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The Spectacular Ribosomal Architecture: Linking Positional Catalysis To Antibiotics Synergism

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Ribosomes, the universal cellular organelles catalyzing the translation of genetic code into proteins, are giant asymmetric riboprotein assemblies with a striking architecture and inherent mobility, enabling their function. Built of two subunits of unequal size that associate upon the initiation of protein biosynthesis, the ribosome contains the tRNA binding sites: A- of the aminoacylated tRNA, P- of the peptidyl-tRNA, and E- for the exiting free tRNA molecule.

Crystal structures of functional ribosomal complexes of the small ribosomal subunit from *Thermus thermophilus* (Schluenzen et al., 2000) and the large ribosomal subunit from *Deinococcus radiodurans*, D50S, (Harms et al., 2001) revealed that the ribosome is a ribozyme, and that it provides positional, rather than chemical, catalysis. It also showed that precise placement of the tRNA substrates in the peptidyl transferase center (PTC), is crucial for amino acid polymerization, and that this precise placement is dominated by remote interactions (Fig. 1a) (Bashan et al., 2003).

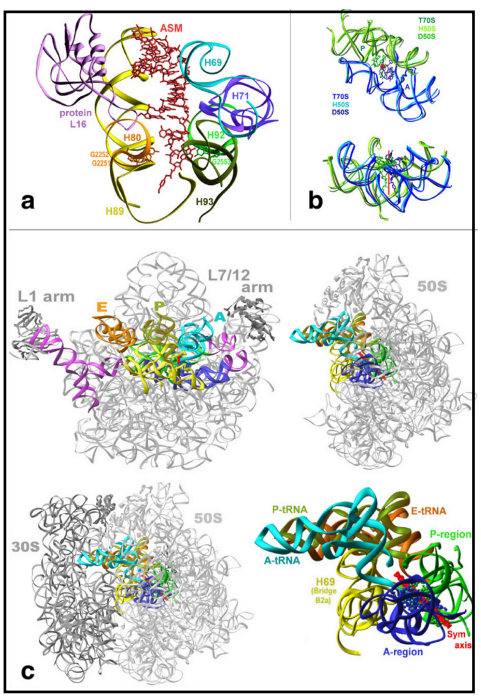

Fig. 1: (a) *The ASM in D50S PTC, showing the remote interactions at its rim, and highlighting the nucleotides basepairing with the tRNAs.* **(b)** *Two perpendicular views of the ribosomal RNA backbone of the symmetry related region, as seen in all known structures. The symmetry axis is red. The bottom is view shows also the ASM 3'end (at the A-site, blue) and the derived P-site 3'end (green).* **(c)** *The symmetry related region within the large ribosomal subunit (rRNA in gray) Its connections with the tRNA entrance and exit regions, and with the small subunit decoding site, are colored (purple). Also shown are ASM, derived P-site tRNA3'end, and three docked tRNAs (Bashan et al., 2003).* **Top: front (left)** *and* **side (right)** *views of D50S. Bottom left includes the small subunit (dark gray). Bottom right: zoom of the colored features in all other views, including the symmetry related region, its axis, ASM, the docked tRNAs and Helix H69 that bridges to the small subunit decoding center. Note the strategic location of H69, consistent with its contribution to A-site tRNA accurate placement, and its function in translocation (Agmon et al., 2003).*

A sizable symmetry-related region was revealed in all known ribosomal structures (Fig. 1b). This region connects all ribosomal functional centers involved in amino-acid polymerization (Agmon et al., 2003), including the PTC in the large subunit and the decoding center in the small one (Fig. 1c). The linkage between this unique architectural design and substrate positioning suggested that translocation occurs by a rotatory motion of the A-site tRNA 3'end, and that this motion is synchronized with the overall mRNA/tRNA shift (Fig. 2a). Facilitated by the mobility of specific nucleotides A2602 and U2585,

and guided by ribosomal components of the PTC rear wall (Fig. 2b), this rotatory motion results in stereochemistry suitable for peptide bond formation and for directing the entrance of the nascent proteins into the ribosomal exit tunnel (Bashan et al., 2003).

Visualized by us almost two decades ago and remaining controversial for almost a decade, this tunnel was found to be a major target for antibiotics from the macrolide-ketolide family (Fig. 2b). Analysis of their binding modes shed light on their selectivity and illuminated resistance mechanisms (Schluenzen et al., 2001). Moreover, synergism induced by an antibiotics mixture, called Synercid©, could be interrelated with the exceptional flexibility nucleotide U2585 anchoring the rotatory motion (Harms et al., 2004).

Finally, although this tunnel was assumed to be a passive path for the growing polypeptides, it does posses dynamic properties. Thus, by correlating tunnel gating, induced troleandomycin, with mutations bypassing elongations arrest, we identified the tunnel dynamics properties allowing alterations in its wall structure, thus providing the structural basis for sequence discrimination and for ribosomal participation in cellular regulation (Berisio et al., 2003) (Fig. 2).

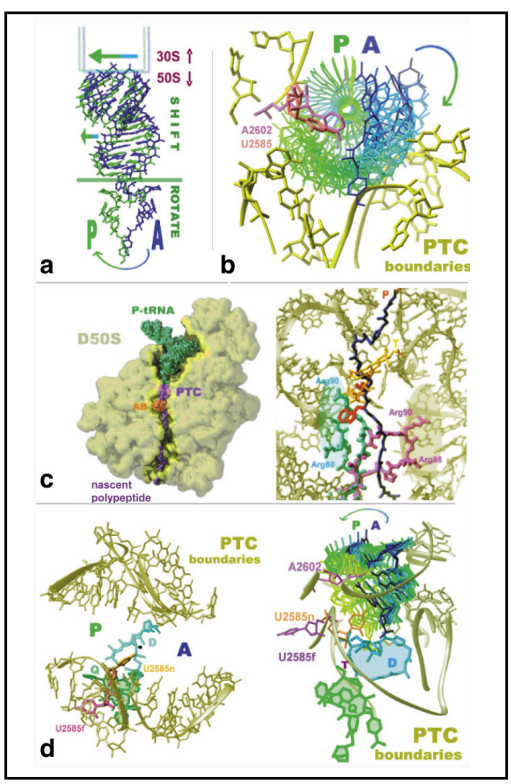

Fig. 2: ***(a)*** *Translocating tRNA from A- (blue) to P- (green) site, based on ASM-D50S structure (Bashan et al., 2003). The dark green line is the division between the shifted double helical region (above) and the rotating 3'end (below). The tRNA regions interacting with the small subunit are represented by blue-green boxes. Straight arrows show the shifting direction. The round one represents the rotatory motion.* ***(b)*** *View form the tunnel into the PTC, showing its boundaries scaffolding the path of the rotatory motion, and the mobile bases anchoring it (A2602, U2585).* ***(c) Left:*** *A section through D50S (in yellow-gray) at the height of the ribosomal tunnel, with docked P-site tRNA and modeled nascent chain. The PTC approximate position and the antibiotics targeted site are marked (violet and orange, respectively).* ***Right: A*** *view parallel to the tunnel long axis (rRNA in olive green) with a modeled polyalanine (blue). Two key residues for nascent protein arrest, Pro and Trp separated by 12-12 residues, are highlighted in red. The tip of the ribosomal protein L22 beta-hairpin at its native and swung conformations, the latter induced by TAO (yellow) binding, are shown in cyan and magenta, respectively. The shaded areas correspond to regions of mutations bypassing elongation arrest (Berisio et al., 2003).* ***(d)*** *The structural basis for Synercid© synergetic effect, shown in two orthogonal views with both Synercid© components: Dalfopristin, D, and Quinupristin, Q (cyan and light-green, respectively).* ***Left:*** *views of the PTC down its two fold axis. Right: view approximately parallel to the symmetry axis, showing also snapshots of the A- to P-site rotation of the alanilayted tRNA3'end, progressing from blue to green (each step =15 degrees rotation).*

Selected Publications

Agmon et al., (2003) *Eur J Biochem*, 270, 2543-56.
Bashan et al., (2003) *Mol Cell*, 11, 91-102.
Berisio et al., (2003) *Nat Struct Biol*, 10, 366-70.
Harms et al., (2001) *Cell*, 107, 679-88.
Harms et al., (2004) *BMC Biology*, 2, online 1st April.
Schluenzen et al., (2000) *Cell*, 102, 615-23.
Schluenzen et al., (2001) *Nature*, 413, 814-21.

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