Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4038–4042, June 1990
Botany

Chlorophyll regulates accumulation of the plastid-encoded chlorophyll apoproteins CP43 and D1 by increasing apoprotein stability

(barley/chloroplast/photosystem II reaction center)

JOHN E. MULLET, PATRICIA GAMBLE KLEIN†, AND ROBERT R. KLEIN‡

Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843–2128

Communicated by Winslow R. Briggs, March 2, 1990 (received for review November 30, 1989)

ABSTRACT **Chlorophyll apoprotein accumulation in higher plant chloroplasts is controlled by light-dependent chlorophyll formation. Dark-grown plants lack chlorophyll and chlorophyll apoproteins. However, the plastid genes encoding the chlorophyll apoproteins are transcribed; chlorophyll apoprotein mRNA accumulates and associates with polysomes in plastids of dark-grown plants. Pulse-labeling assays revealed a population of short-lived proteins in plastids of dark-grown plants. One of these transiently labeled proteins was CP43, a chlorophyll apoprotein associated with photosystem II. Pulse–chase assays showed that newly synthesized CP43 was rapidly degraded in plastids of dark-grown plants, which lack chlorophyll. In contrast, CP43 synthesized in plastids from illuminated plants was stable. The synthesis of D1, a chlorophyll apoprotein of the photosystem II reaction center, was also analyzed in plastids of dark-grown and illuminated plants. Radiolabel accumulation into full-length D1 was only detected in plastids of illuminated plants. However, D1 translation intermediates of 15–25 kDa were detected in both plastid populations. Pulse–chase assays showed that the 15- to 25-kDa D1 translation products were precursors of mature D1 in plastids of illuminated plants. In contrast, in plastids of dark-grown plants, the 15- to 25-kDa translation intermediates were converted into a 23-kDa polypeptide previously suggested to be a proteolytic product of D1. These results indicate that chlorophyll produced in illuminated plants stabilizes D1 nascent polypeptides, which allows accumulation of mature D1.**

Chlorophyll is the key chromophore involved in plant photosynthetic light reactions. This chromophore mediates light-dependent charge separation in photosystem I (PSI) and photosystem II (PSII) and serves as the primary light-harvesting pigment in these photosystems. Chlorophyll is noncovalently associated with at least 12 different membrane-bound proteins of the thylakoid. PSII reaction centers contain two plastid-encoded chlorophyll-binding proteins, D1 and D2, which share structural and sequence homology with the bacterial reaction center subunits L and M (reviewed in refs. 1 and 2). D1 and D2 together bind at least four chlorophylls, two pheophytins, two quinones, and a non-heme Fe (reviewed in ref. 2). Primary charge separation in PSII is mediated by a chlorophyll dimer; the other chlorophylls associated with D1 and D2 serve as light-harvesting antennae and may facilitate electron transfer. In addition to the chlorophyll associated with D1 and D2, PSII has an inner chlorophyll antennae composed of up to 40 chlorophyll a molecules bound to the chlorophyll apoproteins CP43 and CP47 (reviewed in ref. 3). These proteins, like D1 and D2, are encoded by plastid-localized genes (4, 5). A peripheral chlorophyll–protein complex, LHCII, also contributes to the light-harvesting capacity of PSII (reviewed in ref. 3). The reaction center proteins of PSI are chlorophyll apoproteins (P_{700}-A and P_{700}-B) of 82 and 83 kDa. These plastid-encoded chlorophyll apoproteins (6) bind chlorophyll involved in primary charge separation (P_{700}) plus ≈40 chlorophyll a antennae. PSI also has a peripheral light-harvesting complex, termed LHCI (7–9).

During barley leaf biogenesis in illuminated plants, chloroplasts accumulate $\approx 10^6$ PSI and PSII complexes containing 3.7×10^8 chlorophyll molecules during a 36- to 48-hr period (10). However, higher plants grown in darkness lack chlorophyll and chlorophyll apoproteins (10–14), although they accumulate protochlorophyllide, a chlorophyll precursor. When plants are illuminated, protochlorophyllide is reduced to chlorophyllide by protochlorophyllide reductase in a light- and NADPH-dependent reaction. Chlorophyllide is subsequently esterified with geranylgeranyl pyrophosphate in a light-independent step to form chlorophyll (reviewed in ref. 15). Although plastids of dark-grown plants lack chlorophyll apoproteins, most of the soluble proteins and many membrane proteins found in chloroplasts accumulate in these plants (10). Furthermore, the plastid genes encoding chlorophyll apoproteins are transcribed in dark-grown plants (16), and chlorophyll apoprotein mRNA accumulates (10, 12, 14, 17, 18). This mRNA is associated with polysomes (19), although radiolabeling studies failed to detect amino acid incorporation into D1, CP43, CP47, and the P_{700} chlorophyll apoproteins (10). Within 5 min after dark-grown plants are illuminated, amino acid incorporation into D1, CP43, CP47, and the P_{700} chlorophyll apoproteins can be detected, and the chlorophyll apoproteins begin to accumulate (11, 14, 20). The light-dependent activation of chlorophyll apoprotein accumulation is controlled by protochlorophyllide reductase and requires formation of chlorophyll a (11, 20). Furthermore, activation of chlorophyll apoprotein accumulation occurs without large changes in the distribution of chlorophyll apoprotein mRNA in polysomes (19). On the basis of these data, we suggested that chlorophyll a activates chlorophyll apoprotein accumulation either by overcoming a block in translation elongation or by binding to and stabilizing nascent chlorophyll apoproteins (19). In this paper, we show that chlorophyll stabilizes newly synthesized chlorophyll apoproteins D1 and CP43, which results in their accumulation.

MATERIALS AND METHODS

Plant Growth and Plastid Isolation. Barley (*Hordeum vulgare* L. var. Morex) seedlings were grown as described (10).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: PSI and PSII, photosystems I and II.

†Present address: Department of Plant Pathology, University of Kentucky, Lexington, KY 40546-0091.
‡Present Address: Agricultural Research Service, U.S. Department of Agriculture, University of Kentucky, Lexington, KY 40546-0076.

After 4.5 days of growth in darkness, some seedlings were transferred to an illuminated chamber for up to 1 hr before harvest (light fluence rate = 120 $\mu E \cdot m^{-2} \cdot s^{-1}$; 1 E (einstein) = 1 mol of photons; incandescent plus fluorescent bulbs). Plastids were isolated from the top 3 cm of barley primary leaves by using Percoll gradients as described (10, 14). Plastids were isolated from dark-grown plants in the dark except for brief periods when a dim green safelight was used (20).

Plastid Protein Synthesis and Polysome Analysis. ATP-dependent protein synthesis in plastids was assayed as described (14). Pulse-labeling assays were 5 min in duration and utilized 100 μCi (1 Ci = 37 GBq) of [^{35}S]methionine in 75-μl reaction mixtures containing 1.4×10^7 plastids. When chase periods were used, 5 mM unlabeled methionine was added after the pulse-labeling period. Radiolabeled proteins were separated into soluble and membrane fractions (10), solubilized in SDS, and separated on 7.5–15% polyacrylamide gels containing 4 M urea or 12–18% polyacrylamide gels containing 8 M urea. Gels were fixed, fluorographed, and exposed to x-ray film as described (14, 20). Polysomes from isolated plastids were separated on sucrose gradients as described (19). The distribution of RNA in the polysome gradients was determined by fractionating the sucrose gradients containing the polysomes, extracting the RNA, and analyzing the RNA by formaldehyde gel Northern analysis (20). The probe used to detect *psbD–psbC* transcripts was a nick-translated 1.27-kilobase pair *Hin*dIII–*Eco*RI DNA from the barley *psbD–psbC* transcription unit (17).

Preparation and Use of Antibodies. Preparation of antibodies to D2 and CP43 has been described (21). For production of antibodies against D1, a portion of the open reading frame (153 base pairs, amino acids 34–84) was fused, in frame, to the 3′ end of a portion of the *trpE* gene of *Escherichia coli* in the vector PATH10. Growth of RR1 strains containing recombinant plasmids and isolation of *trpE* fusion protein were done as described (21). Prior to immunoprecipitation, plastid proteins were treated with 2% SDS and boiled for 2 min, then diluted 10-fold with 50 mM Tris·HCl (pH 7.5), 0.15 M NaCl, 2 mM EDTA, and 1% Nonidet P-40. Preimmune or immune sera was then added to the sample, and after incubation, IgG was collected by using protein A-Sepharose (22). Immunoprecipitated proteins were separated on SDS gels and detected by fluorography as described above.

Chlorophyll Determination. Chlorophyll a was quantitated after acetone extraction by using HPLC (20). Chlorophyll a used for generating a standard curve was purchased from Sigma.

RESULTS

Distribution of *psbD–psbC* RNA in Plastid Polysomes of Dark-Grown and Illuminated Barley. We previously reported that plastids of dark-grown barley incorporate little radiolabel into CP43 and that illumination of plants prior to plastid isolation greatly stimulates accumulation of radiolabeled CP43 (14). In contrast, the synthesis of D2, a PSII reaction center chlorophyll apoprotein, was not altered by plant illumination (10). The genes encoding D2 and CP43 (*psbD* and *psbC*, respectively) are adjacent to each other in the barley plastid genome (17). Some large transcripts produced from this DNA region encode both D2 and CP43 (RNAs designated a in Fig. 1). Other RNAs only encode CP43 (RNAs designated b in Fig. 1) (17). It is possible that light-induced radiolabeling of CP43 involves selective activation of translation of the transcripts encoding CP43. To examine this possibility, we assayed the distribution of *psbD–psbC* RNAs in plastid polysome gradients of dark-grown and illuminated seedlings (Fig. 1). This experiment showed that RNAs encoding D2 and CP43 were associated

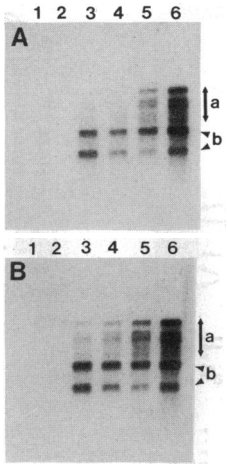

FIG. 1. Northern blots of *psbD–psbC* RNAs isolated from sucrose gradients used to separate plastid membrane-associated ribosomal material from 4.5-day-old dark-grown seedlings (*A*) or 4.5-day-old dark-grown seedlings that were illuminated for 1 hr (*B*). Fraction 1 is from the top of the sucrose gradient, which contained 30S ribosomal subunits. Fraction 2 contained 50S ribosomal subunits, whereas fractions 3–6 contained 70S monomers to polysomes consisting of greater than six ribosomes. a, RNAs encoding both D2 and CP43; b, RNAs encoding only CP43.

with polysomes in dark-grown and illuminated plants. Furthermore, the population of RNAs associated with the polysomes and the relative distribution of the RNAs in the polysome gradients were similar in the two plastid populations. These data were not consistent with selective activation of translation initiation from these RNAs when plants are illuminated. We did note an increase in soluble and membrane polysome content in illuminated seedlings (i.e., membrane polysome to total ribosome ratio increased from 0.45 to 0.61). As reported earlier, this reflects a general stimulation of plastid protein synthesis rather than selective activation of chlorophyll apoprotein synthesis (19).

Pulse–Chase Assays Reveal Transiently Labeled Plastid Proteins. The association of chlorophyll apoprotein mRNAs with polysomes but lack of radiolabel accumulation into the apoproteins could be due to very rapid turnover of newly synthesized full-length chlorophyll apoproteins. Alternatively, nascent chlorophyll apoproteins could be degraded at some intermediate step in their synthesis while still associated with polysomes. Previous attempts to detect chlorophyll apoprotein synthesis in dark-grown plants may have failed because protein turnover occurred during translation of the apoproteins. In this case, it still might be possible to detect apoprotein translation intermediates that had not reached the point of protease sensitivity or breakdown products resulting from chlorophyll apoprotein proteolysis. To test this possibility, plastids were isolated from 4.5-day-old dark-grown barley plants and pulse-labeled with [^{35}S]methionine for 5 min or pulse-labeled for 5 min and then incubated for 10 min in the presence of excess unlabeled methionine. As expected, little [^{35}S]methionine accumulated in mature chlorophyll apoproteins D1, CP43, CP47, P_{700}-A, or P_{700}-B in plastids from dark-grown plants that received the pulse–chase treatments (Fig. 2, lane 2). As previously noted, one chlorophyll apoprotein, D2, is radiolabeled in plastids from dark-grown plants (10, 21). Pulse-labeled plastids were more difficult to analyze due to the large number of radiolabeled proteins in this sample (Fig. 2, lane 1). However, it was clear that little [^{35}S]methionine accumulated in the P_{700} chlorophyll apoproteins in the pulse-labeled plastids from dark-grown plants. The pulse-labeled plastids contained a large number of radiolabeled soluble and membrane proteins that disappeared

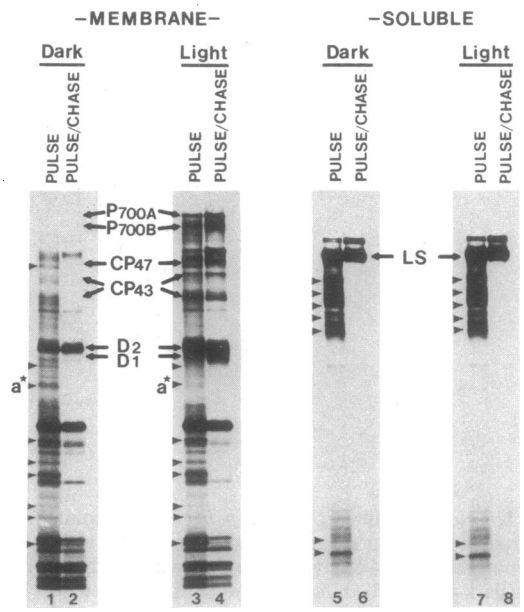

FIG. 2. Autoradiogram of proteins synthesized in plastids of dark-grown or illuminated barley seedlings (lanes marked Dark and Light, respectively). Plastids were isolated from 4.5-day-old dark-grown seedlings or from similar plants that had been illuminated with 120 $\mu E \cdot m^{-2} \cdot s^{-1}$ of white light for 1 hr. Following isolation, plastid proteins were either pulse-labeled for 5 min with [^{35}S]methionine (lanes 1, 3, 5, and 7) or pulse-labeled for 5 min and then incubated in the presence of excess unlabeled methionine for 10 min (lanes 2, 4, 6, and 8). Plastid proteins were then fractionated into membrane and soluble phases, solubilized in SDS, and loaded on 7.5–15% polyacrylamide gels containing 4 M urea (20). Samples were loaded on an equal plastid number basis. Gels were fixed, fluorographed, and exposed to x-ray film for 4 days. The migration of the chlorophyll apoproteins D1, D2, CP43, CP47, P_{700}-A, and P_{700}-B are indicated between lanes 2 and 3. The location of the large subunit of Rubisco (LS) in lanes 5–8 is noted. Arrowheads point to transiently labeled proteins, and the protein marked a* is a transiently labeled protein enriched in plastid membranes of dark-grown plants.

during the 10-min chase (Fig. 2, proteins marked with arrowheads at the left of lanes 1 and 5). Most of these transiently labeled proteins did not comigrate with previously identified, stainable plastid proteins (14, 20). We previously reported that some transiently labeled soluble proteins in pea chloroplasts were paused translation intermediates of the large subunit of Rubisco (23). The transiently labeled proteins observed here could likewise be translation intermediates or could result from the action of plastid proteases.

Illumination could activate translation of the chlorophyll apoproteins or decrease their rate of turnover. These changes might alter the population of transiently labeled plastid membrane proteins. To check this possibility, plastids isolated from illuminated plants were pulse-labeled or labeled in pulse–chase experiments as described for plastids from dark-grown plants (Fig. 2, lanes 3, 4, 7, and 8). As expected, radiolabel incorporation into D1, CP43, CP47, and the P_{700} chlorophyll apoproteins was readily detected in these plastids. In addition, most of the transiently labeled proteins observed in plastids of dark-grown plants were also observed in pulse-labeled plastids from illuminated plants. During the chase, the amount of radioactivity in the transiently labeled proteins decreased, and the amount of radiolabel in some of the chlorophyll apoproteins increased (i.e., D1, CP47, and the P_{700} chlorophyll apoproteins). This could indicate that some of the transiently labeled proteins are precursors of the chlorophyll apoproteins. The abundance of at least one transiently labeled protein (labeled a*) observed in plastids of dark-grown plants was decreased in illuminated plastids.

Chlorophyll Apoprotein CP43 Is Translated But Unstable in Dark-Grown Plants. We tested the relationship between the transiently labeled proteins and D1, D2, and CP43 by immunoprecipitation (Fig. 3). Plastids from dark-grown or 1-hr-illuminated barley seedlings were pulse-labeled for 2 min (Fig. 3, lanes 1, 3, 5, 7, 9, and 11) or pulse-labeled for 2 min and then incubated with an excess of unlabeled methionine for 30 min (Fig. 3, lanes 2, 4, 6, 8, 10, and 12). The plastids were then lysed; membranes were isolated and dissolved in SDS; and proteins were immunoprecipitated with antisera to D2 (Fig. 3, lanes 1–4), CP43 (Fig. 3, lanes 5–8), or D1 (Fig. 3, lanes 9–12). D2 was readily detected in pulse-labeled plastids of dark-grown or illuminated plants, and as previously noted, D2 proteins did not exhibit enhanced radiolabel incorporation in illuminated plants. In both plastid populations, radioactivity in D2 increased during the chase period (compare lane 1 to lane 2 and lane 3 to lane 4 in Fig. 3). This could be explained by readout of nonimmunoprecipitable paused translation intermediates during the chase or if the chases were not totally effective. In contrast, radiolabel incorporation into CP43 is enhanced in plastids isolated from illuminated plants (20). Incorporation of radiolabel into two forms of CP43, which migrate at 43 and 45 kDa, and a series of lower molecular mass proteins was detected in pulse-labeled plastids of dark-grown and illuminated plants (Fig. 3, lanes 5 and 7). During the chase, the lower molecular mass CP43-related proteins disappeared. The radiolabeled 43- and 45-kDa forms of CP43 also disappeared when plastids from dark-grown plants were incubated with excess unlabeled methionine (Fig. 3, lane 5 vs. lane 6). In contrast, these proteins were relatively stable in plastids from illuminated plants (Fig. 3, lanes 7 and 8). We have previously shown by Western blot analysis that barley plastids accumulate two forms of CP43, which have apparent molecular masses of 43 and 45 kDa. On the basis of data from Ikeuchi *et al.* (24), we suggested that the more slowly migrating form of CP43 was

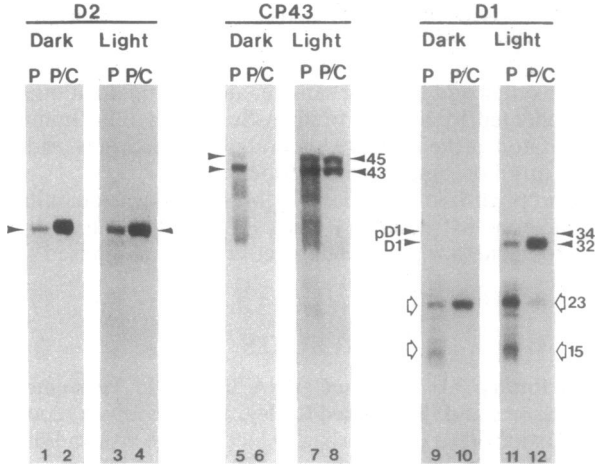

FIG. 3. Immunological analysis of PSII polypeptides radiolabeled in plastids from 4.5-day-old dark-grown seedlings and dark-grown seedlings illuminated for 1 hr (lanes labeled Dark and Light, respectively). Intact plastids were incubated for 2 min in the presence of [^{35}S]methionine and then incubated with excess unlabeled methionine for either 0 (lanes 1, 3, 5, 7, 9, and 11; labeled P) or 30 min (lanes 2, 4, 6, 8, 10, and 12; labeled P/C). Translation products from the membrane fractions were immunoprecipitated (22) with antisera to D2 (lanes 1–4), CP43 (lanes 5–8), or D1 (lanes 9–12), and the products were electrophoresed on a 12–18% SDS/PAGE gel containing 8 M urea (21). The arrowheads at the left of lane 5 and the right of lane 8 indicate two forms (43 and 45 kDa) of CP43. At the left of lane 9, arrowheads mark the location of a 34-kDa precursor of D1 (pD1) and D1. Open arrows at the left of lane 9 and to the right of lane 12 point to radiolabeled D1 translation products of 15 kDa and 23 kDa.

phosphorylated (21). If this interpretation is correct, then the results in Fig. 3 indicate that CP43 is phosphorylated during or shortly after this protein is synthesized.

D1 Translation Intermediates Are Synthesized in Dark-Grown Plants. D1 is initially synthesized as a 34-kDa precursor, which is proteolytically processed at its 3′ end to yield D1 with an apparent molecular mass of 32 kDa (25). When pulse-labeled membrane proteins from dark-grown plants were immunoprecipitated with D1 antisera, the 34-kDa precursor to D1 and mature D1 were not detected, but a series of 15- to 25-kDa proteins were immunoprecipitated (Fig. 3, lane 9; open arrows). During the chase, most of the 15- to 25-kDa D1 translation products disappeared, whereas radiolabel in a 23-kDa protein increased (Fig. 3, lane 10). The 23-kDa protein could be a proteolytic product of D1 as previously suggested (26). These results show that D1 is translated in plastids of dark-grown plants, although D1 intermediates larger than 23 kDa do not accumulate. When pulse-labeled membrane proteins from illuminated plastids were immunoprecipitated with D1 antisera, D1 and a 34-kDa precursor to D1 were detected (Fig. 3, lane 11). In addition, the D1 antisera immunoprecipitated a series of radiolabeled proteins 15–25 kDa in size (Fig. 3, lane 11). During a 30-min chase, the 34-kDa precursor to D1 and most of the 15- to 25-kDa D1 translation products disappeared. At the same time, additional label accumulated in mature D1, suggesting that some of the 15- to 25-kDa proteins are precursors to D1. One 23-kDa D1 translation product remained after the chase as observed in plastids of dark-grown plants. This protein could be a proteolytic product of D1 (26).

Light-Dependent Chlorophyll Formation and Chlorophyll Apoprotein Accumulation. We have previously shown that the light-dependent accumulation of D1, CP43, CP47, and the P_{700} chlorophyll apoproteins is controlled by the protochlorophyllide reductase holochrome and specifically requires chlorophyll a formation (20). This information and the results in Fig. 3 lead us to conclude that stability and, therefore, the accumulation of D1 and CP43 are regulated by the binding of chlorophyll to the chlorophyll apoproteins. When dark-grown plants are first illuminated, accumulation of all the plastid-encoded chlorophyll apoproteins begins within 5 min. Therefore, at least six different plastid-encoded chlorophyll apoproteins, each with multiple chlorophyll-binding sites, compete for chlorophyll when dark-grown plants are first illuminated. This raises the question of whether all chlorophyll-binding sites on a chlorophyll apoprotein need to be filled to increase apoprotein stability or only specific binding sites. Furthermore, the relative affinity of each chlorophyll apoprotein for chlorophyll might play a significant role in determining the final chlorophyll apoprotein composition of the thylakoid. As a first step toward addressing these questions, we illuminated dark-grown barley seedlings with different fluences of red light and examined the relationship between the amount of chlorophyll produced by a pulse of light and [^{35}S]methionine incorporated in the P_{700} chlorophyll apoproteins after pulse–chase labeling (Table 1). The formation of 3.4 pmol of chlorophyll per 10^7 plastids did not result in P_{700} chlorophyll apoprotein accumulation, whereas 13.7 pmol of chlorophyll per 10^7 plastids increased P_{700} chlorophyll apoprotein radiolabeling to 20% of the maximum level observed. When the amount of chlorophyll per 10^7 plastids was increased to 20.9 pmol, P_{700} chlorophyll apoprotein radiolabeling reached maximal levels. Further increases in red light fluence increased chlorophyll levels slightly and caused a small decrease in P_{700} chlorophyll apoprotein labeling. At high light intensities, photon absorption by chlorophyll or protochlorophyllide can generate oxygen radicals, which damage plastids (27). This may cause a decline in chlorophyll apoprotein synthesis at the higher fluence rates used to give 1350 and 2700 $\mu E/m^2$ of red light in this experiment. The results in Table 1 indicate that chlorophyll formation and P_{700} chlorophyll apoprotein accumulation are not linearly related. This may indicate that the P_{700} apoproteins are unstable until their chlorophyll-binding sites are saturated.

Table 1. Chlorophyll-induced P_{700} apoprotein accumulation

Fluence of red light, $\mu E/m^2$	P_{700} apoprotein radiolabeling	Chlorophyll a accumulation, pmol per 10^7 plastids
0	0.0	0
12.5	0.0	3.4
75	0.2	13.7
500	1.0	20.9
1350	0.9	28.5
2700	0.9	24.5

Accumulation of P_{700} chlorophyll a apoproteins is expressed relative to their radiolabeling at 500 $\mu E/m^2$. Apoprotein radiolabeling was quantitated by laser scanning densitometry of autoradiograms. Red light treatments were given as described (20).

DISCUSSION

Primary leaf development is largely light independent in monocots such as barley. Furthermore, the buildup of plastid number per cell, plastid DNA copy number, activation of plastid transcription, and accumulation of most proteins found in chloroplasts occurs in dark-grown seedlings. However, dark-grown plants lack chlorophyll and the chlorophyll apoproteins (13, 17, 18, 21) and are therefore photosynthetically incompetent. In this paper we show that chlorophyll a activates chlorophyll apoprotein accumulation by stabilizing newly synthesized apoproteins. This mechanism is consistent with chlorophyll apoprotein mRNA association with polysomes in dark-grown plants (refs. 10 and 12; Fig. 1) and with pulse–chase assays showing chlorophyll-induced stabilization of newly synthesized CP43. The accumulation of the chlorophyll apoprotein D1 is also stimulated by light-induced chlorophyll formation. In this case, no full-length D1 was detected in plastids of dark-grown plants even though D1 translation intermediates of 15–25 kDa were observed. In plastids of illuminated plants, radiolabel in 15- to 25-kDa D1 translation intermediates could be converted into full-length D1. In contrast, pulse–chase assays in plastids of dark-grown plants yielded only a 23-kDa D1 protein, which probably arises from proteolysis of D1 (26).

The absence of D1 translation products larger than 23-kDa in plastids of dark-grown plants can be explained in the following way. D1 contains five membrane-spanning α-helices (28, 29), and a protease-sensitive 44-amino acid loop is located between α-helix IV and α-helix V (25). D1 translation intermediates containing four α-helices would be $\approx$20–25 kDa in size, which is similar to several of the pulse-labeled polypeptides that are immunoprecipitated with D1 antisera. It is possible that lack of chlorophyll binding to D1 translation intermediates containing α-helices I–IV makes the amino acid loop between α-helix IV and α-helix V highly susceptible to cleavage when it is translated. This would explain the absence of D1 and its 34-kDa precursor in pulse-labeled plastids of dark-grown plants and the accumulation of a 23-kDa putative proteolytic product of D1 in pulse–chase assays of these plastids. In illuminated plants, chlorophyll binding during translation of D1 could stabilize the nascent chains, allowing the formation of mature D1. This model implicates cotranslational binding of chlorophyll to D1 as a necessary step in D1 synthesis.

Regulation of apoprotein stability by cofactor binding is not unique to the plastid-encoded chlorophyll apoproteins. For

example, newly synthesized plastocyanin is unstable in Cu^{2+}-deficient cyanobacteria (30). Furthermore, chlorophyll a and chlorophyll b increase the stability of the nuclear-encoded LHCI and LHCII chlorophyll a/b apoproteins (31, 32). Likewise, bacteriochlorophyll-binding proteins of photosynthetic bacteria turn over rapidly when bacteriochlorophyll levels are limiting (33–35). In higher plant chloroplasts, the regulatory mechanism described here affects the accumulation of a large number of abundant chloroplast proteins, giving it special significance during chloroplast biogenesis. When 4.5-day-old dark-grown barley seedlings are illuminated, chlorophyll biosynthesis results in a 3-fold increase in membrane protein synthesis due almost entirely to increased chlorophyll apoprotein accumulation (14). The increase occurs in the absence of changes in mRNA levels or polysome distribution (19), which is consistent with continual chlorophyll apoprotein synthesis and rapid degradation in dark-grown plants.

The chemical nature of chlorophyll provides a compelling reason to coordinate chlorophyll synthesis and chlorophyll apoprotein accumulation. Chlorophyll is ideal for light-harvesting because its excited singlet state is sufficiently long lived to allow energy transfer from antennae chlorophyll to reaction centers for trapping. However, if photon energy absorbed by chlorophyll is not used for photochemistry, the excited singlet state can convert to a long-lived triplet state. Chlorophyll triplets generated within a chlorophyll apoprotein are rapidly quenched by energy transfer to carotenoids (27). Triplet states generated in free chlorophyll, however, may react with oxygen to generate superoxide and hydroxyl radicals, which can rapidly damage chloroplast membranes. Therefore, mechanisms that generate "free" chlorophyll must be strongly selected against. From this point of view, the light-independent buildup of the capacity for plastid-encoded chlorophyll apoprotein synthesis and synthesis of apoproteins in dark-grown plants ensures that chlorophyll apoproteins are always available to bind chlorophyll when plant illumination occurs. If apoproteins need to be readily available, why don't plastids stockpile these proteins in darkness? The answer to this question may be related to another constraint, the need to have completely filled chlorophyll antennae beds in order to efficiently transfer energy from light-harvesting chlorophyll to reaction centers. If chlorophyll apoproteins were stable in the absence of chlorophyll, then, at subsaturating levels of chlorophyll, some chlorophyll could be too far from other antennae for efficient energy transfer to reaction centers. Energy absorbed in such isolated chlorophyll could cause photodamage similar to that which occurs at high light intensity when reaction centers are closed. Finally, it should be noted that other regulatory mechanisms ensure that chloroplast gene expression and chlorophyll biosynthesis occur in an organ- and cell-specific manner. Once these pathways are activated, however, chlorophyll-induced chlorophyll apoprotein stability plays the central role in coordinating the accumulation of chlorophyll and the chlorophyll apoproteins.

We thank Mrs. Sharyll Pressley for her help in preparing the manuscript. This research was supported by National Institutes of Health Grant GM37987.

1. Feher, G., Allen, J. P., Okamura, M. Y. & Rees, D. C. (1989) *Nature (London)* **339,** 111–116.
2. Mattoo, A. K., Marder, J. B. & Edelman, M. (1989) *Cell* **56,** 241–246.
3. Green, B. R. (1988) *Photosyn. Res.* **15,** 3–32.
4. Alt, J., Morris, J., Westhoff, P. & Herrmann, R. G. (1984) *Curr. Gene.* **8,** 597–606.
5. Westhoff, P., Alt, J. & Herrmann, R. G. (1983) *EMBO J.* **2,** 2229–2237.
6. Fish, L. E., Kuck, U. & Bogorad, L. (1985) *J. Biol. Chem.* **260,** 1413–1421.
7. Mullett, J. E., Burke, J. J. & Arntzen, C. J. (1980) *Plant Physiol.* **65,** 814–822.
8. Mullet, J. E., Grossman, A. R. & Chua, N.-H. (1982) *Cold Spring Harbor Symp. Quant. Biol.* **46,** 979–984.
9. Pichersky, E., Hoffman, N. E., Bernatzky, R., Piechulla, B., Tanksley, S. D. & Cashmore, A. R. (1987) *Plant Mol. Biol.* **9,** 205–216.
10. Klein, R. R. & Mullett, J. E. (1987) *J. Biol. Chem.* **262,** 4341–4348.
11. Laing, W., Kreuz, K. & Apel, K. (1988) *Planta* **176,** 269–276.
12. Kreuz, K., Dehesh, K. & Apel, K. (1986) *Eur. J. Biochem.* **159,** 459–467.
13. Sutton, A., Sieburth, L. E. & Bennett, J. (1987) *Eur. J. Biochem.* **164,** 571–578.
14. Klein, R. R. & Mullet, J. E. (1986) *J. Biol. Chem.* **261,** 11138–11145.
15. Castelfranco, P. A. (1983) *Annu. Rev. Plant Physiol.* **34,** 241–278.
16. Mullet, J. E. & Klein, R. R. (1987) *EMBO J.* **6,** 1571–1579.
17. Berends, T., Gamble, P. E. & Mullet, J. E. (1987) *Nucleic Acids Res.* **15,** 5217–5240.
18. Herrmann, R. G., Westhoff, P., Alt, J., Tittgen, J. & Nelson, N. (1985) in *Molecular Form and Function of the Plant Genome,* eds. van Volten-Doting, L., Groot, G. S. P. & Hall, T. C. (Plenum), pp. 233–256.
19. Klein, R. R., Mason, H. S. & Mullet, J. E. (1988) *J. Cell Biol.* **106,** 289–301.
20. Klein, R. R., Gamble, P. E. & Mullet, J. E. (1988) *Plant Physiol.* **88,** 1246–1256.
21. Gamble, P. E. & Mullet, J. E. (1989) *J. Biol. Chem.* **264,** 7236–7243.
22. Mason, H. S., Guerrero, F. D., Boyer, J. S. & Mullet, J. E. (1988) *Plant Mol. Biol.* **11,** 845–856.
23. Mullet, J. E., Klein, R. R. & Grossman, A. R. (1986) *Eur. J. Biochem.* **155,** 331–338.
24. Ikeuchi, M., Plumley, F. G., Inoue, Y. & Schmidt, G. W. (1987) *Plant Physiol.* **85,** 638–642.
25. Marder, J. B., Goloubinoff, P. & Edelman, M. (1984) *J. Biol. Chem.* **259,** 3900–3908.
26. Greenberg, B. M., Gaba, V., Mattoo, A. K. & Edelman, M. (1987) *EMBO J.* **6,** 2865–2869.
27. Renger, G. & Wolff, C. H. (1977) *Biochim. Biophys. Acta* **460,** 47–57.
28. Trebst, A. (1986) *Z. Naturforsch.* **41c,** 240–245.
29. Sayre, R. T., Andersson, B. & Bogorad, L. (1986) *Cell* **47,** 601–608.
30. Merchant, S. & Bogorad, L. (1986) *J. Biol. Chem.* **261,** 15850–15853.
31. Bennett, J. (1981) *Eur. J. Biochem.* **118,** 61–70.
32. Apel, K. (1979) *Eur. J. Biochem.* **97,** 183–188.
33. Dierstein, R. (1983) *FEBS Lett.* **160,** 281–286.
34. Takemoto, J. & Lascelles, J. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 799–803.
35. Oelze, J., Schroeder, J. & Drews, G. (1970) *J. Bacteriol.* **101,** 669–674.