Blue-Light Regulation of Specific Transcript Levels in *Pisum sativum*¹

Fluence-Response, Time-Course, and Reciprocity Characteristics

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ABSTRACT

The expression of many nuclear genes in plants is light regulated. We have examined the fluence-response, time-course, and reciprocity characteristics of four nuclear, blue-light-regulated transcripts in *Pisum sativum* L. var Alaska: Cab RNA, pEA207 RNA, pEA215 RNA, and pEA25 RNA. To avoid complications due to blue-light-induced transformation of phytochrome, we have adapted the procedure of measuring blue-light-induced changes in steady-state-RNA levels in seedlings grown in continuous red light. The fluence-response curves for Cab and pEA215 RNA are bell-shaped, with peak accumulations at 10¹ and 10³ micromoles per square meter, respectively. No response is observed at 10⁴ micromoles per square meter. pEA25 RNA has threshold and saturation fluences below 10⁻¹ micromoles per square meter. pEA207 RNA has a threshold at 10² micromoles per square meter and saturates above 10⁴ micromoles per square meter. pEA215 and Cab RNA start to increase within 1 hour after the 10³ micromoles per square meter pulse, and finish accumulation by 5 hours. The decrease in pEA207 RNA in response to 10⁴ micromoles per square meter and pEA25 RNA in response to 10⁴ micromoles per square meter starts between 3 and 5 hours after the blue-light pulse. The Bunsen-Roscoe Law of Reciprocity is followed in the Cab, pEE215, pEA25, and pEA207 RNA responses to 10¹ and 10⁴ micromoles per square meter.

Photomorphogenesis occurs in higher plants through the actions of phytochrome, responding primarily to red light, and a family of blue-light receptors, responding to blue and near-UV light (4, 9, 10). Excitation of a blue-light receptor is known to control such processes as inhibition of stem elongation in dicots (7, 21), phototropic curvature in fungi and higher plants (2, 11), chloroplast movement in *Vaucheria* (3) and phototaxis in *Euglena* (6) and *Dictyostelium* (19). While some blue-light responses are well described at the physiological level, very little is known about the effects of blue light on gene expression (16, 22).

As phytochrome can respond to blue light, especially in long-term irradiations (17), it is necessary to control for the possible excitation of phytochrome by blue light. This is accomplished by maintaining the seedlings in continuous red light throughout the experiment (for discussion see 1, 2, 4, 21). Using this growth protocol, Marrs and Kaufman (18) demonstrated that a brief pulse of blue light is capable of altering the steady-state levels of several nuclear-coded transcripts as well as the rate of transcription of the genes coding for these transcripts. Blue-light treatment results in an increase in the steady-state-RNA level and rate of transcription for the *Cab* (*Chl a/b* binding protein) gene family and a gene detected by cDNA clone pEA215 (a partial pea cDNA clone which encodes the C-terminal 14 amino acids of a sequence which has strong similarity to known *Cab* sequences, but which is not a member of the *Cab* gene family; WF Thompson, personal communication; 20). The blue-light treatment resulted in a decrease in the steady-state-RNA level and the rate of transcription of the gene families detected by cDNA clones pEA207 (a pea clone showing strong sequence similarity to those of seed lectins; 8) and pEA25 (the gene product is unknown; 20).

We report herein the fluence-response, time-course, and reciprocity characteristics for the blue-light responses of *Cab* RNA, pEA207 RNA, pEA25 RNA, and pEA215 RNA.

MATERIALS AND METHODS

Plant Growth Conditions

Seeds of *Pisum sativum* L. var Alaska (J. Mollema and Sons; Grand Rapids, MI) were imbibed and grown in continuous red light (0.25 μmol m⁻² s⁻¹) for 7 d in 80% RH at 21°C as described previously (21). Growth in red light is necessary to saturate any phytochrome responses, insuring that any response to the blue-light treatment is not a result of phytochrome excitation (2, 4, 21).

Fluence-Response Experiments

Six d after planting, separate trays of seedlings were irradiated with either 10⁻¹, 10⁰, 10¹, 10², 10³, or 10⁴ μmol m⁻² of blue light. The lengths of the irradiations were as follows: 10⁻¹ to 10² μmol m⁻², 10¹ s; 10² μmol m⁻², 10² s; 10³ μmol m⁻², 10³ s. Seedlings were harvested 24 h after the blue-light pulse. Control seedlings received a mock pulse of blue light. That is, they were treated exactly as for the blue-light-irradiated seed-
lings (moved to light source, etc.) except that the shutter on the light source was not opened. All samples are normalized to the level of rRNA as described previously (13).

**Time-Course Experiments**

Trays of seedlings were pulsed with either $10^1$ or $10^4$ umol m$^{-2}$ of blue light. Seedlings were harvested 0, 1, 3, 5, 11, 18, and 24 h after the blue-light pulse.

**Reciprocly Experiments**

Seedlings were irradiated with a total fluence of $10^1$ or $10^4$ umol m$^{-2}$ of blue light. These fluences were chosen based on the results of the fluence-response experiments. Total fluence of $10^1$ umol m$^{-2}$ irradiations were held constant with the use of neutral density filters (Balzer, Lichtenstein). Irradiation periods tested were $10^0$, $10^1$, $10^2$, $10^3$, and $10^4$ s. Total fluence of $10^4$ umol m$^{-2}$ was held constant with one sheet of GB001 (10$^1$ s) filter paper, or one sheet of GB002 (10$^2$ s) filter paper (Schleicher and Schuell, Keene, NH). Irradiation periods tested were $10^0$, $10^1$, $10^2$, and $10^3$ s.

**Light Sources**

The light sources used for continuous red light irradiation and blue light pulses are described elsewhere (21). Continuous red light was provided by F40/R red fluorescent bulbs (Sylvania, GTE Products; Danvers, MA) covered with a single sheet of Rohm and Haas (Philadelphia) No. 2423 red Plexiglas and two sheets of Roscolux ‘fire’ No. 19 (Rasco; Port Chester, NY). The blue-light source consisted of a Hanixem (Brookvale, NSW, Australia) projector fitted with a Corning (Corning Glass; Corning, NY) blue, 2-60 glass filter. Desired blue-light fluences were obtained with the aid of neutral density filters (Balzer; Lichtenstein). Irradiations of $10^0$ to $10^2$ s for $10^4$ umol m$^{-2}$ of blue light were provided by a 6 inch Fresnel lamp housing a 750 W quartz bulb (Grand Stage Co., Inc., Chicago), covered with a single sheet of Rohm and Haas (Philadelphia) No. 2424 blue plexiglas and two sheets of Roscolux ‘surprise blue’ No. 82 (Rasco; Port Chester, NY). With this light source, desired blue-light fluences were obtained with the aid of GB001 and GB002 filter paper (Schleicher and Schuell, Keene, NH). A mirror was used to direct the illumination to ensure bilateral irradiation.

**cDNA Clones**

The cDNA clones pEA25, pEA207, and pEA215 are derived from those reported by Thompson et al. (20). pEA207 is a pea cDNA clone detecting an apex specific, dark-abundant transcript which shows considerable sequence homology to seed lectins (8); pEA215 is a partial pea cDNA clone which encodes the C-terminal 14 amino acids of a sequence with strong sequence similarity to known Cab sequences, but which is not a member of presently characterized Cab gene families (WF Thompson, personal communication); pEA25 is a pea cDNA clone whose product is unknown. pAB96 detects transcripts of the Chl a/b binding protein gene family, Cab (5); The cDNA clones pEA25, pEA207, and pEA215 were provided by Dr. W. F. Thompson; pAB96 was provided by Dr. N.-H. Chua.

**RNA Isolation and Quantitation**

Total RNA was extracted from tissue apical to the third node by LiCl precipitation as described elsewhere (13, 15). Tissue from approximately 35 seedlings was used per experimental replicate. Three to four independent replicates were performed per experiment. Five ug of total RNA were fixed to nitrocellulose (Schleicher and Schuell, Keene, NH) using a Minifold II slot-blot apparatus (Schleicher and Schuell) and probed with the appropriately labeled cDNA clones as described previously (13, 15). Hybridization conditions, standardization and quantitation of the slot-blot assay are also described elsewhere (13, 15).

**Statistics**

Each point represents the average of at least three independently replicated experiments. Tissue from at least 35 seedlings is used for each replicate. Error bars represent standard errors of the mean.

**RESULTS**

**Fluence-Response**

The steady-state RNA levels for the transcripts corresponding to Cab, pEA215, pEA25, and pEA207 resulting from irradiation with different fluences of blue light are shown in Figure 1. Three fluence-response patterns are observed. Cab and pEA215 RNA have bell-shaped fluence-response curves. Accumulation of Cab RNA has a threshold at or below $10^0$ umol m$^{-2}$ and a peak at $10^2$ umol m$^{-2}$. Accumulation of pEA215 RNA has a threshold below $10^{-1}$ umol m$^{-2}$, and the peak accumulation at $10^1$ umol m$^{-2}$. Both transcripts accumulate to control levels at $10^0$ umol m$^{-2}$. The steady-state levels of transcripts corresponding to pEA25 and pEA207 decrease in response to increasing fluences of blue light, although the respective threshold and saturation fluences differ. The threshold and saturation fluences for pEA25 RNA occur below $10^{-1}$ umol m$^{-2}$. The response in the level of pEA207 RNA is at least $10^3$ times less sensitive to blue light, with a significant decrease in transcript occurring at $10^3$ umol m$^{-2}$. The saturating fluence appears to be greater than $10^4$ umol m$^{-2}$.

**Time-Course**

The steady-state level of pEA215 and Cab RNA 24 h after the blue-light treatment of $10^4$ umol m$^{-2}$ closely approximates the level seen in control seedlings (Fig. 1A). It is possible that a transient change in the transcript level occurs during the 24 h postirradiation period. A similar possibility exists for pEA207 RNA at the $10^1$ umol m$^{-2}$ fluence (Fig. 1B). It was of interest, therefore, to detail the kinetics of accumulation for each of the transcripts in response to $10^1$ and $10^4$ umol m$^{-2}$ fluences of blue light.

The time-course of accumulation for each transcript was measured in response to $10^1$ umol m$^{-2}$ (Fig. 2). Cab and
pEA215 RNA started to increase within 1 h after the blue-light treatment, and accumulation appeared to be complete by 5 h. No significant changes occurred in the steady-state level of pEA207 RNA, indicating that a transient response did not occur. Transcripts corresponding to pEA25 showed a gradual decrease throughout the 24 h postirradiation period. The results for the 10^4 μmol m^-2 time-courses are shown in Figure 2. No significant changes occurred in the steady-state level of Cab or pEA215 RNA during the 24 h period following the blue-light treatment. As expected from the results of the fluence-response experiment (Fig. 1), transcripts corresponding to pEA25 and pEA207 decreased in response to the 10^4 μmol m^-2. Both transcripts start to decrease 3 h after the treatment. pEA207 RNA levels continue to decrease at a lesser rate through the 24 h time point.

Reciprocity

It is possible that the levels we observe for the specific transcripts in response to specific light treatments is not solely a function of the total number of photons delivered (i.e. total fluence), but is due in part to the length of the irradiation period. For example, it is possible that during the longer irradiations a second more efficient receptor is synthesized. If this were the case, the responses observed would not be a function solely of total fluence and the thresholds observed may be incorrect. In order to determine if the length of time over which the irradiation was delivered has an effect on the level of transcript observed, the level of transcript resulting from blue light irradiations of a fixed total fluence, delivered over different lengths of time were compared. The results shown in Figure 3 demonstrate that the transcript levels are not altered as a function of the length of irradiation period for total fluences of 10^3 and 10^4 μmol m^-2. Thus, the Bunsen-Roscoe Law of Reciprocity (the response to a light treatment is a function of the total number of photons in the treatment and is independent of the time over which they are delivered) holds for Cab, pEA215, pEA25, and pEA207 RNA at both 10^3 and 10^4 μmol m^-2.

Total Extractable RNA per Apex

The decrease observed for pEA207 RNA and pEA25 RNA can result from an increase in total RNA with little or no real change in pEA207 RNA and pEA25 RNA levels (i.e. dilution), rather than a decrease in the rate of transcription and/or an increase in the specific rate of degradation for pEA207 RNA and pEA25 RNA. We have recently determined that the rate of transcription for pEA207 and pEA25 does indeed decrease as a result of blue-light excitation (18).

In order to determine if the blue-light treatment results in a large increase in total RNA, total extractable RNA was measured as a function of fluence (10^-1–10^4 μmol m^-2) 24 h after irradiation. No difference is observed between control and blue-light treated seedlings (Fig. 4A). Therefore, the decreases observed for pEA207 RNA and pEA25 RNA are probably not resulting from a blue light-induced increase in total RNA with little or no change in pEA207 RNA and pEA25 RNA levels. The time course for accumulation of total extractable RNA per apex was measured for control seedlings and seedlings treated with 10^4 μmol m^-2 of blue light (Fig. 4B). Both treated and control seedlings showed an increase in total extractable RNA occurring 3 to 5 h after the treatment.

DISCUSSION

We have expanded upon the observation of Marrs and Kaufman (18) who reported on blue-light induced changes in steady-state-RNA level and rate of transcription for several nuclear gene families in pea. In this paper, we report on the fluence-response, time-course and reciprocity characteristics for the steady-state levels of Cab (Chl a/b binding protein), pEA215 (a partial pea cDNA clone whose 14 C-terminal amino acids show considerable homology to known Cab sequences, W. F. Thompson, personal communication), pEA207 (a pea cDNA clone which shows significant sequence homology to seed lectins, 8), and PEA25 (an unidentified gene family represented by cDNA clone pEA25) RNA. The fluence-response curves of Cab and pEA215 RNAs are bell-shaped, with steady-state levels at 10^4 μmol m^-2 approximating those in control plants. These data confirm our earlier observation for Cab RNA wherein no response was observed to 10^6 μmol m^-2 of blue light (12). The threshold and saturation fluences for pEA25 RNA occur below 10^-1 μmol m^-2;
pEA207 RNA has a threshold at $10^2$ µmol m$^{-2}$ and saturation greater than $10^4$ µmol m$^{-2}$. The observed decrease for the steady-state levels of pEA207 RNA and pEA25 RNA can result from the specific degradation of pEA207 and pEA25 transcripts, and/or an increase in other transcripts with little or no real change in pEA207 RNA and pEA25 RNA levels (i.e. dilution). There are no observed differences in total extractable RNA between control and treated seedlings 24 h after blue-light irradiation, indicating that dilution is probably not occurring. This is further supported by the fact that the thresholds for the respective decreases in pEA207 RNA and pEA25 RNA differ by at least 3 orders of magnitude (Fig. 1). It is probable, therefore, that the decrease observed for pEA207 RNA and pEA25 RNA reflects a fluence-dependent, blue-light induced increase in the specific rate of degradation for pEA207 and pEA25 RNAs.

pEA215 and Cab RNA start to increase within 1 h after a pulse of $10^3$ µmol m$^{-2}$. Accumulation is complete by 5 h. The decrease observed for pEA25 and pEA207 RNAs in response to $10^4$ µmol m$^{-2}$ and for pEA25 at $10^6$ µmol m$^{-2}$ starts 3 h after the treatment. Total extractable RNA also increases between 3 and 5 h after a mock pulse (control) or a blue-light pulse of $10^6$ µmol m$^{-2}$. The significance of this coincidence is unknown, although, it is possible that the 3 to 5 h point represents a time when the plant can 'show' a response to a 'stored' signal.

We observe bell-shaped, fluence-response curves for Cab and pEA215 RNAs. Bell-shaped fluence-response curves can be indicative of two antagonistic signal-transduction mechanisms, one with a threshold to low fluences of blue light and a second responsive to high fluences only. It is possible that
the fluence-response curves for pEA25 and pEA207 RNAs, which have thresholds below $10^{-1}$ and above $10^2 \mu$mol m$^{-2}$, respectively, represent these two mechanisms.

In contrast to the bell-shaped, fluence-response curve observed for *Cab* RNA, Chl, and carotenoid accumulation increase with increasing fluence of blue light (21). Thus, a single pulse of high-fluence blue light (i.e. $10^4 \mu$mol m$^{-2}$) results in increased Chl, but control levels of *Cab* RNA. It will be of considerable interest to determine the fluence-response characteristics for *Cab* protein.

Bell-shaped, fluence-response curves have been observed for several blue-light-induced phenomena: chloroplast movements in *Vaucheria* (3), phototropism in *Euglena* (6), phototropic curvature in fungi (11), phototropic curvature in pea, maize, barley, and mung bean grown under continuous-red-light conditions (1, 2, 4), as well as for suppression of epicotyl elongation in peas grown under continuous-light conditions (21). In the latter case, it has been suggested that the bell-shaped fluence-response curve is the product of two antagonistic processes, one with a threshold at or below $10^{-1}$ \mu mol m$^{-2}$ of blue light and resulting in suppression; and a second, responding to high fluences only, alleviating the suppression (21). Based solely on the similarity of these response characteristics, it is possible that the signal-transduction mechanisms regulating steady-state-RNA levels have some early steps in common with cognate transduction mechanisms regulating suppression of elongation.

Previous studies have shown that the steady-state levels of *Cab*, pEA25, and pEA215 RNA are phytochrome regulated (13–16, 20, 22). *Cab* and pEA25 are multigene families (12), and different members may be blue-light and/or phytochrome regulated. A single-copy gene is probably responsible for pEA215 RNA (12), and, therefore, these results indicate that it is possible for a single gene to be both phytochrome and blue-light regulated. Blue-light does affect the rate of transcription of the gene responsible for pEA215 RNA (18) and the same is probably true of phytochrome excitation. This raises the possibility that both signal-response mechanisms might feed into the same transcriptional regulatory factors.

It is interesting that *Cab* and pEA215 (a *Cab*-like gene) RNA which respond similarly to blue light also respond similarly to phytochrome excitation (13–15). Both transcripts appear to be under very rigid photoregulation as there are at least four photocontrols: very low fluence and low fluence (13, 15) phytochrome responses; a low fluence-induced accumulation and high fluence-induced decrease to blue light. Why the *Cab* and pEA215 transcripts need to be so tightly regulated is unknown.

**Figure 4.** Fluence response and time course of accumulation for total extractable RNA per apex. A, Seedlings were grown, irradiated and harvested as for Figure 1. Total RNA was extracted 24 h after the appropriate light treatments. Each point represents the mean of 3 to 5 replicates of approximately 35 seedlings each. Bars represent standard error of the mean. The level of RNA in control plants is set to 1.00. B, Seedlings grown as in Figure 2 were irradiated with $10^4 \mu$mol m$^{-2}$ (○) or no blue light (○). Tissue was harvested and extracted as described in Figure 2. Approximately 35 seedlings were used for each of the 3 to 5 replicates. The level of total extractable RNA at time '0' is set to 1. Bars represent standard error of the mean.

**LITERATURE CITED**


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