Ada Yonath

Agmon Ilana
Auerbach Tamar
Bashan Anat
Kessler Maggie
Zarivach Raz

Antibiotics targeting ribosomes

Department of Structural Biology

Tel. 972 8 934 3028 / 2541 **Fax.** 972 8 934 4154 / 4136
E-mail: ada.yonath@weizmann.ac.il yonath@mpgars.desy.de

Analysis of high resolution structures of complexes of antibiotics with ribosomal particles shed light on antibiotic selectivity and illuminated various modes of action, from reducing of decoding accuracy, via limiting conformational mobility, to interference with substrate binding and hindrance of the progression of growing proteins. Their interactions and the lack of major conformational rearrangements associated with antibiotics binding, support the suggestion that the ribosome provides an accurate frame, rather than enzymatic activity, for peptide bond formation.

Resistance to antibiotics is a major problem in modern therapeutics. Ribosomes of pathogenic bacteria are major targets for antibiotics. Ribosomes are cellular organelle catalyzing the translation of genetic code into proteins. They are protein/RNA assemblies arranged in two subunits that associate for performing protein biosynthesis. The large subunit (1.5 mDa, 3000 nucleotides in two RNA chains and ~35 proteins) creates the peptide bonds and provides the path for emerging nascent proteins. The smaller subunit (0.85 mDa, 1500 nucleotides in one RNA chain and ~20 proteins) has key roles in controlling the fidelity of codon-anti-codon base-pairing and in initiating the biosynthetic process.

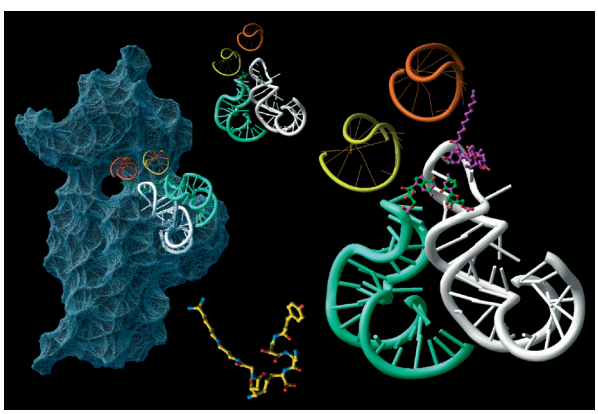

Fig. 1 *Left: The small ribosomal subunit. The mRNA path and the P-(orange) and E-(yellow) sites are shown. The RNA features that are "frozen" by edeine are highlighted in white and cyan. (in the assembled ribosome the large subunit will face the left side of the particle). Middle: top: the free edeine binding site. Bottom: the structure of edeine. Right: Detailed view of edeine (purple) binding site. Note the newly formed base pair (green).*

The high resolution structures of ribosomal subunits from two pathogen-models [1,2], obtained recently by bright synchrotron radiation, were used as reference that allowed unambiguous localization of several antibiotics. Among them six clinically relevant and one of no clinical use are reported here. All were found to bind primarily to ribosomal RNA and their binding did not cause major conformational changes.

Small subunit antibiotics: Tetracycline was found to be a multi-site antibiotic with inhibitory action that stems from its interference with A-site tRNA binding. Edeine, a universal agent, inhibits the initiation of protein synthesis by linking critical features for tRNA, IF3 and mRNA binding, thus imposing constraints on ribosomal mobility that accompany the translation process (Fig. 1). Its universality implies conservation of structural elements important for initiation [3].

Large subunit antibiotics: Chloramphenicol targets the peptidyl transferase cavity close to the amino acceptor group of tRNA. Clindamycin interferes with substrate binding and physically hinders the path of the growing peptide chain. The macrolides: erythromycin, clarithromycin and roxithromycin bind to the entrance of the protein exit tunnel and block the progression of nascent proteins (Fig. 2a). Interestingly, neither of these antibiotics binds to the nucleotides assigned to be crucial for the catalytic mechanism of the ribosome that was proposed based on the 2.4 Å structure of the Haloarcula marismortui large subunit.

Comparative studies illuminated elements that may confer drug selectivity (e.g. Fig. 2b). The antibiotics modes of interactions and the preservation of the active-site conformation, favor the suggestion that the peptidyl transferase center serves as a template for proper positioning of tRNAs to allow for spontaneous, rather than enzymatic, creation of peptide bonds. The ribosomal components constructing the frame for accurate positioning of the tRNA molecules may include the proteins L27 and L16 [4].

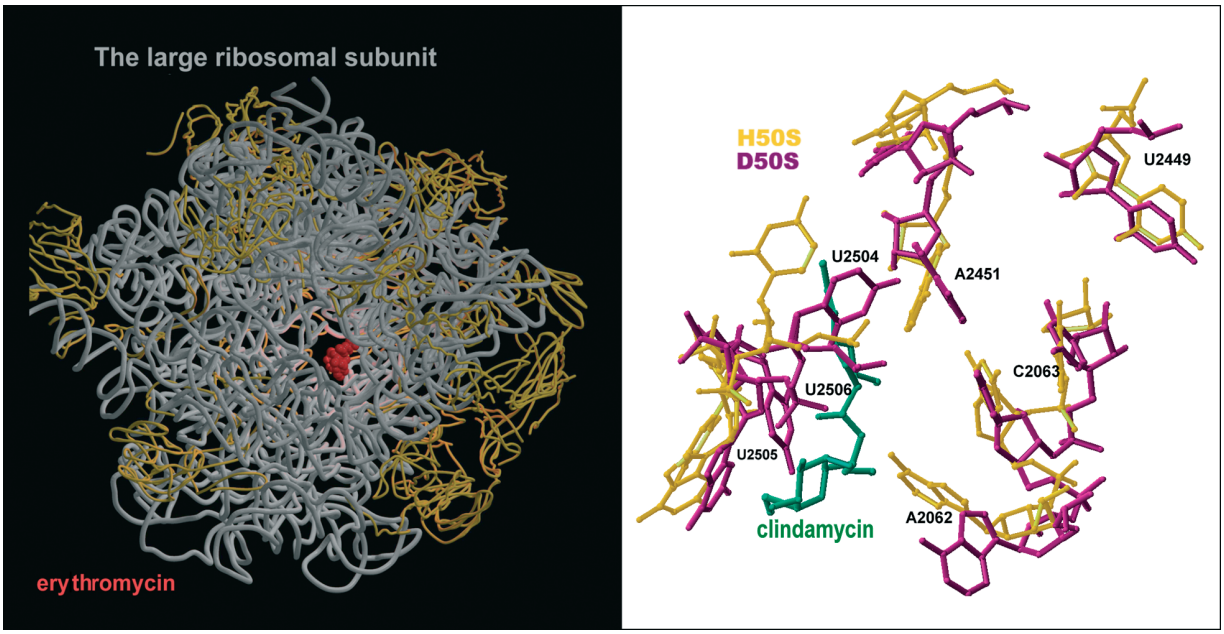

Fig. 2 *a)* ***left:*** *The position of erythromycin (red) within the large ribosomal subunit (RNA=gray, the proteins=yellow). The view is from the active site into the protein exit tunnel. b)* ***right:*** *Clindamycin binding site shown on a superposition of the backbone of the peptidyl transfer ring of a eubacterial pathogen model (D50S) and of its archeal counterpart (H50S) which serves as a model for eukaryotes (E. coli numbering scheme).*

Conclusions

Antibiotics targeting ribosomes are excellent tools for studying ribosomal function and for understanding mechanisms of drug action. Analysis of their modes of action should lead to structural based design of improved antibiotics.

Selected Publications

[1] F. Schluenzen, A. Tocilj, R. Zarivach, J. Harms, M. Glueman, D. Janell, A. Bashan, H. Bartels, I. Agmon, F. Franceschi and A. Yonath (2000) Structure of functionally activated small ribosomal subunit at 3.3 A resolution, Cell, 102, 615-623

[2] J. Harms, F. Schluenzen, R. Zarivach, A. Bashan, S. Gat, I. Agmon, H. Bartels, F. Franceschi and A. Yonath (2001) High resolution structure of the large ribosomal subunit from a mesophilic eubacterium, Cell, 107, 679-688

[3] M. Pioletti, F. Schluenzen, J. Harms, R. Zarivach, M. Glueman, H. Avila, A. Bashan, H. Bartels, T. Auerbach, C. Jacobi, Hartsch T, A. Yonath and F. Franceschi (2001) Crystal structures of complexes of the small ribosomal subunit with tetracycline, edeine and IF3, EMBO J, 20(8), 1829-1839

[4] F. Schluenzen, R. Zarivach, J. Harms, A. Bashan, A. Tocilj, R. Albrecht, A. Yonath and F. Franceschi (2001) Structural basis for the interaction of chloramphenicol, clindamycin, and macrolides with the peptidyl transferase center in eubacteria, Nature, 413, 814-821

Acknowledgments

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Prof. Ada Yonath is the incumbent Martin S. Kimmel professor of Structural Biology, Structural Biology Department, Weizmann Institute of Science, Israel and Max-Planck Research Unit for Ribosomal Structure, Hamburg, Germany.