Salmonella typhimurium rpoB and *rpoC* genes cloned on λ phages

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Salmonella typhimurium rpoB and *rpoC* genes, coding for the RNA polymerase subunits β and β′, respectively, were isolated on λ phages. Like their *E. coli* counterparts the two genes are closely linked and probably share a common promoter. The distribution of the target sites for several restriction enzymes, however, shows considerable divergence from the *E. coli* pattern.

Salmonella rpoB *gene* rpoC *gene* *Cloning* *λ phage*

1. INTRODUCTION

In *E. coli* the RNA-polymerase, the enzyme responsible for transcribing DNA into RNA, consists of the protein subunits α (40 kDa), β (150 kDa), β′ (160 kDa) and σ (70 kDa), in the stoichiometry $\alpha_2\beta\beta'\sigma'$ per complete enzyme molecule. The genes for these subunits are dispersed on the chromosome and are clustered with those for certain ribosomal proteins and DNA-primase (fig.1).

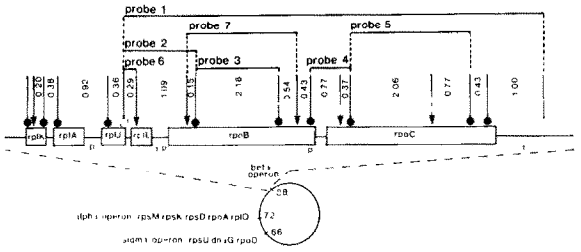

Fig.1. RNA polymerase gene clusters in *E. coli*. Details of the sigma (σ) and alpha (α) clusters are given elsewhere [1–3]. The beta (β) operon [4–6], from left to right, is drawn expanded. The genes *rplK, rplA, rplJ, rplL, rpoB* and *rpoC* code for ribosomal proteins L11, L1, L10 and L7/L12, and the RNA polymerase subunits β and β′, respectively. p, promoters; t, terminators. Relevant restriction sites: (♦) *Pst*I; (↓) *Eco*RI; (↑) *Hin*dIII. Sizes of restriction fragments are given in kilobase pairs. Probes featuring in this work are indicated.

We have begun a study of the arrangement of these genes in a variety of Enterobacteria, and since Southern blots had previously shown that *E. coli* probe 1 (fig.1) hybridises well to certain chromosomal fragments from *Salmonella typhimurium* [7], we used this probe to screen a pool of λ phages containing random inserts of *S. typhimurium* chromosomal DNA. The phages were provided by Dr L. Bossi, and were prepared by the methods of Karn et al. [8] by ligating and packaging *Bam*HI-cleaved DNA from vector λ 1059 and size-fractionated *Sau*3a partial digests of chromosomal DNA from *S. typhimurium*. Here we describe 3 hybrid phages derived from positive plaques.

2. EXPERIMENTAL

All procedures, unless otherwise stated, are from [9]. Expression of λ phages inside UV-treated bacteria was studied essentially as described in [10]. The host bacteria, kindly provided by Professor M. Nomura and Dr. N.E. Murray, were *E. coli* S159 (*uvrA sup*$^-$ *gal*$^-$ *str*^{R}) and its λ lysogen (S159 λ *ind*$^-$). Proteins were labelled with [^{35}S]-methionine during a period of 8 min immediately after infection, and chased for 4 min with unlabelled methionine. Equal amounts of labelled protein in SDS sample buffer were electrophoresed

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in an SDS gel containing a 7–15% gradient of acrylamide.

3. RESULTS AND DISCUSSION

Fig.2 shows the physical maps for phages derived from two positive plaques and for the *rpoBC* region of the *Salmonella* chromosome.

To look for a closer correspondence between the cloned *Salmonella* DNA and the different regions of the *E. coli rpoBC* region, single (not shown) and multiple restriction digests of these phages were electrophoresed in agarose, blotted onto nitrocellulose, and tested for their ability to hybridise to probes 2–5 (see fig.1 for probes). The hybridisation pattern for a *Hin*dIII/*Eco*RI digest of λ *S.ty-rpoBC*$_{14}$ is shown in fig.3a. These data are transposed onto a linear map in fig.3b. The results indicate that the regions on the *Salmonella* DNA showing homology to *E. coli rpoB* and *rpoC* regions are closely linked.

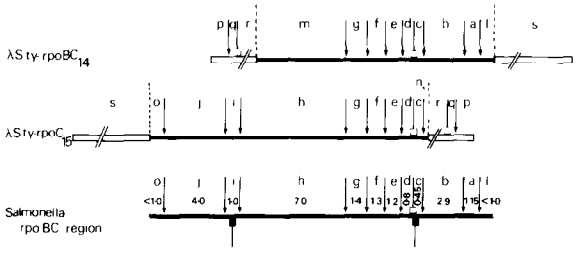

Fig.2. Physical maps constructed from a restriction analysis of two λ phages (λ *S.ty-rpoBC*$_{14}$ and λ *S.ty-rpoC*$_{15}$) carrying DNA from the *S. typhimurium* beta cluster. The lettering identifies the DNA fragments, and the numbers their sizes in kilobase pairs. Heavy lines indicate bacterial DNA, and the boxes λ DNA. Restriction sites: (↓) *Eco*RI; (|) *Hin*dIII; (▼) *Sal*I.

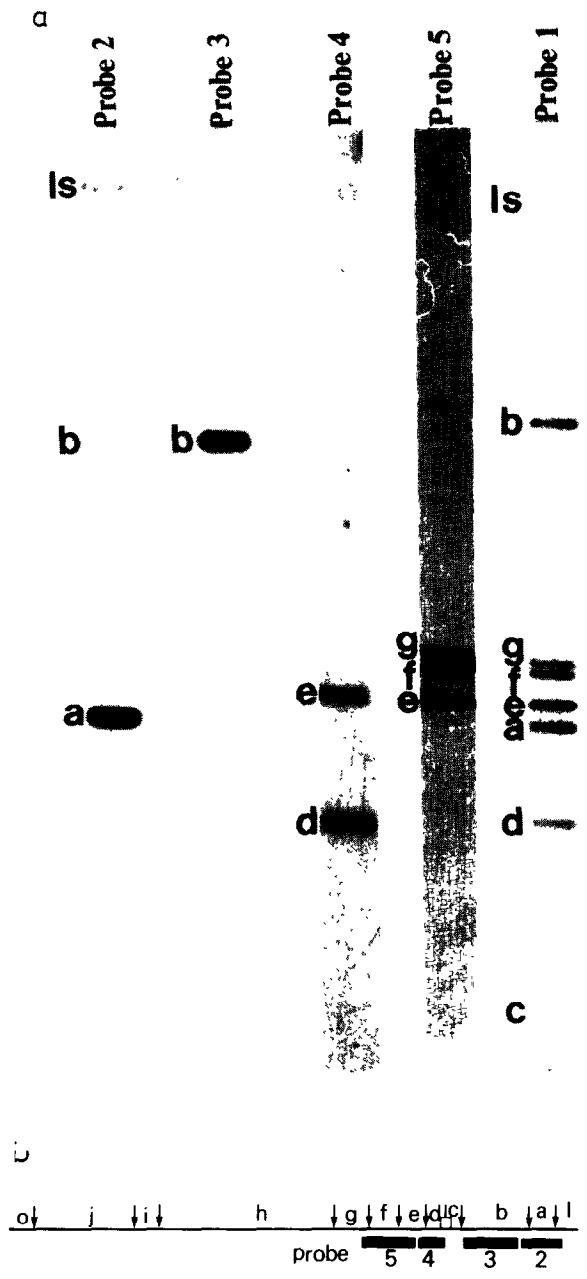

Fig.3. (a) Souther blot analysis of λ *S.ty-rpoBC*$_{14}$ DNA using probes from the *E. coli rpoBC* cluster. In all tracks a complete *Hin*dIII/*Eco*RI digest of the phage DNA was electrophoresed. After blotting, the DNA from each track was hybridized to [^{32}P]GTP-labelled probes derived from the *E. coli rpoBC* cluster. (Before nick-translation the probes were purified by twice passaging in low-melting agarose.) The probes are identified above each track with the numbers assigned to them in fig.1. The hybridising bands from λ *S.ty-rpoBC*$_{14}$ are identified with letters corresponding to the fragments in fig.2. In the 'probe1' track, bands ls and c are not visible in the reproduction. (b) Map of the *Salmonella rpoBC* cluster interpreting the hybridisation data. The *E. coli* probes are drawn in thick line and are aligned with the λ *S.ty-rpoBC*$_{14}$ restriction fragments to which they hybridise. The restriction fragments are lettered and demarcated as in fig.2.

The hybrid phages were used to study gene expression inside *E. coli* that had previously received a heavy dose of UV radiation. Fig.4 shows the results of such an experiment with 3 phages; λ *S.ty-rpoC*$_6$, λ *S.ty-rpoC*$_{15}$ and λ *S.ty-rpoBC*$_{14}$. Restriction and Southern data strongly suggest that λ *S.ty-rpoC*$_6$ (not shown) and λ *S.ty-rpoC*$_{15}$ (fig.2) carry an intact β′ gene (*rpoC*), and that λ *S.ty-rpoBC*$_{14}$ (figs 2,3) carries both the β and β′ genes (*rpoB* and *rpoC*, respectively). This is clearly confirmed by the pattern of β and β′ synthesis in fig.4. Subunit synthesis is considerably reduced, however, when the phages infect the λ lysogen. The simplest explanation is that none of the phages includes a major promoter for *rpoB* and *rpoC*, the expression of these genes depending on read-through transcription from λ (which is much reduced in the lysogen). In λ *S.ty-rpoC*$_6$ and λ *S.ty-rpoC*$_{15}$ this may well be the case (assuming that the general features of the *E. coli rpoBC* control system [5] apply here) because little DNA upstream of *rpoC* is cloned. In λ *S.ty-rpoBC*$_{14}$, even if a major promoter is included in the fragment cloned, its activity may be contained, say by an attenuator (which in *E. coli* is known to lie immediately upstream of *rpoB* [5]).

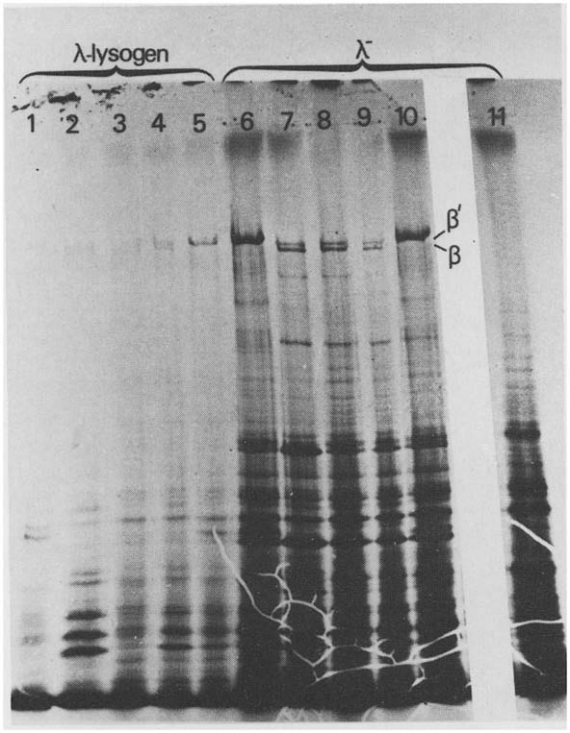

Fig.4. Expression of λ clones inside UV-treated bacteria. See section 2 for procedures. The figure shows the autoradiograph of a dried gel. As markers we used (among others) *E. coli* RNA polymerase core enzyme (a gift from Boehringer-Mannheim). Note that β and β′ from *E. coli* co-migrate with their *Salmonella* counterparts under these conditions [12]. Tracks 1 and 6, λ *S.ty-rpoC*$_{15}$; tracks 5 and 10, λ *S.ty-rpoC*$_6$; tracks 2–4,7–9, λ *S.ty-rpoBC*$_{14}$; track 11, uninfected control.

None of the 15 λ phages we characterized had much DNA from upstream of *rpoB* (i.e., from the region to the right of fragment 'a' in fig.2), λ *S.ty-rpoBC*$_{14}$ being the one that had the most. Thus some feature in this part of the *Salmonella* chromosome must resist successful isolation, packaging and growth in λ. Furthermore, of the 15 phages analysed, 13 had the orientation shown for λ *S.ty-rpoC*$_{15}$, whereas only λ *S.ty-rpoBC*$_{14}$ and one other had the opposite orientation. We cannot explain this bias at present.

DNA extracts from *S. typhimurium* bacteria yield an approx. 5-kb *Hin*dIII fragment showing homology to DNA derived from tl e *E. coli rpoBC* cluster (fig.5). Further studies indicate (not shown)

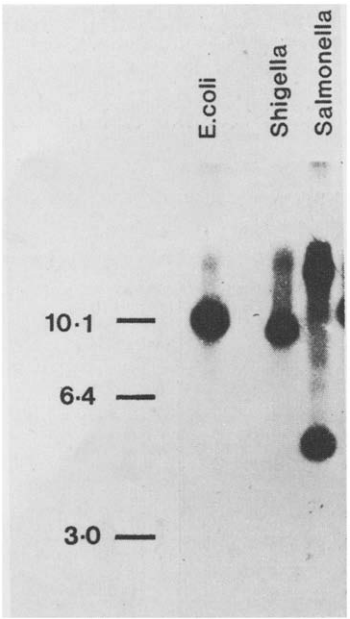

Fig.5. Southern blot analysis of *Hin*dIII chromosomal digests of *S. typhimurium* (and other enterobacteria) using ^{32}P-labelled *E. coli* probe 1 (fig.1). Marker sizes are given in kilobase pairs.

that this fragment hybridises to *E. coli* probes 6 and 7, but not 5. In none of the 15 phages examined was there a 5-kb *Hin*dIII fragment with homology to these *E. coli* probes. It is very likely that the *Salmonella* chromosome has a *Hin*dIII site just to the right of fragment '1' (fig.2) that has not been included in the DNA cloned. Other, less likely possibilities, however, such as the fragment deriving from a plasmid inhabiting *S. typhimurium*, or from a chromosomal duplicate of a part of the *rpoBC* operon, must not be overlooked.

The β and β' subunits of *S. typhimurium* and *E. coli* are very similar in size and function [11,12] and can even be exchanged between the two species to produce a viable enzyme [13]. The distribution of restriction sites on *rpoBC*, however, has diverged considerably between the two species. A comparison of these two systems should prove useful in understanding the limits within which DNA sequences may drift while preserving the functional characteristics of their products.

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