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Differential Synthesis of Photosystem Cores and Light-Harvesting Antenna during Proplastid to Chloroplast Development in *Spirodela oligorrhiza*[1]

Dennis J. McCormac and Bruce M. Greenberg*

Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

ABSTRACT

Proplastids and etioplasts are common starting points for monitoring chloroplast development in higher plants. Although proplastids are the primary precursor of chloroplasts, most proplastid to chloroplast systems are cumbersome to study temporally. Conversely, the etioplast to chloroplast transition is initiated by light and is readily examined as a function of time. Etioplasts, however, are found mostly in plants germinated in the dark and are not an obligatory step in chloroplast development. We have chosen to study chloroplast ontogeny in *Spirodela oligorrhiza* (Kurtz) Hegelm (a C_3-monocot) because of its unique ability to grow indefinitely in the dark. Ultrastructural, physiological, and molecular evidence is presented in support of a temporal, light-triggered proplastid to chloroplast transition in *Spirodela*. The dark-grown plants are devoid of chlorophyll, and upon illumination synchronously green over a 3- to 5-day period. Synthesis of chloroplast proteins involved in photosynthesis is coincident with thylakoid assembly, chlorophyll accumulation, and appearance of CO_2 fixation activity. Interestingly, the developmental sequence in *Spirodela* was slow enough to reveal that biosynthesis of the D1 photosystem II reaction center protein precedes biosynthesis of the major light-harvesting antenna proteins. This, coupled with the high chlorophyll *a*/*b* ratio observed early in development, indicated that reaction center assembly occurred prior to accumulation of the light-harvesting complexes. Thus, with *Spirodela* one can study proplastid to chloroplast conversions temporally in higher plants and follow the process on a time scale that enables a detailed dissection of plastid maturation processes.

A dominant factor influencing plant growth and development is light. Typical responses include hypocotyl growth, flower development, chloroplast ontogeny, and photosynthesis (9). Chloroplast development is particularly fascinating because it encompasses many key photomorphogenic processes and results in a photosynthetic apparatus that requires light for activity. Plastids exist in several interconvertible forms such as proplastids, amyloplasts, etioplasts, chloroplasts, and chromoplasts (12, 24, 28, 29). The conversion of immature plastids (proplastids and etioplasts) to chloroplasts requires light. The photoreceptors for this developmental process are phytochrome and a blue light receptor(s), and the process is regulated at the levels of transcription and translation (3, 5, 6, 16, 22, 23, 26).

Chloroplast development can be followed from two starting points: proplastids or etioplasts (12, 16, 28, 29). Proplastids are small (0.5–1 μm in diameter), about 10 to 20% of the size of a chloroplast (12, 20). They are considered the most immature plastid form and are the direct precursor to several plastid types (12, 16, 29). Devoid of Chl and internal membrane structure, they are found in meristematic tissue, as well as in very young, dark-grown cotyledons (5, 16, 20). In both higher plants and algae, proplastid to chloroplast conversions take 3 to 5 d (2, 10, 20, 21). This developmental program is seen along the axis of a light-grown monocot leaf as a linear array of developmental stages with only a few intermediates (12, 20, 29). The stages include proplastids (or eoplasts), amyloplasts, immature chloroplasts with stromal thylakoids, and mature chloroplasts with both stromal and granal thylakoids. Absent from the proplastid to chloroplast pathway are etioplast-like structures. A drawback of the above monocot systems is that plants are generally grown in the light, negating the possibility of using light to trigger synchronized development.

Etioplasts are about 5 to 10 times larger than proplastids and contain a characteristic prolamellar body (12, 20, 24, 29). Prolamellar bodies are paracrystalline membrane structures that hold basal levels of protochlorophyllide and thylakoid proteins (28). Etioplasts develop from proplastids in dark-grown monocots and dicots (20, 29). Light induces synchronous conversion of etioplasts to chloroplasts in a process that takes 12 to 24 h (11, 18, 27, 28). During this period, the prolamellar body dissociates and the thylakoid membrane network develops (28). The etioplast system is important experimentally because chloroplast development can be controlled by light quality and quantity. For example, the greening process is readily initiated by a short red-light pulse and is halted by far-red light, which has revealed that phytochrome is a key photoreceptor for chloroplast development (3, 6, 16, 22, 25). Moreover, because etiolated plants green synchronously, it has been possible to isolate cDNAs for many of the genes that are activated during chloroplast development (16, 26). However, etioplasts are absent from light-grown monocots in which normal chloroplast development is seen to occur directly from proplastids (11, 20, 24). Thus, there is some discussion concerning whether etioplasts are integral to the primary chloroplast developmental pathway.

It would be useful to identify a higher plant system that

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combined the advantages of proplastid systems (slow development from an early stage) with the etioplast systems (light induction and temporal control). Interestingly, *Spirodela oligorrhiza*, a C_3-monocot, can grow continuously in the dark as white tissue and it greens slowly upon exposure to light with coordinated appearance of thylakoid proteins (4, 14, 19). We have investigated the possibility that this plant can be used to follow synchronized proplastid to chloroplast development. In this paper, we describe structural, physiological, and molecular changes that occur during light-induced chloroplast biogenesis in *Spirodela*.

MATERIALS AND METHODS

Growth of Plants

Axenic cultures of *Spirodela oligorrhiza* (Kurtz) Hegelm were grown heterotrophically on half-strength Hutner's medium supplemented with 1% (w/v) sucrose (14, 19). Plants were grown for 3 months in the dark at approximately 25°C (doubling time: 2 weeks). This assures sufficient experimental tissue and full adaptation to dark growth. Chloroplast development was initiated by transfer of the plants to medium without sucrose and exposure to continuous visible light (cool-white fluorescent, 70 μmol m^{-2} s^{-1} of photosynthetically active radiation [400–700 nm]). Plastid development could be followed over a 5-d period, as there was little plant growth during this time (Fig. 1).

EM

Spirodela plants were prepared for transmission electron microscopy according to Porath (19) and Tobin (25) with some modifications. Whole leaves were vacuum infiltrated with fixative (2.5% glutaraldehyde, 20 mM sodium phosphate [pH 7.0], 0.3 M sucrose), and incubated in the fixative for 24 h at 4°C. The fronds were postfixed in 2% (w/v) osmium tetroxide for 1 h at room temperature and prestained with 5% (w/v) uranyl acetate for 1 h. Thin sections were prepared, stained with lead citrate (4 mg/mL) and viewed on a Philips 300 transmission electron microscope.

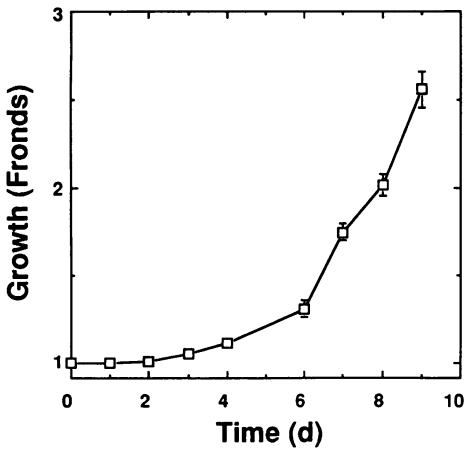


Figure 1. Growth of *Spirodela* during light-induced chloroplast development. Dark-grown plants were transferred to the light at time zero and growth was determined by counting the number of fronds at the time points indicated. Growth was normalized to the number of leaves that were present at time zero. Data are presented as means and the bars are standard errors (n = 5).

Chl Determination and Carbon Fixation

Approximately 20 mg of *Spirodela* fronds were placed in 0.5 mL of *N,N*-dimethylformamide and kept at 4°C in the dark for 24 h (15). The optical density of the sample was measured at 664 and 647 nm, and total Chl, Chl *a* and Chl *b* were calculated.

Dark-grown fronds were exposed to visible light for various times. The plants were then incubated for 1 h in growth medium containing 4 μCi/mL [^{14}C]bicarbonate (Amersham, 50–60 mCi/mmol) (7) under the same light conditions used to promote chloroplast development (cool-white fluorescent lamps, 70 μmol m^{-2} s^{-1}). Background incorporation was established with a sample incubated in the dark, which accounted for $^{14}CO_2$ taken up by the tissue but not fixed into photosynthate. Because the assays were performed in unsealed Petri dishes, each sample had a parallel light-grown (green) control plant to account for possible variability in ambient CO_2. After incorporation, the plants were washed three times with ice-cold growth medium. The Chl in the tissue was then bleached by light incubation in 2.5 mL of 80% acetone for 24 h in a fumehood. Incorporation of radiolabel was quantified by liquid scintillation counting. To remove the contribution of unincorporated $^{14}CO_2$, the signal from the sample incubated in the dark was subtracted from that of the sample incubated in the light. Each time point was normalized to its parallel green plant control.

In Vivo Radiolabeling and Analysis of Proteins

Dark-grown *Spirodela* plants were greened in the light for various lengths of time and then placed on 0.5 mL of media containing 50 μCi/mL [^{35}S]methionine (Amersham, >800 Ci/mmol) (13). Radiolabeling was carried out for 2 h under the light conditions used for greening. The plants were washed three times with cold, sterile water and kept at −70°C until homogenized.

Plant tissue (100 mg) was homogenized in 300 μL of grinding buffer (2.5 mM Tris-HCl, pH 8.5, 50 mM NaCl, and 0.1 mM PMSF) using a motor-driven glass/glass homogenizer. The homogenate was separated into soluble and membrane fractions by centrifugation (12,000*g* for 15 min). The supernatant (300 μL) was recentrifuged at 12,000*g* for 15 min and added to 150 μL of 3 × sample buffer (13). The membrane pellet was washed with 300 μL of 2.5 mM Tris/glycine (pH 7.8) containing 50 mM NaCl and repelleted at 12,000*g* for 15 min. The resulting membrane fraction was washed with Tris/glycine, collected by centrifugation (12,000*g*) and resuspended in 300 μL of 1 × sample buffer. All steps were performed at 4°C.

SDS-PAGE, Coomassie staining, and fluorography were performed as described by Marder *et al.* (13). For immunological analysis, proteins were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose (0.2 μm, Schleicher and Schuell). Protein blots were probed with anti-

sera as previously described (8), except that the blocking solution contained nonfat dry milk (1%, w/v) instead of BSA and detection was with alkaline phosphatase conjugated to anti-rabbit immunoglobulin G (Bio-Rad). Prior to use, the D1 and LHCII[2] antisera were titered for both an optimal level of specificity and a high degree of sensitivity. The immunoblots were quantified with a computerized laser scanning densitometer (Molecular Dynamics).

RESULTS

Ultrastructural Analysis

Transmission EM is one of the most effective techniques for determining the types of plastids in plant cells (12, 20, 28, 29). Thus, we performed ultrastructural analysis on dark-grown *Spirodela* (Fig. 2). Plastids of approximately 0.5 μm in diameter with very little internal membrane structure were observed. Although osmiophilic globules (commonly found in proplastids [20, 28, 29]) were present, none of the microscope fields showed evidence of prolamellar bodies, characteristic of etioplasts. Many of the plastids also contained starch granules. Thus, based on composition and size, these organelles are reminiscent of proplastids and amyloplasts. Interestingly, amyloplasts are one of the steps in the proplastid to chloroplast transition, and except for the starch grains, amyloplasts are structurally quite similar to proplastids (29).

After exposing the plants to 12 or 21 h of light, the plastids grew to approximately 1 μm in diameter (Fig. 2). Only proplastid- and amyloplast-like structures without prolamellar bodies were observed. Furthermore, significant amounts of internal membranes were not present and the plastids were still circular in appearance. Thus, the proplastids did not go through an etioplast stage before appearance of immature chloroplasts.

After 36 h of exposure to light, the dark-grown plants took on a light green appearance. The plastids continued to grow (approximately 1 μm by 2 μm) and were ovoid in shape (Fig. 2). Thylakoid membranes were present throughout the plastids and were the first identifiable new internal membranes. Typical of immature chloroplasts developing directly from proplastids (20), the membranes were mostly single lamellar thylakoids with only a few granal stacks. In an etioplast to chloroplast conversion, the first thylakoids appear to grow out of the prolamellar body (28).

By 54 h of light exposure, the plastids contained both stromal and granal thylakoids (Fig. 2). The regions of membrane appression were of only modest length. At 72 h, the plants had a normal green appearance. The regions of appression of the granal thylakoids now appeared to be longer than they were at 54 h (Fig. 2). Although not fully mature after 72 h of light exposure, the plastids were similar in size and membrane structure to chloroplasts from light-grown control plants (Fig. 2). Thus, dark grown *Spirodela* plants develop chloroplasts with a normal appearance after exposure to 3 d of continuous light. The ultrastructural data (size of plastids, internal structure, and mode of thylakoid appearance) suggest that light-initiated chloroplast development in *Spirodela* begins at the proplastid stage, and does not employ an etioplast step.

Chl Accumulation

When chloroplasts develop from proplastids, Chl synthesis is gradual, showing a steady build-up over a 3- to 5-d period (2, 20, 21). However, if etioplasts are the starting point, Chl accumulation is very rapid, saturating after 24 h of light exposure (18, 20, 27). Thus, we monitored Chl accumulation in dark-grown *Spirodela* after exposure to light to determine if this parameter was consistent with chloroplast ontogeny from proplastids (Fig. 3). A 24 h lag was observed before Chl synthesis ensued. The pigments were then accumulated over the next 4 d (Fig. 3A) to a level similar to that found in other proplastid to chloroplast developmental systems (20). Thus, Chl formation in *Spirodela* was analogous to proplastid/chloroplast developmental systems.

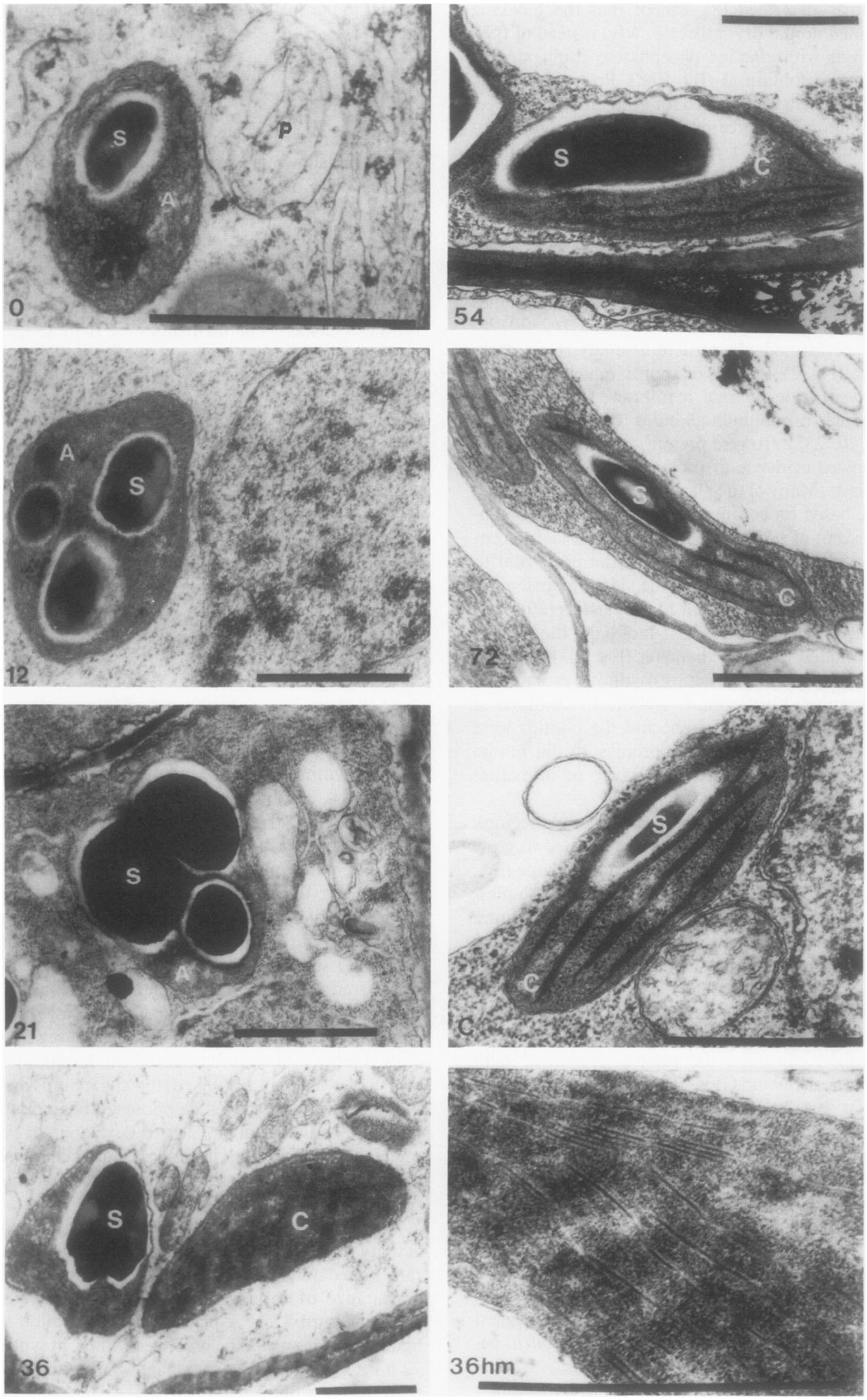
It was found that the Chl *a*/*b* ratio was high early in development (approximately 15) and decreased to a level typical for mature chloroplasts (approximately 4) (Fig. 3B). This adjustment in the Chl *a*/*b* ratio is typical for proplastid to chloroplast development in C_3-monocots (2). The high Chl *a* levels could be indicative of intense photosystem core synthesis early in development (photosystem cores do not contain Chl *b*), whereas the subsequent increase in Chl *b* might reflect antenna accumulation during chloroplast maturation.

Photosynthetic Activity

Photosynthesis develops rapidly in etioplasts upon exposure to light (18, 27), but it appears more gradually in proplastids (2, 3, 20, 21). To determine whether the appearance of photosynthetic activity in *Spirodela* is consistent with proplastid to chloroplast transitions, carbon fixation was monitored *in vivo* as a function of greening (Fig. 4). Incorporation of carbon dioxide was first detected after 24 to 30 h of exposure of dark-grown *Spirodela* to light. Consistent with a proplastid starting point, this initial lag phase was followed by a gradual increase in photosynthetic activity. It then reached a plateau at 72 h. Because an increase in light intensity could alter the chloroplast developmental regimen, the moderate fluence rate used for chloroplast development (70 μmol m^{-2} s^{-1}) was also employed for the photosynthesis assay. Nonetheless, the steady-state photosynthetic rate under these lighting conditions after 72 h of development was approximately 75% of the activity in continuous light-grown plants, showing that chloroplasts with a high proportion of normal photosynthetic capacity were generated.

A comparison of ultrastructure, Chl, and photosynthetic activity (Figs. 2–4) reveals the three parameters to be temporally linked during chloroplast development. Interestingly, the appearance of carbon fixation activity only mirrored Chl synthesis until 72 h. A plateau in CO_2 fixation at moderate light intensities would be predicted if the Chl synthesized after 72 h is targeted mostly for the light-harvesting antenna rather than the reaction center cores.

[2] Abbreviation: LHCII, light-harvesting Chl *a*/*b* binding protein.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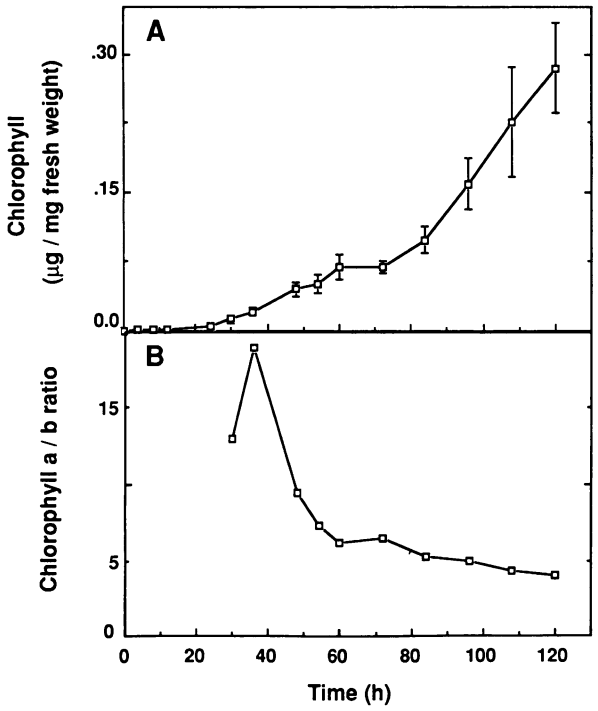

Figure 3. Chl accumulation during chloroplast development in *Spirodela*. Dark-grown *Spirodela* plants were exposed to light and, at the times indicated, Chl was analyzed as described in "Materials and Methods." A, Appearance of total Chl (on a fresh weight basis) as a function of time. Control light-grown plants contained approximately 1 μg Chl/mg fresh weight of tissue. Data are presented as means and bars represent the standard errors (n = 5). B, Chl a/b ratios as a function of chloroplast development. Note, the 30 h time point was the first time point with enough Chl b to give a reliable Chl a/b ratio.

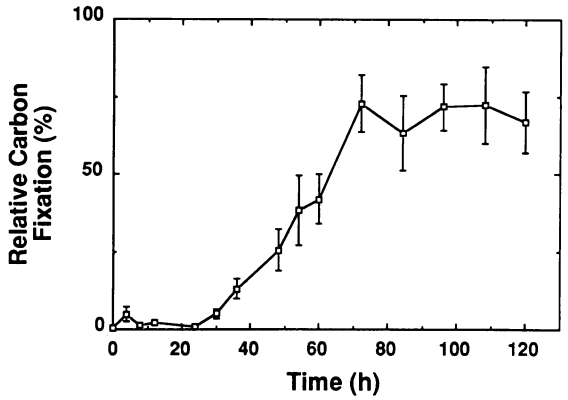

Figure 4. Appearance of photosynthetic activity as *Spirodela* chloroplast development proceeds. Dark-grown *Spirodela* plants were transferred to the light for the times indicated. Net photosynthetic activity was measured by light-dependent incorporation of [^{14}C]bicarbonate. Activity is expressed as a percentage of a continuous light-grown plant control. Data are presented as means and the bars represent the standard errors (n = 3–5).

Protein Accumulation

Because membrane assembly, Chl synthesis, and appearance of photosynthetic activity are temporally linked during proplastid to chloroplast development in *Spirodela,* chloroplast protein accumulation should follow a similar program. To determine if this was indeed the case, soluble and membrane proteins were isolated from *Spirodela* at several stages of chloroplast development and analyzed by SDS-PAGE (Fig. 5). Although there may be a small amount of the large subunit of Rubisco present in dark-grown tissue, the small subunit was almost undetectable. Starting at 12 to 24 h, there was a steady increase in both subunits of this enzyme (Fig. 5A), consistent with the appearance of thylakoids, Chl, and photosynthetic activity. Accumulation of several other unidentified soluble proteins (*e.g.* a 36-kD polypeptide) also began after about 24 h of light exposure and then continued in a manner linked to chloroplast development. Decreases in the levels of some polypeptides during development were also noted, perhaps having functions not required for light growth.

The membrane protein pattern also changed during chloroplast development, reflecting the growing thylakoid membrane network (Fig. 5B). Again, increases and decreases of various proteins were observed. Interestingly, the LHCII associated with PSII was not detected until 36 to 48 h of light exposure. The relative amount of LHCII then increased with chloroplast maturation in a manner coincident with the drop in the Chl a/b ratio (see Figs. 3B and 5B). However, LHCII synthesis lagged behind the initial appearance of photosynthetic activity and Rubisco, indicating that a full antenna complement is not required for the carbon fixation observed early in development.

Membrane Protein Synthesis

To examine the synthesis of thylakoid proteins during chloroplast biogenesis in more detail, *Spirodela* proteins were radiolabeled at several time points during greening (Fig. 6). Whereas biosynthesis of LHCII began at approximately 36 to 48 h (as observed in Fig. 5B), radiolabeling of the D1 PSII reaction center protein was detected slightly above background after only 24 h of continuous light exposure. This is a further indication that synthesis of photosystem cores was initiated prior to the light-gathering antenna. However, because of the high rate of D1 protein turnover (7, 14), these data cannot be used as a measure of net accumulation of the D1 protein *versus* the LHCII protein.

Synthesis of other thylakoid membrane proteins (*e.g.* CP43

Figure 2. Plastid ultrastructure during chloroplast development in *Spirodela*. Thin sections were prepared from leaves of dark-grown *Spirodela* plants exposed to 0, 12, 21, 36, 54, and 72 h of light and analyzed by transmission EM. A control chloroplast (panel C) from continuous light-grown *Spirodela* is also shown. As well, a higher magnification of the 36 h micrograph (36hm) is presented. Bars represent 1 μm. Abbreviations are amyloplast (A), proplastid (P), chloroplast (white C), and starch granule (S). The larger black particles in the immature plastids have the appearance of osmiophilic globules, commonly found in proplastids (20, 28, 29).

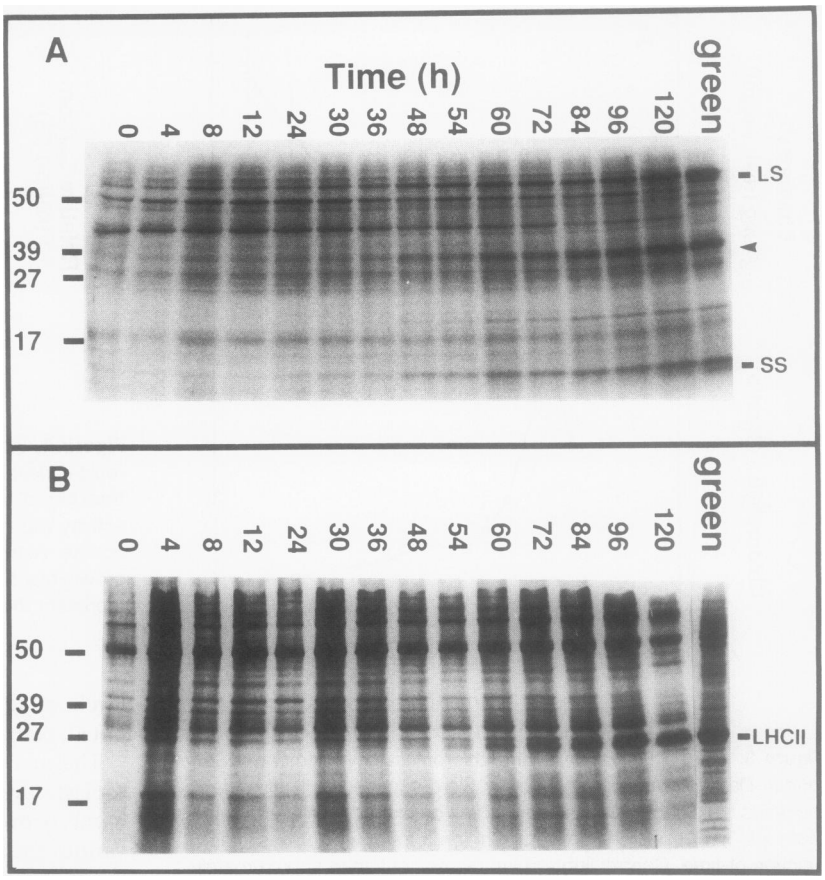

Figure 5. Protein accumulation following exposure of dark-grown *Spirodela* to light. Soluble and membrane proteins were extracted from plants after exposure to visible light for the time periods indicated. Proteins were also extracted from continuous light-grown plants (green). Protein fractions were separated by SDS-PAGE. Gels were loaded on an equal protein basis (10 μg/lane) and analyzed by staining with Coomassie brilliant blue R-250. A, Soluble proteins; B, membrane proteins. Positions of the large subunit (LS) and small subunit (SS) of Rubisco, and LHCII are labeled on the right. Immunological analyses were used to positively identify the positions of large and small subunits of Rubisco (data not shown) and LHCII (see Fig. 7). The position of a 36-kD light-regulated soluble protein is marked with an arrow. Molecular mass standards, in kD, are indicated on the left.

of the PSII core complex) could also be followed during development. However, due to their low radiolabeling levels, they were hard to detect above background. Also, the background was probably high due to the overall changes in protein populations that occur during a major developmental sequence. Interestingly, synthesis of the detectable thylakoid proteins began about 1 d after light exposure and increased for 3 to 4 d. Therefore, all of the aspects of *Spirodela* chloroplast development we assayed were temporally linked, showing up-regulation at around 24 to 36 h, and were consistent with the time frame for proplastid to chloroplast transitions.

Differential Appearance of the PSII Reaction Center and LHCII Proteins

It is clear from the appearance of CO_2 fixation activity, accumulation of Rubisco, and synthesis of the D1 protein that developing *Spirodela* chloroplasts became photosynthetically competent within 24 h of light exposure. However, the data also showed that Chl *a* appeared prior to Chl *b*, and synthesis of the D1 PSII reaction center protein preceded that of LHCII, indicating that reaction center accumulation began prior to the corresponding antenna. To assess not only when protein synthesis was initiated but also when the steady-state levels of the proteins were achieved, accumulation of the D1 protein and the LHCII protein was compared at the immunological level (Fig. 7). To aid in the observation of differential appearance of the proteins, an immunoblot was probed simultaneously with antisera to both polypeptides (Fig. 7A).

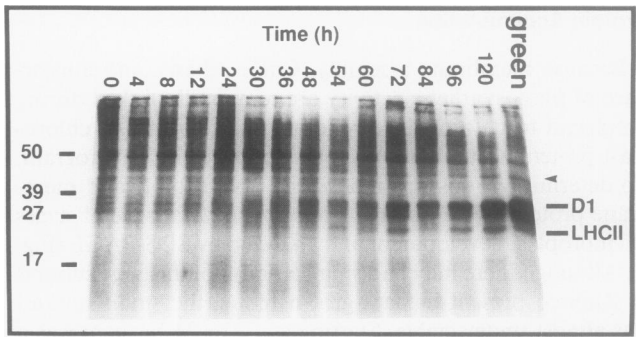

Figure 6. Biosynthesis of membrane proteins during light-induced chloroplast development. Dark-grown *Spirodela* plants were incubated in the light for the time periods indicated. At each time point, proteins were radiolabeled *in vivo* with [^{35}S]methionine as described in "Materials and Methods." Membrane proteins were isolated, separated by SDS-PAGE, and detected by fluorography. Gels were loaded on an equal protein basis (10 μg/lane). Positions of the D1 PSII reaction center protein and LHCII are indicated on the right, and the molecular mass standards are shown on the left. The putative position of CP43 is marked by the arrow.

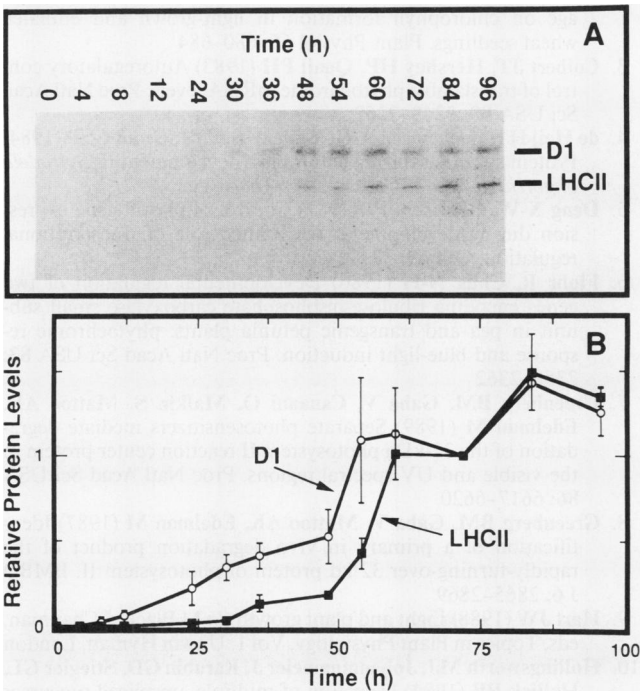

Figure 7. Differential accumulation of the D1 PSII reaction center and LHCII proteins during chloroplast development. Dark-grown *Spirodela* plants were greened in the light, membranes isolated, and proteins separated by SDS-PAGE as in Figure 5. The proteins were electrophoretically transferred to nitrocellulose and probed with antisera to the D1 and LHCII proteins. Hybridization was detected colorimetrically with alkaline-phosphatase conjugated to anti-rabbit immunoglobulin G (Bio-Rad). A, Photograph of a representative immunoblot; B, quantification of immunoblots. Blots were scanned using a computerized scanning laser densitometer. The data were normalized at the 72 h time point so D1 and LHCII could be shown on the same scale. (The 72 h point was chosen because photosynthetic activity developmentally saturated at that time point.) Data are presented as means and the bars represent the standard errors ($n = 3$).

When filters were probed with the antisera independently, the same results were achieved (these data were incorporated into Fig. 7B). Visual examination of the immunoblot indicated that the D1 protein was synthesized prior to LHCII (Fig. 7A) and densitometric analysis of the filters confirmed this observation (Fig. 7B). The level of the D1 protein increased steadily with time and was coincident with the appearance of thylakoids, photosynthetic activity, Rubisco, and Chl observed in the early phases of development. The decrease in the Chl *a/b* ratio, however, appeared to be concomitant with LHCII synthesis, supporting the hypothesis that a high proportion of Chl synthesized later in development is targeted for the antenna. Thus, the chloroplast becomes fully functional at an intermediate developmental phase (between 36 and 72 h), but the maturation process proceeds after this point, as evidenced by the accumulation of light-harvesting antenna.

DISCUSSION

We have shown that dark-grown *Spirodela* maintains its plastids at the proplastid/amyloplast stage. Upon exposure to light, the plastids synchronously develop into chloroplasts over a 5-d period. Several facets of *Spirodela* chloroplast development are coincident, including membrane assembly, Chl accumulation, appearance of photosynthetic activity, and synthesis of chloroplast proteins. Chloroplast development in *Spirodela* is kinetically slower than that found in typical etiolated plant systems, allowing us to observe reaction center assembly prior to LHCII synthesis.

An important finding of this work is that *Spirodela* can be used as a higher plant model system to temporally study light-initiated chloroplast development from proplastids and amyloplasts. Evidence in favor of the presence of proplastids in dark-grown *Spirodela* is derived from the EM analysis and the time frame for chloroplast development. The plastids are approximately 0.5 μm in size and do not contain prolamellar bodies. This structure is typical for proplastids (12, 20, 29). Many of the plastids also contain starch (probably accumulated due to the high sucrose in the growth medium), making them amyloplasts. Developmentally, amyloplasts are related to proplastids. In fact, Whatley (29) has suggested that the term proplastid be extended to include all prelamellar stages of the organelles (*i.e.* eoplasts, amyloplasts, and amoeboid plastids). After 36 h of light exposure, stromal lamellae with only a few granal stacks were present, a characteristic typical of early membrane synthesis during direct proplastid to chloroplast conversions (20). Conversely, when chloroplasts develop from etioplasts, the first thylakoids appear to grow out of the prolamellar body (28).

Chloroplast development in *Spirodela* takes 3 to 5 d. This is consistent with the time frame for proplastid to chloroplast conversions, whether based on exposure time to light or age of the plastid estimated from position in the leaf (*cf.* 2, 10, 20, 21). Several parameters, including ultrastructure, CO_2 fixation, Chl accumulation, and protein synthesis, follow these developmental kinetics. Also, as observed in proplastid to chloroplast development in other C_3-monocots (2), the Chl *a/b* ratio in *Spirodela* starts high and decreases as development proceeds.

Interestingly, etioplasts can be formed in *Spirodela* under certain conditions. For example, Porath (19) found etioplasts with prolamellar bodies in the early phases (4 d) of dark growth. However, after longer periods (11 d) in the dark, amyloplasts become evident. To assure uniform dark adaptation, we grew the cultures 3 months in the dark before use. Tobin (25) found etioplasts in dark-grown *Lemna gibba,* a close relative to *Spirodela,* exposed to intermittent pulses of red light. Thus, these plants are quite versatile, as by simply changing the growth conditions one can follow chloroplast development from proplastids or etioplasts.

A key advantage of etioplast systems is that exposure to light induces synchronous chloroplast development and it is possible to obtain large amounts of material at different stages of development. This has allowed the characterization of the photoreceptors involved in chloroplast development, as well as the isolation of key genes for chloroplast proteins (5, 16, 17, 25). However, the etioplast to chloroplast transition is so rapid that it is difficult to dissect many of the developmental events. Because the proplastid to chloroplast transition in *Spirodela* was slow, it was possible to detect synthesis of photosystem cores prior to the light-harvesting complexes (see

Figs. 3B and 7). The reaction center cores synthesized early in development were active because CO_2 fixation is detectable within the time frame of D1 protein appearance. Additionally, D1 protein accumulation and carbon fixation reached a plateau at approximately the same developmental phase (*cf.* Figs. 4 and 7), indicating that by 72 h the reaction center content was sufficient to support a high proportion of normal photosynthetic activity under moderate light intensities.

Although the LHCII antenna protein appeared about 12 h later than the D1 protein, both polypeptides were accumulated in a parallel fashion. Moreover, LHCII synthesis was coincident with the drop in the Chl *a/b* ratio. This would result in an increase in the light-gathering efficiency of the photosynthetic apparatus as the chloroplast matures, which is consistent with the plateau in photosynthetic activity. We also point out that the presence of LHCII has been associated with thylakoid stacking (1) and we observed that stacking increased only after 36 h of light exposure, as did LHCII accumulation (see Figs. 2 and 7). Furthermore, the data reveal that all components of the PSII complex need not be synthesized simultaneously, but instead can be assembled as active elements from the inside out (*i.e.* reaction center synthesis preceding that of antenna synthesis as the chloroplast matures). This developmental pattern was predicted for etioplast to chloroplast systems based on biophysical measurements (18), but was difficult to confirm at the protein level because LHCII appears very early in development (*cf.* 11, 17). It is interesting to note that the D1 protein gene (*psbA*) is chloroplast encoded (30), whereas the LHCII genes (the *Cab* family) are nuclear encoded (16, 26), raising the possibility that the two genomes are differentially regulated during proplastid to chloroplast maturation.

Considering that dark-grown *Spirodela* contains proplastids/amyloplasts and that differential expression of photosynthetic proteins can be observed during greening, these plants offer a powerful system with which to study chloroplast development in higher plants. It couples the advantages of etioplast model systems (temporal control, synchronization, and light induction) with the benefits of proplastid model systems (slow maturation, earlier developmental stage as starting point, and relevance to light-grown tissue). With the relative ease of obtaining large amounts of *Spirodela* tissue at any stage of development, it will be possible to study light-induced, synchronous chloroplast development from proplastids in greater detail.

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