Iron-sulfur Clusters in RNA Polymerase: A New Role for an Ancient Prosthetic Group M. E. Jennings[1], F. H. Lessner[1], E. A. Karr[3], and D. J. Lessner[1,2]
[1]Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701; [2]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR 72701; [3]Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019

Introduction Iron-sulfur (Fe-S) clusters are ubiquitous prosthetic groups that serve essential catalytic, structural, and regulatory functions in a wide variety of proteins in modern organisms. The utilization of Fe-S clusters within cells likely originated very early when the Earth was largely oxygen-free, since Fe-S clusters are typically oxygen-labile. Similarly, all species from the three domains of life utilize a multi-subunit RNA polymerase (RNAP) to synthesize RNA from a DNA template, indicating RNAP also originated very early. Interestingly, subunit D of RNAP from numerous archaea and the homologous Rpb3/AC40 subunits of RNAP from several eukaryotes contain a [4Fe-4S] ferredoxin-like domain predicted to bind one or two [4Fe-4S] clusters. The ferredoxin-like domain is absent from RNAP from all sequenced bacteria. The function(s) of the [4Fe-4S] clusters is unknown. We hypothesize that acquisition of the ferredoxin-like domain by RNAP in early anaerobic archaea provided a mechanism to correlate information processing (i.e. transcription) with energy conserving metabolism, and this function was maintained in modern archaea and eukaryotes. Specifically, subunit D forms a heterodimer with subunit L, which is the first step in the assembly of multi-subunit RNAP. As such, the [4Fe-4S] cluster(s) may affect formation or stability of the D/L heterodimer and therefore serve to regulate assembly of RNAP in response to factors such as oxygen, iron, or sulfur levels. Methanogens are an ancient lineage of anaerobic archaea whose metabolism is heavily dependent on [4Fe-4S] clusters; thus, an understanding of the role of [4Fe-4S] clusters in the assembly and activity of RNAP in modern methanogens may provide insight into the origins and evolution of RNAP. We have previously demonstrated that the methanogen *Methanosarcina acetivorans* possesses a subunit D that contains two oxygen-labile [4Fe-4S] clusters [1]. Using the *M. acetivorans* genetic system we have probed the *in vivo* role of the clusters and provide evidence that the [4Fe-4S] cluster binding regions of subunit D are important, but not essential, for assembly of RNAP, consistent with a regulatory role. Specifically, we show that the assembly of subunit D with subunit L is unaffected by the absence of cluster 1, cluster 2, or the ferredoxin-like domain, indicating the clusters do not impact the *in vivo* assembly of the D/L heterodimer. However, assembly of subunit D with subunits B' and A'', which are part of the RNAP catalytic region, is impacted by the absence of [4Fe-4S] clusters, revealing that the clusters are important for the assembly of RNAP post D/L heterodimer formation. In particular, loss of [4Fe-4S] cluster 1 had a greater impact on RNAP assembly than the loss of cluster 2, consistent with cluster 1, rather than cluster 2, being conserved in RNAP subunits from numerous archaea and eukaryotes. Gene replacement experiments also revealed that cluster 1, cluster 2, and the ferredoxin-like domain are not essential for functional RNAP in *M. acetivorans*. However, mutant strains with RNAP containing variant subunit D exhibited a slow growth phenotype. Western blot analysis indicated the slow growth phenotype is not due to significant differences in RNAP levels. Partial purification of RNAP followed by Western blot analysis and non-specific transcription assays indicated that RNAP containing subunit D lacking cluster 1, cluster 2, or the ferredoxin-like domain is less stable than wild-type RNAP. Overall, these results support the hypothesis that the presence of the [4Fe-4S] clusters is required for optimal assembly of RNAP and for the stability of holo RNAP in *M. acetivorans*. This mode of regulation may be conserved in other archaea and eukaryotes. The ferredoxin-like domain of RNAP may be a surviving example of one of the earliest transcription regulatory mechanisms.

References:

[1] Lessner FH, Jennings ME, Hirata A, Duin EC, Lessner DJ. *Journal of Biological Chemistry* 2012, 287(22):18510-18523.