Photocontrol of the Expression of Genes Encoding Chlorophyll a/b Binding Proteins and Small Subunit of Ribulose-1,5-Bisphosphate Carboxylase in Etiolated Seedlings of Lycopersicon esculentum (L.) and Nicotiana tabacum (L.)

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ABSTRACT

Phytochrome and the blue ultraviolet-A photoreceptor control light-induced expression of genes encoding the chlorophyll a/b binding protein of photosystem II and photosystem I and the genes for the small subunit of the ribulose-1,5-bisphosphate carboxylase in etiolated seedlings of Lycopersicon esculentum (tomato) and Nicotiana tabacum (tobacco). A ‘high irradiance response’ also controls the induction of these genes. Genes encoding photosystem II- and I-associated chlorophyll a/b binding proteins both exhibit a transient rapid increase in expression in response to light pulse or to continuous irradiation. In contrast, genes encoding the small subunit exhibit a continuous increase in expression in response to light. These distinct expression characteristics are shown to reflect differences at the level of transcription.

Higher plants have several photoreceptors which detect light quality and intensity. The major photoreceptors are phytochrome (26), which control induction and ‘high irradiance responses’ (HIR2) (10), a blue/UV-A photoreceptor (27), and a UV-B photoreceptor (34).

Studies of expression of nuclear genes encoding CAB, SSU, and CHS proteins revealed that photoregulation of gene expression in higher plants occurs at transcriptional and posttranscriptional levels (33). The dependence of gene expression on light quality and intensity varies for different genes and different species. For instance, in parsley cell suspension cultures excitation of the UV-B photoreceptor is essential for maximal expression of CHS, while the excitation of the blue/UV-A photoreceptor and phytochrome control the level of the UV-B-induced gene expression (22). Expression of CAB and SSU genes, which are encoded by multigene families in monocots and in dicots (4, 5, 24, 31), is controlled by phytochrome (2, 33). The regulation of these genes is mediated, at least in part, at the level of transcription (3, 22, 33). For etiolated pea seedlings, it was demonstrated that the kinetics of red light induction are quite different for CAB and SSU genes. In addition, CAB gene expression occurs in the very low fluence and low fluence ranges whereas SSU gene expression occurs only in the fluence range (14).

It is well established that the development of both PSI and PSII is controlled by light quality and intensity (1). Thus, it is expected that an interaction of photoreceptors controls the accumulation of CAB and SSU mRNA. The purpose of the present research was to determine which photoreceptors control the expression of these genes and whether the CAB genes of the two photosystems are regulated differently. Etiolated tomato and tobacco seedlings were chosen because these plants can be used for subsequent transgenic studies. Some light-induced gene expression studies have been reported for tomato (29, 31), but there are no detailed studies of light-induced gene expression for either species. The results presented in this paper will be useful as a basis for a detailed combined photobiological and molecular study of the promoters of CAB and SSU genes using seedlings from transformed tomato and tobacco plants.

MATERIALS AND METHODS

Plant Material and Growth

Lycopersicon esculentum cv Hilds Matina (tomato) seedlings were grown for 4 d in darkness (25°C) in a box (8 × 8 × 5.5 cm) containing four sheets of filter paper (Schleicher & Schüll, Dassel, FRG) and 3.5 mL water. Nicotiana tabacum cv Virginia (tobacco) seedlings were grown for 5 d in darkness (25°C) on Hoagland medium (containing 0.6 g agar/100 mL). Immediately upon harvest plant material was frozen in liquid nitrogen and stored at −80°C.
Light Treatments of Etiolated Seedlings

Light sources were as follows. UV light was obtained from three Osram (Berlin, FRG) L 40 W/73 tubes ($\lambda_{\text{max}} = 350$ nm, half bandwidth 40 nm), fluence rate was $1.9 \times 10^4$ $\mu$mol m$^{-2}$ s$^{-1}$. Blue light ($\lambda_{\text{max}} = 436$ nm, $1.9 \times 10^4$ $\mu$mol m$^{-2}$ s$^{-1}$, half bandwidth 43 nm), far-red light ($\lambda_{\text{max}} = 730$ nm, $6.0 \times 10^3$ $\mu$mol m$^{-2}$ s$^{-1}$) and red light ($\lambda_{\text{max}} = 658$ nm, $1.9 \times 10^4$ $\mu$mol m$^{-2}$ s$^{-1}$, half bandwidth 15 nm) were obtained from standard sources (19). Long wavelength far-red light was obtained from a Leitz Prado projector combined with a far-red glass filter (Schott RG 9, 5 mm thick, $\lambda_{\text{max}} = 775$ nm, $6.2 \times 10^3$ $\mu$mol m$^{-2}$ s$^{-1}$, Schott, Mainz, FRG). White light was obtained from 6 Osram HQ IL bulbs. The fluence rate was $1.4 \times 10^4$ $\mu$mol m$^{-2}$ s$^{-1}$.

Plasmids

The following plasmids were used. PSII CAB, a 0.9 kbp EcoRV EcoRV fragment of the genomic cab-1A gene of tomato, including ca. 50 bp of the 5' upstream region and ca. 160 bp of the 3' downstream region, cloned into the vector pUC 18 (23). PSI CAB, a 0.9 kbp SstI-XbaI fragment of the cDNA of the tomato cab-6 gene, cloned into the vector pGEM 4 (11). SSU, a 0.8 kbp Psfl-Psil fragment of the cDNA of the tomato rbcS-3A gene, cloned into the vector pUC 9 (24). Actin, a 3.0 kbp HindIII-HindIII fragment of the soybean actin gene, cloned into the vector pBR 322 (28).

Chemicals

Restriction endonucleases and nick translation kits were purchased from Bethesda Research Laboratory; radioactive materials were purchased from Amersham Buchler.

RNA Preparation

RNA was extracted from tomato cotyledons or whole tobacco seedlings using an extraction buffer of 5 M guanidinium thiocyanate, 25 mM sodium citrate, 0.5% Sarkosyl, 2 mM EDTA and 0.1 M β-mercaptoethanol. After phenol/chloroform (1:1 [v/v]) extraction, nucleic acids were precipitated. Final DNA contamination was removed by selectively pelleting the RNA in 2 M LiCl. One g of tissue yielded about 1 mg of total RNA (tomato cotyledons) or 250 µg of total RNA (etiolated tobacco seedlings).

Northern Blot Analysis

For Northern blots, 10 µg of RNA was denatured in 40% formamide, 15% formaldehyde, 1× Mops buffer (= 20 mM Mops (pH 7.0), 5 mM sodium acetate, 1 mM EDTA) at 65°C for 10 min and separated on an agarose-formaldehyde gel (1.2% agarose (w/v) in 1× Mops buffer containing 15% formaldehyde) (17). The RNA was transferred in 10× SSC (1× SSC = 0.15 M NaCl, 0.015 M sodium citrate). The filter bound RNA was hybridized to cab-1A 32P-nick-translated DNA. Hybridization was at 42°C in a buffer consisting of 5

Figure 1. PSII CAB, PSI CAB, and SSU mRNA accumulation after treatment with light pulses. Graphs of the relative mRNA amounts of etiolated tomato (A) and tobacco (B) seedlings. RNA was extracted 1, 2, 4, 6, and 8 h after onset of a 5 min red light pulse (r), 5 min red light pulse followed by 5 min long-wavelength far-red light (RG light) pulse (fr) and 5 min long-wavelength far-red light (RG9 light) pulse (fr). Time = 0 represents the mRNA amount in darkness.
× SSC, 5 × Denhardt solution, 0.5% (w/v) SDS, 20 μg/mL salmon sperm DNA and 50% (v/v) formamide. The stringency of the final wash was 2 × SSC, 1% (v/v) SDS at 65°C. Filters were exposed to x-ray film for 16 h with intensifying screens.

**Dot Blot Analysis**

For preparation of dot blots, a dilution series of 0.3 to 5.0 μg denatured RNA was dotted onto nitrocellulose as described elsewhere (9). The nitrocellulose bound RNA was hybridized to either cab-1A, cab-6, or rbcS-3A 32P-nick-translated DNA. Hybridization was at 42°C in a buffer of 5 × SSC, 50 mM sodium phosphate (pH 7.0), 10 × Denhardt solution, 1% (w/v) SDS, 10 μg/mL salmon sperm DNA, and 50% (v/v) formamide. The stringency of the final wash was 2 × SSC, 1% SDS at 65°C. Filters were exposed with intensifying screens for 16 h for hybridization to cab-1A and rbcS-3A, and for 42 h for hybridization to cab-6. For quantification, dots were cut out and filter-bound radioactivity was determined in liquid scintillation counter. Data are averages of at least two independent experiments except for one (see “Results”). Differences between replicates were 10 to 15%.

**Isolation of Chromatin and Run-On Transcription**

Chromatin was isolated from whole etiolated tobacco seedlings as described elsewhere (30). A preparation starting from 50 g of plant material yielded chromatin which typically contained 4 × 10^7 nuclei. Preparative nuclear run-on transcription assays were performed as described (22) using 2 × 10^7 nuclei per assay in a total volume of 1.2 mL. The nitrocellulose bound DNA (cab-1A, cab-6, rbcS-3A, actin, and pSP64) was hybridized to in vitro, 32P-labeled RNA transcripts. Hybridization, washing procedure, and quantitation were carried out as described for the dot blot analysis. Data were averaged from two independent experiments. Replicates differed by 10 to 15%.

**RESULTS**

**Phytochrome Mediates the Expression of CAB Genes**

Etiolated seedlings were irradiated with a light pulse and then transferred to darkness to test whether the expression of CAB and SSU genes is controlled by phytochrome. The seedlings were harvested after variable periods of darkness, and the total RNA was extracted and assayed for specific mRNAs during the dot blot technique. Figure 1 (A and B) shows the time course for PSI II CAB, PSI CAB and SSU mRNA accumulation in tomato and tobacco seedlings after red, red followed by far-red, and far-red light pulses. No cross-hybridization between PSI II CAB and PSI CAB occurred (data not shown). The reversibility of a red light pulse by a subsequent far-red light pulse clearly indicates that phytochrome controls the expression of PSI II CAB and PSI CAB in both species. Expression of both genes reaches a maximum 4 h after the light pulse.

In contrast to the CAB mRNA, phytochrome regulation of SSU mRNA cannot be demonstrated as easily. The accumulation of SSU mRNA increased continuously after a red light pulse and after a far-red light pulse.

**Light of Different Spectral Distribution Has Similar Effect on Expression of CAB and SSU Genes**

The effects of continuous irradiations of different spectral distribution but equal photo fluence-rates was analyzed to test

![Figure 2](https://example.com/figure2.png)

Figure 2. PSI II CAB, PSI CAB, and SSU mRNA accumulation during irradiation with different wavelength (equal photo fluence rate). Graphs of the relative mRNA amount of etiolated tomato (top) and tobacco (bottom) seedlings measured after 1, 2, 4, 6, 8 and 16 h of continuous irradiation with red (r), blue (b), ultraviolet (uv) and far-red (fr) light. Time = 0 represents the mRNA amount in darkness. (A) Accumulation of PSI II CAB mRNA; (B) accumulation of PSI II CAB mRNA; (C) accumulation of SSU mRNA.
the role(s) of the blue/UV-A and the UV-B photoreceptor in controlling the CAB and SSU mRNA accumulation. In both species similar kinetics for PSII CAB and PSI CAB mRNA accumulation were observed during irradiation with red, far-red, blue, and UV light (Fig. 2, A, B, and C). No significant differences in the kinetics of accumulation was observed for the PSII and PSI CAB mRNAs. A minor difference between tomato and tobacco seedlings was that maximum mRNA levels occurred between 4 and 6 h in tobacco and between 6 and 8 h in tomato seedlings.

In contrast to the rapid transient accumulation of CAB mRNA, the SSU mRNA accumulated continuously in both species and was highest during irradiation with blue light. These results suggest that multiple photoreceptors may control CAB and SSU gene-expression in these species. Therefore, more detailed experiments were performed to further examine the possible role(s) of the blue/UV-A and UV-B photoreceptors.

**Fluence Rate Response Measurements Indicate HIR and Involvement of the Blue/UV-A and UV-B Photoreceptor in the Expression of CAB Genes**

To identify photoreceptors which cause mRNA accumulation in the above experiments, photon fluence rate response curves were determined for red, blue, UV, and far-red light for 4 h irradiation (Fig. 3, A and B). The lowest given photo fluence rate was 1.9 × 10⁻³ µmol m⁻²s⁻¹ so these experiments would be unable to detect a very low fluence response. Using the PSII CAB probe for tomato and tobacco seedlings, a fluence rate dependence was shown for these mRNAs for all light sources tested. For the expression of the PSII CAB genes in tomato seedlings, blue light was most effective (indicating the involvement of the blue/UV-A photoreceptor), followed by red and far-red light, with lowest effectiveness for UV light. In tobacco seedlings, blue and UV light were the most effective (indicating the involvement of the blue/UV-A and a UV-B photoreceptor), followed by far-red and red light.

Beside the phytochrome induction response, analyzed by red/far-red reversibility, phytochrome responses to continuous irradiation also have been described (10). This so called HIR is characterized by a fluence rate dependence, even at high fluence rates (above 10¹ µmol m⁻²s⁻¹), and a strong responsiveness to far-red light (10). According to these criteria, an HIR controls the expression of PSII CAB genes in both tomato and tobacco seedlings.

As the SSU mRNA accumulation was only weakly regulated, we focused on CAB mRNA induction for further studies.

### Responsiveness to Pfr following Continuous Light Pretreatments Confirms the Results of the Fluence Response Experiments

Another method to test the action of multiple photoreceptors is to test the effect of different Pfr levels following different light pretreatments (18). Etiolated seedlings were preirradiated for 4 h with red, far-red, blue, or UV light followed by either a saturating red or a long wavelength far-red (RG 9) light pulse to establish high (red; ca. 80% Pfr) or low (far-red; ca. 0.1% Pfr) levels of Pfr. The amount of PSII CAB mRNA was measured after an additional 4 h dark period. Higher expression was obtained when red light was given after far-red, blue, and UV preirradiation, but not after red light preirradiation (Table I). This confirms that an HIR controls expression of

### Table I. PSII CAB mRNA Accumulation in Etiolated Tomato and Tobacco Seedlings after Different Light Treatments

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>Tomato, PSII CAB</th>
<th>Tobacco, PSII CAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>d</td>
<td>5.5</td>
<td>26.2</td>
</tr>
<tr>
<td>5 min r, 4 h</td>
<td>30.7</td>
<td>108.5</td>
</tr>
<tr>
<td>5 min r, 5 min fr, 4 h</td>
<td>14.5</td>
<td>75.0</td>
</tr>
<tr>
<td>5 min fr, 4 h</td>
<td>10.0</td>
<td>41.2</td>
</tr>
<tr>
<td>4 h r</td>
<td>65.0</td>
<td>135.0</td>
</tr>
<tr>
<td>4 h r, 4 h</td>
<td>24.5</td>
<td>53.5</td>
</tr>
<tr>
<td>4 h r, 5 min fr, 4 h</td>
<td>20.0</td>
<td>38.0</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>15.5</td>
</tr>
<tr>
<td>4 h fr</td>
<td>45.0</td>
<td>210.0</td>
</tr>
<tr>
<td>4 h fr, 5 min r, 4 h</td>
<td>30.5</td>
<td>109.5</td>
</tr>
<tr>
<td>4 h fr, 5 min fr, 4 h</td>
<td>19.5</td>
<td>55.0</td>
</tr>
<tr>
<td></td>
<td>11.0</td>
<td>54.5</td>
</tr>
<tr>
<td>4 h b</td>
<td>80.0</td>
<td>160.0</td>
</tr>
<tr>
<td>4 h b, 5 min r, 4 h</td>
<td>30.0</td>
<td>89.0</td>
</tr>
<tr>
<td>4 h b, 5 min fr, 4 h</td>
<td>19.5</td>
<td>45.7</td>
</tr>
<tr>
<td></td>
<td>10.5</td>
<td>43.3</td>
</tr>
<tr>
<td>4 h uv</td>
<td>50.0</td>
<td>175.0</td>
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<td>4 h uv, 5 min r, 4 h</td>
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<td>94.7</td>
</tr>
<tr>
<td>4 h uv, 5 min fr, 4 h</td>
<td>17.0</td>
<td>51.7</td>
</tr>
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</table>

a d, darkness; r, red; fr, far-red; b, blue; uv, ultraviolet.
PSII CAB genes. Furthermore, the results indicate that the blue/UV-A photoreceptor controls expression of PSII CAB genes in tomato seedlings. The possible involvement of the UV-B photoreceptor in the expression of these genes was not determined because the excitation of the blue/UV-A photoreceptor may cover the effect of the UV-B receptor.

Increase in Transcription Rate Causes the Increase in Expression of CAB and SSU Genes

The distinct expression characteristics for the CAB genes (rapid transient increase) and SSU genes (continuous increase) also occur during irradiation with white light (Fig. 4, A and B). To test whether these differences are due to different mRNA stabilities or to different transcription rates, nuclear run-on assays were carried out with nuclei isolated from etiolated tobacco seedlings (Fig. 5, A and B). The results show a transient increase in transcription rate for PSI CAB and more clearly for PSII with a maximum of transcriptional activity 4 h after onset of white light. In contrast, the transcription rate of the SSU gene family continuously increased for up to 16 h of white light. These results indicate that the accumulation kinetics of all three mRNAs is to some extent controlled by increases in transcription rates.

PSII CAB Genes Show Maxima of Expression 4 and 20 h after Single Red Light Pulse

When seedlings are given a single red light pulse and then darkness, CAB mRNA accumulation reached maxima 4 h and 20 h after a red light pulse (Fig. 6). This result suggests that light regulation of this gene may also be controlled by a circadian clock.

DISCUSSION

Using classical red (induction) and far-red (reversion) experiments, we demonstrated that phytochrome controls accumulation of CAB mRNAs (PSII and PSI) in etiolated seedlings of tomato and tobacco (Fig. 1). For these mRNAs, far-red light did not completely reverse the red light induction to the level obtained by far-red light alone (Fig. 1). For SSU mRNA accumulation, far-red light only partially reverses the effect of red light. However, phytochrome is the only known photoreceptor to be excited by red light and therefore phytochrome likely controls the expression of SSU genes.

This process may be caused by a rapid loss of full reversibility due to a fast coupling of Pfr to the transduction chain as has been shown for the SSU genes of *Leucaena gibba* (33). Alternatively, it could be because SSU gene expression can be stimulated even by the very low levels of Pfr created by a far-red light pulse. The latter possibility is the most probable, because other data indicate that the accumulation of all three mRNAs tested is very sensitive to low levels of Pfr.

The analysis of the expression of CAB/PSII mRNA by fluence-rate response measurements (Fig. 3), and the effect of preirradiation with different lights on the responsiveness to subsequent red and far-red light pulses (Table I), show several distinctive features. First, the accumulation of CAB/PSII mRNA in etiolated tomato and tobacco seedlings is controlled by a phytochrome induction response and by phytochrome-mediated HIR. Second, the blue/UV-A photoreceptor and possibly the UV-B photoreceptor (27, 34) also control CAB/PSII mRNA accumulation. Responsiveness to blue light is higher than to red light for both species. Because the Pfr/Ptot ratio is about 30 times higher in red light than in blue light (12, 15), these results clearly demonstrate that a blue light photoreceptor controls CAB/PSII mRNA accumulation in

Figure 4. PSII CAB, PSI CAB, and SSU mRNA accumulation during irradiation with white light. Graphs of the relative mRNA amount of etiolated tomato (A) and tobacco (B) seedlings measured after 1, 2, 4, 6, 8, and 16 h of continuous irradiation with white light (empty symbols), and measured after 4 and 6 h of continuous irradiation with red light (filled symbols). Time = 0 represents the mRNA amount in darkness.
both species. The contribution of the UV-B photoreceptor could not be demonstrated unequivocally because of the high responsiveness of the systems to blue light. Nevertheless, the responsiveness to Pfr after pretreatment with UV light suggests that a UV-B photoreceptor also controls expression of PSII CAB mRNA in tomato. It was previously shown that all three photoreceptors control anthocyanin synthesis in tomato seedlings (20).

Photoregulation of CAB/PSI mRNA and SSU mRNA is rather weak in both etiolated tomato and tobacco seedlings leading to a high variance of the measurable signals. Irrespective of this, fluence-rate response curves and analysis of responsiveness to Pfr after a light pretreatment showed similar tendencies as observed for CAB/PSII mRNA indicating that also in these cases HIR, blue light/UV-A, and a UV-B photoreceptor control the expression of these genes in addition to phytochrome action in darkness after light pulses. When the accumulation kinetics of the mRNA of PSII CAB and PSI CAB were compared, no strong differences were observed (Figs. 1 and 2). The major differences observed for these etiolated seedlings was a higher sensitivity to very low levels of Pfr for the PSI CAB gene expression compared to the PSII CAB gene expression.

A prominent difference between CAB (both PSII and PSI) and SSU mRNA accumulation was that induction of the former was transient after a light pulse (Fig. 1) or continuous irradiation (Fig. 2), whereas the latter showed a continuous increase (Figs. 1 and 2). Such kinetic differences were obtained for all light qualities tested including continuous white light (Fig. 4), a situation where all photoreceptors are excited and may interact in controlling mRNA accumulation. The higher expression of RNAs in etiolated tobacco seedlings during irradiation with continuous red light compared with the one in white light is probably due to different overall induction in independent experiments. Different transcription rates appear to be a major mechanism for varying steady state levels of the mRNAs from these two gene families (Fig. 5). For etiolated Arabidopsis thaliana seedlings it was recently demonstrated that CAB genes are transiently expressed (13), although the time of maximal expression, occurring 2 h after a red light pulse, was shorter than we have observed here for tomato and tobacco seedlings. The transient expression that we have observed for the CAB genes in tomato and tobacco, like that observed in Arabidopsis (13), probably represents the first cycle of a light-regulated circadian rhythm (see Fig. 6) (8, 16, 19).
25). Related observations have been made for the induction of CAB gene expression in etiolated barley seedlings and recently for etiolated bean seedlings (32). The different transcription rates for CAB and SSU genes (CAB genes are transcribed three to four times higher than SSU genes) have also been demonstrated for the expression of these genes in isolated pea leaf nuclei (7).

Previous studies on the wheat cab-I promoter in transgenic tobacco plants identified a 268 bp region responsible for phytochrome action (21). Similarly, the rbcS-3A promoter of pea can confer phytochrome response and blue light induction in transgenic petunia plants (6). However, the specific promoter sequences through which this phytochrome action occurs remain obscure, as there is little homology between these two promoter fragments. The results presented here will be useful in studies with transgenic plants aimed at characterizing the CAB and RBCS regulatory sequences and/or factors which mediate the transcriptional responses to different photoreceptors. Similarly, it will be of interest to determine which regulatory sequences and/or factors are responsible for mediating the distinct expression characteristics of the CAB and RBCS genes.

LITERATURE CITED

and genetic mapping of two clusters of genes encoding chlorophyll a/b binding proteins in *Lycopersicon esculentum* (tomato). Gene 40: 247–258


