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DNA Sequence and Transcriptional Organization of Essential Cell Division Genes *ftsQ* and *ftsA* of *Escherichia coli*: Evidence for Overlapping Transcriptional Units

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The DNA sequence of a cloned segment of the *Escherichia coli* chromosome containing *ftsQ*, *ftsA*, and part of the *ftsZ* gene was determined and interpreted for genetic complementation and promoter fusion data for the region. The contiguous genes *ftsQ*, *ftsA*, and *ftsZ* were transcribed in the same direction (clockwise on the genetic map) and each had at least one associated promoter which allowed it to be transcribed independently of neighboring genes. *ftsA* and *ftsZ* possessed promoters within the coding sequences of the juxtaposed upstream structural genes, and a promoter element for *ftsA* was surrounded by a region of twofold symmetry which corresponded closely to a symmetrical element in the region of a putative *ftsZ* promoter. The structural gene of *ftsQ* consisted of 838 nucleotides, encoding a 276-residue amino acid polypeptide of molecular weight 31,400; the structural gene of *ftsA* consisted of 1,260 nucleotides, encoding a 420-residue amino acid polypeptide of molecular weight 45,400. The observation that the termination codon of *ftsQ* overlaps with a potential initiation codon for *ftsA* suggested that these two genes may be translationally coupled when transcription is initiated upstream of the *ftsQ* coding sequence.

Little is known about the molecular events which constitute or control cell division in *Escherichia coli*. A genetic analysis of division mutants has resulted in the identification of genes specifically involved in the division process, and these studies have been aided, in recent years, by the introduction of in vitro recombination methodologies and the ability to transform cells with novel genetic constructions.

Many genes that are involved in the morphogenesis and function of the *E. coli* cell envelope (13) are located in two clusters: one at 2 minutes and one at 14 minutes on the genetic map (1). The arrangement of genes in the 2-minute region is as follows: (*mraA mraB*) *pbpB murE murF murG murC ddl ftsQ ftsA ftsZ envA secA azi*. A number of temperature-sensitive, filament-forming mutants (designated *fts*) have been isolated, and the mutations in many instances were found to map within this cluster (K. J. Begg, unpublished data). Such *fts* cells continue to grow but are unable to divide at the restrictive temperature. The essential cell division genes which are considered in this communication are *ftsQ*, *ftsA*, and *ftsZ* (4, 25). Temperature-sensitive mutants of *ftsQ*, *ftsA*, and *ftsZ* form multinucleated filaments at restrictive temperatures, but there are differences in their phenotypes. Mutations in *ftsA* result in filaments with indentations along their length (presumably partially completed septa), whereas mutations in *ftsQ* and *ftsZ* result in the formation of filamentous cells with no such constrictions.

Earlier work in this laboratory identified the product of the *ftsA* gene as a protein with a molecular weight of 50,000 as measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (24). It was demonstrated that if cell division is to occur, synthesis of the *ftsA* protein is required during the 10- to 15-min period before the cells divide (12). It is not known whether the synthesis of *ftsA* protein occurs throughout the cell cycle or only during the period immediately before division. There is some evidence (K. J. Begg, personal communication) that synthesis of the *ftsQ* protein also may be required during this critical period, which prompts speculation as to whether the expression of these two genes may be coordinately regulated.

Lutkenhaus (23) has recently shown that *sulB* is an allele of *ftsZ* and that the product of the *ftsZ* gene (which is required for an essential early step in division) is itself a target for an inhibitor of cell division. This inhibitor is believed to be the product of the *sulA*(*sfiA*) gene (18) and is produced as part of the SOS response (22) in cells which have suffered damage to their DNA. Lutkenhaus and Wu (26) reported that an element required for full *ftsZ* expression appears to be located within the *ftsA* structural gene, and Sullivan and Donachie (46) have shown that the *ftsZ* gene is preceded by at least two promoters. At least one of these was reported to lie within the coding sequence of *ftsA*. The DNA sequence reported in the present work permits us to identify putative promoters for *ftsQ*, *ftsA*, and *ftsZ* and to confirm that the element required for full *ftsZ* expression lies within the *ftsA* structural gene. We also conclude that a promoter or promoters for *ftsA* must lie within the structural gene of *ftsQ*.

MATERIALS AND METHODS

Bacterial strains. *E. coli* strains ED8654 (8) and NFS6 (46) were used as hosts for plasmid constructions. *E. coli* JM101 (28) was employed for growth of M13 phage and its recombinants. Unless otherwise stated, cultures were routinely grown in L broth, which consists of Difco tryptone (10 g/liter, Difco yeast extract (5 g/liter), and NaCl (5 g/liter).

Enzymes and biochemicals. Restriction enzymes, with the exception of *Bam*HI and *Eco*RI, were purchased from Bethesda Research Laboratories. Deoxyribonucleotide and

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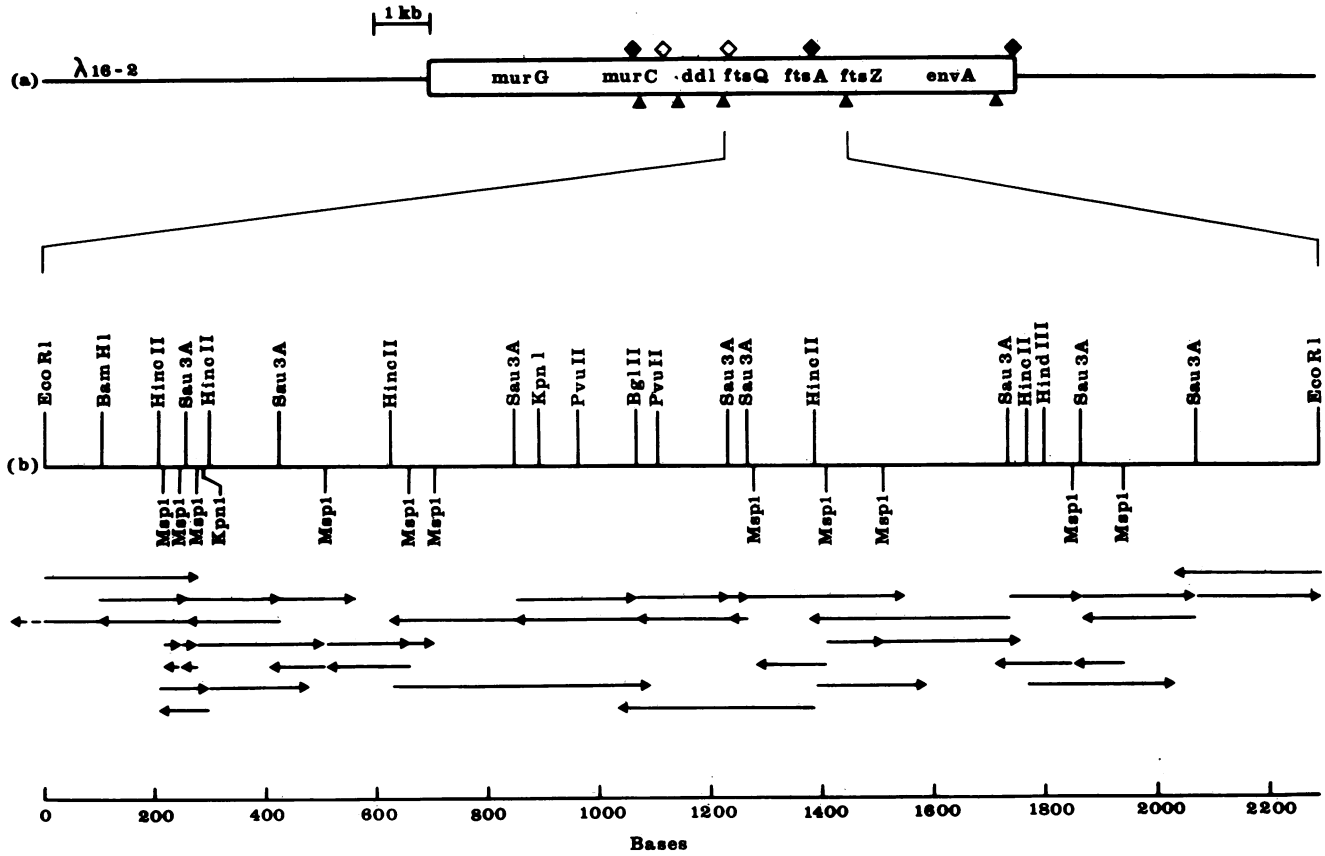

FIG. 1. Restriction enzyme cleavage sites and strategy for determining the sequence of the *ftsQ ftsA* region. (a) Genetic map of λ16-2 (25) showing cleavage sites for *Eco*RI (▲), *Hin*dIII (◆), and *Bam*HI (◇). (b) Restriction map of the 2.3-kb *Eco*RI fragment from the *ftsQ ftsA* region cloned into pBR325 (7). Restriction enzyme sites were determined as described in the text and verified by the established nucleotide sequence. The arrows beneath the map show the direction of sequencing and the length of the sequence determined. The broken arrow extending leftwards from the left-hand *Eco*RI site indicates sequence that was determined from an adjacent, overlapping *Bam*HI fragment. The numbering of bases corresponds to that described in the legend to Fig. 4.

dideoxyribonucleotide triphosphates, DNA polymerase I (Klenow enzyme and Kornberg polymerase), calf intestinal phosphatase, polynucleotide kinase, and restriction endonucleases *Bam*HI and *Eco*RI were supplied by Boehringer Corp., Ltd. T4 DNA ligase was obtained from New England Biolabs. The [α-^{32}P]dCTP, [α-^{32}P]dTTP, [α-^{35}S]dATP (specific activities, >400 Ci/mmol), and [γ-^{32}P]ATP (specific activity, >5,000 Ci/mmol) were purchased from Amersham International.

DNA isolation and ligation. Covalently closed circular plasmid DNA was isolated from cleared lysates (21) and subsequently purified by two consecutive centrifugations in ethidium bromide-cesium chloride density gradients (9). Specific DNA restriction fragments were isolated by separation on 1% agarose gels in Tris-acetate buffer (5 mM sodium acetate, 1 mM EDTA, 40 mM Tris [pH 7.5], followed by electroelution into a solution of 5 mM Tris–2.5 mM sodium acetate. After dialysis against a low-salt buffer (0.2 M NaCl, 1 mM EDTA, 20 mM Tris-hydrochloride [pH 7.4]), the DNA was concentrated by binding to an Elutip-d column (Schleicher and Schuell), eluted in high-salt buffer (1.0 M NaCl, 1 mM EDTA, 20 mM Tris-hydrochloride [pH 7.4]), and ethanol precipitated. Ligations were carried out in buffer conditions recommended by the manufacturer at 14°C overnight for fragments with protruding 5′ or 3′ ends and at 20°C overnight for fragments with blunt ends. Transformations were performed by conventional calcium heat shock procedure.

Construction of plasmids carrying cloned chromosomal fragments. A 2.3-kilobase (kb)-pair *Eco*RI restriction fragment derived from phage λ16-2 (25) was cloned into vector pBR325 (7). This plasmid was designated pGH4. This same 2.3-kb fragment was inserted into the *Eco*RI cloning site of the pBR322 derivative pKO-6 (27) to give pNS27. pNS27 has the fragment oriented such that transcription initiated within it would also transcribe the *galK* gene of the pKO-6 vector (see Fig. 2). pNS27 was in turn used to construct pNS28 (by deletion of a *Bam*HI fragment), pNS29 (by deletion of a *Bam*HI-*Bgl*II fragment from pNS27), and pNS30 (by deletion of a *Hin*dIII fragment from pNS27). pNS54 was obtained by deleting a *Hin*dIII-*Eco*RI fragment from pNS29. Both pGH300 and pDK302 carry the same 2.3-kb *Eco*RI fragment as does pNS27, but in these cases inserted into the *Eco*RI cloning site of pKO-1 (27). pDK302 carries this chromosomal insert in the same orientation as pNS27 does, but pGH300 carries it in the reverse orientation (see Fig. 2). pGH300 was used to construct pNS45 (by deletion of a *Hin*dIII fragment). pDK302 was used to produce pNS36 and pNS37 (by *Pvu*II and *Sma*I digestion) and pDK340 (by *Hin*dIII digestion). pGH301, which contains the same chromosomal insert as pDK302 does, was used to construct pGH305 (by *Bam*HI and *Bgl*II digestion), pGH360 (by *Kpn*I

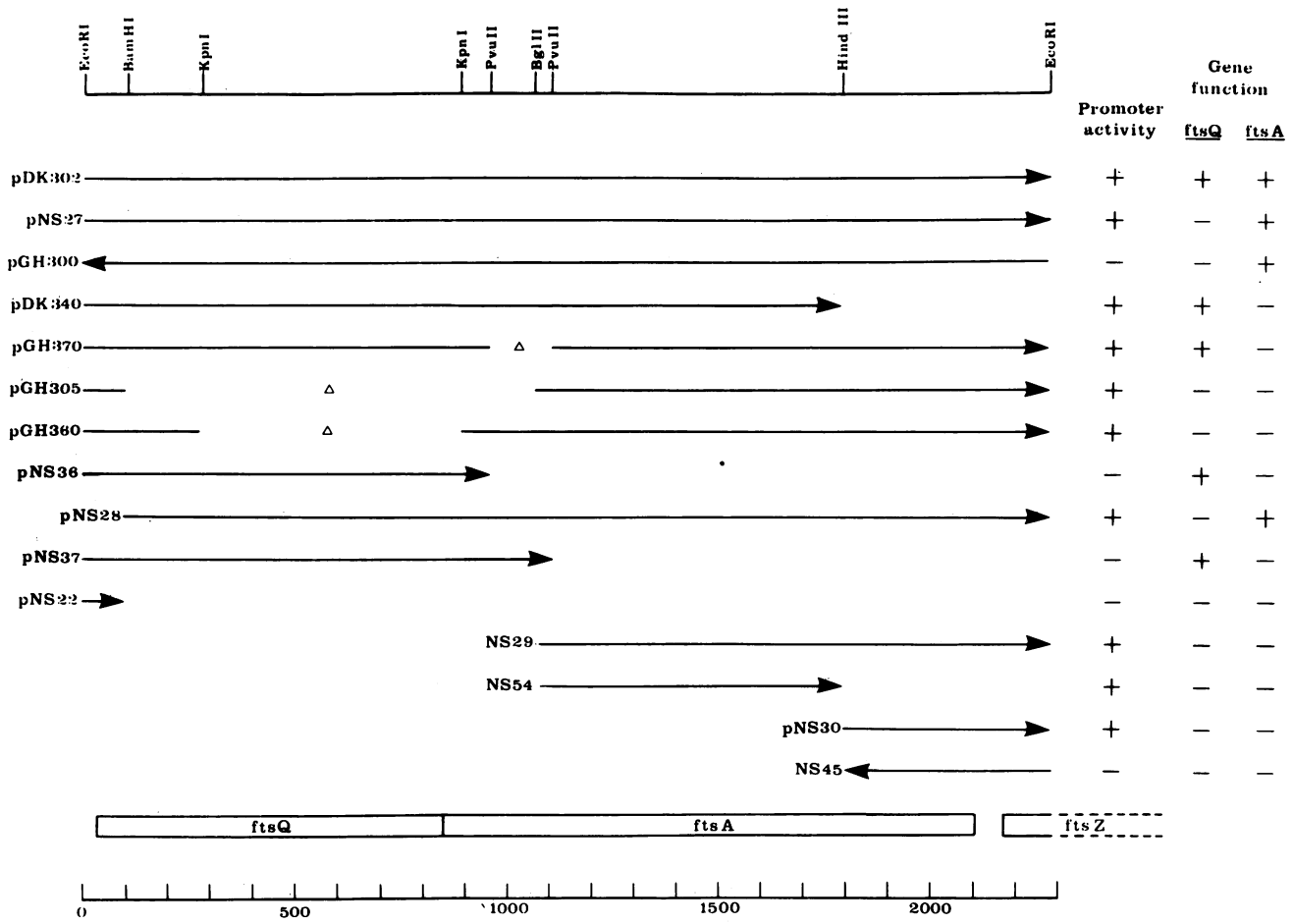

FIG. 2. Location of coding sequences and promoters within the *ftsQ ftsA ftsZ* region. Top, locations of restriction sites used for plasmid constructions. Horizontal lines beneath the map denote fragments cloned into pKO vectors upstream of *galK*. At one end of each insert is an arrowhead which shows the orientation of the fragment relative to the *galK* coding sequence in the complete plasmid (see the text for construction details). The columns to the right of the figure show the results of the tests for promoter activity (column 1) and gene function (columns 2 and 3). Column 1, *galK*$^-$ cells carrying the plasmid which form red (+) or white (−) colonies on galactose indicator plates; columns 2 and 3, positive or negative plasmid complementation of temperature-sensitive mutants of the gene. Normal gene function is defined as both the ability to form colonies on plates at the restrictive temperature and to allow normal growth and morphology of the cells at this temperature. Bottom, locations of the coding sequences of the genes as determined from this data and the nucleotide sequence of the region (see the legend to Fig. 4).

digestion), and pGH370 (by *Pvu*II digestion). pNS20 (data not shown) again carries the same 2.3-kb *Eco*RI fragment as pNS27 does, but cloned into the *Eco*RI site of pKO-4 (27). pNS20 was used to construct pNS22 (by *Bam*HI digestion).

Restriction mapping and DNA sequencing. The restriction enzyme map of pGH4 was determined by the use of partial restriction enzyme digests of singly 5′ ^{32}P-labeled DNA fragments (40). Restriction fragments from the 2.3-kb *Eco*RI fragment and an overlapping upstream 1.2-kb *Bam*HI fragment, derived from phage λ16-2, were subcloned into phage vectors M13mp7, M13mp8, and M13mp9 (28, 29). Radioactive probes were prepared by DNA nick translation in the presence of [α-^{32}P]dCTP by the method of Rigby et al. (34), and phage plaques were screened after blotting onto nitrocellulose filters (5). The recombinants were sequenced by the dideoxy chain termination method of Sanger et al. (36, 37). Initially, all sequencing reactions were carried out with ^{32}P-labeled deoxynucleotide triphosphates. Subsequently, [α-^{35}S]dATP was employed for all reactions (6). Reverse sequencing was carried out by the method of Hong (16). Computer analysis of DNA sequence data employed programs developed by Staden (41–43) and Devereux et al. (11).

Genetic tests for gene expression. Plasmids to be tested were introduced by transformation into either TOE-1 *ftsQ*(Ts) (4) or TOE-13 *ftsA13*(Ts) (K. J. Begg, unpublished data). Transformants were selected for ampicillin resistance at 30°C (27). Individual colonies were streaked for purification and then checked for the presence of plasmid DNA of the expected size and restriction pattern. Single colonies were then either inoculated into Oxoid nutrient broth and grown to an optical density at 540 nm of 0.2 at 30°C before shifting to 42°C or patched onto solid medium and incubated at either 30 or 42°C. Expression of cloned wild-type alleles was judged by the growth of cells of normal rod shape after 2 to 3 generations at 42°C in liquid medium and by the formation of colonies on solid medium after overnight growth at 42°C. In the absence of wild-type gene expression from the cloned DNA, the *fts* mutant strains at 42°C formed populations of long filamentous cells (4, 12). None of the mutant strains formed colonies on plates at 42°C.

Tests for the presence of promoters on cloned DNA fragments. Assay for the presence of transcriptional promoters

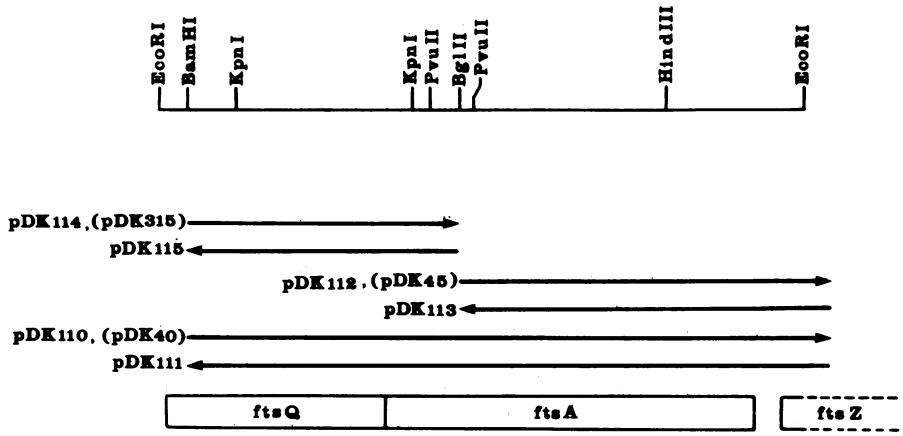

FIG. 3. Chromosomal segments cloned between P*gal* and *galK* to test for the presence of transcriptional terminators. Top, restriction sites in the chromosomal DNA as described in the legend to Fig. 2; bottom, location of coding sequences. The horizontal lines beneath the map show the extent of cloned inserts, and the arrowheads show their orientation relative to the *galK* coding sequence. The plasmid designations are shown on the left; those designations shown in parentheses refer to the same chromosomal segment cloned into pKO-4. The short rightward extension beyond the right-hand *Eco*RI site is the ca. 100-base-pair *Eco*RI-*Bam*HI chromosomal segment covering the start of *ftsQ*. See the text for details of these constructions.

on chromosomal fragments cloned into pKO vectors was carried out as described in the procedures of McKenny et al. (27). The vectors constructed by these authors and employed in the present work (pKO-1, pKO-4, and pKO-6) each contain three unique restriction sites for cloning. These sites vary in different pKO plasmids, but they are located at the same relative positions: *Eco*RI, *Hin*dIII, and *Sma*I in pKO-1; *Eco*RI, *Hin*dIII, and *Bam*HI in pKO-4; *Bam*HI, *Hin*dIII, and *Eco*RI in pKO-6. The cloning sites are located in the plasmid such that transcription originating within DNA which has been cloned in the correct orientation in one of these locations will also transcribe a *galK* coding sequence in the plasmid. To prevent possible translational coupling between transcripts originating in cloned DNA and *galK*, translational stop codons have been inserted upstream of *galK* in all three reading frames, and polar effects on the translation of *galK* have been minimized by the inclusion of a 150-base-pair leader sequence which contains a ribosome binding site (38, 45). The presence of a correctly oriented promoter in a cloned segment gives rise to the Gal^+ phenotype (red colonies on McConkey agar-galactose indicator plates) after transformation of *E. coli* C600 $galK^-$ (46), whereas Gal^- colonies are white. Galactokinase activities were also measured directly for all constructs by the methods of McKenney et al. (27). Those constructs which gave rise to red colonies were found to have consistently higher galactokinase activities compared with controls (data not shown).

Tests for the presence of terminators on cloned DNA fragments. Plasmid pHR9 (30) is a derivative of pKO-4 which carries the *Eco*RI-(*gal* promoter)-*Hin*dIII fragment from the terminator-monitoring plasmid pKG1800 (27). Expression of *galK* in pHR9 is therefore dependent on transcription initiated at the *gal* promoter (P*gal*). Insertion of fragments bearing transcriptional terminators between this promoter and the *galK* gene results in a reduction of *galK* expression and permits a quantitative estimate to be made of the extent of transcriptional readthrough. A search for the presence of transcriptional terminators within the *ftsQ ftsA ftsZ* region was made by performing the following constructions (see Fig. 3). The 2.3-kb *Eco*RI fragment was first purified, ligated to form a circle, and then cut with *Bam*HI. The resulting fragment with *Bam*HI cohesive termini was inserted into pHR9 at the *Bam* site between P*gal* and *galK*. Two clones (pDK110 and pDK111) were selected with this insert in the two possible orientations. pDK111 was digested with *Bam*HI and *Bgl*II and religated to give pDK112, pDK113, pDK114, and pDK115. A complication in these constructions is that fragments from the *ftsQ-ftsZ* region themselves carry promoters which are capable of expressing *galK* (see Fig. 2). An estimate of the extent of readthrough from P*gal* therefore requires a quantitative measurement of galactokinase activities in pairs of plasmids containing the same chromosomal fragments, in the same position relative to *galK*, but with only one of the pair possessing an upstream P*gal*. This was achieved by cloning the equivalent restriction fragment into pKO-4 (from which pHR9 is derived). The pairs of plasmids constructed are as follows (the pHR9 derivative is given first, and the pKO-4 derivative is given second): pDK110 and pDK40, pDK114 and pDK315, and pDK112 and pDK45. Galactokinase activities were measured in *E. coli* C600 $galK^-$ cells (46) and expressed as a percentage of the activity determined for pHR9-carrying cells. The relative amount of transcriptional readthrough in each cloned fragment was calculated as the difference between the galactokinase activities found in cells carrying the fragment in the same orientation in pHR9 and pKO-4 vectors.

RESULTS

Restriction mapping, genetic complementation tests, and identification of DNA fragments bearing transcriptional promoters. A physical map of the 2.3-kb *Eco*RI fragment was constructed for the enzymes *Bam*HI, *Bgl*II, *Hin*cII, *Hin*dIII, *Kpn*I, *Msp*I, *Pvu*II, and *Sau*3AI (Fig. 1). Figure 2 shows the locations and extents of the sequences subcloned from the 2.3-kb fragment into pKO vector plasmids. The ability of specific restriction fragments to complement mutations at a chromosomal locus was tested by introducing each plasmid into host cells carrying a temperature-sensitive allele of the appropriate gene on the chromosome and by determining whether these cells were normal sized and able to form colonies at the restrictive temperature of 42°C. Mutant cells

carrying a particular plasmid showed either the typical mutant phenotype at 42°C or apparently normal cell growth and division (Fig. 2).

The coding sequence for *ftsQ* can be assigned to lie within the *Eco*RI-*Pvu*II segment carried by pNS36 (position 0 to position 970), but its expression depends upon the orientation of the cloned segment in the plasmid (compare pDK302 with pGH300). *ftsQ*(Ts) cells carrying pDK302 exhibited a wild-type phenotype at 42°C, and such cells carrying pGH300 exhibited the typical *ftsQ*(Ts) mutant phenotype. These results suggested that *ftsQ* was probably being transcribed (from left to right [Fig. 2]) by readthrough transcription from the P4 promoter of the pKO vector (27). To investigate this possibility, the plasmid pNS27 was constructed in which the 2.3-kb fragment was cloned into the *Eco*RI site of pKO-6 rather than the *Eco*RI site of pKO-1, as in pDK302 and pGH300. The difference between the two kinds of constructions lies in the location of a weak terminator sequence (27) relative to the cloned *Eco*RI fragment. In pDK302 and pGH300, this terminator lies between the cloned segment and *galK* and therefore does not affect readthrough transcription from the plasmid P4 promoter into the cloned segment. In pNS27, this terminator lies upstream of the cloned insert, i.e., between the *ftsQ* coding sequence and the P4 promoter. pNS27 does not show expression of *ftsQ*, thereby showing that the *ftsQ* sequence on the 2.3-kb fragment was being expressed from this plasmid promoter.

The maximum size of the *ftsA* functional unit defined by deletion derivatives was shown to be the 2.2-kb *Bam*HI-*Eco*RI sequence carried by pNS28, and the minimum size must be greater than 900 base pairs (including the sequence between the *Kpn*I site at position 900 and the *Hin*dIII site at position 1800). Since neither the orientation of the 2.3-kb fragment, as in pDK302 and pGH300, nor the presence of an upstream terminator, as in pNS27, has any effect on *ftsA* expression, we concluded that this segment includes a promoter capable of expressing *ftsA*. This finding is consistent with the ability of phage λJFL41 (26), which carries the 2.3-kb *Eco*RI fragment, to complement mutations in *ftsA*.

The plasmids shown in Fig. 2 were also used to locate promoter sites and to determine the directions of transcription originating at these sites. The genetic evidence discussed above clearly demonstrates the existence of a promoter capable of expressing *ftsA* which must lie within the 2.2-kb *Bam*HI-*Eco*RI fragment (pNS28). In addition, the reported molecular weight of the *ftsA* protein of 50,000 (24) requires that promoter activity lie either within the 800-base-pair *Bam*HI-*Kpn*I fragment (position 100 to position 900) and transcribe from left to right or within the 500-base-pair *Hin*dIII-*Eco*RI fragment (position 1800 to position 2300) and transcribe from right to left. The analysis of the nucleotide sequence reported below shows that *ftsA* is transcribed from left to right; hence, the promoter which is responsible for *ftsA* expression should be carried by pNS36. A very low level of transcription attributable to this region has been reported previously (46). Although this level is not sufficient to give Gal^+ phenotype on indicator plates, it is evidently sufficient to complement an *ftsA* mutation (Fig. 2). The fact that we were unable to detect this clearly by *galK* expression may be attributed to the limited sensitivity of that method of detection.

The identification of promoter activity upstream of the *ftsZ* coding sequence (pNS29, pNS30, and pNS54) has been reported elsewhere (46), but these results are included in Fig. 2, for completeness. Promoter activity is associated with the 720-base-pair *Bgl*II-*Hin*dIII fragment (pNS54) and the 500-base-pair *Hin*dIII-*Eco*RI fragment (pNS30). Transcription is from left to right in both cases (Fig. 2).

TABLE 1. Transcription from P*gal* through cloned segments of the *ftsQ* to *ftsZ* region[a]

Plasmid	Vector	Activity (%) of:	
		Galactokinase (relative to pHR9)	Readthrough transcription
pKO1		7	
pHR9		100	100
pDK315	pKO4	3	48
pDK114	pHR9	51	
pDK115	pHR9	0	0
pDK45	pKO4	32	
pDK112	pHR9	59	27
pDK113	pHR9	0	0
pDK40	pKO4	35	ND
pDK110	pHR9	ND	ND
pDK111	pHR9	0	0

[a] Restriction fragments (Fig. 3) were cloned into pKO-4 in the correct orientation to transcribe the *galK* gene in the plasmid, and the same fragments were also inserted, in both orientations, into the *Bam*HI site of pHR9. See the text and legend to Fig. 3 for details of constructions and for calculation of percentage of readthrough transcription. ND, Not determined.

Transcriptional termination. A search was carried out for the presence of transcriptional terminators by cloning restriction fragments into the *Bam*HI site of pHR9 and pKO-4 (Fig. 3). The results (Table 1) show that the extent of readthrough transcription from the *Bam*HI site to the *Bgl*II site (clones pDK114) and pDK315) was 48%, and from the *Bgl*II site to the *Bam*HI site (clones pDK112 and pDK45) was 27%, when the fragments were oriented such that any internal promoters would initiate transcription towards *galK*. When these same fragments or the entire 2.3-kb fragment were inserted in the opposite orientation, as in pDK115, pDK113, and pDK111, no expression of *galK* was detectable. No role for transcriptional termination in the anti-clockwise sense was evident, since all downstream promoters (in the *ftsZ envA secA* region) have been found to initiate transcription in the clockwise sense (31; Sullivan and Donachie, submitted for publication).

It was impossible to test for the extent of transcriptional readthrough in the clockwise sense for the whole 2.3-kb fragment (covering the *ftsQ-ftsZ'* sequence) because cells carrying this fragment cloned downstream of P*gal* grew very poorly when the chromosomal fragment was oriented such that P*gal* would transcribe the coding sequences within it (as in pDK110; Fig. 3). Since the *ftsQ* coding sequence was interrupted by the rearrangement necessary to insert the 2.3-kb fragment into pHR9, and since neither pDK112 nor pDK114 (which do not carry an intact *ftsA* coding sequence) had deleterious effects on cell growth, the effect with pDK110 might be attributable to overexpression of *ftsA* by P*gal*. In contrast, pDK111 (which carries the same 2.3-kb fragment as pDK110 does, but in the opposite orientation) had no effect on cell growth but was capable of complementing a chromosomal *ftsA*.ts mutation. Opposing transcription initiated at P*gal* does not, therefore, prevent expression of *ftsA* from its own promoter (internal to *ftsQ*), despite the apparently low levels of transcription from this promoter (see above).

DNA nucleotide sequence and assignment of open reading frames. The strategy used for sequencing the 2.3-kb *Eco*RI fragment (and part of the overlapping upstream 1.2-kb *Bam*HI fragment) is shown in Fig. 1. With the exception of

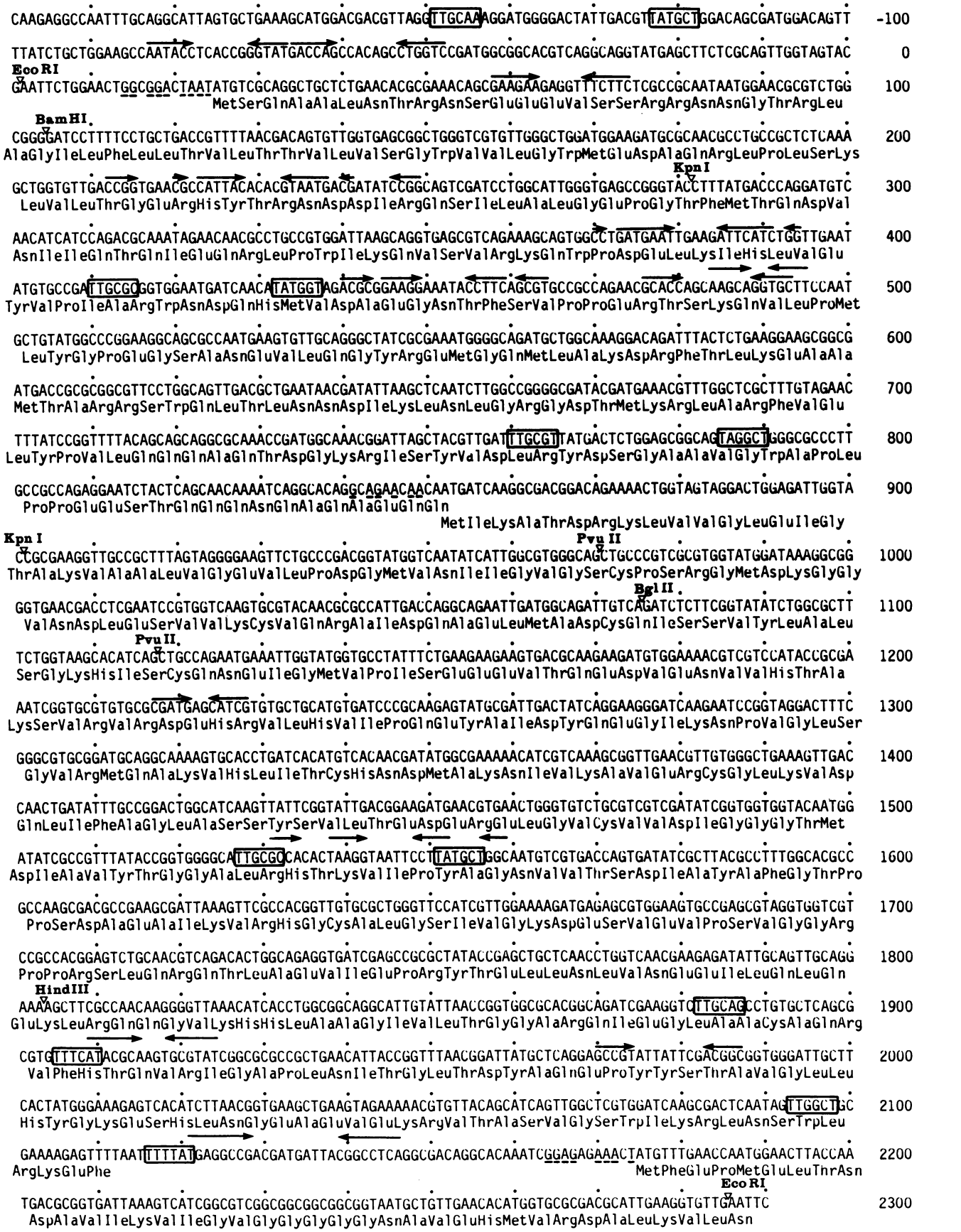

FIG. 4. DNA nucleotide sequence of the noncoding strand of the *ftsQ ftsA ftsZ* region. The amino acid sequences deduced for the products of *ftsQ* (nucleotides 25 to 852), *ftsA* (nucleotides 852 to 2111), and *ftsZ* (nucleotide 2175 onwards) are shown beneath the sequence. Bases are numbered arbitrarily from the left-hand *Eco*RI site of the 2.3-kb *Eco*RI fragment (see Fig. 1). Putative promoter sequences are shown in boxes (see Table 2), and inverted repeat sequences are overlined with arrows. Bases upstream of ATG codons which match the consensus for ribosome binding are underlined. The cutting sites for the restriction enzymes shown in Fig. 2 and 3 are also depicted.

the 200 bases immediately upstream of the left-hand *Eco*RI site, the sequence of the entire region was determined in both orientations from recombinant M13 phage generated by random cloning of restriction fragments and forced cloning of specific restriction fragments where necessary.

The nucleotide sequence of 2,490 base pairs thus determined was numbered arbitrarily from the left-hand *Eco*RI site (Fig. 4). Analysis of this sequence for potential polypeptide coding regions showed that it contains two large open translational reading frames, both of which are in the left-to-right sense (clockwise on the *E. coli* genetic map). The first possible initiation codons for these large open reading frames were at nucleotides 25 to 27 and 852 to 854. The first frame, which from the genetic complementation data must correspond to the structural gene of *ftsQ*, coded for a protein of 276 amino acid residues with a calculated molecular weight of 31,400 and was terminated by the nonsense triplet TGA (nucleotides 853 to 855). The first potential translational initiation codon of the second large open reading frame was found to overlap with the termination codon of the upstream *ftsQ* reading frame. The second frame coded for a 420-residue amino acid protein with a calculated molecular weight of 45,400 and was terminated by the nonsense triplet TAA at nucleotides 2112 to 2114. This must correspond to the structural gene of *ftsA*. The ATG codon at position 25 is immediately preceded by a sequence showing strong homology to a ribosome binding site (38, 45). We concluded that this was the probable start site for *ftsQ* translation since this was the only ATG codon within the proposed *ftsQ* structural gene which showed such homology. There was no close adherence to the consensus of preferred bases for ribosome binding upstream of the ATG codon at position 852, although this was not surprising, given the constraints imposed upon this sequence by its being within the upstream structural gene. An inspection of the sequences immediately preceding the other seven ATG codons within this open reading frame revealed less homology to the consensus than that found upstream of position 852. We concluded that this was the probable start site for *ftsA* translation. The 10% discrepancy between the calculated molecular weight of the *ftsA* product (see Table 4), determined from the deduced amino acid sequence, and the molecular weight determined by gel electrophoresis (24) might be due to anomalous migration (2) of this protein on sodium dodecyl sulfate-polyacrylamide gels.

Ward and Lutkenhaus (47) reported the isolation of an in-frame *lacZ-ftsZ* gene fusion and showed that the *ftsZ* coding sequence begins within the 2.3-kb *Eco*RI fragment. An inspection of the DNA sequence downstream of the termination codon TAA (nucleotides 2112 to 2114) of the proposed *ftsA* structural gene revealed only one open reading frame which extends to the *Eco*RI site. Since the ATG codon at position 2175 was preceded by a sequence showing a strong resemblance to a ribosome binding site, we concluded that this was the probable translational start site for the *ftsZ* structural gene.

Identification of potential transcriptional regulatory sequences. The nucleotide sequence was analyzed for sequences known to be involved in interactions with RNA polymerase and which are well conserved in promoters of *E. coli* genes (15, 35). A total of six possible promoters were identified from consensus considerations and their respective −10 and −35 are shown in Table 2. Their locations and orientation (all in the clockwise sense) were in full agreement with genetic complementation and promoter fusion data, and in all cases the separation of the −35 and −10 regions was within the range of 15 to 21 base pairs observed for other *E. coli* promoters (15).

TABLE 2. Comparison of −35 and −10 regions of putative promoters for *ftsQ*, *ftsA*, and *ftsZ* with consensus sequences for *E. coli* promoter[a]

Promoter	Sequence		Separation (base pairs)
	−35	−10	
ftsQ p_1	TTGCAA	TATGCT^{-118}	20
ftsA p_1	TTGCGT	TAGGCT^{790}	19
ftsA p_2	TTGCGC	TATGGT^{437}	16
ftsZ p_1	TTGGCT	TTTTAT^{2121}	17
ftsZ p_2	TTGCAG	TTTCAT^{1910}	17
ftsZ p_3	TTGCGC	TATGCT^{1556}	18
Consensus	TTGACA	TATRAT	17

[a] The separation of these two regions is also shown, since this is known to influence promoter efficiency (44). The position of the conserved T of the −10 sequence is given as a superscript. R, Purine.

A potential promoter was identified upstream of the presumed start site for translation of *ftsQ* and was designated *ftsQ* P_1. Since the genetic evidence described above demonstrated that *ftsA* was able to be expressed independently of neighboring genes in this cluster, a search was made for promoter-like elements upstream of *ftsA* (i.e., within the *ftsQ* structural gene). Two potential promoters were identified and designated *ftsA*p_1 and *ftsA*p_2. The *ftsA* P_2 promoter sequence was of particular interest since it was surrounded by extensive regions of twofold symmetry and followed by a decanucleotide sequence, TGCCGCCAGA (nucleotides 464 to 473), which was repeated 10 nucleotides downstream from the −10 region of *ftsA*p_1 (nucleotides 800 to 809).

Lutkenhaus et al. (25) and Lutkenhaus and Wu (26) previously located the *ftsZ* gene between *ftsA* and *envA* and inferred its direction of transcription to be in the same sense as that of *ftsA* (i.e., clockwise on the *E. coli* genetic map). They found that expression of the *ftsZ* gene product from a phage that carried only a 3.5-kb *Hin*dIII fragment (λ *envA*$^+$) was weak. This is the region downstream from the *Hin*dIII site (nucleotides 1803 to 1808) within the structural gene of *ftsA*. Furthermore, the addition of bacterial DNA upstream of the *Hin*dIII site was found to restore expression to normal levels, which suggests that some element required for full *ftsZ* expression might be located within the *ftsA* structural gene. Since both the 487-base-pair *Hin*dIII-*Eco*RI fragment (nucleotides 1804 to 2290) and the 727-base-pair *Bgl*II-*Hin*dIII fragment (nucleotides 1076 to 1803) showed promoter activity in the left-to-right sense (46), a search was made for consensus promoter sequences in these fragments. Three putative promoters (designated *ftsZ*p_1, *ftsZ*p_2, and *ftsZ*p_3) were identified, all of which were located within the proposed *ftsA* coding sequence. This designation supercedes that given previously (46). Both *ftsZ*p_1 and *ftsZ*p_2 were associated with twofold symmetry, and an unusual 17-base sequence of alternating purine-pyrimidine residues, which encompassed a region of twofold symmetry, overlapped the −10 sequence of *ftsZ*p_2. A region of twofold symmetry was again found to be coincident with the only promoter-like element, which is upstream of the *Hin*dIII site, within the structural gene of *ftsA*. This putative promoter is designated *ftsZ*p_3. A comparison of the inverted repeat sequences within the regions of *ftsA*p_2 and *ftsZ*p_3 revealed a strong degree of correspondence: of the 13 base pairs that surround the centers of symmetry, 11 were identical. There are no

TABLE 3. Codon usage in *ftsQ* and *ftsA*

Amino acid	Codon	Codon usage in		Amino acid	Codon	Codon usage in	
		ftsQ	*ftsA*			*ftsQ*	*ftsA*
Gly	GGG	2	7	Trp	UGG	7	2
Gly	GGA	3	5	End	UGA	0	0
Gly	GGU	3	18	Cys	UGU	0	5
Gly	GGC	9	10	Cys	UGC	0	4
Glu	GAG	3	11	End	UAG	1	0
Glu	GAA	16	22	End	UAA	0	1
Asp	GAU	9	11	Tyr	UAU	5	11
Asp	GAC	4	7	Tyr	UAC	2	1
Val	GUG	9	19	Leu	UUG	10	6
Val	GUA	3	11	Leu	UUA	2	3
Val	GUU	5	10	Phe	UUU	3	4
Val	GUC	3	10	Phe	UUC	2	0
Ala	GCG	10	12	Ser	UCG	2	7
Ala	GCA	5	10	Ser	UCA	1	3
Ala	GCU	3	6	Ser	UCU	4	3
Ala	GCC	1	7	Ser	UCC	1	2
Arg	AGG	0	0	Arg	CGG	5	3
Arg	AGA	2	1	Arg	CGA	1	2
Ser	AGU	0	4	Arg	CGU	5	9
Ser	AGC	7	5	Arg	CGC	8	7
Lys	AAG	8	8	Gln	CAG	16	11
Lys	AAA	2	13	Gln	CAA	9	8
Asn	AAU	9	5	His	CAU	3	6
Asn	AAC	4	8	His	CAC	0	7
Met	AUG	9	8	Leu	CUG	13	18
Ile	AUA	1	2	Leu	CUA	0	0
Ile	AUU	5	10	Leu	CUU	4	6
Ile	AUC	5	16	Leu	CUC	2	3
Thr	ACG	6	7	Pro	CCG	8	9
Thr	ACA	2	4	Pro	CCA	3	2
Thr	ACU	2	1	Pro	CCU	1	2
Thr	ACC	8	7	Pro	CCC	1	1

recognizable transcriptional terminators (33) in the entire region of the sequence (Fig. 4).

Codon usage and amino acid composition of *ftsQ* and *ftsA* proteins. Codon usage in *ftsQ* and *ftsA* is shown in Table 3. The following codons, corresponding to weakly interacting or minor tRNAs (19; reviewed by Grosjean and Fiers [14]), occurred very infrequently or not at all in both *ftsQ* and *ftsA*: CUA for leucine, AUA for isoleucine, and CGA/AGA/AGG for arginine. In accordance with the general trend for efficiently expressed genes of *E. coli*, the following codons were found to predominate: CUG for leucine, GGPy (Py is pyrimidine) for glycine, AUPy for isoleucine, CCG for proline, GCG for alanine, GAA for glutamine, and CGPy for arginine. Exceptions to the trend were found in *ftsQ* (use of AAG for lysine rather than AAA, and AAU for asparagine rather than AAC). Both *ftsQ* and *ftsA* showed a predominant use of the UAU codon for tyrosine, whereas the trend for highly expressed genes was to favor UAC, and both genes utilized the CGG codon for arginine (which corresponds to a minor tRNA species).

The amino acid composition of the predicted *ftsQ* and *ftsA* proteins is shown in Table 4, together with the calculated average composition of *E. coli* proteins (10). No significant differences are apparent in either case.

DISCUSSION

The results reported here identify the coding sequences for the essential cell division genes *ftsQ* and *ftsA*, together with part of the *ftsZ* gene. All of the DNA sequence shown in Fig. 4, with the exception of the 200 bases immediately upstream of the *Eco*RI site at position 1, was determined in both orientations from overlapping clones to avoid potential errors. Owing to technical difficulties, such as stacking of DNA fragments on gels, it is important to apply several criteria in judging the validity of lengthy nucleotide sequences. If portions of a DNA sequence are translated in an incorrect frame, then regions with many uncommon amino acids are found, even if no nonsense codon is encountered. The open reading frames assigned to *ftsQ*, *ftsA*, and *ftsZ* contain no detectable regions of this sort. Termination codons are found to be distributed uniformly throughout the alternative reading frames (data not shown). Furthermore, an analysis of codon usage in *ftsQ*, *ftsA*, and the N-terminal part of the *ftsZ* gene shows a codon preference associated with efficient gene expression in *E. coli* (14). The amino-terminal sequences of the *ftsQ*, *ftsA*, and *ftsZ* proteins, and hence the positions of translational starts, are unknown. However, an analysis of the genetic data (Fig. 2) and DNA sequence (Fig. 4) permits the identification of structural genes and potential ribosome binding sites associated with translational start codons.

The observation that the termination codon TGA of *ftsQ* overlaps with a potential initiation codon for the *ftsA* gene suggests that these two genes may be translationally coupled. Translational coupling in *E. coli* has previously been

TABLE 4. Amino acid composition of polypeptides encoded by *ftsQ* and *ftsA*

Amino acid	Protein				*E. coli* (% of total)[a]
	ftsQ		*ftsA*		
	No. of residues	% Of total	No. of residues	% Of total	
Alanine	19	6.9	35	8.3	8.6
Arginine	21	7.6	22	5.2	4.9
Aspartic acid	13	4.7	18	4.3	5.5
Asparagine	13	4.7	13	3.1	4.3
Cysteine	0	0.0	9	2.1	2.9
Glutamic acid	19	6.9	33	7.9	6.0
Glutamine	25	9.1	19	4.5	3.9
Glycine	17	6.2	40	9.5	8.4
Histidine	3	1.1	13	3.1	2.0
Isoleucine	11	4.0	28	6.7	4.5
Leucine	31	11.2	36	8.6	7.4
Lysine	10	3.6	21	5.0	6.6
Methionine	9	3.3	8	1.9	1.7
Phenylalanine	5	1.8	4	1.0	3.6
Proline	13	4.7	14	3.3	5.2
Serine	15	5.4	24	5.7	7.0
Threonine	18	6.5	19	4.5	6.1
Tryptophan	7	2.5	2	0.5	1.3
Tyrosine	7	2.5	12	2.9	3.4
Valine	20	7.3	50	11.9	6.6
% Nonpolar	42		42		39
% Polar, uncharged	34		26		36
% Polar, charged	24		32		24
Calculated molecular weight	31,434		45,416		

[a] Data from Dayhoff et al. (10).

reported in the tryptophan operon (32), galactose operon (39), and ribosomal protein operons (3, 48). Although the mechanisms of translational coupling of two adjacent genes are poorly understood at present, it is thought that the close proximity or overlapping of translational termination and initiation codons ensures the coordinated expression of genes of related function. The significance of this finding in relation to *ftsQ* and *ftsA* is unclear at present, since *ftsA*(Ts) mutant cells which carry a cloned fragment of the wild-type *ftsA* gene, without a juxtaposed, intact, upstream *ftsQ* structural gene, appear to grow and divide normally (clone pNS28).

The designation of the ATG codon at position 2175 as the proposed start site for translation of *ftsZ* is consistent with the conclusion of Ward and Lutkenhaus (47) that the *ftsZ* coding sequence begins no more than 300 bases upstream of the *Eco*RI site in *ftsZ*. The DNA sequence presented here offers no alternative start site for this gene.

Although a precise identification of the various components of promoters (i.e., polymerase binding sites and regulatory sites) is uncertain in the absence of additional data, the locations of the putative promoters that we have identified are entirely consistent with the genetic and promoter fusion results presented here. The identification of the putative promoter $ftsQp_1$ is consistent with the detection of promoter activity within the overlapping, upstream 900-base-pair *Eco*RI-*Bam*HI fragment which does not complement mutations in the upstream *ddl* gene (D. Kenan, unpublished data). We are able to conclude that *ftsQ*, *ftsA*, and *ftsZ* are each preceded by at least one promoter, such that they may be expressed from this promoter in the absence of expression of any upstream or downstream neighboring genes. The DNA sequence reveals that the promoter(s) of *ftsA* must lie within the coding sequence of *ftsQ*; this therefore represents a second overlapping transcriptional unit within the gene cluster, similar to the case of *ftsZ* in which an element required for full *ftsZ* expression lies within the coding sequence for *ftsA* (46). Indeed, the three putative *ftsZ* promoters identified here are all found to overlap or lie within the *ftsA* structural gene.

Since *ftsQ*, *ftsA*, and *ftsZ* are all transcribed in the same direction, it is conceivable that the majority of the transcription of this region originates upstream of this cluster, and we are currently investigating this possibility. Further work, currently in progress, will also permit the precise transcriptional start sites to be determined. The existence of overlapping transcriptional units such as those we have identified here implies that there can be no strong transcriptional terminators, and our results bear this out. Transcription from an exogenous promoter (P*gal*) is not blocked by the insertion downstream of fragments from the *ftsQ-ftsZ'* region. The possibility exists that the dissection of the region with *Bam*HI, *Eco*RI, and *Bgl*II may have destroyed termination signals, and we cannot rule this out at present. Some reduction in downstream *galK* expression results from insertion of these fragments (Table 1), but this may be due to polarity effects rather than partial termination signals. Such transcriptional polarity would provide an explanation for the existence of the internal promoters within the cluster. The occurrence of promoters and regulatory sequences within bacterial structural genes is unusual but not unprecedented. The *trpD* gene of *E. coli* has been shown to contain an internal promoter which may confer a bypass function that is advantageous to the cell under conditions of severe nutritional deprivation (17), and an internal operator has been identified within the *galE* gene (20).

It is intriguing that there should be such a strong correspondence between the inverted repeat sequences surrounding $ftsAp_2$ and $ftsZp_3$. The correspondence is especially remarkable when considered in the context of the constraints imposed upon these sequences by their being located within structural genes. These and other regions of twofold symmetry identified here may be binding sites for proteins which regulate the transcription of *ftsA* and *ftsZ*. Alternatively, they may reflect secondary structure in the mRNA and be involved in posttranscriptional regulation.

An interesting point to come out of this work is that apparently normal cell growth and division take place even when essential genes from this cluster are being expressed from internal promoters in cloned fragments, either in multicopy plasmids as described here or in single copy in chromosomally inserted λ vectors (24–26). Coordinate transcription of the cluster is not, therefore, essential for biologically effective function of the genes under normal laboratory conditions.

In conclusion, the analysis presented here forms the basis of our current investigation into the organisation of the *ftsQ ftsA ftsZ* region. The observation that transcriptional units within this cluster overlap is most interesting and potentially important in understanding the expression of these genes. Further experiments in progress should reveal how the expression of this region is related to the complex process of cell division.

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