ANALYSIS OF RIBOSOMAL RNA STRUCTURE MAY PROVIDE INSIGHT TO EARLY EVOLUTION

Q. Tran[1], M.C. Jacobsen[1], G. E. Fox[1],
[1]Dept Biology & Biochemistry, University of Houston, 3455, Cullen Blvd, Houston, TX, 77204-5001, USA (rdirective@gmail.com; mari3jacobsen@gmail.com; fox@uh.edu).

Introduction: The ribosome is a complex and dynamic RNA/Protein machine that synthesizes proteins according to the genetic code. Most of its components are already present at the time of the Last Universal Common Ancestor which indicates an even earlier origin. Recent works have focused on developing a timeline of major events in ribosome evolution and when possible relating them to the development of other cellular systems (1). Central to these efforts was the realization that the large ribosomal RNAs frequently grew by accretion events that largely preserved preexisting structure. Thus, by reversing these events and taking into account long range A-minor interactions, it became possible to develop a moderately detailed model that provides a relative age for each helical region in both subunits (2).

With a timeline in hand for the origin of different regions of the RNAs one can begin to associate other elements to the time lime. Thus, ribosomal proteins that primarily interacted with older regions of the RNA are likely old, whereas those that primarily interacted with newer areas are likely recent. An unexpected insight into protein evolution was that proteins that bind to the oldest regions are frequently all beta sheets whereas alpha helices showed up in the newer region (3).

Separately, an examination of the dynamic aspects of the ribosome was undertaken. As it goes through a cycle of peptide addition facilitated in part by GTPase cleavage, the ribosome structure changes in a reproducible manner. By comparing these changes, it was possible to determine sites where motions originated in the RNA. These pivot points are typically found at weak sites in the structure (4). Moreover, the pivot points can be assigned a relative age based on their position in the RNA, analogously to how proteins are classified. So in the current study we are examining the detailed structure of the RNA at various pivot points to see if there are unique features associated with the older pivots that distinguish them from those that are newer. Our analysis has begun with the 5S rRNA.

Methods: The hydrogen bonding networks of five high resolution 5S rRNAs structures from *Escherichia coli, Thermus thermophilus, Haloarcula marismortui*, *Deinococcus radiodurans,* and *Saccharomyces cerevisiae* were examined to make a global structural comparison of ribosomes in the classical state. Separately, the hydrogen bonding networks of 22 *E. coli* 5S rRNAs from ribosomes in various states were also examined. Possible hydrogen bonds were first found using the What If (5) package, then compared with results from DSSR (6) and HBPLUS (7) for consensus. Interactions were characterized using the Leontis-Westhoft nomenclature. Subsequently, an area in the same 22 *E. coli* 5S rRNA structures, which had low B-factor in all structures, encompassing residues 74-86 and 90-102, was selected for superposition using the P and C1' coordinate. Pair-wise RMSDs over the same atoms in residues 3-43, 45-86, and 90-117 were then computed and the results were clustered using the Phylip (8) average-neighborhood joining algorithm.

Preliminary Results: 5S rRNA is thought to be involved in a signaling pathway that connects the peptidyl transferase center to the decoding center. Our immediate interest was to determine whether the 5S rRNA undergo structural changes as the ribosome went through its various states. The 5S rRNAs from the classical state all showed a structural core that is extremely conserved. Likewise, the 22 *E. coli* 5S rRNAs from ribosomes in various translational states all showed a conserved set of 34 Watson Crick base pairs and 16 non-standard pairs. Thus, in all structures there was no sudden structural change associated with base pairing. However, the before and after GTPase cleavage structures when superimposed suggest a meaningful structural change has occurred and is corroborated by the clustering of RMSDs. Thus, in this case the origin of the change appears to be associated with the backbone.

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