Signatures of Low-Mo Ancient Ocean May be Preserved in Cyanobacterial Genomes J. B. Glass[1], Felisa L. Wolfe-Simon[2,3], A. T. Poret-Peterson[1] and A. D. Anbar[1,4], [1]Arizona State University School of Earth and Space Exploration (PO Box 871404 Tempe, AZ; jennifer.b.glass@asu.edu; amisha.poretpeterson@asu.edu; anbar@asu.edu), [2]Harvard University Department of Earth and Planetary Sciences (20 Oxford St. Cambridge, MA 02138; wolfe@eps.harvard.edu). [3]U.S. Geological Survey (345 Middlefield Rd. Menlo Park, CA 94025). [4]Arizona State University Department of Chemistry and Biochemistry (PO Box 871604 Tempe, AZ 85287)

Molybdenum (Mo) is an essential trace element for all life, and is particularly important for key enzymes that drive the biogeochemical N cycle. While Mo is the most abundant transition metal in the ocean today, it was present at much lower concentrations before 800 Ma [1,2]. Cyanobacteria evolved several billion years prior [3], and so may have struggled to acquire sufficient Mo. This problem might have been particularly acute when fixing N_2.

Today, organisms possess a variety of strategies to cope with the scarcity of iron (Fe) in the oceans. Fe storage proteins (ferritins) have been discovered in marine cyanobacteria [4] and diatoms [5]. Ferritins sequester Fe when ambient concentrations rise, and donate Fe to metalloenzymes when Fe drops. Marine microorganisms also produce siderophores to chelate Fe, thus making it available for uptake [6]. The possibility that ancient marine microorganisms adopted a similar strategy for Mo is supported by the recent discovery of bacterial "molybdophores" [7]. We are investigating whether bacteria also store Mo in ways analogous to Fe.

The Mo storage protein Mop was first discovered in the anaerobic N_2-fixing bacterium *Clostridium pasteurianum* [8]. Crystal structures revealed that Mop binds eight atoms of Mo as the oxyanion molybdate (MoO_4^{2-}) [9]. Mop is known to be expressed by the heterocystous cyanobacterium *Nostoc* sp. PCC 7120 when grown on nitrate at >1000 nM Mo [10]. We are interested in whether Mop is also important when *Nostoc* is fixing N_2. We are exploring this idea through physiological and genomic studies.

Physiologic studies were performed using *Nostoc* because (1) heterocystous cyanobacteria are likely the best modern analogues available for primary producers in the low-Mo Proterozoic ocean [11]; (2) they can grow on a range of N sources, including those that require Mo: N_2 and nitrate; and (3) they are fast-growing, with growth rates up to 1-2 doublings per day.

Our experiments show 10-fold higher cellular Mo when N_2-fixing *Nostoc* was grown at high Mo (1500 nM) vs. low Mo (1-100 nM) [12]. This is consistent with Mo storage and suggests Mop expression when Mo is abundant. At high Mo, N_2-fixing cultures contained 1.5-3 times more cellular Mo than nitrate-assimilating cultures [12]. We hypothesize that more Mop is produced when *Nostoc* is N_2-fixing than assimilating nitrate. Mop gene and protein expression studies are underway to test these hypotheses.

Although we are studying modern cyanobacteria, Mop is probably an ancient protein. This theory is supported by three lines of evidence. First, Mop is widespread in prokaryotes, and appears to be especially prevalent in methanogenic archaea and N_2-fixers, both of which have high Mo requirements [12, 13]. Second, Mop is not present in eukaryotes, perhaps because Mo storage is not necessary for higher life forms that evolved after Mo rose ~800 Ma. Third, the Mop domain has been incorporated into many other proteins through gene duplication and fusion that requires long timespans.

If Mop is ancient, we might expect that it would have been lost from marine cyanobacteria due to higher Mo (100 nM) today, but retained or adopted by freshwater cyanobacteria living where Mo is scarce (<5 nM in most freshwaters). Consistent with this expectation, we have done a genomic survey which shows just this. We identified Mop genes in all ten N_2-fixing freshwater cyanobacteria with sequenced genomes, but in neither of the two N_2-fixing marine cyanobacteria with sequenced genomes [12].

Thus, Mop genes may be signatures of an ancient imprint of Mo limitation from Archean and Proterozoic oceans.

References:

[1] Anbar A. D. et al. (2007) *Science, 317,* 1903-1906. [2] Scott C. et al. (2008) *Nature, 452,* 456-459. [3] Knoll A. H. (2007) In *Evolution of Primary Producers in the Sea,* 133-163. [4] Castruita M. et al. (2006) *AEM*, *72,* 2918-2924. [5] Marchetti A. et al. (2009) *Nature, 457*, 467-470. [6] Butler A. (2005) *BioMetals, 18,* 369-374. [7] Bellenger et al. (2008) *Nature Geosci,. 1,* 243-246. [8] Hinton S. M. & Merritt B. (1986) *J. Bact., 168,* 688-693. [9] Schüttelkopf et al. (2002) *JMB, 326,* 761-767. [10] Sazuka T. (2003) *Photosyn. Res., 78,* 279-291. [11] Tomitani et al. (2006) *PNAS, 103*, 5442-5447. [12] Glass J. B. et al. (in press) *Limnol. Oceanogr.* [13] Schöneit et al. (1979) *Arch. Microbiol., 123,* 105-107.