# The Import and Export Business in Plastids: Transport Processes across the Inner Envelope Membrane[1]

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Plastids are the characteristic organelles of plant and algae cells and are, like mitochondria, of endosymbiontic origin. They can be traced back to a single cyanobacterial ancestor that was engulfed by a primitive protist about 1.5 billion years ago (Yoon et al., 2004). Higher plants possess different types of plastids, among them undifferentiated proplastids, colorless etioplasts, green photosynthetically active chloroplasts, colored chromoplasts that are responsible for the yellow, orange, and red colors of flowers and fruits, starch-storing amyloplasts, and lipid-storing elaioplasts. The differentiation into these plastid types depends on the tissue and the developmental stage of the plant. Plastids show a tremendous biochemical versatility. They are the site of carbon, sulfur, and nitrogen assimilation, and they synthesize fatty acids, many amino acids, and a plethora of other primary and secondary metabolites. It is, therefore, reasonable to consider plastids as metabolic factories of plant cells. Plastids of red and green algae and of higher plants are enclosed by two membranes, the outer and inner envelope membranes that separate the stroma from the cytosol. The integration of plastids into the metabolism of plant cells is achieved by a large set of proteins transporting numerous inorganic and organic molecules across both membranes. Transport across the outer envelope membrane is enabled by a set of $\beta$-barrel proteins that form selective pores (Bölter and Soll, 2001). In contrast, the transport across the inner envelope is catalyzed by a large set of $\alpha$-helical membrane transporters, which are highly specific for their substrates.

In this Update, I will give a short overview of the current knowledge of transport processes across the plastid inner envelope membrane and of the approaches used for the identification and characterization of plastid membrane transporters. Furthermore, I will discuss the evolution of these proteins and future challenges and opportunities in the field. For more detailed information about particular transporters, the reader is referred to several comprehensive reviews covering plastid transport processes (e.g. Weber and Fischer, 2007; Linka and Weber, 2010).

## FROM THE BEGINNINGS OF PLASTID TRANSPORT TOWARD THE PERMEOME OF PLASTIDS

A first direct hint at the transport processes across the inner envelope membrane and especially at the metabolites that are exported from chloroplasts during photosynthesis was obtained from transport experiments with isolated plastids. It could be shown, for example, that chloroplasts export assimilated carbon in the form of triose phosphates (TPs), that they import ATP, and that they have a sophisticated system for the transport of dicarbonic acids. For a detailed and personal overview about the first two decades of transport business I refer the reader to the excellent articles by Heldt (2002) and Walker (2003).

With the cloning of the first cDNA encoding a plastid transporter, the TP/phosphate translocator (TPT)—one member of the family of plastid phosphate translocators (pPTs)—the molecular age of plastid transport began (Flügge et al., 1989). Since then, 35 transporters of the inner envelope membrane have been characterized at the molecular level and the physiological function of most of them have been elucidated. These proteins transport numerous inorganic and organic molecules into and out of plastids (Fig. 1; Table I). A combination of data from in silico analyses and proteomics projects has led to the estimate that as much as 120 to 150 proteins (Weber et al., 2005; Armbruster et al., 2010) might be involved in plastid transport processes. If these estimates are correct, approximately 75% of transporters are yet unknown. Thus, a major goal in future plastid research remains to determine the permeome, the complete set of transport proteins, of chloroplasts and of all other types of plastids.

### The Hard Way: Biochemical Approaches

Biochemical approaches were the first used to identify and isolate plastid transporters. They were based on methods for the isolation of chloroplasts developed in the 1960s and on techniques for the isolation of envelope membranes. Both purified organelles and membranes were the starting material for the isolation of transporters by two different strategies. The TPT and later the plastid Glc transporter (pGlcT) were identified by using radiolabeled inhibitors that bind to and inhibit a particular transporter protein. The cor-

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responding cDNA was subsequently cloned, taking advantage of peptide sequences obtained from labeled proteins (Flügge et al., 1989; Weber et al., 2000). Another strategy that was applied to analyze transporters was the determination of the particular transport activity of a protein by reconstitution of envelope membrane proteins into artificial liposomes. Based on the transport measurements, the transporters were purified to apparent homogeneity by different chromatographic methods. Peptide sequence information was then used to clone cDNAs encoding the transporters. Using these biochemical methods, the dicarboxylate transporter DiT1 and two members of the pPT family, the PPT (transporting phospho*enol*pyruvate [PEP]), and the GPT (transporting Glc 6-P [Glc6P]), were isolated and characterized (Weber et al., 1995; Fischer et al., 1997; Kammerer et al., 1998).

### The Mendelian Way: Forward Genetics

One tool is classical or forward genetics, i.e. the identification of mutants with specific phenotypes followed by the identification and analysis of the mutated genes. Primary forward genetics screens, suppressor mutant screens, and other screening strategies have led to the identification of a large number of genes encoding plastid proteins, many of them involved in photosynthesis (Armbruster et al., 2010). The first transport protein from plastid envelopes was identified almost 30 years ago in a pioneering forward screen aimed at identifying proteins involved in photorespiration or the photorespiratory C2 cycle. The screen was based on the assumption that photorespiratory mutants are unable to survive in air but are viable at high $CO_2$ concentrations that prevent photorespiration. Besides a number of enzymes of the C2 cycle, an envelope membrane protein was identified that is involved in dicarboxylate transport, a process that is part of the refixation of ammonia released during photorespiration (Somerville and Ogren, 1983). In this case, however, the corresponding gene DiT2 was identified only 20 years later because of its homology to DiT1 and shown to be the Glu/malate transporter that is indeed involved in ammonia refixation (Taniguchi et al., 2002; Renné et al., 2003).

The first gene encoding a plastid transporter isolated from a plant mutant was *brittle-1* (*bt-1*). The maize (*Zea mays*) *bt-1* mutant was identified back in 1926 and is characterized by a severely decreased amount of starch in the endosperm. The *bt-1* locus in the mutant is disrupted by an integrated transposable element that was used to identify the *bt-1* locus and to clone the corresponding cDNA (Sullivan et al., 1991). Based on extensive biochemical evidence it has been proposed that the BT1 protein is involved in the transport of ADP-Glc, the substrate for starch biosyn-

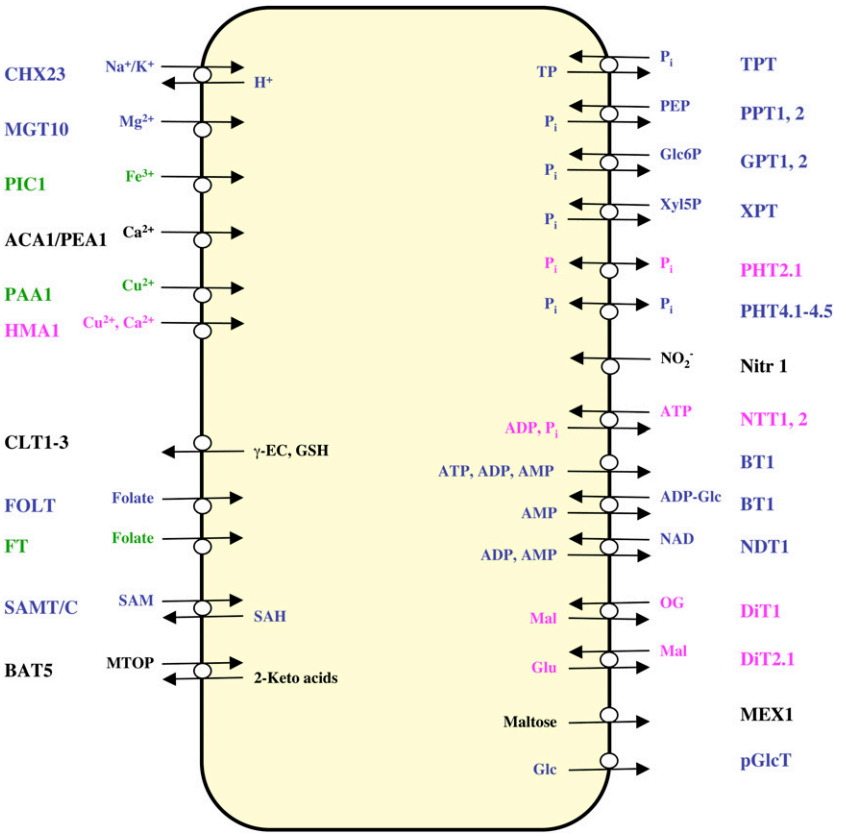


**Figure 1.** Transport processes across the inner envelope membrane of plastids from higher plants. Only transporters that were characterized at the molecular level are shown. The names of the transport proteins are explained in Table I. All names refer to the proteins in Arabidopsis, except for the BT1 protein from maize. The origin of the transporters is indicated by a color code. Blue, Host cell; green, cyanobacterium; pink, horizontal gene transfer; black, unknown. GSH, Glutathione; Mal, malate; MTOP, 4-methylthio-2-oxobutanoate; OG, 2-oxoglutarate; $P_i$, inorganic phosphate; SAH, *S*-adenosylhomo-Cys; SAM, *S*-adenosyl-Met; Xyl5P, xylulose-5-P; γ-EC, γ-glutamyl-Cys.

**Table I.** *Transporters of the inner envelope membrane of plastids from higher plants*

Only transporters with well-defined physiological functions are included. HGT, Horizontal gene transfer.

| Name | Activity | Gene ID | Origin | Identification | Reference |
|---|---|---|---|---|---|
| TPT | TrioseP/phosphate translocator | At5g46110 | Host cell | Protein labeling | Flügge et al. (1989) |
| PPT1 | PEP/phosphate translocator 1 | At5g33320 | Host cell | Protein purification | Fischer et al. (1997) |
| PPT2 | PEP/phosphate translocator 2 | At3g01550 | Host cell | Sequence homology | Knappe et al. (2003) |
| GPT1 | Glc6P/phosphate translocator 1 | At5g54800 | Host cell | Protein purification | Kammerer et al. (1998) |
| GPT2 | Glc6P/phosphate translocator 2 | At1g61800 | Host cell | Sequence homology | Knappe et al. (2003) |
| XPT | Xyl5P/phosphate translocator | At5g17630 | Host cell | Sequence homology | Eicks et al. (2002) |
| PHT4.4 | Phosphate transporter | At4g00370 | Host cell | Sequence homology | Guo et al. (2008) |
| PHT4.2 | Phosphate transporter | At2g38060 | Host cell | Sequence homology | Guo et al. (2008) |
| PHT4.3 | Phosphate transporter | At3g46980 | Host cell | Sequence homology | Guo et al. (2008) |
| PHT4.5 | Phosphate transporter | At5g20380 | Host cell | Sequence homology | Guo et al. (2008) |
| PHT2.1 | Phosphate transporter | At3g26570 | HGT | Sequence homology | Versaw and Harrisson (2002) |
| Nitr1 | Nitrite transporter | At1g68570 | Unknown | cDNA Isolation | Sugiura et al. (2007) |
| NTT1 | Nucleotide transporters | At1g80300 | HGT | Sequence homology | Reiser et al. (2004) |
| NTT2 | Nucleotide transporters | At1g15500 | HGT | Sequence homology | Reiser et al. (2004) |
| BT1 | Brittle 1 protein (maize) | BT016796 | Host cell | Forward genetics | Sullivan et al. (1991) |
| BT1 | Brittle 1 protein | At4g32400 | Host cell | Sequence homology | Leroch et al. (2005) |
| NDT1 | NAD transporter | At2g47490 | Host cell | Sequence homology | Palmieri et al. (2009) |
| DiT1 | Dicarboxylate transporter 1 | At5g12860 | HGT | Protein purification | Weber et al. (1995) |
| DiT2.1 | Dicarboxylate transporter 2.1 | At5g64290 | HGT | Sequence homology | Taniguchi et al. (2002) |
| DiT2.2 | Dicarboxylate transporter 2.2 | At5g64280 | HGT | Sequence homology | Renné et al. (2003) |
| pGlcT | Plastid Glc transporter | At5g16150 | Host cell | Protein labeling | Weber et al. (2000) |
| Mex1 | Maltose exporter 1 | At5g17520 | Unknown | Forward genetics | Niittylä et al. (2004) |
| CHX23 | Cationic proton exchanger 23 | At1g05580 | Host cell | Sequence homology | Song et al. (2004) |
| MGT10 | Magnesium transporter 10 | At5g22830 | Host cell | Sequence homology | Li et al. (2001) |
| PIC1 | Permease in chloroplasts 1 | At2g15290 | Cyanobacterium | Sequence homology | Duy et al. (2007) |
| ACA1/PEA1 | Arabidopsis calcium ATPase | At1g27770 | Unknown | Antibody Screening | Huang et al. (1993) |
| PAA1 | Copper P-type ATPase | At4g33520 | Cyanobacterium | Forward genetics | Shikanai et al. (2003) |
| HMA1 | Heavy metal ATPase 1 | At4g37270 | HGT | Proteomics | Seigneurin-Berny et al. (2006) |
| CLT1 | CRT like transporter 1 | At5g19380 | Unknown | Forward genetics | Maughan et al. (2010) |
| CLT2 | CRT like transporter 2 | At4g24460 | Unknown | Forward genetics | Maughan et al. (2010) |
| CLT3 | CRT like transporter 3 | At5g12160 | Unknown | Forward genetics | Maughan et al. (2010) |
| FOLT1 | Folate transporter 1 | At5g66830 | Host cell | Sequence homology | Bedhomme et al. (2005) |
| FT | Folate transporter | At2g32040 | Cyanobacterium | Sequence homology | Klaus et al. (2005) |
| SAMT/C | SAM transporter/carrier | At4g39460 | Host cell | Sequence homology | Bouvier et al. (2006) |
| BAT5 | Bile acid transporter like 5 | At5g12030 | Unknown | Coexpression | Gigolashvili et al. (2009) |

thesis. In cereals, this metabolite is synthesized in the cytosol and therefore has to be imported into amyloplasts. This theory was finally proved to be correct by direct measurement of the transport activity of the maize BT1 protein after heterologous expression in *Escherichia coli* (Kirchberger et al., 2007; but notice the different function of BT1 in plants other than cereals [Fig. 1; Table I]). A second transporter being involved in starch metabolism was identified in a screen for starch excess mutants. One mutant showed, in addition, elevated levels of maltose and was shown to be impaired in the export of maltose generated in the plastid during starch degradation at night. The mutated locus that was named *mex-1* (maltose excess) was shown to encode the plastid maltose transporter Mex-1 (Niittylä et al., 2004). A loss of Mex-1 leads to an accumulation of maltose in amyloplasts that in turn severely inhibits starch breakdown. Other transporters that were also identified by mutant analysis are listed in Table I. These examples clearly indicate that forward genetics screens are valuable tools for plastid research.

### Modern Times: Bioinformatics and Omics Revelations

The enormous amount of data emerging from the numerous genome sequencing projects offers unique opportunities for the identification of plastid transporters. Indeed, more than half of the transporters listed in Table I were identified based on their sequence homology to transporters of known function in other organisms, namely cyanobacteria (e.g. the folate transporter FT), parasitic bacteria (e.g. the ATP transporter NTT), yeast (e.g. the NAD transporter NDT or the cationic ion exchanger CHX23), plants (e.g. the XPT), or mammalian cells (the folate transporter FOLT). These homology searches can be combined with in silico analyses of their subcellular localization using several algorithms that have been developed to identify targeting signals within proteins. The wealth of data

garnered is publicly available in (plant) protein databases such as ARAMEMNON (http://aramemnon.botanik.uni-koeln.de/) or SUBA (http://suba.plantenergy.uwa.edu.au/).

Transcriptome projects offer a new analytic approach, the guilty-by-association approach, which is based on the concept that genes with similar function often display similar transcription profiles, meaning genes can be identified based on their coexpression with genes of known function (Armbruster et al., 2010). Recently, using this new in silico approach, a first plastid transporter was identified. BAT5 is a member of a small family of five proteins in Arabidopsis (*Arabidopsis thaliana*) having homology to bile acid carriers from mammalian cells. BAT5, but not the other members of the family, shows coexpression with several genes involved in the synthesis of aliphatic glucosinolates (Gigolashvili et al., 2009), a pathway that is divided between the cytosol and the stroma. This specific compartmentation implies the need for the transport of metabolites of this pathway across the envelope membranes. In a comprehensive analysis of *bat5* mutants it could be shown that BAT5 is catalyzing the import of 4-methylthio-2-oxobutanoate, which is produced from Met in the cytosol. In chloroplasts, 4-methylthio-2-oxobutanoate is then converted to homoketo acids of different chain length, followed by the export into the cytosol, most likely also by BAT5 (Gigolashvili et al., 2009). These first results demonstrate that transcriptome analysis is a powerful tool for the identification of genes in plants.

Another widely used high-throughput method is proteomics, i.e. the analysis of the total protein content of a particular type of cell or of a cellular compartment. Proteome studies of purified envelope membranes from chloroplasts of spinach (*Spinacia oleracea*), Arabidopsis, pea (*Pisum sativum*), and maize (from both mesophyll and bundle sheath cells) and also from proplastids of cauliflower (*Brassica oleracea*; e.g. Ferro et al., 2003; Fröhlich et al., 2003; Bräutigam et al., 2008; Majeran et al., 2008; Bräutigam and Weber, 2009) have been performed. These studies not only confirmed the number of 300 to 400 envelope membrane proteins identified by in silico analysis, but also caught a first glimpse of the dynamics of the envelope proteome, i.e. the similarities and differences between different plants species and between different types of plastids. Various proteins of unknown function have been identified in these studies. A first protein (HMA1) has been analyzed in more detail and shown to represent a copper transporter involved in the import of copper into plastids (Seigneurin-Berny et al., 2006; a second copper transporter [PAA1] has been identified by forward genetics [Table I]).

## THE MEANING OF PLASTID TRANSPORTERS

Knowledge of the substrate specificities of a transporter is the prerequisite for understanding its physiological function and its role in plant metabolism. Therefore, several techniques have been developed for the measurement of the transport activities of membrane proteins. These include the expression of cDNAs encoding a particular transporter in heterologous systems such as *E. coli* or yeast (*Saccharomyces cerevisiae*), followed by the measurement of the transport activity of the recombinant protein. The bacterial system has the advantage that the plant transport proteins are integrated into the bacterial plasma membrane. This enables the determination of the transport activity by measuring the import or export of radiolabeled substances into or out of intact *E. coli* cells. Several plastid transporters have been analyzed using this approach including the NTTs and BT1 from maize and Arabidopsis (Tjaden et al., 1998; Kirchberger et al., 2007). As the production of membrane proteins in *E. coli* is in some cases difficult to achieve, yeast cells have been widely used for this purpose as an alternative. As yeast cells do not possess plastids, plastid proteins are targeted to other internal membranes, i.e. to mitochondrial membranes and/or to the endoplasmic reticulum (ER; Loddenkötter et al., 1993). For further characterization, the recombinant protein is isolated from yeast membranes, in most cases by affinity chromatography, and reconstituted into artificial membranes to measure its transport activity (Loddenkötter et al., 1993). All proteins of the pPT family and several other plastid transporters have been characterized at the molecular level by this approach (Flügge et al., 2003).

### Reverse Genetics: A Powerful Tool

The in vivo function of a transporter, however, cannot be deduced from its in vitro transport activity alone. Reducing gene expression in planta either by knockout (by insertion of a T-DNA or a transposon) or knockdown approaches (by RNA interference), or increasing gene expression by overexpression of an introduced second copy of the gene that is under the control of a specific promoter clarify the in vivo roles of transport proteins. I want to illustrate the enormous potential of these reverse genetics approaches by two examples, the analysis of the GPTs and the NTTs (for a broader overview covering many more proteins see, for example, Linka and Weber, 2010 and refs. therein).

Plastids of nongreen tissues from multicellular plants import carbon mainly in the form of Glc6P as a source for biosynthetic pathways, and ATP as source of energy. Several studies of carbon metabolism in plants indicate that Glc6P is used for three different purposes. It is the substrate for starch synthesis and fatty acid synthesis in nongreen plastids but is also fed into the oxidative pentose phosphate pathway for the production of reducing equivalents (NADPH). The import of Glc6P into heterotrophic plastids is mediated by the GPT (Kammerer et al., 1998). As Arabidopsis possesses two different functional GPTs, GPT1 and 2, the contribution of these two transporters to plant metabolism was originally not known.

Therefore, *gpt1* and *gpt2* mutants were analyzed. The deletion of the *gpt1* gene turned out to be lethal (Niewiadomski et al., 2005). A detailed analysis of heterozygous *GPT1/gpt1* plants revealed that the development of both the female and the male *gpt1* gametophyte are impaired. Two more recent investigations showed that GPT1 is also involved in embryo development and seed maturation (Rolletschek et al., 2007; Andriotis et al., 2010). A reduction of *GPT1* expression of about 50% in seeds of *Vicia narbonensis* by an antisense approach with a seed-specific promoter led to a shift in assimilate partitioning from starch/lipids into storage proteins, indicating that GPT1 exerts a significant control on seed filling and maturation (Rolletschek et al., 2007). A reduction of more than 80% of *GPT1* expression in Arabidopsis seeds is lethal because embryo development stops at the globular stage (Andriotis et al., 2010). The reason why a defect in Glc6P transport leads to such severe phenotypes is not clear so far. The existence of several starch-free mutants of Arabidopsis that retain full fertility demonstrates that starch accumulation per se is not a prerequisite for development. As the lipid content in Arabidopsis seeds with a reduced GPT1 activity is only slightly reduced, an interruption of fatty acid synthesis is not the cause of the lethality. Therefore, it has been suggested that a reduction in the production of NADPH via the oxidative pentose phosphate pathway impairs several NADPH-dependent processes necessary for plastid development and differentiation, loss of which inhibits gametophyte and embryo development (Niewiadomski et al., 2005; Andriotis et al., 2010). However, a direct proof of this hypothesis is still lacking.

In contrast, the *gpt2* knockout line shows no detectable phenotype. Only recently it could be shown that *GPT2* expression is up-regulated in mutants impaired in starch synthesis (Kunz et al., 2010). In an extensive analysis of *gpt2* plants and several double knockout lines, the contributions of both GPT1 and GPT2 to metabolism in vegetative tissues were dissected. Whereas the GPT1 is constitutively present in particular cells such as stomatal guard cells of leaves or cells of the root tip, GPT2 is induced when carbohydrate metabolism is impaired, e.g. at higher concentrations of soluble sugars (Kunz et al., 2010). Thus, the two GPT proteins, even though showing the same transport activity, have different physiological functions in Arabidopsis.

ATP represents the universal energy currency of all living cells. It is mainly synthesized in mitochondria and, in plant cells, also in chloroplasts. An ATP transport activity has been shown to be present in envelope membranes of chloroplasts and nongreen plastids (Heldt, 1969). Within the past decade, the physiological functions of the two ATP transporters (NTTs) in Arabidopsis have been characterized. Extensive analysis of *NTT* antisense, RNA interference, and knockout plants revealed distinctive physiological roles of the NTTs in different types of plastids (Reiser et al., 2004). In nongreen plastids, the function of the NTTs, especially NTT2, is the supply of ATP for several anabolic pathways. These transporters exert significant control on starch synthesis in amyloplasts of potato (*Solanum tuberosum*) tubers and on lipid biosynthesis in developing Arabidopsis seeds (Geigenberger et al., 2001).

In contrast to nongreen plastids, the function of ATP transport in chloroplasts remained elusive. Chloroplasts show a high level of ATP synthesis by photophosphorylation but the ATP transport activity in these organelles is low. Therefore, it has been proposed that the function of ATP transport is to energize unknown processes in the stroma during the night, rather than to export ATP during photosynthesis (Heldt, 1969). Only very recently, this hypothesis could be validated through the analysis of Arabidopsis lines lacking both NTTs (Reinhold et al., 2007). These mutants showed a dwarf phenotype with necrotic lesions on the leaves under short-day conditions at low-light intensities, a phenotype that is caused by photooxidation due to the accumulation of protoporphyrin IX. The increased protoporphyrin IX levels are most likely due to a reduced activity of magnesium-chelatase, which requires ATP for catalysis and also for stabilization of the holoenzyme. Thus, one important ATP-dependent process during the night is magnesium-chelatase assembly (Reinhold et al., 2007). Whether the inhibition other energy-dependent processes in chloroplasts in the dark are also impaired in the mutant is not known so far.

## TRANSPORT PROCESSES IN SECONDARY PLASTIDS: A NEW AND PROMISING FIELD

Compared to primary plastids, almost nothing is known about transport processes in secondary plastids that are derived from at least three different secondary endosymbiotic events, in which either a green or a red algae was taken up by a protist and subsequently reduced to a plastid. Although there is an ongoing debate about the complicated phylogeny of the different groups of algae and protists (with the possibility of tertiary endosymbiotic events; for more information see Archibald, 2009; Keeling, 2010) it can be clearly stated that these different endosymbiotic events gave rise to an enormous diversity of algae and nonphotosynthetic protists like the diatoms and the parasitic *Apicomplexa* (Gould et al., 2008). Secondary plastids are surrounded by three or four membranes representing the two envelope membranes of the primary plastid (the two innermost membranes), the plasma membrane of the endosymbiont and a fourth membrane that is continuous with the ER of the host cell. Thus, solute transport is much more complicated compared to primary plastids as solutes have to cross up to four membranes.

Recently, two transporters from the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* showing homology to plastid NTTs from higher plants

were identified and analyzed (Ast et al., 2009). By expression of these genes in *E. coli* cells it was shown that NTT1 transports different adenine nucleotides including dATP, while NTT2 transports a range of different nucleotides and deoxynucleotides. Thus, the substrate specificities of the algal transporters are much broader than those of their counterparts from higher plants (Fig. 1), indicating a different physiological function, namely the import of nucleotides into the plastids from the cytosol where their synthesis takes place. Thus, the permeability of the envelope membranes clearly reflects differences in the metabolism of diatoms and higher plants, here the distinct localization of nucleotide biosynthesis in the cytosol and in plastids.

Another important group of organisms bearing secondary plastids are the *Apicomplexa*, a phylum comprising several thousand obligate intracellular protozoan parasites. Several of these species are causing important human and animal diseases. Malaria caused by species of the genus *Plasmodium* remains one of the most devastating diseases worldwide, leading to more than 1 million deaths per year and imposing a massive economic burden on developing countries (Breman, 2001). *Toxoplasma gondii* causes severe toxoplasmosis in immunocompromised individuals while other species are important pathogens in wild and domestic animals (Wong and Remington, 1993). The discovery that *Apicomplexa* harbor a single secondary plastid, called apicoplast, more than a decade ago represented a major breakthrough in this field (McFadden et al., 1996). It is now widely believed that the apicoplast was derived from the uptake of a red algal cell and that the *Apicomplexa* are part of the eukaryotic supergroup *Chromalveolata* (Gould et al., 2008). The identification of a photosynthetic alga, *Chromera velia*, which belongs to the alveolates and is closely related to the *Apicomplexa* corroborates this phylogeny (Moore et al., 2008). Furthermore, *Apicomplexa* possess a number of plant-specific metabolic pathways, many of them associated with the apicoplast. Like plants, some apicomplexan species synthesize fatty acids via a bacterial type II fatty acid synthesis pathway located in apicoplasts (Mazumdar and Striepen, 2007). Second, isopentenyl diphosphate, the precursor of isoprenoids, is synthesized via the nonmevalonate or 1-deoxy-xylulose 5-phosphate pathway found only in plastids and bacteria. Several inhibitor and gene knockout studies have provided strong evidence that these plant-like metabolic pathways and the apicoplast where they are located in are essential for survival of the parasites and thus provide new possibilities for drug development (e.g. Jomaa et al., 1999).

A comprehensive in silico analysis of the genome of *Plasmodium falciparum* and other *Apicomplexa* revealed that their genomes encode less than 200 membrane transporters, which is about 10% of the transporters found in other, nonparasitic eukaryotes (Martin et al., 2005; R. Schwacke and K. Fischer, unpublished data). Obviously, these cells are minimalistic with regard to transporters. Only a few transporters homologous to plastid transporters from higher plants have been identified, and only the apicoplast phosphate translocator (APT) has been characterized in detail (Brooks et al., 2010; Lim et al., 2010). While *Plasmodium* species possess two APTs, *T. gondii* has only one. The APTs show a significant similarity with the pPT family from higher plants and are located in the apicoplast envelope membranes, most likely in the innermost but also in the outermost membrane. A detailed analysis of the transport activities of the APTs revealed that they are able to transport TPs, 3-phosphoglycerate, PEP, and inorganic phosphate (Brooks et al., 2010; Lim et al., 2010). Thus, these transporters have the combined substrate specificities of a TPT and a PPT from higher plants. The physiological function of the APTs is the import of carbon in the form of TPs and PEP that are used as substrates for fatty acid synthesis and most likely also for the 1-deoxy-xylulose 5-phosphate pathway (Brooks et al., 2010). A disruption of the APT gene in *T. gondii* is lethal, clearly showing the importance of this transporter for parasitic metabolism.

The few examples of transporters from secondary plastids indicate that it is an important goal in the next decade to characterize their transport processes in more detail. Many of these organisms are of enormous medical importance while unicellular algae like the diatoms represent a major part of the phytoplankton and are responsible for a large part of the primary productivity in the ocean (Roberts et al., 2007).

## WHERE DO THEY COME FROM? NEW INSIGHTS INTO THE EVOLUTION OF PLASTID TRANSPORTERS

After the uptake of a cyanobacterium by a host cell, an endosymbiotic relationship (or an enslavement of the bacterium, as suggested by some authors [Cavalier-Smith, 2000]) was established. A major factor in the process of bacterial integration into the host cell was the endosymbiotic gene transfer, i.e. the massive transfer of genes from the endosymbiont to the nucleus of the host cell. The endosymbiotic gene transfer had to be closely accompanied by the evolution of a plastid protein import machinery for the reimport of proteins that were now synthesized in the cytosol. Both processes are extensively discussed in the literature (e.g. Bhattacharya et al., 2007; Archibald, 2009). A second important process, the change in membrane permeability during the transformation of the bacterial membranes into the organellar envelope membranes, is, in contrast, rarely mentioned in the literature. This process was achieved by the loss of many bacterial transporters and by the insertion of new transporters with different transport characteristics. It has been proposed that such a system of new metabolite transporters was a critical and very early step in plastid evolution (Cavalier-Smith, 2000; Weber et al., 2006) based on the following arguments: The establishment

of an endosymbiotic relationship is driven mainly through complementation of the host cell's limited metabolic capabilities by the biochemical versatility of the endosymbiont (Nowack and Melkonian, 2010). Therefore, the integration of at least one or more transporters into the bacterial membranes to establish a connection between both cells would enable the export of bacterial metabolites and allow the host cell to benefit immediately from bacterial metabolism, i.e. photosynthesis and other processes, thereby stabilizing and sustaining endosymbiosis early on (Cavalier-Smith, 2000; Bhattacharya et al., 2007; Tyra et al., 2007).

In modern plants, carbon assimilated in photosynthesis is exported from chloroplasts via two different routes. During the day, carbon is exported in the form of TPs by the TPT (Flügge et al., 2003) while at night chloroplasts export mainly maltose and—to a lesser extent—Glc, both derived from starch breakdown (Weber et al., 2000; Niittylä et al., 2004). An extensive analysis of the phylogeny of the pPT family has clearly revealed that these transporters are related to a large superfamily of transporters, the nucleotide sugar transporters that are localized in the Golgi or ER membranes and transport nucleotide sugars like GDP-Man or UDP-Gal into the ER and Golgi lumen (Knappe et al., 2003; Weber et al., 2006). The pPTs are derived from a single nucleotide sugar transporter of the host cell that was rerouted from its location, the endomembranes, to the bacterial plasma membrane. This hypothesis is supported by recent evidence of membrane contact sites between the ER membranes and plastids that allow the exchange of lipids between both organellar membranes but that might also explain the rerouting of proteins from endomembranes to plastids during organellar evolution (Andersson et al., 2007). Thus, it is tempting to speculate that the first metabolites exported from the endosymbiont were nucleotide sugars like UDP-Gal or, as has been suggested very recently, ADP-Glc (Colleoni et al., 2010). During the evolution of plastids the substrate specificities of the pPTs later changed from nucleotide sugars to phosphorylated metabolites like TPs and, after the split into the different pPT subfamilies, to PEP and sugar phosphates.

To get more insight into the evolution of plastid envelope transporters, Bhattacharya and colleagues performed a detailed phylogenetic analysis of more than 80 plastid transporters commonly found in plants (Tyra et al., 2007). It turned out that about 60% of all transport proteins were derived from existing host membrane proteins. These include, besides the pPT family, phosphate transporters and transporters for *S*-adenosyl-Met and folate (Fig. 1; Table I; Tyra et al., 2007). In contrast, only 12% of the membrane proteins were contributed by the cyanobacterial endosymbiont, for example a second folate transporter (Klaus et al., 2005). For about 20% of the proteins their origin could not be conclusively determined. These include Mex1 that is found only in higher plants and a number of transporters of unknown function. Finally, a number of proteins were identified having homologs only in obligate intracellular bacterial parasites like *Chlamydia* and *Rickettsia* (Tyra et al., 2007). These are the two NTTs, the metal ATPase HMA1, the phosphate transporter PHT2, and the dicarboxylate translocators (DiT1, 2). These surprising findings have recently been explained by a horizontal gene transfer between plants and bacteria. Although the direction of the transfer is a matter of discussion, phylogenetic analysis supports a *Chlamydia*-to-*Plantae* transfer (Tyra et al., 2007 and refs. therein).

The data discussed here clearly corroborate the assumption that the integration of plastid and host metabolism was mainly driven by the host cell and that their interrelationship is rather slavery than symbiosis.

## CONCLUSION

In the last 20 years enormous progress has been achieved in understanding the transport processes across both the inner and outer envelope membranes of plastids and their role in connecting the metabolism of plastids with that of the other parts of a plant cell. However, we are far away from having completed the puzzle. The omics techniques (genomics, proteomics, transcriptomics, metabolomics, etc.) that have been developed in the last decade and that are now collectively called systems biology have had and will have an enormous impact on plant science. Several plastid transporters have been identified and characterized by these methods in combination with biochemistry and molecular biology. One goal ought to be the description and functional characterization of the proteome of a model plastid, namely the chloroplast of Arabidopsis, including the function of all transporters in both the inner and outer envelope membrane. A second goal would be to look at the differences of the proteomes of different types of plastids and at changes of the permeability of the plastid membranes during plastid differentiation. Studying the adaptation of the transport processes to different types of photosynthesis, namely $C_3$, $C_4$, and crassulacean acid metabolism and studying the transport into secondary plastids will lead to a better understanding of adaptations to changing environments and of the evolution of intracellular connections.

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