Complexity and Abundance of Ribonucleic Acid Transcribed from Restriction Endonuclease Fragments of *Euglena* Chloroplast Deoxyribonucleic Acid during Chloroplast Development†

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ABSTRACT: Chloroplast DNA from *Euglena gracilis* was used to construct a partial library of recombinant plasmids representing 45% of the DNA. Each plasmid was radioactively labeled in vitro by nick translation and hybridized in liquid to a vast excess of total cellular RNA isolated either from cells grown continually in the dark or from cells containing developing chloroplasts. The complexity and abundance of the RNA that hybridized to the different chloroplast restriction endonuclease DNA fragments were calculated from the RNA–DNA hybridization saturation values and the pseudo-first-order hybridization rate constants, respectively. The complexity of these transcripts showed little change during chloroplast development. In several cases, the complexity of the RNA was greater than expected for asymmetrical transcription, suggesting the possibility that transcription may be symmetrical in some regions of chloroplast DNA. The abundance of the transcripts ranged from 0.0001% to nearly 10% of the total cellular RNA, and in some cases changed by as much as 5–10-fold during chloroplast development.

The unicellular alga *Euglena gracilis* is a simple eukaryotic organism in which the expression of both chloroplast and nuclear DNA can be studied during chloroplast development. When *Euglena* is grown in a heterotrophic medium in the dark, the cells are devoid of functional chloroplasts. If dark-grown *Euglena* cells are illuminated, a succession of macromolecular events occur which lead to the formation of a fully developed chloroplast. Rawson & Boerma (1976), Chelm & Hallick (1976), and Chelm et al. (1978, 1979) have measured by RNA–DNA hybridization the complexity and abundance of RNA derived from the entire chloroplast genome and RNA from several large chloroplast restriction endonuclease DNA fragments. These studies showed that (1) a large fraction of the transcripts derived from chloroplast DNA are present in dark-grown cells and throughout the development of the chloroplast, and (2) the abundance of the RNA derived from certain regions of the chloroplast DNA is developmentally regulated. The purpose of the following experiments is to determine the complexity and abundance of RNA derived from more narrowly defined regions of the chloroplast DNA. Chloroplast restriction endonuclease DNA fragments ranging in size from 2.7 to 8.7 kilobase pairs (kbp) and all together representing 45% of the chloroplast genome were cloned into bacterial plasmids and used in RNA–DNA hybridization experiments to develop a detailed transcriptional map of the chloroplast genome.

Materials and Methods

Cell Growth. Euglena gracilis Klebs (Z strain, The Culture Collection of Algae at the University of Texas at Austin, no. 753) cells were grown in a heterotrophic medium (Rawson & Boerma, 1976; Curtis & Rawson, 1979). Chloroplast development proceeded in cells which were first grown in the dark to stationary phase [$(5–6) \times 10^6$ cells/mL] and then transferred to the light (2500 lx) and maintained for various periods of time with constant shaking. The cell concentration and chlorophyll content of the cells were monitored during chloroplast development (Rawson & Boerma, 1976).

RNA Isolation. Total cell RNA was isolated according to Rawson & Boerma (1976). RNA was isolated from cells grown continuously in the dark (0-h RNA) and from cells which had been illuminated for either 24 (24-h RNA) or 48 (48-h RNA) h.

Chloroplast DNA Isolation. Supercoiled chloroplast DNA was prepared from *Euglena* cells grown continually in the light in a heterotrophic medium (Chelm & Hallick, 1977).

Construction of Recombinant Plasmids. The chloroplast and plasmid vector DNAs were digested with the appropriate restriction endonuclease and ligated by using T_4 DNA ligase as described earlier (Rawson & Andrews, 1981). Recombinant plasmids containing *Eco*RI chloroplast restriction endonuclease DNA fragments were constructed by using the plasmid vector pACYC184 (Tc^R, Cm^R) (Chang & Cohen, 1978). A *Sal*I chloroplast restriction endonuclease DNA fragment was cloned by using the plasmid vector pBR322 (Tc^R, Cm^R) (Bolivar et al., 1977). The resulting recombinant molecules constructed with the vector pACYC184 and *Eco*RI DNA fragments were transformed into the *Escherichia coli* strain SK2267 (*hsm*$^+$ *hsr*$^-$ *endo*I$^-$ *exo*I$^-$ *rec*A1$^-$) as described by Kushner (1978). The recombinant plasmid constructed by using the vector pBR322 and the *Sal*I DNA fragment was transformed into the *E. coli* strain SK1590 (*hsm*$^+$ *hsr*$^-$ *endo*I$^-$ *exo*I$^-$). Both strains of *E. coli* were supplied by Dr. Sidney Kushner of the University of Georgia. The presence of bacterial colonies containing recombinant plasmids was determined by the appropriate antibiotic sensitivity. The transformed strains of *E. coli* carrying putative recombinant DNA molecules were screened for the presence of chloroplast restriction endonuclease DNA fragments by a modification of a procedure used for screening λ recombinants (Blattner et al., 1978; Rawson & Andrews, 1980). The construction of the plasmid pVK52 has been described previously (Rawson & Boerma, 1976). All experiments were carried out under P2/EK1 conditions as specified by the *NIH Guidelines for Research Involving Recombinant DNA* (1978).

Isolation of Recombinant Plasmids. Bacterial strains containing recombinant plasmids were grown in Luria broth

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(Kushner, 1974) supplemented with the antibiotic (20 μg/mL) appropriate for maintaining a selective pressure upon the plasmids. The bacterial strains were grown to a final density of 5×10^8 cells/mL, and chloramphenicol was added to final concentration of 250 μg/mL. The cells were shaken overnight at 37 °C to permit amplification of the plasmid. Plasmid DNA was isolated from a cleared cell lysate by chromatography on an agarose A-50 column and centrifugation in CsCl–ethidium bromide gradients (Rawson et al., 1978). The ethidium bromide was removed from the supercoiled plasmid DNA by extraction with 2-propanol, and then the DNA was dialyzed against several changes of TE [10 mM Tris-HCl (pH 7.6) and 10 mM EDTA].

Nick Translation of Plasmid DNA. Plasmid DNA was radioactively labeled by the method of nick translation (Rigby et al., 1977). The reaction mixture (50–100 μL) contained 50–200 ng of DNA, 25 μM dCTP, dGTP, and dTTP, 50 μCi of [α-^{32}P]dATP (400 Ci/mmol), 5 mM $MgCl_2$, 50 mM Tris-HCl (pH 8), 10 mM 2-mercaptoethanol, 50 μg/mL bovine serum albumin, and 5 units of *E. coli* DNA polymerase I (Boehringer Mannheim). The reaction mix was incubated overnight at 14 °C and then adjusted to 0.5 mL with 12 mM sodium phosphate (pH 6.8). The DNA was boiled for 5 min, incubated at 60 °C for 30 s, and adsorbed onto 0.2 g of hydroxylapatite in 12 mM sodium phosphate (pH 6.8) at 60 °C. Unincorporated nucleotides were eluted with 50–100 mL of 12 mM sodium phosphate (pH 6.8), and then the single-stranded DNA was collected by wasing the column with several milliliters of 0.12 M sodium phosphate. The self-complementary regions of DNA generated by the DNA polymerase I remained bound to the column. The ^{32}P-labeled single-stranded plasmid DNAs were concentrated by the addition of 10 μg of λ DNA and centrifugation in a Beckman type 65 rotor at 50 000 rpm overnight. The DNA pellet was suspended in 0.1–0.2 mL of TE buffer. The specific activity of the ^{32}P-labeled plasmid DNA before removal of the self-complementary sequences was $(0.5–1.5) \times 10^8$ cpm/μg of DNA.

Hybridization Reaction. A vast excess of RNA was hybridized in liquid to ^{32}P-labeled plasmid DNA [$(1–5) \times 10^6$:1] at 60 °C in 100 mM Tris-HCl (pH 8.0) and 0.3 mM NaCl. In order to minimize degradation of the nucleic acids during the hybridization reaction, these buffers were passed over a Chelex 100 (Bio-Rad Labs) column to remove possible contaminating heavy metals. Reaction mixtures of 250 μL were prepared in 3-mL glass conical tubes and overlayed with mineral oil. The hybridization reactions were started by boiling the mixture for 5 min and then transferring the mix to a 60 °C bath. At various times, 10-μL samples were removed from the reaction mixture and analyzed by S1 nuclease digestion.

The fraction of ^{32}P-labeled plasmid DNA in the form of a double-stranded structure was determined by its resistance to S1 nuclease. The S1 nuclease assay was performed by a modificatin of the DEAE filter-paper technique of Maxwell et al. (1978). Aliquots from the hybridization reactions were diluted into a final volume of 150 μL of S1 nuclease assay buffer [1 mM $ZnSO_4$, 25 mM sodium acetate (pH 4.5), 0.20 M NaCl, and 5 mM 2-mercaptoethanol] containing 200 units of S1 enzyme (Sigma) and 1.5 μg of calf thymus DNA. A 50-μL sample was immediately spotted onto a DE-81 filter disk (Whatman) to determine the total amount of ^{32}P-labeled plasmid DNA in a given volume. The remaining volume was incubated at 37 °C for 2 h, and a second 50-μL aliquot was then spotted onto a similar disk to assay for ^{32}P-labeled plasmid DNA present as double-stranded structures. The filters were washed 3 times with 0.48 M sodium phosphate (pH 6.8) to remove S1 digestion products, dried, and counted in a Packard liquid scintillation counter (Rawson & Boerma, 1976).

Table I: Properties of Recombinant Plasmids Containing Chloroplast DNA[a]

recombinant plasmid	chloroplast restriction endonuclease DNA fragment[b]	size of chloroplast DNA fragment (kbp)
pVK52	*Bam*HI-E	5.48
pECS1	*Sal*I-C	4.2
pECR1	*Eco*RI-I	4.7
pECR2	*Eco*RI-F	7.3
pECR4	*Eco*RI-J[c]	3.6
pECR11	*Eco*RI-O	2.7
pECR13	*Eco*RI-M	3.1
pECR16	*Eco*RI-G	7.0
pECR17	*Eco*RI-D	8.77
pECR18	*Eco*RI-H	5.5

[a] Construction of recombinant DNA molecules with pBR313 and pBR322 by using the restriction endonuclease *Bam*HI and *Sal*I produced plasmids which are $Tc^S Amp^R$. When recombinants are constructed with pACYC184 and the enzyme *Eco*RI, the resulting plasmids are $Tc^R Cm^S$. [b] The different chloroplast restriction endonuclease fragments are referred to by the restriction endonuclease used to generate that fragment and a letter which refers to the relative descending size of the fragments produced by that enzyme. [c] There are two *Eco*RI DNA fragments designated J in an *Eco*RI digest of the *Euglena* chloroplast DNA. One has a *Bam*HI site; the other does not. This *Eco*RI-J DNA fragment contains the *Bam*HI site.

The double-stranded and single-stranded activity of the S1 nuclease was monitored throughout this series of experiments (Curtis & Rawson, 1979). No double-stranded activity was ever detected, and the single-stranded activity was greater than 96%. The extent of self-renaturation of each plasmid DNA during the hybridization reactions was determined in parallel experiments by using the same hybridization mixture minus the driver RNA. The fraction of plasmid DNA present as an RNA–DNA hybrid was calculated by subtracting the extent of self-renaturation of the DNA from the fraction of the plasmid DNA present as a double-stranded duplex. The fraction of plasmid DNA in the form of an RNA–DNA hybrid was measured as a function of the initial RNA concentration (R_0, M nucleotides) multiplied by the time (t, s). The R_0t values were reported as equivalent R_0t (ER_0t) values, indicating the correction for acceleration of the rate of renaturation in other than standard Na^+ concentrations (Britten et al., 1974).

Analysis of Data. The hybridization reactions were assumed to follow pseudo-first-order kinetics (Galau et al., 1974) and were fit to an appropriate curve by using a nonlinear least-squares regression (Pearson et al., 1977).

Results

Characterization of Recombinant Plasmids Carrying Chloroplast DNA. Ten recombinant plasmids were constructed which contained sequences representing approximately 45% (61 kbp) of the chloroplast DNA. The plasmids were each mapped by using a variety of restriction endonucleases to verify the presence of the appropriate chloroplast DNA fragment. Table I summarizes the characteristics of the recombinant plasmids used in these studies. The plasmid pECR17 was unstable in a variety of strains of *E. coli* used in other studies (W. H. Andrews and J. R. Y. Rawson, unpublished experiments) and was, therefore, carefully mapped each time it was isolated and compared to the map of the original isolate.

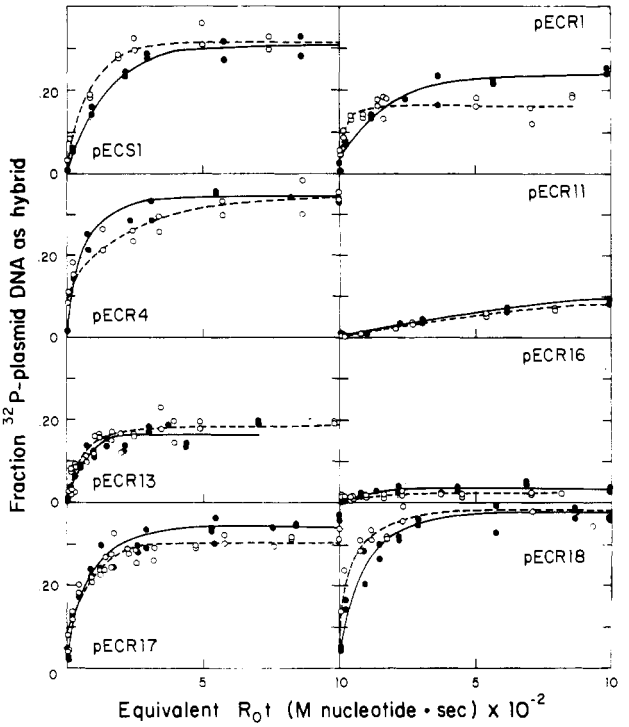

FIGURE 1: Hybridization of ^{32}P-labeled plasmid DNAs to total cell RNA from *Euglena* cells at different stages of chloroplast development. The curves represent the hybridization of ^{32}P-labeled plasmid DNAs to an excess of total cell RNA [(1–5) × 10^6:1] isolated from cells grown continuously in the dark (0-h RNA) (●—●) or from cells undergoing chloroplast development (25- or 48-h RNA) (O---O). The plasmid DNAs pECS1, pECR1, pECR4, pECR11, pECR16, pECR17, and pECR18 were hybridized to 48-h RNA. The plasmid DNA pECR13 was hybridized to 24-h RNA.

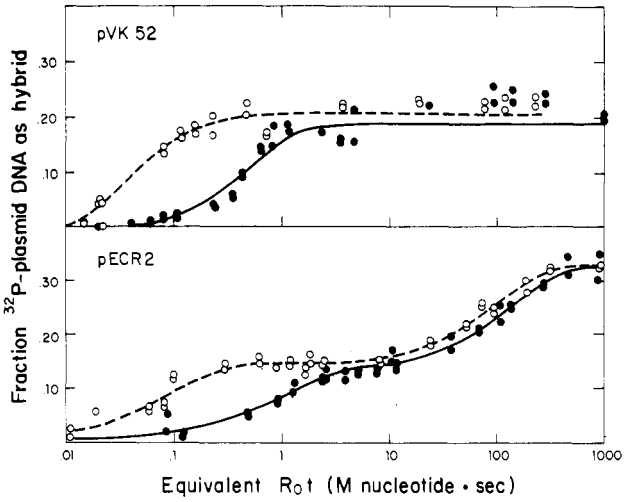

FIGURE 2: Hybridization of ^{32}P-labeled plasmid DNAs containing ribosomal DNA sequences to total cell RNA from *Euglena* cells at different stages of chloroplast development. The curves represent the hybridization of ^{32}P-labeled plasmid DNA containing the entire ribosomal DNA (pVK52) or part of the 23S ribosomal DNA (pECR2) to an excess of total cell RNA (10^4–10^6:1) isolated from cells grown continuously in the dark (0-h RNA) (●—●) or from cells undergoing chloroplast development (48-h RNA) (O---O).

Complexity of RNA Sequences Transcribed from Chloroplast Restriction Endonuclease Fragments. The ten recombinant plasmids listed in Table I were each nick translated and hybridized to total cellular RNA from dark-grown cells (0-h RNA) and from cells undergoing chloroplast development (24- or 48-h RNA). The extent of hybrid formation as a function of R_0t was assayed by using S1 nuclease. Figures 1 and 2 show the kinetics of hybridization of the ten different recombinant plasmids to the various RNA preparations. The saturation hybridization value obtained for each recombinant plasmid DNA was used to determine the complexity [in kilobases (kb)] of the transcripts derived from that chloroplast restriction endonuclease DNA fragment contained on the plasmid. The complexity of the RNAs derived from the different chloroplast restriction endonuclease DNA fragments during chloroplast development is summarized in Table II.

All of the DNA fragments analyzed in these experiments were observed to be transcribed to some degree. The complexity of the RNA derived from the fragments *Bam*HI-E, *Eco*RI-D, -F, -G, -H, -J, and -M, and *Sal*I-C did not change significantly when dark-grown cells were placed in the light. The *Bam*HI-E fragment, which contains a complete ribosomal RNA cistron (Rawson et al., 1978), the 5S gene (Gray & Hallick, 1979), and several tRNA genes (Orozco & Hallick, 1980), is completely transcribed. The *Eco*RI-F fragment, which contains part of a 23S ribosomal RNA gene and DNA coding for unknown RNA products, is also completely transcribed. Only a small percentage (less than 10%) of *Eco*RI-G, approximately two-thirds of *Eco*RI-D, and 80% of *Eco*RI-M are transcribed.

Part of both the *Eco*RI-I and -O DNA fragments which are transcribed in the dark is not represented by RNA transcripts 48 h after the initiation of chloroplast development. The complexity of the RNA derived from *Eco*RI-I is 4.2 kb in dark-grown cells and drops to 2.5 kb in cells which had been in the light for 48 h. The complexity of RNA derived from *Eco*RI-O drops by approximately one-third (0.7 kb) during this same period.

The transcription of the DNA fragments *Sal*I-C and *Eco*RI-H and -J is unusual in that the complexity of the RNA (in kilobases) is greater than the complexity of the individual DNA fragments (in kilobase pairs) themselves. This indicates that at least a fraction of these DNA fragments are symmetrically transcribed.

Abundance of RNA Transcripts from Chloroplast Restriction Endonuclease Fragments. The fraction of the total mass or the abundance of the RNA which drives a hybridization reaction can be calculated from the observed rate constant for the reaction by comparing it to a known kinetic standard (Galau et al., 1974). By use of similar conditions described earlier, the rate constant for the reassociation of our standard (*E. coli* DNA) is 0.20 M^{-1} s^{-1}. The observed pseudo-first-order rate constant for the hybridization of each recombinant plasmid DNA to the different RNAs was calculated from the experiments shown in Figures 1 and 2 and is summarized in Table III. All the plasmid DNAs except pECR2, which carries the DNA fragment *Eco*RI-F, hybridized to the different RNAs as a single pseudo-first-order reaction (Figures 1 and 2). The hybridization of the plasmid pECR2 to both 0- and 48-h RNA shows two distinct pseudo-first-order reactions whose rate constants were easily distinguishable from one another (Figure 2, bottom panel).

The percent of the total cell RNA mass driving these hybridization reactions [$(0.5K_{obsd}/K_{expected})100$] was calculated from these data. The average number of diverse transcripts from each chloroplast DNA fragment was calculated by dividing the complexity of the RNA transcribed from each fragment into the product of the total cellular RNA content (12 pg or 2.1 × 10^7 kb; Parenti et al., 1969) and the fraction of the total RNA driving the reaction (Galau et al., 1974). These numbers are also summarized in Table III.

The abundance of the transcripts from these different chloroplast restriction endonuclease DNA fragments varies as much as 5 orders of magnitude. The abundance of the

Table II: Complexity of RNA Transcribed from Chloroplast Restriction Endonuclease DNA Fragments

plasmid	chloroplast restriction endonuclease DNA fragment	RNA preparation[a] (h)	fraction of plasmid DNA as RNA-DNA duplex ± SE[b]	fraction of chloroplast restriction endonuclease DNA fragment transcribed[c]	complexity of RNA transcribed (kb)[d]
pVK52	*Bam*HI-E	0	0.186 ± 0.008	0.97	5.3
		48	0.204 ± 0.007	1.05	5.8
pECS1	*Sal*I-C	0	0.307 ± 0.007	1.17	5.0
		48	0.316 ± 0.009	1.22	5.1
pECR1	*Eco*RI-I	0	0.241 ± 0.013	0.89	4.2
		48	0.164 ± 0.006	0.60	2.8
pECR2	*Eco*RI-F	0	0.120 ± 0.008	0.37	2.7
			0.194 ± 0.008	0.60	4.4
		48	0.137 ± 0.013	0.42	3.1
			0.184 ± 0.009	0.57	4.2
pECR4	*Eco*-RI-J	0	0.343 ± 0.014	1.44	5.2
		48	0.347 ± 0.019	1.45	5.3
pECR11	*Eco*RI-O	0	0.149 ± 0.013	0.74	2.0
		48	0.106 ± 0.010	0.52	1.4
pECR13	*Eco*RI-M	0	0.164 ± 0.006	0.75	2.3
		24	0.182 ± 0.006	0.83	2.6
pECR16	*Eco*RI-G	0	0.036 ± 0.003	0.07	0.8
		48	0.027 ± 0.003	0.05	0.6
pECR17	*Eco*RI-D	0	0.340 ± 0.008	0.68	6.0
		48	0.301 ± 0.006	0.60	5.3
pECR18	*Eco*RI-H	0	0.367 ± 0.011	1.26	7.0
		48	0.376 ± 0.016	1.29	7.1

[a] Total cell RNA was prepared from cells grown continuously in the dark to stationary phase (0-h RNA) and from cells which were grown in the dark to stationary phase and then transferred and maintained under continued illumination for either 24 (24-h RNA) or 48 (48-h RNA) h. [b] Fraction of plasmid as RNA-DNA hybrid = (total ^{32}P-labeled plasmid DNA resistant to S1 nuclease in presence of RNA) − (total ^{32}P-labeled plasmid DNA resistant to S1 nuclease in absence of RNA) ± SE. [c] Fraction of chloroplast restriction endonuclease DNA fragment transcribed = [fraction of plasmid as RNA-DNA hybrid × 2(molecular weight of plasmid)](molecular weight of chloroplast restriction endonuclease DNA fragment). [d] Complexity of RNA transcribed = (fraction of chloroplast restriction endonuclease DNA fragment transcribed) × (molecular weight of chloroplast restriction endonuclease DNA fragment).

Table III: Abundance of RNA Transcripts from Chloroplast Restriction Endonuclease DNA Fragments

plasmid	chloroplast restriction endonuclease DNA fragment	RNA preparation[a] (h)	K_{obsd} ± SE (M^{-1} s^{-1})[b]	% total cell RNA driving reaction[c]	no. of copies of transcripts/cell[d]
pVK52	*Bam*HI-E	0	1.85 ± 0.25	0.43	1.7×10^4
		48	14.2 ± 2.24	3.63	1.3×10^5
pECS1	*Sal*I-C	0	$7.4 \times 10^{-3} \pm 0.6 \times 10^{-3}$	1.6×10^{-3}	70
		48	$1.1 \times 10^{-2} \pm 0.2 \times 10^{-2}$	2.5×10^{-3}	100
pECR1	*Eco*RI-I	0	$5.2 \times 10^{-2} \pm 1.1 \times 10^{-3}$	9.5×10^{-4}	50
		48	$3.6 \times 10^{-2} \pm 1.2 \times 10^{-2}$	4.5×10^{-3}	350
pECR2	*Eco*RI-F	0	0.91 ± 0.15	0.56	4.3×10^4
			$6.9 \times 10^{-3} \pm 0.81 \times 10^{-3}$	1.1×10^{-2}	500
		48	10.1 ± 1.91	7.1	4.8×10^5
			$9.4 \times 10^{-3} \pm 1.19 \times 10^{-3}$	1.2×10^{-2}	600
pECR4	*Eco*RI-J	0	$1.5 \times 10^{-2} \pm 0.4 \times 10^{-2}$	3.3×10^{-3}	130
		48	$3.8 \times 10^{-3} \pm 1.1 \times 10^{-3}$	8.8×10^{-4}	40
pECR11	*Eco*RI-O	0	$1.1 \times 10^{-3} \pm 0.15 \times 10^{-3}$	9.2×10^{-5}	10
		48	$1.3 \times 10^{-3} \pm 0.20 \times 10^{-3}$	8.0×10^{-5}	20
pECR13	*Eco*RI-M	0	$1.6 \times 10^{-2} \pm 0.2 \times 10^{-2}$	1.6×10^{-3}	1500
		24	$1.4 \times 10^{-2} \pm 0.9 \times 10^{-2}$	1.6×10^{-3}	1100
pECR16	*Eco*RI-G	0	$8.3 \times 10^{-3} \pm 2.9 \times 10^{-3}$	2.9×10^{-4}	80
		48	$2.7 \times 10^{-3} \pm 1.2 \times 10^{-3}$	7.1×10^{-4}	250
pECR17	*Eco*RI-D	0	$1.1 \times 10^{-2} \pm 0.2 \times 10^{-2}$	2.9×10^{-3}	100
		48	$1.1 \times 10^{-2} \pm 0.1 \times 10^{-2}$	2.6×10^{-3}	100
pECR18	*Eco*RI-H	0	$9.2 \times 10^{-3} \pm 1.4 \times 10^{-3}$	2.8×10^{-3}	80
		48	$1.2 \times 10^{-2} \pm 0.3 \times 10^{-3}$	3.8×10^{-3}	110

[a] Total cell RNA was prepared from cells grown continuously in the dark to stationary phase (0-h RNA) and from cells which were grown in the dark to stationary phase and then transferred and maintained under constant illumination for either 24 (24-h RNA) or 48 (48-h RNA). h. [b] The observed K for hybridization of ^{32}P-labeled plasmid DNA to total cell RNA was determined by assuming pseudo-first-order kinetics. [c] Percent of total RNA driving the reaction = $(0.5\ K_{obsd}/K_{pure}) \times 100$. [d] Number of copies of transcripts/cell = (2.1×10^{10} nucleotides of RNA/cell × fraction of RNA driving reactions)/(complexity of RNA transcribed).

RNA from the DNA fragments *Sal*I-C and *Eco*RI-D, -H, -M, and -O shows no change during chloroplast development. The abundance of the RNA derived from *Eco*RI-J shows a 4-fold decrease when cells are removed from the dark and exposed to light for 48 h. The transcripts from the DNA fragments *Eco*RI-G, -I, and *Bam*HI-E (rDNA genes) show a 4-, 7-, and 10-fold increase, respectively, in their abundance 48 h after the initiation of chloroplast development. The abundance of

the transcripts from the DNA fragment *Eco*RI-F varies considerably. Approximately 40% (2.3 kbp) of this DNA fragment contains 70% of the 23S rRNA nucleotide sequence (Rawson et al., 1978) and represents that fraction of the plasmid hybridizing to RNA sequences at the low R_0t values. The observed K values of this part of the reaction for both 0- and 48-h RNA are similar to the observed K values for the plasmid carrying *Bam*HI-E (Table III and Figure 2, top panel). The function of the remaining 5 kbp of this DNA fragment is unknown and does not seem to be developmentally regulated during chloroplast development. Only about 0.01% of 0- and 48-h total cellular RNA is derived from this portion of the *Eco*RI-F DNA fragment.

Discussion

Rawson & Boerma (1979) have shown by pulse labeling of RNA during chloroplast development that most of the *Eco*RI chloroplast restriction endonuclease DNA fragments are transcribed both in the dark and throughout chloroplast development. These observations suggested that the transcripts from these individual DNA fragments might show considerable change in either their complexity or their abundance. However, the experiments described here show that no significant increase in the complexity of the RNA transcripts derived from chloroplast DNA fragments used in these studies occurred when dark-grown cells are allowed to undergo chloroplast development for 48 h. The complexity of the transcripts from *Eco*RI-I and -O actually showed a decrease in complexity of 1.4 and 0.7 kb, respectively, during this time.

More surprising is the observation that the transcription from three restriction endonuclease DNA fragments (*Sal*I-C and *Eco*RI-H and -J) is at least to some degree symmetrical. It is not possible from these experiments to calculate how much of one strand of each DNA fragment is transcribed, though one can make a minimum estimate as to the fraction of the DNA fragment that is symmetrically transcribed. If one assumes that all of one strand of each DNA fragment is transcribed, then at least 0.8, 1.5, and 1.6 kbp of the DNA fragments *Sal*I-C and *Eco*RI-H and -J, respectively, are symmetrically transcribed. It is not likely that these measurements are the result of experimental error because the sensitivity of the single-stranded radioactive plasmid DNA to S1 nuclease digestion is measured concurrently with each hybridization experiment. Similar observations of symmetrical transcription of the chloroplast DNA in *Chlamydomonas* have been made by Howell & Walker (1977). They found that the overall complexity of the RNA from the chloroplast DNA was greater than the overall complexity of the DNA. An even more intriguing observation is the recent report (Ikegami & Fraenkel-Conrat, 1979) that tobacco leaves contain a small fraction of double-stranded RNA, the expected product of symmetrical transcription, and that this material is enriched for by isolating chloroplasts. In order to actually demonstrate the symmetrical transcription of a *Euglena* chloroplast DNA fragment, RNA should be isolated from an RNA–DNA hybrid formed with such a fragment and analyzed for its ability to reassociate with itself.

The chloroplast restriction endonuclease DNA fragments contained on the recombinant plasmids used in these experiments represent approximately 45% or 61 kbp of the chloroplast DNA. (The size of *Bam*HI-E is included 3 times in these calculations, because there are three rDNA repeats. Only 5.0 kbp of *Eco*RI-F, which is not rDNA, are added into these numbers.) The total unique complexity of the RNA in dark-grown cells derived from these DNA fragments is 42.2 kb. The total unique complexity of RNA from these DNA fragments during chloroplast development is 40.2 kb. These estimates include the complexity of the symmetrical transcripts.

The abundance of the transcripts derived from the different DNA fragments used in these studies varies tremendously. The least abundant transcripts originate from the DNA fragment *Eco*RI-O and are present as only about ten copies per cell. The most abundant transcripts are those from the rDNA (*Bam*HI-E) and are present between 10^4 and 10^5 copies per cell, depending on the stage of chloroplast development. These numbers for the transcription of the rDNA are in agreement with those of Chelm et al. (1977, 1978). The abundance of the transcripts from the DNA fragment *Eco*RI-I also increases considerably (7-fold) after 48 h of illumination, while *Eco*RI-G only increases about 3-fold during this same time. On the other hand, the transcripts from the fragment *Eco*RI-J decrease approximately 4-fold after 48 h of illumination of the cells. The abundance of the transcripts from the remainder of the fragments shows little change from that in the dark to that in cells which are undergoing chloroplast development.

An interesting number to calculate is how many times a given restriction endonuclease DNA fragment in a single chloroplast DNA molecule is represented as an RNA transcript in the cell. With the assumption that there are approximately 500–600 chloroplast DNA molecules per cell (Rawson & Boerma, 1976), the majority of the restriction endonuclease DNA fragments used in our experiments were represented as RNA transcripts in the cell on the average of somewhat less than once per chloroplast DNA molecule. The two exceptions to this statement are the rDNA genes and the fragment *Eco*RI-M which are represented as RNA transcripts 20–200 times and 2-3 times per chloroplast genome, respectively.

Chelm et al. (1979) recently depicted a transcriptional program of the chloroplast genome of *Euglena* by using a slightly different approach. They took advantage of the fact that the chloroplast DNA could be subdivided into large restriction endonuclease DNA fragments with the enzyme *Pst*I. They then used these *Pst*I DNA fragments to hybridize to RNA isolated from cells undergoing greening in a photoautotrophic media. The resolution of the experiments of Chelm et al. (1979) was limited by the large size of the DNA fragments they use in their experiments, although by this approach they were able to consider the entire chloroplast genome. When our data are compared to those of Chelm et al. (1979), there is general agreement in the abundance of transcripts from various regions of the DNA. Certain data can be compared directly. Chelm et al. (1979) report that the complexity of the RNA derived from *Eco*RI-H in cells grown in the dark is 0.8 kb. We find that the complexity of the RNA derived from this same DNA fragment is actually greater (7 kb) than the DNA fragment (5.5 kbp) itself, indicating that a fraction of this region of the chloroplast DNA produces symmetrical transcripts. The complexity of the RNA that we have measured from the fragments *Eco*RI-D and -H (12–13 kb) would argue that Chelm et al. (1979) should have detected RNA with a greater complexity from *Pst*I-E if this DNA fragment is expressed similarly in cells undergoing chloroplast development in a heterotrophic and a photoautotrophic media. It is not unlikely that the metabolic processes involved in chloroplast development in a heterotrophic and a photoautotrophic media require the expression of different regions of the chloroplast genome. Link et al. (1978) have shown that chloroplasts in mesophyll and bundle sheath cells, which are carrying out different modes of CO_2 fixation, show selective expression of

the restriction endonuclease DNA fragment containing the gene for the large subunit of ribulose-1,5-bisphosphate carboxylase.

In summary, we have measured the complexity and the abundance of RNA derived from chloroplast restriction endonuclease DNA fragments representing 45% of this genome. The DNA fragments used in these experiments range in size from 2.7 to 8.8 kbp and, therefore, each represent an average of 5–16 genes. The expression of these DNA fragments can be categorized as follows: (1) some show no change in the complexity or the abundance of transcripts, (2) some show a decrease in the complexity of the RNA transcribed after chloroplast development has been allowed to proceed for 48 h, and (3) the transcripts from some DNA fragments show significant increases in their abundance while at least one fragment (*Eco*RI-J) shows a decrease in the abundance of its transcripts. Overall, it appears that the selected regions of the chloroplast genome in *Euglena* are constitutively transcribed in the dark and during chloroplast development while other regions are differentially regulated during chloroplast development.

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