

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

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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.