

Proteome-Wide Identification of Metalloproteins in *Saccharomyces cerevisiae*

Nathan H. Zahler¹, Andrea Stoddard¹, Matthew J. Kidd², James E. Penner-Hahn² and Carol A. Fierke¹

Department of Chemistry¹ and Biophysics Research Division², The University of Michigan, Ann Arbor, Michigan 48109-1055

It is widely estimated in the literature that metalloproteins and metalloenzymes constitute a large fraction of functional cellular proteins. In addition, naturally occurring trace metals play diverse roles in protein structure and biological catalysis. To date, metalloenzyme active sites have been identified which contain V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Mo, I, and, more recently, Cd [1,2]; however, a complete accounting of metalloproteins and metalloenzymes (the "metallome") is not currently available for any organism. To better understand the role of metal ions in cells and expand our understanding of metalloproteins in general, we are conducting a proteome-wide survey of metalloproteins in *Saccharomyces cerevisiae* using a commercially available (Open Biosystems) expression library developed in the laboratories of Dr. Eric Phizicky (University of Rochester) and Dr. Michael Snyder (Yale University). Proteins in this library contain a C-terminal affinity tag comprised of a His₆ tag, an HA epitope tag, a C3 protease cleavage site and a protein A IgG binding domain. Initial experiments using positive controls, including superoxide dismutase (*Sod1p*), indicate that proteins in this library can be rapidly isolated in a manner which allows for the copurification of tightly-bound Cu and Zn, and results in minimal contamination with Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb, despite the presence the His₆ tag. Current efforts are focusing on the adaptation of these techniques for use in high-throughput x-ray fluorescence analysis. Finally, using the background in metal metabolism available for *S. cerevisiae*, we are analyzing specific sets of proteins that may bind xenobiotic metals, including Cd(II). These studies are likely to identify proteins involved in the origins of both metal toxicity and tolerance, and together with the analysis of naturally occurring metals will provide a more detailed understanding of the biological roles of metal ions.

1. Frausto da Silva, J.J.R. and Williams, R.J.P. (1993) "The Biological Chemistry of the Elements", Clarendon Press, Oxford.
2. Lane, T.W., and Morel, F.M. (2000) "A biological function for cadmium in marine diatoms" *Proc. Natl. Acad. Sci. USA* **97**: 4627-31