Abstract – NSF Project IOS-1456361

a. Nontechnical description

Some plants have the fascinating property that they hyperaccumulate a particular toxic element to levels that are 100-1000 fold higher than surrounding vegetation. Prince’s plume (Stanleya pinnata), from the mustard family, is such a plant. This native to the Western USA can hyperaccumulate selenium up to 0.5% of its dry weight. Selenium is a micronutrient for many life forms including humans, but toxic at high levels. It is very similar to sulfur, which is why most life forms cannot discriminate between the two elements. This hyperaccumulator, however, has the unique capacity to distinguish selenium (selenate) from sulfur (sulfate), and preferentially accumulates selenate. Hyperaccumulators also distinguish themselves by accumulating organic forms of selenium (aminoacids) where non-hyperaccumulator relatives accumulate inorganic selenium. This capacity likely explains their selenium tolerance. Questions addressed in this project are: what happened in evolution that let hyperaccumulation arise? Has S. pinnata evolved a selenate-specific transporter? That would be a first for any organism. In what way is such a transporter different? And which gene is key for its capacity to produce organic selenium? Based on experiments so far, a candidate selenate transporter has been identified, as well as a possible key gene for the accumulation of organic selenium. These genes will be investigated in this project by expressing them in both yeast and in the model plant species Arabidopsis: a nonaccumulator from the mustard family. Why is this important? The results from this project are expected to provide better insight into mechanisms of Se hyperaccumulation: selenate/sulfate discrimination as well as enhanced Se uptake, assimilation and tolerance. Also, the genes from the hyperaccumulator may be used to breed plants with superior capacity to clean up selenium-polluted land or water (phytoremediation) and that can also serve as selenium-fortified crops with higher nutritional value. From a broader perspective, this project may serve as an evolutionary model to study plant adaptation to extreme soil conditions, and as a biochemical model to study mechanisms by which specificity of transporters and enzymes is controlled. These mechanisms are at the basis of metabolism and membrane transport.

b. Technical description

In a transcriptome sequencing study using selenium hyperaccumulator Stanleya pinnata and non-accumulator Stanleya elata, two genes stood out because their transcript levels were much higher in the hyperaccumulator: a high affinity sulfate/selenate transporter homologous to Arabidopsis thaliana Sultr1;2 (44x higher) and an ATP sulfurylase homologous to AtAPS2, key enzyme of sulfur/selenium assimilation (479x higher). These findings led to the hypotheses that (I) S. pinnata (Sp) Sultr1;2 is responsible for selenium hyperaccumulation and selenate-specific uptake in S. pinnata, and (II) SpAPS2 is key to the selenate assimilation into organic forms, and thus Se tolerance. The research objectives of this project are: (1) To determine whether the elevated expression levels of SpSultr1;2 and SpAPS2 are associated with elevated uptake rates of selenate and/or sulfate and elevated levels of ATP sulfurylase activity; (2) To analyze the intracellular location of the SpSULTR1;2 and SpAPS2 proteins, as well as their tissue-specific expression patterns; (3) To functionally analyze the sulfate and selenate transport properties of the S. pinnata and S. elata SULTR1;2 transporters in yeast (Saccharomyces cerevisiae); (4) To determine the physiological effects of expression of S. pinnata and S. elata Sultr1;2 and APS2 in Arabidopsis thaliana. Parameters that will be measured include uptake kinetics of selenate and sulfate (alone and in combination), concentration, forms and locations of selenium accumulated (using ICP-OES, LC-MS, XAS), and selenium tolerance. In yeast, structure-function studies will also be carried out, to study the importance of selected aminoacids for selenate specificity.