

Transcription of the *Escherichia coli* *dcw* cluster: Evidence for distal upstream transcripts being involved in the expression of the downstream *ftsZ* gene

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(Received 10 October 2000; accepted 28 November 2000)

Abstract — *Escherichia coli* strains VIP596 and VIP597 have been constructed to compare the amount of transcription of the *ftsZ* gene derived from proximal promoters in the *ddlB-ftsZ* region with that originating in the upstream regions of the *dcw* cluster. Both strains have in common a β -galactosidase reporter fusion located at the *ddlB* locus, but differ in that VIP597 has a transcription terminator Ω interposon located downstream from *lacZ*. In addition, these strains have the *ddlB*, *ftsQ*, *ftsA* and *ftsZ* genes under the control of the IPTG-inducible promoter P_{tac} , allowing to control artificially *ftsZ* expression for normal cell division to take place. When β -galactosidase activity was measured in VIP596 and VIP597 and compared to the levels measured in strain VIP407, in which the *lacZ* reporter fusion is located in the *ftsZ* gene, they were found to account for nearly 66% of the total transcription entering into *ftsZ*. This result indicates that the reduction in *ftsZ* transcription observed when the promoters in the *ddlB-ftsA* region are disconnected from the upstream sequences of the *dcw* cluster (as observed by Flårdh et al., Mol. Microbiol. 30 (1998) 305–316) in strain VIP490) is the direct consequence of the interruption in the transcription originated upstream and not due to the effect of such sequences on the promoters proximal to *ftsZ*. © 2001 Société française de biochimie et biologie moléculaire / Éditions scientifiques et médicales Elsevier SAS

cell division / regulation / transcription / *dcw* cluster / *Escherichia coli*

1. Introduction

The *ftsZ* gene maps at 2.5 min of the *Escherichia coli* chromosome immediately after two other cell-division genes, *ftsQ* and *ftsA*, at the distal end of the *dcw* cluster. *ftsA* and *Z* share a high degree of homology with other bacterial species and their products, together with FtsQ, are essential for *E. coli* cell division. The *E. coli* *dcw* cluster is formed by a total of 16 genes involved either in cell division or in peptidoglycan biosynthesis [2]. FtsZ is an essential protein for cell division in many organisms including bacteria, archaea and eukaryotic organelles, both chloroplasts [3, 4] and mitochondria [5]. Gene expression regulation in the *E. coli* *dcw* cluster is very complex, as it occurs as well in other clusters containing essential genes (reviewed in [6, 7]). Given the absence of transcriptional terminators the whole *dcw* cluster may function in *E. coli* as a single transcriptional unit [8]. This is not the case in other microorganisms such as *Neisseria gonorrhoeae* [9] in which the presence of transcriptional terminators divides the *dcw* cluster in at least five distinct transcriptional units.

Similarly, transcriptional organisation of the distal end of the *E. coli* *dcw* cluster is complex, marked by the existence of at least seven promoters, located within the

coding regions of the immediately adjacent upstream *ddlB*, *ftsQ* and *ftsA* genes, to regulate *ftsZ* expression [2, 10]. Previous studies demonstrated that these promoters provide only 33% of the total *ftsZ* transcription [1]. A number of regulatory factors (SdiA, σ^s , ppGpp, RcsB) have been demonstrated to act on these promoters [11–14]. As a further complexity the accumulation of transcripts for *ftsZ* has been reported to oscillate in a cell-cycle-dependent manner [15].

In this work we have constructed strains in which a reporter β -galactosidase gene is placed in the *E. coli* chromosome at a location suitable to report the transcriptional activity that, having originated in the upstream regions of the *dcw* cluster, is able to progress into the distal *ddl-fts* region. We find that a substantial amount of transcripts originating in the upstream *dcw* sequences enter into the *ddl-fts* region. It seems then unlikely that the upstream *dcw* sequences have an enhancing effect on the downstream *ddl-fts* region promoters.

2. Materials and methods

2.1. Bacterial strains

E. coli K12 strains MC1061 (*araD139* Δ (*ara-leu*)7697 Δ (*lac*)X74 *galU galK strA*; [16]); DH5 α (*supE44* Δ *lacU169* Φ *lacZM* Δ 15 *hsdR17 recA1 endA1 gyrA96*)

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thi-1 relA1; [17]) and CC118(λ *pir*) (Δ (*lac-pro*) *argE*(Am) *recA56 nalA* Rif^R (λ *pir*); [18]) were grown on Luria broth (LB) either liquid or solidified with 1.5% agar [19] at 37 °C and supplemented when required with thiamine (thi) at 5 μ g mL⁻¹, ampicillin (Amp) at 50 μ g mL⁻¹, isopropyl- β -D-thiogalactopyranoside (IPTG) (20 or 100 μ M, except when stated otherwise), and 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal) at 40 μ g mL⁻¹.

2.2. DNA manipulations

General DNA manipulations were performed according to standard protocols [20, 21]. Transformations were performed either as described by Hanahan [17], or using the BioRad *E. coli* electroporation pulser at the manufacturer's specifications. Non-radioactive labeling of probes, hybridization, and colorimetric detection of Southern blots were carried out using Boehringer (Roche Molecular Biochemicals) Nucleic Acid Labelling and Detection kit accordingly to the manufacturer's instructions.

2.3. Plasmid constructions

Plasmid pPFV1 was constructed by cloning a *Hind*III-*Pst*I 729 bp fragment containing part of *ddlB* gene and part of *murC* from plasmid pKFV117 in the *Hind*III and *Pst*I sites of plasmid pTL61T which contains the *lacZ* reporter gene and an Rnase III processing site at the start of *lacZ* [22]. This resulted in the *murC-ddlB* segment being placed upstream the *lacZ* gene. The resulting pPFV1 plasmid was then propagated in DH5 α .

To obtain plasmid pAFV1 a 4115 bp *Sal*I-*Nru*I fragment from plasmid pPFV1, containing the C-terminal part of *murC* and a fusion Φ (*ddlB-lacZ*), was cloned between the *Xho*I and the Klenow treated *Bgl*II sites of plasmid pKFV115 [1], so that the *P*_{*tac*} promoter of pKFV115 is able to direct transcription from the truncated *murC* region. Plasmid pAFV2 was constructed as pAFV1 but inserting the transcription terminator Ω as a 2.0 kb *Sma*I fragment from plasmid pHP45 Ω [23] downstream the *lacZ* gene. Both plasmids were propagated in the strain CC118 (λ *pir*).

2.4. Construction of the reporter strains VIP596 and VIP597

Plasmids pAFV1 and pAFV2 do not replicate except in hosts expressing the π protein [24]. To select for strains in which the desired constructions had integrated into the chromosome, pAFV1 and pAFV2 were electroporated into MC1061. Amp^R Lac⁺ colonies were then selected on LB plates containing IPTG at different concentrations (ranging from 20–100 μ M) and 50 μ g mL⁻¹ ampicillin. Their IPTG growth dependence was tested and the correct location of the integrated plasmid in the chromosome was

confirmed using PCR amplification and sequencing. Primer pairs used and sizes of the expected amplified fragments were: KF7(*murC*) - TG17(*lacZ*) (2.8 kb); TG20 (*tac* promoter) - MA1(*ddlB*), (600 bp); and KF8 (*aadA*) - MA1(*ddlB*) (6.0 kb) (see figure 1) [1]. Detection of the Ω fragment in strain VIP597 was also confirmed by Southern blot analysis of *Eco*RV digested chromosomal DNA using an 2.0 kb Ω probe from plasmid pHP45 Ω [23] yielding an 8.0 kb fragment as expected.

2.5. β -Galactosidase assays

Measurement of β -galactosidase activities was done in samples obtained from LB liquid medium cultures supplemented with IPTG (5, 20, 60 and 100 μ M), thi (5 μ g mL⁻¹), and Amp (50 μ g mL⁻¹ when necessary). The cultures were maintained in exponential growth phase at an OD₆₀₀ lower than 0.25 by diluting into pre-warmed medium for at least 10 doubling times. For assays samples were taken at OD₆₀₀ = 0.2 and β -galactosidase activities were measured as described by Miller [19], with the modifications described by Masters et al. [25]. The activities are expressed in Miller units.

2.6. Cell volume measurements

Portions of exponentially growing cultures of VIP596 and VIP597 in medium containing 20 μ M IPTG were transferred to medium containing different IPTG concentrations and grown, maintaining the OD₆₀₀ below 0.3 by suitable dilutions, for at least three doublings. After this time the median cell volume of each portion was measured using a ZM Coulter Counter with a 30 μ m orifice connected to a Coulter Channelyzer 256 (both from Coulter Electronics) as described previously [26].

2.7. Measurement of FtsZ and FtsA concentrations

FtsZ and FtsA contents were determined as described previously [26, 27], except that blots were revealed using a luminescence detection kit from Boehringer Mannheim (Roche Molecular Biochemicals). The FtsZ and A levels were measured in the same blot using polyclonal antisera MVJ9(anti-FtsZ) and MVJ1(anti-FtsA).

3. Results

3.1. Determination of the transcription potential required to produce levels of FtsQ, A and Z able to sustain cell viability

According to Flärth et al. [1], 66% of the transcription potential that reaches the *dcw* cluster gene *ftsZ*, located almost at the end of the cluster, is lost when a strong transcriptional terminator (the Ω interposon) is placed in

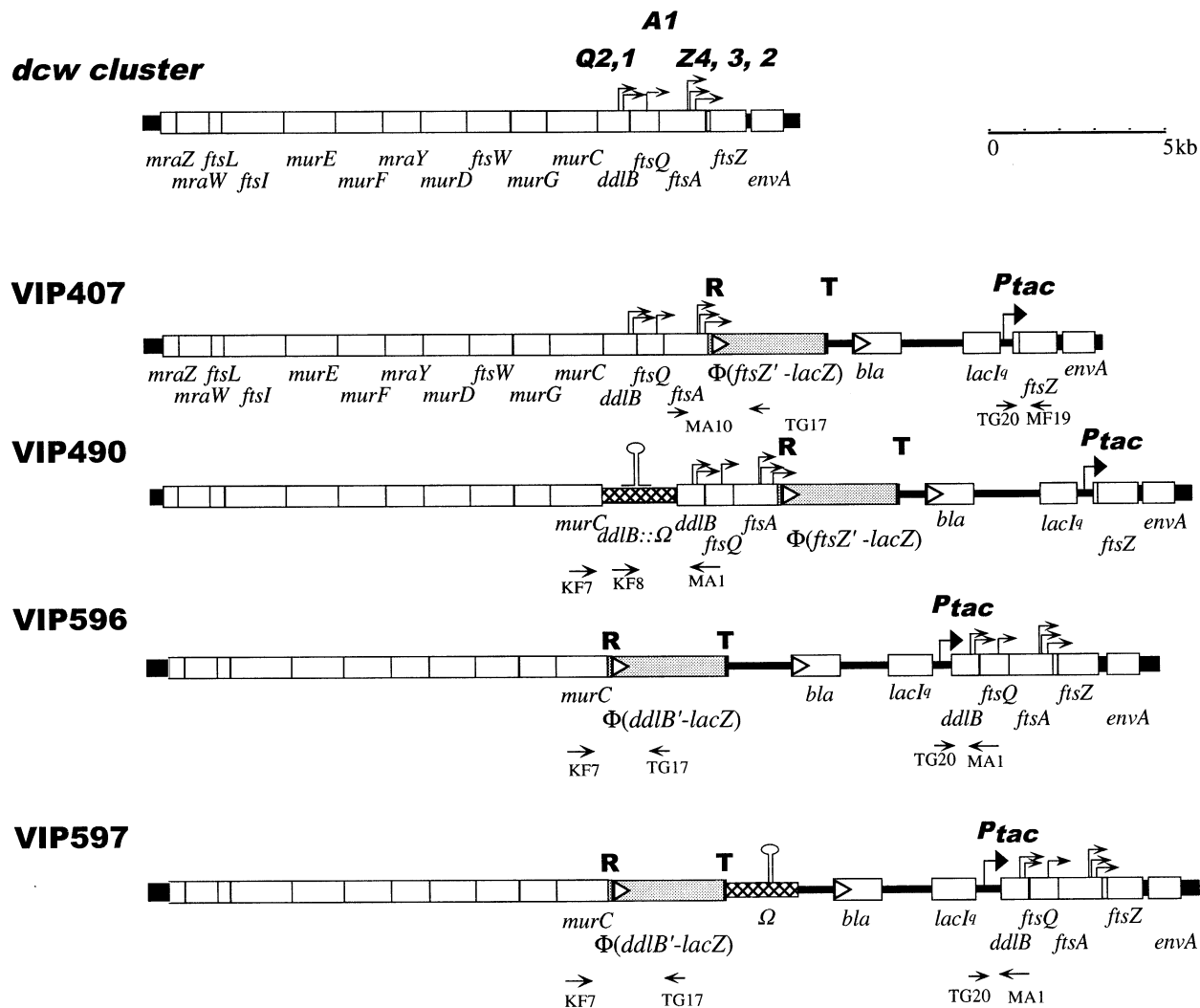


Figure 1. β -Galactosidase reporter fusions in the *dcw* cluster constructed in the *E. coli* chromosome. The arrangement of genes in the *E. coli* *dcw* cluster is shown in the top row. Arrows show the position of known promoters in the *ddlB*-*ftsQAZ* region (their abbreviated names, gene and promoter number are shown). The *lacZ* reporter is shaded in grey. Direction of transcription of *bla* and *lacZ* is indicated by an arrowhead inside them. The Ω interposon is represented by a double cross-hatched box. The hairpin symbol represents one of the transcriptional terminators contained in the Ω element. R marks the RNaseIII processing site located upstream the *lacZ* gene. T represents the transcriptional terminator *rrnBT* in the *lacZ* gene [22]. The position of the *tac* promoter used to modulate the expression of essential cell division genes is also highlighted. The approximate locations of relevant oligonucleotides primers used to verify the constructions [1] are indicated.

ddlB in strain VIP490. To find if this reduction is actually due to the physical interruption of the transcripts, a reporter gene was placed in the bacterial chromosome inside the non-essential gene *ddlB*. For this purpose *ddlB* was replaced by a transcriptional fusion $\Phi(ddlB-lacZ)$ by integrating plasmid pKPFV2 into the chromosome of strain MC1061 to create strain VIP596 (see figure 1). Additionally strain VIP597 was constructed by integrating

in the chromosome a transcriptional fusion $\Phi(ddlB-lacZ)$ containing the strong transcriptional terminator Ω located downstream the *lacZ* reporter. The chromosome of this strain contains exactly the same genes as VIP490, but arranged in a different order. In this way the *lacZ* gene inVIP490 reports the transcription that is originated within the *ddl*-*fts* region, while the same reporter in VIP596 and VIP597 reports the transcription that, originating in the

Table I. Effect of IPTG concentration on cell volume, FtsA and Z levels and β -galactosidase activity in strains VIP596 and VIP597.

Strain	IPTG concentration (μ M) ^a	Cell volume ^b	FtsA ^b	FtsZ ^b	β -Galactosidase activity (Miller units)
VIP596	5	1.40	0.26	0.57	784
	20	0.90	0.70	0.90	712
	60	1.20	1.80	1.48	785
	100	1.50	2.24	1.82	894
VIP597	5	1.90	0.10	0.45	833
	20	0.80	0.45	0.81	816
	60	1.10	0.89	1.40	727
	100	1.03	0.98	1.60	792

^a Exponentially growing cultures of VIP596 and VIP597 in LB thi, Amp, 20 μ M IPTG were washed and divided into media with different IPTG concentrations. Cultures were kept in exponential growth phase and sampled after 3 h.

^b The values are relative to the ones present in MC1061 growing in the same medium lacking antibiotics and IPTG.

upstream regions of the *dcw* cluster, is able to reach into the *ddlB* gene. As the *ddl-fts* region contains three essential genes, *ftsQ*, *A* and *Z*, a *tac* promoter, together with a *lacI^q* gene was provided in front of *ddlB* to regulate their transcription in a controlled manner by the addition of IPTG to VIP596 and VIP597 (VIP490 contains plasmid pKFV122 to ensure a sufficient supply of FtsQ and A [1]). The chromosomal structure of VIP596 and VIP597 was confirmed by PCR amplification and sequencing of the engineered region (results not shown).

As expected both strains VIP596 and VIP597 are ampicillin resistant, Lac⁺ and, due to the presence of P_{*tac*} and *lacI^q* to transcribe essential genes, they are also IPTG dependent. While VIP597 and VIP490 do not grow at all when inoculated into LB plates without IPTG, VIP596 grows poorly under the same conditions. This result indicates that the promoters found inside the *ddl-fts* region do not provide enough transcription potential to produce sufficient levels of the FtsQ, A or Z proteins to sustain cell viability. Sufficient Fts protein levels (FtsQ, A and Z in VIP596, or FtsZ in VIP490) could only be attained when P_{*tac*} is induced by IPTG (note that FtsQ and A in VIP490 are supplied by a plasmid). Moreover, results from an experiment designed to determine the IPTG concentration required by each strains VIP596 and VIP597 to maintain a volume similar to the wild type (table I) indicated that both strains require an optimal concentration near 20 μ M IPTG. Microscopical examination of VIP596 and VIP597 cells grown at 20 μ M IPTG and transferred to 5 μ M IPTG shows that they form long filaments as expected (data not shown). At 5 μ M IPTG VIP597 cells failed to divide altogether (data not shown). These results show that if any leakage of transcription termination is allowed by the less powerful *rrnBT* terminator element [22] in VIP596 it is not sufficient to increase the transcription of the promoters inside the *ddl-fts* region to levels able to supply sufficient amounts of the Fts Q, A and Z proteins.

3.2. Determination of the transcription potential derived from upstream *dcw* cluster sequences that is able to enter into the *ddl-fts* region

In order to measure the amount of upstream transcription potential that is able to enter into the *ddl-fts* region, the β -galactosidase activity of strains VIP596 and VIP597 growing in the presence of several IPTG concentrations was measured (table II) and compared with the activities measured in strains VIP407 and VIP490 ([1] and table II). Strain VIP407, accordingly to previous estimates, is considered to report the full potential transcription reaching the *ftsZ* gene, while VIP490, containing the interposon in *ddlB* and the *lacZ* reporter in *ftsZ*, reports the transcription potential originated at the promoters found in the *ddl-fts* region that was found to be 33% of the total [1]. The results (tables I, II) indicate that the transcription values reported by the *lacZ* gene placed immediately upstream from *ddlB* at different IPTG concentrations amount up to 74% (with an average of 61%) of the total activity reported when the *lacZ* gene is placed immediately upstream from *ftsZ*. We conclude then that transcription termination is the major cause of the decrease in the transcription potential that enters into the *ftsZ* gene observed when a transcription terminator is inserted in *ddlB* (VIP490). Any *cis*-activation effects on the promoter activities located in the *ddl-fts* from the upstream sequences, if at all present, are only a minor element in the regulation of gene expression in this region.

3.3. Effect of the FtsA and FtsZ concentrations on the expression of the upstream *dcw* cluster promoters

The presence of a P_{*tac*} promoter in front of *ddlB* in strains VIP596 and VIP597 allows the induction of the expression of *ftsQ*, *A*, and *Z* at different levels depending on the concentration of the inducer. An experiment was done in which these strains were grown in the presence of

Table II. β -Galactosidase activities in strains containing single-copy *lacZ* reporter fusions in the *dcw* cluster.

Strain	Relevant genotype	Doubling time (min) ^a	β -galactosidase activity (Miller units) ^a
VIP407 ^b	MC1061 <i>ftsZ</i> ::pKfV116 [Φ (<i>ftsZ-lacZ</i>)]	26	1318 \pm 152 (100)
VIP490 ^b	VIP406 ^b <i>ftsZ</i> ::pKfV116 [Φ (<i>ftsZ-lacZ</i>)] <i>ddlB</i> :: Ω pKfV122 [<i>ftsQ</i> ⁺ , A ⁺]	30	435 \pm 92 (33)
VIP596	MC1061 <i>ddlB</i> ::pAFV1 [Φ (<i>ddlB-lacZ</i>)]	25	712 \pm 100 (54)
VIP597	MC1061 <i>ddlB</i> ::pAFV2 [Φ (<i>ddlB-lacZ</i>):: Ω]	30	816 \pm 100 (62)

^a Cultures were grown for more than 10 doubling times at 37 °C in LB liquid medium supplemented with 20 μ M IPTG, thi and Amp. Figures between brackets are percentages of values relative to VIP407.

^b [1].

different IPTG concentrations (5 to 100 μ M). The FtsA and Z cell contents were quantified by Western blotting and the level of reporter *lacZ* expression was measured. The results shown in *table I* indicate that the level of the transcription originated in the upstream regions of the *dcw* cluster that enters into the *ddl-fts* region is not modified significantly by the cellular amounts of the FtsA or FtsZ proteins. Given the low concentration of FtsQ in the wild type strain [28, 29], it has not been possible to titrate it reliably. As the constructions used for this experiment also contain the *ftsQ* gene under the control of P_{*tac*}, it seems reasonable to assume as well that the levels of FtsQ should not affect transcription of the upstream *dcw* promoters. The differential effects of IPTG induction on the expression of *fts* and Z are also interesting to note. If we consider the VIP597 induced levels of *ftsA* and Z as a result of the induction from P_{*tac*}, then the levels found in VIP596 under the same induction conditions should result from the addition of P_{*tac*} directed transcription and any other possible *ftsA* expression induction. Subtraction of the *ftsA* and Z levels obtained in VIP597 from those in VIP596 at each IPTG concentration shows that in response to IPTG induction the levels of FtsA in VIP596 seem to increase at a higher rate than those of FtsZ.

4. Discussion

Expression of the essential cell division gene *ftsZ* is driven in *E. coli* by a complex set of promoters. Some of them have been experimentally identified in the proximal and distal upstream regions of the *dcw* cluster. Flärth et al. [1] reported that at least 66% of the transcription of *ftsZ* was dependent on sequences upstream from *ddlB*. They could not discriminate if such sequences were in itself promoters or conversely if they played an enhancer-like role on the promoters within the *ddl-fts* region. We have now placed a *lacZ* reporter gene inside the dispensable *ddlB* gene and used it to measure the transcription activity originating at upstream sequences that enters into the *ddl-fts* region. Flärth et al. [1] considered that a similar

reporter construction placed after the end of *ftsZ* was reporting 100% of the transcription activity that entered into *ftsZ*. However, insertion of an interposon transcriptional terminator in the *ddlB* gene upstream from *ftsQ2p* resulted in a decrease of 66% of the measured activity. Our results show that the upstream transcription that enters into the *ddlB* gene accounts for approximately 60% of the total. This result indicates that the decrease observed by Flärth et al. [1], is largely a consequence of the blocking of incoming transcripts that originated at sequences of the *dcw* cluster located upstream from *ddlB*.

Hara et al. [30] found that the most upstream promoters of the *E. coli dcw* cluster are required for the proper expression of genes downstream to *ftsW*. Similarly Mengin-Lecreux et al. [31] have shown that expression of *ftsZ* is dependent (although not exclusively) on the promoters found at the 5' end of the *dcw* cluster. It is not known if the messengers that originate at the head of the cluster are able to reach the *ddl-fts* region, what would result in a polycistronic transcript containing information for 16 genes. If this were the case the regulation of gene expression in the *E. coli dcw* cluster may offer some intricate examples of postranscriptional control.

Studying the complementation properties of a lambda phage vector (λ 16-2) that carries the terminal half of the *dcw* cluster (from the mid part of *ftsW*) Dai and Lutkenhaus [32] found that it failed to complement a null mutation in *ftsZ* estimating then the contribution of upstream-directed expression of *ftsZ* to be 30 to 40%. Our results similarly show that the transcription originated in the *ddl-fts* region (33% of the total) is not sufficient to sustain growth and division in VIP596 and VIP597 under normal experimental conditions. This does not exclude that under specific circumstances some of the promoters contained in this region may have a more important role, for example upon entrance into stationary phase the relative contribution of *ftsQ1p* increases [13], SdiA induction of *ftsQ2p* is able to raise the levels of *ftsZ* transcription [33] and *ftsA1p* may be induced by RcsB [14].

We also find that different levels of *ftsQ*, A, and Z expression obtained when adding different IPTG concen-

tration to cultures of VIP596 or VIP597 do not significantly modify the activity obtained from the reporter gene, while on the other hand the levels of the FtsA and Z proteins are induced. This confirms that FtsZ has no regulatory role in the expression of the promoters located within the *dcw* cluster [1]. Moreover, as *ftsQ* is also controlled in VIP596 and VIP597 by the P_{lac} promoter we can conclude that neither the levels of FtsA, FtsZ, nor (possibly) FtsQ are likely to have an important effector role on the overall transcription activity of the *dcw* cluster genes in *E. coli*. Results obtained by Dewar et al. [34], indicate that the β -galactosidase levels reported by a construction carrying the *ftsAp*, *ftsZ3p* and *4p* promoters in a lambda prophage in a Ts suppressor background increased at 42 °C when the levels of FtsA16 (Am) diminished as a consequence of the loss of suppressor activity. Our results would suggest that the modulation of expression observed in their experiments do not extend to the promoters found upstream from *ddlB*, those that under normal growth conditions supply 66% of the transcription potential for *ftsZ*.

On the other hand, a different effect of IPTG concentration on the rate of FtsA and Z production can be observed when comparing the levels of both proteins obtained in VIP596 and VIP597. As the *dcw* cluster promoters present in both strains are exactly the same, this observation should be interpreted as a possible effect of the FtsA protein on postranscriptional events affecting the artificial transcript generated in VIP596 by the combined effect of RNase III cleavage upstream *lacZ* and leakiness of the transcriptional terminator immediately downstream.

Although there is a fair degree of gene and gene order conservation among the *dcw* cluster of different organisms [8] it seems that regulation of its expression in different species may adopt different patterns, depending perhaps on the presence of transcriptional terminators in the cluster [9]. The physical arrangement of the *E. coli* *dcw* cluster raises some interesting questions on how such a complexity has been conserved during evolution instead of the cluster being, or the isolated genes remaining, dispersed. The dispersion of the *dcw* cluster genes seems to be a rare event [8], but the separation into different transcriptional units occurs in some organisms [9]. This is not the case in *E. coli* in which these complex mechanisms that regulate transcription of the *dcw* cluster seem to be required for the proper performance of septation [26].

Acknowledgments

The excellent technical assistance of Mercedes Casanova is acknowledged. We thank Klas Flårdh for his expert advice, Jesús Mingorance for his useful comments and critical reading of the manuscript, and Larry Rothfield for stimulating discussion. This work was supported by grant BIO 97-1246 from Ministerio de Educación y Cultura (Spain).

References

- [1] Flårdh K., Palacios P., Vicente M., Cell division genes *ftsQAZ* in *Escherichia coli* require distant *cis*-acting signals upstream of *ddlB* for full expression, *Mol. Microbiol.* 30 (1998) 305–316.
- [2] Ayala J.A., Garrido T., de Pedro M.A., Vicente M., Molecular biology of bacterial septation, in: Ghuyssen J.M., Hakenbeck R. (Eds.), *Bacterial cell wall*, Elsevier Science B.V., Amsterdam, 1994, pp. 73–101.
- [3] Osteryoung K.W., Vierling E., Conserved cell and organelle division, *Nature* 376 (1995) 473–474.
- [4] Strepp R., Kalman S., Kruse S., Speth V., Reski R., Plant nuclear gene knockout reveals a role in plastid division for the homolog of the bacterial cell division protein FtsZ, an ancestral tubulin, *Proc. Natl. Acad. Sci. USA* 95 (1998) 4368–4373.
- [5] Gray M.W., Evolution of organellar genomes, *Curr. Opin. Genet. Dev.* 9 (1999) 678–687.
- [6] Vicente M., Chater K.F., de Lorenzo V., Bacterial transcription factors involved in global regulation, *Mol. Microbiol.* 33 (1999) 8–17.
- [7] Vicente M., Errington J., Structure, function and controls in microbial division, *Mol. Microbiol.* 20 (1996) 1–7.
- [8] Vicente M., Gómez M.J., Ayala J.A., Regulation of transcription of cell division genes in the *Escherichia coli* *dcw* cluster, *Cell. Mol. Life Sci.* 54 (1998) 317–324.
- [9] Francis F., Ramirez-Arcos S., Salimnia H., Victor C., Dillon J.A.R., Organization and transcription of the division cell wall (*dcw*) in *Neisseria gonorrhoeae*, *Gene* 251 (2000) 141–151.
- [10] Flårdh K., Garrido T., Vicente M., Contribution of individual promoters in the *ddlB-ftsZ* region to the transcription of the essential cell-division gene *ftsZ* in *Escherichia coli*, *Mol. Microbiol.* 24 (1997) 927–936.
- [11] García-Lara J., Shang L.H., Rothfield L.I., An extracellular factor regulates gene expression of *sdia*, a transcriptional activator of cell division genes in *Escherichia coli*, *J. Bacteriol.* 178 (1996) 2742–2748.
- [12] Aldea M., Garrido T., Pla J., Vicente M., Division genes in *Escherichia coli* are expressed co-ordinately to cell septum requirements by gearbox promoters, *EMBO J.* 9 (1990) 3787–3794.
- [13] Ballesteros M., Kusano S., Ishihama A., Vicente M., The *ftsQ1p* gearbox promoter of *Escherichia coli* is a major sigma S-dependent promoter in the *ddlB-ftsA* region, *Mol. Microbiol.* 30 (1998) 419–430.
- [14] Carballès F., Bertrand C., Bouché J.P., Cam K., Regulation of *Escherichia coli* cell division genes *ftsA* and *ftsZ* by the two-component system *resC-resB*, *Mol. Microbiol.* 34 (1999) 442–450.
- [15] Garrido T., Sánchez M., Palacios P., Aldea M., Vicente M., Transcription of *ftsZ* oscillates during the cell cycle of *Escherichia coli*, *EMBO J.* 12 (1993) 3957–3965.
- [16] Casadaban M.J., Cohen S.N., Analysis of gene control signals by DNA fusion and cloning in *Escherichia coli*, *J. Mol. Biol.* 138 (1980) 179–207.
- [17] Hanahan D., Studies on transformation of *Escherichia coli* with plasmids, *J. Mol. Biol.* 166 (1983) 557–580.
- [18] Herrero M., de Lorenzo V., Timmis K.N., Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in Gram-negative bacteria, *J. Bacteriol.* 172 (1990) 6557–6567.
- [19] Miller J.H., A short course in bacterial genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1992.
- [20] Sambrook J., Fritsch E.F., Maniatis T., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, N.Y., 1989.
- [21] Wilson K., Miniprep of bacterial genomic DNA, in: Ausubel F.M., Brent R., Kingston R.E., Moore D.D., Seidman J.G., Smith J.A., Struhl K. (Eds.), *Current Protocols in Molecular Biology*, Vol. 1, John Wiley and Sons Inc., New York, N.Y., 1998.

- [22] Linn T., St-Pierre R., Improved vector systems for constricting transcriptional fusions that ensures independent translation of *lacZ*, *J. Bacteriol.* 172 (1990) 1077–1084.
- [23] Prentki P., Krisch H.M., In vitro insertional mutagenesis with a selectable DNA fragment, *Gene* 29 (1984) 303–313.
- [24] Miller J.H., Mekalanos J.J., A novel suicide vector and its use in construction of insertion mutations: osmoregulation of outer membrane proteins and virulence determinants in *Vibrio cholerae* requires *toxR*, *J. Bacteriol.* 170 (1992) 2575–2583.
- [25] Masters M., Patterson T., Popplewell A.G., Owen-Hughes T., Pringle J.H., Begg K.J., 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 (1989) 475–483.
- [26] Palacios P., Vicente M., Sánchez M., Dependency of *Escherichia coli* cell-division size, and independency of nucleoid segregation on the mode and level of *ftsZ* expression, *Mol. Microbiol.* 20 (1996) 1093–1098.
- [27] Pla J., Sánchez M., Palacios P., Vicente M., Aldea M., Preferential cytoplasmic location of FtsZ, a protein essential for *Escherichia coli* septation, *Mol. Microbiol.* 5 (1991) 1681–1686.
- [28] Carson M.J., Barondess J., Beckwith J., The FtsQ protein of *Escherichia coli*: Membrane topology, abundance, and cell division phenotypes due to overproduction and insertion mutations, *J. Bacteriol.* 173 (1991) 2187–2195.
- [29] Dopazo A., Palacios P., Sánchez M., Pla J., Vicente M., An amino-proximal domain required for the localization of FtsQ in the cytoplasmic membrane, and for its biological function in *Escherichia coli*, *Mol. Microbiol.* 6 (1992) 715–722.
- [30] Hara H., Yasuda S., Horiuchi K., Park J.T., 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 (1997) 5802–5811.
- [31] Mengin-Lecreux D., Ayala J., Buhss A., van Heijenoort J., Parquet C., Hara H., Contribution of the *Pmra* promoter to expression of genes in the *Escherichia coli mra* cluster of cell envelope biosynthesis and cell division, *J. Bacteriol.* 180 (1998) 4406–4412.
- [32] Dai K., Lutkenhaus J., *ftsZ* is an essential cell division gene in *Escherichia coli*, *J. Bacteriol.* 173 (1991) 3500–3506.
- [33] Wang X., de Boer P.A.J., Rothfield L.I., 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 (1991) 3363–3372.
- [34] Dewar S.J., Kagan-Zur V., Begg K.J., Donachie W.D., Transcriptional regulation of cell division genes in *Escherichia coli*, *Mol. Microbiol.* 3 (1989) 1371–1377.