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Light Represses Transcription of Asparagine Synthetase Genes in Photosynthetic and Nonphotosynthetic Organs of Plants

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Asparagine synthetase (AS) mRNA in *Pisum sativum* accumulates preferentially in plants grown in the dark. Nuclear run-on experiments demonstrate that expression of both the AS1 and AS2 genes is negatively regulated by light at the level of transcription. A decrease in the transcriptional rate of the AS1 gene can be detected as early as 20 min after exposure to light. Time course experiments reveal that the levels of AS mRNA fluctuate dramatically during a "normal" light/dark cycle. This is due to a direct effect of light and not to changes associated with circadian rhythm. A novel finding is that the light-repressed expression of the AS1 gene is as dramatic in nonphotosynthetic organs such as roots as it is in leaves. Experiments demonstrate that the small amount of light which passes through the soil is sufficient to repress AS1 expression in roots, indicating that light has a direct effect on AS1 gene expression in roots. The negative regulation of AS gene expression by light was shown to be a general phenomenon in plants which also occurs in nonlegumes such as *Nicotiana plumbaginifolia* and *Nicotiana tabacum*. Thus, the AS genes can serve as a model with which to dissect the molecular basis for light-regulated transcriptional repression in plants.

Light is the primary energy source for all living organisms on earth, excluding chemotrophs. In plants, light influences morphogenic features (phototropism and photomovement) as well as metabolic reactions (photosynthesis). Plants contain several photoreceptors that can detect light of specific wavelengths; some of these have been characterized (phytochrome), while others remain elusive (cryptochrome). The mechanism by which light signals are subsequently transferred from the photoreceptors to the target sites remains unknown.

While light may act to alter plant proteins or membranes directly, at least some of the effects of light on plant development and metabolism are the results of changes in gene expression. Light has been shown to induce or repress the expression of specific photoregulated genes. Light-induced genes which have been well characterized at the molecular level include the gene families encoding the small subunit of ribulose bisphosphate carboxylase (*rbcS*) (6, 10–12, 14, 15, 20, 21) and the chlorophyll *a/b* binding protein (Cab) (15, 21, 29, 34). The light-induced expression of both the *rbcS* and Cab genes is regulated via phytochrome at the transcriptional level (1, 28, 33). Light also positively regulates the expression of plant genes which encode enzymes for nitrogen metabolism, including the nuclear genes for the chloroplast form of glutamine synthetase (GS2) (7, 40), nitrate reductase (13, 27), and nitrite reductase (35). Other genes whose expression is regulated by light at the transcriptional or posttranscriptional level are ferredoxin genes (8, 9) and genes encoding enzymes involved in flavonoid pigment formation, such as chalcone synthase (18, 44).

Light has been shown to regulate gene expression in a negative fashion for only a few identified genes (phytochrome [3, 5, 19, 23, 30, 31] and protochlorophyllide reductase [28]), the best studied of which is phytochrome. Recently, we have described a family of genes (AS1 and AS2) in *Pisum sativum* which encode asparagine synthetase (AS), whose expression is negatively regulated by light (42). The negative effect of light on AS gene expression is mediated via the chromophore phytochrome (42). These molecular findings on the photoregulation of AS gene expression demonstrate a dramatic correlation with the physiological role of asparagine as a nitrogen transport compound in higher plants. In particular, the preferential expression of AS genes in dark-adapted plants correlates with the finding that asparagine (which has a high nitrogen-to-carbon ratio) is an efficient nitrogen transport compound that is utilized when carbon skeletons are limiting. Our findings on AS gene expression indicate that the expression of AS genes in higher plants is regulated by multiple factors (e.g., light, organ type, and development) (42).

Here, we further examine the dark-induced expression of AS1 and AS2 genes in peas to determine the molecular mechanisms involved in the negative regulation of plant gene expression by light. The results presented herein demonstrate that the photoregulation of AS1 gene expression has physiological significance in a normal day/night growth cycle. Further studies demonstrate that these changes in AS1 gene expression are regulated by light and not by a circadian rhythm. A novel feature of AS gene regulation by light is that the photoregulation of AS1 gene expression occurs independent of organ type. The light-repressed expression of AS genes is regulated at the transcriptional level. Moreover, our results indicate that light repression of AS expression occurs in other species and represents a model for dissecting the molecular mechanisms by which light represses gene transcription in plants.

MATERIALS AND METHODS

Growth of plant material. Seeds of *P. sativum* (var. Sparkle) were obtained from Rogers Brothers Seed Co. (Twin Falls, Idaho). Peas were grown as described previously (42). The light/dark cycle for plant growth was 16 h of light (1,000 microeinsteins/m^2/s) at 21°C and 8 h of dark at 18°C. Dark-adapted plants were grown in continuous white

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light for more than 21 days and transferred to the dark for 3 days. For the experiments in Fig. 4B, pea plants were grown in soil (Metro Mix type 200; A. L. Saffer, Parkchester, N.Y.) mixed with Osmocote fertilizer formulation 14-14-14 according to the manufacturer's instructions (A. H. Hummert Seed Co., St. Louis, Mo.). The pots were covered at the base immediately above the soil with two layers of black plastic (weight, 50 mg/in^2 [ca. 50 mg/2.5 cm^2]). Small slits (8 mm) were made in the plastic in a cross pattern, and the seeds were planted so that the axes of germinating seeds penetrated the center of each cross. Plants were then grown in continuous light for 28 days and transferred to the dark for 3 additional days. *Nicotiana plumbaginifolia* was grown in a normal day/night cycle for 21 days and then transferred to continuous light (light grown) or continuous dark (dark adapted) for 4 days.

Southern and Northern (RNA) blot analysis. Pea nuclear DNA was digested, and Southern blots were performed as described previously (42). Total RNA and polyadenylated RNA were isolated as described previously (41). For Northern blot analysis, 20 μg of total RNA or 1 μg of polyadenylated RNA was denatured by glyoxal treatment (4), separated in 1.3% agarose gels containing 10 mM sodium phosphate (pH 6.5), and transferred to nitrocellulose filters (38). For homologous probes, hybridization was performed in 50% formamide hybridization buffer (41) at 42°C for 16 h and the filters were washed in 0.1× SSC–0.1% sodium dodecyl sulfate (SDS) at 55°C (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). For heterologous probes, hybridization was performed in 30% formamide hybridization buffer (39) at room temperature for 16 h and the filters were washed in 0.5× SSC–0.1% SDS at 45°C. Gene-specific probes for AS genes correspond to a *Bam*HI-*Eco*RI DNA fragment containing the 3′ noncoding region of pcAS1 or a *Hin*cII-*Eco*RI DNA fragment containing the 3′ noncoding region of pcAS201 (42).

Quantitation of steady-state mRNA in various organs. Duplicate RNA samples (20 μg of total RNA) from leaves of dark-grown peas and light-grown peas as well as from roots, root nodules, and cotyledons of pea plants grown in a "normal" light/dark cycle were electrophoresed on the same gel and transferred to a nitrocellulose filter. One set of samples was hybridized with two probes at once; one probe was specific for the AS1 gene, and the other was specific for cytosolic glutamine synthetase (GS) (GS299) (40), each with known specific activities. The replicate Northern blot was hybridized with an AS2 gene-specific probe of known specific activity and the same cytosolic GS probe used in the first set of samples. After autoradiography, the results were quantified by densitometry with a Beckman DU-8 spectrophotometer. The relative levels of AS1 and AS2 mRNA were determined by standardizing them against the amount of cytosolic GS mRNA. The relative levels of AS1 and AS2 mRNA in various organs are plotted graphically in Fig. 2B, and the values represent the average of two independent experiments.

Nuclear run-on assays. Nuclei were isolated from pea plants following the protocol described by Hagen and Guilfoyle (16). All the steps for isolating nuclei were performed at 4°C. Between 50 and 100 g of green tissue, including leaves and stems, from 11- or 28-day-old pea plants was rinsed with cold distilled water and submerged in ice-cold diethyl ether for 1 min. After the ether was poured off, 4× volumes of nucleus isolation buffer (10 mM Tris-HCl [pH 7.2], 5 mM $MgCl_2$, 1 M sucrose, 10 mM 2-mercaptoethanol) were added, and the sample was homogenized in a blender on a medium setting. The homogenate was filtered through 80-μm nylon mesh and centrifuged at 2,500 × *g* for 10 min. The pellet was gently resuspended in 30 ml of nucleus isolation buffer containing 0.25% Triton X-100. The nuclei were pelleted by centrifugation, resuspended in 10 ml of nucleus isolation buffer, and layered over a discontinuous Percoll gradient containing 10 ml of 50% and 10 ml of 25% Percoll in nucleus isolation buffer. The gradient was then centrifuged for 30 min at 7,000 rpm in a Beckman JS-13 rotor. The nuclei banded between the two layers of Percoll were collected with a Pasteur pipette, diluted with 2× volume of nucleus isolation buffer, pelleted again by centrifugation at 2,500 × *g* for 10 min, and resuspended in 1 to 2 ml of nucleus isolation buffer.

For the nuclear run-on assays, 200 μl of nucleus suspension was pelleted by centrifugation at 1,500 × *g* for 5 min at 4°C. The pellet was carefully resuspended in 200 μl of reaction buffer (20 mM Tris-HCl [pH 7.4], 140 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 20% glycerol) containing 250 μCi of [α-^{32}P]UTP and 1 mM each ATP, CTP, and GTP and incubated at 32°C for 15 min. Then, 1 ml of HSB (10 mM Tris-HCl [pH 7.4], 500 mM NaCl, 50 mM $MgCl_2$, 2 mM $CaCl_2$) was added to the reaction suspension, followed by 10 μl (23 U/ml) of RNase-free DNase I. After incubation at room temperature for 5 min, the reaction solution was mixed with 2 ml of 50 mM sodium acetate (pH 5.0), 0.1 ml of 20% SDS, and 0.1 ml of 0.5 M EDTA. Phenol extraction (incubated at 60°C for 15 min) and phenol-chloroform extraction (at room temperature) were then performed. The RNA in the aqueous layer was ethanol precipitated with 100 μg of carrier tRNA and resuspended in 200 μl of TE buffer. The RNA solution was mixed well with 4 ml of a 10% trichloroacetic acid solution, incubated on ice for 30 min, and filtered through a 0.45-μm nitrocellulose filter. The RNA on the filter was then eluted in 0.8 ml of ETS buffer (10 mM Tris [pH 7.4], 10 mM EDTA, 0.2% SDS) at 65°C for 15 min. The final RNA solution was adjusted to accommodate the hybridization conditions.

DNA slot-blot hybridization. pTZ18U (5 μg) and cDNAs for AS1 (pcAS1), AS2 (pcAS201) (42), chloroplast GS2 (cGS185), and cytosolic GS (cGS299) (40) (see Fig. 5A), or 5 μg of pTZ18U and genomic clones containing similar lengths of transcribed region for AS1, AS2, and cytosolic GS (unpublished data) (see Fig. 5B) were slot-blotted onto nitrocellulose filters with a Bio-Rad slot-blot apparatus according to the manufacturer's instructions (Bio-Rad Laboratories, Richmond, Calif.). Filters were dried and baked prior to hybridization. Hybridization was performed at 65°C for 24 h in a solution containing 2× SSC, 0.1% SDS, 100 μg of yeast tRNA per ml, and 5 × 10^6 cpm of ^{32}P-labeled RNA per ml. The filters were washed in 2× SSC–0.1% SDS at 65°C for 30 min and then in 0.5× SSC–0.025% SDS at 65°C for 20 min. The filters were then treated with 2.5 μg of RNase A per ml in 2× SSC at 37°C for 30 min and then rinsed in 0.5× SSC–0.025% SDS at room temperature. The filters were air dried and prepared for autoradiography.

RESULTS

DNA probes from the 3′ noncoding region are specific for AS1 and AS2. Previously, we showed that the coding regions of AS1 and AS2 cDNAs are 81% homologous at the DNA level, while their 3′ noncoding regions are completely divergent (42). DNA fragments from the 3′ noncoding regions of AS1 and AS2 cDNAs were used to detect their cognate DNA sequences in pea nuclear DNA digested with four different

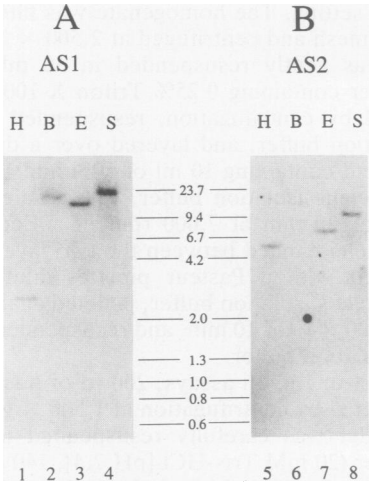

FIG. 1. Gene-specific probes from AS1 and AS2 genes hybridize to unique DNA fragments in the pea nuclear genome. Pea nuclear DNA was digested with *Hin*dIII (H, lanes 1 and 5), *Bam*HI (B, lanes 2 and 6), *Eco*RI (E, lanes 3 and 7), or *Sst*I (S, lanes 4 and 8). Probes from the 3′ noncoding regions of cDNAs encoding AS1 (pcAS1) (A) or AS2 (pcAS201) (B) were used to detect homologous genes on the Southern blot (42). See Materials and Methods for 3′ probe descriptions. Sizes are shown in kilobases.

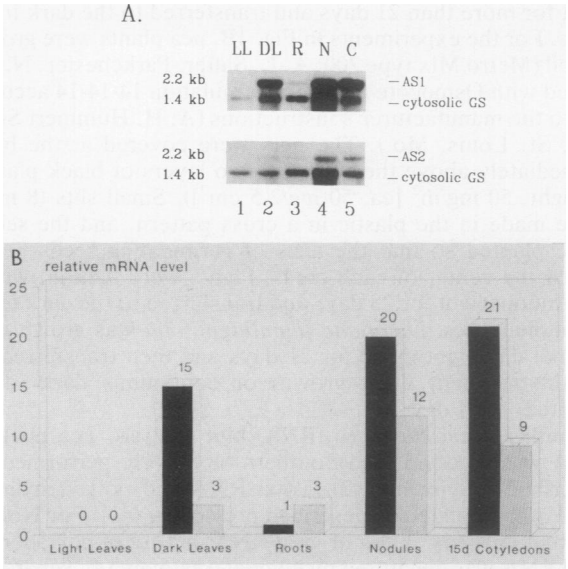

FIG. 2. Quantitation of AS1 and AS2 mRNA in different pea organs. (A) Total RNA (20 μg) was extracted from leaves of 31-day-old peas grown in continuous light (light leaves, LL), leaves of peas grown in continuous light for 28 days followed by dark treatment for 4 days (dark leaves, DL), roots of 32-day-old peas (R), root nodules from 32-day-old *Rhizobium*-infected peas (N), and cotyledons of 15-day-old germinating pea seedlings (C). AS1 and AS2 mRNAs were detected on replicate Northern blots by using gene-specific DNA probes from the 3′ noncoding region of either AS1 cDNA or AS2 cDNA (42). As an internal control, mRNA for cytosolic GS (1.4 kb) was also detected by using the 3′ noncoding region of pGS299 (40) as a probe. (B) Intensities of the radioactive signals detected in panel A were quantified by densitometer. AS1 (solid bars) and AS2 (shaded bars) mRNA levels were compared by using cytosolic GS mRNA levels as a standard. Relative levels of AS1 and AS2 mRNA are shown, with the AS1 mRNA levels in root set at 1. Results shown are an average of two separate experiments.

restriction enzymes. In each case, the 3′ noncoding probe from AS1 and AS2 cDNAs detected distinct DNA fragments in pea nuclear DNA (Fig. 1A and B). These 3′ noncoding probes were used to discriminate between mRNAs for each AS gene in the experiments detailed below.

Quantitation of AS1 and AS2 mRNAs in various organs. The AS gene family in peas consists of at least two members, AS1 and AS2, which encode homologous but distinct glutamine-dependent asparagine synthetase polypeptides (42). Both AS1 and AS2 are expressed at highest levels in nitrogen-fixing root nodules and in cotyledons of germinating seedlings, two organs from which large amounts of nitrogen are transported (42). The expression of both AS1 and AS2 in leaves is induced by dark treatment, albeit at different levels (42). To compare the relative levels of AS1 and AS2 mRNAs which accumulate in various organs, gene-specific Northern blot analysis was performed (Fig. 2A), and the results were analyzed quantitatively (Fig. 2B) as described in Materials and Methods. A comparison revealed that the levels of AS1 mRNA were fivefold higher than AS2 mRNA levels in dark-adapted leaves (Fig. 2A, lane 2, and Fig. 2B). In other organs, AS1 expression was twofold higher than AS2 expression in nodules (Fig. 2A, lane 4, and Fig. 2B) and in cotyledons of germinating seedlings (Fig. 2A, lane 5, and Fig. 2B). In roots, however, the AS2 mRNA level was threefold higher than that of AS1 mRNA (Fig. 2A, lane 3, and Fig. 2B). Both AS1 and AS2 mRNAs were below detection in leaves of light-grown peas (Fig. 2A, lane 1). Dark induced expression of the AS1 gene in leaves was 15-fold, while the induction for the AS2 gene was only 3- to 5-fold (Fig. 2B).

Dark-induced expression of AS1 gene is detectable in a normal light/dark growth cycle and is not a function of circadian rhythm. Circadian rhythms regulate the expression of some light-induced genes (e.g., Cab), while the expression of other light-induced genes is unaffected by circadian rhythm (*rbcS*) (29). The accumulation of AS mRNA can be induced by dark treatment and repressed by light treatment in peas (42). We next determined whether the light regulation of the AS1 gene is significant in a normal day/night growth cycle. The expression of the AS1 and AS2 genes was monitored in pea leaves collected at different time points during a 24-h cycle of 16 h light and 8 h dark. Peas were initially grown under a normal day/night cycle (16 h light and 8 h dark) for 18 days. One-third of the plants were kept in this normal day/night cycle (Fig. 3A), one-third were transferred to the dark for an extended dark treatment (Fig. 3B), and the remaining third were kept in the light for an extended light treatment (Fig. 3C). Figure 3A shows that the steady-state levels of AS1 mRNA fluctuate within a normal day/night cycle in response to the light/dark growth conditions. The accumulation of AS1 mRNA increased after 5 h in the dark (Fig. 3A, lane 5) and reached a peak level after 8 h in the dark (Fig. 3A, lane 6). A subsequent decrease in the AS1 mRNA level was detected after only 3 h of exposure to light (Fig. 3A, lane 7), and it decreased further after 6 h in the light (Fig. 3A, lane 8). When the duration of the dark cycle was extended, high levels of AS1 mRNA were maintained (Fig. 3B, lanes 7 to 9) despite the time of a day at which these samples were collected. In contrast, when a normal light period was followed by an extended light treatment, the AS1 mRNA levels were maintained at a basal low level (Fig. 3C, lanes 4 and 5). As a control, the levels for cytosolic GS mRNA were determined and shown to be relatively unchanged in response to the various light/dark regimens (Fig.

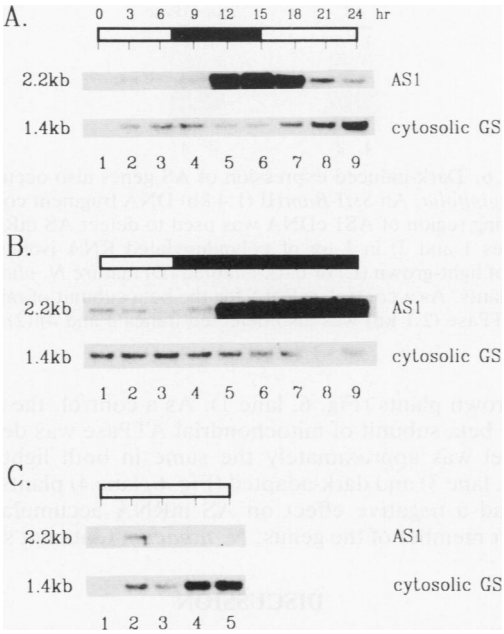

FIG. 3. Fluctuations in AS mRNAs occur in a normal light/dark growth cycle and are not due to circadian rhythm. Pea plants were grown under a normal light/dark cycle (16 h of light and 8 h of dark) for 18 days. On day 19, some of the plants were kept in the normal light/dark cycle (A), some of the plants were kept in the dark for a total of 18 h (B), and others were kept in an extended light period (C). Leaves were collected every 3 h, and RNA was isolated. Gene-specific Northern blot analysis was performed to detect the steady-state levels of AS1 mRNA. As a control, Northern blots were also probed for cytosolic GS mRNA.

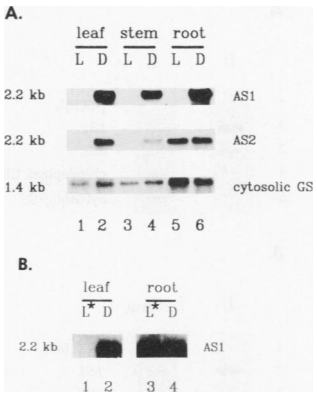

FIG. 4. Light-repressed expression of AS occurs independent of organ type. (A) Gene-specific Northern blot analysis was performed to detect the effect of light on the steady-state levels of AS1 and AS2 mRNAs in leaves, stems, and roots. Pea plants were grown in continuous light for 28 days (L) and then transferred to the dark for 3 days (D). Total RNA was isolated from the leaves (lanes 1 and 2), stems (lanes 3 and 4), and roots (lanes 5 and 6) of either light-grown (L) or dark-adapted (D) plants. Gene-specific probes from the 3′ noncoding regions of AS1 and AS2 (42) were used to detect specific AS mRNA (2.2 kb). As a control, cytosolic GS mRNA (1.4 kb) was also detected on the Northern blot (40). (B) AS1 mRNA levels were detected in leaves (lanes 1 and 2) and roots (lanes 3 and 4) of pea plants grown in soil covered with two layers of black plastic as described in Materials and Methods. These plants were grown for 28 days in continuous light (L*, lanes 1 and 3) and were then transferred to the dark for 3 days (D, lanes 2 and 4).

3A, B, and C, lower panels). These results show that dark-induced or light-repressed changes in the accumulation of AS1 mRNA are physiologically significant in a normal day/night cycle. These changes are the result of AS1 expression in response to the light/dark growth conditions and not to an internal clock or circadian rhythm, because they do not persist in continuous-light regimens. Similar results were obtained when a gene-specific probe for AS2 mRNA was used (not shown).

AS1 mRNA accumulation is repressed by light in both photosynthetic and nonphotosynthetic organs. Expression of most of the light-regulated genes has been studied in leaves (e.g., *rbcS* and Cab). Here, we have examined the effect of light on AS gene expression in other plant organs than leaves (Fig. 4A). The steady-state levels of AS1 and AS2 mRNAs in the leaves, stems, and roots of light-grown (Fig. 4A, lanes 1, 3, and 5) or dark-adapted (Fig. 4A, lanes 2, 4, and 6) pea plants were determined by gene-specific Northern blot analysis. These results show that AS1 mRNA levels are regulated by light in all vegetative organs (Fig. 4A, upper panel). In contrast, AS2 mRNA accumulated independently of light in the roots of both light-grown and dark-adapted plants (Fig. 4A, middle panel, lanes 5 and 6). As a control, the mRNA levels for cytosolic GS were shown to be relatively unaffected by the light/dark treatments in all vegetative organs (Fig. 4A, lower panel).

The absence of AS1 mRNA in the leaves and stems of light-grown plants could be explained as a direct negative effect of light on AS gene expression in these aerial organs. For roots, however, there are two possible explanations for the specific accumulation of mRNA in the dark. Light may act to repress AS1 gene expression in roots directly via light which penetrates the soil, or light may act indirectly on roots via a signal transmitted from the aerial portions of the plant (25, 26). To determine whether light has a direct effect on AS1 gene expression in roots, AS1 mRNA levels were monitored in plants whose roots were shielded from direct exposure to light. Peas were sown in soil which was covered with two layers of black plastic to block light from penetrating the soil directly (see Materials and Methods). After 28 days of growth in continuous light, the plants were transferred to the dark for an additional 3 days. RNA was isolated from the leaves and roots, and Northern blot analysis was performed (Fig. 4B). These experiments revealed that blocking the majority of light from penetrating the soil abolished the negative effect of light on AS1 gene expression in roots. The level of AS1 mRNA in shielded roots (Fig. 4B, lane 3) was equivalent to the AS1 mRNA level in the roots of dark-grown plants (Fig. 4B, lane 4). Light still acted to repress AS1 mRNA accumulation in leaves in the plants whose roots were covered (Fig. 4B, lane 1). These results demonstrate that light acts directly on AS1 gene expression in roots and furthermore that the small amount of light which normally passes through the soil is sufficient to repress expression of the AS1 gene in roots.

Light-repressed expression of AS genes in pea plants is regulated at the level of transcription. Nuclear run-on assays were performed to determine whether the effects of light on AS gene expression in peas is regulated at the level of transcription (Fig. 5). Nuclei were isolated from the leaves and stems of peas which were grown in continuous white light for 12 days and dark-adapted for 1 day. The results of nuclear run-on experiments demonstrate that the AS1 gene is transcribed at high levels in the leaves of dark-adapted

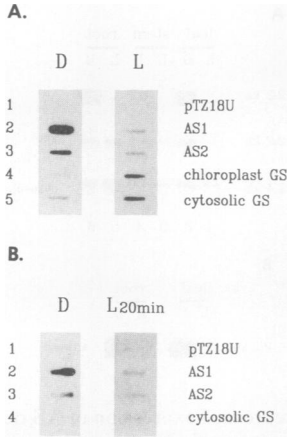

FIG. 5. Light-repressed expression of AS genes is regulated at the level of transcription. (A) Nuclei isolated from leaves of 12-day-old peas dark-adapted for 1 day (D) and subsequently treated with white light for 6 h (L) were used to perform nuclear run-on assays with [^{32}P]ATP. Slot-blot filters containing DNA from plasmid pTZ18U (negative control) (row 1) or the genes for AS1 (row 2), AS2 (row 3), chloroplast GS2 (row 4), and cytosolic GS (row 5) were used to detect specific ^{32}P-labeled transcripts generated in the nuclear run-on reactions. (B) Nuclei isolated from 27-day-old pea plants dark-adapted for 3 days (D) and subsequently treated with white light for 20 min (L20min) were used to perform a nuclear run-on assay. The transcription rates of AS1 (row 2), AS2 (row 3), and cytosolic GS (row 4) were analyzed by slot blots. Plasmid pTZ18U was used as a negative control (row 1).

peas (Fig. 5A, 2D). When the dark-adapted plants were transferred back to white light for 6 h, the transcription rate of the AS1 gene decreased dramatically (Fig. 5A, 2L). The transcription rate of the AS2 gene was also negatively affected by light (Fig. 5A, row 3). However, the transcription rate of the AS2 gene was significantly lower than that of the AS1 gene in the leaves of dark-adapted peas (Fig. 5A, compare 2D and 3D). As a control, the transcription rate for the nuclear gene encoding chloroplast GS was shown to be induced by the light treatment (Fig. 5A, row 4), while the transcription rate for cytosolic GS was relatively unaffected by light or dark growth conditions (Fig. 5A, row 5).

Nuclear run-on experiments were also performed on dark-adapted plants which were reexposed to light for only 20 min (Fig. 5B). A decrease in the rate of transcription of AS1 was detected within 20 min of continuous exposure to light (Fig. 5B, row 2). While there was a slight decrease in AS2 transcripts in the light-treated plants (Fig. 5B, 3L20min) compared with that in dark-adapted plants (Fig. 5B, 3D), transcript levels were too low for an accurate quantitation. The transcriptional rate of cytosolic GS gene was monitored as a control in these experiments (Fig. 5B, row 4). These results demonstrate that light-repressed expression of both the AS1 and AS2 genes is regulated at the transcriptional level and that the transcription of AS1 is rapidly turned off in response to light treatment.

Dark-induced expression of AS gene also occurs in members of the family *Solanaceae*. To determine whether the dark-induced expression of AS gene occurs in species other than *P. sativum*, AS mRNA was detected on Northern blots of RNA isolated from *Nicotiana plumbaginifolia* grown in the light and transferred to the dark for 4 days (Fig. 6). AS mRNA in *N. plumbaginifolia* was detected in the leaves of dark-adapted plants (Fig. 6, lane 2) but not in the leaves of

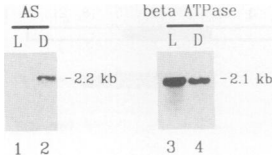

FIG. 6. Dark-induced expression of AS genes also occurs in *N. plumbaginifolia*. An *Sst*I-*Bam*HI (1.4 kb) DNA fragment containing the coding region of AS1 cDNA was used to detect AS mRNA (2.2 kb, lanes 1 and 2) in 1 μg of polyadenylated RNA isolated from leaves of light-grown (L) or dark-adapted (D) mature *N. plumbaginifolia* plants. As a control, mRNA for the beta subunit of mitochondrial ATPase (2.1 kb) was also detected (lanes 3 and 4) (2).

light-grown plants (Fig. 6, lane 1). As a control, the mRNA for the beta subunit of mitochondrial ATPase was detected; its level was approximately the same in both light-grown (Fig. 6, lane 3) and dark-adapted (Fig. 6, lane 4) plants. Light also had a negative effect on AS mRNA accumulation in another member of the genus, *N. tabacum* (data not shown).

DISCUSSION

Here we examine in detail the photoregulation of asparagine synthetase gene expression in plants. Asparagine, which has a high nitrogen-to-carbon ratio, is the predominant nitrogen transport compound utilized when carbon sources are relatively limited (e.g., in the dark) (22, 32). Previously, we showed that the two genes encoding asparagine synthetase in peas (AS1 and AS2) are expressed preferentially in etiolated and dark-adapted pea plants (42). The specific expression of AS genes in the dark may be the result of dark-induced gene activation or repression of gene expression by light. We have also shown that phytochrome mediates the effect of light (42). Since phytochrome is only active in the light, at least part of the effect of light must be due to light-mediated repression. In addition, dark-induced enhancement of AS gene expression may also occur. Until these two models for gene regulation are distinguished at the molecular level, we consider both possibilities and refer to both mechanisms herein.

Previously, we showed that AS mRNAs accumulate to high steady-state levels specifically in dark-grown or dark-adapted plants (42). Here, nuclear run-on experiments reveal that the negative effect of light is due at least in part to changes in transcription. Light represses the transcription of the AS1 gene, and this effect can be detected as early as 20 min after exposure to light. Time course experiments indicate that changes in steady-state levels of AS mRNA occur within only 3 h; thus, mRNA stability may also play a role in the photoregulation of AS gene expression. The results of time course experiments indicate that AS1 and AS2 mRNA levels fluctuate in a normal day/night cycle in an expected fashion (e.g., high at night, low during the day) and thus reflect changes in asparagine biosynthesis. This pattern of expression does not persist in the absence of a photoperiod and is therefore not due to circadian rhythm.

A novel finding of these studies is that light dramatically affects AS1 gene expression in nonphotosynthetic organs (e.g., roots), equal to the regulation observed in leaves. While light has been shown to affect the expression of genes which are positively regulated by light (e.g., *rbcS*) in nonphotosynthetic organs (such as root), the effects of light on expression of these genes in roots were much lower than in leaves (6, 36). For the two other genes whose expression in

leaves is negatively regulated by light (phytochrome and protochlorophyllide reductase), no light regulation has been observed in roots (19, 23, 28, 30). When the light regulation of AS1 and AS2 genes is compared, a dramatic difference occurs in the roots, where AS2 mRNA accumulation is unaffected by light. The constitutive expression of AS2 in roots presumably provides a constitutive source of asparagine for plant growth, while the specific expression of high levels of AS1 mRNA in the roots of dark-grown plants may provide a boost of asparagine synthesis to accommodate the metabolic demands.

Light which passes through the soil may exert a direct effect on AS1 gene expression in roots, or light could act indirectly via a signal transmitted from the aerial portions of the plant. Here we show that shielding soil-borne roots from direct exposure to light abolishes the negative effect of light on AS1 gene expression in roots, even though the aerial portions of the plant were exposed to light. These findings indicate that light acts directly to repress AS1 gene expression in roots. It has been shown that the geotropism in roots is influenced by red light via phytochrome (24, 37). It is possible that light regulates AS1 gene expression in roots via phytochrome. The effect of light on AS1 gene expression in roots is sensitive, since the small amount of light that normally penetrates the soil (25, 26) is sufficient to repress the expression of the AS1 gene in pea roots. These findings demonstrate that subsoil light affects molecular processes, as was predicted by earlier studies on light penetration of soil (26).

Traditionally, nitrogen metabolism has been intensively studied in legumes because of their importance as nitrogen-rich crops. Although asparagine is a major nitrogen transport compound in both legumes and nonlegume plants (22), it is possible that the genes encoding AS are regulated by different factors in different plant species. We observed that the negative regulation by light of AS gene expression occurs in *N. plumbaginifolia* and *N. tabacum*. These results demonstrate that the photoregulation of AS gene expression is a general phenomenon not limited to legumes and that transgenic tobacco plants may be used as a vehicle by which to dissect the regulatory elements of the pea AS genes.

Future studies will be directed at a dissection of the AS1 and AS2 promoters by in vitro and in vivo approaches. These types of studies should uncover the molecular mechanisms which mediate the organ-specific and/or light-repressed expression of AS genes in higher plants. Since light regulation of AS gene expression occurs in nonphotosynthetic organs as well as in leaves, it may be possible to separate for the first time light-regulatory elements from organ-specific *cis*-acting DNA elements. For other light-regulated genes which are expressed predominantly in leaves (e.g., *rbcS* and Cab), it has not been possible to make mutations which affect light regulation but maintain expression (15). Our continuing molecular studies on AS gene regulation will serve to uncover not only the mechanism of light-repressed gene expression in plants, but also the mechanisms which regulate asparagine biosynthesis in plants.

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