Nucleotide Sequence Homology Exists between the Chloroplast and Nuclear Ribosomal DNAs of *Euglena gracilis*¹

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ABSTRACT

The nuclear and chloroplast ribosomal DNAs from *Euglena* were shown to have specific regions of nucleotide sequence homology. The regions of homology were identified by hybridization of restriction endonuclease DNAs of cloned chloroplast and nuclear ribosomal DNAs to one another. The regions of homology between these two ribosomal DNAs were in part the genes that code for the 3' end of the small rRNAs (16S and 18S) and near or at the DNA sequences coding for the 5S rRNAs. The nucleotide sequence homology between these regions was estimated to be approximately 94% by the melting point depression of a hybrid formed between the two ribosomal DNAs.

Nucleotide sequence homology between chloroplast and nuclear rRNAs has been explored in a variety of plants (1, 12, 26, 28). All of these studies have shown hybridization between the cytoplasmic rRNA and chloroplast DNA or reciprocally between the chloroplast rRNA and the nuclear DNA. These results have been interpreted in three ways: (a) nucleotide sequence homology exists between the nuclear and chloroplast rRNAs; (b) the cytoplasmic and/or chloroplast rRNAs used for these studies were contaminated with one another; or (c) the chloroplast and nuclear DNAs to which the different rRNAs were hybridized were contaminated with one another.

Final resolution of the question of nucleotide sequence homology between nuclear and chloroplast rDNAs required the use of absolutely pure preparations of nuclear and chloroplast rDNAs. Both the nuclear and chloroplast rDNAs from *Euglena gracilis* have been cloned and individually isolated as homogenous recombinant DNA molecules (4, 20). These cloned rDNAs were used in hybridization studies to demonstrate the presence of specific nucleotide sequence homology between chloroplast and nuclear rDNAs and to localize those regions on restriction endonuclease maps of the two rDNAs.

MATERIALS AND METHODS

Construction of Recombinant Plasmids. *Euglena* chloroplast rDNA was cloned into the *Bam HI* restriction endonuclease site of the bacterial plasmid vector pBR313 (20) to yield a recombinant plasmid pVK52 containing a complete chloroplast rDNA repeat 6.2 kbp in length (Fig. 3). This plasmid was used to construct the plasmid pECRB1 which contains a 0.85-kbp nucleotide sequence delineated by *Eco RI* and *Bam HI* restriction endonuclease sites (W. H. Andrews, personal communication). This DNA fragment represents the spacer sequence between the rDNA repeat unit on the chloroplast genome and contains part of the 5S rRNA gene (14, 15). An *Eco RI* chloroplast DNA fragment (*Eco RI*-P) containing the complete 16S rRNA gene, two tRNA genes, and approximately 6% 23S rRNA was cloned into the vector RSF2124 (14, 15, 20) to yield the recombinant plasmid pVK49. This *Eco RI* chloroplast DNA fragment is completely contained in pVK52.

Construction of λ Recombinants. The nuclear rDNA was plaque purified from a library of recombinant DNA molecules consisting of the λ phage Charon 9 and *Eco RI* endonuclease DNA fragments of *E. gracilis* DNA (4). The library was screened using ¹²⁵I-labeled cytoplasmic rRNA (4). The recombinant phage Ch9-ERD8 DNA was purified and extensively mapped with restriction endonucleases (4).

Propagation and Isolation of Plasmids and λ Recombinant DNAs. Recombinant plasmids were propagated and isolated from *Escherichia coli* as described earlier (17, 19). λ Recombinant phage were propagated in *E. coli* K802 (4, 19) and isolated as described by Williams and Blattner (29). Phage DNA was prepared by the method of Bovre and Szybalski (3).

Restriction Endonuclease Digestion of DNAs and Gel Electrophoresis. All restriction endonucleases were purchased from New England Biolabs and were used as recommended by the supplier. Restriction endonuclease DNA fragments were separated on the basis of mol wt by electrophoresis in agarose slab gels. The gels were prepared in E buffer (30 mm Tris, 30 mm NaH₂PO₄, and 1 mm EDTA) and run at room temperature at 1.5 to 2.0 V/cm for 12 to 16 h. The gels were stained and photographed as described earlier (20). HindIII λ DNA digestion products were used as mol wt markers.

Preparation of Southern Imprints and Hybridization of Nucleic Acids. DNA was eluted from agarose gels onto strips of Millipore paper (HA, 0.45 mm) according to Southern (24). The Southern imprints were preincubated in 6 × SSC (SSC: 0.15 M NaCl and 15 mm Na-citrate), 5 × Denhardt's solution (5), and 0.5% SDS for 2 to 3 h at 60°C. Radioactive DNA in 6 × SSC, 0.5% SDS, and Denhardt's solution was hybridized to the Southern imprints. Following hybridization, the Southern imprints were washed three times for 5 min each, twice for a total of 30 min, and three more times for 5 min each in an excess of 2 × SSC and 0.5% SDS at 60°C. In 2 × SSC, the Tm of a DNA duplex with a base composition of 50% is 94°C. Autoradiographs were prepared of the Southern imprints by exposing the filters to film (Dupont Cronex-2DC) in the presence of intensifier screens at −80°C (27).

In Vitro Labeling of DNA and RNA. DNA was labeled in vitro with [α-³²P]dATP by nick translation (21). Following the reaction, the volume of the mixture was increased to 0.50 ml with 10 mm Tris-HCl (pH 8.0) and 1 mm EDTA and 100 μg *E. coli* tRNA were added. The DNA was precipitated with 0.1 volume of 3 M sodium acetate plus 2 volumes of ethanol and collected in a ¹ Supported by research grants PCM79-01596 and PCM80-07507 from the National Science Foundation to J. R. Y. R. and by National Institutes of Health Predoctoral Traineeship 5-T37-GM07103-0 to S. E. C. ² Abbreviations: rDNA, ribosomal DNA; kbp, kilobase pairs.
The intense bands were detected by autoradiography. [\(^{32}P\)]Ch9-ERD8 DNA did not hybridize to any DNA fragment (data not shown). A small amount of hybridization of Ch9-ERD8 was detected in the region of the 8.7-kbp Bam HI DNA fragment (pBR313). This must be due to trapping of the larger 6.2-kbp DNA fragment by the larger DNA fragments as evidenced by the fact the Ch9-ERD8 did not hybridize to pBR313 DNA (data not shown).

**RESULTS**

Hybridization of Nuclear rDNA to *Euglena* Restriction Endonucleases DNA Fragments. Studies on the nuclear rDNA of *Euglena* (4) initially suggested the possibility of the existence of nucleotide sequence homology between the chloroplast and nuclear rDNAs. In these studies, a cloned nuclear rDNA repeat unit (Ch9-ERD8) was hybridized to Southern imprints of *E. gracilis* var. Z DNA to test the arrangement of rDNA in the nuclear genome. Ch9-ERD8 DNA hybridized to restriction endonuclease DNA fragments which coincided with those predicted from the map of the nuclear rDNA insert (4) and, in addition, to several other DNA fragments (Fig. 1). Since there is no nucleotide sequence heterogeneity in the nuclear rDNA (4) and Charon 9 DNA does not hybridize to *Euglena* DNA (data not shown), the possibility existed that these other DNA fragments might be explained by sequence homology of the nuclear rDNA to organelle DNA (chloroplast and/or mitochondrial DNA).

**Nucleotide Sequence Homology between Nuclear and Chloroplast rDNAs.** The most likely region of nucleotide sequence homology between the nuclear rDNA and the chloroplast DNA was the chloroplast rDNA. The possibility of such homology was tested by hybridizing [\(^{32}P\)]Ch9-ERD8 DNA and the two individual [\(^{32}P\)]cytoplasmic rRNAs (25S and 19S) to restriction endonuclease DNA fragments of the plasmid pVK52. Both Ch9-ERD8 DNA and the 19S cytoplasmic rRNA hybridized to specific regions of the chloroplast rDNA. These data are shown in Figure 2 and summarized in Table I. The 25S cytoplasmic rRNA showed no sequence homology to the chloroplast rDNA (data not shown).

The 19S cytoplasmic rRNA hybridizes to a region of the chloroplast rDNA (pVK52) which is most narrowly defined by a 1.4-kbp Eco RI-Hind III DNA fragment (Table I and Fig. 3A). This region of the chloroplast rDNA encompasses the 3′-end of the 16S rRNA gene and the 5′-end of the 23S gene. The nuclear rDNA (Ch9-ERD8) also hybridizes to the 1.4-kbp Eco RI-Hind III fragment as well as to the 0.8-kbp *Bam HI*-Hind III and 0.8-kbp *Bam HI*-Eco RI fragments. The 0.8-kbp *Bam HI*-Hind III fragment contains the 3′-end of the 23S rRNA gene and a part of...
the 5S rRNA. The 0.8-kbp Bam HI-Eco RI fragment contains the other part of the 5S RNA gene and approximately 0.7 kbp of DNA of unknown function. Since the 25S cytoplasmic rRNA does not hybridize to the 23S chloroplast rDNA gene, we concluded that the region of homology existing between this 0.8-kbp Bam HI-Hind III DNA fragment and the nuclear rRNA must be in the region near or at the DNA sequence coding for 5S RNA genes contained on both of these DNA sequences.

The converse experiments were performed by hybridizing the cloned chloroplast rDNA (pVK52) to Southern imprints of the cloned nuclear rDNA (Ch9-ERD8). Two subclones of pVK52 (pVK49 and pECRB1) were also used. The hybridization of pVK52, pVK49, and pECRB1 DNAs to Ch9-ERD8 DNA fragments is summarized in Table II and shown diagrammatically in Figure 3B. The chloroplast rDNA probes show homology with regions of the nuclear rDNA map which represent the genes for 19S and 5S rRNAs.

Measurement of Tm of DNA-DNA Hybrid Formed between Chloroplast and Nuclear rDNAs. The degree of homology that exists between the chloroplast and nuclear rDNA genes was determined by measuring the Tm of DNA-DNA hybrids formed between the two rDNA sequences. Trace amounts of [3H]pVK52 were hybridized with a vast excess of chloroplast DNA or Ch9-ERD8 DNA. The hybrids were bound to and thermally eluted from hydroxylapatite in 0.12 M sodium phosphate buffer (Fig. 4). The Tm of the hybrid formed between pVK52 (chloroplast rDNA) and the chloroplast DNA and between pVK52 and the nuclear rDNA were 84°C and 78°C, respectively. Inasmuch as there is no sequence homology between the two vectors (pBR313 and Charon 9) used to construct the recombinant DNA molecules pVK52 and Ch9-ERD8, the only possible region of homology between these 2 recombinant DNA molecules must be the two rDNAs. The specificity of the nucleotide sequence homology between the chloroplast and nuclear rDNAs was determined from the difference in the melting temperatures of these duplexes. Since a 1°C drop in Tm indicates approximately 1% base pair mismatch of a reassocitated DNA duplex (7), those regions of the two rDNAs which hybridize must be approximately 94% homologous to one another.

**DISCUSSION**

The existence of nucleotide sequence homology between the chloroplast and nuclear rDNAs has been suggested in studies of these genes in both algae and higher plants (1, 12, 26, 28). These experiments were all performed using either cytoplasmic and/or chloroplast rRNAs as hybridization probes and were subject to criticism because the possibility of contamination between the two rRNA species or the two DNAs species had not been excluded. The most direct means of resolving this problem was to study the sequence homology of cloned nuclear and chloroplast rDNA sequences. The cloned nuclear and chloroplast rDNAs of *Euglena* were hybridized to one another, two distinct regions of nucleotide sequence homology were observed. These two regions of homology were not contiguous with one another and were each 1 kbp or less in size. One region of homology was shown to exist between the cytoplasmic 19S rRNA gene and that part of the gene coding for the 3' end of the chloroplast 16S rRNA. This observation is not surprising in that the 3' terminus of eukaryotic 18S rRNA molecules, although lacking the Shine-Dalgarno sequence, are highly conserved and exhibit strong homology with the 3'-terminus of the *E. coli* 16S rRNA (10, 22). The 3'-terminus of the chloroplast 16S rDNA in *Euglena* has been shown to be similar to the 3'-terminus of *E. coli* 16S rRNA (8) and probably functions in mRNA recognition and binding during formation of an initiation complex during protein synthesis as it does in *E. coli* (23, 25). Undoubtedly much of the nucleotide sequence homology existing between what is probably the 3'-end of the 19S cytoplasmic rRNA gene and the 3'-end of the 16S chloroplast rRNA gene is related to the functional significance of these sequences in the ribosome.

The second region of the homology that exists between the chloroplast and nuclear rDNAs occurs near or at the DNA sequences that code for the 5S RNAs. The nucleotide sequence homology between these regions of the rDNAs is more difficult to explain. Chloroplast 5S RNAs from several plants have been sequenced (6) and compared to cytoplasmic 5S RNA (16) from the same plant. There is considerable difference between the nucleotide sequence of the chloroplast and cytoplasmic 5S RNAs from these plants. Approximately 50% residues are different and there are few long stretches of nucleotide sequence homology between the chloroplast and cytoplasmic 5S RNAs of the same plant. Although there are differences between these 5S RNAs, it is much less than the expected 75% if the two RNAs were completely unrelated random sequences (6). Despite these findings in higher plants, the present results from *Euglena* tentatively suggest a greater degree of homology between the nuclear and
Fig. 3. Restriction endonuclease map of chloroplast and nuclear rDNAs showing regions of nucleotide sequence homology between the two rDNAs. The plasmid pVK52 consists of a 6.2-kbp Bam HI Euglena chloroplast DNA fragment and the vector pBR313. The Bam HI fragment contains a single gene for the 16S, two different tRNAs, 23S and 5S rRNAs. The plasmid pVK49 contains a 2.6-kbp Eco RI chloroplast DNA fragment carrying the complete 16S rRNA gene, two tRNA genes, approximately 6% 23S rRNA, and the vector RSF2124. The Eco RI DNA fragment carried on pVK49 is completely contained in the Bam HI DNA fragment of pVK52. The chloroplast restriction endonuclease DNA fragment carrying part of the 5S rRNA gene and contained on pECRB1 is also located on the Bam HI DNA fragment in pVK52. The recombinant phage Ch9-ERD8 consists of Ch9-arms, a Ch9 stuffer fragment, and a complete nuclear rDNA repeat containing the 19S, 25S, 5.8S, and 5S rRNA genes. The minimum region defined by various restriction endonuclease sites to which each of these recombinant DNAs hybridize are indicated by arrows above those DNA fragments.

Chloroplast 5S rRNA sequences. Confirmation of this suggestion awaits sequencing of these two 5S rRNAs or their respective genes.

Although homology between nuclear and chloroplast rDNA sequences has been previously reported, the present study is the first to demonstrate unequivocally that such homology is not due to impure preparations of rRNA or rDNA species. The sequence homology between the chloroplast and nuclear rDNAs of Euglena is generally limited and may be attributed to the functional properties of these sequences. Earlier studies on Euglena nuclear and chloroplast DNAs (6) indicated an absence of rDNA sequence homology. These findings may reflect the limited degree of Euglena nuclear and chloroplast rDNA homology which probably would have been undetectable given the resolution of the techniques used by Groul et al. (9).

When the possibility of nucleotide sequence homology was explored in the alga Chlamydomonas, using cloned nuclear and chloroplast rDNAs, no homology was detected between these two DNA sequences (13). At this time, it is not possible to determine whether the variance in our observations using Euglena rDNA with those made in Chlamydomonas is due to a difference in the stringency of the hybridization conditions or is actually a true

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<th>+ Eco RI</th>
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* Size of DNA fragments in kbp.
\text{abc} DNA fragment hybridized to [\text{32P}]pVK52 DNA.
\text{bcd} DNA fragment hybridized to [\text{32P}]pVK49.
\text{de} DNA fragment hybridized to [\text{32P}]pECRB1.
\text{ef} DNA fragment not detected in our gel system.

**Fig. 4.** Thermal elution profile of the hybrid between nuclear rDNA and chloroplast rDNA. Radioactive pVK52 DNA was hybridized to a vast excess of either Ch9-ERD8 (\text{–––}C) or chloroplast DNA (\text{––––}O), adsorbed to hydroxylapatite columns and thermally eluted. The Tm of the hybrid formed between [\text{32P}]pVK52 and chloroplast DNA or Ch9-ERD8 DNA was 74°C and 78°C, respectively.

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**LITERATURE CITED**