An Early Origin for Molybdenum-Nitrogenase Eric S. Boyd[1], Ariel D. Anbar[2], Scott Miller[3], Trinity L. Hamilton[1], Matthew Lavin[4], and John W. Peters[1], [1]*Department of Chemistry and Biochemistry and the Astrobiology Biogeocatalysis Research Center, Montana State University, Bozeman, Montana 59717,* [2]*School of Earth & Space Exploration and Department of Chemistry & Biochemistry, Arizona State University, Tempe, AZ, 85287, USA,* [3]*Department of Biological Sciences, University of Montana, Missoula, Montana, 59812,* [4]*Department of Plant Sciences, Montana State University, Bozeman, Montana 59717*

The taxonomic distribution and phylogenetic relationships of proteins required for molybdenum (Mo)-nitrogenase that arose by gene fusion and duplication reveals that Mo-nitrogenase was not associated with LUCA, but rather emerged in the strictly anaerobic methanogenic archaea and was acquired in bacteria via lateral gene transfer in an anoxic environment. These findings strongly suggest that this process emerged in an anaerobic environment early during the evolution of life, perhaps prior to the emergence of oxygenic photosynthesis.

To test this hypothesis, we examined the evolutionary relationships of paralogous proteins required for the biosynthesis of the nitrogenase active site cofactor and bacteriochlorophyll (Bch). The results, in the context of evolutionary analyses of additional nitrogenase and Bch proteins, collectively indicate an origin for Mo-nitrogenase that predates oxygenic photosynthesis. Importantly, the age of nodes delineating the major diversification of Mo-dependent nitrogenase is similar to the maximum age for the emergence of oxygenic photosynthesis, suggesting that the diversification of Mo-nitrogenase may have been promoted by the emergence of oxygenic photosynthesis, most likely through the widespread oxidation of Mo-sulfides and subsequent increases in Mo bioavailability. These findings imply that Mo-dependent biological nitrogen fixation emerged prior to the transition from the Archean to the Proterozoic and the widespread oxidation of the atmosphere and ocean.