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Overlapping Functional Units in a Cell Division Gene Cluster in *Escherichia coli*

N. F. SULLIVAN AND W. D. DONACHIE*

Department of Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR, United Kingdom

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The *ftsZ* (*sulB*) coding sequence is preceded by two promoters, at least one of which lies within the coding sequence of the neighboring gene, *ftsA*. This region of the *ftsA* gene is required for full biological activity of *ftsZ*.

The cell division genes *ftsA* and *ftsZ* (*sulB* [5]) are neighbors within a large cluster of contiguous genes of related function in the genome of *Escherichia coli* (1, 6, 7, 14). In cloned DNA fragments, each may be expressed separately, with their own promoters (7). However, biologically functional levels of *ftsZ* protein are obtained only from cloned DNA segments which extend into the upstream *ftsA* coding sequence (8). We describe here gene-fusion experiments which show that the *ftsZ* gene has two promoters, at least one of which lies within the coding sequence of *ftsA*. High levels of transcription of *ftsZ* are shown to require both promoters. Thus, although the coding sequences of *ftsA* and *ftsZ* are separate, the functional genetic units do overlap.

immediate neighbors is also shown (2, 7, 8), together with the three DNA fragments which were cloned into lambda phage (6, 7). All three phages are capable of directing the synthesis of some *ftsZ* protein, and each is capable of partial complementation of a chromosomal *ftsZ*(Ts) mutation in lysogens (7). Lutkenhaus and Wu (7) showed that a single copy of either λ16-2 or the deletion derivative λΔB was sufficient to provide full *ftsZ*$^+$ expression in *ftsZ84*(Ts) cells, but that λ *envA*, carrying a 3.6-kb *Hin*dIII restriction fragment, provided a very low level of *ftsZ*$^+$ expression, which was sufficient to give only partial complementation of the *ftsZ84*(Ts) mutation. Because the *Hin*dIII restriction site was known to lie within the *ftsA* gene (6, 8), this provided

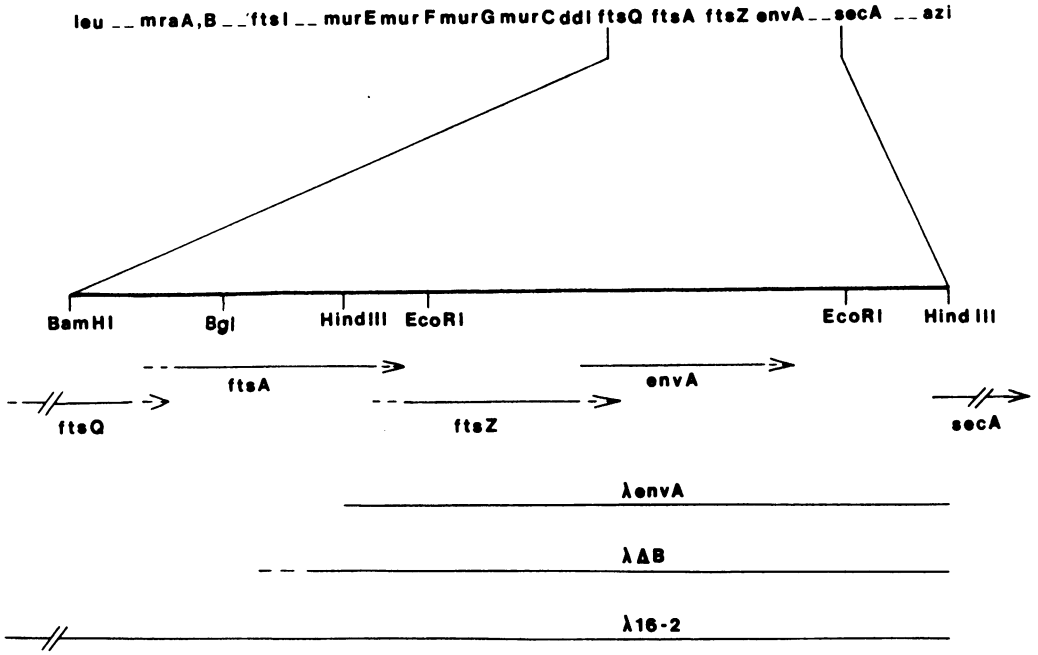

FIG. 1. The gene order of the 2-min cluster. The expanded region shows a 5.2-kb DNA fragment which contains the complete structural genes of *ftsA*, *ftsZ*, and *envA*. The locations and direction of transcription of *ftsA* and *ftsZ* have been described previously (7, 8). Contrary to published evidence (8), the *envA* gene has been shown to transcribe clockwise on the *E. coli* map (N. F. Sullivan and W. D. Donachie, manuscript in preparation), as does the *ftsQ* gene (N. F. Sullivan, D. J. Kenan, and G. F. Hatfull, unpublished observations). The lambda-transducing phages are as described (7).

Figure 1 shows the order of genes within the cell envelope gene cluster located at 2 min on the genetic map of *E. coli* (1). A more detailed map of the 5.2-kilobase (kb) section containing the cell division genes *ftsA* and *ftsZ* and their preliminary evidence that there is a sequence lying within *ftsA* which is necessary for full levels of transcription of the *ftsZ* gene.

To provide direct evidence for this interaction, both the promoter of *ftsZ* and the sequence responsible for the enhancement were cloned upstream of the galactokinase

* Corresponding author.

gene (9) (Fig. 2). It should be noted here that the pKO cloning vectors themselves show a low level of transcription of *galK* (Fig. 3). This is probably due to read-through from the P_4 promoter of pBR322, which normally functions to produce RNA-1, a molecule involved in control of replication (10). In the pKO vectors the extent of this read-through is reduced by a transcription terminator (from the λ *o* gene) inserted between P_4 and *galK*. In the case of pKO6 (Fig. 2), this terminator lies between the *Bam*HI and *Hin*dIII sites (M. Rosenberg, personal communication). This terminator remains in place in pNS30 but is deleted during the construction of pNS28, pNS29, and pNS54 (see Fig. 2). Consequently, the extent of galactokinase production in cells harboring these plasmids may include a contribution from P_4 which is greater than that in either pK06 or pNS30. To measure this effect, a control plasmid was constructed in which the λ*o* terminator fragment was deleted. (To do this, the pKO1 vector was restricted with *Eco*RI, and the ends were filled in with Klenow. Subsequent *Sma*I digestion and blunt-end ligation gave pKOC1.) pKOC1 does indeed have a slightly higher level of *galK* expression than pKO1 (Fig. 3). To estimate the relative strengths of promoters in cloned chromosomal fragments, therefore, the values obtained from either pKO1 (which is identical in this respect to pKO6) or pKOC1 strains were subtracted as appropriate (Fig. 3).

The plasmid pNS30 shows promoter activity associated with the *Hin*dIII-*Eco*RI fragment (Fig. 3). This is attributable to a promoter (PZ_1) and is probably responsible for the low level of *ftsZ* expression from λ *envA*. If a contiguous upstream DNA fragment is added, as in pNS29, then the

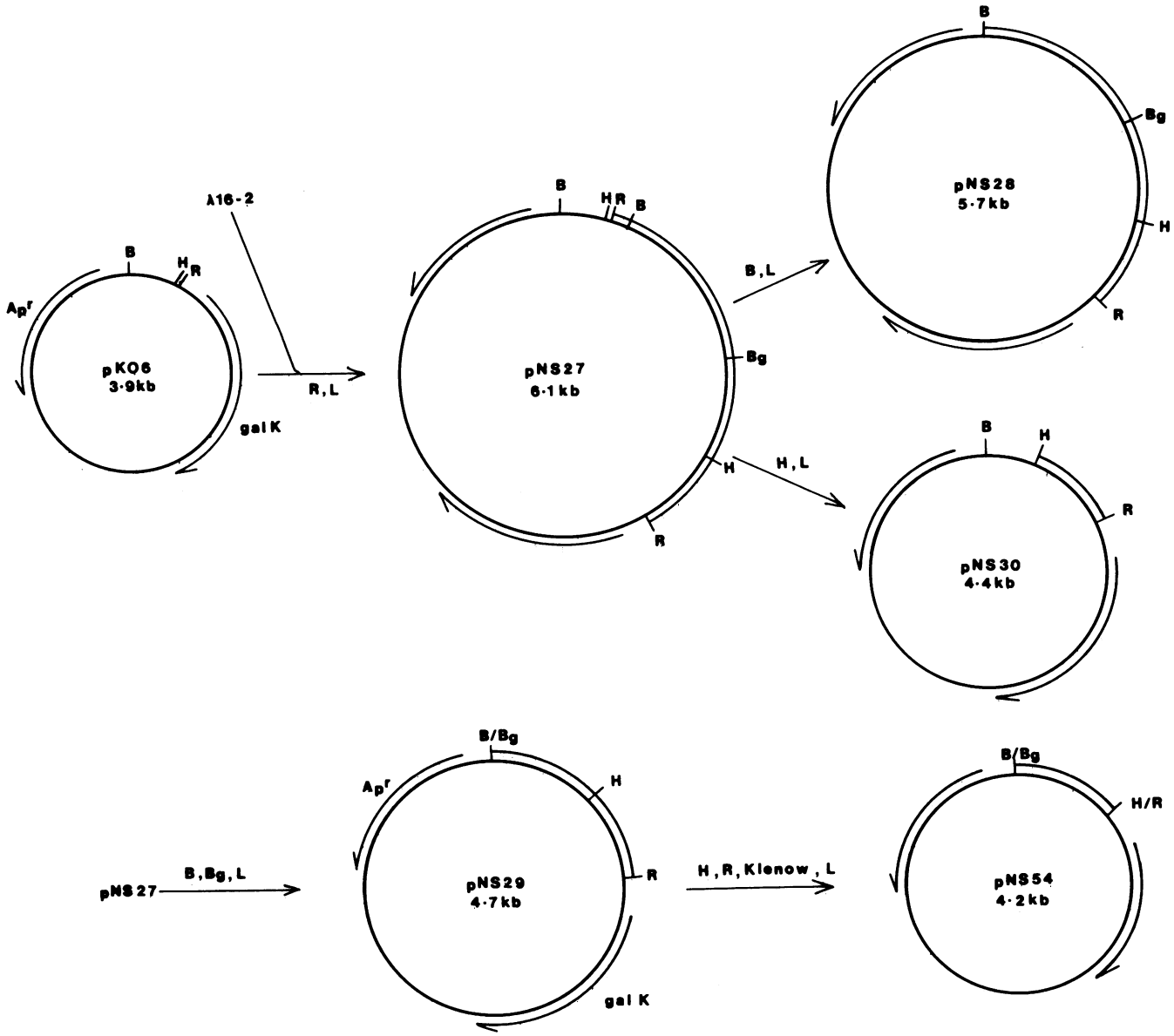

FIG. 2. Construction of the plasmids that fuse chromosomal fragments to the *galK* structural gene. Plasmid pNS27 has the 2.3-kb *Eco*RI *ftsA*-containing fragment of λ16-2 (6) inserted into pK06 (obtained from M. Rosenberg). Plasmids pNS28, pNS29, and pNS30 were constructed from pNS27 by *Bam*HI, *Bam*HI-*Bgl*II, and *Hin*dIII deletions, respectively. pNS54 was constructed by restricting pNS29 with *Hin*dIII and *Eco*RI, filling in the 5′ extensions with the Klenow fragment of DNA polymerase I and religating. Abbreviations used for restriction enzymes and nucleases and their target sites are: B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; L, T4 DNA ligase; R, *Eco*RI.

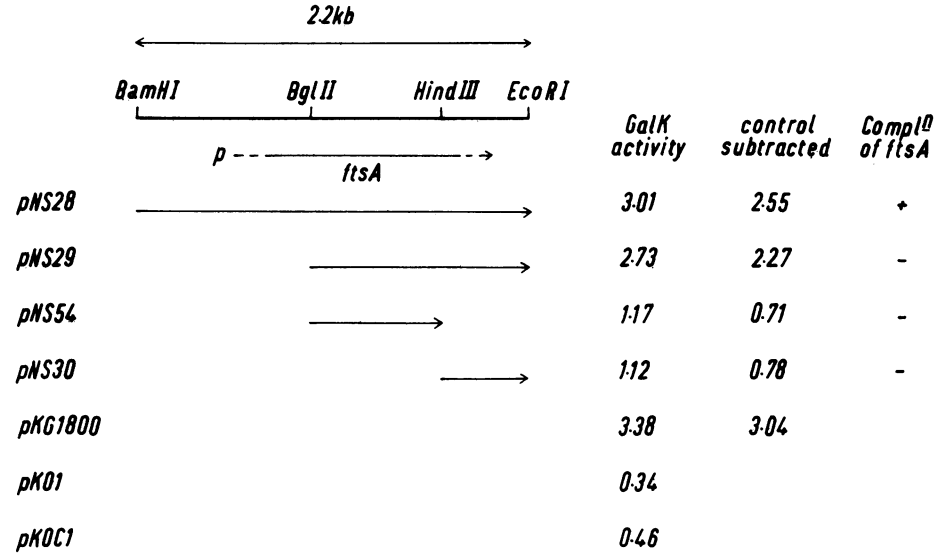

FIG. 3. Promoter strengths and locations within the 2.2-kb *Bam*HI-*Eco*RI fragment. A partial restriction map of the fragment is shown, together with the extent of cloned fragments and their orientation relative to the galactokinase gene. The plasmid pKG1800 was used to determine the activity of the *gal* promoter and is given for comparison (13). Plasmids were classified as able to complement (Compl[n]) *ftsA* if they allowed colony formation by TOE13, an *ftsA13*(Ts) derivative of AB2497 (K. J. Begg, unpublished results), at 42°C on nutrient agar plates containing 50 μg of ampicillin per ml. Galactokinase activities were determined as described by McKenney et al. (9) and were initially expressed as nanomoles of galactose phosphorylated per minute at an optical density of 650 nm. For each strain a minimum of four separate extracts were prepared and assayed twice, and the results were normalized. The observed variation among replicates was less than 10% in all cases. The *E. coli* host strain used was NFS6, a *galK recA* Tc[r] *gyrA* derivative of strain C600 (N. F. Sullivan, unpublished results). β-Lactamase assays were also carried out as a measure of plasmid copy number. No significant differences between strains were found. To estimate the relative strengths of the cloned promoters, the galactokinase activities of strains carrying appropriate vector plasmids (pKO1 for pNS30 and pKG1800; pKOC1 for pNS28, pNS29, and pNS54) were subtracted as shown.

promoter activity increases 2.9-fold. This plasmid contains only part of *ftsA*, since it cannot complement the *ftsA* mutation in our *ftsA13*(Ts) strain. If further upstream DNA is added, as in pNS28, this time to include the entire *ftsA* structural gene and promoter, then there is a 12% increase in promoter activity over that of pNS29, which is probably attributable to the weak *ftsA* promoter. Since pNS28 begins within the *ftsQ* structural gene (N. F. Sullivan, unpublished results), there can be no contribution from the *ftsQ* promoter. The plasmids pNS30, pNS29, and pNS28 all have the same downstream fusion junction, so any translation from *ftsZ* entering the *galK* leader region and hence into the *galK* gene will be in the same frame, making it unlikely that the observed enhancement of *galK* activity results from a differential translation efficiency (11).

When cloned separately, as in pNS54, the DNA responsible for the enhancement of *ftsZ* transcription itself shows promoter activity equivalent to that of PZ_1, making it likely that the sequence responsible for the enhancement of *ftsZ* expression is a second promoter (PZ_2) located within the coding sequence of *ftsA*. These measurements are indicative of relatively weak *E. coli* promoters (9, 13), but they are strong enough to be unlikely to result from a promoter split by *Hin*dIII restriction. In vitro studies of transcriptional start points (N. F. Sullivan, unpublished observations) and nucleotide sequencing of the region (12) have confirmed the existence of promoter sequences in the predicted positions and also that the *Hin*dIII site does indeed lie within the *ftsA* open reading frame.

The level of transcription from pNS29 (2.27) is greater than the sum of the separate activities of PZ_1 and PZ_2 (1.49), and thus there appears to be cooperative enhancement of transcription initiation betweeen these two promoters. The mechanism of enhancement is not known, but it is interesting to note that a probable pause site (3) in the PZ_2-initiated mRNA overlaps the −10 region of PZ_1 (12). Transient pausing of the RNA polymerase at this site might induce a conformational change in the DNA (4), thus allowing extra RNA polymerase molecules to bind at the PZ_1 promoter.

Thus, the *ftsZ* gene has two promoters, at least one of which lies within the coding region of the neighboring *ftsA* gene. The upstream promoter acts to cooperatively enhance the transcription of *ftsZ* to a level which can support normal septation. How this unusual overlap affects the expression of the *ftsA* or *ftsZ* genes in vivo remains to be determined.

Normal expression of the *ftsZ* gene requires the presence of a region of the neighboring *ftsA* coding sequence but requires neither the transcription of that *ftsA* sequence nor even the presence of a complete coding sequence. Expression of *ftsZ* depends upon neighboring genes in a novel way which may reflect the evolution of transcriptional regulation within a gene-dense region rather than a dependence of gene expression on transcription of neighboring genes as is found, for example, in classical bacterial operons.

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