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Ribosomal Protein Structures and Sequences Define the Prokaryotic Tree of Life. William. L. Duax, Robert Huether, David Dziak. *Structural Biology, Hauptman-Woodward Medical Research Institute, USA.* E-mail: duax@hwi.buffalo.edu

Search vectors composed primary of Gly, Ala, Arg, and Pro residues (GARP) distributed across the entire protein sequence retrieve 98% of each of the ribosomal proteins in prokaryotic species with no false ³hits². Different combinations of G, A, R and P and insertions or deletions differentiate each ribosomal protein from all others. Specific combinations of amino acids in two sequence positions in perfectly aligned L1 ribosomal proteins from 1600 different prokaryotic species in the gene bank separate all Gram positive from Gram negative bacteria. We are able to identify site mutations that subdivide each ribosomal protein ensemble into the individual phylum of bacteria. Further subdivision into orders, families, genus, and species is trivial. For example, specific residues in three positions in the alignment of prokaryotic L1 ribosomal proteins isolate 44 L1 proteins from cyanobacteria and 17 L1 proteins from chloroplasts unequivocally supporting the postulated evolution of the latter from the former. While there are significant differences between the sequences of the ribosomal proteins in different classes and orders of prokaryotes, within each order the amino acid sequences have remained highly conserved since divergence and speciation. We have found that the total GARP content of the ribosomal proteins of each class and order is a marker of the order of evolution and that the last universal common ancestor (LUCA) appears to have been an Actinobacteria. Perfect alignment of thousands of members of a protein family is essential to determining the molecular level details of its evolution, the evolution of protein fold and function and the evolution of bacterial species. Three dimensional structural information played an essential role in developing a new GARP based technique to achieve perfect sequence alignment. In retrospect it is possible to understand why GARP residues are 100% conserved in specific positions in families of proteins present in all species.

Keywords: ribosome, evolution, bioinformatic

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Investigation of the protein synthesis machinery at different levels of organization through integrative cryo-EM. Alexander G. Myasnikov[a], Zhanna A. Afonina[c], Angelita Simonetti[a], Stefano Marzi[b] & Bruno P. Klaholz[a] , [a] *IGBMC (Institute of Genetics and of Molecular and Cellular Biology), Department of Structural Biology and Genomics, Illkirch, F-67404 France.* [b] *Architecture et Réactivité de l'ARN, UPR 9002 CNRS, IBMC (Institute of Molecular and Cellular Biology), 15 rue R. Descartes, 67084 Strasbourg, France.* [c]*Institute of Protein Research, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia*

Studying the structure and function of the ribosome increasingly requires integrating complementary tools which together provide detailed insights into the mechanism of action of this dynamic macromolecular complex. We are interested in the function of the molecular machinery that the ribosome is when it catalyzes protein synthesis.

Protein synthesis can be divided in 4 steps – initiation, elongation, termination and recycling. The most complex and most regulated step is initiation. In prokaryotes it can be regulated by structured mRNA through the formation of the so-called "pre-initiation" complex. In order to investigate such a complex we have used a comprehensive approach with a combination of different approaches: biochemistry, modeling, sequence analysis and cryo-EM [1]. We did a series of cryo-electron microscopy snapshots of ribosomal complexes directly visualizing either the mRNA structure blocked by repressor protein S15 or the unfolded, active mRNA. In the stalled state, the folded mRNA prevents the start codon from reaching the peptidyl-tRNA (P) site inside the ribosome. We have also undertaken the cryo-EM analysis of the translation initation complex of the small ribosomal subunit with initiatior tRNA and initiation factors IF1 and IF2 that has revealed a key cooperativity between IF2 and the tRNA in the stabilisation of the initiator tRNA on the 30S subunit [2]. Large conformational changes have been observed during the transition from the 30S to the assembled 70S ribosome [2,3,4,5].

During protein synthesis several ribosomes may bind to a messenger RNA (mRNA) molecule and thereby parallelize protein synthesis by the formation of a large macromolecular assembly – polyribosome (polysome). Using cryo electron tomography (CET) we reveal the organization of ribosome in eukaryotic polysomes [6]. We found out that within a row the ribosomes point into the same direction in accordance with the path and polarity of the mRNA chain being decoded. Inside double-rows, the ribosomes are oriented in an anti-parallel manner, consistent with the idea that in eukaryotic polysomes the 5'- and 3'-ends of the mRNA are in close proximity.

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Drug-stabilized and drug-free complexes of topo IV from *S. pneumoniae* shed light on the mechanisms of reversible DNA scission and selective drug resistance. Ivan Laponogov [a,b], Xiao-Su Pan[b], Dennis A. Veselkov[a], Katherine McAuley[c], L. Mark Fisher[b], Mark R. Sanderson[a]. [a]*Randall Division of Cell and Molecular Biophysics, King's College London, 3rd Floor New Hunt's House, Guy's Campus, University of*