Regulation of Carbon Metabolism in Relation to Plant Productivity

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Abstract

Sustainable agriculture will be required to maintain the environment and to feed the growing world population. In addition to improved cultural practices (e.g., crop rotation, use of crop residues to build the soil, biological pest control), improved crops will certainly be part of the answer and genetically engineered plants will be an approach of choice. Early goals of genetic engineering were to transfer single genes that could influence pest resistance or herbicide resistance. Future goals will need to focus on multiple genes or global factors that impact pathways, and will also shift from gene identification to consider the impact of proteomics on phenotype. In addition, in order to impact on agriculture it will be necessary to involve teams of scientists that span traditional disciplinary boundaries. The University can truly function as an 'Architect of the New Century' by assembling such teams and providing the "seed" money needed to initiate programs that conduct fundamental research of applied problems that impact on agriculture. How might primary plant metabolism be manipulated to improve productivity or crop value? In general, we are trying to identify biological mechanisms controlling important plant processes that may provide opportunities for manipulation to produce 'improved' plants for agriculture. This paper briefly mentions a few examples from literature and focuses on some possible avenues for control of metabolism that are related to current research activities in the author's laboratory.

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Control of enzyme intracellular localization

Sucrose synthase (SuSy) is known to be an important enzyme involved in the initial metabolism of sucrose in many sink tissues. Recent results from several laboratories indicate that SuSy may be localized in several cellular compartments: 1) soluble in the cytosol (as traditionally assumed); 2) associated with membranes; and 3) associated with the actin cytoskeleton (Winter and Huber, 2000). The physiological significance of the enzyme in these different subcellular locations is not known, but may be important for directing the products of sucrose cleavage into specific biosynthetic pathways. Consequently, we are attempting to identify the lipid- and actin-binding domains on SuSy. As well, we are interested in what mechanisms control the distribution of enzyme among the three locations. In particular, efforts are focused on a possible role for phosphorylation of SuSy at the Ser-15 site. Controlling the intracellular localization of an enzyme may be one approach whereby manipulation of a single gene product can influence flux through metabolic pathways.

Control of enzyme degradation

The steady state level of an enzyme will reflect the balance between its synthesis and degradation. However, little is known about the mechanisms involved in degradation of plant metabolic enzymes. Recent studies of protein phosphorylation in our laboratory suggest that phosphorylation of certain sites may be a trigger for protein degradation via the 20S/26S proteasome. Phosphorylation of these 'cryptic sites' is somehow blocked in the native protein. Once they become accessible to protein kinases (perhaps in response to release of a binding protein or proteolytic nicking of the N- or C-terminus), the site(s) is phosphorylated and the protein is targeted for degradation. This appears to be the case for two different enzymes—maize SuSy and soybean cytosolic pyruvate kinase (PyrKinC). In the case of SuSy, phosphorylation of Ser-170 was predicted by sequence analysis. Sequence-and phosphorylation-state specific antibodies demonstrated that phosphorylation of Ser-170 occurs in vivo, but was primarily contained on fragments of SuSy that were associated with the 20S proteasome (Hardin et al. 2002). Similarly, phosphorylation of two sites on soybean PyrKinC occurred, but again

was contained on fragments associated with the 26S proteasome (Tang et al. 2002). Understanding the mechanisms that target metabolic enzymes for degradation may provide new strategies to control the steady state level of important enzymes and thereby influence flux through metabolic pathways.

14-3-3 proteins: factors that interact broadly.

The '14-3-3 proteins' are highly conserved eukaryotic proteins that function as binding proteins, usually targeting the sequence: R-x-x-phosphoS-x-P. They are involved in many important plant processes; including the regulation of nitrate reductase activity in leaves in response to light/dark transitions (Kaiser and Huber, 2001) and subsequently have been shown to interact with a broad array of metabolic enzymes and trans factors. Because of their broad interactions, manipulation of 14-3-3s may offer the possibility to up- or down-regulate an entire pathway. We have been studying the regulation of 14-3-3 binding to target proteins, using phosphorylated nitrate reductase (pNR) as a model system. Binding of 14-3-3s to pNR requires a divalent cation or polyamine, which first bind to the 14-3-3 protein in the region of loop 8 (located near the C-terminus between helix 8 and 9; Athwal and Huber, 2002). The resulting conformational change partially involves displacement of the C-terminal tail, which functions as an autoinhibitor (Shen et al. 2002). Activation mutants are currently being expressed in transgenic plants to investigate the significance of cation regulation in vivo. Recent evidence also indicates that binding of 14-3-3s to pNR can influence its degradation. We are speculating that uncomplexed pNR may be selectively degraded, which would provide a mechanism to insure balance between the level of 14-3-3s and their target proteins. This is especially critical for NR, but if generally applicable, suggests that manipulation of 14-3-3 expression may impact the level of target proteins by affecting their degradation.

How these, and other, biological mechanisms can be exploited to increase plant performance in sustainable systems is a continuing challenge for the future.

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