Interaction of cytosolic and plastidic nitrogen metabolism in plants

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Abstract

In angiosperms, the assimilation of ammonia resulting from nitrate reduction and from photorespiration depends on the operation of the plastidic GS/GOGAT cycle. The precursor for ammonia assimilation, 2-oxoglutarate, is synthesized in the mitochondria and in the cytosol. It is imported into the plastid by a 2-oxoglutarate/malate translocator (DiT1). In turn, the product of ammonia assimilation, glutamate, is exported from the plastids by a glutamate/malate translocator (DiT2). These transport processes link plastidic and cytosolic nitrogen metabolism and are essential for plant metabolism. DiT1 was purified to homogeneity from spinach chloroplast envelope membranes and identified as a protein with an apparent molecular mass of 45 kDa. Peptide sequences were obtained from the protein and the corresponding cDNA was cloned. The function of the DiT1 protein and its substrate specificity were confirmed by expression of the cDNA in yeast cells and functional reconstitution of the recombinant protein into liposomes. Recent advances in the molecular cloning of DiT2 and in the analysis of the in vivo function of DiT1 by antisense repression in transgenic tobacco plants will be discussed. In non-green tissues, the reducing equivalents required for glutamate formation by NADH-GOGAT are supplied by the oxidative pentose phosphate pathway. Glucose 6-phosphate, the immediate precursor of the oxidative pentose phosphate pathway is generated in the cytosol and imported into the plastids by the plastidic glucose 6-phosphate/phosphate translocator.

Key words: Glucose 6-phosphate/phosphate translocator, glutamate/malate translocator, glutamine/glutamate translocator, GS/GOGAT, photorespiration, 2-oxoglutarate/malate translocator.

Subcellular localization of the main ammonia assimilation pathway in plants

In green leaves of most C3-type angiosperm plant species, ammonia is assimilated into nitrogen compounds via the plastidic glutamine synthetase/glutamate synthase (GS/GOGAT) cycle (Miflin and Lea, 1980) with the plastidic GS2 being the predominant isozyme in these tissues (McNally et al., 1983). The plastidic GS2/GOGAT cycle has to cope with ammonia produced by nitrate/nitrite reduction and, to a larger extent, with ammonia released in the photorespiratory cycle. In several achlorophyllous plant parasites, however, cytosolic GS1 is the only detectable isozyme and in several C4-type and CAM plants, the contribution of GS1 to total GS-activity is about 35–80% (McNally et al., 1983).

The predominant role of the GS/GOGAT-system for the reassimilation of ammonia released during the regeneration of 3-phosphoglycerate in the photorespiratory pathway was further established by the isolation of mutants defective in the ferredoxin-dependent glutamate synthase (Fd-GOGAT) and in GS2. An A. thaliana mutant that is defective in Fd-GOGAT activity has been described (Somerville and Ogren, 1980). This mutant is unable to grow under ambient CO2 conditions and is only viable under high CO2 conditions that suppress...
photorespiration. Barley mutants defective in GS2 have been described previously (Blackwell et al., 1987; Wallsgrove et al., 1987). These mutants developed normally when grown under conditions that minimized photorespiration. In air, however, they were unable to grow. The studies using mutants of Arabidopsis and barley conclusively demonstrated that both GS2 and Fd-GOGAT are absolutely necessary for the reassimilation of ammonia released during photorespiration in higher plants.

Fusion of the reporter gene GUS to the promoter regions of the GS1 and GS2 genes from pea and subsequent transformation of the reporter constructs into tobacco plants revealed a cell-specific expression pattern of the $GS2::GUS$ fusion in photosynthetic cells, whereas expression of the $GS1::GUS$ construct was restricted to phloem cells. Immunocytochemical studies also demonstrated that GS2 was localized to the plastids of mesophyll cells whereas GS1 could be detected mainly in the cytosol of phloem companion cells (Carvalho et al., 1992). Since GS1 is also induced by wounding and during senescence, differential roles for GS1 (synthesis of amino acids for long-distance transport) and GS2 (synthesis of amino acids for protein biosynthesis) have been suggested (Masclaux et al., 2000).

Because the precursor for ammonia assimilation is synthesized in the cytosol and/or in the mitochondria (for a recent review on the synthesis of 2-oxoglutarate, see Lancien et al., 2000), plastids rely on a transport system for the import of 2-oxoglutarate into the plastids and for the export of the product of ammonia assimilation, glutamate, from the plastids into the cytosol.

**Physiological characterization of plastidic dicarboxylate translocators in C₃-plants**

Lehner and Heldt first reported on the characterization of plastidic dicarboxylate transporters in spinach chloroplasts (Lehner and Heldt, 1978). As demonstrated by silicone-oil-filtration-centrifugation, these proteins catalyse the uptake of $^{14}$C-labelled dicarboxylic acids (e.g. 2-oxoglutarate, malate, succinate, aspartate, and glutamate) in an antiport manner. The uptake showed saturation kinetics and was stimulated by light. The measurements of the $K_m$ and $K_i$ values for the individual dicarboxylates suggested the existence of more than one translocator with overlapping substrate specificities. Woo and Osmond analysed the ammonia and 2-oxoglutarate-dependent O$_2$ evolution in isolated chloroplasts and found a strong stimulation of the (ammonia, 2-oxoglutarate)-dependent O$_2$ evolution by dicarboxylates (e.g. malate, succinate and fumarate) (Woo and Osmond, 1982). The calculated maximum rates of ammonia assimilation in isolated chloroplasts were found to be sufficient to cope with the reassimilation of ammonia generated in the photorespiratory pathway in vivo. Using a reconstituted spinach chloroplast system, Anderson and Osmond found no significant effect of malate on the (glutamine, 2-oxoglutarate)-dependent O$_2$ evolution, thereby supporting the suggestion that the observed stimulation of (ammonia, 2-oxoglutarate)-dependent O$_2$ evolution of isolated chloroplasts by malate may be due to a promotion of 2-oxoglutarate uptake into chloroplasts by malate (Anderson and Walker, 1983). However, Lehner and Heldt had shown that the uptake of 2-oxoglutarate is competitively inhibited by malate (Lehner and Heldt, 1978). Thus, the observed malate-stimulation of ammonia assimilation by isolated chloroplasts would require an entirely new mechanism for the transport of 2-oxoglutarate across the chloroplast envelope membrane.

**The two-translocator model for the transport of dicarboxylic acids and glutamate in plastids**

The mechanism of 2-oxoglutarate uptake into chloroplasts has been investigated further (Woo et al., 1987a; Flügge et al., 1988) and it was shown that the chloroplast envelope membrane contains at least two distinct dicarboxylate transporters with partially overlapping substrate specificities, the 2-oxoglutarate/malate-translocator, (DiT1) and the glutamate/malate-translocator (DiT2). DiT1 was demonstrated to be specific for dicarboxylates (malate, succinate, fumarate, glutarate, and 2-oxoglutarate) whereas DiT2, in addition to the substrates transported by DiT1, also accepts the amino acids glutamate and aspartate. A two-translocator model for the transport of 2-oxoglutarate and glutamate was proposed (Fig. 1A) that explained the stimulation of 2-oxoglutarate transport into isolated chloroplasts and the stimulation of the (ammonia, 2-oxoglutarate)-dependent oxygen evolution in isolated chloroplasts by malate (Woo and Osmond, 1982). According to this model, 2-oxoglutarate is imported into the plastid in counter-exchange with malate by DiT1 and is subsequently converted to glutamate by GS; GOGAT. Glutamate is then exported to the cytosol by DiT2, again in counter-exchange with malate. In summary, this results in a counter-exchange of 2-oxoglutarate with glutamate without a net malate transport. By inhibiting DiT2 with high concentrations of glutamate, the kinetic properties of DiT1 could be analysed in more detail (Yu and Woo, 1992a, b). The $K_m$ for the uptake of malate into isolated spinach chloroplasts was found to be 2.7 mM and 0.6 mM for DiT1 and DiT2, respectively. The different affinities of DiT1 and DiT2 for malate and the other substrates provide the kinetic basis of the ‘push and pull’ mechanism for the transport of dicarboxylates that was proposed by the two-translocator model. In potato leaves, the concentration of malate at the end of the light...
period was found to be 3.2 mM in the cytosol and 2.4 mM in the stroma. The concentrations of glutamate in the cytosol and the stroma were determined to be 41 mM and 26.4 mM, respectively (Leidreiter et al., 1995). Consequently, the export of glutamate from the plastid stroma to the cytosol occurs against a concentration gradient and is driven by the inverse concentration gradient for malate. For 2-oxoglutarate, no data on the subcellular concentration are available. However, it can be speculated that the concentration of 2-oxoglutarate in the cytosol is in the low micromolar range and even lower in the stroma (because GS/GOGAT represents a strong sink for 2-oxoglutarate). Therefore, the import of 2-oxoglutarate is driven by a concentration gradient towards the stroma and this, in turn, drives the export of malate to the cytosol against a concentration gradient. The question arises, why two transporters need to work in concert. Considering the high concentration of glutamate in the cytosol in comparison with the low concentration of 2-oxoglutarate (at least three orders of magnitude difference in concentration), it is obvious that the transport of 2-oxoglutarate by one single transporter with affinities for both glutamate and 2-oxoglutarate, would always be out-competed by glutamate and no transport of 2-oxoglutarate would occur. However, the presence of two transporters with overlapping substrate specificities allows the import of 2-oxoglutarate into the plastid stroma by DiT1 even in the presence of high cytosolic concentrations of glutamate.

**The plastidic glutamine translocator and the three-translocator model**

Using a double silicone layer filtering centrifugation system, Yu and Woo characterized a specific glutamine translocator from spinach and oat chloroplasts (Yu and Woo, 1988). The transport of glutamine into the plastid stroma was greatly affected by the endogenous dicarboxylate pools of plastids. In glutamine-preloaded chloroplasts, the uptake of glutamine was competitively inhibited by glutamate only, but not by other dicarboxylates that are substrates of DiT2, thus demonstrating that the glutamine transport is mediated by a translocator different from DiT2. The combined action of this putative glutamine/glutamate translocator and both DiT1 and DiT2 opens the way to export glutamine in exchange for 2-oxoglutarate, with no net glutamate and malate transport, thereby providing the cytosol with glutamine as a precursor, for example, for biosynthesis of asparagine (Fig. 1B). The coupling of glutamine export from plastids to the availability of glutamate in the cytosol may act as a control mechanism circumventing glutamine-depletion of the GS/GOGAT-cycle. In summary, it is likely that, in vivo, the transport of dicarboxylates, glutamate and glutamine occurs according to the three-translocator model.

**Ammonia assimilation and dicarboxylate transport in gymnosperms**

By contrast to the situation in C3-type angiosperms (McNally et al., 1983), there is increasing evidence that at least in some gymnosperm species GS1 is the dominant isoform of GS and therefore most of the GS activity is found in the cytosol and not in the plastid stroma (Cantón et al., 1993). An immunolocalization study of GS1 showed that GS1 is localized not only in phloem cells of pine seedlings, but also in mesophyll cells (García-Gutiérrez et al., 1998). A detailed analysis of GS1

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**Fig. 1.** The two-translocator model for transport of dicarboxylic acids and glutamate (A) and the three-translocator model for the transport of dicarboxylates, glutamate and glutamine (B) into chloroplasts (1, DiT1; 2, DiT2; 3, glutamine/glutamate translocator). Cycling of substrates between chloroplast stroma and the cytosol is indicated by broken lines whereas net fluxes are indicated by solid lines.
expression levels in Scots pine seedlings (Cantón et al., 1999) showed that GS1 mRNA levels increased upon transfer of seedlings into the light. These findings suggested that glutamine synthesis in pine seedlings by glutamine synthetase mainly takes place in the cytosol rather than in the plastid stroma. Since the glutamine required for the de novo synthesis of glutamate by plastidic Fd-GOGAT is supplied by cytosolic GS1, an active glutamate/glutamine exchange able to cope with the flux of ammonia from nitrate reduction and photorespiration has to be postulated. Two possible models for the transport of 2-oxoglutarate, glutamate and glutamine can be proposed.

Possible models for the transport of 2-oxoglutarate, glutamate and glutamine into gymnosperm plastids

One possible mode of transport could involve DiT2 and the glutamine translocator working in concert as a double translocator system for 2-oxoglutarate, glutamate and glutamine (Fig. 2A). DiT2 would import 2-oxoglutarate into the plastid stroma in counter-exchange with an export of glutamate. Glutamate is converted to glutamine in the cytosol by GS1 and imported into the plastid in a counter-exchange for glutamate. In summary, this would result in a net flux of 2-oxoglutarate to glutamate whereas, in parallel, glutamine and glutamate would cycle on DiT2 and the glutamine translocator, respectively. In this scenario, the activity of DiT1 would not be required.

The other possible model would combine DiT1, DiT2 and the glutamine translocator to a three-translocator model (Fig. 2B). In this model, glutamine formed by GS1 in the cytosol is imported into the chloroplast in a 1:1 counter-exchange with glutamate. The import of 2-oxoglutarate by DiT1 and the export of the second molecule of glutamate resulting from Fd-GOGAT action by DiT2 is coupled to malate counter-exchange in the cascade-like ‘push-and-pull’ mechanism already described for the double translocator system.

In order to check which of the above-described models describes best the in vivo situation, it will be necessary to analyse the expression patterns of DiT1 and DiT2 in gymnosperms, such as pine seedlings, as well as the transport characteristics of pine seedling chloroplasts. It might be speculated that the three-translocator model best describes the situation in vivo.

Role of the plastidic glucose 6-phosphate/phosphate translocator during ammonia assimilation

Non-green plastids of heterotrophic tissues rely on the provision of carbohydrates from the cytosol. The imported carbon is used as a substrate for biosynthetic pathways, for example, for the biosynthesis of starch or fatty acids. Carbon can also be used to drive the oxidative pentose phosphate pathway (OPPP), which is the major source of reducing power required for the reduction of nitrite and the biosynthesis of fatty acids and of amino acids. The immediate substrate for the OPPP is glucose 6-phosphate (Glc6P) that is imported into the plastids by the Glc6P/phosphate translocator (GPT; Kammerer et al., 1999).
et al., 1998). The GPT thus links reactions located in the cytosol, the conversion of sucrose to hexoses and Glc6P, with the metabolism inside the plastid. Contrary to photosynthetic tissues, the main source of ammonium is not photorespiration but nitrate assimilation. Previous studies have shown that nitrite reduction and the subsequent synthesis of glutamate via GS/NAD(P)H-GOGAT is strongly dependent on the provision of the plastids with Glc6P and a functional OPPP that delivers reductants for assimilatory processes in non-green plastids (Bowsher et al., 1989, 1992; Fig. 3).

**Purification and identification of the plastidic 2-oxoglutarate/malate translocator**

Menzlaff and Flügge reported on the identification of the plastidic 2-oxoglutarate/malate translocator as a component of the plastid envelope membrane (Menzlaff and Flügge, 1993). Detergent solubilized envelope membrane proteins were separated by two successive column chromatography steps and the activity of DiT1 was monitored by reconstitution of column fractions into liposomes followed by transport measurements. DiT1 transport activity could be assigned to a protein with an apparent molecular mass of 45 kDa.

Purified, reconstituted DiT1 accepted malate, succinate, tartrate, glutarate, 2-oxoglutarate, and fumarate, but not the amino acids aspartate and glutamate, as counter-substrates for the exchange of malate. Thus, the substrate specificity of purified, reconstituted DiT1 was in accordance with the substrate specificity determined for DiT1 in isolated spinach chloroplasts (Woo et al., 1987a, b; Flügge et al., 1988) and therefore in accordance with the function of DiT1 proposed in the two-translocator model for the transport of dicarboxylates.

**cDNA cloning, expression in yeast cells and functional characterization of DiT1**

The identification of DiT1 as a component of the chloroplast envelope membrane (Menzlaff and Flügge, 1993) was instrumental for cloning of the corresponding cDNA. Weber et al. developed a preparative purification procedure for the 45 kDa protein based on its solubility in chloroform (Weber et al., 1995). The protein could be purified by preparative SDS-PAGE in amounts sufficient for subsequent cleavage with the endoproteinase Lys-C. This approach yielded two peptide sequences of DiT1 that were used to synthesize corresponding oligonucleotides and subsequently to screen a spinach leaf cDNA library.

A full-length cDNA was obtained, encoding a highly hydrophobic protein of 569 amino acids with a predicted molecular mass of 60.3 kDa and a calculated isoelectric point of 9.6. A plastid targeting signal sequence of 90–100 amino acid residues in length was predicted from the protein sequence. The in vitro-translated protein was found to be imported into intact isolated chloroplasts and processed to its mature form.

By contrast to other known translocator proteins of the plastid envelope membrane at that time (e.g. the triose phosphate/phosphate translocator; Flügge et al., 1989) that were found to form dimers of two identical sub-units with six α-helices each, the DiT1 protein possesses 12 highly hydrophobic membrane-spanning segments. Such a 12 transmembrane helix topology is found in many bacterial transporters or transporters of the plasma membrane. Later, a similar topology was also found for other plastidic transporters, for example, the adenylate translocator (Neuhaus et al., 1997) and the hexose translocator (Weber et al., 2000). Currently, it seems more likely that the phosphate translocator family is rather the exception than the rule in terms of its transmembrane topology (for a recent review on plastid transporter proteins see Neuhaus and Wagner, 2000).

Surprisingly, no significant homologies were found to dicarboxylate transporter proteins from mitochondria (Walker and Runswick, 1993; Taniguchi and Sugiyama, 1996) although both transporters share similar substrate specificities and functions.

In order to confirm the identity of the DiT1 cDNA with the proposed function, the cDNA was expressed in yeast cells. Membrane proteins from yeast cells harbouring the transgene were isolated and reconstituted into liposomes. The malate uptake into liposomes containing the recombinant protein was found to exceed the transport activity of control liposomes by at least two orders of magnitude. It could also be shown that the recombinant protein possessed substrate specificities that were identical to those of the authentic protein (Weber et al., 1995).
Related sequences to DiT1 in plants and bacteria

Related sequences in plants

A sequence homology search of the GenBank database (Viridiplantae subsection) was performed to identify genes that are homologous to DiT1 gene from spinach. These homologues can be grouped into two related, but distinct classes. The first group contains sequences from *A. thaliana*, spinach, corn, and tobacco and is closely related to spinach DiT1 (70–85% identity at the protein level). The second group (DiT2-group), containing sequences from *A. thaliana*, *Sorghum bicolor*, *Flaveria bidentis*, tobacco, and spinach shares only about 45–55% identity to the spinach DiT1 protein, but 70–80% identical amino acid residues with each other (see phylogenetic tree, Fig. 4). The *A. thaliana* genome contains three genes homologous to spinach DiT1, all located on chromosome 5. The DiT1 homologue is a single copy gene whereas the two other genes fall into the DiT2-group.

**Fig. 4.** Unrooted phylogenetic tree of the dicarboxylate transporter family in plants and eubacteria. The first two letters of the acronyms indicate the species (At, *Arabidopsis thaliana*; Bs, *Bacillus subtilis*; Cm, *Chlamydia muridarum*; Cp, *Chlamydia pneumoniae*; Ct, *Chlamydia trachomatis*; Ec, *Escherichia coli*; Fb, *Flaveria bidentis*; Hi, *Haemophilus influenzae*; Nt, *Nicotiana tabacum*; Sa, *Staphylococcus aureus*; So, *Spinacia oleracea*; Zm, *Zea mays*). DiT, dicarboxylate transporter; TST, tartrate succinate transporter; CST, citrate succinate transporter.
of sequences. The *A. thaliana* DiT2 genes are arranged in tandem with a head to tail orientation. No other significant homologies were found to other plant proteins.

A cDNA representing DiT2 from spinach has recently been isolated and this cDNA was expressed in yeast. Preliminary experiments showed that the reconstituted recombinant DiT2 protein has a substrate specificity comparable to DiT1 but, in addition to the substrates transported by DiT1, accepts glutamate and aspartate as counter-substrates for malate. It is therefore very likely that the DiT2 group of genes encodes the general dicarboxylate translocator (glutamate/malate-translocator) of the plastidic double-translocator system for dicarboxylates (P Renne´, UI Fl¨ugge, A Weber, unpublished results).

The *E. coli* citrate transporter CitT is related to DiT1

Most *Escherichia coli* strains are unable to grow on citrate under oxic conditions. Under anaerobic conditions, however, *E. coli* is able to grow on citrate in the presence of an oxidizable co-substrate (e.g. glucose or glycerol). Citrate is taken up into the cell and split into acetate and oxaloacetate by citrate lyase (Lütgens and Gottschalk, 1980). Oxaloacetate is then reduced to succinate by concerted reactions of malate dehydrogenase, fumarase and fumarate reductase. The required reducing equivalents are generated by the oxidation of the co-substrate (e.g. glucose or glycerol). Two enzymes are specifically required for the fermentation of the tricarboxylic acid, a citrate uptake system and citrate lyase. Pos *et al*. reported on an open reading frame (designated CitT) located at 13.90 min on the *E. coli* chromosome between the RNA (RNase I) and the citrate lyase genes that encodes a citrate lyase (Lu¨tgens and Gottschalk, 1980). Since succinate is the end-product of citrate fermentation in *E.coli*, it is likely that CitT catalyses either an homologous exchange of citrate or a heterologous exchange with succinate, fumarate, or tartrate (Pos *et al.*, 1998). *E. coli* cells transformed with a plasmid expressing CitT were able to grow on citrate under aerobic conditions. It could be also shown that CitT catalyses either an homologous exchange of citrate or a heterologous exchange with succinate, fumarate, or tartrate (Pos *et al.*, 1998). Since succinate is the end-product of citrate fermentation in *E.coli*, it is likely that CitT functions in *vivo* as a citrate/succinate antiporter. CitT is a highly hydrophobic protein (487 amino acids, 53.1 kDa) with 12 putative transmembrane helices. It is related to the 2-oxoglutarate/malate translocator from spinach chloroplasts (SoDiT) with the amino acid identity of about 34%.

Proteins related to DiT1 in other bacterial species

Related proteins are also encoded by the genomes of other bacteria like *Helicobacter pylori*, *Staphylococcus aureus*, *Chlamydia trachomatis*, *Chlamydia pneumoniae*, and *Bacillus subtilis*. The protein from *B. subtilis* shows the highest amino acid identity (53%) followed by the proteins from *C. trachomatis* and *C. pneumoniae* with about 48% amino acid identities. DiT2 displays a somewhat lower identity with the bacterial transporters, suggesting that DiT1 is more closely related to the common ancestor of the bacterial and plant transporters while DiT2 might have evolved from DiT1 during the evolution of plants. The primary function of DiT1 in early plant cells might have been the indirect exchange of redox equivalents between the endosymbiont and the host cell. For example, in *Chlamydia*, 2-oxoglutarate is taken up from the host cell by a DiT1 homologue in exchange for malate. The oxidation of 2-oxoglutarate to malate leads to the generation of reductants and of energy in the form of GTP. Since no significant homologies are found to proteins from cyanobacteria, it is tempting to speculate that DiT1 might trace back to the genome of the host cell of the endosymbiotic event that eventually led to the evolution of a eukaryotic plant cell. No significant homologies with proteins from fungi or animals were found. Together with the bacterial transporters, DiT1 and DiT2 form a class of plant and eubacterial transporters involved in the transport of di- and tricarboxylic acids (Fig. 4).

**In vivo function of plastidic dicarboxylate translocators**

Sommenville and Ogren isolated an EMS-mutagenized photorespiratory mutant of *A. thaliana* (Sommenville and Ogren, 1983) that was shown to be defective in the plastidic glutamate/malate-translocator (dct, Somerville and Somerville, 1985). A corresponding mutant from barley has also been described (Wallsgrove *et al*., 1986).

The *A. thaliana* dicarboxylate translocator mutant dct

The *A. thaliana* mutant was shown to be not viable under ambient CO2-conditions, but grew like the wild type under elevated CO2 concentrations that suppress photorespiration. The phenotype was attributed to the inability of the mutant to carry out a functional photorespiratory pathway. A defect in a plastidic dicarboxylate translocator would lead to a deficiency in the reassimilation of ammonia resulting from glycine-decarboxylase activity, or a decreased export capacity for glutamate (or both). In addition, either case would lead to a shortage in glutamate supply for the formation of glycine from glyoxylate in the peroxisomes, leading to a build-up of toxic photorespiratory intermediates in the mutant. Furthermore, a block in photorespiratory cycling causes a depletion of Calvin cycle intermediates. From the physiological characterization, it is difficult to decide whether the mutant is deficient in the activity of either
DiT1 or DiT2. Since the complete sequence of the *Arabidopsis* genome is now available, it should be possible to search for mutations in the three genes related to spinach DiT1 in the *Arabidopsis* genome in order to identify the gene defective in *dct*.

**The barley dicarboxylate transport mutant RPr 79/2**

Wallsgrove *et al.* isolated a barley mutant (RPr 79/2) that is defective in chloroplast dicarboxylate transport (Wallsgrove *et al.*, 1986). This mutant resembles in many aspects the *Arabidopsis* mutant *dct*, including the labelling pattern of serine, glycine, glycerate, and malate following fixation of $^{14}$CO$_2$, the rapid rise in glutamine content and the fall in glutamate content in leaves upon transfer from non-photorespiratory conditions to air. In contrast to *dct*, the barley mutant does not die in ambient air unless the temperature and irradiance are high. The survival of RPr 79/2 in ambient air might be due to the build-up of 2-oxoglutarate levels (up to 10-fold in comparison to the wild type) that might overcome the deficiency in chloroplast dicarboxylate transport (probably in DiT1) by allowing DiT2 to operate as a 2-oxoglutarate/glutamate exchanger under these conditions.

**Knockout lines for plastidic dicarboxylate translocators**

The availability of large collections of T-DNA tagged *A. thaliana* insertion mutants (Krysan *et al.*, 1999; Sussman *et al.*, 2000) enabled a PCR-based reverse genetic screen to be set up to identify mutants that are defective in either of the three DiT-related genes. A homozygous knockout line for *A. thaliana DiT2.2* was isolated (Ü Kolukisaoglu, B Schulz, A Weber, unpublished results) but, surprisingly, the mutant does not display an obvious phenotype. This might be due to a redundancy in gene function with DiT2.1 or to a tissue or cell specific expression pattern of the DiT2.2 gene that does not lead to a visible phenotype in the aerial parts of the plant. As judged from the availability of *A. thaliana* ESTs for DiT2.1 and DiT2.2, it can be speculated that DiT2.1 is expressed at higher levels and more ubiquitous as compared to DiT2.2. A search for insertional mutants in DiT1 and DiT2.1 is currently underway.

**Antisense repression of DiT1 in transgenic tobacco plants**

Antisense repression of gene expression has turned out to be a successful approach to study the *in vivo* function of plant genes. Transgenic tobacco plants showing antisense repression of DiT1 gene expression have been generated (A Weber, J Schneiderreit, U Flügge, W Kaiser, unpublished results). These tobacco plants show a distinct phenotype, characterized by bleached veins, altered leaf shape, stunted growth and delayed flowering (Fig. 5). The transformants possess lower levels of amino acids and protein, but increased levels of ammonia and glyoxylate, suggesting a defect in the reassimilation of ammonia generated in the photorespiratory pathway and in the regeneration of 3-phosphoglyceric acid from glyoxylate. This assumption is supported by the observation that the phenotype of DiT1 antisense repression can be at least partially suppressed by cultivation of the transgenic plants under elevated CO$_2$ levels. In addition to the aspects of the phenotype related to the photorespiratory pathway, profound effects on the accumulation of nitrate were found in the transgenic plants and a striking discrepancy between the *in vivo* activity of nitrate reductase and the *in vivo* rate of nitrate reduction.

Taken together, the findings by Somerville and Somerville (Somerville and Somerville, 1985), Wallsgrove *et al.* (Wallsgrove *et al.*, 1986), and the authors’ observations on antisense repression of DiT1 in transgenic tobacco plants suggest an important role of plastidic dicarboxylate translocators in the co-ordination of the plastidic and the cytosolic C and N-metabolism by controlling the flux of 2-oxoglutarate into the plastid stroma and of glutamate to the cytosol.

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**References**


