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**SUCROSE SYNTHASE: ELUCIDATION OF COMPLEX
POST-TRANSLATIONAL REGULATORY MECHANISMS
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S.C. Huber, USDA/ARS and Department of Plant Biology, University of Illinois

Final Report

The overall goals of the project are to determine the molecular bases for the localization of sucrose synthase (SUS) within heterotrophic cells, the role of phosphorylation of specific sites on the enzyme, and to identify the requisite protein kinases that phosphorylate this important enzyme. Progress has been made in several areas during FY2005, as detailed below.

SUS isoforms

Zea mays has three known sucrose synthase isoforms: SUS1, SH1, and SUS3. The *Sus1* and *Sh1* genes are functionally redundant and the encoded proteins are known to be very similar biochemically. The *Sus3* gene was only recently identified and little is known about the encoded SUS3 protein. Recent evidence using a peptide antibody that specifically recognizes the SUS3 isoform suggests that it is a ubiquitous protein. Abundant SUS3 protein is found in developing kernels, leaf midvein, internode cortex tissue, and etiolated roots, and thus, it will be important to characterize its properties. One difference among the isoforms is that SUS3 is not associated with cellular membranes *in vivo*, whereas SUS1 and SH1 are partially membrane bound. This suggests that SUS3 may play a unique role in the metabolism of sucrose in the plant cell cytoplasm, and if confirmed would be the first significant functional difference among the SUS isoforms.

Membrane-binding domains of SUS

As noted above, SUS is a soluble protein but two of the isoforms are also partially associated with plant membranes. It is possible that the subcellular localization of the enzyme affects how carbon is partitioned among competing pathways and thus, we would like to understand the basis for its association with cellular membranes. To do this, we are investigating the structural requirements for interaction of maize SUS1 with membranes using site-directed mutants of full length SUS1 and a series of truncation mutants, fused at the N-terminus to the maltose-binding protein (MBP). Collectively, the results suggest that two regions are involved in membrane binding: the non-catalytic N-terminus of SUS1 (residues 1 to 360) and a region that is likely to reside at the beginning of the catalytic domain (residues 360 to 460). During the past year, we have used site-directed alanine substitution mutants of residues within the catalytic domain to identify specific amino acids that may function in membrane binding. This ~100 amino acid

region within the catalytic domain was originally identified based on significant sequence similarity to the C-terminal pleckstrin homology (PH) domain in human pleckstrin, which is known to interact with membranes. Several Ala-substitutions within this region of SUS1 reduced membrane association with plant microsomes *in vitro* without reducing enzymatic activity, suggesting that it may be possible to control the localization of the enzyme *in vivo*.

SUS is generally considered to be tetrameric protein but recent evidence suggests that SUS can also occur as a dimeric protein. The formation of tetrameric SUS is regulated by sucrose concentration *in vitro* and this could also be an important factor in the cellular localization of the protein. We found that high sucrose concentrations, which promote tetramer formation, also inhibit the binding of SUS1 to actin filaments *in vitro*. Previously, high sucrose concentrations were shown to promote SUS association with the plasma membrane. The specific regions of the SUS molecule involved in oligomerization are not known, but we identified a region of the SUS1 molecule by bioinformatics analysis that was predicted to form a coiled coil. We demonstrated that this sequence could, in fact, self-associate as predicted for a coiled coil, but truncation analysis with the full-length recombinant protein suggested that it was not responsible for formation of dimers or tetramers. However, the coiled coil may function in binding of other proteins to SUS1. Overall, sugar availability may differentially influence the binding of SUS to cellular structures, and these effects may be mediated by changes in the oligomeric nature of the enzyme.

Possible Signaling function of SUS

Analysis with isoform-specific antibodies identified SH1 and SUS1 proteins in maize root and shoot mitochondria (mitoSUS). Interestingly, the SUS3 protein was not detectable in mitochondrial preparations despite its presence in the soluble fraction. Mitochondrial SUS was a minor fraction (<1%) of the total SUS, but may be significant and have important non-catalytic (and presently unrecognized) functions. Unlike the glycolytic enzymes that have been reported to be bound to the outside of mitochondria, SUS was intramitochondrial because the protein was thermolysin resistant in intact organelles but labile after Triton X-100 lysis. This observation, along with the lack of sucrose-phosphate synthase (another cytoplasmic protein) in mitochondrial preparations, rules out the possibility of cytoplasmic contamination as a source of mitoSUS. In maize primary roots, the mitochondrion-associated SUS was present mostly in the root tip, indicating a developmental regulation of SUS compartmentation. We also observed that SUS was co-immunoprecipitated with the voltage dependent anion channel (VDAC), which is the mitochondrial porin that is the most abundant protein of the outer mitochondrial membrane. This interaction may contribute to the association of SUS with mitochondrial membranes. The maize SH1 protein has a putative mitochondrial targeting peptide, whereas SUS1 and SUS3 do not; because both SUS1 and SH1 are apparently taken up into mitochondria, the presence or absence of a mitochondrial targeting peptide cannot be used to predict localization. However, in several plant species, at least one of the SUS proteins possesses a putative mitochondrial targeting peptide suggesting that the results are not unique to maize. While many questions remain to be answered, the preliminary results suggest that SUS may be associated with mitochondria, where it may

function not in sucrose catabolism but perhaps in signaling. If confirmed, SUS may be one of the growing number of “moonlighting” proteins that also have non-catalytic functions and contribute in unexpected ways to mediate sophisticated biological functions.

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