Evolution of Protein Folding. N. A. Kovacs[1] , A. S. Petrov[1], C. R. Bernier[1], and L. D. Williams[1], [1]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332

Introduction: The recent availability of 70S and 80S ribosomal crystal structures of Bacteria [1], Archea [2], and Eukarya [3][4] have allowed for the construction of a model of ribosomal evolution at atomic resolution [5][6]. Comparisons of ribosomal RNA (rRNA) of representative species across the tree of life reveals the existence of a common core that deviates in sequence, but is conserved in secondary and 3D structure [7]. Mapping ribosomal size onto a phylogenetic tree containing species throughout life's 3 domains reveals that ribosomal size correlates very well with species complexity [6][8]. The progression of ribosomal large subunit (LSU) and small subunit (SSU) rRNA size closely follows the trend Bacteria ≤ Archea < Simple Eukaryotes < Complex Eukaryotes.

Comparisons of rRNA secondary structures between bacteria and eukaryotes led to the discovery of expansion segments in eukaryotic rRNAs [9]. Observing ribosomes in 3D has revealed that expansion segments branch off of common core rRNA helices at distinct structures we call *insertion fingerprints*. Expansion segments do not perturb the helices of the common core. Using the same method to define expansion segments in 3D, we have revealed *ancestral expansion segments* within the common core.

Our model describes the stepwise accretion of ancestral expansion segments onto the original rRNA helices of the LSU and SSU of the ribosome [6]. Focusing on the peptide-bond catalyzing function of the LSU, we use our model of ribosomal evolution to detail the evolution of protein folding.

The formation of a primitive LSU began with the accretion of ancestral expansion segments that constitute the peptidyl-transferase center (PTC), onto the very first rRNA helix [8]. The formation of the PTC allowed the primitive ribosome to synthesize very simple peptides [10]. Nestled between ancestral expansion segments, on the surface of the common core, and between the expansion segments of eukaryotes, are ribosomal protein (rProteins). Residues of rProteins found deep within the common core are unstructured, residues found directly under the common core surface have rudimentary secondary structure, and residues on the surface of the common core form globular domains. As rRNA ancestral expansion segments were accreted onto the surface of the primitive ribosome, so were peptide fragments of rProteins. As more ancestral expansion segments were accreted onto the surface of the ribosome over billions of years, the size and complexity of rProteins accreted onto the surface increased.

Over approximately 4 billion years, the ribosome evolved from being able to synthesize small peptides, to synthesizing complex proteins necessary for extant life. Comparing differences in protein folding parameters such as dihedral angle, intramolecular hydrogen bonds, and surface area of individual resides of rProteins to their distance to the PTC will elucidate the evolution of protein folding.

References: [1] Cate, J. H., Yusupov, M. M., Yusupova, G. Z., Earnest, T. N., and Noller, H. F. (1999) X-ray crystal structures of 70S ribosome functional complexes. Science 285, 2095–2104. [2] Armache, J. P., Anger, A. M., Márquez, V., Franckenberg, S., Fröhlich, T., Villa, E., Berninghausen, O., Thomm, M., Arnold, G. J., Beckmann, R., and Wilson, D. N. (2013) Promiscuous behaviour of archaeal ribosomal proteins: Implications for eukaryotic ribosome evolution. Nucleic Acids Res. 41, 1284–1293. [3] Jenner, L., Melnikov, S., Garreau de Loubresse, N., Ben-Shem, A., Iskakova, M., Urzhumtsev, A., Meskauskas, A., Dinman, J., Yusupova, G., and Yusupov, M. (2012) Crystal structure of the 80S yeast ribosome. Curr. Opin. Struct. Biol. 22, 759–67. [4] Anger, A. M., Armache, J.-P., Berninghausen, O., Habeck, M., Subklewe, M., Wilson, D. N., and Beckmann, R. (2013) Structures of the human and Drosophila 80S ribosome. Nature 497, 80–85. [5] Petrov, A. S., Bernier, C. R., Hsiao, C., Norris, A. M., Kovacs, N. A., Waterbury, C. C., Stepanov, V. G., Harvey, S. C., Fox, G. E., Wartell, R. M., Hud, N. V, and Williams, L. D. (2014) Evolution of the ribosome at atomic resolution. Proc. Natl. Acad. Sci. 111, 10251–10256. [6] Petrov, A. S., Gulen, B., Norris, A., Kovacs, N. A., Bernier, C. R., Lanier, K. A., Harvey, S. C., Fox, G. E., Wartell, R. M., Hud, N. V., and Williams, L. D. (2015) The History of the Ribosome and the Origin of Translation. Submitted. [7] Melnikov, S., Ben-Shem, A., Garreau de Loubresse, N., Jenner, L., Yusupova, G., and Yusupov, M. (2012) One core, two shells: bacterial and eukaryotic ribosomes. Nat. Struct. Mol. Biol. 19, 560–7. [8] Petrov, A. S., Bernier, C. R., Hsiao, C., Norris, A. M., Kovacs, N. A., Waterbury, C. C., Stepanov, G., Harvey, S. C., Fox, G. E., Wartell, R., Hud, N. V, and Williams, L. D. Evolution of the Ribosome at Atomic Resolution Submission PDF I. [9] Michot, B., and Bachellerie, J.-P. (1987) Comparisons of large subunit rRNAs reveal some eukaryote-specific elements of secondary structure. Biochimie 69, 11–23. [10] Fox, G. E. (2010) Origin and evolution of the ribosome. Cold Spring Harb. Perspect. Biol. 2, a003483.