**Analysis of Fe-S Cluster Formation in Plant Chloroplasts**

Marinus Pilon*, Salah E. Abdel-Ghany, Hong Ye and Elizabeth A.H. Pilon-Smits.
Department of Biology, Colorado State University, Fort Collins, CO 80523, USA

Iron-sulfur clusters are essential components for photosynthesis, the process unique for plants and algae that drives life on earth. Nuclear-encoded chloroplast Fe-S proteins like ferredoxin (Fd) acquire their cofactor after import into the organelle. We investigated the presence of Suf or Isc related machineries in plastids. CpNifS is the only plastid protein with Cys-desulfurase activity. Several other potential members of a plastid Fe-S cluster formation machinery can be identified including 3 NFU proteins and homologues of SufA, B, C, D and E and Hcf101 protein. CpNifS is in the stroma of *Arabidopsis* chloroplasts and is expressed in all major plant tissues at about equal levels. The purified enzyme had both Cys desulfurase and SeCys lyase activity. Compared to other NifS-like proteins the preference for SeCys over Cys was high and the Cys desulfurase activity low, indicating that perhaps the enzyme is subject to activation in vivo (Pilon-Smits *et al.* 2002, Plant Physiology 130: 1309-18). A recently discovered SufE homolog of Arabidopsis is expressed in chloroplasts and stimulated CysD activity of CpNifS 40 fold. KO analysis indicates that both CpNifS and CpSufE are essential for photo-autotrophic growth. To test whether CpNifS is involved in Fe-S cluster formation for photosynthetic proteins, an *in vitro* reconstitution assay was developed for ferredoxin. The reconstitution activity requires an intact PLP-cofactor, and CpNifS protein with a mutation of the conserved active site cysteine ($Cys_{418}$-Ser) is inactive, indicating that ferredoxin reconstitution involves the cysteine desulfurase activity of CpNifS. Stromal proteins at 300 □g/ml showed activity comparable to 10 □g/ml CpNifS. Based on a quantification of CpNifS we estimated that the reconstitution activity of stroma was 50-80 times more than that of pure CpNifS protein. Thus, stromal components activate CpNifS. Interestingly, gelfiltration experiments indicate that CpNifS interacts with other proteins *in vivo* and may form a transient complex. Depletion experiments indicated that CpNifS is absolutely required for Fe-S formation in ferredoxin (Ye *et al*. 2005, Planta 220: 602-08). Our initial functional studies of the potential scaffold proteins have focused on CpSufA (CpIscA), which was shown to be plastidic by GFP-fusion studies. Pre-incubation of pure CpNifS and purified CpSufA gives a 2x stimulation of apo-Fd reconstitution compared to CpNifS alone. Gel filtration experiments indicated that upon incubation with CpNifS purified CpSufA acquires a transient Fe-S cluster. This cluster can subsequently be transferred to apo-Fd to form holo-Fd. Thus CpSufA can function as an assembly scaffold for Fe-S clusters (Abdel-Ghany *et al*. 2005 Plant Physiology 138, *in press).*