CMLS, Cell. Mol. Life Sci. 54 (1998) 317–324
1420-682X/98/040317-08 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Regulation of transcription of cell division genes in the *Escherichia coli dcw* cluster

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Abstract. The *Escherichia coli dcw* cluster contains cell division genes, such as the phylogenetically ubiquitous *ftsZ*, and genes involved in peptidoglycan synthesis. Transcription in the cluster proceeds in the same direction as the progress of the replication fork along the chromosome. Regulation is exerted at the transcriptional and post-transcriptional levels. The absence of transcriptional termination signals may, in principle, allow extension of the transcripts initiated at the upstream promoter (*mraZ1p*) even to the furthest downstream gene (*envA*). Complementation tests suggest that they extend into *ftsW* in the central part of the cluster. In addition, the cluster contains other promoters individually regulated by *cis*- and *trans*-acting signals. Dissociation of the expression of the *ftsZ* gene, located after *ftsQ* and *A* near the 3′ end of the cluster, from its natural regulatory signals leads to an alteration in the physiology of cell division. The complexities observed in the regulation of gene expression in the cluster may then have an important biological role. Among them, LexA-binding SOS boxes have been found at the 5′ end of the cluster, preceding promoters which direct the expression of *ftsI* (coding for PBP3, the penicillin-binding protein involved in septum formation). A gearbox promoter, *ftsQ1p*, forms part of the signals regulating the transcription of *ftsQ*, *A* and *Z*. It is an inversely growth-dependent mechanism driven by RNA polymerase containing σ^s, the factor involved in the expression of stationary phase-specific genes. Although the *dcw* cluster is conserved to a different extent in a variety of bacteria, the regulation of gene expression, the presence or absence of individual genes, and even the essentiality of some of them, show variations in the phylogenetic scale which may reflect adaptation to specific life cycles.

Key words. Cell division; regulation; *Escherichia coli*; *dcw* cluster; *ftsQ*; *ftsA*; *ftsZ*; gearbox promoter.

Cell division

The division processes observed in present-day prokaryotic cells are the result of many millions of years of adaptation to specific environments. Inefficient modes of division may have been superseded during those millions of years, so that nowadays we may only find exquisite examples of cell division adapted to certain specific environments. But even if that is not the case, the physiological and molecular processes which constitute division are so complex that impairment of any of the genes involved in cell division, and in general in essential processes, leads to alterations in the behaviour of the cell. These alterations make life more difficult even for bacteria which are kept under well-controlled laboratory conditions (e.g. VIP205, see below). In living bacteria most of the genes involved in cell division are well conserved, not only in their encoded amino acid sequence but also in their relative positions in the chromosome.

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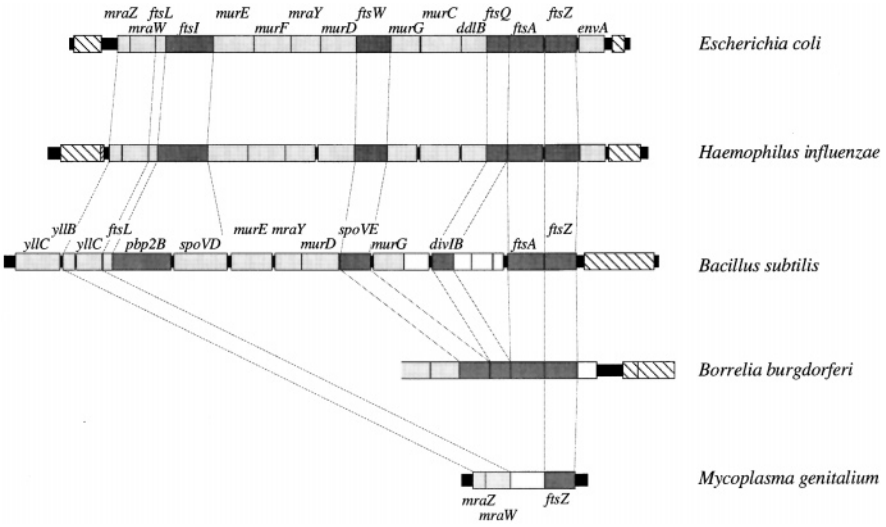

Figure 1. Conservation of the *dcw* cluster in several microorganisms. Each line shows a scheme of the organization of the cluster in the species indicated at the right-hand side. Black boxes indicate noncoding regions. Dark grey shaded boxes represent genes involved in septation during cell division. Light grey shaded boxes are genes involved in cell wall synthesis or with no well-defined function. The *spoVD* gene of *B. subtilis* is a duplicate of the *ftsI* ($\equiv$ *pbp2B*) septation gene with a function specific for sporulation. White boxes represent genes with no obvious homologies. Cross-hatched boxes indicate genes not belonging to the cluster. See further comments and exceptions in the text.

The *dcw* cluster

Many bacterial species contain a cluster of cell division genes and, in those bacteria which possess a cell wall, genes coding for enzymes that participate in its synthesis [1–9]. The cluster has been named the *dcw* cluster [1]. Strong conservation of the general structure of the cluster (fig. 1) is found in many microorganisms. This conservation has been deduced from sequence homology comparison of the genes of the cluster among most of those bacteria whose complete genome sequence is known, namely: *Haemophilus influenzae* [5], *Methanococcus janaschii* [3], *Mycoplasma genitalium* [6], *Mycoplasma pneumoniae* [7] and *Synechocystis* sp. [8], those in which the *dcw* cluster has been fully sequenced: *Escherichia coli* [9], *Bacillus subtilis* [2], and *Enterococcus hirae* [9a] and those where a *dcw* cluster homologue was detected by sequence homology search: *Mycobacterium tuberculosis* [10], *Neisseria gonorrohoeae* [11], and *Streptococcus pyogenes* (contig 178, Streptococcal genome sequencing project, B.A. Roe, S. Clifton, M. McShan and J. Ferretti, unpublished observations). In addition to the conservation of individual genes in the cluster, the ordering of such genes, except when one or more genes are absent, is also conserved (but see the case of *Helicobacter pylori* below; [12]).

The biological reasons, if any, for this ordering are unknown at present. In the absence of proof, we can speculate on the need to have all these genes physically adjacent to impose on them a specific regulatory mechanism for their coordinated expression, but we could equally well propose that the genes have been conserved together because they have been inherited as a genetic unit. It is also unknown if the genes emerged as a complex cluster which lost genes along the course of microbial evolution, in which case the organisms containing a voluminous *dcw* cluster, such as *E. coli*, would be primitive. Equally well it could happen that the cluster may have been formed by accretion of different genes from different sources, and in later developments either lost or gained other genes, in which case both the organisms containing a fat or a slim *dcw* cluster would be the result of separate evolutionary trends. Whatever reasoning is correct, present-day organisms contain versions of the *dcw* cluster ranging from the most luxuriant one present in many peptidoglycan-containing eubacteria (e.g. *E. coli*, *H. influezae*, *B. subtilis*), to the most austere cluster present in mycoplasmas and archaebacteria.

Another insight into this question derives from the *dcw*-gene arrangement found in *H. pylori*, a prominent exception to the presence of a *dcw* cluster [12]. In this species the *mraZ*, *mraW*, *ftsL*, *ddlB*, *ftsQ* and *envA* genes are absent. Moreover the rest of the genes that are found in the *E. coli dcw* cluster are placed at seven different chromosomal locations in *H. pylori*. Some remnants of the clustering found in *E. coli* are nevertheless conserved in the *H. pylori mraY-murD* and *ftsA-ftsZ* genes (these two are preceded by a gene encoding a secreted protein). The *ftsI* and *ftsW* genes, although separated by three genes encoding flagellar proteins, are also linked, but they are transcribed in opposite orientations. The *murF* gene is separated from the *ddlA* gene by another gene encoding 2-hydroxy-6-oxohepta-2,4-dienoate hydrolase. The *murG* gene is followed by two genes encoding outer

membrane proteins. Why is the *dcw* cluster conserved in bacteria distantly related to *E. coli*, such as *B. subtilis*, but is not in the more closely related *H. pylori*? There is a relatively low abundance of global regulatory mechanisms for gene expression in *H. pylori*. In these circumstances, assuming that clustering of related genes responds to the need for their coordinated regulation, the clustering of the *dcw* genes may offer no particular advantage. Clustering may either be the outcome of convergent evolution from a simple common ancestor through a cluster similar to the one present in *E. coli*, or the result of a divergent adaptation to the environmental conditions, but whatever the case, it has given rise to a set of complex regulatory mechanisms that control cell division in this species.

Some genes and gene products of the *dcw* cluster

Different bacteria differ as well in their essential requirement for some of the genes located in the cluster, for example the *ftsZ* gene, coding for a GTPase able to form transient rings at the time and the site of division [13–16], is present in all the bacteria studied so far, and in the genome of plants where it is required for the division of chloroplasts [17]. Although *ftsZ* is essential in most bacteria, it seems that actinomycetes, such as *Streptomyces*, can survive and divide in the mycelial vegetative phase even in the absence of *ftsZ* [18]. This gene is nevertheless required for septation of the aerial hyphae during the sporulation phase. A similar case occurs for *ftsA*, a gene which codes for a protein whose structure resembles that of eukaryotic adenosine triphosphate (ATP)-binding proteins of the actin, Hsc70, hexokinase family [19]. The FtsA protein is required for *E. coli* division, and for the formation of the septum during sporulation of *B. subtilis*, but in its absence some vegetative division, enough to render the defective cells viable, can occur in this latter organism [20]. In the peptidoglycan-containing bacteria the *ftsI* ($\equiv$ *pbpB*) gene product, a penicillin-binding protein involved in the synthesis of septal peptidoglycan, is found to be essential in all circumstances [21]. In this kind of bacteria cell, lysis occurs when the level of lipid II is rapidly depleted either in conditional mutants or, in some cases, by the addition of a specific antibiotic [22]. It is thus considered that, although not rigorously proven by null allele experiments, the genes that code for the enzymes responsible for the biosynthesis of the precursor lipid II (*murE*, *murF*, *murC* and *murD*) are required for cell viability.

Genetic organization in the *dcw* cluster of *E. coli*

DNA sequence analysis of the *dcw* cluster in different bacteria reveals that the genes in the cluster are arranged in close proximity to each other and that all are transcribed in the same direction, which in *E. coli* is coincident with the progression of the replication fork. In *E. coli* the *dcw* cluster ($\equiv$ *mra* region) is located at min 2.5 of the genetic map [23] (in *B. subtilis* it is located in the 133–135° region. [2]). Most of its genes code for proteins with a well-established enzymatic activity and function [*ftsI*, *murE*, *murF*, *murD*, *mraY*, *murG*, *murC*, *ddlB*, *ftsZ* and *envA*($\equiv$ *lpxC*)]; for some others either a function or an enzymatic activity has been predicted [*mraW*, *mraR*($\equiv$ *ftsL*), *ftsW*, *ftsQ* and *ftsA*], but the functionality of one gene (*mraZ*) remains unknown [1].

Promoter regions for each gene in the cluster, some of which have been characterized and others predicted, are located inside the preceding structural gene. The exception would logically be the 5′ proximal *mraZ* gene whose promoter is located 38 bp upstream of the structural gene inside a noncoding region of the chromosome (see below). Two Rho-independent transcription termination sites, one at the 5′ end of the *mraZ* gene (also found in the *B. subtilis dcw* cluster; [24]) and another at the 3′ end of *envA*, have been predicted. An additional terminator sequence found in *B. subtilis* after the *ftsI* homologue ($\equiv$ *pbpB*) is not found in *E. coli.*

The sequential organization of the first 12 genes identified (flanked upstream by the *shl* gene and downstream by the *ftsQAZ* region), together with the absence of a Rho-independent transcription termination sequence, as deduced by nucleotide sequence search, suggests that these genes may transcribed as a polycistronic messenger RNA (mRNA). Moreover, the existence of a large mRNA comprising the whole cluster has been postulated [25]. This putative mRNA molecule would be transcribed from the first upstream promoter (*mraZ1p*) and subsequently processed to achieve the differential expression of the different genes. The transcriptional organization of the 16 genes involved in the cluster has not been described in detail, except for the *ftsQAZ* [26, 27] and *mraZWR-ftsI* regions [25, 28, 28a]. The instability of several of the mRNA species produced, as occurs for the *ftsI* mRNA [28a], could be partly responsible for this lack of data.

Although promoter-like sequences are found for any individual gene on the cluster, a chromosome insertion of an IPTG-inducible-promoter cassette at the *Hind*III site that lies 75 bp apart from the 5′ end of *mraZ* makes growth of the cells dependent on the addition of isopropyl β-D-thiogalactopyranoside (IPTG) to the medium. Growth cannot be made IPTG-independent unless the strain is transformed with a plasmid containing at least all the genes of the cluster up to *ftsW* [28]. This result indicates that transcription from the most distal promoter of the cluster (*mraZ1p*) may proceed up to the *ftsW* gene. The description of the regulation of gene expression

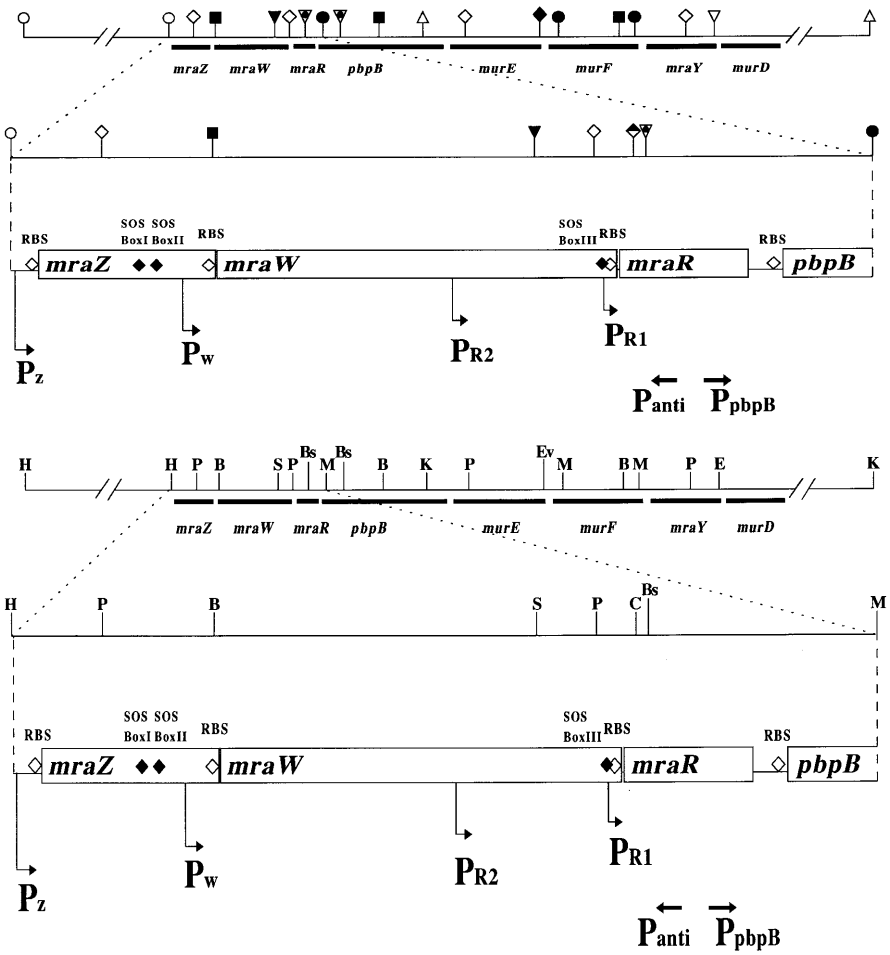

Figure 2. Transcription start sites identified by S1 nuclease mapping analysis at the 5′ end of the *dcw* cluster. Five transcription start sites in the sense strand: *mraZ1p* (Pz), *mraW1p* (Pw), *mraR2p* (Pr2), *mraR1p* (Pr1) and *fstI1p* (PpbpB), and a start site on the antisense strand (Panti) have been identified when using the S1 nuclease mapping technique with probes for the corresponding mRNAs. The predicted ribosome-binding sites (RBS) and the putative LexA-binding regions (SOS box I, II and III) are also represented. On the top of the figure the symbols correspond to the main restriction sites for endonucleases at the beginning of the cluster (B, *Bam*HI; Bs, *Bst*EII; C, *Cla*I; E, *Eco*RI; Ev, *Eco*RV; H, *Hind*III; K, *Kpn*I; M, *Mlu*I; P, *Pvu*II; S, *Sma*I).

at the 5′ end of this cluster is then an important component in understanding the regulation of transcription of the whole cluster and, consequently, the regulation of bacterial cell division.

Promoters and regulatory signals at the 5′ end of the *dcw* cluster

Five transcription start sites have been identified by S1 nuclease mapping analysis at the 5′ end of the *dcw* cluster, and promoter-like sequences have been found at the appropriate distance from each of these sites. They lie at the following positions (fig. 2): *mraZ1p*, 38 bp upstream from the *mraZ* structural gene; *mraW1p*, 84 bp upstream from *mraW*; *mraR1p* and *mraR2p*, 344 bp and 20bp upstream from *mraR*; and *ftsIp* which, although it has not been located with precision, has to be close to the end of the *mraR* gene. The contribution of each promoter to the level of expression of the subsequent genes has not been tested.

Three LexA-binding sites have been identified by homology sequence analysis at the 5′ end of the cluster. Ishino et al. [29] pointed out the existence of an SOS box (SOS box III), in the vicinity of *mraR*, which is highly homologous to the consensus. Two additional SOS boxes have also been found upstream of *mraW* [28a]. The SOS box of *mraR* overlaps the −10 region of the promoter in a fashion similar to that of the *sulA* gene and the cloDF13 plasmid [30]. However, the SOS boxes found in the *mraW* promoter are arranged in a pattern that has not been previously described in any other gene. They are located in positions −64 to −87

(SOS box I) and −129 to −150 (SOS box II) from the transcription initiation site. Location of SOS boxes upstream of the −35 region has only been reported for a single SOS box in the *ssb* gene. The three *dcw* SOS boxes I–III are highly homologous to each other, all of them containing one more nucleotide than the consensus. A band-shift mobility experiment shows that LexA actually binds to SOS box I (M.J. Gómez, M. Carrión and J.A. Ayala, unpublished observations).

The natural complexity in the regulation of gene expression is required for normal cell division

The *E. coli* VIP205 strain, in which the sole change relative to the parental strain is the modification of the natural control of expression of the essential cell division gene *ftsZ*, has been used to reveal some of the complexities of gene expression at the 3′ end of the cluster. The expression of *ftsZ* in VIP205 has been placed under the control of an IPTG inducible *tac* promoter. This abolishes the oscillation in *ftsZ* transcription observed in the wild type [31], allowing different levels of *ftsZ* expression. This construction does not affect the expression of other genes, and has no effect on replication or nucleoid segregation. However, VIP205 cells present genetic instability problems both upon prolonged storage and after entrance into stationary phase. A shift in IPTG from 30 μM, that supports division at wild type sizes, to lower (6 μM) or higher (100 μM) concentrations, indicates that VIP205 cells can divide within a broad range of FtsZ concentrations. Analysis of the morphological parameters during the transition from one IPTG concentration to another suggests that the correct timing of *ftsZ* expression, and the correct FtsZ concentration, are required for division to occur at normal sizes. After a transient division delay during the transition to lower IPTG concentrations, cells in which *ftsZ* is expressed continuously (yielding 80% of the wild-type FtsZ levels), divide with the same division time as the wild type, but at the expense of becoming 1.5 times larger [32]. A precise control of *ftsZ* expression is then required for normal division.

Promoters at the 3′ end of the *dcw* cluster

The *ftsZ* gene is transcribed from at least six promoters found within the coding regions of the upstream *ddlB*, *ftsQ* and *ftsA* genes (fig. 3). The contribution of each one to the final yield of *ftsZ* transcription has been estimated using transcriptional *lacZ* fusions. The most proximal promoter, *ftsZ2p*, contributes less than 5% of the total transcription from the region that reaches *ftsZ*. The *ftsZ4p* and *3p* promoters, both located inside *ftsA*, produce close to 37% of the transcription. An *ftsAp* promoter within the *ftsQ* gene yields nearly 12% of total transcription from the region. A large proportion of transcription (approximately 46%) derives from *ftsQ2p* and *1p*, which are located inside the upstream *ddlB* gene [27]. Thus, *ftsQAZ* are to a large extent transcribed as a polycistronic mRNA. However, we find that the *ftsZ* proximal region is necessary for full expression, which is in agreement with the report that mRNA cleavage by RNase E at the end of the *ftsA* cistron has a significant role in the control of *ftsZ* expression [33]. As the *dcw* cluster contains no transcriptional terminator before the end of the cluster, transcription from other promoters located upstream from *ddlB* can have a significant effect on the transcription of *ftsZ*. Results from chromosomal fusions able to report the transcriptional activity from all the promoters in the cluster, both upstream and downstream of the *ddlB* gene, are required to obtain a precise picture of how the mechanisms of global regulation of gene expression influence the cell division cycle.

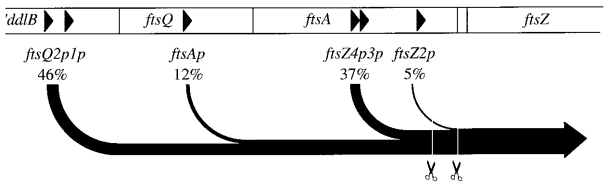

Figure 3. Model for the contribution of *ftsQAZ* promoters to the expression of *ftsZ*. The relative contribution of each promoter or promoter pair to the total transcription of *ftsZ* that starts downstream of the *Pst*I site in the *ddlB* gene has been estimated from results obtained in single-copy-number chromosomal fusions comprising different stretches of the *fts* region of the *dcw* cluster with a β-galactosidase reporter gene [27]. Most of the data were obtained from fusions containing the same sequence at the junction between the 3′ end of the *dcw*-derived portion and the reporter vector. Independent of the particular fusion involved, and due to the presence of an RNase III cutting site immediately downstream of the junction, the β-galactosidase message was mainly formed by the same mRNA molecule. Scissors indicate RNase E processing sites.

The *ftsQ1p* promoter belongs to a distinct class of *E. coli* promoters, which have been named gearbox promoters because their final yield is constant per cell and cell cycle, independent of the growth rate of the population. Together with another gearbox promoter, *bolA1p*, *ftsQ1p* is recognized by both RNA polymerase sigma factors σ^D and σ^S, and depends on the latter for growth rate regulation ([34]; M. Ballesteros, S. Kusano, A. Ishihama and M. Vicente, unpublished observations).

The *ftsQ2p* promoter is a housekeeper promoter which is transcribed by RNA polymerase containing σ^D. Transcription from this promoter is regulated by SdiA, a protein which may form part of a quorum-sensing mechanism (see below; [34, 35]).

The existence of a weak specific promoter for *ftsA* was inferred from genetic complementation tests [36–38], but it was not detected in S1 nuclease protection assays [26]. These reports were based on methods which tend to overestimate (complementation) and underestimate (mapping) promoter activities. Reporter fusions with β-galactosidase confirmed the existence of *ftsAp* [27], but its exact location in the *dcw* sequence is still unknown.

Similar experiments from reporter fusions showed that *ftsZ4p3p* are the most important of the promoters specific for *ftsZ* [27]. When examined in isolation from both upstream and downstream promoters, they are found to be inversely growth rate-dependent and moderately induced in stationary phase [27].

Overexpression of the *rcsB* gene, which encodes an activator of colanic acid biosynthesis, causes significant induction of a *lacZ*-fusion carrying *ftsZ4p3p2p* [39]. A number of additional studies have addressed the regulatory behaviour of these promoters [31, 40–42], but there is no conclusive information about any regulatory mechanisms controlling them. Evidence from additional *lacZ* fusions indicates that *ftsZ4p* is the stronger of the two, while *ftsZ3p* is very weak, at least during rapid growth (unpublished observations). This is in agreement with the relative strengths of the signals from the two promoters in runoff transcription assays (M. Ballesteros, S. Kusano, A. Ishihama and M. Vicente, unpublished observations).

It remains to be seen whether the weak intragenic promoters *ftsAp*, *ftsZ3p* and *ftsZ2p* have any significant roles in cell division. It is in fact possible that they represent vestigial elements that are conserved, not because they have a specific function, but rather because they are fixed within the sequences of essential genes. Alternatively, they may be upregulated during specific conditions as is the gearbox promoter *ftsQ1p*.

Being essential for the survival of the cell, the *fts* genes in the *dcw* cluster may have acquired a complex set of regulatory pathways such as the gearbox promoter, a quorum-sensing promoter [34, 35] and a cell cycle-dependent mode of gene expression [31, 43], both as a way to ensure that cell division products are correctly synthesized during the normal cell cycle, and as fail-safe mechanisms able to prevent a division catastrophe when the cells are under stress.

Antisense transcription and *cis*- and *trans*-regulatory signals in the *dcw* cluster

The product of *dicF* (a gene contained in a vestigial element located at the replication terminus) has been characterized as an antisense RNA able to inhibit cell division by impeding translation of *ftsZ* [44].

Antisense transcription of *ftsZ* can be initiated within the *ftsZ*-coding region extending through the junction between *ftsA* and *ftsZ*. This segment contains a potential antisense promoter and an antisense transcription terminator, and it may inhibit cell division when present in very high copy number [45].

An antisense transcript has been identified at the junction between *mraR* and *ftsI* by means of S1 mapping (fig. 4), and antisense promoter activities have been detected using transcriptional *lacZ* gene fusions at the *Sma*I-*Mlu*I and *Mlu*I-*Pst*I segments, including the junction between *mraR* and *ftsI* genes and an internal fragment of *ftsI*, respectively (M. J. Gómez, M. Carrión and J.A. Ayala, unpublished observations).

SdiA is a member of the quorum-sensing LuxR family of transcriptional regulators and has been shown to positively regulate cell division by specific *trans*-activation of transcription of the *ftsQAZ* region, acting on the *ftsQ2p* promoter [46].

Two 9-bp putative regulatory elements (named O1 and O2) may be involved in the regulation of expression of *ftsA* from the weak promoter *ftsA1p*. They have been identified using deletions of the preceding gene *ftsQ* carried on a plasmid to complement *ftsA* [36].

Amplification of a 2.6-kb chromosomal fragment comprising *mraR* and *ftsI* inhibits the growth of *ftsQ*, *ftsA* and *ftsZ* mutants at the permissive temperature, suggesting that some balance between expression of genes at the 5′ end and 3′ end of the *dcw* cluster may be required for normal cell growth [47]. Alternatively, some unknown cis-acting mechanism connected with the expression of the gene products of the 5′ end of the *dcw* cluster might also be involved in regulation of the expression of the *ftsQAZ* region.

Evidence for the existence of additional promoters in the cluster

Only two reports showing the existence of promoters other than those described at the 5′ and 3′ ends of the cluster have been published. The DNA upstream from *ftsW*, encompassing the 3′ end of *mraY* and the full coding sequence of *murD*, was found to be needed to complement a temperature-sensitive allele of *ftsW* in the absence of an exogenous promoter, indicating that the promoter activity for *ftsW* transcription is located in the *mraY* structural gene [48]. When expression of the whole cluster is driven under the control of the P*lac* promoter, it has been shown to proceed not further than *ftsW* [28]. This result indicates that other independent promoters, in addition to *mraZ1p*, should also be responsible for the expression of the rest of the cluster. It would not be surprising to find promoters

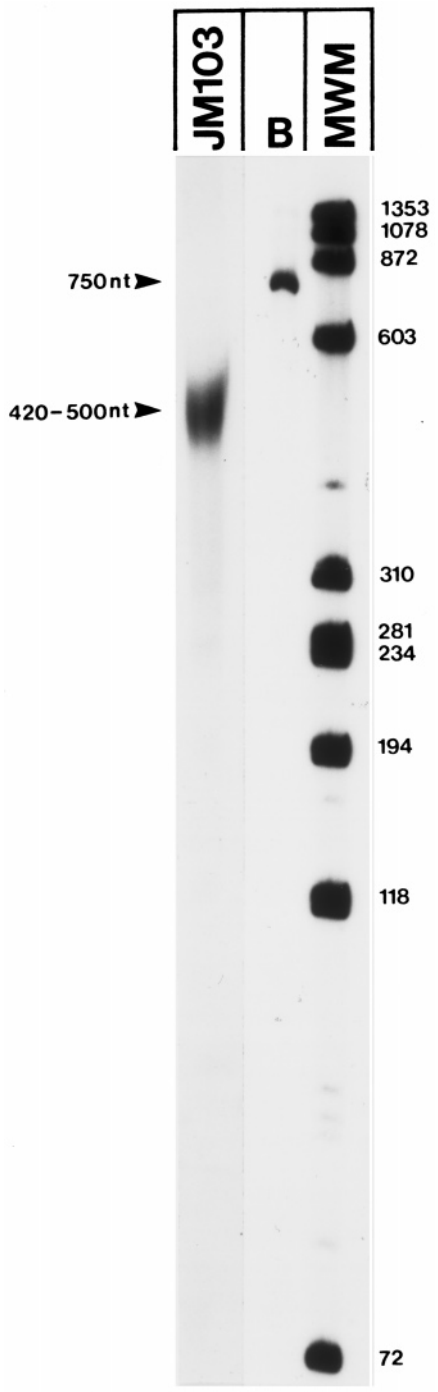

Figure 4. Antisense transcription at the 5′ end of the *dcw* cluster. Low-resolution S1 nuclease mapping of the mRNA isolated from an exponential phase culture of the *Escherichia coli* strain JM103 (lane JM103) was carried out by using as probe the 750-nt ^{32}P-labelled fragment between the *Sma*I and *Mlu*I endonucleases sites at the 5′ end of the *dcw* cluster (lane B). The *Hae*III digested fragments of ^{32}P-labelled ΦX174 DNA were used as markers (MWM). The transcription product originating at an antisense promoter in an internal region of the *mraR* gene (see fig. 2) is detected as a 420–450 nt fragment protected by the probe.

responsible for expression of *murG*, *murC* and *ddlB* lying upstream of these genes, as has been already found for the expression of *ftsQ*, *A* and *Z*. Some or all of these promoters upstream of the *ddlB-envA* region may have a significant effect on *ftsZ* expression (see above).

In conclusion, gene expression of the *dcw* cluster is regulated in *E. coli* by a variety of sophisticated mechanisms which together contribute to the precise timing of cell division. The presence of all these regulatory signals makes regulation of the cluster more like the sort of regulation found in eukaryotic genes, rather than the simple mechanisms used for bacterial metabolic operons like the paradigmatic *lac* operon [49].

1 Ayala J. A., Garrido T., de Pedro M. A. and Vicente M. (1994) Molecular biology of bacterial septation. In: New Comprehensive Biochemistry. Bacterial Cell Wall, vol. 27, pp. 73–101, Hakenbeck R. and Ghuysen J. M. (eds), Elsevier, Amsterdam

2 Buchanan C. E., Henriques A. O. and Piggot P. J. (1994) Cell wall changes during bacterial endospore formation. In: New Comprehensive Biochemistry. Bacterial Cell Wall, vol. 27, pp. 167–183, Hakenbeck R. and Ghuysen J. M. (eds), Elsevier, Amsterdam

3 Bult C. J., White O., Olsen G. J., Zhou L., Fleischmann R. D., Sutton G. G. et al. (1996) Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. Science **273:** 1058–1073

4 Doherty H. M. and Adams D. G. (1995) Cloning and sequence of *ftsZ* and flanking regions from the cyanobacterium *Anabaena PCC 7120*. Gene **163:** 93–96

5 Fleischmann R. D., Adams M. D., White O., Clayton R. A., Kirkness E. F., Kerlavage A. R. et al. (1995) Whole-genome random sequencing and assembly of *Haemophilus influenzae Rd*. Science **269:** 496–512

6 Fraser C. M., Gocayne J. D., White O., Adams M. D., Clayton R. A., Fleischmann R. D. et al. (1995) The minimal gene complement of *Mycoplasma genitalium*. Science **270:** 397–403

7 Himmelreich R., Hilbert H., Plagens H., Pirkl E., Li B. C. and Herrmann R. (1996) Complete sequence analysis of the genome of the bacterium *Mycoplasma pneumoniae*. Nucleic Acids Res. **24:** 4420–4449

8 Kaneko T., Sato S., Kotani H., Tanaka A., Asamizu E., Nakamura Y. et al. (1996) Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. DNA Res. **3:** 109–136

9 Yura T., Mori H., Nagai H., Nagata T., Ishihama A., Fujita N. et al. (1992) Systematic sequencing of the *Escherichia coli* genome: analysis of the 0–2.4 min region. Nucleic Acids Res. **20:** 3305–3308

9a Duez C. and Coyette J. (1997) From peptidoglycan biosynthesis to antibiotic resistance. Present and future, University of Liège, Liège

10 Philipp W. J., Poulet S., Eiglmeier K., Pascopella L., Balasubramanian V., Heym B. et al. (1996) An integrated map of the genome of the tubercle bacillus, *Mycobacterium tuberculosis* H37Rv, and comparison with *Mycobacterium leprae*. Proc. Natl. Acad. Sci. USA **93:** 3132–3137

11 Dempsey J. F., Litaker W., Madhure A., Snodgrass T. L. and Cannon J. G. (1991) Physical map of the chromosome of *Neisseria gonorrhoeae* FA1090 with locations of genetic markers, including *opa* and *pil* genes. J. Bacteriol. **173:** 5476–5486

12 Tomb J.-F., White O., Kerlavage A. R., Clayton R. A., Sutton G. G., Fleischmann R. D. et al. (1997) The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:** 539–547

13 de Boer P., Crossley R. and Rothfield L. (1993) The essential bacterial cell-division protein FtsZ is a GTPase. Nature **359:** 254–256
14 Mukherjee A., Dai K. and Lutkenhaus J. (1993) *Escherichia coli* cell division protein FtsZ is a guanine nucleotide binding protein. Proc. Natl. Acad. Sci. USA **90:** 1053–1057
15 RayChaudhuri D. and Park J. T. (1993) *Escherichia coli* cell-division gene *ftsZ* encodes a novel GTP-binding protein. Nature **359:** 251–254
16 Bi E. F. and Lutkenhaus J. (1991) FtsZ ring structure associated with division in *Escherichia coli*. Nature **354:** 161–164
17 Osteryoung K. W. and Vierling E. (1995) Conserved cell and organelle division. Nature **376:** 473–474
18 McCormick J. R., Su E. P., Driks A. and Losick R. (1994) Growth and viability of *Streptomyces coelicolor* mutant for the cell division gene *ftsZ*. Mol. Microbiol. **14:** 243–254
19 Sánchez M., Valencia A., Ferrándiz M.-J., Sander C. and Vicente M. (1994) Correlation between the structure and biochemical activities of FtsA, an essential cell division protein of the actin family. EMBO J. **13:** 4919–4925
20 Beall B. and Lutkenhaus J. (1992) Impaired cell division and sporulation of a *Bacillus subtilis* strain with the *ftsA* gene deleted. J. Bacteriol. **174:** 2398–2403
21 Curtis N. A., Eisenstadt R. L., Turner K. A. and White A. J. (1985) Inhibition of penicillin-binding protein 3 of *Escherichia coli* K-12. Effects upon growth, viability and outer membrane barrier function. J. Antimicrob. Chemother. **16:** 287–296
22 van Heijenoort J. (1994) Biosynthesis of the bacterial peptidoglycan unit. In: New Comprehensive Biochemistry. Bacterial Cell Wall, vol. 27, pp. 39–54, Hakenbeck R. and Ghuysen J. M. (eds), Elsevier, Amsterdam
23 Berlyn M. K. B., Low K. B. and Rudd K. E. (1996) Linkage map of *Escherichia coli* K-12, edition 9. In: *Escherichia coli* and *Salmonella*, pp. 1715–1912, Neidhardt F. C. (ed.), ASM Press, Washington DC
24 Daniel R. A., Williams A. M. and Errington J. (1996) A complex four-gene operon containing essential cell division gene *pbpB* in *Bacillus* subtilis. J. Bacteriol. **178:** 2343–2350
25 Hara H. and Park J. T. (1993). A far upstream region is required for expression of the *ftsI* gene coding for penicillin-binding protein 3 of *Escherichia coli*. In: Bacterial Growth and Lysis: Metabolism and Structure of the Bacterial Sacculus, pp. 303–308, de Pedro M. A., Höltje J. V. and Löffelhardt W. (eds), Plenum, London
26 Aldea M., Garrido T., Pla J. and Vicente M. (1990) Division genes in *Escherichia coli* are expressed coordinately to cell septum requirements by gearbox promoters. EMBO J. **9:** 3787–3794
27 Flärdh K., Garrido T. and Vicente M. (1997) Contribution of individual promoters in the *ddlB-ftsZ* region to the transcription of the essential cell divison gene *ftsZ* in *Escherichia coli*. Mol. Microbiol. **24:** 927–936
28 Hara H., Yasuda S., Horiuchi K. and Park J. T. (1997) A promoter for the first nine genes of the *Escherichia coli mra* cluster of cell division and cell envelope biosynthesis genes, including *ftsI* and *ftsW*. J. Bacteriol. **179:** 5802–5811
28a Gomèz M. (1991) Funcionalidad de la PBP3 de *Escherichia coli*: estudio de la actividad, localización y regulación de la proteína, Ph.D. thesis, Universidad Autónoma de Madrid
29 Ishino F., Jung H. K., Ikeda M., Doi M., Wachi M. and Matsuhashi M. (1989) New mutations *fts-36*, *lts-33* and *ftsW* clustered in the *mra* region of the *Escherichia coli* chromosome induce thermosensitive cell growth and division. J. Bacteriol. **171:** 5523–5530
30 Schnarr M., Oertel-Buchheit P., Kazmaier M. and Granger-Schnarr M. (1991). DNA binding properties of the LexA repressor. Biochimie **73:** 423–431
31 Garrido T., Sánchez M., Palacios P., Aldea M. and Vicente M. (1993) Transcription of *ftsZ* oscillates during the cell cycle of *Escherichia coli*. EMBO J. **10:** 3957–3965
32 Palacios P., Vicente M. and Sánchez M. (1996) Dependency of *Escherichia coli* cell-division size, and independency of nucleoid segregation on the mode and level of *ftsZ* expression. Mol. Microbiol. **20:** 1093–1098
33 Cam K., Rome G., Krisch H. M. and Bouché J.-P. (1996) RNase E processing of essential cell division genes mRNA in *Escherichia coli*. Nucleic Acids Res. **24:** 3065–3070
34 Sitnikov D. M., Schineller J. B. and Baldwin T. O. (1996) Control of cell division in *Escherichia coli*: regulation of transcription of *ftsQA* involves both *rpoS* and SdiA-mediated autoinduction. Proc. Natl. Acad. Sci. USA **93:** 336–341
35 García-Lara J., Shang L. H. and Rothfield L. I. (1996) An extracellular factor regulates gene expression of *sdiA*, a transcriptional activator of cell divison genes in *Escherichia coli*. J. Bacteriol. **178:** 2742–2748
36 Dewar S. J. and Donachie W. D. (1990) Regulation of expression of the *ftsA* cell division gene by sequences in upstream genes. J. Bacteriol. **172:** 6611–6614
37 Robinson A. C., Kenan D. J., Hatful G. F., Sullivan N. F., Spiegelberg R. and Donachie W. D. (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
38 Yi Q.-M., Rockenbach S., Ward Jr. J. E. and Lutkenhaus J. (1985) Structure and expression of the cell division genes *ftsQ*, *ftsA* and *ftsZ*. J. Mol. Biol. **184:** 399–412
39 Gervais F. G., Phoenix P. and Drapeau G. R. (1992) The *rcsB* gene, a positive regulator of colanic acid biosynthesis in *Escherichia coli*, is also an activator of *ftsZ* expression. J. Bacteriol. **174:** 3964–3971
40 Masters M., Patterson T., Popplewell A. G., Owen-Hughes T., Pringle J. H. and Begg K. J. (1989) The effect of DnaA protein levels and the rate of initiation at *oriC* on transcription originating in the *ftsQ* and *ftsA* genes: in vivo experiments. Mol. Gen. Genet. **216:** 475–483
41 Robin A., Joseleau-Petit D. and D'Ari R. (1990) Transcription of the *ftsZ* gene and cell division in *Escherichia coli*. J. Bacteriol. **172:** 1392–1399
42 Smith R. W. P., McAteer S. and Masters M. (1996) The coupling between *ftsZ* transcription and initiation of DNA replication is not mediated by the DnaA-boxes upstream of *ftsZ* or by DnaA. Mol. Microbiol. **21:** 361–372
43 Zhou P. and Helmstetter C. E. (1994) Relationship between *ftsZ* gene expression and chromosome replication in *Escherichia coli*. J. Bacteriol. **176:** 6100–6106
44 Tétart F. and Bouché J.-P. (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
45 Dewar S. J. and Donachie W. D. (1993). Antisense transcription of the *ftsZ-ftsA* gene junction inhibits cell division in *Escherichia coli*. J. Bacteriol. **175:** 7097–7101
46 Wang X., de Boer P. A. J. and Rothfield L. I. (1991) A factor that positively regulates cell division by activating transcription of the major cluster of essential cell division genes of *Escherichia coli*. EMBO J. **10:** 3363–3372
47 Jung H. K, Ishino F. and Matsuhashi M. (1989) Inhibition of growth of *ftsQ*, *ftsA* and *ftsZ* mutant cells of *Escherichia coli* by amplification of a chromosomal region encompassing closely aligned cell division and cell growth genes. J. Bacteriol. **171:** 6379–6382
48 Boyle D. S., Khattar M. M., Addinall S. G., Lutkenhaus J. and Donachie W. D. (1997) *ftsW* is an essential cell-division gene in *Escherichia coli*. Mol. Microbiol. **24:**1263–1273
49 Vicente M. 1993. A simple expression? Complexities of genetic regulation in microorganisms. World J. Microbiol. Biotechnol. **9:** 401–402