The amazing ribosome and its antibiotics: from genetic code to partially folded proteins

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For other citations please see Bashan and Yonath 2008 and Yonath 2005)

Translation of the genetic code into proteins is performed by a complex apparatus us comprising the ribosome, messenger RNA (mRNA), transfer RNAs (tRNAs) and accessory protein factors. The ribosome, a universal dynamic cellular ribonucleoprotein complex, is the key player in this process. The recently emerged high resolution structures revealed that ribosomes are precisely engineered machines that utilize conformational variability for optimizing their functional efficiency.

Ribosomes are built of two ribonucleoprotein subunits (Figure 1a) that associate to form the functional ribosome. While elongation proceeds they operate cooperatively: the small subunit provides the mRNA binding machinery, the path along which the mRNA progresses, the decoding center and components controlling translation fidelity. The large subunit performs amino acid polymerization and provides the protein exit tunnel. The interface surfaces of the two subunits are composed predominantly from ribosomal RNA (rRNA). Both the decoding center and the site of peptide bond formation, called peptidyl transferase center (PTC) reside in proximity to the intersubunit interface. Hence, unlike typical polymerases, which are protein enzymes, RNA is the major player in ribosome activities. tRNAs, the molecules decoding the genetic information and carrying the amino acids (Figure 1a) to be incorporated in the growing protein, are the non-ribosomal entities combining the two subunits. At each elongation cycle both subunits participate in translocating the mRNA and the tRNA molecules attached to it by precisely a single codon from the A- to the P- and then to the E-site. While a peptide bond is formed [Gindulyte et al., 2006]

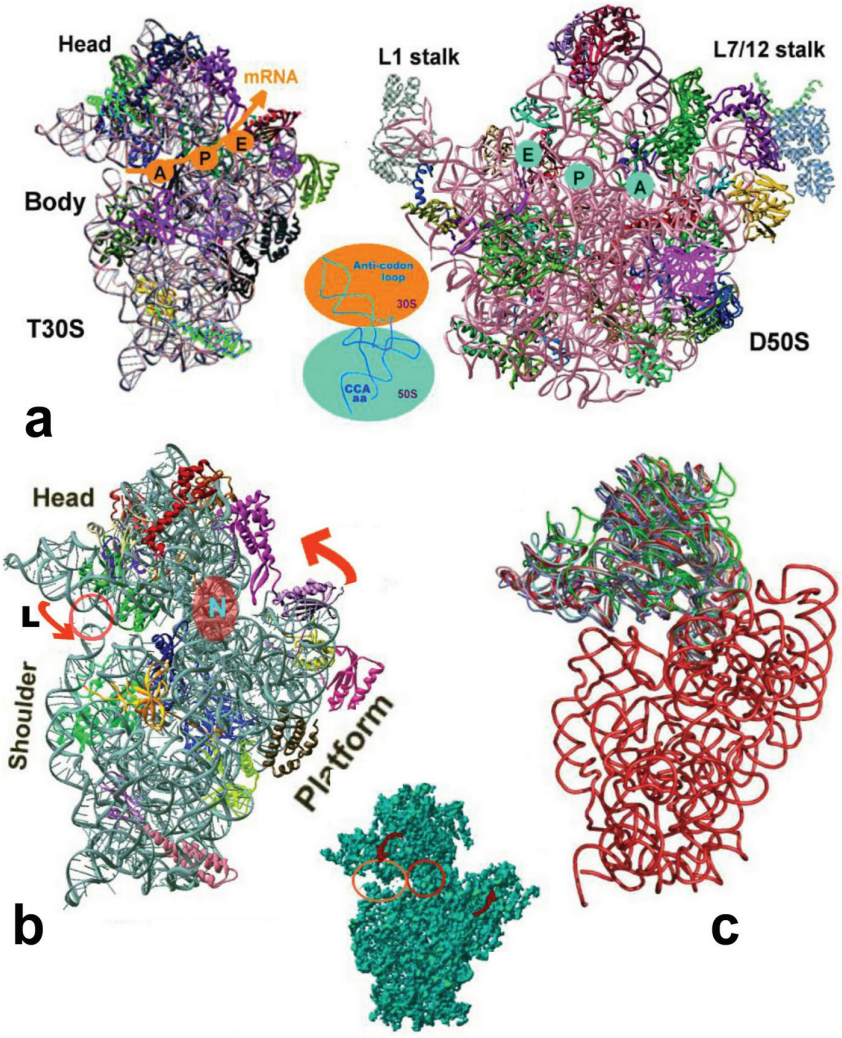

Fig. 1 *Functional mobility of the ribosomal subunits*

(a) *The interface surfaces of the high resolution structures of the small (left) and the large (right) ribosomal subunits from Thermus thermophilus [Schluenzen et al., 2000] and Deinococcus radiodurans [Harms et al., 2001], respectively, with their main structural regions. The approximate mRNA channel on the small subunit and the positions of the three tRNA sites on both subunits are shown, with a representative tRNA molecule placed between the two subunits, showing the regions interacting with each of the subunits.*

(b) *The small subunit with its functionally relevant motions, the latch (L), the neck (N) that seems to act as the structural element facilitating the head motions. The pink circle indicates the position that closes and opens during the latch motion, thus creating a pore for the incoming mRNA.*

(c) The ensemble of conformations of rRNA backbone of the small subunit. The body, shown in red, is almost identical in all known crystal structures, whereas various "head" folds have been detected and are shown in different colors.

Insert: the 7Å resolution structure of the small subunit. Possible motions are indicated by arrows. The left red circle indicates the position of the latch and the right circle is positioned on the neck.

A-tRNA is translocated to the P-site, a new aminoacylated tRNA enters the A-site, and the deacylated tRNA moves from the P-site to the exit (E)-site on its way out from the ribosome.

The ribosome is a dynamic molecular machine, utilizing structural rearrangements as integral components of the translation machinery. Various motions were detected by interpolations between crystal structures representing various functional states, the structure of unbound large subunit [Harms et al 2001] and that of the entire ribosome in complex with 3 tRNAs, or by investigating the reasons for disorder in functionally relevant regions in crystals grown under far from physiological conditions (e.g. those of the large ribosomal subunits from the Dead Sea bacterium, determined at Yale). Among the so far identified motions are the movements of the two large subunit mobile stalks (Figure 1a) for the entrance and release of the A- and E-tRNAs; the small subunit coordinated head-shoulder and head-platform motions (Figure 1b,c) upon mRNA entrance to its groove on the small subunit, resembling a latch like closing/opening mechanism.

The ribosome is a polymerase

The PTC is positioned within a universal pseudo 2-fold symmetrical region (Figure 2a,b), which relates the rRNA fold and nucleotide orientations highly conserved ~180 nucleotides, but not nucleotides sequences, and is called the symmetrical region. The central location of the symmetrical region and its link to all ribosomal features involved in amino acid polymerization (Figure 2c) indicates that it can serve as the element signaling between remote ribosomal locations (up to 200Å away from each other) and thus can coordinate translation processes.

The high conservation of the symmetrical region, the linkage between the elaborate PTC architecture and the position of an A-site tRNA mimic observed crystallographically [Bashan et al., 2003] indicates that the translocation of the A-site tRNA to the P-site is performed by a combination of two independent, albeit synchronized motions: a sideways shift, performed by the overall mRNA/tRNA translocation, and a rotatory motion of the A-tRNA 3'end. This motion progresses along a path confined by the PTC walls (Figure 2d), of which all nucleotides were shown to be essential by a comprehensive genetic selection analysis [Sato et al., 2006]. Hence, the rotatory motion is navigated and guided by the ribosomal architecture, which provides all of the structural elements for ribosome function as an amino acid polymerase and for directing the nascent protein into the exit tunnel [Agmon et al., 2004]. Remarkably, the position of the 3'end of P-site, derived by the rotatory motion based on the mode of binding of a tRNA mimic to unbound large ribosomal subunit [Bashan et al., 2003], overlaps the positions of full-size tRNAs bound to the entire ribosome (Figure 2e). Hence, the ribosome provides positional, rather than functional catalysis, which also allows for rate enhancement by its substrate, the t-site tRNA.

The preservation of the three-dimensional structure of the two halves of the ribosomal frame regardless of the sequence demonstrates the rigorous requirements of accurate substrate positioning in stereochemistry supporting peptide bond formation. The conservation of the symmetrical region is consistent with its vital functions in intra-ribosomal signaling,

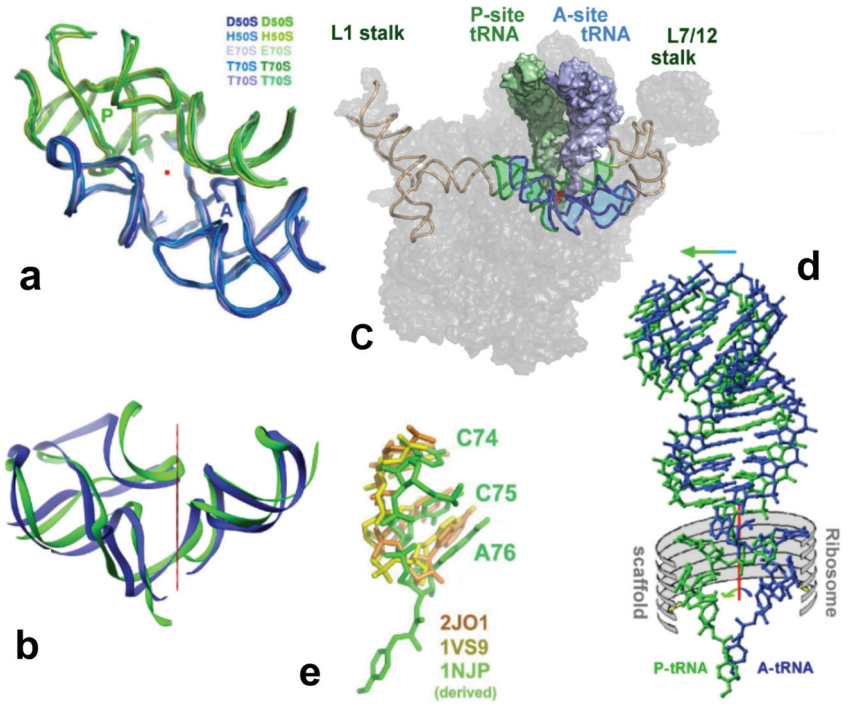

Fig. 2 *The symmetrical region and peptide bond formation. In all, the A- and P- sub-regions are shown in blue and green, respectively. The imaginary symmetry axis is shown in red.* ***(a)*** *Superposition of fold of the 180 nucleotides comprising the symmetry region in all known structures, shown as ribbons. The center of the PTC lies roughly on this axis.* ***(b)*** *Superposition of the backbones of the rRNA comprising the A- and P- sub-regions of the symmetrical region, as obtained by a 180 degrees rotation around the imaginary symmetrical axis, indicating the level of the ribosomal internal symmetry.* ***(c)*** *the central location of the symmetrical region(in blue and green) within the large ribosomal subunit. The extensions of the symmetrical region are shown in gold.* ***(d)*** *The two components of the tRNA translocation motion: the sideways shiftin the direction of the horizontal arrow and the rotatory motion of the A-tRNA 3'end along a path confined by the PTC grey walls, shown as ribs.* ***(e)*** *Superposition of the derived P-site CCA by the rotatory motion on the crystallographically determined locations of the P-site CCA in crystals of entire ribosome complexes. The PDB accession codes are indicated.*

peptide bond formation and aminoacid polymerization, led to the assumption that the ancient ribosome was made of a pocket confined by RNA chains and that the ribosome evolved by gene fusion or duplication [Agmon et al, 2006].

The voyage of the nascent protein

Nascent polypeptides progress through their exit tunnel (Figure 3a), a universal feature of the large ribosomal subunit that lies adjacent to the PTC and lined primarily by rRNA with a few r-proteins (Figure 3b). This tunnel (~120 Å in length and varying diameter, 10-25Å) possesses dynamics required for gating and discriminating, i.e. elongation arrest due to cellular unfavorable conditions [Berisio et al., 2003] and plays an active role in sequence-specific arrest of nascent chains. Nascent proteins emerge from their protective exit tunnel into the crowded cellular environment before gaining sufficient length to acquire the final fold. Molecular chaperones support correct folding within the crowded cells. In eubacteria, at the tunnel opening, the first chaperone encountered by the emerging nascent chain, called trigger factor (TF), binds to the translating ribosome at ~1:1 stoichiometry by interacting with ribosomal proteins L23 and L29. Protein L23 belongs to the small group of ribosomal proteins that display a significant evolutionary divergence from conservation, and although its globular domain is rather conserved only in eubacteria does it possess a sizable elongated loop, which extends from ribosome exterior all the way into the tunnel walls. At this position the L23 extended loop can undergo allosteric conformational changes that, in turn, can modulate the shape of the tunnel, implying trafficking of the nascent protein [Baram et al., 2005].

Comparison of the structures of unbound TF to that seen in the complex of its binding domain with the eubacterial large ribosomal subunit, shows that TF binding domain (TFa) undergoes conformational rearrangements that expose a sizable hydrophobic region (Figure 3c) thus acquiring a configuration suitable to adhere to hydrophobic patches on the nascent chain (Figure 3d) [Baram et al., 2005]. Consistent with dynamic studies, it appears that TFa prevents the aggregation of the emerging nascent chain by providing a hydrophobic surface that can transiently mask exposed hydrophobic regions of the elongating polypeptide chains until they become buried in the interior of the mature protein.

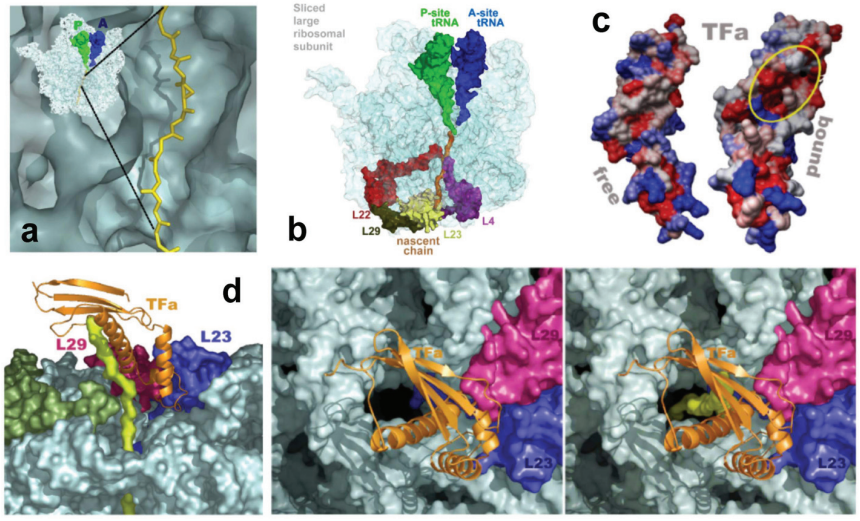

Fig. 3 *The nascent protein exit tunnel and chaperoning the emerging proteins.* ***(a)*** *The position, curvature and varying diameter of the protein exit tunnel within the large ribosomal subunit, are indicated by a modeled polyalanine (yellow).* ***(b)*** *Proteins reaching the tunnel's walls from the large subunit exterior. The tunnel interior is marked by a modeled nascent chain (in orange). The large subunit is shown in blue-grey.* ***(c)*** *Conformational differences between free and ribosome bound TFa. The yellow ellipse delineates the sizable hydrophobic region that becomes exposed upon its binding to the ribosome.* ***(d)*** *Space filling representation of ribosomal RNA (in grey) and r-proteins (in blue, dark red and dark green) at the tunnel opening. TFa is shown as gold ribbons, and a modeled nascent chain as yellow ribbons. Left: the emerging protein (modeled polyalanine) enters the shelter provided by TFa. The proteins associated with the trigger factor, L23 and L29, are shown. Note L23 extension reaching the tunnel wall. Middle and Right: a perpendicular view of the tunnel opening. Middle: empty tunnel. Right: A modeled polyalanine chain is emerging from the tunnel. Note that in this crystal structure the tunnel was empty.*

Antibiotics targeting ribosomes

Despite ribosome conservation many of the antibiotics targeting ribosome are clinically relevant. As so far ribosome from pathogens could not crystallize, structural information can so far being extracted only from the crystallizable eubacterial ribosome [Schluenzen et al., 2001]. These were found suitable to represent pathogens and yielded unmatched relevant clinical information, and alongside rationalizing many genetic, biochemical and medical observations, revealed expected and unexpected inhibitory modes, and showed exploitation of divergence strategies with common denominators. All bind to functionally relevant regions, and each prevents a crucial step in the biosynthetic cycle: causing miscoding; minimizing essential functional mobility; inhibiting translation initiation; interfering with tRNA substrate binding at the decoding center; preventing the interactions of the ribosomal recycling factor; blocking the protein exit tunnel (Figure 4a) and hindering tRNA substrate accommodations at the PTC (Figure 4b).

Elucidation of the various mode of action of antibiotics targeting ribosomes and a careful analysis of the ribosomal components comprising the binding pockets confirms that the imperative distinction between eubacterial pathogens and mammalian ribosomes hinges on subtle structural difference within the antibiotics binding

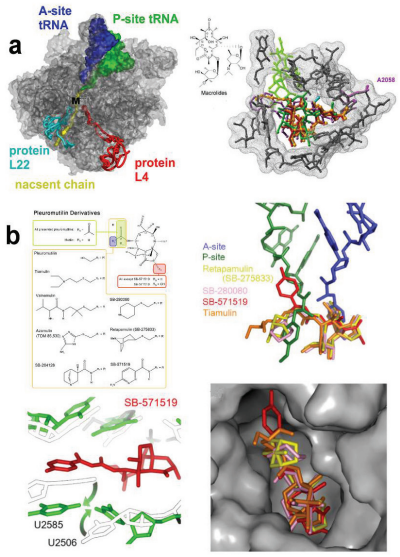

Fig. 4. *Ribosomal antibiotics action.* ***(a)*** *Left: The location of the macrolide binding pocket within the ribosomal tunnel. Right: Superposition of the binding modes of a few macrolides within a section of the ribosomal tunnel (in grey). Nucleotide 2058 is shown in pink.* ***(b)*** *Clockwise: various pleuromutilins; the modes of their binding at the active site (A- and P-site tRNAs are included for orientation); the shape of the binding pocket; the motions (shown by arrows) exploited for the induced fit mechanism and the remote interactions. The original positions of the flexible nucleotides are shown as white, and the new positions in green.*

pockets [Yonath, 2005]. For example, adenine in position 2058 was found crucial for macrolide binding (Figure 4a). Consistently, macrolide resistance is acquired by pretranslational A->G mutation or post translational methylation, both at the critical position 2058, as well as insertion and/or deletion of components into the tunnel walls (mainly proteins L22 and L4).

Such mutated ribosome is currently being analyzed crystallographically, and initial evidence for modification of the tunnels diameter and shape have been obtained. Importantly, factors discriminating pathogens vs. eukaryotes, which are of crucial clinical importance, were identified by comparisons between antibiotics binding sites in ribosomes from eubacteria and those from an archaeon that shares properties with eukaryotes. Specifically, these comparisons indicated that a single nucleotide identity determines solely the antibiotics' binding, whereas proximal stereochemistry governs the antibiotics orientation within the binding pocket [Yonath, 2005] and consequently it's therapeutical effectiveness, thus highlighted the distinction between binding and inhibitory activity, in accord with recent mutagenesis studies showing that mutation from guanine to adenine in 25S rRNA at the position equivalent to E. coli A2058 does not confer erythromycin sensitivity in Sacchromyces cerevisae.

Among the ribosomal antibiotics, the pleuromutilins (Figure 4b) are of special interest since they bind to the almost fully conserved PTC, yet they discriminate between eubacterial and mammalian ribosomes. To circumvent the high conservation of the PTC the pleuromutilins exploit the PTC inherent functional mobility and trigger a novel induced-fit mechanism that involves a network of remote interactions between flexible PTC nucleotides and less conserved nucleotides residing in the PTC-vicinity. These interactions reshape the PTC contour and trigger its closure on the bound drug [Davidovich et al., 2007]. The uniqueness of pleuromutilins mode of binding led to new insights into ribosomal functional flexibility, as it indicated the existence of an allosteric network around the ribosomal active site. Furthermore, the value of these findings is far beyond their perspective clinical usage, as they highlight basic issues, such as the possibility of remote reshaping of binding pockets and the ability of ribosome inhibitors to benefit from the ribosome functional flexibility.

The elucidation of common principles of the mode of action of antibiotics targeting ribosomal, combined with variability in binding modes, the revelation of diverse mechanisms acquiring antibiotic resistance, and the discovery that remote interactions can govern induced fit mechanisms enabling species discrimination even within highly conserved regions, justify expectations for structural based improved properties of existing antibiotics as well as for the development of novel drugs. Furthermore, efforts at designing additional compounds that can be used as eubacterial ribosome inhibitors, based on attachment of antisense DNA oligomers to exposed, albeit functionally relevant RNA regions, are in progress.

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