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DNA: Recombination, Interactions and Repair

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DNA RECOMBINATIONS
IN VITRO

STRUCTURAL AND FUNCTIONAL ANALYSIS OF CLONED BACTERIAL rRNA GENES

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ABSTRACT

The seven rRNA operons (genes) of E. coli were physically mapped by restriction endonucleases, using the Southern blotting technique. Two complete rRNA operons (rrnB and rrnD)were isolated by the recombinant DNA method, using the pBR322 plasmid cloning vehicle. The rRNA operon carried by the transducing phage λdaroE152 was found to be a hybrid of the rrnD and rrnE operons. The DNA sequence of the promoter region for rrnB was determined. The RNA-polymerase binding sites on the cloned rRNA operons were localized by filter-binding and electron-microscopic techniques. rRNA transcription was investigated by electron microscopy and hybridization analysis of the in vitro transcripts. All these findings were correlated with the DNA sequence, and a tentative model was proposed to explain the unusual properties of rRNA promoters.

INTRODUCTION

One of the main advantages of the recombinant DNA technology is that it allows the detailed molecular analysis of genes which are not amenable to classical genetic methods. The ribosomal RNA genes of Escherichia coli belong to this group, no mutant has ever been isolated in them. The three stable rRNA (16 S, 23 S, S S) components of the bacterial ribosome are synthetised as a 30 S precursor, which is subsequently processed through a complex series of reactions. The genes (operons) coding for this 30 S transcript are redundant, several copies are located scattered on the bacterial chromosome.

These are the most active genes of bacteria. Although they make up only about 0.8 % of the genome, more than 50 % of all transcription takes place on them in exponentially growing cells. This high rate of transcription is subject to several intricate control mechanisms. The problems raised by this interesting system can ultimately be solved only by a detailed molecular analysis of the rRNA genes, especially their regulatory regions. In this report we summarize our work, done in the past few years on this system.

RESULTS

By using the so-called Southern-blotting technique (Southern, 1975) we were able to determine the copy number of E. coli rRNA genes. Briefly: E. coli DNA was digested with BamHI restriction endonuclease, which does not cleave into the rRNA operon, and the DNA was hybridized to rRNA after electrophoresis. As seven distinct hybridizing bands of approximately equal intensity were detected, it was concluded that the number of rRNA operons is seven (Kiss, Sain, Venetianer, 1977). This approach was then extended, and E. coli DNA was similarly analyzed with 9 different restriction endonucleases and 36 different double and triple combinations of these enzymes (Boros, Kiss, Venetianer, 1979). These results unambiguously established that the copy number is indeed seven, and allowed the determination of the physical map of the vicinity of all seven genes. This map, shown on Fig 1. was very helpful in the further analysis of rRNA genes. First: it helped to identify cloned rRNA genes (see below); second: it allowed the detection of hitherto unknown rearrangements. For instance, it was supposed that the transducing phage λdaroE152 carries the bacterial rrnD operon (Jorgensen, 1976). A comparison of the physical map of this phage with the E. coli map suggests that the RNA operon of this phage is a "hybrid", resulting from recombination between the rrnD and rrnE operons.

For detailed analysis of individual rRNA genes, and especially their promoter regions we decided to clone them. First the rrnB gene was cloned, starting from the transducing phage λdrif^{d}18 (Kiss and coworkers, 1978). Electronmicroscopic R-loop mapping and in vitro transcription experiments verified that the 7.1 kB BamHI fragment cloned in plasmid pBR 313 indeed carries the intact rRNA gene of the phage.

Then we attempted to clone all seven genes starting from the bacterial chromosome, but this attempt had been only

partially successful. Out of 2000 screened recombinants only seven clones contained bacterial rRNA genes. By using the physical map shown on Fig 1. it was easy to establish that six of these clones carried the rrnD gene and one contained rrnB. This latter clone however proved to be rather unstable, the maintenance and large-scale preparation of the recombinant plasmid (termed pBK 17) was difficult. It is interesting to note that the loss of the plasmid appeared to be gradual, through spontaneous deletions starting from the cloned bacterial fragment, but going into the vector plasmid itself. Another interesting observation: in several cases the disappearance of an rRNA gene carrying plasmid was accompanied by the appearance of a new, eighth rRNA gene in the chromosome. These extra copies were also unstable.

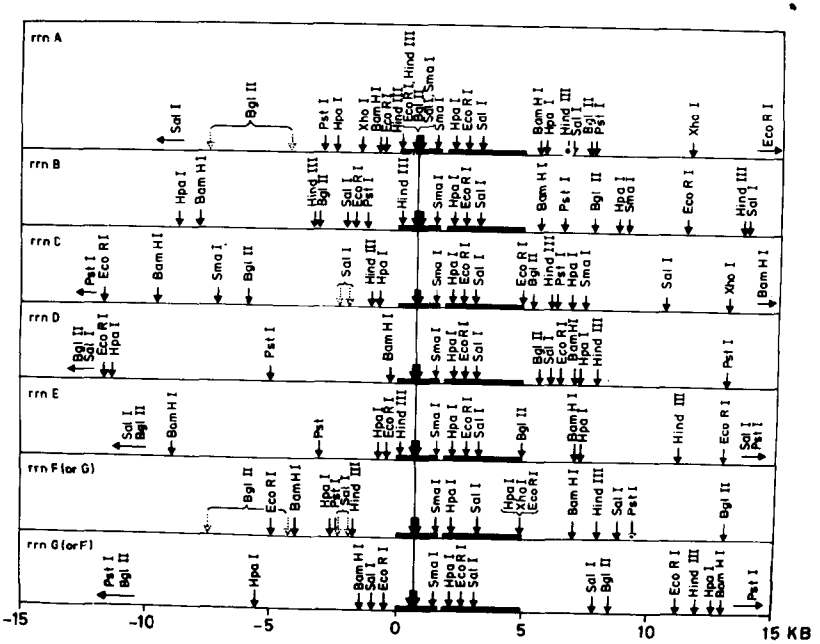

Fig.1. Physical map of the seven rRNA operons of E. coli

For further analysis we chose the rrnB gene. Fig 2. shows the physical map of the two recombinant plasmids carrying rrnB. For practical reasons the sequencing work was done on plasmid 2/12. An 1.6 kB long Hind III-BamHI fragment was prepared from this plasmid. It contained all the DNA of bacterial origin from the att region till the 80th nucleotide of the mature 16 S rRNA sequence, thus it must have contained the promoter region. After the determination of the detailed physical map of this region, sequencing was carried out by the technique of Maxam and Gilbert (1977), according to the strategy outlined on Fig 2.

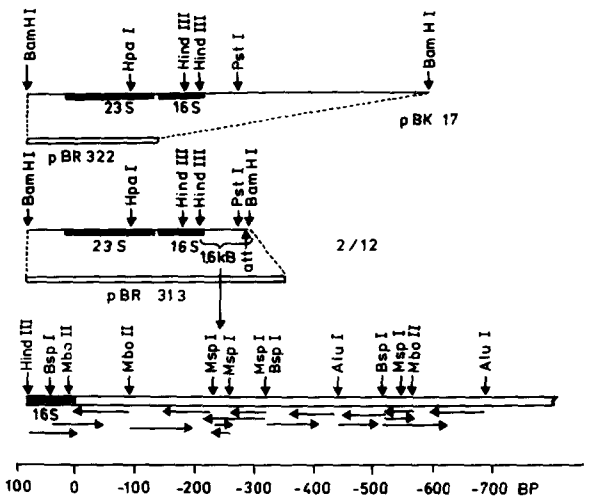

Fig. 2. Physical map of the recombinant plasmids pBK 17 and 2/12 and outline of the sequencing strategy

(Incidentally, it must be noted that at the other end of this fragment the sequence was also determined and this sequence defined a secondary attachment site for phage lambda, Csordás-Tóth, Boros, Venetianer, submitted for publication). The 700 bp long sequence immediately preceding the mature rRNA sequence thus presumably including the entire promoter region is shown on Fig 3.

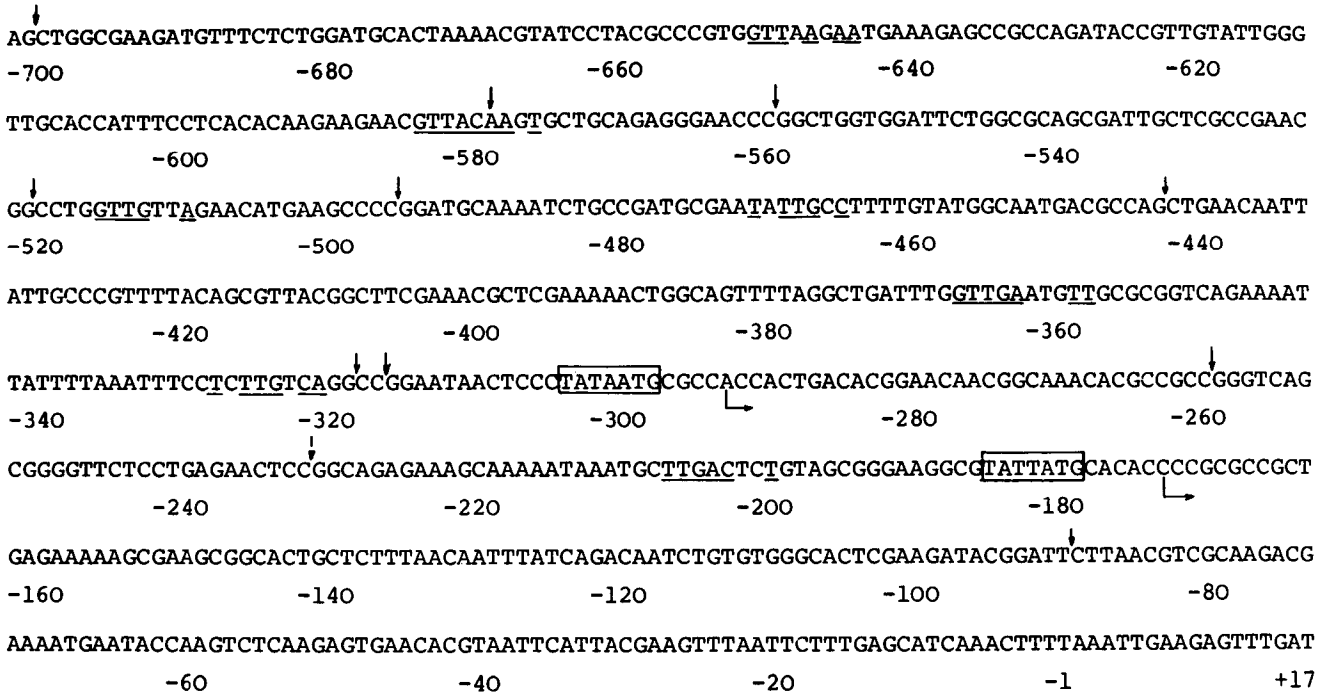

Fig 3. The rrnB promoter sequence. Numbering starts at the beginning of the mature 16 S rRNA sequence. Only one strand is shown. Arrows indicate the restriction endonuclease cleavage sites used in sequencing. The Pribnow-sequences corresponding to the two promoters are boxed, broken arrows indicate the sites of initiation. The polymerase recognition sequences are underlined.

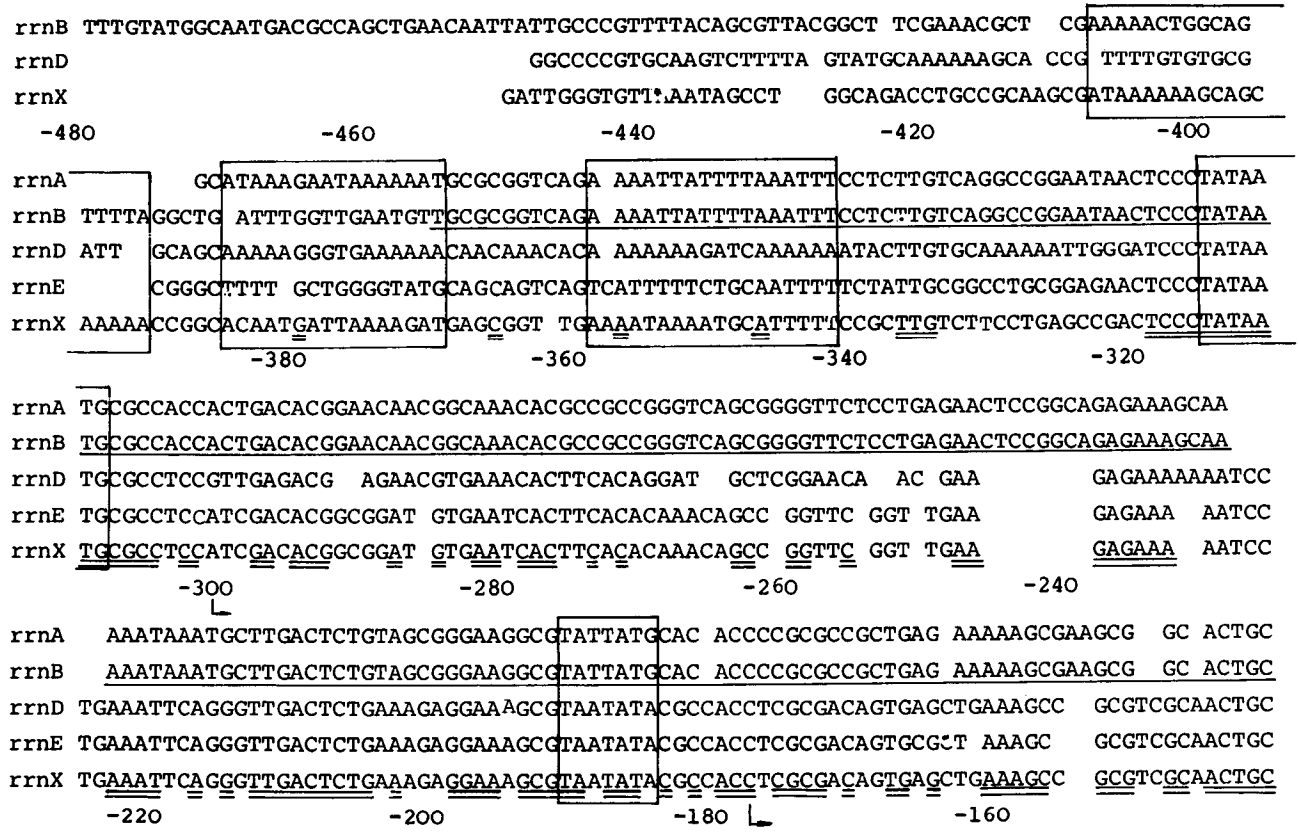

Fig 4. The five rrn promoter regions aligned to maximize homologies.

As during the past year the sequence of four other rrn promoter regions had been determined in other laboratories (deBoer, Gilbert and Nomura, 1979, Young and Steitz, 1979) it is now possible to compare all five sequences. These are shown on Fig 4. The sequences are aligned by the introduction of a few gaps in such a way that their homologies should be maximal. All five sequences are completely identical up to position -145, this region is not shown (the numbering begins at the beginning of the mature 16 S rRNA sequence). Long stretches of complete homology between all five regions exist up to position -320. The rrnB and rrnA sequences are completely identical up to -370, and some partial homologies can be detected even further upstream. These farreaching homologies are unlikely to be fortuitous. These sequences are probably parts of the promoter region.

Sequence information in itself is not sufficient to explain the mechanism of action of these promoters, it must be correlated with other experimental results. Several years ago, on the basis of in vitro transcription experiments with total E. coli DNA, we proposed a model to explain the extremely high rate of rRNA transcription (Sümegi, Udvardy, Venetianer, 1977). According to this model each rRNA operon has multiple promoters, which are able to store 4-5 RNA polymerase molecules in a heparin-resistant form. Similar proposals have been made by other authors as well (Travers, 1976, Mueller, Oebbecke, Förster, 1977). In an effort to verify this model we performed electron-microscopic polymerase binding experiments with the cloned rRNA genes or with appropriate restriction fragments derived from such clones.

Unfortunately, for technical reasons it was not possible to carry out these binding experiments at polymerase concentrations sufficiently high to saturate all the proposed binding sites. Thus only one or at most two polymerase molecules could be observed on any individual DNA fragment. However the statistical evaluation of a large number of pictures revealed that the rRNA operon promoter region is much wider than a simple binding site. As shown on Fig 5. this region must represent at least two but possibly four or five sites, the width of the region is 200-300 base pairs.

The number of transcription initiation sites can best be studied by rifampicine challenge experiments, or by the analysis of the in vitro transcription products. By the former method we found two initiation sites for rrnB (Kiss and co-workers, 1978). Recently Gilbert, deBoer and Nomura (1979), Glaser and Cashel (1979), Young and Steitz (1979) found two distinct initiation sites for rrnA, rrnB,