Organization of Ribosomal Protein Genes of *Escherichia coli* as Analyzed by Polar Insertion Mutations

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Several mutants of λ*spcl* and λ* fus3* have been isolated carrying DNA insertion elements that were selected for their ability to reduce the expression of the *spc* gene. The sizes and locations of the insertions on the phage genomes were determined by heteroduplex analysis. They were found to be located at different positions in the *spc* transcription unit. The effect of these insertions on the expression of the ribosomal protein genes carried by these phages in ultraviolet light-irradiated bacteria was investigated. The insertions at intermediate positions in the transcription unit reduced the expression of some of the genes in the unit but not others. Assuming that the genes whose expressions were reduced are distal to the insertion, it was possible to determine the relative position of most of the genes in the unit. The results indicate the order of genes in the *spc* transcription unit is: promoter, L14, L24, L5, S14, S8, L6, L18, (S5, L15, L30).

We have isolated several transducing phages carrying bacterial DNA from the *str-spc* region of the *Escherichia coli* chromosome (*1, 2*). The phage with the largest substitution of bacterial DNA, λ* fus2* (and λ* fus3*), carries genes for 27 ribosomal proteins (r-proteins) (*2, 3*), EF-G (*4*), EF-TU (*4*), and the α subunit of RNA polymerase (*5, 6*). Another phage is λ* spcl*, which contains part of the bacterial DNA of λ* fus2* (*2, 6*), and consequently it carries a subset of the genes on λ* fus2* (*2, 3*). One of our goals is to determine the order of the r-protein genes on the * fus2* genome and therefore on the *E. coli* chromosome.

The r-protein genes on λ* fus2* appear to be organized into at least four transcription units (*2, 4, 5, 7*). One of these units is the *spc* transcription unit, which appears to contain genes for 10 r-proteins (*cf. Fig. 1* (*7* and this paper)). Evidence that these genes are co-transcribed has come from the isolation of two polar insertion mutants of λ* spcl*, called λ* spcl*1-115 and λ* spcl*1-116 (*7*). These mutants were selected as phages on which the expression of the *spc* gene had been reduced. We found that these mutations resulted from the insertion of a piece of foreign DNA similar to the IS elements into the bacterial DNA of λ* spcl* (*see Ref. 13 for a review of IS elements*). The best studied IS elements are IS1 and IS2. When either IS1 or IS2 elements are inserted into the middle of a transcription unit, such as the lac or gal operons, they have a polar effect on the expression of the genes that are distal to the site of the insertion, similar to the effects caused by nonsense mutations (*13*). The molecular nature of the mechanism causing this polarity is not clear. Presumably it is similar to the mechanism which results in polarity caused by nonsense mutations. Both IS1 and IS2 can be inserted with two orientations relative to the direction of transcription. For IS1, both orientations are polar. However, for IS2, only orientation I is polar.

It has been established that the insertion in λ* spcl*1-115 is an IS1 in orientation II and the insertion in λ* spcl*1-116 is an IS2 in orientation I (*14*), i.e. the polar orientation. Analysis of the expression of the r-protein genes on λ* spcl*1-116 revealed that the expression of 10 genes, including the *spc* gene, had been reduced to less than 10% of the level of expression in the parent phage (*7 and see below*). These genes coded for S5, S8, S14, L5, L6, L14, L15, L18, L24, and L30. The *spc* gene is the structural gene for S6 (*15*). Since it was known that these genes as a group were located downstream in the direction of transcription from the site of the insertion, we concluded that they were part of a single transcription unit, which we have named the *spc* transcription unit.

Genes for S13, S11, S4, L17, and α, which are also distal to the insertion, did not appear to be affected, at least not to the same extent (*7, and see below*). Thus, we concluded these genes were transcribed from one or more different promoters. We later found that at least the genes for S4, S11, L17, and α appeared to be transcribed from a promoter which is located distal to the *spc* transcription unit (see Fig. 1 of the preceding paper, *2*; and Fig. 1) (*5, 8*). The gene for S13 may also be in this unit.

We have now isolated several more independent insertion mutants of λ* spcl* and λ* fus3* that were selected for their inactivation of the *spc* gene. The sizes and positions of the insertions were determined by heteroduplex analysis and their effects on the expression of the genes in the *spc* unit.
were measured. From the results it has been possible to deduce the relative order of most of the genes in this unit. Preliminary accounts of the present work have been described (16).

**MATERIALS AND METHODS**

Isolation of Insertion Mutants — The methods used to isolate insertion mutants of \( \lambda s p c \) and \( \lambda f u s 3 \) that reduce the expression of the \( \text{spc} \) gene have been described previously (7, 16). Briefly, the methods were as follows. Stable lysogens were constructed in which a \( \text{spc} \) allele was on the chromosome and a \( \text{spc} \) allele was on the plasmid. These lysogens were sensitive to spectinomycin (Spc) since the \( \text{spc} \) allele is dominant (17). Mutants were then isolated that were resistant to Spc. We screened these mutants by measuring the buoyant density of their transducing phages. We found that approximately 40 to 50% of the mutants had resulted from an insertion into the transducing phage genome. The mutant transducing phages were purified and separated from helper phages by standard techniques (18).

Heteroduplex Techniques — Heteroduplex analysis was done as described previously (19). The standard for double strand measurements was PM2 circular DNA which is 9500 base-pairs in length (20-22). The standard for single strand measurements was \( \Delta \) X 174 single strand circular DNA which is 9500 base-pairs in length (21). The results from at least 10 molecules were averaged in each case.

Materials — Antiserum to purified proteins were prepared in this laboratory (23, cf. Ref. 3). \[^1\mathrm{H}]\text{Leucine and } \[^1\mathrm{C}]\text{Leucine were obtained from Amersham/Searle.}

Other Methods — They are described in the preceding paper (2).

**RESULTS**

The physical structures of the genomes of \( \lambda s p c 1 \) and \( \lambda f u s 3 \) are given in the upper part of Fig. 1. \( \lambda f u s 2 \) and \( \lambda f u s 3 \) are the same except \( \lambda f u s 2 \) carries a \( \text{str} \) allele and \( \lambda f u s 3 \) carries a \( \text{str} \) allele (2). All the \( \text{r-protein} \) genes on \( \lambda f u s 2 \) appear to be transcribed leftward on the phage genome (7). We have previously suggested that the \( \text{r-protein} \) genes on \( \lambda f u s 2 \) are organized into at least four transcription units (2, 4, 5, 7; and an accompanying paper, 8). The approximate positions of these units are given in Fig. 1. \( \lambda s p c 1 \) carries only the \( \alpha \) and Spc transcription units (see introduction). The experiments reported here are primarily directed at determining the relative order of the 10 genes in the Spc transcription unit with the use of insertion mutants.

Heteroduplex Analysis of Spc Insertion Mutants — Several mutants of \( \lambda s p c 1 \) and \( \lambda f u s 3 \) have been isolated as outlined under "Materials and Methods" on which the \( \text{spc} \) gene has been at least partially inactivated by the insertion of an IS element. The sizes of the insertions and their locations on the phage genomes have been determined by heteroduplex analysis.

\( \lambda s p c 1 \) and \( \lambda f u s 2 \) (or \( \lambda f u s 3 \)) are homologous from their left end to the right bacterial-\( \lambda \) DNA junction (called the "junction" in this paper) in \( \lambda s p c 1 \) (see Fig. 1) (6). They also have a \( \lambda \)-DNA homology with the length of 50-\% of units at their right ends. Thus, heteroduplex molecules of \( \lambda s p c 1 \) and \( \lambda f u s 2 \) have a long double strand tail at one end and a short double strand tail at the other end separated by a large bubble (cf. Fig. 2).

Spc insertion mutants of \( \lambda s p c 1 \) were heteroduplexed with \( \lambda f u s 3 \) and Spc insertion mutants of \( \lambda f u s 3 \) were heteroduplexed with \( \lambda s p c 1 \). In all cases the insertion loop was found in the long double strand region (see Fig. 2, A and B). The sizes of the insertion loops and the distances between the junction and the insertion loop (\( \text{D in Fig. 2B} \)) are given in Table 1.

The locations of many of the insertions are also given schematically in Fig. 1 along with the gene order that was deduced from the analysis of these mutants (see below).

Most of the insertions were the size of ISI, approximately 800 base pairs, or the size of IS2, approximately 1300 base-pairs. None of the insertions have been identified except for the IS1 in \( \lambda s p c 1-115 \) and the IS2 in \( \lambda s p c 1-116 \) (14).

We found the insertions were scattered throughout the region between the junction and 4.3 kilobases to the left of the junction. This indicates that each of the insertions is located within the bacterial DNA that is common to both \( \lambda s p c 1 \) and \( \lambda f u s 3 \). There seems to be a larger number of insertions near the junction in \( \lambda s p c 1 \), which is the approximate location of the presumed promoter for the Spc transcription unit (2, 7).

Expression of \( \text{r-Protein} \) Genes of Mutant Phages in UV-Irradiated Bacteria — The effect of some of the insertions on the expression of the \( \text{r-protein} \) genes in UV-irradiated bacteria has been examined. Table II presents the results of experiments in which the host bacteria were prelabeled with \( \[^1\mathrm{H}]\text{Leucine, irradiated, infected with a purified phage, and the proteins synthesized after phage infection were labeled with } \[^1\mathrm{C}]\text{Leucine. The ratio of } \[^1\mathrm{H}]\text{C to } \[^1\mathrm{C}]\text{H was determined. The data was normalized to the expression of one of the genes on the phage that do not seem to be affected by the insertions (see legend to Table II). The normalized expression of each gene of the mutant phage is then expressed in Table II relative to the expression of that gene on the parent phage.}

The purpose of the internal normalization is to correct for differences in the multiplicity of infection from one phage preparation to another. For derivatives of \( \lambda f u s 3 \), the gene for S7 was used for this normalization. It is located on the 11% EcoRI fragment at the right end of \( \lambda f u s 3 \) (see Fig. 1) (3, 8). It appears to be in a transcription unit with the \( \alpha \) gene and genes for EF-G and EF-Tu, which we call the Str transcription unit (2, 7). None of the insertions in the Spc transcription unit affect the expression of any of the \( \text{r-protein} \) genes to the right of the Spc unit on \( \lambda f u s 3 \), including the genes in the Str unit. Thus, the use of the S7 gene for the internal standardization is justified. \( \lambda s p c 1 \) does not carry any of the \( \text{r-protein} \) genes of \( \lambda f u s 3 \) that are located to the right of the Spc transcription unit. Thus, for derivatives of \( \lambda s p c 1 \) we have used the S4 gene for normalization. This gene appears to be in the \( \alpha \) transcription unit, which is located distal to the Spc transcription unit (see Fig. 1) (5). Our earlier experiments had indicated that the genes in this unit were only weakly affected by the insertions in the Spc unit (7). The experiments reported below with the Spc insertion mutants of \( \lambda f u s 3 \) indicate that the genes in the \( \alpha \) unit are partially affected by these insertions, but not to the same extent as the genes in the Spc unit (see "Discussion"). Nevertheless, it is still possible to use the S4 gene for the standardization in comparing the various derivatives of \( \lambda s p c 1 \).

Previously we had found that the IS2 insertion in \( \lambda s p c 1-116 \) reduced the expression of the genes for S5 (spc), S8, S14, L5, L6, L14, L15, L18, L24, and L30, i.e., all of the genes in the Spc transcription unit. Similar results were seen for the experiment reported in Table II. The insertions in \( \lambda s p c 1-126 \), and \( \lambda s p c 1-133 \), which are located at approximately the same position as the insertion in \( \lambda s p c 1-116 \), also had the same qualitative effect. However, the insertion in \( \lambda s p c 1-133 \) seemed to be less polar.

In contrast to these results, the insertions located farther from the junction reduced the levels of expression of only some of the genes in the Spc transcription unit. For example, in \( \lambda s p c 1-140 \), in which the insertion is located farther to the
FIG. 1. Physical structure of λspc1 and λfus3 and the order of genes in the Spc transcription unit. The physical structure of λspc1 and λfus3 (3, 6) and the transcription map of the r-protein genes on λfus3 (2, 4, 5, 7, 8) are shown in the upper part. The open bars are bacterial DNA and the hatched bars are λ-DNA. The structure of λspc1 is drawn to emphasize the homologies with λfus3 at both ends. λspc1 is actually smaller than λfus3 (6). The EcoRI restriction endonuclease map of λfus3 (3) is also shown. The size of EcoRI fragments is indicated in %A units (1%A is 465 base-pairs). The relative order of genes in the a (3, 5) and Spc transcription units is shown in expanded scales. The genes are identified by their gene products. Each insertion in the Spc unit is represented by a vertical arrow and the identity of the insertions is given below the arrow. (The insertions used to locate genes in in vitro experiments (3) are underlined.) Insertions in the 200 series were isolated on λfus3 and all the others were isolated on λspc1. The positions of the insertions are given in terms of their distance from the coli-λ junction in λspc1, i.e. the D value of Table I. The junction is at 0. 1 Kilobase = 1000 base-pairs. The genes in the Spc unit are represented by boxes which approximate the size of the genes, as determined from the known molecular weights of the gene products. (The molecular weights of r-proteins are taken either from the values calculated from amino acid sequence data (for S8 (9), L18 (10), and L30 (11)) or from the values obtained from SDS-gel electrophoresis (for other proteins (12)). In the latter case, 90% of the values given by the Berlin group were used, because the molecular weights of proteins calculated from sequence data are on the average about 90% of the values given by the SDS method.) The relative positions of the genes and the insertions were determined as described in the text.

The positions of the genes for S5, L15, and L30 relative to the 129, 123, and 1210 insertions are not known, but 1201 is probably very close to or within the S5 gene (3). The genes for L15 and L30 are in brackets because their relative order is not known. The horizontal arrows indicate the direction of transcription.

### Table I

Heteroduplex analysis of λspc1 and λfus3 “Spc-insertion” mutants

| Mutant | Distance (D) from Junction* | Insertion Size (Base Pairs ± S.D.**) |
|--------|-----------------------------|-------------------------------------|
| λspc-116 | 270 ± 30                    | 2170 ± 110                          |
| λspc-126 | 250 ± 60                    | 2160 ± 120                          |
| λspc-129 | 180 ± 40                    | 1130 ± 100                          |
| λspc-130 | 450 ± 40                    | 1130 ± 130                          |
| λspc-133 | 390 ± 50                    | 850 ± 110                           |
| λspc-112 | 840 ± 40                    | 800 ± 80                            |
| λspc-122 | 1,000 ± 70                  | 770 ± 110                           |
| λspc-128 | 1,150 ± 60                  | 820 ± 90                            |
| λspc-132 | 1,590 ± 60                  | 850 ± 110                           |
| λspc-127 | 1,370 ± 70                  | 710 ± 70                            |
| λspc-140 | 2,090 ± 250                 | 840 ± 70                            |
| λspc-139 | 2,460 ± 150                 | 870 ± 100                           |
| λspc-124 | 2,660 ± 50                  | 870 ± 110                           |
| λspc-129 | 3,160 ± 90                  | 810 ± 70                            |
| λspc-125 | 3,570 ± 250                 | 770 ± 110                           |
| λspc-123 | 3,540 ± 160                 | 800 ± 90                            |
| λfus3-1220 | 1,580 ± 110               | 1,280 ± 120                         |
| λfus3-1211 | 1,880 ± 90              | 1,250 ± 130                         |
| λfus3-1214 | 2,870 ± 110           | 1,250 ± 130                         |
| λfus3-1201 | 3,160 ± 120           | 1,320 ± 120                         |
| λfus3-1210 | 4,250 ± 180             | 1,260 ± 130                         |

* See Fig. 2.

** S. D. = standard deviation.

left than it is in λspc-116, the expression of genes for L24, L5, S14, and possibly the genes for L14, or L15, or both, were not affected. The expression of other genes was reduced at least partially. For λspc-129, in which the insertion is located even farther to the left, the expression of the gene for S8, in addition to the genes for L24, L5, S14, and possibly L14 (or L15, or both), was not reduced. The simplest interpretation of
| Phage     | Distance (0) From Junction* (base pairs) | Proteins | Other Proteins |
|----------|----------------------------------------|----------|---------------|
|          |                                        | S4       | S11 | S15 | L18 | L6 | S8 | S14 | L5 | L24 (L14)** | S7 | Others |
| __       |                                       | L17      | 54  | S11 | 513 | L30 | s5 | L18 | L6 | S8 | Others |
| __       |                                       | L5       | 98  | L24 | (L15+L14)** | S7 | Others |
| __       |                                       | Hspcl-116 | 270 | L18 | 100 | 1.01 | 1.07 | 0.88 | 0.07 | 0.10 | 0.14 | 0.14 | 0.06 | 0.08 | 0.07 | 0.04 | 0.05 | 0.03 | 0.08 | 0.24 |
| __       |                                       | Hspcl-126 | 250 | L18 | 1.00 | 1.0 | 0.82 | 0.87 | 0.04 | 0.21 | 0.14 | 0.23 | 0.06 | 0.10 | 0.05 | 0.03 | 0.08 | 0.08 |
| __       |                                       | Hspcl-133 | 390 | L18 | 0.98 | 1.0 | 0.91 | 0.84 | 0.25 | 0.18 | 0.25 | 0.29 | 0.24 | 0.26 | 0.23 | 0.21 | 0.24 |
| __       |                                       | Hspcl-115 | 840 | L18 | 1.01 | 1.0 | 1.00 | 0.87 | 0.52 | 0.47 | 0.30 | 0.53 | 0.55 | 0.54 | 0.48 | 0.02 | 0.61 |
| __       |                                       | Hspcl-128 | 1,000 | L18 | 1.02 | 1.0 | 1.00 | 0.96 | 0.44 | 0.35 | 0.43 | 0.47 | 0.46 | 0.40 | 0.01 | 0.68 | 0.63 |
| __       |                                       | Hspcl-140 | 2,100 | L18 | 0.98 | 1.0 | 0.89 | 1.00 | 0.41 | 0.33 | 0.25 | 0.38 | 0.08 | 2.02 | 2.45 | 1.15 | 0.43 |
| __       |                                       | Hspcl-139 | 2,460 | L18 | 1.04 | 1.0 | 1.05 | 1.06 | 0.40 | 0.34 | 0.25 | 0.13 | 1.37 | 1.66 | 1.76 | 1.22 | 0.86 |
| __       |                                       | Hspcl-124 | 2,660 | L18 | 1.01 | 1.0 | 1.00 | 1.00 | 0.44 | 0.34 | 0.34 | 0.12 | 1.43 | 1.78 | 1.04 | 1.21 | 0.02 |
| __       |                                       | Hspcl-129 | 3,160 | L18 | 0.92 | 1.0 | 0.89 | 0.98 | 0.39 | 0.11 | 0.97 | 1.21 | 1.52 | 1.74 | 1.83 | 1.21 | 0.75 |
| __       |                                       | Hspcl-123 | 3,540 | L18 | 0.97 | 1.0 | 0.88 | 1.35 | 0.33 | 0.21 | 1.56 | 1.63 | 2.04 | 2.15 | 1.79 | 0.07 | 0.07 |
| λfus3   |                                        | ---      | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| λfus3-1220 |                                    | 1,380 | 0.48 | 0.45 | 0.76 | 0.45 | 0.27 | 0.27 | 0.26 | 0.30 | 0.18 | 0.24 | 0.06 | 0.58 | 0.36 | 1.06 |
| λfus3-1211 |                                    | 1,580 | 0.51 | 0.48 | 0.56 | 0.52 | 0.24 | 0.25 | 0.22 | 0.27 | 0.13 | 0.13 | 1.23 | 0.65 | 0.81 | 1.14 |
| λfus3-1214 |                                    | 2,870 | 0.48 | 0.31 | 0.57 | 0.00 | 0.25 | 0.19 | 0.27 | 0.50 | 0.50 | 1.02 | 1.23 | 0.64 | 0.37 | 0.98 |
| λfus3-1201 |                                    | 3,160 | 0.66 | 0.72 | 0.90 | 0.85 | 0.41 | 0.25 | 0.82 | 0.81 | 1.19 | 1.19 | 1.35 | 1.15 | 0.65 | 1.05 |

*See Fig. 2 and Table I.
**As described in the text, L14 and L15 were not separated completely in the two-dimensional system used, and, therefore, the proteins were analyzed as a mixture. The outline for the experiment is described in the text. The bacterial host was S159 (λ). Further details are given in the preceding paper (2) (in the Methods section and in the legend to Table II). The data in the table was calculated from the equation:

## Relative expression of protein P1

\[
\frac{\left(\frac{3H}{14C}\right)_M - \left(\frac{3H}{14C}\right)_P}{\left(\frac{3H}{14C}\right)_M - \left(\frac{3H}{14C}\right)_\text{Std}} = \frac{\left(\frac{3H}{14C}\right)_P - \left(\frac{3H}{14C}\right)_\text{Std}}{\left(\frac{3H}{14C}\right)_M - \left(\frac{3H}{14C}\right)_\text{Std}}
\]

The subscripts M, P, and λ refer to the mutant phage, parent phage (λpol or λfus3) and λ1857Δ7, respectively. The subscript "Std" refers to the standard protein. For derivatives of λpol, the standard was S4 and for derivatives of λfus3, the standard was S7. The 1.0's in boxes are the result of the normalization. The genes whose expression seemed to be reduced by the insertions are underlined. The column under "Others" gives the average of the results for S17, L16, L29, S3, S19, L22, L2, L23, L4, L3, S10 and S12. None of these genes nor the gene for S7 is present on λpol, but they are present on λfus3 (cf. Fig. 1 of the preceding paper and ref. 3).
these observations is that the insertions only reduced the expression of genes located distal to the insertion, relative to the promoter. Since the direction of transcription is leftward (in Fig. 1), these genes would be located to the left of the insertion. The genes whose expressions were not reduced would presumably be located between the insertion and the promoter, i.e. to the right of the insertion. Thus, analysis of the effect of insertions located at different positions in the transcription unit should tell us the relative order of the genes, much as would the analysis of a collection of nonsense mutations.

The genes whose expression seemed to be reduced by the insertion are underlined in Table II. For several of the mutants there is one protein whose synthesis is reduced considerably more than the others, reduced to what appears to be zero. Such is the case for L5 in λspc-128 and S8 in λspc-140. It is possible that the insertion is actually located within the structural gene for these proteins. (In vitro experiments using DNA from λspc-140 showed that the synthesis of S8, but not other proteins, was abolished by this insertion, confirming this possibility (3). For further discussion on the in vitro experiments using this and other insertions, see an accompanying paper (6).) From the results of Table II and the physical location of the insertions, the gene order appears to be promoter, L24, L5, L14, S8, L6, L18, (S5, L30).

The position of the genes for L14 and L15 in the unit is not clear from the results given in Table II because the two-dimensional gel system used to separate the r-proteins for these experiments did not give a clean separation of these two proteins. However, it was possible to separate them from each other and from the other r-proteins whose genes are carried by λspc1 by electrophoresis on a one-dimensional polyacrylamide-urea gel at pH 4.5. This can be seen in the experimental results shown in Fig. 3. In this experiment, the proteins synthesized after λspc1 infection of UV-irradiated bacteria were labeled with [3H]leucine, extracts of these cells were electrophoresed on the one-dimensional polyacrylamide-urea gel, and the r-proteins synthesized from λspc1 were detected by fluorography. The bands have been identified by comparison with the mobility of purified reference r-proteins and, for some of the proteins, by demonstrating that they could be precipitated with antisera to that particular r-protein. It can be seen that proteins S5, L6, L14, L15, and L24 were synthesized in infected cells and that these five proteins can be separated in the gel system used, except that separation of L6 and S8 is not complete. (We note that the S5 made from λspc1 in the UV-irradiated bacteria appeared to have a slightly faster mobility in these gels than the reference S5. The significance of this is not known.)

Fig. 4 gives the results of an experiment in which the r-proteins synthesized in UV-irradiated bacteria after infection with several different insertion mutants of λspc1 were analyzed by electrophoresis on a one-dimensional urea slab gel. Several of the bands are identified on the left (see also the figure legend). The distance between the insertion and the junction for the various mutants used in this experiment (see Table I) increases as one goes from left to right across the gel. The last sample is for λspc1-123, which has the insertion farthest from the junction of all the λspc1 mutants.

The following observations can be made from the experiment shown in Fig. 4. The synthesis of S5, L15, and L30 was greatly reduced for all the mutants tested, indicating that the genes for these proteins are toward the end of the transcription unit. In addition to these proteins, the synthesis of L5, L14,
were determined in a similar fashion. In general, the results and 140 insertions as indicated in Fig. 1. The positions of the right of the 140 insertion, but "cut" by the 1211 insertion.

relative to the insertions were determined from the data in Table II. For example, the L5 gene appears to be to the right shown in Table II and Fig. 4, as well as the relative positions other genes in the transcription unit relative to the insertions. Since the 514 gene seems to be to the left of the L5 gene, we note that the order S5, (L15, L30) obtained from the in vitro experiments is consistent with our failure to isolate any insertion mutants which inactivate S5 without inactivating L15, or L30, or both. Thus, the agreement between the present mapping results and the in vitro mapping results is excellent and supports the validity of the present method based on the polarity caused by various insertions.

The only exceptional insertion we have found is I210 (see Table I and Fig. 1). This insertion is located the farthest from the junction of all the insertions, and is in the 3% EcoRI fragment which is to the left of the 10% EcoRI fragment (cf. Fig. 1). If the physical arrangement of genes in the Spc unit is like that shown in Fig. 1, as we believe, then the I210 insertion is distal to the spc gene. In our preliminary experiments, this insertion did not seem to decrease significantly the expression of any of the genes in the Spc unit. Yet this insertion mutant was isolated as the one affecting the expression of the spc gene on λpSC1. It is possible that only a small reduction in the expression of a spc allele makes it recessive to a spc allele. Since IS2 elements carry a promoter (13), one could imagine that if the direction of transcription from the insertion was opposite to the direction of transcription from the unit in which it is inserted, then the "collision" of the two polymerases could result in a decrease in the expression of promoter proximal genes.

The four insertions in λfas3 that were investigated seemed to decrease the expression of the r-protein genes in the α unit to about 50% (45 to 90%). The expression of the genes in the Spc unit that appear to be affected was reduced to 15 to 30% in most of the cases. This suggests the genes in the α unit are part of the Spc transcription unit to some degree. Nevertheless, it appears these genes can be expressed from another promoter that is distal to the Spc unit, i.e. the α promoter (5, 8). Several models could accommodate this complication. For example, the genes in the α unit may be expressed approximately 50% of the time from the α promoter and 50% of the time from the Spc promoter. Or the α promoter may be a "secondary" promoter which is used in the Spc insertion mutants.

The effect of the Spc insertions on the expression of the genes in the α unit is not apparent in Table II for the λpSC1 mutants because all the data for these mutants was normalized to the expression of S4, one of the genes in the α unit. If the expression of S4 is actually about 50% of its expression from the parent phage, as it is for the λfas3-Spc insertion mutants, then the data in Table II for all the λpSC1 mutants should be divided by 2. This would help explain how some of the genes that are promoter proximal to the insertion appear to be expressed at twice their normal rate, e.g. S14 and L5 in λpSC1-140.

In principle, we could have mapped the genes in the Spc unit with a collection of nonsense mutants. However, the use

- L. Post and M. Nomura, unpublished experiments.
of insertions is more powerful because the insertions can be mapped relatively precisely by heteroduplex techniques. At present we cannot easily map nonsense mutants in r-protein genes. In addition, isolation of insertions is easier in the present system. We also note that genes in which the insertions took place can be identified using a DNA-dependent in vitro protein-synthesizing system (see accompanying paper, Ref. 3), at least in certain instances. Insertion mutations may be useful in studying the organization of other r-protein transcription units.

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