THE INFLUENCE OF OXYGEN ON THE IRON CONTENT OF BACTERIAL RIBOSOMES. M. S. Bray[1], E. B. O'Neill[2], L. D. Williams[2], P. L. Morton[3], and J. B. Glass[1,4*], [1]School of Biology, Georgia Institute of Technology, Atlanta, GA, USA, [2]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, USA,,[3]Department of Earth, Ocean and Atmospheric Science, Florida State University, Tallahassee, FL, USA, [4]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA, USA, *Jennifer.Glass@eas.gatech.edu

The ribosome is the most ancient and evolutionarily conserved macromolecular assembly in all biology. Since the last universal common ancestor, the core structure of the ribosome has not changed in four billion years. Only through surface accretion over evolutionary time has the ribosome increased in complexity. The ancient Hadean ocean contained abundant dissolved Fe(II), which likely played a major role in prebiotic chemistry, including the development of the translational system. Although modern ribosomes bind Mg(II) ions, it is likely that Fe(II) could have been the first cofactor for the protoribosome, with Mg(II) fully or partially replacing Fe(II) when Fe(II) levels fell precipitously during the Great Oxidation Event.

Based on previous *in vitro* experiments [1, 2], we hypothesize that extant bacterial ribosomes retain the capacity to bind Fe(II) when grown under anoxia. In this study, we grew *Escherichia coli* K12 aerobically and anaerobically in media containing 100 μM total Fe. Cells were lysed in 10 mM Mg(II) buffer, ribosomes were isolated using chromatography [3], and digested in acid-washed Teflon using ultrapure nitric acid and hydrogen peroxide. The metal content of the ribosomes was analyzed using inductively coupled plasma mass spectrometry. Preliminary data show that ribosomes isolated from anaerobically grown cells contain higher total Fe than those from aerobically grown cells. Our initial findings lend support to the hypothesis that early translational machinery may have depended on Fe(II). We are now in the process of optimizing the purification method by lowering the Mg(II) concentration of the buffer to enable more precise quantification of ribosomal Mg(II) content. We will also attempt to directly observe evidence for Fe(II)-binding to rRNA by testing for Fenton-based cleavage in the presence of O_2, as well as footprinting of ribosomes to identify sites of Fe(II)-binding, measuring activity and fidelity of translation by *in vitro* translation, and profiling of ribosomal proteins by 2D electrophoresis and mass spectrometry compared to a commercial control.

References: [1] Athavale S. S. et al. (2012) *PLoS One, 7,* e38024. [2] Hsiao C. et al. (2013) *Nature Chem. 5,* 525-528. [3] Maguire, B. A. (2008) *RNA,* 14, 188-195.