A Truncated Version of an ADP-Glucose Pyrophosphorylase Promoter from Potato Specifies Guard Cell-Selective Expression in Transgenic Plants

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ADP-glucose pyrophosphorylase (AGPase) is a key regulatory enzyme in starch biosynthesis in higher plants. A 3.2-kb promoter of the large subunit gene of the AGPase from potato has been isolated and its activity analyzed in transgenic potato and tobacco plants using a promoter-β-glucuronidase fusion system. The promoter was active in various starch-containing cells, including guard cells, tuber parenchyma cells, and the starch sheath layer of stems and petioles. No expression was observed in mesophyll cells. Analysis of various promoter derivatives showed that with respect to expression in petioles and stems, essential elements must be located in the 5' distal region of the promoter, whereas elements important for expression in tuber parenchyma cells are located in an internal fragment comprising nucleotides from positions - 500 to - 1200. Finally, a 0.3-kb 5' proximal promoter fragment was identified that was sufficient to obtain exclusive expression in guard cells of transgenic potato and tobacco plants. The implications of our observations are discussed with respect to starch synthesis in various tissues and the use of the newly identified promoter as a tool for stomatal biology.

INTRODUCTION

ADP-glucose pyrophosphorylase (ATP: α-glucose-1-phosphate adenyllyl transferase, EC 2.7.7.27; AGPase) plays a central role in the biosynthesis of starch in both photosynthetic and non-photosynthetic plant tissues (for review, see Preiss, 1991). Within chloroplasts and amyloplasts, the enzyme catalyzes the synthesis of ADP-glucose and PPi from glucose-1-phosphate and ATP. ADP-glucose functions as the glucosyl donor for α-glucan synthesis by various starch synthases (Preiss, 1991).

The importance of AGPase in starch biosynthesis has been shown by mutants deficient in AGPase activity in storage organs and leaves (e.g., Tsai and Nelson, 1966; Lin et al., 1988a, 1988b), by kinetic models (Pettersson and Ryde-Pettersson, 1989), by control analysis of photosynthetic partitioning (Neuhaus and Stitt, 1990), and by the use of transgenic plants in which AGPase activity has either been decreased by an antisense RNA approach (leading to reduced starch levels; Müller-Röber et al., 1992) or increased by ectopic expression of a bacterial AGPase (leading to higher starch levels; Stark et al., 1992). AGPase is assumed to be a tetramer composed of two different but similar polypeptides (e.g., see Morell et al., 1987; Lin et al., 1988b). The size of the two small subunits ranges from 50 to 55 kD, and the size of the two large subunits is from 51 to 60 kD, depending on the plant species (Kleckowski et al., 1991). cDNA clones encoding the different subunit polypeptides have been isolated from a number of monocot and dicot plant species (e.g., see Anderson et al., 1989; Olive et al., 1989; Bae et al., 1990; Bhave et al., 1990; Müller-Röber et al., 1990; du Jardin and Berhin, 1991; Nakata et al., 1991; Villand et al., 1992a, 1992b; Ainsworth et al., 1993). By comparing the primary structures of 11 plant AGPase proteins, Smith-White and Preiss (1992) recently pointed out the existence of at least three types of AGPase polypeptides: (1) small subunit, that is present in photosynthetic and non-photosynthetic tissues; (2) large subunit, that is found in nonphotosynthetic tissues; and (3) large subunit, that is present in photosynthetic cells.

The complexity of the plant's AGPase system is further underscored by the observation of multiple AGPase polypeptides in potato tubers (Okita et al., 1990) and the rice and pea endosperm (Hylton and Smith, 1992; Nakamura and Kawaguchi, 1992). Developmental (coarse) control of AGPase mRNA expression has been observed in several plant species (Krishnan et al., 1986; Reeves et al., 1986; Müller-Röber et al., 1990; Anderson et al., 1991; Villand et al., 1992a). In potato, the small (i.e., AGPase B) subunit was strongly expressed in a variety of tissues tested, including leaves and tubers. By contrast, expression of the large (i.e., AGPase S) subunit was high in tubers and generally weak to moderate in leaves (Müller-Röber et al., 1990). Incubation of excised leaves in sucrose led to a strong accumulation of the AGPase S transcript (Müller-Röber et al., 1990). By contrast, expression of the AGPase B mRNA was only slightly influenced by the presence of external sucrose.

Starch synthesis takes place in several different cells and organs of the plant and is of major importance for carbon...
partitioning. One of the key regulatory mechanisms is the allosteric activation of the AGPase by 3-phosphoglycerate and the inhibition by Pi (see Preiss, 1991). This manner of regulation has been found in a wide range of AGPases from photosynthetic and nonphotosynthetic tissues, including guard cells (Robinson et al., 1983; Outlaw and Tarczynski, 1984; Preiss, 1988). Recently, Kiecowski et al. (1993) showed the insensitivity of the barley endosperm AGPase to 3-phosphoglycerate and Pi regulation, demonstrating heterogeneity within the allosteric regulatory properties of the plant enzyme.

Understanding regulation of carbon partitioning and starch biosynthesis in higher plants not only requires knowledge about biochemical characteristics of the enzymes involved, but also of the cellular and organ-specific expression of the genes encoding these enzymes. Here, we report the isolation of a genomic DNA fragment containing regulatory elements of the large subunit of the potato AGPase. The promoter was fused to the β-glucuronidase (gus) reporter gene, and its expression pattern was analyzed in transgenic potato and tobacco plants.

RESULTS

Isolation of a Potato ADP-Glucose Pyrophosphorylase Large Subunit Promoter

A genomic clone homologous to the large subunit cDNA of the potato AGPase (AGPase S; Müller-Rüber et al., 1990) was isolated from a genomic library prepared from the monohaploid potato line AM 80/5793 (Liu et al., 1991). DNA sequences were obtained for several subclones and compared with the 5′ end of the full-length AGPase S cDNA of plasmid S9-D (see Methods). A 5.2-kb HindIII fragment was found to comprise a 3.2-kb promoter fragment and part of the AGPase coding region. A schematic alignment of the cDNA and the genomic fragment is shown in Figure 1. With the exception of two introns (corresponding to positions 190/191 and 426/427 of the cDNA), complete identity was found between nucleotides 1 to 484 of the cDNA and the genomic clone. (No effort was made to sequence further downstream regions of the genomic clone.)

The DNA sequence of the 1.2-kb HindIII-BgIII genomic segment (see Figure 1) is shown in Figure 2A. Comparison with the full-length cDNA S9-D revealed a 56-bp overlap with the 5′ untranslated leader of the cDNA. A TATA-box-like sequence element ("TATAATA") is present at position −66/59 upstream of the 5′ end of the corresponding cDNA. Two direct repeats (each repeating sequence 50 nucleotides long, with five mismatches) were identified (Figure 2A).

The 3.2-kb Promoter of the AGPase S Gene Is Most Active in Nodal and Internodal Stem Sections of Transgenic Potato Plants

To analyze the expression pattern of the AGPase S promoter, the 3.2-kb promoter fragment was transcriptionally fused to the gus reporter gene of the binary vector pBI101.1 (Jefferson et al., 1987). The structure of the chimeric AGPase S promoter–gus gene is shown in Figure 2B. This plasmid was used to transform potato plants using the Agrobacterium-mediated gene transfer technique. Approximately 50 independent transgenic plants (B-GUS-1 transformants) were analyzed.

To gain insight into quantitative aspects of the expression of the AGPase S–gus construct, various tissues of transgenic potato plants were homogenized and analyzed by way of the fluorescence assay. As shown in Figure 3, the highest activities were found in stem sections, reaching more than 7000 pmol of 4-methylumbelliferone (4-MU) produced per min per mg of protein in some of the transformants. The activity in nodes was higher by a factor of 1.2 to 2 compared to that in internodes (data not shown). Strong GUS activity (up to about 6000 pmol of 4-MU produced per min per mg of protein) was also found in petioles (sampled with basal parts of the leaf midrib) and in tubers of all transformants tested, whereas much lower activity, which was only slightly above background (between 50 and 120 pmol of 4-MU produced per min per mg of protein as compared to 2 to 20 pmol of 4-MU produced per min per mg of protein in nontransgenic control plants), was found in leaves from which the first- (midrib) and second-order veins had been removed.

The level of expression varied over a wide (more than 10-fold) range, as shown in Tables 1 and 2; this observation has been made in the case of many other transgenes (e.g., Peach and Velten, 1991). When DNA gel blot analysis was performed on various transgenic lines differing in their expression levels, no correlation was observed between AGPase S–gus gene copy number and expression level, which again is in agreement with many other cases where this problem has been analyzed (for a review, see Weising et al., 1988).
Histochemical Localization of Chimeric AGPase S-\textit{gus} Gene Expression in Transgenic Potato Plants

Histochemical GUS stainings of various tissues were performed to study the expression of the AGPase S-\textit{gus} construct in a more detailed manner. Potato tubers harvested from plants grown in soil or vermiculite showed weak blue GUS staining in storage parenchyma cells after 1 to 1.5 hr in staining solution. After overnight incubation, these cells showed intense color formation. An example is shown in Figure 4A. Vascular strands remained unstained when the incubation time was restricted. No staining could be detected in the periderm of tubers (Figure 4B). In stolons of the same plants, a much weaker, and often also nonuniform staining, was observed (see Figure 4C). No staining was seen in roots including the root cap (Figure 4D).

When potato flowers of different developmental stages were analyzed, intense color formation was seen in the ovaries of young flower buds (Figure 4E). Staining intensity decreased when flowers became older. GUS activity was also detected in storage parenchyma cells after 1 to 1.5 hr in staining solution. After overnight incubation, these cells showed intense color formation. An example is shown in Figure 4A. Vascular strands remained unstained when the incubation time was restricted. No staining could be detected in the periderm of tubers (Figure 4B). In stolons of the same plants, a much weaker, and often also nonuniform staining, was observed (see Figure 4C). No staining was seen in roots including the root cap (Figure 4D).
in cross-sections of anthers (Figure 4F) and in mature pollen grains (data not shown). Immature pollen grains remained completely unstained even after more than 24 hr of incubation.

In cross-sections prepared from stems of potato B-GUS-1 transformants, GUS staining was restricted to a single cell layer surrounding the vascular tissue. A representative example is shown in Figure 4G. This cell layer was identified as a starch sheath, which has been described for a variety of plant species, including potato and tobacco. Figure 4H shows that starch granules can easily be detected in this cell type. Due to a higher density of vascular tissues and corresponding starch sheath cells, GUS staining was more intense in nodal than in internodal stem regions (data not shown). The only other cells stained in the stem were guard cells, which frequently appeared in the stem epidermis, as shown in Figure 5A. All other cells, even parenchyma cells, which very often contained starch granules, remained completely unstained.

When leaves were incubated for 15 to 24 hr in staining solution, a strong blue staining was visible along the petiole and the midrib (first-order vein), with the intensity decreasing from the tip region of the leaf. In addition, second-order veins and often also third-order veins showed staining, again with an acropetal decrease in intensity. A representative example is shown in Figure 5B. No GUS staining was seen in the tip region of the major veins or in any of the smaller leaf veins even after prolonged (up to 48 hr) incubation. Figure 5C shows that in cross-sections strong GUS staining appeared in a single cell layer adjacent to the vascular tissues. This cell layer was very similar to the starch sheath observed in the stem (see above). An investigation for the presence of the starch sheath showed that this cell layer is present only in the petiole and in the basal regions of major veins. No starch sheath was detected in any of the minor veins of the leaf. The distribution of the starch sheath therefore corresponded well to the decrease of GUS staining intensity toward the tip region of the veins and to the absence of GUS staining in small (i.e., higher order) veins. Guard cells distributed within the epidermis of the major veins were easily stained. No GUS activity was seen in any other cell type of the petiole (and midrib), including parenchyma cells.

Mesophyll cells that accumulated massive amounts of transitory starch were completely devoid of any GUS staining, irrespective of the developmental stage of the leaf or length of incubation (see Figure 5D for an example). Also, leaf trichomes never showed any GUS staining (Figure 5E). Most interestingly, stomatal guard cells of both the abaxial and the adaxial leaf surface were stained after incubation overnight. An example is shown in Figure 5F. The fact that guard cells are the only cell type (apart from the major veins) that is stained in leaves is in agreement with the very low levels of GUS activity determined by the fluorescence assay (see above). Guard cells are estimated to represent only about 1% of the cellular population of a leaf (Hsiao, 1976).

Among the transgenic plants analyzed in about 10 to 20% of the cases, GUS staining observed in leaf guard cells was classified as strong or medium, whereas in about 30 to 40% of the plants harboring the full-length AGPase S promoter, staining in guard cells was classified as weak. In the remaining 30 to 40% of the plants, no staining was found in guard cells after incubation overnight.

Expression or nonexpression in guard cells correlated in a strict manner with expression/nonexpression in other tissues (such as tubers or petioles), suggesting that this is a typical position effect. In the few cases where quantitative numbers are given with respect to nonexpressing transgenic plants, a similar proportion of plants was identified as nonexpressers.
Figure 4. Histochemical Localization of GUS Activity in Potato Plants Transformed with the AGPase S-gus Construct (B-GUS-1 Transformants).

(A) Transverse sections through potato tubers. The unstained tuber slice is from a wild-type plant.
(B) Tuber section stained for 3 hr.
(C) Stolons of an untransformed plant (top) and a transgenic plant (other stolons).
(D) Root tip stained for 24 hr.
(E) Longitudinal section through a flower bud (length of 13 mm).
(F) Cross-section through an anther.
(G) Cross-section through a stem. Staining was performed for 2 hr.
(H) Cross-section through a stem. The tissue was stained with Lugol's solution to show starch granules within the starch sheath.

Unless otherwise indicated, GUS staining was performed for 15 hr.

In (B), bar = 0.5 cm; in (D), (G), and (H), bars = 200 μm; in (F), bar = 0.5 mm. an, anther; fi, filament; lo, locule; ov, ovary; p, periderm; pa, parenchyma cells; ss, starch sheath; st, stolon; vs, vascular strand; xy, xylem.
Figure 5. Localization of GUS Activity in Potato Plants Transformed with the AGPase S-gus Construct.

(A) Guard cell in the stem epidermis.
(B) Close-up of a leaf.
(C) Cross-section through the leaf midrib (central region). Staining was performed for 30 min.
(D) Cross-section through the upper part of a leaf.
(E) Trichome.
(F) Leaf guard cells in an epidermal strip.

Unless otherwise indicated, staining was performed for 15 hr. In (A), (D), and (F), bars = 20 μm; in (B), bar = 0.5 cm; in (C), bar = 200 μm; in (E), bar = 50 μm. e, epidermis; gc, guard cell; m, mesophyll; mr, midrib; pa, parenchyma cells; ss, starch sheath; sv, second-order vein; tr, trichome; xy, xylem.
(20 to 50%; cf., e.g., Sanders et al., 1987; Keil et al., 1990; Peach and Velten, 1991).

A Truncated Derivative of the AGPase S Promoter Leads to a Guard Cell–Specific Expression in Transgenic Potato Plants

As described above, the AGPase S promoter is able to induce strong gus expression in various starch-producing cells and tissues of transgenic potato plants, including the guard cells. To determine whether promoter regions important for the expression in the petiole, stems, and tubers can be separated from the expression in guard cells, a series of deletions was generated in the 3.2-kb promoter fragment. These deletions were fused to the gus reporter gene (see Methods) and were transformed in potato plants. A schematic representation of the different deletion constructs is given in Figure 6. With the exception of S1-Δ5 transformants, weak GUS activity was observed in leaves (sampled without first- and second-order veins) of many transformants. Construct S1-Δ5, which does not contain the TATA box and the basal promoter, led to background levels of GUS activity. The results for tubers are shown in Table 1. Highest GUS activity was observed in plants containing the 3.2-kb promoter fragment. This activity decreased by ~60% when 1.2 kb of the most distal 5' end was deleted (construct S1-Δ1). Further 5' deletions down to ~1.2 kb (construct S1-Δ2) led to a decrease of ~90%. Finally, a 5' deletion down to position ~0.3 kb (construct S1-Δ4) resulted in a complete loss of GUS activity in tubers.

The fluorometric data were supported by histochemical analysis. Whereas clear blue staining was observed in tubers of S1-Δ1 transformants, a much weaker staining could be seen in S1-Δ2 transgenic plants. No staining was observed in S1-Δ4, S1-Δ13, and S1-Δ14 transgenic tubers. (This conclusion is based on the analysis of three to five tubers from each transgenic plant; each test included 10 independent transgenic lines.)

The results obtained for the fluorometric GUS determination in petioles are summarized in Table 2. Highest GUS activity was maintained in plants transformed with the internal deletion constructs S1-Δ13 and S1-Δ14. On the other hand, deletion of the 5'-most 1.2-kb fragment (S1-Δ1) resulted in a complete loss of GUS activity in petioles. When histochemical stainings were performed, no difference was seen between B-GUS-1 and S1-Δ13 or S1-Δ14 transformants, that is, dark blue staining occurred in cells of the starch sheath (data not shown). The same was true for stem sections of these transformants (data not shown). Stems, petioles, and veins from all the transgenic lines remained completely unstained, even when the incubation time was extended to 48 hr.

With respect to expression in leaves, none of the S1-Δ5 transformants showed any GUS staining in guard cells. In all of the other transformants, a similar distribution of plants showing strong, middle, weak, or no expression in leaf guard cells was observed for the different constructs, as described above for the full-length construct B-GUS-1. This distribution was also found for S1-Δ4 transgenic plants that did not show staining in tubers and petioles (see above).

Transgenic Tobacco Plants Harboring the B-GUS-1 or the S1-Δ4 Constructs Display Expression Patterns Similar to the Ones Observed in Potato

To analyze whether the expression profile conferred by the AGPase S promoter is maintained in other species, two chimeric genes, that is, the full-length promoter construct B-GUS-1 and the truncated promoter construct S1-Δ4 resulting in a guard cell–selective expression, were introduced into tobacco plants. As shown in Figures 7B and 7C, GUS activity was observed in the major veins of leaves and within the petiole. Cross-sections showed strong staining in the starch sheath surrounding the vascular tissue. The same was true for the stem (data not shown). In addition, guard cells showed an intense staining
Figure 7. Histochemical Localization of GUS Activity in Tissues of Transgenic Potato and Tobacco Plants.
(A) Close-up of a leaf of potato transformant S1-Δ4-43.
(B) Leaves of tobacco B-GUS-1 transformants.
(C) Cross-section through a leaf petiole of a tobacco B-GUS-1 transformant.
(D) Leaf guard cell of a tobacco B-GUS-1 transformant. The leaf was not bleached before photography.
Staining time in (A), (B), and (D) was 15 hr; in (C), staining was performed for 30 min. In (A), bar = 1 mm; in (C), bar = 0.5 mm; in (D), bar = 50 μm. Abbreviations are as given in Figure 5.

(Figure 7D), thus being in complete agreement with the data obtained for potato. The expression pattern in flowers was very similar to the one observed for potato. Furthermore, histochemical analysis did not reveal any activity in roots, including the root cap. When tobacco plants harboring the deletion derivative S1-Δ4 were analyzed, GUS staining was restricted exclusively to guard cells. Thus, the elements necessary and sufficient to give rise to a guard cell–selective expression in potato also function in transgenic tobacco plants.

DISCUSSION

AGPase is one of the major regulatory enzymes in starch biosynthesis in higher plants. We isolated genomic DNA fragments representing the large subunit of the AGPase from potato. The 5′ upstream regulatory region of one of the fragments was fused to the gus reporter gene and transferred into potato plants. The AGPase is a plastidic enzyme and, hence, contains targeting signals at its N-terminal end. To avoid interference of plastidial targeting information with GUS activity, we decided to perform transcriptional instead of translational gus fusions. Expression of the AGPase–gus construct in leaves and stems of transgenic plants was restricted to stomatal guard cells and to a specialized cell layer, the starch sheath. In addition, strong expression was observed in mature pollen grains and developing tubers. Thus, the AGPase S promoter is active in many but not all starch-containing cells. Most surprising in this respect is the observation that no expression was observed in the cells representing the major starch reservoirs in leaves (i.e., mesophyll cells). Also, no staining was detected in parenchymatous cells of the petiole and stem.

Currently, there is no explanation for the inactivity of this promoter in leaf mesophyll cells. However, because the genomic clone described here, with the exception of the two introns, is completely homologous to a cDNA recently isolated by us, it is likely that this promoter originates from an expressed...
gene. Thus, with respect to the clear requirement for a promoter driving AGPase S gene expression in mesophyll cells, two possibilities remain: (1) the promoter fragment isolated and used in this study is devoid of some regulatory elements (i.e., expression in mesophyll cells requires elements located in the coding or 3′ untranslated region), or (2) there is a second AGPase S gene, with a different promoter, driving expression in mesophyll cells. We cannot distinguish between the two possibilities.

Recently, restriction fragment length polymorphism mapping experiments revealed two loci in the potato genome homologous to the AGPase S cDNA (Gebhardt et al., 1993). This observation is compatible with the putative existence of a second gene with a different promoter specificity. The latter interpretation would also offer an explanation for the fact that the expression of the gus gene driven by the promoter described here is not inducible by sucrose (data not shown). This observation is seemingly in contrast to our previous results. In this study, we observed a sucrose-dependent accumulation of AGPase S homologous mRNA in detached leaves (Müller-Röber et al., 1990). The exact number of genes encoding AGPase polypeptides in any plant is not known. In potato, at least four different AGPase genes are expressed (Müller-Röber et al., 1990; U. La Cognata, L. Willmitzer, and B. Müller-Röber, manuscript in preparation).

Starch biosynthesis occurs in many different cell types. With respect to photoassimilates, these can be divided into exporters and importers. It is interesting to note that the expression pattern displayed by the AGPase promoter used in this study is restricted to various cell types that are all net importers of photoassimilates, such as tubers, the starch sheath cells of the stem and petioles, pollen grains, ovaries, and guard cells. No activity was observed in the main exporters of photoassimilates (i.e., the mesophyll cells of leaves). It is conceivable, although not certain, that starch biosynthesis is regulated in a different manner in photoassimilate importers versus exporters. One way to achieve such regulations could occur by way of the fusion of different promoters to the key enzyme of starch biosynthesis (i.e., the AGPase). We are currently pursuing this possibility by isolating other genomic clones homologous to the AGPase S cDNA.

By analyzing the expression pattern of the gus gene fused to various derivatives of the AGPase S promoter in transgenic potato plants, different regions responsible for the expression in the petioles/stems, the tubers, and the guard cells were distinguished. With respect to expression in petioles and stems, essential elements must be located in the distal 5′ upstream region of this promoter, because a 5′ deletion devoid of only the most distal 1.2 kb was completely inactive in the starch sheath of the vascular tissue. In tubers, a more gradual decrease in activity was seen upon deleting 5′ regions of the promoter. However, when a 0.7-kb-long internal fragment encompassing nucleotides from position –0.5 to –1.2 kb was removed (construct S1−Δ13), a more than 90% decrease in GUS activity occurred, indicating that a sequence element essential for expression in tubers is present in the corresponding region of the AGPase promoter. Finally, the most proximal 0.3 kb of the promoter are necessary and sufficient to give full expression in stomatal guard cells. In addition, this expression is highly specific because no other tissue showed activity upon histochemical analysis. To the best of our knowledge, this represents the first functional identification of a guard cell–specific promoter.

While this manuscript was in preparation, the promoter of the Arabidopsis rha1 gene (encoding a small GTP binding protein) was reported to be active in developing guard cells and several other tissues (including flowers and root tips) of Arabidopsis plants (Terry et al., 1993). No deletion analysis was performed on the rha1 promoter. Therefore, it is currently not known whether expression in guard cells is conferred by a promoter element different from the ones driving expression in the other cell types.

Stomatal guard cells of higher plants play an important role in gas exchange and transpiration in the leaves of higher plants. Opening and closing of stomates are under the control of a large variety of plant endogenous and exogenous (environmental) factors, including light intensity, CO₂ concentration, humidity, and hormones (Zeiger et al., 1987). Stomatal opening is caused by the accumulation of both inorganic and organic compounds in guard cells (for a recent review, see Tallman, 1992).

Thus far, no mutants have been described that are specifically changed with respect to guard cell metabolism or signal transduction. We believe that the truncated AGPase S promoter from potato will be a valuable tool in creating transgenic plants that are altered in various biochemical pathways, specifically those in guard cells. These plants will allow us to test in vivo the importance of certain biochemical pathways or signal transduction steps for stomatal functioning.

METHODS

Plants, Bacterial Strains, and Growth Conditions

Potato (Solanum tuberosum L.) cv Desiree was obtained through Saat- zucht Fritz Lange KG (Bad Schwartau, Germany). Plants in tissue culture were maintained under a 16-hr light/8-hr dark regime on Murashige and Skoog medium (Murashige and Skoog, 1962) containing 2% (w/v) sucrose. Plants in the greenhouse were cultivated in soil or vermiculite under a regime of ~16 hr of light and 8 hr of darkness. Plants were grown in individual pots (200 cm², 15 cm deep) and were watered daily.

Escherichia coli DH5a (Bethesda Research Laboratories) was cultured using standard techniques (Sambrook et al., 1989). Agrobacterium tumefaciens C58C1 containing plasmid pGV2280 (Deblaere et al., 1985) was cultured in YEB medium (Verzijl et al., 1975).

Enzymes and Chemicals

DNA restriction and modification enzymes were purchased from Boehringer Mannheim and New England Biolabs (Beverly, MA).
5-Bromo-4-chloro-3-indolyl β-D-glucuronide was obtained from Biosynth AG (Staad, Switzerland). Other chemicals were purchased from Sigma or Merck.

**DNA Manipulations**

DNA manipulations were performed essentially as described by Sambrook et al. (1989). DNA sequences were determined by the dideoxy method (Sanger et al., 1977) using supercoiled plasmids (Chen and Seeburg, 1985). Either commercial sequencing primers (Pharmacia) or specifically synthesized oligonucleotides (DNA Synthesizer 380A; Applied Biosystems, Foster City, CA) were used for sequencing with a T7 DNA polymerase sequencing kit (Pharmacia).

**Isolation of the Potato ADP-Glucose Pyrophosphorylase Large Subunit Promoter**

To isolate the ADP-glucose pyrophosphorylase large subunit (AGPase S) promoter, a genomic library prepared from the monohaploid potato line AM 80/5793 (Liu et al., 1991) was screened with a partial AGPase S cDNA (EcoRI insert of plasmid S25-1; Müller-Röber et al., 1990). Several hybridizing phage clones were obtained. The >12-kb-long SalI fragment of clone gSF-6 was subcloned into pUC19. The resulting plasmid SF-6 was analyzed by restriction analysis and DNA gel blot hybridization using an ~200-bp-long 5' fragment of the AGPase S cDNA. A 5.2-kb HindIII subfragment strongly hybridizing to the probe was subcloned into pUC19, resulting in plasmid pH6-1 (see Figure 1). DNA sequences obtained for several subfragments were compared with the full-length potato AGPase S cDNA S9-D. This cDNA was obtained from a tuber-specific cDNA library (provided by J. Köhnmann, Institut für Genbiologische Forschung, Berlin, Germany) using the previously isolated cDNA S25-1 (Müller-Röber et al., 1990) as a probe.

**Construction of Promoter-gus Fusions**

Binary plasmids containing chimeric AGPase-β-glucuronidase (gus) gene fusions were constructed as follows. In all cases, the binary vector pBl101.1 (Jefferson et al., 1987) was used to obtain plasmids for the transformation of potato and tobacco plants.

For all fusions, the AGPase S promoter fragment was cloned into the Smal site of pUC19 (5' end oriented to the EcoRI site of pUC19). The resulting 3.2-kb promoter fragment was subsequently introduced as an EcoRI (blunt-ended)-BamHI fragment into pBl101.1, which was previously cleaved with HindIII, blunt-ended, and recut with BamHI.

Creating S1-Δ13 required an internal 0.7-kb promoter fragment to be deleted by cleaving plasmid pPSA with Clal and subsequent religation of the remaining vector. The resulting plasmid pPSA-Δ13 was cut with EcoRI, blunt-ended with T4 polymerase, and cut with BamHI. The 2.5-kb-long promoter fragment was subsequently cloned into pBI101.1, which was previously cleaved with HindIII, blunt-ended, and recut with BamHI.

To construct S1-Δ14, a 1.5-kb internal fragment of the AGPase promoter needed to be deleted. To this end, plasmid pPSA was cut with Ncol and Clal, blunt-ended, and religated. The 1.7-kb-long promoter fragment from the resulting plasmid pPSA-Δ14 was inserted as an EcoRI (blunt-ended)-BamHI fragment into pBI101.1, which was previously cleaved with HindIII, blunt-ended, and recut with BamHI.

**Plant Transformations**

Binary vectors were used to transform potato (Solanum tuberosum cv Desiree) and tobacco (Nicotiana tabacum cv Samsun NN) by way of Agrobacterium C58C1:pGV2260, as described by Rocha-Sosa et al. (1989) and Rosahl et al. (1987).

**Histochemical Localization of GUS Activity**

Histochemical staining for GUS activity was performed using 5-bromo-4-chloro-3-indolyl β-D-glucuronide as chromogenic substrate (Jefferson et al., 1987). Freshly cut plant tissues were incubated in GUS staining solution (for 30 min to 24 hr at PC, depending on staining intensity) and rinsed with water before photography. Photosynthetic tissues were bleached with ethanol. Microscopic analysis was performed using an Axioptit microscope (Zeiss, Oberkochen, Germany).

**Fluorometric GUS Assay**

Transgenic lines showing strongest histochemical GUS staining in leaf guard cells were used for fluorometric GUS assays. Tissue samples were homogenized in extraction buffer (50 mM sodium phosphate buffer, pH 7.0, 10 mM β-mercaptoethanol, 10 mM EDTA, 0.1% SDS, 0.1% Triton X-100) in a microcentrifuge tube and centrifuged for 10 min at 13,000 rpm in a microcentrifuge (Biofuge A; Heraeus Instruments, Hanau, Germany). Aliquots of the supernatant were taken for enzyme assay using 4-methylumbelliferyl β-D-glucuronic acid as substrate (Jefferson, 1987) and for protein determination (Bradford, 1976). The product of the reaction (4-methylumbelliferyl [4-MU]) was measured fluorometrically (SFM 25 Fluorescence Spectrophotometer; Kontron Instruments, Hamburg, Germany).

**Iodine Staining**

To visualize starch granules, tissues were incubated for 2 min in Lugol's solution (5 g KI, 2 g I₂ in 100 mL H₂O), washed in H₂O for 30 sec, and subsequently viewed microscopically.

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ADP-Glucose Pyrophosphorylase Promoter from Potato

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A truncated version of an ADP-glucose pyrophosphorylase promoter from potato specifies guard cell-selective expression in transgenic plants.

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