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Antisense repression of the chloroplast triose phosphate translocator affects carbon partitioning in transgenic potato plants

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ABSTRACT **The major chloroplast envelope membrane protein E29 is central for the communication between chloroplasts and cytosol. It has been identified as the triose phosphate translocator (TPT) exporting the primary products of the Calvin cycle (i.e., triose phosphates and 3-phosphoglycerate) out of the chloroplast in a strict counter exchange for P_i. To study the *in vivo* role of the TPT, transgenic potato plants were constructed that have a reduced expression of the TPT at both the RNA and protein level due to antisense inhibition. Chloroplasts isolated from these plants show a 20–30% reduction with respect to their ability to import P_i. The reduced TPT activity leads to a reduction of maximal photosynthesis by 40–60%, to a change in carbon partitioning into starch at the expense of sucrose and amino acids, and to an increase of the leaf starch content by a factor of ≈3. At early developmental stages the inhibited plants are retarded in growth compared to the wild type.**

During photosynthesis, triose phosphates (triose-*P*) represent the net product of the Calvin cycle. Only one-sixth of the triose-*P* formed during the CO_2 fixation reaction can be withdrawn from the Calvin cycle. Triose-*P* can be shuttled into several biosynthetic pathways within the chloroplast such as starch formation or lipid biosynthesis or can be exported from the chloroplasts to the cytosol and there converted into sucrose or amino acids. A specific transport system located at the inner membrane of the chloroplast envelope catalyzes the export of triose-*P*. The spinach triose-*P* translocator (TPT) with an apparent molecular mass of 29 kDa (E29) is the major protein of the inner envelope membrane accounting for 10–15% of the total protein content of this membrane (1). TPT mediates transport of triose-*P*, 3-phosphoglycerate (3-PGA), and P_i in a strict counter-exchange mode (2). In the cytosol, P_i is released during biosynthetic processes and is made available as the counterion for triose-*P* export. When the export of triose-*P* from the chloroplasts is limited by shortage of P_i (e.g., due to decreased sucrose synthesis), fixed carbon can be deposited in chloroplasts in the form of starch (3). The TPT gene is nuclear-encoded and respective cDNAs have been cloned from spinach (4), pea, and potato (5, 6).

The export of triose-*P* into the cytosol is of central importance with respect to assimilate partitioning between various organs and allocation between various pathways such as sucrose and starch biosynthesis. To study these processes, we create transgenic plants that are modulated at steps in the allocation or partitioning of photoassimilates by using antisense RNA repression or (ectopic) expression of (alien) enzymes (7–9). Specifically, we created transgenic plants with reduced TPT activity due to antisense repression to test the effect of reduced TPT activity on photosynthesis, metabolism, and assimilate partitioning. Since the TPT governs export of photoassimilates from the chloroplasts, one would expect phenotypic changes as a result of the downregulation of the TPT activity. This includes the reduction of triose-*P* export and P_i import, which should affect both photosynthetic rates and allocation of photoassimilates.

MATERIALS AND METHODS

Recombinant DNA. The 1.35-kb *Hin*cII–*Eco*RI fragment of the potato TPT cDNA pTP2 (6) was ligated in reverse orientation between the *Eco*RI and *Sma* I restriction sites of pRT101 (10). The *Hin*dIII fragment containing the TPT antisense gene under control of the cauliflower mosaic virus (CaMV) 35S promoter and terminator was excised and cloned into pBin19 (11).

Transformation and Analysis of Transgenic Plants. Transfer of the chimeric construct into *Agrobacterium tumefaciens* GV2260 and transformation of potato were performed as described (12). Transgenic plants were transferred to soil and analyzed under greenhouse conditions. Experiments were repeated independently using *in vitro* propagated clones of the transformants. For Northern blot analysis, RNA was isolated from mature leaves of greenhouse-grown transformants and wild-type plants after 4–6 h of light as described (6). As a control, filters were rehybridized with a cDNA encoding the chloroplastic fructose 1,6-bisphosphatase, showing that equal amounts of RNA were present (data not shown). To ensure reproducibility, RNA was isolated from both young and mature plants and from different leaves. Similar levels of reduction in TPT mRNA were observed irrespective of plant age.

Chloroplast Membrane Proteins and P_i Uptake. Chloroplasts from leaves of wild-type and antisense potato plants were prepared essentially as described (13). Mature leaves from one individual greenhouse-grown plant were harvested after an extended period of darkness (12–14 h), mid ribs were excised and discarded, and the remaining material was homogenized in 0.33 M sorbitol/5 mM ascorbate/1 mM $MnCl_2$/1 mM $MgCl_2$/5 mM EDTA/0.5 mM KH_2PO_4/4 mM dithiothreitol/0.2% bovine serum albumin/0.1% polyvinylpyrrolidone/25 mM Hepes/25 mM Mes, pH 6.8. Uptake of [^{32}P]P_i and [^{14}C]malate was determined by silicone-oil-layer centrifugation (14) at 10°C in 0.33 M sorbitol/50 mM Hepes·KOH, pH 7.6, and was terminated after 20 sec by

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Abbreviations: 3-PGA, 3-phosphoglycerate; chl, chlorophyll; Glc-6-*P*, glucose 6-phosphate; triose-*P*, triose phosphate; TPT, triose-*P* translocator; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; RbuP_2, ribulose bisphosphate; CaMV, cauliflower mosaic virus.

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centrifugation. The transport of [^{14}C]malate was used to normalize [^{32}P]P_i uptake activities of different chloroplast preparations from wild-type and transgenic plants, respectively.

Envelope membranes from potato chloroplasts were isolated (15) and labeled with 5 μM [^{3}H]DIDS, where DIDS is 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (16). Identical amounts of envelope proteins from both wild-type and transgenic chloroplasts were analyzed by SDS/PAGE (17), silver staining, and fluorography (18). ^{3}H radioactivity incorporated into the 31-kDa polypeptide was quantitated by densitometry.

Physiological Measurements. Maximal photosynthesis rates were measured from leaf discs of greenhouse-grown plants in a Hansatech leaf-disc electrode (Kings Lynn, Norfolk, U.K.) under saturating light conditions. Each measurement was reproduced independently at least twice (19). Photosynthesis under ambient conditions was measured in growth chambers with a portable infrared gas analyzing system LCA3 (Analytical Development, Hoddesdon, U.K.). Starch determination was performed as described (20) and metabolites were analyzed (21). The data shown for 3-PGA and starch levels are representative for multiple repetitions of the analysis from both greenhouse- and growth-chamber-grown plants with samples taken from different leaves. For determination of $^{14}CO_2$ incorporation, detached leaves were placed into plastic cuvettes (10 × 15 cm) and a pulse of 1 MBq of $^{14}CO_2$ was added by injecting $NaH^{14}CO_3$ into a solution of 1 M perchloric acid. After 15 min, leaves were frozen in liquid N_2 and incorporated ^{14}C radioactivity was measured (19).

RESULTS

Reduced Levels of TPT mRNA Due to Antisense Inhibition. To reduce the level of TPT expression in transgenic potato plants by virtue of transfer of a chimeric gene expressing TPT antisense RNA, a 1.35-kb *Hin*cII–*Eco*RI fragment spanning most of the potato TPT coding region (6) was cloned in antisense orientation under control of the CaMV 35S promoter into pBIN19 (Fig. 1). *Solanum tuberosum* cv. Désirée was transformed via *Agrobacterium*-mediated gene transfer. The levels of TPT mRNA were analyzed in leaves of transgenic plants after transfer to the greenhouse. Eight (out of 50) transformants revealed a strong reduction of TPT mRNA in both young and old leaves. Even after overexposure, no or only very low levels of transcript were detectable. Transformants 1, 7, 9, 39, and 43 showing a strongly reduced level of expression were chosen for further analysis (Fig. 1).

Reduced Levels of TPT mRNA Affect Phosphate Transport Activity. To check whether the strong reduction of TPT mRNA also affected the amount of TPT protein, chloroplasts were isolated from spinach, wild-type potato, and transgenic potato plants 7 and 43. Envelope membranes were isolated from chloroplasts, labeled with [^{3}H]DIDS, a specific inhibitor for the TPT (22), and analyzed by SDS/PAGE and fluorography. In spinach envelopes, E29 is the major polypeptide and is the only protein that is labeled by micromolar concentrations of [^{3}H]DIDS (Fig. 2; ref. 22). Under these conditions, one molecule of [^{3}H]DIDS is incorporated into the dimeric E29 polypeptide (16). The major polypeptide from potato chloroplasts with an apparent molecular mass of 31 kDa is also strongly labeled with [^{3}H]DIDS, which is consistent with the potato protein being six amino acid residues larger than the spinach protein (6). Two of the transgenic potato plants (plants 7 and 43) that showed a strong reduction in levels of the TPT mRNA were analyzed by [^{3}H]DIDS-labeling of chloroplast envelope proteins. In agreement with the data from Northern blot analyses, a 20–40% reduction in the amount of [^{3}H]DIDS label associated with the 31-kDa polypeptide was measured compared to wild-type plants.

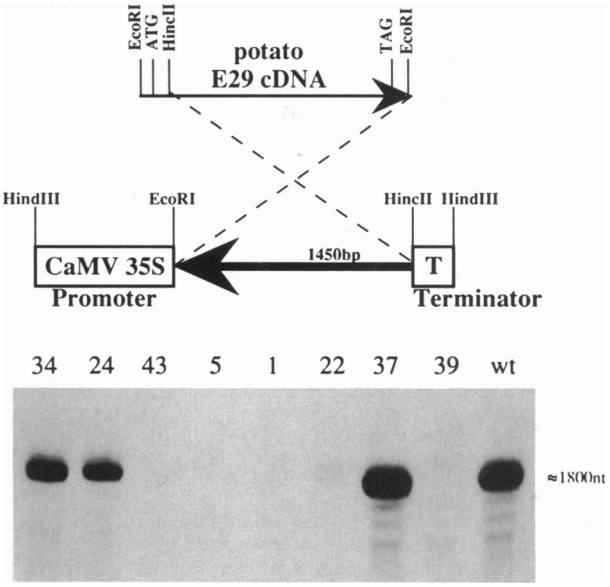

FIG. 1. Construction of TPT antisense plants and Northern blot analysis of TPT mRNA in leaves from transformed potato plants. (*Upper*) Structure of the chimeric TPT antisense gene. The 1.35-kb *Hin*cII–*Eco*RI fragment from the potato TPT cDNA clone pTP2 was cloned in antisense orientation into pBin19. (*Lower*) Northern blot analysis of TPT antisense plants. Total leaf RNA from 50 wild-type and TPT antisense plants was separated on formaldehyde/agarose gels (50 μg per lane), blotted on nylon membranes, and probed with a ^{32}P-labeled TPT cDNA [transformants are indicated as numbers above the lanes; wild type (wt), approximate transcript size is given to the right]. Transformants with a similar reduction in TPT mRNA levels were plants 1, 5, 22, 39, and 43 and plants 7, 9, and 31 (data not shown).

Chloroplast envelope membrane preparations from antisense plants 1 and 39 showed a similar degree of reduction of the labeled 31-kDa polypeptide. The extent of the reduction with respect to the protein amount is far less in quantitative terms than the reduction of the corresponding mRNA levels. The explanation for the difference between the reduction of respective RNA and protein levels is presently unclear.

To determine directly the chloroplast phosphate transport activity in the transgenic plants, chloroplasts were isolated from these plants and the kinetic constants for uptake of

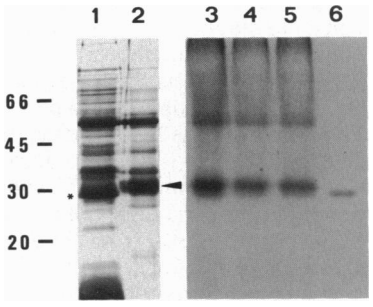

FIG. 2. [^{3}H]DIDS labeling of the chloroplast TPT. Envelope membranes from potato chloroplasts were isolated, labeled with 5 μM [^{3}H]DIDS, separated by SDS/PAGE, and stained in Coomassie blue or fluorographed. Lanes 1 and 2 show Coomassie blue-stained envelope membranes from spinach and potato chloroplasts, respectively. Spinach TPT (E29), asterisk; potato TPT, arrowhead. Lanes 3–6 are fluorographic analyses of [^{3}H]DIDS-labeled chloroplast envelope membrane polypeptides from wild-type plants (lane 3) and antisense potato plants 7 (lane 4) and 43 (lane 5). Identical amounts of membrane proteins were subjected to SDS/PAGE. Lane 6 shows E29 from spinach chloroplast envelope membranes labeled with 1 μM [^{3}H]DIDS. Relative molecular mass calibration (in kDa) is shown on the left.

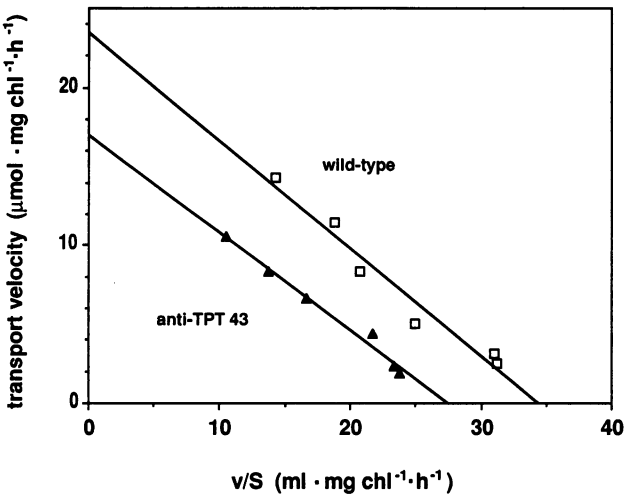

FIG. 3. Transport of [^{32}P]P_i into chloroplasts from wild-type (□) and antisense (▲) potato plants as determined by silicone-oil-layer centrifugation. The calculated transport data are shown in an Eadie–Hofstee presentation. chl, Chlorophyll.

[^{32}P]P_i were determined. Chloroplasts from plants with reduced TPT RNA showed a 20–30% reduction in V_{max} with the K_m remaining unaltered (Fig. 3 and Table 1). A similar range of reduction in V_{max} was observed for all transformants that showed a strong decrease in TPT RNA. The reduction in P_i transport activity is in good agreement with the reduction in TPT protein amount as determined by [^{3}H]DIDS labeling (Fig. 2).

Reduced Plant Vigor Due to Decreased TPT Activity. As outlined above, a reduced export of triose-*P* due to an inhibition of the TPT activity should result in a reduced supply of the cytosol with fixed carbon and thus should lead to a growth retardation due to decreased nourishment of import-dependent (sink) organs with photoassimilates. To test this hypothesis, nontransgenic control plants and transgenic plants with a high degree of inhibition of TPT expression or with largely no inhibition (control transgenics) were transferred from tissue culture to the greenhouse. At early growth stages, plants with strongly reduced levels of TPT mRNA reproducibly showed a severe retardation in growth and development (Fig. 4). This difference, however, became less evident upon further development, and mature plants showed only a slight reduction in height compared to wild-type control plants. No reduction in tuber yield was observed under these conditions (data not shown).

Decreased TPT Activity Affects Photosynthesis and Assimilate Partitioning. The TPT is supposed to play a central role in the control of photosynthesis by exporting triose-*P* to the cytosol in exchange for P_i that is released during sucrose or amino acid synthesis. To determine the effect of reduced TPT activity on photosynthesis, maximal photosynthetic rates were measured using leaf discs from adult plants under saturating light and CO_2 conditions. A 40–60% reduction in maximal rates compared to untransformed controls was observed for plants with reduced TPT expression levels (Table 1). The TPT transport activity, which was reduced by only 20–30%, thus strongly affects the maximal photosynthetic capacity. However, under ambient CO_2 and light conditions (i.e., when CO_2 assimilation of leaves from intact plants was continuously measured during a 16-h light period), no significant difference was observed between wild-type and antisense plants. Under these conditions the photosynthetic rates of wild-type and antisense plants were one-third of the maximum rates measured in the antisense plants and thus at most one-sixth of the maximum wild-type rates (Table 2). This is in line with the finding that the phenotype of mature plants is only marginally affected and no significant alteration in tuber yield is observed. No difference in chlorophyll content was detectable in mature plants between antisense and control plants (data not shown).

FIG. 4. Phenotype of TPT antisense plants. Five replicas of the transformants 1 (front), 9 (middle), and wild-type (rear) potato plants of the same size and age were transferred from tissue culture to soil and grown for 4 weeks in the greenhouse. All replicas of both transformants show a strong growth retardation.

To study the effects of reduced TPT activity on carbon partitioning under ambient conditions (i.e., when assimilation rates are unaffected), the total content of metabolites, sucrose, amino acids, and starch was analyzed. In addition, the partitioning of fixed $^{14}CO_2$ into starch, sucrose, and amino acids was determined. As shown in Table 2, 3-PGA, which is mainly localized inside the chloroplast (21), was increased by a factor of 1.4–1.8 in the transgenic plants, whereas the mainly cytosolic intermediates triose-*P* and glucose 6-phosphate (Glc-6-*P*) are decreased. Together with the increased level of ribulose 1,5-bisphosphate (RbuP_2), this indicates an inhibition of the export of chloroplastic products of CO_2 fixation. Whereas steady-state concentrations of sucrose and amino acids remained almost unchanged, the content of malate was reduced 40–60% in three antisense plants tested (Table 2). The strongest effect was observed for starch, which showed a 2- to 3-fold higher level in antisense plants at the end of the light period. In contrast to wild-type plants, where the endogenous starch pool was almost entirely depleted after 8 h of darkness, the TPT antisense plants possessed an up to 6-fold higher starch level (Table 2 and data not shown). A high correlation between increased starch content and reduced maximal photosynthesis rate was observed only in plants showing reduced TPT RNA levels, whereas transgenic plants with unaltered TPT mRNA amounts showed no difference compared to wild-type plants (data not shown). An increased carbon partitioning in favor of starch was also observed when the rates for biosynthesis

Table 1. Maximal rates of photosynthesis and P_i transport of wild-type and TPT antisense plants

Parameter	Plant				
	Wild type	1	9	39	43
Uptake of [^{32}P]P_i into chloroplasts, % of V_{max}	100	72 ± 3	69 ± 5	83 ± 4	72 ± 4
Maximal photosynthetic rates mmol of O_2 per m^2 per h	113.0 ± 17.0	44.3 ± 11.1	51.1 ± 5.1	51.9 ± 4.3	52.8 ± 11.1

[^{32}P]P_i uptake into isolated chloroplasts from wild-type and TPT antisense plants. The 100% control value was 29 μmol per mg of chl per h. Photosynthesis rates of excised leaf discs were measured under saturating light and CO_2 conditions.

Table 2. Metabolite levels in leaves from wild-type and TPT antisense potato plants

	Plant			
	Wild type	1	7	39
Photosynthetic rate, ambient conditions, mmol of O_2 per m^2 per h	17.4 ± 4.5	21.9 ± 5.3	14.3 ± 0.9	19.8 ± 3.8
3-PGA, nmol/mg of chl	254 ± 56	452	356	396
Triose-*P*, nmol/mg of chl	45 ± 9	25	21	30
3-PGA/triose-*P*	5.6	18.1	16.9	13.2
RbuP_2, nmol/mg of chl	45 ± 15	113	101	88
Glc-6-*P*, nmol/mg of chl	171 ± 39	124	121	86
Sucrose, nmol/mg of chl	4850 ± 2860	5700	3900	4500
Malate, nmol/mg of chl	4480 ± 2540	2650	2350	2300
Total amino acids, nmol/mg of chl	5370 ± 1360	5180	6410	7000
Starch (end of the day), μmol of hexose per mg of chl	64 ± 30	211	191	106
Starch (end of the night), μmol of hexose per mg of chl	1.6 ± 1.0	9.8 ± 3.6	ND	4.2 ± 1.4

Photosynthetic (assimilation) rates measured the light are reported as mean (±SD) values from five (wild-type plants) and three (TPT antisense plants) measurements. Metabolite analyses were performed from eight leaves of four wild-type plants or four leaves from two antisense plants harvested at the end of the light period. ND, not determined.

of starch, sucrose, and amino acids were measured after exposure of leaves to pulses of $^{14}CO_2$ in the light. In TPT antisense plants, a decrease in the relative rates of synthesis of sucrose to starch and of amino acids to starch was observed (Table 3). Thus, even under ambient conditions where photosynthesis rates are almost unaffected, the reduced TPT activity results in a significant change in partitioning of photosynthate into starch at the expense of sucrose and amino acids.

DISCUSSION

Physiological Effects of the TPT Inhibition. Communication between chloroplasts and the cytosol is essential for adjusting the rates of photosynthesis to the demands of various parts of the plant for photoassimilates in form of reduced CO_2. This communication is occurring via the membrane system of the chloroplasts. Two membranes enclose the plastidic lumen, an outer membrane that is permeable with an exclusion limit of 10 kDa (23) and an inner membrane that controls the substances entering and leaving the plastid. The most prominent protein of the inner envelope membrane is the 29-kDa protein (E29). Based on biochemical evidence, this protein has been identified as the TPT, which plays a central role in exchanging metabolites between chloroplasts and the cytosol.

To assess the *in vivo* function of the protein, potato plants were transformed with an antisense construct of the TPT cDNA under control of the CaMV 35S promoter. In several transformants, reduced amounts of TPT mRNA and protein were found. The reduction is specific, as other proteins such as RbuP_2 carboxylase were not significantly reduced (data not shown). The decrease in TPT protein fits well with the 20–30% reduction of the P_i transport activity. The simple kinetics of the P_i transport system in both wild-type and antisense plants plus the fact that no related gene was found in potato (6) make it very unlikely that the remaining activity in the antisense plants is due to a second P_i-translocator protein. Apparently, there is no proportionality between the levels of mRNA and protein. We therefore have to assume that small amounts of RNA still yield sufficient protein to secure most of the TPT activity. The TPT mRNA thus seems to be produced in large excess relative to the amount needed for the steady-state level of the protein. Possibly, the TPT protein is made in surplus and only a fraction is incorporated into the chloroplast envelope membrane.

Table 3. Labeling pattern of sucrose, starch, and amino acids in wild-type and TPT antisense potato leaves after exposure to $^{14}CO_2$

Plant	Sucrose/ starch	Amino acids/starch	*n*
Wild-type	1.63 ± 0.32	0.57 ± 0.21	6
1	1.31 ± 0.31	0.56 ± 0.32	3
7	1.13 ± 0.06	0.38 ± 0.04	3
39	0.82 ± 0.06	0.29 ± 0.05	3

Detached leaves from wild-type and TPT antisense plants were exposed to a 15-min pulse of $^{14}CO_2$ and analyzed. *n*, Number of leaves analyzed.

The reduction in TPT activity led to marked effects such as a dwarf phenotype at young stages and to a 40–60% reduction of photosynthesis rates under saturating CO_2 and light conditions. This indicates that under these conditions the activity of the TPT can become rate-limiting for maximal photosynthesis. During further development, the difference between wild-type and antisense plants became less drastic, and under the experimental conditions, no significant effect on tuber production could be observed. Under ambient CO_2 and light conditions, when the rate of photosynthesis of the wild-type plants was only 20% of the maximal rate, the photosynthetic rates of antisense plants were not altered. Although under ambient conditions, the reduced TPT activity did not limit photosynthesis, it markedly affected the allocation of carbohydrates and the distribution of metabolites between chloroplasts and cytosol. The reduction in export of triose-*P* led to an increased accumulation of starch. The observed effects can be explained by P_i limitation of photosynthesis due to the decreased TPT activity.

Antisense Effects and P_i Limitation of Photosynthesis. P_i in addition to light and CO_2 represents a prime substrate for photosynthesis in higher plants. Photosynthesis is strictly limited by light intensity and the availability of CO_2, whereas the role of P_i limitation for photosynthesis under *in vivo* conditions is less clear (24, 25).

The assumption that P_i limits photosynthesis comes mainly from an analysis of the response of isolated chloroplasts to external P_i and the effects of P_i-sequestering agents. Isolated chloroplasts incubated under conditions allowing photosynthesis showed that maximal rates of photosynthesis depend on an optimal concentration of P_i in the medium (3, 26). Likewise, the incubation of detached leaves in solutions containing D-mannose, 2-deoxyglucose, or glycerol strongly affected photosynthesis. These compounds are phosphorylated in the plant but cannot be further metabolized (27, 28).

As a result cytosolic P_i is sequestered into organic compounds and becomes limiting.

General responses of detached leaves to P_i limitation include a reduction of light-saturated rates of photosynthesis, an increased allocation of photoassimilates into starch, decreased pools of phosphorylated intermediates, and an increase in stomatal opening (29).

A comparison of the effects of P_i limitation with those observed in TPT antisense plants reveals striking similarities. As in chloroplasts performing photosynthesis under P_i limitation (3), the level of 3-PGA in total leaf extracts from antisense plants was increased whereas triose-*P* was decreased, resulting in an ≈3-fold increase in the ratio of 3-PGA/triose-*P*. Also, the level of Glc-6-*P* was decreased. According to subcellular compartmentation analyses, 3-PGA is mainly localized in the chloroplast whereas Glc-6-*P* is a cytosolic metabolite (21). Due to the allosteric activation of ADP-glucose pyrophosphorylase by 3-PGA and its inhibition by P_i, the elevated stromal ratio of 3-PGA/P_i explains the observed increase in starch formation (30). As a consequence of the reduced TPT activity, less triose-*P* is available in the cytosol for biosynthetic pathways—e.g., for the synthesis of sucrose and amino acids. Indeed, the amount of malate as a precursor for the biosynthesis of amino acids or as a substrate for oxidative pathways is decreased in three antisense plants tested. Also, the rates of sucrose and amino acids biosynthesis were diminished even though the steady-state concentrations of both sucrose and amino acids remained almost constant. This implies that the decrease in formation of sucrose and amino acids has to be accompanied by a decreased export of these assimilates from the leaves to other parts of the plant. Despite the fact that control and antisense plants show about equal rates of photosynthesis under ambient conditions, carbon partitioning of photosynthates is affected in TPT antisense plants resulting in an increased formation of starch. Thus the TPT antisense plants essentially display the phenotype described for P_i limitation.

TPT as a Rate-Limiting Factor. One of the most unexpected results is the observation that a decreased chloroplast P_i-import capacity of only 20–30% leads to strong effects on maximal photosynthetic rates and assimilate partitioning. Even under ambient conditions, metabolite levels are changed compared to wild-type plants, although photosynthesis under these conditions is not affected. The TPT has been shown to exert a kinetic limitation on photosynthesis *in vivo* under certain conditions (21), although the total activity of the translocator exceeds by far the rate of photosynthesis (1). The strong effects were also found *in vitro* and could be due to the fact that the TPT activity is largely occupied by synonymous exchanges (31).

The Antisense Effects Are Compatible with the Function as TPT. It may be noted that the E29 gene has also been suggested as the major receptor for import of nuclear-encoded precursor proteins into chloroplasts (32). As no reduction in RbuP_2 carboxylase protein levels were observed, which would be expected if the number of import receptors is reduced, the data presented in this study are in full agreement with the role of E29 as the TPT. Rather complicated models would be necessary to bring the effects observed in antisense plants in line with E29 being the chloroplast import receptor. In addition, experimental evidence for the identity of E29 as the TPT has been provided biochemically and by proof of P_i transport activity in transformed yeast strains (22, 33).

Concluding Remarks. The *in vivo* data shown in this study indicate that a 20–30% reduction of the TPT activity leads to a change in carbon partitioning of photoassimilates under ambient conditions and can significantly limit photosynthesis under saturating CO_2 and light conditions. The TPT thus represents a possible target for increasing the flow of carbon by overexpression of the transporter. This can be tested by experiments designed to increase TPT activity—e.g., by overexpression of heterologous TPT genes.

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