Molec. gen. Genet. 179, 539–545 (1980)

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Homologies Among Ribosomal RNA and Messenger RNA Genes in Chloroplasts, Mitochondria and *E. coli*

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Summary. Labelled chloroplast rRNAs from *Spinacia oleracea* were hybridized to restriction endonuclease digests of chloroplast DNA from *Oenothera hookeri* and *Euglena gracilis,* to mitochondrial DNA of *Acanthamoeba castellanii,* and to DNA of the *E. coli* rrn B operon in the transducing phage lambda rif^{d}18. The degree of homology is greatest for the 16S rRNA gene. Greater than 90% occurs between the two higher plant genes, 80% homology to the lower plant gene, 60%–70% homology to the bacterial gene, and 20% homology to the mitochondrial gene. The degree of hybridization varied considerably for the 23S and the 5S rRNA genes. Very high homology exists between the two higher plant genes, only about 50% homology for both the Euglena and bacterial genes, and no significant homology for the mitochondrial genes. These results show that any chloroplast (or *E. coli*) rRNA may be used as a probe to identify rRNA genes in other ctDNAs.

Two RNA populations, each enriched for a different ctDNA-encoded mRNA, proved useful in the location of these genes on both higher plant ctDNAs. No significant hybridization was obtained using these probes to the *Euglena* ctDNA which seems to be too distantly related.

Introduction

Mitochondria and plastids are genetically semi-autonomous organelles, containing their own DNA and ribosomes and being able to carry out transcription and translation. These genetic systems are regarded as procaryotic because several of their features are analogous to those of *E. coli* and blue-green algae. All plastid ribosomes studied are 70S in size, with subunits of 50S and 30S. These subunits contain large rRNA molecules of 23S and 16S, respectively (see Whitfeld 1977 for review). The large ribosomal subunit contains also a 5S rRNA (Payne and Dyer 1971). One difference between the bacterial and plastid ribosomes is the existence of additional small RNA molecules (4S to 7S which is species dependent) in addition to the above-mentioned 5S rRNA (Whitfeld et al. 1977). In all species studied these extra rRNAs are also found in the large ribosomal subunit.

Mitochondrial ribosomes display a greater diversity of size. One group is approximately 70S and occur in fungi, ciliates (see Borst and Grivell 1971), and Acanthamoeba mitochondria (Bohnert 1972). The subunits of these ribosomes contain rRNAs of similar sizes to those found in *E. coli.* Another group of mitochondrial ribosomes (miniribosomes) which have been found in higher animals is smaller in size (Borst and Grivell 1971). Their large rRNAs sediment considerably faster than those of the *E. coli* ribosomes. It is not yet clear whether or not mitochondrial ribosomes contain low molecular weight rRNAs of similar function to 5S rRNA in *E. coli* or, for example, the 4.5S rRNA in plastids. Such 5S rRNA has been found only in plant mitochondria to date (Leaver and Harmey 1976).

Physical maps of the rDNA regions have been constructed for all these DNAs (Rawson et al. 1978; Lund et al. 1976; Gordon et al. 1980; Crouse et al. 1978; Bohnert and von Gabain to be published). The *E. coli* rrn B rDNA cistron and the three ctDNA have the same order of rRNA genes, i.e. 16S-spacer-23S-5S. That they form a transcriptional unit has been shown for *E. coli* (Lund et al. 1976), spinach (Bohnert

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Abbreviations: Md = megadalton, 10^6 dalton; bp, kbp = base pair, kilo base pair; SSC = Standard saline citrate, 1 times SSC is 0.15 M sodium, chloride, 0.015 M trisodium citrate, pH 6.8; mtDNA = mitochondrial DNA; ctDNA = chloroplast DNA; ctrRNA = chloroplast ribosomal RNA

0026-8925/80/0179/0539/$01.40

et al. 1976) and Euglena (Wollgiehn and Parthier 1979).

By using DNA/RNA hybridization we have, in the present study, determined homologies between the ctDNA from the dicotyledons *Spinacia oleracea* and *Oenothera hookeri*, from the alga *Euglena gracilis*, and from the *E. coli* rrn B rDNA cistron (Nomura et al. 1977) in the transducing phage lambda rif^{d}18 (Lund et al. 1976). To include a comparison to a mtDNA encoding rRNAs for 70S ribosomes, the mtDNA from *Acanthamoeba castellanii* was used in the experiments.

To further examine sequence homologies between genes in the three ctDNAs, two chloroplast RNA fractions from spinach, each enriched for a specific mRNA, have been used. The mRNAs code for the 55 kd large subunit of ribulose bisphosphate carboxylase (E.C. 4.1.1.39) (Driesel and Schmitt unpublished work) and for a 32 kd protein probably from the thylakoid membrane (Driesel 1979). The genes for both these proteins have been tentatively located on the physical map of spinach ctDNA (Driesel 1979; Herrmann and Possingham 1980). Their location is comparable to those already determined for *Zea mays* ctDNA (Bedbrook et al. 1978; Coen et al. 1977) and, in the case of the putative membrane protein, in *Chlamydomonas reinhardii* ctDNA (Malnoe et al. 1979).

Material and Methods

Isolation of DNAs

Chloroplast DNA was isolated from *Spinacia oleracea* var. Monopa as described in Driesel et al. (1979), from *Oenothera hookeri* var. Johansen (plastome I) as described in Gordon et al. (1980), and from *Euglena gracilis* strain Z as described in Kopecka et al. (1977). Mitochondrial DNA from *Acanthamoeba castellanii* was isolated as described in Bohnert and von Gabain (to be published). The *E. coli* rrn B rDNA unit in the transcluding phage lambda rif^{d}18 was a gift of Drs. I. Kiss and P. Venetianer, Szeged, Hungary.

Isolation of Spinach Chloroplast rRNAs

Usually 1 kg of spinach leaves, longer than 5 cm, were washed, surface sterilized and used to isolate chloroplasts. Conditions were selected to obtain a high yield of structurally intact chloroplasts which could be washed free of contaminating cytosolic ribosomes by raising the EDTA concentration to 20 mM. The chloroplasts were lysed by the addition of 20 mM Tris/HCl, pH 7.8, 50 mM potassium chloride, 10 mM magnesium acetate, 3 mM dithiothreitol and 2% Triton X-100 in the cold. After centrifugation at 30 krpm (20 min, 0° C) to remove membrane remnants, the ribosomes in the supernatant were pelleted through a cushion of 20% sucrose in the above buffer. The yellowish clear pellet was resuspended in 20 mM Tris/HCl pH 7.8, 50 mM potassium chloride, 10 mM magnesium acetate, 1 mM dithiothreitol and incubated for 1 h at 37° C in the presence of 2 mM ATP, 4 mM phosphoenolpyruvate, and 20 µg/ml pyruvate kinase to facilitate run-off synthesis of mRNA ribosome complexes. After the addition of 1 volume 0.4 M ammonium acetate in the above buffer the suspension was chilled and any precipitate was removed by centrifugation at 10 krpm (10 min, 0° C). The ribosomes were then pelleted once more, either differentially to obtain pellets enriched for first the large and then the small ribosomal subunit, or totally by centrifugation through a 20% sucrose cushion at 55 krpm in a Beckman 75Ti rotor (5 h, 0° C). Ribosomal subunits were purified by two cycles of preparative 10%–35% sucrose gradient centrifugation (20 h at 24 krpm, 4° C, SW 25.2 rotor). The rRNAs were isolated from the purified ribosomal subunits by phenol extraction followed by two chloroform extractions. The small ribosomal subunit contains only the 16S rRNA which was recovered from a 5%–25% sucrose gradient in 50 mM Tris/HCl, pH 7.8, 150 mM potassium chloride, 1.5 mM magnesium acetate, 1 mM EDTA (16 h at 24 krpm, 4° C, SW 25.2 rotor).

The large ribosomal subunit contains the 23S rRNA and both the small rRNAs, 4.5S rRNA and 5S rRNA (Bohnert et al. 1976). The 23S rRNA was recovered after sucrose gradient centrifugation as above. The low molecular weight rRNAs were separated on 8% preparative polyacrylamide gels (Loening 1968). The individual bands were cut out of the gel and the RNA was eluted and phenolized again. All rRNAs were precipitated with ethanol and the precipitates were washed thoroughly and stored dry. The rRNAs were iodinated according to Commerford (1971) with modifications as described by Driesel et al. (1980).

Isolation of Chloroplast RNAs Enriched for mRNAs

The 14S and 19S RNA fractions containing mRNAs were obtained from chloroplasts of 10-day-old seedlings which had been kept in the dark for 2 days and then illuminated for 2 h immediately before plastid isolation. The total RNA from these plastids was fractionated on sucrose gradients as described in Driesel et al. (1980).

Restriction Endonuclease Cleavage

Up to 2 µg of the individual DNAs were fragmented using a variety of restriction endonucleases as stated in the figure legends. The enzymes were purchased from Boehringer (Mannheim) or Biolabs (Beverly) and were used as recommended. The DNA fragments were separated by electrophoresis on 0.8%–1.6% horizontal agarose slab gels in 40 mM Tris/HCl, pH 7.8, 20 mM sodium acetate, 1 mM EDTA, and 0.5 µg/ml ethidium bromide for up to 20 h at 10° C. The DNA bands were visualized with short wave length UV light and the fluorescence was photographed using a Polaroid camera and 665 film.

Fragment Transfer and Hybridization

The DNA in the agarose gels was denatured, neutralized, and transferred to nitrocellulose filters by slight modification of the method of Southern (1975). The filters were then incubated in sealed plastic bags for 24 h in 3 × SSC at 60 or 69° C in the presence of the individual iodinated RNAs. After hybridization, the filters were washed in 2 × SSC for 20 min at 50° C, treated with pancreatic RNase (20 µg/ml in 1 × SSC at 37° C) for 2 h, washed briefly in H_2O, dried, and exposed to Kodak XR-5 X-ray film for 4 days and up to 2 months. The films were sometimes preflashed.

Quantification of Hybridization Results

Negatives of the agarose gels and autoradiograph plates were scanned using a Joyce-Loebl densitometer. The areas of the peaks were determined using a Numonics graph calculator. With the

molecular weights of the DNA fragments and of the RNAs known, the amount of radioactivity, determined from not overexposed plates, could be correlated with the DNA amount. These ratios have been compared to a ratio obtained from the homologous spinach DNA/RNA hybrid which was always present as a standard on the same gel and plate.

Results

Cleavage of DNAs in Relation to rRNA Genes

All DNA samples have been cleaved by restriction endonucleases so as to separate the individual rRNA genes on different fragments as far as possible. Cleavage of spinach ctDNA with Eco RI yields most of the 16S rRNA gene on a 2.3 Md fragment and the remainder, along with the complete 23S rRNA gene, on a 2.9 Md fragment. The 5S rRNA gene is located on a 1.2 Md fragment (see maps in Crouse et al. 1978; Whitfeld et al. 1978; Figs. 1 and 2). Using the enzyme Bam HI, the 16S rRNA gene is located on a 2.4 Md fragment, the 23S and 5S rRNA genes on a 3.3 Md fragment (Fig. 1).

By cleaving Euglena ctDNA with Eco RI, the 16S and the 23S rRNA genes are located on a 2.4 Md and a 1.6 Md fragment. The 5S rRNA gene is found on the same fragment as the 16S rRNA gene (see map in Rawson et al. 1978; Hallick et al. 1979; Fig. 2). A fourth 16S rRNA gene, or a part of such a gene, is found on a 13 Md fragment (Jenni and Stutz 1979). This fragment is also labelled when spinach 16S rRNA is used (Figs. 1 and 2).

Eco RI cleavage of the rDNA units of Oenothera ctDNA is similar to that in spinach (see map in Gordon et al. 1980; Fig. 2). The 16S rDNA fene is mostly located on a 2.5 Md fragment. Some of the 16S rRNA gene and most of the 23S rRNA gene is on a 3.0 Md fragment. A 0.45 Md piece contains the rest of the 23S rRNA gene and a 0.75 Md fragment carries the 5S rRNA gene.

The 16S rRNA gene from lambda rif^{d}18 is located on Eco RI fragments of 3.8 Md and 1.35 Md, with the 23S rRNA gene being partly located in the latter of the fragments and partly on a 5.7 Md DNA fragment. The 5S rRNA gene is contained in this latter fragment too (see map in Lund et al. 1976; Fig. 1).

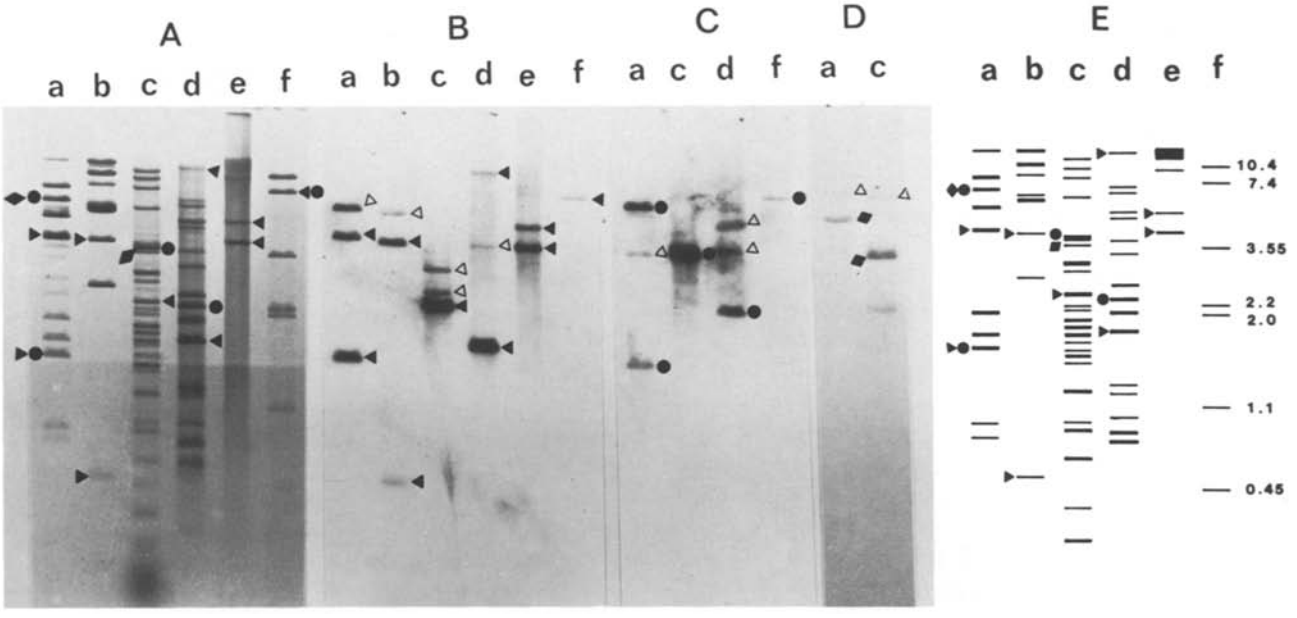

Fig. 1A–E. Hybridization of spinach chloroplast rRNAs to organellar DNA and DNA of lambda rif^{d}18 after restriction endonuclease cleavage. Part **A** shows the DNA fragment patterns of (a) lambda rif^{d}18 (digested with Eco RI), (b) lambda rif^{d}18 (Sma I), (c) spinach ctDNA (Bam HI), (d) *Euglena* ctDNA (Eco RI), (e) *Euglena* ctDNA (Bam HI), (f) *Acanthamoeba* mtDNA (Eco RI). Part **B** shows the hybridization of spinach chloroplast 16S rRNA to the pattern shown in Part **A**. In Part **C** is shown hybridization of spinach chloroplast 23S rRNA to patterns of the four different DNAs as shown in (a), (c), (d) and (f) of Part **A**. In Part **D** spinach chloroplast 5S rRNA has been hybridized to patterns as shown in (a) and (c) of Part **A**. Part **E** shows a schematical presentation of the DNA digestions given in Part **A**. The molecular weights (in Md) of the Eco RI DNA fragments from the mtDNA are given as markers in Part **E**. The DNA fragments were separated on a 0.8% agarose gel. – In **A** and **E** the bands to which the individual rRNAs hybridize are labelled; ▶ 16S RNA, ● 23S rRNA, ◆ 5S rRNA. Some intermediate bands in the autoradiographs which are not visible on the fragment pattern are also labelled (▷). The weak DNA bands in the lambda rif^{d}18 DNA pattern are due to some contamination with lambda DNA. – With *Euglena* ctDNA (Part **B**-d) hybridization of spinach chloroplast 16S rRNA also occurs to fragment Eco RI-B (13 Md), in addition to fragments of the rDNA units, (see text). As a control these rRNAs have been hybridized to fragments of lambda DNA and of T5 and T7 DNA. Essentially no hybridization was found with all different hybridization conditions

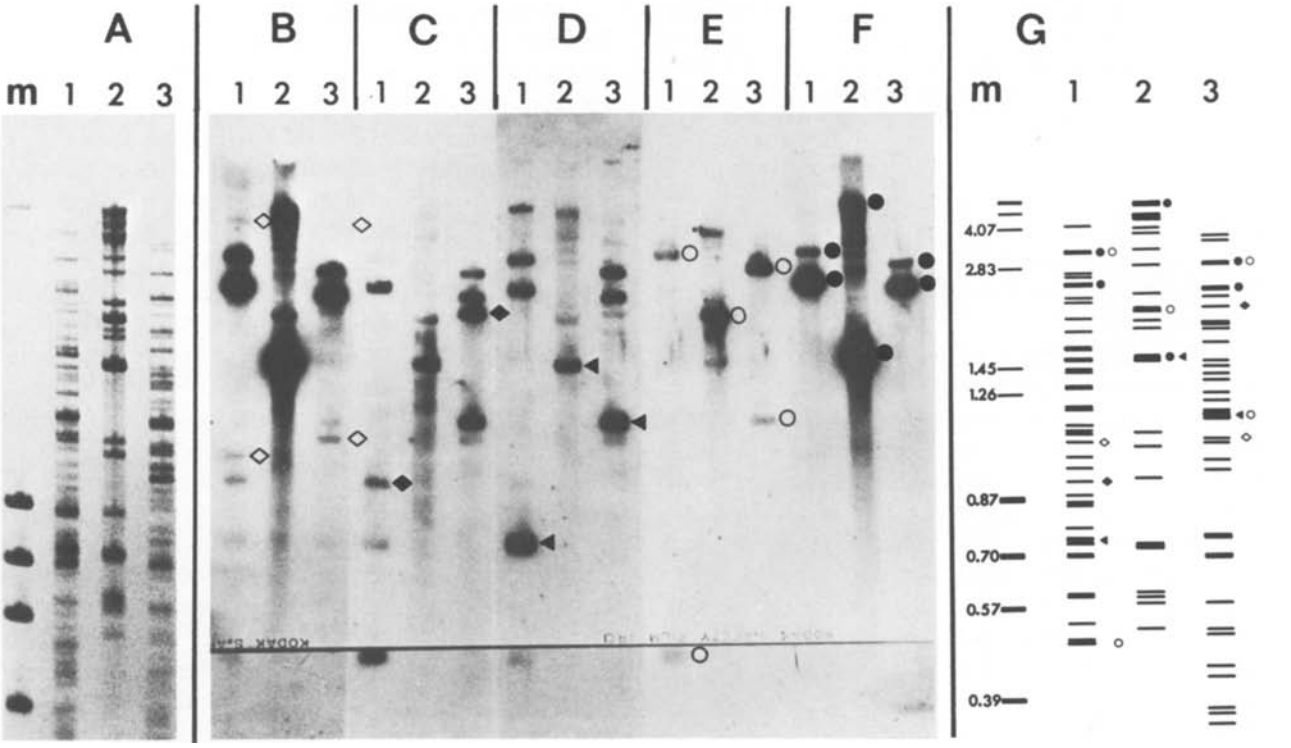

Fig. 2A–G. Hybridization of spinach chloroplast RNAs to ctDNAs of *Oenothera* (1), *Euglena* (2), and *Spinacia* (3) fragmented by the restriction endonuclease Eco RI. A photograph of the 1.2% agarose gel is shown in **A**. The DNA used as a marker (m) is DNA from phage lambda digested with Hind III (15–1.26 Md) and DNA of ϕX174 digested with Hae III (0.87–0.01 Md). The different RNAs hybridized to these DNA fragments were '55 kd enriched' mRNA (**B**), '32 kd enriched' mRNA (**C**), 5S rRNA (**D**), 23S rRNA (**E**), and 16S rRNA (**F**). The schematic presentation of the DNA fragments larger than approximately 0.4 Md produced in these digestions is given in Part **G**

The remainder of the *E. coli* genes on lambda rif^{d}18 are located on fragments of 1.35 Md, 0.80 Md, 1.84 Md, 1.54 Md, 0,86 Md, and 4.9 Md which are labelled in Fig. 1E. According to the physical map of the lambda rif^{d}18 DNA, a small fragment of 0.43 Md generated by Sma I is located entirely within the 16S rRNA gene. The other fragments covering this gene are of 3.75 Md and 5.3 Md, with the latter also containing the 23S and 5S rRNA genes. Hybridization of spinach ctrRNAs was observed to these fragments as predicted (data not shown).

For the amoeba mtDNA, the 16S rRNA gene is located on a 7.4 Md fragment, which also includes about 70% of the 23S rRNA gene. The rest of this latter gene is located on a 10.4 Md DNA fragment (Bohnert and von Gabain to be published).

Conditions for Hybridization of rRNAs

The G + C content of the rRNA genes of spinach ctDNA is approximately 52% (Crouse et al. 1978) and so the homologous hybridization was carried out at 69° C. The DNA/RNA hybrids, once formed, are stable up to approximately 85° C under our conditions. The optimum temperature for the heterologous hybridizations was found to be 60° C. This temperature was also used for hybridizations to the *E. coli* rRNA genes although the higher temperature (69° C) is suitable as expected from the similar average base composition of *E. coli* (Stanley and Bock 1965) and spinach (Rossi and Gualerzi 1970) rRNAs. Similarly, strong specific hybridization of spinach chloroplast rRNAs to Oenothera ctDNA was observed at 60° C or 69° C. Since the G + C content of Euglena ct rRNA genes is 47% (Crouse et al. 1978), there is weaker hybridization at 69° C of the spinach ctrRNAs and the lower temperature (60° C) employed proved more favorable for specific hybridization. In the Acanthamoeba mtDNA the G + C content of the rRNAs has not been determined; however the base composition of a large DNA fragment which contains the rRNA genes is 38% G + C (Crouse and Bohnert unpublished observation). While the G + C content of the rRNA genes may be even somewhat higher there is no hybridization of the spinach ctrRNAs at 69° C. Hybridization of the spinach rRNA to the 16S rRNA gene in the mtDNA was observed at lower

temperatures (60° C). With the 23S rRNA from spinach some weak hybridization occured to the DNA fragment which contained about 70% of the 23S rRNA gene, but no hybridization could be observed, even after long exposure, to the fragment carrying the rest of the 23S rRNA gene. The validity of the hybridization resulted is however demonstrated by the fact that unlabelled *E. coli* 16S or 23S rRNAs competed efficiently with the spinach rRNAs. (In 50-fold excess these bacterial rRNAs prevented hybridization to all organelle and the bacterial rRNA genes.)

After RNase removal of unhybridized rRNA, the intensities of the hybrid bands have been measured and quantitated using the known molecular weights for the rRNAs (see Whitfeld 1977 for review), the quantitatively measured DNA amount per band and the positions of the cleavage sites. The values expressed as percent of the homologous hybridization (spinach ctDNA/rRNAs) are given in Table 1. The highest degree of homology is evident in hybridization to the ctDNA of Oenothera: for all three rRNAs used sequence homology is over 90%. Euglena ctDNA also shows a high level of homology for the 16S rRNA gene, but half the homology for the 23S and 5S rRNA genes. A similar result to Euglena ctDNA is obtained for the *E. coli* rRNA cistrons: homology to the 16S rRNA from spinach is also high (60%–70%) but about half for the 23S and 5S rRNAs genes. The rRNA genes on Acanthamoeba mtDNA show least homology: the 16S rRNA is most pronounced; the 23S rRNA gene is poorly recognized, if at all (approx. 5%); and no 5S rRNA gene could be detected.

Table 1. Percentage of homology between rRNA genes of organellar DNAs and *E. coli* (rrn B) as determined by hybridization with spinach chloroplast RNAs

Source	DNA	16S	23S	5S	'mRNAs'[a]
Spinach	ct	100	100	100	+
Oenothera	ct	>90	>90	>90	+
Euglena	ct	80	50	50	+ (?)
Acanthamoeba	mt	20	5 (?)	0	–
E. coli (rif^{d}18)	chromosomal	60–70	40–50	40	–

The quantitation has been performed as described. Only experiments were several different DNAs were present on one gel have been used. The results were consistently the same in three experiments (± 10% if no range is indicated). Virtually no hybridization occurred to lambda DNA or to fragments of T5 DNA which were sometimes included as marker DNAs

[a] Only the three ctDNAs were compared; + indicates the hybridization to additional DNA fragments besides to fragments which carry the rRNA genes

Hybridization of mRNA – enriched RNA Fractions

Two spinach ctRNA fractions enriched for mRNAs were used in a further analysis of the sequence homology between ctDNA genes. One of the fractions (14S RNA), when used to program a rabbit reticulocyte translation system. yielded as a major product a polypeptide approximately 32 kd in size and another fraction (19S RNA) yielded an approximately 55 kd polypeptide (Driesel 1979; Herrmann and Possingham 1980). While not being pure, these RNAs could be labelled and used for hybridization to Eco RI cleaved ctDNAs of spinach, Oenothera and Euglena (Fig. 2A and B). As a result of the way in which these RNAs were isolated from sucrose gradients of total ctRNA most of the RNA present in each component was composed of 16S and 23S rRNA sequences. In Fig. 2 are shown the hybridizations of the three ctDNAs with 16S (F), 23S (E) and 5S rRNA (D) in addition to the hybridization of the mRNA enriched fractions (B and C). Elimination of the rRNA hybridization was used to determine which hybridization bands represented each of the mRNAs. Again there is the highest level of homology between spinach and Oenothera ctDNAs whereas with Euglena ctDNA any specific hybridization of the mRNAs remains doubtful. Even between spinach and Oenothera ctDNAs the mRNAs seem to be considerably different as one might see from the mRNA 32 kd hybridization to spinach ctDNA (arrow in Fig. 2C) while to Oenothera ctDNA one extra band has much less label.

Discussion

This study has revealed varying levels of homology in the sequences of rRNA genes, depending both on the type of rRNA and the source of the DNA. As might be expected, the highest degree of homology is observed between ctDNAs from dicotyledenous plants. Similar observations have been made with some other higher plants (*Beta vulgaris* and *Anterrhinum majus*) and with Spirodela (van Ee personnal communication). The degree of homology comparing spinach ctRNA and Euglena ctrDNA is smaller and about equal to the homology observed when comparing spinach ctrDNA and the *E. coli* rrn B cistron. For the spinach-*E. coli* comparison we find 60%–70% homology for the 16S rRNA. This value is in good agreement with the 74% homology reported between the maize chloroplast 16S rRNA gene (Schwarz and Kössel 1980) and the *E. coli* 16S rRNA (Carbon et al. 1979) or its gene (Brosius et al. 1979). Even when a gross homology exists there may, however, be cer-

tain regions of a gene where sequence divergence has occurred.

For the 16S rRNA gene on amoeba mtDNA, a low level of sequence homology (20%), which can be competed out by *E. coli* 16S rRNA, is found with the 16S rRNA from spinach chloroplasts. The overall homology for the 16S, 23S, and 5S rRNA genes is comparable to that found for cross-hybridization of Euglena mitochondrial rRNAs to Euglena ctDNA (Crouse et al. 1974). Homology has also been demonstrated between the mtDNA of maize and the *E. coli* rrn B rDNA unit or the *E. coli* rRNAs (Koncz and Sain 1980). It would thus be of interest to determine if any sequence homology can be determined to rRNAs in miniribosomes.

For all the DNAs examined, the 16S rRNA genes show the highest degree of homology to the gene on spinach ctDNA. The 23S and 5S rRNA genes in contrast vary significantly (exept in the case of the spinach-Oenothera hybridizations). This suggests that the structure and function of the small ribosomal subunit do not allow changes to occur in the primary structure of its rRNA as readily as may happen to the 23S and 5S rRNAs in the large subunit. The high degree of homology among 16S rRNA genes suggests that such rRNAs from *E. coli* or spinach chloroplasts should be useful tools in locating at least the 16S rRNA gene in other systems. The hybridization conditions used in this study should allow formation of stable hybrids once approximately 20 bases have paired. However, with the 5S rRNA, approximately 120 nucleotides in length, this may show a low level of hybridization even when there is a high degree of sequence conservation (Schwartz and Dayhoff 1978). Hence the sequence of 23S rRNA may be a more useful tool in assessing phylogenetic relationships than the highly conserved 16S rRNA or possibly the 5S rRNA sequences. The gross homologies measured in this study do not give any information as to the linear distribution of conserved sequences. A parallel electron microscopic study on heteroduplexes between lambda rif^{d}18 DNA and *Vicia faba* or spinach ctDNA has shown extensive homologies throughout the rRNA operons (Delius and Koller personal communication).

The results reported for the hybridization of mRNAs to ctDNA show that considerable homology is present between more closely related species, e.g. spinach and Oenothera. Some homology was also found for protein genes on Spirodela ctDNA compared to spinach (van Ee personal communication). No homology could be detected for the distantly related Euglena ctDNA, at least with the present degree of mRNA purification. In contrast, Hallick et al. (1979) using a DNA fragment coding for the 55 kd large subunit of ribulose bisphosphate carboxylase from the unicellular alga *Chlamydomonas reinhardii,* obtained hybridization to Euglena ctDNA. Whereas it is useful to take mRNAs from plants where they can be relatively easily isolated and use these as probes for genes in other plants, this is only possible between more closely related plants. This approach is also of interest in determining to what extent the positions of genes have been conserved between species.

In a similar study, chloroplast tRNAs from the two dicotyledons (spinach and bean) a moncotyledon (maize) and Euglena were hybridized to bean ctDNA (Mubumbila et al. 1980). High homologies were again found for the most closely related plants with spinach and bean chloroplast tRNAs hybridizing equally well to the ctDNA. Maize chloroplast tRNAs show less homology (about 75%) and the Euglena tRNAs very little (under 10%). Hybridization of individual chloroplast tRNAs shows further similarity between some chloroplast rDNA units and those of *E. coli.* As is known for the rDNA units of *E. coli* (Nomura et al. 1977), a gene for an isoleucine tRNA has been identified in the spacer between the 16S and 23S rRNA genes of spinach ctDNA (Bohnert et al. 1979), a possible fossil tRNA sequence in the same spacer of maize ctDNA (Kössel personal communication) and genes for isoleucine and alanine tRNA in this spacer in Euglena ctDNA (Keller 1979). The length of the spacer is considerably greater in ctDNAs (Bedbrook and Kolodner 1979) than on the *E. coli* DNA (Young et al. 1979) and may vary in closely related ctDNAs, e.g. in Oenothera (Gordon et al. 1980). It will be of interest to determine whether other sequences, e.g. rRNA processing sites, are preserved in and near the rDNA units of ctDNA and *E. coli.*

Acknowledgements. We are indebted to Dr. H. Delius, EMBL Heidelberg, and P. Venetianer, Szeged, for providing us with lambda rif^{d}18 and to Prof. Dr. R.G. Herrmann, Düsseldorf, for permission to use equipment in his laboratory. We are very grateful to Prof. J.H. Weil, Strasbourg, for critically reading the paper and to Miss Christine Michalowski for her excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft, the Deutscher Akademischer Austauschdienst, and the European Molecular Biology Organization.

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Communicated by E. Bautz

Received March 4 / May 27, 1980