Biochemical-genetic analysis of [Fe-S] cluster biosynthesis in *Azotobacter vinelandii*

Mihaela-Carmen Unciuleac[1], Deborah C. Johnson[1], Patricia C. Dos Santos[1], Ina Puleri[1], Estella C. Raulfs[1], Timothy J. Larson[1], Archer D. Smith[2], Kala Chandramouli[2], Michael K. Johnson[2], Sunil Naik[3], Boi Hanh Huynh[3] and Dennis R. Dean[1*]

[1]Department of Biochemistry, Virginia Tech, Blacksburg, Virginia 24061, [2]Department of Chemistry and Center for Metalloenzyme Studies, University of Georgia, Athens, Georgia 30602, and [3]Department of Physics, Emory Univesity, Atlanta, Georgia 30322

The nitrogen-fixing bacterium *Azotobacter vinelandii* has two different systems (Nif and Isc) that have been shown to have functions related to the biogenesis of [Fe-S] clusters. We are interested in exploring the biochemical mechanism for [Fe-S] cluster assembly directed by these systems and how target specificity is selected. We are also interested in the potential for functional cross-talk between the Nif and Isc systems as well as the functions of other possible [Fe-S] cluster biosynthetic proteins that are genetically unlinked to either the Nif or the Isc components. These other systems include remnants of the Suf system, a second IscA, and an Nfu protein. Our strategy has involved the development of a controlled expression system for the evaluation of the physiological and biochemical effects that occur upon depletion of a specific targeted component. In addition we are developing methods for the *in vitro* activation of nitrogenase components, and various [4Fe-4S] and [2Fe-2S] cluster-containing proteins. Our biochemical and genetic studies are also complemented by biophysical strategies aimed at elucidating the nature of Fe-S cluster intermediates and gaining insight into how cluster transfer to target proteins is accomplished. Recent progress on the development of an *in vitro* system for the activation of aconitase and the *in vivo* consequences of loss of [Fe-S] assembly factor functions on maturation of a Rieske-type [2Fe-2S] cluster-containing protein will be discussed.