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Chen Davidovich

Targeting Functional Centers of the Ribosome

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Targeting Functional Centers of the Ribosome

Doctoral Thesis accepted by
Weizmann Institute of Science (WIS), Rehovot, Israel

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Author
Dr. Chen Davidovich
Department of Structural Biology
Weizmann Insitute of Science (WIS)
Herzl Street
76100 Rehovot
Israel
e-mail: chend@weizmann.ac.il

Supervisor
Prof. Ada Yonath
Department of Structural Biology
Weizmann Insitute of Science (WIS)
Herzl Street
76100 Rehovot
Israel
e-mail: ada.yonath@weizmann.ac.il

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C. Davidovich, A. Bashan, A. Yonath, "Structural basis for cross-resistance to ribosomal PTC antibiotics", *Proc Natl Acad Sci U S A*, (2008) 105 (52), pp. 20665–70.

I. Wekselman, C. Davidovich,[1] I. Agmon, E. Zimmerman, H. Rosenberg, A. Bashan, R. Berisio and A. Yonath, "Ribosome's mode of function: myths, facts and recent results", *J Pept Sci*, (2008) 15 (3), pp. 122–30.

C. Davidovich, A. Bashan, T. Auerbach-Nevo, R. D. Yaggie, R. R. Gontarek, A. Yonath, "Induced-fit tightens pleuromutilins binding to ribosomes and remote interactions enable their selectivity", *Proc Natl Acad Sci U S A*, (2007) 104 (11), pp. 4291–96.

[1] Wekselman and Davidovich contributed equally.

Supervisor's Foreword

Ribosomes, the key players in the translation process, are universal ribozymes performing two main tasks: decoding genetic information and polymerizing amino acids. Hundreds of thousands of ribosomes operate in each living cell due to the constant degradation of proteins through programmed cell death, which is matched by simultaneous production of proteins. For example, quickly replicating cells, e.g. liver cells, may contain a few million ribosomes. Even bacterial cells may contain to 100,000 ribosomes during their log period. Other constituents are the mRNA chains, produced by the transcription of the segments of the DNA that should be translated, which carry the genetic information to the ribosomes, and tRNA molecules bring the cognate amino acids to the ribosome. To increase efficiency, a large number of ribosomes act simultaneously as polymerases, synthesizing proteins by one-at-a-time addition of amino acids to a growing peptide chain, while translocating along the mRNA template, and producing proteins on a continuous basis at an incredible speed, namely up to 20 peptide bonds per second.

Ribosomes are giant assemblies composed of many different proteins (r-proteins) and long ribosomal RNA (rRNA) chains. Among these, the RNA moieties perform the two ribosomal main functions. The ratio of rRNA to r-proteins ($\sim$2:1) is maintained throughout evolution, except in mitochondrial ribosome (mitoribosome) in which $\sim$ half of the bacterial rRNA is replaced by r-proteins. Nevertheless, the active regions are almost fully conserved in all species. In all organisms ribosomes are built of two subunits, which associate to form the functionally active ribosomes. In prokaryotes, the small subunit, denoted as 30S, contains an RNA chain (16S) of $\sim$1500 nucleotides and $\sim$20 different proteins. The large subunit (50S in prokaryotes) has two RNA chains (23S and 5S RNA) of about 3000 nucleotides in total, and different >31 proteins. The available three dimensional structures of the bacterial ribosome and their subunits show that in each of the two subunits the ribosomal proteins are entangled within the complex rRNA conformation, thus maintaining a striking dynamic architecture that is ingeniously designed for their functions: precise decoding; substrate mediated peptide-bond formation and efficient polymerase activity.

While the elongation of the nascent chain proceeds, the two subunits perform cooperatively while the tRNA molecules are the non-ribosomal entities combine the two subunits. The small subunit provides the path along which the mRNA progresses, the decoding center and the mechanism controlling translation fidelity. Translation initiation is the rate-limiting step of the entire process. It starts by the correct selection and placement of the mRNA reading frame, and proceeds through a tightly regulated decoding at the P-site. The large subunit contains the site for the main ribosomal catalytic function, namely polymerization of the amino acids and provides the dynamic protein exit tunnel. Simultaneously with the advancement of the mRNA along the path in the small subunit, peptide bonds are being formed. This inherently dynamic process requires small and large-scale motions of the ribosomal substrates coupled to conformational rearrangements of its components and substrates. The nascent proteins progress along a dynamic tunnel and emerge from the large subunit into a shelter formed by ribosome-bound trigger-factor, acting as a chaperone preventing aggregation and misfolding. The current consensus view is consistent with ribosomal positional catalysis, namely providing suitable stereochemistry for peptide bond formation accompanied by appropriate geometrical means for substrate mediated catalysis, and not by acid/base mechanism.

Chen Davidovich joined our group after the molecular structures of the two bacterial ribosomal subunits had been determined and their functional regions identified and localized. In his PhD thesis Chen aimed to reveal the structural basis for the catalytic function of the ribosome as a polymerase, namely as the cellular machine that forms peptide bonds successively, thus elongating the nascent chains. When he started, this approach was rather neglected since most ribosomologists assumed that understanding the formation of a single peptide bond was sufficient to describe the ribosomal function, and hence disregarded the processivity and elongation.

Chen investigated in detail the mode of function, inhibition, and evolution of the Peptidyl Transferase Center (PTC) by a combination of X-ray crystallography, biochemistry, molecular biology and theoretical studies. Among his major achievements was the determination of the crystal structures of complexes of ribosomal particles with antibiotics that target the PTC. These are clinically useful, despite the extremely high conservation of this region in all forms of life. Chen revealed the modes of action of these antibiotics, thus shedding light on the discrimination principles between ribosomes of eubacterial pathogens and the eukaryotic hosts that are based on remote interaction networks and induced fit. In parallel, he investigated the mechanisms acquiring resistance to antibiotics. Furthermore, by thorough comparative structural and genetic analyses, he determined the structural basis for crossresistance between all of the clinically useful PTC-binding antibiotics.

Chen also attempted to elucidate the origin of the ribosome. He was focused on the ribosomal substructure that may represent the minimal entity capable of performing peptide bond formation, namely the proto-ribosomes. Remarkably, despite the ribosome asymmetric structure, in all of the structures determined so

far the PTC and its environs are situated within a highly conserved region of internal structural symmetry that connects all ribosomal functional centers involved in amino-acid polymerization, hence can serve as the central signaling feature. The high level of conservation of the symmetrical region suggests that the modern ribosome evolved from a simpler entity that can be described as a proto-ribosome that was formed by gene fusion or gene duplication and contained a pocket confined by two self folded RNA chains, which associated to form a pocket like dimer with functional capabilities. As RNAs can act as gene-like molecules coding for their own reproduction, it is conceivable that the surviving pockets became the templates for the ancient ribosomes. In a later stage these RNA genes underwent initial optimization to produce a more defined rather stable pocket, in which each of the two halves was further optimized for its task in distinction between the amino acid and the growing peptidyl sites, so that their sequences evolved differently. Indeed, the preservation of the three-dimensional structure of the two halves of the ribosomal frame regardless of their sequence demonstrates the superiority of rigorous functional requirements over sequence preservations.

Two symmetry-related RNA entities seem to have not only the capability to self fold, but can also undergo self dimerization. Based on the assumption that dimerization is the minimal requirement for formation of catalytic active pockets, Chen designed and synthesized RNA chains corresponding to the sequence of this pocket in the contemporary ribosome and determined their tendency to dimerize. He found, surprisingly, a marked preference of specific sequences to dimerize, whereas other, very similar RNA chains did not. This sequence preference for self dimerization, indicate that the principles of 'survival of the fittest' may have played a major role on the molecular level, although these terms are usually used for species, hence suggesting pre Darwinian Darwinisim.

Chen's thesis has already led to attempts at improving the PTC antibiotics, performed locally as well as by companies. Additionally, a significant effort is being made by two graduate students and a postdoctoral fellow, to try to design a functional construct mimicking a functional proto-ribosome.

December 2010 Ada Yonath