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Physical map of the seven ribosomal RNA genes of Escherichia coli

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ABSTRACT

Escherichia coli DNA was digested with restriction endonucleases BamHI, PstI, EcoRI, SalI, HindIII, XhoI, BglII, SmaI, HpaI and with selected double and triple combinations of the same enzymes. The digests were electrophoresed and hybridized with ^{32}P-labelled ribosomal RNA by using the Southern blotting technique. The resulting bands could be arranged into seven groups, and it was possible to construct a unique physical map of the seven rRNA genes (operons) of the bacterial chromosome. Mapping information obtained on several transducing phages and recombinant plasmids carrying rRNA genes, and mapping data published in the literature helped to determine the final map. The results suggest that phage λ daroE152 carries a "hybrid" rRNA gene which was probably formed by recombination between two different chromosomal rRNA genes.

INTRODUCTION

The powerful technique known as Southern blotting (1) has been successfully used by several investigators to study the structure and organization of unique eukaryotic genes (2,3,4,5). Here we report that by this method it was possible to determine the physical map of a group of redundant but scattered homologous genes, the transcription units (operons) coding for ribosomal RNA on the bacterial chromosome. We have shown earlier by using the Southern method that the number of such units is seven (6). The same conclusion has been reached by Morgan et al. on the basis of the structural analysis of recombinant plasmids carrying rRNA genes (7). In the following these units will be termed operons (not quite correctly) because each codes for three different stable rRNA and several tRNA species. They are designated rrnA, etc. till rrnG. The positions of six operons have been determined

by genetic means. Several different rrn operons are available in the form of transducing bacteriophages, such as: λ darol52 (8), λ drif^{d}18 (9), λ d279rbs (10), λ dmetA20 (11), and λ dilv5 (10). Table I. summarizes the nomenclature of these operons on the basis of the review of Nomura et al (12). For the construction of the map presented below published data on these phages have also been used.

METHODS

Enzymes

All restrictrion enzymes were purified in this laboratory according to established protocols. BglII (13), KpnI(14), PstI(14), XhoI(15), SalI(16), XbaI(17). HpaI, SmaI, and HindIII were purified according to unpublished methods provided by Dr. R.J. Roberts. EcoRI and BamHI were purified as described in (18) by Dr. J. Sümegi.

DNA

For all experiments described here, the same batch of DNA was used, prepared from E.coli JC5466 (K12) as described earlier (19). The defective transducing phages λ drif^{d}18, λ dilv5, and

Table I.

Nomenclature of the rrn operons

The table is based on ref. 12. Roman numerals refer to groups of recombinant plasmids carrying different parts of the same operon (7)

Gene designation	Map position	Plasmid group	Transducing phage
rrnA	85 min	III	-
rrnB	88 min	IV	λ drif^{d}18
rrnC	83 min	VI	λ d279rbs (distal part of dilv5)
rrnD	71 min	II	λ daroE152
rrnE	89 min	VII	λ dmet A20
rrnF	74 min	I or V	
rrnG	?	I or V	(I is on the proximal part of λ dilv5)

λ daroE152 were grown, and their DNA purified as described in Miller's manual (20). Plasmid DNA was purified by a rapid procedure involving hydroxylapatite chromatography (Udvardy et al. manuscript in preparation).

RNA

^{32}P-labelled ribosomal RNA was prepared from Escherichia coli MRE600 by Dr. A. Udvardy as described (19). For the preparation of 16S and 23S rRNA, the ribosomal subunits were separated by repeated sucrose gradient centrifugation and rRNA was prepared from the purified subunits. Freshly prepared ^{32}P-rRNA had a specific activity of about 10^6 cpm/μg.

Digestion:

Usually 50 μg DNA was digested in 300 μl volume at 37 °C in 10 mM tris-HCl pH 7.4, 10 mM $MgCl_2$, 50 mM NaCl, 7 mM 2-mercapto-ethanol. These conditions have been altered in the case of EcoRI (100 mM tris-HCl) and Sma I (15 mM KCl instead of NaCl, pH 9 and 30 °C). Double or triple digestions were performed subsequently and not simultaneously. Digestion times varied between 4 and 12 hours. Completeness of digestion was checked by electrophoresis of small aliquot samples.

Electrophoresis

Aliquots of the digests (30 μl per slot) were electrophoresed on 200 x 200 x 3 mm agarose (Sigma) slab gels as described by Helling et al. (21). Agarose concentration varied between 0.6 - 1 % according to the size of the fragments. Appropriately chosen digests of various phages and recombinant plasmids carrying rRNA genes (22) served as molecular weight markers.

Blotting, hybridization and autoradiography

It was carried out according to Southern (1), using Sartorius nitrocellulose filters and Kodak X-Omat R film. The concentration of ^{32}P-rRNA in the hybridization mixture varied between 0.5 - 1 μg/ml (in 2 x SSC). In experiments where separated 16S or 23S rRNA probes were used, the other ribosomal RNA species was added in 3-5-fold excess in unlabelled from as competitor.

RESULTS

As a first step to determine the physical map of all seven rRNA operons of the E. coli chromosome, we wanted to know in great detail the structure of one of these operons, available on a transducing phage. Therefore the physical map of λdrif^{d}18 (9) (rrnB) was determined with respect to cleavage by eleven restriction endonucleases: EcoRI, SalI, BamHI, PstI, KpnI, HpaI, HindIII, XhoI, XbaI, BglII, and SmaI, As part of this map has already been published with the full description of the methods employed (23), here we show only the final map (Fig.1.).

Further information was provided in the literature about the locations of several cleavage sites in and around operons rrnA and rrnE (7,11), rrnD (8,25), and rrnC (10). These data are

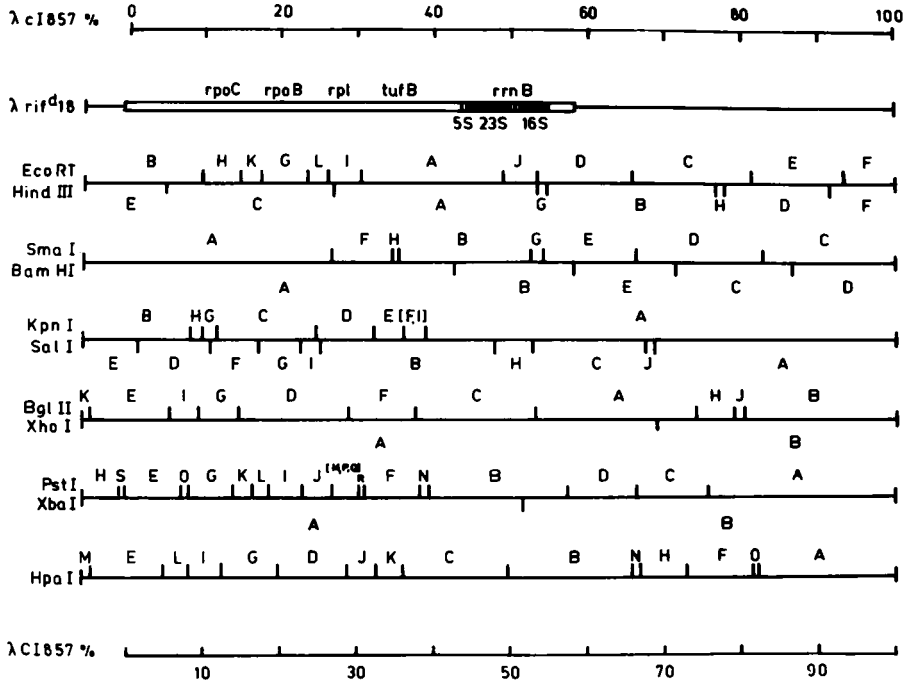

Fig. 1. Physical map of the phage λdrif^{d}18

The HindIII, SmaI and EcoRI map had been determined by Lindahl et al. (24). The relative positions of KpnI fragments F,I and PstI fragments M,P,Q had not been established.

summarized on Fig.2. (Part A), together with additional mapping data obtained in this laboratory by analyzing the same phages (Part B).

Recently we constructed a series of recombinant plasmids carrying rRNA operons by cloning BamHI digested E.coli chromosomal DNA with the pBR322 cloning vehicle (Boros et al. manuscript in preparation). Fig.3. shows the physical map of two such plasmids, pBK 8 and pBK 17. These results have also been used in compiling the E.coli map.

For this purpose we digested high molecular weight total E.coli DNA with various combinations of restriction endonucle-

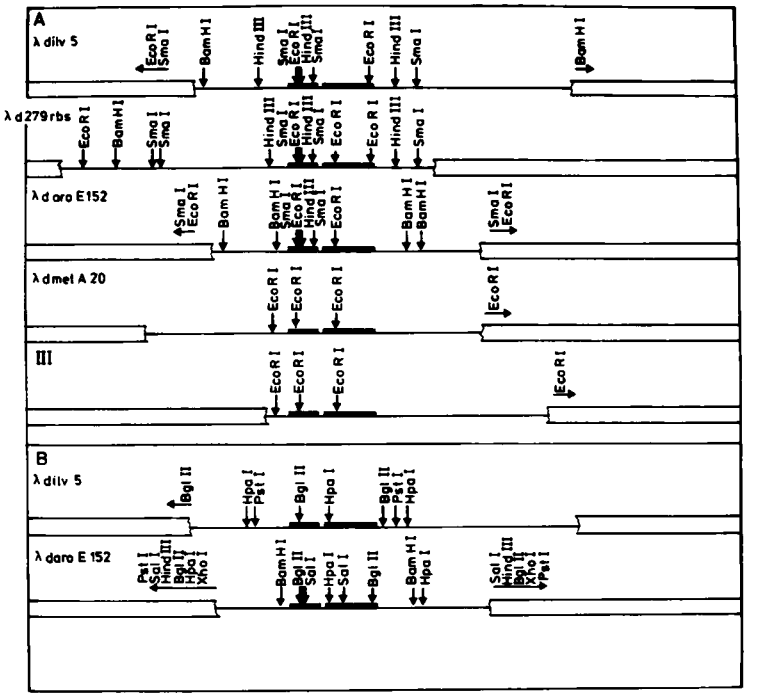

Fig.2.

Part A. Mapping information published in the literature on transducing phages and recombinant plasmids, carrying rRNA operons (7,8,10,11,24). Part B. Mapping information on λdaroE152 and λdilv5 obtained in this laboratory. Thick open bars: DNA of phage or plasmid origin. Solid lines: DNA of bacterial origin. Thick black bars: DNA sequences corresponding to 16S and 23S rRNA.

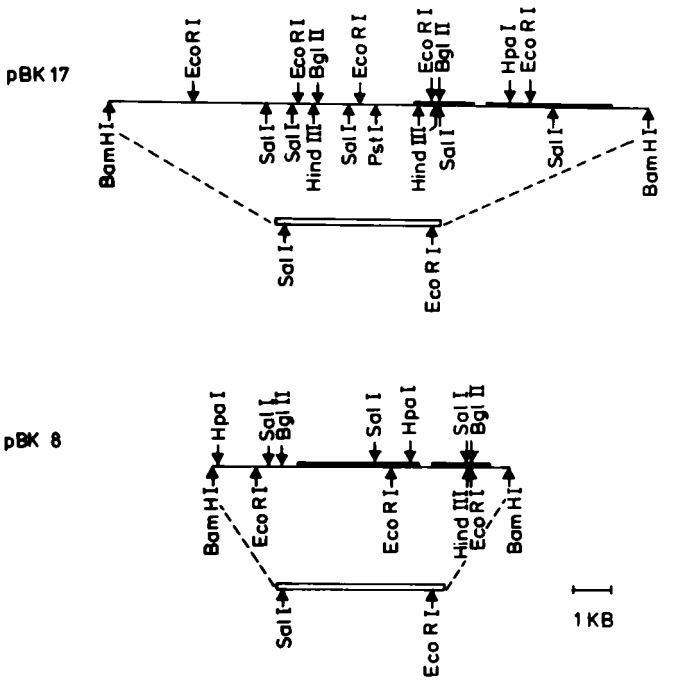

Fig.3.

Physical map of recombinant plasmids pBK 8 and pBK 17. Open bar: pBR322. Solid line: bacterial insertion.

ases. The following digestion experiments have been performed and analyzed.

Single digestions: EcoRI, SalI, BamHI, HpaI, BglII, PstI, HindIII, SmaI, XhoI.

Double digestions: BglII, BamHI, BglII-PstI, BglII-EcoRI, BglII-HpaI, BglII-HindIII, BglII-XhoI, BamHI-PstI, BamHI-EcoRI, BamHI-HpaI, BamHI-HindIII, BamHI-XhoI, BamHI-SalI, PstI-EcoRI, PstI-HpaI, PstI-HindIII, PstI-XhoI, PstI-SalI, EcoRI-XhoI, HpaI-XhoI, SmaI-XhoI, HindIII-XhoI.

Triple digestions: HpaI-BamHI-PstI, BamHI-PstI-HindIII, BamHI-BglII-HindIII, BamHI-BglII-PstI, BamHI-PstI-XhoI, BamHI-SalI-PstI.

The digests were electrophoresed in agarose gels, blotted

to nitrocellulose filters, hybridized to ^{32}P-labelled E.coli ribosomal RNA and autoradiographed. In the case of the underlined digestion experiments hybridizations were performed with separated 16 S and 23 S rRNA probes.

Fig.4. and Fig.5. show the results of some of these experiments to illustrate the types of banding patterns obtained. The construction of the map started with the EcoRI fragments. This enzyme cleaves at the 673rd base of the 16 S sequence (26) and 2 kB downstream, within the 23 S sequence, thus splitting the operon to three pieces. In one of the operons the second EcoRI site is missing (7,10) and in rrnC the middle fragment is shorter than elsewhere (10). On Fig.4. the two different spacer fragments (2, 1.9 kB), the proximal fragments (12.3, 12.3, 5.7,

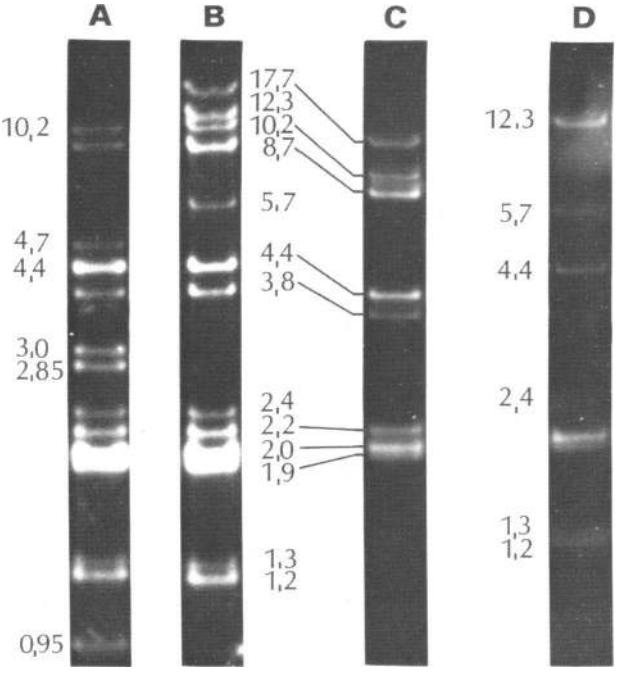

Fig.4.

A: EcoRI-BamHI digestion. B-D: EcoRI digestion. A-B: hybridization with total rRNA. C: hybridization with 23 S rRNA. D: hybridization with 16 S rRNA. C and D are independent experiments, so mobilities cannot be directly compared.

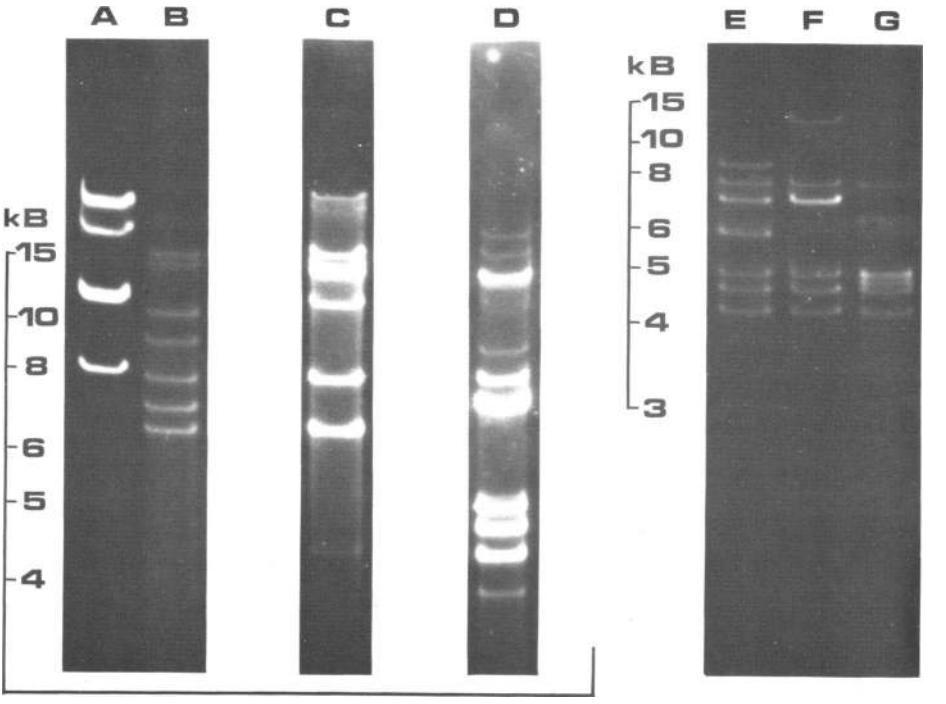

Fig.5.

A: PstI digestion. B: PstI-BamHI digestion. C: BamHI digestion. D: BglII digestion. E: BglII-PstI digestion. F: BglII digestion. G: BglII-BamHI digestion. All hybridizations were done with total rRNA.

2.35, 1.3, 1.2, 1.2 kB) and the distal fragments (17.7, 8.7, 8.7, 10.2, 4.4, 3.8, 2.2 kB) can be clearly distinguished. Double digestions where EcoRI is used with another enzyme (Fig.4. A) define several similar series of fragments which can be connected with the simple EcoRI pattern. Similarly one can start with BglII (it cleaves at the 703rd base of the 16S sequence generating a set of strongly hybridizing distal and very faintly hybridizing proximal fragments) and double digestions involving BglII and other enzymes. The EcoRI-BglII pattern helps two connect the two series. The procedure can be continued with HpaI which cleaves once, near the beginning of the 23 S sequence, HindIII which cleaves once at the 647 base within the 16 S sequence, and SalI

which cleaves at the 820th and the 873rd base of the 16S and 2.35kB downstream within the 23S sequence. This way most of the cleavage sites of those enzymes (BamHI, PstI, XhoI) which do not cleave the operon can be located, and the BamHI, etc fragments assigned. All the fragments can be arranged in seven independent groups, each group corresponding to an rrn operon. Table II. illustrates part of the results, the molecular weights of the fragments shown on Fig.4. and Fig.5. arranged in seven columns.

After this arrangement has been completed each group of fragments was assigned to an operon on the basis of the phage and plasmid maps. Thus the second column is rrnB because the pattern is identical with that of phage λdrif^{d}18. Plasmid pBK 17 also carries rrnB. The third column is rrnC, the map is identical with that of phage λd279rbs and the distal half of λdilv5. The first column can only be rrnA because in this operon the distal EcoRI site is at least 15kB away (7). The fourth column is rrnD because the map is identical with the proximal half of the

Table II.

Arrangement of fragments into seven "families"

Only those results are included which are shown on Fig.4. and 5. Fragment sizes are in kB. The first numbers in each box refer to the proximal, the second numbers to the distal fragment.

	1.	2.	3.	4.	5.	6.	7.
EcoRI	1.3 17.7	2.4 8.7	12.3 2.2	12.3 3.8	1.2 8.7	5.7 4.4	1.2 10.2
EcoRI-BamHI	1.3 2.85	2.4 3	10.2 2.2	0.95 3.8	1.2 8.7	4.7 4.4	1.2 4.4
BglII	? 7	3.9 7	6.9 4.6	>12 5	>12 7.7	? 12.3	>12 4.4
BglII-BamHI	1.6 4.8	3.9 5	6.9 4.6	1 5	2 7.7	5 6.2	9.8 4.4
BglII-PstI	3.8 7	1.9 5.9	6.9 4.6	5.7 5	13 7.7	3 8.6	3.9 4.4
BamHI	6.2	13.5	30	7.4	14.3	11	16
PstI	10.8	7.7	>20	18	>20	11.3	>20
BamHI-PstI	6.2	6.8	15.8	7.4	14.3	9	10.2

λdaroEl52 phage. The distal part is different. In contrast, the map of plasmid pBK8 is entirely consistent with the data of the fourth column (Fig.6.). We conclude that plasmid pBK8 carries operon rrnD, whereas λdaroEl52 is a "hybrid" like λdilv5, a recombination product of rrnD and another operon. From the map it follows that this other operon corresponds to the seventh column. The sixth column corresponds to the operon carried by the proximal part of the λdilv5 phage. rrnE must correspond to the fifth column because Kennerley et al. (27) reported that a phage carrying this operon does not have any homology outside rrnE with any other phage or recombinant plasmid.

The resulting map of the seven ribosomal RNA genes and their flanking regions is shown on Fig.7.

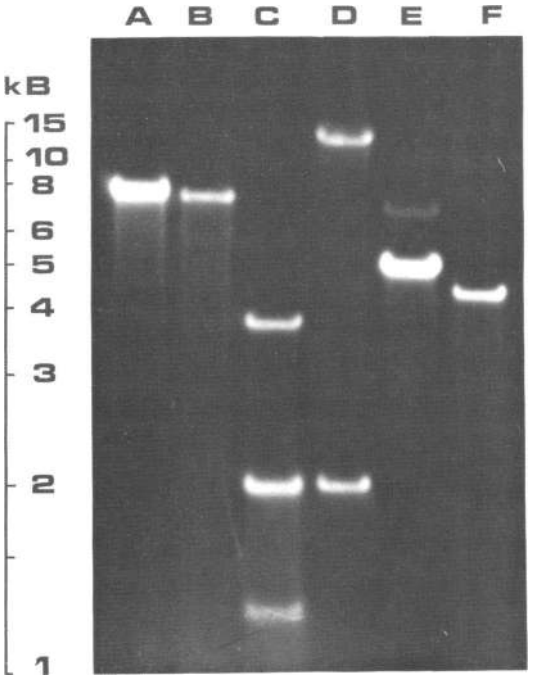

Fig.6. Comparison of λdaroEl52 and pBK 8

A,C,E: pBK 8. B,D,F: λdaroEl52. A,B: BamHI digestion. C,D: EcoRI digestion. E,F: BglII digestion. Hybridizations with total rRNA.

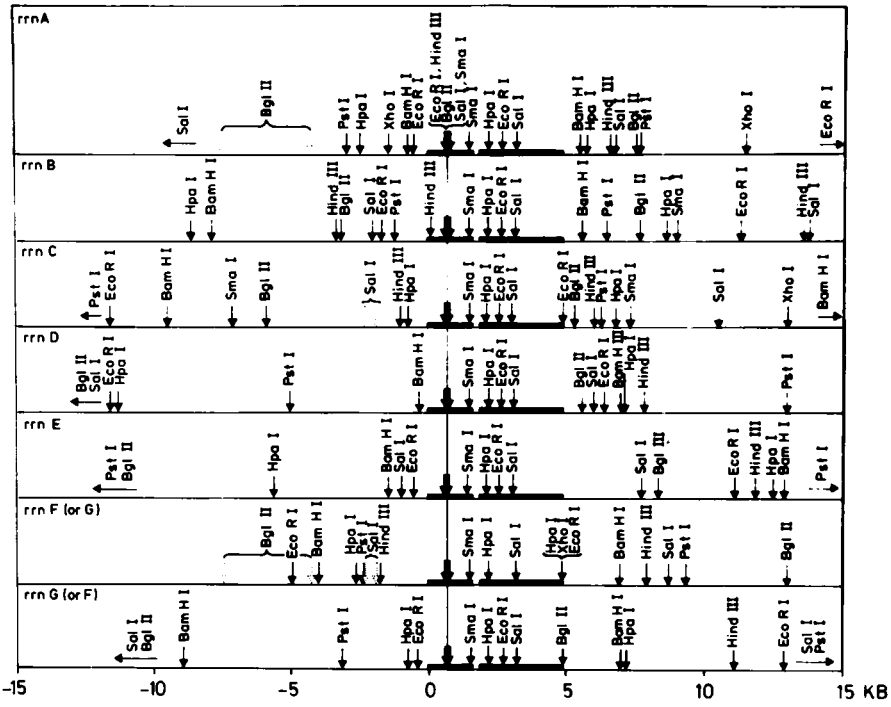

Fig.7. Physical map of the seven rRNA operons of E.coli

Thick solid bars represent the 16S and 23S rRNA sequences. The beginning of the 16S sequence was chosen as point of reference and distances in kilobases are related to this point. The vertical line in the middle of the 16S rRNA sequence denotes a 0.1 kB long region where all five enzymes written in the top row have cleavage sites. Dotted arrows represent undecided alternatives.

DISCUSSION

The approach taken in this investigation has several inherent drawbacks. In the Southern technique, very large fragments are eluted from agarose gels with low yield. On the other hand, small fragments are efficiently eluted, but not completely retained on the nitrocellulose filter. The method is not strictly quantitative even in the medium range of molecular weights, therefore assumptions about double bands must be carefully controlled. In more complicated digestion patterns it is not always easy to exclude the possibility of partial digestions. The estimation of

molecular weights especially in the high molecular weight range is quite uncertain, even with a wide range of molecular weight standards as used in this work. In spite of all these difficulties, we believe that the derived map is correct because the large number of digestions allowed the careful cross-checking of every single result. The map is internally consistent and unambiguous.

It is obvious that this type of "in situ" approach should give more reliable information on the possitions of chromosomal cleavage sites than any data obtained from the analysis of phages and recombinant plasmids, where the possibility of rearrangements can never be excluded. In fact such rearrangements have been shown to exist. The λdilv5 phage contains a "hybrid" of the rrnC and rrnF (or G) operons. The results presented above demonstrate another such rearrangement: the λdaroE152 phage carries a hybrid of the rrnD and rrnG (or F) operons. Although it could be argued that the phage λdaroE152 is the intact rrnD and plasmid pBK 8 carries a hybrid operon, this is extremely unlikely. Plasmid pBK 8 is one example of several independently isolated plasmids carrying a similar 7.4 kB BamHI fragment, and each of these have the same structure as pBK 8. The detection of this hitherto unknown rearrangement serves to illustrate the value of the map. It has helped, and will help to identify new recombinant plasmids and transducing phages carrying rRNA operons (or parts of them) and to verify their integrity, or detect rearrangements.

The redundancy of rRNA genes poses an interesting problem: to what extent are they heterogeneous, and what (if any) is the functional role of the heterogeneity. According to RNA sequencing data (26) and heteroduplex studies (27) the seven genes are essentially homologous, but several minor heterogeneities have long been known, such as the missing EcoRI site in rrnF (or G) and the shorter internal EcoRI fragment in rrnC (7, 10). We detected a second HindIII site in rrnB. It is located at the 80th base of the 16 S sequence (confirmed by direct sequencing by E. Csordás-Tóth, unpublished) and its presence can be detected in the chromosome, as well as in plasmid pBK17. It is probably a unique heterogeneity, because it did not show up in the RNA

sequence or in the map of any other phage, or recombinant plasmid. Other possible candidates for heterogeneity are the distal HpaI, XhoI and EcoRI sites of rrnF (or G), the distal EcoRI site of rrnC, and the distal BglII site of rrnG (or F). These sites may or may not be within the operon, according to the reliability of the molecular weight estimations.

The flanking regions of all seven operons are quite heterogeneous. As the exact site of the beginning or the end of the operon is not known, it is hard to tell whether this heterogeneity has any significance with respect to function. However in the case of rrnD the proximal BamHI site is so close to the 16S sequence that it quite likely lies within or near the promoter. If so, this means a heterogeneity in the promoter sequence because the other six operons do not have this site.

The map positions of six rrn operons are known. The seventh operon cannot be very closely linked to any other rrn operon because the maps do not overlap and the unassigned rrn operon is located individually on a very large (around 30 kB) PstI fragment.

Finally, it must be noted that the map presented here was derived from one K12 E. coli strain. A single similar digestion experiment performed by B. Sain with MRE 600 resulted in a strikingly different EcoRI pattern, indicating that considerable differences exist between the rRNA gene maps of different E.coli strains.

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