Journal of PeptideScience

Received: 30 June 2008 Revised: 19 August 2008 Accepted: 1 September 2008 Published online in Wiley Interscience: 3 December 2008

(www.interscience.com) DOI 10.1002/psc.1077

Ribosome's mode of function: myths, facts and recent results[‡]

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Ribosomes translate the genetic code into proteins in all living cells with extremely high efficiency, owing to their inherent flexibility and to their spectacular architecture. During the last 6 decades, extensive effort has been made to elucidate the molecular mechanisms associated with their function, and a quantum jump has been made in recent years, once the three dimensional structures of ribosomes and their functional complexes have been determined. These illuminated key issues in ribosome function, confirmed various biochemical, genetic, and medical findings, and revealed mechanistic details beyond previous expectation, thus leading to conceptual revolutions, and turning old myths into actual facts. **Copyright © 2008 European Peptide Society and John Wiley & Sons, Ltd.**

Keywords: ribosome; peptide biosynthesis; RNA; X-ray crystallography; antibiotics; tunnel; nanomachine

Introduction

Ribosomes, the universal riboprotein assemblies, are the nanomachines which translate the genetic code into proteins. These giant organelles, of a molecular weight of about 2.5 MDa in bacteria and up to 4 MDa in higher organisms, are composed of many different proteins and long RNA chains, accounting for two-thirds of the mass, except for mitochondria, where the ratio of RNA to proteins is ~1 : 1. All ribosomes are constituted by two unequal subunits, which associate during the initiation step of protein biosynthesis. In prokaryotes, the small subunit, denoted as 30S, contains an RNA chain (16S) of about 1500 nucleotides and 20–21 different proteins, whereas the large subunit (called 50S in prokaryotes) has two RNA chains (23S and 5S RNA) of about 3000 nucleotides in total, and different 31–35 proteins.

The mRNA chains carry the genetic information that is translated by the ribosome, and tRNAs bring the cognate, the amino acids to the ribosome. The ribosome contains three sites for hosting its tRNA substrates, each residing on both the subunits. The A-site hosts aminoacyl-tRNA (aa-tRNA) molecules, while the P-site hosts the growing peptidyl-tRNA (pept-tRNA). E denotes the site of the exiting tRNA. The decoding center resides on the small subunit, hence the mRNA and the tRNA anticodon loops are attached to it, whereas catalytic site of the ribosome, the peptidyl transferase center (PTC), resides on the large subunit (Figures 1 and 2). During the formation of the peptide bond, nucleophilic attack of the amino group of the aa-tRNA in the A site on the carbonyl carbon of pept-tRNA produces a pept-tRNA that is elongated by one amino acid residue and the deacylated tRNA moves into the E site and then exits the ribosome. The decoding and the formation of the peptide bonds occur in an iterative manner, resulting in a polypeptide chain with a sequence dictated by the mRNA sequence.

Recent reviews demonstrate the current understanding of the ribosomal functions, based on the correlation between the functional data and their high resolution crystal structures (e.g. [1,2]) obtained after 20 years [3] of extensive systematic explorations of crystal growth, refinements of bacterial growth pathways [4], and ribosome separations (E. Zimmerman, data not shown) and requiring the development of innovative methodologies, such as bio-crystallography at cryogenic temperatures [5], and an unconventional use of multiheavy atom clusters [6–9]. Accompanied by advances in the cryo electron microscopy that revealed elements of ribosome's functional dynamics, e.g. Ref. 10–13, these structures revolutionalized many issues concerning ribosome mode of function. Time has come to shed light on selected points in the history of ribosome structural research.

'Palade particles', suggested to be involved in genetic expression were detected already in the 1950s [14], and later located within RNA rich regions, in close association with the membrane of the endoplasmic reticulum [15]. However, as the detailed structural information of such a big organelle was hard to be elucidated, a series of myths were built over the years, based on common wisdom combined with results of biochemical experiments. For example, originally, the common view was that the ribosomal RNA is the scaffold holding the numerous ribosomal proteins in the stereochemistry allowing for performing their tasks, including catalyzing peptide bond formation, and that nascent polypeptide chains leave the ribosome while advancing on its surface [16,17].

Here, we discuss several recent results from the analysis of high-resolution crystal structures, EM reconstructions of ribosomes and of their complexes with substrate analogs or inhibitors with emphasis on those that challenged early conceptions and shed light on ribosome function.

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‡ 11th Naples Workshop on Bioactive Peptides.

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Biography

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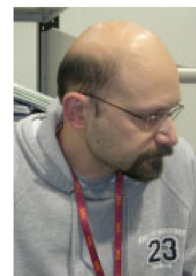
Biography



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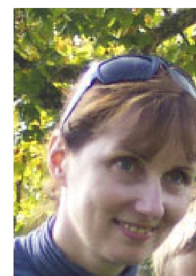

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Dr Rita Berisio earned her B.Sc. degree in chemistry at the University of Naples Federico II, Italy, in 1995. From 1996 to 1998 she worked at the European Molecular Biology Lab in Hamburg and in 1999 she received her Ph.D. at the University of Naples. In 2000, she was a postdoctoral fellow of Uni Naples and in 2001 she became a permanent researcher at the Institute of Biostructures and Bioimaging of the Italian Research National Council, in Naples. In 2002–2004 she joined Ada Yonath's group at the Max-Planck-Institute in Hamburg, Germany. In 2006 she was awarded the Italian Crystallography Association (AIC) Prize for her contribution to structural biology. The research activities of Rita Berisio are focused on aspects of structure/function relationship in macromolecules of biological interest, for which she is combining macromolecular crystallography with other physical–chemical techniques (CD spectroscopy, molecular modeling, molecular dynamics and statistical analyses).

Polypeptide Synthesis by the Ribosome

In the mid 1980s, the importance of the ribosomal RNA (rRNA) became evident [18] and in the 1990s, the dominance of rRNA in ribosome functional activity was proven biochemically [19]. All the available crystal structures verified this notion [20–24]. However, the mode of ribosome function and the nature of its contribution to peptide bond formation remained controversial for quite some time. Thus, based on the early high resolution structure of the large ribosomal subunit from *Haloarcula marismortui* (H50S), four universally conserved rRNA nucleotides of the PTC were identified as the main players in peptide bond catalysis [20,21], through a general acid–base-mechanism, an idea that was soon disputed by a battery of methods (e.g. [25,26]).

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Biography

Dr Ada Yonath graduate earned her B.Sc. (1962) and M.Sc. (1964) degrees in chemistry at the Hebrew University in Jerusalem and her Ph.D. (1968) at the Weizmann Institute of Science. She conducted her postdoctoral studies at Carnegie Mellon University and at the Massachusetts Institute of Technology and in 1970 she established the first protein crystallography laboratory in Israel. Currently she is a professor of structural biology at the Weizmann Institute, incumbent of the Martin S. and Helen Kimmel Professorial Chair, and the director of the Helen and Milton A. Kimmelman Center for Biomolecular Structure and Assembly. During 1989–1994 she chaired the Departments of Structural Chemistry and Structural Biology at the Weizmann Institute. Between 1986 and 2004, she headed a Max-Planck Research Unit in Hamburg, Germany, in parallel to her activities at the Weizmann Institute.

The choice of substrate analogs is partially responsible for the misinterpretation. Amino-acylated tRNA molecules are the natural substrates of ribosomes for the production of nascent proteins. However, for matters of simplicity, substrate analogs commonly used are 'minimal substrates' or 'fragment reaction substrates' (mainly puromycin derivatives), capable of producing single peptide bonds. The complexes of H50S with such minimal substrates obtained under far from physiological conditions and showing disorder in all of the functionally relevant regions, led to the above misinterpretation. Furthermore, additional crystallographic studies on complexes of H50S with similar, albeit more sophisticated substrate analogs illuminated several aspects of peptide-bond formation. In contrast, crystals obtained and maintained under conditions that are more similar to those optimal for protein biosynthesis of complexes of the ribosome large subunit from *Deinocuccus radiodurans* (D50S) with the entire fraction of the A-site tRNA that binds to the large subunit, led to a step forward in the comprehension of peptide synthesis and showed that the ribosome contributes positional, rather than chemical catalysis [24]. In these crystals, the substrate mimicked the entire A-site tRNA acceptor-stem (composed of 35 nucleotides) and the aminoacylated 3′ end (called ASM). This D50S–ASM complex is so far the only complex with A-site tRNA mimic extending beyond the tip of the tRNA 3′ end, although crystals of entire ribosomes with two and three tRNA molecules have been subjected to crystallographic analysis [27,28].

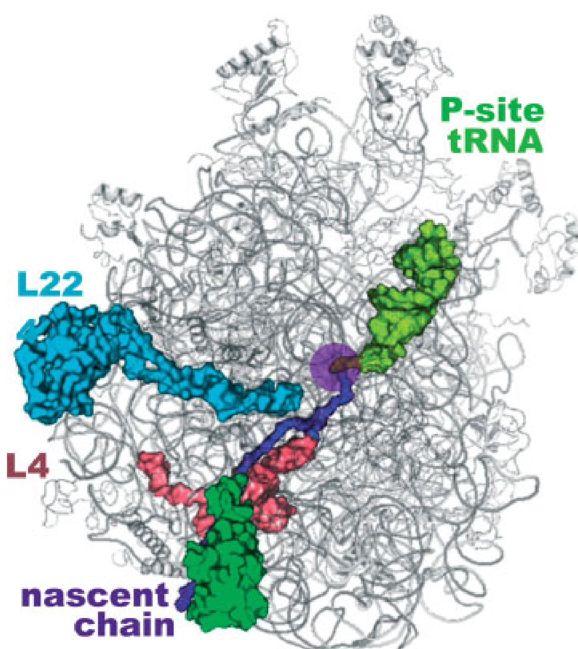

Figure 1. The PTC and the nascent protein exit tunnel in the large ribosomal subunit. The backbones of the ribosomal RNA and the ribosomal proteins are drawn in gray, except for proteins L4, L22, and L23, which contribute to the tunnel wall architecture, and are shown as space-filled bodies colored in blue, pink and light-green, respectively. P-site tRNA is shown in lemon-green, a modeled poly-alanine, representing the nascent chain, is shown in purple, and the PTC is indicated as a transparent purple circle. Coordinates are of the large subunit from *D. radiodurans* (PDB 1NKW).

Different than the 'minimal substrates' that are only capable of the fragment reaction, ASM can ensure peptide synthesis similar to a full length tRNA. Notably, whereas in analyzing the complexes including the 'minimal substrates' the sole attention is paid to the PTC; the complex D50S–ASM demonstrated the importance of the large cavity located above the PTC and hosting the substrate during catalysis. Furthermore, key interactions that occur between the acceptor stem of A-site tRNA and the ribosome cavity were identified in the ASM–D50S complex (Figure 2). These seem to govern the accurate substrate positioning and consequently efficient nascent protein elongation [29].

A sizable intraribosomal region, located in and around the PTC and connecting all ribosomal components involved in protein biosynthesis, has been identified in the crystals of the ASM–D50S complex. This region contains 180 nucleotides of which the RNA backbone fold and base orientation are internally related by a pseudo twofold symmetry (Figure 2) [29]. This striking architectural element was later revealed in all known structures of the large ribosomal subunit, regardless of its source (namely mesophilic, thermophilic, and halophilic bacteria), its functional state (assembled ribosome or unbound subunit, as well as complexes of either with substrate analogs or inhibitors or an), or its kingdom of life (eubacteria and archaea).

As the bond connecting the ASM 3′end with the rest of the molecule was found to overlap the twofold rotation axis, this structure shows that the A-site tRNA translocation occurs by a motion composed of two components: a rotatory motion of the A-site tRNA 3′end in concert with the sideways translocation of mRNA, and the helical portions of the A-site tRNA [24]. The architectural design of the symmetrical region navigates and guides the translocation of the A-site tRNA 3′end towards the P-site. Simulation studies indicated that during this motion the rotating moiety interacts with ribosomal components confining the rotatory path, along the 'PTC rear wall' [24,29]. Consistently, the quantum mechanical calculations indicated that the transition state (TS) for this reaction is formed during the rotatory motion, and is stabilized by the interactions of the rotating 3′end with the ribosome components of the rear wall [30]. The location of the computed TS is similar to that observed crystallographically for mimics of the TS in the large ribosomal subunit from another source, namely H50S [31], and the significance of the interactions between the rotating moiety and the PTC walls was verified by comprehensive mutagenesis analysis [32].

Analysis of the substrate location within the PTC showed that the rotatory motion positions the proximal 2′-hydroxyl of P-site tRNA

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A76 in the same position and orientation found in the crystals of the entire ribosome with mRNA and tRNAs, as determined independently in two laboratories [27,28]. This position allows for chemical catalysis of the peptide bond formation, in accord with the biochemical observations showing a substrate-assisted catalysis of peptide bond formation by full size tRNA [33]. Remarkably, substrate catalysis was not implicated in the crystal structure of the complexes of H50S with 'minimal substrates', in accord with the differences between the formation of the nascent chain by full-size tRNAs and 'minimal substrates' observed by biochemical and kinetics studies, as well as, by mutagenesis [31,33,34].

The current consensus view is that the ribosome supply positional catalysis provides the path along which the translocation from the A- to the P-site occurs and promotes substrate-mediated chemical catalysis. The ability of the symmetrical region to provide all structural elements required for performing polypeptide elongation and the high level of conservation of components of the symmetrical region, which was detected even in mitochondrial ribosomes, in which half the ribosomal RNA is replaced by proteins, suggest that the ribosome evolved by gene fusion or duplication [29].

Essential Contributions of Ribosomal Proteins

As discussed above, although in the late 1980s, the importance of the rRNA became evident, and in the 1990s its dominance in ribosomal functional activity was proven [19], it is clear that the ribosomal proteins make crucial contributions to selected ribosomal activities. Among these, protein S12 of the small subunit is responsible to a conformational switch in the rRNA during decoding of the mRNA [35] and monitors the accuracy of decoding by the ribosome [36]. Proteins of the large subunit that play an important role in PTC activities are proteins L16 [22,24] and L27 [37], which appears to assists proper substrate positioning, as well as protein L2 [38,39] that has been shown to be required for smooth elongation of the polypeptide chain. In addition, long internal loops or external termini of a few r-proteins (L4, L22, L23, and L29) penetrate the RNA that lines the nascent polypeptides exit tunnel (Figure 1) and seem to be involved in the progression of the nascent chains through it. Among those proteins, L22 is involved in elongation arrest [40] and protein L23 is located so that it can influence the trafficking of the nascent proteins near the tunnel opening [41].

The Ribosome Tunnel: from the PTC to the External Cellular Environment

The exit tunnel is a conserved feature of the large ribosomal subunit that extends from the PTC to the ribosome exterior, for a total length of about 120 Å and of a diameter varying in the range of 10–25 Å. Historically, nascent polypeptide chains were assumed to leave the ribosome while advancing on its surface, although pioneering experiments, performed during the 1960s, showed that the most recent synthesized segments of nascent chains are resistant to proteolytic degradation [42,43].

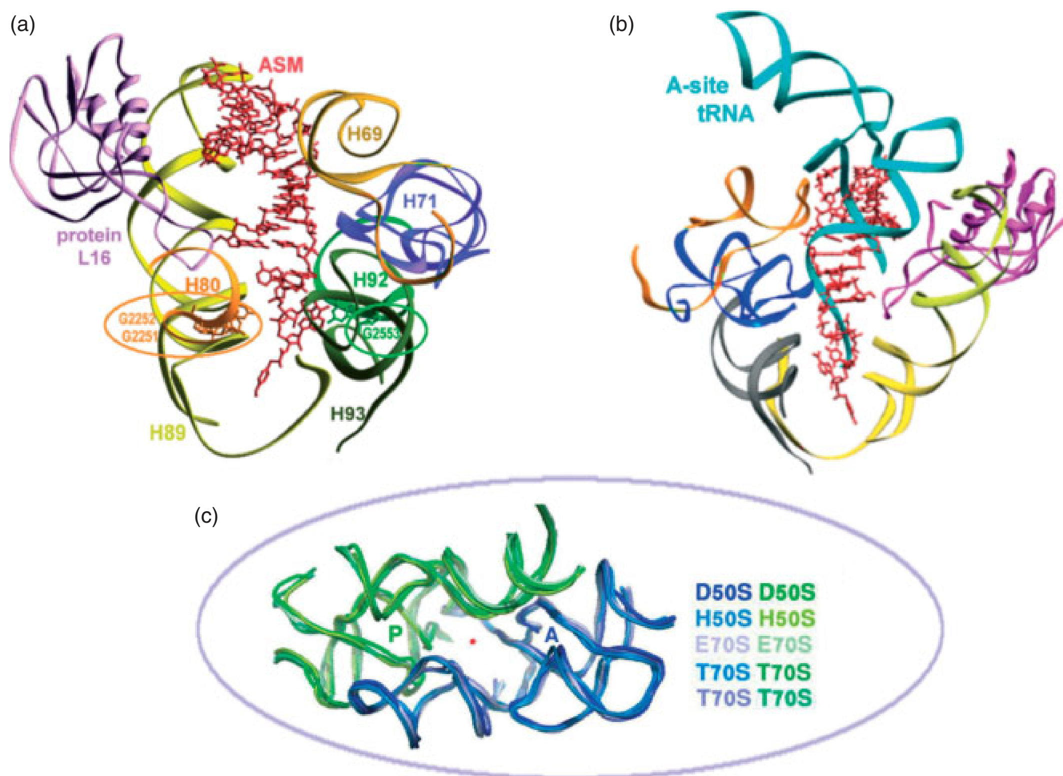

Figure 2. The PTC pocket and the universal symmetry related region. (A and B) Two views of the pocket, with the tRNA Acceptor stem mimic (ASM) shown in red. (A) Highlights the PTC guanines involved in base pairing with A- and P-site tRNAs (depicted by circles). The two base-pair donors at the P-site (compared to the single donor at the A-site), indicate the reason for the preference of the initial P-site tRNA for binding in the P-site, half of the PTC at the rotated orientation. A-site tRNA as seen in Yusupov *et al*., 2001 (PDB 1GIY) is docked on the right image (B). Color code for both sides is as on the left image (A). (C) Superposition of the backbones of the symmetrical region in all known structures of the large subunit (in each it includes 180 nucleotides).

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Even after an elongated tunnel in the large ribosomal subunit has been visualized in the mid-1980s by a three-dimensional image reconstruction from two-dimensional arrays of the entire ribosomes [44] and of the large subunit [45], these findings were met with skepticism, since even then the common perception was that all proteins are produced as α-helices and thus resist degradation even when partially exposed on the ribosome surface [46]. Only after verification, almost a decade later by cryo EM [47,48], the notion that the ribosomes possess a tunnel that can protect emerging nascent chains from degradation became commonly accepted. Nevertheless, even after accepting the existence of the tunnel, its involvement in the passage of the nascent chains and in transmitting information to the cell, as found biochemically [49,50], was hard to comprehend. Hence, when seen at high resolution in the structure of H50S the tunnel was considered as a passive, 'Teflon-like' path [20,21].

Currently, not only is the existence of a protein exit tunnel commonly accepted it is also well established that this tunnel possesses dynamic features [40,51] which enable it to play active roles in the sequence-specific arrest of nascent chains in response to the cellular signals [52,53] and in controlling the operational mode of the translocon at the ER membrane [54]. Fluorescence resonance energy transfer (FRET) measurements [54,55] and computational analyses [56–58] have indicated that some extent of protein folding may happen within the ribosome exit tunnel. In support to these findings, a crevice adjacent to the tunnel wall that can provide space for co-translational transient folding has been identified [59], thus hinting at intratunnel ribosomal chaperon activity. Nevertheless, whether events of cotranslational folding of nascent proteins occur within the ribosome is still under debate. An additional issue to be further investigated is the mode of action of the extended loop of protein L23 that spans the tunnel wall in a fashion allowing for trafficking nascent chains [41,60]. As protein L23 belongs to the small group of ribosomal proteins that display significant evolutionary divergence and this loop is a eubacterial unique feature, higher organisms must possess alternative pathways, which so far have not been identified.

A smooth entrance of nascent proteins into the crowded cellular environment is ensured by molecular chaperones, which support protein proper folding. This is required since nascent chains are exposed to the cellular environment when they reach a length that is not sufficient to allow for proper folding. In eubacteria, trigger factor (TF) is the first chaperone encountered by the emerging polypeptides at the tunnel opening. The various functions of TF are still being explored, as in addition to minimization misfolding and aggregation, it may provide a cradle for the newly born protein [61,62], or provide a sheltered region in which small proteins can obtained their mature fold [63].

TF binds to the ribosome via a few contacts with rRNA and the external globular domains of ribosomal proteins L23 and L29. A comparison of the crystal structures of free TF from *Escherichia coli* [61] with the structure of the binding domain of TF from its homologue *D. radiodurans* bound to the 50S subunit of *D. radiodurans* has shown that TF undergoes conformational rearrangements once bound to the ribosome [41,64]. Notably, these rearrangements expose a sizable hydrophobic region, which confer TF the ability to mask hydrophobic patches occurring on non–fully synthesized proteins, thus stabilizing their temporary conformations. Consistently, FRET studies have shown that the TF bound to the translating ribosomes interacts with nascent chains containing exposed hydrophobic segments after its chemical activation [65], which can be described as the exposure of its hydrophobic regions by conformational rearrangement [66]. Gradual burial of the hydrophobic regions may be accompanied by TF release.

Ribosome Inactivation by Antibiotics

Ribosomes are the target of many clinically relevant antibiotics. As no structural information is available for the ribosomes of pathogenic organisms, crystallizable eubacterial ribosomes, proved to provide suitable models for understanding the mode of action of various antibiotics, are the only sources for structural information of antibiotics binding. As a rule of thumb, all major sites of antibiotic binding are within functional regions of the ribosome. In the large ribosomal subunit, these are the PTC (e.g. chloramphenicol, clindamycin, pleuromutilins, streptogramin$_A$, and oxazolidinones), the ribosome exit tunnel (e.g. macrolides) and the base of the L7/L12 stalk (e.g. thiostrepton, evernimicin).

Antibiotics targeting the PTC [20,24,67–75] hamper either the binding of A-site tRNA or interfere with the formation of the peptide bond. Chloramphenicol blocks only the A site, whereas clindamycin, tiamulin, and streptogramin A bind to both the A and the P sites. Notably, most of these drugs are rather selective despite the high conservation of the PTC. Pleuromutilin and its various derivatives are of great interest for their clinical relevance, as well as for their action mechanism, which demonstrates how selectivity (and resistance) can be acquired despite almost full conservation [70,71]. These drugs have been shown to bind through an induced-fit mechanism that exploits the flexibility of two specific nucleotides, U2585 and U2506, to tighten the antibiotic binding (Figure 3) accompanied by the creation of a network of contacts that involve remote nucleotides, which are less conserved, hence allow for drug selectivity [71].

A different mechanism of action is displayed by the exit tunnel targeting antibiotics, which block the ribosome exit tunnel at a specific latitude and hamper the nascent chain progression. Macrolides are typical exit tunnel targeting antibiotics. They share common chemical characteristics, as they are composed of a derivatized macrolactone ring. The first widely used macrolide drug was erythromycin, a 14-member lactone ring, derivatized with a desosamine and cladinose sugar (Figure 4). All currently available crystal structures of complexes of macrolides and their advanced derivatives with large ribosomal subunits [40,59,67,76–82] show that most of the interactions of macrolides with the ribosome exit tunnel involve the main constituents of the macrolide-binding pocket, nucleotides A2058–A2059, which reside on one side of the tunnel wall, and provide the major elements allowing for drug selectivity (see below) and drug resistance. Erythromycin resistance can be acquired also by mutations in protein L22 tip, as well as in protein L4 that forms, together with L22, a constricted region of the tunnel, and can meet it in its swung conformation (Figure 4). As neither of these proteins interacts directly with the bound drug, it is conceivable that in addition to the change of the protein conformation [83], these mutations may trigger alterations in the structure of the tunnel walls. Indeed, preliminary results of high-resolution structural studies verified previous biochemical [84] and electron microscopical [51] findings, indicating such alterations.

Among exit tunnel targeting drugs, a peculiar inhibitory mechanism has been observed in the crystal structure of D50S complex with the macrolide troleandomycin (TAO). This structure evidenced a novel mechanism where an exit tunnel blockage

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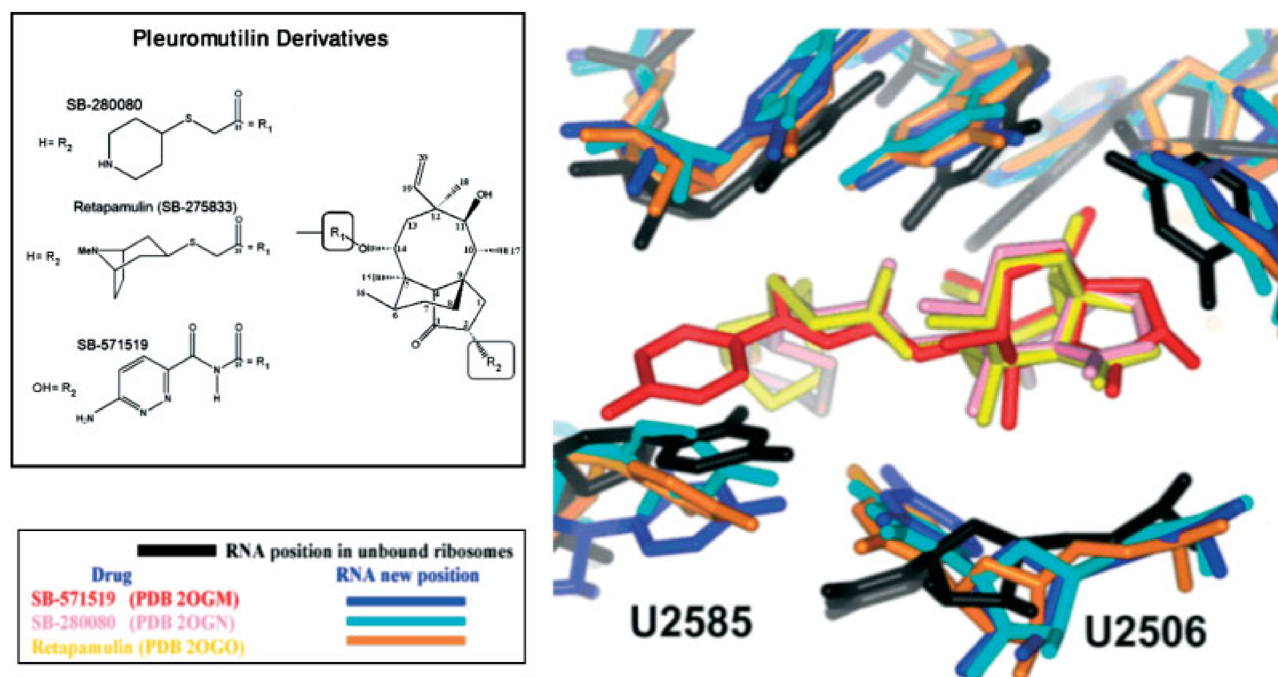

Figure 3. Induced fit and remote interactions in pleuromutilins binding. Remarkable conformational reorganization observed for nucleotides U2506 and U2585. U2506 is tilted toward the pleuromutilins tricyclic cores and thus tightens up the binding pocket on the bound drugs, while U2585 is shifted away from all C14 extensions for avoiding hindrances. Pleuromutilins antibiotics SB-571519 (PDB 2OGM), SB-280080 (PDB 2OGN), and retapamulin (PDB 2OGO) are shown in red, pink, and yellow, respectively. Nucleotides of the unbound large subunit, D50S (PDB 1NKW), are in black and their positions observed in the presence of the antibiotics are in blue, cyan, and orange, respectively.

is achieved by novel interactions with the macrolide binding pocket alongside conformational changes in the protein L22, whose β-hairpin tip is swung across the tunnel (Figure 4). In its swung conformation, the L22 β-hairpin tip gates the tunnel [40]. TAO's interactions with nucleotides of the tunnel wall across the macrolide binding pocket are correlated with mutations bypassing tunnel arrest [52], hence providing the structural basis for conformational dynamics of the tunnel and validating tunnel discrimination properties, obtained biochemically [52,53]. These results have been successively corroborated by the studies of drug-dependent ribosome stalling during translation, which identified the β-loop of L22 as a major molecular player involved in a translation arrest [85] and by mutagenesis studies indicating that in addition to protein L22, protein L4 is also involved in the translational arrest [86].

Ketolides are derivatives of macrolides with an alkyl–aryl or quinollyallyl arm bound to the macrolactone ring and a keto group replacing the cladinose sugar at its C3 position. These chemical modifications have proved to confer a stronger binding. Indeed, biochemical and crystallographic studies [82,87] have shown that they bind to both the sides of the tunnel and that this binding mode exploits an extensive and precise network of interactions [88], at both sides of the tunnel, and therefore can bind to macrolide resistant bacterial strains.

To BE or Not to BE a Pathogen Model

The crystal structures of either of the ribosomal subunits in complex with antibiotics have provided a plethora of information on antibiotic inhibitory mechanisms. Additionally, by revealing the structural factors that discriminate between the ribosomes from eubacteria and those of an archaeon representing eukaryotes, these structures illuminated basic issues in antibiotics susceptibility, specificity, selectivity, and toxicity [1,75,80,89]. As an example, the key nucleotide in position 2058, which is crucial for macrolides binding, plays a major role in macrolide selectivity. In eubacteria, namely in all pathogens, it is an adenine, whereas in eukaryotes and in the archaeon *H. marismortui*, it is a guanine, thus requiring a large drug excess for facilitating some macrolide binding [76]. As this nucleotide is also involved in most macrolides resistance mechanism, advanced compounds that can bind to resistant stains (e.g. with A2058G mutation or A2058 methylamine) were designed. An example is azithromycin that was designed for increased flexibility by an addition of a member to the macrolactone ring (15 rather than 14 members). Indeed, this modification facilitated the binding of the drug also to the ribosomes from the archaeon *H. marismortui* [76], albeit without inhibitory action, since its mode of binding is different from that observed for eubacteria [81]. Thus, azithromycin binding to H50S blocks only a small part of the tunnel, whereas in D50S the binding yields an effective blockage of the exit tunnel [70,80].

Consistently, G2058A mutation in ribosomes of *H. marismortui* [77], aimed at mimicking the binding properties of eukaryotic ribosome, led to macrolide and ketolides binding to H50S at clinically relevant concentrations. However, careful comparisons between the antibiotic-binding sites in ribosomes from the eubacterium *D. Radiodurans* and those from the archaeon *H. marismortui* highlighted a neat distinction in the nucleotide sequence and orientation, leading to substantial differences in macrolide binding modes [2,75,80]. Hence, it was concluded that although A2058 is the main macrolide binding 'anchor', it is not the sole nucleotide determining drug positioning and, therefore, effectiveness. In support of these conclusions is the study showing that mutagenesis from guanine to adenine in the

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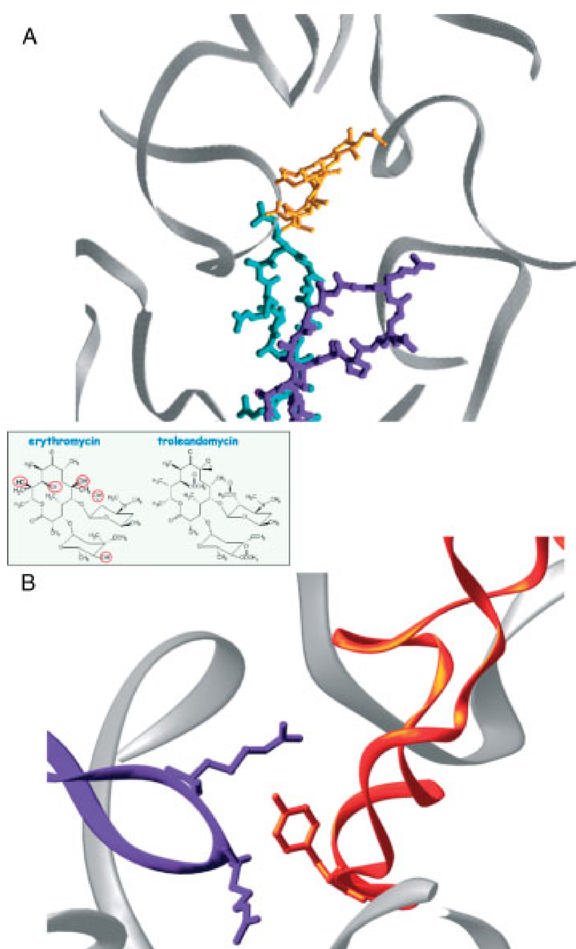

Figure 4. The location of the macrolide troleandomycin (TAO) in the ribosome exit tunnel. The tunnel is represented by the backbone of its RNA, shown as gray ribbons. (A) TAO binding to the ribosome exit tunnel triggers a motion of the tip of the β-hairpin of protein L22 from its native conformation (cyan), lining the tunnel wall, to the other side of the tunnel (purple). (B) In its swung conformation, L22 occludes the tunnel and forms interactions with the RNA components of the tunnel wall, as well as with ribosomal protein L4 (red). Insert: the chemical structures of erythromycin and troleandomycin. In Erythromycin, the depicted OH moieties are those involved in interactions with the binding pocket.

yeast *Saccharomyces cerevisiae* rRNA at the position equivalent to *E. coli* A2058, allows erythromycin binding but does not confer erythromycin sensitivity [90].

Conclusions

The intricate ribosomal architecture positions its substrates in an orientation that promotes peptide bond formation and provides the machinery required for enabling the reiteration of this reaction, which results in amino acid polymerization. The current consensus is that the ribosome contributes positional catalysis to peptide-bond formation and provides the path along which A-to-P-site translocation occurs, whereas chemical catalysis is provided by the proximal 2′-hydroxyl of P-site tRNA A76. Although several structural and biochemical studies have been so far carried out, a rational view of the protein manufacture process and its regulation in cells is still to come. The picture that emerges from the available studies is that the ribosome is a stunning autonomous machinery with outstanding sensory elements. Indeed, not only ribosomes synthesize proteins in a cell-free environment but they also auto-control and adjust the rate of polypeptide synthesis to the specific expression requirements [85,91].

Acknowledgements

Support was provided (to RB) by the MIUR (FIRB-Contract number RBRN07BMCT) and (to AY) by the US National Inst. of Health (GM34360), and the Kimmelman Center for Macromolecular Assemblies. Thanks are due to all former members of the ribosome group at the Weizmann Institute for their scientific contributions and constant interest. Particular thanks are due to Shoshana Tel-Or, Dina Levy, Miriam Laschever, Moshe Peretz, Yaakov Halfon, Ofir Falk-Sade, Giosuè Sorrentino and Maurizio Amendola for their technical assistance. CD is supported by the Adams Fellowship Program of the Israel Academy of Sciences and Humanities and AY holds the Martin and Helen Kimmel Professorial Chair.

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