Insight into the Evolution of Cellular Redox Systems: Molecular Characterization of the Thioredoxin System in Methanogenic Archaea. A. C. McCarver[1,2] and D. J. Lessner[2,3], [1] Cellular and Molecular Biology Program University of Arkansas Fayetteville (email:acmccarv@uark.edu), [2] Department of Biological Sciences University of Arkansas Fayetteville, [3]Arkansas Center for Space and Planetary Sciences University of Arkansas Fayetteville (email:dlessner@uark.edu)

Introduction: All cells require mechanisms to maintain a reduced intracellular environment and to provide reducing equivalents for biosynthetic processes. In modern organisms, the thioredoxin system is the most ubiquitous reducing system, found in species from all three domains of life. The thioredoxin system is composed of a thioredoxin reductase (TrxR) that utilizes electrons from NADPH to reduce thioredoxin (Trx), which reduces a variety of target proteins. In addition to acting as an electron donor to biosynthetic enzymes such as ribonucleotide reductase, Trx in modern organisms serves a key role in protecting cells from the deleterious effects of oxygen (i.e. oxidative stress) by reducing protein disulfides. Strictly anaerobic methane-producing archaea (methanogens) likely originated prior to the great oxygenation event (GOE) [1]. After the GOE, oxygen likely restricted the growth of methanogens to anoxic ecological niches. However, modern methanogens occupy a diverse range of habitats, including those often exposed to oxygen, suggesting that some methanogens evolved to cope with oxidative stress. Thus, an understanding of the role of the thioredoxin system in methanogens may provide insight into the evolution of the role(s) of Trx. Recent evidence supports that Trx homologs exist in nearly all methanogens and Trx can reduce proteins involved in methanogenesis and other processes in *Methanocaldococcus jannaschii*, a hyperthermophilic methanogen [2]. Our recent work has shown that *Methanosarcina acetivorans*, a mesophilic methanogen, encodes seven Trx homologs (MaTrx1-7) from five distinct phylogenetic clades and a single TrxR (MaTrxR) [3]. Moreover, evidence supports MaTrx2, MaTrx6, and MaTrx7 are disulfide reductases and that *M. acetivorans* contains a complete thioredoxin system composed of MaTrxR and MaTrx7. Interestingly, NADPH serves as the electron donor for MaTrxR, despite $F_{420}H_2$ and ferredoxin being the primary electron donors generated by methanogenesis. We show here that of the remaining MaTrxs, only MaTrx3 has disulfide reductase activity, albeit low, indicating MaTrx1, MaTrx4, and MaTrx5 may have alternative functions. Protein BLAST searches for TrxR within methanogen genomes reveals that nearly all methanogens have a TrxR. However, the presence of a conserved NADPH binding motif in the TrxR homologs is primarily restricted to members of the *Methanomicrobia* and *Methanobacteria*, indicating that the NADPH-dependent system evolved within or was acquired by only a subset of methanogens. Compared to other methanogens, *Methanosarcina* spp. are more often found in aerated environments, supporting an increased demand for oxidative stress defense systems. To understand how NADPH is supplied to MaTrxR *in vivo*, we tested *M. acetivorans* cell lysate for $F_{420}H_2$:NADP oxidoreductase (Fno) activity and MaTrx-dependent $F_{420}H_2$ oxidation. *M. acetivorans* cell lysate contains robust Fno activity and the addition of oxidized MaTrx7 to cell lysate resulted in the concomitant oxidation of $F_{42O}H_2$. Overall, these results support that *M. acetivorans* contains a functional NADPH-dependent thioredoxin system, with $F_{420}H_2$ generated during methanogenesis serving as a primary electron donor to the system. The acquisition of a NADPH-dependent Trx system in *M. acetivorans* and likely additional methanogens, followed by functional integration of the system, may have been in response to the increased levels of oxygen in our modern atmosphere. The additional *M. acetivorans* Trx homologs may play roles that are possibly ancestral, but are currently unknown.

References: [1] Ueno Y. *et al.* (2006) *Nature*, 440, 516-519. [2] Susanti D. *et al*. (2014) *PNAS*, 111, 2608-2613. [3] McCarver A. *et al*. (2014) *FEBS*, 281, 4598-4611.