JOURNAL OF BACTERIOLOGY, Nov. 1990, p. 6611–6614
0021-9193/90/116611-04$02.00/0
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Vol. 172, No. 11

Regulation of Expression of the *ftsA* Cell Division Gene by Sequences in Upstream Genes

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Received 10 April 1990/Accepted 27 August 1990

The essential cell division genes *ftsQ* and *ftsA* overlap by 1 bp (A. C. Robinson, D. J. Kenan, G. F. Hatfull, N. F. Sullivan, R. Spiegelberg, and W. D. Donachie. J. Bacteriol. 160:546–555, 1984; Q.-M. Yi, S. Rockenbach, J. E. Ward, and J. F. Lutkenhaus. J. Mol. Biol. 184:399–412, 1985). We have previously shown that *ftsA* can be expressed from a weak promoter located within the *ftsQ* gene (Robinson et al., J. Bacteriol. 160:546–555, 1984). We report here the effects on *ftsA* expression of a series of deletions within *ftsQ*. We find that two regions upstream of the promoter are important in its expression. When both are present, *ftsA* is expressed, as is also the case when both are absent. The two regulatory elements (O_1 and O_2) have 9-bp sequences, of which 8 bp are identical.

Figure 1 shows plasmid pSZ24, which carries the 2.3-kb *Eco*RI chromosome fragment containing the *ftsQ* and *ftsA* coding sequences (together with a few bases from the upstream *ddl* and the downstream *ftsZ* sequences). This plasmid expresses *ftsA* but not *ftsQ*, as previously reported for similar constructs (5, 10).

Sequential, controlled degradation (1) of the 5′-terminal end of the *'ddl ftsQ ftsA ftsZ'* insert was used to produce the set of deletions shown in Fig. 2 and 3. Double digestion with *Xba*I and *Sph*I was used to release a 14-bp fragment from the vector and create a suitable substrate for exonuclease III digestion. Exonuclease III acts solely on termini with recessed 3′ ends, and the nuclease therefore degraded the vector only from the *Xba*I end. The extent of the deletions was controlled by varying either the nuclease concentration or the reaction time. The long 5′-terminal extensions were removed with mung bean nuclease, any remaining overhangs were filled in with DNA polymerase I in the presence of all four deoxynucleoside triphosphates, and the molecules were finally religated on themselves. The end points of the deletions were determined by dideoxy sequencing (7).

pSZ24 and its deletion derivatives were transformed into TOE13 [*ftsA13*(Ts)] grown with ampicillin at 30°C, and single colonies were tested for the ability to form colonies on plates at 30 and 42°C. At 42°C, TOE13 cells grow as long, aseptate filaments and are unable to form colonies. TOE13(pSZ24) cells divide and form colonies normally at 42°C.

Deletions of up to 13 bp from the *Eco*RI site at the 5′ end of the insert had no effect on *ftsA* expression, but deletion of 16 bp inactivated the *ftsA* gene, which is located more than 800 bp downstream. Deletions of increasing length, up to 244 bp, were alike in showing no expression of *ftsA*. However, longer deletions, from 251 bp up to as much as 419 bp, caused the reactivation of *ftsA* expression (Fig. 2).

Because a deletion of all of the cloned DNA up to bp 419 did not prevent *ftsA* expression, a promoter must be located between bp 419 and the beginning of the *ftsA* gene (Fig. 3). Deletion of 245 bp between the *Nru*I (bp 548) and *Nar*I (bp 793) sites did not affect *ftsA* expression, but deletion of 111 bp between the *Acc*I (bp 437) and *Nru*I sites prevented *ftsA* expression. This latter deletion includes an inverted repeat which is similar to a sequence at a promoter consensus sequence (4) (P_{Z3}) within the *ftsA* gene (5). The promoter near P_{Z3} is much stronger than that (P_A) within *ftsQ* (5), and therefore its much closer resemblance to the consensus might be expected (Fig. 4). The inverted repeat also suggests the possibility of common regulation of these two promoters. The possibility remains, however, that the weak promoter in *ftsQ* is elsewhere within or overlapping this deletion.

The deletion analysis also identifies two upstream regions which are important for transcription. The first overlaps the sequence between bp 13 and 16 within the *ddl* gene. Disruption of this sequence inactivates expression of *ftsA*. The second overlaps the sequence between bp 244 and 251 within the *ftsQ* gene. Disruption of this sequence restores *ftsA* expression in the absence of the first regulatory sequence. Examination of the base sequence in these two regions (O_1 and O_2) shows that they have 8 of 9 bp identical in inverted repeat.

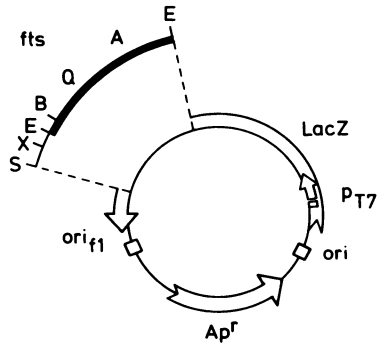

FIG. 1. pSZ24, showing the locations of the cloned 2.3-kb chromosomal fragment (fts, heavy bar) within the *lacZ* open reading frame of pTZ18R (Pharmacia). Q, *ftsQ*; A, *ftsA*; E, *Eco*RI; B, *Bam*HI; S, *Sal*I; X, *Xba*I. P_{T7} is a phage T7 promoter; ori_{f1} is the phage M13 replication origin; Ap^r is the β-lactamase gene.

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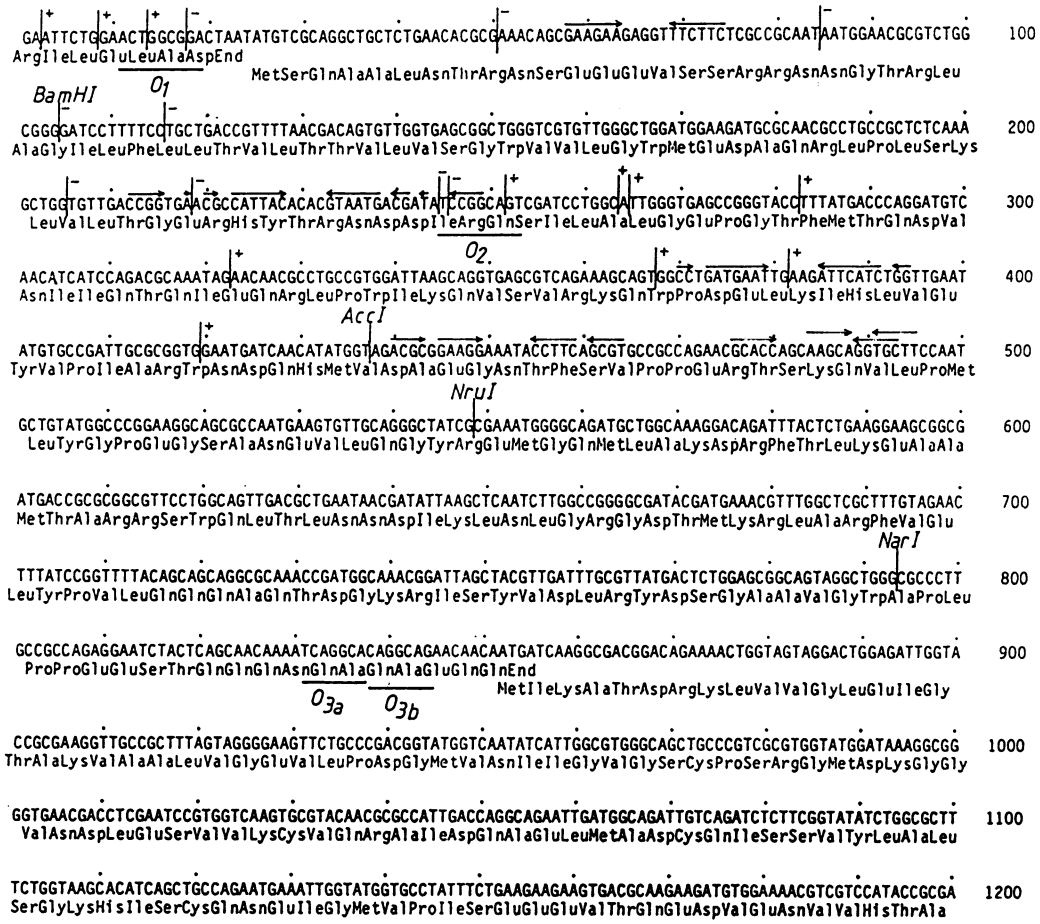

FIG. 2. Nucleotide sequence of the region upstream of *ftsA*. The sequence starts at the *Eco*RI site near the end of the *ddl* gene. The *ddl* sequence ends in a stop codon at bp 23 and is immediately followed by the start of the *ftsQ* coding sequence at bp 25. The *ftsQ* sequence ends in a stop codon at bp 855, overlapping the beginning of *ftsA* at bp 852. Inverted repeat sequences are indicated by arrows. Vertical lines show the end points of deletions starting at the initial *Eco*RI site. A + indicates that the downstream *ftsA* gene is expressed when the deletion is present, and a − indicates that it is not (Fig. 3). O_1, O_2, O_{3a}, and O_{3b}, proposed operator sequences discussed in the text. The cleavage points of the *Bam*HI, *Acc*I, *Nru*I, and *Nar*I endonucleases are also shown.

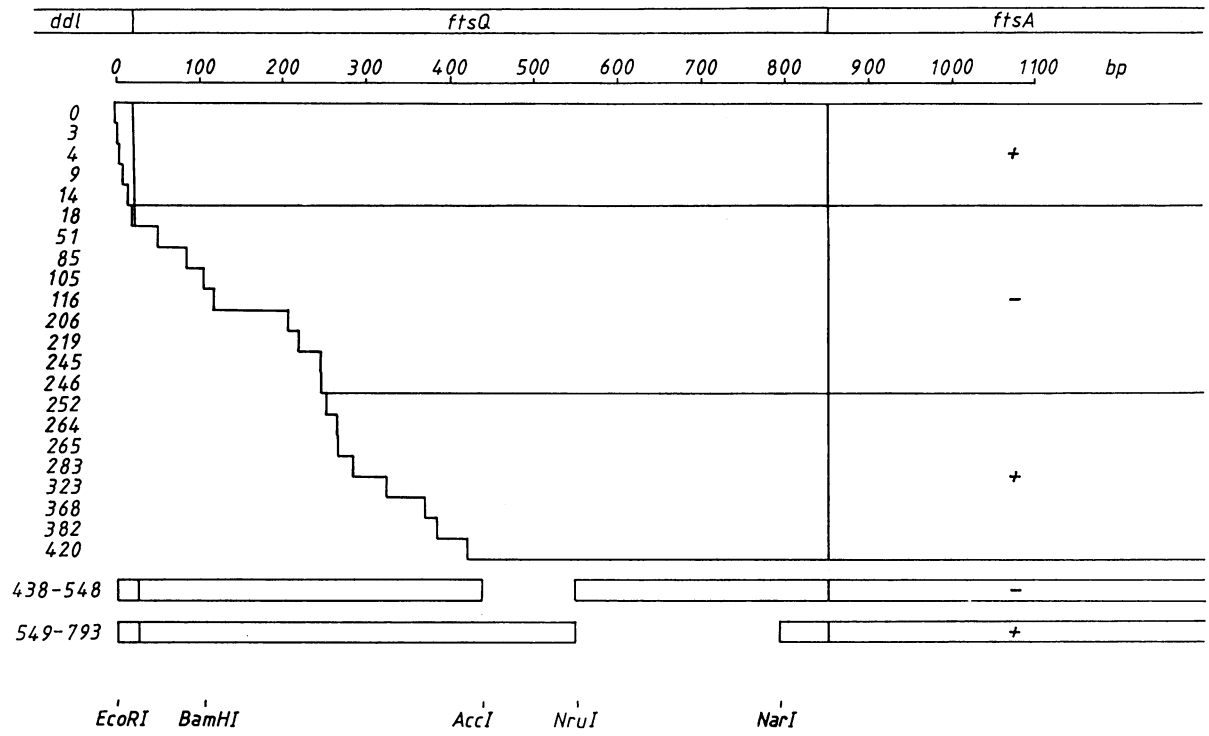

FIG. 3. Deletions in the *ddl ftsQ* genes and their effects on expression of *ftsA*. At the top is the *ftsQ* gene (coding sequence) and parts of the adjacent *ddl* and *ftsA* genes. A scale (in base pairs) starts at the *Eco*RI restriction site in *ddl* (6). The numbers on the left give the positions of the end base pairs in the deleted fragments which are drawn on the right. The effect of each deletion on the expression *ftsA* is shown as + (the plasmid complements the chromosomal *ftsA13* mutation) or − (no complementation). The insert at the top (0) represents the undeleted insert in pSZ24. Some reference restriction sites are shown at the bottom.

	O_1		O_2	
bp	11	19	245	253
5′	ACTGGCGGA		TCCGGCAGT	3′
3′	TGACCGCCT		AGGCCGTCA	5′

If these regions were transcribed, then the O_1 sequence (which includes the Shine-Dalgarno ribosome-binding sequence for *ftsQ*) could base pair with the O_2 sequence and prevent translation of *ftsQ*. However, we have shown elsewhere (3a) that deletion of the O_1 region has no effect on translation of *ftsA* when the whole region is transcribed in a single mRNA. Together with the evidence presented here and earlier (5) that transcription of *ftsA* in these constructs starts within *ftsQ* (downstream of O_1 and O_2), this effectively eliminates any explanation which might involve RNA base pairing between O_1 and O_2. We therefore think that O_1 and O_2 affect transcription from the downstream promoter within *ftsQ*. In an attempt to see how this might work, we looked for sequences resembling O_1 and O_2 elsewhere in the region.

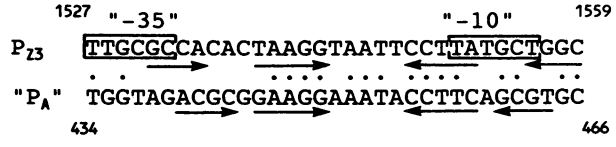

FIG. 4. P_{Z3} and P_A sequences.

Two overlapping sequences (O_{3a} and O_{3b}) in the same orientation as O_2 were found at the opposite end of *ftsQ* (between bp 832 and 852) (Fig. 5).

One explanation of how these sequences might control expression of *ftsA* is given in Fig. 6. We propose that a dimeric protein can recognize and bind pairs of sites. Binding of O_1 and O_2 would produce a DNA loop which would prevent the expression of *ftsQ* but would allow transcription of *ftsA*. An alternative DNA loop could form between O_1 and O_3 (a or b), and this would prevent transcription of both *ftsQ* and *ftsA*. In our experiments, deletion of O_1 allows the formation of a loop between O_2 and O_3, which would also prevent transcription of *ftsA*, but deletion of both O_1 and O_2 prevents all loop formation and allows *ftsA* to be transcribed once more.

We are at present trying to identify the putative regulatory protein and find direct evidence for our model. How such a regulatory system is involved in the control of cell division is unknown. However, this is yet another example in this complex gene cluster of sequences being used both for

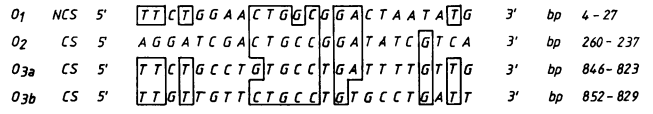

FIG. 5. Alignment of bases in the noncoding strand (NCS) or coding strand (CS) centered around the O_1, O_2, O_{3a} and O_{3b} sequences (see text). Identical bases are boxed.

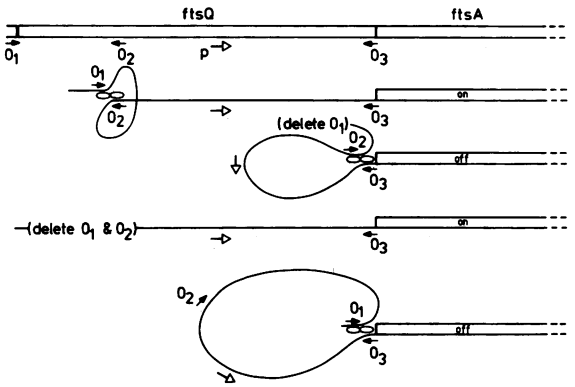

FIG. 6. Model for DNA-loop formation. The locations and orientations of the proposed protein-binding sequences O_1, O_2, O_3 and O_{3b} not being differentiated in this diagram) are shown, together with the promoter (P) in the *ftsQ* gene. In the second line a dimeric protein is shown binding O_1 and O_2 to form a 234-bp loop. In this configuration, RNA polymerase can bind to the promoter, and downstream sequences, including *ftsA*, are transcribed (ftsA, on). In the third line, the protein binds O_2 and O_3 because O_1 has been deleted. The 586-bp loop thus formed contains the promoter. Transcription of *ftsA* does not then take place (either because it is blocked by the bound protein or because the configuration of the DNA in the loop prevents polymerase binding). The fourth line shows the deletion of both O_1 and O_2. Transcription of *ftsA* takes place because no loop formation is now possible. The bottom line shows a possible alternative pairing of O_1 and O_3 which could take place in undeleted DNA and which would also prevent *ftsA* transcription. Three possible states of this region in the intact chromosome would therefore be as follows: (i) no protein bound, both *ftsQ* and *ftsA* transcribed; (ii) protein bound to O_1 and O_2, *ftsA* expressed but *ftsQ* not; and (iii) protein bound to O_1 and O_3, *ftsA* and *ftsQ* not expressed. (Binding of O_2 and O_3 might also take place in the intact chromosome.)

coding (*ddl* and *ftsQ*) and as promoters, ribosome-recognition sequences, and regulatory sites (2, 3, 3a, 5, 6, 8–10).

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