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Differential Translation of Cell Division Proteins

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Cloned division genes (*ftsQ* and *ftsA*) and the gene for β-lactamase (*bla*) were transcribed in vivo from a bacteriophage T7 promoter under conditions which blocked the use of other promoters. The different coding regions of single mRNAs were translated with widely different efficiencies, such that the ratio of β-lactamase production to FtsQ production was about 75:1. The relative rates of translation of the division proteins reflected their relative rates of production from normal chromosomal promoters (FtsA > FtsQ). We show that the low rates of production of FtsQ and FtsA proteins are due to their ribosome-binding sequences and that there is no obligatory translational coupling between them, despite the close proximity of the genes. Levels of translation of FtsA are shown to be proportional to levels of transcription, and therefore there is no evidence of variable regulation of translation.

The essential cell division genes *ftsQ*, *ftsA*, and *ftsZ* are tightly linked within a large cluster of genes concerned with peptidoglycan synthesis and septation (Fig. 1). There are numerous promoters within the cluster, several of which are found within coding sequences (12, 13, 17, 18, 21, 22). Figure 1 shows the arrangement in the *ddl-envA* region. There are no transcriptional terminators within this group, but there is a strong terminator after *envA* (1). (This terminator separates transcription of the cell division and other upstream genes from that of downstream genes concerned with transport of proteins across membranes [1, 9].) When cloned segments of this region are expressed in vivo, it is found that quite different amounts of the different proteins are produced and that these amounts are in reverse order from the transcriptional order of the genes on the chromosome (1, 7, 12, 13, 22). Thus, more EnvA protein than FtsZ is produced (7), while FtsZ is produced in much greater amounts than FtsA (7). The FtsQ protein has only recently been identified (15), but it was noted that it was produced in small amounts from cloned DNA. Presumably this differential expression reflects the relative requirements for the four proteins in cell division. Although the contributions of the different promoters to transcription of the region are not known for normal conditions, the lack of terminators between them should lead to progressively higher levels of transcription of downstream genes (Fig. 1). This might therefore explain their different levels of expression. However, we show here that differential translation of the different coding sequences in a common mRNA (produced from a cloned bacteriophage T7 promoter [16]) gives similar relative rates of production for FtsQ and FtsA (translation of *envA* has not yet been studied in this way). We also have evidence which suggests that FtsZ is translated more efficiently than either FtsQ or FtsA. Mechanisms for the strong differential expression of the division proteins therefore appear to have evolved at both the transcriptional and translational levels.

We have earlier provided evidence that transcription from promoters in this region is regulated by the requirements of the cell for FtsA protein during division (2). We have therefore looked for evidence of similar regulation of the frequency of translation of FtsA. Our results suggest, however, that there is no such independent control.

MATERIALS AND METHODS

Strains. *Escherichia coli* K-12 NM275 (an *hsdR trpR* derivative of W3350), obtained from Noreen Murray, was used in most experiments, and *E. coli* BL21(DE3) (16) was used in experiments using isopropyl-β-D-thiogalactopyranoside (IPTG) induction.

Measurement of rates of synthesis of polypeptides from mRNAs transcribed from the φ10 promoter of phage T7. The method used was essentially that described by Studier and Moffat (16) and Tabor and Richardson (19). Cells carrying the various plasmids (described below) were grown overnight with shaking at 37°C in M9 salts with 0.4% maltose, thiamine (1 μg/ml), and ampicillin (50 μg/ml). These cultures were diluted into fresh medium to give an optical density at 540 nm of between 0.06 and 0.1 and were grown with shaking at 37°C. When the optical density reached 0.8, 10 mM $MgSO_4$ was added. Part of the culture was then infected with λCE6 (16) at a multiplicity of infection of about 7. The uninfected portion served as a control. After 30 min, samples were withdrawn from both the infected and uninfected cultures, and rifampin was added to a final concentration of 200 μg/ml. After 45 min, 500-μl samples of the four portions of each culture were pulse-labeled with 5 μCi of [^{35}S]-methionine for 5 min. These were then chilled in an ice water bath before being centrifuged at 4°C for 2 min. The supernatants were decanted, and the pellets were suspended in 60 μl of cracking buffer (5) and placed in a boiling water bath for 4 min. Portions (20 μl each) of each sample were loaded and electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gels. The gels were stained with Coomassie blue for 30 min, destained, dried, and exposed to Du Pont X-ray film for 12 h. The autoradiographs were developed in an automatic film developer. The labeled bands were located with the autoradiograms, cut out of the dried gel, placed on polyethylene, rehydrated with 20 μl of water for 20 min, dropped into 3 ml of Petri solubilizing scintillant (11), swirled overnight at 37°C, and then counted in a scintillation counter.

Construction of plasmids. The parent plasmids pT7-4 and pT7-5 were obtained from Chris Boyd, and pET-3C was

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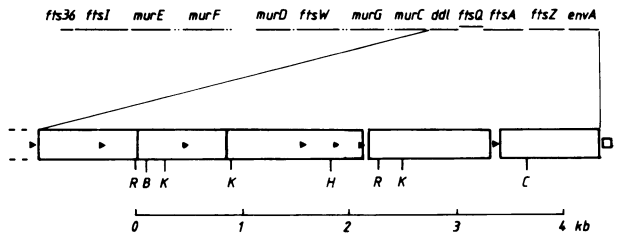

FIG. 1. Arrangement of genes in the major morphogene cluster (3) and the promoters ▶ and terminator (□) known in the *ddl-envA* segment. The locations of the recently described *fts36*, *ftsW* (4), and *murD* (8) are included. Some reference restriction sites are shown: *R*, *Eco*RI; *B*, *Bam*HI; *K*, *Kpn*I; *H*, *Hin*dIII; and *C*, *Cla*I.

obtained from W. Studier. These plasmids carry the ϕ10 promoter of phage T7 upstream from a polylinker and the *bla* gene (Fig. 2) (19). pT7-4QA was made by cloning the 2.3-kilobase (kb) *Eco*RI fragment from pGH4 (12) into the *Eco*RI site of pT7-4. This fragment carries the coding sequences of *ftsQ* and *ftsA* together with short flanking sequences from the upstream *ddl* gene and the downstream *ftsZ* gene (12). pT7-4A was made by digesting pT7-4QA with *Bam*HI to delete the region between the *Bam*HI site on the polylinker and the *Bam*HI site on the 2.3-kb fragment, followed by religation. This deletes part of *ftsQ* but leaves the *ftsA* sequence intact. pT7-5QA was made by cloning the 2.3-kb fragment into the *Eco*RI site of pT7-5. pT7-5Q was made by digesting pT7-5QA with *Hin*dIII to delete the region between the *Hin*dIII site on the polylinker and the *Hin*dIII site on the 2.3-kb fragment, followed by religation. This removes the C-terminal coding sequence of *ftsA* but leaves *ftsQ* intact. pET-3CQA was made by cloning the *Bam*HI fragment of pT7-5QA (from the *Bam*HI site in *ftsQ* to the *Bam*HI site on the polylinker) into the *Bam*HI site of

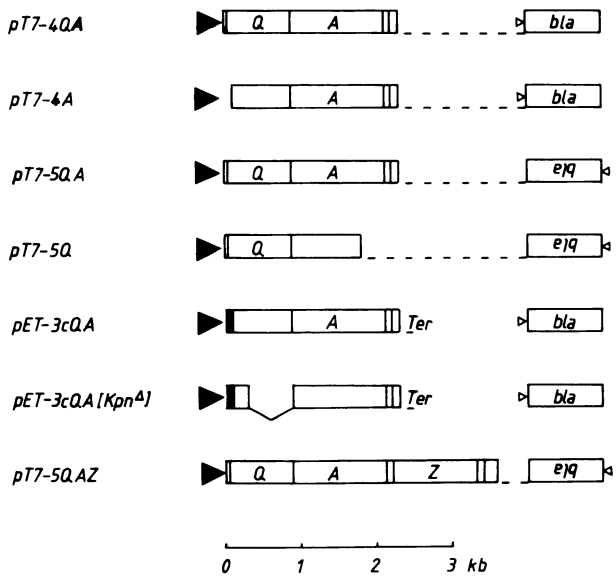

FIG. 2. Chromosomal inserts in the plasmids used in this work. The boxes represent coding sequences (*Q*, *ftsQ*; *A*, *ftsA*; and *Z*, *ftsZ*). The large black triangle represents the ϕ10 promoter, the small triangle represents the *bla* promoter, and Ter indicates the T7 terminator. The distance between the cloned *fts* and *bla* segments is not to scale. The black segment represents the RBS and first codons of the T7 gene 10 (see text).

pET-3C. This creates an in-frame translational fusion between the N-terminal codons of gene 10 (from phage T7) and the C-terminal codons of *ftsQ*, while leaving the *ftsA* sequence unchanged. pET-3CQA(*Kpn*IΔ) was made by digesting pET-3CQA with *Kpn*I and religating. This procedure creates an in-frame translational fusion between the N-terminal codons of the gene 10::*ftsQ* fusion and the C-terminal sequence of *ftsA*. pT7-5QAZ was made by digesting pT7-5QA, first with *Hin*dIII and then with *Cla*I, to remove the fragment between the *Hin*dIII site in *ftsA* and the *Cla*I site in the polylinker and then religating with a 1.87-kb *Hin*dIII-*Cla*I fragment (obtained from pSUZ donated by Susan Dewar) to replace the deleted portion of *ftsA* together with the complete *ftsZ* downstream coding sequence.

RESULTS

Differential production of β-lactamase and FtsA protein from a single mRNA. The 2.3-kb *Eco*RI chromosomal fragment carrying the complete *ftsQ* and *ftsA* coding sequences was cloned into the plasmid pT7-4 (14) to form pT7-4QA (Fig. 2). Restriction analysis was used to show that the fragment was inserted in the correct orientation between the phage T7 promoter (ϕ10) and the β-lactamase gene (*bla*). The ϕ10 promoter is inactive in the absence of the highly specific T7 RNA polymerase (14, 16, 19), and cells carrying pT7-4QA express *ftsA* and *bla* from their own promoters, which are transcribed by the *E. coli* RNA polymerase. *ftsQ* is not expressed because the cloned fragment does not include its promoter (12, 13).

Transcription from ϕ10 can be induced by the production of T7 RNA polymerase, either by induction of a repressed T7 polymerase gene already present in the cell or by infection of the cell with a λ phage carrying this gene (14). Transcription from all other promoters in the cell can be blocked at the same time by addition of rifampin, which inhibits *E. coli* RNA polymerase but not T7 RNA polymerase (14). Under these conditions, cells carrying pT7-4QA should produce only a single mRNA species and the FtsQ, FtsA, and β-lactamase proteins encoded by it.

Figure 3 shows the result of such an experiment. Log-phase cells of *E. coli* K-12 NM275, carrying either pT7-4 or pT7-4QA, were infected with λCE6 (carrying the gene for T7 RNA-polymerase [14]). After 30 min, rifampin was added to inhibit host RNA polymerase. Forty-five minutes later, the cells were labeled for 5 min with [^{35}S]methionine (see Materials and Methods). Cells carrying pT7-4 produced only the three bands of β-lactamase (10) under these conditions (Fig. 3A, lane 8). (The three polypeptides correspond to a precursor protein, mature β-lactamase, and a lower-molecular-weight [MW] product which is always seen under these conditions [10].) Cells carrying pT7-4QA produced an additional polypeptide with a mobility corresponding to that of FtsA protein (6, 7) (Fig. 3B, lane 4). FtsQ protein was not resolved in this case, presumably because its MW caused it to migrate together with one of the β-lactamase bands. To confirm that this labeled band is FtsA protein, pT7-5Q was constructed by deletion of a *Hin*dIII fragment from pT7-5QA. This deletion removes the C-terminal 102 codons of *ftsA* and forms an in-frame fusion with the downstream vector sequence to give a predicted run-on polypeptide with a molecular mass of 37.15 kilodaltons (kDa). A band of this mobility is seen to replace the FtsA band (45.4 kDa) in Fig. 3C, lane 8. (The lower-molecular-mass band is presumed to be the FtsQ protein; see below.)

It is clear from these autoradiograms that the rate of FtsA

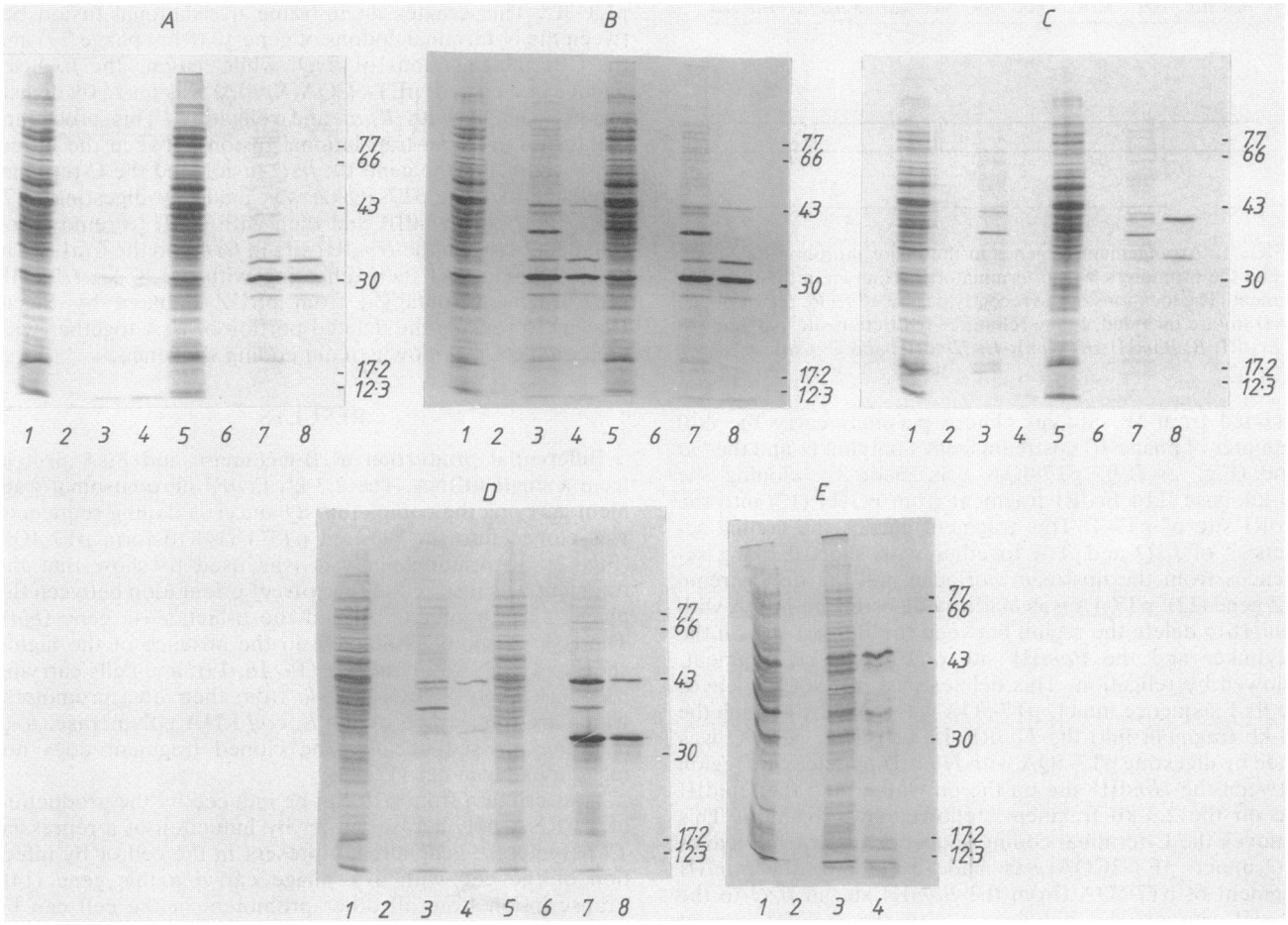

FIG. 3. Autoradiograms of polypeptides pulse-labeled with [^{35}S]methionine and separated by polyacrylamide gel electrophoresis. For each set of cultures, lane 1 shows the control culture (i.e., uninfected and not treated with rifampin), lane 2 shows the uninfected, rifampin-treated culture, lane 3 shows the infected culture without rifampin, and lane 4 shows the infected culture with rifampin added. (A) Lanes 1 to 4, pET3C; lanes 5 to 8, pT7-4. (B) Lanes 1 to 4, pT7-4QA; lanes 5 to 8, pT7-4A. (C) Lanes 1 to 4, pT7-5; lanes 5 to 8, pT7-5Q. (D) Lanes 1 to 4, pT7-5QA; lanes 5 to 8, pET3CQA. (E) Lanes 1 to 4, pET-3CQA(*Kpn*IΔ). Theoretical molecular masses (in kilodaltons) from DNA sequences are as follows: FtsA, 45.4; FtsQ, 31.4; FtsA run-on polypeptide, 37.15; T7 protein 10-FtsQ hybrid, 29.6; T7 protein 10-FtsQ-FtsA hybrid, 52; and β-lactamase polypeptides, 32, 29, and 26. Molecular mass markers are given to the right of each panel in kilodaltons.

synthesis is very much lower than that of β-lactamase from the same transcript. Direct measurement of the radioactivity in the β-lactamase and FtsA bands gave a β-lactamase synthesis to FtsA synthesis ratio of 23:1.

Differential production of FtsQ and FtsA from a common mRNA. In order to resolve the FtsQ polypeptide from the β-lactamase bands, the 2.3-kb *Eco*RI fragment was recloned into pT7-5 to form pT7-5QA (Fig. 2). In this plasmid the *bla* gene is in the orientation opposite from its orientation in pT7-4 and is therefore not transcribed from ϕ10 (14). After the same treatment as before, cells carrying pT7-5QA expressed only two polypeptides (Fig. 3D, lane 4). Since the cloned segment being expressed from ϕ10 carries only the *ftsQ* and *ftsA* coding sequences, we infer that the polypeptide with the higher mobility corresponded to FtsQ, although its mobility under our conditions gave a slightly higher MW than that reported earlier (15). This conclusion was confirmed by modification of the *ftsQ*′ sequence to produce a lower-MW product (see below). The band corresponding to FtsQ was less intense than FtsA. The bands were cut out of the gel, and the relative amounts of label were measured. The ratio of FtsA to FtsQ was 3.3:1. This ratio was confirmed in similar experiments.

High level of production of a hybrid FtsQ protein from a different RBS. The differential rates of synthesis of FtsQ and FtsA and β-lactamase from common mRNAs suggest that the different coding regions are translated independently from separate ribosome-binding sites (RBS). To test this hypothesis, we constructed pET-3CQA (Fig. 2). In this plasmid the RBS and first 27 codons of *ftsQ* have been replaced by the RBS and first 12 codons of gene 10 of phage T7 to make an in-frame translational fusion resulting in a new polypeptide of 29.6 kDa. Cells carrying pET-3CQA produced large amounts of a new, lower-MW polypeptide (Fig. 3D, lane 8). (Like the normal FtsQ protein itself, this band moved more slowly than predicted from its MW alone.) The amount of label in this band was similar to that found in β-lactamase in other experiments (Fig. 3A, lane 8; Fig. 3B, lanes 4 and 8). This demonstrates that the original band was indeed FtsQ and also shows that the low level of synthesis of this protein was probably due to the nature of the RBS present in the original chromosomal DNA.

Figure 3D (lanes 7 and 8) also shows that there was increased production of FtsA protein by pET-3CQA. This presumably resulted from the increased translation of the upstream *ftsQ* sequence. The effect might result either from

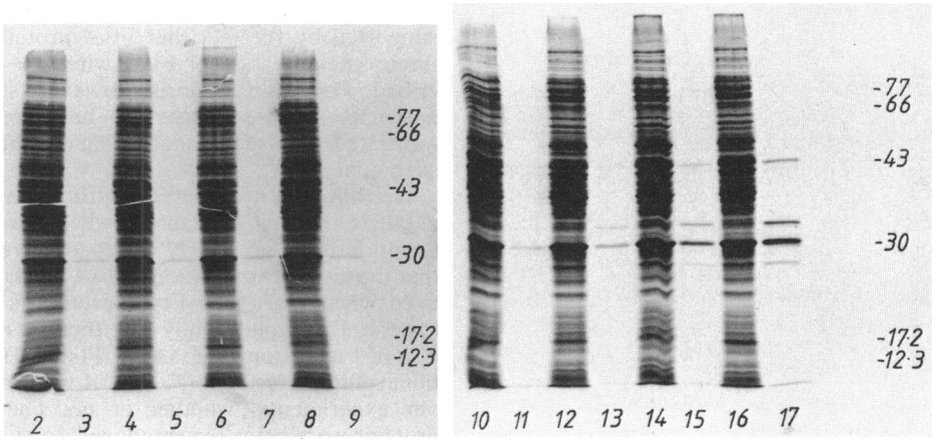

FIG. 4. Coordinate induction of transcription and translation of *ftsQ* and *bla*. Increasing concentrations of IPTG were added to induce transcription of the T7 RNA polymerase gene (gene 1) from the *lac*UV5 promoter in cells of strain BL21(DE3), which also carried the plasmid pT7-4QA. (For induction and labeling procedures, see text.) The first of each pair of lanes is an autoradiogram of pulse-labeled cells in the absence of rifampin, and the second shows the polypeptides labeled in the presence of rifampin. The IPTG concentrations were as follows: lanes 2 and 3, no IPTG; lanes 4 and 5, 1 μM; lanes 6 and 7, 5 μM; lanes 8 and 9, 10 μM; lanes 10 and 11, 20 μM; lanes 12 and 13, 50 μM; lanes 14 and 15, 100 μM; and lanes 16 and 17, 400 μM. The gels were exposed for 96 h. Molecular mass markers (in kilodaltons) are shown to the right of each panel.

increased stability of the mRNA in this state or from translation coupling (12, 22). It should be noted that less FtsA than hybrid FtsQ protein was produced in this situation.

High level of production of a hybrid FtsA protein. To test whether the relatively low level of production of FtsA (compared with β-lactamase) was also due to the nature of the proximal RBS (lying within *ftsQ*), a new in-frame fusion was constructed; in this fusion, the N-terminal 16 codons of *ftsA* were replaced with the RBS and the region coding for the beginning of the protein 10-FtsQ hybrid. To do this, pET-3CQA DNA was digested with *Kpn*I and religated to produce pET-3CQA(KpnIΔ) (Fig. 2). The new hybrid polypeptide has the first 12 amino acids of the gene 10 product, 59 amino acids of FtsQ, and 370 amino acids of FtsA. Under conditions in which transcription originates only at φ10, pulse-labeled cells carrying this plasmid showed a strongly labeled band with the mobility expected for the new hybrid protein (52 kDa) (Fig. 3E, lane 4), together with a number of lower-MW bands which we suppose represent degradation products. We interpret this result to mean that the low rates of synthesis of FtsA protein from pT7-4QA, pT7-5QA, and pET-3CQA were due to the nature of the RBS within *ftsQ*.

Lack of obligatory coupling between translation of *ftsQ* and *ftsA*. The fact that the reading frames of *ftsQ* and *ftsA* overlap by 2 base pairs has suggested that translation of these two genes might be coupled (12, 22). That there can be some coupling is demonstrated by the observation that FtsA protein was made in larger amounts when the upstream *ftsQ* sequence was translated more efficiently (Fig. 3D, lanes 7 and 8). However, the fact that FtsA protein was produced at a higher rate than FtsQ when normal low-level FtsQ translation was occurring (Fig. 3D, lane 4) suggests that coupling is not complete and translation of *ftsA* takes place independently of translation of *ftsQ*. We showed that this is so by constructing pT7-4A by *Bam*HI digestion and religation of pT7-4QA (Fig. 2). This removes the RBS and N-terminal codons of *ftsQ* so that the mRNA upstream of *ftsA* is no longer translated. Figure 3B shows that this had no effect on the rate of production of FtsA protein (compare lanes 4 and 8; the ratio of ^{35}S label in these bands was 0.98:1).

Translation of *ftsA* is proportional to the rate of transcription and is not independently regulated. We have earlier shown that the level of transcription from the promoters within the 1.7-kb *Eco*RI-*Hin*dIII fragment (covering all of *ftsQ* and part of *ftsA*) appears to be regulated in response to the need of the cell for division proteins (perhaps only FtsA) (2). We therefore wished to determine whether translation of *ftsA* was also regulated.

To try to test this, we constructed a strain in which the rate of transcription from φ10 was controlled experimentally. We assumed that if translation was independently regulated, then the rate of translation would not be directly proportional to the rate of transcription over a range of transcription rates. We therefore introduced pT7-4QA into cells of strain BL21(DE3) in which the level of transcription of φ10 is controlled by IPTG (16). This is because this strain is lysogenized with a phage (λDE3) which carries the gene for T7 RNA polymerase under the control of the *lac*UV5 promoter. Addition of IPTG is therefore required for T7 polymerase production, which is in turn required for transcription of *ftsQ*, *ftsA*, and *bla* from the φ10 promoter. The strategy was to induce different levels of transcription by using different concentrations of IPTG and then to determine whether the rate of synthesis of FtsA protein was proportional to that of β-lactamase from the φ10 transcripts. (We assumed that the rate of β-lactamase translation would be proportional to the rate of transcription.)

The rates of production of FtsA and β-lactamase polypeptides increased together over the range of IPTG levels used (0, 1, 5, 10, 20, 50, 100, and 400 μM) (Fig. 4). There is therefore no evidence in this experiment of independent translational control of FtsA production. (FtsQ could not be resolved from β-lactamase in this experiment.)

Translation of *ftsZ* transcripts. pT7-5QAZ was constructed by removing a *Hin*dIII-*Cla*I fragment from pT7-5QA (which deletes the C-terminal coding sequence from *ftsA* together with some plasmid sequence) and replacing it with a *Hin*dIII-*Cla*I segment consisting of the same C-terminal part of *ftsA* together with the downstream *ftsZ* gene. In this plasmid, the *ftsQ*, *ftsA*, and *ftsZ* sequences are in their normal chromosomal order and can be cotranscribed from φ10. Restriction

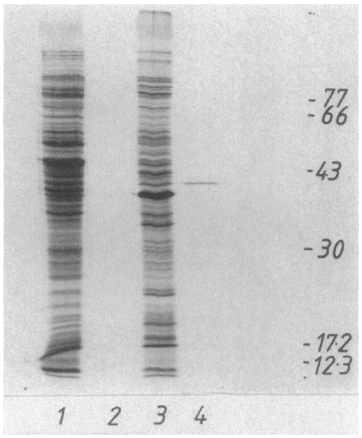

FIG. 5. Pulse-labeled polypeptides in cells carrying pT7-5QAZ (samples were treated as described in the legend to Fig. 3). The gel was exposed for 7 days. Molecular mass markers (in kilodaltons) are shown to the right of the gel.

analysis confirmed the construct, and the presence of active *ftsA* and *ftsZ* genes was checked by complementation of mutants.

Cells carrying pT7-5QAZ produced numerous minicells, in addition to normally dividing cells, as previously reported for plasmids carrying functional *ftsZ* (20). Some difficulty was experienced in maintaining this plasmid in minimal medium long enough to carry out the labeling protocol used for all the other plasmids. A labeled polypeptide corresponding to the molecular mass (40 kDa) of FtsZ was seen, but the expected FtsA and FtsQ bands were not detected (Fig. 5). Genetic testing of the treated population showed that this was due to the low percentage of cells which still carried the plasmid at the time of labeling. Although we have been unable to overcome this problem of stability, this result nevertheless suggests that much more FtsZ protein than either FtsA or FtsQ is made when these proteins are made from a single transcript.

DISCUSSION

The experiments described above show differential translation of the *ftsQ*, *ftsA*, *ftsZ*, and *bla* coding sequences when they are transcribed as polycistronic mRNAs. The ratio of rates of synthesis of β-lactamase, FtsA, and FtsQ is estimated to be about 75:3:1 (by direct measurement of the radioactivities of eluted polypeptides), although the *bla* sequence was always transcribed last and might therefore have been expected to be transcribed and translated at lower levels than upstream genes. It is clear, therefore, that the FtsA and FtsQ proteins are very inefficiently produced. (These proteins are stable [15; unpublished data], and the low levels of labeled polypeptides are not due to rapid degradation.) Very inefficient translation of mRNA must therefore be the cause of their low rates of synthesis. This was confirmed by replacing the upstream sequences containing the putative RBS with the RBS and initial few codons of an efficiently translated gene, gene 10 of phage T7 (14). The resulting hybrid polypeptides (containing 90% of the FtsQ polypeptide or 96% of the FtsA polypeptide) were produced at rates similar to that of β-lactamase (as measured by the radioactivity of eluted bands).

We have shown that the *ftsA* coding sequence is translated independently of *ftsQ*, confirming the existence of ribosome-binding sequences within the *ftsQ* coding frame (12). This is supported by the fact that FtsA protein is made at about three times the rate of FtsQ when the genes are cotranscribed. FtsA translation is, however, stimulated when the upstream *ftsQ* sequences are being much more actively translated. Translational coupling can therefore occur under such conditions.

We have shown that the production of FtsA protein parallels that of β-lactamase when the frequency of transcription is progressively increased. We take this to show that the level of translation of *ftsA* per transcript is constant even when FtsA (and FtsQ) protein is being produced in excess. This would imply that there is no feedback regulation of translation by FtsA (or FtsQ). The fact that translational efficiencies of *ftsQ* and *ftsA* were largely unchanged in our experiments, whether or not one or both of these proteins were being overproduced or whether (in the case of FtsA) a normal or an abnormal runoff protein was being produced, also argues against any regulation of translation efficiency by either of these proteins. This is in contrast to transcription of *ftsA*, which has been shown to be regulated in response to the requirements of the cell for FtsA protein (2).

The arrangement of the *ftsQ-envA* group of division genes within a larger cluster of genes containing many internal promoters but no known intervening transcription terminators is expected to produce progressively increasing numbers of transcripts for progressively later genes. This is in accord with published observations on the relative levels of the EnvA, FtsZ, FtsA, and FtsQ polypeptides expressed from cloned DNA with some or all of these promoters (7, 15). Our present work shows that this differential transcription of different division genes is reinforced by strong differential translation of the different segments of mRNA. We presume that these controls on division protein production have evolved in response to the requirements of the cell for particular amounts of the different proteins at a particular stage in cell growth. The result is a very complex arrangement in which sequences allowing independent transcription and translation of adjacent genes are superimposed on the coding sequences themselves.

LITERATURE CITED

1. **Beall, B., and J. Lutkenhaus.** 1987. Sequence analysis, transcriptional organization, and insertional mutagenesis of the *envA* gene of *Escherichia coli*. J. Bacteriol. **169**:5408–5415.
2. **Dewar, S. J., V. Kagan-Zur, K. J. Begg, and W. D. Donachie.** 1989. Transcriptional regulation of cell division genes in *Escherichia coli*. Mol. Microbiol. **3**:1371–1377.
3. **Donachie, W. D., K. J. Begg, and N. F. Sullivan.** 1984. The morphogenes of *Escherichia coli*, p. 27–62. *In* R. Losick and L. Shapiro (ed.), Microbial development. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
4. **Ishino, F., H. K. Jung, M. Ikeda, M. Doi, M. Wachi, and M. Matsuhashi.** 1989. New mutations *fts-36*, *lts-33*, and *ftsW* clustered in the *mra* region of the *Escherichia coli* chromosome induce thermosensitive cell growth and division. J. Bacteriol. **171**:5523–5530.
5. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
6. **Lutkenhaus, J. F., and W. D. Donachie.** 1979. Identification of the *ftsA* gene product. J. Bacteriol. **137**:1088–1094.
7. **Lutkenhaus, J. F., and H. C. Wu.** 1980. Determination of transcriptional units and gene products from the *ftsA* region of *Escherichia coli*. J. Bacteriol. **143**:1281–1288.
8. **Mengin-Lecreulx, D., C. Parquet, L. R. Desviat, J. Plá, B.**

Flouret, J. A. Ayala, and J. van Heijenoort. 1989. Organization of the *murE-murG* region of *Escherichia coli*: identification of the *murD* gene encoding the D-glumatic-acid-adding enzyme. J. Bacteriol. **171**:6126–6134.

9. **Oliver, D. B., and J. Beckwith.** 1982. Identification of a new gene (*secA*) and gene product involved in secretion of envelope proteins in *Escherichia coli*. J. Bacteriol. **150**:686–691.
10. **Oudega, B., and F. R. Mooi.** 1983. The use of minicells and maxicells to detect the expression of cloned genes, p. 241–255. *In* J. Walker and W. Gaastra (ed.), Techniques in molecular biology. Croom Helm Ltd., London.
11. **Petri, W. H.** 1972. Discontinuous polyacrylamide-gel electrophoresis of RNA. Anal. Biochem. **48**:442–448.
12. **Robinson, A. C., D. J. Kenan, G. F. Hatfull, N. F. Sullivan, R. Spiegelberg, and W. D. Donachie.** 1984. DNA sequence and transcriptional organization of essential cell division genes *ftsQ* and *ftsA* of *Escherichia coli*: evidence for overlapping transcriptional units. J. Bacteriol. **160**:546–555.
13. **Robinson, A. C., D. J. Kenan, J. Sweeney, and W. D. Donachie.** 1986. Further evidence for overlapping transcriptional units in an *Escherichia coli* cell envelope-cell division gene cluster: DNA sequence and transcriptional organization of the *ddl ftsQ* region. J. Bacteriol. **167**:809–817.
14. **Rosenberg, A. H., B. N. Lade, D.-S. Chui, S.-W. Lin, J. J. Dunn, and F. W. Studier.** 1987. Vectors for selective expression of cloned DNAs by T7 RNA polymerase. Gene **56**:125–135.
15. **Storts, D. R., O. M. Aparicio, J. M. Schoemaker, and A. Markovitz.** 1989. Overproduction and identification of the *ftsQ* gene product, an essential cell division protein in *Escherichia coli* K-12. J. Bacteriol. **171**:4290–4297.
16. **Studier, F. W., and B. A. Moffatt.** 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. **189**:113–130.
17. **Sullivan, N. F., and W. D. Donachie.** 1984. Overlapping functional units in a cell division gene cluster in *Escherichia coli*. J. Bacteriol. **158**:1198–1201.
18. **Sullivan, N. F., and W. D. Donachie.** 1984. Transcriptional organization within an *Escherichia coli* cell division gene cluster: direction of transcription of the cell separation gene *envA*. J. Bacteriol. **160**:724–732.
19. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82**: 1074–1078.
20. **Ward, J. E., Jr., and J. F. Lutkenhaus.** 1985. Overproduction of FtsZ induces minicell formation in *Escherichia coli*. Cell **42**: 941–949.
21. **Yi, Q.-M., and J. F. Lutkenhaus.** 1985. The nucleotide sequence of the essential cell division gene *ftsZ*. Gene **36**:241–247.
22. **Yi, Q.-M., S. Rockenbach, J. E. Ward, and J. F. Lutkenhaus.** 1985. Structure and expression of the cell division genes *ftsQ*, *ftsA* and *ftsZ*. J. Mol. Biol. **184**:399–412.