Bacterial Ribosomes Incorporate Iron Under Pre-GOE Conditions. M. S. Bray[1], C. M. Albalate[2], E. B. O'Neill[1,2], A. R. Reddi[2], P. L. Morton[3], J. B. Glass[4*], L. D. Williams[2*]. [1]School of Biology, Georgia Institute of Technology, Atlanta, GA, USA *Jennifer.Glass@eas.gatech.edu; Loren.Williams@chemistry.gatech.edu, [1]School of Biology, [2]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA, USA, [3]Geochemistry, National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL, USA, [4]School of Earth and Atmospheric Sciences, Georgia Institute of Technology, Atlanta, GA, USA

Hypothesis: Divalent ions are essential for nucleic acid function during replication, transcription and translation. Although modern nucleic acids associate with Mg^{2+}, we hypothesize that Fe^{2+} was their original cofactor, with Mg^{2+} fully or partially replacing Fe^{2+} when seawater Fe^{2+} levels fell after the Great Oxidation Event (GOE; 2.4 Ga). In the ribosome, deeply buried divalent cations can be traced to the origin of life. Because the ribosome evolved under anoxic, Fe^{2+}-rich ("pre-GOE") conditions, and Fe^{2+} catalyzes RNA folding and catalysis *in vitro* [1, 2], we hypothesize that extant ribosomes retain the *in vivo* capacity to bind and function with Fe^{2+} under pre-GOE conditions.

Methods: *Escherichia coli* K12 cells were grown with and without oxygen with 0.01 mM ("low") and 1 mM ("high") Fe^{2+}. Cell growth was measured by OD_{600}. At the transition from log to stationary growth phase, cells were harvested, washed, and lysed in 1 mM Mg^{2+} buffer. Ribosomes were isolated by chromatography [3] and quantified by A_{260}. The metal content of purified ribosomes was measured by inductively coupled plasma mass spectrometry (ICP-MS) and total reflection x-ray fluorescence (TXRF). Ribosomal proteins were analyzed by SDS-PAGE.

Growth rates: The doubling time of *E. coli* was identical at low and high Fe^{2+} (oxic: 0.5 OD_{600} hr^{-1}; anoxic: 0.4 OD_{600} hr^{-1}), suggesting that ribosomal function is basically equivalent regardless of Fe^{2+} availability in the growth medium.

Ribosomal iron content: Both ICP-MS (**Fig. 1A**) and TXRF (**Fig. 1B**) data showed that ribosomes isolated from cells grown under pre-GOE conditions contained significantly more Fe (~2 Fe^{2+} mol^{-1} ribosome by TXRF and ~6 Fe^{2+} mol^{-1} ribosome by ICP-MS) than cells grown in the presence of O_2 with low or high Fe (<1 Fe^{2+} mol^{-1} ribosome), consistent with our previous identification of eight tightly-bound divalent metal cations per ribosome based on structural modeling [2]. These Fe^{2+} ions must be tightly-bound and long-lived because repeated Mg^{2+} washes did not displace them.

Ribosomal proteins: SDS-PAGE of the same ribosomes analyzed for Fe content showed similar banding patterns as highly purified ribosomes (**Fig. 1C**). Additional bands likely represent tightly-bound, ribosome-associated proteins. Differences in banding patterns between the four treatments suggest that O_2 and Fe^{2+} affect ribosomal protein expression and/or folding.

Evolutionary implications: These findings support our hypothesis that extant ribosomes retain the ability to incorporate and function with Fe^{2+} *in vivo* (e.g. maintain the same growth rates as cells with ribosomes lacking Fe^{2+}) under pre-GOE conditions.

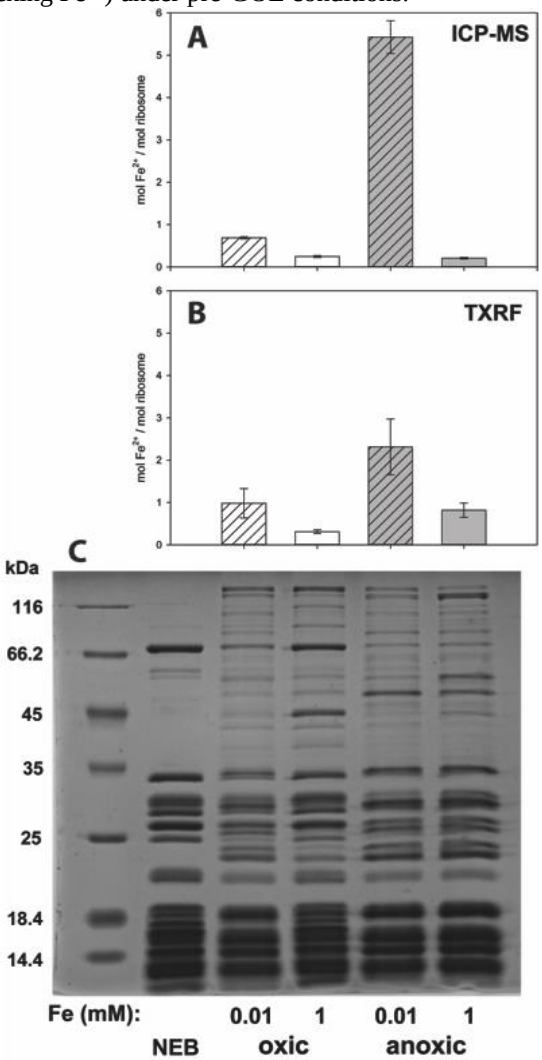

Fig. 1. Purified *E. coli* ribosomal Fe content determined by (A) ICP-MS and (B) TXRF. (C) SDS-PAGE protein gel for the four growth conditions. NEB refers to commercially purified ribosomes.

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.