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Transcriptional Regulation of the *Arabidopsis thaliana* Chalcone Synthase Gene

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We have cloned an *Arabiodpsis thaliana* chalcone synthase (CHS) gene on the basis of cross-hybridization with a *Petroselinum hortense* CHS cDNA clone. The protein sequence deduced from the *A. thaliana* CHS DNA sequence is at least 85% homologous to the CHS sequences from *P. hortense*, *Antirrhinum majus*, and *Petunia hybrida*. Southern blot analysis indicated that CHS is a single-copy gene in *A. thaliana*. High-intensity light treatment of *A. thaliana* plants for 24 h caused a 50-fold increase in CHS enzyme activity and an accumulation of visibly detectable levels of anthocyanin pigments in the vegetative structures of these plants. A corresponding increase in the steady-state level of CHS mRNA was detected after high-intensity light treatment for the same period of time. The accumulation of CHS mRNA in response to high-intensity light was due, at least in part, to an increased rate of transcription of the CHS gene as demonstrated by nuclear runoff experiments.

The accumulation of flavonoid compounds, a group of secondary metabolic products which includes the flavones, flavonols, isoflavonoids, and anthocyanins, is highly regulated in response to environmental stimuli and is thought to play an important role in protecting plants from environmental stress (26). For example, UV light treatment of parsley tissue culture cells resulted in the accumulation of flavone and flavonol glycosides (44). These flavonoid compounds absorbed UV light and were proposed to protect the cells from UV-induced damage (16). In legumes, fungal pathogens and elicitors induced the production of isoflavonoid phytoalexins that inhibited the growth of the pathogenic fungi (3, 11, 39). Similarly, light, temperature, and availablity of nutrients and water affected the accumulation of anthocyanins, pigments which are responsible for the red and blue coloration of various plant structures, in many plant species (23, 45). The light-induced production of anthocyanins in the epidermis may have evolved to protect plants against solar radiation (7, 12).

The accumulation of flavone, flavonol, and isoflavonoid compounds in response to UV light and pathogen stress has been shown to be due to an increase in the rate of transcription of the chalcone synthase (CHS) gene, which encodes the first enzyme unique to flavonoid biosynthesis, and other flavonoid biosynthetic genes (9, 10, 20). By analogy with the production of these flavonoid compounds, the accumulation of anthocyanins in response to specific environmental conditions might also be due to transcriptional regulation of CHS and other genes in the biosynthetic pathway.

CHS genes, or cDNA copies of these genes, have been isolated from several plant species including *Antirrhinum majus*, *Zea mays*, *Petunia hybrida*, *Petroselinum hortense*, and *Phaseolus vulgaris* (19, 33, 35, 40, 46). CHS has been shown to be regulated transcriptionally in response to two environmental signals, UV light (in *P. hortense* and *A. majus*) and fungal pathogen treatment (in *P. vulgaris*) (9, 10, 17, 20). To identify and characterize putative *trans*-acting regulatory factors that control CHS expression, it would be advantageous to be able to isolate mutants altered in these factors. However, the systems in which environmental regulation of CHS has been best studied, *P. hortense* and *P. vulgaris*, are not readily amenable to genetic analyses. In contrast, *Arabidopsis thaliana* is particularly well suited to genetic studies: it has the smallest known genome size (7×10^7 base pairs) of any dicotyledonous plant and a generation time of only 6 weeks, produces up to 10,000 seeds per plant, is self-fertile, and is very small in size (14, 26).

This report establishes that high-intensity light conditions, which induce the accumulation of anthocyanin pigments in the leaves and stems of *A. thaliana* plants, cause a concomitant increase in the level of CHS enzyme activity. We demonstrate that the increase in CHS activity is due, at least in part, to an increased rate of transcription of CHS.

MATERIALS AND METHODS

Plasmids. The plasmid pLF15 is a 1.5-kilobase (kb) *P. hortense* CHS cDNA cloned into pBR322 (33). B. Lalonde provided the plasmid pBL104, which is an *Eco*RI genomic fragment containing a portion of the gene coding for the *A. thaliana* acetolactate synthase enzyme in pUC18. pCAB1.8 is a pUC8 plasmid derivative that contains a 1.8-kb fragment which includes one of the three *A. thaliana* chlorophyll *a/b* binding protein (*cab*) genes (22).

Isolation of CHS. Standard plaque hybridization procedures were used to isolate *A. thaliana* CHS clones from a λ EMBL4 library (24). All radiolabeled DNA probes were generated by nick translation (34). Plaque hybridizations and Southern blot hybridizations with the heterologous parsley cDNA probe were done in 0.5 M Tris hydrochloride (pH 7.5)–1 M NaCl–10% dextran sulfate–1% sodium dodecyl sulfate–0.2% polyvinylpyrrolidone (40,000 daltons)–0.2% bovine serum albumin–0.2% Ficoll (400,000 daltons)–0.1% sodium pyrophosphate–100 μg of salmon testis DNA per ml at 65°C, and washes were in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–1% sodium dodecyl sulfate at 65°C for 1 h. Southern hybridizations with the homologous probe were as described above, except that 25% formamide was added to the hybridization solution, the hybridization was at 45°C, and washes were at 50°C.

Light treatment. All plants were grown in a climate-controlled greenhouse at 22 ± 5°C. Seeds were planted at a density of 2,000 per 28- by 55- by 7-cm flat in Terralite

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Metromix 200 growing medium. Plants were grown under natural photoperiod for 3½ weeks, from November to December, and received an average of 25 μE of sunlight per m^2 per s during daylight hours. Sets of flats were then placed under high-intensity sodium lamps (General Electric LU1000) suspended at a height of 30 in. (ca. 75 cm) and received additional illumination, (total light intensity, 1,500 $\mu E/m^2s$) for 6, 12, or 24 h. The ambient temperature under the high-intensity sodium lamps was approximately 5°C higher than normal greenhouse conditions, and therefore plants exposed to the high-intensity lights were watered more heavily. Plants were harvested immediately after the light treatment; 100 g of fresh whole plant tissue was used to prepare nuclei, and the rest was frozen in liquid nitrogen for use in either RNA preparations or enzyme assays. Control plants remained under natural photoperiod and were harvested simultaneously with the 24-h light treatment time point. Anthocyanin levels were determined from a separate set of plants which were treated in an identical fashion.

Anthocyanin extraction. Frozen plant tissue (0.5 g) was ground in a mortar and pestle, total plant pigments were extracted in 1.5 ml of 1% HCl in methanol, and 1.0 ml of distilled H_2O was added. Chlorophyll was separated from the anthocyanins by back-extraction with chloroform. The quantity of anthocyanin pigments in control and high-light-treated plants was determined by spectrophotometric measurements of the aqueous-methanol phase ($A_{530} - A_{657}$ was used as a measure of anthocyanin content) and normalized to the dry weight of tissue used in each sample (31).

RNA analysis. Isolation of RNA was by a modification of the phenol-sodium dodecyl sulfate method (2). Poly(A)$^+$ RNA was selected on oligo(dT)-cellulose columns (24). RNA was separated in formaldehyde-agarose gels (15), and Northern blot hybridization and washing were as stated above for Southern hybridizations with the heterologous probe, except that 50% formamide was added and the temperature of hybridization was 42°C. The 5′ end of the mRNA was mapped by primer extension and S1 nuclease analyses (2). A synthetic oligonucleotide that corresponded to the first 30 nucleotides of the CHS-coding sequence was used in the primer extension reaction. For the S1 probe, this same oligonucleotide was hybridized to a single-stranded M13 mp19 clone of CHS (60CHS) and extended with DNA polymerase I Klenow fragment. A specific 570-nucleotide probe that extended 5′ of the beginning of the CHS-coding sequence was generated by cutting the products of the Klenow reaction with *Ssp*I. The S1 and primer extension reaction products were compared to the sequence of 60CHS generated by the Sanger et al. sequencing method (36). The primer for the sequencing reactions was also the 30 base oligonucleotide. The sequencing primer did not have a terminal 5′ phosphate; however, this should have minimal effect on the mobility of the fragments greater than 50 bases in length (42).

Enzyme assays. A crude extract made from whole plants was used in all enzyme assays. For each set of plants assayed, 0.5 to 1.0 g of frozen plant tissue was ground with 0.1 g of Dowex 1×2 (Sigma Chemical Co., St. Louis, Mo.) equilibrated with 0.2 M Tris hydrochloride (pH 8.0) in a mortar and pestle. Two milliliters of 0.2 M Tris hydrochloride (pH 7.8)–14 mM β-mercaptoethanol was then added to the frozen tissue, and the sample was homogenized until it melted. The slurry was stirred for 20 min on ice, and the cellular debris was removed by centrifugation. A 10-μl sample of this plant extract was used in each enzyme assay (37) in a final volume of 100 μl. The malonyl-2-[^{14}C]coenzyme A reaction substrate was purchased from New England Nuclear Corp., Boston, Mass., and the other substrate, *p*-coumaroyl coenzyme A, was synthesized by the method of Stockigt and Zenk (41). Assays were incubated at 30°C for 15 min, and then 30 μg of naringenin (Roth; high-pressure liquid chromatography grade) was added to terminate the reaction. Reaction products were extracted with 200 μl of ethylacetate, and 40 μl from each reaction was analyzed by chromatography on a silica gel thin-layer chromatography plate in 75% chloroform–12.5% acetic acid–12.5% formic acid. The naringenin was visualized by illumination of the thin-layer chromatography plate with UV light, autoradiography, or both. The amount of naringenin produced by the enzyme reaction was determined by scraping the thin-layer chromatography plates and quantifying the amount of ^{14}C comigrating with the unlabeled naringenin by scintillation counting. To obtain a measurement of specific activity (counts per minute of labeled naringenin per microgram of protein), the amount of protein added to each reaction was determined by using the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.). In each case, before the specific activity was calculated, the number of counts per minute comigrating with naringenin obtained from reactions in which no coumaroyl-coenzyme A was added was subtracted from the amount of labeled naringenin produced.

Nuclear assay runoff. Nuclei from 3 ½-week-old *A. thaliana* plants were prepared by a modification of the method of Olszewski et al. (29). Leaf tissue (100 g) was washed with cold distilled H_2O and chilled to 4°C. All of the following steps were carried out at 4°C unless indicated. The tissue was chopped into small pieces, submerged in diethyl ether for 3 min, rinsed with cold distilled H_2O, homogenized in 3 volumes of nucleus isolation buffer (1 M sucrose, 10 mM Tris hydrochloride [pH 7.2], 5 mM $MgCl_2$, 10 mM β-mercaptoethanol) with a Brinkmann Polytron PT20 ST (Brinkmann Instruments, Inc., Westbury, N.Y.), and filtered through four layers of cheesecloth and one layer of Miracloth (Calbiochem-Behringer, La Jolla, Calif.). The homogenate was subjected to centrifugation at 9,000 rpm for 15 min in a Beckman JA-10 rotor (Beckman Instruments, Inc., Fullerton, Calif.). The pellet was suspended with a Dounce homogenizer in 20 ml of nucleus isolation buffer. The volume of suspended nuclei was carefully measured, and 100% Percoll solution (34.23 g of sucrose, 1.0 ml of 1 M Tris hydrochloride [pH 7.2], 0.5 ml of 1 M $MgCl_2$, 34 μl of β-mercaptoethanol, and Percoll to 100 ml) was added to generate a final concentration of 35% (vol/vol) Percoll. Nuclei suspended in 35% Percoll were layered over 7.5 ml of 60% (vol/vol) Percoll to create four discontinuous Percoll gradients. The gradients were subjected to centrifugation in a JS-13 rotor at an initial speed of 2,000 rpm for 10 min and then at 8,000 rpm for an additional 20 min. The crude nuclei were harvested from between the 35% and 60% layers and diluted with 5 volumes of nucleus isolation buffer. Nuclei were collected by centrifugation at 8,000 rpm for 10 min in a JS-13 rotor, suspended in 10 volumes of nuclei isolation buffer, and then collected by centrifugation. The crude nuclear pellet was suspended in 1.0 ml of storage buffer (20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [pH 7.2], 5 mM $MgCl_2$, 2 mM dithiothreitol, 50% glycerol) and stored at −70°C. The number of nuclei in each preparation was determined by quantitating their DNA content with diphenylamine analysis. To prepare nuclei for diphenylamine analysis, 50 μl of nuclei in storage buffer was pelleted by centrifugation, and the storage buffer solution was removed. Contaminating chloroplast

pigments were extracted once with 200 μl of acetone. The nuclei were recovered from the acetone extraction by centrifugation and suspended in 0.5 N perchloric acid. The perchloric acid solution of nuclei was boiled for 10 min and then assayed as described previously (6). A sample of 10^7 nuclei (~2 μg of DNA by diphenylamine analysis) was used in each nuclear run-on experiment. Nuclear run-on experiments were performed as described previously (2), except that the NaOH hydrolysis step was omitted, hybridizations were in 4 ml rather than 2 ml for a period of 72 h instead of 36, and washes were in 0.4× SSC–0.1% sodium dodecyl sulfate at 65°C.

RESULTS

Cloning of the *A. thaliana* CHS. Fifteen genomic equivalents of an *A. thaliana* λ EMBL4 library (obtained from E. Meyerowitz) were screened for cross-hybridization with a *P. hortense* CHS cDNA clone (pLF15, provided by K. Hahlbrock). Nine positive genomic clones were detected; restriction mapping and Southern blot analyses showed that these nine clones fell into two classes. Both classes contained a common region of homology to the *P. hortense* CHS cDNA, and there was significant overlap in sequences between the two classes (Fig. 1). The entire region of homology to the *P. hortense* CHS cDNA was contained on two adjacent *Hin*dIII fragments, 2.6 and 1.3 kb, respectively. This region was subcloned as a 3.9-kb partial *Hin*dIII fragment into pUC13 to generate pCHS3.9 (Fig. 1).

Genomic blots verified that pCHS3.9 is colinear with sequences in the *A. thaliana* genome. Digests of *A. thaliana* DNA with *Hin*dIII, *Eco*RI, *Dra*I, and *Bgl*II were probed with nick-translated pCHS3.9 DNA under low-stringency hybridization conditions (data not shown). The restriction fragments that hybridized with the pCHS3.9 probe agreed with those predicted from the restriction map of the original genomic clone, indicating that pCHS3.9 represented a single-copy gene in *A. thaliana*.

DNA sequence analysis of pCHS3.9. The DNA sequence of the 2.6- and 1.3-kb *Hin*dIII fragments was determined by the Sanger et al. dideoxy sequencing method (36). To obtain a complete double-stranded sequence of this region, nested sets of deletions were generated from the two *Hin*dIII fragments and the 3.9-kb partial *Hin*dIII fragment by using *Bal*31 exonuclease (30); these subclones were the substrates for the sequencing reactions. The DNA sequence of the entire 3.9-kb fragment is presented in Fig. 2. Comparison of the CHS protein sequence deduced from the *P. hortense* CHS cDNA with the *A. thaliana* genomic sequence suggested the presence of a single intron within the *A. thaliana* CHS gene. This putative intron is bordered by the appropriate 5′-GT and 3′-AG splice junction consensus sequences (5, 38) and is located at a position within the *A. thaliana* gene very similar to that of the first intron of the *A. majus* CHS gene (40). The deduced protein sequence of the *A. thaliana* CHS is 90% homologous to that of *P. hortense*, 87% homologous to that of *P. hybrida*, and 85% homologous to that of *A. majus*.

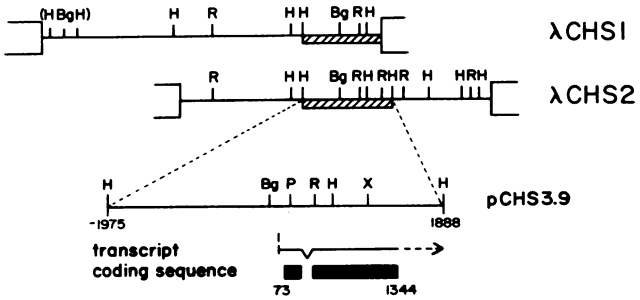

FIG. 1. Map of *A. thaliana* CHS genomic clones and pCHS3.9. λ CHS1 and λ CHS2 are representative of the two classes of genomic phage isolated from the *A. thaliana* library by cross-hybridization with the *P. hortense* cDNA. The hatched regions define the limits of the subclone pCHS3.9 and the sequence reported in this paper. An expanded restriction map of pCHS3.9 and the location of the CHS transcript and coding sequence within pCHS3.9 are shown in the lower portion of the figure. Numbers indicate the ends of pCHS3.9 (positions −1975 and 1888), the start of transcription (position 1), and the extent of the CHS coding sequence (positions 73 and 1344). Restriction enzymes: H, *Hin*dIII; R, *Eco*RI; Bg, *Bgl*II; P, *Pst*I; and X, *Xba*I. The order of the restriction enzyme sites shown in brackets was not determined.

Induction of CHS enzyme activity and mRNA by high-intensity light. A change in plant growth conditions, from natural photoperiod (25 μE/m²s during daylight hours) to continuous illumination with high-intensity light (total illumination, 1,500 μE/m²s), resulted in the accumulation of purple anthocyanin pigments in the leaves and stems of wild-type *A. thaliana* plants (data not shown). The accumulation of anthocyanin pigments in the leaves of *A. thaliana* plants was visually detectable after 24 h of high-intensity light treatment. This visible change in leaf color represented a greater than 15-fold increase in the level of anthocyanins (pigments were extracted and measured spectrophotometrically) in these high-intensity light-treated plants. To determine whether the accumulation of anthocyanin pigments was associated with increased CHS activity, *A. thaliana* plants were grown and subsequently treated with high-intensity light for 6, 12, or 24 h as described above. Plant material from each time point was divided into three parts and used for preparing protein extracts, RNA [poly(A)$^+$ and total], or nuclei.

The yield of naringenin, the product of the CHS and subsequent chalcone isomerase reactions, was used as a measure of CHS activity. The level of CHS activity, measured in vitro by an enzyme assay, increased by 50-fold after exposure of plants to high-intensity light conditions for 24 h. The specific activity of CHS was 5 cpm of naringenin per μg of protein in controls and 14, 64, and 240 cpm of naringenin per μg of protein after light treatment for 6, 12, and 24 h, respectively. The significant amount of CHS activity detectable in plants treated for only 12 h with high-intensity light preceded visual detection of anthocyanin pigments in the leaves.

Poly(A)$^+$ RNA isolated from plants that had been light treated as described above was examined by Northern blot analysis for the induction of CHS mRNA. RNA blots were probed with nick-translated pCHS3.9. A large increase in the level of mRNA coding for CHS was detected in RNA isolated from plants exposed to high-intensity light for 24 h compared with that in RNA isolated from the control plants (Fig. 3a; compare lane 24h with lane c). (Overexposure of the autoradiogram depicted in Fig. 3a revealed the presence of a low level of CHS mRNA in control tissue [data not shown].) Densitometric measurements determined that the level of CHS mRNA was more than 25-fold higher in plants treated with high-intensity light for 24 h than in plants which remained under normal daylight conditions. A 2.5-fold increase in CHS mRNA levels was detectable after exposure of the plants to high-intensity light conditions for only 6 h (lane 6h). The increase in the level of CHS mRNA was not

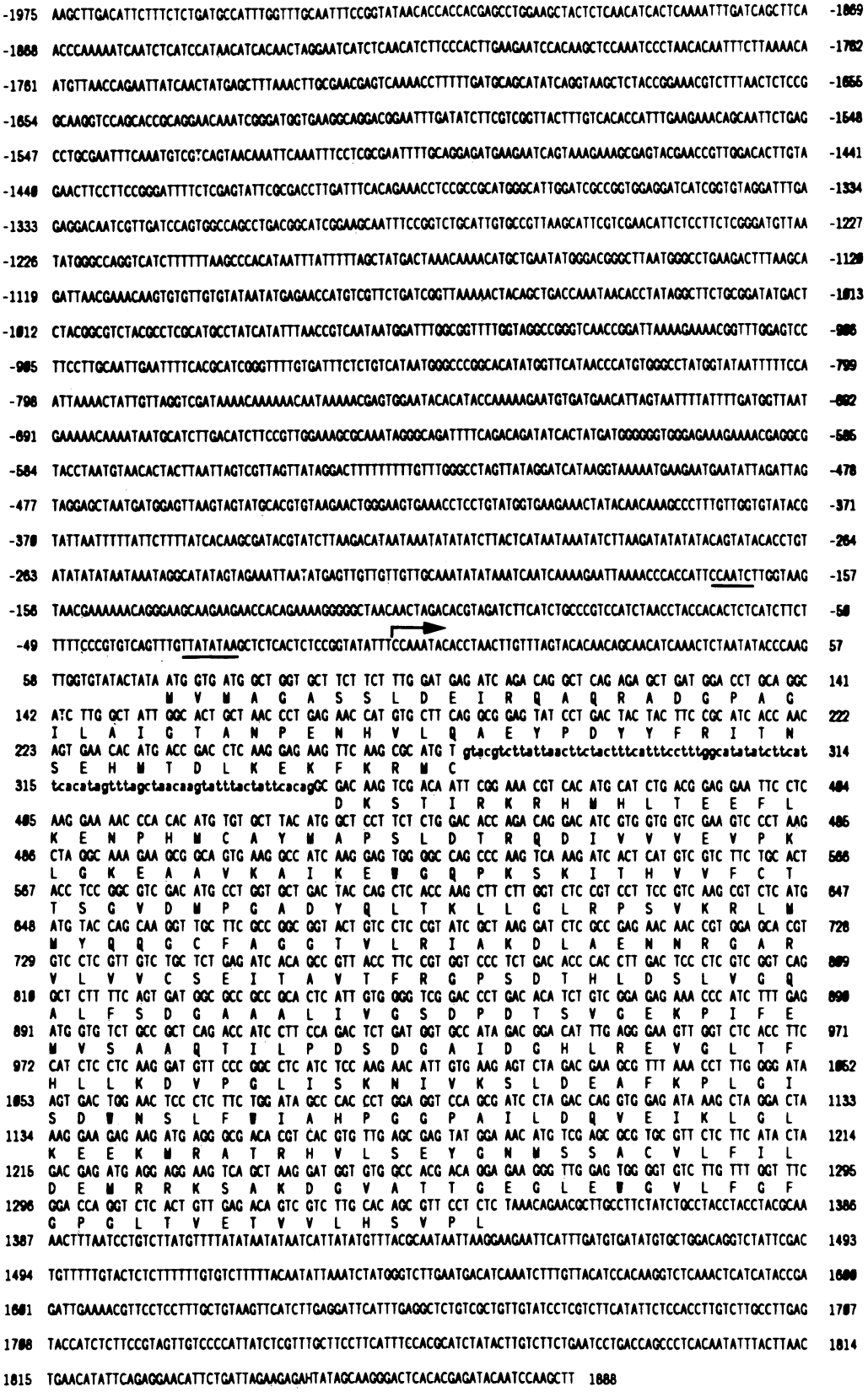

FIG. 2. Sequence of pCHS3.9 which includes the CHS-coding sequence as well as 5′- and 3′-flanking regions. The transcriptional start site (+1) is marked by an arrow, and putative promoter elements are underlined. The presence of an intron (positions 265 to 351) in the *A. thaliana* CHS gene was suggested by comparison with the *P. hortense* cDNA and is shown in lowercase letters.

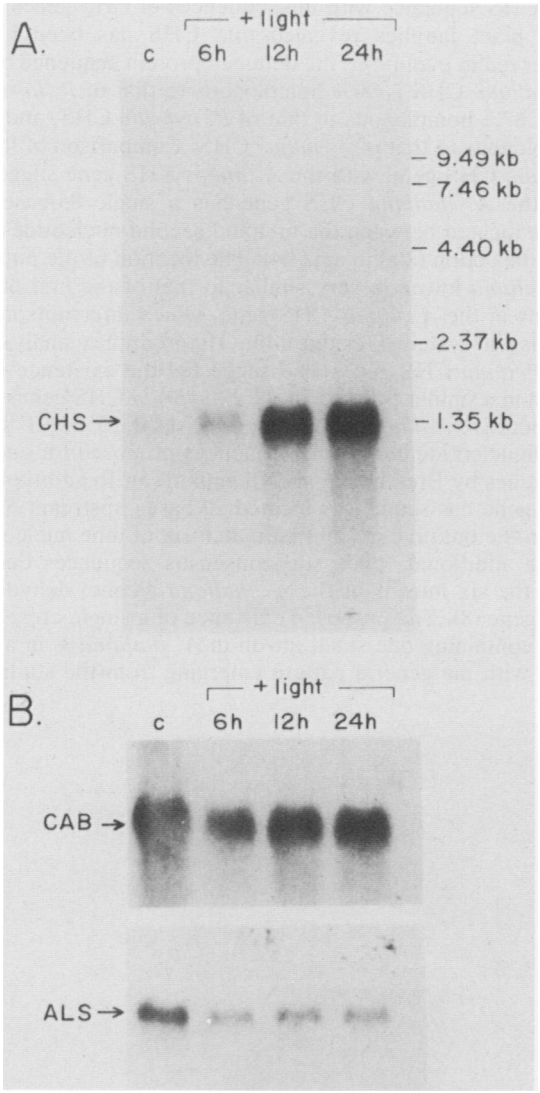

FIG. 3. Northern blot analysis of CHS mRNA levels in plants exposed to high-intensity light treatment. Poly(A)$^+$ RNA (1 μg) isolated from control plants and plants treated with high-intensity light for 6, 12, and 24 h was separated on agarose-formaldehyde gels and blotted onto GeneScreen filters. Blots were probed with nick-translated (A) pCHS3.9 (CHS) and (B) pCAB1.8 (chlorophyl *a/b*) or pBL104 (ALS). The positions of RNA size standards (BRL RNA ladder; Bethesda Research Laboratories, Inc., Gaithersburg, Md.) are indicated to the right in A.

due to a general accumulation of mRNA under high-intensity light conditions, because neither the total level of mRNA from the three chlorophyll *a/b*-binding protein genes (*cab*) nor the level of mRNA from the gene coding for acetolactate synthase (ALS), an enzyme involved in branched chain amino acid biosynthesis, was increased by the alteration in light conditions (Fig. 4b). In fact, the level of ALS mRNA appeared to decrease upon light treatment, which may be due to a negative effect of the high-intensity light treatment on expression of this gene. On the other hand, the rate of transcription of the ALS gene remained constant under these conditions (see below).

Rate of CHS transcription increased by light. The rate of CHS transcription was examined by nuclear runoff assays using nuclei isolated from plants that had been treated in parallel with the plants used for the CHS enzyme assays and steady-state mRNA analysis. In nuclear runoff assays, RNA transcripts are not initiated, but elongation of transcripts started before disruption of the plant cells occurs, providing a measure of the number of transcripts produced from a gene template at the time of nuclei isolation. Radiolabeled RNA isolated from nuclei of plants exposed to high-intensity light treatment (6-, 12-, 24-h time points) or control plants was used to probe slot blots containing DNA of various cloned plant genes.

Nuclei isolated from plants exposed to high-intensity light conditions for 6 h showed an increase in the level of transcription of the CHS gene over the nondetectable rate of CHS transcription in control nuclei (Fig. 4, compare lane 6h with lane c). The rate of CHS transcription appeared to continue to increase in nuclei isolated from plants treated with high-intensity light for 12 and 24 h (Fig. 4, lanes 12h and 24h).

It is likely that the increase in the rate of CHS transcription is a specific response to the alteration in light conditions, because the rate of transcription of neither the *A. thaliana* gene coding for ALS nor the *cab* genes was increased by the same treatment. The level of transcription of the gene coding for ALS, although very low, appeared to remain constant in all of the time points assayed. In contrast, the total rate of transcription of the three *A. thaliana cab* genes appeared to decrease in the plants treated with light for 6 h. It is unclear why this occurred, although *cab* mRNA levels are thought to fluctuate in a diurnal manner (18) and this could have affected the levels of *cab* transcription detected in this experiment. On the other hand, it may be that transcription of one or more of the *cab* genes is down-regulated by the high-intensity light conditions of this experiment. Finally, the level of transcription in the nucleus preparations from the different time points remained approximately the same; the total counts incorporated into RNA varied less than twofold between time points (data not shown). Therefore, exposure of *A. thaliana* plants to high-intensity light conditions resulted in a specific increase in the rate of transcription of the CHS gene concomitant with the accumulation of CHS mRNA and preceding the increase in CHS enzyme activity.

Identification of the 5′ end of the CHS mRNA. Primer

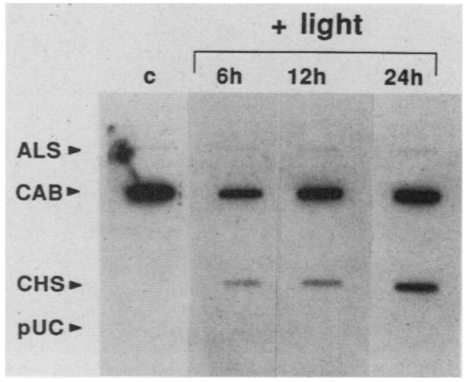

FIG. 4. Transcriptional analysis of the CHS gene after treatment of *A. thaliana* plants with high-intensity light. ^{32}P-labeled runoff transcripts from nuclei isolated after 6, 12, or 24 h of high-intensity light treatment and from control nuclei were used to probe slot blots of pBL1.04 (ALS), pCAB1.8 (chorophyll *a/b*), pCHS3.9 (CHS), and pUC12 (pUC). Each slot contained 5 μg of plasmid DNA that had been linearized with a unique restriction enzyme. All plasmids are derivatives of pUC.

extension and S1 nuclease analyses were used to map the 5′ start site of the CHS mRNA produced under both control and high-intensity light conditions. There were three major primer extension products generated in the analysis of CHS mRNA from plants treated for 24 h with high-intensity light (Fig. 5a, lane 24h). These transcripts mapped to the 2 C's and the T in the sequence CCAAAT located −69 to −74 nucleotides from the ATG start codon. In control plants, a low level of initiation at the same two C residues was also detectable. The light-independent bands which terminate a few bases downstream of these initiation sites may be due to premature termination by the reverse transcriptase as a result of secondary structure near the 5′ end of the message. S1 nuclease analysis confirmed that the two C residues at positions −73 and −74 correspond to the in vivo transcriptional initiation points for both control and high-intensity light-treated plants (Fig. 5b and c). In the S1 analysis, transcripts also appeared to start at the four bases, AAAT, downstream of the two C's. This may be due to artifactual S1 clipping within A+T-rich regions.

DISCUSSION

We isolated the CHS gene from *A. thaliana* and demonstrated that it is regulated transcriptionally in response to high-intensity light conditions. Comparison of the *A. thaliana* CHS sequence with the sequences of CHS genes from three plant families revealed that CHS has been highly conserved in evolution; the deduced protein sequence of the *A. thaliana* CHS is 90% homologous to that of *P. hortense* CHS, 87% homologous to that of *P. hybrida* CHS, and 85% homologous to that of *A. majus* CHS. Comparison of the *A. thaliana* CHS gene with the *A. majus* CHS gene suggested that the *A. thaliana* CHS gene has a single 85-base-pair intron located between the first and second nucleotides of a cysteine codon (amino acid 64). The location of the putative *A. thaliana* intron is very similar to that of the first of two introns in the *A. majus* CHS gene, which interrupts codon 60, also a cysteine residue (40). Heteroduplex analysis of two *Petunia* CHS genes also suggested the existence of an intron in a similar position in the *P. hybrida* CHS genes (32). The putative *A. thaliana* intron possesses the 5′ and 3′ splice site dinucleotide consensus sequences proposed for eucaryotic genes by Breathnach and Chambon (5). In addition, the CHS gene has sequences located 20 bases upstream of and within the putative intron that match six of nine nucleotides of the additional splice site consensus sequences derived from the six introns of the *A. thaliana* alcohol dehydrogenase gene (8). The proposed existence of a single-copy CHS gene containing one small intron in *A. thaliana* is in agreement with the general pattern emerging from the studies of

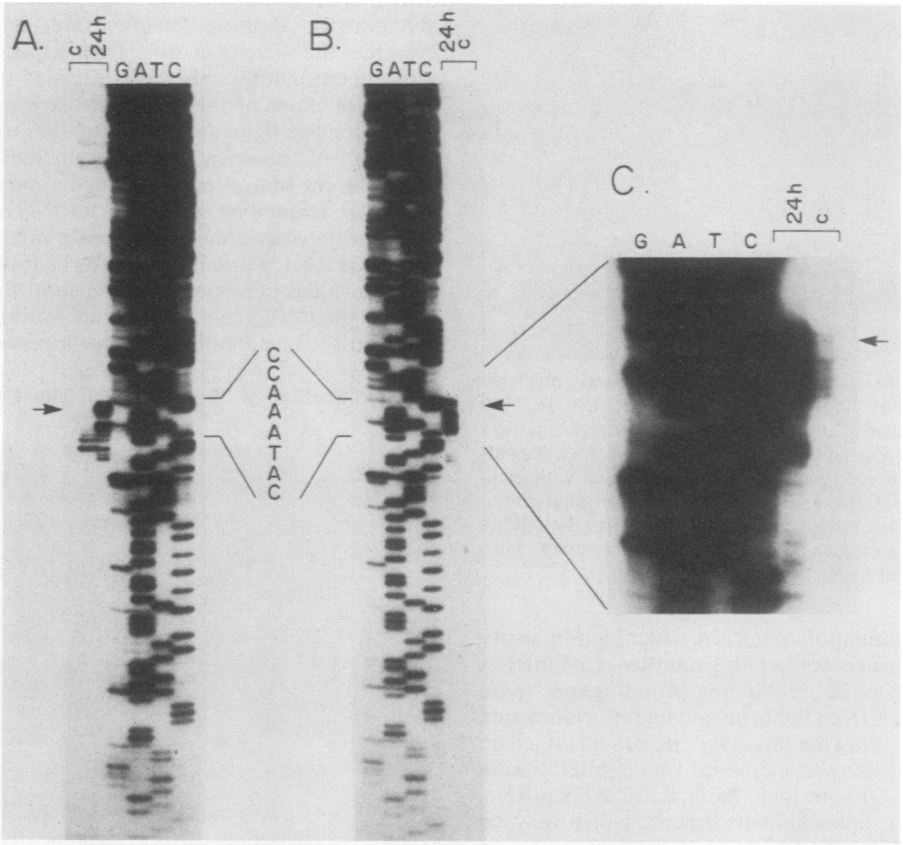

FIG. 5. Identification of the 5′ end of the CHS mRNA. (A) Primer extension analysis of 50 μg of total RNA isolated from both control plants and plants treated for 24 h with high-intensity, continuous light. The oligomer primer used included the first 30 bases of the CHS coding sequence (positions 73 through 102). Products were run on a 6% polyacrylamide sequencing gel and identified by autoradiography for 3 h. (B) S1 nuclease analysis of the same RNA used in the primer extension experiments with a probe that extended from positions −469 to +102. Products were separated and identified as in A. (C) Overexposure (12 h versus 3 h) of the autoradiogram shown in B to emphasize that the S1 products from control and plants treated with light for 24 h are identical. The arrows indicate transcripts that map to 2 C residues located −74 and −73 bases from the ATG codon.

the *A. thaliana* genome and gene structures; it appears that genes from *A. thaliana*, when compared with similar genes in other organisms, are represented as single or small gene families and have a minimal number of introns which are small in size (21, 27).

The 5′ transcriptional start site for the *A. thaliana* CHS mRNA was mapped to a site −74 bases from the translation start codon. The sequence upstream of this site was analyzed for potential promoter elements. There is an A+T-rich sequence, TATATAAG, located at positions −28 to −19 that may represent a TATA box (5). In addition, a perfect CCAATC box is present −175 bases from the transcriptional initiation site; however, for most eucaryotic genes, this particular sequence element is found within approximately 100 base pairs of the transcriptional start site (13, 25). In addition to potential TATA and CAAT boxes in the 5′ region of the *A. thaliana* CHS gene, there are several repeated sequences in the 2 kb of 5′-flanking region that might be involved in controlling expression of the CHS gene; the most striking is a repeat that occurs at −340 and −299 base pairs upstream from the transcriptional start site in which 16 of 19 bases are identical.

To identify sequences potentially important in the transcription of the *A. thaliana* CHS gene, the 5′-flanking sequence of this gene was compared with that of the CHS gene from *A. majus*, where deletion studies have identified two regions that are important in determining the level of CHS expression (17). However, these sequence comparisons failed to identify any regions of homology between the promoter regions of the *A. thaliana* and *A. majus* CHS genes. The only similarity was the presence of a sequence reminiscent of the simian virus 40 enhancer core, gTGG(A/T)(A/T)(A/T)g, in both promoters (43). At position −611 from the transcriptional start site, the *A. majus* CHS promoter contains the sequence GTGGTTAG, which is identical to the simian virus 40 core enhancer sequence. In a similar, although not identical, position in the *A. thaliana* CHS promoter (position −650 from the transcriptional start site) is the sequence TTGGAAAG, which has seven of eight bases in common with the simian virus 40 consensus sequence. Further experimentation will be required to define the functional units of the *A. thaliana* CHS promoter.

We have demonstrated that the *A. thaliana* CHS gene is induced transcriptionally by a change in light conditions. It has been previously shown that, in parsley tissue culture cells and in tobacco teratomas carrying a chimeric CHS gene, transcription of the CHS gene is induced by UV B light (280 to 320 nm) (9, 17). However, the induction of transcription of the *A. thaliana* CHS gene reported here is probably not due to the effect of UV light, for several reasons. First, very little UV B light should be produced by the high-intensity sodium lamps used in our experiments; the maximum emission of these lamps is in the yellow-orange range of the visible spectrum. Second, all plants were grown under sunlight before the high-intensity light treatment, and control plants remained under sunlight conditions. Therefore, all plants were exposed to some UV irradiation throughout their life cycle. This is in contast to previous experiments demonstrating UV induction of CHS where plant tissue was grown in a UV-free environment before induction with UV light. Thus, the induction of CHS transcription observed here may not be the same as that in previously reported responses.

Whether or not the high-intensity light response that we observed resulted from UV exposure, it is apparent that the *A. thaliana* plants are stressed under the conditions used. One observation that supports this conclusion is that plants placed under the high-intensity lamps remain small and bolt quickly. Both of these phenotypes are also observed when *A. thaliana* is planted densely or becomes desiccated, suggesting that they are general stress responses. In addition, the production of anthocyanins and other flavonoids is induced in a variety of plants by various environmental stresses such as cool temperatures, insufficient nutrients, and ionizing radiation (26). Therefore, the increase in CHS transcription observed in these experiments may be due to a high-intensity light-induced stress response.

An alternative explanation involves the role of light in the regulation of anthocyanin production. Light is known to stimulate anthocyanin production through a combination of mechanisms: (i) the red-far red phytochrome system, (ii) a blue or far-red high light intensity response (>1 J/cm^2 of light), (iii) a UV light response, and (iv) the availability of fixed carbon derived from photosynthesis that is used for production of precursors (1, 4, 23, 28). Thus, it is possible that the induction of CHS transcription in response to treatment with high-intensity light is mediated by one of the known photoreceptors.

Despite some uncertainty concerning the exact nature of the inducing signal, the expression of the *A. thaliana* CHS gene clearly is regulated at the transcriptional level. We are interested in identifying *trans*-acting regulatory factors responsible for the transcriptional regulation of this gene and hope to utilize the potential of *A. thaliana* as a genetic system for the isolation of mutants in such transcriptional factors. Ultimately, the identification and characterization of *trans*-acting regulatory factors will help to elucidate how plants perceive and respond to changes in their environment.

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