the *cIlac*Z mRNA was also able to form complexes with 40S ribosomal subunits in the presence of all factors, the binding efficiency was virtually the same as in the case of the binding to whole 80S ribosomes in the absence of the factors. Note that the 80S complexes formed on the *cIlac*Z mRNA in the absence of the initiation factors were completely competent for further elongation of the polypeptide when supplemented with both elongation factors, EF1H and EF2 [26]. Thus, it was demonstrated that the canonical initiation pathway via the 40S ribosomal subunit at least does not have advantages over the 80S initiation pathway in the case of leaderless mRNAs. Presumably, similar to the bacterial systems, this is the main, if not the only, pathway under conditions of natural competition with standard leader-containing mRNAs.

The similarity in the mechanism of translation initiation on leaderless mRNAs by 70S and 80S ribosomes in the future should be supported by a comparison of structural organizations of the corresponding mRNA-binding tunnels, especially parts that interact with the mRNA 5'-coding region. Intense research on this problem is currently in progress at the lab of G.G. Karpova in Novosibirsk (Russia); however, final conclusions have yet to come.

CONCLUSIONS

The properties displayed by certain noncanonical mRNAs when programming mammalian and bacterial ribosomes suggest that the basic principles of mRNA binding in eukaryotic and prokaryotic ribosomes have far more similar features than could be assumed 5–10 years ago. As in the case for leaderless mRNAs, there is a complete coincidence in the mechanisms of their binding to prokaryotic and eukaryotic ribosomes. It cannot be excluded that such mRNAs represent an evolutionary relict of the translational machinery. These data must confirm to us the opinion that the apparently ponderous eukaryotic translation initiation machinery exists mainly to operate with intricate 5'-UTRs of eukaryotic mRNAs, concurrently providing for the regulation of their activities. The tremendous diversity of 5'- and 3'-UTRs of eukaryotic mRNAs, especially, mammalian mRNAs, requires that many protein factors, both common for all mRNAs and specific for individual eukaryotic mRNAs, were involved in translational control. Presumably, some of them connect translation to other processes, ensuring transmission and realization of genetic information in the eukaryotic cell (transcription, splicing, mRNA transport, and biogenesis of ribosomal subunits).

It is logical to assume that these specific protein factors can contact not only mRNAs, but also structural ribosomal proteins or additional domains of eukaryotic rRNAs. Many of the structural proteins of the ribosomal subunits and additional domains of eukaryotic rRNAs may not be directly related to the main functions of the ribosome as a protein-synthesizing machine. The discovery that protein RACK1, an integral component of the 40S ribosomal subunit, is able to bind protein kinase C illustrates well the diversity of functions of eukaryotic ribosomal components [27]. In this case, the ribosomal protein appears to provide an interlink between the signaling and translational machineries of the cell. Another striking example is L13a of the 60S subunit. In a nonphosphorylated state, this protein is stably integrated into the subunit structure; while in a phosphorylated state L13a binds to the 3'-UTR of the ceruloplasmin mRNA to suppress its translation [28]. In fact, examples demonstrating the functions of ribosomal proteins that are not directly connected with polypeptide synthesis are as of yet scarce. However, their number will grow in due course, taking into account that even in the yeast small ribosomal subunit only 15 out of the 32 structural proteins are homologous to bacterial proteins, whereas 17 have no analogs. The mammalian 40S subunit is even more complex. On the other hand, only six to seven of the 22 proteins found in the 30S subunit have no analogs in the eukaryotic ribosome. Numerous data on the changes in the expression of individual ribosomal proteins in mammalian cancer cell lines are available; however, the functional significance of such changes is as of yet unknown.

When conceiving this paper, we assumed that continuous comparisons of not only the structures of ribosomes or individual translation factors (which is a common situation) but also of the bacterial and eukaryotic translation mechanisms themselves may be most useful for a deeper insight into the fundamental process of translation. A detailed analysis of these mechanisms allows a more distinct differentiation between what is similar and what is disparate, therefore the focus of researchers needs to be on the problems that have yet to be studied.

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REFERENCES

1. Hershey J.W.B., Merrick W.C. 2000. Pathway and mechanism of initiation of protein synthesis. In: *Translational Control*. Eds. Sonenberg N., Hershey J.W.B., Mathews M.B. Cold Spring Harbor, New York: Cold Spring Harbor Lab. Press, pp. 33–88.
2. Hellen C.U.T., Sarnow P. 2001. Internal ribosome entry sites in eukaryotic mRNA molecules. *Genes Dev.* **15**, 1593–1612.
3. Sizova D.V., Shatsky I.N. 2000. Internal ribosome etry sites in viral and cellular RNAs, *Mol. Biol*. **34**, 181–192.
4. Boni I.V., Issaeva D.M., Musychenko M.L., Tzareva N.V. 1991. Ribosome–messenger recognition: mRNA target sites for ribosomal protein S1. *Nucleic Acids Res*. **19**, 155–162.
5. Pestova T.V., Lomakin I.B., Lee J.H., Choi S.K., Dever T.E., Hellen C.U. 2000. The joining of ribosomal subunits in eukaryotes requires eIF5B. *Nature*. **403**, 332–335.
6. Battiste J.L., Pestova T.V., Hellen C.U., Wagner G. 2000. The eIF1A solution structure reveals a large RNA-binding surface important for scanning function. *Mol. Cell*. **5**, 109–119.
7. Pestova T.V., Kolupaeva V.G. 2002. The roles of individual eukaryotic translation initiation factors in ribosomal scanning and initiation codon selection. *Genes Dev.* **16**, 2906–2922.
8. Lomakin I.B., Shirokikh N.E., Yusupov M.M., Hellen C.U., Pestova T.V. 2006. The fidelity of translation initiation: Reciprocal activities of eIF1, IF3, and YciH. *EMBO J*. **25**, 196–210.
9. Sorensen M.A., Fricke J., Pedersen S. 1998. Ribosomal protein S1 is required for translation of most, if not all, natural mRNAs in *Escherichia coli* in vivo. *J. Mol. Biol*. **280**, 561–569.
10. Komarova A.V., Tchufistova L.S., Supina E.V., Boni I.V. 2002. Protein S1 counteracts the inhibitory effect of the extended Shine–Dalgarno sequence on translation. *RNA*. **8**, 1137–1147.
11. Dahlquist K.D., Puglisi J.D. 2000. Interaction of translation initiation factor IF1 with the *E. coli* ribosomal A site. *J. Mol. Biol*. **299**, 1–15.
12. Takyar S., Hickerson R.P., Noller H.F. 2005. mRNA helicase activity of the ribosome. *Cell*. **120**, 49–58.
13. Wells S.E., Hillner P.E., Vale R.D., Sachs A.B. 1998. Circularization of mRNA by eukaryotic translation initiation factors. *Mol. Cell*. **2**, 135–140.
14. Kozak M. 1989. The scanning model for translation: An update. *J. Cell. Biol*. **108**, 229–241.
15. Dmitriev S.E., Terenin I.M., Dunaevsky Y.E., Merrick W.C., Shatsky I.N. 2003. Assembly of 48S translation initiation complexes from purified components with mRNAs that have some base pairing within their 5' untranslated regions. *Mol. Cell. Biol*. **23**, 8925–8933.
16. Dresios J., Chappell S.A., Zhou W., Mauro V.P. 2006. An mRNA–rRNA base-pairing mechanism for translation initiation in eukaryotes. *Nature Struct. Mol. Biol*. **13**, 30–34.
17. Kozak M. 2005. A second look at cellular mRNA sequences said to function as internal ribosome entry sites. *Nucleic Acids Res*. **33**, 6593–6602.
18. Shatsky I.N. 2001. Reconstruction of initiation complexes *in vitro* for analyzing molecular mechanisms of translation initiation in mammals. *Mol. Biol*. **35**, 628–637.
19. Woolaway K.E., Lazardis K., Belsham G.J., Carter M.J., Roberts L. 2001. The 5' untranslated region of *Rhopalosiphum padi* virus contains an internal ribosome entry site which functions efficiently in mammalian, plant, and insect translation systems. *J. Virol*. **75**, 10,244–10,249.
20. Terenin I.M., Dmitriev S.E., Andreev D.E., Royall E., Belsham G. J., Roberts L.O., Shatsky I.N. 2005. A cross-kingdom internal ribosome entry site reveals a simplified mode of internal ribosome entry. *Mol. Cell. Biol*. **25**, 7879–7888.
21. Tzareva N.V., Makhno V.I., Boni I.V. 1994. Ribosome–messenger recognition in the absence of the Shine–Dalgarno interactions. *FEBS Lett*. **337**, 189–194.
22. Pestova T.V., Shatsky I.N., Fletcher S.P., Jackson R.J., Hellen C.U.T. 1998. A prokaryotic-like mode of cytoplasmic eukaryotic ribosome binding to the initiation codon during internal translation initiation of hepatitis C and classical swine fever virus RNAs. *Genes Dev.* **12**, 67–83.
23. Boni I.V., Artamonova V.S., Tzareva N.V., Dreyfus M. 2001. Non-canonical mechanism for translational control in bacteria: Synthesis of ribosomal protein S1. *EMBO J*. **20**, 4222–4232.
24. Balakin A.G., Skripkin E.A., Shatsky I.N., Bogdanov A.A. 1992. Unusual ribosome binding properties of mRNA encoding bacteriophage lambda repressor. *Nucleic Acids Res*. **20**, 563–571.
25. Moll I., Grill S., Gualerzi C.O., Blasi U. 2002. Leaderless mRNAs in bacteria: Surprises in ribosomal recruitment and translational control. *Mol. Microbiol*. **43**, 239–246.
26. Andreev D.E., Terenin I.M., Dunaevsky Y.E., Dmitriev S.E., Shatsky I.N. 2006. A leaderless mRNA can bind to mammalian 80S ribosomes and direct polypeptide synthesis in the absence of translation initiation factors. *Mol. Cell. Biol*. (in press).
27. Nilsson J., Sengupta J., Frank J., Nissen P. 2004. Regulation of eukaryotic translation by the RACK1 protein: A platform for signalling molecules on the ribosome. *EMBO Rep*. **5**, 1137–1141.
28. Mazumder P., Sampath P., Seshadri V., Maitra R.K., DiCorleto P.E., Fox P.L. 2003. Regulated release of L13a from the 60S ribosomal subunit as a mechanism of transcript-specific translational control. *Cell*. **115**, 187–198.