Determination of Transcriptional Units and Gene Products from the ftsA Region of Escherichia coli

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Lambda transducing phage λ16-2 carries the genes enaA, ftsZ, ftsA, ddl, and murC and directs the synthesis of six unique proteins in ultraviolet-irradiated cells. Various derivatives of λ16-2 carrying smaller segments of the bacterial deoxyribonucleic acid have also been analyzed for their capacity to direct protein synthesis in ultraviolet-irradiated cells. These results, in combination with genetic results, have allowed the gene product of each of these genes to be assigned. In addition, an unidentified gene was located counterclockwise to murC between murC and murF. Analysis of the direction of transcription indicates that murC, ddl, ftsA, and ftsZ are transcribed clockwise on the Escherichia coli genetic map, and enaA is transcribed counterclockwise. In addition, it is shown that each of the genes enaA, ftsZ, and ftsA can be expressed independently.

The 2-min region on the Escherichia coli K-12 genetic map contains a cluster of genes involved in the normal functioning of the cell wall. These include the cell division genes ftsA (16), ftsZ (7), and sep (pBP, fts1) (1, 2); the cell permeability gene enaA (12); and genes for murein biosynthesis, murE, murC, murF, and ddl (16). It is known that the products of the murein genes are enzymes involved in the biosynthesis of murein: the amino acid-adding enzymes and D-alanyl-D-alanine ligase (3–5). The functions of the remaining gene products are unknown. However, it is possible that these gene products also affect murein metabolism, especially that part which must be distinct for cell division. Thus, mutants in enaA have a reduced level of N-acetylmuramyl-l-alanine amidase activity (18), ftsZ mutants have a lower level of DD-carboxypeptidase at the restrictive temperature (8, 9), and sep codes for penicillin-binding protein 3 (2, 11, 13, 14). It is of interest, therefore, to see how these genes are organized on the chromosome.

Recently, a specialized λ transducing phage was isolated that carries the genes murC, ddl, ftsA, ftsZ, and enaA (6). This led to the identification of the ftsA gene product. This phage was also used to isolate new transducing phages carrying smaller segments of the bacterial DNA from this region. From complementation tests and marker rescue experiments, it was possible to draw some conclusions concerning the organization of these genes (7). In this report this work is extended by examining protein synthesis directed by these transducing phages in UV-irradiated cells. It has been possible to identify the gene products of the enaA, ftsZ, murC, and ddl genes. We also find that enaA, ftsA, and ftsZ can be expressed independently. In addition, murC and ddl are in an operon which may include an unidentified gene that maps between murC and murF.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial strains used for protein synthesis after UV irradiation were E. coli K-12 159 (uraA gal rpsL) and two lysogenic, recA derivatives, 159 recA (dim13) and 159 recA (imm2 supF). The recA mutation was introduced from JC5088 (7) by conjugation with a spontaneous thyA derivative of 159 (selecting for thyA+). For the phage cross the strains NEM43 (suppressor-free) and NEM325 (groN supF tonA), obtained from Noreen Murray (17), were employed. Also, a lysogenic derivative, NEM325 (A16-2), was used. Other strains carrying mutated alleles of genes which map in the ftsA region have been described earlier (6, 7).

The transducing phage strains used in this investigation were isolated previously (7), and their structures are presented in Fig. 1. Phage λNM770 (h80 trp46 Nam7 am53 p+ c1+ p4m55 SupF) was used as a donor of hybrid immunity and Nam7 and was obtained from Noreen Murray (17).

Phage cross involving hybrid immunity. The use of hybrid immunity phages to obtain derepressed transcription of λ from pl has been described elsewhere (10, 17). In such a phage, the cro gene product (from phage 434) cannot turn off expression originating from pL (from phage λ). This expression can be controlled experimentally if the phage also carries an Nam mutation. In a suppressor-free host, transcription from pL will terminate at the end of the N gene, whereas in a host containing a suppressor, transcription will continue beyond this point.

Cells of strain NEM43 in exponential phase were centrifuged, resuspended in 10 mM MgSO4, and infected with a multiplicity of infection of 5 for each of the two phages, λ16-2 and ANM770. After phage ad-
sorption, the mixture was diluted fivefold into L-broth and incubated with shaking for 2 h at 37°C. Chloroform was then added, and recombinants were selected by plating on NEM325(λ16-2). Plaques were purified on NEM325. The phage cross is diagrammed in Fig. 2.

**Protein synthesis in UV-irradiated cells.** Strain 159 and its lysogenic derivatives were grown in minimal medium (M9) supplemented with 0.2% maltose as the carbon source (6). At an optical density at 600 nm of 0.5, cells from 50 ml of culture were centrifuged and resuspended in 5 ml of the above medium supplemented with 20 mM MgSO₄. The suspension was transferred to a glass petri dish and irradiated with a UV dose of 8,000 ergs per mm². In a typical experiment, 75 µl of cells was infected with 10 µl of phage (absorbance at 260 nm of 1.0), which gives a multiplicity of infection of 10. In experiments involving infection of a nonlysogen or a heteroimmune lysogen, the phage were absorbed for 10 min at 37°C, followed by the addition of 200 µl of prewarmed medium. After a

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**Fig. 1.** Diagram of the central region of the phages used in this investigation. Sizes of the restriction nuclease fragments are given in kilobases. The thin line represents λ DNA, and the open rectangle represents bacterial DNA. In some cases, the endpoints of the deletions are close to or at restriction sites. However, in others the endpoints are not known precisely, and this is indicated by dotted lines. The two phages at the bottom were obtained by cloning the EcoRI fragments from λ16-2 into a λ-replacement vector and were described previously (7).

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**Fig. 2.** Construction of the transducing phage carrying hybrid immunity. The thin line represents λ DNA, the rectangles indicate substitutions: shaded rectangles indicate bacterial DNA and open rectangles indicate phage DNA other than λ, and the gaps indicate deletions. The construction and use of the hybrid immunity phages have been described elsewhere (10). Briefly, in such phages transcription originating from the promoter p₁ cannot be turned off (by the cro gene) after infection, since the cro gene originates from phage 434. In addition, in the presence of a suppressing host, this transcription will continue into the bacterial DNA.
further 20 min of incubation, 75 μCi of [35S]methionine was added, and incubation continued for another 10 min. In experiments involving infection of a homologous lysogen, the phage were absorbed for 5 min at 37°C, followed by the addition of 200 μl of prewarmed medium. Five minutes later, 7.5 μCi of [35S]methionine was added, and the incubation continued for another 5 min. The shorter incubation periods were necessary to avoid the appearance of proteins expressed from phage promoters. After the labeling, the cells were chilled on ice and collected by centrifugation. The samples were then analyzed for radioactive protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously (6).

RESULTS

The bacterial DNA present in the transducing phage A16-2 has been shown previously to direct the synthesis of at least four proteins in UV-irradiated cells (6). One of these, with a molecular weight of approximately 50,000, was identified as the ftsA gene product. To determine which genes were responsible for the synthesis of the remaining proteins and to locate the positions of the promoters, the transducing phages diagrammed in Fig. 1 were used to infect UV-irradiated cells. Figures 3 and 4 show the proteins synthesized when these transducing phages were used to infect nonlysogenic and λimm91-lysogenic cells, respectively. Protein synthesis in infected nonlysogens can result from transcription starting from either phage or bacterial promoters or both, whereas in infected lysogens protein synthesis results only from transcription initiating at the bacterial promoters. In nonlysogenic cells A16-2 directed the synthesis of five proteins in addition to the phage proteins (compare lanes 2 and 3, Fig. 3). The protein with a molecular weight of 30,000 has not been seen previously (6). Each of the transducing phages derived from A16-2 had a unique pattern of synthesis for these five proteins (Fig. 3, lanes 4 to 11). Infection of lysogenic cells by A16-2 revealed only three clear bands (compare lanes 1 and 2, Fig. 4); however, upon prolonged exposure and in other experiments, the 50,000- and 48,000-molecular-weight protein bands were also seen (data not shown), indicating the presence of promoters on the bacterial DNA present in A16-2. Comparison of these two figures revealed a protein at a molecular weight of 31,000 in infected lysogens which was not seen in infected nonlysogens, where its position coincided with several phage bands. These results bring the total number of proteins coded for by the bacterial DNA present in A16-2 to six. To determine the gene that is coding for each protein and the location of the promoters, these results must be examined along with Fig. 1 and Table 1, which show the results of complementation tests with these phages.

The 65,000-molecular-weight protein. The protein with a molecular weight of 65,000 was synthesized after infection of cells by A16-2 and ΔR2, but was not synthesized after infection by any of the remaining phages (Fig. 3). These results placed the corresponding gene towards the left end of the bacterial DNA fragment. The phage ΔR1 directed the synthesis of a unique polypeptide with a molecular weight of approximately 45,000 (Fig. 3, lane 9, indicated by the arrow). In a longer gel this polypeptide migrated
more slowly than the darker band seen at this position in several of the other samples (data not shown). An explanation for the appearance of this polypeptide is that ΔR1 contains only the DNA coding for the N-terminus of the 65,000-molecular-weight protein. The juxtaposition of bacterial and λ DNA in this phage must result in the premature termination of the synthesis of this protein. The genetic results indicate that the 65,000-molecular-weight protein is the product of the murC gene, since ΔR2 can complement a strain carrying a murC(Ts) mutation (Table 1) and ΔR1 can rescue this same mutation (7). Such an interpretation indicates that the direction of transcription of the murC gene is clockwise, as drawn both in Fig. 1 and 2 and on the E. coli genetic map.

The 50,000-molecular-weight protein. The 50,000-molecular-weight protein is the ftsA gene product identified earlier by means of a phage carrying an ftsA(Am) mutation (6). In this gel system, though, the ftsA gene product migrated more slowly than the adjacent bacterial protein (48,000 molecular weight). The phages that directed the synthesis of this protein, λ16-2, λ16-25, and λJFL41, were also the phages that complemented ftsA mutants. This places the ftsA gene along with its promotor on the 2.3-kilobase (kb) EcoRI fragment. λJFL41 directed the synthesis of two new proteins, the origin of which is unknown, but must result from the juxtaposition of the bacterial and λ DNAs in this phage. Previously, the ftsA12(Ts) mutation had been located to the right end of the 3.2-kb HindIII fragment (7). Therefore, ΔR2 was of interest since it directed the synthesis of a unique protein with a molecular weight of approximately 46,000 (indicated by the arrow, Fig. 3, lane 8). Since ΔR2 contains only part of the ftsA gene, it could be argued that this novel polypeptide resulted from premature termination of the ftsA gene product, which is a consequence of the juxtaposition of bacterial and λ DNAs at the central HindIII site in this phage. This would indicate that the direction of transcription of the ftsA gene is the same as that for the murC gene, although, as argued previously (7), they must have separate promoters.

The 31,000-molecular-weight protein. The 31,000-molecular-weight protein was seen only after infection of lysogenic cells, since its position coincided with several phage bands in nonlysogenic cells. Analysis of Fig. 4 and Table 1 reveals that all phages that are able to complement the envA mutation are able to direct the synthesis of this protein and that this protein is the only candidate for the envA gene product. The envA structural gene and its promotor must lie within the 2.5-kb EcoRI fragment, since the phage λJFL40 containing just this bacterial DNA fragment can complement an envA mutation and direct the synthesis of the 31,000-molecular-weight protein in lysogenic cells (Fig. 4, lane 10).

The 48,000-molecular-weight protein. The phage ΔR1, in which the two HindIII fragments have been deleted, still retained the ability to direct the synthesis of the 48,000-molecular-weight protein. This phage, however, did not complement any of the known mutations that map in this area (7; Table 1). Therefore, this protein must be the product of an unidentified gene.

The 30,000-molecular-weight protein. The gene for the 30,000-molecular-weight protein is located on the 3.2-kb HindIII fragment, since this protein was seen only after infection by those phages (λ16-2, ΔR2, and λ16-25) that have this fragment in common (Fig. 3). The only
gene known to be entirely contained on this fragment is \textit{ddl}. Both \(\lambda 16-2\) and \(\Delta R2\) can complement a \textit{ddl} mutation (Table 1). Although \(\lambda 16-25\) cannot complement, it can marker rescue a \textit{ddl} mutation (7). It was previously argued that the promoter for \textit{ddl} is to the left of \textit{murC} and therefore would not be carried by \(\lambda 16-25\). The expression of the 30,000-molecular-weight protein from this phage must occur from the phage promoter, \(p\text{IV}^\prime\). This could be argued, since \textit{ddl} is in the proper orientation (7) and it is known that transcription initiating at \(p\text{IV}^\prime\) can proceed into bacterial DNA located in this region (10). \(\lambda 16-25\) would not be expected to complement \textit{ddl}, since this expression would be repressed in a lysogen by phage repressor. After infection of lysogenic cells (Fig. 4), this protein was not seen due to its weak expression and the background of cellular proteins.

The 45,000-molecular-weight protein. The protein with a molecular weight of 45,000 was synthesized after infection of both lysogenic and nonlysogenic cells with the phages \(\lambda 16-2\), \(\Delta B\), and \(\lambda 16-25\). In \(\lambda \text{envA}^+\)-infected cells (especially lysogenic cells) this protein was also synthesized, but the amount was lower. The synthesis of this protein was not detectable after infection by any of the remaining phages. Thus, the region of DNA essential for the synthesis of this protein is around the central \textit{HindIII} site, where the \textit{ftsZ} gene has been located (7). \(\lambda \text{envA}^+\) did not complement the \(\textit{ftsZ84}\)'s mutation (7; Table 1), whereas the phages showing a stronger expression of the 45,000-molecular-weight protein do complement. It is possible that this protein is the \textit{ftsZ} gene product but that a high level of expression of this protein is required for complementation. To investigate this further, lysogens of a \textit{recA} derivative of an \(\textit{ftsZ84}\)'s mutant were constructed with the phages shown in Table 1. These lysogens were then tested for their ability to grow at the restrictive temperature on nutrient agar plates supplemented with various concentrations of NaCl (Table 2). At 0.4% NaCl, \(\lambda \text{envA}^+\) complemented, indicating that the low-level expression of the 45,000-molecular-weight protein is sufficient under these conditions. This provides further evidence that this protein is the \textit{ftsZ} gene product.

\textbf{Direction of transcription and location of promoters.} The results presented here and earlier (7) indicated that the direction of transcription for \textit{murC}, \textit{ddl}, and \textit{ftsA} is clockwise. To independently determine the orientation of transcription of these and the remaining genes, advantage was taken of the effect of the phage promoter \(p_1\) on their expression. Ward and Murray (15) have shown that transcription from \(p_1\) can block transcription of genes located downstream but expressed in the opposite direction. The phage \(\lambda JFL50\) was constructed by the phage cross as indicated in Fig. 2 and then used to infect UV-irradiated cells. In a strain carrying \textit{supF}, transcription initiating at \(p_1\) will proceed into the bacterial DNA, whereas in a suppressor-free strain this transcription will terminate at the termination site just beyond the \(N\) gene.

\begin{table}[h]
\centering
\caption{Correlation of the ability of the transducing phages to complement various mutations and direct protein synthesis in UV-irradiated cells}
\begin{tabular}{|c|c|c|c|c|}
\hline
Phage & \textit{murC} (Ts) & \textit{ddl} (Ts) & \textit{ftsA} (Ts) & \textit{ftsZ} (Ts) & \textit{envA} \\
& (65K) & (30K) & (50K) & (45K) & (31K) (48K) \\
\hline
\(\lambda 16-2\) & + & + & + & + & + \\
\(\Delta J\) & - & - & - & - & + \\
\(\lambda \text{envA}^+\) & - & - & - & + & + \\
\(\Delta B\) & - & - & - & + & + \\
\(\lambda 16-25\) & - & - & - & - & - \\
\(\Delta R1\) & - & - & - & - & - \\
\(\Delta R2\) & + & + & + & - & - \\
\(\lambda JFL40\) & - & - & + & - & - \\
\(\lambda JFL41\) & - & - & + & - & - \\
\hline
\end{tabular}
\end{table}
When transcription starting at \( p_L \) was allowed to proceed into the bacterial DNA, the synthesis of those proteins with molecular weights of 65,000, 50,000, 48,000, and 45,000 was reduced, whereas the synthesis of the 31,000-molecular-weight protein was enhanced (compare lanes 2 and 3, Fig. 5). These results indicate that the transcription of the genes for the 65,000-, 50,000-, 48,000-, and 45,000-molecular-weight proteins is opposed to \( p_L \), whereas transcription of the gene for the 31,000-molecular-weight protein is in the same orientation as \( p_L \). The 30,000-molecular-weight protein cannot be detected in this experiment. These results are summarized in Fig. 6.

**DISCUSSION**

In this report we have examined the ability of various transducing phages carrying various chromosomal segments from the \( ftsA \) region to direct protein synthesis in UV-irradiated cells. From these results and the genetic results presented here and earlier (7), it has been possible to identify the \( envA, ftsZ, murC, \) and \( ddl \) gene products and to verify the identity of the \( ftsA \) gene product. It has also been possible to determine the approximate location of their respective promoters and the direction in which RNA is transcribed from them (Fig. 6). From the genetic results, it was concluded that \( ftsA \) and \( envA \) were expressed independently (7). From these results, it is possible to conclude that \( ftsZ \) can also be expressed independently.

The \( ftsA \) gene product was identified earlier with the aid of an amber mutation in the gene, and this designation was confirmed here. For the other genes, the gene products were identified based on a correlation between the genetic localization of a gene to a DNA segment and the ability of that DNA segment to direct the synthesis of the gene product in UV-irradiated cells. Since this is only indirect evidence, these designations are only tentative. However, there are several reasons for believing them to be correct. First of all, as shown in Fig. 6, the proteins identified here occupy most of the coding capacity of the bacterial DNA in \( \lambda 16-2 \). Second, the variation in expression of the 45,000-molecular-weight protein from different phages correlates with the ability of these phages to complement

**TABLE 2. Effect of NaCl on growth of lysogens of JFL101 [ftsZ84(Ts) recA] at 42°C**

<table>
<thead>
<tr>
<th>Lysogen</th>
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<th>0.4</th>
<th>0.5</th>
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<td>Nonlyso-</td>
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<td>( \lambda 16-2 )</td>
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<tr>
<td>( \lambda envA^+ )</td>
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<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>( \lambda 16-25 )</td>
<td>+</td>
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Fig. 5. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of proteins synthesized after infection of UV-irradiated cells. Lane 1, 159 recA(\( \lambda \)imm\(^{21} \)) infected with \( \lambda 16-2 \); lane 2, 159 recA(\( \lambda \)imm\(^{21} \)sup\(^{F} \)) infected with \( \lambda \)JFL50; lane 3, 159 recA(\( \lambda \)imm\(^{21} \)) infected with \( \lambda \)JFL50; and lane 4, uninfected 159 recA(\( \lambda \)imm\(^{21} \)). An autoradiogram of the gel is presented as described in Fig. 3. The sizes of the proteins of interest are indicated on the left of the figure. In the sample in lane 1, only the bacterial promoters are active; in the sample in lane 2, the presence of the suppressor allows \( p_L \) transcription to proceed into the bacterial DNA; and in the sample in lane 3 there is no suppressor, so \( p_L \) transcription terminates before reaching the bacterial DNA.
ftsZ under different conditions. And third, we have recently been able to isolate independent Tn5 insertions into ftsZ and envA with a corresponding loss of the 45,000- and 31,000-molecular-weight proteins, respectively. The expression of the other proteins was not affected by these insertions (manuscript in preparation).

The expression of the ftsZ gene product is weak from a phage carrying only the 3.5-kb HindIII fragment (λ envA'); however, the addition of bacterial DNA to the left of the central HindIII site (ΔB) restores the expression to normal levels. This increased expression occurs even though this additional DNA does not contain the ftsA promoter, as evidenced by the failure of this phage to complement ftsA (Table 1). It is possible that the juxtaposition of phage and bacterial DNA at the left HindIII site in λ envA' has fortuitously created a weak promoter or that the juxtaposition of these DNAs has affected the activity of the normal promoter. If the latter case is true, then in the phage ΔB the promoter would be restored. Alternatively, a positive regulatory site might be located near or at this HindIII site. This raises a question as to how close the promoter (or regulatory site) for the ftsZ gene is to the 3' end of the ftsA structural gene. From the size of the polypeptide fragment synthesized after infection by the phage carrying DNA to the left of this HindIII site (ΔR2), we can calculate that the ftsA gene must extend at least 135 nucleotides past this HindIII site. This indeed suggests that some element required for full ftsZ expression is located within the ftsA structural gene, and this is under investigation.

It is likely that the 48,000-molecular-weight protein is a product of a gene involved in murein biosynthesis, since the gene is located between murF and murC. It is therefore of interest that the proposed gene (murD) for the D-glutamyl acid-adding enzyme has not been located. It is plausible that this protein may be the murD gene product.

The identification of these gene products and the determination of the organization of the transcriptional units in this area should open the way for an investigation into the role of these gene products in the cell and how their expression is regulated.

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