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Antisense Transcription of the *ftsZ-ftsA* Gene Junction Inhibits Cell Division in *Escherichia coli*

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A 490-bp DNA segment spanning the junction between the *ftsA* and *ftsZ* genes inhibits cell division when present in high copy number. We show that this segment contains an antisense promoter and an antisense transcription terminator which define a new gene, *stfZ*.

High copy numbers of the 2.3-kbp *Eco*RI (*'ddlB*) *ftsQ ftsA* (*ftsZ'*) chromosomal segment (Fig. 1) block cell division (5, 6, 8, 11). This inhibition of division has previously been ascribed to excess production of FtsA protein (5, 6, 8, 11). The block is to the earliest stage of division, that which requires FtsZ protein, and can be reversed by increasing the number of copies of the *ftsZ* gene (4, 5). However, several observations have made us question whether the cause of the division block is excess FtsA protein alone. Firstly, Tétart and Bouché (10) have shown that the product of the *dicF* gene (a member of the *dicB* operon, apparently a relict phage operon, without known function in the cell cycle [1, 2, 7]) is an antisense RNA which is complementary to the sequence around the ribosome-binding site of *ftsZ* mRNA and that *dicF* RNA blocks translation of *ftsZ* mRNA. Secondly, as we have reported earlier (8), the 2.3-kbp chromosomal fragment contains within it a strong transcription terminator which, however, is oriented in such a way that it could terminate only transcription opposing that of *ftsA* or *ftsZ*. A reverse RNA transcript of this segment would include an 8-base, GC-rich inverted repeat, separated by 10 bases and followed by an 19-base AU-rich sequence. This therefore resembles a rho-independent transcription terminator that, however, could operate to stop only reverse transcription (8) (Fig. 2). The location of this possible reverse terminator is such that reverse transcription of the *ftsA-ftsZ* junction region starting from within *ftsZ* would produce an antisense RNA that was complementary to the same mRNA sequence as is *dicF* RNA.

To test the idea that transcription from the antisense strand of *ftsZ*, rather than overproduction of FtsA protein alone, is responsible for the inhibition of cell division by pSZ24, we removed the 1.8-kbp *Eco*RI-*Hin*dIII segment (containing the *ftsQ* gene and most of *ftsA*) to produce pSZ25 (Fig. 1). We found that cells carrying pSZ25, like those carrying pSZ24 (5), are completely blocked in cell division at 42°C (Fig. 3), although the 490-bp chromosomal fragment remaining in this plasmid contains only the 3′ end of the *ftsA* gene and the 5′ end of *ftsZ* (Fig. 1). Therefore, excess FtsA protein cannot be the cause of the inhibition of cell division by pSZ25.

To see whether the "reverse terminator" is within the 490-bp *Hin*dIII-*Eco*RI fragment, as suggested by the DNA sequence (Fig. 2), we cloned this piece in both orientations between the *gal* promoter and the *galK* gene in the termination assay vector pJW30 (12) (to give pSD55 and pSD56 [Fig. 1]). Host cells with the *galK* mutation carrying the parent vector form red colonies on MacConkey-galactose plates. Colonies of cells with the *galK* mutation carrying P_{gal}::[*Hin*dIII-*Eco*RI]::*galK*$^+$ also are red, but those carrying P_{gal}::[*Eco*RI-*Hin*dIII]::*galK*$^+$ are white. Therefore there is indeed a strong reverse transcription terminator that is able to block P_{gal} transcription of this part of the *ftsA-ftsZ* complementary strand.

The same 490-bp piece of DNA was cloned in the orientation [*Eco*RI-*Hin*dIII]::*galK*$^+$ into the promoter assay vector, pKO1. Colonies of host cells with the *galK* mutation carrying pNS45 or pKO1-500 are white on MacConkey-galactose plates (8), confirming that there is no promoter between the reverse terminator and the *Hin*dIII end of this piece of DNA. To test whether there is an internal reverse promoter at the *Eco*RI end of this fragment, a 342-bp *Hin*dIII-*Bsu*36I section was removed to give pAS142. The *Bsu*36I restriction site is located within one of the inverted-repeat sequences of the proposed terminator (Fig. 2), and removal of the 342-bp fragment was therefore expected to abolish transcription termination. Colonies of host cells with the *galK* mutation carrying pAS142 are deep red on MacConkey-galactose plates. Restriction analysis confirmed the structure of pAS142 to be *Eco*RI-*Bsu*36I::*galK*$^+$. The 148-bp *Eco*RI-*Bsu*36I sequence therefore does indeed contain a strong reverse promoter.

All the plasmids shown in Fig. 1 (except pAS142) contain the reverse promoter and reverse terminator that together delimit a new antisense gene, *stfZ*. However, of the plasmids containing intact *stfZ* sequences, only pSZ24 and pSZ25 cause detectable inhibition of cell division (at 42°C). The reason that pSZ24 and pSZ25 inhibit division most strongly is probably because they have copy numbers of more than 200 per cell, whereas the remaining plasmids have copy numbers in the range of 20 to 50 per cell. Cells carrying pAS142 are also filamentous at 42°C, perhaps because this plasmid produces a hybrid *stfZ'*::*galK* transcript which may fold into a configuration which more readily pairs with *ftsZ* mRNA than does *stfZ* RNA itself.

The experiments described here show that there are both a strong promoter and a strong transcription terminator within the 490-bp *Hin*dIII-*Eco*RI segment. This therefore defines a new antisense gene, *stfZ*, which would be expected to produce an antisense RNA of not more than about 140 bases in length (allowing a minimum spacing of 40 bp between the *Eco*RI site and the beginning of such an RNA and assuming that the

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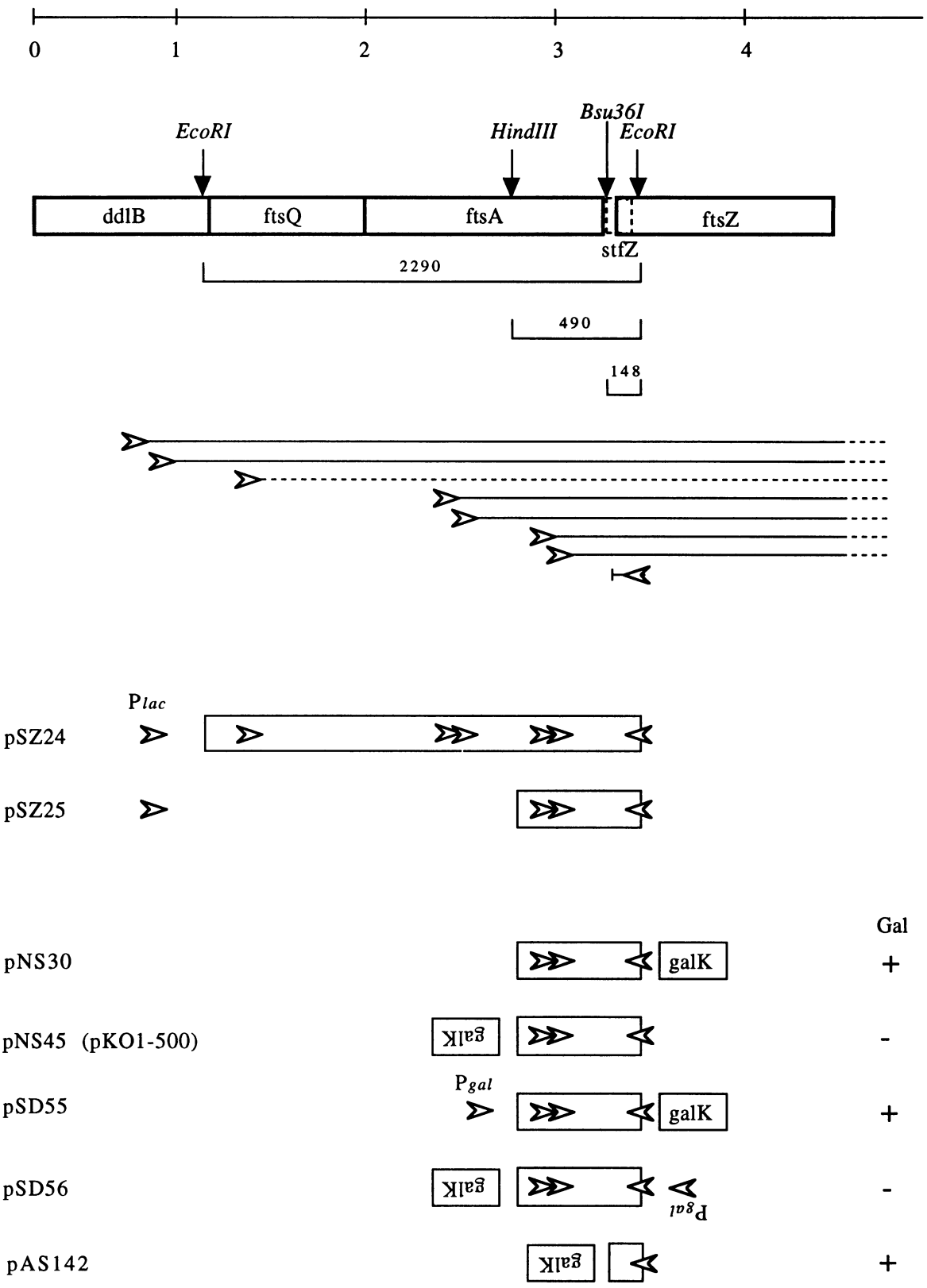

FIG. 1. The *ddlB*-to-*ftsZ* section of the *mra* operon. The open reading frames of the four genes in this segment are shown as boxes, and at the top the scale is given in kilobase pairs. The positions of four restriction sites are shown, together with three subcloned segments (sizes in base pairs) relevant to this paper. Beneath this are shown the known promoters (arrowheads) and the deduced transcripts for this region. All sense transcripts are assumed to continue through *ftsZ* and end at the terminator following *envA* (the next gene); the short antisense transcript (*stfZ*) is assumed to end at the proposed antisense terminator (see text). Gal phenotypes of strains carrying the plasmids, are indicated at right.

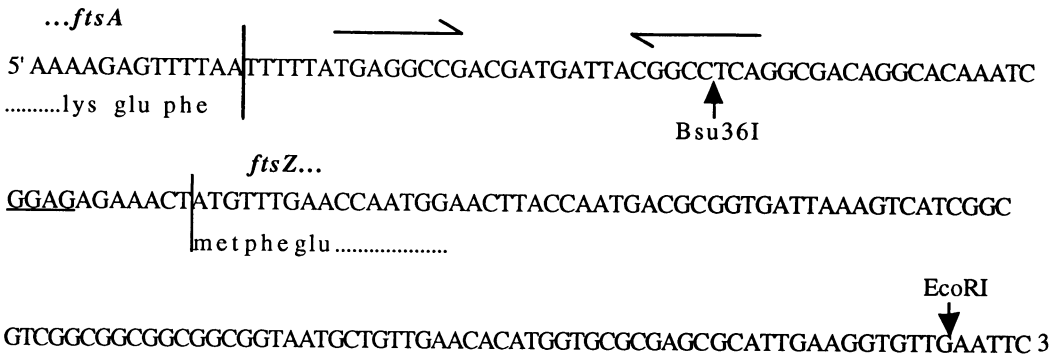

FIG. 2. 5′-to-3′ sequence of the *ftsA-ftsZ* junction. A 60-bp stretch separates the stop codon of *ftsA* from the start codon of *ftsZ* (vertical bars). The proposed "antiterminator" is the GC-rich 8-bp inverted repeat (horizontal arrows) preceded by the 19-bp AT-rich sequence. The putative ribosome-binding Shine-Dalgarno sequence for *ftsZ* is underlined. The "antipromoter" must lie within the 148-bp *Bsu*36I-*Eco*RI segment.

transcript would end just beyond the inverted-repeat sequence). Our observations suggest that it is likely to be this transcript which is responsible for the inhibition of division in cells which carry this DNA segment in high copy number.

Although it is attractive to suppose that the *stfZ* reverse transcript acts, like *dicF* RNA, by hydrogen bonding to the ribosome-binding site of *ftsZ* mRNA, it is nevertheless possible that *stfZ* RNA is translated and that it is the translation product which inhibits cell division. There is one possible open reading frame within the region of the *stfZ* RNA transcript. This is preceded by a possible Shine-Dalgarno motif and would code for a 25-amino-acid polypeptide. Further work is required to show whether this polypeptide is produced and whether it has a role in division control.

We have shown that the segment of DNA which spans the region from the end of *ftsA* through the 60-bp spacer sequence into the beginning of *ftsZ* blocks cell division. We have also shown that this short segment contains both a strong reverse promoter and a strong reverse terminator, which should produce an antisense transcript of this region. However, a puzzling feature of both the *dicF* and *stfZ* systems is that neither of them appears to be able to inhibit cell division except when greatly overexpressed at high temperature. A possible explanation for this is that each of these sense-antisense interactions requires the participation of a further factor, similar to the Rom-Rop protein which mediates the RNAI-RNAII hybridization of ColE1 plasmids (3, 9). When either *dicF* RNA or *stfZ* RNA alone is overproduced, there may not be enough of this second factor to allow hybridization or to stabilize the RNAs. Inhibition of *ftsZ* mRNA translation would therefore take place to a significant extent only when either of the antisense RNAs is present in great excess and when the temperature is high enough to encourage melting of the secondary structure and hybridization with *ftsZ* mRNA. Future work will be directed to a search for this possible hybridization factor. Finally, we should emphasize that our present observations do not contradict earlier reports which showed that induction of excess FtsA protein also inhibits the activity of FtsZ (4, 6, 11).

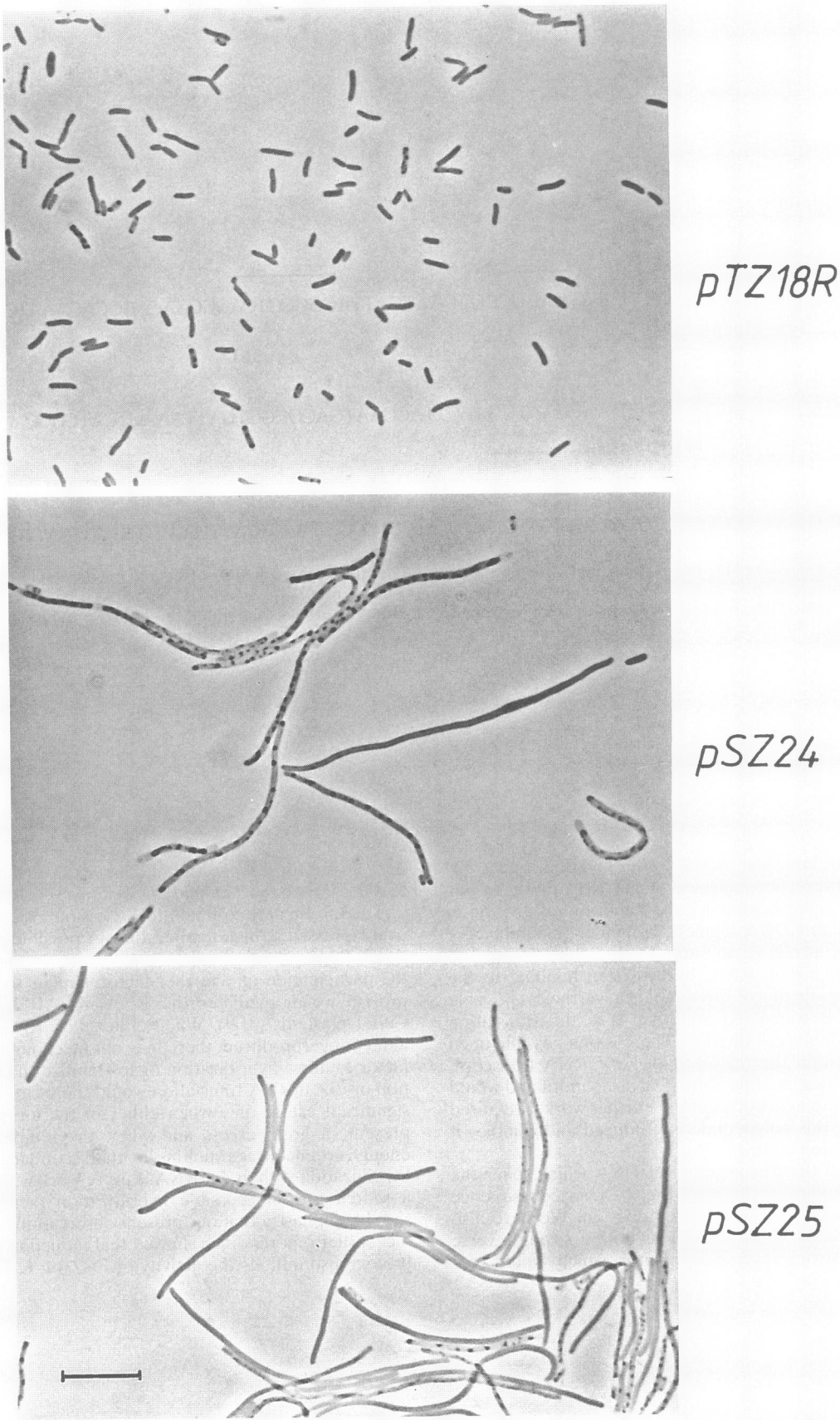

FIG. 3. Cells carrying pTZ18R (vector), pSZ24, or pSZ25 after growth overnight on nutrient agar plates at 42°C. Bar, 10 μm.

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REFERENCES

1. **Béjar, S., and J.-P. Bouché.** 1985. A new dispensable genetic locus of the terminus region involved in control of cell division in *Escherichia coli*. Mol. Gen. Genet. **201:**146–150.
2. **Bouché, F., and J.-P. Bouché.** 1989. Genetic evidence that DicF, a second division inhibitor encoded by the *Escherichia coli dicB* operon, is probably RNA. Mol. Microbiol. **3:**991–994.
3. **Cesareni, G., M. Helmer-Citterich, and L. Castagnoli.** 1991. Control of ColE1 plasmid replication by antisense RNA. Trends Genet. **7:**230–235.
4. **Dai, K., and J. Lutkenhaus.** 1992. The proper ratio of FtsZ to FtsA is required for cell division to occur in *Escherichia coli*. J. Bacteriol. **174:**6145–6151.
5. **Dewar, S. J., K. J. Begg, and W. D. Donachie.** 1992. Inhibition of cell division by an imbalance in the ratio of FtsA to FtsZ. J. Bacteriol. **174:**6314–6316.
6. **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.
7. **Faubladier, M., K. Cam, and J.-P. Bouché.** 1990. *Escherichia coli* cell division inhibitor DicF-RNA of the *dicB* operon. Evidence for its generation *in vivo* by transcription termination and by RNaseIII and RNaseE-dependent processing. J. Mol. Biol. **212:**461–471.
8. **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.
9. **Simons, R. W., and N. Kleckner.** 1988. Biological regulation by antisense RNA in prokaryotes. Annu. Rev. Genet. **22:**567–600.
10. **Tétart, F., and J.-P. Bouché.** 1992. Regulation of the expression of the cell cycle gene *ftsZ* by DicF antisense RNA. Division does not require a fixed number of FtsZ molecules. Mol. Microbiol. **6:**615–620.
11. **Wang, H., and R. C. Gayda.** 1990. High-level expression of the FtsA protein inhibits cell septation in *Escherichia coli* K-12. J. Bacteriol. **172:**4736–4740.
12. **Wright, J. J., A. Kumar, and R. S. Hayward.** 1992. Hypersymmetry in a transcriptional terminator of *Escherichia coli* confers increased efficiency as well as bidirectionality. EMBO J. **11:**1957–1964.