SYNTHESIS OF CHLOROPLAST MEMBRANE POLYPEPTIDES DURING SYNCHRONOUS GROWTH OF *CHLAMYDOMONAS REINHARDTII*

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ABSTRACT

The synthesis of the major chloroplast membrane polypeptides has been studied during synchronous growth of *Chlamydomonas reinhardtii*. Under these conditions, chlorophyll is synthesized during the latter part of the light period and cell division takes place during the dark period. The profile of the chloroplast membrane polypeptides of *C. reinhardtii* has been well characterized and shown to contain two major classes by size (Hoober, J. 1970. *J. Biol. Chem.* **245**:4327). Polypeptides of group I have a mol wt range of 50,000-55,000 daltons. The second region consists of at least three polypeptide groups, IIa, IIb, and IIc, having mol wt of 40,000, 31,000, and 27,000 daltons, respectively. The synthesis of these polypeptides has been measured using a double-labeling technique and a computer-aided statistical analysis. The rate of labeling of group I polypeptides is highest during the early light period and decreases after 6 h of growth. Group IIa is labeled from the beginning of the light period, but little synthesis of IIb occurs before 3 h, and significant amounts of label are not found in IIc before 5 h of growth. After approximately 8 h of light, groups IIb and IIc are synthesized at rates significantly greater than those of the other membrane polypeptides. The synthesis of the major polypeptide groups ceases in the dark. We conclude that the biosynthesis of the chloroplast membranes is a sequential or stepwise process.

Much information has recently become available concerning the physical properties of biological membranes. Less, however, is known about the processes of synthesis and integration of components required for the production of functional membranes, especially in eukaryotic cells. First, it is not known whether there exists an obligatory coupling of the synthesis of one or more of the proteins and lipids involved in the formation of membranes under conditions of normal cell growth, and, second, it is not known whether the association of specific lipid and protein components is required for their integration into the membrane. With regard to the first question, experiments with bacterial mutants have indicated that the synthesis of membrane lipids and proteins is closely coordinated (1-4). On the other hand, the turnover of lipids and proteins of rat liver cell endoplasmic reticulum appears to occur at different rates (5, 6), and studies with the y-1 mutant of *Chlamydomonas reinhardtii* have shown that the ratios of various chloroplast membrane components vary during the greening process (7-9). These results sug-

gest that the association between lipids and proteins is less rigid in hepatocyte and chloroplast membranes and that new membrane components can be inserted into the membranes independently. Evidence relevant to the second question comes from the observations (10, 11) that the integration of chlorophyll into the chloroplast membranes of the alga C. reinhardtii does not occur until after the pigment has been complexed with protein newly synthesized on cytoplasmic ribosomes.

In view of the accumulated knowledge regarding the structure, function, and composition of the chloroplast membranes of C. reinhardtii (11-14), a study of the synthesis of the components of the chloroplast membranes has the potential for providing answers to the important questions concerning the relationship between the synthesis of membrane lipids and proteins and concerning a specific association of lipids and proteins before integration into the membrane.

C. reinhardtii can be grown in a synchronous 12-h light, 12-h dark cycle, and an entire culture can be treated as a single cell under these conditions. Changes in many enzyme activities, including several membrane-associated activities, and the synthesis of the several cellular nucleic acids throughout the synchronous cycle have been measured (15, 16). The electrophoretic profile of polypeptides from C. reinhardtii chloroplast membranes has been characterized by Hoober (17) and shown to contain two major size classes. We have found that group I polypeptides lie in the mol wt range of 50,000-55,000 daltons and that group II polypeptides have mol wt between 27,000 and 40,000 daltons. Previous results from this laboratory with digitonin fractionation of chloroplasts from both spinach and C. reinhardtii (18) have indicated that the group I polypeptides are concentrated in a membrane fraction enriched for photosystem I activity and that group II polypeptides are associated with a membrane fraction enriched for photosystem II activity. For the most part, the function of these polypeptides is unknown. It appears, however, that some of those of group II are related to the stacking of the chloroplast membranes into grana (19, 20). Furthermore, during electrophoresis of green, lipid-containing chloroplast membrane preparations, chlorophyll runs coincidently with several of the group II polypeptides and with a high molecular weight polypeptide fraction which, when extracted with acetone, runs in group I (21). It is thought that at least two of the polypeptides of group II are part of the light-harvesting chlorophyll-protein complex (21-24) and that polypeptides of group I are derived from the chlorophyll-protein complex of photosystem I (21-23, 25).

We report in this paper the results of studies of the synthesis of group I and II polypeptides during the synchronous cycle. We have used a double-labeling technique and polyacrylamide gel electrophoresis to identify the membrane polypeptides. The data indicate that the synthesis of the group I polypeptides precedes the bulk of chlorophyll synthesis and that the initiation of synthesis of the various group II polypeptides occurs at different times. We conclude that the biosynthesis of the chloroplast membranes of C. reinhardtii is a stepwise process.

MATERIALS AND METHODS

A clone from wild type strain 137c of C. reinhardtii was selected for its suitability for synchronous growth by the method of Surzycki (15). Cells were grown in 3-liter cultures of minimal medium (26) and synchrony was induced by a 12-h light, 12-h dark cycle according to the procedure of Armstrong et al. (16). Cell density was estimated with the aid of a hemacytometer. Chlorophyll was measured by a modification (27) of the method of Mackinney (28). Protein was determined by use of the Folin reagent (29). Ribulose-1,5-diphosphate (RuDP)-carboxylase activity was measured as $^{14}CO_2$ fixed by the various fractions in the presence and absence of RuDP, as described previously (30).

Synthesis of membrane proteins was measured by a double-labeling technique. In general, such techniques provide a high level of sensitivity for experiments measuring the synthesis or turnover of various cellular components (31). We have used two double-labeling procedures to examine the synthesis of membrane polypeptides in C. reinhardtii. In the first procedure, cells were labeled for 1 h during the synchronous cycle simultaneously with [^{14}C]acetate (1 μCi/μmol) and [^{3}H]arginine [5 μCi/μmol (5)] or with [^{3}H]acetate (17 μCi/μmol) and [^{35}S]sulfate (0.5 μmole/ml), (1.2 mCi/mmol). These experiments were designed to compare the labeling of polypeptides by acetate with that of other labels for proteins. In a second double-labeling experiment designed to measure the synthesis of new membrane polypeptides, cells were labeled to uniform specific activity with [^{14}C]acetate (0.3 μCi/ml growth medium) before growth in synchronous cultures. At various times during the synchronous cycle, these cells were labeled for 1 h with [^{3}H]acetate (17 μCi/μmol). In this experi-
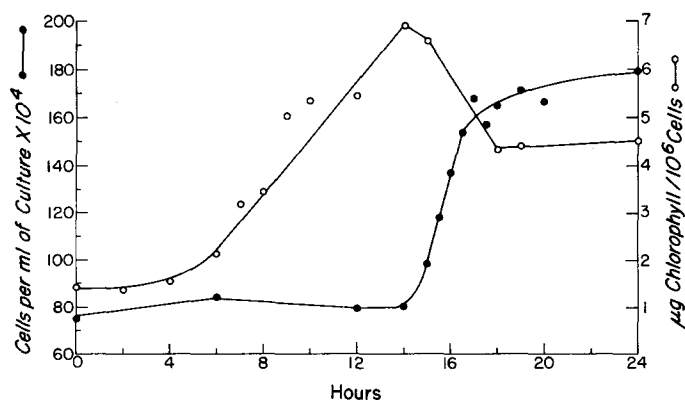
FIGURE 1   Chlorophyll concentration of the cells and cell concentration of the culture during synchrony. The light period is 0–12 h and the dark period is 12–24 h.

ment, the synthesis of new material was measured by the [³H]acetate incorporated; the labeling profiles obtained were analyzed by the computer method described below. In all acetate pulse experiments the final acetate concentration was 100 μM. The incorporation of acetate never exceeded 2% of the total acetate added in these experiments. All radiochemicals were obtained from New England Nuclear, Boston, Mass.

After labeling during the synchronous cycle, samples of cells were harvested, washed, and disrupted by sonic oscillation, and the membranous fraction was obtained (32). Membrane polypeptides were analyzed by polyacrylamide gel electrophoresis by the methods described by Hoober, including extraction with 90% acetone to remove lipids and addition of mercaptoethanol to prevent oxidation of sulfhydryl groups (17). Gels were divided into 1-mm slices and radioactivity in the slices were measured by counting in toluene-Omnifluor containing 3% Protosol (New England Nuclear). The data were analyzed by a computer program (33) which carried out a regression analysis of the counts of one isotope on the counts of the second isotope for each gel and which then plotted the results.

Acrylamide, methylenebisacrylamide (both electrophoresis grade), and urea were obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis. Sodium lauryl sulfate was obtained from Sigma Chemical Co., St. Louis, Mo.

RESULTS

Synchronous Growth

Under the light-dark regime we employed, cells of C. reinhardi synthesized chlorophyll between hour 6 and hour 12[1] (Fig. 1). A small

[1] For convention, the light period is denoted 0–12 h and the dark period 12–24 h of growth during the third 24-h light-dark cycle.

increase in chlorophyll was observed after the dark period had begun (12–14 h). Cell number remained relatively constant in those cultures throughout the light and early dark periods, and cell division occurred during a short interval of approximately 3 h, between hours 15 and 18. This rapid increase in cell number was reflected in the sharp decrease in the ratio of chlorophyll per cell. The degree of synchrony of these cultures could be assessed by one or both of the parameters described. Cultures which showed little or no chlorophyll synthesis before the 6th h of growth or whose curve of cell number showed that the period of cell division was closely confined to a 3-h period were accepted for use.

Labeling of Membrane Proteins

Since we chose to measure synthesis of proteins by introducing an isotope into the cells during a relatively short time interval, it was necessary to find a carrier molecule which could meet several criteria: first, it must enter the cells rapidly and be incorporated into protein at a high rate; second, its concentration must be high enough so that it would not be exhausted during the pulse interval but, most importantly, not so high that it would disrupt the dependence of cell growth on phototrophic metabolism. Cells of C. reinhardtii were known to have a high affinity for acetate, and we chose this molecule for use in labeling studies. Preliminary experiments had indicated that the rate of acetate utilization by C. reinhardtii changes during growth in the light, so we chose growth times when we expected maximal differences in acetate uptake and determined the dependence of incorporation on acetate concentration at those

times (Fig. 2). For cells 2 and 10 h old, incorporation of acetate into protein was highly dependent on concentration below 100 μM acetate. Incorporation was nearly maximal at 100 μM acetate in cells of the ages tested and only a small increase in labeling was found at higher acetate concentrations. Accordingly, all pulse labeling with acetate was carried out at a concentration of 100 μM.

During synchronous growth of *C. reinhardtii*, the majority of the membrane protein was made in the light period (Fig. 3). The highest rates of incorporation of [3H]acetate occurred between hours 4 and 10, and the rates of incorporation in the dark were only 25% of the maximal rates in the light. Synthesis of membranes proteins during the light period was also reflected in the dilution by newly formed protein of the 14C label which was present for the double-labeling experiments described below. The specific activity of 14C in the membrane protein decreased continuously during the light period to approximately one third the value of the 1st h.

Electrophoretic Analysis of Membrane Proteins

We subjected labeled membrane polypeptides to separation on polyacrylamide gels by the method of Hoober (17) and analyzed the gels by slicing, counting, and a computer-aided statistical analysis. A comparison of the two labels in double-labeled samples was obtained from the regression of counts of one isotope on counts of the other isotope (3H on the background 14C in the pulse experiments) and by plotting the deviations from the regression line versus slice number (33). Differences between the levels of the isotopes in a given slice appear as positive or negative deflections on a plot of the deviations (e.g., Fig. 4 B). A pair of horizontal lines is drawn on the plot at ± 1.96 σ for arbitrary reference points.

For vegetative cells of *C. reinhardtii*, acetate was an efficient label, and it was readily transported and metabolized at all stages of the synchronous cycle. In order to determine whether acetate was an appropriate label for membrane proteins, labeling experiments were carried out in which cells were pulsed simultaneously with [3H]acetate and [35S]sulfate or with [3H]acetate and [14C]arginine. The membrane proteins were isolated and separated on gels, and the
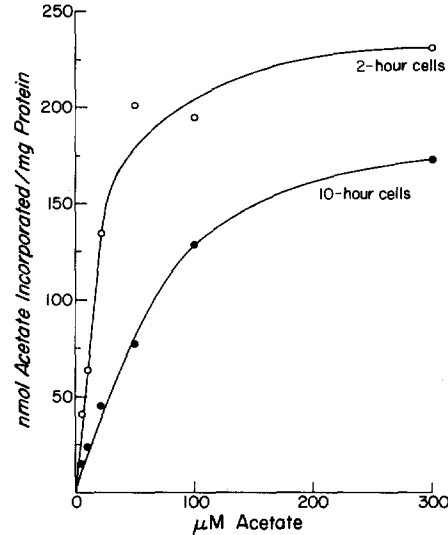

FIGURE 2 Dependence of acetate incorporation into protein on acetate concentration. Cells of 2 and 10 h old were incubated for 1 h with various concentrations of [3H]acetate of constant specific activity. After harvesting the cells, cellular protein was precipitated by acetone, assayed by the method of Lowry et al. (29) and counted in a Beckman Liquid Scintillation Counter.
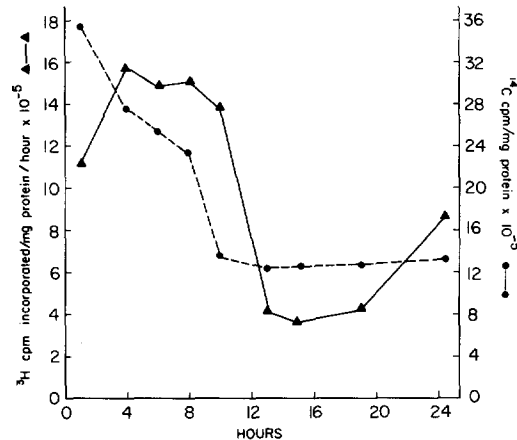

FIGURE 3 Synthesis of chloroplast membrane protein during the synchronous cycle. Cells of *C. reinhardtii* were double labeled with [3H]acetate and [14C]acetate, the chloroplast membranes were isolated, and the membrane protein was extracted and counted as described in Materials and Methods.

results were analyzed with the aid of a computer as described above. Fig. 4 shows an example of a dual pulse experiment with acetate and arginine at 4 h of growth.[1] Similar profiles of the crossover- and background-corrected
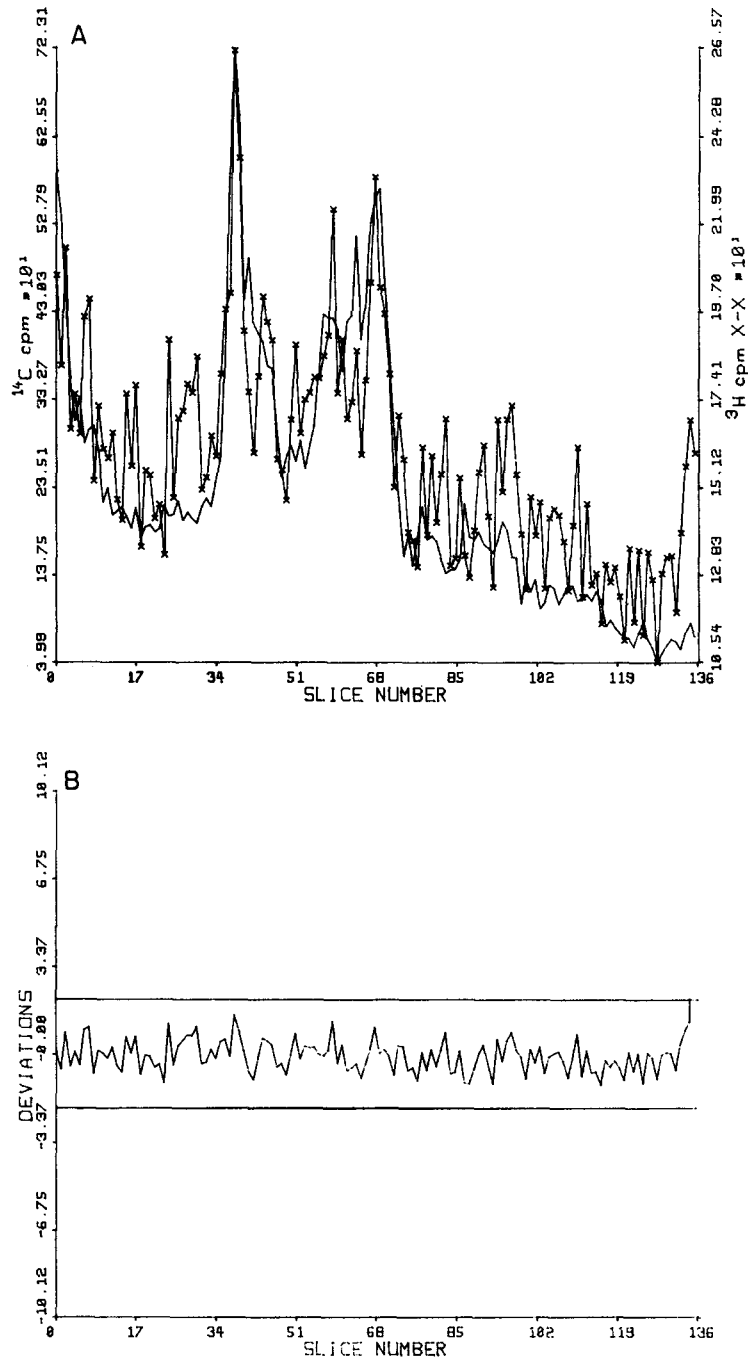
FIGURES 4–8 Computer-generated traces of double-labeled polypeptides on polyacrylamide gels. Part A of each figure shows the background- and crossover-corrected counts for the ^3H and ^{14}C labels. Part B of each of these figures shows a plot of the deviations from the regression line versus slice number. Differences between the levels of the isotopes in a given slice appear as a positive or negative deflection on a plot of the deviations. A pair of horizontal lines is drawn on the plot at ± 1.96 σ (the 95% confidence levels for significant deviation) for arbitrary reference points.

FIGURE 4 Labeling of membrane polypeptides during simultaneous pulses with [^{14}C]acetate and [^3H]arginine. Cells were grown for 4 h and then incubated for 1 h with the labeled precursors as described in Materials and Methods.

counts were obtained (Fig. 4 A). The statistical analysis (Fig. 4 B) shows that there are no significant differences between the amounts of ^{3}H and ^{14}C in the various polypeptide bands on this gel. At the several growth times examined, the same labeling pattern was obtained with sulfate and arginine as had been obtained with acetate, and the regression analysis indicates that there were no significant differences between acetate-and arginine-labeled polypeptides.

Since acetate is a nonspecific label, it was necessary to consider the possibility that cellular components other than protein, which might also become labeled, would not interfere with the radiometric profiles of our gels. In one experiment, we added ^{32}P-labeled lipids to a protein sample and subjected an aliquot to gel electrophoresis. As expected, all the lipid ran with the dye front and no ^{32}P counts were found elsewhere in the gel. In a second experiment, we examined the behavior of starch added to acrylamide gels. Gels on which had been loaded protein samples or protein samples containing added starch were run under the normal electrophoresis procedure and stained with a solution of 0.01 M KI and 0.01 M I_2. No starch was found in the gels containing only the membrane polypeptide sample, and all the starch from the sample containing added starch remained at the top of the gel. It appeared that any lipid remaining after acetone extraction and any starch remaining after differential centrifugation would not interfere with our gel profiles. Furthermore, one would not expect significant penetration of 8% gels by ribosomal RNA's (34), which would represent the major type of nucleic acid contamination.

Experiments designed to indicate the time of synthesis of the various membrane polypeptide groups were carried out as follows: a starter culture of cells was labeled to uniform specific activity with [^{14}C]acetate and then transferred to experimental flasks in the growth chamber; at the appropriate times in the synchronous cycle, [^{3}H]acetate was added to the flasks for 1 hr; cells were then harvested and the membrane proteins prepared. Radiometric profiles of the gels containing these proteins are shown in Figs. 5-8. In these experiments, the uniform ^{14}C label is equivalent to an optical scan of a gel stained for total protein with Coomassie blue. The major membrane peaks are indicated in Fig. 5 A; group

I is seen as at least one peak having a mol wt of 55,000 daltons; group II consists of at least three peaks, IIa, IIb, and IIc, which have mol wt of 40,000, 31,000, and 27,000 daltons, respectively. Several molecular weight calibrations have been carried out with different sets of protein standards. The locations of one set of standards is shown in Fig. 5 A.

Results concerning the time of synthesis of membrane polypeptides were obtained by examination of several series of computer plots; several plots from one of these series are shown in Figs. 5-8. Fig. 5 A shows the labeling of membrane polypeptides between 0 and 1 h in the light. ^{3}H is incorporated into I, into a shoulder on I, and into IIa. A small amount of label appears in IIb and almost no ^{3}H is observed under IIc. The statistical analysis shown in Fig. 5 B indicates that more label is located under I and its shoulder at this time than under any of the other peaks on the gel, that an average amount of label appears in IIa, and that the labeling of IIb and IIc is markedly lower than the rest of the polypeptides. By hour 6 (Figs. 6 A and 6 B), the rate of labeling of IIb had reached the mean rate for the remainder of the gel, and group I continued to be labeled at high rates although its shoulder is no longer seen. The labeling of IIc remained significantly lower than that of the other polypeptides. At 8 h (Figs. 7 A and 7 B), significant changes in the labeling profile appear. The labeling of I dropped nearly to the mean level and the incorporation into IIb and IIc was proceeding at markedly higher rates than for the remainder of the gel. Incorporation into IIa continued at a mean level. By hour 10 (Fig. 8 A and 8 B), there was a marked deficiency in the labeling of I and the relative rates of IIb and IIc had reached even higher levels.

Pulse labeling of membrane proteins between hours 12 and 13, the 1st h in the dark, showed a marked decrease in the synthesis of all the major chloroplast membrane polypeptides as well as several minor ones. Labeling of polypeptides greater than approximately 60,000 daltons, however, continued at a relatively high rate.

Data from these curves are summarized in Fig. 9. We have plotted the deviations from the least squares regression line for group I, IIa, IIb, and IIc for several experiments. These plots indicate the maximum differences between the newly labeled polypeptides and the background
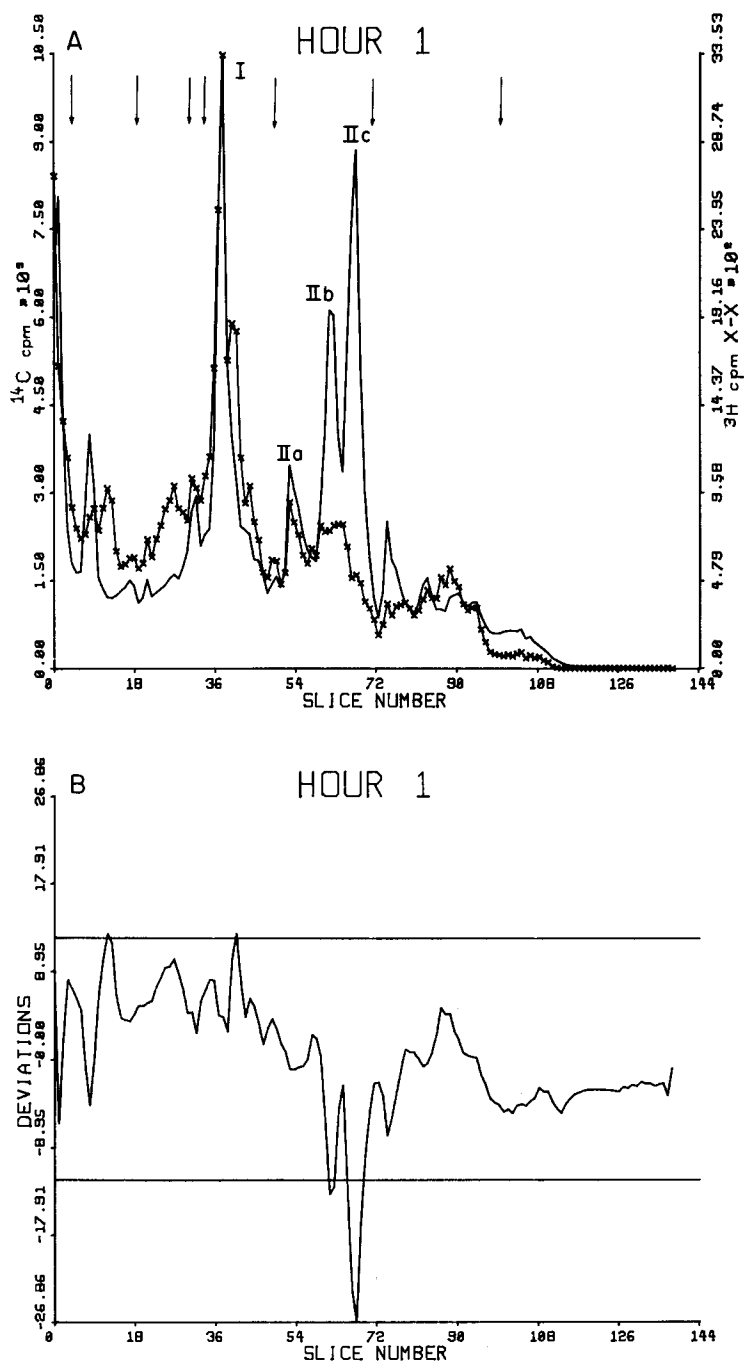
FIGURE 5 Membrane polypeptides pulse labeled between 0-1 h. Arrows at the top of the figure indicate position reached by proteins of known molecular weight run on a companion gel. These proteins and their mol wt were, left to right, β-galactosidase (130,000), phosphorylase a (93,000), bovine serum albumin (68,000), pyruvate kinase (57,000), aldolase (40,000), chymotrypsin (25,000), and cytochrome c (11,700).
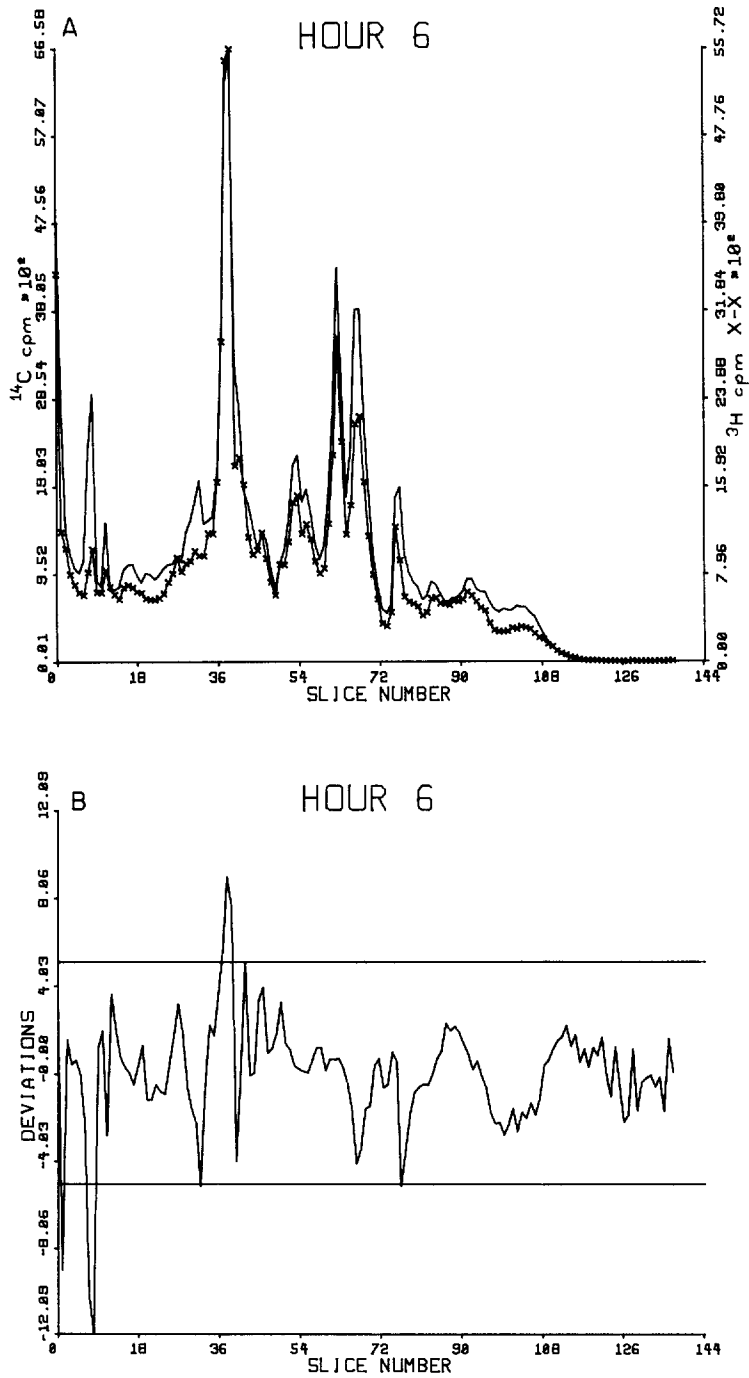
FIGURE 6   Membrane polypeptides pulse labeled between 5 and 6 h.

label for a series of 1-h pulses, and thus show the changes in the rates of synthesis of the membrane polypeptides. Fig. 9 A shows the rates of synthesis of the group I polypeptides superimposed on a plot of the chlorophyll content of the culture. The rate of synthesis of group I was highest at hour 4, decreased steadily after this time, and proceeded more slowly than the
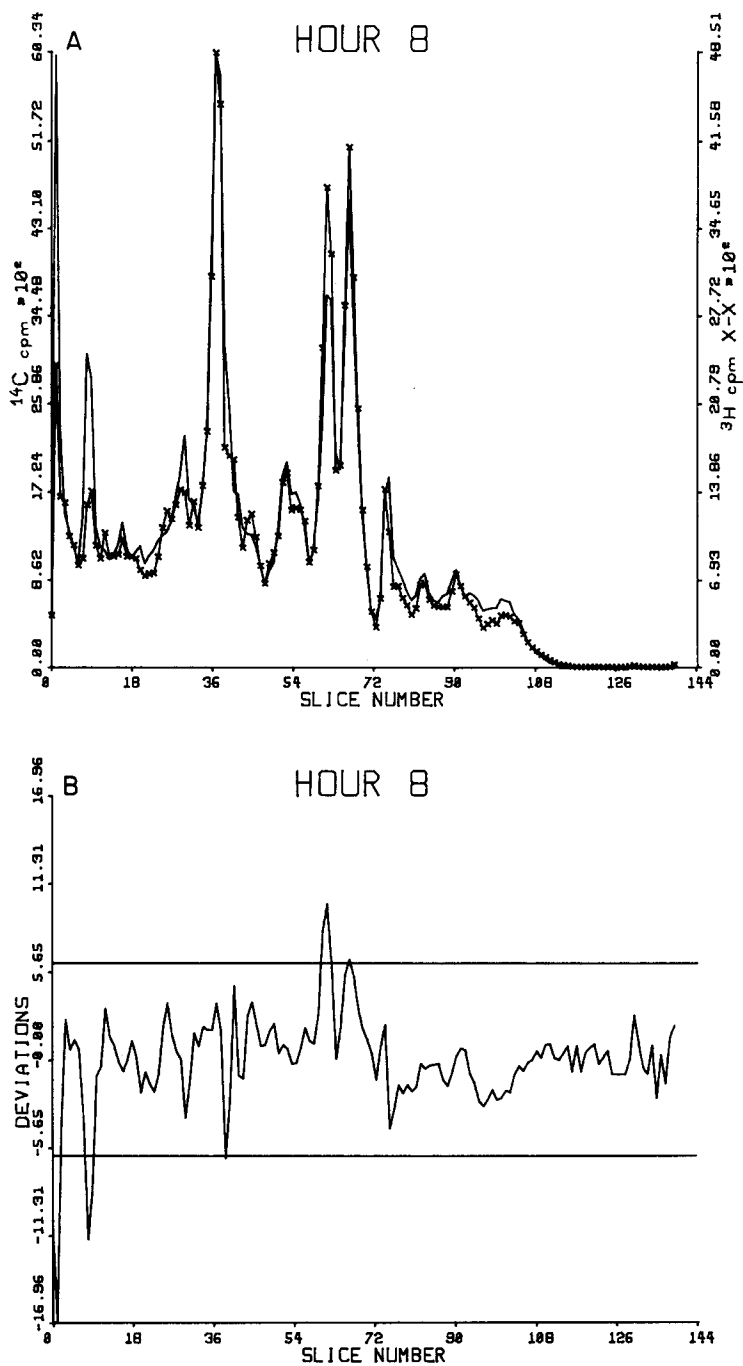
FIGURE 7   Membrane polypeptides pulse labeled between 7 and 8 h.

group II polypeptides after 7 h. The chlorophyll content of the culture remained constant for the first 5–6 h and then increased continuously for the remainder of the light period. Labeling of group IIb polypeptides (Fig. 9 B) occurred at a low but constant level from the beginning of the light period, although significant amounts of label were not incorporated until after 4 h of growth. The rate of synthesis of IIb increased continuously for the remainder of the light period.
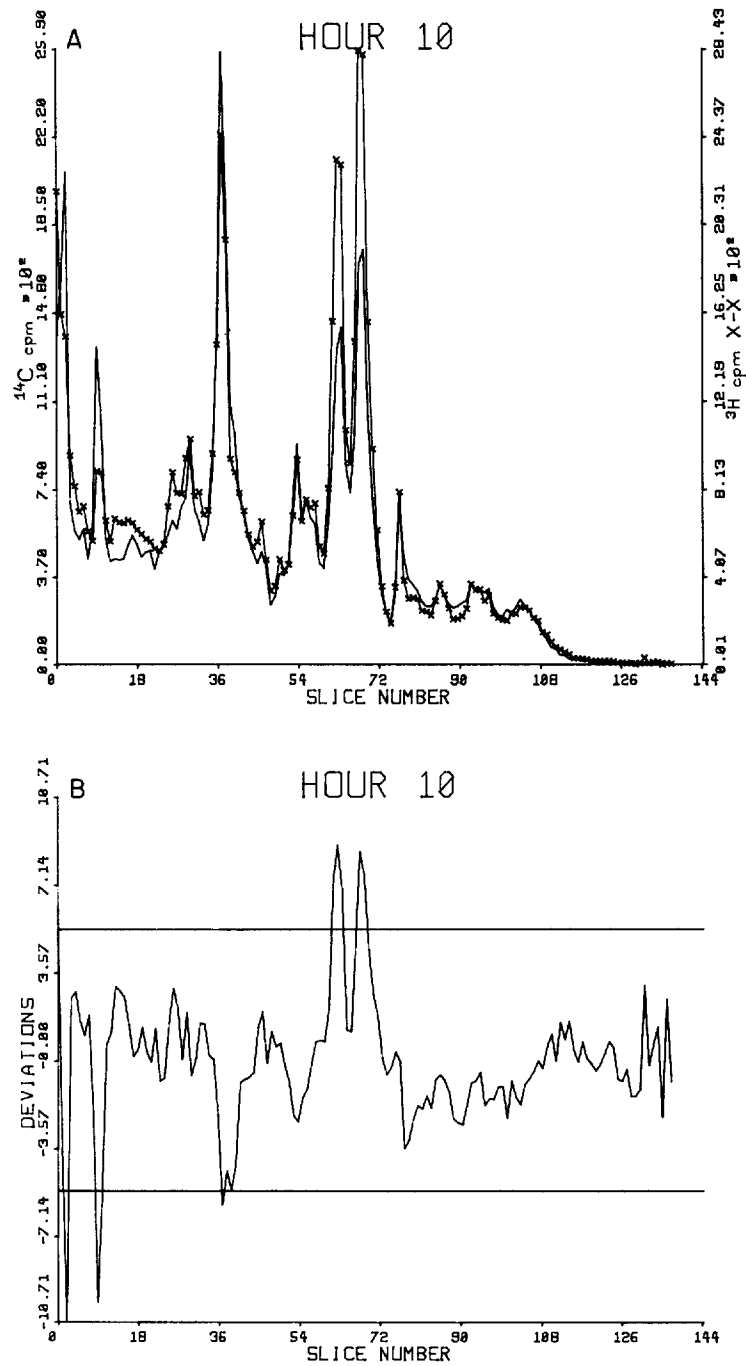
FIGURE 8   Membrane polypeptides pulse labeled between 9 and 10 h.

Synthesis of group IIc polypeptides proceeded at a rate even lower than that of IIb for the first 4 h; but in the 5th h its rate of labeling increased rapidly, reaching its maximal value between 10 and 12 h. The regression analyses indicate that the labeling of the group IIa polypeptides proceeded at the mean rate throughout the light period.

We have carried out two experiments concerning the fate of the newly synthesized mem-
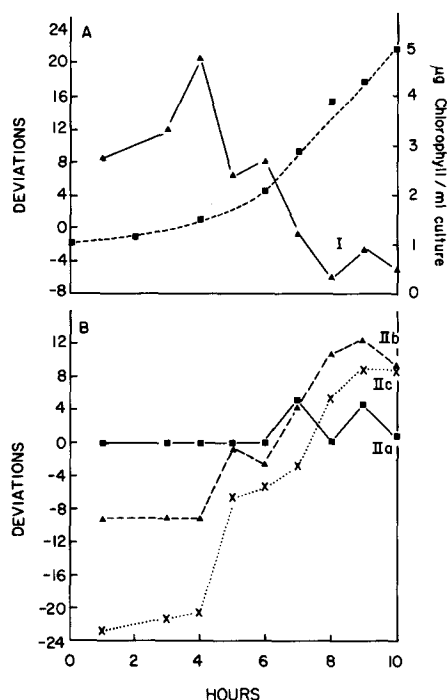
FIGURE 9 Summary of maximum deviations from least squares regression line for membrane polypeptides during synchrony. Deviations from several series of experiments are plotted together. Since the values of the deviations express the relationship between the pulse label and the background label after a 1-h pulse, they may be viewed as expressions of the relative rates of synthesis of the various membrane polypeptides. Part A shows group I polypeptides and the chlorophyll content of the culture. Part B shows the group II polypeptides.

brane polypeptides. In order to determine if a newly synthesized membrane polypeptide appears initially in a soluble form before integration into the membrane, we have examined the fraction of soluble proteins for the presence of the major membrane polypeptides. After sonic disruption of the cells, all membranous material was sedimented by centrifugation at 144,000 g for 2 h, and the protein was precipitated from the supernate with 10% trichloroacetic acid (TCA). At no time during the synchronous cycle were we able to detect any newly synthesized (labeled) group II polypeptides in a soluble form. Group I polypeptides run on these gels in the position of the large subunit of RuDP-carboxylase (mol wt 55,000), a readily solubilized protein. The presence of the carboxylase subunit material effectively obscured the group

I position in the soluble fraction, and we could thus gain no information about group I in this experiment. In a second experiment, we followed the fate of newly synthesized and incorporated membrane polypeptides by carrying out pulse-chase experiments throughout the synchronous cycle. Cells were labeled for 1 h and then washed and reincubated in the light. Once labeled and incorporated into the membrane, the polypeptides appeared to be highly stable. They retained for at least several hours the same total number of counts that they had at the end of the pulse period.

DISCUSSION

We conclude from the results described above that the assembly of the chloroplast membranes proceeds asynchronously. Not only does the synthesis of the various polypeptides begin at different times, but the rates of their synthesis change in different ways. Group I polypeptides are made early in the light period, while groups IIb and IIc are made at maximal rates somewhat later. Furthermore, although the increase in the rate of synthesis of IIc polypeptides begins at the same time as that of IIb, it proceeds more rapidly between hours 4 and 10 than the increase in synthesis of IIb.

The polypeptides of Groups I and II are observed in a membrane fraction from sonically disrupted cells, and we believe that they are integral membrane components. We have considered the possibility that Group I represents solely the large subunit of RuDP-carboxylase, which has a mol wt of 55,000 (11). However, carboxylase has been shown to be removed from the chloroplast membranes of spinach by washing with water (35) and is routinely prepared from homogenates obtained with the aid of a French pressure cell without further extraction of the membranes (11, 36). Furthermore, we have performed several experiments which bear on the question of the nature of the Group I polypeptides. (a) We have carried out an immunological assay for carboxylase protein in our membrane preparations. Under the same conditions in which an antibody to RuDP-carboxylase from spinach[2] will form a precipitate with the supernate from the membrane preparation and with an aliquot of membrane polypeptides to which has been added purified carboxyl-

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[2] The antibody to spinich RuDP-carboxylase was obtained from Dr. L. Bogorad.

ase, no precipitate will form with a sample of membrane polypeptides alone. (b) RuDP-carboxylase activity has been assayed in the several fractions obtained in a membrane preparation. We have found that 93% of the enzyme activity recovered is in the supernate, while only 7% is in the membrane fraction. (c) Very little material appears on our gels (Figs. 4–8) in the region where the small subunit of the carboxylase would be expected to appear. (d) The polypeptides of Group I are observed in the membrane fraction even after several washes with 3 mM EDTA.

In addition, gel electrophoresis of membrane preparations of C. reinhardtii which have not been extracted with 90% acetone show that chlorophyll runs coincidently with several polypeptide bands, one of which is at a high molecular weight position (21). This band has been excised, extracted, and rerun; the polypeptides of this band now appear in the mol wt range of 55,000, in coincidence with the group I polypeptides of acetone-extracted preparations. Finally Hoober[3] has found that washing membranes with 8 M urea containing 1 M KCl will remove group I polypeptides.

For the above reasons, we believe that the material in the group I region is closely associated with the chloroplast membranes, and that the loss of RuDP-carboxylase activity and small subunits from the membranes indicates that the carboxylase itself is lost from the membranes during their isolation. It may be suggested that during isolation the carboxylase dissociates and that a large amount of the large subunit alone remains tightly bound to the membranes; however, this does not appear to be a likely explanation since this polypeptide peak is not removed by washing with 3 mM EDTA. We conclude, then, that the isotopic label observed in the membranes is a measure of both the synthesis and integration of the polypeptides into the membranes. Therefore, these measurements of the labeling of membrane polypeptides show that the concentration of major membrane components can be varied in a completely functional membrane.

Similar variations in the times and rates of synthesis have been observed for several membrane lipids in synchronously grown Chlamydomonas (37). Although there are coincident periods of synthesis of the various membrane polypeptide

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[3] Hoober, J. K. Personal communication.

and lipids as described in this study (37), the data do not yet allow conclusions to be drawn about obligatory associations between lipids and proteins before or during integration into the membrane. However, data obtained in our laboratory (21) have demonstrated that, during electrophoresis of unextracted (chlorophyll-containing) membrane polypeptides, chlorophyll runs coincidently with groups IIb and IIc and that chlorophyll is also associated with high molecular weight polypeptides which run at the group I position after extraction with 90% acetone. We conclude from these observations that a specific association exists in the membrane between certain of the major membrane polypeptides and at least one of the membrane lipids, chlorophyll. Furthermore experiments on membrane lipid-protein associations are being carried out.

The association of group I polypeptides with photosystem I activity and group II polypeptides with photosystem II activity (18), and the association of both major polypeptide groups with chlorophyll (21) suggests that a sequential, stepwise assembly of the photosynthetic apparatus may occur during membrane biogenesis. Group I, which may be related to the photosystem I chlorophyll-protein complex and to the photosystem I photochemical reaction center (21, 38), may be inserted into the membrane structure before chlorophyll and before the components of photosystem II in C. reinhardtii. It has also been reported (39) that photosystem I units are synthesized and inserted into the membrane ahead of photosystem II units during greening of etiolated Phasoleus leaves. A possible function of such an arrangement is the synthesis of ATP via cyclic photosynthetic phosphorylation sensitized by photosystem I sites before the development of the entire photosynthetic apparatus is complete.

We have carried out several experiments to determine the fate of membrane polypeptides subsequent to their synthesis. In experiments designed to determine if any of the major chloroplast membrane polypeptides appeared first in a soluble form before their integration into the membrane, we examined the electrophoretic profile of the nonmembranous polypeptides and found no detectable group II polypeptides in the soluble fraction. This result suggests that, although it is likely that IIb and IIc exist in a nonmembranous form since they are synthesized in the cytoplasm (11, 17), the lamellar polypeptides must be integrated

into the membrane at high rates and that their nonmembranous existence must be extremely brief. Once incorporated into the membrane, however, the new polypeptides are very stable.

Group IIa polypeptides are clearly different from the other membrane polypeptides. Their synthesis is only partially inhibited by chloramphenicol and cycloheximide (17), they do not appear to be associated with chlorophyll (21), and, unlike I, IIb, and IIc, their rate of labeling is relatively constant during the light period. These data indicate that, while IIa is an integral membrane component, its role in the membrane may be substantially different from the other membrane polypeptides.

Finally, since these studies have employed synchronously grown cells of wild type *C. reinhardtii*, caution should be used in comparing directly the results obtained with greening of the *y-1* mutant of *C. reinhardtii*. For example, since the wild type cells are capable of synthesizing chlorophyll in the dark (40), the same controls over chlorophyll and membrane polypeptide synthesis may not be apparent in the two strains. Furthermore, at the beginning of the greening process, *y-1* cells contain only few membranes, and the composition of these membranes is considerably different from that of the normal green membranes (8, 41, 42). Therefore, the rates of synthesis of new components may reflect the demand for bringing the markedly altered composition in line with that required for a fully functional membrane. Thus, these rates may not indicate solely the synthesis required for growth of functional chloroplast lamellae. Greening of *y-1* cells does, however, proceed through continuously changing membrane compositions (42), suggesting that membrane assembly occurs in these cells by a sequential or multistep process. Such a conclusion is consistent with the findings of Schor et al. (9) and with these and other results (37) from our laboratory.

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