Parker, F. S., & Irvin, J. L. (1952) *J. Biol. Chem. 199,* 897.
Radloff, R., Bauer, W., & Vinograd, J. (1967) *Proc. Natl. Acad. Sci. U.S.A. 57,* 1514.
Schmitz, H., Crook, K. E., Jr., & Bush, J. A. (1967) *Antimicrob. Agents Chemother. 1966,* 606.
Sequin, U., Beford, C. T., Chung, S. K., & Scott, A. I. (1977) *Helv. Chim. Acta 60,* 896.
Szybalski, W. (1968) *Methods Enzymol. 12B,* 331.
Waring, M. J. (1966) *Biochim. Biophys. Acta 114,* 234.
White, H. L., & White, J. R. (1966) *Biochim. Biophys. Acta, 123,* 648.
White, H. L., & White, J. R. (1969) *Biochemistry 8,* 1030.

Mapping of Transcribed Regions of *Euglena gracilis* Chloroplast DNA†

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ABSTRACT: RNA transcription from defined regions of the *Euglena gracilis* chloroplast genome has been characterized by hybridization of total cell RNA to ^{3}H-labeled chloroplast DNA restriction endonuclease fragments. Chloroplast DNA was digested into five fragments of 53, 35, 25, 10, and 6.9 kilobase pairs (kbp) with *Pst*1. The 53-kbp DNA was also subfractionated by *Bam*HI digestion. The extent of transcription of the *Pst*1 fragments was found to be 30, 17, 15, 2.2, and 2.3 kb of RNA, respectively. The total amount of RNA transcription of 67 kb represents 26% of the double-strand information content of the genome. Transcribed regions are dispersed throughout the DNA. The RNA transcripts are present in two major abundance classes in the cell. High abundance transcripts of approximately 10^6 copies/cell were mapped in the rRNA gene region of the 53-kbp fragment and in the 35-kbp fragment. Low abundance transcripts of approximately 1000–4000 copies/cell were mapped in all five *Pst* fragments.

The chloroplast genome of *Euglena gracilis* is a covalently closed superhelical double-strand DNA of 130–140 kbp (Manning & Richards, 1972; Gray & Hallick, 1978a). This DNA is extensively transcribed in *Euglena* at all stages of chloroplast development (Chelm & Hallick, 1976; Rawson & Boerma, 1976). In rapidly growing cells containing fully developed chloroplasts 23% of the DNA or approximately 60 kb of RNA is transcribed (Chelm & Hallick, 1976). Two classes of RNA transcripts are known to be encoded on the chloroplast DNA. These are the 16S and 23S rRNA of chloroplast ribosomes (Scott & Smillie, 1967; Stutz & Rawson, 1970) and approximately 25 tRNAs (Schwartzbach et al., 1976; McCrea & Hershberger, 1976). A detailed restriction endonuclease map of *Euglena* chloroplast DNA has been described (Gray & Hallick, 1977, 1978a). The rRNA gene region was located on the genome as three tandemly repeated 5.6-kbp[1] segments, each coding for a 16S and 23S rRNA (Gray & Hallick, 1978a; Rawson et al., 1978). The repeated segment also contains a 5S ribosomal RNA gene (P. W. Gray & R. B. Hallick, manuscript in preparation). Transcription from the rRNA genes accounts for 14–15 kb of the 60-kb total RNA transcript. Twenty-five tRNAs, which have not been mapped, represent an RNA transcript totaling approximately 2.5 kb. The remaining 43 kb of RNAs are of unknown function and genome map position.

In an effort to better understand the function and organization of chloroplast DNA transcription units, we have physically mapped the transcribed regions of the chloroplast genome. Specific restriction fragments from chloroplast DNA were purified, radioactively labeled to high specific activity in vitro, and utilized as hybridization probes for defined regions of the chloroplast genome. This study has led to a quantitative transcription map of *Euglena* chloroplast DNA. We have measured both the fraction of each chloroplast DNA region transcribed and the abundance to which the RNA transcripts from each region accumulate in the cell.

Materials and Methods

Materials. The sources of all materials have been described (Chelm & Hallick, 1976; Gray & Hallick, 1978a).

Preparation of DNA and RNA. Covalently closed, superhelical *Euglena* chloroplast DNA was isolated by centrifugation of chloroplast lysates in CsCl–ethidium bromide as previously described (Chelm et al., 1977b). The recombinant DNA plasmid, pPG5, constructed from a ligation of *Eco*R1 digested pMB9 and an *Eco*R1 fragment of chloroplast DNA (P. W. Gray, R. J. Hall, & R. B. Hallick, manuscript in preparation) was isolated by the cleared lysate procedure (Guerry et al., 1973) and purified by equilibrium centrifugation in a CsCl–ethidium bromide gradient (Cohen & Miller, 1970). P2, EK1 recombinant DNA procedures were used. *Euglena* total cellular RNA was isolated from cells grown to 4 or 72 h of chloroplast development, as previously described (Chelm & Hallick, 1976).

Preparative Scale Restriction Endonuclease Digestion of DNA. Intact chloroplast DNA was digested with *Pst*I, or double digested with *Pst*I and *Bam*HI. The plasmid pPG5 was double digested with *Eco*R1 and *Sal*1. Limit restriction en-

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[1] Abbreviations used: kbp, kilobase pair; kb, kilobase; SSC, 0.15 M NaCl, 0.015 M sodium citrate; *Pst*A–E, BP 3–4, and *Eco*H, restriction endonuclease fragments of *Euglena* chloroplast DNA produced following digestion with *Pst*1, *Bam*H1–*Pst*1, or *Eco*R1, respectively. For nomenclature, see Gray & Hallick (1978b).

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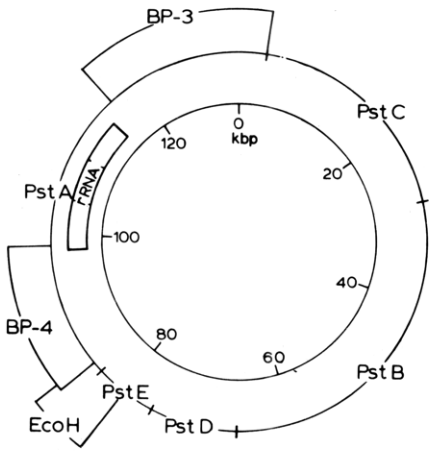

FIGURE 1: Restriction endonuclease fragment map of *Euglena* chloroplast DNA. The location of the chloroplast rRNA genes within the fragment *Pst*A is also shown. Each of the designated fragments, *Pst*A–E, BP 3–4, and *Eco*H, were isolated and converted to the corresponding [^{3}H]DNAs for hybridization studies. BP-4 is identical with *Bam*C (Gray & Hallick, 1977, 1978a).

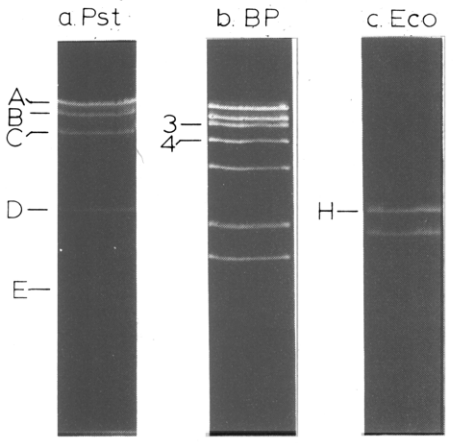

FIGURE 2: Preparative agarose gel electrophoresis of *Euglena* chloroplast DNA digested with various restriction endonucleases. (a) *Pst*I; (b) *Bam*HI plus *Pst*I; (c) *Eco*RI plus *Sal*I digested pPG5. Fragments *Pst*A–E, BP 3–4, and *Eco*H were used for hybridization studies.

donuclease digestion of supercoiled DNA was obtained by a two-step procedure. Supercoiled DNA was first digested at a DNA concentration of 10–15 μg/mL for 5 h. The DNA was collected by ethanol precipitation. Following the addition of 0.1 volume of 2 M sodium acetate and 2 volumes of 95% ethanol the DNA was pelleted at 15 000*g* for 30 min, redissolved to a DNA concentration of 50–75 μg/mL, and redigested for an additional 5 h after the addition of a fresh aliquot of enzyme. This procedure assured complete digestion of the DNA and resulted in the expected fragment patterns following electrophoresis.

The resulting DNA fragments were then separated by electrophoresis in preparative 0.7% agarose gels (12 × 14 × 0.3 cm) as previously described (Gray & Hallick, 1977).

Extraction of DNA Fragments from Agarose Gels. DNA fragments were extracted from agarose gel slices by a modification of the procedure of Blin et al. (1975). A slice of agarose containing the appropriate DNA fragment was excised and dissolved in 7 mL of saturated KI. The KI had previously been decolorized with activated charcoal. The solution was then adjusted with H_2O to a density of 1.49 g/mL ($\eta_{20}^D = 1.420$). Ethidium bromide, 5 mg/mL, was added to a final concentration of 50 μg/mL and the mixture was centrifuged to equilibrium in a Spinco Type 65 rotor at 44 000 rpm and 20 °C for 40 h. The fluorescent DNA band was visualized under ultraviolet light and collected through the side of the cellulose nitrate tube with a syringe fitted with an 18-gauge needle. The equilibrium density centrifugation was repeated to remove residual agarose. The DNA containing fraction from the second density gradient was extracted twice with 1 volume of *n*-butanol to remove the ethidium bromide and then dialyzed extensively against 0.1 × SSC to remove KI and dialyzable contaminants. The DNA was then dialyzed against 50 mM triethylammonium bicarbonate and lyophilized. The dried DNA was washed with cold 95% ethanol, dried, and dissolved in buffer for the labeling reaction.

Preparation of ^{3}H-Labeled Restriction Fragments. DNA restriction fragments, isolated as described above, were labeled in vitro by the nick-translation reaction of *E. coli* DNA polymerase I as previously described (Chelm & Hallick, 1976). [*methyl*-^{3}H]TTP (64.5 Ci/mmol) was the labeled substrate. Specific activities of 1.1–3.6 μCi/μg of DNA were achieved for all the hybridization probes used in these studies. This corresponds to 1.4–5.0% substitution in the labeling reaction. The removal of rapidly renaturable material following nick-translation, fractionation by centrifugation in an alkaline sucrose gradient, and collection of the [^{3}H]DNAs by ethanol precipitation has been described (Chelm et al., 1977a).

DNA–DNA renaturation and DNA–RNA hybridization reactions were incubated in 0.3 M NaCl, 1 mM EDTA, 0.025 M Tris-HCl, pH 7.4, and analyzed by the S1 nuclease sensitivity of resulting products (Chelm & Hallick, 1976; Chelm et al., 1977a).

Results

Preparation of Chloroplast DNA Restriction Fragments. From previous work in this laboratory, an extensive restriction endonuclease cleavage map of *Euglena gracilis* chloroplast DNA has been established (Gray & Hallick, 1978a). For the initial studies on the expression of the chloroplast genome, the restriction fragments produced by the enzyme *Pst*I isolated from *Providentia stuartii 164* (Smith et al., 1976) were chosen. This enzyme cleaves chloroplast DNA into five fragments, designated *Pst*A–E, which have been ordered as shown in Figure 1. The location of the tandemly repeated rRNA genes in *Pst*A is also shown. These *Pst*I fragments are well resolved after electrophoresis in 0.7% agarose gels. A preparative scale agarose gel of a *Pst*I digest of chloroplast DNA is shown in Figure 2a. The DNA bands were sliced out of the slab gel. The DNA was extracted as described above. *Pst* fragments C, D, and E were extremely well separated and therefore were assumed to be pure. *Pst* fragments A and B were not well separated and therefore the possibility of cross-contamination between these two fragments was considered. The level of cross-contamination can be quantitated since *Pst*A and *Pst*B have been shown to have different buoyant densities. From equilibrium buoyant density analysis of the DNA extracted from each of these bands, the *Pst*A preparation was found to be 76% *Pst*A and 24% *Pst*B, while the *Pst*B preparation was 91% *Pst*B and 9% *Pst*A (P. W. Gray & R. B. Hallick, 1978b). These levels of contamination will be taken into consideration when calculating the extent of transcription of these fragments, described below.

*Pst*A is a rather large fragment, encompassing 41% of the *Euglena* chloroplast genome. In order to increase the sensitivity and resolution of hybridization studies in this region, *Pst*A was subfractionated. Two additional fragments, BP-3 and BP-4, which are 40 and 26% of *Pst*A, respectively, but do not contain the rRNA gene region, were obtained from the double digestion of chloroplast DNA with the enzymes *Bam*H1 from *Ba-*

TABLE I: Fraction of Chloroplast DNA Restriction Fragments Transcribed.

restriction fragment	size (kbp)	% RNA-DNA duplex[c]	RNA transcribed (kb)
(a) *Pst* fragments			
*Pst*A	53	21.8 ± 0.7	29.8[a]
*Pst*B	35	27.5 ± 1.2	17.4[b]
*Pst*C	25	30.0 ± 0.7	15.0
*Pst*D	10	11.1 ± 1.0	2.2
*Pst*E	7	16.2 ± 0.6	2.3
total *Pst*	130	26	67
(b) *Eco*R1 and *Bam*H1-*Pst* fragments			
*Eco*H	5.4	21.1 ± 0.6	2.3
BP-3	21	17.1 ± 0.8	7.1
BP-4	13	24.0 ± 0.7	6.2

[a] Corrected for the 24% contamination of the *Pst*A probe with *Pst*B and the base compositional heterogeneity of the *Pst*A (P. W. Gray & R. B. Hallick, 1978b). [b] Corrected for the 9% contamination of the *Pst*B probe with *Pst*A (P. W. Gray & R. B. Hallick, 1978b). [c] Average for 6-12 determinations ± SEM.

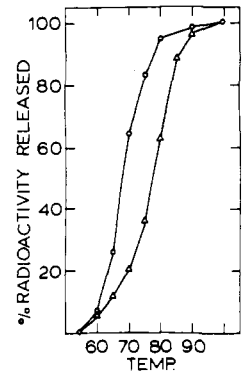

FIGURE 3: Thermal stability of [^{3}H]*Pst*C DNA-RNA hybrids. Hybrids were collected on hydroxylapatite columns in 0.12 M sodium phosphate and 0.4% sodium dodecyl sulfate (pH 6.8). Thermal stability was determined by raising the temperature in increments and subsequently eluting any material which had denatured. Shown for comparison are [^{3}H]*Pst*C DNA-RNA (open circles) and a [^{3}H]ct rDNA-rRNA standard (open triangles) which has previously been described (Chelm et al., 1977a).

cillis amyloliquefaciens H (Wilson & Young, 1975) and *Pst*1. The positions of these fragments on the chloroplast genome are indicated on Figure 1. A preparative 0.7% agarose gel of a *Bam*H1-*Pst*1 double digest of chloroplast DNA is shown in Figure 2b. These two DNA fragments were well resolved from other fragments and therefore assumed pure when isolated from the agarose gel.

The fragment *Eco*H resulting from the digestion of *Euglena* chloroplast DNA with the enzyme *Eco*R1 from *E. coli* was also prepared for utilization as a hybridization probe. This fragment has been positioned as illustrated in Figure 1 (P. W. Gray, R. J. Hall, & R. B. Hallick, manuscript in preparation). Fragment *Eco*H was chosen to test the applicability of these procedures to small DNA fragments capable of coding for only a few genes. It was readily available as a cloned recombinant DNA molecule. The plasmid pPG5 contains fragment *Eco*H and the vector pMB9. Both the vector and the inserted *Eco*H are approximately the same size. To purify fragment *Eco*H from the plasmid pPG5, pPG5 was double digested with both *Eco*R1 and *Sal*1. *Sal*1 does not cleave the *Eco*H fragment but cleaves the vector pMB9 into two smaller fragments. *Eco*H is then well resolved after electrophoresis in 0.7% agarose gels as is illustrated in Figure 2c. *Eco*H is assumed to be pure when isolated from the gel.

The sizes of all the restriction fragments utilized in this study are listed in Table I. Their location on a chloroplast DNA restriction nuclease map is shown in Figure 1.

Preparation and Characterization of Hybridization Probes. The *Euglena* chloroplast DNA restriction fragments *Pst*A, B, C, D, E, BP-3, 4, and *Eco*H were radioactively labeled in vitro by the nick-translation reaction of *E. coli* DNA polymerase I as described above. The specific activities achieved ranged from 1.1 to 3.6 μCi/μg of DNA with [^{3}H]TTP (64.5 Ci/mmol) as the radioactive substrate, corresponding to 1.4-5.0% substitution in the labeling reaction.

Several of the ^{3}H-labeled restriction fragments were tested for their ability to renature with an excess of sheared, unfractionated chloroplast DNA. All of the hybridization probes tested renatured with the same $C_0t_{1/2}$ of 0.23 M s, in excellent agreement with previously measured values for the renaturation of excess chloroplast DNA with ^{3}H-labeled total chloroplast DNA labeled by similar procedures (Chelm & Hallick, 1976). In renaturation reactions with nonradioactive chloroplast DNA driver, the hybridization probes utilized in these studies were consistently saturated to >60% by sheared chloroplast DNA as expected for reactions in which the driver DNA has a significantly higher single-strand size than the hybridization probe (550-660 bases for the driver vs. 150-200 bases for the probe).

In order to test the fidelity of base pairing of the ^{3}H-labeled restriction fragments to RNA preparations, the thermal stabilities of [^{3}H]*Pst*C:RNA duplexes were measured using thermal elution chromatography on hydroxylapatite. As shown in Figure 3 [^{3}H]*Pst*C:RNA hybrids exhibited mean thermal stabilities of 68 °C and cooperative melting behavior with 80% of the duplexes melting over a 16 °C temperature range consistent with well matched duplexes (Kohne & Britten, 1971). This mean thermal stability is lower than the value of 77.5 °C for chloroplast rRNA:chloroplast [^{3}H]rDNA hybrids (Figure 3; Chelm et al., 1977a). This result is not unexpected since *Pst*C has a 21 mol % G + C content while the rRNA gene region is 44 mol % G + C (Gray & Hallick, 1978b).

Hybridization of Total Cell RNA to Chloroplast [^{3}H]-DNA Restriction Fragments. ^{3}H-labeled chloroplast restriction fragments were used as hybridization probes in RNA-driven hybridizations in order to determine the fraction of each restriction fragment which is transcribed. The RNA used in these hybridization reactions was obtained from whole cells harvested 72 h after the onset of light-induced chloroplast development (Chelm & Hallick, 1976). These cells have fully developed chloroplasts. All hybridization saturation reactions were incubated to R_0ts (initial RNA concentration × time) of greater than 600 M s which has previously been shown to be sufficient to drive the hybridization of a total chloroplast DNA probe to completion. This conclusion has been confirmed for individual restriction nuclease fragments in the present study.

The results of the saturation hybridizations for *Pst*A, B, C, D, and E, BP-3, 4, and *Eco*H are listed in Table I. The percent of each fragment transcribed and the corresponding extent of RNA synthesis are reported. The DNA-DNA renaturation contribution to the saturation values were determined with identical reactions both minus RNA and with yeast RNA replacing *Euglena* RNA. This value was subtracted from the saturation values observed with the *Euglena* RNA driven reactions and the resultant RNA-DNA contribution is that reported in Table I.

The RNA transcripts from *Pst*A-E, which represent the

TABLE II: Analysis of Hybridization Kinetics of *Euglena* RNA to Chloroplast DNA Restriction Fragments *Pst*A and *Pst*B.

probe	transition	$R_0t_{1/2}$(obsd) (mol/(s L))	complexity[a] (kb)	$R_0t_{1/2}$(calcd)[b] (mol/(s L))	% total RNA[c]	no. copies/cell[d]
*Pst*A	1	0.015	13.8[e]	0.0026	17	1.3×10^6
	2	5.4	16.0[e]	0.0090	0.17	3.7×10^3
*Pst*B	1	0.012	5.8[f]	0.0037	28	1.7×10^6
	2	12	11.6[f]	0.0065	0.054	1.6×10^3

[a] Calculated from the % duplex for each transition × the complexity of the probe. [b] Calculated using the relationship $(R_0t_{1/2}(\text{calcd}))(\text{complexity std}) = (R_0t_{1/2}\text{ std})(\text{complexity unknown})$ (Chelm & Hallick, 1976). The standard utilized in these calculations was a $R_0t_{1/2} = 2.7 \times 10^{-3}$ M s for a complexity = 4.8 kb as determined with chloroplast rRNA. [c] Calculated using the relationship (% total RNA) = $[(R_0t_{1/2}(\text{calcd}))/(R_0t_{1/2}(\text{obsd}))]100$ as previously described (Chelm & Hallick, 1976). [d] Calculated using 19.0 pg of RNA/cell as previously determined (Chelm et al., 1977a). [e] Corrected for 24% contamination with *Pst*B. Also corrected for the different specific activities when labeling with [^{3}H]TTP of the rRNA region, which is 44 mol % GC, compared with the remainder of the genome which is 20–22 mol % GC (Gray & Hallick, 1978b). [f] Corrected for 9% contamination with *Pst*A as described in the text.

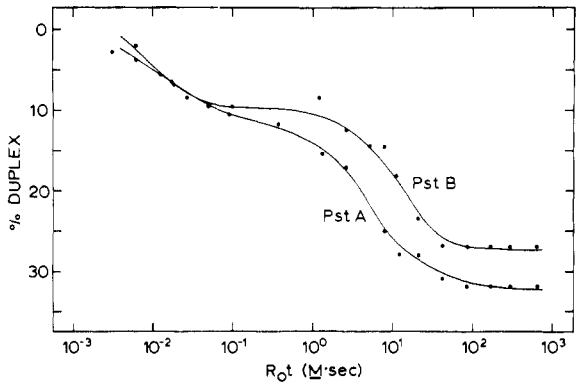

FIGURE 4: Hybridization kinetics of total *Euglena* RNA to [^{3}H]*Pst*A (●) and [^{3}H]*Pst*B (○).

entire genome, total 67 kb, or 26% of the double-strand information content of the genome. This result is in excellent agreement with the previously reported value of 23% determined using a total chloroplast DNA hybridization probe (Chelm & Hallick, 1976). The transcribed regions are dispersed throughout the genome, as evidenced by the RNA–DNA hybrids formed by each probe. Both the BP3 and BP4 probes, which are contained within *Pst*A (Figure 1), also formed RNA–DNA hybrids. The 13-kb extent of BP 3 and 4 transcription is 45% of the *Pst*A total. The remaining *Pst*A transcript is mainly chloroplast rRNA, as described below. Also described below is the relationship between the 2.3 kb of transcript of *Eco*H to the *Pst*E transcript of 2.3 kb.

Hybridization Kinetics. In order to test for differences in the cellular abundance of RNA transcripts from each region of the chloroplast genome, the kinetics of the RNA driven hybridization reactions to restriction fragment probes were analyzed.

The kinetics of the total cell RNA driven hybridization to restriction fragments *Pst*A and *Pst*B are shown in Figure 4. The data are a plot of the fraction of the hybridization probe which is duplex vs. the logarithm of the product of the initial RNA concentration times the hybridization time, R_0t. The fraction of the hybridization probe in the duplex form was measured by its insensitivity to the single-strand specific S1 nuclease. The DNA–DNA contribution to the duplex fraction was subtracted as described above. Reactions were carried out with RNA in either 75 or 30 000-fold molar excess over the [^{3}H]DNA. Reaction mixtures were incubated at 68 °C for varying times.

The RNA populations complementary to the *Pst*A and *Pst*B regions of the chloroplast genome are kinetically similar. Both of these regions of the genome are transcribed into RNAs of at least two distinct abundance classes as indicated by the two distinct transitions evident in the R_0t curves (Figure 4). The transitions which are completed at lower R_0t values represent transcripts which accumulate to higher abundance in the cell while the transitions at higher R_0t values represent transcripts which have accumulated to significantly lower abundance. The data for both *Pst*A and *Pst*B can be analyzed in terms of two pseudo-first-order components in each hybridization reaction. These results are quantitatively represented in Table II. From these calculations, it can be seen that both *Pst*A and *Pst*B code for high abundance classes of RNA which accumulate to approximately 10^6 copies per cell. It has been shown that the *Pst*A region of the chloroplast genome contains three sets of genes coding for chloroplast 16S and 23S rRNA (Gray & Hallick, 1978a; Rawson et al., 1978). The extent of the high abundance *Pst*A transcripts of 13.8 kb is in agreement with its identification as those rRNAs, which from restriction nuclease mapping are known to comprise 14.4 kbp of *Pst*A. Furthermore, the abundance of this class as determined with the *Pst*A probe of 17% of the cell RNA is in agreement with that determined using a more sensitive rRNA probe of 26% of the cell RNA (Chelm et al., 1977a). The high abundance transcripts in *Pst*B have not been identified. These RNAs are of low complexity (4–6 kb) compared to the hybridization probe used to detect them. Therefore the exact quantitation of this RNA must await more detailed studies with a more sensitive [^{3}H]DNA probe. Nevertheless it can be seen that this RNA is a major chloroplast transcription product that accumulates to a level comparable to that of chloroplast rRNAs. The identities of the low abundance transcripts from *Pst*A and B are unknown. These RNAs are present at approximately 1000–5000 copies/cell.

The kinetics of the total cell RNA driven hybridization to restriction fragments BP-3 and BP-4 are illustrated in Figure 5. These hybridization reactions can both be approximated as single pseudo-first-order reactions. The analysis of these results is shown in Table III. The sum of the complexities of the transcripts from these regions is 13.3 kb, in agreement with the complexity of the *Pst*A low abundance transcripts determined with the *Pst*A probe. The BP-3 and BP-4 transcripts accumulate to approximately 3000 copies/cell, also in agreement with the *Pst*A low abundance transcripts.

The kinetics of the total cellular RNA driven hybridization to restriction fragments *Pst*C, *Pst*D, and *Pst*E are illustrated in Figure 6. These reactions were carried out with RNA in 30 000-fold molar excess over the [^{3}H]DNA. The kinetics of hybridization to these three regions of the chloroplast genome can all be approximated as a single pseudo-first-order reaction.

TABLE III: Analysis of Hybridization Kinetics of *Euglena* RNA to Chloroplast DNA Restriction Fragments BP-3, BP-4, *Pst*D, *Pst*E, and *Eco*H.[a]

probe	transition	$R_0t_{1/2}$(obsd) (mol/(s L))	complexity (kb)	$R_0t_{1/2}$(calcd) (mol/(s L))	% total RNA	no. copies/ cell
BP-3	1	6.5	7.1	0.0040	0.062	3.0×10^3
BP-4	1	7.4	6.2	0.0035	0.047	2.6×10^3
*Pst*C	1	9.8	15.0	0.0084	0.086	2.0×10^3
*Pst*D	1	9.6	2.2	0.0012	0.012	1.9×10^3
*Pst*E	1	8.1	2.3	0.0013	0.015	2.4×10^3
*Eco*H	1	6.8	2.3	0.0013	0.019	2.8×10^3

[a] Calculations are described in the footnotes to Table II.

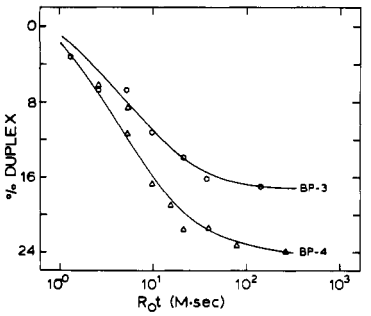

FIGURE 5: Hybridization kinetics of *Euglena* total cell RNA to [^{3}H]BP-3 (○) and [^{3}H]BP-4 (△).

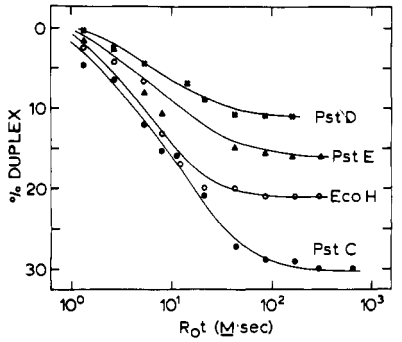

FIGURE 6: Hybridization kinetics of *Euglena* total cell RNA to [^{3}H]*Pst*C (●), [^{3}H]*Pst*D (X), [^{3}H]*Pst*E (▲), and [^{3}H]*Eco*H (○).

The quantitative analysis of these data is shown in Table III. These regions are transcribed into low abundance transcripts which accumulate to levels ranging from approximately 2000 to 3000 copies per cell. The identities of these transcripts are unknown.

Also illustrated in Figure 5 are the kinetics of the hybridization of total cellular RNA to the chloroplast DNA restriction fragment *Eco*H. This fragment has been positioned overlapping the *Pst*A-*Pst*E junction (see Figure 1). It can be seen from the analysis of these data (Table III) that the complexity of the *Eco*H transcripts are the same as the complexity of the *Pst*E transcripts. The rate constants for the hybridization of this RNA (as reflected by the $R_0t_{1/2}$ observed) to both *Pst*E and *Eco*H are not significantly different. Since these restriction fragments have a greater overlap than that needed to encode this RNA, and since the transcripts accumulate similarly, the transcripts are assumed to be identical. The transcribed region can therefore be located to within approximately 1 kbp (Figure 6). Data on the transcription of this region during chloroplast development is consistent with this interpretation (B. K. Chelm, P. W. Gray, and R. B. Hallick, in preparation).

These transcription mapping results are summarized in

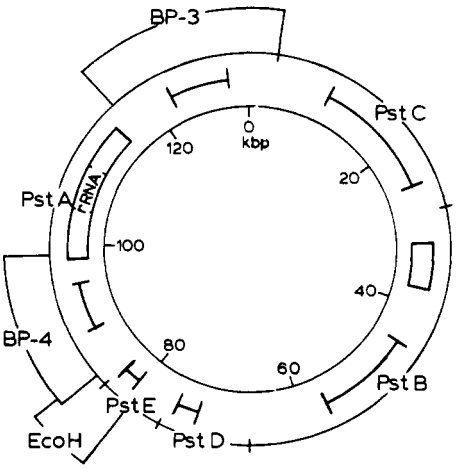

FIGURE 7: Transcription map of *Euglena gracilis* chloroplast DNA. The extent of transcription for a region of the DNA is depicted inside the restriction fragment map. High abundance transcripts (▭) and low abundance transcripts (⊢—⊣) are depicted.

Figure 7. The physical positions of the restriction fragments studied and the total size of the transcribed regions within the boundaries of the restriction endonuclease cleavage sites are shown. The high and low abundance transcription regions of *Pst*A and *Pst*B are shown as separate units. Transcription units are arbitrarily shown as blocks, but they may very well be interspersed with nontranscribed regions.

Discussion

In this paper studies aimed at defining transcription of the *Euglena* chloroplast genome in terms of the structural organization of the transcribed regions are described. A procedure is described for preparing high molecular weight DNA restriction fragments in good yield and with template activity for the nick-translation reaction of *E. coli* DNA polymerase I. These fragments were labeled in vitro to high specific activity and used as hybridization probes in RNA driven reactions to characterize the transcription of the region of the genome represented by each restriction fragment. Using this procedure, a quantitative, highly sensitive analysis of the transcription of this genome could be undertaken. It is our experience that many of the transcribed regions which yield low abundance RNA classes could not be detected by the use of hybridization of RNA labeled either in vivo or in vitro to restriction fragments by the method of Southern (1975).

From the results of this work it can be estimated that 26% of the *Euglena* chloroplast genome is transcribed in cells containing fully developed chloroplasts. This value is in good agreement with the previously reported value of 23% of the genome transcribed (Chelm & Hallick, 1976). The transcribed regions are scattered throughout the genome and must there-

fore be interspersed by regions which are either not transcribed at all or are transcribed into RNA of extremely low cellular concentration. The RNA-driven hybridization technique used in this study is sensitive enough that RNA from any appreciable region of chloroplast genome which has accumulated to approximately 20 copies per cell could be detected. This level is two orders of magnitude less abundant than the lowest abundance transcripts described above (Tables II and III). Since there are 2500 copies of the chloroplast genome per cell (Chelm et al., 1977b), there must be less than 0.008 copy per genome accumulating for these "silent" regions of DNA.

The RNA transcripts of the *Euglena* chloroplast genome are characterized in a general sense as a population of two abundance classes. Fragments *Pst*A and *Pst*B both code for abundant transcripts which accumulate to $1-2 \times 10^6$ copies per cell. The abundant *Pst*A transcripts can be identified as chloroplast rRNA by their abundance and complexity, consistent with both the physical mapping of these genes (Gray & Hallick, 1978a; Rawson et al., 1978) and results from previous hybridization studies with a [^{3}H]rDNA hybridization probe (Chelm et al., 1977a). The identities of the abundant *Pst*B transcripts are as yet unknown. Possible candidates for at least part of this RNA complexity are the chloroplast tRNA genes. *Euglena* chloroplast DNA has been reported to contain coding sequences for approximately 25 tRNAs (Schwartzbach et al., 1976; McCrea & Hershberger, 1976). If tRNA genes were clustered one might expect to find a fast renaturating, low complexity component in RNA driven hybridizations as was observed for *Pst*B. In preliminary experiments, we have observed *Euglena* tRNA hybridization to [^{3}H]*Pst*B and to other restriction nuclease fragments. In addition to the abundant RNAs, all the *Pst* fragments code for low abundance RNA transcripts which accumulate in the range of $1.6-3.6 \times 10^3$ copies per cell. The low abundance and high complexity of these transcripts are consistent with a possible mRNA functional role but no positive identifications have been made.

The results described above should provide both the framework and the methodology for much more detailed, quantitative transcription mapping of this genome. Relatively large restriction nuclease fragments have been employed in this study. A more exact location and quantitation of even individual transcription units is approachable with lower complexity probes. The use of cloned *Eco*R1 fragments, such as the [^{3}H]*Eco*H probe, is a good example of such an experiment.

References

Blin, N., Gabain, A. V., & Bujard, H. (1975) *FEBS Lett. 53,* 84.

Chelm, B. K., & Hallick, R. B. (1976) *Biochemistry 15,* 593.

Chelm, B. K., Hoben, P. J., & Hallick, R. B. (1977a) *Biochemistry 16,* 776.

Chelm, B. K., Hoben, P. J., & Hallick, R. B. (1977b) *Biochemistry 16,* 782.

Cohen, S. N., & Miller, C. A. (1970) *J. Mol. Biol. 50,* 671.

Gray, P. W., & Hallick, R. B. (1977) *Biochemistry 16,* 1665.

Gray, P. W., & Hallick, R. B. (1978a) *Biochemistry 17,* 284.

Gray, P. W., & Hallick, R. B. (1978b) *Biochim. Biophys. Acta* (in press).

Guerry, P., LeBlanc, D. J., & Falkow, S. (1973) *J. Bacteriol. 116,* 1064.

Kohne, D. E., & Britten, R. J. (1971) *Proc. Nucleic Acids Res. 2,* 500.

Manning, J. E., & Richards, O. C. (1972) *Biochim. Biophys. Acta 259,* 295.

McCrea, J. M., & Hershberger, C. L. (1976) *Nucleic Acids Res. 3,* 2005.

Rawson, J. R. Y., & Boerma, C. (1976) *Proc. Natl. Sci. U.S.A. 73,* 2401.

Rawson, J. R. Y., Kushner, S. K., Vapnek, D., Alton, N. K., & Boerma, C. L. (1978) *Gene 3,* 191.

Schwartzbach, S. D., Hecker, L. I., & Barnett, W. E. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73,* 1984.

Scott, N. S., & Smillie, R. N. (1967) *Biochem. Biophys. Res. Commun. 28,* 598.

Smith, D. I., Blattner, R. F., & Davies, J. (1976) *Nucleic Acids Res. 3,* 343.

Southern, E. M. (1975) *J. Mol. Biol. 98,* 503.

Stutz, E., & Rawson, J. R. (1970) *Biochim. Biophys. Acta 209,* 16.

Wilson, G. A., & Young, F. E. (1975) *J. Mol. Biol. 97,* 123.