Light Effects on Several Chloroplast Components in Norflurazon-Treated Pea Seedlings

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ABSTRACT

Changes occurring in several chloroplast components during Norflurazon-induced photobleaching of Pisum sativum seedlings were investigated. mRNA steady state levels of the chlorophyll a/b-binding protein of photosystem II, ferredoxin I, the small and large subunits of ribulose 1,5-bisphosphate carboxylase, and pEA214 and pEA207, two other light-responsive genes, were determined during chlorophyll photooxidation. Relative transcription rates were assayed in isolated nuclei. The results illustrate a complex set of interactions regulating expression of the nuclear and chloroplast genomes. Photobleaching was found to affect the expression of the various genes in different ways. While transcript levels of the chlorophyll a/b-binding protein decreased by more than 80% under photooxidative light conditions in carotenoid-deficient peas, levels of ferredoxin, the small and large subunits of ribulose 1,5-bisphosphate carboxylase, and pEA214 mRNAs were reduced by less than 50%. pEA207 mRNA levels, on the other hand, were resistant to the effects of photobleaching. Analyses of chlorophylls a and b and the chlorophyll a/b-binding protein suggest that accumulation of the protein and its mRNA are coordinated with chlorophyll abundance at several steps. In addition to post-transcriptional regulation at the level of mRNA and protein stability, there may exist coordination at the transcriptional stage.

Since most (>80%) chloroplast proteins are nuclear encoded (14), the nuclear and chloroplast genomes must coordinate gene expression to bring about normal development of a green plant. The messenger RNAs of the nuclear-encoded chloroplast proteins are translated on cytosolic ribosomes and transported into the plastid where they are incorporated into the photosynthetic machinery. The nuclear-chloroplast interaction can be studied both during greening of etiolated tissue and by the use of mutants lacking Chl. In addition, one can study plants that are blocked in carotenoid biosynthesis either because of mutations or due to growth in the presence of herbicides such as Norflurazon [NF; 4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-m-tolyl)-3(2H)-pyridazinone] (7). In the absence of protecting carotenoids in these plants, high light intensities cause "bleaching" of the green parts (1, 19).

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4 Abbreviations: Cab, Chl a/b-binding protein of the photosystem II light harvesting complex; Fed I, ferredoxin I; RbcS and rbcL, small and large subunits of ribulose 1,5-bisphosphate carboxylase; NF, Norflurazon; PMSF, phenylmethylsulfonyl fluoride.

In plants lacking carotenoids, as a result of nuclear mutation or herbicide treatment, under high light growth conditions plastids are arrested at a rudimentary stage in development (4). By contrast, in low fluence-rate light, carotenoid-deficient chloroplasts develop normally and are similar to plastids from normal plants grown under low light (5, 6, 42). mRNAs coding for Cab failed to accumulate in high light-grown, carotenoid-deficient plants. This reduction in Cab mRNA, related to photobleaching, has been reported for maize (Zea mays) (27, 37), barley (Hordeum vulgare) (8), and mustard (Sinapis alba) (31). Furthermore, Cab gene transcription measured in isolated nuclei also decreased in barley and maize (8, 37). Normal mRNA levels were found in NF-treated, carotenoid-deficient plants grown in low light (8, 31), indicating that the presence of NF or absence of carotenoids per se did not prevent Cab mRNA accumulation. Moreover, since Chl-deficient mutants of barley and maize were found to have normal amounts of Cab mRNAs (8, 27), lack of Chl also does not appear to be responsible for absence of these transcripts.

Besides affecting Cab (both RNA and protein), Chl photobleaching was found to cause an alteration in mRNA and protein levels of the small subunit of ribulose 1,5-bisphosphate carboxylase (26, 30, 31). In maize, the accumulation of several cytosolic mRNAs besides Cab and RbcS was affected (13), although the effect seen on the different mRNA species varied widely. Similarly, chloroplast proteins exhibited varying degrees of sensitivity to damage caused by photooxidation (26). Cytosolic mRNAs or proteins not associated with the plastid were not affected by this treatment (32). The existence of a signal from the plastid to the nucleus (absent during photodestruction of the chloroplast) was therefore postulated as a factor regulating the accumulation of nuclear-encoded chloroplast proteins (30, 37).

We are interested in various aspects of light-regulated gene expression in Pisum sativum (garden pea). In the present study we report on the interaction between the nucleus and the chloroplast during photobleaching in NF-treated, carotenoid-deficient peas. To gain an understanding of regulatory signals coordinating gene expression between the developing plastid and the nucleus, we examined the effects of Chl photooxidation on the levels of mRNAs for several chloroplast proteins for which cloned probes are available in the laboratory. Five probes represent nuclear-encoded mRNAs: Cab, Fed I, RbcS, pEA214, pEA207; one, rbcL, is encoded by the chloroplast genome. pEA214 and pEA207 both represent as yet unidentified nuclear-encoded, light-responsive genes (24, 38), but whereas RNA levels corresponding to pEA214 increase in light, pEA207 mRNA levels decrease in white light as compared to the level in etiolated pea buds. Changes in the rates of transcription in isolated nuclei were determined for the photooxidation-sensitive nuclear genes, and steady state mRNA levels were analyzed for all six genes. The relationship between levels of Chl (a and b) as well as levels of Cab protein and its mRNA were also examined. The results illustrate the existence of a complex relationship between nuclear
and chloroplast genomes. Mechanisms coordinating gene expression appear to exist at several different steps—transcriptional as well as post-transcriptional—at the level of mRNA and protein stability.

**MATERIALS AND METHODS**

**Plant Materials and Light Treatments.** *Pisum sativum* cv Alaska (W. Atlee Burpee Co., Warrenster, PA) seeds were imbibed for 2 h in 100 μM Norflurazon (Sandoz 9789; NF-treated) or water (control), briefly washed with a 1:10 dilution of commercial bleach, rinsed, and planted in vermiculite watered with or without 100 μM NF. Plants were grown at 24 to 25°C in (a) absolute darkness, (b) continuous dim red light (0.7 μmol m⁻² sec⁻¹), or (c) continuous white light (100 μmol m⁻² sec⁻¹, cool white fluorescent) for 5.5 d prior to harvest. At this stage one set of plants was harvested for analyses of RNA levels and Chl determinations and an identical set was placed in white light (100 μmol m⁻² sec⁻¹) for 24 h.

**Chl Determinations.** Buds were harvested (five buds assay), weighed, and placed in glass vials on ice. N,N-Dimethylformamide was added (20 mL/g fresh weight), and the vials were covered and kept in the dark at 4°C for 48 h (28). Chl levels were determined by measuring absorbance at 664 and 647 nm for Chl a and b, respectively. Chl concentrations were calculated using the Moran formula (28). Since measurement of fluorescence emission provides a more sensitive measure of Chl amounts, changes in Chl ab ratios were also assayed by measuring fluorescence emission maxima using a Perkin-Elmer MFP 3L Fluorescence Spectrophotometer (Perkin-Elmer, Coleman Instrument Division, Oak Brook IL). Light at 435 and 465 nm was used for excitation of Chl a and b, respectively; fluorescence emission was measured at 672 nm for Chl a and at 659 nm for Chl b.

**RNA Extraction and Analyses.** Following the various light treatments, terminal buds were harvested on ice and frozen in liquid N₂. Tissue was stored at −70°C until used for RNA extraction. RNA extraction was as described (24). The RNA was fixed to Gene Screen membranes (New England Nuclear, E.I. dUffont de Nemours, Boston MA) using a MiniII slot blot apparatus (Schleicher and Schuell, Keene, NH) according to previously described protocols (24, 38). Each experimental slot contained 2.5 μg of RNA. The blots were hybridized in 6 X SSC (1 X SSC is 0.15 M NaCl, 0.015 M Na acetate) at 65°C for 16 to 20 h with 32P-labeled cDNAs (25). Filters were washed briefly in 2 X SSC at room temperature, followed by two washes in 0.3 X SSC at 65°C for 1.5 h. Hybridization was quantitated by autoradiography and densitometry using an LKB Ultra Scan (LKB Instruments, Pleasant Hill, CA).

The isolation and properties of the clones used to analyze mRNA levels have been previously described (38). pE4A6 has been identified as a ferredoxin I cDNA (16), pAB96 and pSS15 (cDNAs corresponding to Cab and RbcS mRNAs) (12) were obtained from N.-H. Chua. The probe used for determination of rbcL mRNA levels is a 685 base pair fragment from the 5’ end of the pea rbcL gene (44).

A concentration series of pBR322 DNA was included in each slot blot. Densitometric scanning of this DNA was used to construct a standard curve for each probe to correct for differences in hybridization efficiency between experiments. Signal strengths for hybridization to RNA are therefore expressed in “pBR322 equivalents” and have been corrected for differences in probe lengths.

**Protein Analyses.** Pea buds were harvested on ice and frozen in liquid N₂ until used. Frozen tissue was ground using a tight-fitting Dounce homogenizer in buffer containing 50 mM Tris (pH 8.0), 5 mM β-mercaptoethanol, 1 mM each of PMSF and benzamidine, and 5 mM ε-aminoacaproic acid. The membrane pellet, obtained after filtering the extract, was sonicated in the presence of 0.1 M each of DTT and Na₂CO₃, boiled in 5% SDS, and electrophoresed through a 7.5 to 15% polyacrylamide gradient gel. Equal amounts of sample were loaded on each lane, and several prominent stained proteins that did not change due to the light or herbicide treatment were scanned densitometrically to verify even loading across the lanes.

Following electrophoresis, one section of the gel was stained and photographed, the other section was electrophorotized onto nitrocellulose paper (41) (Hoefer Scientific Instruments, San Francisco, CA) in buffer containing 40 mM Tris, 87 mM glycine, 20% methanol, and 0.05% SDS. The blot was prewashed in a solution containing 10 mM Tris, 0.1% SDS, and 3% BSA for 2 h at room temperature and then was incubated with light harvesting-Chl-protein complex II antibody (kindly provided by Dr. W. Taylor) in the same buffer (containing 2% BSA) overnight at 4°C. After reacting with antibody, the filter was washed several times in Tris and NaCl as above and then treated with protein A-peroxidase conjugate in the presence of 2% BSA for 2 h. Antigen-antibody complex was visualized by a color reaction with 4-chloronaphthol (3 mg/mL) in methanol and hydrogen peroxide (0.01%) (21). For quantitation of the antibody complex, an identical blot in which the color reaction was kept in the linear range was scanned densitometrically using an LKB Ultra Scan densitometer. Control and NF-treated samples were blotted on the same nitrocellulose sheet in order to permit direct comparison.

**Isolation of Nuclei.** Pea nuclei were isolated from seedlings grown in continuous red light, and after further exposure of these red light-grown seedlings to 24 h white light. Nuclei isolation was carried out using a modification of published protocols (43). Terminal buds were harvested on ice, washed briefly in ice-cold ether, and then rinsed with extraction buffer (containing 1 M hexylene glycol, 10 mM Pipes [pH 7.0], 10 mM MgCl₂, 5 mM βME, 0.1 mM PMSF). Washed tissue was disrupted for 1 min in extraction buffer using a Polytron homogenizer (Brinkmann, Westbury, NY), and the homogenate was filtered through 500- and 300-μm nylon-mesh (Nixet; Tetko, Elmsford, NY). Triton X-100 was added slowly to the filtrate (with constant swirling) to a final concentration of 0.5% before filtering sequentially through 100-, 50-, and 20-μm nylon mesh. The final filtrate was layered onto a 35/70% Percoll step gradient (gradients were made in buffer identical to the extraction buffer except that it contained 0.5 M hexylene glycol and 0.5% Triton X-100) and was centrifuged for 30 min at +2000g in an IEC clinical benchtop centrifuge running at 4°C. Nuclei sedimented through the Percoll tape and were gently resuspended, loaded onto a second step gradient, and the centrifugation and resuspension steps were repeated. Following centrifugation through a third and final 35/70% Percoll step gradient, the purified nuclei were suspended in storage buffer (0.5 M sucrose, 20% glycerol, 50 mM Tris [pH 8.0], 5 mM MgCl₂, 0.1 mM EDTA, 1 mM DTT) at a final concentration of 2 mg/mL. Nuclei were frozen in liquid N₂ and stored at −70°C until further use.

**Transcription in Isolated Nuclei.** Nuclei (at a concentration of 50 μg of DNA/mL) were incubated for 15 min at 27°C in 100 μL of buffer containing 50 mM Tris (pH 7.9), 10 mM MgCl₂, 10% (v/v) glycerol, 500 μM each of ATP, CTP, and GTP, 75 mM NaCl, 0.8 mM DTT, 100 μCi of [32P]UTP (400 Ci/mmol), and 75 units of RNAse (Promega Biotech, Madison, WI).

The reaction was stopped by addition of 20 μg of yeast tRNA, 10 nMol of UTP, and 12 μg of RNAase-free DNase I (25). This mixture was then incubated for an additional 5 min, and a 200-μL volume of 7.5 M urea, 5% SDS, 20 mM EDTA, 100 mM LiCl, and 10 mM aurin tricarboxylic acid (ATA; pH 7.0) was added. The resulting mixture was extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). The 32P-labeled, ethanol-precipitated RNA was washed twice with 70% ethanol, dissolved in 100 μL of 50 μM ATA, and centrifuged through a
RESULTS

Chl Abundance and mRNA Accumulation under Different Light Regimes. In order to determine the most appropriate light treatment for an analysis of NF effects on chloroplasts, pea seedlings were grown for 5.5 d in absolute darkness, dim red light, or continuous white light. Seedlings grown in darkness or dim light were subsequently transferred to white light for 24 h. Total Chl levels were measured in terminal buds of seedlings grown in the presence or absence of NF. RNA extracted from the buds of plants grown under similar conditions was analyzed by hybridization of slot blots with 32P-labeled probes for Cab, Fed I, RbcS, rbcL, pEA214, and pEA207.

Figure 1 depicts Chl levels observed in untreated and NF-treated pea buds under the light regimes described above. When the dark-grown seedlings were transferred to white light for 24 h, a low level of Chl accumulation occurred. This white light-induced accumulation was largely suppressed by the NF treatment. In seedlings grown continuously in red light a significant amount of Chl accumulation occurred in both treated and untreated seedlings, although the amount in plants grown in the presence of NF was found to be lower (about 70% of the control). Upon transfer of the red-grown plants to white light, there was an increase in Chl in the control seedlings while the levels in the NF-treated seedlings were reduced several fold. Finally, in plants grown continuously in white light, Chl levels were almost fourfold higher than those grown in red light. NF treatment almost completely prevented Chl accumulation under these conditions.

Steady state levels of mRNAs corresponding to the various light-regulated genes under the conditions described above are summarized in Table I. In dark-grown pea buds the mRNAs for all six genes/gene families were present at a “basal” level. Transcript levels were similar in herbicide-treated and untreated plants (except Cab, rbcL, and pEA207, where the levels were somewhat higher in the NF-treated plants). Transfer to white light of untreated seedlings produced a dramatic increase in the mRNA in each case except pEA207 where, as expected, there was a decrease (24, 38). This reduction in pEA207 mRNA occurred both in control and NF plants, indicating that NF treatment did not affect the accumulation of this transcript. Cab, Fed I, RbcS, and pEA214 mRNAs each increased six- to eightfold, while for rbcL the increase was about threefold. Noticeably smaller increases occurred in the NF-treated plants, although the magnitude of the NF effect varied from one RNA species to the next.

In plants grown in continuous red light, RNA amounts were similar in both untreated and NF-treated seedlings (although several fold higher than in the dark-grown plants, in all cases except pEA214; Table I). When red-grown control plants were placed in white light, RbcS mRNA increased most dramatically, while the increases in pEA214, Fed I, and rbcL were less. In contrast to the increase in Chl observed under the same conditions (Fig. 1), levels of Cab mRNA remained unchanged. However, when red light-grown, NF-treated plants were exposed to white light, Cab mRNAs declined about six- to sevenfold, consistent with the large decrease seen in Chl. RbcS, rbcL, and pEA214 mRNAs decreased much less (about 1.5-fold), while Fed I RNA abundance did not appear to change. In control plants grown continuously under white light, mRNAs corresponding to the various genes were at the highest levels measured (lowest for pEA207). These levels were reduced by different amounts by the herbicide treatment. Again, as for the other light treatments, pEA207 mRNA in NF-treated plants was similar to that in the control plants.

FIG. 1. Chl levels in herbicide-treated and untreated Pisum seedlings under three different light treatments. Seedlings grown for 5.5 d either in absolute darkness or in low-fluence red light were transferred to continuous white light for 24 h. Buds were harvested before and following the white light treatments. For the white light-grown plants, germination and growth were in white light for 6.5 d, at which time buds were harvested. Chl was assayed spectrophotometrically as described. Chl concentrations were calculated using the Moran formula (28). Bars indicate the mean ± se; 1.0 A664 corresponds to 270 μg of total Chl per g fresh weight.

Table I. RNA Levels under Various Light Conditions in Control (C) and NF-treated Plants

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<td>NF</td>
<td>C</td>
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<td>206</td>
<td>400</td>
<td>143</td>
<td>366</td>
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*RNA levels are expressed in relative pBR322 units per microgram of total cellular RNA.
Although under all the experimental light conditions studied, only the light-enhanced, chloroplast-related components appeared to be affected by the herbicide treatment as indicated by the unchanged pattern of pEA207 expression in control and NF-treated peas (Table I), the most useful light treatment to study nuclear-chloroplast interactions during photobleaching appeared to be growth in continuous dim red light followed by transfer to high light. Unlike growth in the dark or strong white light, the low fluence-rate red light permits Chl accumulation—although at somewhat reduced levels (Fig. 1). Furthermore, under these conditions chloroplast development appears to be normal (5, 42). Chloroplast ribosomal RNA levels in NF-treated pea buds were similar to those in untreated plants (not shown). Similarly mRNA of at least one chloroplast-encoded protein (rbcL) was present at normal levels (Table I). In order to study the kinetics of RNA abundance changes in the high light during which photobleaching occurs, a protocol consisting of growth in red light for 5.5 d followed by 1 d of continuous white light was selected. We addressed the following questions: (a) What was the relationship between Chl photooxidation and changes in the abundance of several mRNAs, at least four of which are known to encode chloroplast proteins involved in photosynthesis? (b) Were the changes in RNA accumulation regulated transcriptionally or at a post-transcriptional step?

Photobleaching of Chl a and b. In order to assess the changes that had occurred within the 24-h high-light-treatment period in both NF-treated and untreated seedlings, terminal buds were harvested every 2 h and either frozen in liquid N₂ to be used for RNA analyses (24) or extracted with dimethylformamide for Chl assay (28). Figure 2 depicts the levels of Chl a and b in control (panel A) and NF-treated (panel B) plants. Levels of both Chl a and Chl b increase in white light in the control plants. The Chl a:b ratio (panel C) was about 6 in the red light-grown plants and decreased to about 5 during the next 48 h of white light. Similar a:b ratios were reported for peas grown in darkness for 7 d and transferred to white light for 24 h (10). In the NF-treated plants, both Chl a and Chl b decreased in the white light (Fig. 2, panel B), although kinetics of Chl b degradation were faster and a:b ratios were >8 within 8 h of the white light treatment.

Analysis of Changes in mRNA Abundance and Transcription. The changes in mRNA levels due to white light stimulation (control) and photooxidation (NF) depicted in Table I could be controlled either transcriptionally or post-transcriptionally at a later stage of RNA processing. Although NF-induced changes in steady state translatabile mRNA have been determined for several chloroplast protein genes (8, 27, 31, 33), Cab is the only gene for which any transcription data are available. Light is known to affect transcription of Cab genes in NF-treated plants in both barley and maize (8, 37). To determine the steps at which the RNA abundance changes were regulated in pea, we first analyzed the time course of mRNA accumulation for Cab, Fed I, RbcS, rbcL, and pEA214 in red light-grown, control, and NF-treated plants, during the 24-h white light treatment period. Relative rates of transcription were also determined for the nuclear-encoded genes by nuclear “run off” assays (as detailed under “Materials and Methods”). Transcription in isolated nuclei is generally believed to represent, at least in part, an elongation of previously initiated RNA molecules. These experiments should thus reflect the change in number of polymerase molecules associated with the genes under study during the white light treatment. The results of the kinetic and transcription data are shown in Figure 3 and 4, respectively.

Panel A of Figure 3, shows the time course of Cab mRNA changes. Comparison of Chl (see Fig. 2) with Cab mRNA in control plants indicated that, despite enhanced Chl accumulation, there was no change in Cab mRNA abundance during the white light treatment. Furthermore, while Chl levels in herbicide-treated plants were already somewhat lower than in the untreated

![Figure 2](https://example.com/figure2.png)
treated plants showed an increase in mRNA levels for both, although the increase in RbcS was greater than 2.5-fold while that in rbcL was only 1.2-fold. The large increase seen in RbcS mRNA in control seedlings was associated with a dramatic stimulation in rates of transcription of these genes (Fig. 4). This enhancement of RbcS transcription and mRNA abundance following transfer from dim red to high fluence-rate white light is likely to be mediated by a photoreceptor other than phytochrome since the transfer should not cause any change in Pfr.

In both maize (26, 27) and mustard (30, 31), RbcS mRNA and protein were markedly affected by the herbicide treatment. In barley (8), although mRNA abundance was not affected, RbcS protein levels were reduced. In NF-treated peas, RbcS transcript levels showed a transient increase within the first 8 h of white light, followed by a decrease to 65% of the initial mRNA amounts by 18 h (panel B, Fig. 3). Transcription in isolated nuclei indicated that the white light-stimulated increase in transcription seen in control peas is suppressed by the NF treatment (Fig. 4). Because of the low hybridization signal, however, it is not possible to say whether there is an actual decrease in transcription.

Transcripts of rbcL were reduced by 50% within 14 h of white light treatment. These final levels, still surprisingly high considering the almost complete photooxidation of Chl, suggest that either rbcL mRNA has a long half-life or that the photobleached plastids are still capable of some rbcL RNA synthesis under our experimental conditions. Presumably, longer light treatments would further reduce rbcL amounts as suggested by the lower levels present in white light-grown seedlings (Table I).

**Fig. 3.** Kinetics of NF-induced mRNA abundance changes of several light-sensitive genes. Light treatments were as described for Figure 2. Each time point is the average of at least three independent experiments. The 0 h time point was designated as 100% in both control and NF-treated plants; all other data points were normalized to this level. The open symbols in each panel represent the control, and the filled squares show the mRNA levels in NF-treated plants.
following growth in continuous red light were subjected to Western blot analysis as described. Results of a representative experiment are shown in the top panel in Figure 5. The lower panel shows a quantitation of the blot.

At 0 h when the seedlings had not been exposed to any white light, the Chl a/b-binding protein in control plants was present at about 30% of the maximum level reached. Unlike Cab transcripts, which remained unchanged, the protein level increased with increasing time of white light treatment, reaching a maximum by 12 h. In NF-treated seedlings, on the other hand, at 0 h Cab protein was barely detectable (the blot shown here was highly overexposed), although the mRNA level was normal and the Chl slightly reduced. Within the first 4 h in white light the protein level increased and then remained at a steady state (20% of the maximum) for the next 20 h, again in contrast to Chl a

Fed I mRNA (panel D, Fig. 3) showed a slow 1.2-fold increase in the control seedlings. The steady state mRNA levels in NF-treated seedlings showed an initial rapid increase followed by a decline. The levels were reduced by about 30% within 14 h of white light. Analyses of the transcription rates in isolated nuclei were inconclusive.

pEA214 represents an unidentified phytochrome-regulated nuclear gene (24, 38). The mRNA corresponding to this cDNA clone shows a low fluence response and increases linearly following a pulse of red light (24). Its sensitivity to the NF treatment suggests that the pEA214 gene product may also be a nuclear-encoded protein destined for the chloroplast, since cytosolic proteins and mRNAs do not appear to be affected by NF (32). Proof of this hypothesis will ultimately reside in identification at the protein level. pEA214 is an example of a gene whose mRNA steady state levels (Table I) and transcription rates (see Fig. 4) were similar in control and NF-treated plants grown in red light, a pattern that was also found in the case of Cab. Again, as for Cab genes, transfer to white light strongly stimulated pEA214 transcription in control plants, and NF treatment inhibited this increase (Fig. 4). In contrast to the decreased transcription of the herbicide-treated Cab genes, transcription before and after the white light treatment was unchanged in the case of pEA214 (see Norflurazon 0 and 24 h, Fig. 4). The transcriptional regulation of pEA214 may represent another type of gene regulation during NF-induced photooxidation—decreased RNA steady state levels (panel E, Fig. 3), without a detectable change in transcription.

**Regulation of Chl a/b-binding Protein Levels during Photooxidation.** The appearance of Cab mRNA is induced by phytochrome stimulation in pea (15, 23), barley (2), and *Lemna* (36, 39). However, although a pulse of red light is sufficient to induce an increase in mRNA in etiolated barley, it is not sufficient for Cab polypeptide integration in the plastid membrane (3). Since Cab protein was found to turn over rapidly in etiolated or light-treated seedlings transferred to the dark, Chl accumulation has been postulated to be a necessary step in stabilization of the Chl a/b-binding protein (3, 10).

We therefore investigated the relationship between Chl, Cab mRNA, and Cab protein during photobleaching. Membrane proteins extracted from buds of control and NF-treated pea seedlings exposed to white light for varying lengths of time were analyzed by SDS-PAGE and Western blot analysis (Fig. 4). The upper panel in Figure 4 shows representative blots of replicas of the same nitrocellulose filter as a result of the photobleaching treatment with pEA214- and anti-LHCP antibody staining. The lower panel is a densitometric scan of the blots, demonstrating a strong correlation between the level of photobleaching and the level of pEA214 mRNA (see Fig. 3). These results provide strong evidence for a positive interaction between Cab and pEA214 during photooxidation. The data also suggest that Cab mRNA levels may be a good indicator of the level of photooxidation in the plastid membrane, which is consistent with the hypothesis that Cab protein is involved in the stabilization of the Chl a/b-binding protein.
and b (Fig. 2) as well as Cab mRNA (panel A, Fig. 3), which declined dramatically during that period. These results suggest the existence of an array of regulatory mechanisms controlling accumulation of Chl, Cab protein, and its mRNA.

**DISCUSSION**

A green plant regulates the expression of its nuclear genes in a tissue-and stage-specific manner, as well as coordinating expression of the nuclear and plastid genomes during development. We have studied the regulation of several nuclear-encoded chloroplast proteins in pea seedlings made carotenoid deficient by growth in the presence of the herbicide Norflurazon. Growth in low-fluence red light permitted normal seedling development in both control and NF-treated plants, although Chl accumulation was slightly reduced in the latter, possibly as a consequence of some photooxidation (Fig. 1). In the low light conditions used, RNA levels of the five light-stimulated genes analyzed were similar in untreated and herbicide-treated seedlings. Upon transfer to high fluence-rate white light, the herbicide-treated seedlings showed a dramatic reduction in Chl within 6 h in contrast to untreated plants whose Chl abundance increased twofold within the same period. Concomitant with this increase in Chl, there was an increase in Cab protein. Surprisingly, in contrast to the steady state decline in Chl in the NF-treated plants, there was an initial increase in Cab protein during the first 4 h in white light. A similar change has also been observed in maize (26). This increase, and maintenance of the Cab protein level (although low) while Chl levels decreased, was unexpected since Chl and Cab a/b-binding protein are thought to be coregulated (3, 10). Possibly as a result of rapid integration in the thyakloid membrane following transfer to white light, Cab protein may be stabilized as a Chl a complex (17) in these NF-treated plants. Chl photooxidation at a later stage may then be unable to destabilize these proteins.

Transcript abundance analyses depicted in Figure 3 for the three nuclear genes encoding known chloroplast proteins (Cab, RbcS, and Fed I), as well as pEA214, indicated that although in each case the final RNA amounts were distinctly lower in NF-treated peas as compared to the control plants, chloroplast photoxidation affected the steady state level of these mRNAs in different ways. While Cab mRNA levels decreased by >80%, RbcS, Fed I, and pEA214 mRNAs were decreased by <50%, pEA207 mRNA is not increased in light and did not vary between control and herbicide-treated plants, thus illustrating a normal pattern of cytosolic RNA expression not sensitive to photooxidation. Interestingly, when dark-grown plants are exposed to light, mRNAs corresponding to the chloroplast proteins increase both in the controls and the NF-treated plants, although the latter show a somewhat smaller increase (see Table I), depicting perhaps a component of the induced RNA that is photooxidation insensitive.

At least in the cases for which we have clear answers from analyses of transcription in isolated nuclei (Cab and pEA214) (Fig. 4), the decrease in transcript abundance when red light-grown plants are transferred to photooxidative white light may be brought about by different mechanisms. In the case of Cab, NF not only inhibited the light-induced increase in transcription observed in control nuclei, it also decreased transcription below the red control level. For pEA214 transcription, however, NF blocked the light-induced increase but did not cause the rate to fall below that seen in the red control. An alternative explanation for the pEA214 result could be the existence of two differentially regulated genes coding for an enzyme activity present both in the cytoplasm and the plastid. The photooxidation-resistant component would then presumably belong to the cytoplasmic compartment.

Regulation of Cab mRNA and its protein appears to be quite complex, with control occurring at several different steps, transcriptional as well as post-transcriptional. Transcriptional regulation of Cab genes is known to be under phytochrome control in numerous different systems (18, 29, 34, 40). The experiments described in this report demonstrate an increase in Cab transcription in white light over the relative rate in continuous red light (in control plants). Since this transcriptional increase is not followed by an increase in steady state mRNA (compare Cab expression in Fig. 3 and 4), there must exist a post-transcriptional regulation of these transcripts. The transcription rates and the steady state RNA levels measured here represent the sum total of transcription from various members of a multigene family (the cDNA probe used here does not distinguish between the different members). It is possible, therefore, that it is only the rate of transcription of an unstable member of the Cab gene family that increases.

Although the increase in Cab mRNA accumulation was not accompanied by increased Cab mRNA, the transfer to white light did lead to an increase in Cab gene transcription. Similarly, the white light-induced decrease in Chl (in NF-treated plants) was paralleled by a decreased rate of transcription of the Cab genes. The possibility of a correlation between Chl accumulation and Cab gene transcription is intriguing, although such a relationship cannot be a simple one, since in pea Cab mRNA accumulation appears to be more light sensitive than Chl induction (22).

However, it may be that transcription of different members of the Cab gene family is controlled by different mechanisms, with one of them being coregulated with Chl induction. This is a question that we hope to address with the use of gene-specific probes.

Finally, Cab regulation also appears to occur at a translational or post-translational step. We observed an increase in Cab protein in both control and NF-treated peas upon transfer to white light, although the mRNA level did not change in the former and was actually decreasing in the latter. Regulation at a translational step has been reported for *Lemma* (35) and *Amaranth* (11).

Whatever the mode of communication between the two genomes in a green plant, the observations described in this study indicate a diverse set of signals that coordinate expression of the nucleus and the chloroplast in a variety of ways. It will be of interest in further experiments to dissect this question biochemically and with the use of mutants.

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