Post-Transcriptional Control of Plastid mRNA Accumulation during Adaptation of Chloroplasts to Different Light Quality Environments

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The adaptation of germinating spinach seedlings to yellow and red light was studied and compared with plants grown in white light. Spinach chloroplasts isolated from cotyledons and leaves of yellow and white light-grown plants showed similar membrane structures and compositions, while chloroplasts from plants grown in red light have significant adaptive changes. Based on an equal amount of chlorophyll, these changes include a reduction in the number of photosystem I complexes, an increase of photosystem II antenna size, and an increased ratio of stacked to unstacked membranes in red light-adapted chloroplasts. The decrease in the number of photosystem I complexes per unit of chlorophyll in these chloroplasts was qualitatively correlated with an approximately 10-fold decrease in the level of the psaA mRNA encoding the photosystem I 65-kilodalton to 70-kilodalton chlorophyll apoprotein, as well as with a differential decrease in mRNA levels of other photosynthetic proteins. Light quality adaptations do not significantly affect the plastid to nuclear DNA ratio or the overall chloroplast transcription activity. The relative transcriptional activities of 10 plastid genes, as determined by run-on transcription assays, are similar in chloroplasts from cotyledons and leaves of plants grown under the three light qualities. Only the psaA gene shows a 30% to 40% decrease in transcription activity in chloroplasts of plants adapted to red light. This decrease in psaA transcription activity, however, cannot fully account for the decrease of its mRNA level. We conclude, therefore, that post-transcriptional mechanisms are primarily responsible for the control of differential chloroplast mRNA accumulation in light quality adaptations.

INTRODUCTION

Chloroplasts of higher plants can adapt to different environmental light conditions that allow the plants to succeed in a variety of habitats (for review see Boardman, 1977; Baker and Markwell, 1985; Melis et al., 1985). These adaptations are marked by characteristic morphological and biochemical changes in the chloroplasts (Boardman, 1977; Buschmann et al., 1978; Melis and Harvey, 1981; Lichtenhager et al., 1982; Leong and Anderson, 1983; Melis, 1984; Baker and Markwell, 1985). For example, there are significant differences in the ultrastructure of chloroplasts and in the relative amounts of pigmented complexes in plant thylakoids adapted to different light quality environments (Buschmann et al., 1978; Melis and Harvey, 1981; Melis, 1984). The chloroplasts from plants grown in photosystem II (PS II)-sensitizing (yellow) light are relatively enriched in photosystem I (PS I) complexes, while plants grown in light preferentially absorbed by PS I (red) are relatively enriched in PS II complexes (Melis and Harvey, 1981; Melis, 1984; Glick, McCauley, and Melis, 1985; Melis et al., 1985; Melis and Deng, 1987). As a result, plants that are grown in red light show an increase in stacked thylakoid membrane regions, which contain primarily PS II complexes (Melis, 1984; Melis et al., 1985), whereas the distribution and size of stacked membrane regions is similar in plants grown in yellow or white light. This difference in thylakoid structure between plants grown in yellow and red light is thought to be a response to the imbalance in light absorption between PS I and PS II and the subsequent rebalance in electron flow between the two photosystems (Glick, McCauley, and Melis, 1985; Melis et al., 1985; Deng and Melis, 1986). In pea plants, changes in the relative amounts of the thylakoid membrane complexes are reported to reflect different steady-state levels of the plastid mRNAs that encode specific proteins of these complexes (Glick et al., 1986). This suggests that light quality can affect either the synthesis or stability of specific plastid mRNAs. However, it is not known whether or not adjustment of chloroplast mRNA levels of photosynthetic proteins during adaptation to different light environments is a general mechanism in higher plants, and
whether these adjustments are controlled at the level of transcription or mRNA stability.

We and others have recently demonstrated that post-transcriptional regulatory mechanisms rather than transcriptional control are mainly responsible for the differential accumulation of several plastid mRNAs during plant development and organ differentiation (Deng and Gruissem, 1987, 1988; Gruissem et al., 1987; Mullet and Klein, 1987; Kuntz et al., 1989). These studies showed that specific mRNA levels for many plastid genes are established in different plant organs as part of the developmental program, and that light affects the stability, but not the relative transcription rate, of several mRNAs. Since changes in plastid mRNA levels have been observed in pea plants growing under different light quality conditions (Glick et al., 1986), it is possible that differences in mRNA accumulation during adaptation are also regulated by post-transcriptional processes.

In this report we wanted to determine whether or not the adaptive changes observed in pea leaves reflect a general mechanism that allows plants to adapt to different light quality environments. We also wanted to establish the molecular mechanisms by which plants regulate the expression of chloroplast genes for proteins that are directly involved in such adaptive changes. We have therefore analyzed spinach chloroplasts at the ultrastructural and biochemical levels in plants grown in yellow or red light environments, and compared them with white light-grown plants. The results show that chloroplasts in spinach cotyledons and leaves in red light undergo significant morphological adjustments, as indicated by their differences in ultrastructure and relative amounts of thylakoid membrane pigment-protein complexes. These changes are also reflected by differences in the levels of plastid mRNAs encoding protein components of the photosystem I and II pigment-protein complexes. Transcriptional analysis demonstrates that the relative transcription activity of the plastid genes encoding these mRNAs, with the exception of the psaA gene (for the 65-kD to 70-kD chlorophyll a-apoprotein of photosystem I), is not affected by different light qualities. Therefore, adaptation of chloroplasts to different light qualities appears to be primarily controlled at the level of mRNA stability.

RESULTS

Ultrastructural and Biochemical Adaptations of Spinach Chloroplasts to Different Light Quality Environments

Three light quality conditions were chosen for our study: red, yellow, and white light. The red light preferentially excited photosystem I complexes, while the yellow light preferentially excited photosystem II complexes. White light, used as a control, resembled most closely the natural light condition. Thus, red and yellow light conditions should result in an imbalance in the rate of photon absorption by the two photosystems compared with the normal (white) light condition (Glick, McCauley, and Melis, 1985; Glick et al., 1986). In pea, it has been shown that the response of the plant to yellow or red light was a reorganization of the components of the thylakoid membrane such that chloroplasts from plants grown in yellow light were enriched in PS I complexes, while plants grown in red light were enriched in PS II complexes (Glick et al., 1986). Such differences are manifested in morphological changes in the structure of the thylakoid membrane system. To examine whether similar changes occur in spinach cotyledons and leaves grown under the different light qualities, we compared the ultrastructure of chloroplasts from spinach plants grown in red, yellow, and white light. Figure 1 shows the corresponding electron micrographs of cotyledon chloroplasts. Two significant differences in membrane structure are readily noted when the chloroplasts of red and yellow light-grown cotyledons are compared. In chloroplasts of cotyledons grown in red light, the ratio of stacked to unstacked thylakoid membranes is significantly increased and the average number of membrane layers in each stacked region is greater than in control plants. The ultrastructure of chloroplasts from cotyledons grown in yellow light does not differ significantly from that of chloroplasts of white light-grown cotyledons. These results are comparable to earlier observations made with pea plants (Melis, 1984), which also showed more drastic structural

![Figure 1. Ultrastructural Changes in Thylakoid Membrane Structure of Chloroplasts from Spinach Plants Germinated and Grown in Different Light Quality Conditions.](image)

(A) Thin sections of chloroplasts in cotyledons of plants grown in white light for 10 days.
(B) Thin sections of chloroplasts in cotyledons of plants grown in yellow light for 10 days.
(C) Thin sections of chloroplasts in cotyledons of plants grown in red light for 10 days.

The magnification is indicated in (B). Abbreviations: G, stacked membrane region; U, unstacked membrane region; S, starch granule.
changes in thylakoid membrane organization in plants grown in far-red enriched light than those grown in far-red depleted light.

The different photosynthetic complexes in the thylakoid membrane are arranged so that photosystem I is located in unstacked membrane regions, whereas the majority of photosystem II is located in stacked membrane regions (Andersson and Anderson, 1980; Anderson and Melis, 1983). Thus, a change in the extent of stacking of the thylakoid membrane system in response to different light qualities indicates an adjustment in the relative amount of the different photosynthetic complexes. To correlate the observed ultrastructural changes in chloroplasts from spinach cotyledons with the biochemical adaptation of the thylakoid membrane system, we quantitated the amount of chlorophyll that is associated with the different thylakoid membrane pigment-protein complexes isolated from chloroplasts of cotyledons grown under different light quality conditions. By solubilizing the thylakoid membrane with surfactant and separating the pigment-protein complexes by Deriphat-PAGE, different chlorophyll-containing complexes were resolved (Figure 2) without significant loss of protein of PS II; LHC II, a minor light-harvesting complex; LHC III, a minor light-harvesting complex; LHC IV, photosystem I; CC II, core complex of photosystem II subcomplex, composed of core complex and light-harvesting complex; LHC II, light-harvesting complex of photosystem II; PSI, photosystem I; CC II, core complex of photosystem II; LHC I, monomer, putative connector light-harvesting complex of photosystem II; LHC II, major light-harvesting complex of photosystem II; LHC I, minor light-harvesting complex of photosystem II; LHC I, a minor light-harvesting pigment-protein of PS II; FP, free pigment.

Figure 2. Non-denaturing Deriphat-PAGE of Pigment-Protein Complexes of Chloroplast Membranes Isolated from Plants Grown in Three Different Light Qualities.

Thylakoids membranes containing 25 µg of chlorophyll from cotyledons grown in white, red, or yellow light were solubilized with 0.1% SDS, 0.9% glycosidic surfactant at 1 mg of chlorophyll/ml final chlorophyll concentration and loaded in each lane. The gel was electrophoresed at 20 mA for 1 hr at room temperature and photographed without staining. Abbreviations: PS II sub, photosystem II subcomplex, composed of core complex and light-harvesting complex; LHC II, light-harvesting complex of photosystem II; PSI, photosystem I; CC II, core complex of photosystem II; LHC I, monomer, putative connector light-harvesting complex of photosystem II; LHC II, major light-harvesting complex of photosystem II; LHC I, minor light-harvesting complex of photosystem II; LHC I, a minor light-harvesting pigment-protein of PS II; FP, free pigment.

Differential Accumulation of Plastid mRNAs in Different Light Quality Environments

To address the role of plastid gene expression in the adaptation process of chloroplasts to different light quality conditions, we first analyzed the steady-state mRNA levels of several plastid genes that encode the reaction center polypeptide of photosystem I (psaA), the 32-kD (D1) polypeptide of photosystem II (psbA), the 47-kD polypeptide of photosystem II (psbB), the ATP synthase β- and ε-subunits (atpBE), and the large subunit of ribulose-1,5-bisphosphate carboxylase (rbcL). The gene-specific probes used in this study are described in Methods. Total RNA from cotyledons grown in white, yellow, or red light containing equal amounts of plastid 16S rRNA was used for RNA blot quantitation analysis (Table 2). The changes...
in mRNA accumulation for the five genes can be categorized into three groups: The first group is represented by *psbA*, for which the mRNA level does not change significantly in white, red, or yellow light. The *psbB*, *rbcL*, and *atpBE* mRNAs represent a second group, in which the mRNA levels are similar in white and yellow light, but are decreased approximately 50% in red light. The *psaA* mRNA falls into a third group, in which the mRNA level shows a small increase in yellow light, but decreases more than 10-fold in red light when compared with a plant grown in white light. When total RNAs were extracted from spinach leaves of plants grown for 15 days after germination in identical light quality conditions, and the mRNAs for the same genes (except *psbB*) were subjected to a similar quantitative analysis, changes in mRNA levels fell into only two groups (Figure 4). Now the *psbA*, *atpBE*, and *rbcL* mRNA levels are similar in leaves from plants grown under the three light quality conditions. In contrast, the change in leaves of the *psaA* mRNA level is comparable with that in 10-day-old cotyledons. It is interesting to note that specific mRNAs respond differently to red light in spinach cotyledons and leaves. Although the mechanism is not understood, such differences may reflect the developmental and/or physiological states of cotyledons and leaves. Despite these differences, the *psaA* mRNA level was reduced significantly in red light both in leaves and cotyledons, which qualitatively correlates with the reduced level of PS I complex and its core polypeptides encoded by the *psaA-psaB* genes. Taken together, the results indicate that adaptive changes in the chloroplast to different light qualities appear to be regulated, at least in part, at the level of RNA accumulation.

### Table 1. Biochemical Analyses of Thylakoid Membranes and of PS I and PS II Pigment-Protein Complexes in Chloroplasts of Spinach Cotyledons Grown in Different Light Quality Conditions

<table>
<thead>
<tr>
<th>Light Quality</th>
<th>Total Thylakoid Membranes</th>
<th>Percentage of Chlorophyll Contained in Each Photosynthetic Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chlorophyll $\alpha/b$</td>
<td>Chlorophyll/Protein $P700$</td>
</tr>
<tr>
<td>White</td>
<td>2.61</td>
<td>560</td>
</tr>
<tr>
<td>Red</td>
<td>2.51</td>
<td>645</td>
</tr>
<tr>
<td>Yellow</td>
<td>2.58</td>
<td>580</td>
</tr>
</tbody>
</table>

Values are the average of at least three independent measurements. CC II is a sum of the pigment in three bands: PS I$_{sub}$, CC II dimer, and CC II monomer (see Figure 2). The percentages were calculated from 560 nm scans of gels (Ziegler and Egle, 1965) similar to that shown in Figure 2, and closely agree with those obtained by electrophoresis and quantification in 80% acetone (Arnon, 1949). Abbreviations are defined in the legend to Figure 2.

Plastid DNA Levels and Overall Rates of Transcription Are Maintained in Different Light Quality Environments

The differential accumulation of plastid mRNAs in cotyledons and leaves grown in red, yellow, or white light could result from changes in gene copy number, transcription activity, or mRNA stability. To distinguish among these possibilities, we have first examined changes in chloroplast genome copy number, organization, and transcription activity in cotyledons grown under three light quality conditions.

The ratio of plastid to nuclear DNA was quantitated by molecular hybridization (Deng and Gruissem, 1987). Briefly, total DNA from spinach cotyledons was extracted, digested with EcoRI, and blotted onto nylon membrane. The membrane was hybridized with DNA probes specific for the plastid *atpB* and nuclear ribosomal DNA genes. After hybridization, the radioactivity in the plastid DNA band and nuclear DNA band were quantitated by scintillation counting and their ratio was calculated (Table 3). The plastid DNA level is increased approximately 20% in cotyledons grown in yellow light, whereas there is no difference between the levels in cotyledons from plants grown in red and those grown in white light. Moreover, comparison of DNA restriction enzyme fragment profiles of chloroplast DNA from these cotyledons does not show any differences (data not shown), indicating that there are no major rearrangements or regional amplifications of plastid DNA fragments in the chloroplast genome that could be attributed to the changes observed at the mRNA level. Incorporation of nucleotide triphosphates into trichloroacetic acid-precipitable RNA during run-on transcription in isolated chloroplasts shows that the overall rate of transcription activity of the chloroplast genomes in cotyledons grown in red, yellow, or white light differs only by 10% or less. Thus, changes in genome structure or in overall rate of transcription are not determinants for the observed differences in mRNA accumulation.

Relative Transcriptional Activities of Plastid Genes Are Maintained in Cotyledons Grown in Different Light Quality Conditions

To test whether modulation of the relative transcriptional activities of individual genes causes the observed differ-
Light Qualities Affect Plastid mRNAs

Figure 3. Two-Dimensional Gel Analysis of Thylakoid Pigment-Proteins from Cotyledons of Plants Grown in White, Red, or Yellow Light.

The directions of the first-dimensional separations of thylakoid pigment-protein complexes are indicated by arrows above each electrophoresis profile. The first dimension gel was run at conditions identical to those described in Figure 2. The green gel slices from the first-dimension gel were excised and denatured, and the proteins were analyzed by second-dimension SDS-PAGE. The SDS-polyacrylamide gel was stained with Coomassie Blue. Details of the identification of individual protein components are presented elsewhere (Peter and Thornber, 1989). The chloroplast proteins for which the expression of their corresponding plastid genes was analyzed are: CC I a/b, 65-kD to 70-kD proteins encoded by psaA/B; CF/β, protein encoded by atpB; CC IIa, the 47-kD protein encoded by psbB; CC Ile, the 32-kD (D1) protein encoded by psbA. Abbreviations: CC I (a to h), core complex of photosystem I; CF I (α, β, γ), coupling factor; LHC I (a and b), light-harvesting complex of photosystem I; PS II17 and PS II11, peptides of photosystems I and II, respectively; OEE, O2 evolution complex; other abbreviations are defined in Figure 2.

Table 2. Changes in Relative Plastid mRNA Levels in Spinach Cotyledons Growing in Different Light Qualities

<table>
<thead>
<tr>
<th>Light Quality</th>
<th>psaA</th>
<th>psbA</th>
<th>psbB</th>
<th>rbcL</th>
<th>atpBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Yellow</td>
<td>106</td>
<td>94</td>
<td>94</td>
<td>109</td>
<td>112</td>
</tr>
<tr>
<td>Red</td>
<td>8</td>
<td>90</td>
<td>49</td>
<td>44</td>
<td>56</td>
</tr>
</tbody>
</table>

Total RNAs (20 μg) containing equal amounts of 16S plastid RNA were blotted to nylon membranes and probed with gene-specific probes for psaA, psbA, psbB, rbcL, and atpBE. The levels were quantitated by scintillation counting of the gene-specific probes hybridized to the RNA in the blots. The levels of mRNAs for each gene in white light-grown cotyledons were arbitrarily set to 100% (control). The numbers presented are the average of three independent measurements.
Atriplex triangularis has shown that eightfold differences in chloroplast mRNA levels are reduced when plants are grown in red light that is preferentially absorbed by PS I, while in leaves, these mRNA levels are similar in yellow, red, and white light. However, in cotyledons and leaves, psaA mRNA accumulation shows a small increase in yellow light and a more than 10-fold decrease in red light. Although the mechanism is not known, it is interesting to note that red light, but not yellow light, affects atpB, rbcL, and psbB mRNA levels in cotyledons, but not in leaves. Moreover, when compared with the level of the psbA mRNA, it is apparent that this effect is not a general decrease in plastid mRNA levels, but may be specific for individual mRNA populations. Quantitatively, there is also a discrepancy between mRNA levels and the levels of the photosynthetic protein complexes containing one or more of the transcribed mRNA products. For example, in cotyledons and leaves, the change in psaA mRNA level was significantly greater than the changes in the amount of PS I complexes or the level of the 65-kD to 70-kD chlorophyll a-apoprotein between chloroplasts from plants grown in red, yellow, and white light. Also, two-dimensional gel analysis has shown that the 47-kD chlorophyll a-binding protein (CC IIa in Figure 3, encoded by psbB) is always present in equimolar amounts with the 32-kD D1 protein of PS II (CC IIe in Figure 3, encoded by psbA) under all light conditions tested (data not shown), although the psbB mRNA level, but not that of psbA, is clearly reduced in cotyledons grown in red light. Thus, although different light qualities significantly affect the level of mRNAs that encode proteins of the same photosynthetic complex, this does not necessarily alter the steady-state level of the proteins derived from them. It is apparent, therefore, that the adaptation process of spinach plants to different light quality environments is far more complex than a simple adjustment of mRNA levels for photosynthetic proteins.

The experimental results suggest that different light qualities can affect the stability of individual plastid mRNAs, and therefore differential mRNA accumulation may be a post-transcriptional control step in the regulation of plastid genes in plants growing in different light quality environments. 

DISCUSSION

Our results demonstrate that spinach seedlings germinated and grown in different light qualities develop chloroplasts with apparent adaptive morphogenetic changes, most significantly when grown under red light. Specifically, when chloroplasts of red light-grown plants are compared with those grown in yellow or white light, the amount of PS I complexes is decreased and the antenna size of PS II is increased (Table 1). These adaptive changes can, in principal, minimize the imbalance of electron flow generated by the preferential absorption of red light by photosystem I (Melis et al., 1985). Such adaptive compositional changes in the thylakoid membrane would contribute, in part, to the observed morphological changes in the chloroplast of spinach cotyledons grown in red light (Figure 1, Table 1). Similar adaptations to red and yellow light were reported for leaves of germinating pea plants (Melis, 1984; Glick, McCauley, and Melis, 1985; Glick et al., 1986).

Comparison of data on pea and spinach reveals that, under similar light intensities, the response to different light qualities is greater in pea than in spinach. Light intensity per se is most likely not responsible for these differences in response since earlier work in Phaseolus vulgaris and Atriplex triangularis has shown that eightfold differences in light intensity resulted only in a 12% to 14% change in PS II/PS I ratio (Melis and Harvey, 1981; Melis et al., 1985).

Qualitatively, differences in the relative amount of PS I and PS II complexes in spinach plants grown in different wavelengths of light paralleled differences in the steady-state level of chloroplast mRNAs coding for PS I and PS II polypeptides. The responses differ in 10-day-old cotyledons and 15-day-old leaves. In cotyledons, the mRNA levels of rbcL, atpBE, and psbB are reduced when plants are grown in red light that is preferentially absorbed by PS I, while in leaves, these mRNA levels are similar in yellow, red, and white light. However, in cotyledons and leaves, psaA mRNA accumulation shows a small increase in yellow light and a more than 10-fold decrease in red light. Although the mechanism is not known, it is interesting to note that red light, but not yellow light, affects atpB, rbcL, and psbB mRNA levels in cotyledons, but not in leaves. Moreover, when compared with the level of the psbA mRNA, it is apparent that this effect is not a general decrease in plastid mRNA levels, but may be specific for individual mRNA populations. Quantitatively, there is also a discrepancy between mRNA levels and the levels of the photosynthetic protein complexes containing one or more of the transcribed mRNA products. For example, in cotyledons and leaves, the change in psaA mRNA level was significantly greater than the changes in the amount of PS I complexes or the level of the 65-kD to 70-kD chlorophyll a-apoprotein between chloroplasts from plants grown in red, yellow, and white light. Also, two-dimensional gel analysis has shown that the 47-kD chlorophyll a-binding protein (CC IIa in Figure 3, encoded by psbB) is always present in equimolar amounts with the 32-kD D1 protein of PS II (CC IIe in Figure 3, encoded by psbA) under all light conditions tested (data not shown), although the psbB mRNA level, but not that of psbA, is clearly reduced in cotyledons grown in red light. Thus, although different light qualities significantly affect the level of mRNAs that encode proteins of the same photosynthetic complex, this does not necessarily alter the steady-state level of the proteins derived from them. It is apparent, therefore, that the adaptation process of spinach plants to different light quality environments is far more complex than a simple adjustment of mRNA levels for photosynthetic proteins.

The experimental results suggest that different light qualities can affect the stability of individual plastid mRNAs, and therefore differential mRNA accumulation may be a post-transcriptional control step in the regulation of plastid genes in plants growing in different light quality environments.

<table>
<thead>
<tr>
<th>Light Quality</th>
<th>ctDNA/nuDNA</th>
<th>Overall Transcription Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>arbitrary unit</td>
<td>cpm x 10⁴/μg DNA</td>
</tr>
<tr>
<td>White</td>
<td>0.87</td>
<td>2.21</td>
</tr>
<tr>
<td>Yellow</td>
<td>1.0</td>
<td>2.30</td>
</tr>
<tr>
<td>Red</td>
<td>0.82</td>
<td>2.47</td>
</tr>
</tbody>
</table>

The quantitation of relative chloroplast DNA (ctDNA) to nuclear DNA (nuDNA) ratios is described in Methods, and the highest ratio was defined as one arbitrary unit.
Equivolot amounts of 10 different gene-specific DNA fragments were blotted to nylon membranes. Purified labeled run-on transcripts from isolated chloroplasts of white, yellow, or red light-germinated spinach seedlings were hybridized to three identical blots. After washing, the filters were exposed to x-ray film at -70°C for approximately 20 hr. Abbreviations for plastid genes are: rnr, rRNA; rpoA, putative α-subunit of the plastid RNA polymerase; rpl2, ribosomal protein L2; psbA, 32-kD quinone-binding protein of photosystem II reaction center; rbcL, large subunit of ribulose-1,5-bisphosphate carboxylase; atpB, β-subunit of chloroplast ATPase; psaA, 65-kD to 70-kD chlorophyll a-apoprotein of photosystem I; psbB, 50-kD chlorophyll a-binding protein of photosystem II; petB and petD, cytochrome b6f complex, respectively. The construction of specific probes for the above genes has been described previously (Deng and Gruissem, 1987).

ments. Similar conclusions of post-transcriptional control have been drawn from earlier studies examining the regulation of spinach plastid gene expression during plant development and plastid differentiation (Deng and Gruissem, 1987, 1988). The conclusion in this report is supported by our observations that chloroplast DNA levels and overall transcription activities are similar whether plants are grown in white, yellow, or red light (Table 3). Furthermore, the relative transcription activities of several plastid genes, including seven genes encoding mRNAs for photosynthetic proteins, are also similar under those conditions. One exception may be the gene for the 65-kD to 70-kD chlorophyll a-apoprotein of photosystem I (psaA), for which we can consistently measure by run-on transcription a decrease in activity of 30% to 40% (Figure 5). A similar decrease of psaA transcription has also been observed in young as compared with mature spinach leaves and overall transcription activities are similar whether plants are grown in white, yellow, or red light (Deng and Gruissem, 1987).

Figure 5. Relative Transcription Activities of 10 Different Plastid Genes in Chloroplasts from Cotyledons Grown in White (W), Yellow (Y), or Red (R) Light.

The experiments reported here have established a correlation between differential excitation of the two photosystems and post-transcriptional mechanisms that modulate the stability of mRNAs that encode the proteins for the two photosystems. It will be of great interest to establish the transduction pathway and the molecular mechanism that result in an alteration of plastid mRNA stability and that allow chloroplasts to adapt to different light quality environments.

METHODS

Plant Materials

Spinach (Spinacia oleracea cv Marathon hybrid) seeds were planted in sterile soil and germinated in growth chambers at 25°C under a 12-hr light/12-hr dark cycle. The white light was provided by a combination of 30-W General Electric cool-white fluorescent lights and 50-W incandescent bulbs. The yellow and red light were provided either by 30-W General Electric cool-white lights filtered through yellow Plexiglas (Rohm & Haas, no. 2208) or by 50-W incandescent bulbs filtered through red Plexiglas (Rohm & Haas, no. 2423). The energy fluence rate in the visible region for all three growth chambers was 50 μE·m⁻²·s⁻¹ at the soil surface, and was measured using Li-Cor 185 photometer (Li-Cor, Inc.), which only senses the visible light (380 nm to 700 nm). As previously reported (Glick et al., 1986), the red and yellow illuminations used were selected to maximize differential absorptions of the two photosystems and to minimize for light intensity effects on chloroplast membranes. Spinach cotyledons or leaves were harvested 10 days or 15 days after planting, respectively, and used immediately for the isolation of chloroplasts or thylakoid membranes.

Electron Microscopic Analysis

Spinach cotyledons grown under different light quality conditions were fixed for 2 hr at room temperature in 0.5% (w/v) glutaraldehyde containing 10 mM phosphate buffer (pH 7.2) and 0.3 M sucrose. After five washings in the same buffer without glutaraldehyde, samples were post-fixed in 10 mM phosphate buffer (pH 7.2) containing 1% OsO₄ for 2 hr at room temperature. The samples were subsequently washed four times in 0.1 M sodium cacodylate buffer (pH 7.2) and incubated in 1% (w/v) tannic acid in 0.1 M sodium cacodylate buffer (pH 7.2) at room temperature for 10 min. The samples were washed again in 0.1 M sodium cacodylate buffer (pH 7.2) three times and dehydrated in an ethanol series (once with 30%, 50%, 70%, 85%, 90%; twice with 100%), followed by propylene oxide, and subsequently embedded in a sample capsule with Araldite 6005:dodecenylsuccinic anhydride:benzylidimethylamine (v:v: 13.5:11.5/0.5) at 60°C for 5 hr. The samples were sectioned using an ultramicrotome (model MT 6000-XL from RMC Inc.). The sections were then post-stained.
with 5% (w/v) uranyl acetate in water for 20 min and counter-
stained with 3% (w/v) lead citrate for 10 min. After the sections were
dried, they were examined and photographed using a Zeiss
109 transmission electron microscope.

**Isolation of Thylakoid Membranes from Spinach Cotyledons**

For the isolation of thylakoid membranes, cotyledons or leaves were
homogenized in a Waring blender in homogenization buffer (50 mM
HEPES, pH 6.8, 1 mM sodium pyrophosphate, 0.33 M
sorbitol, 10 mM DTT, 1 mM MgCl₂, 1 mM MnCl₂, 2 mM EDTA for
2 sec at low speed and 8 sec at high speed. The homogenate was
filtered through four layers of sterile miracloth. The filtrate
was centrifuged at 250 g for 1 min to remove debris. Chloroplasts
were collected from the supernatant by centrifugation at 3,000g
for 30 sec. The chloroplast pellet was resuspended and washed
once in 40 ml of the homogenation buffer, followed by centrifu-
gation at 3,000g for 30 sec. Following resuspension of the chlo-
roplast pellet in a solution of 6.2 mM Tris, 48 mM glycine (pH 8.3)
to disrupt the chloroplast envelope by osmotic shock, the broken
chloroplast suspension was then centrifuged at 10,000g for 5
min. The pellet consisting primarily of thylakoid membranes was
resuspended to a final concentration of 1.1 mg of chlorophyll/ml
in the Tris-glycine buffer containing 10% (v/v) glycerol, aliquoted
and frozen at −70°C. The concentration of P700 in thylakoid
membranes was quantitated using the light-induced differential
absorption at 700 nm and an extinction coefficient of 59 mM⁻¹
(Dietrich and Thornber, 1971).

**Thylakoid Membrane Solubilization**

Surfactant stock solutions were typically prepared by mixing 1%
(w/v) SDS with 9% (w/v) of a glycosidic surfactant to give a 10%
(w/v) stock solution. Membranes were thawed quickly at 20°C
but not allowed to reach room temperature and were chilled on
ice. The membrane and surfactant stocks were then mixed, 1
volume surfactant and 9 volumes membranes, to yield a final 10:1
weight ratio of surfactant to chlorophyll. The surfactant extracts
were spun in a microcentrifuge for 2 min to remove starch, and
the green supernatant was immediately applied to Deriphat-PAGE.
Solubilizations and incubations were carried out on ice. Chloro-
phyll concentration was estimated according to Arnon (1949).

**Deriphat-PAGE**

The procedure was essentially that used previously (Peter and
Thornber, 1989). Gels contained 12.4 mM Tris, 48 mM glycine
(pH 8.3), and 8% acrylamide (acylamide to bis-acylamide ratio,
33.5/0.3), and were polymerized by adding 0.1% ammonium persulfate and 0.005% N,N,N′N′-tetramethylethylenediamine
(TEMED). Typically, 25 µg of chlorophyll in the surfactant solution
was loaded per lane for 2-mm-thick gels. The electrophoresis
reservoir buffer was 12.4 mM Tris, 96 mM glycine (pH 8.3)
containing 0.2% Deriphat 160, which was precooled on ice. SDS
was added (0.01% final concentration) to the buffer immediately
before electrophoresis. The timing is necessary to prevent precip-
itation of Deriphat before the sample enters the gel. Gels were
electrophoresed at 100 V (constant voltage) for 35 min. Longer
electrophoresis times decreased the resolution of complexes due
to decreasing pH within the gel. The initial current was 20 mA,
which decreased to approximately 15 mA after 35 min.

**Denaturing SDS-PAGE**

Linear gradient gels of 11% to 16% acrylamide were used ac-
cording to Laemmli (1970), except that the separating gel con-
tained 4 M urea and twice the ionic strength (0.755 M Tris-HCl,
pH 8.8). Gel slices were incubated for 30 min in 75 mM Tris-HCl
(pH 6.8), 5.0 mM EDTA, 100 mM DTT, and 4% SDS at 25°C.
Gels were run overnight at a constant current of 25 mA. They
were fixed and stained for 2 hr in 50% methanol, 10% acetic acid,
40% water, 0.1% Coomassie Brilliant Blue, and destained in the
same solution without Coomassie.

**Chloroplast RNA Levels**

Total RNA was isolated from spinach cotyledons and leaves
according to a published procedure (Deng and Gruissem, 1987).
Briefly, 10 g of fresh cotyledons were quickly frozen in liquid
nitrogen and ground with a mortar and pestle. The powdered
tissue was suspended in 25 ml of 50 mM Tris-HCl (pH 8.0)/0.35
M sorbitol/25 mM EDTA/15 mM 2-mercaptoethanol/10 mM dihi-
thiothreitol/25 mM aurin tricarboxylic acid. The homogenate was
filtered by two layers of Miracloth (Behring Diagnostics), and
chloroplasts were lysed by the addition of 2.5 ml of 5% (w/v)
sodium sarcosinate/50 mM Tris-HCl (pH 8.0)/25 mM EDTA. The
lysed suspension was extracted three times with an equal volume of
phenol/chloroform/isooamyl alcohol (25:25:1, v/v/v) at room
temperature. After centrifugation at 10,000g for 10 min, the
aqueous phase was collected and precipitated with 1/10 volume
of 5 M ammonium acetate and 2 volumes of ice-cold ethanol at
−20°C for 1 hr. After centrifugation at 10,000g for 15 min at 4°C,
the pellet was suspended in 10 mM Tris-HCl (pH 7.5)/1 mM
EDTA. High molecular weight RNA was precipitated with 2 M LiCl
overnight at 4°C. The RNA was collected by centrifugation at
10,000g for 15 min at 4°C, suspended in RNase-free water, and
stored at −20°C. RNAs from the different preparations were
denatured and fractionated in 1.1%
M urea and twice the ionic strength (0.755 M Tris-HCl,
pH 6.8). Gel slices were incubated for 30 min in 75 mM Tris-HCl
(pH 6.8), 5.0 mM EDTA, 100 mM DTT, and 4% SDS at 25°C.
Gels were run overnight at a constant current of 25 mA. They
were fixed and stained for 2 hr in 50% methanol, 10% acetic acid,
40% water, 0.1% Coomassie Brilliant Blue, and destained in the
same solution without Coomassie.
Total DNA from 10 g of cotyledons or leaves grown in yellow, red, or white light was isolated by using a procedure similar to the RNA isolation except that the powdered tissue was suspended in a buffer without aurin tricarboxylic acid and the homogenate was not filtered through Miracloth. In addition, after ethanol precipitation, the nucleic acid pellet was suspended in 10 mM Tris-HCl (pH 7.5)/1 mM EDTA, and DNA was purified by CsCl density gradient centrifugation. The isolated DNA was digested with EcoRl to completion. Equal amounts of digested DNA were electrophoretically separated and transferred to nylon membranes. DNA fragments specific for the peak cytoplasmic 25S and 18S ribosomal RNA genes and the spinach plastid atpB gene were labeled to similar specific activities, mixed, and hybridized to the DNA blots (Deng and Gruissem, 1987). The radioactivity in the probes hybridized to the restriction fragments was determined by scintillation counting. The ratio of the radioactivity in the plastid DNA and nuclear DNA bands was calculated and used as an indication of the ratio of plastid DNA to nuclear DNA.

**Chloroplast Run-On Transcription**

Cotyledons and leaves from seedlings grown in yellow, red, or white light were harvested approximately 2 hr after the beginning of the light period and used immediately for chloroplast isolation (Deng and Gruissem, 1987). The chloroplast run-on transcription followed a procedure described previously (Deng et al., 1987). The run-on transcripts from different chloroplast samples were hybridized to nylon membranes blotted with stoichiometric amounts of gene-specific DNA probes. The detailed description of gene-specific probes has been published previously (Deng and Gruissem, 1987). After hybridization, the amount (cpm) of transcript hybridized to individual gene probes was determined by scintillation counting, and was regarded as a measure of the transcription activity of this gene.

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