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Regulation of Flavonoid Biosynthetic Genes in Germinating Arabidopsis Seedlings

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Many higher plants, including Arabidopsis, transiently display purple anthocyanin pigments just after seed germination. We observed that steady state levels of mRNAs encoded by four flavonoid biosynthetic genes, *PAL1* (encoding phenylalanine ammonia-lyase 1), *CHS* (encoding chalcone synthase), *CHI* (encoding chalcone isomerase), and *DFR* (encoding dihydroflavonol reductase), were temporally regulated, peaking in 3-day-old seedlings grown in continuous white light. Except for the case of *PAL1* mRNA, mRNA levels for these flavonoid genes were very low in seedlings grown in darkness. Light induction studies using seedlings grown in darkness showed that *PAL1* mRNA began to accumulate before *CHS* and *CHI* mRNAs, which, in turn, began to accumulate before *DFR* mRNA. This order of induction is the same as the order of the biosynthetic steps in flavonoid biosynthesis. Our results suggest that the flavonoid biosynthetic pathway is coordinately regulated by a developmental timing mechanism during germination. Blue light and UVB light induction experiments using red light- and dark-grown seedlings showed that the flavonoid biosynthetic genes are induced most effectively by UVB light and that blue light induction is mediated by a specific blue light receptor.

INTRODUCTION

Flavonoids are 15-carbon compounds derived from phenylalanine and malonyl coenzyme A that are found in all higher plants. This large group of compounds serves as UV protectants (Schmelzer et al., 1988), signal molecules in plant–microbe interactions (Long, 1989), and antibiotics in plant defense responses (Dixon, 1986; Lamb et al., 1989). Subclasses of flavonoid compounds (tannins, anthocyanidins, and anthocyanins) are the brown, red, and purple pigments in flowers, fruits, seeds, and other plant tissues and organs (Brouillard, 1988). Genes required for flavonoid biosynthesis (flavonoid genes) are regulated in a tissue-specific manner during plant development as well as by a variety of environmental signals including blue and UV light, wounding, fungal elicitors, and infection by pathogenic fungi and bacteria (Mancinelli, 1983; Beggs et al., 1986; Bruns et al., 1986; Dixon, 1986; Senger and Schmidt, 1986; Lamb et al., 1989; Dangl, 1991).

We have been investigating the transient accumulation of purple flavonoid pigments around the rims of the cotyledons and in the epidermal layers of the hypocotyls in Arabidopsis seedlings. This coloration is under strict temporal control. It is most noticeable 3 to 4 days after germination and then dissipates to very low levels in 5-day-old seedlings. Although the transient accumulation of flavonoid pigments has been observed in germinating seedlings in many plants (Beggs et al., 1986; Ehmann et al., 1991), the function(s) of flavonoid compounds in seedlings is unknown. The ubiquity of this phenomenon would suggest that it is of some fundamental importance to the plant. One possibility is that flavonoids are produced prophylactically to protect seedlings against pathogens or UV light.

As part of the backdrop to a comprehensive molecular-genetic analysis of flavonoid gene regulation in Arabidopsis, we have monitored the light-induced accumulation of mRNAs encoded by four Arabidopsis flavonoid genes during the first week following germination. As illustrated in Figure 1, these four genes encode phenylalanine ammonia-lyase (PAL1), the first enzyme in the general phenylpropanoid pathway; chalcone synthase (CHS), the first enzyme in the flavonoid pathway; chalcone isomerase (CHI), the second enzyme in the flavonoid pathway; and dihydroflavonol reductase (DFR), the first enzyme leading to the production of anthocyanidins. Arabidopsis contains at least three genes that encode phenylalanine ammonia-lyase; the gene studied here, *PAL1*, is induced by wounding and pathogen attack (Davis and Ausubel, 1989; Davis et al., 1991; Dong et al., 1991; G.-L. Yu and F. Ausubel, unpublished results) and is expressed in a tissue-specific manner in Arabidopsis seedlings (Ohl et al., 1990). Previous studies from our laboratory have shown that Arabidopsis has a single *CHS* gene and that its transcription is induced by blue light in seedlings and by high-intensity white light stress in mature plants (Feinbaum and Ausubel, 1988; Feinbaum et al., 1991).

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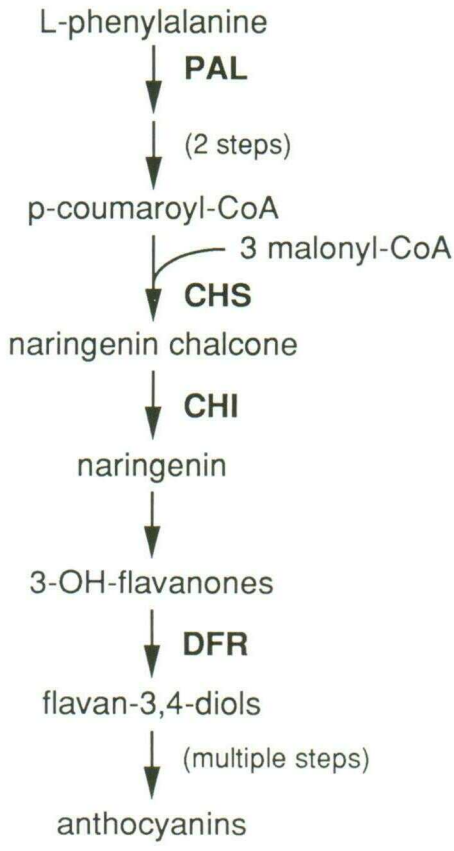

Figure 1. Simplified Schematic Representation of the Phenylpropanoid and Flavonoid Pathways.

The genes examined in this study are *PAL1*, *CHS*, *CHI*, and *DFR*. CoA, coenzyme A.

Unlike in legumes, the Arabidopsis *CHS* gene is not induced by pathogens in mature plants (Dong et al., 1991). Arabidopsis contains single *CHI* and *DFR* genes which are induced by high-intensity white light (Shirley et al., 1992).

Our results indicate that flavonoid gene expression in developing seedlings is under strict temporal control. This makes the study of phenylpropanoid gene regulation in Arabidopsis seedlings an attractive model for studying the relationships between developmental and environmental signals in gene regulation, the coordinate transcriptional regulation of biosynthetic pathways, and the role of flavonoids in plant development.

RESULTS

Transient Accumulation of Flavonoid Pigments in Arabidopsis Seedlings

As illustrated in Figure 2, Arabidopsis seedlings during the first few days following germination exhibit a characteristic pattern of purple pigmentation on the distal edges of the cotyledons and in the epidermal layers of the hypocotyls. This coloration is maximal in 4-day-old seedlings and then declines to almost nondetectable levels in 5-day-old seedlings. As shown in Figure 3, extraction and quantitation of anthocyanins from seedlings grown under continuous white light showed that accumulation of these compounds begins between days 2 and 3, reaches maximal levels near day 6, and starts to decline on day 7. For this figure, anthocyanin content was normalized per seedling, indicating that anthocyanin pigments were not simply being diluted during the rapid cell expansion that occurs at this stage of development.

Comparison of Figures 2 and 3 shows that the loss of purple coloration preceded the loss of anthocyanins as measured spectrophotometrically in 1% HCl/methanol extracts. This apparent discrepancy is reconciled by the fact that colorless leucoanthocyanidins are converted to pink anthocyanidins by the acid/methanol extraction procedure (Stafford, 1990). This result suggests that the disappearance of colored pigments in the seedlings could be caused by their conversion to colorless anthocyanidins.

Transient Accumulation of Flavonoid mRNAs in Arabidopsis Seedlings

To determine if the accumulation of anthocyanin pigments in Arabidopsis seedlings was accompanied by an increase in the levels of mRNAs corresponding to flavonoid genes, we isolated total RNA from seedlings grown under continuous white light or darkness on 6 consecutive days following germination and determined the steady state levels of *PAL1*, *CHS*, *CHI*,

Figure 2. Developing Arabidopsis Seedlings at 2, 3, 4, and 5 Days of Age.

The seedlings display the characteristic pattern of anthocyanin accumulation. They were grown under continuous white light on MS agar plates.

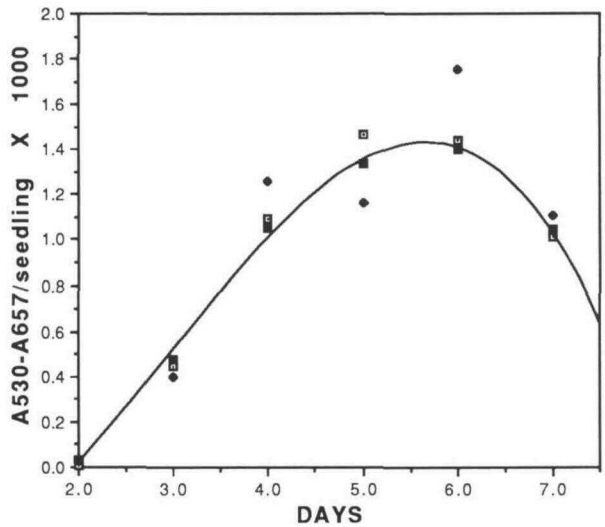

Figure 3. Quantitation of Anthocyanin Levels in Germinating Arabidopsis Seedlings.

Anthocyanins were extracted from Arabidopsis seedlings as described in Methods. Each day shows three independent experiments. Absorbance is at 530 nm minus absorbance at 657 nm.

and *DFR* mRNA by RNA gel blot hybridization analysis. The seedlings were grown under continuous light conditions to avoid circadian effects on patterns of gene expression. Germination frequencies approached 100% and growth of the seedlings was mostly synchronous with only a small percentage of the seedlings varying noticeably in developmental stage. For comparison, we also determined mRNA levels corresponding to one of the three Arabidopsis genes encoding chlorophyll *a/b* binding protein (CAB) because *CAB* gene mRNA accumulation is also regulated by light and should correlate with chloroplast development in seedlings (Karlin-Neumann et al., 1988). When Arabidopsis seedlings were grown in continuous white light (60 μmol m^{-2} sec^{-1}), steady state transcript levels corresponding to all four genes (*PAL1*, *CHS*, *CHI*, and *DFR*) were highest in 3-day-old seedlings, as shown in Figure 4A.

We had previously shown that *CHS* mRNA accumulation in Arabidopsis seedlings is dependent on light (Feinbaum et al., 1991). To determine whether *PAL1*, *CHI*, and *DFR* mRNA accumulation were also light-dependent, we isolated total RNA from Arabidopsis seedlings germinated and grown in total darkness. As shown in Figure 4B, only *PAL1* mRNA was readily observed in dark-grown seedlings. As in seedlings grown in light, *PAL1* mRNA levels reached a maximum in 3-day-old, dark-grown seedlings. Hybridization signals for *CHS*, *CHI*, and *DFR* mRNAs were only detected in RNA samples isolated from 3-day-old seedlings in autoradiographs exposed for several days (data not shown).

Because the same amount of total RNA was loaded into each lane in the experiment shown in Figure 4A, one explanation for the rapid decrease in hybridization signals for *PAL1*, *CHS*, *CHI*, and *DFR* mRNAs in 4-day-old seedlings is that the transcript levels of other genes increased dramatically, thus decreasing the relative proportion of flavonoid gene mRNAs. However, several lines of evidence suggest that flavonoid mRNA levels are down regulated. First, in contrast to the *PAL1*, *CHS*, *CHI*, and *DFR* transcript levels, *CAB* transcript levels were still increasing after flavonoid transcripts reached their maximum levels (Figure 4A). This result argues against a general decrease in mRNA levels relative to rRNA levels (the bulk of total RNA) and indicates that these two sets of genes are regulated by different mechanisms. Second, the total amount of RNA isolated from seedlings between the ages of 3 and 7 days varied at a maximum only two- to threefold, whereas the steady state transcript levels of the flavonoid genes varied by 10-fold or more. Third, when the same RNA gel blot was probed with an Arabidopsis actin gene, steady state transcript levels remained constant as a fraction of total RNA throughout the developmental time course (data not shown).

Evidence for a Specific Blue Light Receptor

To characterize which wavelengths of light are responsible for inducing flavonoid gene expression, light induction experiments were carried out using 3-day-old seedlings. For blue light induction experiments, seedlings were grown in either darkness, as shown in Figure 5A, or continuous red light, as shown in Figure 5B, at a sufficient fluence rate (3 μmol m^{-2} sec^{-1}) to saturate the phytochrome response (Frankland,

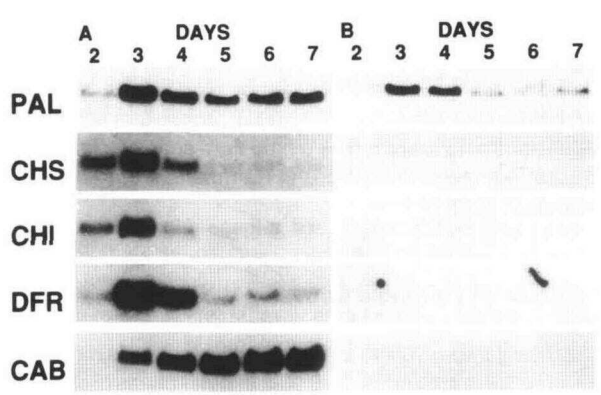

Figure 4. Accumulation of Flavonoid Gene mRNAs in Arabidopsis Seedlings.

Steady state transcript levels of *PAL1*, *CHS*, *CHI*, *DFR*, and *CAB* mRNAs were determined by RNA gel blot analysis as a function of seedling age (days 2 through 7) and light condition. The same RNA gel blot was probed with the indicated gene in each panel.

(A) Grown in continuous white light.

(B) Grown in continuous darkness.

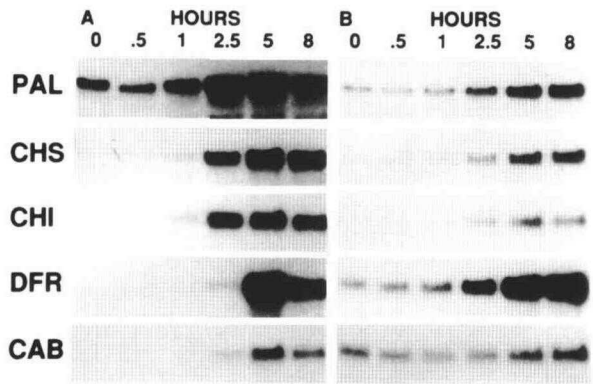

Figure 5. Kinetics of Flavonoid Gene Induction by Blue Light.

Steady state transcript levels of *PAL1*, *CHS*, *CHI*, *DFR*, and *CAB* were determined by RNA gel blot analysis in dark- or red light-grown, 3-day-old seedlings. Lane labeling indicates the number of hours after the beginning of blue light treatment that seedlings were harvested. The same RNA gel blot was probed with the indicated gene in each panel.

(A) Three-day-old, dark-grown seedlings.

(B) Three-day-old, red light-grown seedlings.

1986; see Methods for details). In both cases all four genes studied were induced by blue light. Flavonoid gene induction by blue light in red light-grown seedlings suggests a specific blue light receptor because any additional enhancement in transcript levels by blue light would indicate a photoreceptor other than phytochrome (Briggs and Iino, 1983; Warpeha and Kaufman, 1989, 1990). Compared to dark-grown seedlings, *CAB* mRNA levels were high in the red light-grown seedlings prior to blue light induction (Figure 5B). This was the expected result because *CAB* has been shown to be under phytochrome regulation (Karlin-Neumann et al., 1988). *CAB* transcript levels were also moderately induced by blue light in 3-day-old, red light-grown seedlings (Figure 5B), consistent with recent results showing that there is a blue light-specific component to *CAB* induction (Warpeha and Kaufman, 1990; Marrs and Kaufman, 1991).

Induction of Flavonoid Genes by UVB Light

Because genes involved in flavonoid biosynthesis have been shown to be regulated by UVB light in other plants (Schmelzer et al., 1988), we investigated the developmental dependence of flavonoid gene expression under continuous white light (60 μmol m^{-2} sec^{-1}) supplemented with 2 μmol m^{-2} sec^{-1} of UVB light (see Methods for details). The highest levels of mRNA accumulation for all four genes were observed in 3-day-old seedlings as in white light-grown seedlings, as shown in Figure 6. However, somewhat higher transcript levels were observed with UVB-supplemented white light rather than with white light alone. In the data presented here, the enhancement as a consequence of the added UVB light was most noticeable in 3-day-old seedlings.

We also carried out UVB light induction studies on dark-grown, 3-day-old seedlings. For these experiments, fluorescent lights were used that emit approximately half of their photons in a narrow band centered at 312 nm and half at several wavelengths in the blue region of the electromagnetic spectrum. The fluence rates of both UVB and blue light were 6 μmol m^{-2} sec^{-1}. To compare the effects of the UVB and blue light components of these lights on flavonoid gene expression, we induced 3-day-old, dark-grown seedlings under the UVB emitting lights with, as shown in Figure 7A, or without, as shown in Figure 7B, UVB-absorbing Mylar sheeting (see Methods for details).

Figures 7A and 7B show that expression of the flavonoid genes was highly induced by UVB light and that only a small part of this induction could be attributed to the wavelengths in the blue region of the spectrum (Figure 7A). Because fluence rates were approximately fivefold lower for UVB light than blue light, these results indicate that the expression of flavonoid genes is significantly more responsive to UVB light than to blue light. It is not clear from these experiments whether a separate UVB receptor is involved or whether there is a single blue/UVB receptor with an absorption maximum in the UV portion of the spectrum. In these experiments, UVB light did not induce *CAB* mRNA accumulation, whereas the blue portion of these UVB lights did result in some induction.

Sequential Induction of the *PAL1*, *CHS*, *CHI*, and *DFR* Genes

Within the resolution of the experiments illustrated in Figure 4, *PAL1*, *CHS*, *CHI*, and *DFR* mRNA accumulation appeared

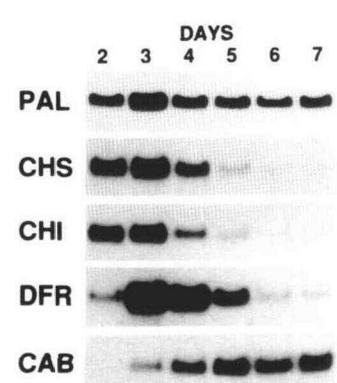

Figure 6. Accumulation of Flavonoid mRNAs in Arabidopsis Seedlings Grown under White Light Supplemented with UVB Light.

Steady state transcript levels of *PAL1*, *CHS*, *CHI*, *DFR*, and *CAB* mRNAs were determined by RNA gel blot analysis as a function of seedling age (days 2 through 7). The same RNA gel blot was probed with the indicated gene.

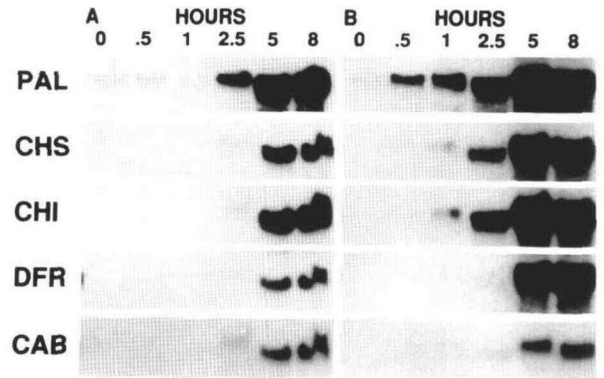

Figure 7. Kinetics of Flavonoid Gene Induction by UVB Light.

Steady state transcript levels of *PAL1*, *CHS*, *CHI*, *DFR*, and *CAB* were determined by RNA gel blot analysis in dark-grown, 3-day-old seedlings. Because UVB bulbs emit light in the blue range as well, parallel experiments were done with or without UVB-absorbing Mylar sheeting to determine the contribution of blue light alone. Lane labeling indicates the number of hours after the beginning of irradiation that seedlings were harvested. The same RNA gel blot was probed with the indicated gene in each panel.

(A) Three-day-old seedlings, with Mylar sheeting.

(B) Three-day-old seedlings, no Mylar sheeting.

to be coordinately regulated. However, the smaller time intervals used in the blue and UVB light induction experiments on etiolated seedlings illustrated in Figures 5A and 7B uncovered a sequential pattern of induction of these genes. Although mRNA levels of all four genes reached a maximum by 5 hr, the order of induction of the four genes is the same as the order in which the gene products are synthesized (Figure 1).

DISCUSSION

Flavonoid Gene Expression in Arabidopsis Seedlings Is under Precise Developmental Control

We have initiated a long-term study of flavonoid gene regulation in Arabidopsis seedlings by determining the steady state transcript levels corresponding to the *PAL1*, *CHS*, *CHI*, and *DFR* genes as a function of seedling age and light quality. We observed that accumulation of *PAL1*, *CHS*, *CHI*, and *DFR* mRNAs rose and fell during a 5-day period, reaching a maximum in 3-day-old, white light-grown seedlings. These same mRNA levels were very low in wild-type seedlings grown in darkness (Figures 4A and 4B). These data suggest that the potential for flavonoid gene expression to be induced by light during germination is controlled by a developmental timing mechanism that links seedling development with the synthesis of flavonoid compounds.

Because Arabidopsis *CHS* null mutants appear to grow and flower normally, it seems likely that flavonoids are nonessential for growth and development. Nevertheless, the precise regulation of flavonoid biosynthesis during seedling development suggests that flavonoids afford Arabidopsis some selective advantage, such as prophylaxis against pathogens or UV light. Because flavonoids are likely to be most useful as UV protectants just as the cotyledons expand and before the chloroplasts are fully developed, it is logical that flavonoid production and cotyledon expansion are coordinately regulated. Thus, when a seedling first perceives light, setting off cotyledon expansion and chloroplast development, the plant simultaneously begins to synthesize flavonoid compounds.

Flavonoid Biosynthetic Genes Are Induced by a Blue/UVB Light Receptor

The light induction experiments illustrated in Figures 5 and 7 showed that blue light and UVB light were effective in inducing the accumulation of *PAL1*, *CHS*, *CHI*, and *DFR* mRNAs. Furthermore, blue light induced additional accumulation of these transcripts in red light-grown plants (Figure 5B). These experiments indicate that a blue light-specific receptor is involved in the induction of the flavonoid biosynthetic genes. In our experiments, UVB light was much more efficient than blue light for inducing *PAL1*, *CHS*, *CHI*, and *DFR* mRNA accumulation in Arabidopsis seedlings. This result supports the hypothesis that flavonoid compounds act as UVB protectants (Schmelzer et al., 1988).

Induction of flavonoid and anthocyanin biosynthesis by various environmental stimuli including blue and UV light is a common theme among higher plants (Wellmann, 1971; Oelmüller and Mohr, 1985; Beggs et al., 1986; Rabino and Mancinelli, 1986; Sponga et al., 1986; Marrs and Kaufman, 1989, 1991). Several studies, mostly on *CHS* gene expression, have shown that the production of flavonoid and anthocyanin compounds in response to light is controlled at least in part at the level of transcription (Feinbaum and Ausubel, 1988; van Tunen et al., 1988; Taylor and Briggs, 1990; Feinbaum et al., 1991). For example, in parsley tissue culture cells *CHS* gene expression has been shown to be regulated by a UVB light receptor, a blue light receptor, and phytochrome (Bruns et al., 1986; Ohl et al., 1989). Our results are consistent with previous studies on *CHS* gene regulation and go on to confirm that other genes within the pathway are similarly regulated. Considering the utility of Arabidopsis in genetic and molecular studies and the fact that at least three flavonoid genes (*CHS*, *CHI*, and *DFR*) exist as single copies in its genome, Arabidopsis provides a powerful system with which to dissect flavonoid gene regulation.

Sequential Induction of Flavonoid Genes

Coordinate induction of flavonoid biosynthetic genes has been demonstrated in snapdragon flowers (Martin et al., 1991). The experiments shown in Figures 5 and 7 suggest that flavonoid

genes are sequentially induced in the order of the biosynthetic steps in the flavonoid pathway. The sequential induction of flavonoid biosynthetic enzymes may permit sufficient levels of precursor molecules to accumulate to ensure efficient enzyme function. This level of regulation may be achieved by feed-forward or feedback mechanisms utilizing phenylpropanoid intermediates themselves. For example, *trans*-cinnamic and *trans-p*-coumaric acid have been shown to regulate a bean *CHS* promoter in alfalfa protoplasts that have been induced by a fungal elicitor (Loake et al., 1991). In addition, the independent regulation of the genes or transcripts encoding these enzymes could also provide flexibility for the synthesis of a wide variety of compounds in response to different internal and external stimuli.

METHODS

Plants and Growth Conditions

Arabidopsis thaliana ecotype Landsberg *erecta* was used in these studies. Seeds were soaked in water for approximately 1 hr, then surface sterilized by soaking in 30% bleach, 0.1% Triton X-100 for 7 min, washed 5 times in sterile distilled water, and suspended in 0.1% agar. Seeds were spread in 2 mL of soft agar on MS (Murashige and Skoog, 1962) agar (0.8%) containing 2% sucrose in 8-cm-diameter Petri plates. The plates were covered and sealed with surgical tape (Transpore 1527-0; 3M Co., St. Paul, MN) that allows the passage of gases. Plants were grown in Percival (Boone, IA) or Conviron (Asheville, NC) growth chambers at 22°C under various light conditions.

For experiments utilizing white light supplemented with UVB light, four white fluorescent (40CW; General Electric Co., Wilmington, MA) lamps and one UVB (UV, TL40W; Philips Electronic Instruments, Mahwah, NJ) fluorescent lamp were mounted in the growth chamber. Fluence rates were 60 μmol m^{-2} sec^{-1} of visible light and 2 μmol m^{-2} sec^{-1} UVB light. The white light fluence rate was empirically chosen because it supported healthy plant growth. Light levels were measured using a light meter (model Li-1858; Li-Cor, Lincoln, NE) and a spectral emission detector (model 742; Optronix, Chelmsford, MA). White light was obtained using this same apparatus except that a polyester sheet (Mylar, 5 mil thickness; Cadillac plastics 2299001, Baltimore, MD) was placed between the plants and the lights to filter out UVB light. Dark conditions were achieved by wrapping plates in three to six layers of aluminum foil and/or placing the samples in a light-tight box. Red light conditions were achieved using white lights (Sylvania, Danvers, MA) filtered through two layers of red plexiglass (Shinkolite 102; Argo Plastics, Los Angeles, CA). UVB induction experiments utilized three UV lamps (UV, TL40W; Philips Electronic Instruments, Mahwah, NJ) that emitted 6 μmol m^{-2} sec^{-1} in the blue light and 6 μmol m^{-2} sec^{-1} in the UVB range. No UVC light was detected in the emission spectrum of these lamps. However, to ensure the absence of UVC wavelengths, seedlings were grown in covered Petri plates because the lids absorb UVC wavelengths. For blue light induction experiments, blue lights (F24T12/247/HO; Sylvania) were filtered through royal blue plexiglass (Plexiglas 2424; Future Plastics, Inc., Watertown, MA). The blue light fluence rate was 30 μmol m^{-2} sec^{-1}.

Anthocyanin Extraction and Quantitation

Anthocyanins were extracted using 1% HCl/methanol as described previously (Feinbaum and Ausubel, 1988). In these experiments 200 to 300 seedlings were grown on MS agar Petri plates under continuous white light. The number of seedlings on each plate was counted and seedlings were harvested by scraping the seedlings, including the roots, off the agar with a microscope slide. One plate was harvested on each day from day 2 to day 7 after planting (the day of planting being day 0). Harvested seedlings were placed in 15-mL plastic tubes, immediately frozen in liquid nitrogen, and stored at −80°C. Anthocyanins were extracted in 1 mL of 1% HCl/methanol overnight at room temperature using the method of Rabino and Mancinelli (1986). Absorbance at 530 nm minus absorbance at 657 nm was used as a measure of anthocyanin content and was normalized per seedling. The experiment was performed in triplicate.

Developmental Time Course and Light Induction Experiments

All developmental time courses and light induction experiments utilized MS agar Petri dishes planted with approximately 800 seeds each. In the developmental studies, seedlings from one Petri plate were harvested each day (as described above) from day 2 to day 7 after planting (the day of planting being day 0). Red light- and dark-grown samples were transferred in a light-tight box to a dark room and harvested under a dim green safe light (15 watt incandescent light filtered through a Kodak Safelight No. 7). All other samples were harvested in the light. Each light induction experiment included six time points. Each point consisted of one Petri plate containing approximately 800 seedlings.

Blue light induction experiments were performed using plants grown for 3 days in darkness or red light. Plates were transferred to the blue light chamber using a light-tight box and harvested after 0.5, 1.0, 2.5, 5, and 8 hr of light treatment. The plants used for the 0 time point were never exposed to blue light and were harvested under a dim green safe light. Seedlings were harvested as described above. UVB induction experiments on 3-day-old, dark-grown seedlings were carried out similarly to the blue light experiments. Because UVB lights also emit at wavelengths in the blue region, parallel inductions were performed with and without UVB-absorbing Mylar sheeting. In all cases, harvested seedlings were placed in 15 mL-plastic tubes, immediately frozen in liquid nitrogen, and stored at −80°C prior to RNA extraction. Each experiment was repeated at least once.

Photographs of Arabidopsis seedlings were made using a dissection microscope (Zeiss, Oberkochen, Germany) equipped with an illuminator (Fiberlite, series 180; Dolan-Jenner, Woburn, MA; with a General Electric MR-16Q bulb) filtered through a blue filter (model 3202; Rosco, Port Chester, NY).

RNA Isolation and RNA Gel Blot Analysis

RNA was isolated by phenol-SDS extraction and LiCl precipitation (Ausubel et al., 1987). RNA samples (6 μg) were electrophoresed in formaldehyde-agarose gels, stained with ethidium bromide to check for uniformity of gel loading by comparing the intensity of rRNA bands, and then transferred to nylon filters (BioTrans; ICN, Irvine, CA) in 10 × SSC (1 × SSC, is 0.15 M NaCl, 0.015M sodium citrate). Filters were treated with UV light using a Stratalinker (Stratagene) to cross-link the RNA to the filters and then baked at 80°C for 1 hr. Plasmid inserts

from pAtPa1R1 (Dong et al., 1991), pCHS3.8 (Feinbaum and Ausubel, 1988), pCHI4.8 (Shirley et al., 1992), and pDFR4.8 (Shirley et al., 1992) were isolated on low-melting agarose-TAE (Tris-acetate-EDTA) gels. Probes were prepared from the inserts using a random primer labeling kit (Boehringer Mannheim) with α-^{32}P-dCTP (*PAL* and *CHS*) or using the random primer method (Feinberg and Vogelstein, 1983) with α-^{32}P-dATP (*CHI* and *DFR*), which were separated from unincorporated nucleotides on a 1-mL Sephadex G-50 spin column (Pharmacia) and denatured either by boiling (*PAL* and *CHS*) or by using a microwave oven (*CHI* and *DFR*; Stroop and Schaefer, 1989). The filters were prehybridized (1 hr) and hybridized (14 to 16 hrs) in 0.5 M Na_2HPO_4, pH 7.2, 7% SDS, 10 μg/mL BSA (Church and Gilbert, 1984), then washed twice, 45 min each time in 2 × SSC, 1% SDS at 65°C (*PAL* and *CHS*) or 30 min each time in 40 mM $NaHPO_4$, pH 7.2, 1 mM EDTA, 1% SDS at 65°C (Church and Gilbert, 1984). The damp filters were autoradiographed with Kodak XAR film at −80°C with an intensifying screen. Filters were stripped in 2 mM Tris, pH 8.0, 2 mM EDTA at 70°C for 15 min prior to reprobing (Church and Gilbert, 1984).

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REFERENCES

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K. (eds) (1987). Current Protocols in Molecular Biology. (New York: Greene Publishing Associates/Wiley-Interscience).

Beggs, C.J., Wellmann, E., and Griesbach, H. (1986). Photocontrol of flavonoid biosynthesis. In Photomorphogenesis in Plants, R.E. Kendrick and G. M. H. Kronenberg, eds (Dordrecht, The Netherlands: Martinus Nijhoff/Dr. Junk), pp. 467–491.

Briggs, W.R., and Iino, M. (1983). Blue light absorbing photoreceptors in plants. Phil. Trans. R. Soc. Lond. B. Biol. Sci. **303,** 19347–19359.

Brouillard, R. (1988). Flavonoids and flower colour. In The Flavonoids: Advances in Research Since 1980, J.B. Harborne, ed (London: Chapman and Hall), pp. 525–538.

Bruns, B., Hahlbrock, K., and Schäfer, E. (1986). Fluence dependence of the ultraviolet-light-induced accumulation of chalcone synthase mRNA and effects of blue and far-red light in cultured parsley cells. Planta **169,** 19393–19398.

Church, G.M., and Gilbert, W. (1984). Genomic sequencing. Proc. Natl. Acad. Sci. USA **81,** 1991–1995.

Dangl, J.L. (1991). Regulatory elements controlling developmental and stress induced expression of phenylpropanoid genes. In Plant Gene Research, Vol 8: Genes Involved in Plant Defense, T. Boller and F. Meins, eds (New York: Springer-Verlag).

Davis, K.R., and Ausubel, F.M. (1989). Characterization of elicitor-induced defense responses in suspension-cultured cells of *Arabidopsis*. Mol. Plant–Microbe Interact. **2,** 363–368.

Davis, K.R., Schott, E., and Ausubel, F.M. (1991). Virulence of selected phytopathogenic pseudomonads in *Arabidopsis thaliana*. Mol. Plant–Microbe Interact. **4,** 477–488.

Dixon, R.A. (1986). The phytoalexin response: Eliciting, signaling and control of host gene expression. Biol. Rev. **61,** 239–291.

Dong, X., Mindrinos, M., Davis, K.R., and Ausubel, F.M. (1991). Induction of Arabidopsis defense genes by virulent and avirulent *Pseudomonas syringae* strains and by a cloned avirulence gene. Plant Cell **3,** 61–72.

Ehmann, B., Ocker, B., and Schäfer, E. (1991). Development- and light-dependent regulation of the expression of two different chalcone synthase transcripts in mustard cotyledons. Planta **183,** 416–422.

Feinbaum, R.L., and Ausubel, F.M. (1988). Transcriptional regulation of the *Arabidopsis thaliana* chalcone synthase gene. Mol. Cell Biol. **8,** 1985–1992.

Feinbaum, R.L., Storz, G., and Ausubel, F.M. (1991). High intensity and blue light regulated expression of chimeric chalcone synthase genes in transgenic *Arabidopsis thaliana* plants. Mol. Gen. Genet. **226,** 449–456.

Feinberg, A.B., and Vogelstein, B. (1983). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132,** 6–13.

Frankland, B. (1986). Perception of light quantity. In Photomorphogenesis in Plants, R.E. Kendrick and G.M.H. Kronenberg, eds (Dordrecht, The Netherlands: Martinus Nijhoff/Dr. Junk), pp. 219–234.

Karlin-Neumann, G.A., Sun, L., and Tobin, T.M. (1988). Expression of light-harvesting chlorophyll *a/b*-protein genes is phytochrome-regulated in etiolated *Arabidopsis thaliana* seedlings. Plant Physiol. **88,** 1323–1331.

Lamb, C.J., Lawton, M.A., Dron, M., and Dixon, R.A. (1989). Signals and transduction mechanisms for activation of plant defenses against microbial attack. Cell **56,** 215–224.

Loake, G.L., Choudhary, A.D., Harrison, M.J., Mavandad, M., Lamb, C.J., and Dixon, R.A. (1991). Phenylpropanoid pathway intermediates regulate transient expression of a chalcone synthase gene promoter. Plant Cell **3,** 829–840.

Long, S. (1989). *Rhizobium*-legume nodulation: Life together in the underground. Cell **56,** 203–214.

Mancinelli, A.L. (1983). The photoregulation of anthocyanin synthesis. Encyclopedia of Plant Physiology, Vol. 16B, W. Shropshire, Jr. and H. Mohr, eds (New York: Springer-Verlag), pp. 640–661.

Marrs, K.A., and Kaufman, L.S. (1989). Blue-light regulation of transcription for nuclear genes in pea. Proc. Natl. Acad. Sci. USA **86,** 4492–4495.

Marrs, K.A., and Kaufman, L.S. (1991). Rapid transcriptional regulation of the *Cab* and pEA207 gene families in peas by blue light in the absence of cytoplasmic protein synthesis. Planta **183,** 327–333.

Martin, C., Prescott, A., Mackay, S., Bartlett, J., and Vrijlandt, E. (1991). Control of anthocyanin biosynthesis in flowers of *Antirrhinum majus*. Plant J. **1**, 37–49.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**, 473–479.

Oelmüller, R., and Mohr, H. (1985). Mode of coaction between blue/UV light and light absorbed by phytochrome in light-mediated anthocyanin formation in the milo (*Sorghum vulgare* Pers.) seedlings. Proc. Natl. Acad. Sci. USA **82**, 6124–6128.

Ohl, S., Hahlbrock, K., and Schäfer, E. (1989). A stable blue-light-derived signal modulates ultraviolet-light-induced activation of the chalcone-synthase gene in cultured parsley cells. Planta **177**, 228–236.

Ohl, S., Hedrick, S.A., Chory, J., and Lamb, C.J. (1990). Functional properties of a phenylalanine ammonia-lyase promoter from *Arabidopsis*. Plant Cell **2**, 837–848.

Rabino, I., and Mancinelli, A.L. (1986). Light temperature and anthocyanin production. Plant Physiol. **81**, 922–924.

Schmelzer, E., Jahnen, W., and Hahlbrock, K. (1988). *In situ* localization of light-induced chalcone synthase mRNA, chalcone synthase, and flavonoid end products in epidermal cells of parsley leaves. Proc. Natl. Acad. Sci. USA **85**, 2984–2993.

Senger, H., and Schmidt, W. (1986). Cryptochrome and UV receptors. In Photomorphogenesis in Plants, R.E. Kendrick and G.M.H. Kronenberg, eds (Dordrecht, The Netherlands: Martinus Nijhoff/Dr. Junk), pp. 137–156.

Shirley, B. W., Hanley, S., and Goodman, H.M. (1992). Effects of ionizing radiation on a plant genome: Analysis of two Arabidopsis *transparent testa* mutations. Plant Cell **4**, 333–347.

Sponga, F., Deitzer, G.F., and Mancinelli, A.L. (1986). Cryptochrome, phytochrome, and the photoregulation of anthocyanin production under blue light. Plant Physiol. **82**, 952–955.

Stafford, H.A. (1990). Flavonoid Metabolism (Boca Raton, FL: CRC Press, Inc.).

Stroop, W.G., and Schaefer, D.C. (1989). Comparative effect of microwaves and boiling on the denaturation of DNA. Anal. Biochem. **182**, 222–225.

Taylor, L.P., and Briggs, W.R. (1990). Genetic regulation and photocontrol of anthocyanin accumulation in maize seedlings. Plant Cell **2**, 115–127.

van Tunen, A.J., Koes, R.E., Spelt, C.E., van der Krol, A.R., Stuitje, A.R., and Mol, J.N.M. (1988). Cloning of two chalcone flavanone isomerase genes from *Petunia hybrida*: Coordinate, light-regulated, and differential expression. EMBO J. **7**, 1257–1263.

Warpeha, K.M.F., and Kaufman, L.S. (1989). Blue-light regulation of epicotyl elongation in *Pisum sativum*. Plant Physiol. **89**, 544–548.

Warpeha, K.M.F., and Kaufman, L.S. (1990). Two distinct blue-light responses regulate the levels of transcripts of specific nuclear-coded genes in pea. Planta **182**, 553–558.

Wellmann, E. (1971). Phytochrome-mediated flavone glycoside synthesis in cell suspension cultures of *Petroselinum hortense* after pre-irradiation with ultraviolet light. Planta **101**, 283–286.