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Expression of the Chloroplast Ribosomal RNA Genes of *Euglena gracilis* during Chloroplast Development†

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ABSTRACT: The cellular content and transcription program of the chloroplast ribosomal RNA genes of *Euglena gracilis Z* have been determined during the light-induced development of chloroplasts by hybridization of total cell DNA or RNA to purified ^{3}H-labeled chloroplast ribosomal DNA ([^{3}H]ct rDNA). Pancreatic DNase activated, partially purified chloroplast rDNA was enzymatically labeled in vitro by *E. coli* DNA polymerase I with [^{3}H]TTP as a substrate. The [^{3}H] DNA was denatured and hybridized with a vast excess of purified chloroplast 16 and 23S rRNA. The rRNA-[^{3}H]ct rDNA hybrid was isolated by chromatography on hydroxylapatite. The [^{3}H]ct rDNA was purified and characterized by the kinetics of its renaturation with chloroplast DNA and rRNA, and by the thermal stability of [^{3}H]DNA-DNA and [^{3}H]DNA-RNA hybrids. [^{3}H]ct rDNA was hybridized in trace amounts to cellular RNA or DNA isolated from *Euglena* cells 0, 4, 8, 12, 24, 48, and 72 h after the onset of chloroplast development. From a comparison of the kinetics of hybridization with hybridization of standards of known kinetic complexity quantitative estimates of the cellular rRNA and rDNA gene content were made. Chloroplast rRNA increases from 2 to 26% of the cellular RNA during development, while the percentage of cellular DNA represented by ct rDNA increases two- to threefold. Correcting for the change in cellular RNA and DNA content during development, the number of copies of the rRNA gene increases less than twofold, while the number of copies of rRNA per cell increases sixfold. The results are consistent with either a transcriptional activation of the ribosomal genes or an increased rRNA stability during development.

Euglena chloroplasts represent an excellent model system for studying the temporal control of RNA synthesis during a developmental process. The chloroplasts undergo dramatic changes in both structure and physiology during light-induced development (Schiff et al., 1967; Ben-Shaul et al., 1964). The developmental process is accompanied by both qualitative and quantitative changes in the transcription of the chloroplast DNA (Chelm and Hallick, 1976; Rawson and Boerma, 1976). The *Euglena* chloroplast DNA exists as a covalently closed, circular duplex molecule of molecular weight 92×10^6 (Manning and Richards, 1972). The best characterized chloroplast RNAs are the 16 and 23S (0.6 and 1.1×10^6) ribosomal RNAs. These rRNAs are encoded by chloroplast DNA (Scott and Smillie, 1967; Stutz and Rawson, 1970) and represent the most abundant chloroplast DNA transcripts of the cell.

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Previously the possibility has been raised that the expression of the chloroplast rRNA genes varies during chloroplast development. Brown and Haselkorn (1971) could not detect chloroplast rRNA in dark-adapted cells, but found that both the 16 and 23S species increase in abundance during chloroplast development. There has recently been a report (Cohen and Schiff, 1976) that chloroplast rRNA increases from 2 to 25% of the cellular rRNA during light-induced greening.

In an effort to more fully understand the regulation of the chloroplast rRNA genes, we have measured both the chloroplast rRNA and chloroplast rRNA gene levels in *Euglena* cells undergoing chloroplast development. A hybridization probe specific for the chloroplast rRNA sequences was prepared. From the kinetics of molecular hybridization of total cellular RNAs and DNAs to this probe, quantitative estimates of both chloroplast rRNA and rRNA gene content of *Euglena* cells were made. When these results are analyzed throughout chloroplast development, a model for the regulation of the chloroplast rRNA gene emerges involving both increased gene activity and a gene dosage effect.

Materials and Methods

Materials. Ribonuclease T1 was purchased from Sigma Chemical Co. The sources of other materials have been described (Chelm and Hallick, 1976).

Growth of Cells. The preparation of dark-adapted cultures, the protocol for greening cultures, and the procedure for harvesting cells at various stages of chloroplast development have previously been described (Chelm and Hallick, 1976). Cellular DNA and RNA contents were determined using diphenylamine (Burton, 1968) and orcinol (Schneider, 1957) reactions, respectively, on cell extracts (Pogo et al., 1966).

Preparation of RNA and DNA. Chloroplast ribosomal RNA was prepared by the method of Zablen et al. (1975). Total cell RNA was extracted from *Euglena* as previously described (Chelm and Hallick, 1976). DNA was extracted from *Euglena* cells and purified by preparative CsCl density gradient centrifugation by the method of Richards et al. (1971). Following centrifugation and fractionation of the gradient all DNA-containing fractions were pooled and dialyzed against SSC.[1] The DNA was collected by ethanol precipitation, redissolved in 0.1 × SSC, and sheared by passage three times through an Amico 4-3398A French pressure cell at 12 000 psi. The resulting DNA fragment size was 500–600 bases, as determined by the method of Vinograd et al. (1963).

Preparation of ^{3}H-Labeled Chloroplast Ribosomal DNA. *Euglena* chloroplast DNA, enriched for the chloroplast rRNA sequences by the method of Rawson and Haselkorn (1973), was a gift from Oliver C. Richards. This DNA was radioactively labeled in vitro as previously described (Chelm and Hallick, 1976). Following purification by gel filtration, the DNA was dissolved in 3 ml of annealing buffer (0.3 M NaCl–1 mM NaEDTA–0.05 M Tris-HCl, pH 7.4) and denatured by heating at 94 °C for 10 min, followed by quick cooling. The solution was dialyzed against 0.12 M sodium phosphate–0.4% sodium dodecyl sulfate (pH 6.8). Single-strand DNA was separated from rapidly renaturable DNA by chromatography on hydroxylapatite (Kohne and Britten, 1971). The resulting single-strand DNA was annealed to a R_0t value of 0.115 M s at 68 °C in 0.12 M sodium phosphate buffer–0.4% sodium dodecyl sulfate (pH 6.8) with an excess of purified chloroplast rRNA. The C_0t value for the [^{3}H]DNA was 0.02 M s. Ribosomal RNA–[^{3}H]ct DNA hybrids were separated from single-strand [^{3}H]ct DNA by chromatography on hydroxylapatite (Kohne and Britten, 1971). The hybrids were treated with 0.3 N KOH at 37 °C for 16 h. The reaction mixture was neutralized and the resulting [^{3}H]rDNA was collected by ethanol precipitation. The DNA was redissolved in 0.1 × SSC and fractionated by centrifugation in an alkaline sucrose gradient (Chelm and Hallick, 1976). To further purify the [^{3}H]rDNA, the cycle of hybridization of the [^{3}H]rDNA with rRNA, hydroxylapatite chromatography, alkali digestion, alkaline sucrose gradient fractionation, and ethanol precipitation was repeated. The resulting DNA was redissolved in 0.1 mM NaEDTA and used for subsequent hybridization experiments.

DNA–RNA and DNA–DNA Hybridizations. Hybridization of total cell RNA or DNA to [^{3}H]ct rDNA in solution, and detection of hybrids, using an S1-nuclease criteria, have previously been described (Chelm and Hallick, 1976).

Data from hybridization experiments carried out with RNA excess were analyzed by a least-squares standard deviation fit to the pseudo-first-order rate equation $C/C_0 = e^{-kR_0t}$ with the value of C/C_0 at completion of the reaction as a variable. R_0 is the initial RNA concentration in moles of nucleotides/liter, t is the time in seconds, and C/C_0 is the fraction of the initial concentration (C_0) of the [^{3}H]ct rDNA remaining single stranded. The rate constant was then determined as the average rate constant for all the data points so normalized. Experiments carried out with nonradioactive DNA in excess of the hybridization probe were analyzed in the same way except the equation $C/C_0 = 1/(1 + kC_0t)$ (Britten and Kohne, 1968) was used.

RNase Resistance of DNA–DNA Hybrids. The sensitivity of DNA–DNA duplexes to ribonucleases at low ionic strength was used as a test for RNA–DNA hybrid contamination (Firtel, 1972). S1 nuclease was used for the analysis of RNase resistant duplexes.

Preparation and Renaturation of [^{3}H]pSC101 DNA. pSC101 DNA was isolated from a transformant of *E. coli* C600 $r_k^-m_k^-$ by the cleared lysis procedure (Guerry et al., 1973) and purified by equilibrium centrifugation in a CsCl–ethidium bromide gradient (Cohen and Miller, 1970), followed by centrifugation in a 5–20% sucrose gradient (Guerry et al., 1973). [^{3}H]pSC101 DNA, specific activity 3 Ci/mmol of nucleotides, was prepared by a previously described procedure (Chelm and Hallick, 1976). Renaturation of [^{3}H]pSC101 DNA with excess unlabeled pSC101 was performed as described above.

Results

[^{3}H]Chloroplast Ribosomal DNA Preparation and Properties. Chloroplast DNA, enriched as a heavy density "satellite" (ρ = 1.700 g/cm^3) for chloroplast ribosomal DNA sequences by equilibrium buoyant density centrifugation in CsCl, and activated by brief pancreatic DNase digestion was labeled in vitro by the action of *E. coli* DNA polymerase I with [^{3}H]TTP as a substrate. The specific activity of the ^{3}H-labeled satellite DNA was 10 μCi/μg of DNA, or 3.3 mCi/μmol of DNA nucleotide. At the completion of a ct rRNA driven hybridization (R_0t = 0.115 M s) to the [^{3}H]DNA, 8.3% of the [^{3}H]DNA were rRNA–[^{3}H]ct rDNA hybrids as assayed by S1-nuclease resistance. This 8.3% value is in agreement with a previous estimate (Rawson and Haselkorn, 1973) that ap-

[1] Abbreviations used are the following: rRNA, ribosomal RNA; SSC, 0.15 M NaCl–0.015 M sodium citrate; ctDNA, chloroplast DNA; rDNA, ribosomal DNA; ct rRNA, chloroplast ribosomal RNA; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

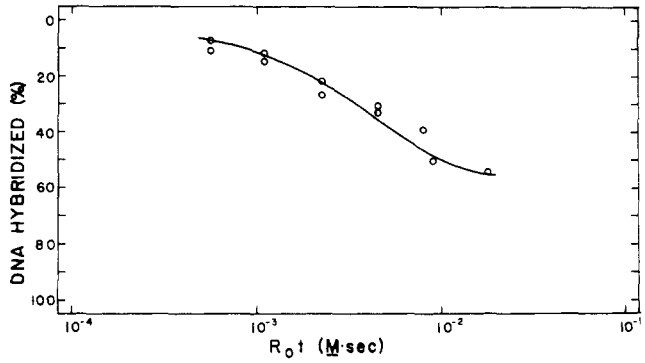

FIGURE 1: Hybridization of [^{3}H]chloroplast ribosomal DNA with excess, purified chloroplast ribosomal RNA. The experimental data are compared with the curve for the function $C/C_0 = e^{-kR_0t}$, with a best fit k = 220 l. mol^{-1} s^{-1}

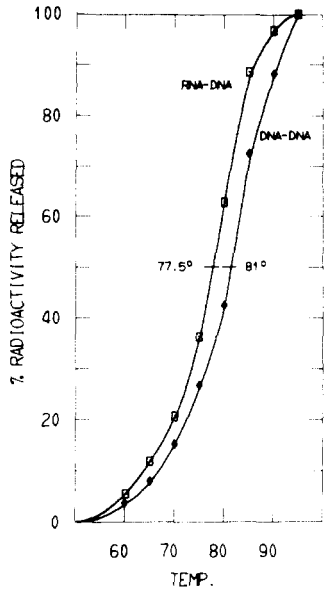

FIGURE 2: Thermal stability of [^{3}H]ct rDNA-DNA and [^{3}H]ct rDNA-RNA hybrids. The double-strand nucleic acid hybrids were collected on 1-ml hydroxylapatite columns in 0.12 M sodium phosphate and 0.4% sodium dodecyl sulfate (pH 6.8). Thermal stability of the hybrids was determined by raising the temperature and buffer in increments and subsequently eluting any material which had denatured with 2.0 ml of 0.12 M sodium phosphate and 0.4% sodium dodecyl sulfate (pH 6.8).

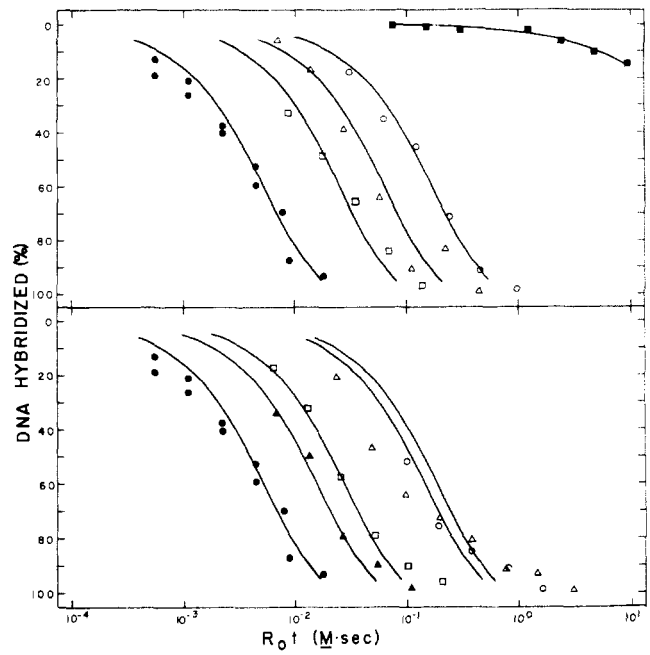

FIGURE 3: Hybridization of [^{3}H]ct rDNA with RNA extracted from *Euglena* cells at different stages of chloroplast development. Top panel: (●) pure chloroplast rRNA; (○) 4 h of development; (△) 12 h of development; (□) 48 h of development; (■) W$_3$BUL RNA. Bottow panel: (●) pure chloroplast rRNA; (○) 0 h of development; (△) 8 h of development; (□) 24 h of development; (▲) 72 h of development. Each point represents the average of triplicate reactions. Solid lines are those determined by computer fit as described in the Materials and Methods.

proximately 10% of the chloroplast satellite DNA is composed of rRNA genes.

The specific activity of the purified [^{3}H]ct rDNA hybridization probe should be less than that of the ^{3}H-labeled satellite DNA. The substrate for DNA labeling was [^{3}H]TTP, specific activity 54.3 mCi/μmol. The ^{3}H-labeled satellite DNA contains 30 mol % T calculated from the buoyant density, while the rRNA gene contains only 24.2 mol% T, as estimated from the rRNA base composition (Rawson and Stutz, 1969). Therefore the calculated specific activity of the [^{3}H]ct rDNA is 2.7 mCi/μmol of DNA nucleotide, representing a 20% substitution during the DNA polymerase reaction.

The kinetics of hybridization of [^{3}H]ct rDNA with excess purified chloroplast rRNA is shown in Figure 1. The renaturation reaction follows pseudo-first-order kinetics (Figure 1), with a best fit rate constant, k = 220 l. mol^{-1} s^{-1}. This corresponds to a $R_0t_{1/2}$ value of 2.74 × 10^{-3} M s for a kinetic complexity of 1.6 × 10^6 daltons, consistent with previously published values (Bishop et al., 1974; Levy and McCarthy, 1975). The [^{3}H]ct rDNA was consistently saturated by rRNA at 55–60% [^{3}H]DNA hybridized, suggesting that, even after two purifications of the probe as an rRNA-[^{3}H]rDNA duplex, there may be some non-rDNA sequence content.

In order to test the fidelity of base pairing of the [^{3}H]ct rDNA to RNA and DNA preparations, the thermal stability of RNA-DNA and DNA-DNA duplexes were measured using thermal elution chromatography on hydroxylapatite. The data are shown in Figure 2. DNA-DNA hybrids, prepared by hybridizing [^{3}H]rDNA with total chloroplast DNA to C_0t = 9.29 m s, exhibited a mean thermal stability of 81 °C. RNA-DNA hybrids, prepared by hybridizing [^{3}H]ct rDNA with purified chloroplast rRNA to R_0t = 0.067 M s, had a mean thermal stability of 77.5 °C. The 3.5 °C lowering of the thermal stability of RNA-DNA hybrids as compared with the homologous DNA-DNA duplex has been previously observed (Chelm and Hallick, 1976; Kohne, 1968; Brown and Church, 1971; Davidson and Hough, 1971; Firtel, 1972). The observed thermal stabilities for both RNA-DNA and DNA-DNA duplexes are consistent with those of well-matched duplexes (Chelm and Hallick, 1976).

Cellular Chloroplast rRNA Content during Chloroplast Development. To determine the fraction of cellular RNA represented by chloroplast rRNA, RNA-driven hybridizations to [^{3}H]ct rDNA were performed. The kinetics of hybridization of total cellular RNA, extracted from *Euglena* cells 0, 4, 8, 12, 24, 48, and 72 h after the onset of light-induced chloroplast development, were determined. The R_0t curves for all seven RNA preparations and also for purified ct rRNA are shown in Figure 3. For ease of comparison, the data are normalized to 100% hybridization. With increasing time of chloroplast development, a temporal increase in the rate of hybridization of total cellular RNA to [^{3}H]ct rDNA is observed, indicating that the ct rRNA sequences represent an increasingly larger

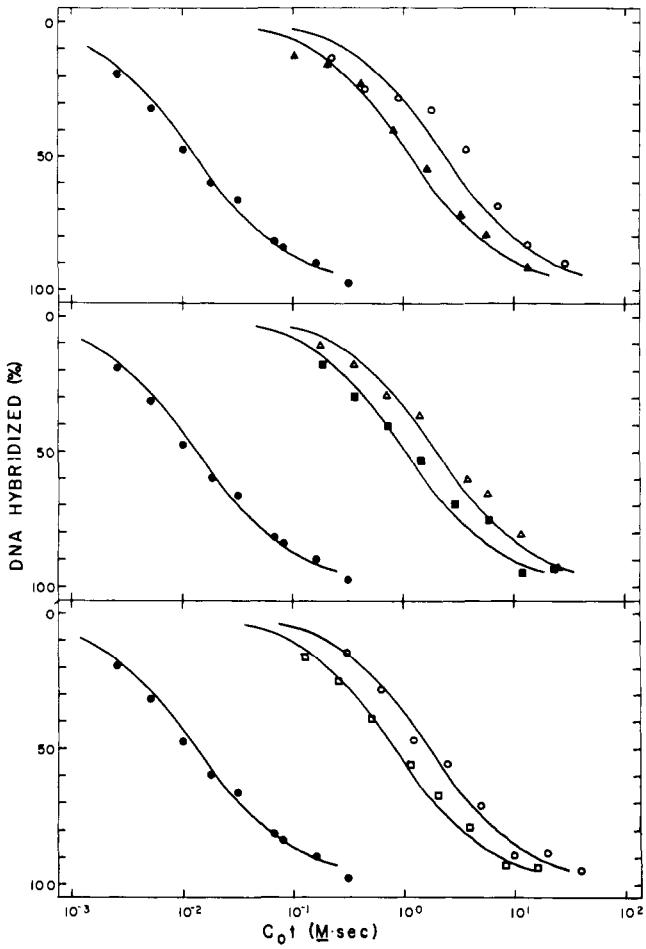

FIGURE 4: Hybridization of [^{3}H]ct rDNA with DNA extracted from *Euglena* cells at different stages of chloroplast development. Top panel: (●) pSC101 DNA; (○) 8 h of development; (▲) 12 h of development. Middle panel: (●) pSC101 DNA; (△) 4 h of development; (■) 48 h of development. Bottom panel: (●) pSC101 DNA; (○) 0 h of development; (□) 72 h of development. Each point represents the average of triplicate reactions. Solid lines are those determined by computer fit as described in the Materials and Methods.

TABLE I: Cellular Chloroplast rRNA Content during Chloroplast Development.

Hours of Chloroplast Development	Pseudo-First-Order[a] Rate Constant (l. mol^{-1} s^{-1})	ct rRNA Fraction[b] (%)
0	5.02 ± 1.04	2.28
4	5.49 ± 0.46	2.50
8	6.31 ± 1.56	2.87
12	13.8 ± 2.0[c]	6.27
24	32.6 ± 2.7	14.8
48	36.5 ± 7.7	16.6
72	58.3 ± 7.8	26.5
ct rRNA	220 ± 26	100

[a] Determined by best fit to the pseudo-first-order equation $C/C_0 = e^{-kR_0t}$. [b] Calculated using the equation ct rRNA fraction = k_{obsd}/k_{ctrRNA} pure × 100. [c] Values for 12, 24, 48, and 72 h are significantly different than 0-h value: $p < 0.01$, $p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively.

TABLE II: Cellular Chloroplast rDNA Content during Chloroplast Development.

Hours of Chloroplast Development	Second-Order[a] Rate Constant (l. mol^{-1} s^{-1})	ct rDNA Fraction[b] (%)
0	0.570 ± 0.055	0.44
4	0.474 ± 0.042	0.36
8	0.413 ± 0.068	0.32
12	0.846 ± 0.078[c]	0.65
48	0.908 ± 0.116	0.70
72	1.21 ± 0.088[d]	0.93
pSC101 DNA	71.6 ± 5.1	

[a] Determined from the data in Figure 4 by best fit to the equation $C/C_0 = 1/(1 + kC_0t)$. [b] Calculated as described in the text. [c] Significantly different from 0 h, $p < 0.05$. [d] Significantly different from 0 h, $p < 0.001$.

fraction of the total cell RNA. Also shown for comparison is the hybridization of RNA from the permanently bleached *Euglena* strain, W_3BUL, which lacks chloroplast DNA. No hybridization to [^{3}H]ct rDNA is observed. Quantitative estimates of the fraction of total cell RNA which is ct rRNA can be determined from the ratio of the rate constant for hybridization of total cell RNA to that for hybridization of pure ct rRNA. These results are compiled in Table I.

Chloroplast rRNA increases from a minimum of 2.3% of the total cellular RNA in dark-adapted *Euglena* to 26% of the total cellular RNA after 72 h of chloroplast development, an increase of 12-fold.

Cellular Chloroplast Ribosomal DNA Content during Chloroplast Development. One mechanism to increase chloroplast rRNA content during development would be to increase the rDNA gene content. It has been reported that light-grown *Euglena* cells contain more chloroplast DNA than dark-adapted cells (Rawson, 1975; Chelm, Hoben, and Hallick, 1977). In order to determine how much of the increase in ct rRNA abundance during chloroplast development is due to a gene dosage effect, the cellular content of chloroplast ribosomal DNA was measured during chloroplast development. By comparison of the rate of hybridization of cellular DNA from different developmental stages with [^{3}H]ct rDNA, quantitative estimates of the fraction of the cellular DNA which represents ct rDNA sequences can be made. The results for hybridizations of a large excess (>10^4-fold) of total cellular DNA, extracted from *Euglena* cells 0, 4, 8, 12, 48, and 72 h after the onset of chloroplast development, to trace amounts of [^{3}H]ct rDNA are shown in Figure 4. Second-order rate constants for the hybridization of each DNA fraction were determined by computer fit to the second-order renaturation equation, $C/C_0 = 1/(1 + kC_0t)$ (Britten and Kohne, 1968), as described in Materials and Methods. The second-order rate constants are compiled in Table II. From a comparison of these rate constants with that of a DNA sample with a known kinetic complexity, the fraction of the cellular DNA represented by ct rDNA sequences can be determined. The *E. coli* plasmid pSC101 DNA, whose base composition and kinetic complexity (51% G + C, 8.8 kbp) are similar to that of ct rDNA (51% G + C, 4.8 kbp), was used as a hybridization standard. [^{3}H]-pSC101 DNA, specific activity 3 mCi/μmol of DNA nucleotide, was renatured in the presence of excess, sheared non-radioactive pSC101 DNA. The second-order rate constant was 71.6 ± 5.1 l. mol^{-1} s^{-1}. The fraction of the cellular DNA that is ct rDNA is given by the equation (Chelm et al., 1977)

$$f = \frac{(k_{obsd})(X_{ctrDNA})}{(k_{pSC101})(X_{pSC101})}$$

TABLE III: Ribonuclease Treatment of [^{3}H]ct rDNA-DNA Duplexes.

Duplex[a]	Ribonuclease Treatment	[^{3}H]ct rDNA S1 Insensitive (cpm)
DNA-[^{3}H]ct rDNA	−	375
DNA-[^{3}H]ct rDNA	+	437
RNA-[^{3}H]ct rDNA	−	409
RNA-[^{3}H]ct rDNA	+	0

[a] In DNA-driven reactions, 72-h DNA (Table II) was incubated with [^{3}H]ct rDNA to a C_0t of 17 M s. In RNA-driven reactions, 72-h RNA (Table I) was incubated with [^{3}H]ct rDNA to a R_0t of 0.41 M s.

TABLE IV: Nucleic Acid Content of *Euglena gracilis* during Light-Induced Chloroplast Development.

Hours of Chloroplast Development	DNA[a] Content	RNA[a] Content
0	4.58 ± 0.41	39.30 ± 1.99
4	4.50 ± 0.46	34.48 ± 1.22
12	3.02 ± 0.12	28.10 ± 1.73
24	3.12 ± 0.22	24.62 ± 0.50
48	3.02 ± 0.25	19.38 ± 0.92

[a] Values are expressed as pg/cell. Each value is the average of four determinations ± SEM.

where X_{ctrDNA} and X_{pSC101} are the kinetic complexities of the chloroplast rRNA cistrons and pSC101 DNA, respectively. Calculated values of f for different times of chloroplast development are listed in Table II.

In dark-adapted *Euglena* cells, chloroplast ribosomal DNA is a significant fraction of the cellular DNA, 0.44%. After an initial lag period of at least 8 h at the onset of development, ct rDNA steadily increases as a cellular component, reaching 0.93% of the total cell DNA after 72 h of light-induced chloroplast development.

Since chloroplast rRNA is a large fraction of the cellular RNA as demonstrated in the previous section, extremely low levels of RNA contamination in the DNA extracts would significantly alter the results of DNA-driven reactions to [^{3}H]ct rDNA. We tested for this possibility by hybridizing total cellular DNA to [^{3}H]ct rDNA. Then, following a pretreatment of the hybrids with a mixture of ribonucleases A and T1, the S1-nuclease resistance of the [^{3}H]ct rDNA was determined. These results are shown in Table III. Treatment with ribonucleases did not affect the amount of [^{3}H]ct rDNA detected as hybrid in a DNA-driven reaction. However, in a control RNA-driven reaction, pretreatment with ribonucleases rendered all [^{3}H]ct rDNA sensitive to S1 nuclease. Therefore the hybrids detected in DNA-driven reactions are not contaminated by RNA-DNA hybrids.

Changes in the Number of Copies of Chloroplast Ribosomal RNA and Ribosomal RNA Genes during Chloroplast Development. In order to convert the hybridization data on cellular ct rRNA and rDNA content into estimates of the mass, or number of copies, of these species per cell, it was necessary to determine the total mass of RNA and DNA per *Euglena* cell. Cellular RNA and DNA contents were determined colorimetrically on extracts of cells that had previously been titered by hemocytometer cell counting. These results, for various times of chloroplast development, are shown in Table IV. Under our growth conditions, dark adaption in a heterotrophic medium followed by a switch to a photoautotrophic medium with the beginning of light growth, there is an initial decrease in cellular DNA content with the onset of light growth. However, after 12 h of chloroplast development, the cellular DNA content remains constant at 3 pg of DNA/cell, comparable to previously reported values (Brawerman, 1968). In contrast there is a gradual decrease in cellular RNA content throughout the developmental period.

From the data in Table I on the fraction of total cellular RNA as ct rRNA and the data in Table IV on total cell RNA content, we have calculated the number of copies of ct rRNA per cell at various developmental stages (Table V). During the first 8 h of development, the ct rRNA is at a relatively constant 3.2×10^5 copies/cell. This is followed by an increase to 1.9×10^6 copies/cell after 72 h of light growth, representing a sixfold increase in the accumulation of these rRNAs.

From the data in Tables II and IV, we have calculated the number of copies of ct rDNA per cell (Table V). Chloroplast ribosomal RNA genes increase from 1900 copies per cell following 8 h of chloroplast development to 5200 copies/cell after 72 h of chloroplast development. The maximum increase in the rRNA gene content is twofold, significantly lower than the observed sixfold increase in rRNA content. It is noteworthy that the ct rDNA content is higher than one would have estimated from the reported values for chloroplast DNA in *Euglena* (Rawson, 1975).

Also shown in Table V are values for the average number of copies of ct rRNA that accumulate per copy of the rRNA genes. This ratio increases from 90 copies per gene in dark-adapted cells to 370 copies per gene after chloroplast development is complete.

TABLE V: Changes in the Number of Copies of Chloroplast Ribosomal RNA and Chloroplast Ribosomal RNA Genes in *Euglena* during Chloroplast Development.

Hours of Chloroplast Development	Copies ct rRNA per Cell	Copies ct rDNA per Cell	Copies ct rRNA per Gene
0	3.4×10^5	3800	89
4	3.2×10^5	3000	110
8	3.2×10^5[a]	1900[c]	170
12	6.6×10^5	3700	180
48	1.2×10^6	4000	300
72	1.9×10^6[b]	5200[d]	370

[a] Calculated assuming 30.8 pg of RNA/cell. [b] Calculated assuming 19.0 pg of RNA/cell. [c] Calculated assuming 3.2 pg of DNA/cell. [d] Calculated assuming 3.0 pg of DNA/cell.

Discussion

We have described a quantitative and sensitive procedure to follow changes in the cellular content of individual genes and their transcript, 16S and 23S chloroplast ribosomal RNA, during the process of chloroplast development. This procedure was particularly applicable to the study of cell organelle transcription because the limited complexity and ease of isolation of the chloroplast genome facilitated preparation of the [^{3}H]ct rDNA hybridization probe. This probe had a high specific activity, formed duplexes with both rRNA and rDNA with faithful base pairing, and obeyed the expected kinetics in molecular hybridization reactions.

The major experimental finding of this work is that there is a temporal change in the cellular content of chloroplast ribosomal RNA in *Euglena gracilis* during light-induced chloroplast development. The 16S and 23S rRNAs increase from 2.3 to 26.5% of the cell RNA. Since there is a parallel decrease in the mass of RNA per cell, there is only a sixfold increase in the number of copies of each rRNA/cell. The increased rRNA content could reflect either increased rRNA transcription or rRNA stability. Since the rRNA transcripts are normally considered to be very stable RNA species, it is likely that there is increased transcription of the chloroplast rRNA genes during development. The postulated increase in transcription can not be due solely to a gene dosage effect, because the content of rRNA genes increases less than twofold during the time the rRNA content increases sixfold.

A second finding of this work is the high content of ct rRNA in dark-adapted cells, 3.4×10^5 copies per cell. It has previously been reported that ct rRNA could not be detected in dark-grown cells (Brown and Haselkorn, 1971; Heizmann, 1970). These previous studies were based on detection of ct rRNA by the criteria of size on polyacrylamide gels or sucrose gradients. Molecular hybridizations provide a much more sensitive test for RNA transcripts. The high content of rRNA in dark-grown cells is not surprising. Proplastid maintenance must require membrane biosynthesis, DNA replication, and presumably RNA transcription (Chelm and Hallick, 1976). If any of these functions are expressed via chloroplast protein synthesis, chloroplast ribosomes would be required.

Euglena seems to contain many more chloroplast rRNA genes than would be expected to be necessary for accumulation of rRNA at the observed levels. During light-induced development, the cells have a 24-h generation time and accumulate a maximum of 370 copies of rRNA per gene. If one assumes that the rRNA is stable, and that rRNA synthesis is continuous throughout the cell cycle, the in vivo average transcription rate is approximately 21 nucleotides per second per gene. In dark-adapted cells the rate is 7 nucleotides per second per gene. These rates are slower than the estimated transcription rate for rRNA synthesis in vivo in *E. coli* of 50–80 nucleotides per second per RNA polymerase (Pace, 1973), suggesting that chloroplast rRNA synthesis may occur during one part of the cell cycle, rather than being continuous at a low transcription level.

The procedures we have described for studying the expression of individual genes should be directly applicable to the study of other *Euglena* chloroplast transcripts. Although chloroplast rRNA did not prove to be a light-inducible transcript, it is possible that the transcription of other chloroplast genes may only be activated during chloroplast development. Further experiments will hopefully provide answers to this intriguing question.

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