The SOS promoter *dinH* is essential for *ftsK* transcription during cell division

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The formation of the *Escherichia coli* division septum has been well characterized and the majority of the genes involved have been shown to map to the *dcw* cluster. One exception is *ftsK*, which lies at 20 minutes, immediately downstream of the global response regulatory gene, *lrp*. The promoter for *ftsK* has not yet been assigned. Here, it is reported that *ftsK* is transcribed from two promoters; the first is located within the *lrp* reading frame and is dispensable whilst the second is essential and corresponds to *dinH*, previously characterized as an SOS promoter regulated by LexA. *ftsK* is the first essential gene to be described that is controlled by an SOS-inducible promoter. A possible mechanism by which *dinH* may be activated in *recA* minus strains, or in strains with uncleavable LexA, is discussed.

Keywords: cell division, FtsK, *dinH*, LexA, transcription

INTRODUCTION

Polymerization of the tubulin-like GTPase FtsZ into a cytokinetic ring at midcell has been identified as one of the earliest steps in the formation of the bacterial division septum (Bi & Lutkenhaus, 1991). The mature septation complex comprises the FtsZ ring associated with an ordered complement of accessory division proteins, including the recently characterized FtsK. Immunofluorescence studies have provided detailed evidence of the order in which the division proteins are recruited to the division site. Thus, ZipA and FtsA target the Z-ring shortly after its formation, with FtsK localizing immediately after this (Addinall & Lutkenhaus, 1996; Addinall *et al.*, 1997). *ftsK* does not belong to the *dcw* cluster at 2 minutes on the *Escherichia coli* chromosome (Vicente *et al.*, 1998), but maps to 20 minutes, next to the leucine-responsive regulatory gene *lrp*.

The *ftsK* gene encodes a 1329 amino acid protein that is essential for septation (Begg *et al.*, 1995; Wang & Lutkenhaus, 1998). Sequence analysis suggests that the gene encodes a tripartite protein with strong sequence similarity in its C terminus to a family of DNA-translocating proteins that includes *Bacillus* SpoIIIE. Significantly, only the N-terminal hydrophobic domain (the first 202 amino acids) is essential for cell division (Diez *et al.*, 1997; Yu *et al.*, 1998a; Draper *et al.*, 1998), whilst a role in chromosome partition and the resolution of chromosome dimers has recently been suggested for the C terminus (Liu *et al.*, 1998; Yu *et al.*, 1998b; Steiner

et al., 1999; Recchia *et al.*, 1999). The N-terminal domain is predicted to contain four membrane-spanning regions, as does SpoIIIE, which are likely to localize FtsK to the division site (Begg *et al.*, 1995). Localization of FtsK to the septum is dependent on the prior localization of FtsZ and FtsA, but not on localization of FtsI or FtsQ nor, by inference, FtsN (Yu *et al.*, 1998a). Cells depleted in FtsK form smooth-sided filaments, which in combination with a *rodA* mutation exhibit the swollen, partially constricted filaments typical of division block that occurs after formation of the Z-ring (Begg & Donachie, 1985).

Whilst the *ftsK* promoter has not yet been identified, an SOS-inducible promoter designated *dinH* (damage inducible; Lewis *et al.*, 1992) has been located within the 134 bp upstream gap between *ftsK* and the global regulator gene *lrp*. Transcription of the SOS regulon is controlled by the LexA repressor (Little & Mount, 1982), which binds with high affinity to a consensus operator, the LexA box (Berg & Von Hippel, 1987), located within or very close to the promoter. RecA is activated in the presence of ssDNA (Anderson & Kowalczykowski, 1998) and greatly enhances LexA self-cleavage (Slilaty & Little, 1987) to allow high level expression of the SOS proteins. As DNA damage is repaired, LexA autodigestion stops and the system returns to a quiescent state in which the SOS promoters are repressed.

The SOS response has been shown to induce *dinH* promoter activity and FtsK expression is increased in a *recA*⁺*lexA*⁺-dependent manner during the SOS response

(Wang & Lutkenhaus, 1998). We present experimental evidence that *dinH* is the primary *ftsK* promoter during normal cell growth. Primer extension, RT-PCR and site-directed mutagenesis confirmed the location of the primary *ftsK* promoter ($P1_{ftsK}$, previously designated *dinH*) and indicated the presence of a second promoter, $P2_{ftsK}$, within *lrp*. Transcriptional fusions demonstrated that transcription of *ftsK* from $P1_{ftsK}$ is *recA*-independent and occurs in the absence of DNA damage, whilst complementation data indicated that *dinH* is essential for *ftsK* transcription during the division process.

METHODS

Bacterial strains and growth conditions. *E. coli* DH5 α (*recA1*; Low, 1968), was used to harbour all fusion vectors; *E. coli* TG1 is *recA*⁺ *r*_k[−] *m*_k[−]. *E. coli* C600/44/*pcn*, obtained from K. Begg, was constructed by transferring the temperature-sensitive *ftsK44* mutation from TOE44 (AB2497 *ftsK44*; Begg *et al.*, 1995) and the plasmid-copy-number mutation (*pcn*) into the C600 background. Strains were grown overnight in Luria–Bertani (LB) medium at 37 °C with the exception of C600/44/*pcn*, which was grown at 30 °C. Where necessary, media were supplemented with ampicillin (100 µg ml^{−1}) and X-Gal (40 µg ml^{−1}). Assays for β -galactosidase were performed and the specific activity was calculated as described by Miller (1972). Comparison of promoter activities showed negligible fluctuations between constructs prepared in different experiments and the data presented are means of three different experiments from two separate isolates. Complementation tests were carried out on L broth (no salt) plates supplemented with 50 µg thymine ml^{−1} and appropriate antibiotics. The *lrp* coding sequence of the strain CV1008 (Haney *et al.*, 1992) was amplified, cloned into pUC18 and sequenced by Cambridge BioScience.

Construction of transcriptional fusions. The reporter vector pDDZ100 was constructed by fusing *lacZ* in-frame with the

first 38 amino acids of the promoterless chloramphenicol acetyl transferase (CAT) gene in pKK232-8 (Brosius, 1984). The vector retains translational stops in all three reading frames between the polycloning site and the *lacZ* gene, ensuring that cloned inserts are in transcriptional fusion with *lacZ*. Whilst transcription of *lacZ* is dependent on the introduction of a promoter within a cloned insert, it is translated from its own RBS, independent of the insert.

Construction of *ftsK* reporter vectors for complementation studies. The parental plasmid pDDK20 was constructed using oligonucleotides P1*Bam*HI and Frag1P2*Sal*I to amplify the upstream regulatory region and the first 337 codons of *ftsK*. Plasmids with upstream deletions were derived by restriction digestion at internal sites.

Site-directed mutagenesis. Site-directed mutagenesis of *ftsK* was carried out using the *Pfu* protocol described by Stratagene. Oligonucleotides Δ −10 P1 and Δ −10 P2 deleted the *dinH* −10 consensus and generated pDDK30 and pDDK31, whilst oligonucleotides −35 P1 and −35 P2 modified the −35 sequence to generate pDDK35 and pDDK36. Constructs generated by site-directed mutagenesis were sequenced by Cambridge BioScience to verify the accuracy of the procedure. Mutated inserts were excised from the vector and recloned into pDDZ100 to ensure that secondary mutations in *lacZ* were not responsible for the changes observed in their activity.

RT-PCR and primer extension analyses. Total RNA was extracted from DH5 α , treated with DNase I, precipitated after extraction with phenol/chloroform and resuspended in ultra-pure water. RNA was incubated at 60 °C for 1 h, then, with the radiolabelled oligonucleotides, for 20 min at room temperature. Primer extensions were performed at 48 °C for 45 min in the presence of 1 unit AMV reverse transcriptase. Extension products were resolved on an 8% denaturing polyacrylamide gel alongside ϕ X174/*Hinf*I size standards.

RT-PCR was performed on cDNA synthesized from total DH5 α mRNA. Forward primers were RT0, RT1, RT2, RT3 and RT4. Reverse primers were EXT1, EXT2 and EXT3 (Table 1, Fig. 2). PCR was also carried out on mRNA

Table 1. Primers used

Primer	Sequence (5′–3′)	Position of 3′ end relative to <i>ftsK</i> TTG (bp)
P1 <i>Bam</i> HI	CAAGGCAACGGGATCCTCACCG	−753
P4 <i>Hind</i> III	CCAGGTTACAAGCTTTTCCTG	−31
Frag1P2 <i>Sal</i> I	ACAGGGTCGACCGGCGCAGCCAG	+995
Δ −10 P1	CGGCGTATTTTGATCCTGTTAATCCATACAGC	−78
Δ −10 P2	GCTGTATGGATTAAACAGGATCAAAAATACGCCG	−115
−35 P1	CTAACACGGAACAGATCCAGAATCGGCGTATTTTG	−104
−35 P2	CAAAATACGCCGATTCTGGATCTGTTCCGTGTTAG	−138
EXT1	CCTGGCTCAAGAAAGGCTCTCCAG	−14
EXT2	CAGGACAATAAGGATCAGCAACCG	+74
EXT3	CTGTGTAGCAGTGGTTGTAGCG	+457
RT0	CGCGTGCCGATATGTCAG	−252
RT1	GGAACAGGTGCAAAATCGGC	−112
RT2	CAAGCAGAGTAATCGTCTGG	−152
RT3	CACGGACATACGTTGTTATG	−180
RT4	GAGTGTCATTTAGTATCCGGT	−298
EXT8	TCCATTTTAGCTTCCTTAGCTCCTG	On plasmid sequence

preparations to ensure the absence of contaminating chromosomal DNA and on *E. coli* TG1 DNA to confirm the amplification conditions.

Gel shift assay. A 160 bp DNA fragment carrying the LexA-2 box was prepared using P1*Bam*HI labelled with [γ -³²P]ATP and P4*Hind*III. The PCR fragment was digested with *Bgl*II to release the end-labelled fragment. Reaction mixtures (20 μ l each) containing approximately 100 ng labelled probe, excess competitor DNA (PCR-generated fragments lacking a recognizable LexA binding site) and *E. coli* TG1 crude extract were incubated with or without anti-LexA antibodies (100 ng) at room temperature for 20 min in binding buffer (50 mM Tris/HCl, 5 mM EDTA, 5% glycerol, 0.2 mg BSA ml⁻¹). Protein-DNA complexes were separated in native polyacrylamide gels. Gels were dried and subsequently exposed to X-ray film.

RESULTS

Construction of a plasmid complementing *ftsK44*

Previous work (Begg *et al.*, 1995) has demonstrated that, in addition to the first 1170 bp of *ftsK*, a 750 bp fragment of DNA upstream of the gene is required to complement the *ftsK44* chromosomal mutation. The 750 bp sequence includes a short non-coding sequence upstream of the *lrp* gene, the *lrp* gene itself, and a further 134 bp non-coding region immediately upstream of the *ftsK* reading frame (Fig. 1). Deletion of the first 154 bases of this sequence (the non-coding region upstream of *lrp* and the first 30 bp of *lrp*) abolishes complementation of the *ftsK44* mutant, whilst deletion of most of *lrp* has no effect on complementation. From these results, it was concluded that the 154 bp *Acc*I-*Bgl*II fragment upstream of *lrp* was required for transcription of *ftsK* and that the two genes were likely to be co-transcribed.

These preliminary conclusions were not fully supported by data from other studies. For example, it has been reported that a transposon insertion in *lrp* had no effect on cell division (Haney *et al.*, 1992); also, the *lrp* promoter has been shown to lie 177 bp upstream of the *Acc*I-*Bgl*II fragment. To examine the patterns of expression that might be expected *in vivo*, we investigated transcription from plasmids containing part of the *ftsK*

coding sequence and varying amounts of upstream DNA.

A plasmid (pDDK20) was constructed that contained 1011 bp of the *ftsK* ORF and 750 bases of upstream DNA, sufficient to complement *ftsK44* and provide an *in vivo* assay for functional FtsK expression (Fig. 1). Translational stops present in the vector between the end of *ftsK* and the *lacZ* cassette ensure independent translation of the gene products. This plasmid exhibits a β -galactosidase activity of 2600 Miller units. The DNA in pDDK20 was deleted between the *Acc*I site and a *Sex*AI site 750 and 50 bp, respectively, upstream of *ftsK* to allow the *ftsK* coding sequence to be tested in isolation. β -Galactosidase activity from this plasmid (pDDK21) was just 100 units and, crucially, this construct does not complement the *ftsK44* mutant. The basal activity of pDDK21 was subsequently subtracted from the values obtained from the other pDDK derivatives to standardize their activities.

Location of a dispensable promoter (P_{2_{ftsK}}) for *ftsK* inside *lrp*

RT-PCR of chromosomal RNA was used to identify mRNA species transcribed from the *Acc*I/*ftsK*' sequence (Fig. 2). Primers EXT1, EXT2 and EXT3 (Fig. 2a), generate the predicted PCR products when used in combination with forward primers RT0, 1, 2 and 3 (Fig. 2b), indicating that a transcript initiates from sequences upstream of RT0, beyond the *Mlu*I site. In contrast, no product was generated using RT4. The mRNA therefore must initiate from a position between RT0 and RT4 in *lrp*, less than 40 bp upstream of the *Mlu*I site. The RT-PCR results also indicate that the *Acc*I/*Bgl*II fragment does not contain a promoter and that *lrp* and *ftsK* are not cotranscribed. Primer extension analysis was performed on mRNA extracted from DH5 α cells transformed with plasmid pDDZ3 (Fig. 1) containing the *Bgl*II/*Mlu*I sequence to allow enrichment of P2-specific mRNA. The primer EXT8 (Fig. 2d) generates a cDNA of between 85 and 90 bases in length (Fig. 2c). This locates the start of the mRNA to between 18 and 23

Fig. 1. *ftsK/lacZ* complementation vectors. The 5' end of *ftsK* and its upstream DNA was cloned in pDDZ100 to give pDDK20. Deletions of pDDK20 generated the derivatives shown. The P1_{ftsK} promoter (*dinH*) is shown as a striped rectangle. The *lacZ* gene is shown as a rectangle shaded with squares. The β -galactosidase activities (' β -Gal'; Miller units) of pDDK and pDDZ vectors shown were standardized against the pDDK21 control (set to zero units), and the ability of each construct to complement the C600/44*pcn* mutant [*ftsK*(ts)] ('Comp') is also indicated. NA, Not applicable.

Fig. 2. Localization of the non-essential promoter *P_{2ftsK}*. (a) Position of RT-PCR and primer extension oligonucleotides used. The linear map depicts the *ftsK* gene and its regulatory region (not drawn to scale). The position of each primer is indicated by an arrow and the location of the 3' end of each primer is given relative to the *ftsK* start codon. A band of around 45 bp is also present in the RT-PCR products. Since a similar-sized product is found with other primers of the same length but which initiate at different positions, these bands are likely to represent primer concatemers. (b) Table showing the primer combinations used in RT-PCR reactions. Plus or minus symbols indicate the presence or absence, respectively, of the predicted PCR product. (c) Primer extension of mRNA extracted from DH5 α transformed with pDDZ3. ϕ X174/HinfI size standard (lane 1) and the extension product (lane 2) were run on an 8% denaturing polyacrylamide gel. The position of the transcript is indicated by an arrow with the estimated size of the transcript given above. (d) Diagram showing part of the sequence of pDDZ3. *lrp* sequence is shown in lower case, the plasmid sequence is capitalized, and the *Hind*III fusion site is in bold. The start site of the transcript lies between the nucleotides marked with an asterisk (boxed). The consensus -10 and -35 sequences for the *P_{2ftsK}* promoter are indicated by lines above the sequence and the EXT8 binding site is shown by an arrow.

bases from the *Mlu*I site, in keeping with the RT-PCR results. The presence of well-conserved -35 and -10 sequences upstream of the predicted mRNA start suggests that this is the location of the *P_{2ftsK}* promoter (Fig. 2d).

A LexA-controlled promoter regulates expression of *ftsK*

The above results identify a promoter for *ftsK* within *lrp*, but previous work (Begg *et al.*, 1995) has demonstrated that the *Bgl*III/*Mlu*I region that would contain this promoter can be deleted without affecting *ftsK* transcription. Primer extension was therefore used on total cellular mRNA to reveal whether shorter trans-

cripts, obscured in the RT-PCR analysis, were initiated within the sequences upstream of *ftsK*. When annealed at the *ftsK* initiation codon (Fig. 3b), the primer EXT1 generates a single cDNA product of between 93 and 96 bp in length (Fig. 3a). The 5' end of this mRNA delimits the inducible promoter that transcribes *ftsK*, *P_{1ftsK}*. Unexpectedly, this locates the 5' end of the *P_{1ftsK}* transcript to within 12 bp of the consensus -10 sequence previously defined for the SOS-inducible promoter, *dinH*. Although the transcription studies were carried out in a *rec* minus background, which precludes SOS-induced transcription from *P_{dinH}*, we still considered the possibility that transcription of *ftsK* might initiate from *P_{dinH}* and that *P_{dinH}* and *P_{1ftsK}* represent the same sequence.

Fig. 3. Localization of the primary *ftsK* promoter. (a) Primer extension of total DH5α mRNA incubated with EXT1 end-labelled with [³⁵S]ATP_γS by standard procedures. The extension product (lane 1) and ϕ X174/*Hinf*I size standard (lane 2) were run on an 8% denaturing polyacrylamide gel. The position of the signal corresponding to the *ftsK* transcript is indicated by an arrow. (b) The approximate start of the mRNA, calculated from the primer extension result, lies between the nucleotides indicated by asterisks. The arrow shows the position of the EXT1 primer. The -10 and -35 consensus sequences for the *dinH* promoter are indicated by lines above the sequence and the LexA operator is boxed. The *ftsK* coding sequence is capitalized.

We constructed a set of plasmids modified within the *dinH* promoter (Fig. 4). Deletion of the *dinH* -10 sequence (pDDK30) resulted in a 90% reduction in transcription of *lacZ* compared to the unmodified sequence in pDDK20 (2600 ± 266 Miller units; Fig. 1). The remaining 275 units of activity in pDDK30 were contributed primarily from P2_{*ftsK*}, as deletion of P2_{*ftsK*} by *Bgl*II/*Mlu*I digestion of the plasmid reduced transcription to just 47 units (pDDK31). Modification of three nucleotides within the *dinH* -35 sequence caused a dramatic reduction in transcription (pDDK35 and pDDK36). None of the derivatives complemented C600/44/*pcn*. These results provided strong evidence that the *dinH* promoter is essential for transcription of the *ftsK* gene during unperturbed cell growth.

To evaluate the contribution of the two putative promoter regions P1 and P2 in *ftsK* transcription shown by pDDK20, we constructed pDDK22 (deleted for P2) and pDDK24 (deleted for P1). The 1845 units of transcription in pDDK22 derive solely from P1 and represent 70% of *ftsK* transcription. The 755 unit (30%) reduction in β -galactosidase activity from pDDK22 can be attributed to P2 as the *Bgl*II/*Mlu*I sequence in isolation gives a β -galactosidase activity of 1041 (pDDZ3). In the absence of P1, transcription from P2 in pDDK24 is reduced to levels that are insufficient to complement the C600/44/*pcn* strain (102 units) whilst both pDDK20 and pDDK22 complement this mutant.

The construct pDDK23 (Fig. 4) lacks both P1 and P2 and does not express *lacZ* to assayable levels or complement C600/44/*pcn*, providing additional confirmation that there is no promoter within the *Acc*I/*Bgl*II fragment. These data show that P1 (*dinH*) is the principal *ftsK* promoter. P2 does not transcribe *ftsK* to significant levels except in the presence of P1; consistent with the *Bgl*II/*Mlu*I sequence being dispensable, as noted by Begg *et al.* (1995).

The *Acc*I/*Bgl*II region is essential for *ftsK* transcription and binds LexA

Expression of genes controlled by LexA has a genetic dependence on *recA* function, yet our observations show transcription of *ftsK* from *dinH* in a *recA* minus background. Moreover, the existence of non-cleavable LexA mutants such as LexA3 indicates that activation of *dinH* occurs by a mechanism other than *rec*-induced LexA cleavage. This is not without precedent; Dri & Moreau (1994) reported that transcription of LexA-regulated genes could be increased without LexA cleavage by inducing changes in the intracellular pH.

Despite the absence of a promoter within the *Acc*I/*Bgl*II DNA sequence [confirmed by RT-PCR and the absence of detectable levels of β -galactosidase activity in pDDZ5 (Fig. 1)], deletion of this region results in the abolition of transcription from the downstream promoters (Fig. 4, pDDK26). This was unexpected and suggests that there

Fig. 4. Site-directed mutagenesis of the *dinH* promoter consensus. The nucleotide sequence of the *dinH* region is illustrated. The -10 and -35 consensus sequences are capitalized and the LexA operator is boxed. Site-directed mutagenesis was used to modify three bases within the -35 sequence (shown in bold), and to delete the -10 region (indicated). The position of the modified sequences are shown on the plasmid derivatives by a filled triangle (-10 deletion) and by an open rectangle (modified -35). The *lacZ* gene is shown as a rectangle shaded with squares. β -Galactosidase activities and complementation data are given as in Fig. 1.

Fig. 5. Gel shift assays showing LexA binding to the *AccI*/*BglII* fragment. (a, b) The labelled *AccI*/*BglII* PCR fragment was incubated with *E. coli* TG1 crude extract in the absence (lane 2) or in the presence (lanes 3 and 4) of 100 ng anti-LexA antibodies. The complexes were separated in a 6.5% polyacrylamide gel. Lane 1 is the control, free DNA probe (P) without extract or antibodies. C1 indicates the primary complex of DNA-LexA whilst C2 indicates the secondary (supershift) complex of DNA-LexA-anti-LexA. When the ratio of TG1 extract to competitor DNA is increased (lane 4), only C2 is detected. (c) The primary shift is best seen on a 5% polyacrylamide gel (lanes 5 and 6). The presence or absence of protein extract and anti-LexA antibodies is indicated by plus and/or minus symbols under each lane.

may be a regulatory element present within the *AccI*/*BglII* sequence.

Closer examination of the *AccI*/*BglII* region reveals a 19 bp motif, 5'-CTG AAcAgTcATgTTT CAG-3' (uppercase letters represent bases which show identity

with the LexA operator consensus; bold letters represent the invariable palindrome found within the LexA operator sequence), from -671 to -689 bp that shows similarity to the consensus sequence of a LexA operator. We will refer to this sequence as LexA-2, and the LexA box associated with *dinH* as LexA-1. As the homology

to the consensus LexA operator is only partial, we carried out gel shifts to assay directly for LexA binding. The 160 bp *AccI/BglII* sequence containing the LexA-2 site was radiolabelled and incubated with *E. coli* TG1 protein extract, then subjected to electrophoresis in a 6.5 % polyacrylamide gel. Fig. 5(a) shows the retardation of the LexA-2 operator sequence (lane 2) compared to the mobility of the free probe (lane 1). The shift is also shown on electrophoresis in 5 % polyacrylamide (Fig. 5c, lane 5) where the retardation can be seen more clearly. The presence of LexA within the primary retardation complex was confirmed by carrying out the binding reactions in the presence of anti-LexA antibodies. The specific interaction of anti-LexA antibody with the DNA–LexA complex results in the further retardation or ‘supershift’ seen in lanes 3 and 4. Western analysis of the gel shifts confirmed the presence of LexA in both the primary and secondary retarded complexes. No comparable retardation was observed when the binding reactions were performed with a control protein extract prepared from a *lexA* null mutant strain (data not shown). These results demonstrate that the LexA-2 operator-like sequence can be recognized and bound by LexA.

DISCUSSION

The results of this study demonstrate that the cell division gene *ftsK* is transcribed from two promoters. $P2_{ftsK}$ is dispensable under our experimental conditions, and is located within the *lrp* coding sequence, whilst $P1_{ftsK}$ is essential and corresponds to *dinH*, an SOS-inducible promoter located upstream of *ftsK* (Lewis *et al.*, 1992). The intracellular concentration of FtsK increases on induction of the SOS response (Wang & Lutkenhaus, 1998), and it has been suggested that this may be linked with its proposed role in chromosome partition under these conditions (Liu *et al.*, 1998; Yu *et al.* 1998b). This study establishes that *dinH* ($P1_{ftsK}$) is not activated exclusively in response to SOS stimuli, but that it is also active during unperturbed growth in cells that lack RecA protein. As a consequence, *ftsK* is the first essential gene to be identified that is under the direct control of an SOS promoter.

The LexA repressor controls transcription of the genes of the SOS regulon. LexA binds with high affinity to a consensus operator, the LexA box, located within or very close to the promoter of each SOS gene (Berg & Von Hippel, 1987). Once activated, RecA enhances LexA self-cleavage to prompt high level expression of the SOS proteins (Slilaty & Little, 1987). As DNA damage is repaired, LexA autodigestion stops and the system returns to a quiescent state in which the SOS promoters are repressed. In this study, gene expression was monitored by assaying for β -galactosidase in a DH5 α *rec* minus background, which does not support a mechanism for LexA-mediated regulation of *ftsK*. Whilst it is possible that basal transcription from LexA-repressed *dinH* may supply sufficient *ftsK* to support cell division, an alternative explanation is that *ftsK* is regulated by a RecA-independent mechanism.

Plasmid pDDK26 carries the *dinH* promoter and produces only 13 units of β -galactosidase, about 0.5 % of the level produced by pDDK20 and insufficient to allow complementation of C600/44/*pcn*. If basal transcription of *ftsK* was being initiated from *dinH*, it might reasonably be expected that this construct would exhibit enzyme levels comparable to pDDK20. The very low enzyme levels associated with this plasmid are more likely to reflect that LexA binds with high affinity to the *dinH* promoter, in keeping with the observation that LexA-1 is highly homologous to the *sfiA* LexA operator, which is known to bind LexA very strongly (Preobrajenskaya *et al.*, 1994). Furthermore, strains containing uncleavable LexA do not exhibit the expected filamentation phenotype, suggesting that even when LexA is irreversibly bound to its operators, *ftsK* transcription is unaffected and cell division proceeds normally. Rec-independent regulation, whilst novel, fits better with the experimental observations. Closer examination of the *AccI/BglII* region reveals a sequence within it that shows similarity to the LexA consensus operator motif and that this putative operator (LexA-2) is able to bind LexA *in vitro*. This suggests a potentially straightforward mechanism that would account for the requirement of the promoterless *AccI/BglII* region in the Rec-independent regulation of *ftsK*. According to this model, LexA would ordinarily repress *ftsK* expression by binding its cognate operator at *dinH*, transient displacement of LexA from LexA-1 to LexA-2, in response to an uncharacterized cellular signal, would free *dinH* and allow transcription of *ftsK* in response to the cell's requirement for FtsK protein. The poorer homology of LexA-2 to the consensus binding motif might reflect the lower differential binding affinity of the site for LexA and ensure that the repressor binds preferentially to LexA-1 at *dinH*.

A second promoter, $P2_{ftsK}$, lies in the *lrp* coding sequence and is able to transcribe *ftsK*. In the absence of SOS induction, transcription from $P2_{ftsK}$ alone is insufficient to support cell division in C600/44/*pcn*. $P2$ transcripts were only detected by RT-PCR or with primer extension with mRNA extracted from a strain transformed with a multicopy plasmid containing the *lrp* coding sequence. This suggests that $P2$ mRNA is either expressed at a very low level or that it has a rapid turnover. It seems likely that this promoter is dispensable, although it is not clear if it might have a role under other growth conditions; its presence within the *lrp* ORF may be of importance in *lrp* expression studies.

In the original isolation of *ftsK* (Begg *et al.*, 1995), the promoter for *ftsK* was tentatively located within the *AccI/BglII* sequence upstream of *lrp*, suggesting that *lrp* and *ftsK* might be cotranscribed. However, this was challenged by the existence of strain CV1008 which, despite having transposon Tn10 inserted within the *lrp* coding region, had no apparent cell growth defect (Haney *et al.*, 1992). We used PCR to accurately locate the position of the transposon within CV1008 and found, unexpectedly, that it does not lie within *lrp*, but is located upstream of it, close to the *lrp* promoter.

Sequencing revealed that a 49 bp insertion sequence corresponding to part of the IS10-L element of the transposon remains within the *lrp* reading frame, (the insertion introduces a stop codon at the 78th codon). It is not possible to determine when this second transposition event occurred, but since *ftsK* synthesis is likely to have been restricted with the transposon in its original location, transposed derivatives might be expected to have a selective advantage. The transposition event may have occurred shortly after its initial isolation since the phenotype of the strain, *lrp* minus, would appear unchanged. The 49 bp CV1008 insertion and the 300 bp deletion in pPDDK22 show that the LexA-2 box can be relocated relative to *dinH*/LexA-1 without significantly altering *ftsK* expression. However, there is an upper limit to the distance over which the two LexA sites are functional; complementation is lost when LexA-2 is positioned 3 kb from *dinH*/LexA-1 (data not shown). The loss of the transposon from *lrp* in CV1008 may reflect this. The insertion of an intact Tn10 in *lrp* would impair *ftsK* transcription and allow selection of a derivative in which the LexA boxes are within functional distance. Additionally, the LexA-2 box does not function in *trans* since a chromosomal copy of LexA-2 does not compensate for the absence of the region on a plasmid.

Finally, these results may have implications for the regulation of the genes in the *dcw* cell division cluster. Three LexA-binding sites have been identified within the first three genes at the 5' end of the gene cluster; two lie upstream of *mraW* whilst the third lies close to the promoter for *ftsL* (Ishino *et al.*, 1989; Vicente *et al.*, 1998; Gomez, 1991). The arrangement of SOS box I and SOS box II lying upstream of the *mraW* initiation site is of particular note, since it resembles the arrangement seen in the *ftsK* regulatory region.

This report demonstrates that LexA has an important and unexpected general role in cellular regulation. Our results support a model for the regulation of *ftsK* by LexA in a *rec*-independent manner. Future experiments will characterize the molecular basis of the regulation and examine the significance of LexA control with respect to cell division gene expression in normal growth conditions, and on SOS induction.

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