Ribosome Paleontology. Chiaolong Hsiao[1] and Loren Dean Williams[1]
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Molecular paleontology is the detection and study of ancient biological molecules and their assemblies. We focus on the molecular paleontology of the ribosomes of *H. marismortui* and *T. thermophilus*. These are the highest resolution ribosome structures available and represent lineages that diverged at the LUCA, several billions of years ago.[1] Paleo-RNA along with paleo-magnesium ions are conserved in position, conformation and coordination in these ribosomes.

We have sectioned the superimposed Large Subunits (LSUs) into concentric shells, like an onion (Figure 1), using the site of peptidyl transfer as the origin (the PT-origin). This spherical approximation, combined with a shell-by-shell comparison, captures significant information about ribosomal evolution, suggesting that the conformation and interactions of both RNA and protein are changing over evolutionary time. The tendency of macromolecules (protein and RNA) to assume regular secondary structural elements such as A-form helices with Watson–Crick base pairs (RNA) and α-helices and β-sheets (protein) is low at early time points but increases as time progresses. The non-secondary structures of ribosomal protein segments near the PT-origin suggest that they may be molecular fossils of the peptide ancestors of ribosomal proteins. The rRNA in the inner shells suggests that initial formation of the PTC may have involved Mg^{2+}-mediated assembly of at least partially single-stranded RNA oligomers or polymers. As one moves from center to periphery, proteins appear to replace magnesium ions.

Paleo-magnesium ions are observed primarily in the form of "Mg^{2+}-microsclusters" (Mg^{2+}-μc's). These Mg^{2+}-μc's compose a recurrent RNA-based magnesium-binding motif.[2,3] A Mg^{2+}-μc contains two Mg^{2+} ions bound to a common phosphate group. Mg^{2+}-μc's are unique structural entities with rigid and forced dispositions of functional groups that are impossible to achieve by RNA alone or by RNA in association with Group I cations. A schematic representation of a generic Mg^{2+}-μc is shown in Figure 2. A Mg^{2+}-μc extracted from a ribosomal structure is shown in Figure 3.

In the Large Subunit (LSU) of both *H. marismortui* and *T. thermophilus* ribosomes, three Mg^{2+}-μc's flank the PTC. These Mg^{2+}-μc's are not directly involved in catalysis, and do not form the innermost layer of the PTC, but provide the framework and supporting structure for the RNA that does. Mg^{2+}-μc's lend critical support to function by forming convoluted binding surfaces and providing rigid frameworks for attachment and buttressing of catalytic residues.

The Mg^{2+}-μc's in the LSU are highly conserved over evolution: they are unchanged in position and coordination in bacteria (*T. thermophilus*) and archaea (*H. marismortui*). Mg^{2+}-μc's are found in association with the most conserved rRNA secondary structures. Mg^{2+}-μc's link these conserved secondary structural elements via 'electrostatic tertiary interactions', which are composed of phosphate-Mg^{2+}-phosphate interactions. The 2D elements of the 23S rRNA that are linked by Mg^{2+}-μc's are conserved between the rRNAs of bacteria, archaea, and eukarya,[4] the LSU rRNA of mitochondria,[5,6] and in a proposed minimal LSU rRNA.[7] The exception is Mg^{2+}-μc D3, which has been dispensed of in some mitoribosomes (such as that of *C. elegans)* by conversion of the RNA-based polypeptide exit tunnel to a protein-based tunnel.[6]

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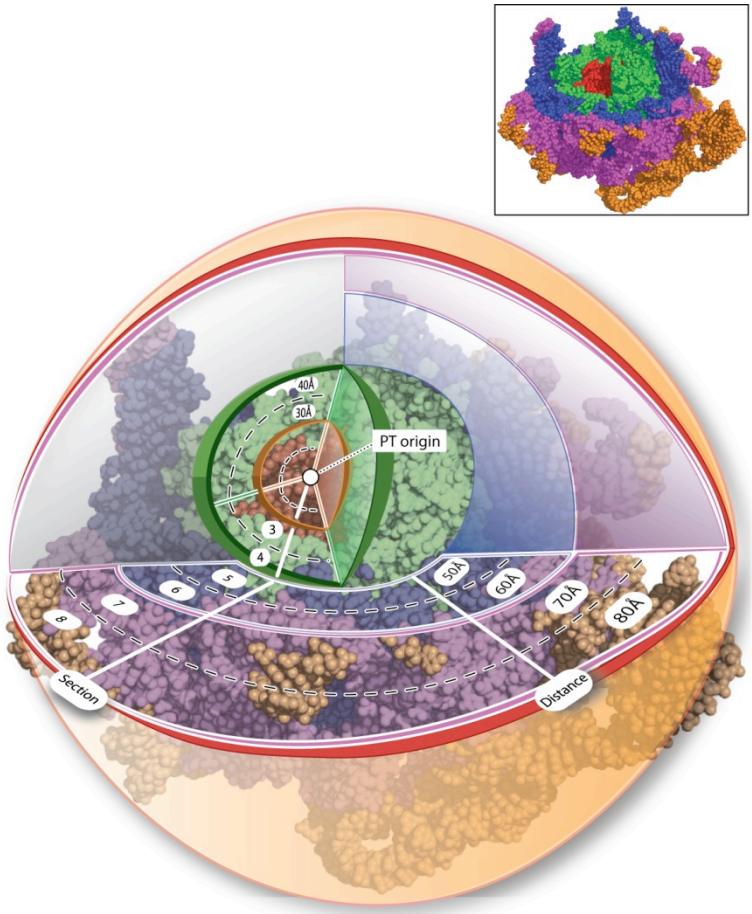

Figure 1. Ribosomal paleontology. The *Haloarcula marismortui* (and *Thermus thermophilus*, not shown) LSUs have been sectioned into concentric shells, with the origin at the site of peptidyl transfer (the PT-origin). The radius of any shell is 10 greater than the radius of the preceding shell. 23S rRNA^{HM} is red in shells 1 and 2, green in shells 3 and 4, blue in shells 5 and 6, and purple in shells 7 and 8. Additional shells are shown in orange. Atoms are represented in spacefill. Ribosomal proteins, ions, and water molecules are omitted for clarity.

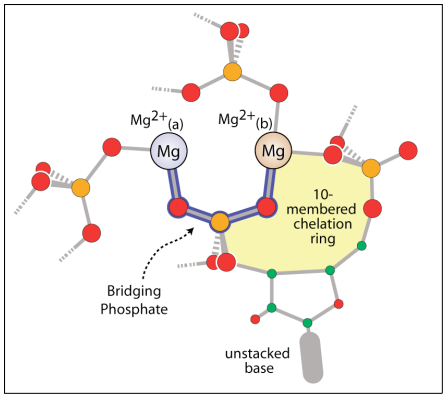

Figure 2. A schematic diagram of common features of Mg^{2+}-µc architecture. Shown is the $Mg^{2+}_{(a)}$-(O1P-P-O2P)-$Mg^{2+}_{(b)}$ bridge (outlined in blue), the 10-membered ring (yellow), an unstacked base, and the additional RNA ligands that enter the Mg^{2+} first shell at variable positions. Carbon is green, oxygen is red, and phosphorous is orange.

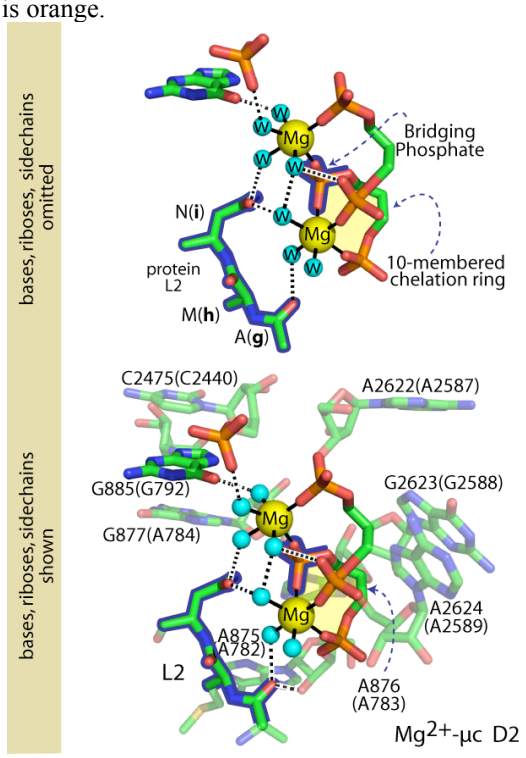

Figure 3. A Mg^{2+}-µc shown at two levels of detail. Both *H. marismortui* and (*E. coli*) residue numbers are shown.