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The ribosome filter hypothesis and specialized ribosomes

Kamil Filipek[a], **Kamil Deryło**[b], **Barbara Michalec-Wawiórka**[c]

Molecular Biology Department, Faculty of Biology and Biotechnology,
Maria Curie-Skłodowska University, 19 Akademicka Str., Lublin, Poland

[a-c]E-mail address: kamilfilipek@wp.pl , kamil@hektor.umcs.lublin.pl ,
basiam@hektor.umcs.lublin.pl

ABSTRACT

The ribosome is a macromolecular complex of proteins and RNA, which plays a key role in every living organism, being a heart of the process of translation. Recent findings have shown that it can be also regarded as a regulatory element that adjusts cellular proteome to highly variable environmental conditions. The ribosome is believed to possess the ability to "filter" populations of mRNAs for choosing their appropriate set to meet current demands of the cell. The filter mechanism is based on a specific interaction between mRNA and rRNA or mRNA and ribosomal proteins. The ribosome "filtering activity" is reflected by the ribosomal particles heterogeneity, which originates mainly from variations or modifications within particular components of translational apparatus. Alternations of ribosomal proteins or/and rRNA generate a specific class of ribosomes called specialized ribosomes, which having unique composition can display selectivity toward particular mRNAs representing an additional step of gene expression regulation at the translational level. This work describes a basis of ribosome filter hypothesis illustrated by interesting examples from different domains of life.

Keywords: ribosome, ribosomal proteins, rRNA, filter hypothesis, specialized ribosomes

1. INTRODUCTION

The central dogma of molecular biology states that genetic information, stored in DNA, is transcribed into messenger RNA (mRNA) and later expressed in the form of proteins. Translation of mRNA is catalyzed by the ribosomes, which are ribonucleoprotein 'nanomachine' known mainly for its involvement in protein synthesis in all living organisms. However, recently ribosomes have gained more attention as particles with regulatory properties, which results largely from differences in their composition [1].

All ribosomes are built of two subunits, which are composed of RNA and proteins. Prokaryotic 70S ribosomes are 2.3 MDa complexes and contain a small 30S subunit and a large 50S subunit. The small subunit consists of 16S rRNA and 21 proteins, while the large subunit is built of two rRNAs – 5S rRNA, 23S rRNA and 33 proteins [2-3]. By contrast, eukaryotic 80S ribosomes are composed of a small 40S subunit and a large 60S subunit. They are larger than bacterial and vary in size from 3.3 MDa in yeast to 4.3 MDa in multicellular organisms like humans. The small eukaryotic subunit contains 18S rRNA and 33 proteins [4]. In the case of large subunits there are some differences between species. For example, in yeast the 60S subunit is made up of 46 proteins with three rRNAs: 5S, 5,8S and 25S, while in humans it is composed of 49 proteins with 5S, 5.8S and 28S rRNA [5].

The translational activity of the ribosome is carried out within its three active sites responsible for different molecular events during the process of protein synthesis: the decoding center (DC), the peptidyl transferase center (PTC) and GTPase associated center (GAC). The decoding center, located within the small subunit is the place where an incoming aminoacyl-tRNA anticodon is matched with an mRNA codons [7-11]. The peptidyl transferase center (PTC), a catalytic heart of the ribosome is placed within the large subunit and is involved in the peptide bond formation between new incoming amino acid and amino acids in growing peptide and additionally it catalyzes the hydrolysis of peptydylo-tRNA leading to release of the newly synthesized peptide [12-15]. The third site GAC, is also located within the large ribosomal subunit and is responsible for the stimulation of GTP hydrolysis by translational GTPases during each step of protein synthesis [16-19] Despite the constitutive nature of aforementioned regions of the ribosome, several ribosomal components seem to be variable and/or activated by additional layers of regulation that depend on the environmental context [20].

2. THE RIBOSOME FILTER HYPOTHESIS

The pattern and the quantity of proteins expressed in living cells are dependent on their mRNA levels, which are regulated by mechanisms controlling transcription, degradation of different mRNAs, alternative splicing of particular mRNAs and localization of mRNAs subpools in the specific regions of the cell [21]. Additionally, there are multiple mechanisms of translation regulation, but the ribosome itself has not been regarded as a regulatory element so far. Interestingly, recent studies suggest that ribosomes have the ability to influence and selectively affect translation rate of various mRNAs. The ribosome filter hypothesis proposes that interactions between mRNA and rRNA and ribosomal proteins in both small and large ribosomal subunits are important in the translation control. These interactions depend on specific sequences found in different groups of mRNA, which compete for binding sites on ribosomal subunits [22]. The interaction profile of mRNAs and ribosomes might be modified

by the ribosomal heterogeneity, which means that differences in ribosome composition may affect the affinity for mRNAs of various sites on the ribosome and thus as a consequence structurally different populations of ribosomes might vary in their ability to translate specific subsets of mRNAs (Fig. 1) [23]. It is speculated that the ribosome heterogeneity can probably lead to differential rates of mRNA translation in various cells or even in the same cell, but in distinct conditions. The basis of filter hypothesis was built on four postulates, presenting different aspects and explanations of the phenomenon of ribosome selectivity toward particular mRNA [23-24].
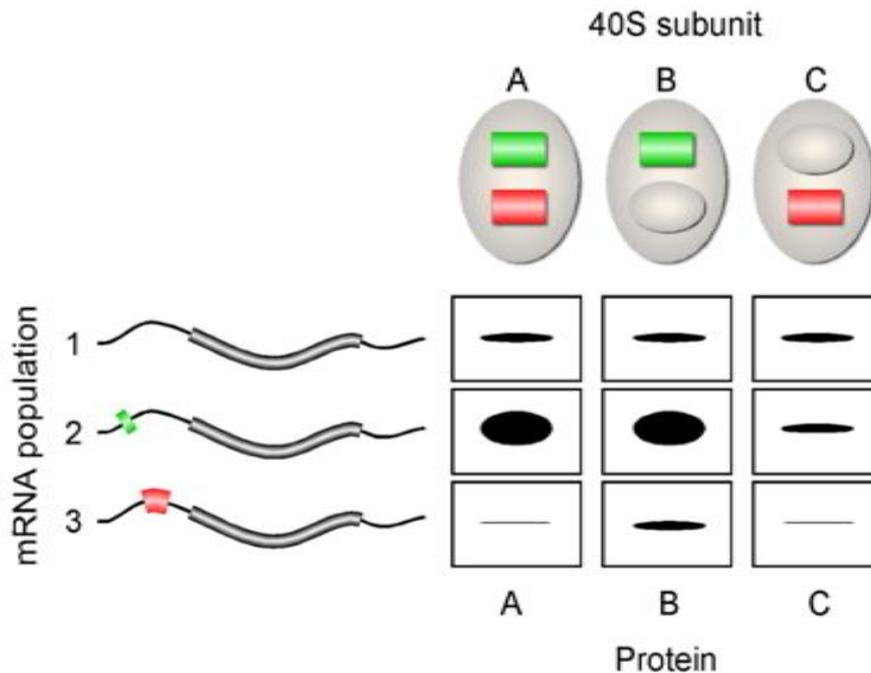

Figure 1. Schematic illustration of ribosomal filtering phenomenon. 40S ribosomal subunits are shown as gray ovals. Subunit A has two mRNA binding sites indicated as green and red boxes. The red mRNA binding site is masked in subunit B, in turn the green binding site is masked in subunit C. The mRNAs are shown in three types, where the first population lacks specific ribosomes binding sites elements. The second population have mRNA-element (green bar), which increases its translation after binding to the green site on 40S subunit. The third mRNA population has an element (red bar) that blocks its translation initiation when it binds to the red site on 40S subunit. The size of black bands corresponds to the amount of protein expression. Translation of first mRNA population occurs by a cap-dependent manner with the same efficiency in A, B and C ribosomal subunits. On the other hand, translation of the second mRNA population is higher when conducted by ribosomal A and B subunits, in which the green binding site is accessible. The translation of third mRNA population is stopped when it comes to ribosomal A and C subunits, in which the red binding site is available [23].

The first postulate says that the ribosome is a regulatory element, which preferentially translates various groups of the mRNA. The ability of ribosomes to modulate translation of mRNA depends on specific interactions between mRNA and rRNA or ribosomal proteins, but these interactions include direct or indirect base pairing between complementary segments of

messenger RNA and ribosomal components [24]. A good example that supports this statement is a difference in cap-independent mRNA translation conducted by mammalian and yeast ribosomes. It has been observed that the level of translation of HCV (hepatitis C virus) genome mediated by IRES (Internal Ribosome Entry Site) differs in yeast and mammalian cells. Numerous studies have determined that HCV mRNA binds to the 40S small subunit's proteins and rRNA (Fig. 2A). Researchers examined the HCV mRNA binding by IRES in mammalian (HeLa cell line) and in yeast (*Saccharomyces cerevisiae)* cells. This study has shown that IRES sequence binds to three ribosomal protein designated as uS2 (SA), uS5 (S2), eS10 (S10) [25] in mammalian cells, but is not able to bind to its yeast counterparts within ribosomal small subunit.

Figure 2. Specificity of HCV IRES binding to the human ribosomal protein eS10 from 40S ribosomal subunit. (A) The structure of HCV IRES bound to the human 40S small subunit. IRES domains are marked by different colors. Domain II is shown in green color, domain IIIac is pink, domain IIIb is red, domain IIIef is cyan, domain IIId is purple [27]. (B) The amino acid sequence alignment of yeast *S. cerevisiae* and human ribosomal eS10 protein. Identical amino acids are presented in black boxes and marked with "*", similar amino acids are presented in gray boxes and marked with." [26].

This failure is caused by differences in amino acid sequence and structure of these ribosomal proteins [23], which implies that the translation initiation via the HCV IRES depends on its ability to bind to human-specific regions of uS2, uS5 and eS10 proteins. Differences between yeast and human amino acid sequence of eS10 protein, representing aforementioned phenomenon, are shown in Figure 2B.

The second postulate concerns that the ribosome as a filter might show a continuum of regulatory effects ranging from interactions responsible for recruitment of particular mRNAs, which enhance translation to interactions that isolate certain mRNAs and decrease their translation rates [24]. This can be explained by the fact that the translation of some mRNA groups can be modified due to the presence of given interactions between mRNA sequences and ribosomal proteins or rRNA [23-24]. An example that supports this postulate is the 9-nt *Gtx* element in the expression of reporter gene. This 9-nt *Gtx* element is an IRES module, which is located in the *Gtx* homeodomain of mammalian mRNA and is perfectly complementary to the 9-nucleotide sequence found within 18S rRNA [28]. It was shown that *Gtx* elements enhance protein synthesis by increasing the translation rate. Introduction of *Gtx* elements in the 5' side of monocistronic mRNA increased translation rate up to 8-fold compared to cap-structure mRNA translation. These results indicated that the *Gtx* sequence influences translation by a base pairing to complementary nucleotides in 18S rRNA [29].

The third postulate proposes that the competition between mRNAs for binding sites placed in ribosomal proteins or rRNA might alter the ratio of translation of certain mRNAs [23-24]. This suggests that certain segments of mRNAs, which can bind to ribosomal subunits may "fight" together in different cells or during special environmental conditions. This mRNAs competition for binding sites can influence the rate of translation of some mRNAs, which are able to bind to the same ribosomal sites. For instance, the tests conducted by Jason Pinkstaff's group have indicated that five dendritical mRNA contain IRES module, which mediates cap-independent translation. The translation of one of them, the neurogranin (RC3) in cultured dendrites from hippocampal neurons, is set by cap-dependent and cap-independent (IRES) mechanisms, but in the case of IRES the manner is more efficient in dendrites than in the cell body. Additionally, in the cellular stress like ischemia or apoptosis the translation of mRNAs with IRES module is still working, while the translation of cap-dependent mRNA is inhibited [30].

The last fourth, postulate points out that the ribosome "filtering activity" might be altered by changing or masking particular binding sites. This might occur as a result of ribosome heterogeneity due to various amount of ribosomal proteins or interaction with extrariboosomal factors like proteins or noncoding RNAs. These differences in ribosome composition may affect the translation rate of some groups of mRNAs [23-24]. The example describing the structural change in the ribosomal mRNA binding site was found in *Dictyostelium discoideum* life cycle (Fig. 3). At the start of slime mold development ribosomal protein eS24 (S24) is methylated. This modification is required for ribosomal proteins mRNAs binding. During this step of life cycle, the translation of these mRNAs is running on a low level. When vegetative growth of *Dictyostelium* starts, the eS24 protein undergoes demethylation and mRNA of ribosomal proteins are effectively translated, because cells need new ribosomes during the development stage. Later, when the slime mold creates a tip-aggregates the eS24 protein loses a methyl group but the other methyl group is attached to the ribosomal eS31 (S31) protein. It was shown that the methylation of eS31 protein is required for the release of ribosomal proteins mRNAs. Cells probably degrade these mRNAs, because they recognize these mRNAs as not useful [31].

These results suggest that during slime mold development a ribosomal filtering mechanism regulates the translation of ribosomal protein mRNAs themselves [24].
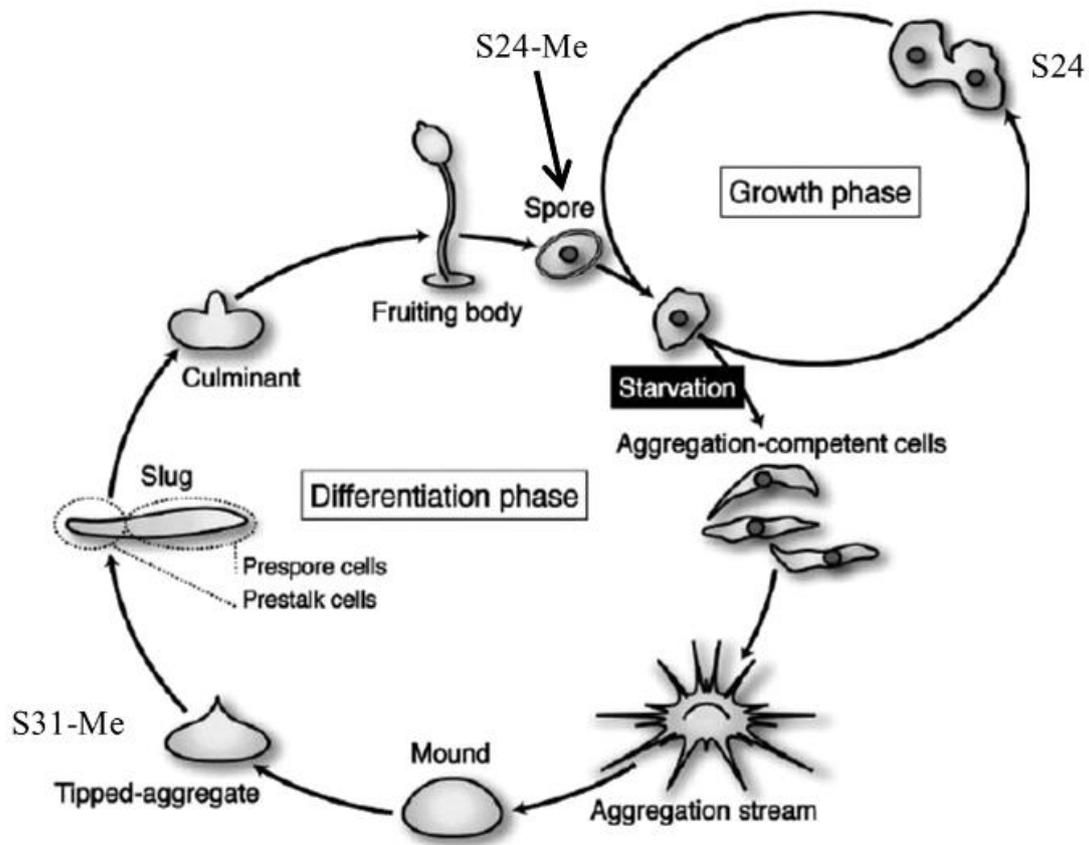

Figure 3. The life cycle of *Dictyostelium discoideum* [32] with indicated methylation (Me) of eS24 and eS31 ribosomal proteins [31].

3. SPECIALIZED RIBOSOMES

Recent studies have highlighted that ribosomes are not static and monolithic machines and they can differ structurally in distinct environmental conditions. Based on these observations the term specialized ribosomes was put forward describing the heterogeneity of translational machinery [1]. This heterogeneity can be associated with differences in the rRNA and protein composition, post-translational modification of ribosomal proteins, variations and modification of rRNA, tRNA or translation factors [20]. To this day researchers have reported the presence of specialized ribosomes in eukaryotic, prokaryotic and archaea cells.

In mammal cells most ribosomal proteins are encoded by a single gene, but there are some exceptions. The first example illustrating specialized ribosomes is connected with small subunit protein eS4 (S4). This protein is encoded by three genes (*RPS4X, RPS4Y1, RPS4Y2*), which are located on X and Y chromosome. In males *RPS4X* and *RPS4Y1* genes are expressed in almost all cells, but *RPS4Y2* is expressed only in testis and prostate [20].

Detailed examination has shown that S4Y2 protein differs from S4X protein about eleven amino acids. Crucial amino acid substitution is located in 171 position, where aspartic acid in S4X is exchanged into serine in S4Y2. This substitution allows the serine 171, located in the central domain, to create a new hydrogen bond with asparagine 232, located in the C-terminus. This type of interaction causes S4Y2 conformational rearrangement (Fig. 4) and affects the position of its domains, which is probably required for unique interactions with different, perhaps specific translation factors or other ribosomal proteins [33].
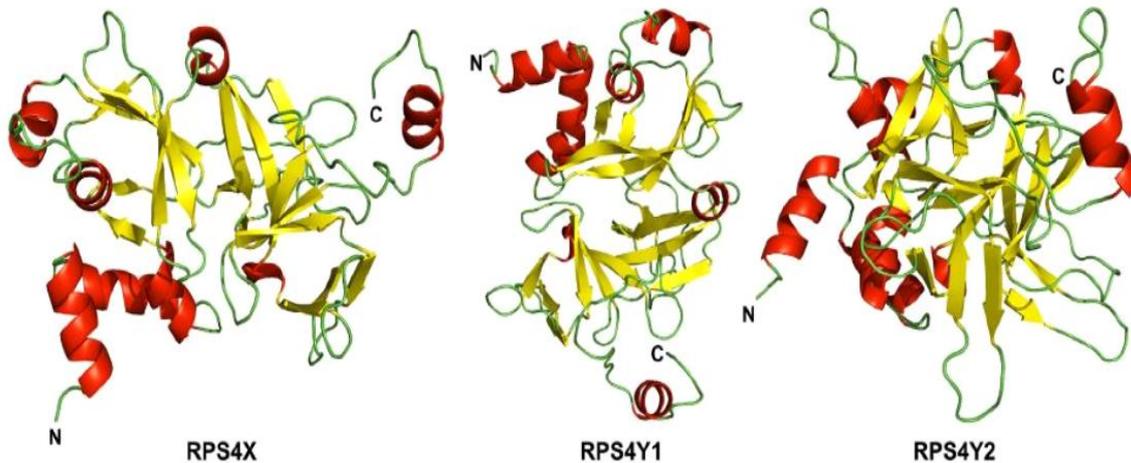
Figure 4. The model of tertiary structure of the ribosomal eS4 protein. α-helices are shown in red, β-strands are shown in yellow and loops are shown in green. The letter "N" means N-terminus and "C" means C-terminus of a protein [33].

Another interesting example, which shows a ribosome heterogeity in eukaryotic cells, was found in *Dictyostelium discoideum*, where different modifications and number of ribosomal proteins appear depending on devopmental stage. This phenomenon could be named ribosome 'switching'. At the vegetative stage amoebae ribosomal eS19 (S19) protein is phosphorylated, uL2 (L2) protein is methylated and uS10 (S20) protein is dephosphorylated. When amoebae aggregates to form a fruiting body the eS19 protein is dephosphorylated uL2 undergoes demethylation and uS10 (S20) protein is phosphorylated. Interestingly, during aggregation ribosomes are depleted of eL18 (L18) protein, which might indicate that this protein is not necessary for certain stages of growth [34]. All aforementioned alternations of ribosome composition are schematically depicted in Figure 5.

Another surprising situation which illustrates the ribosome heterogeneity was found in mice, where loss of one the ribosomal protein can alter a translation of some groups of mRNAs and change the anatomical body structure. In mutant mice depletion of ribosomal protein eL38 (L38) causes inhibition of homeobox (Hox) mRNAs translation, but does not significantly affect global protein synthesis.
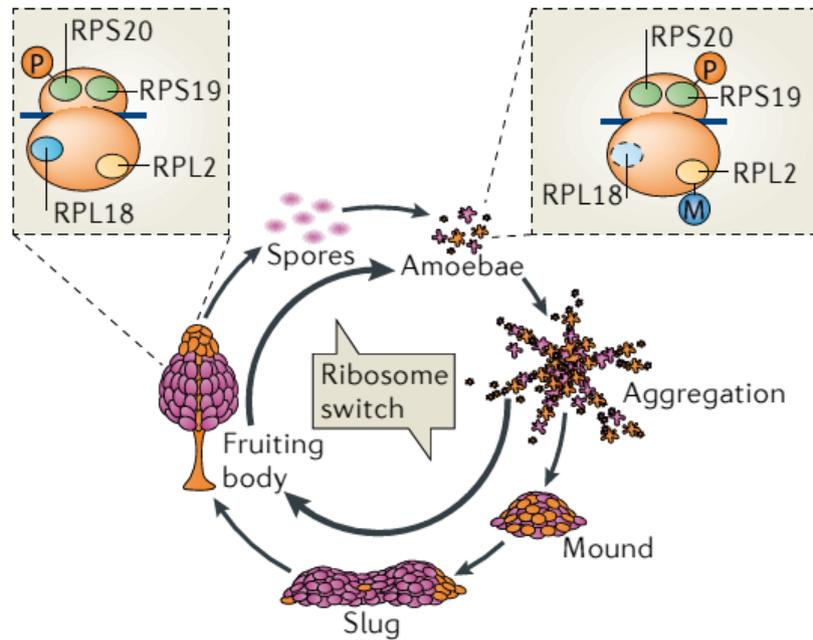
Figure 5. Ribosome alterations during *Dictyostelium discoideum* life cycle [20].
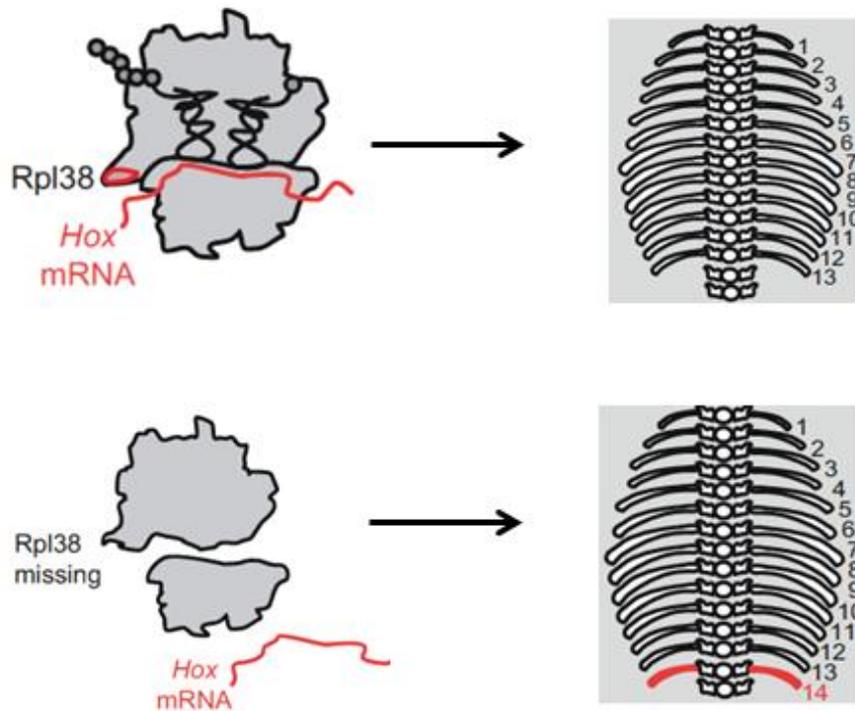

Figure 6. The relationship between the presence of ribosomal eL38 (RPL38) protein and the mice rib cage structure. In the presence of eL38, ribosomes can translate Hox mRNAs and mice have 13 rib pairs. When eL38 is lacking, ribosomes cannot synthetize the Hox protein and mice have an additional rib pair [35].

The homeobox is a DNA sequence within genes, which are involved in anatomical development (morphogenesis) and their products are transcriptional factors that bind to DNA.

The lack of eL38 results in a different pattern of mice rib cage, where mice have fourteen pairs of ribs and unusually kinked tail instead of thirteen found in wild type (Fig. 6) Another important feature of eL38 is its involvement in the tRNA movement during translation and positioning of rRNA, which may contribute to the translation control of specific mRNAs [35].

The ribosome heterogeity is not only based on a different composition of proteins, but also on a variety of ribosomal RNA. This situation can be observed in halophilic red archeon *Haloarcula marismortui*, from the Dead Sea. Its genome encodes three types of rRNA operons, which are paralogues and may increase its fitness at low and high temperatures [1]. Operons *rrnA* and *rrnC* have identical sequences and are expressed at low temperatures, the *rrnB* operon is a highly divergent, has 135 nucleotides polymorphisms among the three 16S, 23S and 5S rRNA genes and is expressed in high temperatures and repressed in low temperatures. Deletion of *rrnB* operon causes impared cell growth in high temperatures. It was observed that expression of *rrnB* operon at 50 °C was four times higher, but three times lower at 15 °C than expression of *rrnA* and *rrnC*. The *rrnB* operon contains much more GC base pairs, whereas *rrnA* and *rrnC* have more AU base pairs. GC pairs have one more hydrogen bond, which increases the structural stability of such DNA region in high temperature. This result supports the idea that divergent rRNA genes can be adaptive with different variants being functional under different environmental conditions [36].

The population of heterogenous ribosomes can be also found amog prokaryotes. The pool of specialized ribosomes are formed in *Escherichia coli* after kasugamycin treatment. Kasugamycin is an aminoglycoside antibiotic, which inhibits the binding of initiator fMet-tRNA^{fMet} to the ribosome. Additionally, the kasugamycin induces formation of specific types of ribosomes, so-called 61S ribosomes, which lacks the bS1 (S1), uS2 (S2), bS6 (S6), uS12 (S12), bS18 (S18) and bS21 (S21) core proteins. The most important protein in this group is bS1 protein, which is necessary for mRNAs binding. Without bS1 the ribosome cannot bind prokaryotic mRNAs and the translation of canonical mRNAs is stopped. Furthermore, certain mRNAs are resistant to the kasugamycin treatment. These mRNAs are leaderless, which means that they do not have a 5'-UTR sequence and begin with 5' AUG codon. These new types of ribosomes preferentially translate leaderless mRNA. These findings have shown that in specific organisms and particular conditions not all ribosomal proteins are needed for translation of mRNAs [37].

4. CONCLUSIONS

The ribosome filter hypothesis and specialized ribosomes are terms, which complement each other to a certain extent. In this review some examples from three domains of life were presented, which makes this hypothesis more credible. In the light of recent findings, ribosomes cannot be considered as homogenous and inflexible molecular machines anymore. The ribosome filter hypothesis posits that translational machinery apart from its main function in protein synthesis can be regarded also as a regulatory element influencing the translation of particular mRNAs. However, the idea that specialized ribosomes are adapted to translate different sets of mRNA in various tissues or in different growth conditions is largely speculation, it is an important and exciting area for scientific exploration.

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