



# Medical Implications of Functional and Destructive Cellular Motions: Curiosity-Driven Open Issues

Andre Rivalta, Disha-Gajanan Hiregange, Tanaya Bose, Gil Fridkin, K. Shanmugha Rajan, Ada Yonath, Ella Zimmerman, Anat Bashan, and Hagith Yonath

## 1 Introduction

Albert Einstein alleged “I have no special talent. I am only passionately curious,” and Albert Szent-Gyorgyi added: “Discovery consists of looking at the same thing as everyone else and thinking something different.” These Nobel Prize laureates were right. Curiosity has long been the driving force of the biological and biomedical sciences, and curiosity-driven research has contributed significantly to our understanding of both simple and complex life processes. Consequently, sophisticated lessons originated from seemingly simple questions, pushed by the natural and insatiable human thirst for knowledge. In fact, curiosity yielded huge dividends in both the short and the long run: we wouldn’t enjoy the benefits of the GPS (or Global Positioning System), if not for the very theory of relativity Einstein proposed, to name an example. Indeed, in research, as a fundamental human impulse, curiosity is a never-ending source of new, head-scratching questions. Thus, several basic issues, some of which with medical implications, are still puzzling us.

Here, we focus on very few selected biomedical non-resolved questions, by attempting to enlighten the broad topic of the relation between structural flexibility and functional appropriateness. Specifically, in the context of our efforts to engage the results of the emerging scientific efforts toward solutions for human health and wellbeing, we are relating to several open questions and focus specifically on a few of them, all associated with protein biosynthesis in healthy as well as in sick cells.

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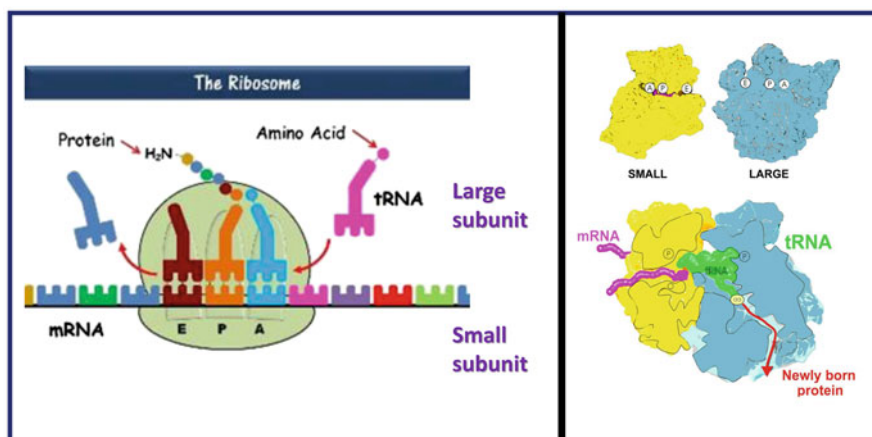
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## 2 Linking Structural Flexibility to Functional Suitability

### 2.1 The Main Protein Biosynthetic Machine, the Ribosome

Ribosomes are the universal multi-RNA-protein cellular assemblies that translate the genetic code into proteins (Fig. 1). They are present in massive numbers in all living organisms, including proliferating human cells, which may contain over 3.3 million ribosomes each. Mammalian ribosomes consist of about 81 r-proteins (ribosomal proteins) and 4 rRNA (ribosomal RNA) chains containing over 6000 rRNAs, many of which participate directly in the protein production process. Their primary functions, namely, efficient genetic code decoding, peptide bond formation, protein elongation, and tRNA release, are performed mostly by the rRNA while being assisted or controlled by ribosomal proteins.

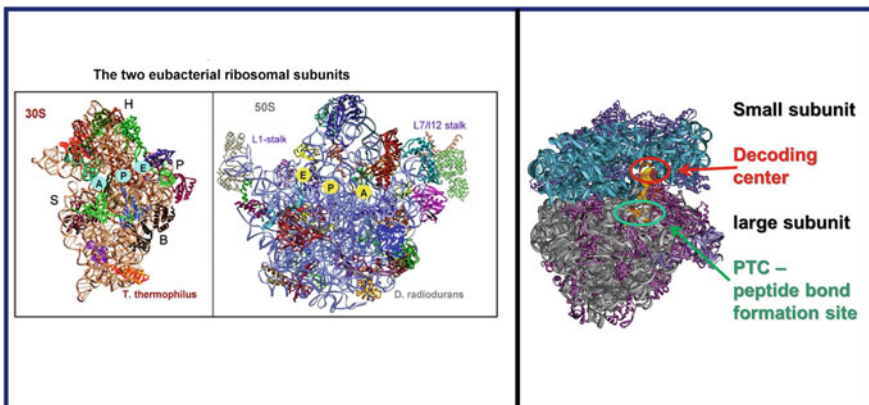
All ribosomes consist of two riboprotein subunits of unequal size, which associate upon the initiation of protein biosynthesis (Figs. 1 and 2) and dissociate once it is terminated. The protein biosynthesis process is performed cooperatively by the two ribosomal subunits and requires signaling between the various functional sites, which are located within the ribosome rather far from each other. The small ribosomal subunit plays a key role in facilitating the initiation of the translation process and in the accurate decoding of the genetic message of the mRNA by controlling the fidelity of codon-anticodon interactions. The large ribosomal subunit catalyzes peptide bond formation and guarantees the elongation of nascent proteins by channeling them into their exit tunnel. This mode of operation is valid even in the presence of several types of RNA modifications, for example, in the highly modified *Leishmania* ribosome [1, 2].



**Fig. 1** An overall description of the ribosome's actions. Left – as a cartoon. Right relates to the ribosome's structure

During each cycle of the elongation event, a new peptide bond is formed by a multi-component cooperative event. mRNA carries the genetic instructions for the amino acid sequence of the produced proteins to the ribosome, and amino-acylated tRNA molecules, each specific to a natural amino acid, are delivering the amino acids to the ribosome. All tRNA molecules share a similar L-shape structure, and although they are built mainly of double helices, their functional sites are located within their single-stranded regions. These include the tRNA anticodon stem-loop that participates in the decoding by base-pairing with the mRNA, and the 5' end of the universal CCA sequence, which carries the amino acids at its other end.

The ribosome possesses three tRNA binding sites, called A, P, and E, each located on both ribosomal subunits (Figs. 1 and 2). The A-site hosts the aminoacylated tRNA, the P-site is the peptidyl tRNA location, and the E-site designates the exiting deacylated tRNA path. The elongation of the polypeptide chain is associated with A- > P- > E translocation of the mRNA chain, together with the tRNA molecules associated with it. Once a peptide bond is created, the peptidyl chain is detached from its tRNA, and the deacylated tRNA molecule exits the ribosome through the E-site, while the A-site tRNA is translocated, presumably by a rotatory motion [3, 4] to the P-site. The so-obtained nascent proteins exit from the ribosome through the protein exit tunnel, which is actively involved in the process of co-translational protein folding (e.g., [5]) as it possesses discriminating properties, and hence can participate in regulating the intracellular co-translational processes. Interestingly, this tunnel could be biochemically and structurally detected even before the structure of ribosome was determined [6–9], and despite initial hints indicating post-translational folding [10, 11], until recently it was assumed to be a rather passive protected path for the nascent peptides.



**Fig. 2** The structures of bacterial ribosomal subunits with designations of the tRNA binding sites. In both parts – the ribosomal RNA is shown by lines, and the proteins as coils. Left – the two subunits, Right – the assembled ribosome

Currently, thanks to thoughtful studies, it is clear that the quality control of ribosomes creation and function is rather complicated. Also, we understand better the initial framework of origin of life and have the ability to focus on the contribution of selected structural features to the creation of several ribosome's sub-regions [12]. Still, the complete picture of the ribosome's evolution is not fully clear.

### 2.1.1 Open Major Issues

- While the overall ribosomes' function is rather well understood for all kingdoms of life, our current understanding of all factors controlling and regulating the processes involved in [cellular protein production](#), including the dynamics of protein turnover within cells and tissues, is still not complete. Thus, only a part of the complex regulatory network that controls the overall process has been thoroughly studied. Consequently, our understanding of the signaling pathways that initiate and terminate protein biosynthesis is only partially uncovered [13].
- Currently, thanks to thoughtful studies, it is clear that the quality control of ribosomes creation and function is rather complicated. Also, we understand better the initial framework of origin of life (see below) and have the ability to focus on the contribution of selected structural features to the creation of several ribosome's sub-regions [12]. Still, the complete picture of the ribosome's evolution is not fully clear.
- As cell vitality requires fast and smooth processing of protein formation, the ribosome must possess features participating in processes allowing response to cellular signals. Do we expect to completely discover and understand these features?
- Efficient processivity of the ribosome catalytic activities depends on accurate positioning of the ribosomal substrates, and it has been suggested that disorder of the PTC may have a functional role in this process. Is this a result of a natural strategy to minimize cell function under hostile conditions?
- RNA modifications are common in biology and are particularly prevalent in rRNA. The specific function of each of these modifications is not fully understood. It is thought that they may play a role in the structure and stability of ribosomes as well as in the regulation of protein synthesis. In addition, they may be involved in ribosome maturation [14], or in the evolution and adaptation to different environments as well as to "enemies," such as antibiotics, by acquiring resistance, e.g., A2058G in bacteria ([15]; [16]; [17]; [18]), but most of them are still elusive.

## 2.2 Bacterial Growth Under Stress

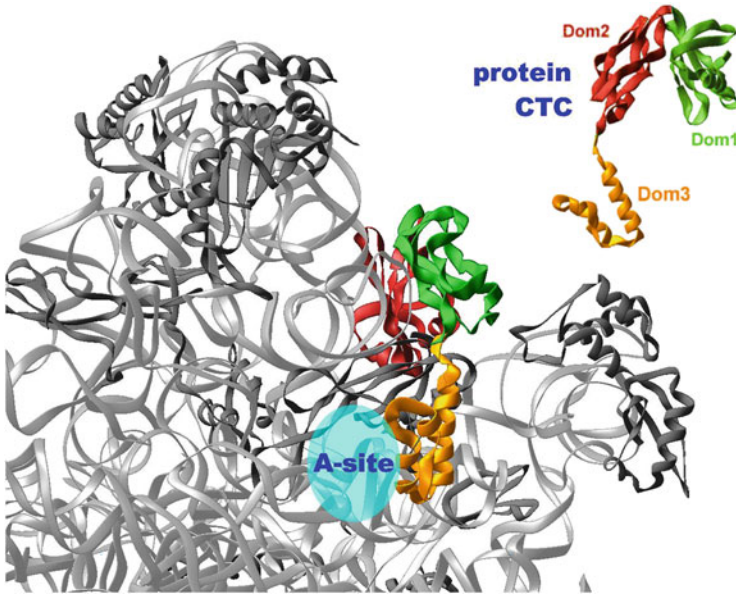
Bacteria devised various mechanisms for responding to hostile growth environments. For example, they can maintain their existence under stressful conditions by temporarily stopping their normal life, thanks to a specific "hibernation mode" which temporarily stops ribosomal activity [19, 20]. Other survival mechanisms showing adaption to harsh surrounding environments have also been

uncovered. For example, *Deinococcus radiodurans* (*D. radiodurans*) is an extremely robust Gram-positive mesophilic eubacterium that nevertheless shares extensive similarities with *Escherichia coli* and *T. thermophilus*. It was originally identified as a contaminant of irradiated canned meat. Currently it is isolated from environments that are either very rich or extremely poor in organic nutrients. As this bacterium lives under stress, its ribosomes are more stable than those of other bacteria, and therefore their large subunits (called D50S) could be among the first ribosomal particles to crystallize, and yielded high resolution structural details, especially of the PTC [21–23] which facilitated further sophisticated studies that shed light on the origin of life [24–28].

*D. radiodurans* contains a few stress-related features, among them the general stress protein CTC, which undergoes conformational changes upon binding the substrate analog ASM (tRNA acceptor stem mimic) to the ribosome, although it does not interact directly with the bound ASM. CTC is a ribosomal protein that regulates tRNA binding to ribosomes [29]. Among the known CTC proteins, the *D. radiodurans* CTC is the longest. In D50S it has three domains. One is located on the ribosome's solvent side and is similar to the single domain *E. coli* protein L25. The combination of this and the second *D. radiodurans* CTC domain resembles the *T. thermophilus* homologue, TL5. The third, which seems to control tightly the A-site tRNA binding, is unique to *D. radiodurans* (Fig. 3). In fact, in D50S each of the domains of protein CTC has a defined task. The N-terminal domain stabilizes the intersubunit-bridge confining the A-site-tRNA entrance. The middle domain protects the intersubunit B1a bridge even at harsh conditions, like elevated temperatures, and the C-terminal domain that can undergo substantial conformational rearrangements upon substrate binding indicates that CTC participates in biosynthesis-control under stressful conditions. Thus, the interactions of CTC with the solvent side of the large subunit central protuberance, its ability to enhance the stability of the B1b intersubunit bridge, and its involvement in controlling the A-site tRNA binding by space exclusion seem to indicate a part of the mechanisms that *D. radiodurans* developed for its survival under stress.

### 2.2.1 Open Major Issues

- What were the driving forces that created the *D. radiodurans* robust bacteria? When was it created?
- How was the natural biosynthesis of the general stress protein CTC controlled by various alternative factors?
- Do the structures of the *D. radiodurans* CTC domains indicate the *D. radiodurans* development path? Dose the structure of CTC of *D. radiodurans* have any bearing on its development path?
- Is protein CTC part of the stress response of *D. radiodurans*?
- Are there other stress-related features in the biology of *D. radiodurans*?

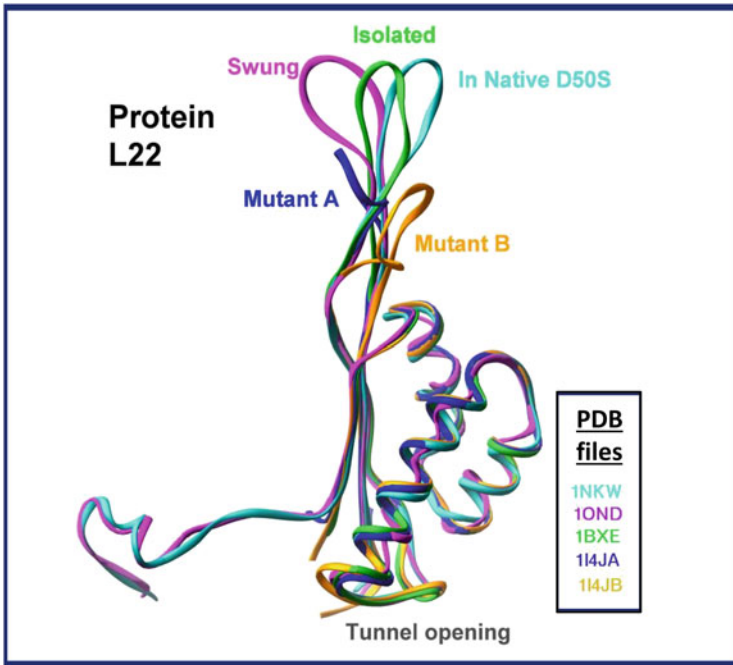


**Fig. 3** (a) A zoom into part of D50S, in which the proteins and the RNA backbones are shown in gray, except for protein CTC, which undergoes substantial conformational changes upon ASM binding, although it does not interact with it. CTC domain-1 resembles protein *E. coli* L25h is shown in red; domain-2, which together with domain 1 resembles protein TL5 of *T. thermophilus* is shown in green; and domain-3, which is unique to *D. radiodurans*, is shown in gold. The position of PTC is shown in cyan. The top-right insert shows the structure of protein CTC in *D. radiodurans*.

## 2.3 Functional Motions and Protective Flexibility

### 2.3.1 Post-Peptide-Bond Formation: Gating and Discrimination

The nascent proteins exit from the ribosome was assumed to be an automatic process after their termination. Actually, it was also assumed to be a fast process, required for efficient overall cellular function. However, conversely it was found that not only the cells include features facilitating control of this event; even the ribosomes are capable of controlling it. Thus, residing on the exit tunnel walls near its exit, and stretching around its opening, ribosomal protein L22 seems to mediate ribosome response to cellular regulatory signals, since it can swing across the tunnel, and cause gating as well as elongation arrest [21, 23]. Protein L22 consists of a single globular domain and a highly conserved hairpin that has a unique twisted conformation [30, 31]. Within the ribosome, it is positioned with its globular domain on the surface of the large subunit, where it can sense the conditions on the ribosome's periphery, whereas its hairpin lines the protein exit tunnel wall and extends approximately 30 Å away from the protein core (Fig. 4). The rather flexible tip of protein L22 can flip across the tunnel and interact with its both sides,



**Fig. 4** Protein L22 and its flexible tip, as found in various bacterial ribosomes. The PDB file numbers are given in the inserted box

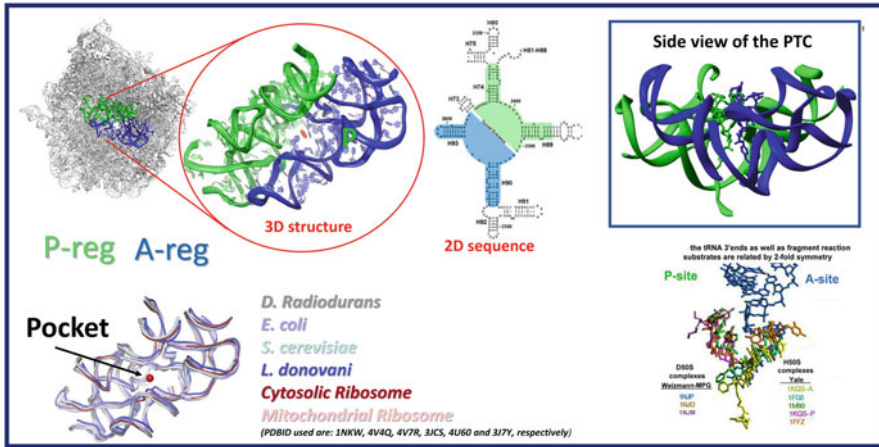
suggesting that a similar swing is involved in the regulatory role assigned to the tunnel.

**2.3.2 Open Major Issues**

- Is a single protein sufficient for maintaining and performing gating of the nascent proteins exit tunnel? Logically, it seems that a single protein is not sufficient for such a complicated concerted role.
- The nascent protein exit tunnel is rather long in molecular terms. Why is such a long path needed to protect the nascent protein while being synthesized?
- Is the discrimination at the tunnel exit obtained by the flexibility of the long arm of protein L22, the only mean for controlling the pace of protein biosynthesis?

**2.4 Origin of Life: The Proto Ribosome—Symmetry at the Active Site of the Ribosome: Structural and Functional Implications**

The PTC, namely, the peptidyl transferase center, is the rRNA region residing within the ribosome large subunits where peptide bonds are being formed. This universal semi-symmetrical region, comprising of about 200 rRNA nucleotides, has been



**Fig. 5** The protoribosome concept. The upper part shows the top shows the structure and the sequence of the contemporary PTC with its two subregions A and P. the lower part indicates its universality

identified in and around the PTC in the large ribosomal subunits of ribosome from all kingdoms of life. Apart from confirming that the ribosome is an RNA machine, namely, a ribozyme, this finding evoked the suggestion that this region was originally a site useful for RNA world interactions, in which amino acids could also dimerize. It later evolved to become the ribosome active center, where the ASMs of A and P tRNAs meet at the entrance to the nascent proteins exit tunnel.

Thus, the ribosomal active site, where the peptide bonds are being formed, is situated within a universal semi-symmetrical region that is embedded in the otherwise asymmetric ribosome structure. This highly conserved region may be the remnant of the protoribosome, which seems to be a dimeric prebiotic machine that initially catalyzed prebiotic reactions, including the rather late formation of chemical bonds (Fig. 5).

In laboratory experiments aimed at imitating the formation of such pockets, namely, constructions meant to mimic the protoribosome, a marked preference of pockets composed of two identical chains, resembling the current PTC P-site, was observed. These pockets were obtained by dimerization of RNA chains of sequences resembling mainly of the P-region of the contemporary PTC, and thus may indicate that the protoribosome was originally a symmetrical homodimer, namely, a pocket made of RNA chains of the same sequence in each of its parts [32]. This concept is in line with the assumption that originally the protoribosome provided almost equal RNA interactions to both its substrates, located at each side of the pocket and created the peptide bonds [26, 27].

Later, with the evolving preference of initial oligopeptides, like those that seem to be able to stabilize the protoribosome, alongside the evolving optimization of the protoribosome into an RNA machine, each of its two parts was independently



adjusted to fulfill its role in peptide bond formation, namely, the entrance to the amino acid charged ASM to A-site and the pushing out the uncharged ASM from the P-site. In this way, the protoribosome adapted to the specific polypeptides' formation requirements, a process through which the protoribosome matured into the PTC contemporary form. Thus, the transition from homo- to hetero-dimers occurred by optimizing the functionality of the protoribosome toward its development to a molecular machine, and the protoribosome conception indicated how the RNA world could be linked to modern life [32].

Furthermore, seemingly crucial features to the polymerase activity of the early ribosome were also identified [33], and models of the PTC activity with initial peptides as larger substrates have indicated which of them could be the initial amino acids [34].

### 2.4.1 Open Major Issues

- Did the protoribosome appear spontaneously in the prebiotic world? Why did it appear?
- Was the protoribosome a symmetrical pocket? How was it produced?
- How did the transition from the initial homo- to the later hetero-dimers occur?
- How was the functionality of the protoribosome optimized?
- What are the evolutionary roots of the ribosome?

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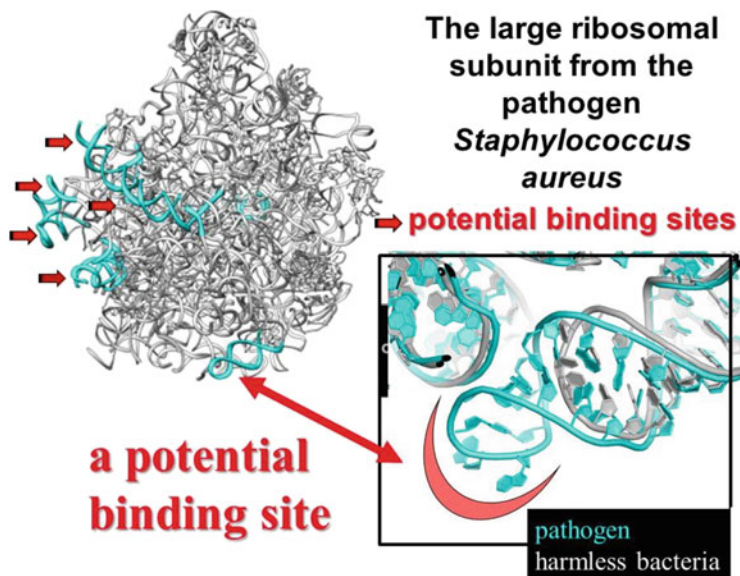
## 3 Protein Biosynthesis in Medicine

### 3.1 Ribosomal Antibiotics: Contemporary Challenges in Medical Usage of Antibiotics

Owing to the vital role played by the ribosomes, many antibiotics target them and successfully obstruct the key ribosomal functions [35–43]. However, the extremely fast increase in antibiotic resistance of many pathogenic bacteria alongside the slow progress (actually negligible) in developing new antibiotics by pharma companies worldwide causes serious medical issues [44–47], including inability to use the antibiotics for treating infections. This leads to severe consequences, including prolonged illness, disability, and even death. Furthermore, most antibiotics do not distinguish between pathogens and nonpathogenic bacteria [48] which may, in turn, affect overall health.

About half of the currently used antibiotics target the process of protein biosynthesis, as it is a key process of life, by blocking the internal ribosome's active sites, such as the mRNA decoding path, the PTC, or the protein exit tunnel. In contrast, instead of targeting the internal ribosomal active for reducing and/or controlling antibiotics resistance, pathogens peripheral species-specific sites, are (Fig. 6), selected [39, 49]. These peripheral sites are identified in various steps, the first of which is performed by structural comparisons to non-pathogenic bacteria.

In principle, as each pathogen tends to contain more than a single such site, several matching molecules may be designed. Hence, for each pathogen several lead



**Fig. 6** Pathogens unique ribosomes peripheral species-specific structural sites, which may be selected to attach environmentally friendly compounds for the development of novel anti-pathogens drugs

compounds that have the potential to be further developed into a next-generation antibiotic drug are being identified. Thus, although not understanding the reasons for the existence of the pathogen-specific peripheral exposed regions, selective binding of antisense oligonucleotides (ASOs) or their chemical imitations [50] could be achieved.

It was shown that lipid nanoparticles (LNPs) are suitable for broad-spectrum nucleic acid delivery [51], as well as means for human vaccination, for example, in the COVID-19 Pfizer-BioNTech vaccine [52]. Combining ASO approaches with the nanoparticles-based nucleic acid delivery can potentially lead to the design of “pathogen-specific antibiotics,” in contrast to the current preference for broad-spectrum antimicrobial drugs that target the highly conserved functional sites of many different species, including helpful bacteria.

In short, the inhibition of protein biosynthesis by the newly identified potential sites for binding of molecules composed of nucleic acids, oligopeptides, etc., which can be optimized in terms of their antibiotics action, chemical properties, poisonous level, modes of penetration, delivery style, and biodegradability.

### 3.1.1 Open Major Issues

- What is the origin of the differences between the ribosomes surfaces of pathogenic and benign bacteria?

- Will the alternative approach, namely, using for antibiotic design pathogen-specific ribosomal peripheral features instead of the ribosomal active regions, lead to a slower pace of resistance appearance?

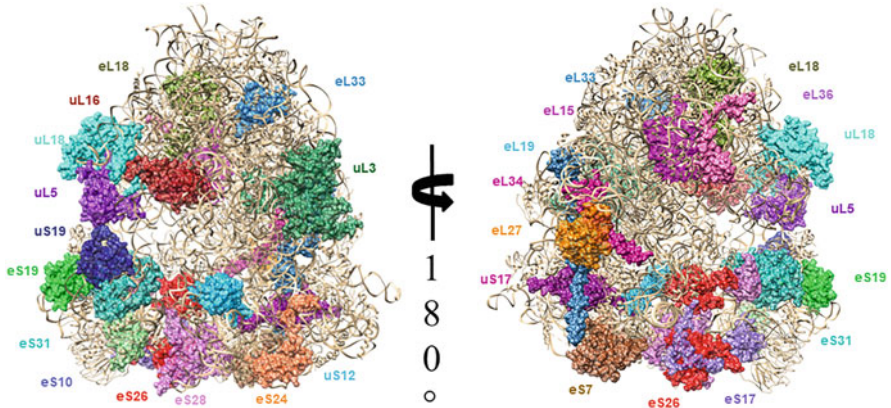
### **3.2 The Eukaryotic Ribosome as a Medicinal Target**

Insight into the structural mechanisms of the pathways of disease of protein biosynthesis should provide the basis for innovative structure-based drug discovery, as well as for the development of novel therapeutic approaches for several human diseases, such as cancers, genetic disorders, and infectious diseases. Thus, owing to the immense importance of protein biosynthesis, intense studies on the ribosome's structural elements and the mechanisms and on the dynamics of biological and disease-linked cellular pathways involved in protein biosynthesis are being performed. For example, it was shown that most natural rRNA modifications cluster around functionally important regions of the ribosome, including the decoding center and the peptidyl transferase center, where they are thought to stabilize folding and the tertiary structure of RNA at these functionally important sites [53–57]. Nevertheless, despite the intensive scientific activity, the natural creation of ribosomes is still considered a difficult puzzle [14, 58, 59].

The investigations on non-coding RNAs that are implicated in human disease yielded some less expected results, including mRNA usage for vaccination by the design of nano-chemically stabilized chains that allows for sufficient tissue and cellular penetration. Among the unforeseen results, it was found that even a single RNA modification can play a critical role in the assembly of the ribosome. For example, a single methylation of ribosomal RNA was found to gate the correct assembly of functional ribosomes [14]. An additional example concerns ribosomes involvement in normal and “ill” expression of proteins in a rather complicated and still not fully investigated manner. Thus, tissue-specific regulation of protein expression pattern in mammals was discovered even in highly complicated events, connected to male fertility [60]. Additionally, since rRNA does not act only as the central scaffold for ribosomal subunits but also, in fact mainly, serves as the center for catalytic activity in ribosome biogenesis, abnormal pre-rRNA processing may cause defects in ribosomal functions, such as unusually early aging, disrupted cardiac protein balance, and induced cardiac hypertrophy, blood and neurodegenerative diseases.

### **3.3 Ribosomopathies and Somatic Mutations**

Ribosomopathies are congenital ribosome-malfunction-related diseases, connected to malfunctioning or reduced function of translating ribosomes, which are characterized by defects in RPs, rRNA processing, or in the assembly of the ribosomes [13, 61, 62]. They include blood diseases like Diamond-Blackfan anemia (DBA) and the Shwachman-Diamond syndrome (SDS), X-linked dyskeratosis



**Fig. 7** Shows all DBA genetically mutated ribosomal proteins. Note their location on or close to the ribosomes surface

congenita (DC), cartilage hair hypoplasia (CHH), and Treacher Collins syndrome (TCS) [61]. Among them, *RPS19* is the first ribosomal gene that was implicated in human disease [63]. It is the most frequently mutated gene in DBA with a total of over 77 mutations that are mostly either whole gene deletion, translocation, or truncation [64].

The molecular pathogenesis studies of DBA patients showed that approximately half of all DBA cases are attributed to mutations in the genes of the ribosomal proteins *RPL35A*, *RPS36*, *RPS2*, *RPS7*, *RPS10*, *RPS15*, *RPS17*, *RPS19*, *RPS26*, *RPS24*, *RPS27A*, *RPS28*, *RPL2*, *RPL5*, *RPL7*, *RPL9*, *RPL14*, *RPL19*, *RPL23A*, *RPL26*, *RPL35*, *RPL36*, *RPS7*, *RPS8*, *RPS10*, *RPL11* [62, 65], all located on or close to the ribosomes surface (Fig. 7). Among those, in ~50% of DBA patients, the genes coding for proteins *RPS19* (called also *eS19*), *RPL5* (*uL18*), *RPL11* (*uL5*), and *RPS10* (*eS10*) are the most frequently mutated [66].

Many unanswered questions are associated with ribosomopathies, for example, so far, the mutations of the DBA encoding genes have been identified only at the genetic level. Also, a search for common denominators among the various mutations, performed at the genes as well as the proteins level, did not reveal any clear linkage between clinical syndromes and the types of their associated mutations or their locations. Furthermore, still there is no answer for the fascinating query: providing DBA disease originates from the ribosomal mutations, how can the mutations triggered by these mutations cause such significant medical problems only in selected parts of the body, although the ribosomes are necessary in all tissues?

These findings corroborate the view that the actual basis for the DBA and other ribosomopathies is caused by the existence of fewer ribosomes. In fact, currently it is not known if the gene-products are indeed expressed and incorporated in the ribosomes of the patients, or if the many mutated genes of the ribosomal proteins

exist only in the genes of their ribosomes. It is commonly suggested, but not proven, that the structural alterations caused by the mutations prevent their incorporation into partially pre-formed ribosomes, which consequently hinder ribosome assembly, thus resulting in a severe reduction in the number of functioning ribosomes.

However, conflicts between this general view and the observed clinical diversity, namely, the observed connections between the patients' medical symptoms and the r-proteins mutations, point at mutated r-proteins involvement in protein biosynthesis, and challenge the ribosome-deficiency common view. In fact, this opinion overlooks several unambiguous indications of explicit connections between specific mutations and specific types/appearances of the diseases (e.g., the previously noted links between particular ribosome's mutations and several types of cancer, as well as of the various DBA's individual tissue-specific symptoms, and their associated unique physical personal abnormalities, like short height, or cleft palate, that are connected to mutated *RPL5* and *RPL11*, or the specific genetic connections to defected heart, or renal anomalies, or bone marrow failure syndromes).

Moreover, this common view opposes findings confirming the existence of ribosome-incorporated mutated r-proteins, like (a) those related to the connection between ribosomes regulation and the under expression of ribosomal protein *RPL22* [67]; (b) those regulating the expression of stress-response regulating genes, like *RPL3* [68]; (c) the uS12-mutant ribosomes that were shown to promote dysmorphism in a recently described ribosomopathies diseases, via increased levels of amino acid disincorporation [69]; (d) those connected to the finding that ribosomal ambiguity mutation Rps2-A226Y that is involved in mice early aging [70]; (e) the connection between modified rRNA base 1248 that is related to common cancer-connected genes, such as p53 [71]; (f) those containing laboratory engineered selected mutation R116D in *RPS3*, which led, as originally planned, to faulty mRNA translation [72]; (g) significant changes that were identified by ribosome footprinting or polysomal RNA sequencing [73].

Other members of this group include blood diseases like 5q- myelodysplastic syndrome. These diseases are associated with genetic mutations of the biosynthetic machinery, including mutations residing in ribosomal components, which may cause mistakes in the process of genetic-code translation, or lead to problems in ribosomes biogenesis, namely, the creation of partially assembled ribosomes owing to mutations in their components.

It was also found that many somatic mutations occur in different ribosomal parts in about 25% of all cancers. Examples include the recurrent R98S mutation of ribosomal protein *RPL10*, which normally facilitates the IRES-dependent translation [74], or mutations in *RPS15* in chronic lymphocytic leukemia (CLL) patients [75] and deletion of *RPS14* in 5q-myelodysplastic syndrome [76, 77].

### 3.3.1 Open Major Issues

- Despite the impressive recent advances in understanding ribosomopathies, several basic intriguing questions still exist and require further conceptual and/or practical studies. Among them, those relating to the possible generalization vs. ribosome specialization need further attention.

- Are all the genetic mutations expressed? In other words, do the mutations dysregulate the ribosome or just lead to a smaller number of correctly functioning ribosome? Or, do both suggested mechanisms exist?
- How can self-renewing tissues happen in cells that are assumed to have a combination of malfunctioning and well-functioning ribosomes?
- What are the bases of the tissue-specificity of DBA and the other ribosomopathies?
- Are there any common denominators among the various cancer mutations?
- What is the linkage between clinical syndromes and the types of the mutations or their locations?
- A highly intriguing point: although the mutations in DBA are expected to create very small fluctuations in the ribosomes structure, they were shown to exceedingly influence its assembly or perform its functions. How does this happen? How can such small alterations cause such significant medical problems? How are these problems expressed only in a part of the body, although the ribosomes are necessary and function in all tissues?

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## 4 Conclusions

This manuscript describes studies that were mainly driven by curiosity, which opened various paths, including medical research, which were not reachable otherwise. It also highlights the need of more research in almost all points discussed here, including origin of life on earth, the roles played by rRNA modifications, the relationship between disorder and ribosome function, and the origins of ribosomopathies.

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