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Division genes in *Escherichia coli* are expressed coordinately to cell septum requirements by gearbox promoters

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The cell division *ftsQAZ* cluster and the *ftsZ*-dependent *bolA* morphogene of *Escherichia coli* are found to be driven by gearboxes, a distinct class of promoters characterized by showing an activity that is inversely dependent on growth rate. These promoters contain specific sequences upstream from the mRNA start point, and their −10 region is essential for the inverse growth rate dependence. Gearbox promoters are essential for driving *ftsQAZ* and *bolA* gene expression so that the encoded products are synthesized at constant amounts per cell independently of cell size. This mode of regulation would be expected for the expression of proteins that either play a regulatory role in cell division or form a stoichiometric component of the septum, a structure that, independently of cell size and growth rate, is produced once per cell cycle.

Key words: cell division/*Escherichia coli*/*ftsQAZ* cluster/gearbox promoter/inverse growth rate dependence

Introduction

Division is a cellular process in which very accurate controls of timing and positioning work together to ensure the appropriate partition of cellular components. The molecular nature of these controls has remained largely unknown. We find that at least four genes, involved in cell division and morphogenesis, are under the control of gearboxes, a name that we propose to designate promoter sequences that ensure that the products under their control are produced at fixed amounts per cell independently of the particular growth rate of the cell.

The cluster of genes located at the 2.5 min region of the standard *Escherichia coli* genetic map has been identified by the block in cell division caused by mutations. In this cluster the *fts* genes have an essential role in the initial (*ftsQ* and *ftsZ*) and final (*ftsA*) steps of septation (Donachie *et al.*, 1984). The gene product of *ftsA* is a structural component of the septum (Tormo and Vicente, 1984) that interacts with PBP3 (Tormo *et al.*, 1986), the penicillin-binding protein required for septation. FtsA has been proposed as the key factor of a termination pathway, the TER pathway of cell division (Tormo *et al.*, 1985). The *ftsZ* gene product is the target of the inhibitory mechanism in cell division induced by the SOS response (Lutkenhaus, 1983; Jones and Holland, 1984), and overexpression of the *ftsZ* gene product leads to minicell formation (Ward and Lutkenhaus, 1985). Aside from being essential in cell division (Begg *et al.*, 1980), very little is known about the role played by the product of the *ftsQ* gene. While in normal cells spherical murein is only produced at the septum during cell division, overexpression of *bolA*, a morphogene located at 9.5 min of the *E. coli* genetic map, produces an *ftsZ*-dependent spherical morphology (Aldea *et al.*, 1988b).

The genetic structure of the *ftsQAZ* region reveals the existence of overlapping transcriptional units where promoters for each gene would be located in the corresponding upstream gene (Robinson *et al.*, 1984, 1986). Since no transcriptional terminators have been found, expression of the cluster could mainly be driven by additive transcription. A higher level of expression of the *ftsQAZ* cluster has been observed during cessation of growth and this fact has been attributed to the presence of promoters similar to those first identified in a plasmid-borne gene, *mcb* (Connell *et al.*, 1987), and in the *bolA* morphogene (Aldea *et al.*, 1989).

Here we report the identification and characterization of promoters in the *ftsQAZ* cluster. One of these promoters, located upstream from the structural gene of the first *fts* gene, is growth phase and growth rate dependent and shares some structural features with the −35 and −10 regions of the *bolA* promoter, which is also shown to be dependent on growth rate. We find that the −10 region of a gearbox is essential to maintain the inverse dependence of expression on growth rate.

Results

Transcription from the ftsQ and bolA promoters is inversely dependent on growth rate

We have recently shown that expression from the *ftsQ* promoter region is growth phase dependent and have proposed that this mode of regulation could be due to the presence of promoters similar to *bolA1p* (Aldea *et al.*, 1989). Promoter locations in the *ftsQAZ* cluster were previously unknown and had been inferred solely from analysis of the expression of cloned fragments in promoter-probe vectors (Robinson *et al.*, 1984, 1986; Yi *et al.*, 1985). From these data promoter activity has been attributed to a 0.7 kb *Pst*I−*Eco*RI fragment located upstream from *ftsQ*, and a 1.2 kb *Pvu*II−*Eco*RI fragment upstream from *ftsZ* (Figure 1). No significant promoter activity has been observed for the 1.0 kb *Eco*RI−*Pvu* II fragment which contains sequences immediately upstream from *ftsA*, suggesting that *ftsA* would mainly be expressed from the *ftsQ* promoters.

To analyze expression levels from the *ftsQ* and *bolA* promoter regions, we have used transcriptional fusions to *lacZ* in a lambda vector, which are described in Figure 1. Typically, β-galactosidase activity produced by the promoter region of *ftsQ* in λTGV13 increased 5- to 6-fold in the stationary phase compared to cultures growing exponentially. A transcriptional fusion of the *bolA1p* promoter to *lacZ* in λMAV103 that only contains 50 bp upstream from the

mRNA start point produced an 18-fold increase in stationary phase cultures, whereas *bolA* expression driven by its two promoters in λMAK400 (Aldea *et al.*, 1989) showed an intermediate increase of 7- to 9-fold. The increase in expression levels always started as soon as the growth rate began to fall below the maximum growth rate supported by the medium, suggesting that these promoters could in fact be regulated by the growth rate. Accordingly, β-galactosidase activity driven by the *bolA* promoters in λMAK400 decreased to 35% during a growth rate upshift from 1.1 to 2 doublings per hour (results not shown).

Table I shows the expression levels from *ftsQ* (λTGV13), *bolA* (λMAV103) and *lacUV5* (λRS74, Simons *et al.*, 1987) promoters during exponential growth at different growth rates. The *lacUV5* promoter has been shown previously to be growth-rate independent (Wanner *et al.*, 1977; Gourse *et al.*, 1986). β-Galactosidase activities produced by the *ftsQ* and *bolA* promoters increase at lower growth rates so that values obtained at 0.25 doublings per hour are almost equal to those corresponding to the stationary phase of growth in the richest medium. Moreover, the relative increase in β-galactosidase activity, plotted versus instantaneous growth rate during a transition to the stationary phase, attain similar values to those obtained from exponential cultures growing at different growth rates (Figure 2). Thus, expression from these promoters is in fact inversely correlated to growth rate, and the increase observed during the entrance into the stationary phase of growth would be a consequence of the gradual reduction in growth rate that takes place during this growth phase transition.

Since cell mass is directly correlated to growth rate in *E.coli* (Nanninga and Woldringh, 1985), any gene product maintained at constant amounts per cell should show an inverse dependence on growth rate. Results in Figure 2 indicate that transcription levels driven by the *ftsQ* promoter region agree with the curves predicted assuming that the encoded gene products are expressed at constant amounts per cell. Although expression from the *bolA1p* promoter exhibits a stronger dependence on growth rate, the two *bolA* promoters contained in λMAK400 when acting together produce a lower increase (7- to 9-fold) resembling that obtained from the *ftsQ* promoter region in λTGV13.

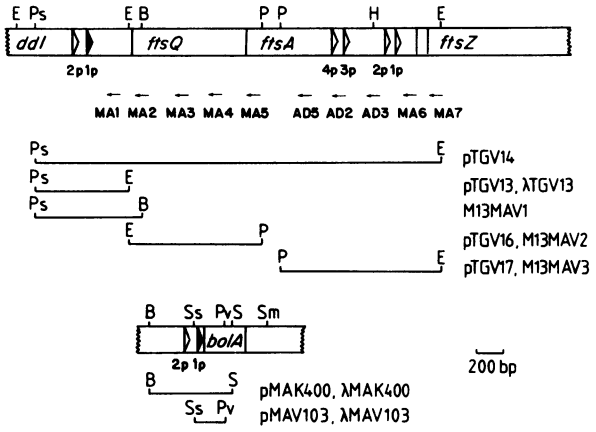

Fig. 1. Maps of the *ftsQAZ* cluster and the *bolA* gene. The top drawing shows the entire *ftsQAZ* cluster indicating the position of the open reading frames for each gene in the cluster. Arrowheads indicate the promoters identified in the present work. The *ftsQ1p* gearbox promoter is indicated by a black arrowhead. Small arrows symbolize primers used for promoter identification. The drawing at the bottom shows the *bolA* gene (Aldea *et al.*, 1989), and the position of the two *bolA* promoters is indicated by arrowheads. The *bolA1p* gearbox promoter is represented by a black arrowhead. Thin lines indicate fragments carried by plasmid and phage vectors used in this work (see Materials and methods for details of construction). Relevant restriction sites are abbreviated (B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; Ps, *Pst*I; Pv, *Pvu*I; P, *Pvu*II; S, *Sca*I; Sm, *Sma*I; Ss, *Ssp*I.

FtsZ and FtsA levels follow inverse growth rate dependence

Since no transcriptional terminators have been found within the *ftsQAZ* cluster, transcription from the *ftsQ* promoters should also affect expression of the downstream *ftsA* and *ftsZ* genes. Figure 3 shows the results of an experiment in which anti-FtsZ antibodies were used to quantify the amounts of FtsZ in total cell extracts obtained from strain W3110 growing exponentially at various growth rates and at different stages during a growth rate transition. Supporting the data obtained with transcriptional fusions of the *ftsQ* promoters to *lacZ*, FtsZ levels increase at lower growth rates following the same kind of dependence.

The amount of FtsA can only be clearly quantified by immunological methods in strains carrying the plasmid pZAQ (results not shown), which contains the whole *ftsQAZ* cluster and a ColE1 replicon. For this reason we measured FtsA and FtsZ levels in W3100 (pZAQ) during a growth phase transition obtaining comparable relative increases for both gene products (Figure 4). Although part of the observed increase (3- to 5-fold) can be ascribed to the increase in the copy number of pBR322 derivative plasmids during the entrance into the stationary phase (Lin and Bremer, 1986), it can be observed that FtsA and FtsZ when expressed in the same construction follow the same growth rate dependence.

Table I. Expression from the *ftsQ* and *bolA* promoters is inversely dependent on growth rate

Promoter region	Phage	β-Galactosidase units					
		LB R = 2.0	Caa R = 1.5	Glu R = 1.1	Gly R = 0.6	Ace R = 0.25	LB Sta
ftsQ	λTGV13	29	56	78	108	137	150
bolA1p	λMAV103	32	60	330	538	544	581
lacUV5	λRS74	958	1053	1201	986	1103	1181

RYC1000 cells lysogenized with phages carrying transcriptional fusions of the promoter regions and promoters indicated to *lacZ* were assayed for β-galactosidase activity as described in Materials and methods. Exponential cultures were grown at different growth rates (R in doublings per hour) in LB and various M9-based media supplemented with either casamino acids and glucose (Caa), glucose (Glu), glycerol (Gly) or acetate (Ace) as described in Materials and methods. Stationary cultures (Sta) were obtained in LB. β-Galactosidase values are the average of five independent assays and coefficients of variation were always <0.1.

The ftsQAZ cluster is driven by a gearbox promoter

We first attempted to map the mRNA start points in the *ftsQAZ* cluster by using an RNA donor strain W3110 transformed with pZAQ, which carries the whole *ftsQAZ* cluster. Despite the theoretical high sensitivity of the method used to map mRNA start points (see Materials and methods), no significant fragments resistant to S1 nuclease were observed. In order to avoid possible repression effects due to a higher gene dosage of the cluster, we decided to use strain RYC1000 transformed with plasmids pTGV13, pTGV16 and pTGV17 (Figure 1) as the RNA donors. These plasmids do not generate any of the cluster products and have the additional advantage of producing hybrid mRNAs where the 3′ end is provided by the *lac* genes fused to the cloned fragments, which could increase and standardize mRNA stability. Finally, the vector used to construct these plasmids includes four copies of a strong transcription terminator immediately upstream from the cloning sites so that read-through transcription from the vector promoters is efficiently blocked (Simons *et al.*, 1987). Figure 5 shows S1 nuclease protections obtained with *ftsQ* and *ftsZ* transcripts when using plasmids pTGV13 and pTGV17 as RNA donors at different stages of a growth phase transition. Two promoters upstream from *ftsQ* and four promoters upstream from *ftsZ* were identified, all of them being found within the open reading frames located immediately upstream from these genes. All promoters identified when using pTGV13 and pTGV17 as RNA donors were also detected when using plasmid pTGV14 (results not shown). It can be seen that transcription from *ftsQ2p* and the four *ftsZ* promoters is almost constant regardless of growth rate. On the other hand, *ftsQ1p* is clearly induced when growth rate decreases during the growth phase transition. This promoter, like *bolA1p* (Aldea *et al.*, 1989), shows a clear induction as soon as the growth rate drops below the maximum value attained during

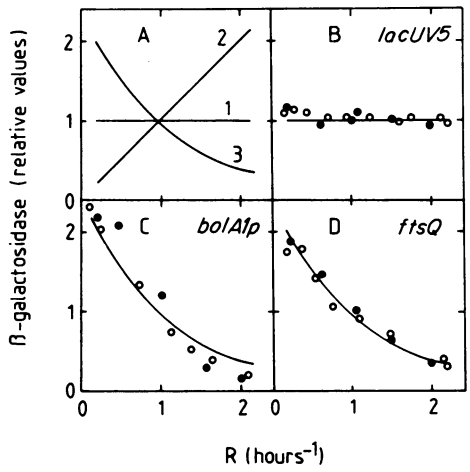

Fig. 2. Inverse growth rate dependence of the *ftsQ* and *bolA* promoters. Gene expression dependence on growth rate may be divided into the three main theoretical classes shown in panel (**A**). (1) Regular promoters produce constant amounts per cell mass independent of growth rate (R). (2) rRNA promoters are more active at higher growth rates so that rRNA content per mass is proportional to growth rate (Gourse *et al.*, 1986). (3) Since cell mass (M) is an exponential function of growth rate ($M = M_0 \times e^{bR}$; M_0 is the cell mass at $R = 0$; $b = 0.85$ for the *E.coli* K-12 strains used), any protein being synthesized at constant amounts per cell would show relative contents (P) to cell mass being inversely dependent on growth rate ($P = P_0 \times e^{-bR}$; P_0 is the relative content at $R = 0$). Other panels show relative β-galactosidase activities produced by the *lacUV5* (panel **B**) and *bolA1p* (panel **C**) promoters and the *ftsQ* promoter region (panel **D**) fused to *lacZ* in phages listed in Table I. Closed circles indicate values obtained from exponential cultures growing in different media (see Table I and Materials and methods). Open circles represent relative values obtained during a transition to the stationary phase in LB medium, which are plotted versus instantaneous growth rate. β-Galactosidase activities were made relative by regression to that corresponding to R = 1.

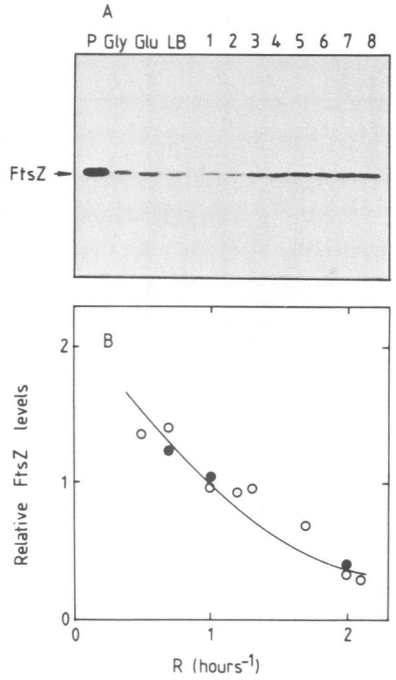

Fig. 3. FtsZ contents are inversely dependent on growth rate. Panel (**A**) shows a Western type blot in which equal amounts of total cell extracts (corresponding to 0.25 OD_{600} units) were electrophoresed in a 10% SDS–polyacrylamide gel and FtsZ was detected with antiserum MVJ4 by the immunoblotting methods described in Materials and methods. W3110 transformed with pZAQ (lane P) was grown exponentially in LB and used to identify FtsZ. W3110 was grown exponentially in LB (lane LB, R = 2) and minimal media supplemented with glucose (lane Glu, R = 1) or glycerol (lane Gly, R = 0.7) as described in Materials and methods. Lane numbers refer to W3100 grown in LB during a transition from the exponential to the stationary phase in order to obtain various instantaneous growth rates (1, R = 2.1; 2, R = 2.0; 3, R = 1.7; 4, R = 1.3; 5, R = 1.2; 6, R = 1.0; 7, R = 0.7; 8, R = 0.5). Panel (**B**) shows the relative FtsZ levels plotted versus growth rate in exponential cultures (closed circles) and during the growth phase transition (open circles). Bands from panel (A) were quantified by densitometry and the values obtained were made relative by regression to that corresponding to R = 1.

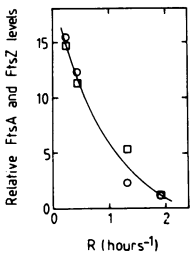

Fig. 4. FtsA and FtsZ levels follow similar inverse growth rate dependence. W3110 transformed with pZAQ was used to quantify FtsA (open squares) and FtsZ (open circles) levels during a transition from the exponential to the stationary phase as described in Figure 3. Values obtained from bands corresponding to FtsA and FtsZ were made relative to the levels found in the exponential phase (R = 1.9).

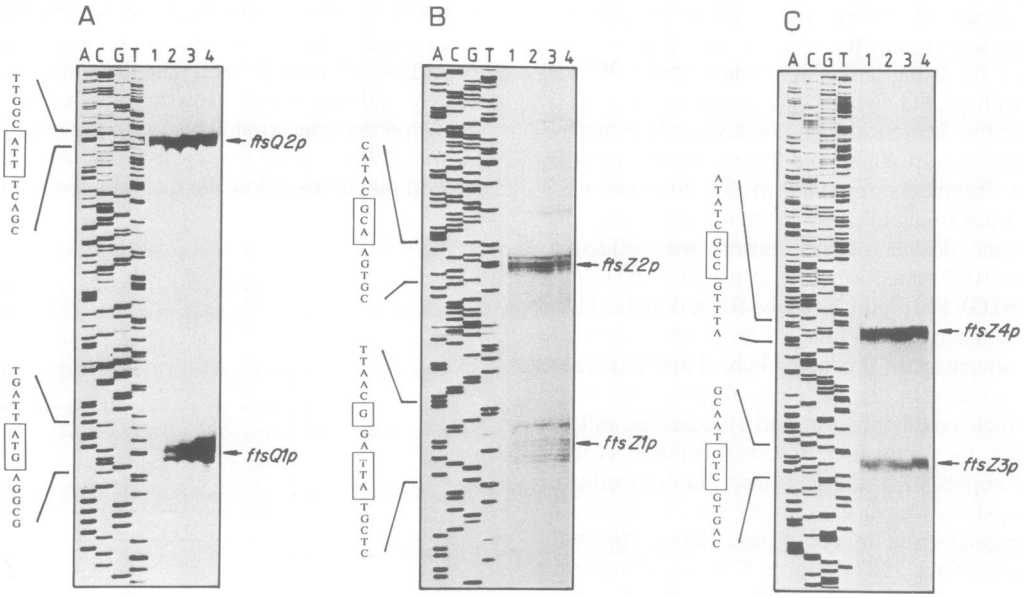

Fig. 5. Promoter mapping in the *ftsQAZ* cluster. Promoters were mapped as described in Materials and methods. *ftsQ1p* and *ftsQ2p* (**A**) were identified by using strain RCY1000 (pTGV13) as RNA donor and DNA probes and sequencing ladders were synthesized with M13MAV1 and primer MA1. All *ftsZ* promoters were mapped by employing RYC1000 (pTGV17) as RNA donor. Sequencing ladders and DNA probes were obtained from M13MAV3 and primer MA6 to map *ftsZ1p* and *ftsZ2p* (**B**), and from M13MAV3 and primer AD3 for *ftsZ3p* and *ftsZ4p* (**C**). Letters refer to the four dideoxynucleotides used for sequencing the coding strand. Four S1 nuclease protections were performed for each set of promoters with total RNA samples obtained at different instantaneous growth rates during a growth phase transition in LB (1, R = 2; 2, R = 0.8; 3, R = 0.6; 4, R = 0.4). Sequences for the non-coding strand at the mRNA start points of each promoter are indicated. Boxed letters indicate the bases corresponding to the identified mRNA start points taking into account that, under used conditions, S1 removes 2–3 additional bases from the DNA probe in the DNA–RNA hybrid (Aldea *et al.*, 1988a).

exponential growth (Figure 6). A transcriptional efficiency value for *ftsQ* can be defined as the sum of transcriptional activities obtained from *ftsQ1p* and *ftsQ2p* referred to that from *ftsQ2p* (the constitutive promoter independent of growth rate), so that the increment expected from increased plasmid copy number is also corrected. Assuming the absence of post-transcriptional regulatory mechanisms, this ratio would be equivalent to the amount of the encoded products relative to total cell mass. Our results show that the transcriptional efficiency for *ftsQ* increases as growth rate decreases during the growth phase transition, following the theoretical curve derived when assuming that encoded products are expressed at constant amounts per cell (Figure 7).

No S1 nuclear protected fragments were observed when analyzing sequences upstream from *ftsA* using pTGV14 and pTGV16 as RNA donors (results not shown). As previously inferred from the analysis of expression by transcriptional fusions (Robinson *et al.*, 1984), the *ftsA* gene does not have then detectable promoters to direct its independent expression.

Sequences containing the transcription start points identified in the *ftsQAZ* cluster are shown in Table II. *ftsQ1p*, the inversely growth-rate dependent promoter in the *ftsQAZ* cluster (Figure 6), does not contain −35 and −10 regions resembling the consensus sequences of the regular *E.coli* promoters (Harley and Reynolds, 1987). Instead, sequences upstream from *ftsQ1p* show a clear homology to the −10 and −35 regions of *bolA1p* and *mcbp*. This class of promoters, which we propose to name gearbox promoters, are therefore defined by two distinctive characteristics: (i) an inverse dependence on growth rate and (ii) specific sequences at the −10 and −35 regions from their transcription start points.

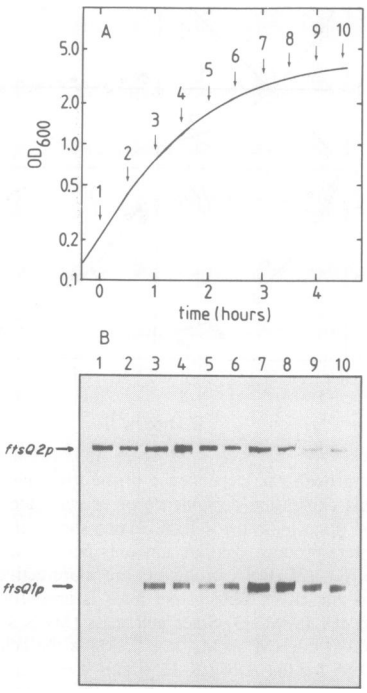

Fig. 6. *ftsQ1p* directs transcription as a gearbox promoter. Total RNA samples from strain RYC1000 (pTGV13) were obtained at different stages of a transition to the stationary phase of growth (**A**), and equal amounts of total RNA were used to determine relative transcription levels (**B**) from *ftsQ1p* and *ftsQ2p* as described in Figure 5.

In addition to the gearbox promoter, the *ftsQAZ* cluster contains five more promoters that are not growth-rate dependent (Figure 5), which show −10 and −35 regions

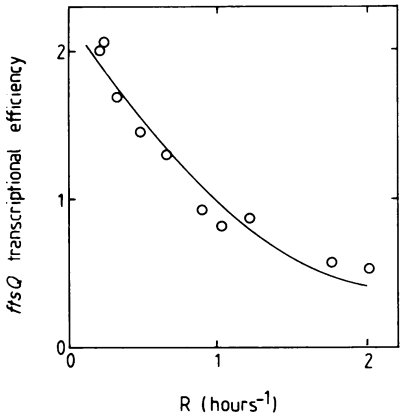

Fig. 7. Transcriptional efficiency of the *ftsQ* promoter region. The transcriptional efficiency for the *ftsQ* promoters is calculated as the sum of transcriptional activities (quantitated by densitometry from bands shown in Figure 6) corresponding to *ftsQ1p* and *ftsQ2p* relative to that corresponding to *ftsQ2p*. This transcriptional efficiency is plotted versus instantaneous growth rate during the growth phase transcription. The line represents the theoretical transcriptional efficiency that would be obtained assuming that the encoded gene product is expressed at constant amounts per cell.

(Table II) with a reasonable degree of homology to the consensus sequences for the regular *E.coli* promoters.

The −10 region of the gearbox promoter is essential for inverse growth rate dependence

The gearbox promoters identified in the *ftsQ*, *bolA* and *mcb* genes share homologous sequences at the −35 and −10 regions. Analysis of the transcriptional fusion contained in λMAV103 (Figure 1) shows that growth rate dependence requires only 50 bp upstream from the mRNA start point in *bolA1p*. We have used the *bolA1p* in λMAV103 as a prototype to obtain two mutated promoters (see Materials and methods), in which sequences upstream from the mRNA start sites are changed, for analyzing the role of the −10 region. Mutation *bolA1p1* was devised to check whether or not the −10 region is required for inverse growth rate dependence, independent of its strength as a promoter. This mutation contains three base substitutions that create the TATAAT consensus sequence for regular *E.coli* promoters and modifies two conserved bases of the −10 region in the gearbox. In the other mutation, *bolA1p2*, the conserved CGG sequence was changed to CCC to evaluate the function of this sequence as a possible determinant of promoter strength. Table III shows the analysis of the expression of these mutated promoters. *bolA1p1* behaves as a constitutive promoter with respect to growth rate; moreover, expression levels obtained from this promoter are similar to those found for the wild-type *bolA1p* at the end of the growth phase transition (Figure 8). In contrast, *bolA1p2* still shows some growth rate dependence but absolute expression levels in stationary cultures show a 20-fold reduction when compared with the wild-type promoters under similar circumstances. Nevertheless, expression kinetics from this mutated promoter are very similar to the wild-type *bolA1p*, being induced as soon as growth rate decreases under the maximum value attained during exponential growth (Figure 8). The two base substitutions in *bolA1p2* produce a −10 region which resembles that found in heat-shock promoters, but no induction was observed when exponentially growing MC1061 cells transformed with pMAV106 were transferred from 30°C to 42°C (results not shown).

We conclude that in association with other structural features present in gearboxes (Table II), the −10 region of the gearbox *bolA1p* not only determines its promoter strength, but also provides specific features that are responsible for inverse growth rate dependence.

Discussion

In the present work we have characterized the pattern of expression of the *ftsQAZ* cluster and have confirmed previous results (Aldea *et al.*, 1989) which suggested that this cluster would be under the control of promoters similar to those first identified in the *mcb* and *bolA* genes (Connell *et al.*, 1987; Aldea *et al.*, 1989). Analysis of expression under several growth conditions allows us to conclude that the *bolA* and *ftsQ* promoter regions are sensitive to growth rate, so that the expression of these genes increases as the growth rate decreases. By measuring amounts of their gene products relative to total protein at different growth rates and during a growth phase transition, this inverse growth rate dependence has also been shown for the other two genes of the cluster, *ftsA* and *ftsZ*. The induction of expression of these genes during the entrance to the stationary phase that we had previously reported can now be interpreted as a consequence of the activation of gearboxes caused by the gradual decrease in growth rate that occurs during this growth phase transition. In agreement with this idea, relative levels of expression plotted versus instantaneous growth rate during a transition to the stationary phase fit with those obtained in cultures growing exponentially at different growth rates.

Two promoters were identified upstream from *ftsQ*, which are found within the open reading frame for *ddl*, and four promoters upstream from the *ftsZ*, which are also found in the open reading frame of the immediate upstream gene, *ftsA*. According to previous results using transcriptional fusions (Robinson *et al.*, 1984), we have found that the *ftsA* gene does not have detectable promoters of its own. Although we cannot exclude the presence of very weak promoters within the *ftsQ* open reading frame, our results indicate that the *ftsA* gene should be expressed mainly from the *ftsQ* promoters since a DNA fragment containing *ftsQ* and *ftsA* but missing the *ftsQ* promoters, when cloned in single copy vectors with proper transcription termination signals, does not complement an *ftsA* chromosomal mutation (to be published elsewhere). Moreover, the fact that open reading frames for the *ftsQ* and *ftsA* gene products share one base suggests that expression of these two genes could be coordinated by coupled translation. Since no transcriptional terminators have been found in this cluster (Robinson *et al.*, 1984, 1986), transcription initiated at the *ftsQ* promoter should also direct expression of the distal *ftsZ* gene. Accordingly, the *ftsA* and *ftsZ* gene products show inverse growth rate dependence as well.

The transcription rate from only one of these promoters, *ftsQ1p*, is inversely dependent on growth rate, a property shared by *bolA1p* (Aldea *et al.*, 1989). This class of promoters, which we name gearbox promoters, presents common features both at the −35 and −10 regions, and shows an A/T-rich stretch upstream from the −35 region (Table II). Two sequences resembling the −10 region of *bolA1p* had previously been found in *ftsQ* and *ftsA* (Aldea

Table II. Sequences and locations of promoters involved in expression of *fts* and *bolA* genes

Promoter	-35	-10	Spacing	Distance to ORF
Gearbox promoters				
ftsQ1p	TGAAGAAATTTTAC	CGTCAA TACGTATT CAACCGTC CGGAACCT TCTATGATTatgAGGCGA	16	286
bolA1p	TATTTGTTGTTAAG	CTGCAA TGGAAACG GTAAAAG CGGCTAGT ATTTaaAGGGATGGATGA	15	9
mcbp	AGTTCAAATTATCA	TTGCAA AATATAG TTAATTA CGGCAAGT AACTagTGTTGGCCAACA	14	62
Consensus	<--A/T rich-->	ctgCAA CGGcaagT		
Regular promoters				
ftsQ2p	GGAATGTCAAAAGTA	GTAGCA GAAAATGCT CTACAAGA TGCATT AAGATTGGCattTCAGCA	17	413
ftsZ1p	ATACGCAAGTGCGTA	TCGGCG CGCCGCTGAACATTACCGG TTTAAC gGAttaTGCTCAGGAGCC	19	215
ftsZ2p	CGCAGATCGAAGGTC	TTGCAG CCTGTGCTC AGCGCGTG TTTCAT ACgcaAGTGCGTATCGGC	17	257
ftsZ3p	TATACCGGTGGGGCA	TTGCGC CACACTAAG GTAATTCCT TATGCT GGCAATgtcGTGACCAGT	18	613
ftsZ4p	ACGTGAACTGGGTGT	CTGCGT CGTCGATAT CGGTGGTGG TACAAT GGATATCgccGTTTATAC	18	670
Consensus		TTGaca TAtaaT		

Transcription start points are indicated in lower case letters (see Materials and methods). A tentative consensus sequence for the gearbox promoters and the consensus for regular promoters (Harley and Reynolds, 1987) are included. Underlined sequences indicate a putative DnaA box. Sequences for *bolA1p* (Aldea *et al.*, 1989) and *mcbp* (Connell *et al.*, 1987) have been described previously.

et al., 1989) but none of them have promoter activity, indicating that some features other than a −10 region must be fulfilled for a region to function as a gearbox, e.g. a −35 region and, perhaps, an upstream A/T-rich stretch. By site specific mutagenesis we have shown that the −10 region is essential in the *bolA1p* gearbox promoter for inverse growth rate dependence. The presence of specific sequences at the −10 region of the gearbox suggests that regulation of this class of promoters could be due either to a sigma factor different from sigma 70, or to a modified form of the RNA polymerase which, in turn, could be activated by some signal dependent on growth rate.

Expression levels driven by the 1.8 kb *Eco*RI−*Hin*dIII fragment, which lacks the *ftsQ1p* gearbox promoter and contains only part of the *ftsZ* promoter region, have also been shown to be inversely dependent on growth rate (Dewar *et al.*, 1989), although to a much lesser extent than that obtained from *ftsQ1*. Unfortunately, their study made use of a transcriptional fusion to *lacZ* in a lambda vector (λJFL100) that even lacks suitable transcription terminators immediately upstream from the cloned fragment being analyzed for transcriptional activity, which is not the case in the present results (Simons *et al.*, 1987). Nonetheless, we have observed that expression levels driven by the whole *ftsZ* promoter region are induced as well during a growth phase transition (Aldea *et al.*, 1989). On the other hand, expression driven by the *ftsZ* promoter region has been found to be partially repressed by DnaA (Masters *et al.*, 1989) and, as DnaA concentration increases at higher growth rates (Chiaremello and Zyskind, 1989), the inverse growth rate dependence attributed to the *ftsZ* promoter region (such as in λTGV3, Aldea *et al.*, 1989) could be due to a superimposed mechanism depending on DnaA. Moreover, promoter *ftsZ1p* (Table II) contains a sequence similar to the consensus DnaA box (Fuller *et al.*, 1984) and shows a very high homology particularly to the DnaA box responsible for autoregulation in the *dnaA* promoter region (Braun *et al.*, 1985). This sequence is located in the non-coding strand and overlaps with the mRNA start point of *ftsZ1p*. As the DnaA protein is able to cause transcription termination when the DnaA box is in the non-coding strand (Messer *et al.*, 1988), DnaA associated with the putative DnaA box in *ftsZ1p* could block transcription initiation from this promoter and cause

Table III. Expression levels from *bolA* mutated promoters

Promoter	−10 region	Phage	Exp	Sta	E_r	S_r	SE_r
bolA1p	CGGCTAGTA	λMAV103	35	596	1.0	1.0	17.1
bolA1p1	CGTATAATA	λMAV104	457	530	13.1	0.9	1.2
bolA1p2	CCCCTAGTA	λMAV106	10	31	0.3	0.05	3.1

Exponential (Exp) and stationary (Sta) cultures of RYC1000 lysogenized with phages carrying transcriptional fusions of listed promoters to *lacZ* were grown in LB and assayed for β-galactosidase activity as described in Materials and methods. Substituted bases in mutated promoters are underlined. Values given are the average of four independent assays and coefficients of variation were always <0.1. Ratios for exponential (E_r) and stationary (S_r) levels are in reference to the wild-type *bolA1p* promoter. Ratios (SE_r) of stationary levels to exponential levels are given for each promoter.

termination of transcription originated at the other promoters located further upstream. The fact that we do not detect any clear growth rate dependence in any of the *ftsZ* promoters by S1 nuclease analysis may be due to the required use of high copy number plasmids as RNA sources, where control by DnaA could be not as effective as in single copy vectors. The DnaA protein has been shown to be the main factor contributing to the so-called initiation mass parameter of *E.coli* (Lobner-Olesen *et al.*, 1989), so that replication initiation depends on DnaA concentration. If DnaA were able to fine-tune expression in the *ftsQAZ* cluster as well, chromosome replication and cell division could share a common regulatory mechanism.

Transcription levels driven by the *ftsQ* and *bolA* promoter regions closely follow what would be expected for gene products that are expressed at a constant amount per cell when considering that cell size relates exponentially to growth rate increases. How a more or less strong dependence on growth rate is attained could be due to intrinsic properties of each gearbox promoter and, more likely, to distinct combinations of gearbox and growth rate independent promoters in a transcriptional unit, as exemplified by the combination of these two classes of promoters in the *ftsQ* and *bolA* promoter regions.

The *ftsQAZ* gene products are essential at different stages of cell division (Donachie *et al.*, 1984). A 4-fold increase in FtsZ causes a 2-fold increase in division frequency, which

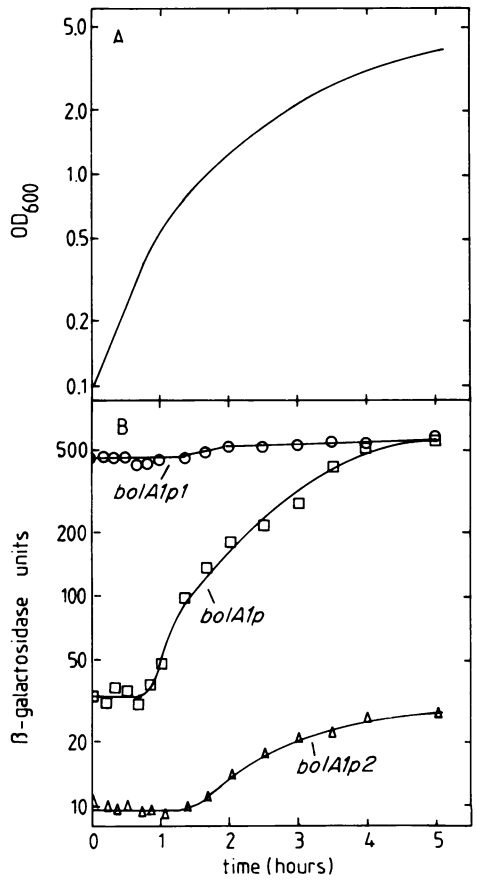

Fig. 8. The −10 region of the gearbox *bolA1p* is essential for inverse growth rate dependence. RYC1000 lysogenized with phages carrying transcriptional fusions to *lacZ* listed in Table III was grown in LB and cultures were allowed to reach the stationary phase of growth (panel **A**). Panel (**B**) shows transcription levels produced by the wild-type promoter *bolA1p* (open squares), and mutated promoters *bolA1p1* (open circles) and *bolA1p2* (open triangles) analyzed by measuring β-galactosidase activity as described in Materials and methods.

leads to minicell production (Ward and Lutkenhaus, 1985), and is able to suppress lethal effects of overexpression of the *minCD* gene products (Lutkenhaus, 1990), the cell division inhibitors (de Boer *et al.*, 1989). Spherical murein in normal cells is only produced at the septum during cell division and, on the other hand, overexpression of *bolA* produces an *ftsZ*-dependent spherical morphology (Aldea *et al.*, 1988b), perhaps by promoting a delocalization of the places active in transversal growth; in accordance with this interpretation, *bolA*, *ftsQ*, *A* and *Z* when overexpressed together, can produce an excess of septation sites that are not limited to the cell poles (this laboratory, unpublished results). If regulated expression of the *ftsQAZ* cluster and the *bolA* gene is to play a role in cell division regulation, synthesis of the encoded gene products should comply with septal requirements relative to all other components of the cell. Our results indicate that gearbox promoters play a role in this regulation by keeping the *ftsQAZ* and *bolA* gene products at stoichiometric levels with respect to the cell septum.

Materials and methods

Bacterial strains, growth media and enzyme assays

All strains used were *E.coli* K-12. Strains MC1061 [*araD139*, Δ(*ara-leu*) *7697*, Δ(*lac*) *X74*, *galU*, *galK*, *strA*; R.L.Gourse] and RYC1000 [*araD139*, Δ(*lac*) *U169*, *rpsL*, *relA*, *thiaA*, *recA56*; F.Moreno] were used to test β-galactosidase activities produced by transcriptional fusions to *lacZ* in plasmid and lambda vectors. JM103 [*supE*, *thi*, *sbcB15*, *rpsL*, *endA*, Δ(*lac-proAB*), F′ (*traD36*, *proAB*$^+$, *lacI*^{q}*ZΔM15*); Messing *et al.*, 1977] was employed for growth of M13 derivatives. W3110 and W3110 transformed with pZAQ (a pBR322 derivative plasmid that contains the whole *ftsQAZ* cluster; Ward and Lutkenhaus, 1985) were used to quantify FtsA and FtsZ by immunological methods. Luria broth and Luria agar were supplemented with antibiotics when required (50 μg/ml ampicillin, 50 μg/ml kanamycin, 20 μg/ml tetracycline, 20 μg/ml chloramphenicol). The M9 medium (Miller, 1972) was supplemented with various carbon sources (0.4% glucose plus 1% casamino acids, 0.4% glucose, 0.4% glycerol or 0.4% sodium acetate), the required amino acids (50 μg/ml) and vitamins (2 μg/ml) to obtain different growth rates. β-Galactosidase activity was measured by the method of Miller (1972) with modifications described by Masters *et al.* (1989). All β-galactosidase activities are referred to cell density and expressed as Miller units.

DNA isolation and cloning techniques

Restriction endonucleases, calf intestine alkaline phosphatase, RNase-free DNase, T4 DNA ligase, S1 nuclease and the Klenow fragment of DNA polymerase I (PolIK) were purchased from Boehringer Mannheim and used according to the manufacturers' instructions. RNase A and lysozyme were purchased from Sigma Chemical Co. Agarose (Pronarose Type D-2) was a gift from Hispanagar S.A. Mutagenic and primer oligonucleotides were synthesized in a Gene Assembler Plus (Pharmacia). Plasmid DNA isolations, cloning techniques and transformation procedures were done as described by Maniatis *et al.* (1982).

Construction of transcriptional fusions

Transcriptional fusions to *lacZ* were constructed by using the high copy number (pRS550) and single copy (λRS45) vectors developed by Simons *et al.* (1987), which contain four copies of a strong growth transcriptional terminator upstream from the cloning sites. Plasmid pMAV103 contains the *bolA1p* promoter in a 225 bp *Ssp*I−*Pvu*I fragment cloned into the *Eco*RI site of pRS550. Plasmid pTGV13 contains the *ftsQ* promoter region in a 0.7 kb *Pst*I−*Eco*RI fragment cloned into pRS550 digested with *Bam*HI and *Eco*RI. In both cases protruding ends were made blunt with PolIK prior to ligation. These transcriptional fusions were subsequently transferred to the single copy vector λRS45 by a double recombination event and the resulting recombinants, λMAV103 and λTGV13 (Figure 1), were lysogenized into RYC1000. Testing for monolysogens was done as recommended by Simons *et al.* (1987). The transcriptional fusion of the two *bolA* promoters to *lacZ* carried by pMAK400 and λMAK400 has already been described (Aldea *et al.*, 1989).

DNA sequencing, oligonucleotide-directed mutagenesis and promoter mapping

DNA sequencing was done by using the Sequenase v 2.0 kit from United States Biochemcal Co. following the instructions supplied by the manufacturer. [^{35}S]dATP (>1000 Ci/mmol) was purchased from Amersham. Point mutations in the *bolA1p* promoter were obtained by oligonucleotide-directed mutagenesis as described by Kunkel (1985) using M13MAV4 as template. M13MAV4 was constructed by cloning the 230 bp *Bam*HI−*Eco*RI fragment from pMAV103 containing the *bolA1p* promoter into M13mp19 digested with *Bam*HI and *Eco*IR. The mutated promoters obtained were cloned back into the transcriptional fusion vector pRS550 and transferred to λRS45 as described above. Total RNA was isolated as previously described (Aldea *et al.*, 1989) from strain MC1061 transformed with plasmids pTGV13 (see above), pTGV14, pTGV16 or pTGV17 (Figure 1). Plasmid pTGV14 contains the whole *ftsQ* and *ftsA* genes in a 3.0 kb *Pst*I−*Eco*RI cloned into pRS550 digested with *Bam*HI and *Eco*RI. Plasmid pTGV16 contains the putative regulatory regions immediately upstream from *ftsA* in a 1.0 kb *Eco*RI−*Pvu*II fragment cloned into the *Eco*RI site of pRS550. Plasmid pTGV17 contains the promoter region of *ftsZ* in a 1.2 kb *Pvu*II−*Eco*RI fragment cloned into the *Eco*RI site of pRS550. Protruding ends were made blunt with PolIK prior to ligation and orientations were chosen so that transcriptional fusions of the *ftsQAZ* genes to *lacZ* were obtained. The promoter mapping method has already been described (Aldea *et al.*, 1988a). Sodium trichloroacetate was purchased from Merck. Primers and M13 derivatives used for synthesizing the DNA probes and sequencing ladders are indicated in Figure 1. M13MAV1 carries a 0.8 kb *Pst*I−*Bam*HI fragment cloned into M13mp19 digested with *Pst*I and *Bam*HI. M13MAV2 contains a 1.0 kb *Eco*RI−*Pvu*II fragment cloned into the *Eco*RI and *Hin*cII sites of M13mp18. M13MAV3 contains a 1.2 kb *Pvu*II−*Eco*RI fragment cloned into the *Hin*cII and *Eco*RI sites of M13mp19. Transcription start

points were corrected taking into account that S1 removes 2–3 additional bases from the DNA probe in the DNA–RNA hybrid (Aldea *et al.*, 1988a).

Immunoblotting procedures

Total proteins electrophoresed on a 10% SDS–polyacrylamide gel were electrophoretically transferred to Immobilon membranes (Millipore) as described (Towbin *et al.*, 1979). FtsZ and FtsA were detected by indirect immunostaining using rabbit polyclonal antisera against FtsZ or FtsA, MVJ4 and MVJ1 respectively, using protein A–horseradish peroxidase conjugate and 4-chloro-1-naphthol as substrate. Quantification of bands was done by densitometry on positive films. Purification of FtsZ and FtsA and isolation of antisera will be described elsewhere.

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