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Photorespiration and Light Act in Concert to Regulate the Expression of the Nuclear Gene for Chloroplast Glutamine Synthetase

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In *Pisum sativum*, distinct chloroplast and cytosolic forms of glutamine synthetase (GS) are encoded by homologous nuclear genes that are differentially expressed in vivo (Tingey, S.V., Tsai, F.-Y., Edwards, J.W., Walker, E.L., and Coruzzi, G.M. [1988]. J. Biol. Chem. 263, 9651–9657). In leaves, light selectively affects the expression of the nuclear gene for chloroplast GS_2. Differences in the maximal levels of GS_2 mRNA in etiolated plants treated with red or white light indicate that only part of the white-light-induced accumulation of GS_2 mRNA is due to a phytochrome-mediated response. The kinetics of GS_2 mRNA accumulation in response to white-light illumination of etiolated or dark-adapted green plants indicates that GS_2 mRNA accumulates more rapidly in plants containing mature, photosynthetically competent chloroplasts. Other evidence that GS_2 mRNA levels are affected by the metabolic status of chloroplasts concerns the selective induction of GS_2 mRNA in plants grown under conditions that result in the production of photorespiratory ammonia. These results indicate that the light-induced accumulation of GS_2 mRNA in leaves results from the action of phytochrome as well as light-induced changes in chloroplast metabolism.

INTRODUCTION

Nitrogen-metabolic enzymes in plants are specialized for the unique functions of a multicellular photoautotroph. This situation is exemplified by the enzyme, glutamine synthetase (GS) (EC 6.3.1.2) that catalyzes the synthesis of glutamine from ammonia, glutamate, and ATP (Miflin and Lea, 1977). Plants possess multiple isoforms of GS that are located in both the cytosol and chloroplast, which function to meet the ammonia-assimilatory demands of various plant organs (McNally and Hirel, 1983; Lara et al., 1983, 1984). In leaves, GS is required to assimilate ammonia produced from nitrate reduction and photorespiration (Wallsgrove et al., 1987), whereas in root nodules of legumes, GS is required to assimilate the ammonia generated by nitrogen-fixing bacteroids (Robertson et al., 1975).

In plants, plastids are the sites of carbon and nitrogen assimilation and thus play an essential role in plant nitrogen metabolism (Wallsgrove et al., 1983). Many amino acid biosynthetic pathways occur in plastids of photosynthetic and nonphotosynthetic tissues (Miflin, 1974). A form of GS located in plastids (GS_2) functions to assimilate ammonia into organic form using compounds derived from carbon skeletons generated during photosynthesis (Miflin, 1974).

Photorespiration, a side reaction of photosynthesis, has important implications in plant nitrogen metabolism. Photorespiration is a complex, multistep process initiated in chloroplasts that results in the production of CO_2 and ammonia in the terminal reaction within mitochondria (Ogren, 1984). The role of GS in the reassimilation of photorespiratory ammonia is crucial since the mass of ammonia generated during photorespiration exceeds that acquired by primary ammonia assimilation (Keys et al., 1978).

Since many higher plant species contain both chloroplast and cytosolic forms of GS in leaves, it has been difficult to establish the relative roles of these GS isoforms in the reassimilation of ammonia generated by photorespiration (Wallsgrove et al., 1983). This longstanding controversy has been resolved recently by the isolation of photorespiratory mutants of barley that are nonviable under photorespiratory conditions but are viable when photorespiration is inhibited with high levels of CO_2 (Wallsgrove et al., 1987). The finding that chloroplast GS_2 is absent in these photorespiratory mutants whereas cytosolic GS is present at normal levels confirms the essential role of chloroplast GS_2 in the reassimilation of photorespiratory ammonia.

In *Pisum sativum*, analysis of the GS isoforms at the nucleic acid level has shown that the distinct chloroplast

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GS_2 and cytosolic GS_1 and GS_n polypeptides are encoded by highly homologous nuclear genes that are differentially expressed in vivo (Tingey et al., 1988). Genes encoding chloroplast GS_2 (Lightfoot et al., 1988) and cytosolic GS (Gebhardt et al., 1986; Tischer et al., 1986; Hirel et al., 1987; Tingey and Coruzzi, 1987) also have been identified in other plant species.

Previously, we have shown for *P. sativum* that GS mRNAs encoding chloroplast GS_2, cytosolic GS_1, and cytosolic GS_n accumulate in an organ-specific manner that correlates with the differential abundance of these distinct GS polypeptides in leaves, roots, and nodules (Tingey et al., 1987, 1988). mRNA for the cytosolic forms of GS accumulates 10-fold to 20-fold in nitrogen-fixing root nodules compared with uninfected roots or leaves (Tingey et al., 1987). In leaves, mRNAs for both cytoslic GS and chloroplast GS_2 are present; however, only the mRNA for chloroplast GS_2 is expressed in a light-dependent fashion (Tingey et al., 1988).

Here we have examined how light-induced chloroplast development affects the expression of the nuclear gene for chloroplast GS_2. We have examined the individual contributions of phytochrome, white light, chloroplast maturity, and photorespiration in modulating the accumulation of chloroplast GS_2 mRNA. These experiments have shown that the light-induced accumulation of GS_2 mRNA is mediated in part by the action of the chromophore, phytochrome, and that light- and CO_2-induced changes in chloroplast metabolism also play a role in modulating the expression of chloroplast GS_2.

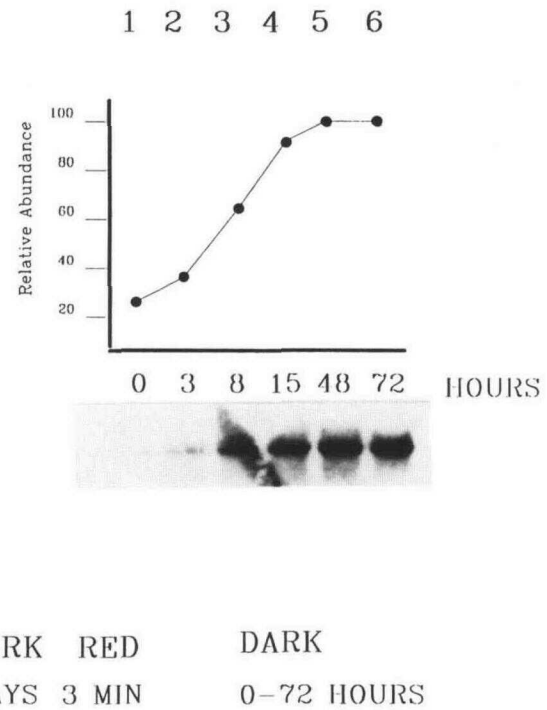

Figure 1. Time course of GS_2 mRNA Accumulation Following Phytochrome Activation.

RNA gel blot of 20 μg of total RNA isolated from etiolated plants 0 hr to 72 hr following a 3-min pulse of red light. GS_2 mRNA (1.5 kb) was detected on RNA gel blots with the cDNA insert of pGS185 (Tingey et al., 1988). The graph depicts the relative abundance of GS_2 mRNA compared with maximum accumulation of GS_2 mRNA at 72 hr. Points on graph reflect the average densitometric scan of two replicate blots.

RESULTS

Phytochrome Mediates a Gradual Induction of GS_2 mRNA

Previous studies have demonstrated that GS_2 mRNA accumulates severalfold when monitored 24 hr following a 3-min pulse of red light. The role of phytochrome in GS_2 mRNA accumulation was demonstrated by the far-red reversibility of this response (Tingey et al., 1988). Here we have examined the kinetics and maxima of the phytochrome-mediated accumulation of GS_2 mRNA.

Etiolated pea seedlings were exposed to a saturating pulse of red-light (3 min) and then transferred to the dark. RNA was isolated after time intervals ranging from 0 hr to 72 hr. Figure 1 depicts an RNA gel blot and graphic representation of the amounts of GS_2 mRNA detected by hybridization to a GS_2 cDNA probe (Tingey et al., 1988). Levels of GS_2 mRNA present in leaves of etiolated plants (Figure 1, lane 1) increased gradually to a maximum of fourfold within 48 hr following red-light illumination, with accumulation beginning as early as 3 hr after illumination (Figure 1, lanes 2 to 6).

The gradual accumulation of GS_2 mRNA following phytochrome activation by red light is similar to the kinetics of mRNA accumulation observed for several phytochrome-responsive genes, including those encoding the small subunit of ribulose-1,5-bisphosphate carboxylase (rbcS) and the chlorophyll *a*/*b* binding protein (cab) (Kaufman et al., 1986). This type of accumulation is characteristic of the most common class of phytochrome-responsive genes examined (Kaufman et al., 1986). However, at least three other distinguishable patterns of mRNA accumulation after red-light irradiation have been reported for light-responsive genes (Kaufman et al., 1986).

Although the maximal red-light-induced accumulation of GS_2 mRNA is fourfold (Figure 1, compare lanes 1 and 6), previous experiments demonstrated that the accumulation of GS_2 mRNA in white light is as high as 20-fold (Tingey et al., 1988). The greater fold induction of GS_2 mRNA levels by white-light illumination may reflect an increase in mRNA stability in continuous white light, the contribution

of other components of white light (e.g. blue light) (Kaufman, et al., 1985; Fluhr and Chua, 1986), or the participation of factors associated with chloroplast maturation (Taylor, 1989).

GS_2 mRNA and Protein Accumulate Slowly during Greening of Etiolated Seedlings

Previous results have demonstrated that white light induces the accumulation of the GS_2 polypeptide and GS_2 mRNA in etiolated pea seedlings (Tingey et al., 1988). Here, the kinetics and maxima of these white-light responses were determined.

Figure 2A shows a protein gel blot analysis of GS polypeptides present in leaf-soluble protein isolated during this "greening" time course of etiolated pea seedlings. The steady-state level of the chloroplast GS_2 polypeptide (44 kD) increased gradually over a period of 3 days, during which time maximal expression was observed (Figure 2A, lanes 1 to 4). In contrast, the steady-state levels of cytosolic GS_1 and GS_n polypeptides (38 kD and 37 kD, respectively) are unchanged by the light treatment (Figure 2A, lanes 1 to 4).

Figure 2 depicts an RNA gel blot analysis of GS_2, GS_1, and GS_n mRNA levels during the greening of etiolated pea seedlings in the same time course described above. The steady-state level of GS_2 mRNA accumulated gradually, reaching a maximum 3 days following white-light illumination (Figure 2B, lanes 1 to 7). This contrasts with the gradual decline in steady-state levels of mRNAs for cytosolic GS_1 and GS_n (Figure 2C, lanes 1 to 7). The decrease in the relative amount of cytosolic GS mRNA in the total RNA population may correspond to a decrease in the absolute number of cytosolic GS transcripts or may reflect the diminished proportion of cytosolic GS mRNAs relative to the increasing number of transcripts for light-responsive genes.

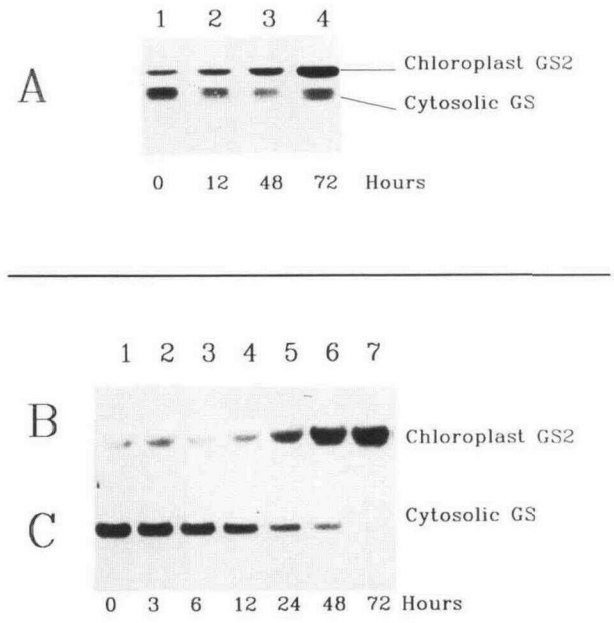

Figure 2. Time Course of the White-Light-Induced Accumulation of GS_2 Protein and mRNA in Etiolated Pea Seedlings.

GS_2 expression was monitored in etiolated pea seedlings exposed to continuous white light for 0 hr to 72 hr.

(A) GS proteins detected in protein gel blot of 20 μg of leaf-soluble protein probed with antibodies to chloroplast GS_2 (Hirel et al., 1984) and cytosolic GS_{n1} (Lara et al., 1984). Chloroplast GS_2 = 44 kD, cytosolic GS_1 = 38 kD, GS_n = 37 kD (Tingey et al., 1987).

(B) and **(C)** RNA gel blots of 20 μg of total RNA probed with cDNA inserts of pGS185 (chloroplast GS_2) **(B)** and pGS299 (cytosolic GS_1) **(C)** (Tingey et al., 1988).

GS_2 mRNA Accumulates Rapidly with Light Treatment in Mature Plants

To determine whether the delayed accumulation of GS_2 mRNA in light-treated, etiolated pea plants reflects the time requirement for chloroplast maturation, we next examined whether the kinetics of light-induced accumulation of GS_2 mRNA were more rapid in plants containing mature chloroplasts. Figure 3 depicts an RNA gel blot and graphic representation of the light regime employed in this experiment. When mature, light-grown pea plants are dark-adapted for 4 days, GS_2 mRNA levels are diminished by at least 20-fold (Figure 3, compare lanes 1 and 2). When the dark-adapted plants are returned to continuous white light, GS_2 mRNA levels rise twofold after 6 hr (Figure 3, lane 3) and regain maximal light-grown levels after only 24 hr of white-light illumination (Figure 3, compare lanes 1 and 4). The rapid kinetics of light-induced accumulation of GS_2 mRNA in mature, green plants (Figure 3) versus the delayed accumulation in etiolated plants (Figure 2) suggests that maximal induction of GS_2 mRNA requires factors associated with the presence of mature chloroplasts.

GS_2 mRNA Accumulates during Photorespiration

Since the studies presented above suggested that maximal levels of GS_2 mRNA accumulation depend on the developmental state of chloroplasts, we next examined whether changes in chloroplast metabolism affect the accumulation of the GS_2 mRNA. Previous studies of barley photorespiratory mutants demonstrated that chloroplast GS_2 functions in the reassimilation of photorespiratory ammonia (Wallsgrove et al., 1987). Here, experiments were designed to test whether growth conditions that

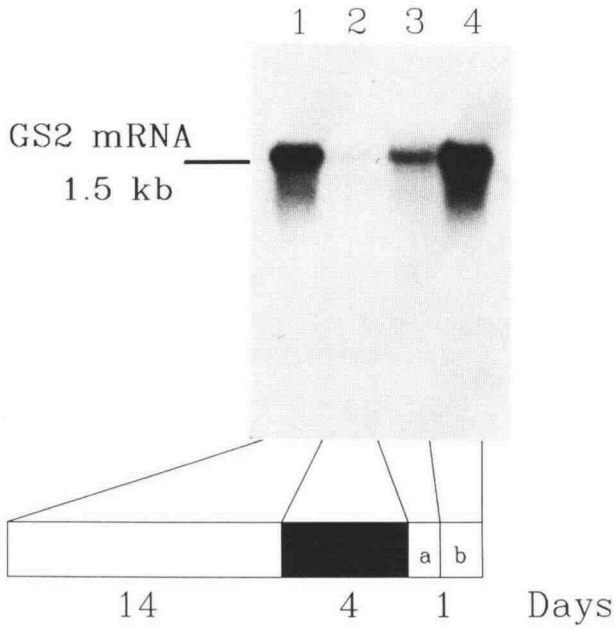

Figure 3. White-Light Induction of GS_2 mRNA in Mature, Dark-Adapted Pea Plants.

RNA gel blot of 20 μg of total RNA isolated from 14-day-old plants grown in continuous white light (lane 1), transferred to the dark for 4 days (lane 2), returned to continuous white light for 6 hr (lane 3) and 24 hr (lane 4). mRNA for chloroplast GS_2 (1.5 kb) was detected on RNA gel blots with the cDNA insert of pGS185 (Tingey et al., 1988).

promote or inhibit photorespiration affect the accumulation of mRNA for chloroplast GS_2 in pea.

In normal air (0.02% CO_2), light-grown plants photorespire and generate photorespiratory ammonia, whereas at elevated levels of CO_2 (2.0% CO_2), CO_2 can effectively compete with O_2 and result in the repression of photorespiration (Somerville and Ogren, 1982). Peas were grown in continuous white light in separate growth chambers under growth conditions that differed only in the CO_2 content of the atmosphere. Chamber 1 contained atmospheric air (0.02% CO_2), which promotes photorespiration, whereas chamber 2 was maintained at a concentration of 2.0% CO_2, which inhibits photorespiration. RNA was isolated from plants spanning stages of development during which photosynthesis and photorespiration rates increase (7 days to 21 days) (Sutcliffe and Pate, 1977). Figure 4 depicts an S1 nuclease protection analysis of chloroplast GS_2 mRNA (Figure 4A) and an RNA gel blot analysis of cytosolic GS mRNA (Figure 4B) isolated during the photorespiration time course experiment. Levels of GS_2 mRNA were monitored by an S1 nuclease protection experiment performed with a GS_2 DNA probe spanning an exon of the GS_2 gene (Figure 4A). The weaker upper signal may represent the presence of a second, similarly regulated, GS_2 transcript (J.W. Edwards and G.M. Coruzzi, work in progress).

In 7-day-old plants, growth conditions that promote or repress photorespiration have little effect on GS_2 mRNA levels (Figure 4A, compare lanes 1 and 2). However, in 14-day-old and 21-day-old plants, conditions that induce photorespiration (0.02% CO_2) increased GS_2 mRNA levels fourfold (Figure 4A, lanes 3 and 5) compared with conditions that inhibited photorespiration (2.0% CO_2) (Figure 4A, lane 4 and 6). In contrast, the levels of cytosolic GS mRNAs remain relatively constant during different photorespiratory growth conditions (Figure 4B, lanes 1 to 6). These results demonstrate that plants grown under photorespiratory conditions maintain higher steady-state levels of GS_2 mRNA than plants grown in nonphotorespiratory conditions. This fourfold increase may reflect the rate of transcription of the GS_2 gene or an increase in mRNA stability under photorespiratory conditions.

DISCUSSION

Chloroplast and cytosolic forms of glutamine synthetase are encoded by a family of homologous nuclear genes that are differentially expressed in developmental contexts

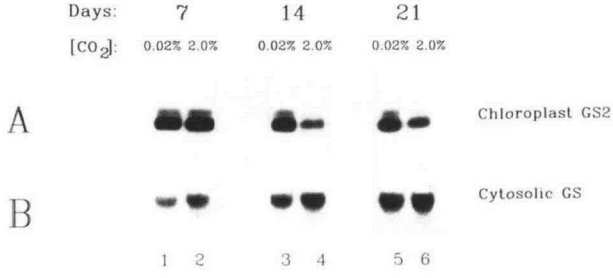

Figure 4. Levels of mRNA for Chloroplast and Cytosolic GS in Plants Grown under Photorespiration-Induced and Photorespiration-Repressed Conditions.

Each lane contains 15 μg of total RNA from plants grown in continuous white light under conditions that induce photorespiration (0.02% CO_2) (lanes 1, 3, 5) or repress photorespiration (2.0% CO_2) (lanes 2, 4, 6).

(A) mRNA for chloroplast GS_2 detected by an S1 nuclease protection experiment performed with a DNA probe corresponding to an exon of the GS_2 gene (J.W. Edwards and G.M. Coruzzi, unpublished results).

(B) mRNA for cytosolic GS_1 mRNA was immobilized on an RNA gel blot and detected by hybridization with the cDNA insert of pGS299 (Tingey et al., 1988). Autoradiographs of two separate experiments were scanned as described in "Methods."

where ammonia assimilation occurs (Tingey et al., 1987, 1988; Lightfoot et al., 1988). In plants where much amino acid biosynthesis occurs in plastids, chloroplast GS_2 plays a crucial role in the reassimilation of ammonia lost via photorespiration (Wallsgrove et al., 1987) and may also function to assimilate ammonia produced from reduced nitrate. Here we have shown that the expression of the nuclear gene for chloroplast GS_2 of pea is modulated in a complex manner during light-induced plastid development.

Light has been shown to induce the accumulation of GS_2 mRNA in leaves, in part via the chromophore, phytochrome (Tingey et al., 1988). Here we show that the kinetics of the phytochrome-mediated accumulation of GS_2 mRNA are similar to the gradual accumulation pattern observed for other phytochrome-responsive genes (e.g. rbcS, cab) (Kaufman et al., 1986). Kaufman et al. (1986) have shown that the phytochrome responsiveness of rbcS and cab genes are distinct when aspects such as photoreversibility are examined. Therefore, although GS_2, rbcS, and cab share kinetic parameters of phytochrome-induced mRNA accumulation, the individual mechanisms of these responses are not necessarily identical. The requirement for ammonia assimilation in plastids of dark-grown and nonphotosynthetic tissues may necessitate a higher basal level expression of GS_2 in the dark than is needed for rbcS or cab. The phytochrome-induced accumulation of GS_2 mRNA may reflect changes in both rates of transcription and mRNA turnover.

Experiments with etiolated plants demonstrated that the accumulation of GS_2 mRNA in response to white-light illumination is twofold to fivefold greater than the accumulation mediated by phytochrome alone. The higher levels of GS_2 mRNA present in plants grown in white light may be the consequence of several nonexclusive phenomena: the involvement of another photoreceptor (e.g. cryptochrome) (Kaufman et al., 1985; Fluhr and Chua, 1986), an increase in mRNA stability, or the involvement of factors associated with white-light-induced chloroplast development (Taylor, 1989).

Light-induced accumulation of GS_2 mRNA was shown to occur more rapidly in plants with mature, photosynthetically competent chloroplasts. Maximal accumulation of GS_2 mRNA in mature, dark-adapted plants occurred within 24 hr of continuous white-light illumination. In contrast, 72 hr of continuous illumination was required to induce maximal levels of GS_2 mRNA in etiolated pea seedlings. The delayed kinetics of GS_2 mRNA accumulation in leaves of etiolated plants suggests that maximal induction of GS_2 mRNA requires factors associated with the presence of mature chloroplasts. That plastid development affects the expression of some light-responsive genes has been documented previously for other nuclear genes encoding chloroplast proteins (Taylor, 1989).

The suggestion that the metabolic status of chloroplasts affects the abundance of GS_2 transcripts was confirmed by a series of photorespiration experiments. Recently, it has been shown that a major role of chloroplast GS_2 is in the reassimilation of photorespiratory ammonia (Wallsgrove et al., 1987). Here we show that, in plants grown under photorespiratory conditions, there was a fourfold induction of GS_2 mRNA, whereas mRNA for cytosolic GS was unaffected by these treatments.

The accumulation of GS_2 mRNA in plants grown under photorespiratory conditions may reflect a metabolic induction of GS_2 gene expression by photorespiratory ammonia. Alternatively, changes in metabolism at high levels of CO_2 (i.e. change in pH) may affect levels of GS_2 mRNA. The fact that GS_2 mRNA induction is most dramatic in plants of a developmental stage where photosynthesis (and hence photorespiration) rates are known to be maximal (Sutcliffe and Pate, 1977) supports the interpretation that the increase in GS_2 mRNA abundance is related most likely to the increase in photorespiration. Since mRNA levels for the cytosolic forms of GS remain unchanged in response to CO_2 treatments, the induction of GS_2 mRNA levels under photorespiratory growth conditions represents a specific response rather than a general effect on cell metabolism.

Metabolic induction of gene expression has been documented for other nitrogen-metabolic genes of plants such as nitrate reductase (Crawford et al., 1986) and nitrite reductase (Back et al., 1988), and many examples have been documented for yeast (Struhl, 1982; Zalkin and Yanofsky, 1982; Silverman et al., 1982; Donahue et al., 1983). The fourfold accumulation of GS_2 mRNA under photorespiratory conditions is comparable to the twofold to fourfold metabolic induction seen for several amino acid biosynthetic genes of yeast (i.e. HIS4, TRP5) (Silverman et al., 1982; Zalkin and Yanofsky, 1982; Penn et al., 1983). The apparent low-level induction of these mRNAs (i.e. twofold to fourfold) may reflect a high basal-level expression at the uninduced state. The fourfold accumulation of the GS_2 mRNA during photorespiration could be the result of an increase in the rate of transcription or an increase in the stability of the GS_2 mRNA.

Although the amino acid biosynthetic genes of yeast are regulated at the level of transcription, in some cases additional control at the level of translation has also been demonstrated (Hope and Struhl, 1985). Secondary structure analysis of the mRNA for chloroplast GS_2 has revealed a stable hairpin (−13.3 kcal) in the 5′-nontranslated leader which includes the translational initiation codon (Figure 5). An RNA hairpin of comparable stability that was artificially introduced into the 5′ leader of the yeast cytochrome *c* gene has been shown to reduce translational efficiency by 90% (Baim and Sherman, 1988). Since peas grow optimally at temperatures 10 degrees lower than laboratory yeast, such secondary structures would be more stable, and hence the effects on translation could be even more dramatic. The in vivo significance of the hairpin in the 5′ leader of the GS_2 mRNA of pea remains to be determined.

A comparative analysis of the promoters for the genes encoding chloroplast GS_2 and cytosolic GS may elucidate

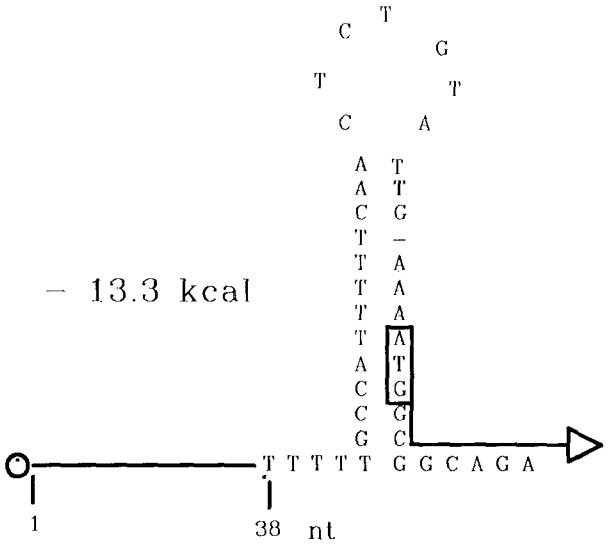

Figure 5. Predicted Secondary Structure in the 5′-Nontranslated Leader of the GS_2 mRNA.

The nucleotide sequence shown corresponds to nucleotides +38 through +78 of cDNA clone pGS185 encoding GS_2 mRNA (Tingey et al., 1988). Secondary structure of the 5′ end of this sequence was analyzed with the software "DNAsis" (LKB for Hitachi). The secondary structure depicted represents the most stable conformation with a calculated energy of −13.3 kcal.

distinguishing features responsible for the differential expression of the distinct members of the GS gene family during plant development. Sequence analysis suggests that these genes arose from a common ancestral gene and that the gene for chloroplast GS_2 evolved traits specialized for function of its gene product in chloroplasts (i.e. transit peptide, light-regulated expression) (Tingey et al., 1988). DNA sequence analysis of the GS_2 promoter has revealed a complex structure that may reflect the evolution of multiple regulatory elements within this gene (J.W. Edwards and G.M. Coruzzi, unpublished results).

Unlike the well-studied gene families for the chloroplast proteins rbcS and cab, the nuclear gene for GS_2 has cytosolic homologs in plants and animals (Smith and Campbell, 1983; Sanders and Wilson, 1984; Hayward et al., 1986; Tingey et al., 1988). Some of the transcription factors that affect regulated expression of the GS_2 gene in plants may have homologs in animals. This possibility is precedented by the recent findings that the GCN4 transcription factor that regulates amino acid biosynthetic genes in yeast has structural homology to the jun oncogene in animals (Vogt et al., 1987; Struhl, 1987). Future studies are aimed at identifying the *cis*- and *trans*-acting factors involved in the regulated expression of the nuclear gene for chloroplast GS_2 in plants.

METHODS

Plant Growth Conditions

Seeds of *Pisum sativum* (var. "Sparkle") were obtained from Rogers Brother Seed Co. (Twin Falls, ID). For etiolated samples, seeds were imbibed and germinated in the dark and grown at 22°C for 7 days in black Lucite boxes within a dark environmental chamber. Light regimes were as follows: Etiolated pea seedlings were exposed to a 3-min flash of red light (GE F20T12R), returned to the dark, and collected after various times for up to 72 hr following illumination. Alternatively, etiolated pea seedlings were exposed to continuous white-light illumination (1000 $\mu E/m^2/sec$) and collected after various times for up to 72 hr. For experiments with mature green plants, seeds were imbibed and germinated in a Conviron environmental chamber with continuous white-light illumination (1000 $\mu E/m^2/sec$), at 21°C. For the dark-adapted plants, 14-day-old plants were transferred to the dark for 4 days. Dark-adapted plants were returned to continuous white light for 6 hr and 24 hr. Photorespiration experiments were conducted in two Conviron environmental chambers maintained under identical conditions of continuous light (1000 $\mu E/m^2/sec$) at 21°C. Chamber 1 contained control plants grown in normal air (0.02% CO_2), whereas chamber 2 was sealed and adapted to accept and maintain a steady concentration of 2.0% CO_2 by introducing a slow stream of 99% CO_2 (Matheson) into the chamber. CO_2 levels were monitored within the chamber using a Bacharach Fyrite CO_2 gas analyzer attached to an outflow valve. Plants were collected from each chamber at 7 days, 14 days, and 21 days after sowing.

Protein Isolation and Protein Gel Blot Analysis

Total soluble protein was extracted from leaves as described previously (Tingey et al., 1987). Polypeptides were separated by SDS-PAGE according to Laemmli (1970) in gels containing a 7.5% to 15% gradient of polyacrylamide. SDS-PAGE gels were processed for protein gel blot analysis according to Blake et al. (1984), using a mixture of antibodies raised against *Nicotiana tabacum* GS_2 (Hirel et al., 1984) and *Phaseolus vulgaris* GS_{n1} (Lara et al., 1984).

Isolation of RNA and DNA

RNA was extracted from pea leaves and stems using guanidine thiocyanate as a protein denaturant (Chirgwin et al., 1979). Plasmid DNA was isolated according to the procedure of Birnboim and Doly (1979).

RNA Gel Blot Analysis

Total RNA (20 μg) was denatured by treatment with glyoxal (Carmichael and McMaster, 1980) and separated by electrophoresis in a 1.2% agarose gel containing 10 mM sodium phosphate, pH 6.5. RNA gel blots were prepared (Thomas, 1980) and hybridized at 42°C with denatured ^{32}P-labeled DNA probes (Feinberg and Vogelstein, 1984) in 50% formamide, 5 × SSC, 1 × Denhardt's solution (Denhardt, 1966), 0.1% SDS, 5% dextran sulfate,

and 100 μg/ml denatured salmon sperm DNA. Filters were washed in 0.1% SSC, 0.1% SDS at 65°C.

S1 Nuclease Protection Experiments

Total RNA (15 μg) was combined with an excess of 5′ end-labeled DNA probe, hybridized overnight in 80% formamide, 40 mM PIPES (pH 6.4), 1 mM EDTA, 0.4 M NaCl at 42°C. S1 digestion and gel electrophoresis was performed according to Maniatis et al. (1982).

Quantitation

Quantitation of autoradiographs was performed by densitometric scanning with a Beckman DU8 spectrophotometer. In each case the results of at least two experiments were averaged.

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