Planta (2006) 223: 248–257
DOI 10.1007/s00425-005-0128-5

ORIGINAL ARTICLE

Kentaro Toyota · Masahiro Tamura · Takashi Ohdan
Yasunori Nakamura

Expression profiling of starch metabolism-related plastidic translocator genes in rice

Received: 3 August 2005 / Accepted: 31 August 2005 / Published online: 14 December 2005

Abstract The genes encoding the major putative rice plastidic translocators involved in the carbon flow related to starch metabolism were identified by exhaustive database searches. The genes identified were two for the triose phosphate/phosphate translocator (*TPT*), five for the glucose 6-phosphate/phosphate translocator (*GPT*) including putatively non-functional ones, four for the phosphoenolpyruvate/phosphate translocator (*PPT*), three for the putative ADP-glucose translocator (or Brittle-1 protein, *BT1*), two for the plastidic nucleotide transport protein (*NTT*), and one each for the plastidic glucose translocator (*pGlcT*) and the maltose translocator (*MT*). The expression patterns of the genes in various photosynthetic and non-photosynthetic organs were examined by quantitative real-time PCR. *OsBT1-1* was specifically expressed in the seed and its transcript level tremendously increased at the onset of vigorous starch production in the endosperm, suggesting that the ADP-glucose synthesized in the cytosol is a major precursor for starch biosynthesis in the endosperm amyloplast. In contrast, all of the genes for *OsTPT*, *OsPPT*, and *OsNTT* were mainly expressed in source tissues, suggesting that their proteins play essential roles in the regulation of carbohydrate metabolism in chloroplasts. Substantial expression of the four *OsGPT* genes and the *OspGlcT* gene in both source and sink organs suggests that the transport of glucose phosphate and glucose is physiologically important in both photosynthetic and non-photosynthetic tissues. The present study shows that comprehensive analysis of expression patterns of the plastidic translocator genes is a valuable tool for the elucidation of the functions of the translocators in the regulation of starch metabolism in rice.

Keywords KOME database · Plastidic translocator · Real-time PCR · Rice · Starch metabolism

Abbreviations BT1: Brittle-1 · DAF: Days after flowering · GPT: Glucose 6-phosphate/phosphate translocator · MT: Maltose translocator · NTT: Plastidic nucleotide transport protein · pGlcT: Plastidic glucose translocator · PPT: Phosphoenolpyruvate/phosphate translocator · TPT: Triose phosphate/phosphate translocator

Introduction

Plastids play essential roles in plant metabolic processes such as photosynthesis, starch biosynthesis, nitrogen assimilation, sulfate assimilation, fatty acid synthesis, and terpenoid synthesis. Plastids are specialized for each metabolic process in plants. For example, chloroplasts of green tissues are specialized for photosynthesis while amyloplasts of non-photosynthetic organs such as tubers and seeds are specialized for storage of a great amount of starch. Starch is a major end-product of photosynthesis in the chloroplast of source organs. Starch metabolism in the chloroplast and amyloplast is closely related to other metabolic events in the cytosol such as sucrose metabolism, glycolysis, and glyconeogenesis. Therefore, a number of translocators on the plastid envelope membrane play important roles in coordinating starch metabolism in the plastid with carbohydrate metabolism in the cytosol (Fig. 1). However, the precise mechanism of how plastidic translocators coordinate the carbohydrate metabolism between plastids and the cytosol is still unknown.

K. Toyota · M. Tamura · T. Ohdan · Y. Nakamura
CREST, Japan Science and Technology Corporation,
Omiya, Saitama, Japan

Y. Nakamura (✉)
Department of Biological Production,
Akita Prefectural University,
241-7 Kaidobata-Nishi, Shimoshinjyo-Nakano,
Akita-city 010-0195, Japan
E-mail: nakayn@akita-pu.ac.jp
Tel.: +81-188-721652
Fax: +81-188-721681

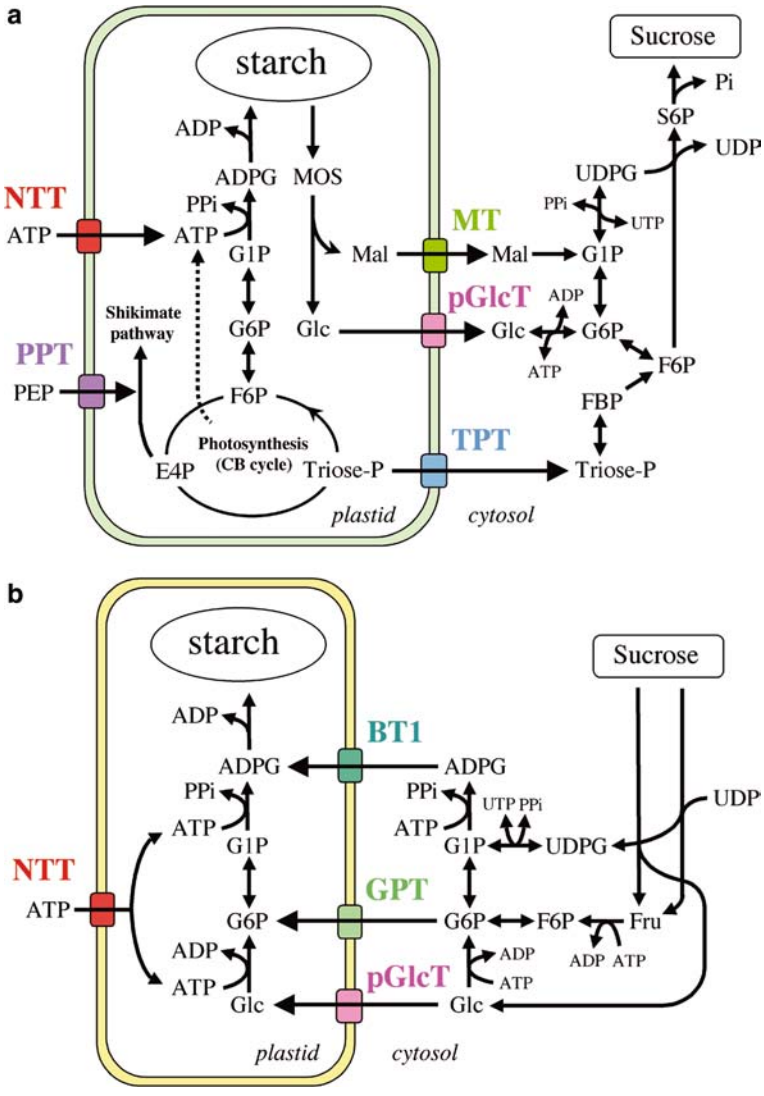

Fig. 1 Schematic representation of starch metabolism-related plastidic translocators in the carbon flow in source (**a**) and sink (**b**) tissues of plants (modified from Neuhaus and Wagner 2000; Fischer and Weber 2002; Weber et al. 2004). *ADPG* ADP-glucose, *CB cycle* Calvin-Benson cycle, *E4P* erythrose 4-phosphate, *FBP* fructose 1,6-bisphosphate, *F6P* fructose 6-phosphate, *Glc* glucose, *G1P* glucose 1-phosphate, *G6P* glucose 6-phosphate, *Mal* maltose, *MOS* malto-oligosaccharides, *PPi* inorganic pyrophosphate, *S6P* sucrose 6-phosphate, *UDPG* UDP-glucose

Phosphate translocators such as the triose phosphate/phosphate translocator (TPT), the glucose 6-phosphate/phosphate translocator (GPT), and the phosphoenolpyruvate/phosphate translocator (PPT), which counter-exchange triose phosphate, glucose 6-phosphate, and phosphoenolpyruvate, respectively, with inorganic phosphate, have been characterized in details (for review, see Flügge 1999). These phosphorylated compounds could be intermediates for starch metabolism. Other plastidic translocators including the putative ADP-glucose translocator (or Brittle-1, BT1), the plastidic nucleotide transport protein (NTT), plastidic glucose translocator (pGlcT), and the maltose translocator (MT) have been characterized (Neuhaus and Wagner 2000; Fischer and Weber 2002; Weber 2004), and are also thought to play important roles in starch metabolism.

The *BT1* gene, which was identified by an analysis of the maize mutant *brittle-1* (Sullivan et al. 1991), was suggested to code for the ADP-glucose translocator on the amyloplast membrane (Sullivan et al. 1995; Shannon et al. 1998), which presumably counter-exchanges ADP-glucose with AMP (Mohlmann et al. 1997; Emes et al. 2003). The *shrunken-2* mutant of maize was found to be defective in the large subunit of cytosolic ADP-glucose pyrophosphorylase (AGPase) in endosperm, and produces shriveled kernel with reduced starch content (Bhave et al. 1990). These observations indicate that the supply of ADP-glucose mediated by cytosolic AGPase and BT1 from cytosol into the amyloplast stroma is essential for starch biosynthesis in maize endosperm. Therefore, BT1 and AGPase are closely related to starch productivity in the maize seed.

NTT in the plastidic membrane, formerly designated as ATP/ADP translocator protein, was characterized in *Arabidopsis* (Kampfenkel et al. 1995; Neuhaus et al. 1997; Reiser et al. 2004). In contrast to the mitochondrial ADP/ATP carrier, the plasitidic NTT imports extra-plastidic ATP from cytosol probably for the biosynthesis of starch or fatty acids in plastids (for review, Winkler and Neuhaus 1999). The importance of plastidic NTT in starch metabolism has been further elucidated by an analysis of transgenic potato plants in which the NTT activity was increased or decreased (Tjaden et al. 1998; Geigenberger et al. 2001).

The gene encoding the pGlcT was cloned from spinach, potato, tobacco, maize, and *Arabidopsis* (Weber et al. 2000), and from olive (Butowt et al. 2003). pGlcT is considered to be crucial for the efflux of glucose produced from the degradation of transitory starch in the chloroplast into cytoplasm in the dark (Weber et al. 2000; Butowt et al. 2003) and is possibly involved in the influx of extra-plastidial glucose into plastids in heterotrophic tissues (Fischer and Weber 2002).

The *MT* gene was cloned only recently in *Arabidopsis* following the analysis of a mutant with an elevated level of maltose (Niittyla et al. 2004), although its protein has been suggested to exist in the plastidic membrane two decades ago (Herold et al. 1981). MT is also considered to be important basically in the same way as pGlcT. MT mediates the export of maltose, the product of starch hydrolysis by α-amylase and/or β-amylase, from source to sink organs (Weise et al. 2004).

The starch metabolic system in plastids consists of a network of reactions catalyzed by numerous enzymes with distinct functions (Nakamura 2002; Ball and Morell 2003). Previous studies have demonstrated that several plastidic translocators are involved in starch metabolism in plants (Neuhaus and Wagner 2000; Fischer and Weber 2002; Weber et al. 2004; Fig. 1). To date, no comprehensive analysis of the expression patterns of genes encoding plastidic translocators involved in starch metabolism has been reported. In the present study, to gain insights into the mechanism of how plastidic translocators regulate starch biosynthesis, the expression patterns of almost all of the plastidic translocator candidate genes identified in the database were analyzed by quantitative real-time PCR in both photosynthetic and non-photosynthetic organs of rice.

Materials and methods

Plant material

Japonica-type rice plants (*Oryza sativa* L. cultivar Kinmaze; MAFF Genebank-Plant, National Agrobiological Sciences, Tsukuba, Japan; http://www.gene.affrc.go.jp/plant/) were grown in a paddy field at Akita Prefectural University, Akita, Japan. Leaf blades, leaf sheaths, and seeds at mid-milking stage [7–9 days after flowering (DAF)] were sampled at noon for total RNA extraction. For preparation of total RNA from root, 2-week-old seedlings were cultured aseptically under continuous illumination (ca. 60 μmol photons m^{-2} s^{-1}) at 28°C in MS media (Murashige and Skoog 1962) with agar.

Gene search

Based on previous reports on starch metabolism-related plastidic translocators in maize and Arabidopsis (i.e., Arabidopsis TPT, Knappe et al. 2003a; maize TPT, Fischer et al. 1994; Arabidopsis GPT, Knappe et al. 2003b; maize GPT, Kammerer et al. 1998; Arabidopsis PPT1, Fischer et al. 1997; Arabidopsis PPT2, Knappe et al. 2003a; maize PPT, Fischer et al. 1997; maize BT1, Sullivan et al. 1991; Arabidopsis NTT1, Neuhaus et al. 1997; Arabidopsis NTT2, Mohlmann et al. 1997; Arabidopsis and maize pGlcT, Weber et al. 2000; Arabidopsis MT1, Niittyla et al. 2004) candidates for plastidic translocator genes in rice were first searched in the rice full-length cDNA (KOME) database (Kikuchi et al. 2003; http://cdna01.dna.affrc.go.jp/cDNA/), and the Gramene database (Ware et al. 2002; http://www.gramene.org/). Subsequently, the protein databases Rice Membrane Protein Library (RMPL; http://www.cbs.umn.edu/rice/), and the ARAMEMNON (http://aramemnon.botanik.uni-koeln.de/) were accessed for confirmation. The data obtained from these databases were scrutinized, compared, and filtered. It was also ensured that none of the genes code for the rice chloroplast genome.

Construction of phylogenetic trees

Multiple alignment of deduced amino acid sequences was performed with the Clustal W program (http://www.ddbj.nig.ac.jp; Thompson et al. 1994) to construct phylogenetic trees which were displayed using the TREEVIEW program (Page 1996).

Total RNA extraction and cDNA synthesis

Approximately 100 mg of leaf, leaf sheath, seed, or root were sampled for total RNA extraction using the RNeasy Plant Mini Kit (QIAGEN) following the attached protocol. Two micrograms of the total RNA were subsequently used to synthesize cDNAs, using the iScript cDNA synthesis kit (Bio-Rad) in total reaction volumes of 40 μl. Sterilized distilled water was added to dilute the cDNA tenfold to obtain 400 μl of cDNA solution.

Quantitative real-time PCR

Expressions of genes were analyzed by using the SYBR Green kit (QIAGEN) and iCycler (Bio-Rad) following the manufacturers' instructions. One microliter of the synthesized cDNA and 1.25 μl of 6.25 μM primer solutions (forward and reverse; summarized in Table 1) were used for each real-time PCR (total volume of 50 μl) with an annealing temperature of around 58–62°C. The melting curve was generated to check the specificity of the amplified fragments. As an internal control gene, rice actin (Accession; X16280) or the α-tubulin (Accession; AK067721) gene was used. Absolute quantification of the transcript number was performed with external calibration standards generated by quantitative real-time PCR from the amplified fragment cloned in

Table 1 Oligonucleotide primers for real-time PCR used in this study

Gene	Sequence	Location on mRNA	Amplified fragment (bp)
OsTPT1	Forward: 5′-ACAACATGGGCGAGGATCAT-3′ Reverse: 5′-CAATCTTACCACCGCAATATGC-3′	3′ UTR	118
OsTPT2	Forward: 5′-TAGTTGGGTAGCTGCTTTGATCGA-3′ Reverse: 5′-AAATGGGATGATGGAGGCTTTG-3′	3′ UTR	210
OsGPT1	Forward: 5′-GCAAGCACTGAGGCCAATTTTG-3′ Reverse: 5′-AAGGAACAAGAAACGAGCAACATAGAC-3′	3′ UTR	114
OsGPT2-1 & 2-2	Forward: 5′-ACTCGGTGCTCGGGATGTAAC-3′ Reverse: 5′-AGAAGAAACATTGGCAGCTACAC-3′	3′ UTR	119
OsGPT2-3	Forward: 5′-GGATTGAAGAAGACTCGATGCGA-3′ Reverse: 5′-GGCTTGAATAATGCCATCTTGG-3′	5′-UTR + CDS	211
OsPPT1	Forward: 5′-ACCGTAGAAGCATTGCCACAC-3′ Reverse: 5′-ACAAAAACCTTGCCACGATTAGC-3′	3′-UTR	145
OsPPT2	Forward: 5′-GCCATCTTATTGTAGCAGTCTTTCCA-3′ Reverse: 5′-CTACACTTAGCAACAACGTGAACATGT-3′	3′-UTR	115
OsPPT3	Forward: 5′-AAACCAAAGAATGCCTGAGAAGAGA-3′ Reverse: 5′-TTGCGCCTGGACACTAATGA-3′	3′-UTR	231
OsPPT4	Forward: 5′-AGGCTAAGACTGCATGAGTCAACA-3′ Reverse: 5′-CAAATTGGAGAGAAGCCAAGTGA-3′	3′-UTR	116
OsBT1-1	Forward: 5′-CTGGAATCGGATGAAACTCGTGTA-3′ Reverse: 5′-TCAGCAGAAATCAGTTATTGGACATG-3′	3′-UTR	149
OsBT1-2	Forward: 5′-TGATTGTGCATGGGTGTGATG-3′ Reverse: 5′-AACAGAGGAAATCGAATCCTACG-3′	3′-UTR	132
OsBT1-3	Forward: 5′-TTGCTAGCGTCGGTCTCAAAG-3′ Reverse: 5′-GCAATGATCAGCGAACGGAA-3′	3′-UTR	94
OsNTT1	Forward: 5′-AGAGAGCGAAGCTACTGCAACTGA-3′ Reverse: 5′-CCCACTGCAATCATTCTCCTACCT-3′	CDS + 3′-UTR	112
OsNTT2	Forward: 5′-CGGAGAAGTCTGGTCAACAATCTC-3′ Reverse: 5′-CAGCTTTTGATTCCACGGAAGA-3′	3′-UTR	83
OspGlcT	Forward: 5′-TGGAGCTTCGAGGGTTGTGA-3′ Reverse: 5′-CGGTGGCATACTGGCATCTA-3′	3′-UTR	84
OsMT	Forward: 5′-GCTGCCAGGCAGGAAGCT-3′ Reverse: 5′-GGTTCCAGTTTCACCACGACAA-3′	3′-UTR	121
Actin	Forward: 5′-CTTCATAGGAATGGAAGCTGCGGGTA-3′ Reverse: 5′-CGACCACCTTGATCTTCATGCTGCTA-3′	CDS	196
α-*tubulin*	Forward: 5′-GGAAATACATGGCTTGCTGCTT-3′ Reverse: 5′-TCTCTTCGTCTTGATGGTTGCA-3′	CDS	89

pGEM-T vector (Promega). The identity of the cloned transcript was calculated as the ratio to actin (Fig. 2) or α-tubulin (Fig. 3) control. All the primers were designed using Primer Express 2.0 (Applied Biosystems).

Results

Starch metabolism-related plastidic translocator genes in rice

Available information on amino acid and genomic sequences of TPT, GPT, PPT, BT1, NTT, pGlcT, and MT of Arabidopsis and maize were used as in silico probes to find their counterparts in rice. The putative rice translocator genes were named according to their *Arabidopsis* and/or maize homologs and annotated using the TIGR loci, thus: *OsTPT1* (LOC_Os01g13770) and *OsTPT2* (LOC_Os05g15160) for the *TPT* genes, *OsGPT1* (LOC_Os08g08840) and *OsGPT2-1*, *2-2*, *2-3* (LOC_Os07g34010, LOC_Os07g33960, LOC_Os07g33910, respectively) for the *GPT* genes, *OsPPT1*,*2*, *3*, *4* (LOC_09g12600, LOC_Os08g25630, LOS_Os01g07730, LOC_Os05g07870, respectively) for the *PPT* genes, *OsBT1-1, 1-2, 1-3* (LOS_Os02g10800, LOC_Os05g07900, LOC_Os06g40050, respectively) for the *BT1* genes, *OsNTT 1, 2* (LOC_Os01g45910, LOC_Os02g11740, respectively) for the *NTT* genes, *OspGlcT* (LOC_Os01g04190) for the *pGlcT* gene, and *OsMT* (LOC_Os04g51330) for the *MT* gene. These genes were considered as starch metabolism-related plastidic translocator genes in rice, based on the high similarities of their amino acid sequences with those of maize and *Arabidopsis*, and of their exon/intron splice sites with their respective counterpart genes in *Arabidopsis*.

One advantage of using the genome databases is that the sequences besides the coding region, such as 5′-upstream and 3′-downstream sequences, are easy to discern. Although the TATA-less promoters were reported to be restricted to photosynthetic and plastid ribosomal genes (Nakamura et al. 2002; Achard et al. 2003), only *OsGPT* and *OsBT1-2* genes had an apparent TATA-box motif (data not shown).

Phylogenetic trees of the rice gene products and their homologs in other plants were constructed (data not

shown). Overall, the rice translocator proteins identified in this study, except for OsBT1-2 and OsBT1-3, were most similar with their homologs in monocots such as maize and wheat.

Expression profiles of starch metabolism-related plastidic translocator genes in rice

Expression profiles of the rice genes were analyzed by quantitative real-time PCR using the oligonucleotide primers listed in Table 1. The actual amounts of each transcript in different organs of rice were calculated. Data were normalized using the expression level of actin (Fig. 2) or α-tubulin (Fig. 3) as internal control because both genes were expressed constitutively in various tissues. Although the amount of transcript is not always correlated with the protein amount or activity, the data in this study could be strong indicators of their physiological roles.

Levels of the *OsTPT1* transcript in all organs examined were by far greater than those of *OsTPT2* (Fig. 2). The expression levels of both genes in source organs such as the leaf blade were more than tenfold greater in comparison to the levels in sink organs such as seeds and roots. The *OsTPT1* transcript levels in the source organs were one order higher than that of the actin gene. These results suggest that OsTPT1 is the major functional form

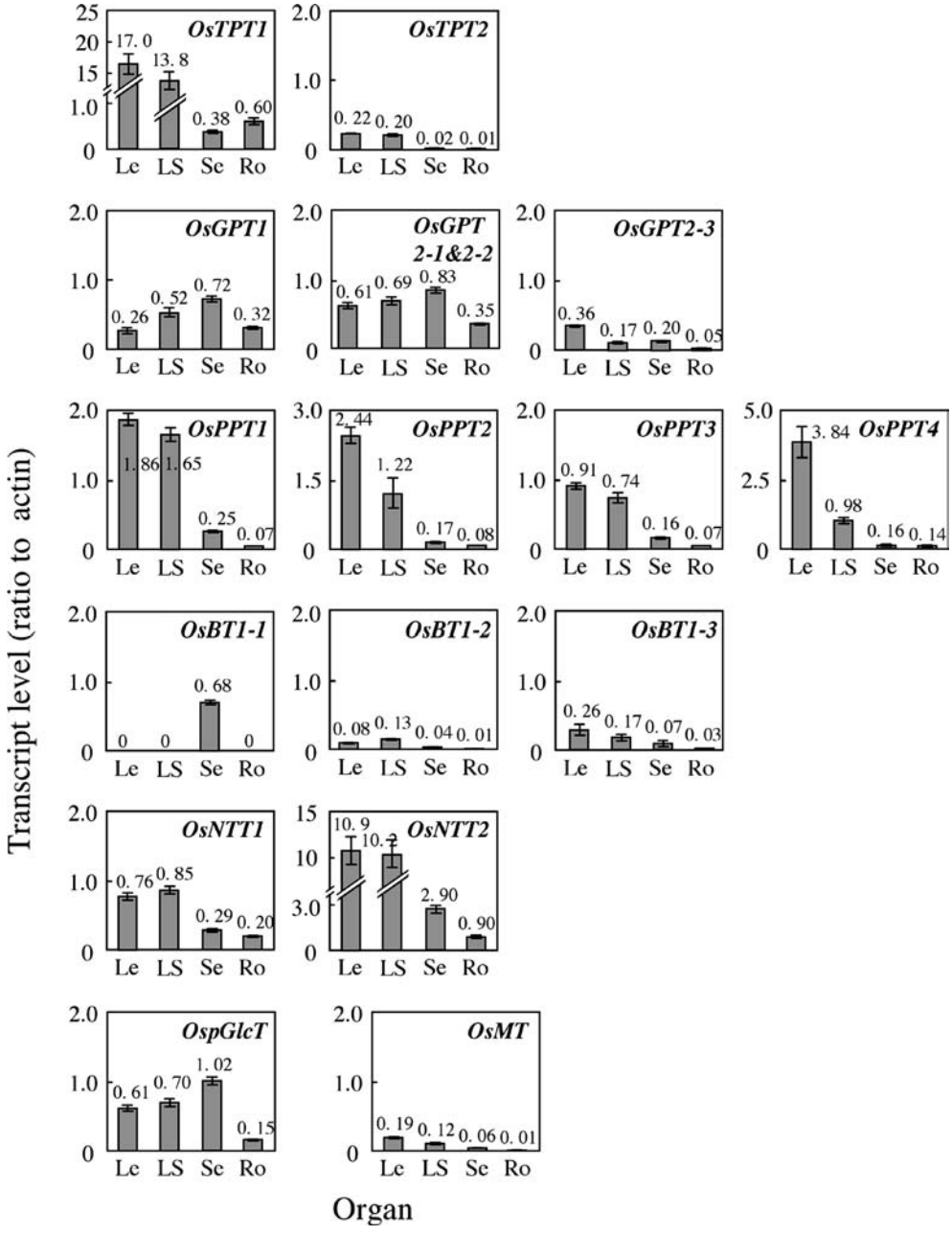

Fig. 2 Expressions of genes possibly encoding starch metabolism-related plastidic translocators in various organs of rice at mid-milking stage of the seed (7–8 DAF). The relative amount of each mRNA (*vertical bars*) was defined as the ratio to that of rice actin gene (Accession no. X16280). Values are means ± SE of at least four replicates. *Le* leaf, *LS* leaf sheath, *Se* seed, *Ro* root

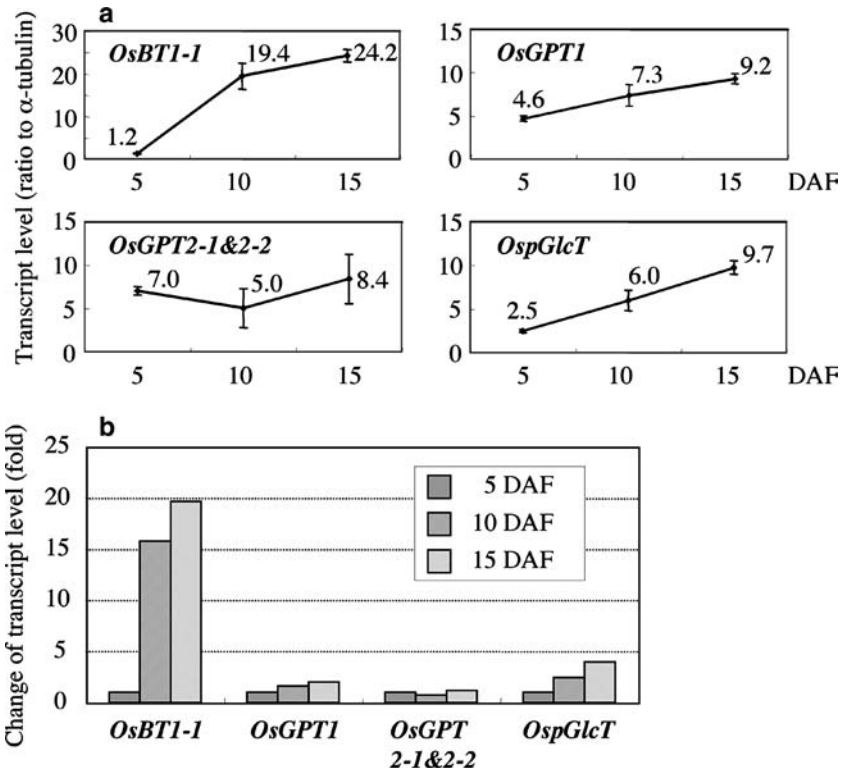

Fig. 3 **a**, **b** Expressions of *OsBT1-1*, *OsGPT1*, *OsGPT2-1* & *2-2*, and *OspGlcT* during seed maturation of rice. Total RNAs from rice seeds at 5, 10, 15 DAF were prepared for real-time PCR analysis. **a** Relative amount of each mRNA was defined as the ratio to that of rice α-tubulin gene (Accession no. AK067721), which was used as the internal control. **b** Expression profile of each gene during seed maturation

playing an important role in the source organs in carbohydrate metabolism in rice plants.

Both *OsGPT1* and *OsGPT2-1*∼*2-3* were expressed in all organs examined, although the amount of *OsGPT1* transcript was higher in the seed than in the leaf (Fig. 2). A similar pattern was also observed for *OsGPT2-1* & *2-2* although it was impossible to independently measure the levels of *OsGPT2-1* and *OsGPT2-2* because of the difficulties in designing the specific primers for each transcript. In contrast, *OsGPT2-3* was expressed the most in the leaf although the overall levels were lower than the other *OsGPT* genes.

The transcript levels of the four *OsPPTs* shared similar patterns in that their transcripts were predominantly expressed in source organs such as the leaf and leaf sheath rather than in sink organs such as the seed and root (Fig. 2). These results suggest that OsPPTs play important parts in carbohydrate metabolism during photosynthesis.

OsBT1-1 gene was expressed almost exclusively in seed while the *OsBT1-2* and *OsBT1-3* were expressed mainly in the leaf and leaf sheath, although the expression level of *OsBT1-1* in seed was markedly higher than those of *OsBT1-2* and *OsBT1-3* in the leaf (Fig. 2). These observations suggest that OsBT1-1 is essential for starch biosynthesis in the rice endosperm by translocating ADP-glucose from the cytosol into the amyloplast.

The *OsNTT2* gene was expressed by far greater than that of the *OsNTT1* gene (Fig. 2). The transcript levels of all the *OsNTT* genes were higher to some extent in the leaf and leaf sheath than in the seed and root.

OspGlcT was mostly expressed in the seed, but lesser in the leaf and leaf sheath (Fig. 2), suggesting that OspGlcT plays some roles in both the photosynthetic and non-photosynthetic tissues of rice. *OsMT* was expressed mainly in the leaf and leaf sheath, but the overall expression of *OsMT* was seemingly quite low (Fig. 2). These results suggest the distinct physiological roles of OspGlcT and OsMT in carbohydrate metabolism in rice plants.

Changes in expression levels of BT1, GPT, and pGlcT during seed development

In an attempt to clarify the possible roles of OsBT1-1, OsGPTs, and OspGlcT, we followed changes in their transcript levels during three different developmental stages of the rice endosperm, as shown in Fig. 3. It was noted that the expression of *OsBT1-1* was greatly higher at 10 and 15 DAF when starch most vigorously accumulates in the endosperm as compared with the very early endosperm development prior to significant starch production in the endosperm (at 5 DAF). The expression of *OsGPT1* gradually increased during endosperm development whereas the transcripts of *OsGPT2-1* and *2-2* were constant throughout the three stages. The expression of *OspGlcT* increased with the progress of endosperm development, but the increase was not dramatic as compared with *OsBT1-1*. All these data strongly suggest a specific role of the OsBT1 protein in starch biosynthesis in the rice endosperm.

Discussion

Arabidopsis has approximately one hundred plastidic translocators (Ferro et al. 2002; Koo et al. 2002; Schwacke et al. 2003). In this study, homologs of these plastidic translocator proteins in rice were searched for in the database. Rice has approximately 32,000–50,000 genes (Goff et al. 2002), and is predicted to have at least a hundred plastidic translocator genes like *Arabidopsis*.

The plastidic translocators TPT, GPT, PPT, BT1, NTT, pGlcT, and MT were examined in this study because of their important roles in coordinating starch metabolism in plastids with carbohydrate metabolism in the cytosol (Flügge 1999; Neuhaus and Wagner 2000; Fischer and Weber 2002; Emes et al. 2003; Weber 2004; Weber et al. 2004). The existence of glucose 1-phosphate/phosphate translocator, which was postulated to be a plastidic translocator, has been reported in wheat (Tetlow et al. 1994, 1996; Tyson and ap Rees 1988) and in potato (Naeem et al. 1997), but its sequence has not been determined. Although the presence of the deduced translocator has been doubted (Huber et al. 1992; Kofler et al. 2000), its existence cannot be ruled out until annotation of the *Arabidopsis* and rice genome is completed. Xylulose 5-phosphate/phosphate translocator (XPT), while present in *Arabidopsis* (Eicks et al. 2002), has not been reported in the TIGR rice database.

Based on the *Arabidopsis* database, Knappe et al. (2003a) showed the gene structures and related data on the plastidic phosphate translocators TPT, GPT, PPT, and XPT. The same strategy was used in this study, which focused on starch metabolism-related plastidic translocators BT1, NTT, pGlcT, and MT, in addition to the phosphate translocators they examined. Although the regulation of starch metabolism, including the structures and functions of numerous enzymes involved, has been extensively studied in various plants, there are no reports on comprehensive gene expressions of plastidic translocators. Having considered that the regulatory mechanism and composition of isoforms in each class of enzymes for starch synthesis are known to be different between dicots and monocots (James et al. 2003), a comprehensive analysis of the expression of plastidic translocator genes in rice as a model monocot plant would generate information for comparison with the available data on plastidic translocators in the model dicot plant *Arabidopsis*.

Figure 2 shows that *OsTPT1* was expressed predominantly in the leaf and leaf sheath, but only slightly in the seed and root, consistent with the results obtained in *Arabidopsis* (Knappe et al. 2003b), cauliflower (Fischer et al. 1997), maize (Fischer et al. 1997; Kammerer et al. 1998), pea (Knight and Gray 1994), potato (Schulz et al. 1993; Schunemann et al. 1996), tobacco (Knight and Gray 1994), and tomato (Schunemann et al. 1996). *OsTPT2* expression pattern was similar to that for *OsTPT1*, although the expression level of the former was much lower (Fig. 2). Thus, the functional TPT in rice might be OsTPT1 only, and TPT2 is probably a non-functional pseudogene. In *Arabidopsis* only a single TPT gene was identified (Knappe et al. 2003a).

The expression of the four *OsPPT* genes was much higher in source organs than in sink organs (Fig. 2), the expression patterns sharply contrasting those in maize and cauliflower (Fischer et al. 1997; Kammerer et al. 1998). RT-PCR analysis demonstrated that the two *ArabidopsisPPTs* exhibit different patterns of expression; *PPT1* is expressed ubiquitously whereas *PPT2* is expressed mainly in the leaf (Knappe et al. 2003b). No marked expression of *OsPPT* genes was detected in the rice seed while the *ArabidopsisPPT1* is significantly expressed in the seed, which may reflect the importance of PPT1 for the shikimate pathway and fatty acid synthesis in *Arabidopsis* seed.

The present observations strongly suggest that both OsTPT and OsPPT fulfill crucial roles in photosynthetic carbon metabolism in source cells. The contribution of *OsTPT1* to the export of triose phosphate synthesized from CO_2 in the chloroplast into cytoplasm might be greater than that of *OsTPT2*, because the transcript level of *OsTPT1* was about 77-fold and 17-fold higher than that of *OsTPT2* and actin transcripts, respectively (Fig. 2).

OsGPT1 and *OsGPT2-1* & *2-2* were most abundantly expressed in the seed, but their transcripts in the leaf and leaf sheath were also substantial (Fig. 2), which is in contrast to the finding of Kammerer et al. (1998) that *GPT* is expressed almost exclusively in non-photosynthetic tissues of maize. One possible explanation for this discrepancy is that the *OsGPT* expression in rice might arise from the probable substantial expression of *GPTs* in leaf guard cells, as observed in pea (Overlach et al. 1993). Alternatively, other translocator(s) might perform the function of GPT in maize, considering that the phosphate translocators, TPT, GPT, PPT, and XPT, often share substrate specificity to some extent (Fischer et al. 1997; Kammerer et al. 1998). In *Arabidopsis*, microarray data demonstrated that *GPT1* (At5g54800) is expressed in both source and sink organs while *GPT2* (At1g61800) is expressed almost exclusively in the seed (Zimmermann et al. 2004). Taken together, the expression pattern of *Arabidopsis GPT1* is similar to that of *OsGPT*s, while that of *Arabidopsis GPT2* resembles that of the maize *GPT* gene.

The expression of *OsBT1-1* was entirely seed-specific, and its expression pattern was very similar to that of maize *BT1* (Fig. 2), which was hypothesized to transport the ADP-glucose produced by cytosolic AGPase into amyloplast for starch biosynthesis in maize and probably all cereal endosperms (Sullivan et al. 1991; Cao and Shannon 1997). However, this postulated function of maize BT1 remains to be proven in direct transport experiments. Results of searches for *cis*-elements in the three *OsBT1* gene promoters (ca. 1.5 kb of the 5′-upstream region from the transcription start site) on the PLACE database (Higo et al. 1999; http://www.dna.af-

frc.go.jp/PLACE/) revealed that the *OsBT1-1* promoter has one *cis*-element for endosperm-specific expression, thus explaining the seed-specific expression of *BT1-1*. Interestingly, microarray data indicate that the putative *ArabidopsisBT1* gene (At4g32400), which seems to be a single copy gene, is expressed in both source and sink organs (Zimmermann et al. 2004). However, the BT1 homolog from potato recently characterized by Leroch et al. (2005) was proposed to be a plastidic ATP uniporter. While the maize plastidic BT1 remains to be elucidated, it appears that the metabolic role and the transport mechanism of BT1 might differ between the two plant species.

Because *OsBT1-1* was expressed specifically in maturing seeds while *OsBT1-2* and *OsBT1-3* were expressed in every tissue in very low levels (Fig. 2), the functions of the three OsBT1 are most likely different.

Based on these observations, we presume that OsBT1-1 and OsGPTs could possibly mediate the transport of ADP-glucose and glucose 6-phosphate, respectively, from the cytosol into the amyloplast in the rice endosperm, where ADP-glucose and glucose 6-phosphate serve as substrates for starch biosynthesis and the pentose phosphate pathway, respectively, or both compounds become the precursor of starch in the amyloplast. However, *BT1*-deficient mutants of maize (Sullivan et al. 1991; Shannon et al. 1998) and barley (Patron et al. 2004), and cytosolic AGPase-deficient mutants from maize (Bhave et al. 1990) and rice (Yano et al. 1984; Satoh et al. 2003; Kawagoe et al. 2005) have shriveled seeds with a markedly reduced starch content, suggesting that the predominant pathway for the supply of ADP-glucose for starch biosynthesis in the amyloplast of cereal endosperm is via cytosolic AGPase and BT1, whereas the contributions of GPT and/or plastidic AGPase are limited, if any. In this connection, it is particularly interesting to note that the expression of *OsBT1-1* sharply increased at 10 DAF and this high expression continued until 15 DAF whereas the increases in transcripts in *OsGPT1*, *OsGPT2-1* & *2-2*, and *OsGlcT* were less marked (Fig. 3). The results indicate that the timing for the increase in starch production in the endosperm is closely related to the level of the BT1 transcript, suggesting a specific role of OsBT1-1 in starch biosynthesis of the rice endosperm.

The expression levels of the two *OsNTT s* were higher in source organs than in sink organs (Fig. 2), which is basically consistent with the data for *Arabidopsis* (Kampfenkel et al. 1995; Reiser et al. 2004) and potato (Tjaden et al. 1998). The result tempts us to speculate that in rice NTT facilitates the transfer of cytosolic ATP derived from mitochondria into chloroplasts at night.

OspGlcT was expressed mainly in the leaf, leaf sheath, and seed (Fig. 2), in agreement with the previous reports in tobacco (Weber et al. 2000). pGlcT may play an important role in both the export and import of glucose through the plastid envelope of rice (Fig. 2). *OsMT* was expressed mainly in the leaf and leaf sheath, but the overall level was relatively lower than those of the other genes (Fig. 2). Despite their possible important roles, pGlcT and MT were revealed to have only one copy each in the rice genome. In *Arabidopsis*, since the MT-deficient mutant (*MEX1*) grows more slowly than wild-type plants and has a reduced amount of chlorophyll, it might have quite an important role(s) in starch metabolism, especially in starch degradation at night (Niittyla et al. 2004). The transcript level of *OspGlcT* was more than threefold greater than that of *OsMT* in every organ examined (Fig. 2). However, microarray data demonstrated that in *Arabidopsis* MT is expressed much more than pGlcT (Zimmermann et al. 2004), suggesting that the mechanism of starch degradation may be different between *Arabidopsis* and rice.

All the results in the present study allowed us to identify the genes involved in, and thus provide insights into, the regulation of the carbohydrate metabolism network encompassing multiple cellular compartments such as the cytosol and chloroplast or amyloplast. The present results strongly suggest the distinct roles of different plastidic translocators in coordinating carbohydrate metabolism in plastids and the cytosol, although these transporters possess varying degrees of importance to the metabolism, and their expression patterns in various tissues seem to be plant species-specific. It is also true that gene transcript levels are merely suggestive of the involvement of the individual gene product in tissue-specific metabolism, and the identification of the gene is undoubtedly an essential initial step prior to its functional characterization. To understand the mechanism of how plastidic translocators coordinate carbohydrate metabolism occurring in plastids and in the cytosol in plants, additional studies such as gene silencing using RNAi methodology and the molecular characterization of these transporters are necessary.

Acknowledgements We are grateful to Dr. Perigio B. Francisco Jr. (Akita Prefectural University, Japan) for his critical reading of the manuscript. Analysis of DNA sequencing was conducted with the CREST-Akita Plant Molecular Science Satellite Laboratory in Life Science Research Support Center in Akita Prefectural University.

References

Achard P, Lagrange T, El-Zanaty AF, Mache R (2003) Architecture and transcriptional activity of the initiator element of the TATA-less RPL21 gene. Plant J 35:743–752

Ball SG, Morell MK (2003) From bacterial glycogen and to starch: understanding the biogenesis of the plant starch granule. Annu Rev Plant Biol 54:207–233

Bhave MR, Lawrence S, Barton C, Hannah LC (1990) Identification and molecular characterization of shrunken-2 cDNA clones of maize. Plant Cell 2:581–588

Butowt R, Granot D, Rodriguez-Garcia MI (2003) A putative plastidic glucose translocator is expressed in heterotrophic tissues that do not contain starch, during olive (*Olea europea* L.) fruit ripening. Plant Cell Physiol 44:1152–1161

Cao H, Shannon JC (1997) BT1, a possible adenylate translocator, is developmentally expressed in maize endosperm but not detected in starchy tissues from several other species. Physiol Plant 100:400–406

Eicks M, Maurino V, Knappe S, Flügge UI, Fischer K (2002) The plastidic pentose phosphate translocator represents a link between the cytosolic and the plastidic pentose phosphate pathways in plants. Plant Physiol 128:512–522

Emes MJ, Bowsher CG, Hedley C, Burrell MM, Scrase-Field ES, Tetlow IJ (2003) Starch synthesis and carbon partitioning in developing endosperm. J Exp Bot 54:569–575

Ferro M, Salvi D, Riviere-Rolland H, Vermat T, Seigneurin-Berny D, Grunwald D, Garin J, Joyard J, Rolland N (2002) Integral membrane proteins of the chloroplast envelope: identification and subcellular localization of new transporters. Proc Natl Acad Sci USA 99:11487–11492

Fischer K, Weber A (2002) Transport of carbon in non-green plastids. Trends Plant Sci 7:345–351

Fischer K, Arbinger B, Kammerer B, Busch C, Brink S, Wallmeier H, Sauer N, Eckerskorn C, Flügge UI (1994) Cloning and in vivo expression of functional triose phosphate/phosphate translocators from C3- and C4-plants: evidence for the putative participation of specific amino acid residues in the recognition of phosphoenolpyruvate. Plant J 5:215–226

Fischer K, Kammerer B, Gutensohn M, Arbinger B, Weber A, Hausler RE, Flügge UI (1997) A new class of plastidic phosphate translocators: a putative link between primary and secondary metabolism by the phosphoenolpyruvate/phosphate antiporter. Plant Cell 9:453–462

Flügge U (1999) Phosphate translocators in plastids. Annu Rev Plant Physiol Plant Mol Biol 50:27–45

Geigenberger P, Stamme C, Tjaden J, Schulz A, Quick PW, Betsche T, Kersting HJ, Neuhaus HE (2001) Tuber physiology and properties of starch from tubers of transgenic potato plants with altered plastidic adenylate transporter activity. Plant Physiol 125:1667–1678

Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, Hadley D, Hutchison D, Martin C, Katagiri F, Lange BM, Moughamer T, Xia Y, Budworth P, Zhong J, Miguel T, Paszkowski U, Zhang S, Colbert M, Sun WL, Chen L, Cooper B, Park S, Wood TC, Mao L, Quail P, Wing R, Dean R, Yu Y, Zharkikh A, Shen R, Sahasrabudhe S, Thomas A, Cannings R, Gutin A, Pruss D, Reid J, Tavtigian S, Mitchell J, Eldredge G, Scholl T, Miller RM, Bhatnagar S, Adey N, Rubano T, Tusneem N, Robinson R, Feldhaus J, Macalma T, Oliphant A, Briggs S (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *japonica*). Science 296:92–100

Herold A, Leegood R, McNeil PH, Robinson SP (1981) Accumulation of maltose during photosynthesis in protoplasts isolated from spinach leaves treated with mannose. Plant Physiol 67:85–88

Higo K, Ugawa Y, Iwamoto M, Korenaga T (1999) Plant cis-acting regulatory elements (PLACE) database: 1999. Nucleic Acids Res 27:297–300

Huber SC, Hanson KR (1992) Carbon partitioning and growth of a starchless mutant of *Nicotiana sylvestris*. Plant Physiol 99:1449–1454

James MG, Denyer K, Myers AM (2003) Starch synthesis in the cereal endosperm. Curr Opin Plant Biol 6:215–222

Kammerer B, Fischer K, Hilpert B, Schubert S, Gutensohn M, Weber A, Flügge UI (1998) Molecular characterization of a carbon transporter in plastids from heterotrophic tissues: the glucose 6-phosphate/phosphate antiporter. Plant Cell 10:105–117

Kampfenkel K, Mohlmann T, Batz O, Van Montagu M, Inzé D, Neuhaus HE (1995) Molecular characterization of an *Arabidopsis thaliana* cDNA encoding a novel putative adenylate translocator of higher plants. FEBS Lett 374:351–355

Kawagoe Y, Kubo A, Satoh H, Takaiwa F, Nakamura Y (2005) Roles of isoamylase and ADP-glucose pyrophosphorylase in starch granule synthesis in rice endosperm. Plant J 42:164–174

Kikuchi S, Satoh K, Nagata T, Kawagashira N, Doi K, Kishimoto N, Yazaki J, Ishikawa M, Yamada H, Ooka H, Hotta I, Kojima K, Namiki T, Ohneda E, Yahagi W, Suzuki K, Li CJ, Ohtsuki K, Shishiki T, Otomo Y, Murakami K, Iida Y, Sugano S, Fujimura T, Suzuki Y, Tsunoda Y, Kurosaki T, Kodama T, Masuda H, Kobayashi M, Xie Q, Lu M, Narikawa R, Sugiyama A, Mizuno K, Yokomizo S, Niikura J, Ikeda R, Ishibiki J, Kawamata M, Yoshimura A, Miura J, Kusumegi T, Oka M, Ryu R, Ueda M, Matsubara K, Kawai J, Carninci P, Adachi J, Aizawa K, Arakawa T, Fukuda S, Hara A, Hashizume W, Hayatsu N, Imotani K, Ishii Y, Itoh M, Kagawa I, Kondo S, Konno H, Miyazaki A, Osato N, Ota Y, Saito R, Sasaki D, Sato K, Shibata K, Shinagawa A, Shiraki T, Yoshino M, Hayashizaki Y, Yasunishi A; Rice Full-Length cDNA Consortium; National Institute of Agrobiological Sciences Rice Full-Length cDNA Project Team; Foundation of Advancement of International Science Genome Sequencing & Analysis Group; RIKEN (2003) Collection, mapping, and annotation of over 28,000 cDNA clones from japonica rice. Science 301:376–379

Knappe S, Flügge UI, Fischer K (2003a) Analysis of the plastidic phosphate translocator gene family in Arabidopsis and identification of new phosphate translocator-homologous transporters, classified by their putative substrate-binding site. Plant Physiol 131:1178–1190

Knappe S, Lottgert T, Schneider A, Voll L, Flügge UI, Fischer K (2003b) Characterization of two functional phosphoenolpyruvate/phosphate translocator (PPT) genes in Arabidopsis–At-PPT1 may be involved in the provision of signals for correct mesophyll development. Plant J 36:411–420

Knight JS, Gray JC (1994) Expression of genes encoding the tobacco chloroplast phosphate translocator is not light-regulated and is repressed by sucrose. Mol Gen Genet 242:586–594

Kofler H, Hausler RE, Schulz B, Groner F, Flügge UI, Weber A (2000) Molecular characterization of a new mutant allele of the plastid phosphoglucomutase in Arabidopsis, and complementation of the mutant with the wild-type cDNA. Mol Gen Genet 263:978–986

Koo AJ, Ohlrogge JB (2002) The predicted candidates of Arabidopsis plastid inner envelope membrane proteins and their expression profiles. Plant Physiol 130:823–836

Leroch M, Kirchberger S, Haferkamp I, Wahl M, Neuhaus E, Tjaden J (2005) Identification and characterization of a novel plastidic adenine nucleotide uniporter from *Solanum tuberosum*. J Biol Chem 280:17992–18000

Mohlmann T, Tjaden J, Henrichs G, Quick WP, Hausler R, Neuhaus HE (1997) ADP-glucose drives starch synthesis in isolated maize endosperm amyloplasts: characterization of starch synthesis and transport properties across the amyloplast envelope. Biochem J 324:503–509

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473–497

Naeem M, Tetlow IJ, Emes MJ (1997) Starch synthesis in amyloplasts purified from developing potato tubers. Plant J 11:1095–1103

Nakamura Y (2002) Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants: rice endosperm as a model tissue. Plant Cell Physiol 43:718–725

Nakamura M, Tsunoda T, Obokata J (2002) Photosynthesis nuclear genes generally lack TATA-boxes: a tobacco photosystem I gene responds to light through an initiator. Plant J 29:1–10

Neuhaus HE, Wagner R (2000) Solute pores, ion channels, and metabolite transporters in the outer and inner envelope membranes of higher plants plastids. Biochim Biophys Acta 1465:307–323

Neuhaus HE, Thom E, Mohlmann T, Steup M, Kampfenkel K (1997) Characterization of a novel eukaryotic ATP/ADP translocator located in the plastid envelope of *Arabidopsis thaliana* L. Plant J 11:73–82

Niittyla T, Messerli G, Trevisan M, Chen J, Smith AM, Zeeman SC (2004) A previously unknown maltose transporter essential for starch degradation in leaves. Science 303:87–89

Overlach S, Diekmann W, Raschke K (1993) Phosphate translocator of isolated guard-cell chloroplasts from *Pisum sativum* L. transports glucose-6-phosphate. Plant Physiol 101:1201–1207

Page RD (1996) Tree view: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358

Patron NJ, Greber B, Fahy BF, Laurie DA, Parker ML, Denyer K (2004) The lys5 mutations of barley reveal the nature and importance of plastidial ADP-Glc transporters for starch synthesis in cereal endosperm. Plant Physiol 135:2088–2097

Reiser J, Linka N, Lemke L, Jeblick W, Neuhaus HE (2004) Molecular physiological analysis of the two plastidic ATP/ADP transporters from Arabidopsis. Plant Physiol 136:3524–3536

Satoh H, Nishi A, Yamashita K, Takemoto Y, Tanaka Y, Hosaka Y, Sakurai A, Fujita N, Nakamura Y (2003) Starch-branching enzyme I-deficient mutation specifically affects the structure and properties of starch in rice endosperm. Plant Physiol 133:1111–1121

Schulz B, Frommer WB, Flugge UI, Hummel S, Fischer K, Willmitzer L (1993) Expression of the triose phosphate translocator gene from potato is light dependent and restricted to green tissues. Mol Gen Genet 238:357–361

Schunemann D, Schott K, Borchert S, Heldt HW (1996) Evidence for the expression of the triosephosphate translocator gene in green and non-green tissue of tomato and potato. Plant Mol Biol 31:101–111

Schwacke R, Schneider A, van der Graaff E, Fischer K, Catoni E, Desimone M, Frommer WB, Flügge UI, Kunze R (2003) ARAMEMNON, a novel database for Arabidopsis integral membrane proteins. Plant Physiol 131:16–26

Shannon JC, Pien FM, Cao H, Liu KC (1998) Brittle-1, an adenylate translocator, facilitates transfer of extraplastidial synthesized ADP-glucose into amyloplasts of maize endosperms. Plant Physiol 117:1235–1252

Sullivan TD, Kaneko Y (1995) The maize brittle1 gene encodes amyloplast membrane polypeptides. Planta 196:477–484

Sullivan TD, Strelow LI, Illingworth CA, Phillips RL, Nelson OE Jr (1991) Analysis of maize brittle-1 alleles and a defective suppressor-mutator-induced mutable allele. Plant Cell 3:1337–1348

Tetlow IJ, Blisset KJ, Emes MJ (1994) Starch synthesis and carbohydrate oxidation in amyloplasts from developing wheat endosperm. Planta 194:454–460

Tetlow IJ, Bowsher CG, Emes MJ (1996) Reconstitution of the hexose phosphate translocator from the envelope membrane of wheat endsperm amyloplasts. Biochem J 319:717–723

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Tjaden J, Mohlmann T, Kampfenkel K, Henrich G, Neuhaus HE (1998) Alterd plastidic ATP/ADP-transporter activity influences potato (*Solanum tuberosum* L.) tuber morphology, yield and composition of tuber starch. Plant J 16:531–540

Tyson RH, ap Rees T (1988) Starch synthesis by isolated amyloplasts from wheat endosperm. Planta 175:33–38

Ware D, Jaiswal P, Ni J, Pan X, Chang K, Clark K, Teytelman L, Schmidt S, Zhao W, Cartinhour S, McCouch S, Stein L (2002) Gramene: a resource for comparative grass genomics. Nucleic Acids Res 30:103–105

Weber A (2004) Solute transporters as connecting elements between cytosol and plastid stroma. Curr Opin Plant Biol 7:247–253

Weber A, Servaites JC, Geiger DR, Kofler H, Hille D, Groner F, Hebbeker U, Flügge UI (2000) Identification, purification, and molecular cloning of a putative plastidic glucose translocator. Plant Cell 12:787–802

Weber A, Schneidereit J, Voll L (2004) Using mutants to probe the in vivo function of plastid envelope membrane metabolite transporters. J Exp Bot 55:1231–1244

Weise SE, Weber A, Sharkey TD (2004) Maltose is the major form of carbon exported from the chloroplast at night. Planta 218:474–482

Winkler HH, Neuhaus HE (1999) Non-mitochondrial ATP transport. Trends Biochem Sci 24:64–68

Yano M, Isono Y, Satoh H, Omura T (1984) Gene analysis of sugary and shrunken mutants of rice, *Oryza sativa* L. Jpn J Breed 34:43–49

Zimmermann P, Hirsch-Hoffmann M, Hennig L, Gruissem W (2004) GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. Plant Physiol 136:2621–2632