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The essential role of plastidial triose phosphate isomerase in the integration of seed reserve mobilization and seedling establishment

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Seed germination and seedling establishment are pivotal for the life cycle of seed plants. Storage reserve mobilization provides energy and carbon to support seedling development. Seedling establishment involves root elongation and plastid transition from a heterotrophic to photoautotrophic state, such that the seedling can attain independence once seed reserves have been depleted. At the biochemical level, this transition is likely complicated as it requires a spatial and temporal shift from degradation to synthesis for many metabolic pathways. The triose phosphate isomerase (TPI) catalyzes the reversible conversion of dihydroxyacetone phosphate (DHAP) to glyceraldehyde-3-phosphate (GAP) and is involved in many pathways including glycolysis, Calvin cycle, and glycerol metabolism. Plants contain both cytosolic and plastid forms of TPI and neither have been extensively characterized, presumably because TPI catalyzes a reversible reaction (i.e., substrate/product are in equilibrium) and is therefore unlikely to be of regulatory importance. In the recent study,[1] we discovered a knockdown mutant for pdTPI that reveals this enzyme plays a crucial, metabolic role during the heterotroph/autotroph transition phase, affecting both chloroplast development and seedling establishment. Inability of a functional cytosolic TPI to compensate for reduced pdTPI expression demonstrates plastid and cytosolic pools of DHAP and GAP are not in equilibrium and may reveal a novel plastid translocator.

Key words: triose phosphate isomerase, chloroplast, DHAP, methylglyoxal, triose phosphate translocator

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TPI Forms are Present in Both the Cytosol and Plastid in Plants

In the Arabidopsis genome there are two genes annotated as TPI: one is localized to the cytoplasm (cytoTPI, At3g55440), and the second copy contains a canonical plastid localization sequence (pdTPI, At2g21170). Two different methods confirmed plastid compartmentalization: biochemical subcellular fraction followed by immunoblotting; and fluorescence microscopic localization of pdTPI-GFP fusion proteins in Arabidopsis.[1] Both results agreed with the conclusion that pdTPI is localized to the plastid. Interestingly, pdTPI was shown to have two different splice variants (At2g21170.1 and At2g21170.2). The At2g21170.2 splice variant contains a nine amino acid deletion compared to At2g21170.1, and the first three of these amino acids are highly conserved among all TPIs. Therefore, an open question is whether the At2g21170.2 splice variant encodes an active TPI protein. To confirm the presence of this alternatively spliced transcript and its expression pattern, RT-PCR was performed.[1] While At2g21170.1 was ubiquitously expressed in roots, stems, leaves, flowers and siliques At2g21170.2 was only present in roots, suggesting perhaps a tissue-specific role.

pdTPI Deficiency Perturbs Multiple Metabolic Pathways in Chloroplasts

pdTPI occupies a strategically important position in plastid metabolism by reversibly isomerizing DHAP to GAP (**Fig. 1**). In the pdTPI knock-down mutant,

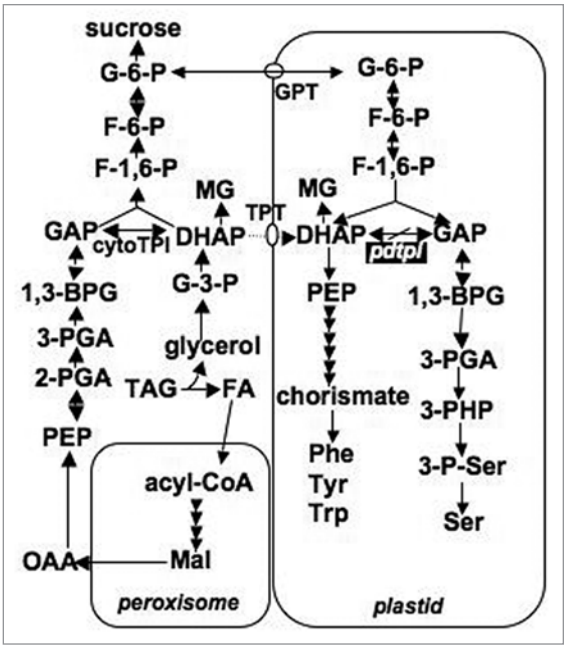

Figure 1. Metabolic pathways affected by a mutation in *pdTPI* during post-germinative seedling transition from heterotrophic to photoautotrophic development. Abbreviations, G-6-P: D-glucose-6-phosphate, F-6-P: D-fructose-6-phosphate, F-1,6-P: fructose-1,6-bisphosphate, DHAP: dihydroxyacetone phosphate, MG: methylglyoxal, GAP: glyceraldehyde-3-phosphate, G-3-P: glycerol-3-phosphate, MG: methylglyoxal, 1,3-BPG: 1,3-diphosphateglycerate, 3-PGA: 3-phosphoglycerate, 2-PGA: 2-phosphoglycerate, PEP: phosphoenolpyruvate, TAG: triacylglycerol, FA: fatty acid, Ac-CoA: acetyl-CoA, Mal: malate, OAA: oxaloacetate, 3-PHP: 3-phosphohydroxypyruvate, 3-P-Ser: 3-phosphoserine, Ser: serine, Phe: phenylalanine, Tyr: tyrosine, Trp: trytophan, TPT: triose phosphate translocator, GPT: glucose 6-phosphate/phosphate translocator.

DHAP and G-3-P pool levels increased up to 5-fold but GAP was reduced by 60%. Since cytosolic TPI activity was unaffected in this mutant and therefore capable of equilibrating DHAP and GAP levels, it is evident that these metabolites can not freely traverse the plastid envelope membrane either by translocator or passive diffusion.

Triose phosphates are the precursors for multiple biosynthesis pathways including glycerolipids and isoprenoids, as well as intermediates for glycolysis and the Calvin cycle. DHAP is also involved in the shikimate pathway for the biosynthesis of tryptophan, tyrosine and phenylalanine in the plastid (**Fig. 1**). Lipid profiling revealed significant changes in glycerolipid composition and content.[1] Gross morphological changes in plastid structure were also observed in the *pdtpi* mutant including a dramatic reduction in starch accumulation.[1] Given the morphological changes to the plastid and overall dwarf stature, it is likely that many (if not all) of the pathways that triose phosphates feed into are affected in this mutant.

A recent characterization of a plastidial glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mutant suggested that GAP can be converted to 3-PGA by the sequential reaction of GAPDH and phosphoglycerate kinase,[2] and 3-PGA is converted to serine via a series of reactions catalyzed by 3-phosphoglycerate dehydrogenase, 3-phosphoserine aminotransferase, and 3-phosphoserine phosphatase in non-photosynthetic tissues (**Fig. 1**).[3] As a result the GAPDH mutant has lower serine content. The *pdtpi* mutant shares a similar general phenotype as the GAPDH mutant including arrested root growth, reduced stature, and sterility. Since the *pdtpi* mutation reduces GAP supply in the plastid, presumably it will also affect Ser biosynthesis although this has not yet been verified. Disruption of amino acid synthesis (e.g., Ser, Trp, Tyr, Phe) in the *pdtpi* mutant could affect plastid protein synthesis, possibly explaining reduced levels of Rubisco large subunit in this mutant.[1]

Does Triose Phosphate Freely Exchange between the Cytoplasm and Plastid?

In the *pdtpi* mutant, cytoTPI protein expression was unaffected.[1] If triose phosphate can freely exchange between these two subcellular compartments, a functional cytoTPI would complement a deficient pdTPI, and the *pdtpi* mutant would not show any severe phenotype. However, this was not the case, therefore favoring the presence of two distinct and non-equilibrated pools for these two metabolites. Supporting this observation, DHAP accumulated while GAP levels decreased in the *pdtpi* mutant suggesting that cytosolic-produced DHAP, likely from the oxidation of glycerol-3-phosphate (i.e., triacylglyerol catabolism), is imported into plastids. The phenotype of this mutant and accumulation of DHAP (presumably the plastid pool of DHAP) suggests that DHAP does not simply passively diffuse across the plastid envelope. A DHAP plastid translocator is necessary to explain these observations; and we discuss some possibilities.

The recent confirmation of TPT expression in proplastids led us to speculate that TPT might assume this role.[4] It is possible that TPT is capable of reversibly transporting both DHAP and GAP with similar efficiency. If true, triose phosphate homeostasis in the plastid should not be influenced by reduced expression of pdTPI and no phenotype would be observed for the *pdtpi* mutant. Therefore, one way to explain the mutant phenotype and the biochemical data is that the TPT translocator prefers DHAP to GAP as a substrate. Interestingly, it was previously observed that cytoTPI has a higher affinity toward GAP than DHAP, which could affect the equilibrium of this reaction and result in higher DHAP content.[5,6]

Another explanation is that an alternative translocator, perhaps one that has yet to be discovered, is responsible for shuttling DHAP into developing seedling plastids. One possible alternative is the glucose 6-phosphate/phosphate translocator (GPT). In an in vitro liposome assay recombinant GPT was capable of transporting glucose-6-phosphate (Glu-6-P) as well as triose phosphate, although this

apparent promiscuity was not confirmed in planta.[7] Also, the GPT has been verified to be expressed in proplastids, in cauliflower meristematic tissues.[4] Whether there is a third, unidentified transporter specific for DHAP is still an open question. The possibility that there are multiple plastid translocators capable of shuttling DHAP could reflect the fact that the demand for DHAP (and GAP) varies among plastid types, and during their transition to a chloroplast, since these metabolites are consumed in diverse metabolic pathways whose activity may differ significantly. This metabolic diversity offers an explanatory role of pdTPI in plastid metabolism, to reversibly control DHAP or GAP supply to meet the dynamic metabolic need for diverse pathways in the plastid.

Accumulation of Methylglyoxal could Exacerbate Disruption of Plastid Metabolism

pdTPI deficiency resulted in a 2-fold increase in cellular methylglyoxal (MG), a non-enzymatic by-product of DHAP.[1] It was previously shown that MG is reactive with Lys and Arg amino acids of certain proteins, forming glycation end products.[8] We demonstrated that seed sown in the present of MG results in stunted plants at concentrations as low as 0.3 mM.[1] Precursors to MG including glycerol, glycerol-3-phosphate, and DHAP were also toxic to seedlings although at higher concentrations; 25 mM, 1 mM and 0.7 mM, respectively. Apparently, the three step shunt from glycerol to DHAP (necessary for the complete breakdown of triacylglycerol) in germinating seeds has the potential to produce sufficient DHAP that it becomes cytotoxic, either directly or as the phosphate elimination product MG (**Fig. 1**). It is therefore possible that DHAP and MG buildup in pdTPI mutants could compound the triose phosphate imbalance in plastids, to produce the severely dwarfed phenotype.

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