IN THIS ISSUE

Peroxisomal Citrate Synthase Provides Exit Route from Fatty Acid Metabolism in Oilseeds

The seed of oilseed plants, including Arabidopsis and several crop species, such as soybean, sunflower, and canola, contain a major store of oil in the form of triacylglycerol (TAG), which provides carbon and energy for seed germination and seedling growth. During germination and seedling development, TAG stored in oil bodies is broken down into fatty acids through the action of lipases. Fatty acids are then transported into specialized organelles called peroxisomes, which contain enzymes that catalyze β-oxidation to produce acetyl-CoA. Fatty acid β-oxidation provides carbon for sucrose synthesis in the cytosol (gluconeogenesis) and also substrates for energy production in mitochondria (respiration).

FATTY ACID β-OXIDATION AND THE GLYOXYLATE CYCLE

Peroxisomal actetyl-CoA is routed into gluconeogenesis through the glyoxylate cycle, a modified form of the respiratory citric acid cycle that bypasses the decarboxylative steps to allow net production of carbon skeletons with no carbon lost as CO₂ (reviewed in Eastmond and Graham, 2001). The five key enzymes of the glyoxylate cycle are considered to be citrate synthase (CSY),aconitase (ACO),isocitrate lyase, malate synthase, and NAD malate dehydrogenase (MDH). Three of these enzymes are located in the peroxisome, and the other two, ACO and MDH, function in the cytosol. Peroxisomal MDH is thought to operate principally in the production of malate from oxaloacetate (the opposite direction of the glyoxylate cycle) to regenerate NAD from NADH produced during β-oxidation (Cornah and Smith, 2001; S. Smith, personal communication). Operation of the glyoxylate cycle therefore requires transport of citrate, isocitrate, malate, and oxaloacetate across the peroxisomal membrane (see figure).

The proportion of TAG that is converted into sucrose or respired varies considerably depending on plant tissue, stage of development, and plant species. The glyoxylate cycle and gluconeogenesis are thought to be of major importance in oilseed plants during seedling emergence and early seedling development. During this period of early seedling growth, there is a concerted increase in the activities of enzymes of TAG breakdown, fatty acid β-oxidation, the glyoxylate cycle, and gluconeogenesis, which decreases as TAG stores decline and the seedling gains photosynthetic competence (Cornah and Smith, 2002). Historically, fatty acids were not considered to be major substrates for mitochondrial respiration, but there is a growing appreciation of the importance of fatty acid respiration in developing seedlings and mature plants. It has been shown that fatty acid respiration is important during the early stage of oilseed germination (Salon et al., 1988; Raymond et al., 1992) and also in senescing and sugar-starved mature tissues (Dieuaide et al., 1992).

HOW DOES CARBON EXIT PEROXISOMES?

However, the transport of the products of β-oxidation out of the peroxisome and into mitochondria is not well understood (Hooks, 2002). One model involves transport of citrate out of the peroxisome and citrate or isocitrate into mitochondria (Raymond et al., 1992; Eastmond and Graham, 2001; Cornah and Smith, 2002). Another possibility is the transport of acetyl units from acetyl-CoA via a carnitine shuttle, which is the major pathway of fatty acid respiration in yeast (van Roermund et al., 1999). Lawand et al. (2002) showed that an acylcarnitine carrier-like protein, named A BOUT DE SOUFFLE (BOU), is located in the mitochondrial membrane in Arabidopsis, and bou mutants exhibit decreased TAG mobilization and stop developing after seed germination, suggesting that a carnitine shuttle similar to that of yeast may operate in plants. In this issue of The Plant Cell, Pracharoenwattana et al. (pages 2037–2048) show that peroxisomal CSY is required for seed germination and the mobilization of TAG and subsequent seedling growth.

PEROXISOMAL CSY IS ESSENTIAL FOR SEED GERMINATION

Arabidopsis has three genes predicted to encode peroxisomal CSY, which the authors called CSY1, CSY2, and CSY3. Gene-specific primers were designed for each of these genes, and the authors show that CSY2 and CSY3 are expressed strongly throughout seedling development and in the mature shoot. Further experiments using the CSY2 and CSY3 peroxisomal targeting sequences fused to green fluorescent protein confirmed peroxisomal targeting of the CSY2 and CSY3 proteins. The authors next constructed knockout csy3 and csy2 single mutants and crossed these mutants to obtain the csy2 csy3 double mutant. The single mutant seedlings were slightly smaller than the wild type but otherwise showed no obvious phenotypic defects, and growth of the mutants beyond the seedling stage was indistinguishable from the wild type. However, no csy2 csy3 double mutant seedlings were observed in the F2 generation of single mutant crosses. A small number of seeds from the single mutant crosses failed to germinate unless surgically disrupted to remove the seed coat and incubated in the presence of sucrose, and molecular analysis of these seedlings confirmed that they were homozygous csy2 csy3 double mutants. The double mutant phenotype could also be complemented by transformation with various CSY3 cDNA constructs, which
confirmed that the phenotype was caused specifically by the lack of peroxisomal CSY. These experiments showed that CSY2 and CSY3 have overlapping or redundant functions, but CSY activity provided by one or both of the proteins together is required for germination and seedling development.

**LACK OF CSY CAUSES A BLOCK IN β-OXIDATION**

The authors next sought to determine if the csy2 csy3 double mutant plants were blocked in fatty acid β-oxidation and TAG mobilization or if the block was specific to the glyoxylate cycle and gluconeogenesis. Transmission electron microscopy was conducted on sections of wild-type and rare csy2 csy3 double mutant seedlings that germinated spontaneously. At 5 d after germination, lipid bodies persisted in mutant seedlings but were no longer present in the wild type, and peroxisomes were enlarged relative to the wild type, consistent with a block in TAG mobilization. Embryos were also removed from wild-type and dormant csy2 csy3 mutant seeds and grown for 5 d and TAG content analyzed by gas chromatography and mass spectrometry. In the wild-type embryos, TAG content declined rapidly and was almost absent by day 5, whereas in the mutant seedlings it remained high. The authors also examined β-oxidation with the use of the proherbicide 2,4-dichlorophenoxybutyric acid (2,4-DB), which is converted by peroxisomal β-oxidation to 2,4-D, leading to severe inhibition of root growth. It has been shown that seedlings blocked in β-oxidation are resistant to 2,4-DB. Homozygous double mutant seedlings grown from embryos removed from dormant seed were found to be resistant to 2,4-DB (but sensitive to 2,4-D), whereas the wild-type seedlings were sensitive. These experiments indicate that a lack of CSY activity causes a block in fatty acid β-oxidation, and there is not an alternate route for the metabolism of acetyl-CoA.

The block in fatty acid β-oxidation is likely the result of a buildup of acetyl-CoA, which is the substrate for CSY. The work of Pracharoenwattana et al. now show that TAG mobilization and transport of the products of fatty acid β-oxidation out of the peroxisome require CSY activity, suggesting that citrate export is the major exit route. Glyoxylate cycle reactions are marked with red arrows.
mobilization. Cytosolic citrate can be converted into isocitrate by ACO and redirected into the peroxisome to enter the glyoxylate cycle, or it can be transported into mitochondria to serve as a substrate for respiration. It will be important to determine if peroxisomal CSY plays a similarly critical role in nonoilseed plants. In cereals, for example, carbon is stored in the endosperm mainly as starch, but a considerable amount of TAG is found in seed aleurone layer and in the scutellum (cotyledon) of developing seedlings (Cornah and Smith, 2002). Dieuade et al. (1992) showed that fatty acid β-oxidation plays a role in respiration in senescing and sugar-starved maize roots. Thus, fatty acid β-oxidation, and likely CSY function, may be important in a variety of plant tissues throughout development in nonoilseed as well as oilseed plants.

The role of BOU also remains to be determined, but the work of Pracharoenwattana et al. suggests that it does not function in the principal pathway of TAG mobilization during germination and seedling development in Arabidopsis. BOU activity is required for seedling establishment in the light but not in the dark, and the bou mutant is blocked in the synthesis of polar lipids (Lawand et al., 2002), suggesting that it may have a more specific function in membrane biogenesis. The work of Pracharoenwattana et al. establishes that citrate is the major route for carbon exiting the peroxisome, and peroxisomal CSY is a key enzyme in fatty acid metabolism leading both to the glyoxylate cycle and to respiration.

Nancy A. Eckardt
News and Reviews Editor
neckardt@aspb.org

REFERENCES
Peroxisomal Citrate Synthase Provides Exit Route from Fatty Acid Metabolism in Oilseeds

Nancy A. Eckardt

*Plant Cell* 2005;17:1863-1865
DOI 10.1105/tpc.105.034843

This information is current as of September 28, 2017