PHOSPHATE TRANSLOCATORS IN PLASTIDS

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ABSTRACT
During photosynthesis, energy from solar radiation is used to convert atmospheric carbon dioxide into intermediates that are used within and outside the chloroplast for a multitude of metabolic pathways. The daily fixed carbon is exported from the chloroplasts as triose phosphates and 3-phosphoglycerate. In contrast, nongreen plastids rely on the import of carbon, mainly hexose phosphates. Most organelles require the import of phosphoenolpyruvate as an immediate substrate for carbon to enter the shikimate pathway, leading to a variety of important secondary compounds. The envelope membrane of plastids contains specific translocators that are involved in these transport processes. Elucidation of the molecular structure of some of these translocators during the past few years has provided new insights in the functioning of particular translocators. This review focuses on the characterization of different classes of phosphate translocators in plastids that mediate the transport of the phosphorylated compounds in exchange with inorganic phosphate.

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INTRODUCTION

Communication between plastids and the surrounding cytosol occurs via the plastid envelope membrane. The inner envelope membrane contains a variety of transporters that mediate the exchange of metabolites between both compartments (19). Carbon fixed during the day can be exported from the chloroplasts into the cytosol for the synthesis of sucrose, which is subsequently allocated to heterotrophic organs of the plant such as roots, seeds, fruits, or tubers. Export of the newly fixed carbon in the form of triose phosphates (and 3-phosphoglycerate) proceeds via the triose phosphate/phosphate translocator (TPT). During the night, the breakdown products of transitory starch are exported in the form of glucose via a glucose translocator (58). Plastids are also involved in nitrogen assimilation, the synthesis of amino acids and fatty acids, and in the synthesis of a series of plant secondary products that are formed via the shikimic acid pathway. This pathway requires the provision of the plastids with phosphoenolpyruvate (PEP) as an immediate precursor. The nature of a corresponding transporter has remained elusive until the recent discovery of the plastidic PEP/phosphate translocator (PPT) (11). Plastids of nonphotosynthetic tissues have to import carbon as a source of energy and for driving biosynthetic pathways, for example, leading to fatty acids, amino acids, or starch. Carbon import into nongreen plastids can proceed in the form of hexose phosphates via a recently discovered glucose 6-phosphate/phosphate translocator (GPT) (37). This review focuses on the characterization of the different classes of phosphate translocators that are present in plastids of various plant tissues.

MEASUREMENTS OF PLASTIDIC PHOSPHATE TRANSPORT ACTIVITIES

Background

A basic technique for measurements of metabolite transport in plastids is the silicone oil filtering centrifugation method (29). Microfuge tubes are filled with a bottom layer of a denaturating solution (e.g. perchloric acid), followed by a layer of silicone oil. The plastids are pipetted on top of the oil, incubated with a radioactively labeled substrate, and subsequently centrifuged through the silicone oil layer, whereby the transport reaction is terminated. A more
advanced technique is the double layer silicone oil centrifugation system, which consists of an aqueous substrate layer sandwiched between two silicone oil layers. This allows measurements of uptake rates in the range of 1 to two seconds (24, 34). Alternatively, transporters, especially antiporters, can be measured by reconstitution into artificial membranes (liposomes) that have been prepared by sonification of phospholipids in a buffered solution containing an exchangeable substrate. Incorporation of detergent-solubilized proteins into the liposomal membrane can be achieved by a freeze/thaw step (40) and the external substrate can subsequently be removed by gel filtration of the proteoliposomes. The reconstituted transport activity can be assessed by measuring the amount of radioactively labeled substrate that is transported into the liposomes. Using various metabolites for preloading of the liposomes, the transport characteristics of an antiporter can be determined, i.e. which countersubstrate can be exchanged by the reconstituted translocator. The reconstitution method can also be used to follow a particular transport activity during purification (11, 18, 37, 46). Not only can highly purified translocator proteins be functionally reconstituted into liposomes but also whole membranes, plastids, or even crude homogenates from different plant tissues (20). The reconstitution system thus allows direct access to antiporters of different plant tissues without the necessity of isolating intact organelles.

The Triose Phosphate/Phosphate Translocator

The triose phosphate/phosphate translocator (TPT) of chloroplasts was the first phosphate translocator to be described in terms of substrate specificities and kinetic constants (12). It mediates the export of fixed carbon in the form of triose phosphates and 3-phosphoglycerate from the chloroplasts into the cytosol. The exported photosynthates are then used for the biosynthesis of sucrose and amino acids and the released phosphate is shuttled back into the chloroplasts via the TPT for the formation of ATP (see Figure 1).

In its functional form, the TPT is a dimer composed of two identical subunits (13, 74). As substrates, the TPT accepts either inorganic phosphate or a phosphate molecule attached to the end of a three-carbon chain, such as triose phosphates or 3-phosphoglycerate. C3-compounds with the phosphate molecule at C-2 position, for example, phosphoenolpyruvate, 2-phosphoglycerate, are only poorly transported (12). Under physiological conditions, the substrates are transported via a strict 1:1 exchange. Transport proceeds via a ping-pong type of reaction mechanism, i.e. the first substrate is transported across the membrane and then leaves the transport site before the second substrate can be bound and transported. The transport site thus alternatively faces either membrane side, thereby transporting substrates in opposite directions (15). Furthermore, only the transport site facing the cytosol is accessible to inhibitors of the TPT, namely
Figure 1 Transport processes mediated by the TPT protein and the PPT protein. 3-PGA, 3-phosphoglycerate; DAHP, 3-deoxy-D-arabino-heptulosonate 7-phosphate; Ery4P, erythrose 4-phosphate; Fru6P, fructose 6-phosphate; Fru1,6P₂, fructose 1,6-bisphosphate; GT, glucose translocator; Pi, inorganic phosphate; PEP, phosphoenolpyruvate; Phe, phenylalanine; PPT, phosphoenolpyruvate/phosphate translocator; RuBP, ribulose 1,5-bisphosphate; TPT, triose phosphate/phosphate translocator; TrioseP, triose phosphates; Tyr, tyrosine; Try, tryptophane. For details, see text.

pyridoxal 5'-phosphate and 4,4'-diisothiocyanostilbene-2,2'-disulfonate. This asymmetry in the structure of the transport site is linked to different transport affinities on both membrane sides; these are about five times lower on the stromal side of chloroplasts (15).

In intact chloroplasts, unidirectional transport of phosphate can be observed but with a \( V_{\text{max}} \) that is two to three orders of magnitude lower than that of the antiport mode (12, 49). Using the reconstituted system in which the concentrations of phosphate in both the internal and the external compartments are accessible to experimental variations, the transport activity of the reconstituted TPT can reach values that exceed those measured for an antiport mode by at least one order of magnitude. It is suggested that transport under these conditions proceeds by a mechanism different from the antiport mode, probably by a (channel-like) uniport mechanism. Evidence for ion channel properties of the TPT is provided (a) by decreasing the activation energy for phosphate transport from 46 kJ/mol (antiport mode) to 18 kJ/mol (uniport mode), a value that is in the
range observed for ion channels and (b) by measuring the TPT-mediated unidirectional transport by the patch-clamp technique (64). It can be concluded from these electrophysiological experiments that the TPT can behave as a voltage-dependent ion channel, preferentially permeable to anions, as well as an antiporter. As suggested by Saier (57), different classes of transporters might share common structural motifs and may have arisen from a common ancestor. A small structural change within the translocation pore might then allow transport via an ion channel mode or might result in strong coupling of substrate binding linked to conformational changes, as observed for transporters operating in the antiport mode.

**The Phosphoenolpyruvate/Phosphate Translocator**

Mesophyll chloroplasts of C₄-plants possess a TPT-like translocator that mediates the export of phosphoenolpyruvate (PEP) from the chloroplasts as substrate for the PEP carboxylase in the cytosol. The resulting inorganic phosphate is shuttled back into the chloroplasts via this translocator. It has been suggested that the phosphate translocator from C₄-mesophyll chloroplasts transports, in addition to the substrates of the TPT, also PEP (7, 10, 24, 35, 56). In view of the recently discovered phosphoenolpyruvate/phosphate translocator (PPT) that transports only PEP and inorganic phosphate but accepts triose phosphates and 3-phosphoglycerate only very poorly, it can now be suggested that mesophyll chloroplasts contain two different phosphate translocators, a TPT that is involved in the triose phosphate/3-phosphoglycerate shuttle and a PPT that transports PEP in exchange with inorganic phosphate.

PEP is only very poorly transported by the TPT of C₃-chloroplasts (11, 12, 24). However, a PEP transport activity is also present in chloroplasts or, at least, in a subtype of plastids that is present in preparations of mesophyll chloroplasts. Reconstitution of chloroplasts or chloroplast envelope membranes (21, 44) always shows a low, but significant transport activity of PEP that cannot be attributed to TPT. A PEP/phosphate exchange activity was also detected in nongreen plastids, including plastids from pea roots (2), tomato fruit plastids (62), cauliflower bud plastids (20), maize kernels (16), and sweet pepper plastids (70).

In all types of plastids, PEP serves different functions, e.g. as precursor for the biosynthesis of fatty acids or of aromatic amino acids. PEP is an immediate substrate for the plastid-localized shikimate pathway leading, via the synthesis of aromatic amino acids, to a large number of secondary metabolites, e.g. alkaloids, flavonoids, and lignins (see Figure 1). Apart from plastids of lipid-storing tissues, most chloroplasts, e.g. from pea, spinach, or Arabidopsis (1, 61, 65, 73), and nonphotosynthetic plastids, e.g. from pea roots (2) or cauliflower buds (36), are unable to convert 3-phosphoglycerate into PEP via the glycolytic pathway. Due to the absence or low activities of phosphoglucomutase and/or enolase,
glycolysis cannot proceed further than to 3-phosphoglycerate (47). Therefore, these plastids rely on the supply of PEP from the cytosol.

**The Hexose Phosphate/Phosphate Translocator**

Nongreen plastids of heterotrophic tissues are carbohydrate-importing organelles and, in the case of amyloplasts in storage tissues, the site of starch and/or fatty acid synthesis. Since these plastids are normally unable to generate hexose phosphates from C3-compounds owing to the absence of fructose 1,6-bisphosphatase activity (9), they rely on the import of cytosolically generated hexose phosphates that are formed from sucrose delivered from source tissues. Sucrose is unloaded from the phloem either via symplasmic connections or via the apoplast and is cleaved by either invertase or sucrose synthase. The resulting hexoses are then converted into hexose phosphates and imported into the plastids as the source of carbon for starch and fatty acid biosynthesis and, in addition, as a substrate for the oxidative pentose phosphate pathway (see Figure 2). This pathway can deliver reductants for nitrogen metabolism and fatty acid biosynthesis (2, 3, 22, 39).

The results of transport measurements with intact organelles or reconstituted tissues from different plants (e.g. pea roots, pea embryos, cauliflower inflorescences, maize endosperm, potato tubers, pepper fruits) suggest that the hexose phosphate transport is mediated by a phosphate translocator that imports hexose phosphates in exchange with inorganic phosphate or C3-sugar phosphates (2, 16, 20, 32, 33, 50, 53, 59, 62). In sink tissues from most plants studied to date, glucose 6-phosphate (Glc6P) is the preferred hexose phosphate taken up

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**Figure 2** Proposed function of the GPT protein in heterotrophic tissues. GPT, glucose 6-phosphate/phosphate translocator; Glc6P, glucose 6-phosphate; Ru5P, ribulose 5-phosphate; P_i, inorganic phosphate; SuSy, sucrose synthase; TrioseP, triose phosphates. For details, see text.
by nongreen plastids. However, in amyloplasts from wheat endosperm, glucose 1-phosphate (Glc1P) rather than Glc6P is the precursor for starch biosynthesis (68, 69, 72).

In conclusion, nongreen plastids appear to possess a phosphate translocator that is specific for the transport of phosphate, phosphorylated C3-compounds and hexose phosphates, which suggests that these transport processes are mediated by a TPT-like phosphate translocator with an extended substrate specificity (31).

A Glc6P transport activity is also present in chloroplasts from guard-cells (51). Like nongreen plastids, these chloroplasts are devoid of fructose 1,6-bisphosphatase activity (27), the key enzyme for the conversion of triose phosphates into hexose phosphates, and, therefore, rely on the provision of hexose phosphates for starch biosynthesis. Starch is mobilized during stomatal opening and converted to malate that is then used as a counterion for potassium.

A hexose phosphate/phosphate transport activity can also be detected in spinach chloroplasts after feeding of detached leaves with glucose (54). The chloroplasts of these leaves are photosynthetically active but contain unusually large quantities of starch. It could be shown that the precursor for starch biosynthesis is imported into the chloroplasts in the form of Glc6P by a hexose phosphate/phosphate translocator, as is the case for heterotrophic plastids in sink tissues. Glucose-feeding has obviously induced a switch in the function of the chloroplasts from carbon-exporting source organelles to carbon-importing sink-organelles and has led to the induction of a sink-linked plastidic hexose phosphate/phosphate transport activity.

MOLECULAR IDENTIFICATION AND CHARACTERIZATION OF PLASTIDIC PHOSPHATE TRANSLOCATORS

We used a biochemical approach to clone cDNAs coding for plastidic transport systems. The particular membrane proteins were purified to homogeneity, cleaved by endoproteases, and the resulting peptides were then used to design oligonucleotides and to generate PCR-fragments for screening of cDNA libraries.

The spinach TPT was the first plant membrane transport system for which the primary sequence could be determined (17). Meanwhile, TPT-sequences are available from various plants, e.g. those from Arabidopsis, pea, potato, maize, Flaveria, and tobacco. All TPT-sequences have a high similarity to each other (10, 11, 41, 77).

More recently, we isolated cDNAs coding for two other plastidic phosphate translocators from heterotrophic tissues: the phosphoenolpyruvate/phosphate
translocator (PPT) from maize endosperm, maize roots, cauliflower buds, tobacco leaves, and Arabidopsis leaves, and the Glc6P/phosphate translocator (GPT) from maize endosperm, pea roots, and potato tubers (11, 37). As is the case for the TPTs, the PPT and the GPT proteins share a high degree of identity with each other (mature proteins about 75–95% identity). A comparison of the phosphate translocator cDNA sequences with entries in the sequence databases revealed no significant homologies with known proteins. This is an indication for the unique transport specificity of the plastidic phosphate translocator proteins. In contrast, the plastidic 2-oxoglutarate/malate translocator as well as the recently discovered plastidic adenylate translocator share some similarities with transporters of bacterial origin (38, 76).

In contrast to the high homologies among the translocators of one class, the overall similarities between the members of the TPT, PPT, and GPT families are about 35% and are restricted in all translocator proteins to five regions, each 15 to 30 amino acid residues in length. A phylogenetic tree constructed by using the distance matrix method confirmed the existence of three different classes of plastidic phosphate translocators and showed that members of each translocator family cluster together but that the three classes of transporters cluster at approximately equal distances from each other (37).

All members of the different classes of phosphate translocators are nuclear-encoded and possess N-terminal transit peptides (about 80 amino acid residues) that direct the adjacent protein correctly to the plastids (4, 11, 37, 42). Import of the translocators into plastids is driven by ATP and depends on the translocation machinery of the envelope membrane. The mature parts of the phosphate translocators consist of approximately 330 amino acid residues per monomer, are highly hydrophobic, and contain information (envelope insertion signals) for the integration of the proteins into the inner envelope membrane. Each monomer is predicted to consist of 5–7 hydrophobic segments, which are assumed to form α-helices that traverse the membrane in zig-zag fashion connected by hydrophilic loops. The phosphate translocators thus belong to the group of translocators with a 6+6 helix folding pattern, as is the case for mitochondrial carrier proteins (45). In contrast, the plastidic 2-oxoglutarate/malate translocator (76) and the ATP/ADP translocator (38) have transmembrane topologies with a 12-helix motif, which resembles that of other plasma membrane transporters from prokaryotes and eukaryotes that presumably all function as monomers.

Based on a tentative model for the arrangement of the TPT in the membrane, it is probable that all 12 α-helices of the phosphate translocator dimer take part in forming a hydrophilic translocation channel through which the substrates could be transported across the membrane (75). Interestingly, two successive charged residues in helix V (Lysine, Arginine) that have been proposed to
be involved in substrate binding (10) are conserved in all phosphate transport proteins. Site-directed mutagenesis of the lysine residue in helix V (Lys273Gln, spinach TPT) led to a complete loss of transport activity (B Kammerer & UI Flügge, unpublished observations), a result suggesting that this residue is indeed essential for the transport reaction.

FUNCTIONAL STUDIES OF PLASTIDIC PHOSPHATE TRANSLOCATORS

**Heterologous Expression of Plastidic Phosphate Translocators in Yeast Cells**

The final proof for the identity of an isolated transporter cDNA is the expression of the corresponding coding region to produce the functional protein in heterologous systems, for example, yeast cells, bacteria, or oocytes. *Escherichia coli*, which is commonly used as a host for the expression of foreign proteins, failed to express plastidic phosphate translocator proteins, because of the toxicity of the gene product. We have demonstrated that yeast (*Schizosaccharomyces pombe*) can be successfully used for the expression of functional plastidic translocators. The recombinant plastidic translocator proteins, representing about 1% of the total protein from transformed cells, were associated with yeast internal membranes, either mitochondrial membranes or membranes of the rough endoplasmic reticulum (44). For yeast transformation, an expression vector containing cDNAs coding for either the whole precursor protein or only the mature translocator protein was used (44, 76). For the subsequent measurements of the transport activities, either total membrane fractions or the transport proteins isolated therefrom were reconstituted into artificial membranes. To facilitate the isolation of the phosphate translocators from the transformed cells, recombinant proteins were engineered to contain a C-terminal tag of six consecutive histidine residues (His6-tag). This allows the purification of the tagged transporter proteins to apparent homogeneity by a single chromatography step on metal-affinity columns (11, 37, 44).

Figure 3 shows the substrate specificities and Table 1 the kinetic constants of the TPT, PPT, and GPT proteins reconstituted into liposomes that had been preloaded with different phosphorylated metabolites that function as exchangeable countersubstrates. It is evident that the purified TPT only transports triose phosphates and 3-phosphoglycerate in exchange with inorganic phosphate, but not PEP and hexose phosphates. In contrast, the PPT transports inorganic phosphate preferentially in exchange with PEP. Triose phosphates and 3-phosphoglycerate, the only substrates of the TPT, are only poorly transported by the PPT, with apparent inhibition constants 1–2 orders of magnitude
Figure 3  Substrate specificities of the TPT, PPT, and GPT. The recombinant and histidine-tagged phosphate transport proteins were isolated from yeast cells by metal affinity chromatography and were reconstituted into liposomes that had been preloaded with the indicated substrates. The phosphate transport activities of the translocators [PPT, phosphoenolpyruvate/phosphate translocator (light gray), left; TPT, triose phosphate/phosphate translocator (dark gray), middle; GPT, glucose 6-phosphate/phosphate translocator (white), right] are given as a percentage of the activity measured for proteoliposomes preloaded with inorganic phosphate. 3-PGA, 3-phosphoglycerate; PEP, phosphoenolpyruvate; Pi, inorganic phosphate; TrioseP, dihydroxyacetone phosphate; Glc6P, glucose 6-phosphate. Data from Reference 37.
higher than that for PEP. The PPT protein thus functions as a PEP/phosphate transporter that is able to provide the plastids with PEP even in the presence of other phosphorylated intermediates.

Unlike the TPT and PPT proteins, the GPT accepts inorganic phosphate, triose phosphates, and Glc6P about equally well as countersubstrates, whereas the affinity of the GPT toward 3-phosphoglycerate is lower. PEP only serves as a poor substrate with an apparent inhibition constant that is three to ten times higher that the apparent $K_m$ values for the transport of phosphate and Glc6P, respectively (37) (Table 1). Glc1P or fructose phosphates are virtually not transported by any of the phosphate transport proteins. The GPT thus links the cytosolically located conversion of sucrose and hexoses to Glc6P with metabolic reactions within the plastid, i.e. the biosynthesis of starch, fatty acids, and the oxidative pentose phosphate pathway that delivers reduction equivalents for nitrogen metabolism and fatty acid biosynthesis. Inorganic phosphate and triose phosphate that are formed during these processes can both be used as counter substrates by the GPT in exchange with Glc6P (see Figure 2).

Until recently, it was accepted that the transport of phosphate, phosphorylated C3-compounds, and hexose phosphates, observed in nongreen plastids, are mediated by a TPT-like phosphate translocator. Our findings clearly show that these metabolites are not transported by a single transport system, but rather by different members of the phosphate translocator family with partially overlapping substrate specificities. Such a system enables the efficient uptake of individual phosphorylated substrates even in the presence of high concentrations of other phosphorylated metabolites, which would otherwise compete for the binding site of a single transport system.

### Table 1

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>TPT $K_m$ (app)</th>
<th>PPT $K_i$ (app)</th>
<th>GPT $K_i$ (app)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td>1.0</td>
<td>0.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Triose phosphate</td>
<td>1.0</td>
<td>8.0</td>
<td>0.6</td>
</tr>
<tr>
<td>3-Phosphoglycerate</td>
<td>1.0</td>
<td>4.6</td>
<td>1.8</td>
</tr>
<tr>
<td>2-Phosphoglycerate</td>
<td>12.6</td>
<td>5.7</td>
<td>—</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td>5.3</td>
<td>0.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Glucose 6-phosphate</td>
<td>&gt;50</td>
<td>&gt;50</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*The $[^{32}P]$phosphate transport activities of the recombinant translocators (TPT, triose phosphate/phosphate translocator from spinach chloroplasts; PPT, phosphoenolpyruvate/phosphate translocator from cauliflower bud plastids; GPT, glucose 6-phosphate/phosphate translocator from pea root plastids), purified from yeast cells, were measured in a reconstituted system using proteoliposomes that had been preloaded with inorganic phosphate. Data from References 11, 37.*
The identity of the hexose phosphate transporter that is specific for the uptake of Glc1P is unknown. The observation that mutants of *Arabidopsis* with a defect in the plastidic phosphoglucomutase are unable to synthesize starch (5, 6) clearly indicates that this plant does not possess a functional Glc1P translocator. Since these mutants possess a cytosolic phosphoglucomutase isoenzyme mediating the conversion of Glc6P into Glc1P, starch biosynthesis should be driven from Glc1P imported via the Glc1P translocator. Furthermore, transgenic potato plants with reduced activity of the plastidic phosphoglucomutase are also defective in starch accumulation and show a phenotype comparable to that found in ADP-glucose pyrophosphorylase antisense lines (R Trethewey, personal communication). This observation indicates that (a) Glc6P is the preferred substrate taken up by plastids and (b) the conversion of Glc6P to Glc1P inside the plastids, catalyzed by phosphoglucomutase, is a prerequisite for starch formation. The dependence of starch formation on the plastidic phosphoglucomutase would also argue against the observation that starch synthesis in potato tubers is driven by cytosolic Glc1P but not Glc6P (48).

**Gene Expression Studies**

The different physiological functions of the phosphate translocator families are linked to differential patterns of expression. The TPT activity is associated with photosynthetic carbon metabolism. Consequently, expression of the TPT gene is observed only in photosynthetically active tissues, but not in unambiguously heterotrophic tissues such as roots or potato tubers (11, 16, 60). As shown by in situ hybridization studies, the TPT gene is present in both mesophyll cells and bundle sheath cells of C₄-plants (P Nicolay & UI Flügge, unpublished observations). The expression level of PPT-specific transcripts could be detected in both photosynthetic and heterotrophic tissues, although transcripts were more abundant in nongreen tissues (11). The level of PPT steady-state RNA in photosynthetic tissues is lower by at least one order of magnitude than is the level of the TPT mRNA, both in C₃- and in C₄-plants. However, we have recently isolated a cDNA from maize that is homologous to the PPT cDNA. The corresponding gene is highly expressed in mesophyll cells of C₄-plants and, presumably, codes for the C₄-PEP/phosphate translocator that exports PEP from the chloroplasts as substrate for the PEP carboxylase in the cytosol (K Fischer & UI Flügge, unpublished observations).

Transcripts of the GPT gene were almost lacking in photosynthetic tissues but are abundant in heterotrophic tissues such as roots, developing maize kernels, potato tubers, or reproductive organs (37). This is in line with the proposed function of the GPT protein in these tissues that utilize Glc6P as a precursor for starch biosynthesis. The slight expression of the GPT observed in photosynthetic
tissues might be due to the presence of the GPT protein in chloroplasts of guard cells (see above).

It has been shown recently that in the seed endosperm of some cereals, the key enzyme for starch synthesis, ADP-glucose pyrophosphorylase, is mainly present in the cytosol and not in the plastids (8, 71). The ADP-glucose formed in the cytosol is presumably transported into the plastids for starch biosynthesis via an ADP-glucose/adenylate antiporter that is functionally and structurally distinct from the recently identified ADP/ATP translocator (38, 63). It is assumed, but not yet proven, that the Brittle-1 protein serves as an ADP-glucose/adenylate transporter, which would thus represent an alternative route to provide the plastids with a precursor for starch biosynthesis (66, 67). In maize, the Brittle-1 protein is expressed during the later stages of endosperm development (66), whereas the level of GPT mRNA reaches a plateau shortly after pollination that was subsequently maintained through 20 days (37). It remains to be established how the activities of both proteins are coordinated in seed development.

**Transgenic Plants with Altered Activities of Plastidic Phosphate Translocators**

The TPT is an important link between metabolism in the chloroplast and the cytosol. Only about 10% of the total transport activity of the TPT can be used for (productive) net triose phosphate export to provide the carbon skeleton for further biosynthetic processes. Since both the cytosol and the stroma contain triose phosphates, 3-phosphoglycerate, and inorganic phosphate competing for transport in either direction, it appears feasible that much of the TPT activity is used for catalyzing (nonproductive) homologous exchanges (14).

From the observation of subcellular metabolite concentrations in intact spinach leaves, it has been proposed that the TPT can exert a kinetic limitation during sucrose biosynthesis in vivo (23). We have assessed the role of the TPT on photosynthetic metabolism by creating transgenic antisense potato plants in which both the amount and the activity of the TPT were reduced to 70% of the controls (55). In ambient CO₂ and intermediate light, there was no significant effect on photosynthetic rates, growth, and tuber development in the transformants. However, moderate reduction of the TPT activity resulted in a marked perturbation of leaf metabolism. Most remarkably, the content of stromal 3-phosphoglycerate was greatly increased compared to the corresponding value in wild-type plants. This should result in a large decrease of the stromal content of inorganic phosphate since the TPT mediates a strict counterexchange of substrates. The increased stromal 3-phosphoglycerate/phosphate ratio should lead to an increase of starch synthesis due to the allosteric activation of the...
ADP-glucose pyrophosphorylase (52) and reflect the situation of chloroplasts in which an increased starch synthesis was observed due to a decreased availability of phosphate within the cytosol (30). Indeed, the starch content in the leaves of the transformants was much higher than in wild-type plants, suggesting that the daily assimilated carbon is mainly maintained within the plastids and directed into the accumulation of starch. Since there was no obvious effect on plant growth, the transformants were obviously able to efficiently compensate for their deficiency in TPT activity.

The transgenic plants mobilize and export the major part of the daily accumulated carbon during the following night, in contrast to wild-type plants that generally export the major part of the fixed carbon during ongoing photosynthesis (28). The altered day-night rhythm of carbon allocation to sink tissues also leads to a change in the diurnal growth pattern of the TPT antisense plants: The growth rate during the night is considerably increased as compared to the growth rate during the day period (J Fisahn & L Willmitzer, personal communication). The transformants likely circumvent the reduced TPT activity by mobilizing the daily accumulated starch via amylolytic starch breakdown. This results in the formation of hexoses that are subsequently exported via a glucose translocator (58; see Figure 1). Interestingly, transgenic antisense TPT plants from tobacco accumulate starch as potato plants do, but start to mobilize the accumulated starch during ongoing photosynthesis. These plants showed increased rates of amylolytic starch mobilization and a higher transport capacity for glucose across the envelope membrane (26).

In further studies, transgenic tobacco plants with gradually decreased or increased TPT activities were utilized to study the control the TPT exerts on the fluxes of carbon into starch and sucrose as well as on the rate of CO₂ assimilation (RE Häsler, NH Schlieben, P Nicolay & UI Flügge, unpublished observations). The data indicate that the TPT exerts a considerable control on the rate of both CO₂ assimilation and sucrose biosynthesis under saturating CO₂. These studies also revealed that the rate of sucrose biosynthesis from glucose (deriving from starch degradation) could account for up to 60–70% of the wild-type rate in the absence of the TPT.

From the experiments with the antisense TPT plants, it can be concluded that the transformants can efficiently compensate for their deficiency in TPT activity provided that a carbon sink (i.e. starch) can be generated during photosynthesis that can subsequently be mobilized. Transgenic potato plants with a reduced ability to synthesize assimilatory starch (e.g. by antisense repression of the ADP-glucose pyrophosphorylase) show also no effect on growth and productivity. Export of the daily fixed carbon via the TPT is obviously so efficient in these plants that heterotrophic tissues can be adequately supplied with reduced carbon. However, if both starch formation and the activity of the TPT
are reduced, the corresponding transformants show a severe phenotype (25). These transformants are unable to export sufficient amounts of fixed carbon during the day, nor do they have a carbon store that they could use during the dark period.

The in planta role of genes can also be studied by analysis of mutants that have been created, for example, via insertion mutagenesis. Recently, *Arabidopsis* mutants with a reduced expression of the chlorophyll a/b binding protein (cab) in responses to pulses of light were isolated [CAB underexpressed, CUE mutants (43)]. The phenotype of the null mutations in *Arabidopsis* is quite severe. The plants underexpress genes for chloroplast components, both in the light and in response to a light pulse. The seedlings are not able to establish photoautotrophic growth and die unless they are germinated on sucrose. The paraveinal regions of the mutant leaves are still green but the interveinal regions are pale green, resulting in a reticulate pattern. Antisense PPT plants from tobacco showed a comparable, but transient, visible phenotype (RE Häusler, A Weber, P Nicolay, L Voll & UI Flügge, unpublished observations). Different alleles of cue1 with reduced light-responsive gene expression were isolated from *Arabidopsis* (cue1-1 to cue1-8) and the corresponding gene was identified in a T-DNA-tagged mutant population. Surprisingly, the cue1 gene corresponds to the PPT (J Chory & S Streatfield, personal communication). Future work will elucidate how this severe phenotype is linked to the role of the PPT in plant metabolism and development.

**CONCLUDING REMARKS**

There has been considerable progress during the past few years in studying plastidic translocators at the biochemical and, more recently, molecular levels. In the near future, the *Arabidopsis* and rice genome sequencing programs will provide the sequences of complete higher plant genomes. To date, 35,000 *Arabidopsis* ESTs and about 11,000 rice ESTs (Expressed Sequence Tags) are already available. Future work will likely concentrate on identifying the functions of genes coding for putative envelope translocators, for example, and on elucidating the specific role of a particular gene in plant metabolism. This will require combined efforts on genetic, molecular, biochemical, and physiological levels.

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