Physical Mapping of the *Euglena gracilis* Chloroplast DNA and Ribosomal RNA Gene Region[+]

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ABSTRACT: *Euglena gracilis* chloroplast DNA is cleaved into 5 fragments by restriction endonuclease *Pst*I, 6 by *Xho*I, and 11 by *Bal*I. These cleavage sites have been mapped with respect to each other and the previously reported (Gray, P. W., and Hallick, R. B. (1977), *Biochemistry 16,* 1665) *Bam*H1 and *Sal*I cleavage sites. The *Bal*I, *Pst*I, and *Xho*I sites were determined by fragment molecular weight analysis, analysis of multiple digestion products, and digestion studies on isolated DNA fragments. The ribosomal RNA gene region has been located on the physical map. The chloroplast genome is found to contain three tandemly repeated 5.6-kbp segments, each of which contains a 16S and 23S ribosomal RNA gene.

Chloroplast DNA of the unicellular alga *Euglena gracilis* is a circular, double-stranded molecule. The estimated molecular weight of the DNA, based on contour length measurements, is 92×10^6, or approximately 140 kbp[1] (Manning and Richards, 1972). The major RNA transcripts of this genome are the 16S and 23S (0.56×10^6 and 1.1×10^6 dalton) chloroplast ribosomal RNAs (Scott and Smillie, 1967; Stutz and Rawson, 1970). The mature rRNA species have precursors of molecular weight 0.64×10^6 and 1.16×10^6 (Scott, 1976). No single common precursor to these molecules has been found. Depending on the stage of chloroplast development, chloroplast rRNA can account for up to 26% of total cell RNA in *Euglena* (Chelm et al., 1977a; Cohen and Schiff, 1976).

One of our goals has been to understand the mechanism for transcriptional control of the rRNA and other genes during development. A detailed knowledge of the location of genes on the chloroplast genome is important to such studies. We recently described a restriction endonuclease cleavage map of the *Euglena* chloroplast genome (Gray and Hallick, 1977). In the present study ambiguities in the earlier map have been resolved. Furthermore, the map has been extended to include the location of 11 *Bal*I, 5 *Pst*I, and 6 *Xho*I cleavage sites.

From the restriction nuclease mapping data it has also been possible to determine the location of the chloroplast rRNA genes. *Eco*RI fragments of *Euglena* chloroplast DNA that hybridize with ct rRNA have been identified in several laboratories (Stutz et al., 1976; Lomax et al., 1977; Mielenz et al., 1977). In this report we describe the location of these *Eco*RI fragments on the physical map. We find that the chloroplast genome contains three 5.6-kbp segments arranged in a tandem repeat, each of which contains a 16S and 23S ribosomal RNA gene.

Materials and Methods

Preparation of Chloroplast DNA. Chloroplast DNA from *Euglena gracilis* Klebs, strain Z Pringsheim cells was isolated as previously described (Chelm et al., 1977b; Gray and Hallick, 1977). Covalently closed, circular DNA was used predominately in this study.

Restriction Endonuclease Analysis. Restriction endonucleases *Sal*I, *Eco*RI, and *Bam*HI were prepared as previously described (Gray and Hallick, 1977). Endonuclease *Pst*I was isolated from *Providencia stuartii* 164 (Smith et al., 1976), *Xho*I was isolated from *Xanthomonas holicola,* and *Bal*I was prepared from *Brevibacterium albidum* by procedures described by R. J. Roberts (personal communication). *Sma*I was provided by Igor Dawid. The cleavage properties and nomenclature of the enzymes used in this study have been reviewed (Roberts, 1976). Electrophoretic analysis of restriction nuclease fragments, elution of DNA from agarose gels, and photography of gels have been described (Gray and Hallick, 1977).

Results

Digestion of Euglena Chloroplast DNA with BalI, PstI, and XhoI. When *Euglena* chloroplast DNA is treated with *Pst*I endonuclease, 5 fragments designated *Pst*A, B, C, D, and E are produced. These fragments can be separated on a 0.7% agarose gel (Figure 1). Digestion of chloroplast DNA with *Bal*I and *Xho*I yields 11 and 6 fragments, respectively. These fragments are designated *Bal*A–J and *Xho*A–E. All of these fragments except *Bal*D and *Bal*E can be resolved on a 0.7% agarose gel (Figure 2). Estimates of the size and stoichiometry of the *Bal*I, *Pst*I, and *Xho*I fragments are presented in Table I. The size estimates for the smaller (<10 kbp) fragments are based on their electrophoretic mobility compared with *Eco*RI digested λ DNA (Figure 2). The size estimates for the larger fragments were determined from multiple digestion experiments. For example, the largest *Pst* fragment (*Pst*A, 53 kbp) is cleaved by *Bam*HI into six fragments which total 53 kbp in size. An important feature of the *Bal*I and *Xho*I digestion data is the fragments *Bal*H and *Xho*D, each of 5.6 kbp and each present twice per genome. When chloroplast DNA is digested with *Bam*HI, a 5.6-kbp fragment (*Bam*E) is also produced in a stoichiometry of two per genome (Figure 2). Evidence will be presented below that these 5.6-kbp fragments arise from cleavage in a tandemly repeated region of the genome containing the rRNA cistrons.

Cleavage Sites in Euglena Chloroplast DNA for Ten Restriction Endonucleases. Data on the number of cleavage sites in *Euglena* chloroplast DNA for ten different site specific endonucleases are summarized in Table II. The observed

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[1] Abbreviations used: kbp, kilobase pair; rRNA, ribosomal RNA; ct, chloroplast; DNA fragments resulting from double digestion of ct DNA are B1B (*Bal*I-*Bam*HI), B1P (*Bal*I-*Pst*I), B1S (*Bal*I-*Sal*I), B1X (*BAL*I-*Xho*I), BP (*Bam*HI-*Pst*I), BS (*Bam*HI-*Sal*I), BX (*Bam*HI-*Xho*I), PS (*Pst*I-*Sal*I), PX (*Pst*I-*Xho*I), and SX (*Sal*I-*Xho*I).

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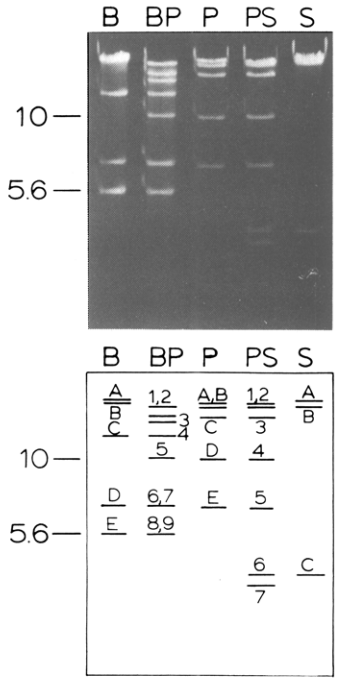

FIGURE 1: Analysis of *Euglena* chloroplast DNA by *Bam*HI, *Pst*I, and *Sal*I restriction endonucleases. Fragments were separated by electrophoresis in a 0.7% agarose gel. Illustrated in the upper panel are the following digestion patterns; B, *Bam*HI; BP, *Bam*HI-*Pst*I; P, *Pst*I; PS, *Pst*I-*Sal*I; S, *Sal*I. Fragment designations are shown in the lower, schematic representation. Size markers of 5.6 and 10 kbp are also given.

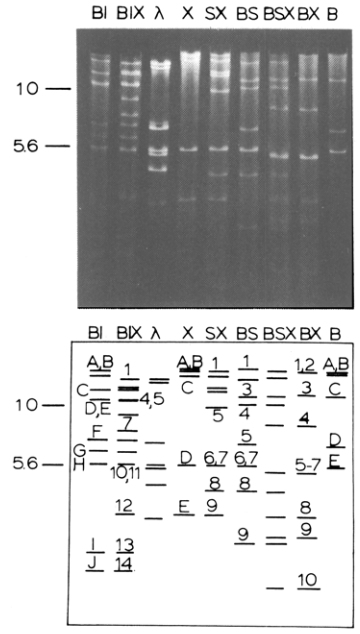

FIGURE 2: Analysis of *Euglena* chloroplast DNA by restriction endonucleases. A photograph of an ethidium bromide stained gel and a schematic representation of the banding pattern for the following digestion experiments are shown: Bl, *Bal*I, BlX, *Bal*I-*Xho*I; λ, *Eco*RI of λ DNA; X, *Xho*I; SX, *Sal*I-*Xho*I; BS, *Bam*HI-*Sal*I; BSX, *Bam*HI-*Sal*I-*Xho*I; BX, *Bam*HI-*Xho*I; B, *Bam*HI. The 5.6 and 10 kbp migration positions are also shown.

TABLE I: DNACleavage Products Resulting from Digestion of *Euglena gracilis* Chloroplast DNA with Restriction Endonucleases *Bal*I, *Pst*I, and *Xho*I.

*Bal*I		*Pst*I		*Xho*I	
Fragment	Length (kbp)	Fragment	Length (kbp)	Fragment	Length (kbp)
*Bal*A	34	*Pst*A	53	*Xho*A	49
*Bal*B	27	*Pst*B	35	*Xho*B	38
*Bal*C	17	*Pst*C	25	*Xho*C	28
*Bal*D	12	*Pst*D	10	*Xho*D[a]	5.6
*Bal*E	12	*Pst*E	6.9	*Xho*E	3.4
*Bal*F	7.1				
*Bal*G	6.3				
*Bal*H[a]	5.6				
*Bal*I	2.3				
*Bal*J	2.0				
Total *Bal*	131	Total *Pst*	130	Total *Xho*	130

[a] All fragments are present in a stoichiometry of one except *Bal*H and *Xho*D, which are each present twice per DNA molecule.

TABLE II: Predicted and Observed Number of Cleavage Sites in *Euglena gracilis* Chloroplast DNA for Various Restriction Nucleases.

Enzyme	Recognition sequence[a]	No. of cleavage sites	Predicted no. of sites[b]
*Sma*I	CCCGGG	0	0.5
*Sal*I	GTCGAC	3	4.5
*Pst*I	CTGCAG	5	4.5
*Bam*HI	GGATCC	6	4.5
*Xho*I	CTCGAG	6	4.5
*Bal*I	TGGCCA	11	4.5
*Eco*RI	GAATTC	27[c]	40
*Hpa*I	GTTAAC	28-34[d]	40
*Hin*dIII	AAGCTT	30-35[d]	40
*Hae*III	GGCC	51-52[e]	32

[a] Roberts, 1976. [b] Assuming 75% A + T base composition and a 130-kbp genome. [c] Gray and Hallick, 1977; Stutz et al., 1976; Mielenz et al., 1977; J. R. Y. Rawson, personal communication. [d] P. W. Gray and R. B. Hallick, unpublished observations. [e] Kopecka et al., 1977.

number of cleavage sites are compared with the number predicted assuming a random distribution of bases in the chloroplast genome. The enzyme *Sma*I does not cleave *Euglena* chloroplast DNA. No fragmentation of chloroplast DNA by *Sma*I either alone or in combination with several other endonucleases has been observed, in agreement with a previous report (Kopecka et al., 1977). The five enzymes described in this study were chosen because of the prediction that chloroplast DNA would contain approximately five cleavage sites for each enzyme. Four of these enzymes, *Sal*I, *Bam*HI, *Pst*I, and *Xho*I, cleave chloroplast DNA at 3 to 6 sites, and the fifth Bal I, at 11 sites. Four other enzymes *Eco*RI, *Hpa*I, *Hin*dIII, and *Hae*III, all cleave *Euglena* chloroplast DNA at 27 or more sites, as would be expected from their recognition sequences. From the data in Table II it may be concluded that there is not a completely random distribution of bases in the chloroplast genome, but the predicted and observed number of cleavage sites for a number of enzymes are in qualitative agreement.

Double Digestion of Euglena Chloroplast DNA with BalI, BamHI, PstI, SalI, and XhoI. The products and resulting cleavage site map for a *Bam*HI-*Sal*I double digestion were the subject of an earlier report (Gray and Hallick, 1977). The remaining nine possible double digestions involving the five enzymes *Bal*I, *Bam*HI, *Pst*I, *Sal*I, and *Xho*I were performed. The analyses of the fragments produced in these experiments are presented in Tables III, IV, and V. In addition, five of the digestion patterns are illustrated in Figures 1 and 2. For a

TABLE III: DNA Cleavage Products Resulting from Double Digestion of *Euglena gracilis* Chloroplast DNA with *Bal*I and *Bam*HI, *Bal*I and *Pst*I, and *Bal*I and *Sal*I.

*Bal*I-*Bam*HI			*Bal*I-*Pst*I			*Bal*I-*Sal*I		
Fragment	Size (kbp)	Equiv fragment	Fragment	Size (kbp)	Equiv fragment	Fragment	Size (kbp)	Equiv fragment
BIB 1	23		BIP 1	16		BIS 1	26	*Bal*B
2	17	*Bal*C	2	15		2	24	
3	14		3	12	*Bal*D	3	12	*Bal*D
4	14		4	12	*Bal*E	4	12	*Bal*E
5	12	*Bal*D	5	10	*Pst*D	5	11	
6	12	*Bal*E	6	10		6	10	
7	10		7	9.5		7	7.1	*Bal*F
8	7.1	*Bal*F	8	7.1	*Bal*F	8	6.3	*Bal*G
9	5.3		9	6.9	*Pst*E	9	5.6	*Bal*H
10	5.3		10	6.8		10	5.6	*Bal*H
11	5.3		11	6.3	*Bal*G	11	4.4	*Sal*C
12	2.3	*Bal*I	12	5.6	*Bal*H	12	2.3	*Bal*I
13	1.5		13	5.6	*Bal*H	13	2.0	*Bal*J
14	1.2		14	3.4		14	1.0	
15	0.5		15	2.3	*Bal*I			
16	0.3		16	2.0	*Bal*J			
17	0.3							
Total	131		Total	131		Total	129	

TABLE IV: DNA Cleavage Products Resulting from Double Digestion of *Euglena gracilis* Chloroplast DNA with *Bal*I and *Xho*I, *Bam*HI and *Pst*I, and *Bam*HI and *Xho*I.

*Bal*I-*Xho*I			*Bam*HI-*Pst*I			*Bam*HI-*Xho*I		
Fragment	Size (kbp)	Equiv fragment	Fragment	Size (kbp)	Equiv fragment	Size Fragment	Equiv (kbp)	Equiv fragment
BIX 1	22		BP 1	35		BX 1	45	*Bam*B
2	18		2	25	*Pst*C[a]	2	38	*Xho*B
3	17	*Bal*C	3	21		3	14	*Bam*C
4	12	*Bal*D	4	14	*Bam*C	4	8.8	
5	12	*Bal*E	5	10	*Pst*D	5	5.3	
6	9.6		6	6.9	*Pst*E	6	5.3	
7	8.0		7	6.9	*Bam*D	7	5.3	
8	7.1	*Bal*F	8	5.6	*Bam*E	8	3.4	*Xho*E
9	6.2		9	5.6	*Bam*E	9	2.8	
10	5.5		10	1.3		10	1.6	
11	5.5		11	0.3		11	0.3	
12	3.4	*Xho*E				12	0.3	
13	2.3	*Bal*I						
14	2.0	*Bal*J						
15	0.1							
16	0.1							
17	0.1							
Total	131		Total	132		Total	131	

[a] The *Bam*HI cleavage site between *Bam*A and *Bam*B is very close to the *Pst*I cleavage site between *Pst*B and *Pst*C. It is possible that BP1 is *Pst*B, and BP2 has a *Bam*HI site at one end and a *Pst*I site at the other end.

double digest, the predicted number of cleavage products is equal to the sum of the products produced by the individual enzymes. In the nine double digestions each of the expected products greater than 0.5 kbp was identified. The size of the very small fragments could be inferred from their map location. Estimates of the molecular weight of each fragment are also presented in Tables III, IV, and V. The location of the internal cleavage sites in double digestions is also indicated in the tables. For example, in the *Bal*I-*Bam*HI digest (Table III), among the products are *Bal*C, D, E, F, and I. Therefore, the six *Bam*HI sites are located in *Bal*A, B, G, H, H, and J.

It is important to note the fate of the 5.6-kbp fragments that are present twice per genome, *Bal*H, *Bam*E, and *Xho*D. There is no evidence for heterogeneity in these fragments. *Sal*I and *Pst*I do not cleave any of the 5.6-kbp segments. However, in all double digestions involving *Bal*I, *Bam*HI, and *Xho*I, the two 5.6-kbp fragments are cleaved in the same manner.

Size of the Euglena Chloroplast Genome. The molecular weight of *Euglena* chloroplast DNA is generally accepted as 92×10^6, or 140 kbp. This result was based on a careful comparison of the contour lengths of circular chloroplast DNA and circular λ DNA (Manning and Richards, 1972). In the present study, the size of the chloroplast genome estimated from the restriction nuclease fragments produced in double digestion experiments is approximately 128–132 kbp. In previous studies, the *Hae*III restriction nuclease fragments of *Euglena* chloroplast DNA have totaled 132 kbp (Kopecka et al., 1977) and the *Eco*RI fragments 128–133 kbp (Gray and Hallick, 1977; Mielenz et al., 1977; Stutz et al., 1976). The reason for the slight discrepancy between the measurements from contour length and from restriction nuclease data may be related to the high A + T base content of the genome (75 mol %). The elec-

TABLE V: DNA Cleavage Products Resulting from Double Digestion of *Euglena gracilis* Chloroplast DNA with *Pst*I and *Sal*I, *Pst*I and *Xho*I, and *Sal*I and *Xho*I.

*Pst*I-*Sal*I			*Pst*I-*Xho*I			*Sal*I-*Xho*I		
Fragment	Size (kbp)	Equiv fragment	Fragment	Size (kbp)	Equiv fragment	Fragment	Size (kbp)	Equiv fragment
PS 1	44		PX 1	32		SX 1	38	*Xho*B
2	35	*Pst*B	2	25	*Pst*C	2	28	
3	25	*Pst*C	3	23		13	17	
4	10	*Pst*D	4	20		4	16	
5	6.9	*Pst*E	5	6.9	*Pst*E	5	12	
6	4.4	*Sal*C	6	6.4		6	5.6	*Xho*D
7	4.1		7	5.6	*Xho*D	7	5.6	*Xho*D
8	0.95		8	5.6	*Xho*D	8	4.4	*Sal*C
			9	3.4	*Xho*E	9	3.4	*Xho*E
			10	3.0				
			11	0.2				
Total	130		Total	131		Total	130	

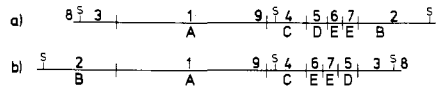

FIGURE 3: *Bam*HI and *Sal*I cleavage map of *Euglena* chloroplast DNA. (a) Previously described (Gray and Hallick, 1977) map. (b) Modified map as described in the text. Letters refer to *Bam*HI fragments and numbers refer to *Bam*HI-*Sal*I (BS) double digestion products. The estimated sizes of fragments BS 1-9 are, respectively: 54, 26, 13, 11, 6.9, 5.6, 5.6, 4.4, and 2.8 kbp. BS 9 is the small fragment between BS 1 and 4.

tron microscope contour length and/or the electrophoretic mobility of DNA fragments in agarose gels may be dependent on base composition. We will assume a size of 130-131 kbp below in describing a physical map of the genome, since our data are based on electrophoresis experiments.

Physical Map of the Euglena gracilis Chloroplast DNA. In the initial restriction nuclease map of *Euglena* chloroplast DNA, the three *Sal*I and six *Bam*HI cleavage sites were located (Gray and Hallick, 1977), but a few unresolved ambiguities were evident in the *Bam*HI-*Sal*I double digestion map. While mapping three additional enzymes, *Bal*I, *Pst*I, and *Xho*I, it becomes apparent how to resolve these ambiguities. Therefore, the earlier map is modified as follows (Figure 3): (1) The 5.6-kbp fragments are now known to be the same and are both designated *Bam*E, rather than E and F; (2) the *Bam* fragment order A-C-E-E-D-B rather than A-C-D-E-E-B has been established; (3) the order of the internal *Bam*HI-*Sal*I double digestion products within *Bam*B (BS 3-8-2) has been inverted; and (4) the estimated fragment sizes are now based on a 130-kbp genome size.

The arrangement of the *Bal*I, *Bam*HI, *Pst*I, *Sal*I, and *Xho*I fragments into a physical cleavage site map was based primarily on the results of the double digestion experiments. A map that is consistent with the 31 limit and 115 double digestion products is shown in Figure 4.

In addition to double digestion data, the *Pst*I mapping was based on studies with isolated fragments. Redigestion of isolated *Pst*A by *Bam*HI yielded *Bam*C, D, E, E, and BP3 as products. Treatment of *Pst*A with *Sal*I gave PS1, 6, and 7 as products. Redigestion of *Bam*A by *Pst*I gave BP1, *Pst*D, *Pst*E, and BP 10. Finally, *Pst*C was the major product when BS2 was redigested with *Pst*I. All of the above results are consistent with the map order predicted from the double digestions.

A third type of evidence for the cleavage site map came from partial digestion experiments. The *Bal*I partials G-H, H-H, and H-J were obtained, consistent with the predicted G-H-H-J

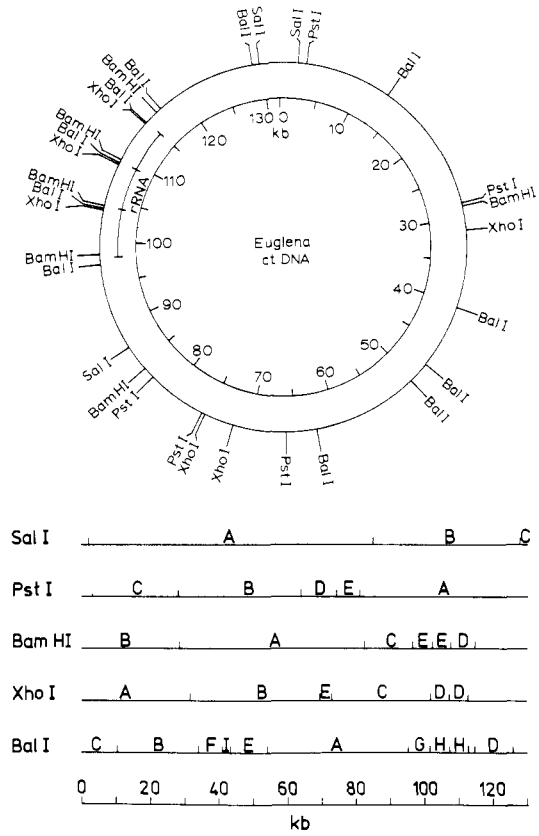

FIGURE 4: Restriction endonuclease cleavage map of *Euglena* chloroplast DNA. The letters refer to cleavage fragments described in the text and in Table III.

fragment order. A *Bal*F-I partial was also found. Partial digestion with *Xho*I gave the D-D partial, but not D-E. The *Pst*I partial corresponding to *Pst*D-E, but not C-D or C-E could be generated.

The relative order of Pst D and E predicted from the double digestion data was confirmed by means of an exonuclease III digestion experiment. Chloroplast DNA was digested with *Sal*I and then treated with exonuclease III for varying lengths of time. Following heat treatment to destroy the nuclease, the DNA was treated with *Pst*I. The fragment *Pst*E was placed closest to the *Sal*I cleavage site since it was found to be more susceptible to exonuclease III than *Pst*D.

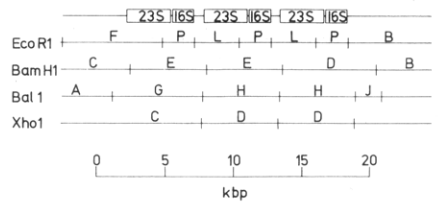

FIGURE 5: Restriction endonuclease cleavage sites and ribosomal RNA cistron locations on the *Euglena* chloroplast genome. Approximately 20 kbp of the 130 kbp genome is shown.

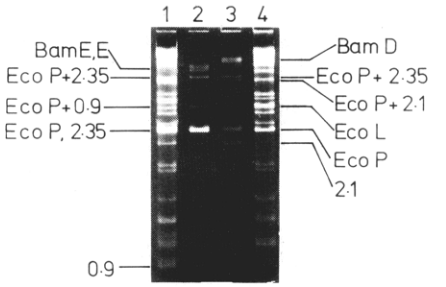

FIGURE 6: Analysis of *Eco*RI fragments in the 5.6 kbp repeated DNA segment. *Bam*D and *Bam*E were redigested with *Eco*RI. The products, which include both limit and partial digestion products, were analyzed by electrophoresis on a 2% agarose gel. (Lane 1) *Bam*HI–*Eco*RI double digestion of ct DNA; (2) *Eco*RI digestion of *Bam*E; (3) *Eco*RI digestion of *Bam*D; (4) *Eco*RI digestion of ct DNA. The interpretation of the fragment patterns for lanes 2 and 3 is given at the left and right of the figure, respectively.

The relative order of the adjacent fragments *Bal*F and *Bal*I could not be determined since these two fragments were not cleaved in any of the double digests. The fragments *Bal*D and E were also not cleaved by any enzyme and cannot be resolved at present.

Physical Map of the Ribosomal RNA Gene Region. An important region of the cleavage site map is the region containing the fragments *Bal*H, *Bam*E, and *Xho*D. These fragments are each 5.6 kbp in size, each present twice per genome, and are paired (*Bal*H-H, *Bam*E-E, *Xho*D-D) as judged by partial digestion and double digestion results. The three sets of paired fragments overlap one another in a 17-kbp segment of the genome, which maps in the region between 97 and 115 kbp on our representation of *Euglena* chloroplast DNA (Figure 4). A detailed map of this region is shown in Figure 5. There are also two adjacent 5.6-kbp *Hpa*I fragments (*Hpa*G) present in this region (P. W. Gray, unpublished observation). The only DNA sequence arrangement that can lead to such a digestion pattern consists of three adjacent, tandemly repeated 5.6-kbp segments. In each 5.6-kbp repeat there is a single cleavage site for each of the enzymes *Bal*I, *Bam*HI, *Hpa*I, and *Xho*I. A final conclusion is that each of the 5.6-kbp restriction nuclease fragments, *Bal*H, *Bam*E, *Hpa*G, and *Xho*D, have a permutation of the same DNA sequence.

Each 5.6-kbp repeated segment contains two *Eco*RI cleavage sites. This finding was based on redigestion studies with isolated *Bam*HI fragments D and E. Both *Bam*E fragments are cleaved into an *Eco*RI fragment P, and two *Bam*HI cleavage products of *Eco*RI fragment L (Figure 6; *Eco*RI fragment nomenclature based on Gray and Hallick, 1977). *Bam*D is cleaved into *Eco*RIP, a 2.35-kbp fragment identical with one of the cleavage products of *Eco*RIL, and a 2.1-kbp product (Figure 6). Therefore, *Eco*RIP and L, which total 5.6 kbp in size, represent the DNA sequence of a single repeat, and are arranged in the 13.5-kbp segment P-L-P-L-P in the repeated region. From double digestion experiments involving *Eco*RI and *Bam*HI and from studies on a recombinant plasmid DNA carrying *Eco*RIF (P. W. Gray and R. J. Hall, unpublished observation), the fragments *Eco*RIF and B have been mapped at either end of the above sequence (Figure 5).

*Eco*RI fragments of approximately 7.3, 3.2, and 2.3 kbp, which correspond to the fragments designated F, L, and P above, have previously been shown to hybridize with 16S and 23S chloroplast ribosomal RNA (Stutz et al., 1976; Mielenz et al., 1977; J. R. Y. Rawson, personal communication). Since these fragments have been mapped in the repeated region, it may be concluded that the 16S and 23S rRNA genes are each present on the 5.6-kbp repeated DNA segment. This rRNA region is present three times as a tandem repeat on the genome and accounts for 17 kbp, or 13% of the 130-kbp DNA.

Discussion

The 5.6-kbp repeated segment of *Euglena* chloroplast DNA is large enough to code for both the 16S and 23S chloroplast ribosomal RNA and a small amount of spacer region. The mature rRNAs are approximately 0.56×10^6 and 1.1×10^6 daltons (Scott and Smillie, 1967; Stutz and Rawson, 1970). DNA coding for these transcripts would be approximately 1.6 and 3.2 kbp respectively, accounting for 4.8 kbp of each 5.6-kbp rRNA repeat. Precursors for the 16S and 23S rRNAs of 0.64×10^6 and 1.16×10^6 daltons have been described (Scott, 1976). At least 5.3 kbp is required to code for the precursor molecules, leaving approximately no more than 300 bp of nontranscribed spacer in the rRNA gene region.

Rawson and colleagues (J. R. Y. Rawson, personal communication) have been independently studying the arrangement of chloroplast rRNA genes, employing bacterial plasmid DNA recombined with fragments *Bam*D or *Bam*E for nuclease digestion and hybridization studies. These workers first recognized the relationship between the *Eco*RI fragments P and L, and the *Bam*D and *Bam*E fragments, and predicted three rRNA cistrons per genome. They also made the important additional observation that 16S rRNA hybridizes only to *Eco*RIP, whereas 23S rRNA hybridizes to both L and P. We have confirmed their physical mapping of the *Eco*RIP and L fragments, and used their hybridization results to locate the 16S and 23S rRNA genes on our cleavage site map (Figure 5). The sequence coding for mature 16S rRNA is placed entirely in *Eco*RIP. This conclusion is based both on the hybridization data (of Rawson and colleagues) and the known RNAse T1 oligonucleotides of *Euglena* chloroplast 16S rRNA. RNA transcription through an *Eco*RI cleavage site, and subsequent digestion of the RNA with RNase T1 would yield an oligonucleotide that would begin 5′AAUUC.... No oligonucleotide with this sequence is among the reported RNase T1 products of 16S rRNA in *Euglena* (Zablen et al., 1975). The *Eco*RI site preceding the 16S rRNA gene must actually be 300–500 bp from the start of the rRNA repeat, since both *Bal*I and *Xho*I sites, which are located 300–500 bp from the *Eco*RI site, map in each 5.6-kbp segment. Following the 16S gene and also beginning in *Eco*RIP is the 23S rRNA precursor, which must occupy almost the entire remainder of the 5.6-kbp repeat.

There have been two reports that mature chloroplast rRNA hybridizes with *Eco*RIB (Stutz et al., 1976; Mielenz et al., 1977). This fragment does not map in the regions identified as coding for 16S and 23S rRNA. Since the 16S rRNA precursor is approximately 300 nucleotides longer than 16S rRNA, the observed hybridization may be due to a rRNA precursor. The transcription of this precursor would be predicted to begin outside the above mentioned *Eco*RI site.

From the restriction nuclease mapping results, it may be concluded that the *Euglena* chloroplast genome contains three cistrons for the 16S and 23S rRNA. There have been several previous determinations of rRNA cistron content based on hybridization experiments, with reported values including one (Rawson and Haselkorn, 1973), two (Vandrey and Stutz, 1973; Groul et al., 1975; Chelm et al., 1977b; Mielenz et al., 1977), three (Stutz and Vandrey, 1971; Kopecka et al., 1977), and three to six (Scott, 1973). Although the recent estimate of Stutz and colleagues of three cistrons per genome was accurate (Kopecka et al., 1977), it is important that this result could be verified by physical mapping of the genes.

In addition to the map of the rRNA genes, a detailed cleavage site map of the entire *Euglena* chloroplast genome has been determined. Thirty-one limit digestion products of the enzymes *Bal*I, *Bam*HI, *Pst*I, *Sal*I, and *Xho*I, and 115 double digestion products have been ordered in a self-consistent cleavage site map. This map represents to the best of our knowledge the most detailed physical analysis of this genome available. However, since additional physical studies of *Euglena* chloroplast DNA are in progress, including buoyant density analysis of fragments, mapping of additional cleavage sites, and transcription mapping experiments, the results presented here will undoubtedly be refined as new data become available.

Acknowledgments

We wish to thank J. R. Y. Rawson for the communication of his rRNA mapping and hybridization results, which enabled us to order the 16S and 23S genes within the 5.6-kbp repeated DNA segment.

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