TEMPORAL PROGRAMMING OF

CHLOROPLAST AND CYTOPLASMIC RIBOSOMAL RNA

TRANSCRIPTION IN THE SYNCHRONOUS CELL CYCLE OF

CHLAMYDOMONAS REINHARDTII

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ABSTRACT

Approximately 90% of the *Chlamydomonas reinhardtii* chloroplast and cytoplasmic rRNAs was transcribed in the nuclear G1 phase, which occurred during the light period under an alternating light-dark synchronization regime of 12 h each. The remaining 10% of chloroplast and cytoplasmic rRNAs was transcribed from its respective DNAs in the dark period, in the midst of an apparent turnover of a limited quantity of rRNA. Thus, the temporal programming of chloroplast DNA transcription appeared to be prokaryotic in sophistication. The transcription was not interrupted during chloroplast DNA synthesis which occurred during the light period. However, transcription of the nuclear DNA was repressed severely during the nuclear S phase in the dark period.

The patterns of incorporation of ^{32}P into chloroplast and cytoplasmic rRNA species in the cell cycle were similar to those of the actual rRNA synthesis as measured optically. However, the quantity of ^{32}P incorporation per unit amount of rRNA synthesized varied considerably during the cell cycle, increasing in all rRNAs during the dark period. ^{32}P incorporation data obtained from continuous and pulse ^{32}P-labeling experiments also revealed a turnover of a small amount of both cytoplasmic and chloroplast rRNAs at the end of the S phase. The ^{32}P incorporation into cytoplasmic and chloroplast rRNAs was well matched temporally with the ^{32}P incorporation into their corresponding ribosomes, indicating that the newly synthesized rRNA molecules are utilized without delay throughout the cell cycle in the assembly of ribosomes.

The distinctive differences in structural organization between the genomes of prokaryotes and eukaryotes is probably related, at least in part, to the degree of flexibility allowed in the transcriptional regulation. Hence, it is not surprising to see that the mechanistic details of transcription of eukaryotes and prokaryotes differ in a number of aspects. Aside from the developmental aspects, it is of interest to ask whether, in a given cell cycle, eukaryotes in general possess a different and, perhaps, a more sophisticated programming capability in RNA transcription than do prokaryotes.

Available experimental evidence has not provided an unequivocal answer to this question at present.

The synthesis of rRNA in the prokaryote *Escherichia coli* has been shown consistently to exhibit an exponentially increasing rate throughout the cell cycle, with no apparent changes or interruption during the DNA synthesis period (29, 11, 12). On the other hand, the RNA synthetic periodicity varies considerably in eukaryotes. A number of studies have shown that transcription of RNA in general and of ribosomal RNA (rRNA) in particular occurs preferentially in the G1 phase and is repressed during the S phase (37, 13, 38, 25, 43, 47, 22, 27). In a few instances, conflicting results have been reported showing that the rate of RNA transcription was enhanced during the S phase (34, 26, 15, 36, 44). Evidence for continuous synthesis of rRNA throughout the eukaryotic cell cycle with an ever-increasing rate has also been documented (42, 33, 39, 14).

These varied results were derived from different cell types, as well as with different techniques to achieve cell synchrony. A variety of different procedures to quantitate RNA transcription were followed. In many instances, either the specific activity of RNA or the rate of RNA synthesis as manifested by the incorporation of labeled RNA precursor was measured, rather than the RNA synthesized being quantitated directly. Unless the specific activity of intracellular immediate nucleotide precursor pools remained essentially unchanged throughout the entire cell cycle (42), these indirect radioactivity measurements may not necessarily mimic the actual RNA synthetic activity at any given time in the cell cycle. Of course, true differences in RNA transcriptional periodicity in the cell cycle may exist among different eukaryotic organisms.

The potential differences and similarities in the eukaryotic and prokaryotic temporal programming of RNA transcription in the cell cycle can probably be further clarified by determining the nuclear and chloroplast RNA synthetic periodicities simultaneously in the same cell, since chloroplast DNA, which replicates semiconservatively and independently of the eukaryotic nuclear genome (6, 24, 7, 8) possesses a prokaryotic-like structural organization (2, 3, 48, 49). Chloroplast ribosomes are also prokaryotic-like (20, 41, 1, 17, 18, 9), and chloroplast RNA is transcribed from the chloroplast DNA (40, 46, 4). Temporal programming of chloroplast RNA transcription in a synchronous cell cycle has scarcely been charac-

terized (5, 45). Such characterization should also be of interest in its own right. Because of the quantity and largely stable nature of rRNA, in both the chloroplast and the nuclear systems, it appears that the synthetic periodicity of rRNA can be unambiguously determined by measuring the increase of rRNA directly throughout the cell cycle rather than relying solely on the quantitation of labeled precursor incorporation.

The unicellular green alga, *Chlamydomonas reinhardtii*, offers some unique advantages for the study of nuclear and chloroplast transcriptional programming in the cell cycle. Firstly, the division of this organism can be effectively synchronized by subjecting cells to an alternating 12-h light–12-h dark regime (23, 24, 6). The time-course of both nuclear and chloroplast DNA synthesis during the synchronous cell cycle has already been determined (6, 24, 7, 8). Secondly, *C. reinhardtii* possesses abundant chloroplast ribosomes (i.e. approximately one-third of total ribosomes), and the chloroplast and cytoplasmic ribosomes and their respective constituent rRNAs can be readily and completely separated for direct quantitation (20, 41, 10). The use of this unicellular organism also makes possible the comparison between the transcription of the nuclear DNA and that of the prokaryotic-like chloroplast DNA under the best possible conditions, since the conditions of growth, synchronization, labeling, and RNA isolation and quantitation are all identical.

MATERIALS AND METHODS

Strains and Culture Conditions

Wild-type *C. reinhardtii*, strain 137c plus mating type were used for all experiments. Depending upon the experiment, 200–3,000-ml cultures were grown under an alternating 12-h light–12-h dark regime with constant stirring and continuous aeration with 3% CO_2 in air at room temperature (23, 6). Illumination was provided by cool white fluorescent lamps at 4,000 lux. Cells were grown in a low phosphate medium containing 20 mM KCl, 9.5 mM NH_4Cl, 0.065 mM $MgSO_4$, 0.41 M K_2HPO_4, 0.027 mM $CaCl_2$, 5 mM 2-(*N*-morpholino)ethane sulfonic acid (Calbiochem, San Diego, Calif.) pH 7.0 (titrated with NaOH) and 1 ml/liter of Hutner's trace metal solution (6). Cell counts were performed with a hemacytometer.

Radioactive Labeling Condition

Carrier-free H_3 $^{32}PO_4$ (Schwarz-Mann Div., Becton, Dickinson & Co., Orangeburg, N. J.) was used to label the culture at an activity of 2 μCi/ml. The uptake of ^{32}P by cells was determined by measuring the difference in

radioactivity of a 50-μl labeled culture with 50 μl of cell-free medium.

Ribosome and Ribosomal RNA Preparation

Cells were harvested by centrifugation at 8,000 g for 15 min at 4°C. All subsequent preparative procedures were performed in the cold. Pelleted cells were washed twice with a buffer containing 25 mM KCl, 25 mM MgCl₂, 25 mM Tris-Cl (pH 7.5), 0.25 M sucrose, with 10 mM mercaptoethanol. After resuspending in the same buffer at 2 × 10⁸ cells/ml, the cells were disrupted by two consecutive passes through a French pressure cell (American Instrument Co., Inc., Silver Spring, Md.) at 7,000 pounds per square inch. This cell lysate was centrifuged for 10 min at 10,000 g, and the supernate (the S-10 preparation) was either frozen at −70°C for later use or processed immediately as follows.

To perform sucrose gradient analysis of ribosomes, the S-10 was made 2.5% in Triton X-100 (Rohn & Haas Co., Philadelphia, Pa.) wt/vol (20) and then layered onto sucrose gradients. To perform polyacrylamide gel electrophoresis of rRNA, the S-10 was made 2% in sodium dodecyl sulfate, incubated for 4 min at 37°C, and subjected to electrophoresis immediately (45).

Sucrose Gradient Sedimentation of Ribosomes

An S-10 preparation (0.3 ml) was layered on a 0.46-1.01 M linear sucrose gradient made in a buffer containing 25 mM KCl, 25 mM Tris-Cl (pH 7.5), 5 mM MgCl₂, and 10 mM mercaptoethanol. The 38-ml gradients were spun in an IEC model B-60 ultracentrifuge with an SB-110 rotor at 25,000 rpm at 4°C for 12 h. 33 fractions were collected from the top with an ISCO model 640 fractionator. Fractions were counted in a Nuclear-Chicago Mark II scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.) utilizing the Cherenkov radiation of the ³²P or by collecting the 5% trichloroacetic acid precipitate of each fraction on Whatman GF83 fiber glass filters and counted in a toluene scintillation cocktail (1 liter toluene, 4 g 2,5-diphenyloxazole, and 0.1 g 1,4-bis[2-(5-phenyloxazolyl)]benzene).

Polyacrylamide Gel Electrophoresis of Ribosomal RNA

50 μl of SDS-treated S-10 samples were electrophoresed through cylindrical gels of 2.8% acrylamide and 0.14% bis-acrylamide (Bio-Rad Laboratories, Richmond, Calif.). The gels were cast in a buffer containing 36 mM Tris, 30 mM NaH₂PO₄, and 1 mM Na₂ EDTA (28). The running buffer was the same as the gel buffer, except that it also consisted of 0.5% Sarkosyl NL-97. (Geigy Chemical Corp., Ardsley, N. Y.). Polymerization conditions have been described (28). All electrophoreses were performed between 2° and 5°C, for 3-4 h.

Quantitation of rRNA and ³²P Incorporation

At the completion of electrophoresis, gels were scanned at 260 nm using a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) equipped with a linear transport device. In an initial experiment, the area under a given absorbancy peak was taken to be a measure of the amount of that rRNA in a constant volume of culture. Since the peak widths for each rRNA are quite similar, the peak height alone was used to calculate the data presented in this paper (Fig. 2 a). Calculations made with peak heights and peak areas produced similar results.

For ³²P-labeling experiments, the gels were dried on Whatman GF83 fiber glass paper strips under an infrared lamp and subsequently autoradiographed, using Kodak medical X-ray film NS-54T developed in D-19. The autoradiograph was used to locate the position of the RNA bands, which were cut out from the dried gel, rehydrated, and counted. The Cherenkov radiation of ³²P detected in these gel slices was used to determine the ³²P incorporation into the various rRNAs.

RESULTS

The rRNA Synthesis during the Synchronous Cell Cycle

To avoid possible complications that might arise from a variation in the rRNA precursor pool size during the synchronous cell cycle, the time of cytoplasmic (25S and 18S) and the chloroplast (23S and 16S) rRNA synthesis was determined by directly measuring the absorbance of these four RNA species after their separation on polyacrylamide gels. Fig. 1 illustrates the growth curve of a synchronous cell culture during the course of the rRNA synthesis experiment in which nine aliquots of this culture were removed at 3-h intervals for the preparation of the S-10 fraction and its subsequent gel electrophoresis. As seen in Fig. 1 a, the cell division in this culture was well synchronized. The four rRNA species present in S-10 preparations were well separated from one another by polyacrylamide gel electrophoresis. A representative scan is shown in Fig. 2 a. The absorbancy ratio between the 25S and 18S rRNAs and between the 23S and 16S rRNAs should equal the ratios of their respective molecular weights, i.e., 1.9 in both of these ribosome types. In the gel scans described below, the values of these absorbancy ratios were always close to this expected value, except during earlier time-points (L0, L3, and L6) when the small amounts of the 18S and 16S rRNAs present in the aliquot of culture made an
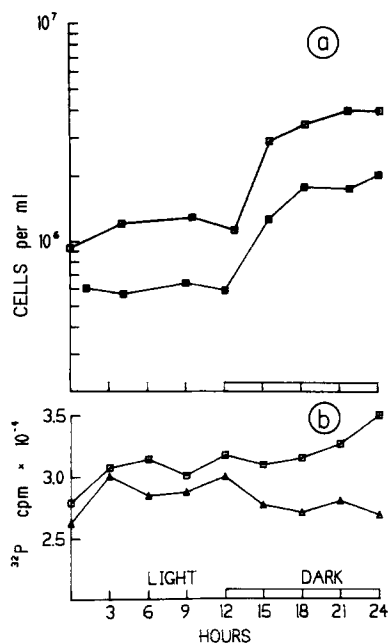
FIGURE 1 The growth and ^{32}P uptake in two synchronous cultures. (a) Growth curves. Cell density was determined by using a hemacytometer. Each point represents an average of three independent cell counts. Growth curve for experiment shown in Figs. 3 and 4, ■. Growth curve for experiment shown in Figs. 6 and 7, □. (b) Uptake of ^{32}P label for experiment shown in Figs. 3 and 4. The radioactivity obtained from a 50 μl aliquot of the culture at each time-point, □. The radioactivity obtained from 50 μl of the medium alone, after removing the cells by centrifugation, △.

accurate determination of their concentration difficult. The 25S-18S ratio and the 23S-16S ratio during these early time-points are therefore higher than expected.

Fig. 3 illustrates the results of the nine S-10 preparations obtained during a complete cell cycle. Most rRNA synthesis, as measured by the increase in the absorbance of the four different rRNA species on a per aliquot basis, occurred during the light period (i.e., L0-L12). The percent of each rRNA made during the light period is tabulated in Table I. A slight yet significant decrease in absorbance of all four species of rRNA was observed towards the end of the light period or at the onset of the dark period. This coincides with the beginning of the nuclear S phase in the cell cycle (6, 24, 7, 8). A limited quantity of rRNA thus appears to have been degraded just before or at the start of the nuclear DNA replication period. In addition, a small amount of net

rRNA synthesis occurred throughout the dark period (D0-D12).

^{32}P Incorporation into the rRNAs during the Cell Cycle

CONTINUOUS LABELING EXPERIMENT: The synchronous culture from which the rRNA synthesis data were obtained by absorbance
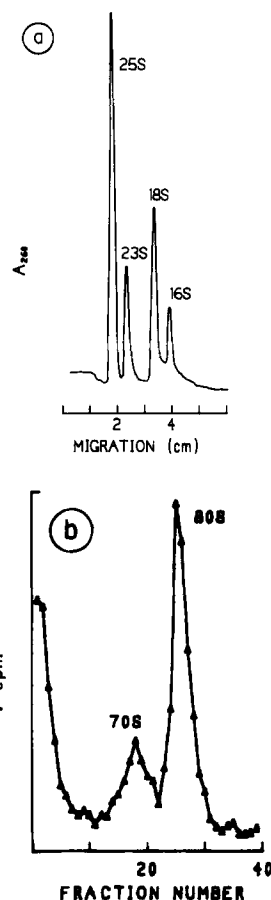

FIGURE 2 (a) The polyacrylamide gel electrophoretic patterns of rRNA. 50 μl of an S-10 preparation was treated with sodium dodecyl sulfate and run on a 2.8% polyacrylamide gel for 4 h. The resulting gel was scanned at 260 nm. Experiment details are explained in Materials and Methods. (b) A typical chloroplast and cytoplasmic ribosome banding pattern in sucrose gradient. 100 μl of an S-10 preparation was treated with Triton X-100 and layered onto 38 ml of 0.46-1.01 M sucrose gradient containing 5 mM $MgCl_2$. After 12-h centrifugation at 110,000 g at 4°C, fractions were collected from the top and counted directly, utilizing the Cherenkov radiation of ^{32}P. Experiment details are explained in Materials and Methods.
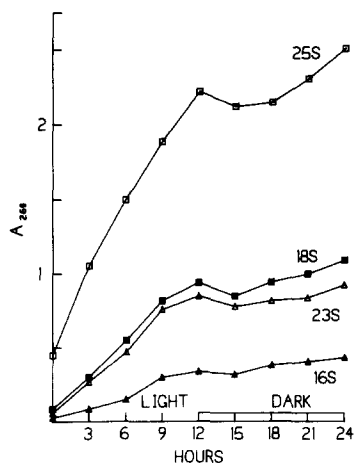
FIGURE 3 The rRNA synthetic pattern as measured optically during the cell cycle. Equivalent amounts of S-10 samples on a per aliquot basis from time-points in the culture shown in Fig. 1 were run on 2.8% polyacrylamide gels. The resulting gels were scanned at 260 nm. The heights of the four rRNA peaks in absorbance unit at 260 nm are plotted vs. time in the synchronous cell culture.

TABLE I

Relative Quantity and Specific Activity of rRNAs Synthesized in a Cell Cycle

rRNA Species	Light period		Dark period	
	Total syn-thesized	Relative sp act*	Total syn-thesized	Relative sp act*
	%		%	
25S	86	1.02	14	2.06
23S	91	1.02	9	3.50
18S	85	1.23	15	3.13
16S	64	1.32	36	2.67

* Given in the same arbitrary units calculated as follows for the light period: (^{32}P cpm at L12 − ^{32}P cpm at L0)/ (A_{260} at L12 − A_{260} at L0). This arbitrary unit is not the same as that shown in Fig. 5.

measurements of the gels was also labeled with ^{32}PO$_4$ at a concentration of 2 μCi/ml 8 h before the onset of the light period L0, the time of the first data point in Figs. 3 and 4. During the course of this continuous labeling experiment, the amount of ^{32}P present in the culture and in the cell-free medium after the cells had been pelleted by centrifugation was determined (Fig. 1 b). Because of the constant aeration with 3% CO$_2$ and the progressive decrease in volume of the original synchronous culture, a slight concentration of the culture by evaporation was unavoidable towards

the end of the cell cycle (Fig. 1 b). As a result, a slight rise in ^{32}P specific activity in the culture was observed at the last time-point of the continuous labeling experiment (Fig. 1 b). It is evident, however, that less than 30% of the phosphate present in the medium at the onset of the labeling was taken up by the cells during the 24-h cell cycle (Fig. 1 b). Hence, throughout the course of the continuous labeling experiment, the phosphate concentration in the medium did not become so low as to be limiting.

The gels which had been used to obtain the absorbancy data presented in Fig. 3 were used to determine the amount of ^{32}P incorporation into each of the four rRNA species as described in Materials and Methods. Consistent with the absorbancy results shown in Fig. 3, most ^{32}P incorporation into all four rRNA species occurred during the light period (Fig. 4). There was a slight decrease in the amount of ^{32}P incorporated at the beginning of the dark period. The relative amount of the ^{32}P incorporation into the rRNAs during the dark period, however, appeared to be significantly more than what the absorbancy data had indicated (cf. Figs. 4 with 3), suggesting that the specific
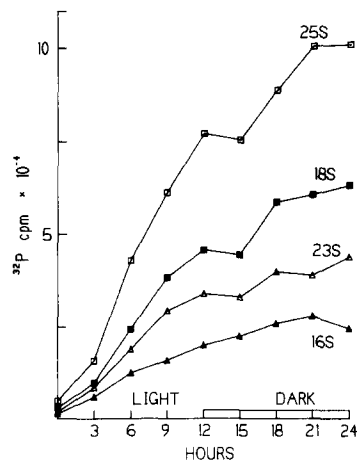

FIGURE 4 The pattern of ^{32}P incorporation into rRNA of continuous labeling experiment during the cell cycle. H$_3$ ^{32}PO$_4$ was added to the synchronous culture grown in a low phosphate medium as shown in Fig. 1 8 h before the onset of the light period. Autoradiograms were made from the dried polyacrylamide gels from which the data shown in Fig. 3 were obtained. The autoradiographs were used to locate the position of the rRNA bands in the dried gel. These bands were excised from the gel, rehydrated, and counted using the Cherenkov radiation of the ^{32}P. Experiment details are explained in Materials and Methods.

activity of *de novo* synthesized RNA was not constant during the entire cell cycle. This situation became more apparent in Fig. 5 where the specific activities of total rRNA were plotted. As would be expected, the ^{32}P specific activities calculated for the total existing rRNA followed a general increasing trend throughout the cell cycle. Most of such increases were detected in the light period except for the 16S rRNA which was the least abundant among the four major rRNA species. Because of its relatively low molecular weight, the 16S rRNA apparently was located on the gel too close to the highly labeled mRNA species and polyphosphates to permit accurate calculation of ^{32}P specific activities.

Although the data in Fig. 5 do show that the accumulated specific activities of the 25S, 23S, and 18S rRNAs continue to rise during the dark period, a calculation of the specific activities of *de novo* synthesized rRNA for each of the eight consecutive 3-h periods (i.e., the ^{32}P count difference between two neighboring rRNA samples over their absorbance difference) should be more revealing. However, based on this calculation procedure, the resulting specific activity variations of the four rRNA samples in the dark period were not as reproducible as those in the light period. This is because, in the dark period, the net increase of rRNA between two neighboring samples taken 3 h apart was too small to permit more accurate absorbance measurements. Hence, the calculation of their differences would suffer much more rela-

tive error resulting from the unavoidable small experimental errors inherited from the rRNA quantitation procedure at different time-points. Such independent experimental errors had relatively little effect on the calculation of *de novo* synthesized specific activity in the light period, where the increase of rRNA amount was large between samples taken 3 h apart.

Nevertheless, a reproducible trend of net rRNA synthesis relative to *de novo* ^{32}P incorporation at different parts of the cell cycle can be deduced by computing over much longer intervals as the difference between rRNA content can be more accurately determined. The result of *de novo* specific activity derived from the data in Figs. 3 and 4 for rRNA's made during the light periods and during the dark periods is presented in Table I. It is evident that the specific activity of rRNA made during the dark period was more than twice that of rRNA made during the light period. Also, in Table I, it is obvious that this high specific activity rRNA constitutes a minor portion of the rRNA made during the entire cell cycle. The fact that the higher specific activity rRNA was made during the dark period — the time of the nuclear S phase — is of considerable interest. The enhanced specific activity most likely represents a sudden alteration of nucleotide pool size and (or) a turnover of previously ^{32}P-labeled RNA which occurs about the time of nuclear DNA synthesis.

PULSE-LABELING EXPERIMENT: To ascertain whether the specific pattern of H_3 ^{32}PO$_4$ incorporation into rRNA species in the cell cycle derived from the continuous labeling experiment (Fig. 4) was affected by either the slight depletion of H_3 ^{32}PO$_4$ (Fig. 1b) or the continuous presence of H_3 ^{32}PO$_4$ in the medium throughout the cell cycle, H_3 ^{32}PO$_4$ pulse-labeling experiments were performed as follows. Starting at the beginning of the light period (L0) and at 3-h intervals thereafter, a 200-ml aliquot was removed from a large culture and pulse labeled for 3 h with 2 μCi/ml H_3^{32}PO$_4$. At L3, an S-10 preparation was made from this aliquot, and a second 200-ml aliquot was removed and pulse labeled for the subsequent 3-h period (from L3 to L6). At L6, an S-10 was made from this second aliquot. Eight samples were obtained in this manner during the 24-h synchronous cell cycle. As illustrated in Fig. 1a, the pulse-labeling experiment was executed with good cell synchrony, although this experiment was performed at a higher cell density (i.e. 0.94–4 × 10^6 cells/ml) than was the continuous labeling experi-
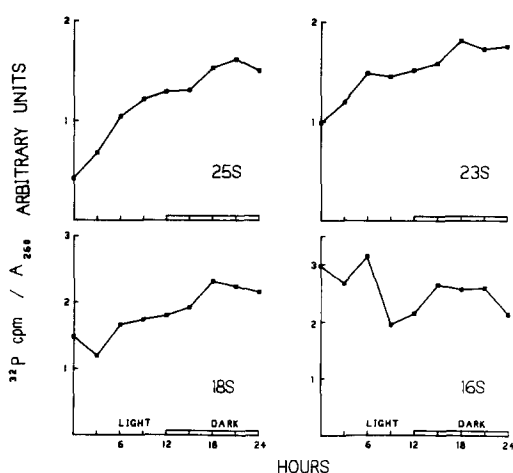

FIGURE 5 The variation of the ^{32}P specific activity of rRNA during the synchronous cell cycle. The accumulative ^{32}P specific activity detected in each rRNA species at a given time in the cell cycle.

ment (i.e., 0.6–2.4 × 10⁶ cells/ml). (Compare the two growth curves in Fig. 1a.)

The pattern of ³²P incorporation into the four rRNA species for each of the eight 3-h periods during the cell cycle is presented in Fig. 6. To readily permit comparison of the results obtained from both of the ³²P labeling experiments, the results of the ³²P pulse-labeling experiment are presented in Fig. 6 as the integral of the actual data obtained at each of the eight 3-h pulse-labeling periods. Hence, the total counts accumulated by an rRNA species up to a given time in the cell cycle were plotted against that particular time in Fig. 6. For example, the ³²P counts plotted for the 25S rRNA at L9 represent the sum of ³²P counts found in the 25S band in samples taken at L3, L6, and L9.

It is apparent that the data obtained from the pulse-labeling experiment (Fig. 6) are consistent and agree with the results of the continuous labeling experiment (Fig. 4). Since turnover or degradation of previously incorporated ³²P was not registered in each of the pulse-labeling periods, con-
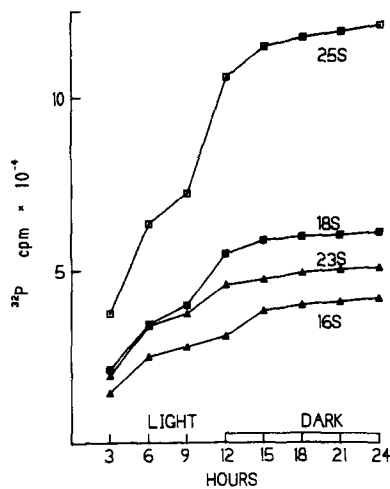

Figure 6   The pattern of ³²P incorporation into rRNA of a pulse-labeling experiment during the cell cycle. Each of eight aliquots taken from a large synchronous culture, whose growth curve is shown in Fig. 1a, at 0, 3, 6, 9, 12, 15, 18, and 21 h was pulse-labeled with H₃ ³²PO₄ for 3 h in a separate, smaller container before harvesting for S-10 preparation. The polyacrylamide gel fractionation of these eight S-10 samples and the subsequent quantitation of ³²P incorporation into each of the four rRNA species were the same as described in the legends to Figs. 3 and 4. The data are plotted as integral or the total accumulative incorporation of ³²P into the four rRNA species up to a given time-point in the cell cycle.

siderably less fluctuation of ³²P incorporation in the dark period was observed for the pulse-labeling experiment than for the continuous labeling experiment. In addition, the total ³²P incorporation in the dark period of the pulse-labeling experiment was somewhat less than that of the continuous labeling experiment. This was apparently related to the age of the synchronous cell culture. In another separate pulse-labeling experiment, in which the cell density was lower and similar to that of the continuous labeling experiment presented in Figs. 3 and 4, more ³²P was incorporated into the four rRNA species in the dark period (50).

³²P Incorporation into Ribosomes during the Cell Cycle

Since an S-10 preparation may be used to analyze rRNA on either gels or ribosomes in sucrose gradients, the pattern of incorporation of ³²P into both the rRNA and the ribosomes was determined with the same S-10 preparation. The chloroplast 70S and the cytoplasmic 80S ribosomes can be readily separated by sucrose gradient centrifugation (20, 41); Fig. 2b illustrates a typical profile of C. reinhardtii ribosomes. Fig. 7 illustrates the pattern of incorporation of ³²P into the two ribosomal species. This ³²P pulse-labeling experiment was the same as that in which the pattern of incorporation of ³²P into the four rRNA species was determined (Fig. 6). As in Fig. 6, the integral of ³²P counts obtained from the 70S and 80S ribosome peaks in the sucrose gradient at each of the eight 3-h pulsing periods was presented in Fig. 7.

From a comparison of Fig. 7 with Fig. 6 it is evident that the pattern of ³²P incorporation into the 80S ribosome is very similar to that into the 25S and 18S rRNAs, while the pattern of ³²P incorporation into 70S ribosome is similar to that into the 23S and 16S rRNAs. To compare data more meaningfully, the normalized sums of the ³²P incorporations into 25S and 18S, as well as 23S and 16S, rRNAs were plotted against the normalized 80S and 70S ribosomes, respectively in Fig. 8. The cytoplasmic ribosomes and their constituent rRNAs exhibited a close resemblance in their incorporation patterns throughout the entire cell cycle. The ³²P incorporation curve of the chloroplast ribosomes is also reasonably close to that of their constituent rRNAs. In three 3-h periods, L3-L6, L6-19, and L9-12, the relative ³²P incorporation into 70S ribosomes was 10% higher than that into the 23S and 16S rRNAs.
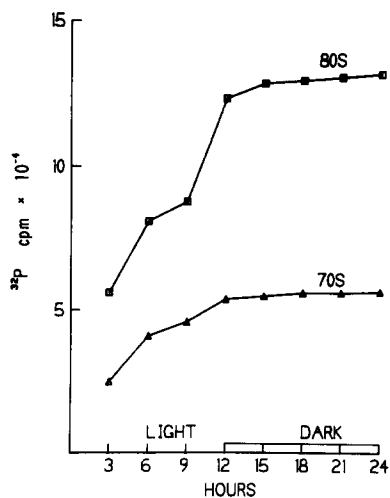
FIGURE 7 The ^{32}P incorporation pattern into ribosomes in the cell cycle. The eight S-10 samples from the ^{32}P pulse-labeling experiment shown in Fig. 6 were centrifuged on sucrose gradients as described in the legend to Fig. 2b. The integrated counts under the 80S and 70S peaks are plotted as integral or total accumulated label up to a given time-point in the cell cycle vs. time during the cell cycle.

This small difference most likely resulted from the experimental difficulty in quantitating the 16S rRNA on the gels as mentioned previously as well as from the greater lability of the 23S rRNA relative to that of the 70S ribosome particles, since the relative quantity of ^{32}P-labeled chloroplast ribosomes invariably exceeded slightly that of the chloroplast rRNA, but never the converse throughout the cell cycle.

Thus, in general, the kinetics of ^{32}P incorporation into both cytoplasmic and chloroplast ribosomes are temporally coincident with those of ^{32}P incorporation into their constituent rRNAs. Because of the relative ease with which all but the 30S rRNA precursors can be isolated in pulse-labeling experiments (50), it appears that rRNA species could exist as free molecules in the S-10 preparation without being extensively degraded. Therefore the composition of rRNA species and their relative content found in S-10 after detergent treatment are not necessarily the same as those found in the ribosome population obtained from the S-10 preparation. Consequently, our measurements of ^{32}P incorporation into ribosomes and constituent rRNAs confirms that there is no long-term lag between rRNA synthesis and the incorporation of those rRNA species into the ribosome

either as a mature rRNA molecule or as one of the slightly larger precursor rRNA molecules described by Miller and McMahon (32) and by Wilson (50).

DISCUSSION

A number of investigators have shown that only very low and relatively constant levels of cytoplasmic ribosomes and almost no detectable chloroplast ribosomes were present in the 10,000 g pellet, which was formed during the preparation of the S-10 fraction (10, 20, 41, 30). Similar to what had been observed in E. coli (16) and chick cells (35), the cooling of Chlamydomonas cells during the harvesting process probably inhibited the initiation of protein synthesis but permitted polypeptide elongation and chain termination to occur, thereby allowing free ribosome monomers to accumulate in the supernate (10). Hence, the synthesis and degradation of rRNA and ribosomes in the cell can be determined efficiently by analyzing the S-10 preparations as performed in the present in-
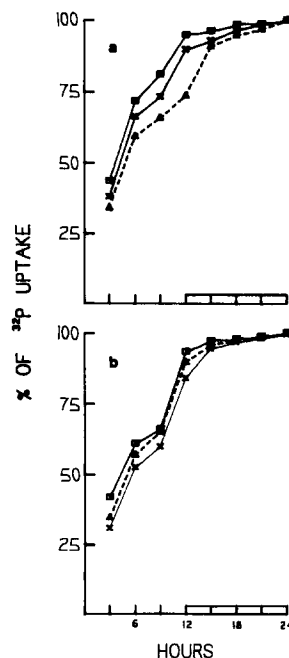

FIGURE 8 Comparative patterns of ^{32}P incorporation into ribosomes and their constituent rRNAs. The data presented in Figs. 6 and 7 with respect to each ribosomal species are normalized and replotted as a function of time in the cell cycle. (a) 70S ribosomes, □; 23S rRNA ×; 16S rRNA, ▲. (b) 80S ribosomes, □; 25S rRNA ×; 18S rRNA, ▲.

vestigation. This experimental design allowed us to monitor the concomitant rRNA as well as ribosome synthesis in the cell cycle and to avoid any possible differential rRNA recovery from different cell samples by phenol extraction procedures.

From the results presented above, it is clear that the rRNA transcriptional activity of both eukaryotic nuclear and prokaryotic-like chloroplast genomes closely approximate each other in a given cell cycle (Figs. 3, 4, and 6). Not only the major synthetic activities but the minor ones, as well as the apparent turnover of both chloroplast and cytoplasmic rRNAs, were coincident in terms of external timing. This is in contrast to the fact that chloroplast and nuclear DNAs were replicated out of phase (i.e. L3-L7 and D15-D21, respectively) in the cell cycle (6, 24, 7, 8). Hence, it is this difference in timing of DNA replication that differentiated the internal transcriptional programming between the nuclear and the chloroplast genome.

The present study indicates that the beginning of the nuclear S phase corresponded roughly to the time when the synthesis of most cytoplasmic rRNA in the cell cycle was completed (Fig. 3). Therefore, transcription of cytoplasmic rRNA in C. reinhardtii occurs preferentially in G1 and becomes severely repressed during S phase, a situation prevalent in many other eukaryotes (37, 27, 13, 38, 25, 43, 47, 22).

Unlike that in the eukaryotic systems, temporal programming of RNA transcription in bacteria has been shown to exhibit exponentially increasing rates during the cell division cycle (11, 12, 29). Dennis (11, 12) reported that bacterial DNA replication does not change the RNA transcription rate in E. coli. Manor and Haselkorn (29), using a different strain, found an enhancement of the RNA synthetic rate during DNA replication. While the experiments presented in the present study were not designed to obtain sufficient resolution to detect small changes in the rate of chloroplast rRNA synthesis during chloroplast DNA replication, it is obvious, nevertheless, that the maximum chloroplast rRNA transcription and chloroplast DNA replication occur simultaneously, a situation similar to bacterial RNA transcription.

Although most chloroplast and nuclear RNA transcription occurs before the onset of nuclear S phase, approximately 15% of the total cytoplasmic and chloroplast rRNA synthesized in the cell cycle was transcribed in the dark period. Recently,

the RNA transcriptional activity in this later period of the cell cycle has been shown to be critical to cell division (21). Since the transcriptional activity in the cell cycle was monitored in the present study by both the actual increases in the amount of stable product rRNA and the accumulated ^{32}P incorporation into these RNA species, the specific activity of ^{32}P incorporated per unit amount of rRNA synthesized in the light and in the dark period in the cell cycle can be calculated (Table I). These ^{32}P specific activity data suggest that an augmentation in the rate of ^{32}P incorporation into a limited amount of rRNAs occurred during the dark period. It would be of interest to ascertain the functional significance of these minor rRNA syntheses with upsurge of ^{32}P incorporation rate and (or) turnover of pre-existing rRNA in the dark period, particularly in view of the fact that all these events occur during the nuclear DNA replication.

The ^{32}P specific activity data presented in Table I also imply that neither the amount nor the rate of the incorporation of precursor into RNA is necessarily a valid measure of the actual amount of RNA being synthesized, since the amount of ^{32}P incorporation per unit amount of RNA synthesized varied significantly between the light and dark periods. This fluctuation in the ^{32}P incorporation pattern may have resulted from a host of physiological conditions that may vary cyclically in the light-dark synchronized cell cycle, such as the size of intracellular nucleoside/nucleotide precursor pool(s), a preferential reutilization of degradative products of labeled rRNA, the rate of nucleoside phosphorylation activity, etc.

The unequal rate of ^{32}P incorporation into rRNA at different times of the cell cycle was also shown to vary somewhat according to the age of the synchronous cell culture (50). The end result of such variation is that in ^{32}P pulse-labeling experiments, the relative amount of ^{32}P detected in each of the eight 3-h periods on a per aliquot basis during the cell cycle was not exactly the same in synchronous cell cultures of different growth phases. Nevertheless the same general trend (i.e. occurrence of the major rRNA synthesis in the light period with additional minor synthesis in the dark period) was in agreement regardless of the culture age (50). The fact that the manifestation of this culture-age effect on ^{32}P incorporation into rRNA was the same for all four rRNA species suggests that physiological conditions associated with different growth phases of the culture may be

influencing the ^{32}P incorporation into rRNA (50).

It is perhaps significant that not only the major synthesis but also the minor synthesis, together with intervening apparent turnover of both cytoplasmic and chloroplast rRNA, all coincide in the cell cycle. This rather precise temporal concomitance suggests that the transcription in both the nucleus and the organelle of *C. reinhardtii* may share a common element which is regulated according to the overall characteristics of the integrated cell cycle as a whole. Recently, the level of activity of RNA polymerase I has been shown to be proportional to the level of rRNA during the cell cycle in yeast (19).

The amount of cytoplasmic and chloroplast rRNA of *C. reinhardtii* was shown, under experimental conditions quite different from those of the present study, to increase in nuclear G1 phase and to decrease to 62-70% of the maximum value in G1 at the time of cell division for all four major rRNA species (5). Although these results parallel our present findings on a qualitative basis, such massive rRNA degradation (i.e. 62-72%) during the vegetative cell cycle of *C. reinhardtii* is in variance with our present study as well as with the previous demonstration that ribosomes are conserved during exponential growth in *E. coli* (31) and that both chloroplast and cytoplasmic ribosomes are conserved during normal vegetative growth in *C. reinhardtii* (41). Since growth curves or cell densities were not presented in the study by Cattolico et al. (5), it is not known whether their observation on the massive rRNA degradation was possibly related to the particular cell growth conditions (e.g., approaching the stationary phase) or a differential recovery of rRNA species by the phenol extraction procedure in different time-points of the synchronous cell cycle.

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