

EMSP Final Report 55041

Project Title:

Recipient Principle Investigator: Julian Schroeder
Recipient Project Director: Julian Schroeder (858)-534-7759
Recipient Business Office and phone number:
DOE Contract Administrator:

Heavy metal toxicity poses major environmental and health problems, and heavy metals are more difficult to remediate than chemical contaminants, which can be degraded by microorganisms. Cadmium and arsenic, for example, are non-essential heavy metals which are toxic to living cells at very low concentrations. Cd^{2+} ions displace Ca^{2+} or Zn^{2+} in proteins and can cause oxidative stress, while arsenic also causes oxidative stress damage and is a well known carcinogen.

Schizosaccharomyces pombe and other fungi as well as plants synthesize phytochelatins which chelate Cd^{2+} , Cu^{2+} and other heavy metal ions. Phytochelatins are thiolate peptides with the primary structure $(-\text{Glu-Cys})_n\text{-Gly}$, which are non-translationally synthesized from glutathione. Phytochelatins play major roles in metal detoxification in plants and fungi and have been proposed to be central to phytoremediation of heavy metal contaminated soils and waters. We were interested in exploring this hypothesis, however, genes encoding phytochelatin synthases had not been identified until DOE-supported results in the P.I.'s lab and parallel research elsewhere (Clemens et al., 1999; Ha et al., 1999; Vatamaniuk et al., 1999).

By screening for plant genes mediating metal tolerance we identified a wheat cDNA, *TaPCS1*, whose expression results in a dramatic increase in cadmium tolerance (Clemens et al, 1999). *TaPCS1* encodes a protein of ~55 kDa with no similarity to proteins of known function. We identified homologues of this new gene family from *Arabidopsis* and fungi. The *Arabidopsis* and fungal genes were also demonstrated to confer substantial increases in metal tolerance upon expression. *PCS*-expressing cells accumulate more Cd^{2+} than controls. *PCS* expression mediates Cd^{2+} tolerance even in mutants that are either deficient in vacuolar acidification or impaired in vacuolar biogenesis. *PCS*-induced metal resistance is lost upon exposure to an inhibitor of glutathione biosynthesis, a process necessary for phytochelatin formation. Disruption in a fungal *PCS* gene results in hypersensitivity to Cd^{2+} and Cu^{2+} and inability to synthesize phytochelatins upon Cd^{2+} exposure. Moreover, cells overexpressing *PCS* produce phytochelatins. These data demonstrate a central role for the *PCS* gene family in phytochelatin synthesis and metal detoxification in eukaryotes and suggests that *PCS* genes could be of central importance for engineering plants or other organisms for remediation of metal contaminated soils and waters. We are continuing to experiment with using *PCS* to increase the metal tolerance of plants.

Another focus of this grant was to identify plant membrane proteins that can facilitate uptake of the toxic metal cadmium. We identified a first plant cDNA, named *LCT1* (Schachtman et al., 1997), that mediates uptake of the toxic metal cadmium (Clemens et al., 1998). *LCT1* was further shown to mediate uptake of calcium into yeast (Clemens et al., 1998). A protocol was further prepared describing newly developed methods for identifying new genes that control plant metal sensitivity (Lee and Schroeder, in press).

In further research we identified a different class of plant proteins that mediates uptake of toxic metals, the *Arabidopsis* Nramp proteins (Thomine et al., 2000). Metal

cation homeostasis is essential for plant nutrition and transport of toxic heavy metals. In this study, we investigated the role of the genes from the Nramp family from *Arabidopsis* (*AtNramps*) in yeast and in *Arabidopsis*. We cloned several *AtNramp* cDNAs and showed that these genes complement the phenotype of a metal uptake deficient yeast strain, *smf1*. This strain fails to grow on synthetic medium containing high concentrations of the divalent cation chelator EGTA. Expression of *AtNramp1*, 3 and 4 restores the growth of *smf1* yeast on synthetic medium containing 20 mM EGTA. Expression of *AtNramp1*, 3 and 4 also increases cadmium sensitivity in wild type yeast. *AtNramp3* and 4 complement an iron uptake mutant in yeast. This suggests a possible involvement in iron transport in plants and reveals heterogeneity in the functional properties of Nramp transporters. In *Arabidopsis*, *AtNramps* are expressed in both roots and aerial parts under metal replete conditions. *AtNramp3* is specifically induced by iron starvation. A plant with a T-DNA insertion in the 3' end of *AtNramp3* gene was identified. RT-PCR experiments show that *AtNramp3* mRNA is not detectable in these mutant plants. Furthermore, the truncated cDNA corresponding to this mutant gene is no longer able to complement the *smf1* yeast strain phenotype. Disruption of *AtNramp3* leads to moderate cadmium resistance of root growth in *Arabidopsis*, likely due suppression of *AtNramp3* mediated cadmium uptake. We selected two independent lines with elevated *AtNramp3* mRNA levels carrying homozygous single T-DNA insertions. Both over-expressing lines display increased cadmium sensitivity of root growth, likely due to an increase in *AtNramp3* mediated cadmium uptake. Our results point to *AtNramp* metal transporters as good candidates to engineer toxic metal sensitivity and metal accumulation in plants (Thomine et al., 2000).

References

- Clemens, S, D.M. Antosiewicz, J.M. Ward, D.P. Schachtman and J.I. Schroeder. *Proc. Natl. Acad. Sci. (USA)*, 95:9773-9778 (1998).
- Clemens, S., Kim, E., Neumann, D., Schroeder, J. *EMBO J.* 18 (1999): 3325-3333.
- Ha, S-B., Smith, A., Howden, R., Dietrich, W., Bugg, S., O'Connell, M., Goldsbrough, P., Cobbett, C. *Plant Cell*, v. 11, n. 6 (1999): 1153-1163.
- Lee, D. A., and Schroeder, J. I. Screening for Increased Arsenic Tolerance in *Arabidopsis*. Submitted for *Arabidopsis Laboratory Manual* (Glazebrook, J., Preuss, D., Weigel, D. ed.) Cold Spring Harbor Laboratory Press. (in press)
- Schachtman, D.P., Kumar, R., Schroeder, J.I. and Marsh, E.L. *Proc. Natl. Acad. Sci. (USA)*, 94:11079-11084 (1997).
- Thomine, S., Wang, R., Ward, J.M., Crawford, N.M. and Schroeder, J.I. *Proc. Natl. Acad. Sci. (USA)* 97:4991-4996 (2000).
- Vatamaniuk, O., Mari, S., Lu, Y-P., Rea, P. *Proc. Nat. Acad. Sci. USA*, v. 96, n. 12 (1999): 7110-7115.