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Light-Dependent and Tissue-Specific Expression of the H-Protein of the Glycine Decarboxylase Complex[1]

R. Srinivasan[2] and David J. Oliver*

Department of Molecular Biology and Biochemistry, University of Idaho, Moscow, Idaho 83843

Glycine decarboxylase is a mitochondrial enzyme complex, which is the site of photorespiratory CO_2 and NH_3 release. Although the proteins that constitute the complex are located within the mitochondria, because of their intimate association with photosynthesis their expression is controlled by light. Comparisons of the kinetics of mRNA accumulation between the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase and the H-protein of glycine decarboxylase during the greening of etiolated *Arabidopsis thaliana* suggest that their expression is controlled in parallel. A genomic clone for the H-protein (*gdcH*) was isolated from Arabidopsis and sequenced. The upstream region from −856 to +62 was fused to the β-glucuronidase (GUS) reporter gene, and this construct was transformed into tobacco. This 5′ upstream regulatory region appears to control GUS expression in a manner very similar to that of the endogenous H-protein gene. Constructs with deletions in the 5′ upstream region were transformed into tobacco. These deletions revealed that light-dependent and tissue-specific expression was largely controlled by a 259-bp region between −376 and −117 bp. This region contains several putative GT boxes with the GGTTAA consensus core sequence. Once these strong light-dependent elements were removed, a second level of control was revealed. In constructs in which the *gdcH* 5′ regulatory region was shortened to −117 bp or less, there was more GUS activity in the roots than in the leaves, and in dark-grown plants than in light-grown plants. This suggests that more proximal control elements may be responsible for the constitutive low levels of gene expression noted in all nonphotosynthetic tissues.

The Gly decarboxylase multienzyme complex is, along with the enzyme Ser hydroxymethyl transferase, responsible for the photorespiratory conversion of Gly to Ser in plant mitochondria (Oliver, 1994). The GDC comprises four different component enzymes: the P-protein (a dimer of 106-kD proteins that is the site of pyridoxal 5-phosphate binding and is formally the amino acid decarboxylase), the H-protein (a 13.8-kD monomer with a bound lipoamide cofactor that forms the core of the complex), the T-protein (a 41-kD monomer that is the tetrahydrofolate transferase), and the L-protein (a dimer of 50-kD subunits that is the flavoprotein, lipoamide dehydrogenase) (Walker and Oliver, 1986a; Bourguignon et al., 1988).

Together these proteins form a multimeric complex. Although instability of the complex has precluded isolation of the intact structure, it has been possible to isolate the individual component proteins and to reconstitute the functional complex. Measurements of the amount of each component protein within the matrix and analysis of the optimal subunit ratios for reconstitution suggest a subunit stoichiometry of 4 P-protein/27 H-protein/9 T-protein/2 L-protein (Oliver et al., 1990b).

The GDC catalyzes the oxidation of Gly to CO_2 and NH_3 with the concomitant reduction of NAD^+ to NADH (Neuburger et al., 1986). The remaining carbon, the methylene carbon of Gly, is then transferred to THF to form N^5,N^{10}-methylene THF. This methylene-THF reacts with a second molecule of Gly in a reaction catalyzed by Ser hydroxymethyltransferase to form Ser.

Gly decarboxylase reaction:

$$Gly+NAD^+ + THF \rightarrow methylene\text{-}THF + CO_2 + NH_3 + NADH$$

Ser hydroxymethyltransferase reaction:

$$Gly + methylene\text{-}THF + H_2O \rightarrow Ser + THF$$

Overall reaction:

$$2Gly + NAD^+ + H_2O \rightarrow Ser + CO_2 + NH_3 + NADH$$

The enzyme is found at low levels in most eukaryotic tissues and has been identified in a broad range of bacteria (Kikuchi, 1973). In the leaves of C_3 plants, however, the enzyme complex takes on an additional role. In this tissue the enzyme is the site of photorespiratory CO_2 and NH_3 release (Husic et al., 1987). To accommodate this additional function, the amount of enzyme within the matrix of mitochondria isolated from the leaves of C_3 plants is several-fold higher than the levels measured in any other tissue. In fact, the component enzymes of the GDC account for between one-third and one-half of the total soluble protein in leaf mitochondria (Day et al., 1985; Oliver et al., 1990a, 1990b).

Because of its role in photosynthesis (photorespiration), Gly decarboxylase is expressed most strongly in leaves in the light. The increase in activity results from the de novo synthesis of three of the four component proteins (Walker

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[2] Present address: Institute of Molecular and Cell Biology, National University of Singapore, 10, Kent Ridge Cresent, Singapore 0511, Republic of Singapore.

* Corresponding author; e-mail doliver@uidaho.edu; fax 1–208–885–6518.

Abbreviations: GDC, Gly decarboxylase complex; MS, Murashige-Skoog; THF, tetrahydrofolate.

and Oliver, 1986b); L-protein is a component of other α-keto acid dehydrogenases in the mitochondria that are not light responsive and exhibit a much smaller light-dependent increase (Bourguignon et al., 1992; Turner et al., 1992c). After the cloning of the cDNAs for the H-protein (Kim and Oliver, 1990; Macherel et al., 1990), P-protein (Kim et al., 1991; Turner et al., 1992b), T-protein (Bourguignon et al., 1993), and L-protein (Bourguignon et al., 1992; Turner et al., 1992c), it was possible to show that the increase in synthesis of the individual component proteins in peas resulted from an increase in the level of the mRNAs for each protein (Srinivasan et al., 1992, 1993; Turner et al., 1993). Run-on transcription experiments have confirmed that the increase in H-protein and P-protein mRNA is controlled at the transcriptional level. In fact, the levels of H- and P-proteins, their mRNA levels, and the transcription rates measured in the run-on experiments, all increase about 8-fold when etiolated pea plants are transferred to the light. This suggests that most of the control of the expression of the *gdcH* and *gdcP* genes is at the level of transcription (Srinivasan et al., 1992).

In this paper we present data suggesting that the light dependence and tissue specificity of the expression of the *gdcH* gene is similar to that for the small subunit of Rubisco, *rbcS*. Promoter analysis of the *gdcH* gene indicates that a 259-bp region of the upstream sequence is largely responsible for the light dependence and tissue specificity of the gene's expression. Once these light-dependent elements are removed, more downstream regions of the regulatory sequence may control a low-level, dark-enhanced (or light-repressed) expression of the H-protein structural gene.

MATERIALS AND METHODS

Plants

Tobacco (*Nicotiana tabacum* var Xanthi) seeds were surface-sterilized and germinated on MS medium in the light or dark. Some seedlings were transferred to soil and grown to maturity. Growth temperature was 25°C, and the light-grown plants had a 16-h photoperiod. During the greening experiments, Arabidopsis seeds were vernalized for 2 d at 4°C and then germinated in the dark for 7 d. The plants were then transferred to the light for the time indicated before total RNA was isolated using the RNaid kit from Bio 101 (La Jolla, CA). The RNA (20 μg) was separated on denaturing agarose gels, blotted to nylon membrane, and probed with the radiolabeled cDNA (Kim and Oliver, 1990). The amount of mRNA was determined by scanning densitometry.

Plasmid Constructions, Promoter Reconstructions, and Promoter Deletions

The *Arabidopsis thaliana* cDNA clone (Srinivasan and Oliver, 1992) was used to screen for a genomic clone (H41) from a λGEM library (a gift from C. Somerville, Carnegie Institute of Washington, Stanford, CA). The *Hin*dIII-*Xba*I fragment of the H41 genomic clone containing the 5′ upstream region of the H-protein gene from *A. thaliana* was subcloned into pBSKS$^+$, restriction mapped, and sequenced using Sequenase according to the manufacturer's procedures (United States Biochemical).

The region from −856 to +62 bp and a series of clones containing exonuclease III-generated deletions were inserted into the promoterless *uidA* (GUS) structural gene in pBI101. These plasmids were amplified in *Escherichia coli*, sequenced to confirm the 5′ end of the deletion, and then transformed into *Agrobacterium tumefaciens* by the freeze-thaw technique of Holsters et al. (1978). Tobacco transformations were performed using the leaf disc procedure described by Horsch et al. (1985).

Assaying for GUS Activity

Preparation of crude extracts of plant parts were assayed for GUS activity as described by Jefferson et al. (1987). GUS levels in different plant tissues were assayed by enzymatic conversion of 4-methylumbelliferyl glucuronide to 4-methylumbelliferone, which was quantified with a fluorimeter (TKO 1000, Hoefer Scientific Instruments, San Francisco, CA). Throughout, GUS activity is expressed as nmol of methylumbelliferone formed per min per mg of protein.

Light Induction of Transgenic T_1 Seedlings

Several hundred T_1 seeds from each of 5 to 10 different independent transformants for each construction were surface-sterilized and sown on solid MS medium with 200 mg/L kanamycin; one batch was grown in the light and the other batch was grown in the dark. The top portions of the seedlings were excised and collected in lots of 75 to 100 seedlings per assay. For each individual transformant, five lots of seedlings were assayed independently. Statistical analyses were done with the Instat computer program (GraphPad Software, Inc., San Diego, CA).

Tissue Specificity of Transgenic T_1 Plants

Several seeds from the individual transformants containing each promoter construction were sown on solid MS media containing 200 mg/L kanamycin. After 4 weeks, the dark-green plants were transferred to soil and grown for another 3 to 4 weeks in the greenhouse. For each construct two independent transformants were grown, and for each transformant five individual plants were dissected into different parts and assayed for GUS activity. Root samples were washed with 10% bleach containing a few drops of Liquinox (Alconox, New York, NY) to minimize bacterial contamination.

RESULTS

The H-protein of Gly decarboxylase and the small subunit of Rubisco have a great deal in common. They are both nuclear encoded and synthesized with an N-terminal presequence for organellar targeting, and they both accumulate to millimolar concentrations within the stroma of chloroplasts or matrix of mitochondria. The first series of experiments was designed to determine if the mechanism

of transcriptional control for *gdcH* is related to those already known for *rbcS* (Tobin and Silverthorne, 1985; Kuhlemeier et al., 1987b; Thompson and White, 1991). This was addressed by examining the light dependence of the mRNA levels for these two genes.

gdcH and *rbcS* Are Regulated in a Similar Manner

The mRNA levels for H-protein and the small subunit of Rubisco are low in etiolated Arabidopsis plants (Fig. 1). After illumination of these etiolated plants there was a 6-h lag before the level of either mRNA species began to increase. After this lag period, the mRNA level for both proteins increased during the remaining 18 h of illumination. In all of these samples there was about 3 times more mRNA for the small subunit of Rubisco than for the H-protein. However, once these differences were removed, the changes in mRNA level for the two proteins were similar (Fig. 1). During the 24 h of light, the level of both H-protein and small subunit mRNA increased 12-fold.

Earlier results suggested that the tissue distribution of the Gly decarboxylase and Rubisco activity were quite similar (Srinivasan et al., 1992, 1993). To extend these observations, tobacco plants were transformed with a transcriptional fusion containing the full-length *gdcH* 5′ upstream regulatory region (−865 to +62 bp) and the GUS reporter gene. GUS activity was measured in different tissues from mature tobacco plants and compared with the Rubisco activity in those tissues (Fig. 2). The tissue distribution of *gdcH*-driven GUS activity and endogenous Rubisco activity was also similar. There was relatively higher *gdcH*:GUS activity in the roots and stem tissues compared to Rubisco, and this is consistent with the nonphotorespiratory role of Gly decarboxylase in all tissues. These results suggest that the temporal and spatial control of *gdcH* and *rbcS* expression are comparable.

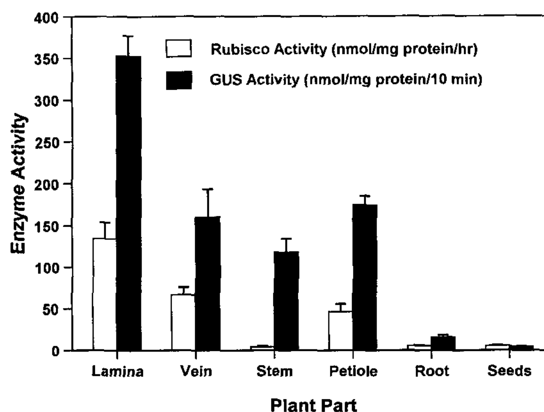

Figure 2. Rubisco and *gdcH*:GUS expression in different parts of a mature tobacco plant. A mature transgenic tobacco plant containing the −856-bp *gdcH*:GUS construct was harvested and the amount of Rubisco (measured in samples of each tissue as ribulose-1,5-bisphosphate-dependent $^{14}CO_2$ fixation) and GUS was measured in each tissue sample. The data are the mean ± SE of five determinations.

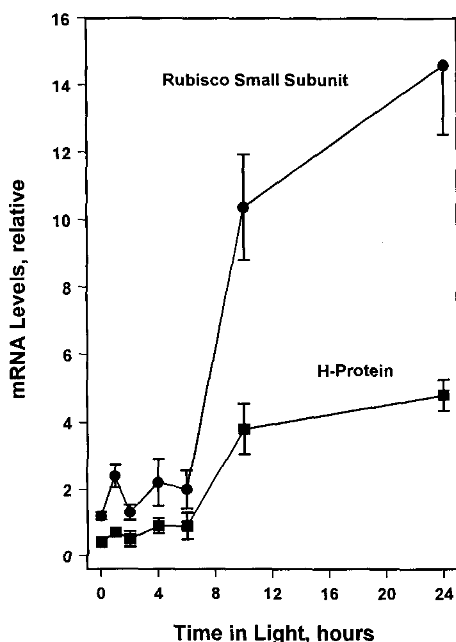

Figure 1. mRNA levels for H-protein and the small subunit of Rubisco during the greening of Arabidopsis plants. Etiolated plants were transferred to the light, samples were taken at the times indicated, and the levels of H-protein and small subunit mRNA were determined by northern blotting and scanning densitometry. The signals were standardized to the actin mRNA signal. The results shown are the mean of two determinations with bars showing the range.

Sequence Analysis of *gdcH*

This preliminary analysis based on mRNA levels suggested that there might be some overlap between the mechanisms controlling Rubisco small subunit expression and H-protein. To begin a more detailed analysis of its promoter, the *gdcH* gene isolated from *A. thaliana* was sequenced. The 2040-bp sequence for the region covering the H-protein structural gene and the upstream regions is presented in Figure 3. Comparison of this sequence with the sequence for the Arabidopsis cDNA published earlier (Srinivasan and Oliver, 1992) reveals the presence of two introns. The sequences for both the coding regions and the untranslated 3′ and 5′ regions of the cDNA clone were identical to those for this genomic clone, demonstrating that this gene was being actively transcribed.

The transcriptional start site of the genomic clone was mapped using primer extension analysis. These data showed a single, major transcriptional start site that was located 81 bp before the translational start site. The "A" at the transcriptional start is designated as +1 in Figure 3. An AT-rich sequence is located at −17 bp, and a potential CAAT sequence is located at −58 bp. Southern analysis suggested that this is a single-copy gene in Arabidopsis.

The structures of the *gdcH* gene from Arabidopsis and peas are compared in Figure 3. The first and second introns are positioned at the same locations in both genes, although the introns from Arabidopsis are longer than those from pea. The equivalent of the third exon from Arabidopsis is broken by a third intron in peas.

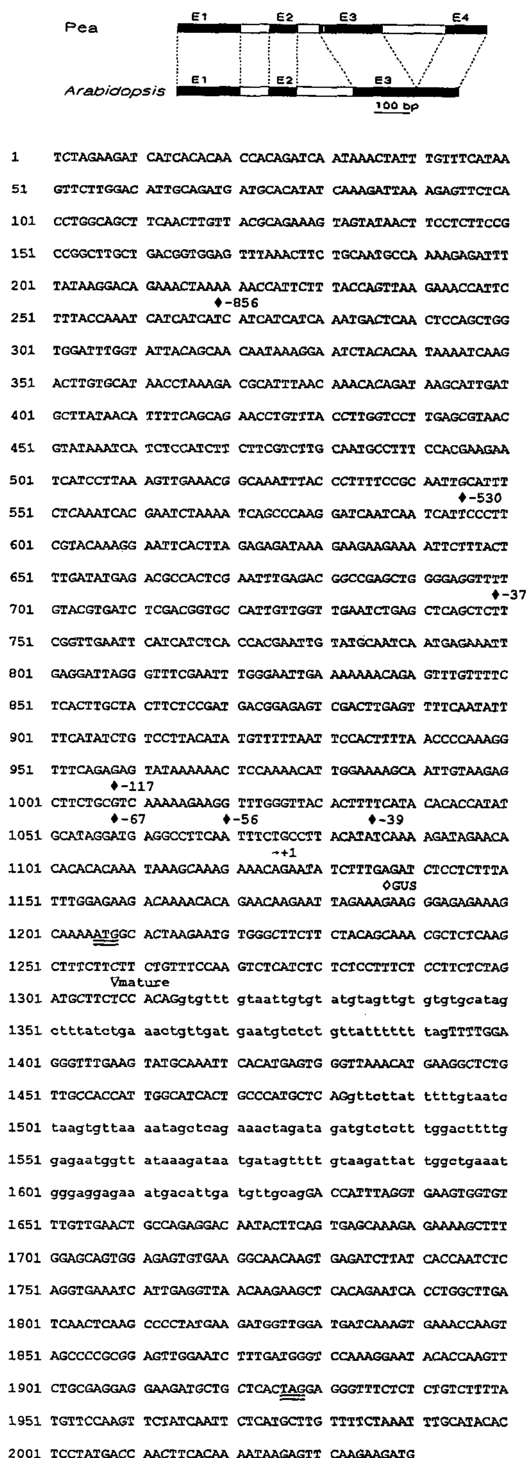

Figure 3. The structure for the *gdcH* gene from *A. thaliana*. The top part of the figure shows the intron-exon structure of *gdcH* from pea and Arabidopsis. The black bars show exons (the two sequences are aligned at the start of transcription), and the open bars show introns. The bottom portion shows the sequence of the *gdcH* gene. The deletions used for the promoter:GUS constructs are indicated at −856, −530, −376, −117, −67, −56, and −39 bp. The transcriptional start is labeled +1. The site of GUS fusion is shown at +62 bp, and the ATG, start of the mature protein, and stop codon are also shown. Introns are shown in lowercase letters.

GUS Expression by the Different *gdcH*:GUS Constructs in the Light and Dark

A group of 5′ deletions for the *gdcH* 5′ upstream region was generated and fused with the GUS structural gene. Five to 10 independent transformants were selected for each *gdcH*:GUS construct and grown to maturity. These transformed plants (T_0) were selfed, and the GUS assays were carried out on the T_1 seedlings. Approximately 500 T_1 seedlings from each of the independent transformants were grown in the light and dark for 9 d. The stems and leaves were then harvested and analyzed for GUS activity (Fig. 4). Tobacco plants containing the full-length construct had 7 times more GUS activity in the light than in the dark (Fig. 4, inset). This light:dark ratio was very similar to the differences measured in H-protein concentration, H-protein mRNA level, and H-protein run-on transcription in peas and approached the differences in H-protein mRNA level measured with Arabidopsis (Fig. 1). This suggests that the 918-bp (−856 to + 62 bp) upstream region contained the elements necessary to drive light-dependent and tissue-specific (see below) expression in a manner that is similar to the endogenous gene.

Shortening the 5′ region down to −530 bp halved the amount of GUS expressed in the light. The amount of activity in the dark actually increased by 50%. Although this increase is statistically significant ($P < 0.05$), it is not as obvious that it represents the deletion of a dark-dependent repressor. Half or more of the light dependence of the *gdcH*:GUS expression is lost upon removal of this 326-bp fragment. Further shortening of the *gdcH* upstream region down to −376 bp again halved the amount of GUS activity in the light (23% of the activity of the −856-bp construct). The amount of GUS expressed in the dark was decreased to about one-half of the full-length activity. The light:dark ratio was not significantly different from that measured for the −530-bp construct. This suggests that the region between −530 and −376 did not contribute significantly to the light-dependent expression of GUS activity, although it does not affect the overall level in the light and dark.

Deletion of the 259-bp fragment of the promoter from −376 to −117 bp had the most striking effect. The GUS activity in light-grown plants decreased 96%. This suggests that this region contains sequences that increase expression in the light over 20-fold when this region is included. The removal of the −376- to −117-bp portion of the *gdcH* promoter had substantially less effect on the amount of GUS expression in dark-grown plants. With the loss of this 259-bp fragment, the amount of expression in the dark decreased only 57%. As a result, the light:dark ratio decreased from 3.11 for the −376-bp construct to 0.32 for the −117-bp construct (Fig. 4, inset). In other words, once the upstream sequence had been deleted down to −117 bp, the *gdcH*:GUS construct showed more activity in the dark than in the light.

Deletion of the −117-bp construct to −67 bp, −56 bp, and even −39 bp had little effect. Whereas the activities of these smaller constructs showed some variability, none (with the exception of the −39-bp construct in the dark) was significantly different from the −117-bp construct. All

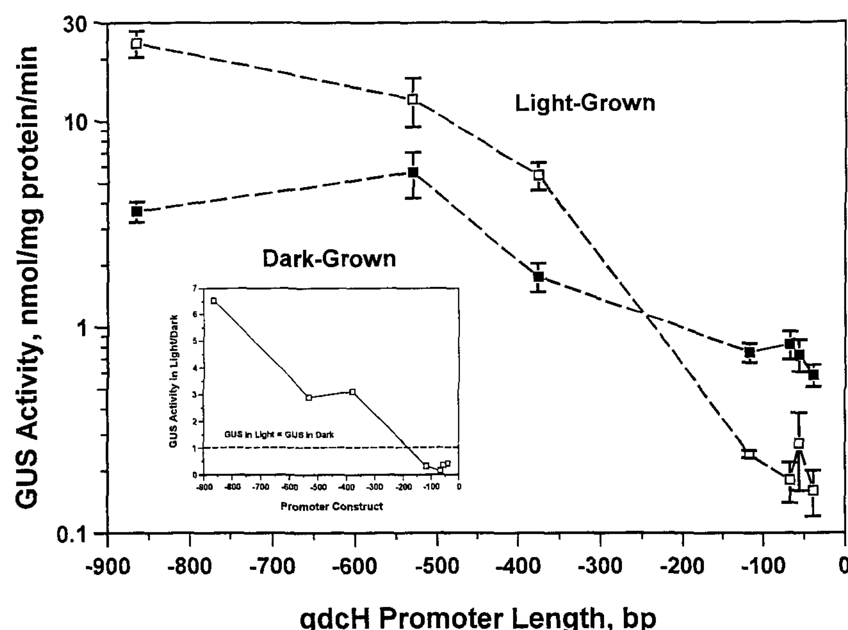

Figure 4. GUS activity in transgenic tobacco seedlings containing *gdcH*:GUS constructs. Tobacco was transformed with the H-protein 5′ upstream region:GUS constructs indicated. Seeds from the transformed plants were dispersed on MS salts containing 200 μg/mL kanamycin and incubated in the light or dark for 9 d. The tops of the plants were harvested and GUS activity was determined. The values presented are the means of the 5 to 10 independent transformants, and the error bars show the SE ranges. The dashed lines are present only to group-related treatments and do not suggest continuity. Inset, The light:dark ratio of GUS activity in transgenic tobacco. The horizontal line represents equal activity in the light and dark. GUS activities in light-grown, wild-type plants was 0.024 nmol mg^{-1} protein min^{-1}, and those transformed with pBI101, a promoterless GUS construct, was 0.034 nmol mg^{-1} protein min^{-1}.

of these constructs expressed 2.5 to 6.5 times more GUS activity in the dark than in the light.

GUS Expression in Different Tissues

Five T_2 plants that showed kanamycin resistance from each construct were grown to a height of about 15 cm in the light. The plants were dissected into leaf lamina, leaf vein, petiole, stem, and root samples, and the amount of GUS activity in each sample was determined. The results of this study are presented in Figure 5A, and the ratio of activity in the roots and leaf lamina are shown in Figure 5B. With the −856-bp construct, the amount of GUS activity was highest in the leaf, intermediate in the green veins, petioles, and stems (37–58% of the leaf lamina), and lowest in the roots. There was 14 times more GUS activity in the leaves than in the roots. This tissue distribution parallels the amount of H-protein enzyme activity we had measured in these tissues earlier (Srinivasan et al., 1992), supporting the conclusion that the −856-bp upstream sequence was functioning like the endogenous promoter.

Deleting the control region from −856 to −530 bp decreased the expression of GUS activity in all parts of the plant 40 to 60%. The leaf:root ratio was 16, which was not significantly different from that measured with the full-length construct. Further deletion of the *gdcH* upstream region from −530 to −376 bp decreased GUS expression little (<26%) in all parts of the plants except the root, where activity was decreased 52%. As a result, the leaf:root ratio was somewhat higher than for the two larger constructs.

Deletion of the *gdcH* upstream region to −117 bp had the strongest effect. Although the activity in the root decreased only by one-half compared to the −376-bp construct, the activity in the leaf decreased by over 99%. Those elements that control tissue-specific expression appear to lie within this 259-bp fragment between −376 and −117 bp. Once the region had been deleted to −117 bp there was actually more activity in the roots than in the leaves. Further deletion of the *gdcH* upstream sequence to −67, −56, and −39 bp did not significantly alter either the amount of GUS activity expressed in the different parts of the plant or the leaf:root ratio.

DISCUSSION

The GDC is one of three light-responsive systems that have been documented in plant mitochondria. The other enzymes are Ser hydroxymethyltransferase (Turner et al., 1992a), another enzyme in the C-2 cycle that reacts with Gly decarboxylase in the conversion of Gly to Ser, and NAD^+-dependent formate dehydrogenase (Francs-Small et al., 1993). Although the control of Ser hydroxymethyltransferase has not received as much study as Gly decarboxylase, analysis of the kinetics of mRNA accumulation after illumination of either etiolated or dark-adapted green peas suggests that it is controlled together with the L-protein and T-protein of Gly decarboxylase (Turner et al., 1992a, 1993). Formate dehydrogenase is present at higher levels in etiolated tissues, and the level decreases upon greening; control appears to be at the level of mRNA (Francs-Small et al., 1993). Whereas a role for formate dehydrogenase in photorespiration has been postulated (Oliver, 1981), its physiological function is unknown. The difference in developmental pattern when compared with the photorespiratory enzymes in mitochondria (Gly decarboxylase and Ser hydroxymethyltransferase) suggests that formate dehydrogenase serves some other function.

Aside from the component proteins of GDC, the only photorespiratory protein in which the control of its expression has been analyzed in detail is hydroxypyruvate reductase. In cucumber seeds hydroxypyruvate reductase mRNA shows a small increase 4 or 5 d after germination.

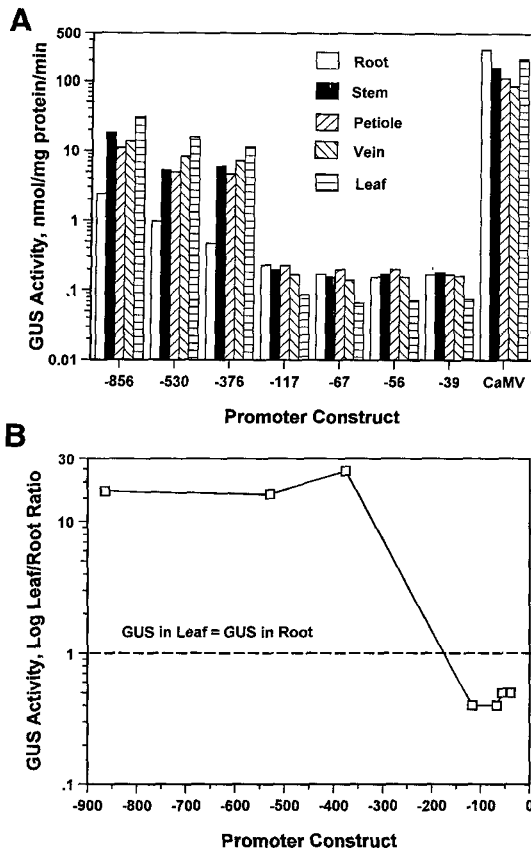

Figure 5. GUS activity in different tissues of transgenic tobacco plants. A, Five transgenic tobacco plants for each construct were grown on soil to a height of about 15 cm. The tissues were harvested from each plant and GUS activity was measured. B, The ratio of GUS activity in the leaves and roots in transgenic tobacco. The horizontal line represents equal activity in the root and leaf.

This increase signals competence for the *hpr* gene, which after, but not before, this transition can undergo a 10-fold increase in expression level following a 24-h exposure to white light (Greenler and Becker, 1990). An analysis of the effects of light fluence and wavelength suggests that *hpr* expression is partially triggered by a red/far-red reversible phytochrome effect and partially controlled by a response more dependent on the length of illumination and less dependent on the light intensity. This latter response was measured in dark-adapted green tissue and may reflect a response to photosynthetic activity and the presence of a "mature chloroplast" signal in these tissues (Bertoni and Becker, 1993).

These authors (Sloan et al., 1993) have also begun an analysis of the cucumber *hpr* promoter in transgenic tobacco. Sequence analysis had identified putative GT box, AT box, I box, and G box regions in the *hpr* upstream region (Schwartz et al., 1991). This construct was expressed in a light-dependent manner in tobacco, and deletion of an 81-bp region between −299 and −218 bp deleted these putative *cis*-elements and resulted in the loss of light-dependent gene expression.

All of the data presented suggests that the mechanisms that control the levels of H-protein are related to those that control the levels of the small subunit of Rubisco. The expression of both *rbcS* and *gdcH* are predominantly controlled at the transcriptional level (Srinivasan et al., 1992, 1993). The time course for the increase in the level of H-protein mRNA during the greening of etiolated Arabidopsis is very similar to that for the small subunit. Earlier work with peas suggests that the expression of both *gdcH* and *rbcS* is dependent on the presence of functional chloroplasts (for a discussion on the role of chloroplast development on nuclear gene expression, see Taylor, 1989; Susek and Chory, 1992). This is illustrated by the faster kinetics of *gdcH* and *rbcS* mRNA accumulation during the reillumination of dark-adapted green plants compared to the greening of etiolated peas. In addition, the light-dependent expression of both genes is inhibited by treatment with Norflurazon, an herbicide that results in the photodestruction of chloroplasts.

It is interesting to note how the function of the mitochondria is controlled by genetic interactions between the chloroplast and the nucleus. The initial light signal triggers nuclear and plastid genes that result in chloroplast greening. The greening of the chloroplast signifies developmental competence. Now a light signal causes high-level expression of nuclear genes needed for conversion of leaf mitochondria into a photorespiratory organelle.

The 918-bp *gdcH* 5′ upstream sequence (−856 to +62) from Arabidopsis appears to contain all of the elements necessary for light-dependent and tissue-specific expression when compared with expression of the endogenous H-protein gene. The −856-bp *gdcH*:GUS fusion in transgenic tobacco expressed 7 times more GUS activity in 9-d-old light-grown compared to dark-grown seedlings. In more mature plants this promoter drove about 14 times more GUS expression in leaves than in roots.

Promoter-deletion experiments suggested that two regions of this sequence contributed to its light dependence. Deletion of the region from −856 to −530 bp caused about a 50% decrease in the light dependence of GUS expression. This change resulted mainly from a loss of activity in light-grown plants and a slightly higher activity in the dark-grown seedlings when compared to the full-length constructs. This decrease, although significant, was less than the decrease in light dependence that resulted from the loss of the region between −376 and −117 bp. Removal of this sequence caused a greater than 95% loss of GUS activity in light-grown plants but a much smaller loss of GUS expression in the dark. Most of the light-dependent control appears to reside in this region.

Sequence analysis of the 259-bp region between −376 and −117 bp reveals several sequences that might be related to elements associated with the light-dependent expression of other genes (Green et al., 1987, 1988; Kuhlemeier et al., 1987a, 1988; Lam and Chua, 1990). There are, for example, six sequences that are similar to the GT box (box II of *rbc-3A*) core sequence (Fig. 6).

This same fragment appeared to be responsible for the tissue specificity of the response. The −856-, −530-, and −376-bp *gdcH*:GUS constructs all exhibited 15 to 20 times more GUS activity in the leaves than in the roots on a

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                 A
Box II   gtgt-GGTTAA-tatg
    1F   cttc-GGTTGA-attc (-377 - -364)
    2R   cgta-TGTTAA-gcac (-353 - -340)
    3R   tagg-AGTTAA-agag (-332 - -319)
    4R   taag-GGTTTA-agct (-311 - -298)
    5R   aaaa-AGTTAA-gggt (-291 - -304)
    6R   aaaa-GGTTAC-aaaa (-138 - -151)
    7R   agaa-TGTTAA-cgaa (-126 - -139)
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Figure 6. Putative GT boxes in the 259-bp fragment of the *gdcH* promoter responsible for light-dependent and tissue-specific expression in transgenic tobacco. The consensus box II sequence from *rbcS-3A* is shown along with sequences from the *gdcH* promoter that share some sequence similarity with the core of the box II sequence. The letters F and R indicate if the sequence faces forward or in reverse, and the location of the sequence is given.

protein basis. When the 259-bp fragment was removed, the −117-bp and shorter constructs had 2 to 5 times more GUS activity in the roots than in the shoots.

Once these strong, light-responsive *cis*-elements located upstream from −117 bp have been deleted, a second light response becomes obvious. In those constructs in which the *gdcH* upstream region had been deleted to −117 bp or smaller (−67, −56, or −39 bp), there was 3 to 7 times more GUS activity in the dark than in the light. The rates of GUS activity with these short constructs are very low, about 1% of the rate with the full-length construct, and it is possible that we are measuring anomalous light:dark activities with these very low-activity promoters. The only reason we do not disregard this dark-enhanced activity is that Gly decarboxylase is not expressed like other photosynthetic enzymes. There is a low level of Gly decarboxylase activity in all tissues in which it is involved in the synthesis of Gly from three carbon glycolytic intermediates (Oliver, 1994). This low level of expression in the dark and in nonphotosynthetic tissues may reflect the processes providing the enzyme for these tissues.

The identification of the functional elements in the −39-bp *gdcH* region is at best tentative. There are, however, two obvious possibilities. Whereas the −39-bp construct has deleted the tentative CAAT sequence, it has left a GATA sequence at −32 bp. A GATA sequence is located between the CAAT and TATA sequence in a number of light-induced genes. Mutations in an upstream GATA sequence (I-box) of the full-length *rbcS-1A* promoter from Arabidopsis has been shown to significantly decrease the light-dependent expression of this promoter (Donald and Cashmore, 1990). The second element is an ACAAAA sequence that Caspar and Quail (1993) have noted in the untranslated 5′ leader of the Fd gene as well as a substantial number of other genes. Deletion of the Fd leader resulted in a 25-fold decrease in the expression in the *FedA* gene in both the light and dark. This element, therefore, may not be involved in light-dependent control. It is not obvious how either the GATA or ACAAAA elements would contribute to the higher levels of expression in the dark. Their presence does, however, open the possibility that they may be involved in or modulate the phenomenon.

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The GenBank accession number for the sequence reported in this article is U27144.

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