Chloroplast-Cytoplasmic Interrelations Involved in Chloroplast Development in *Chlamydomonas reinhardi* y-1: Effect of Selective Depletion of Chloroplast Translates

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ABSTRACT *Chlamydomonas reinhardi* y-1 cells grown in the dark in the presence of chloramphenicol (CD cells) are depleted of photosynthetic membranes and 70S translates. These cells were found to be unable to synthesize chlorophyll in the light until chloroplast protein synthesis was resumed. On the other hand, CD cells acquired the capacity to partially green in the presence of cycloheximide. This greening was characterized by the development of photosynthetic activity, as demonstrated by light-dependent oxygen evolution of whole cells and by measurements of ribulose-1,5-bisphosphate carboxylase and fluorescence kinetics. The chlorophyll synthesized *de novo* during greening in the absence of 80S ribosomal activity was organized in chlorophyll-protein complexes, as ascertained by low-temperature fluorescence-emission spectra. The morphology of these cells appeared to be normal.

A model has been proposed as a working hypothesis, which could account for the phenomena described above and previously reported data pertaining to chloroplast development.

Chloroplast translates have been identified and shown to participate in the formation of multimeric complexes, including the 70S ribosomes (40), ribulose-1,5-bisphosphate carboxylase (RuBPase) (11, 12, 16), coupling factor (12, 32), and the photosynthetic reaction centers of both photosystems I and II (PSI and PSII) (9, 35, 38).

The components of these complexes originating in organelles were detected by the use of mutants impaired in chloroplast functions (6, 9, 19, 38), in vitro protein synthesis by isolated chloroplasts (12), and the selective modulation of chloroplast development by specific inhibitors of protein synthesis (5, 10, 15, 23, 35, 30).

The yellow mutant (y-1) of *Chlamydomonas reinhardi* has been found to be particularly useful for investigating chloroplast participation in the biosynthesis of the photosynthetic apparatus. This is because the chlorophyll synthesis in the y-1 mutant is totally light dependent (33). Growth of the mutant in the dark produces cells containing a plastid that lacks photosynthetic membranes (33). Membrane development can be induced by illumination of the dark-grown cells (34). This process can be modulated by inhibitors of protein synthesis either in the cytoplasm, e.g., cycloheximide (CHI), or in the chloroplast, e.g., chloramphenicol (CAP) (13, 23, 35).

Hoober and Stegeman (25) have suggested that inhibition of 70S translation in dark-grown y-1 cells alters regulatory processes which subsequently influence nucleocytoplasmic gene expression. We have shown that growth of y-1 cells in the dark in the presence of CAP (CD cells) resulted in the loss of at least 10 soluble polypeptides otherwise present in dark-grown cells (17). In view of this fact, selective depletion of these proteins in cells, before their illumination, may allow one to determine whether they play a role in the greening process. The experimental results described in this report indicate that depletion of the cells of 70S translates renders them incapable of chlorophyll synthesis, as long as the resumption of protein synthesis in the chloroplast is prevented. In addition, CD cells are capable of *de novo* synthesis of chlorophyll and functional photosynthetic membranes in the absence of 80S translation.

MATERIALS AND METHODS

Cultivation and Radioactive Labeling of cells

Chlamydomonas reinhardi y-1 cells used throughout this work were grown in either the light or dark, in a semicontinuous culture apparatus, on a mineral medium containing 7 mM acetate as the sole carbon source (33). Cells were degreened by growing them in the dark for four to five cell divisions (33) in the absence or presence of CAP (200 μg/ml) added during the last two cell divisions (48 h). Both the cells degreened in the dark without CAP (D) and those degreened with CAP (CD) contained <2 μg chlorophyll per 10^7 cells and were used for

THE JOURNAL OF CELL BIOLOGY · VOLUME 86 OCTOBER 1980 124-131

greening experiments. The cells were washed in fresh growth medium and resuspended in the same medium to a final concentration of 1–2 × 10^7 cells/ml. At this concentration greening occurred practically in the absence of cell division (34). Greening was carried out in Erlenmeyer flasks filled with the cell suspension to one-fifth of the volume and exposed to white fluorescent light (1.5 × 10^4 erg × cm^{-2} × s^{-1}) at 25°–27°C on a rotary shaker (120 cycles/min). For labeling of chlorophyll, [^{14}C]acetate (1–2 μCi/μmol) was used. For inhibition of 80S ribosomal activity, CHI (2 μg/ml) was added.

Analysis of Radioactive Labeling of Chlorophyll

Total cell lipids and pigments were extracted with a mixture of chloroform: methanol (2:1 vol/vol) containing 0.05% β-hydroxytoluene. The volume of the solvent relative to the volume of the wet packed cells was adjusted so as to adsorb the residual aqueous phase. The extract was dried by evaporation under vacuum and then redissolved in a minimal volume of the same solvent. Chlorophyll, carotenoids, and phospholipids were resolved by thin-layer chromatography (TLC) with silica gel precoated aluminum plates (Woelm Pharma GMBH and Co., Eschwege, W. Germany or Merck Chemical Div., Merck & Co., Inc., Rahway, N. J.) and benzene:isopropanol:water (100:20:0.25 vol/vol/vol) as the developer (21). The radioactivity of the chlorophyllide and phytol moieties of the chlorophyll was determined as follows: The chlorophyll-containing spots were scraped from the TLC plates. The material was extracted in 1 ml of 80% acetone in H_2O (vol/vol), and the amount of chlorophyll was determined spectrophotometrically (3). Chlorophyll was then hydrolyzed by the addition of 20 μl 0.1 N NaOH and heating at 60°C for 1–2 min. The solution was then cooled and 1 ml of petroleum-ether (60–80°C) was added. After phase separation, aliquots were taken from the upper phase, containing the phytol, and the lower phase, containing the chlorophyllide (42), and the distribution of radioactivity of each was determined.

Fluorescence Measurements

Variable fluorescence yield of whole cells was measured in either the absence or presence of 5 × 10^{-5} M 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) with the apparatus described previously (8). The cells were resuspended in growth medium at concentrations that gave 2–3 μg chlorophyll/ml.

Fluorescence emission spectra at 77°K were recorded with a homemade attachment for an Aminco-Chance dual wavelength spectrophotometer (American Instrument Co., Silver Spring, Md.). A glass rod (4 mm Diam) was immersed in a cell suspension (5–20 μg chlorophyll/ml) and then immediately transferred to a Dewar flask containing liquid nitrogen, mounted in the spectrophotometer instead of the light source. Excitation was given by a tungsten-hallogen lamp (150 W) through a Corning 4-96 blue filter (Corning Glass Works, Science Products Div., Corning, N. Y.) (transmission between 340 and 620 nm). The fluorescence emission spectra were recorded by operating the spectrophotometer in the double beam and balance mode scanning from 630 to 750 nm, keeping the reference beam constant at 630 nm. All spectra were normalized to the highest emitting peak by varying the monochromator slit (0.1–1.5 mm).

Electron Microscopic Examination of cells

Whole cells were fixed at 0°C in 2% glutaraldehyde, followed by osmium tetroxide postfixation, and embedded in Epon (Shell Chemical Co., Houston, Tex.), as described (33). Thin sections were cut with an LKB ultrotome III (LKB Instruments, Inc., Rockville, Md.), stained with lead citrate and uranyl acetate, and photographed in a JEOLCO 100 CX electron microscope.

Other Determinations

Respiration and light-dependent oxygen evolution of whole cells were determined polarographically. RuBPase activity of 27,000 *g* supernates of cell homogenates was determined as described by Iwanij et al. (26). Protein concentration was estimated according to Lowry et al. (29). Cells were counted in a hemocytometer, and chlorophyll concentration was estimated in 80% (vol/vol) extracts (3). All chemical reagents were of analytical grade.

RESULTS

Characterization of CAP-degreened Cells (CD cells)

CD cells were found to be motile and to exhibit phototaxis, respiration, no capacity for light-driven oxygen evolution, and extremely low RuBPase activity (Table I).

Examination of thin sections from CD cells showed that, except for lesions seen in the chloroplast, the general ultrastructure of these cells was similar to that of cells grown in the dark in the absence of CAP (cf. reference 33). In CD cells (Fig. 1), well-developed mitochondria and abundant cytoplasmic ribosomes were easily observed. The few plastid ribosomes present were less well defined. The amount and organization of residual plastid membranes were characteristic of CD cells. Relatively reduced numbers of vesicles exist in the plastids, and these are somewhat small and flattened and in close apposition to one another, resembling rows or stacks of beads located close to the inner wall of the chloroplast envelope (Fig. 1). This configuration was found to be extremely prominent in CD cells containing 3–4 μg chlorophyll/10^7 cells. Analysis of thin sections in a goniometric stage (tilting ± 20°) demonstrated that these vesicles were not continuous with the chloroplast envelope (36).

Measurements of chlorophyll fluorescence of CD cells indicated a nonfunctional photosynthetic apparatus. In general, illumination of photosynthetically competent cells leads to a fast rise in fluorescence emission (F_o), which is intrinsic to the system, followed by a high fluorescence transient, which finally drops to a low steady-state level of fluorescence (F_s) (37). On the other hand, when electron flow is blocked (e.g., with DCMU), the steady-state fluorescence reaches a maximal level, F_m, ($F_m > F_s$) (37). The ratio $(F_m - F_s)/F_o$ is, therefore, a convenient "index" of photosynthetic activity in whole cells, reflecting their capacity for electron flow, which is dependent on both H_2O splitting and CO_2 fixation in intact cells. The fluorescence yield ratio, $(F_m - F_s)/F_o$, calculated for CD cells approached zero (Table I).

Further information on the organization of the chlorophyll

TABLE I

Respiratory and Photosynthetic Activities of CD Cells Greened in the Presence or Absence of CHI and CAP

Treatment	Chlorophyll	Fluorescence yield F_o	Fluorescence yield F_m	Fluorescence yield F_s	$\frac{F_m - F_s}{F_o}$	Respiration	Oxygen evolution	RuBPase activity
	μg/10^7 cells		mV/μg Chlorophyll			μmol O_2/h/10^7 cells	μmol O_2/h/10^7 cells	μmol CO_2/min/g protein
CD cells	0.5	313	348	348	—	1.48	—	24
Light	6.6	65	165	90	1.15	1.60	2.87	122
Light + CAP	1.3	368	394	390	0.01	1.72	—	16
Light + CHI	2.3	180	250	220	0.17	1.81	2.09	74

CD cells were washed and allowed to green in the presence or absence of CAP (200 μg/ml) or CHI (2 μg/ml). Respiration and light-induced oxygen evolution of whole cells were measured polarographically. RuBPase activity of 27,000 *g* supernates of cell homogenates was determined according to Iwanij et al. (26). Fluorescence yields of whole cells were measured as described in Materials and Methods.

F_o, initial fluorescence; F_m, maximal fluorescence of cells inhibited with DCMU; F_s, steady state fluorescence of uninhibited cells.

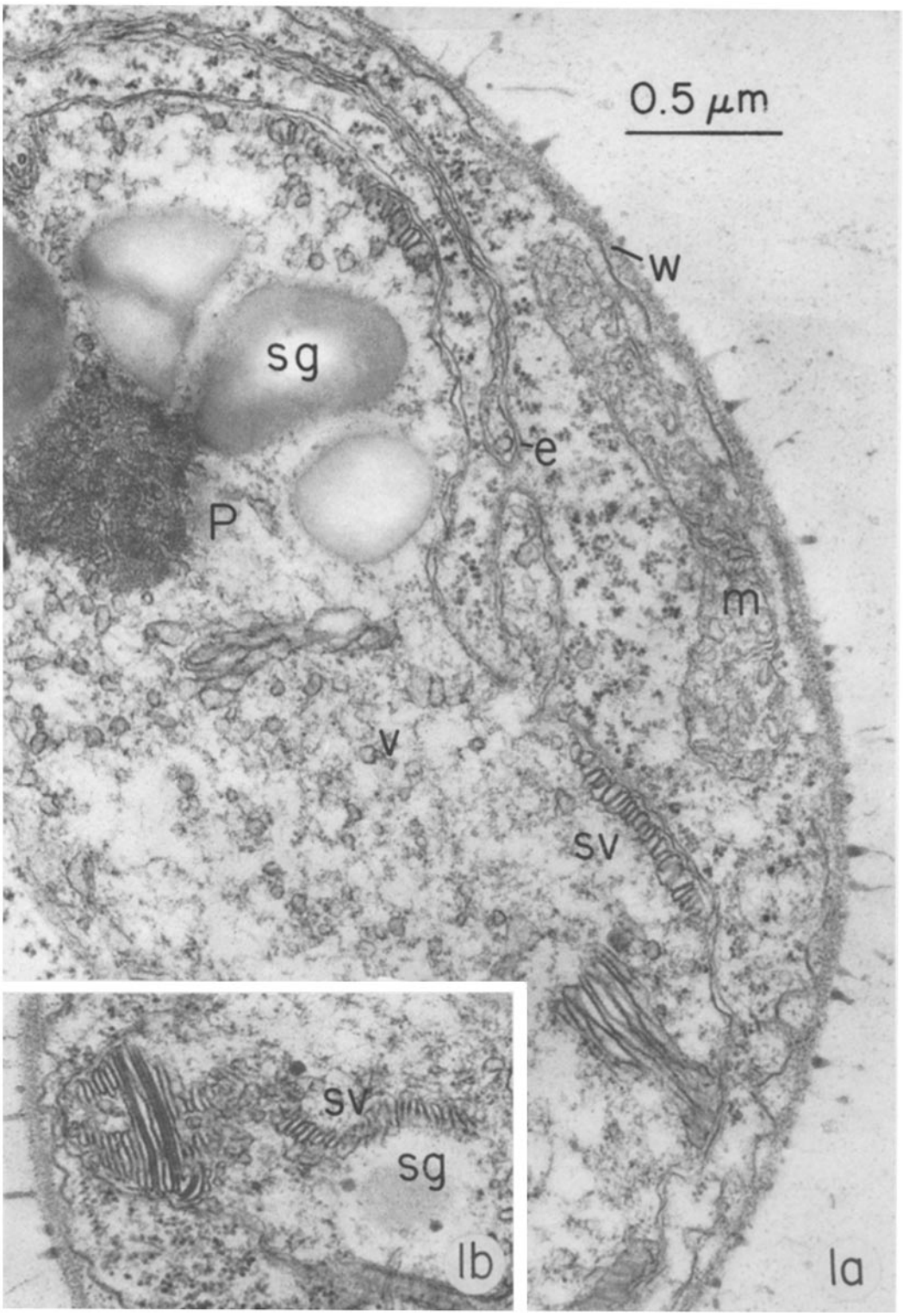

FIGURE 1 Section through a CD cell showing the plastid and part of the surrounding cytoplasm. Notice the presence of stacked rows of small vesicles (*sv*) in close proximity to the inner wall of the chloroplast envelope (*e*); isolated vesicles (*v*) are also present. *p*, pyrenoid; *sg*, starch grain; *m*, mitochondrion; *w*, cell wall. Cytoplasmic ribosomes are well defined and present in clusters; the chloroplast matrix shows only a few well-defined ribosomes. (*b*) Inset showing extensive stacking of vesicles. × 61,000.

in CD cells was obtained by examination of their 77°K fluorescence emission spectra. CD cells, like dark-grown cells, were characterized by a single fluorescence emission peak at 680–682 nm, which was regarded as being that of unorganized chlorophyll (Fig. 2) (7).

Greening of CD Cells in the Presence and Absence of 70S Translation

When CD cells were washed and exposed to light, they greened after a lag of 3–4 h. This process involved chlorophyll

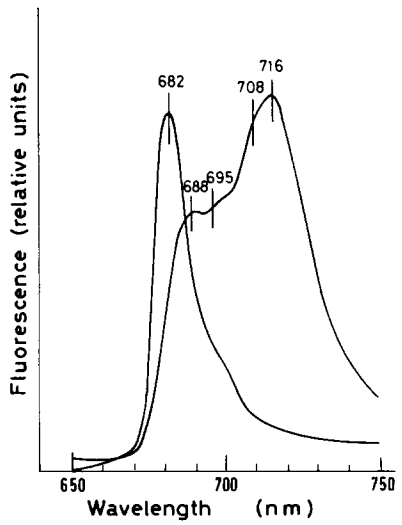

FIGURE 2 Low-temperature fluorescence emission spectra of CD cells and greened CD cells. Fluorescence emission spectra at 77°K of CD cells (0.9 μg chlorophyll/10^7 cells) (peak at 682 nm) and uninhibited greened CD cells (11.4 μg chlorophyll/10^7 cells) (peak at 7 nm) were recorded. These spectra are indistinguishable from those of dark-grown and green cells, respectively (cf. reference 7).

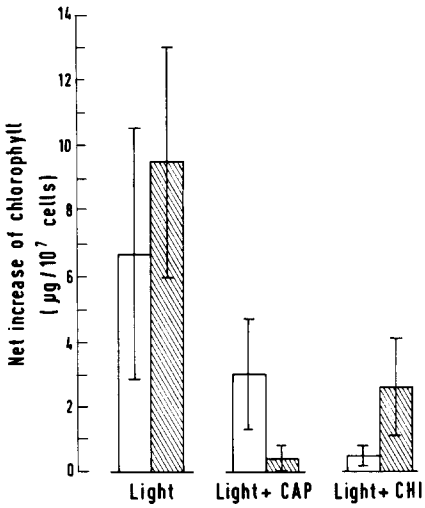

FIGURE 3 Effect of CHI and CAP on chlorophyll synthesis by dark-grown and CD cells. Dark-grown cells and CD cells were exposed to light for 10–15 h in the absence or presence of CAP or CHI. The average net increase in chlorophyll in five such experiments was determined and the values were compared in the histogram above. The average of 25 experiments of greening of CD cells in the presence of CHI was found to be 2.6 ± 1.9. *Open rectangle*, dark-grown cells. *Crosshatched rectangle*, CD cells. *Bar*, standard deviation.

synthesis (Table I and Fig. 3) accompanied by an increase in RuBPase activity, as well as the formation of photosynthetically competent membranes. The latter was demonstrated by high oxygen evolution and a rise in the fluorescence yield ratio ($F_m - F_s)/F_o$ (Table I). The 77°K fluorescence spectrum of the uninhibited, greened CD cells was indistinguishable from that of green cells derived from dark-grown cells (Fig. 2). Three peaks were detected in these cells: 688, 695, and 716 nm, ascribed to the light harvesting chlorophyll of PSII, the chlorophyll of the reaction center complex of PSII, and the chlorophyll associated with PSI, respectively (7, 37).

CD cells exposed to light in the presence of CAP were incapable of chlorophyll synthesis (Fig. 3). Furthermore, they did not develop photosynthetic activity, their fluorescence yield ratio ($F_m - F_s)/F_o$ approached zero, and the residual RuBPase activity continued to deteriorate (Table I). The 77°K fluorescence emission spectrum of CD cells exposed to light in the presence of CAP differed from that of dark-grown cells greened in the presence of this drug. In the latter case, fluorescence emission peaks developed at 688 and 708 nm. The spectrum of the CAP-treated CD cells, however, seemed to be of an "intermediate type." The peak at 682 nm (characteristic of CD cells or dark-grown cells) was retained and that at 708 nm started to develop; no peak at 688 nm was apparent (Fig. 4 *a*).

The inability of CD cells to green in the presence of CAP is unexpected, and it could be attributed to a nonspecific effect

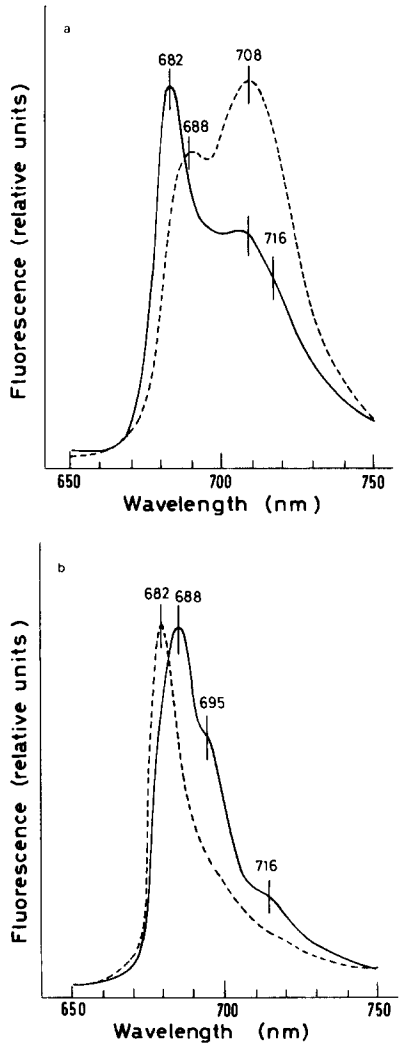

FIGURE 4 Low-temperature fluorescence emission spectra of CD and dark-grown cells greened in the presence of CAP or CHI. CD cells (0.9 μg chlorophyll/10 cells) were washed and transferred directly to the light for 12 h in the presence of CAP or CHI. At the end of the illumination, fluorescence emission spectra at 77°K were recorded. The chlorophyll contents of the cells treated with CAP or CHI were 1.5 and 2.3, respectively. For comparison, spectra of dark-grown cells (1.2 μg chlorophyll/10^7 cells) transferred to the light for 12 h, in the presence of CAP (6.2 μg chlorophyll/10^7 cells) and CHI (1.4 μg chlorophyll/10^7 cells) are shown. (*a*) Cells greened in the presence of CAP. (*b*) Cells greened in the presence of CHI. -----, dark-grown cells. ——, CD cells.

of prolonged exposure of the cells to CAP. To verify that indeed the inhibition of chlorophyll synthesis in CD cells was caused by the lack of 70S ribosomal activity during the greening process, CD cells wre illuminated in the presence of spectinomycin (3 μg/ml) instead of CAP. Once again, and unlike dark-grown cells, which greened in the presence of spectinomycin, CD cells remained incapable of synthesizing chlorophyll in the presence of this inhibitor. Moreover, no photosynthetic activity developed in spectinomycin-inhibited CD cells. Their fluorescence yield ratio remained very low, RuBPase activity was not restored, and the characteristic intermediate type 77°K fluorescence emission spectra of CD cells illuminated in the presence of CAP was obtained.

Greening of CD Cells in the Absence of 80S Ribosomal Translation

In view of the fact that greening of CD cells was found to be dependent on 70S translation, it was of interest to determine whether such a dependency exists for cytoplasmic protein synthesis. CD cells were washed and incubated in the light with the addition of CHI. Unlike dark-grown cells, which do not green at all under similar conditions, CD cells were often found to be able to synthesize chlorophyll (2.5 ± 1.5 μg chlorophyll/10^7 cells) (Fig. 3). The degree of reproducibility of these experiments could be improved if the washed CD cells were incubated in the dark for 0.5–1.0 h before the addition of CHI and the transfer to light. In all these experiments, chlorophyll synthesis was initiated 2–4 h after illumination. Chlorophyll synthesis then proceeded in the presence of CHI at a low rate, extending over a period of 3–6 h.

Inasmuch as δ-aminolevulinic acid (δALA) synthesis is known to be inhibited by CHI (22), it was essential to determine whether the chlorophyll synthesized by CD cells in the presence of this drug was derived from preaccumulated δALA or from δALA synthesized *de novo*. Thus, [^{14}C]acetate was added to the CHI-inhibited cells during the time of actual chlorophyll accumulation. In a typical experiment, it was found that chlorophyll resolved by TLC had a specific activity of 16.7 nmol [^{14}C]acetate/μg chlorophyll. (This is ~60% of the theoretical value, as expected, if all the carbon atoms were derived from [^{14}C]acetate.) To further ascertain whether CD cells incorporate acetate into the porphyrin moiety of chlorophyll, distribution of the radioactivity between the chlorophyllide and phytol of alkaline hydrolyzed chlorophyll was determined. In the four experiments performed, 67 ± 3% of the radioactivity could be ascribed to chlorophyllide. This corresponds well with the fact that 68.6% of the carbons of chlorophyll reside in the porphyrin ring.

Examination of 77°K fluorescence emission spectra of CD cells that greened in the presence of CHI indicated that the chlorophyll synthesized in these cells was organized in PSII reaction centers and chlorophyll-protein complexes. This was apparent from the development of fluorescence peaks at 688, 695, and 716 nm. As a control experiment, dark-grown cells were exposed to light in the presence of CHI. Practically no chlorophyll synthesis occurred and the 77°K fluorescence emission spectrum remained the same as in dark-grown cells (Fig. 4 *b*). The presence of chlorophyll-protein complexes I and II (CPI and CPII) could be demonstrated in CD cells that greened in the presence of CHI, by electrophoresis of membrane preparations from these cells.

Examination of thin sections of CD cells that greened in the presence of CHI showed that in general the cells had a normal appearance comparable to uninhibited cells containing similar amounts of chlorophyll. Long flattened lamellae organized in groups of two to four and, occasionally, more thylakoids could be seen (Fig. 5). Numerous, well-defined ribosomes were visible in the stroma of these cells. The CD cells that greened in the presence of CHI not only exhibited photosynthetic chlorophyll-containing membranes but also light-driven oxygen evolution, and had a fluorescence yield ratio, $(F_m - F_s)/F_o = 0.17$. RuBPase was also restored in these cells (Table I).

DISCUSSION

The aim of this work was to gain further insight into the process of chloroplast-cytoplasm interaction during chloroplast development. The results obtained show that chlorophyll synthesis requires the participation of chloroplast translation products, and that synthesis of chlorophyll and its integration into functional complexes within growing membranes can occur in the absence of concomitant synthesis of 80S translation products.

The greening process of dark-grown y-1 cells was reported to be only partially inhibited by CAP but completely blocked by CHI (35). However, greening of CD cells was shown in this work to be entirely dependent upon 70S translation. Inhibition of protein synthesis by either CAP or spectinomycin, which bind to different sites on the 70S ribosome (1, 10, 23), completely prevented greening of CD cells. In many plant systems, the use of inhibitors of 70S translation has demonstrated that 70S translates are required for chlorophyll synthesis (5, 15, 31, 38, 39), but sites where 70S translates participate are as yet unknown.

It is generally accepted for higher plants that the enzymes necessary for the conversion of δALA to protochlorophyll(ide) are of cytoplasmic origin (22, 31). Therefore, the participation of chloroplast translates in the synthesis of chlorophyll(ide) should occur either before δALA formation or after protochlorophyll(ide) has been formed. Possibilities for such chloroplast activity could include: (*a*) transport of cytoplasmically derived enzymes into the chloroplast; (*b*) synthesis of δALA from five-carbon precursors, a process which has lately been shown to involve numerous enzymatic steps (22, 27); (*c*) regeneration of protochlorophyll(ide)-holochrome complex and its photoconversion to chlorophyll(ide); and (*d*) the integration of the holochrome complex or chlorophyll into the thylakoid, a situation thought to exist for the chlorophyll associated with CPI (28).

Feierabend (14) has shown that temperature-induced chlorosis of rye plants is caused by loss of plastid ribosomes. The plastids were shown to contain the complete spectrum of cytoplasmically derived enzymes and were able to accumulate protochlorophyll(ide) when fed δALA. However, this protochlorophyll(ide) was not photoconverted to chlorophyll but, instead, photooxidized, thus indicating that chloroplast translates participate at a post-protochlorophyll(ide) step. On the other hand, mutants of *Chlamydomonas* lacking plastid ribosomes continue to synthesize chlorophyll (6, 19, 38). It should be noted that these mutant cells maintain the wild-type capacity for enzymatic conversion of protochlorophyll(ide) to chlorophyll(ide) in the absence of light, a process that probably differs from photochemical conversion (20). Therefore, the nuclear mutant y-1 phenotypically resembles higher plants in its inability to enzymatically reduce protochlorophyllide to chlorophyll(ide). In view of this, it seems most likely that 70S translates are involved in the recycling of holochrome and the

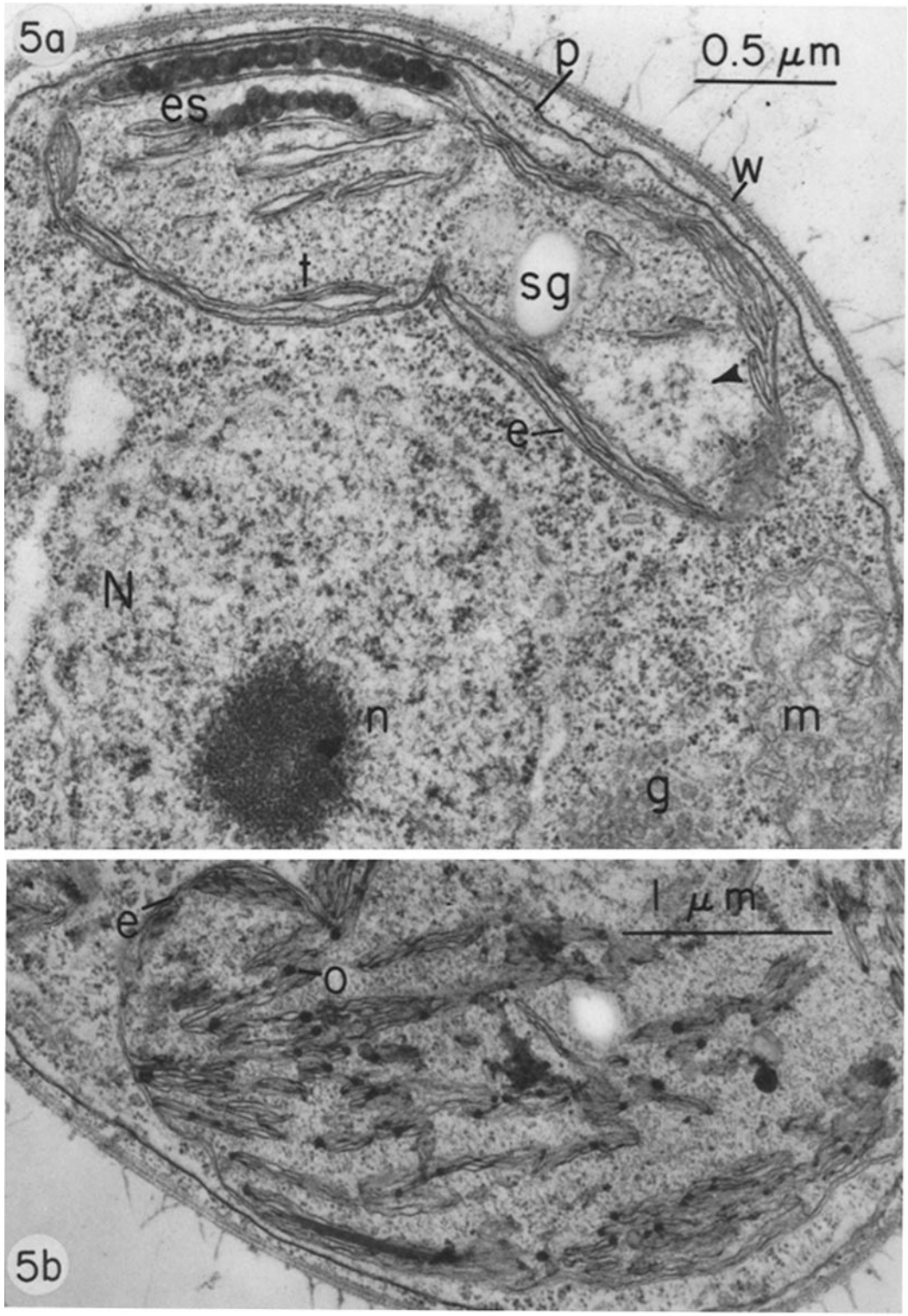

FIGURE 5 Sections through CD cells exposed to the light for 10 h in the presence of CHI. CD cells were washed free of CAP and incubated in the dark for 1 h in the absence (*a*) or presence (*b*) of CHI. Then both treatments were incubated in the light with CHI (2 μg/ml). Notice the dispersion of the stacks of vesicles and growth of membranes to form isolated and paired thylakoids. Well-defined ribosomes are present through the chloroplast stroma (cf. Fig. 1). *N*, nucleus; *n*, nucleolus; *g*, Golgi apparatus; *e*, envelope; *es*, eye spots; *p* plasma membrane; *w*, cell wall; *o*, osmiophilic globules; *m*, mitochondrion; *sg*, starch grain; *t*, thylakoid. Arrow indicates the area of plastid DNA.

formation of the protochlorophyll(ide)-holochrome complex. However, the possible participation of such translates in δALA synthesis cannot be excluded.

Synthesis of chlorophyll and active photosynthetic membranes can occur in the presence of CHI in CD cells, as demonstrated in this study. These cells differ significantly from dark-grown cells pregreened in the presence of CAP (also capable of greening subsequently in the presence of CHI [7,

24, 35]) in that they do not contain comparable amounts of chloroplast membrane and chlorophyll. The morphological appearance of the residual chloroplast membranes of CD cells is abnormal and is reminiscent of that found in *Chlamydomonas* mutants that lack active chloroplast ribosomes (6, 19) or are blocked in the synthesis of chlorophyll, as demonstrated in the Br_s-R1 double mutant (41). However, CD cells greened in the presence of CHI form substantial amounts of membrane, apparently containing antennae of PSI and PSII. This implies that the necessary corresponding polypeptides of cytoplasmic origin of CPII have already been accumulated in CD cells. Therefore, the synthesis and/or preaccumulation of these polypeptides, which is light dependent in y-1 cells, is rendered light independent by depleting the cells of 70S translates. Hoober and Stegeman (25) have proposed that control of the synthesis of a cytoplasmic polypeptide by light is at the level of transcription. The actual repression of the nuclear gene(s) concerned was thought to involve a chlorophyll precursor acting as a corepressor for a postulated 70S translated aporepressor (13, 25).

The experimental results presented in this work can indeed by interpreted according to this hypothesis. However, because 70S translates have been shown to be involved in chlorophyll synthesis, and in view of additional data obtained in several laboratories, an alternative explanation should also be considered. It was recently observed that light seems to induce at least two processes which, once triggered, can proceed in the absence of continuous illumination. The first process involves light for the induction of glutamate-1-semialdehyde aminotransferase, a cytoplasmically synthesized enzyme involved in the formation of δALA from glutamate (27). This pathway (δALA synthesis from a five-carbon precursor) is thought to be the dominant one for chlorophyll synthesis (22). As shown in barley, glutamate-1-semialdehyde aminotransferase is a relatively stable enzyme and, once formed, the presence of CHI may not inhibit δALA synthesis for at least 10 h (27). The second process involves light-induced transcription of the nuclear gene for the apoprotein of CPII (2, 4). The mRNA formed is found to be present in light-induced cells subsequently incubated in the dark for a few hours (4). Nevertheless, the apoprotein itself does not seem to accumulate. It is therefore thought that, in the absence of chlorophyll to bind to the apoprotein, the latter is degraded.

Another observation was that chlorophyll synthesis is negatively regulated by the accumulation of a regulatory form of protochlorophyllide (possibly protochlorophyll(ide)-holochrome complex) in the dark and that this exerts a feedback inhibition on the δALA synthesizing enzymes (22, 31). Upon photoconversion of protochlorophyll(ide), the inhibition is released and subsequently all the chlorophyll formed is complexed with protein (30). Thus, it appears that a free form of chlorophyll is nonexistent. Moreover, it has previously been postulated (28) that in the absence of apoproteins for the chlorophyll-protein complexes, chlorophyll synthesis should come to a stop.

Taking into account all these considerations, one could explain the greening of CD cells in the presence of CHI as follows. After growth of the y-1 mutant in the dark in the presence of CAP (CD cells), chloroplast translates are depleted and the regulatory form of protochlorophyll(ide) is not formed. Therefore, the cytoplasmic enzyme forming δALA remains active. In addition, the level of the cytoplasmic polypeptides binding chlorophyll will be the same as in dark-grown cells, or eventually higher. This latter assumption would be valid if one were to presuppose that the chlorophyll binding polypeptides bind to the regulatory form of protochlorophyll(ide) as well. This protochlorophyll(ide)-apoprotein complex (absent in CD cells) may be more susceptible to the degradative enzymes. Another possibility would be that the degradative enzymes themselves are 70S translates. In both instances, the result would be elevated levels of CPII apoprotein.

Exposing CD cells to the light in the presence of CHI would allow the cells to form the 70S translates required for chlorophyll synthesis. The newly formed chlorophyll could now be complexed with the preaccumulated chlorophyll *a,b*–binding polypeptides of cytoplasmic origin to form the CPII complex, as well as with the chlorophyll *a*–binding polypeptides of chloroplast origin, leading to the appearance of CPI. Thus, a significant amount of functional thylakoids would be formed, the extent of formation being limited by the previous accumulation of cytoplasmic polypeptides.

This model, which should be considered only as a working hypothesis, is, to the best of our knowledge, in agreement with all the experimental results so far reported for *Chlamydomonas* wild-type and mutant strains. Its main feature is that the chloroplast plays an important role in the control of chlorophyll synthesis and, hence, in membrane formation. It also ascribes an important role to turnover processes considered operative in bacteria and eukaryotic organisms as posttranslation regulatory systems (18). The validity of the model remains to be established by future experimentation.

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