SHORT PAPER

Molecular cloning of the genes for ribosomal DNAs from broad bean chloroplast DNA

BY Chong-Rong SUN, Toru ENDO, Mie KUSUDA
and Masahiro SUGIURA

National Institute of Genetics, Mishima, Shizuoka-ken 411

(Received April 9, 1982)

ABSTRACT

Four Eco RI fragments from broad bean chloroplast DNA which were hybridized with chloroplast rRNAs have been cloned into *E. coli* plasmid pBR 325. Physical maps of the recombinant plasmids have been constructed by cleavage with several restriction endonucleases and Southern blot hybridization with chloroplast rRNAs. Plasmids pBC9, pBC14 and pBC52 contain a 16S rRNA sequence on the 3.5 kb Eco RI fragment, a 23S rRNA sequence on the 2.0 kb+1.7 kb Eco RI fragments and 4.5S-5S rRNA sequences on the 1.8 kb Eco RI fragment, respectively.

1. INTRODUCTION

Chloroplast ribosomes of higher plants contain 23S, 16S, 4.5S and 5S rRNAs. Chloroplast DNA exists as a circular molecule with a molecular weight of around 10^8 daltons and codes for all chloroplast rRNAs (Sugiura *et al.* 1980). Majority of higher plant chloroplast DNAs studied contain two sets of rRNA genes, while broad bean chloroplast DNA has only one set of the genes (Koller and Delius 1980). In addition, broad beans contain smaller chloroplast 4.5S rRNA (72 nucleotides) than those from other plants (96-103 nucleotides, Bowman and Dyer 1979).

Previously we reported that the rRNA genes of broad bean chloroplasts are located on the Eco RI fragments with molecular weights of 2.1×10^6 , 1.33×10^6 and 1.16×10^6 daltons (Sugiura 1980a). In this study we have cloned these fragments and constructed their physical maps.

2. MATERIALS AND METHODS

Restriction endonucleases were purchased from Takara Shuzo Co. and (γ - 32 P) ATP from the Radiochemical Center. T4 DNA ligase and T4 polynucleotide kinase were prepared as described (Sugiura 1980b; Sugiura and Takanami 1967). Tobacco chloroplast rRNAs were prepared as described (Sugiura and

Abbreviations: rRNA, ribosomal RNA; kb, kilobase pair.

hybridized to 2.1×10^6 , 1.33×10^6 and 1.16×10^6 , and 1.16×10^6 dalton Eco RI fragments, respectively (Sugiura 1980a).

In order to clone these fragments either separately or together, broad bean chloroplast DNA was digested incompletely with Eco RI. The resultant fragments were ligated to Eco RI-cleaved and phosphatase-treated pBR325 DNA, and the ligated DNA was used to transform cells of *E. coli* HB101. Among 1066 tetracycline- and ampicillin-resistant transformants obtained, 985 transformants were found to be chloramphenicol-sensitive. These 985 transformants were screened for the presence of DNA complementary to chloroplast rRNA using colony hybridization. Fifty eight clones were hybridized with ^{32}P -labeled rRNAs. Plasmid DNAs isolated from the above clones were digested with Eco RI and analyzed by agarose gel electrophoresis. Thirty two plasmids were found to contain 3.5 kb fragments (formerly designated as 2.1×10^6 dalton fragments), ten plasmids were found to contain 2.0 kb and 1.7 kb fragments (1.33×10^6 and 1.16×10^6 dalton fragments), and four plasmids were found to contain 1.8 kb fragments (1.6×10^6 dalton fragments). Plasmids pBC9, pBC14 and pBC52, which contain 3.5 kb, 2.0 kb+1.7 kb and 1.8 kb fragments, respectively, were used for further analysis.

These three plasmid DNAs were cleaved with Eco RI, Pvu II, Bam HI, Hind III, Xho I, Sma I and Kpn I. The resultant fragments were analyzed in agarose gels along with λ DNA Hind III and ϕ X174 RF-DNA Hae III fragments as molecular weight markers. The DNA fragments in the gels were

Table 1. Restriction fragments of the plasmid DNAs (kb)

	Eco RI	Pvu II	Bam HI	Hind III	Xho I	Sma I	Kpn I
pBC9	6.0†	4.4	5.2	9.5*			
	3.5*	2.6	4.3*				
		1.4*					
		1.2					
Σ	9.5	9.6	9.5	9.5			
pBC14	6.0†	6.4**	5.2	6.9	6.2	9.8**	8.9**
	2.0**	2.6	4.0**	1.6	3.0**		0.8**
	1.7	0.6	0.5	1.2**	0.6**		
Σ	9.7	9.6	9.7	9.7	9.8	9.8	9.7
pBC52	6.0†	4.2***	7.7***	7.7***		6.0***	
	1.8***	2.6				1.75***	
		1.1***					
Σ	7.8	7.9	7.7	7.7		7.75	

* fragments hybridized with 16S rRNA

** fragments hybridized with 23S rRNA

*** fragments hybridized with 4.5S+5S rRNAs

† pBR325 DNA

Fig. 2. Physical map of pBC9 DNA. Double lines, chloroplast DNA sequence; solid area, approximate coding region; single line, pBR325 sequence.

transferred to nitrocellulose filter sheets and hybridized with ^{32}P -labeled rRNA. Typical gel patterns and Southern blot hybridization are shown in Fig. 1. Table 1 lists the fragments obtained by single digestion and the results of hybridization experiments. From these data, the data of double digestion (not shown) and the known cleavage map of pBR325 (Bolivar 1978), physical maps of the three plasmid DNAs were constructed (Figs. 2-4).

As shown in Fig. 2, plasmid pBC9 contains a 3.5 kb Eco RI fragment which is hybridized 16S rRNA. Xho I, Sma I and Kpn I did not cleave pBC9, and Hind III cut once the pBR325 part of pBC9.

Plasmid pBC14 contains 2.0 kb and 1.7 kb Eco RI fragments (Fig. 3). ^{32}P -labeled 23S rRNA hybridized strongly to the 2.0 kb fragments and faintly to the 1.7 kb fragment. The 1.7 kb fragment was not hybridized with 4.5S and 5S rRNAs, indicating that this fragment is different, though similar in size, from the 1.8 kb fragment in pBC52. All ten plasmids containing these two Eco RI fragments showed the same restriction patterns by Hind III digestion. This fact indicates that the 2.0 kb and the 1.7 kb fragments are contiguous. The Eco RI site in the 2.0 kb+1.7 kb fragment should be relatively resistant to the enzyme digestion (Note that the incomplete digest of the chloroplast DNA was used for cloning).

Plasmid pBC52 contains a 1.8 kb Eco RI fragment which is hybridized with both 4.5S and 5S rRNAs but not with 23S rRNA (Fig. 4). Xho I and Kpn I

Fig. 3. Physical map of pBC14 DNA. Double lines, chloroplast DNA sequence; solid area, approximate coding region; single line, pBR325 sequence.

Fig. 4. Physical map of pBC52 DNA. Double lines, chloroplast DNA sequence; solid area, approximate coding region; single line, pBR325 sequence.

did not cleave pBC52, and Bam HI and Hind III did not cut the inserted fragment. Pvu II cut once in the middle of the 1.8 kb fragment. Tobacco 4.5S

rRNA gene contains one Pvu II site (Takaiwa and Sugiura 1980b). A partial sequence for broad bean 4.5S RNA which was constructed by superimposing homologous oligonucleotides produced by RNase T₁ digestion (Bowman and Dyer 1979) on the tobacco sequence also contains one Pvu I site. This indicates that the 4.5S and 5S rRNA genes are located in the middle of the 1.8 kb fragment.

Because most higher plants are known to possess two copies of chloroplast rRNA genes, it is of interest to examine in detail the structure of rRNA genes in broad bean chloroplast genome which contains only a single copy of them. The high copy number of these recombinant plasmids in *E. coli* cells allows the production of sizable amounts of pure rRNA gene sequences for further analyses.

We thank Dr. Shin-ya Iyama for his continuous encouragement. This work was supported in part by Grants-in-Aid from the Ministry of Education, Science and Culture, Japan.

REFERENCES

- BOLIVAR, F. (1978) Construction and characterization of new cloning vehicles. III. Derivatives of plasmid pBR322 carrying unique Eco RI sites for selection of Eco RI generated recombinant DNA molecules. *Gene* **4**, 121-136.
- BOWMAN, C. M. and DYER, T. A. (1979) 4.5S ribonucleic acid, a novel ribosome component in the chloroplasts of flowering plants. *Biochem. J.* **183**, 605-613.
- HOLMES, D. S. and QUIGLEY, M. (1981) A rapid boiling method for the preparation of bacterial plasmids. *Anal. Biochem.* **114**, 193-197.
- KOLLER, B. and DELIUS, H. (1980) *Vicia faba* chloroplast DNA has only one set of ribosomal RNA genes as shown by partial denaturation mapping and R-loop analysis. *Mol. Gen. Genet.* **178**, 261-269.
- SUGIURA, M. (1980a) Existence of the gene for 4.5S RNA in broad bean chloroplast DNA. *Current Genetics* **2**, 95-96.
- SUGIURA, M. (1980b) Purification of the T4 DNA ligase by blue sepharose chromatography. *Anal. Biochem.* **108**, 227-229.
- SUGIURA, M. and KUSUDA, J. (1979) Molecular cloning of tobacco chloroplast ribosomal RNA genes. *Mol. Gen. Genet.* **172**, 137-141.
- SUGIURA, M., KUSUDA, J., SHINOZAKI, K. and TAKAIWA, F. (1980) Cloning and characterization of chloroplast ribosomal RNA genes. In *Genetics and Evolution of RNA polymerase, tRNA and ribosomes* (ed. S. Osawa *et al.*), pp. 437-449. Univ. of Tokyo Press.
- SUGIURA, M. and TAKANAMI, M. (1967) Analysis of the 5'-terminal nucleotide sequences of ribonucleic acids, II. *Proc. Natl. Acad. Sci. USA* **58**, 1595-1602.
- TAKAIWA, F. and SUGIURA, M. (1980a) Cloning and characterization of tobacco chloroplast 4.5S and 5S RNA genes. *Gene* **10**, 95-103.
- TAKAIWA, F. and SUGIURA, M. (1980b) Nucleotide sequences of the 4.5S and 5S ribosomal RNA genes from tobacco chloroplasts. *Mol. Gen. Genet.* **180**, 1-4.