HOMOLOGY BETWEEN rRNA OF *ESCHERICHIA COLI* AND MITOCHONDRIAL DNA OF MAIZE

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1. Introduction

Chloroplast and mitochondria of higher plants possess their own DNA coding for organelle-specific RNAs and proteins. The similarity between the translation systems of chloroplast and mitochondria and those of bacteria may serve as a proof of the evolutionary relationship between these semi-autonomous cell organelles and procaryotic organisms [1–3]. Recently, comparison of 16 S rRNA sequences of *Escherichia coli* and maize chloroplast has revealed a 76% homology between the two rRNAs [4]. A similar result was obtained from sequencing data of 16 S rRNA of *Euglena gracilis* chloroplast [5].

We demonstrate here the homology between rRNA of *E. coli* and mitochondrial DNA of maize based on Southern hybridization experiments.

2. Materials and methods

2.1. *RNA and DNA preparation*

Mitochondria and mitochondrial DNA from day 12–14 etiolated leaves were prepared according to [6]. The mitochondrial DNA was not contaminated with chloroplast, chromosomal or *E. coli* DNAs. The ^{32}P-labeled 16 S and 23 S rRNA (spec. act. 10^6 cpm/μg) were prepared from *E. coli* MRE 600 as in [7]. The 2/12 recombinant plasmid DNA containing the *rrnB* rRNA operon of *E. coli* from λrif^d18 transducing phage [8] was purified by a rapid procedure involving hydroxilapatite chromatography [9,10] and was labeled by nick-translation as in [11].

2.2. *Digestion and electrophoresis*

Restriction enzymes were purified according to established protocols: *Bam* HI [12], *Bgl* II [13], *Sal* I [14], *Pst* I [15]. Digestion of mitochondrial DNA (mtDNA) was performed in 20 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl at 37°C for appropriate times to obtain complete cleavage. These conditions were altered in the cases of *Eco* RI (100 mM Tris–HCl (pH 7.5), 200 mM NaCl, 10 mM $MgCl_2$) and *Bam* HI (20 mM Tris–HCl (pH 8.2), 150 mM NaCl, 10 mM $MgCl_2$) to inhibit the *Eco* RI* and *Bam* HII activities. Double digestions were performed subsequently with two enzymes. Aliquots (2 μg) of digested mtDNAs subjected to electrophoresis as in [16]. λcI857 DNA *Hin*dIII, λrif^d18 DNA *Hin*dIII–*Bam* HI, 2/12 DNA *Hin*dIII–*Bam* HI and pBR322 DNA *Hin*dIII–*Pst* I–*Bam* HI–*Sal* I fragments were used to estimate molecular weights.

2.3. *Blotting, hybridization and autoradiography*

DNA fragments were transferred to Sartorius nitrocellulose filters according to [17]. In hybridization experiments ^{32}P-labeled 16 S + 23 S rRNA or 2/12 plasmid DNA (spec. act. 10^7 cpm/μg) were used as a probe. The concentration of r[^{32}P]RNA in the hybridization mixture (2 × SSC) was 1 μg/ml. The DNA–DNA hybridizations were carried out according to [18]. Kodak X-Omat R films and a Dupont screen were used for autoradiography.

3. Results and discussion

The homology of 16 S rRNAs of *E. coli* and maize chloroplast raises the question whether the rDNA of maize mitochondria might be also homologous with the rDNA of *E. coli*. To check this possibility mitochondrial DNA digested with restriction endonucleases

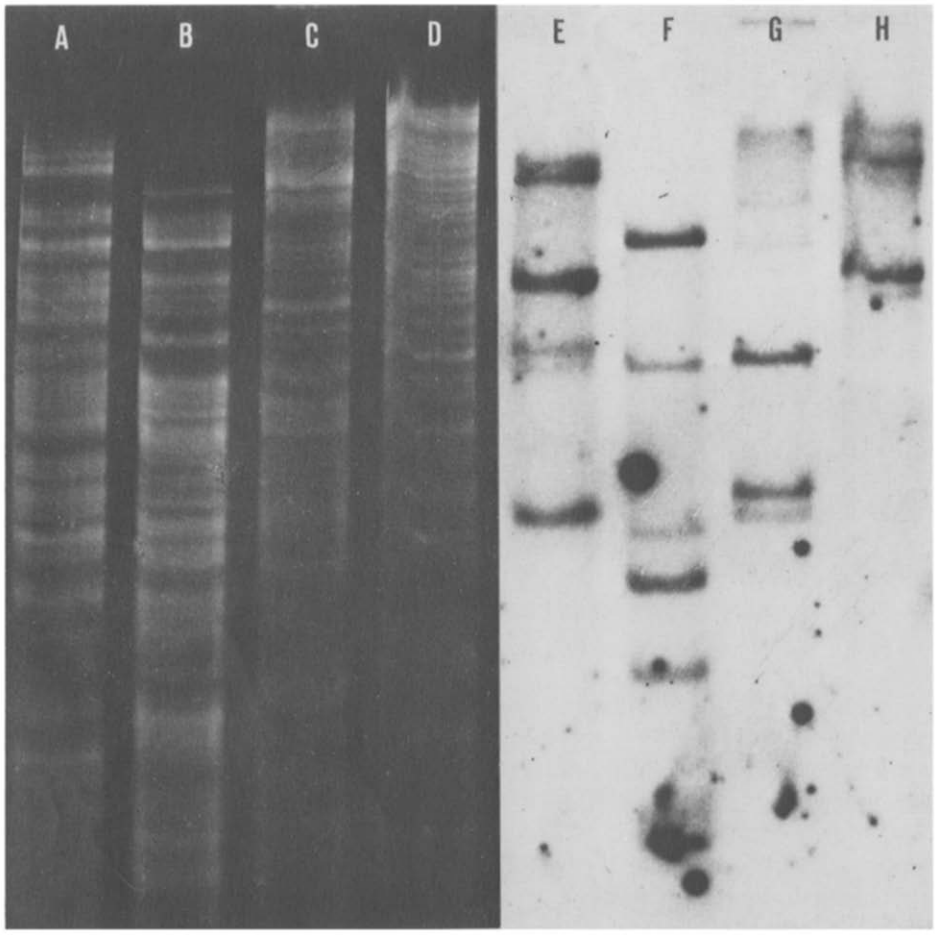

Fig.1. Hybridization of ^{32}P-labeled *E. coli* 16 S + 23 S rRNA to *Bam* HI (A,E), *Hin*dIII (B,F), *Pst* I (C,G) and *Sal* I (D,H) digested mitochondrial DNA of maize. Slots A–D show restriction patterns of digested mitochondrial DNA, while slots E–H illustrate banding patterns obtained after hybridization and autoradiography.

Bam HI, *Pst* I, *Hin*dIII and *Sal*I was hybridized with ^{32}P-labeled 16 S and 23 S rRNAs of *E. coli* (fig.1). In agreement with results [19,24] digestions with restriction endonucleases produced complex but reproducible fragment patterns (fig.1). The Southern hybridization reveals that the mitochondrial DNA of maize carries homologous sequences with 16 S and 23 S rRNAs of *E. coli*. On the basis of restriction patterns the average molecular weight of mitochondrial DNA can be estimated to be 140–160 Mdaltons. The DNA fragments hybridizing with rRNA comprise about 6–10% of the mitochondrial genome which is the maximum limit of the rDNA content of mitochondrial DNA.

We have tried to map the hybridizing region of mitochondrial DNA. In mapping experiments ^{32}P-labeled 2/12 plasmid DNA was used instead of rRNA giving an identical hybridization pattern. The mitochondrial DNA was digested with various combinations of endonucleases (*Bam* HI, *Bgl* II, *Sal* I, *Pst* I, *Hin*dIII, *Bam* HI–*Sal* I, *Bam* HI–*Pst* I, *Bam* HI–*Hin*dIII, *Sal* I–*Pst* I, *Sal* I–*Hin*dIII and *Bgl* II–*Hin*dIII), subjected to electrophoresis in agarose gels, blotted to nitrocellulose filters, hybridized with ^{32}P-labeled 2/12 plasmid DNA and autoradiographed (fig.2). The 2/12 plasmid DNA containing *E. coli* rDNA hybridizes to several fragments of mitochondrial DNA. The hybridizing fragments range from ~0.8–10 Mdaltons. The resulting bands show non-uniform intensity. Upon analysing the *Bam* HI–*Pst* I,

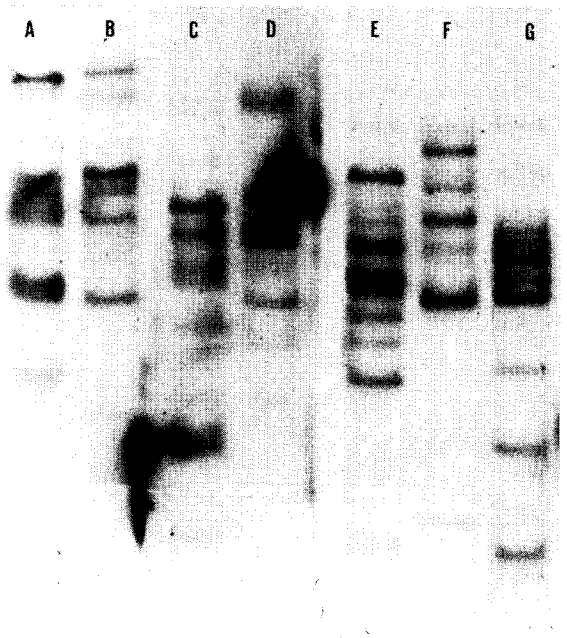

Fig.2. Mapping experiments with ^{32}P-labeled 2/12 plasmid DNA. Slots show the hybridizing fragments of *Bam* HI–*Pst* I (A), *Bam* HI–*Sal* I (B), *Bam* HI–*Hin*dIII (C), *Sal* I–*Pst* I (D), *Sal* I–*Hin*dIII (E), *Bgl* II (F) and *Bgl* II–*Hin*dIII (G) digested mitochondrial DNA of maize with 2/12 plasmid DNA containing rRNA operon of *E. coli*.

Bam HI–*Sal* I or *Sal* I–*Pst* I double digestions we were not able to construct any unambiguous arrangement of fragments similar to rRNA operons of *E. coli* [20] or mammalian mitochondria [21]. The restriction endonuclease cleavage sites probably changed during the evolution of mitochondrial genome, as can be expected. The 16 S and 23 S rRNA genes of maize mitochondria may not be closely linked in contrast to *E. coli* rRNA genes.

Multiplicity and non-uniformity of hybridizing mitochondrial DNA fragments reduced the possibility of physical mapping and suggest the heterogenity of the mitochondrial DNA population. The heterogenity is characteristic for the mitochondrial DNA of higher plants [22,23]. This would mean that the mitochondrial DNA population may consist of physically distinct sets of molecules.

Although we failed to map the hybridizing region on maize mitochondrial DNA the successful hybridization to *E. coli* rRNAs suggests that the cloning procedure of mitochondrial rDNA can be simplified by using pBR322 or λgt clone-banks of mitochondrial DNA [24] and ^{32}P-labeled rRNA of *E. coli*, as a hybridization probe.

In conclusion, the present hybridization experiments suggest that the mitochondrial DNA, similarly to chloroplast DNA of maize, possesses homologous sequences with rDNA of *E. coli*.

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