PERIODIC VARIATIONS IN THE RATIO OF FREE TO THYLAKOID-BOUND CHLOROPLAST RIBOSOMES DURING THE CELL CYCLE OF *CHLAMYDOMONAS REINHARDTII*

NAM-HAI CHUA, G. BLOBEL, P. SIEKEVITZ, and G. E. PALADE

From The Rockefeller University, New York 10021. Dr. Palade's present address is the Section on Cell Biology, Yale University Medical School, New Haven, Connecticut 06510.

ABSTRACT

The ratio of free to thylakoid-bound chloroplast ribosomes in *Chlamydomonas reinhardtii* undergoes periodic changes during the synchronous light-dark cycle. In the light, when there is an increase in the chlorophyll content and synthesis of thylakoid membrane proteins, about 20–30% of the chloroplast ribosomes are bound to the thylakoid membranes. On the other hand, only a few or no bound ribosomes are present in the dark when there is no increase in the chlorophyll content.

The ribosome-membrane interaction depends not only on the developmental stage of the cell but also on light. Thus, bound ribosomes were converted to the free variety after cultures at 4 h in the light had been transferred to the dark for 10 min. Conversely, a larger number of chloroplast ribosomes became attached to the membranes after cultures at 4 h in the dark had been illuminated for 10 min.

Under normal conditions, when there was slow cooling of the cultures during cell harvesting, chloroplast polysomal runoff occurred in vivo leading to low levels of thylakoid-bound ribosomes. This polysomal runoff could be arrested by either rapid cooling of the cells or the addition of chloramphenicol or erythromycin. Each of these treatments prevented polypeptide chain elongation on chloroplast ribosomes and thus allowed the polysomes to remain bound to the thylakoids. Addition of lincomycin, an inhibitor of chain initiation on 70S ribosomes, inhibited the assembly of polysome-thylakoid membrane complex in the light.

These results support a model in which initiation of mRNA translation begins in the chloroplast stroma, and the polysome subsequently becomes attached to the thylakoid membrane. Upon natural chain termination, the chloroplast ribosomes are released from the membrane into the stroma.

Synchronized cells of *Chlamydomonas reinhardtii* (*C. reinhardtii*) which have been treated with chloramphenicol (CAP) at the 4th h of the light period contain two distinct populations of chloroplast ribosomes. Approx. 70% of the chloroplast ribosomes occur free in the chloroplast stroma whereas the remainder are associated with the thylakoid membranes (15). Electron microscope examinations of the latter showed that the bound ribosomes are arranged predominantly as penta- or hexamers on the unstacked regions of the thylakoids. Similar thylakoid-bound ribosomes have also been detected in nonsynchronous cultures of the same organism (28).

Experiments designed to probe the nature of the ribosome-membrane interaction showed that approx. 50% of the bound ribosomes could be removed by a high salt (500 mM KCl) treatment whereas the remaining 50% were released only by a combination of high salt plus puromycin (15, 28). Therefore, in at least half of the cases the nascent chains on the ribosomes were so tightly bound to the membranes that the bound ribosomes resisted removal from the membrane by treatment with high salt alone. These results, together with the observation that thylakoid-bound ribosomes were detected at a time of membrane protein synthesis, strongly suggest that these ribosomes may be involved in the synthesis of thylakoid membrane polypeptides (15, 28). Recently, it has been shown that the nascent chains of thylakoid-bound ribosomes are firmly bound to the membrane after artificial chain termination induced by puromycin treatment (29). Furthermore, thylakoid-bound ribosomes prepared from nonsynchronous cultures of *C. reinhardtii* could incorporate amino acid into proteins in an in vitro system, and the major product, which remains associated with the membranes, has a mol wt of 23,000 in sodium dodecyl sulfate (SDS) gels (30). These recent results are consistent with the suggested role of bound ribosomes mentioned above.

In order to examine the relationship between thylakoid-bound ribosomes and thylakoid membrane polypeptide synthesis in *C. reinhardtii*, we have extended our previous observations (15) and measured the proportion of free and bound 70S ribosomes throughout the entire synchronous cell cycle. We found that maximal amounts of thylakoid-bound ribosomes were detected in the light period when there is synthesis of both chlorophyll and membrane proteins. The effects of light, lincomycin, and rapid cooling of the cells on the proportion of free and bound ribosomes in vivo were also investigated. The results of these experiments will be discussed in relation to a model proposed for the assembly of the chloroplast ribosome-thylakoid membrane complex in vivo.

MATERIALS AND METHODS

Conditions for the Synchronous Cultures of C. reinhardtii

Cells of the wild type strain (137 c, mating type plus) were grown according to Ohad et al. (32), in the minimal medium of Sager and Granick (36) modified by the addition of 10 mg citric acid/liter. Synchronous cultures were obtained by exposing the cells to repeated regimens of 12 h light and dark cycles at 25°C (5). The cultures were continuously bubbled with a moistened gas mixture containing 5% CO_2–95% air. The light intensity at the level of the culture flasks was approx. 4,000 lx. Under these conditions, synchronously dividing populations were obtained after three light-dark cycles. The changes in chlorophyll content and cell number during the synchronous cell cycle were similar to those reported by other workers (2, 26, 38).

Cell Fractionation Procedures

Procedures of cell fractionation were carried out essentially as described previously (15). Experiments were done with cultures which had been exposed to at least three light-dark cycles. The cell number was determined, and the cultures were diluted accordingly 1 day before the experiments were carried out so that the cell density at the time of the experiments was about 1×10^6 cells/ml. Each 2-liter culture was divided into two equal portions: one sample received 1 ml of a CAP stock solution (100 mg/ml ethanol; final concentration 100 μ/ml culture) whereas the control sample received 1 ml of ethanol (final concentration 0.1%). Both samples were further incubated for 10 min before the cells were harvested by centrifugation at 0°C in a Sorvall GSA rotor (DuPont Instruments, Sorvall Operations, Newtown, Conn.) at 2,500 *g* for 5 min. The pelleted cells were washed once in 100 ml of TKMD buffer, which contained 25 mM Tris-HCl (pH 7.5), 25 mM KCl, 10 mM $MgCl_2$, and 5 mM dithiothreitol (DTT), and resuspended in 10 ml of the same buffer to a cell concentration of about 1×10^8 cells/ml. The cell suspension was passed through a chilled French pressure cell (American Instrument Co., Travenol Laboratories, Inc., Silver Spring, Md.) maintained at a constant pressure of 4,800 pounds/inch^2. The efficiency of cell breakage approached 100% as monitored by both light and electron microscopy. All subsequent operations were carried out at 0–4°C.

The homogenate, contained in a 50-ml polypropylene tube (29 × 103 mm, DuPont Instruments, Sorvall Operations), was centrifuged at 17,000 g_{max} for 10 min in a Sorvall SS-34 rotor, and the supernate was saved. For maximal recovery, 5 ml of TKMD buffer was added, and the pellet was dispersed by homogenization directly in the polypropylene tube with a serrated Teflon pestle (diam 1.0 inch; Arthur H. Thomas Co., Philadelphia, Pa.) driven by a top-drive motor. The resulting suspension was centrifuged again at 17,000 g_{max} for 10 min. The supernates from this and the preceding centrifugation were pooled and are referred to hereafter as S17. The volume of S17 was measured and adjusted with TKMD buffer to 15 ml.

For most of the experiments reported in this paper, the 17,000 g_{max} pellet (P17) was resuspended by homogenization in the 50-ml polypropylene tube in about 3–5

ml of TKMD buffer as described above. The volume was measured with a graduated cylinder, and the suspension was analyzed for its ribosomal subunits composition by the high salt puromycin reaction described below.

For other experiments, a fraction consisting primarily of chloroplast thylakoid membranes was purified from P17 by flotation from a heavy sucrose layer (15). To this intent, P17 was resuspended by homogenization in 1.87 M sucrose in TKMD buffer to a chlorophyll concentration of about 300 μg/ml. 4.5 ml of the suspension were overlaid with 5 ml of TKMD buffer and centrifuged in an A321 rotor (International Equipment Co., Needham Heights, Mass. [IEC]) at 320,000 g_{max} for 40 min. The thylakoid membrane band was collected from the sucrose-buffer interface and diluted with 3 vol of TKMD buffer. The membranes were collected by centrifugation at 17,000 g_{max} for 10 min, and the resulting pellet was finally resuspended in TKMD buffer to a chlorophyll concentration of about 1.0–1.2 mg/ml. This fraction will be referred to hereafter as the thylakoid membrane fraction (TMF). Approx. 70% of the total chlorophyll present in the homogenate was recovered in this fraction (Table I).

Analysis of Ribosomal Subunit Composition of the Various Cell Fractions

Total ribosomes associated with each cell fraction were analyzed in terms of their subunits by the high salt sucrose gradient centrifugation technique described earlier (14, 15). Both 70S and 80S ribosomes were first dissociated into their respective subunits by treatment with high salt and puromycin (14, 15), in a reaction mixture which contained 0.5 ml of S17 (9–12 A_{260}), or P17 (1.0–1.2 mg chlorophyll/ml), or TMF (1.0–1.2 mg chlorophyll/ml); 0.1 ml of 5 mM puromycin (adjusted to pH 7.0 with KOH), and 0.4 ml of compensating buffer. The composition of the compensating buffer was adjusted to give, in the final reaction mixture, 50 mM Tris-HCl (pH 7.5), 500 mM KCl, 5 mM $MgCl_2$ and 5 mM DTT. The $MgCl_2$ concentration was raised to 25 mM in samples without puromycin to prevent dissociation of active 80S ribosomes (our unpublished observation). The reaction mixtures were incubated at 37°C for 10 min, and aliquots were layered onto 5–20% linear sucrose gradients containing 50 mM Tris-HCl (pH 7.5), 500 mM KCl, 25 mM $MgCl_2$, and 5 mM DTT. The gradients were centrifuged at 39,000 rpm for 3 h at 18°C in an SB 283 rotor of an IEC centrifuge (B-60). The absorbance of each gradient was continuously monitored with an Instrument Specialties Co. model D gradient fractionator (ISCO, Lincoln, Neb.) and UV analyzer, and the absorbance profile was displayed with a 10-inch Bristol chart recorder (American Chain and Cable Co., Inc., Bristol Div., Waterbury, Conn.).

The S17 and P17 fractions were heterogeneous in their contents. In addition to ribosomes, S17 contained soluble proteins including ribulose-1,5-diphosphate carboxylase, tRNA's, and small membrane vesicles whereas P17 also contained thylakoid membranes, cell-wall materials, pyrenoids, starch granules, and broken and intact nuclei. These contaminants, however, did not interfere with the dissociation of ribosomes into their subunits or

TABLE I

Distribution and Recovery of Chlorophyll and RNA

	Control		Chloramphenicol	
	Chlorophyll	RNA	Chlorophyll	RNA
	%	%	%	%
(a) In S17 and P17 isolated from cultures at L-4				
Total homogenate	100	100	100	100
S17	7.1 ± 0.4	80.5 ± 3.5	10.6 ± 2.1	75.9 ± 1.3
P17	87.0 ± 1.0	16.0 ± 2.4	83.8 ± 2.3	21.6 ± 0.7
Recovery	94.1 ± 0.8	96.5 ± 1.2	94.5 ± 0.21	97.5 ± 1.7
(b) In fractions obtained from the floatation step				
P17	100	100		
TMF	81.0 ± 4.0	50.8 ± 4.2		
1.87 M sucrose layer	7.5 ± 3.0	24.1 ± 5.1		
320,000 g_{max} pellet	3.5 ± 0.8	15.5 ± 5.5		
Recovery	92.0 ± 2.1	90.3 ± 6.0		

Procedures for cell fractionation and measurements of chlorophyll and RNA are given in Materials and Methods. Values given in (*a*) are means from three experiments ±SD of the means. Experiments in (*b*) were carried out with P17 fractions obtained from the CAP-treated cultures at L-4. These values are also means from three experiments ±SD of the means.

with the subsequent separation of these subunits in the sucrose gradient. The soluble proteins and tRNA's sedimented in between the top of the gradient and the small subunit of 70*S* ribosomes (S^{70}) (Fig. 1*a*) whereas membrane vesicles, cell wall materials, pyrenoids, starch granules, and nuclei were all pelleted at the bottom of the tube.

Determinations of Chlorophyll and RNA

Chlorophyll was measured according to Arnon (3) and RNA according to a modification of the Schmidt-Thannhausser procedure (7).

Electron Microscopy

All specimens, cells in suspension or cell fractions, were fixed as pellets in a 0.25% glutaraldehyde solution (in 0.02 M cacodylate buffer, pH 7.0, and 10 mM $CaCl_2$) for 30 min at ~4°C. The pellets were postfixed in 1% OsO_4 (in the same buffer, $CaCl_2$ mixture) for 2-3 h at ~4°C. Sectors, cut to include the whole thickness of the pellets, were stained in block with uranyl acetate (20), before being dehydrated in graded ethanols and embedded in Epon. Sections through the entire depth of the pellets were cut with diamond knives (DuPont Instruments, Wilmington, Del.) on Porter-Blum MT2 microtomes (DuPont Instruments, Sorvall Operations), then stained with uranyl and lead salt solutions and finally examined in a Siemens 101 or 102 electron microscope operated at 80 kV. For each cell fraction, the entire depth of the pellet was systematically examined before taking micrographs from representative fields. The fixation procedure mentioned above was developed to prevent plasmolysis of intact cells and myelin figure formation at the expense of their membranes (9); it was applied to cell fractions to maintain uniformity of preparation procedures.

Chemicals and Solutions

Chemicals were obtained from the following sources: CAP, erythromycin, and DTT from Sigma Chemical Co., St. Louis, Mo.; Ultrapure grade sucrose (ribonuclease-free) from Schwarz/Mann Div., Becton, Dickinson and Co., Orangeburg, N.Y.; puromycin hydrochloride from Nutritional Biochemicals Div., International Chemical & Nuclear Corp., Cleveland, Ohio; and lincomycin hydrochloride from the Upjohn Co., Kalamazoo, Mich.

RESULTS

Cyclic Variations in the Proportion of Thylakoid-Bound 70S Ribosomes During the Synchronous Cell Cycle

We have previously reported (15) that treatment of synchronous cultures with CAP from the 4th to the 5th h in the light, denoted by L-4 to L-5, resulted in a shift of chloroplast ribosomes (70S) from the S17 to P17 which fraction was enriched in 70S ribosomes when compared to the control sample. Analysis of the TMF isolated from P17 by

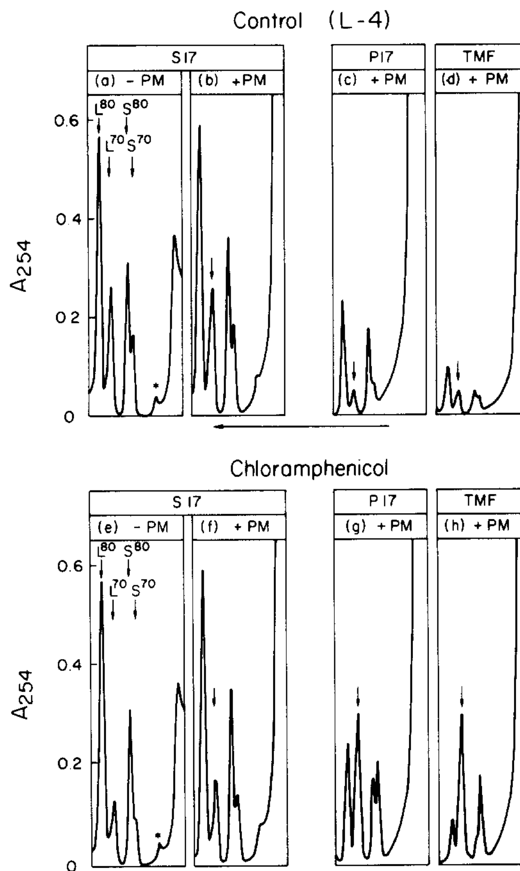


FIGURE 1 Sucrose gradient analysis of ribosomal subunits in S17, P17, and TMF isolated at L-4. The amounts of materials loaded on each gradient are as follows: (*a*) 2.48 A_{260} U; (*b*) 2.48 A_{260} U; (*c*) 250 μg chlorophyll; (*d*) 250 μg chlorophyll; (*e*) 2.48 A_{260} U; (*f*) 2.48 A_{260} U; (*g*) 250 μg chlorophyll; and (*h*) 250 μg chlorophyll. The S17 samples (*a, b, e,* and *f*) were derived from approx. 2.6 × 10^7 cells whereas the P17 samples (*c, d, g,* and *f*) were from approx. 12.1 × 10^7 cells. The direction of sedimentation is indicated by the horizontal arrows. In all cases, the thylakoid membranes were sedimented to the bottom. The small $A_{254\ nm}$ absorbance peak marked by an asterisk in (*a*) and (*e*) is due to ribulose-1,5-diphosphate carboxylase. The vertical arrows in (*b*), (*c*), (*d*), (*f*), (*g*), and (*h*) indicate the position of L^{70}. L^{80} and S^{80}, large and small subunits of 80S ribosomes, respectively; L^{70} and S^{70}, large and small subunits of 70S ribosomes, respectively; *PM*, puromycin.

a flotation procedure showed that it contained most of the 70S ribosomes in the latter fraction. Fig. 1 shows that essentially similar results could be obtained when the time of incubation with the antibiotic was reduced in 10 min. Under these conditions, the concentration of CAP (100 μg/ml) used was saturating as determined by experiments with varying concentrations of the drug (data not shown). The distribution and recovery of chlorophyll and RNA in the various cell fractions derived from the two main steps of the fractionation scheme are presented in Table I (*a*) and (*b*). It can be seen that CAP treatment led to a shift of only 5% of the total RNA from S17 to P17 (Table I [*a*]). Although the magnitude of this CAP effect was small, it was consistently observed in all three experiments (Table I).

In order to estimate quantitatively the effects of CAP treatment on the distribution of 70S ribosomes at L-4, it was assumed that the amounts of 70S ribosomes in S17 and P17 represent the amounts of free and thylakoid-bound ribosomes, respectively, in the homogenate. This assumption is valid only if the amount of cross-contamination between free and membrane-bound ribosomes in the two cell fractions was minimal and if there was no significant loss of ribosomes and thylakoid membranes during cell fractionation. Both these two conditions were met in our experiments. The results in Table I(*a*) show that the recovery of RNA and chlorophyll during cell fractionation of the total homogenate into S17 and P17 was better than 94%, indicating little loss of ribosomes and thylakoid membranes. The amount of 70S ribosomal subunits per unit chlorophyll, and therefore per unit thylakoid membrane, in P17 was approximately the same as that in purified TMF (cf. Fig. 1*c* and *d* and Fig. 1*g* and *h*). Thus, most of the 70S ribosomes that sedimented at 17,000 g_{max} were attached to the thylakoids, and only a few free ribosomes cosedimented with the membranes. Table I(*a*) also shows that more than 84% of the chlorophyll-containing material was recovered in P17 with 10% or less still remaining in S17. Electron microscope examination of the latter fraction revealed mostly free ribosomes and some small membrane vesicles with very few membrane-bound ribosomes. Thus, most of the chlorophyll in S17 could be accounted for by the presence of small thylakoid membrane vesicles that were not pelleted at 17,000 g_{max}.

In the case of 80S ribosomes, it is unlikely that significant amounts of free ribosomes would be sedimented at 17,000 g_{max} after extensive homogenization. However, we have no evidence that all the microsomes were pelleted, and it is possible that under our experimental conditions a certain amount of microsomes still remained in S17 thus leading to an underestimation of the proportion of membrane-bound 80S ribosomes.

Both the 70S and 80S ribosomes in S17 and P17 could be dissociated into their respective subunits with puromycin at high ionic strength (14, 15). Under these conditions the dissociation is 85%–90% complete for both types of ribosomes (14, 15). Using the centrifugation techniques described in Materials and Methods, the dissociated subunits could be displayed on a 5–20% sucrose gradient without interference from any contaminating UV-absorbing materials that were present in S17 or P17. Since the large subunits of the 70S and 80S ribosomes (L^{70} and L^{80}, respectively) were well separated in the gradient (cf. Fig. 1), their relative absorbance was used for quantitative estimation.

Quantitative data relating to the experiments in Fig. 1 are summarized in Table II. At L-4, the P17 of the control sample had 8.6% of 80S ribosomes

TABLE II

Effect of CAP on the Percent of Total 80S and 70S Ribosomes Associated with the P17 Fraction

Experiment	Percent of ribosomes in P17	
	80S	70S
Control (L-4)	8.6 ± 1.5	6.6 ± 1.3
+ CAP (L-4)	8.1 ± 1.4	27.0 ± 3.3
Control (D-4)	11.7 ± 0.8	4.7 ± 2.1
+ CAP (D-4)	11.0 ± 1.5	4.2 ± 0.8

To estimate the relative amounts of 70S ribosomes in S17 and P17, photocopies of the ribosomal subunit profiles of both cell fractions, e.g., Fig. 1*b*, *c*, *f*, and *g*, were made. The L^{70} peak of each profile was cut out and weighed, and the weight multiplied by the appropriate dilution factor in the high-salt puromycin reaction (cf. Materials and Methods) as well as by the volume of the corresponding cell fraction should give the amount of 70S ribosomes in arbitrary units in that fraction. By assuming a 100% recovery for 70S ribosomes during the fractionation into S17 and P17 the proportion of total 70S ribosomes that are bound to the thylakoid membranes, i.e., in P17, could be computed. Similar procedures were followed for the computation of the proportion of total 80S ribosomes in P17. In the later case, it is assumed that the fraction of 80S ribosomes that sedimented a 17,000 g_{max} are all membrane-bound. Values are means of three (D-4) or four (L-4) experiments ±SD of the means.

and 6.6% of 70S ribosomes associated with it. Addition of CAP did not affect the distribution of 80S ribosomes in S17 and P17 but increased the proportion of thylakoid-bound 70S ribosomes from ~7% to 27% of the total. This effect of CAP has been noted before and was attributed to its ability to block chloroplast polysomal runoff which occurred during cell harvesting (15). If this is the case, then the distribution of free and thylakoid-bound 70S ribosomes in the CAP-treated sample should reflect the situation in vivo at L-4. The data in Table II also show that CAP treatment led to an increase of 20% of the total 70S ribosomes in P17 as compared to the control sample. If it is assumed that ribosomal RNA accounts for about 90% of the total cell RNA's in *C. reinhardtii* and since the ratio of 80S to 70S ribosomes is about 2 (10, 14, 24), a shift of 20% of the total 70S ribosomes would correspond to $(90 \times 0.33 \times 0.2)\% = 6\%$ of the total cell RNA. This figure agrees fairly well with the RNA data in Table I (*a*). In contrast to the results obtained at L-4, only ~5% of the total 70S ribosomes were associated with P17 at 4 h after the beginning of the dark period (D-4), and this value was the same for both the control and the CAP-treated cultures (Fig. 2 and Table II).

We have extended our observations at L-4 and D-4 and investigated the variations in the proportion of bound ribosomes throughout the entire light-dark cycle. The results of these experiments are presented in Fig. 3*a*. These data were obtained with cultures that had been treated with CAP for 10 min as described under Materials and Methods. Similar data for the membrane-bound 80S ribosomes are also given for comparison (Fig. 3*b*). It can be seen that thylakoid-bound 70S ribosomes were detected during the entire light period, and this population of ribosomes amounted to 20–30% of the total 70S. Cattolico et al. (12) have recently published data on the cyclic variations of total chloroplast ribosomes in the same strain of *C. reinhardtii* grown under conditions almost identical to ours. If we use their data to calculate the amount of thylakoid-bound chloroplast ribosomes as a function of time in the cell cycle, we find that this amount varies cyclically from a low of 40 relative units at D-4 to a high of 390 relative units at L-7 (Table III). These values should be considered minimal since in the work of Cattolico et al. (12) the increase in 70S ribosomes was smaller than three times over one cycle, and since we do not know whether or not we are obtaining complete inhibition of translation by

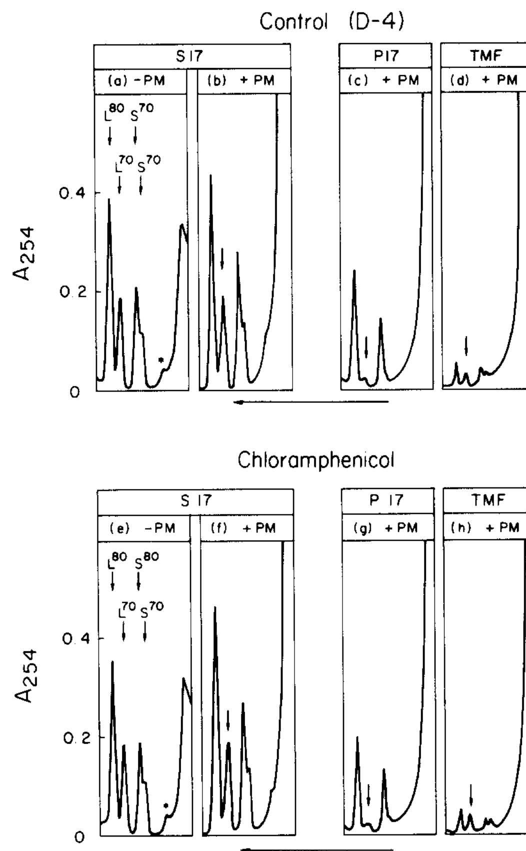


FIGURE 2 Sucrose gradient analysis of ribosomal subunits in S17, P17, and TMF isolated at D-4. The amounts of materials loaded on each gradient are as follows: (*a*) 2.12 A_{260} U; (*b*) 2.12 A_{260} U; (*c*) 250 μg chlorophyll; (*d*) 250 μg chlorophyll; (*e*) 2.12 A_{260} U; (*f*) 2.12 A_{260} U; (*g*) 250 μg chlorophyll; and (*h*) 250 μg chlorophyll. The S17 samples (*a, b, e,* and *f*) were derived from approx. 3.4×10^7 cells whereas the P17 samples (*c, d, g,* and *h*) were from approx 12.5×10^7 cells. For other experimental details, see Fig. 1 and Materials and Methods.

CAP in our system. Table III also shows that the amount of thylakoid-bound ribosomes is highest from L-4 to L-11 during which time there is rapid synthesis of chlorophyll and also membrane polypeptides (4, 9, 38). In contrast to the values obtained in the light, the amount of 70S ribosomes attached to the thylakoids decreased to 4–5% of the total during the dark phase of the cell cycle when there is no change in the chlorophyll concentration. The significance of this low level of remaining bound 70S ribosomes in the dark is not known since this value was not increased by the addition of CAP in all the samples investigated. It

is possible that these ribosomes represent a small fraction of the free 70S which remained entrapped by the thylakoid membranes even after extensive homogenization and subsequent flotation. The transition from dark to light in the synchronous cycle was striking: 70S ribosomes became membrane-bound immediately upon entering the light phase. This result implies that one or more light-dependent factors were involved in the attachment of ribosomes to the membranes. In the case of membrane-bound 80S ribosomes (Fig. 3*b*), no pronounced variations during the light-dark cycle were observed except that the value at D-0 is higher than at the other time-points.

Electron Microscope Observations

Thylakoid membrane fractions prepared from synchronized cultures at selected time-points (L-4, L-7, and D-4) during the cell cycle were examined in a series of successive experiments (five for L-4, two for L-7, and three for D-4). In each experiment, the effect of CAP treatment in vivo and the effects of KCl-puromycin treatment of isolated fractions were systematically studied. Less extensively investigated were chloroplast fragments in P17. A preliminary survey of intact cells after CAP treatment in vivo was also carried out.

Thylakoid membrane fractions isolated from CAP (100 μg/ml for 10 min)-treated cells during the light phase of the cell cycle (L-4 and L-7) consist almost exclusively of thylakoids, many of them swollen and deformed, but most of them still stacked into small, two to three disc grana. Chloroplast envelope fragments, osmiophilic droplets and pyrenoid tubules are rare, and cytoplasmic contaminants (mitochondria, ER-fragments) are only occasionally encountered.

In fractions prepared at both L-4 and L-7, free thylakoids and thylakoids at the end of grana have ribosomal clusters attached to the outer aspects of the exposed (not stacked) domains of their membranes. In normal sections these clusters appear as apparently linear series of two to four particles (Figs. 4–6), but grazing sections reveal that these series correspond to circular polysomes which range in size from pentamers to octamers, hexamers being the predominant form (Figs. 4–6). Open circles of the same sizes (Figs. 4–6) are less frequently found, and extreme forms, e.g., tetramers and clusters larger and more complex than octamers, are only occasionally encountered. The gallery in Fig. 7 illustrates the spectrum of charac-

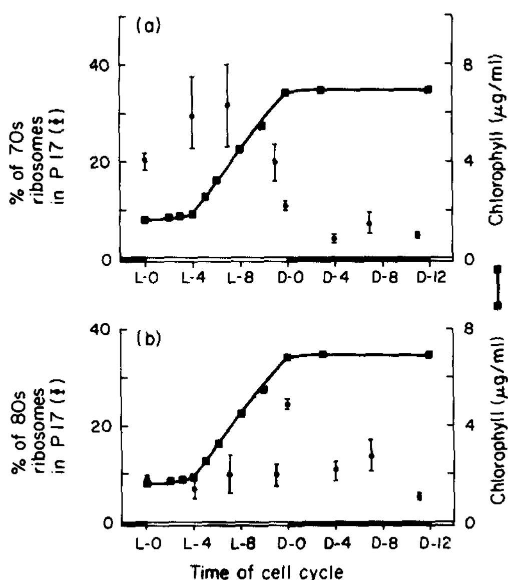


FIGURE 3 Cyclic variations in the proportions of 70S and 80S ribosomes associated with P17 during the synchronous cell cycle. The proportions of 70S and 80S ribosomes were determined as described under Materials and Methods and legend to Table II. The number of experiments carried out for each time-point is shown within parentheses in the following: L-0 (2), L-4 (7), L-7 (3), L-11 (2), D-0 (2), D-4 (4), D-7 (3), and D-11 (2). The average values are represented by the closed circles, whereas the vertical bars indicate the range of variation. The changes in chlorophyll concentration (microgram/milligram culture) during the synchronous cell cycle are represented by the continuous line. Under these conditions, the cell number increased by about four times in the dark. *L* and *D* denote the light and dark period of the synchronous cell cycle, respectively. The number after *L* or *D* refers to the time in hours.

teristic appearances shown by these attached polysomes when seen in full face view.

After treatment of the thylakoid membrane fractions with 25 mM KCl only (15), the polysomal forms described are seen against a variable background of ribosomes scattered singly or in groups 2–4 particles, which may represent the actual distribution *in situ* or may be the result of microtomy artefacts. The polysomes themselves are rather frequent but unevenly distributed: on some exposed thylakoid surfaces, they appear spaced at ~100 nm (center to center); on others, the spacing is five to seven times larger. The background of scattered ribosomes is considerably reduced after treatment of the fractions with 500 mM KCl (15), but in the same time the frequency of attached polysomes is noticeably decreased.

TABLE III

Changes in the Relative Amounts of Thylakoid-Bound Ribosomes per Unit Culture during the Synchronous Light-Dark Cycle

Time of cycle	Amount of total 70S ribosomes per unit culture (relative values)	% of 70S ribosomes bound to thylakoids (mean values)	Amount of bound 70S ribosomes per unit culture (relative values)
L-0	380	20	76
L-4	760	29	220
L-7	900	32	288
L-11	1100	20	220
L-12 (D-0)	1100	11	121
D-4	1000	4	40
D-7	900	7	63
D-11	1000	5	50

Values for the relative amounts of total 70S ribosomes per unit culture were obtained from Fig. 4 of reference 12. The values for the percent of total 70S ribosomes attached to the thylakoid membranes were taken from the mean values presented in Fig. 3.

A more careful examination of the polysomes shows that the extent and type of their attachment to thylakoid membranes is variable. In normal sections, partial detachment is found at the ends of the linear series, as well as in their middle, and attachment limited to a single ribosome, out of a series of three or four is occasionally recognized; in grazing sections, some circular polysomes appear close to, rather than on top of, thylakoid membranes. The gallery in Fig. 8 illustrates the spectrum of appearances interpretable as partial detachment of polysomes. This kind of relationship suggests that polysome assembly precedes attachment to thylakoid membranes.

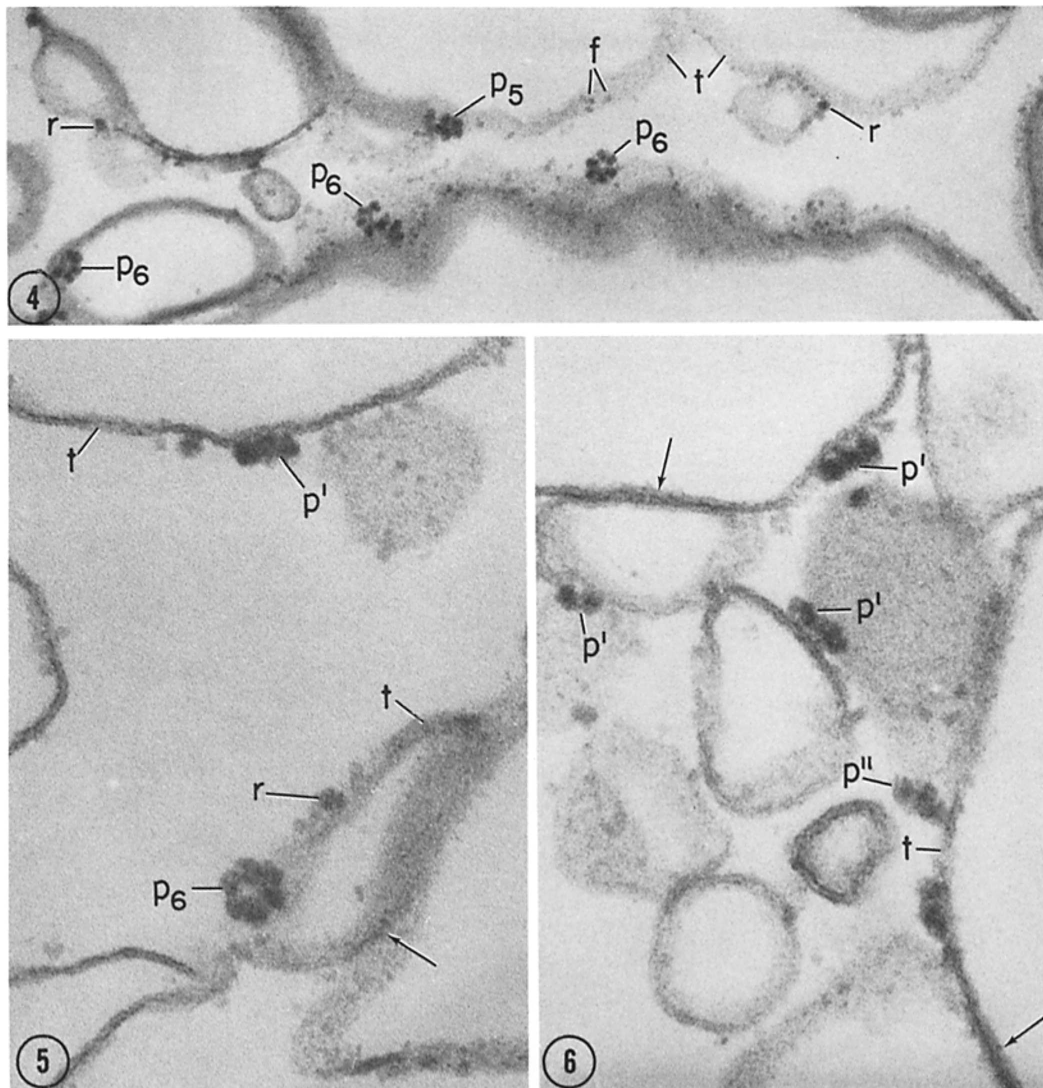
Combined treatment with 500 mM KCl and 1 mM puromycin leads to a quasi-complete removal of all ribosomes from thylakoid membranes: polymers are no longer found, and individually scattered ribosomes are drastically reduced in frequency (Fig. 9). Treatment with 1 mM puromycin at low salt concentration (25 mM KCl) does not remove either the polysomes or the scattered ribosomes.

Thylakoid membrane fractions isolated from the corresponding controls (synchronized cultures (L-4) not treated with CAP) have extremely few attached ribosomes and polysomes (Fig. 10). The polysomes found, however, have the size-range and morphology already described in the case of thylakoid membrane fractions derived from CAP-treated cells. The appearance of control fractions is not changed by subsequent treatments in vitro, e.g. low salt ± puromycin, high salt) except that practically all residual ribosomes are removed by exposure to 500 mM KCl in the presence of 1 mM puromycin. Thylakoid membranes in fractions isolated at D-4 are free of attached polysomes, and ribosomes attached individually or in small groups are rarely found, irrespective of the treatment of the cells in vivo with CAP.

Relatively large chloroplast fragments that contain less disturbed sets of grana and that are still partially surrounded by remnants of the chloroplast envelope were examined in P17 isolated at L-4 from synchronized cells. After CAP treatment in vivo, the frequency of apparently attached polysomes is considerably higher in such fragments than in thylakoid membrane fractions, but their size range, their morphology, and their relationship to the membranes (including partial detachment) are the same (Figs. 11 and 12). These findings suggest that at L-4 in the cycle the proportion of attached polysomes in intact chloroplast is considerably higher than the estimate based on data obtained on isolated thylakoid membrane fractions. This is a reasonable assumption, since CAP inhibition, already known to be incomplete in vivo, may be further reduced by the dilution of the inhibitor during cell homogenization and fractionation. Finally, the same type of circular, predominantly closed polymers were found apparently attached on thylakoid membranes in the chloroplasts of intact cells fixed after 10 min of CAP treatment in vivo (Fig. 13).

Effects of Darkness and Light on the Recovery of Thylakoid-Bound 70S Ribosomes at L-4 and D-4, Respectively

Fig. 3*a* shows that there is only limited polysome-thylakoid membrane association throughout the dark phase of the cell cycle. This low level of thylakoid-bound ribosomes may be attributed to the absence of light since the cells are grown under phototrophic conditions and therefore require light to synthesize ATP and certain amino acids for protein synthesis within the chloroplast. If this explanation were correct, then it should be possible to reduce the amount of thylakoid-bound 70S ribosomes at L-4 by darkness and to increase the amount of thylakoid-bound 70S ribosomes at D-4 by light. Table IV shows that the amount of bound ribosomes at L-4 and D-4 was indeed dependent on light. The proportion of total 70S ribosomes bound to the thylakoid membranes decreased



FIGURES 4–6 TMF's prepared from synchronized cells treated at L-4 with 100 μg/ml CAP for 10 min before cell disruption. Fraction treatment: Fig. 4, 25 mM KCl, no puromycin; Figs. 5 and 6, 500 mM KCl, no puromycin. In all figures, polysomes (p) are seen on the free surface of terminal thylakoids (t). On grazing sections they appear as circular (closed or open) penta- or hexamers (p_5, p_6); on normal sections they are seen in side view (p') as linear series of two to three ribosomes. Note the partial detachment of the polysome marked p''. Coupling factor particles (f) (33) and single ribosomes (r) are less frequent after high-salt treatment (Figs. 5 and 6 as compared to Fig. 4). Arrows mark areas of persisting thylakoid membrane fusion (incompletely dismantled grana). Fig. 4, × 72,000; Figs. 5 and 6, × 150,000.

from 28% to 5% after cultures at L-4 had been transferred to darkness for only 10 min. These results show that the time taken for the polysomes to run off from the membrane is 10 min or less. On the other hand, when cultures at D-4 exposed to light for 10 min, an additional 10% of the chloroplast ribosomes became membrane-bound (Table IV). However, since this value is still lower than those obtained during the light phase and since the proportion of 70S ribosomes bound to the thylakoid membranes varied over twofold during the light (Fig. 3a), factors other than light

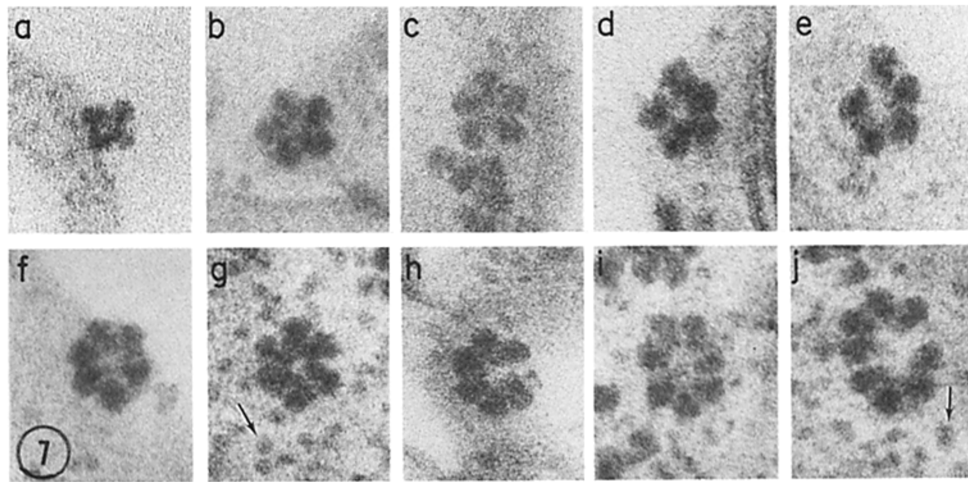


Figure 7 Gallery of chloroplastic polysomes seen in full face view in grazing sections through terminal thylakoids. All micrographs come from either TMF (*a–f* and *h*) or P_{17} (*g, i,* and *j*) preparations obtained from synchronized cells treated with CAP (100 μg/ml) at L-4 for 10 min before cell disruption. A tetramer appears in *a*, closed pentamers in *b* and *c*, open pentamers in *d* and *e*, closed hexamers in *f* and *g*, and open hexamer in *h*, a closed heptamer in *i*, and an open octamer in *j*. Coupling factor particles (33) are indicated by arrows. (*a*) × 150,000; (*b–j*) × 200,000.

must also be important in controlling the assembly of polysome-membrane complex. Such factors may include specific mRNA's which are present only during a restricted period of the cell cycle.

Effects of Rapid Cooling and Other 70S-Specific Antibiotics on the Recovery of Membrane-Bound Ribosomes at L-4

We have previously suggested that the low level of thylakoid-bound ribosomes in the control sample at L-4 (Fig. 1*c* and *d*) is due to the slow cooling of the culture during harvesting (14, 15). It has been shown in both *Escherichia coli* (21) and chick embryo (31) that slow cooling of cells preferentially blocks polypeptide chain initiation but allows chain elongation to continue and chain termination to occur, leading to an accumulation

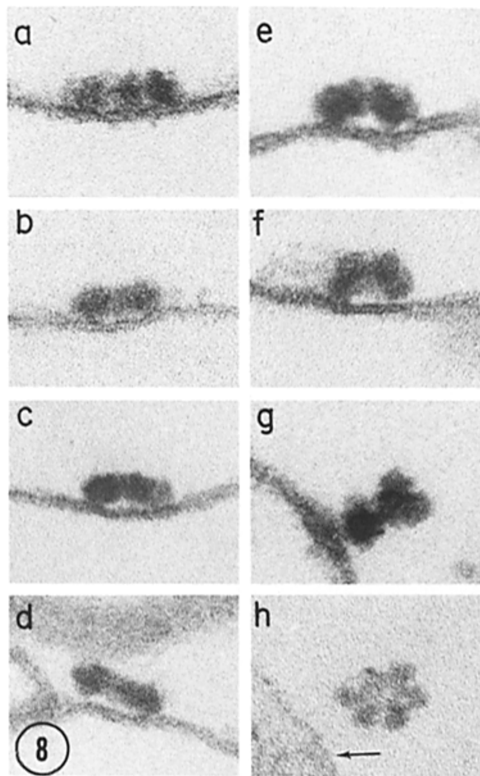


Figure 8 The gallery shows seven (*a–g*) attached polysomes seen in profile (or side view) on normally sectioned membranes of terminal thylakoids. The attachment of the polysomes varies from complete (*a*) to limited to either two ends (*b, c,* and *e*) or one end only (*d, f,* and *g*) of the profile. At *h*, a hexamer is seen in full face view at a distance of 400 Å of the nearest (obliquely sectioned) thylakoid membrane (arrow). All these appearances can be explained by assuming that these polysomes are assembled in the stroma and attach as groups to the membranes. Preparation of the specimens as for Figs. 5 and 6. (*a–g*) × 200,000; (*h*) × 160,000.

of ribosomes devoid of nascent chains. A similar situation might occur in *C. reinhardtii* and, in this case, the nascent chain-free ribosomes become detached from the thylakoid membranes and accumulate as free ribosomes in the chloroplast stroma. Addition of CAP, an inhibitor of peptidyl transferase (cf. reference 34), prevents this runoff and permits the isolation of polysomes-thylakoid membrane complex (Fig. 1*g* and *h*). Similar results (not shown) could also be obtained with erythromycin (50 μg/ml; 1 h incubation), another inhibitor of chain elongation (cf. reference 23).

Were the above interpretation correct, it should be possible to obtain thylakoid-bound polysomes by rapid cooling of the cells without the use of antibiotics that inhibit chain elongation. The results presented in Fig. 14 show that this is indeed possible. In this experiment, rapid cooling of the culture before harvesting increased the proportion of the total 70S ribosomes in P17 from 7% to 20%. The latter value is still lower than that of CAP-treated cultures (27–30%), indicating that limited polysomal runoffs had occurred even under these conditions.

Results obtained with the control sample at L-4 also suggest that polypeptide chain initiation, which is presumably inhibited by low temperatures, is important for the assembly of ribosome-membrane complex. In order to gather direct evidence on the role of initiation in the assembly process, experiments were carried out with lincomycin, a specific inhibitor of chain initiation on 70S ribosomes (11, 34). Cultures at L-4 were first treated with lincomycin (150 μg/ml) for 50 min, the followed by CAP (100 μg/ml) for 10 min. Fig. 15 shows that lincomycin greatly suppressed the amounts of 70S ribosomes in P17, compared to that recovered in the sample treated with CAP alone.

Results similar to these have been reported by Margulies and Michaels (28) with streptogramin, another inhibitor of 70S chain initiation. Our results, together with those of Margulies and Michaels (28), are consistent with a model in which

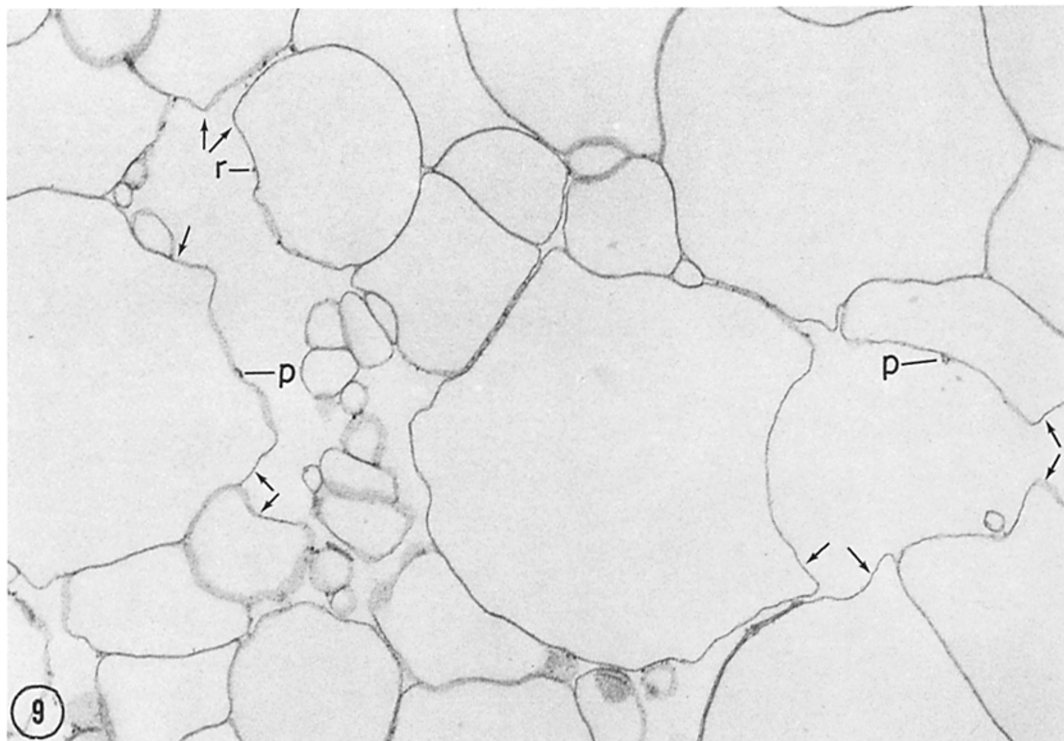


FIGURE 9 TMF isolated from synchronized cells treated at L-4 with CAP (100 μg/ml) for 10 min before cell disruption. The fraction was treated in vitro with 500 mM KCl and 1 mM puromycin. The membranes of terminal thylakoids (arrows) are practically free of attached ribosomes. Possible exceptions are indicated by *r* for isolated ribosomes and *p* for polysomes. × 28,000.

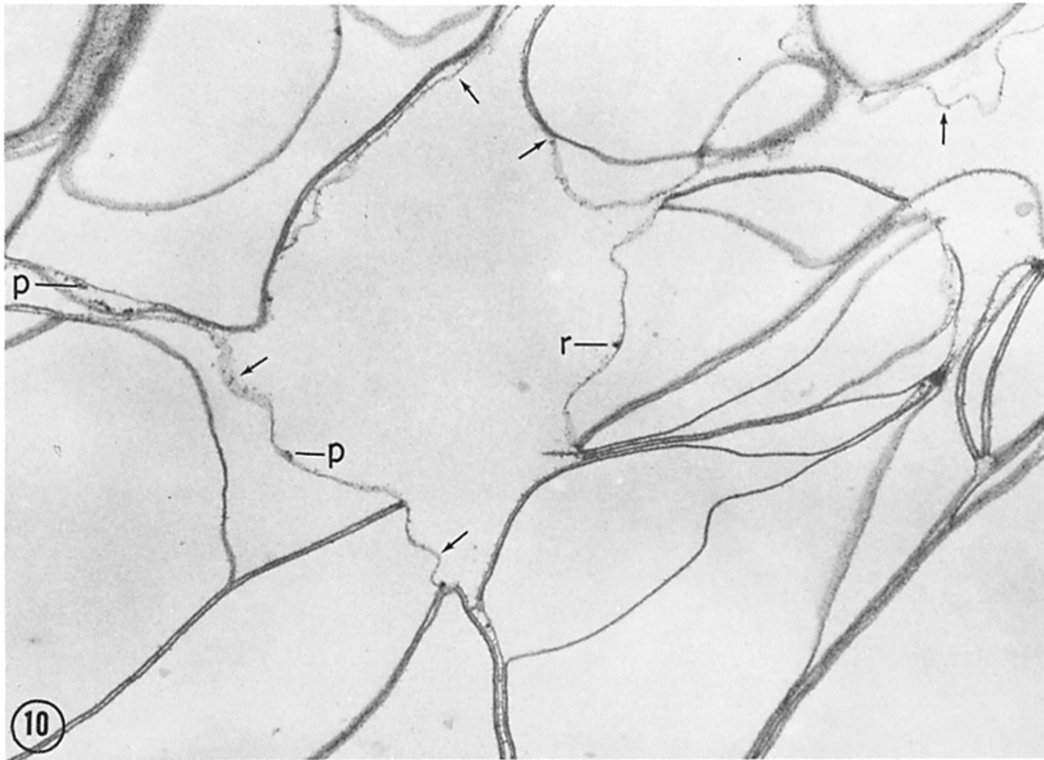


FIGURE 10 TMF isolated from synchronized cells at L-4 without treatment in vivo with CAP. The fraction was treated in vitro with 25 mM KCl without puromycin (*control preparation*). The membranes of terminal thylakoids (arrows) only occasionally show attached ribosomes (*r*) or polysomes (*p*). × 47,000.

translation of membrane-bound mRNA's begins first in the soluble compartment of the chloroplast.

DISCUSSION

The variations in the physiological and biochemical parameters of the synchronous cultures of *C. reinhardtii* have been well characterized by several groups of workers (2, 4, 9, 25, 26, 37). Under our experimental conditions, the cell number remains constant during the light phase but in the dark each cell divides two times giving rise to four daughter cells. The amount of chlorophyll per milliliter culture also increases by about four times but this increase is restricted to the light phase. There is evidence that a group of thylakoid membrane proteins are preferentially synthesized and assembled during this period (2, 4, 9, 32). The synthesis of only a fraction of thylakoid membrane proteins is sensitive to 70S ribosome inhibitors, implying that some of them are made in the chloroplast (2). Although the precise size of this fraction is still unknown, it appears to be a minority. Also, evidence obtained during the re-greening of *Chlamydomonas y-1* mutant indicates that some thylakoid membrane polypeptides are translated on chloroplast ribosomes (18, 23). Results presented in this paper show that during the light phase a sizable fraction of the 70S ribosomes is attached to the thylakoids, and this population of bound ribosomes may account for up to 20–30% of the total. We assume that these bound ribosomes are involved in the synthesis of the membrane proteins mentioned above although we realize that final proof remains to be established by future work.

Schor (37) has previously reported that the incorporation of radioactive precursor into whole cell proteins in the dark phase of the synchronous cell cycle is insensitive to spectinomycin, an inhibitor of chloroplast ribosomes. His results suggest that chloroplast ribosomes, both free and thylakoid-bound, are inactive during this time. Iwanij et al. (25) reported that the rate of synthesis of a soluble chloroplast protein, ribulose-1,5-bisphos-

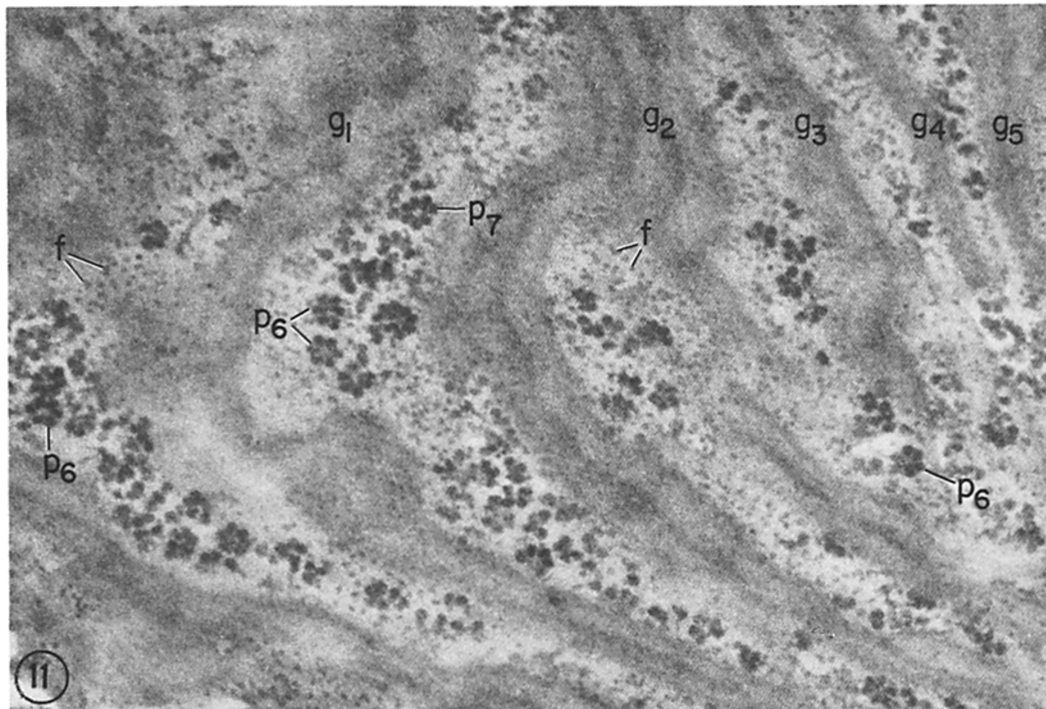


FIGURE 11 Chloroplast fragment in P17 obtained from synchronized cells exposed for 10 min to CAP (100 μg/ml) at L-4. Numerous polysomes (p) appear against the irregular light gray bands which represent oblique sections through terminal thylakoids in a series of grana (the darker composite bands (g_1, g_2, g_3, g_4, g_5) are oblique sections through the central part of these grana). Many of the polysomes are circular (either closed or open) and vary in size from penta- to hexa- (p_6) and heptamers (p_7). As expected, the frequency of recognizable circular polymers decreases towards the left where the thylakoids are less obliquely sectioned. The smaller light bodies associated with the free surface of terminal thylakoids are coupling factor particles (f). × 72,000.

phate carboxylase, whose large subunit is presumably made by the free chloroplast ribosomes (17), is very slow in the dark, and the results in this paper show that there are very few or no thylakoid-bound ribosomes during the same period. Taken together, these results strongly suggest that the chloroplast protein-synthesizing apparatus may be quiescent in the dark.

The ratio of free to thylakoid-bound chloroplast ribosomes in synchronous *Chlamydomonas* cultures is not strictly dependent on the cell cycle since it can be modulated by exposure to light or dark out of phase with the cycle. The proportion of bound 70S ribosomes increased after cells at D-4 were exposed to light for only 10 min and decreased dramatically after cells at L-4 had been transferred to the dark for 10 min. Previous evidence of thylakoid-bound ribosomes in higher plants relied on either electron microscopy (19, 40) or biochemical analyses (13, 39) alone. Estimates of the fraction of thylakoid-bound ribosomes range from 18% (39) to 50% (13). In view of the results presented in this paper, it would be of interest to know whether the attachment of chloroplast ribosomes to thylakoid membranes in higher plants also depends on environmental conditions (light-dark) and perhaps on cell cycle.

The results obtained with the various 70S-specific antibiotics as well as with rapid cooling of the cells have important implications with respect to the mechanism of assembly of the polysome-membrane complex in vivo. These results establish conclusively that the interaction between ribosomes and thylakoid membranes is a transitory process: ribosomes remain bound on the membranes as long as they are active in protein synthesis. The transit time for polysomes on membrane is at most 10 min, since a large fraction of the

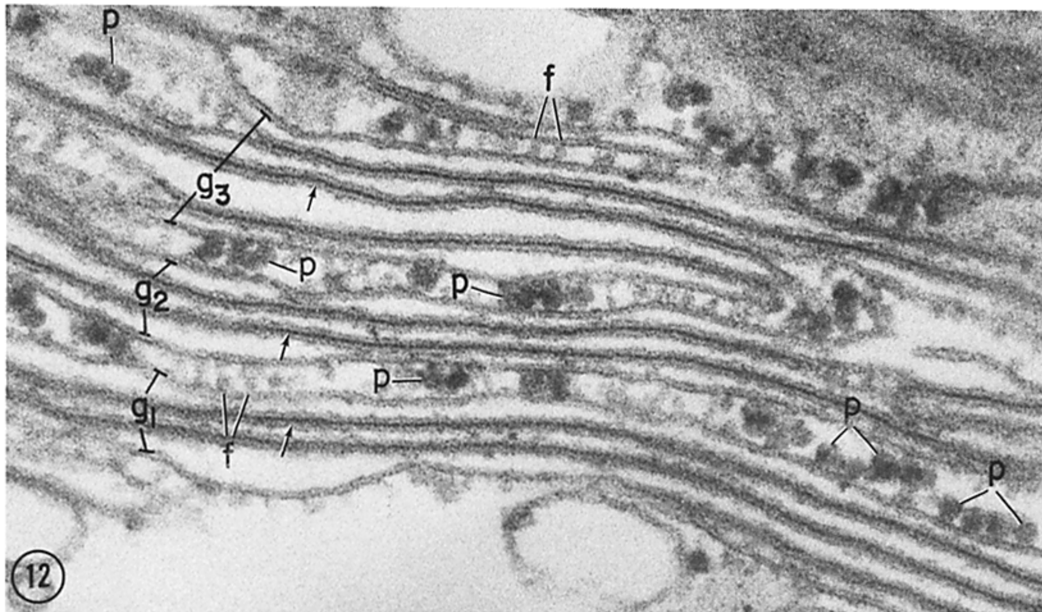


FIGURE 12 Chloroplast fragment in a P17 pellet prepared as for Fig. 11. Three partially disrupted grana (g_1, g_2, g_3) appear in this field. Their terminal thylakoids bear attached polysomes (p) seen in side view as series of four to two ribosomes. Fusion lines between granal thylakoids are indicated by arrows and coupling factor particles by f. × 170,000.

thylakoid-bound ribosomes became free after cultures at L-4 had been transferred to darkness for only 10 min (cf. Results). This period is considerably shorter than the duration of the light phase (12 h), indicating that the polysomes must undergo many rounds of attachment and detachment during this time. The findings so far obtained are consistent with the hypothetical sequence of events indicated in Fig. 16 which is adapted from the model proposed by Blobel and Sabatini (8) and Blobel and Dobberstein (6) to account for the translation of specific mRNA's on the polysome attached to the rough endoplasmic reticulum membrane in eucaryotic cells. We describe the interaction in three distinct steps. Translation of mRNA's coding for a certain class of membrane proteins begins first in the soluble compartment of the chloroplast. After the nascent chain has attained a certain critical length, the ribosomes become attached to the unstacked region of the thylakoid membranes forming the polysome-membrane complex. Upon natural chain termination, the chloroplast ribosomes or their subunits, S^{70} and L^{70}, are released into the chloroplast stroma where they are able to start a fresh round of translation. In the presence of a specific initiation inhibitor, such as lincomycin (this paper) or streptogramin (28), or under conditions in which the cells are slowly cooled, step 1 is blocked, but step 2 and 3 continue, resulting in a low level of thylakoid-bound ribosomes. Step 1 is also blocked during the dark phase of the synchronous cell cycle or when cells in the light phase are interrupted by 10 min of darkness. In both cases, the proportion of 70S ribosomes attached to the thylakoids is reduced to 5% or less. Step 3, on the other hand, is inhibited by antibiotics that prevent chain elongation, e.g. spectinomycin (16); CAP, and erythromycin (this paper), or by rapid cooling of the cells, and under both sets of conditions, the polysomes remain "frozen" on the thylakoid membranes.

In the hypothetical model presented in Fig. 16, we assume that the mRNA is released into the chloroplast stroma after polysomal run-off. However, it is also possible that the mRNA is attached directly to the thylakoid membranes via its 3′ end, as was shown to be the case for the mRNA of membrane-bound polysomes in certain eucaryotic cells (cf. reference 35).

As shown previously (15), approx. 40–50% of the thylakoid-bound chloroplast ribosomes can be

removed by high-salt treatment alone whereas the remainder are released only by high salt and puromycin. Strictly speaking, the scheme in Fig. 16 applies only to the latter fraction which is high-salt resistant. The precise nature of the salt-labile fraction of thylakoid-bound 70S ribosomes is not known, but in the case of microsomes from liver (1) and mouse myeloma cells (22) a similar fraction of the membrane-bound 80S ribosomes has been shown to be predominantly monosomes.

Although our results provide strong experimental evidence for the dynamic aspects of this model, many points remain to be clarified by future work. For example, the molecular events involved in the

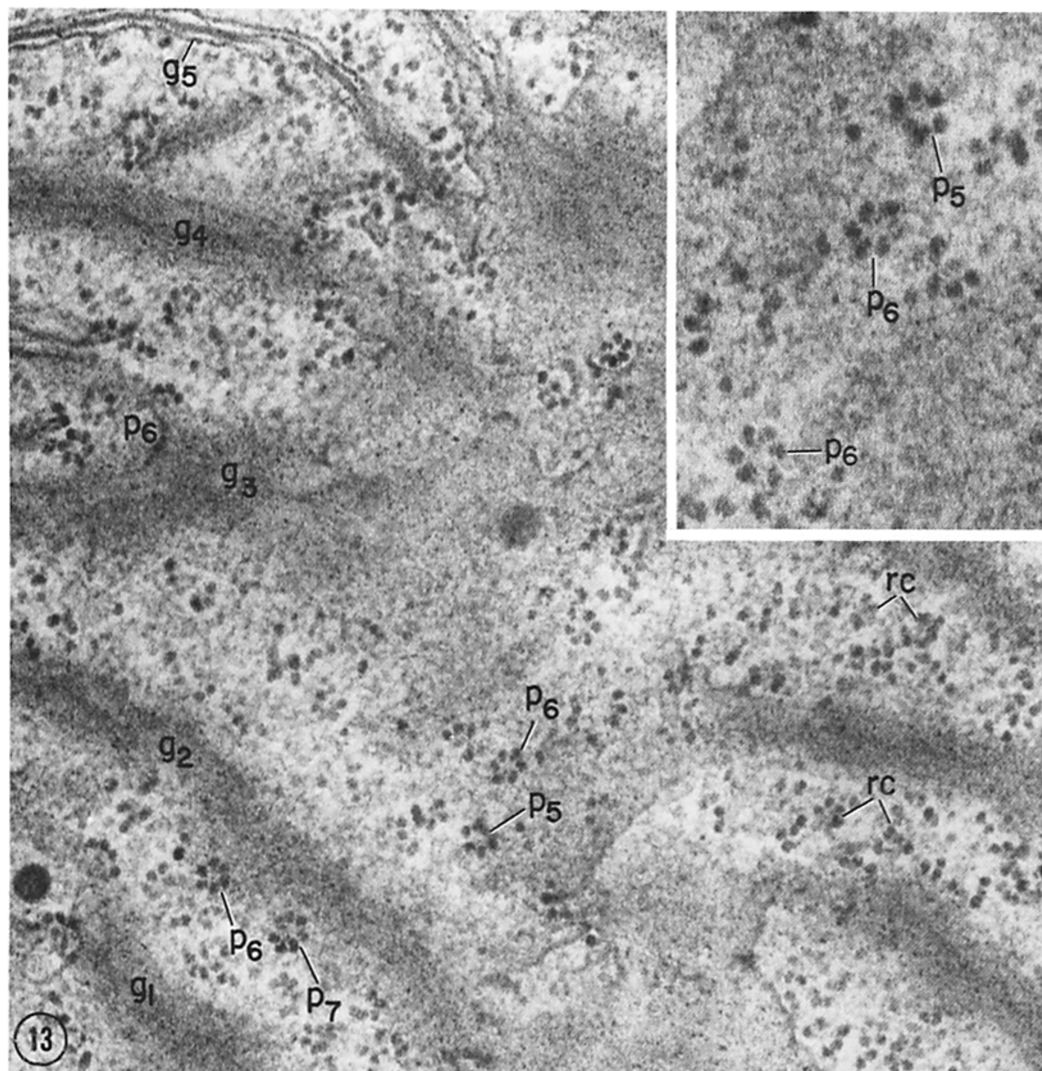


FIGURE 13 Algal cell from a synchronized culture fixed at L-4 10 min after the addition of CAP (100 μg/ml) to the culture medium. The section cuts obliquely through a series of small grana (g_1, g_2, g_3, g_4) and normally through a minimal (two discs) granum (g_5). A series of circular penta-, hexa-, and heptamers (p_5, p_6, p_7) appear within the lighter bands which separate the grana and represent oblique sections through terminal thylakoids. The other ribosomal clusters (*rc*) could be attached, detached, or free chloroplastic polysomes; their irregular appearance may result in part from microtomy. The *inset* shows at a higher magnification a small field in Fig. 13 which contains one pentamer (p_5) and two hexamers (p_6). × 100,000; *inset*, × 160,000.

Table IV

Effect of Darkness and Light on the Proportion of 70S Ribosomes in P17 Isolated at L-4 and D-4, Respectively

Experiment	Percent of ribosomes in P17	
	80S	70S
L-4 + 10 min light + CAP	7.1 ± 1.70	28.0 ± 4.4
L-4 + 10 min darkness + CAP	7.5 ± 1.0	4.8 ± 2.1
D-4 + 10 min darkness + CAP	12.5	5.0
D-4 + 10 min light + CAP	12.5	15.5

Cultures at L-4 were divided into two equal portions: one sample (control) was kept in the light whereas the other sample was darkened by wrapping the culture flask with three layers of aluminum foil. After 10 min, both samples received CAP at a final concentration of 100 μg/ml, and the cells were further incubated with the drug for 10 min before they were harvested. Experiments with D-4 cultures were carried out with similar protocol. One sample (control) was kept in the dark whereas the other sample was exposed to light (intensity = 4,000 1x). After 10 min, CAP (final concentration = 100 μg/ml) was added to both samples, and the cells were further incubated with the inhibitor for 10 min before they were harvested. Procedures for cell fractionation and methods of computation for the distribution of 70S and 80S ribosomes in S17 and P17 are given in Materials and Methods and legend to Table II. Values for L-4 are means from three experiments ±SD for the mean. Values for D-4 represent the average from two experiments.

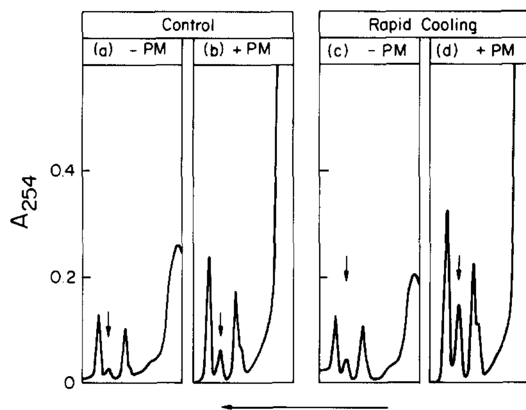


Figure 14 Effect of rapid cooling on the recovery of thylakoid-bound 70S ribosomes in P17 prepared from synchronous cultures at L-4. The rapidly cooled cells were obtained by pouring the culture directly onto 2 vol of crushed ice. The cells were then collected by centrifugation in the cold as described under Materials and Methods. The amounts of materials loaded onto each gradient are as follows: (*a*) 250 μg chlorophyll; (*b*) 250 μg chlorophyll; (*c*) 250 μg chlorophyll; (*d*) 250 μg chlorophyll. *PM*, puromycin. Vertical arrows indicate position of L^{70}. For other experimental details see Fig. 1 and Materials and Methods.

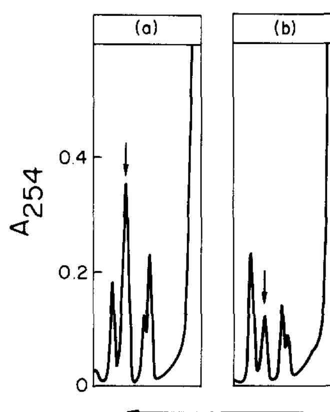


Figure 15 Effect of lincomycin on the amount of thylakoid-bound 70S ribosomes at L-4. (*a*) P17 of CAP-treated sample, 250 μg chlorophyll; (*b*) P17 of sample treated with lincomycin and CAP, 250 μg chlorophyll. Vertical arrows indicate position of L^{70}.

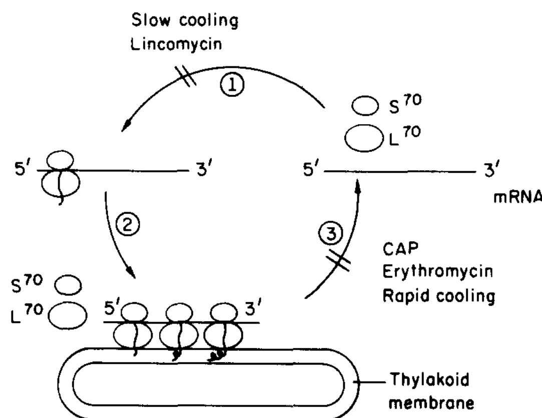


Figure 16 A proposed model for the attachment of chloroplast ribosomes to thylakoid membranes of *C. reinhardtii* in vivo. S^{70} and L^{70} designate the large and small subunits of 70S ribosomes, respectively.

specific interaction between polysomes and thylakoid membrane are largely unknown. It may involve a "signal" as in the case of polypeptides produced for secretion (6), it may depend largely on the hydrophobicity of the membrane polypeptides, or it may involve a preparatory step (proteolysis) as in the case recently studied in reticulocytes (27).

We thank Louise Castle for assistance in electron microscopy.

This work was supported in part by National Institutes of Health grant GM 21060 to Nam-Hai Chua.

Received for publication 8 April 1976, and in revised form 24 June 1976.

REFERENCES

1. Adelman, M. R., D. D. Sabatini, and G. Blobel. 1973. Ribosome-membrane interaction. Nondestructive disassembly of rat liver rough microsomes into ribosomal and membranous components. *J. Cell Biol.* **56:**206–229.
2. Armstrong, J. J., S. J. Surzycki, B. Moll, and R. P. Levine. 1971. Genetic transcription and translation specifying chloroplast components in *Chlamydomonas reinhardtii. Biochemistry.* **10:**692–701.
3. Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta Vulgaris. *Plant Physiol.* **24:**1–15.
4. Beck, D. P., and R. P. Levine. 1974. Synthesis of chloroplast membrane polypeptides during synchronous growth of *Chlamydomonas reinhardtii. J. Cell Biol.* **63:**759–772.
5. Bernstein, E. 1964. Physiology of an obligate photoautotroph (*Chlamydomonas moewusii*). I. Characteristics of synchronously and randomly reproducing cells and a hypothesis to explain their population curves. *J. Protozool.* **11:**56–74.
6. Blobel, G., and B. Dobberstein. 1975. Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J. Cell Biol.* **67:**835–851.
7. Blobel, G., and V. R. Potter. 1968. Distribution of radioactivity between the acid-soluble pool and the pools of RNA in the nuclear non-sedimentable and ribosome fractions of rat liver after a single injection of labeled orotic acid. *Biochim. Biophys. Acta.* **166:**48–57.
8. Blobel, G., and D. D. Sabatini. 1971. Ribosome-membrane interaction in eukaryotic cells. *In* Biomembranes, Vol. 2. L. Manson, editor. Plenum Publishing Corp., New York. 193–195.
9. Bourguignon, L. Y. W., and G. E. Palade. 1976. Incorporation of polypeptides into thylakoid membranes of *Chlamydomonas reinhardtii.* Cyclic variations. *J. Cell Biol.* **69:**327–344.
10. Bourque, D. P., J. E. Boynton, and N. W. Gillham. 1971. Studies on the structure and cellular location of various ribosome and ribosomal RNA species in the green alga *Chlamydomonas reinhardi. J. Cell Sci.* **8:**153–183.
11. Burns, D. J. W., and E. Cundliffe. 1973. Bacterial-protein synthesis: a novel system for studying antibiotic action *in vivo. Eur. J. Biochem.* **37:**570–574.
12. Cattolico, R. A., J. W. Senner, and R. F. Jones. 1973. Changes in cytoplasmic and chloroplast ribosomal ribonucleic acid during the cell cycle of *Chlamydomonas reinhardtii. Arch. Biochem. Biophys.* **156:**58–65.
13. Chen, J. L., and S. G. Wildman. 1970. "Free" and membrane-bound ribosomes, and nature of products formed by isolated tobacco chloroplasts incubated for protein synthesis. *Biochim. Biophys. Acta.* **209:**207–219.
14. Chua, N.-H., G. Blobel, and P. Siekevitz. 1973. Isolation of cytoplasmic and chloroplast ribosomes and their dissociation into active subunits from *Chlamydomonas reinhardtii. J. Cell Biol.* **57:**798–814.
15. Chua, N.-H., G. Blobel, P. Siekevitz, and G. E. Palade. 1973. Attachment of chloroplast polysomes to thylakoid membranes in *Chlamydomonas reinhardtii. Proc. Natl. Acad. Sci. U. S. A.* **70:**1554–1558.
16. Conde, M. F., J. E. Boynton, N. W. Gillham, E. H. Harris, C. L. Tingle, and W. L. Wang. 1975. Chloroplast genes in *Chlamydomonas* affecting organelle ribosomes. *Mol. Gen. Genet.* **140:**183–220.
17. Ellis, R. J. 1975. The synthesis of chloroplast membranes in *Pisum sativum. In* Membrane Biogenesis. A. Tzagoloff, editor. Plenum Publishing Corp., N.Y. 247–278.
18. Eytan, G., and I. Ohad. 1970. Biogenesis of chloroplast membranes. VI. Cooperation between cytoplasmic and chloroplast ribosomes in the synthesis of photosynthetic lamellar proteins during the greening process in a mutant of *Chlamydomonas reinhardi* y-1. *J. Biol. Chem.* **245:**4297–4307.
19. Falk, H. 1969. Rough thylakoids and polysomes attached to chloroplast membranes. *J. Cell Biol.* **42:**582–586.
20. Farquhar, M. G., and G. E. Palade. 1965. Cell junctions in amphibian skin. *J. Cell Biol.* **26:**263–291.
21. Friedman, H., P. Lu, and A. Rich. 1971. Temperature control of initiation of protein synthesis in *Escherichia coli. J. Mol. Biol.* **61:**105–121.
22. Harrison, T. M., G. G. Brownlee, and C. Milstein. 1974. Studies on polysome-membrane interactions in mouse myeloma cells. *Eur. J. Biochem.* **47:**613–620.
23. Hoober, J. K. 1970. Sites of synthesis of chloroplast membrane polypeptides in *Chlamydomonas reinhardi* y-1. *J. Biol. Chem.* **245:**4327–4334.
24. Hoober, J. K., and G. Blobel. 1969. Characterization of the chloroplastic and cytoplasmic ribosomes of *Chlamydomonas reinhardi. J. Mol. Biol.* **41:**121–138.
25. Iwanij, V., Chua, N.-H., and P. Siekevitz. 1975.

Synthesis and turnover of ribulose bisphosphate carboxylase and of its subunits during the cell cycle of *Chlamydomonas reinhardtii*. *J. Cell Biol.* **64**:572-585.

26. JONES, R. F. 1970. Physiological and biochemical aspects of growth and gametogenesis in *Chlamydomonas reinhardtii*. *Ann. N. Y. Acad. Sci.* **175**:648-659.
27. LODISH, H. F., and B. SMALL. 1975. Membrane proteins synthesized by rabbit reticulocytes. *J. Cell Biol.* **65**:51-64.
28. MARGULIES, M. M., and A. MICHAELS. 1974. Ribosomes bound to chloroplast membranes in *Chlamydomonas reinhardtii*. *J. Cell Biol.* **60**:65-77.
29. MARGULIES, M. M., H. L. TIFFANY, and A. MICHAELS. 1975. Vectorial discharge of nascent polypeptides attached to chloroplast thylakoid membranes. *Biochem. Biophys. Res. Commun.* **64**:735-739.
30. MICHAELS, A., and M. M. MARGULIES. 1975. Amino acid incorporation into protein by ribosomes bound to chloroplast thylakoid membranes: formation of discrete products. *Biochim. Biophys. Acta.* **390**:352-362.
31. MORIMOTO, T., G. BLOBEL, and D. D. SABATINI. 1972. Ribosome crystallization in chicken embryos. I. Isolation, characterization, and *in vitro* activity of ribosomes tetramers. *J. Cell Biol.* **52**:338-354.
32. OHAD, I., P. SIEKEVITZ, and G. E. PALADE. 1967. Biogenesis of chloroplast membranes. I. Plastid differentiation in a dark-grown algal mutant (*Chlamydomonas reinhardi*). *J. Cell Biol.* **35**:521-552.
33. OLESZKO, S., and E. N. MOUDRIANAKIS. 1974. The visualization of the photosynthetic coupling factor in embedded spinach chloroplasts. *J. Cell Biol.* **63**:936-948.
34. PESTKA, S. 1971. Inhibitors of ribosome functions. *Annu. Rev. Microbiol.* **25**:487-562.
35. SABATINI, D. D., and G. KREIBICH. 1976. Functional specialization of membrane-bound ribosomes in eukaryotic cells. *In* Enzymes of Biological Membranes, Vol. 2. A. Martonosi, editor. Plenum Publishing Corp., New York. 531-579.
36. SAGER, R., and S. GRANICK. 1953. Nutritional studies with *Chlamydomonas reinhardi*. *Ann. N. Y. Acad. Sci.* **56**:831-838.
37. SCHOR, S. 1971. Regular variations in chloroplast functional activity during the division cycle of *Chlamydomonas reinhardtii*. Ph.D. thesis. The Rockefeller University, New York, N.Y.
38. SCHOR, S., P. SIEKEVITZ, and G. E. PALADE. 1970. Cyclic changes in thylakoid membranes of synchronized *Chlamydomonas reinhardi*. *Proc. Natl. Acad. Sci. U. S. A.* **68**:174-180.
39. TAO, K. L., and A. T. JAGENDORF. 1973. The ratio of free to membrane-bound chloroplast ribosomes. *Biochim. Biophys. Acta.* **324**:518-532.
40. WELLBURN, F. A. M., and A. R. WELLBURN. 1971. Developmental changes occurring in isolated intact etioplasts. *J. Cell Sci.* **9**:271-287.