MOLECULAR EVOLUTION'S "SURPRISE": A RIBOSOME WITH A UNIQUE INSERTION

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Introduction: Halophilic Archaea (or Haloarchaea) are classic extremophiles, thriving under saturated salt concentrations as found in natural brines, alkaline salt lakes, and the Dead Sea, to cite a few examples of extreme environments. They can be regarded as models of living 'fossils' from other eras, as testified by their occurrence worldwide in rock salt deposits of great geological ages, from the Pliocene (5.3–1.8 million years) up to the Silurian (419 million years)[1-4].

Halococcus morrhuae (ATCC® 17082) is an extremely halophilic archaeon, with an obligate growth requirement for 25% salt, which was originally isolated from the Red Sea. Organisms belonging to this species possess unique ribosomes, in that they have a 108 nucleotide insertion in their 5S rRNA thereby increasing the size of that RNA to 228 residues [5]. The *H. morrhuae* 5S rRNA insertion is not shared by other halophiles or Archaea or Bacteria in general. The restricted occurrence of this 5S rRNA insertion in a single genus indicates it is an extremely recent addition. Such ribosomal RNA insertions have previously been used to deduce the relative age of various regions in those RNAs [6]. This 5S rrNA example provides an opportunity to gain a better understanding of how ribosomal RNA insertions have been accommodated in the past and the extent to which they may have induced other changes in the ribosome. This, enhanced understanding in turn, will shed light on how the translation machinery has evolved over time [7, 8].

To better understand how the *H. morrhuae* insert is accommodated, we have succeeded in developing procedures to isolate *H. morrhuae* ribosomes in large quantity and are in the process of visualizing the position of both the unusual insertion in the 5S rRNA as well as the 5S rRNA itself in the ribosome using Cryo-Electron microscopy.

Results: We visualized the ribosomal insertion through cryoEM and single-particle reconstruction of the 50S large subunit. To overcome the ribosome's requirement for high salt concentrations incompatible with cryoEM, we applied an on-grid washing procedure. The subset of particles exhibiting neither aggregation nor self-self-interaction was manually extracted for reconstruction. Initial images of these 70S ribosomes indicated that the insert extends away from the 30S subunit as visualized using 50S particles alone, with the Cryo-EM yielding images with 12.5A resolution. With a larger dataset, we resolved the structure to subnanometer resolution. The lobe projecting off of base 108 of the 5S RNA is clearly visible with sufficient clarity and size. The projecting lobe is large enough to accommodate roughly 40 of the 108 inserted nucleotides, as a single 20 base pair(s) double helix at an acute angle to the underlying 5S core helix. Reconstruction with an increased particle count should resolve the more flexible areas adjoining the well-resolved lobe.

References:

[1] Reiser R. et al (1960) *Trans. Kans. Acad. Sci*, *63*, 31–34.

[2] Stan-Lotter H. et al (1999) *Microbiology, 145*, 3565–3574.

[3] Park J.S. et al (2009) *Geobiology, 7*, 515–523.

[4] Jaakkola, S.T. et al. (2014) *PLoS ONE*, *9*, e110533, doi:10.1371/journal.pone.0110533.

[5] Luehrsen, K.R. et al (1981) *Nature 293*:755-756.

[6] Petrov, A.S. et al (2015) *Proc Natl Acad Sci U S A. 112*(50):15396-401.

[7] Fox, G.E. & Woese, C.R (1975) *Nature 256*:505-507.

[8] Gongadze, G.M. (2011) *Biochemistry Biokhimia 76*:1450-1464.

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