

Mapping of a transcription promoter located inside the *priA* gene of the *Bacillus subtilis* chromosome

Krzysztof Hinc¹, Adam Iwanicki², Simone Seror³ and Michał Obuchowski²✉

¹Department of Molecular Biology, University of Gdańsk, Gdańsk, Poland; ²Department of Medical Biotechnology, Intercollegiate Faculty of Biotechnology, Medical University of Gdańsk, Gdańsk, Poland; ³Institute of Genetics and Microbiology, University of Paris-Sud, Orsay Cedex, France; ✉e-mail: obuchowk@biotech.univ.gda.pl

Received: 05 January, 2006; revised: 18 July, 2006; accepted: 04 August, 2006
available on-line: 09 September, 2006

The genome sequence of the Gram-positive soil bacterium *Bacillus subtilis* was completed in 1997 (Kunst *et al.*, 1998) and the results included the identification of a putative transcription unit encompassing the *yloI* to *yloS* genes. Within this region of the *B. subtilis* chromosome 11 putative open reading frames were found with a wide diversity of probable functions. In this work we have analyzed transcription in the region of the *priA-cpgA* genes and we have mapped a promoter which is located inside the *priA* gene and its activity directs transcription of the *def-yloM* genes. Moreover, this transcript can be extended at low level to the *prpC-priK-cpgA* genes. Analysis of the sequence in proximity of the transcription start site revealed a sequence suitable for the housekeeping σ^A subunit of RNA polymerase. Analysis of the β -galactosidase activity of transcription fusions revealed that the identified promoter is active at low level and its activity is increased during late exponential phase of growth.

Keywords: *Bacillus subtilis*, transcription, weak promoters

INTRODUCTION

Regulation of the transcription is the most common way of controlling gene expression and is necessary for all living organisms to adapt to the continuously changing environmental conditions. Most of this regulation takes part at the level of initiation. Appropriate organization of the genome is therefore very important in order to optimize the transcription process.

The Gram-positive soil bacterium *Bacillus subtilis* encounters various conditions in its natural environment. Its genome has been sequenced and annotated (Kunst *et al.*, 1997) and also an initial functional analysis was performed (Kobayashi *et al.*, 2003). However, many regulation aspects remain undiscovered. From the genome sequencing project, a 28-kbp DNA segment ranging from 138.9° to 142.1° on the genome map (position 1635434 to 1663803) was found to contain 27 ORFs. In this fragment a transcription unit *yloK-yloS* containing 11 ORFs was proposed (Foulger & Errington,

1998). Four of these ORFs have been cloned and their products characterized: PriA, a primosomal replication factor Y (Marians *et al.*, 1999), Def, a protein deformylase (Huntington *et al.*, 2000), PrpC, a protein Ser/Thr phosphatase (Obuchowski *et al.*, 2000), PrkC, a protein Ser/Thr kinase (Madec *et al.*, 2002) and CpgA, a GTP binding protein, essential for viability (Cladiere *et al.*, 2006). The *prpC* gene overlaps by 3 bp with *prkC* and this couple is followed by an essential gene, *cpgA*. The organization of the genes *prpC*, *prkC* and *cpgA* is conserved in several Gram-positive bacteria. Previous analysis of this region revealed that at least two promoters are present in front of the *prpC* and *cpgA* genes (Iwanicki *et al.*, 2005) (see Fig. 1). Interestingly, genes located within this region appear to encode proteins involved in many different functions (Table 1), including *priA* required for restarting replication forks following DNA damage (Marians *et al.*, 1999). This region contains a pair of genes, *prpC* and *prkC*, encoding a protein phosphatase and a protein kinase, respectively (Madec *et al.*, 2002). Autophos-

Table 1. Similarity of the products of genes from the *yloI-yloS* region to other known proteins.

Results of previous BLAST search were taken from Foulger and Errington (1998). Positions of ORF were taken from SubtiList (<http://genolist.pasteur.fr/SubtiList/>).

ORF	Position in <i>B. subtilis</i> chromosome	Proteins with significant homology or similarity	
		Previous BLAST search	BLAST search performed in this work
<i>yloI</i>	1642151 1643368	panthothenate metabolism flavoprotein – <i>E. coli</i>	phosphopanthothenate-cysteine ligase – <i>B. cereus</i>
<i>priA</i> (<i>yloJ</i>)	1643368 1645782	primosomal replication factor Y – <i>E. coli</i>	primosomal replication factor – <i>B. halodurans</i>
<i>def</i> (<i>yloK</i>)	1645812 1646291	N-formylmethionylaminoacyl-tRNA deformylase – <i>E. coli</i>	formylmethionine deformylase – <i>C. acetobutylicum</i>
<i>fnt</i> (<i>yloL</i>)	1646299 1647249	methionylaminoacyl-tRNA formyltransferase – <i>E. coli</i>	methionyl-tRNA formyltransferase – <i>B. halodurans</i>
<i>yloM</i>	1647239 1648579	Fmu protein – <i>E. coli</i>	16S rRNA m (5)C 967 methyltransferase – <i>B. cereus</i>
<i>yloN</i>	1648586 1649674	hypothetical 37.7 kDa protein – <i>E. coli</i>	radical SAM family enzyme – <i>B. cereus</i>
<i>prpC</i> (<i>yloO</i>)	1649684 1650445	Ser/Thr protein phosphatase – <i>M. genitalium</i>	protein PPM phosphatase
<i>prkC</i> (<i>yloP</i>)	1650442 1652385	Ser/Thr protein kinase – <i>M. genitalium</i>	protein Ser/Thr kinase
<i>cpgA</i> (<i>yloQ</i>)	1652409 1653296	hypothetical protein – <i>M. genitalium</i>	GTPase
<i>rpe</i> (<i>yloR</i>)	1653304 1653954	D-ribulose-5-phosphate 3-epimerase – <i>Solanum tuberosum</i>	ribulose-5-phosphate 3-epimerase – <i>B. halodurans</i>
<i>yloS</i>	1654030 1654671	no significant identities found	thiamine pyrophosphokinase – <i>B. cereus</i>

phorylated form of PrkC is efficiently dephosphorylated by the PrpC phosphatase (Obuchowski *et al.*, 2000). Notably, this type of association of the two genes is found in several bacterial species. The conservation of these two adjacent genes encoding enzymes with opposite activities could suggest that they participate together in the regulation of one or more cellular processes. Recently, the structure of CpgA (formerly YloQ) was published and it turned out to be a GTP-binding protein (Levdikov *et al.*, 2003).

The recent work done by de Hoon and co-workers showed a lack of Rho-independent transcription terminators within the *priA-yloS* region (de Hoon *et al.*, 2005). This data supports the hypothesis about the presence of another promoter upstream of *prpC-prkC-cpgA*, which might be responsible for the basal level of transcription. Previously described promoters pO and pQ are activated only at specific conditions and their activities do not explain the ob-

served constant level of the PrpC and PrkC proteins (Madec *et al.*, 2002, Obuchowski *et al.*, 2000, Iwanicki *et al.*, 2005).

Here we present an analysis of a transcription start site within the region of *priA-def* based on a real-time PCR method, β -galactosidase activity assays and primer extension analysis. This is another yet transcription start site which can drive expression of the *prpC-prkC-cpgA* genes (Iwanicki *et al.*, 2005).

MATERIALS AND METHODS

Bacterial strains, plasmids and cultivation.

All strains and plasmids used are listed in Table 2. Bacteria were grown either in Luria-Bertani rich medium (Miller, 1972) or in minimal medium: 0.015 M K_2SO_4 , 0.08 M K_2HPO_4 , 0.044 M KH_2PO_4 , 3.4 mM sodium citrate, 0.8 mM $MgSO_4$, 0.4% glucose, 0.005%

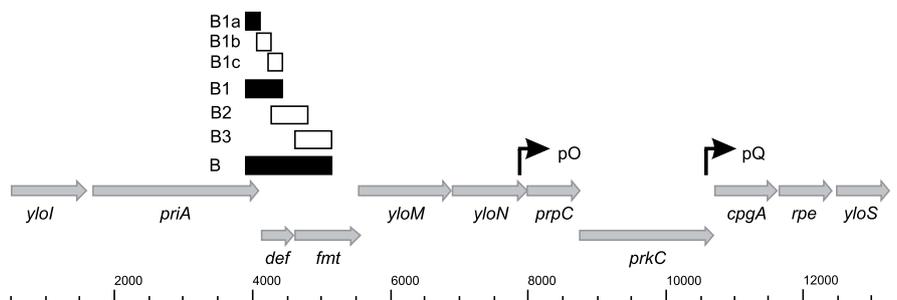


Figure 1. Position of cloned fragments used in this study.

Open boxes, fragments transcriptionally inactive. Black boxes, fragments transcriptionally active. Arrows, promoters identified in previous work (Iwanicki *et al.*, 2005). For details see text and Table 1.

Table 2. Bacterial strains and plasmids.

For detailed information on fragments and restriction sites used for cloning see Table 4.

Strain	Genotype or description	Source or reference
<i>Escherichia coli</i>		
DH5 α	ϕ 80dlacZ Δ M15, <i>endA1</i> , <i>recA1</i> , <i>hsdR17</i> , <i>supE44</i> , <i>thi-1</i>	Hanahan, 1983
<i>Bacillus subtilis</i>		
168	<i>trpC2</i>	<i>B. subtilis</i> Genetic Stock Centre
BFA2820	<i>yloN-lacZ</i> , <i>erm</i> ^R	our collection
BFA2821	<i>prpC-lacZ</i> , <i>erm</i> ^R	our collection
BFA2823	<i>cpgA-lacZ</i> , <i>erm</i> ^R	our collection
MM1105	<i>amyE::lacZ spc</i> ^R	this study
MM1106	<i>amyE::A-lacZ spc</i> ^R	this study
MM1107	<i>amyE::B-lacZ spc</i> ^R	this study
MM1108	<i>amyE::B1-lacZ spc</i> ^R	this study
MM1109	<i>amyE::B2-lacZ spc</i> ^R	this study
MM1110	<i>amyE::B3-lacZ spc</i> ^R	this study
MM1111	<i>amyE::B1a-lacZ spc</i> ^R	this study
MM1112	<i>amyE::B1b-lacZ spc</i> ^R	this study
MM1113	<i>amyE::B1c-lacZ spc</i> ^R	this study
MM1630	<i>yloM::pKH30 erm</i> ^R , <i>cm</i> ^R	this study
MM1631	<i>yloM::pKH30, cpgA-lacZ, erm</i> ^R , <i>cm</i> ^R	this study
MM1632	<i>yloM::pKH30, yloN-lacZ, erm</i> ^R , <i>cm</i> ^R	this study
MM1633	<i>yloM::pKH30, prpC-lacZ, erm</i> ^R , <i>cm</i> ^R	this study
Plasmids		
pDG1728	integration vector for <i>B. subtilis</i> containing <i>E. coli ori</i> , <i>bla</i> , <i>spc</i> , <i>erm</i> , <i>spoVG-lacZ</i>	Guerout-Fleury, Frandsen & Stragier, 1996
pMutin4	vector for systematic gene inactivation	Vagner <i>et al.</i> , 1998
B-lacZ	fragment B cloned into pDG1728 vector	this study
B1-lacZ	fragment B1 cloned into pDG1728 vector	this study
B2-lacZ	fragment B2 cloned into pDG1728 vector	this study
B3-lacZ	fragment B3 cloned into pDG1728 vector	this study
B1a-lacZ	fragment B1a cloned into pDG1728 vector	this study
B1b-lacZ	fragment B1b cloned into pDG1728 vector	this study
B1c-lacZ	fragment B1c cloned into pDG1728 vector	this study
pKH29	fragment C cloned into pMutin4 vector	this study
pKH30	fragment T cloned into pKH29	this study

L-tryptophan, 0.2% L-glutamine, 4 μ g FeCl₃ ml⁻¹, 0.2 μ g MnSO₄ ml⁻¹. Spectinomycin was added for cultivation of *B. subtilis* strains harbouring a spectinomycin resistance cassette up to the final concentration of 100 μ g/ml. X-gal plates contained 12.5 μ g/ml of this compound. *Escherichia coli* DH5 α (Hanahan, 1983) was used as a host for cloning.

Total RNA isolation. Total RNA isolation was performed using a modified hot phenol method described previously (Volker *et al.*, 1994). Cultures were grown in appropriate media and cells were harvested at indicated times. Cell pellets were suspended in 4 ml of 65°C hot TE buffer, then glass beads (125–250 μ m) and 4 ml of hot phenol (65°C, pH 4.8) were added. The mixture was incubated for 2 min at 65°C, vortexed for 2 min and cooled on ice for 3 min. After cooling the mixture was centrifuged and the aqueous phase was collected. Three extractions were made with 1 vol. of phenol, phenol/

chloroform (1:1, v/v) and chloroform, respectively (3 \times 1 min, with 1 min incubation on ice). After each extraction the mixture was centrifuged and the aqueous phase was collected. Finally, 1/10 vol. of 9 M LiCl and 3.5 vol. of cold 95% ethanol were added. RNA was precipitated, pelleted by a 30 min centrifugation, washed with cold 80% ethanol and dried in a vacuum dryer. The dry RNA pellet was dissolved before use in 20 μ l of RNase-free water.

Real-time PCR experiments. Total RNA (50 ng) from late exponential phase cultures grown in rich media was used for real-time PCR. Prior to real-time PCR, RNA was digested with RNase-free DNase I (Roche) and then the enzyme was thermally inactivated. Experiments were performed using the QuantiTect SYBR Green RT-PCR kit (Qiagen) as recommended by the manufacturer. Primers used are shown in Table 3. Products of reactions were analysed for homogeneity by performing a melting

Table 3. Primers used for real-time PCR reactions and RACE PCR.

F, forward primer; R, reverse primer. Primers AAP and AUAP were provided with the RACE system by Invitrogen.

Probe symbol	Gene	Primer sequence 5'→3'	Orientation	Position in <i>B. subtilis</i> chromosome
8-7	<i>priA</i>	GAGCCTGTTCCGCACACTTG	F	1644997
		CGACTGTTTTCTCGACGGTTC	R	1645080
10-9	<i>def</i>	GGAGGGTAACAAATTGGCAG	F	1645799
		CTATACATGCTGTGGTACGAAC	R	1645911
12-11	<i>fmt</i>	GGCTGCATTAACGTTACCGC	F	1646605
		CATCTTTTTAACCTACGCCCG	R	1646716
15-14	<i>yloM</i>	CAATCAAATGAAAGCAGACAGAGC	F	1647790
		GAATTCCCTTGTTAACGGCC	R	1647889
17-16	<i>yloN</i>	GAAAGAAGGGATAAGCAATGGC	F	1648569
		GTCTGTTACCTCTCTTTGGCAAG	R	1648683
18-17	<i>yloN</i>	GACACGAGTATGGCAATTCTGTATG	F	1648917
		GAACTACTTTGCCTACTTGCC	R	1649063
RevT	<i>sigA</i>	GCCTGTCTGATCCACCACGTAGC	F	2599973
		CGGTATGTCGGACGCGGTATG	R	2600109
AAP	<i>priA</i>	GTTAGGAAAAGCTCAAGCTAC	R	1646096
cDAMP	-	GGCCACGCGTCGACTAGTACGGGIIIGGGIIIG	F	-
AUAP	<i>priA</i>	CAATTCTCCCTCTGTCATCC	R	1646022
9d	<i>priA</i>	GGCCACGCGTCGACTAGTAC	F	-
		CAAGCATGGTGTCTGATACATATC	R	1645932

curve after each PCR reaction. The probes were designed as fragments (100–200 bp) complementary to the internal part of the respective ORFs.

Cloning. For the cloning of selected genome fragments the chromosomal DNA from wild type *B. subtilis* was used as a template in PCR reactions (for primers see Table 4). Reactions were performed with *Pwo* polymerase (Roche). The amplified fragment was digested with appropriate enzymes and inserted into the pDG1728 integration vector (Guerout-Fleury *et al.*, 1996). The resulting plasmid was linearized with *XhoI* and used to transform *B. subtilis* 168.

β -Galactosidase measurements. LB cultures were grown at 37°C with shaking. Samples were taken from various growth phases and stored at -20°C until the enzyme assay was carried out. After thawing, bacterial pellets were suspended in buffer Z (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄) containing 1 mM DTT, and 1/100 vol. of lysis solution (1 mg/ml DNase, 10 mg/ml lysozyme) was added. The mixture was incubated for 20 min at 37°C and then centrifuged at 4°C. The supernatant was used for measurement of protein concentration and β -galactosidase assay (Miller, 1972). Protein concentration was measured using the Bradford reagent (Bio-Rad) as recommended by the manufacturer. Supernatant (200 μ l) was mixed with 600 μ l of buffer Z containing 1 mM DTT. Samples were placed in a 37°C water bath and 200 μ l of ONPG (4 mg/ml) was added. After 60 min incubation, the reaction was stopped by the addition of 500 μ l of 1 M Na₂CO₃. The absorbance of samples was measured spectro-

photometrically at 420 nm. The β -galactosidase activity in nmoles of ONP min⁻¹ mg⁻¹ was calculated using the following formula: (420 nm absorbance \times 1.5)/(conc. of protein in mg/ml \times volume of sample in ml \times reaction time in min \times 0.00486).

Primer extension analysis. A total amount of 20 μ g of RNA was mixed with 2.5 μ l of appropriate primer (1 mM) and adjusted to a volume of 15 μ l. The mixture was then incubated at 80°C for 10 min, transferred to 37°C for 30 min and finally left at room temperature for another 30 min. The RNA annealed with the primer was placed on ice and the following reagents were added: reverse transcriptase buffer, a mixture of four dNTPs (final concentration 200 pM), 0.5 μ Ci (1.35 \times 10¹⁰ Bq) of [α -³²P]dATP and 200 units of M-MLV Reverse Transcriptase (Promega). The volume of the reaction was adjusted with water to 25 μ l. The mixture was incubated at 42°C for 1 h and then precipitated with ethanol. Dried pellets were dissolved in 5 μ l of water, mixed with loading buffer and loaded onto a pre-warmed sequencing gel. Sequencing ladders were generated using Reader Sequencing kit (Fermentas) with the use of a control plasmid included in the kit. The gel was run for 120 min, dried and subjected to autoradiography.

RACE PCR. Rapid Amplification of cDNA Ends (RACE) is a procedure for amplification of nucleic acid sequences from a messenger RNA template between a defined internal site and an unknown 5' end. For such an analysis we used the RACE system produced by Invitrogen (version 2.0) and experiments were performed according to the manufactur-

Table 4. Primers used for cloning of selected fragments.

Sequences recognized by restriction enzymes are in bold. F, forward primer; R, reverse primer.

Fragment	Plasmid	Primer sequence 5'→3'	Orientation	Position in <i>B. subtilis</i> chromosome
B	pB-lacZ	GCTGACACGGAATTCCTCGGACC	F	1645585
		CAGACAAAAGCTTCGCGCCAG	R	1646833
B1	pB1-lacZ	GCTGACACGGAATTCCTCGGACC	F	1645585
		TCACCATAAAGCTTAGGAAAGCTCAAG	R	1646107
B2	pB2-lacZ	CTTTCCTAACGAATTCGGTGATGTC	F	1646087
		GCGCAATGAAGCTTCCTTTAC	R	1646468
B3	pB3-lacZ	CCGGTAAAGGAATTCGCATTG	F	1646440
		CAGACAAAAGCTTCGCGCCAG	R	1646833
B1a	pB1a-lacZ	GCTGACACGGAATTCCTCGGACC	F	1645585
		GCCAAATTTGTAAGCTTCCAAGACG	R	1645807
B1b	pB1b-lacZ	CATGTAATGAATTC AATTGATATG	F	1645741
		GTCCGAAGCTTTC CATTTC	R	1645950
B1c	pB1c-lacZ	CTGCTTGATGAATTCACGACACCATG	F	1645902
		CAGACAAAAGCTTCGCGCCAG	R	1646107
C	pKH29	CAGCTTCTTCGTCGACCCCTATATCAAATG	F	1647473
		CCCTTGTTGACCTTAATCTGCGATATG	R	1648068
T	pKH30	CTATGAGTCGACTTTG TAAATTTGG	F	
		GCTCACAAGTCGACACATTATG	R	

er's manual. The specific primers for cDNA synthesis (RevT) and PCR amplification (cDAMP and AAP; 9d and AUAP) are listed in Table 3.

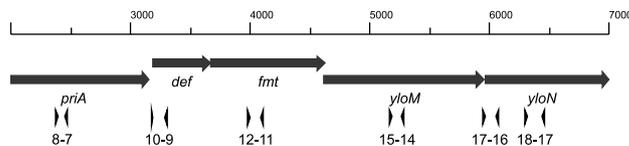
RESULTS

Similarity comparison of ORFs in the putative transcription unit *priA-cpgA*

The sequence of the putative transcription unit *priA-cpgA* was previously analysed for the presence of ORFs that would encode peptides of at least

67 aa (Foulger & Errington, 1998). A BLAST search was repeated in this study in order to compare sequences with current databases (Table 1). Products of three genes — *yloM*, *yloN* and *yloS* share similarity with proteins from other bacteria, however, they have not been analyzed so far in *B. subtilis*. Four genes — *priA* (*yloJ*), *prpC* (*yloO*), *prkC* (*yloP*) and *cpgA* (*yloQ*) have already been cloned and their products identified. The PriA protein is defined as the primosomal factor Y (for a review see Mariani, 1999). PrpC is a protein PPM phosphatase (Obuchowski *et al.*, 2000) and PrkC is a protein Ser/Thr kinase (Maded *et al.*, 2002; 2003). CpgA is a GTPase with perturbed G motifs and is necessary for proper shap-

A



B

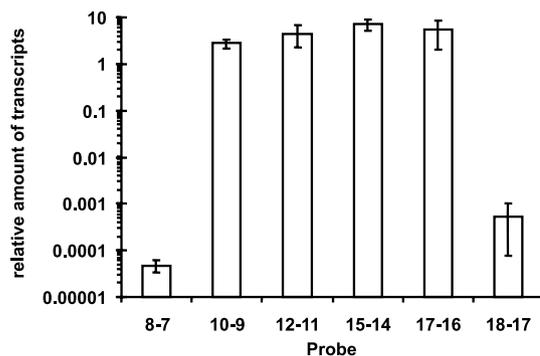


Figure 2. Transcriptional analysis of the *priA-yloN* region by real-time PCR.

Panel A. Positions of primers used in real-time PCR reaction within analysed region are indicated by convergent arrowheads. Panel B. mRNA levels in strain 168 detected by real-time PCR. Total RNA was isolated from cultures grown in rich (LB) medium from late exponential phase of growth. For each reaction 50 ng of total RNA was taken. Each reaction was repeated three times. As a standard we used primers for the *sigA* gene which is transcribed at a relatively constant level. Real-time PCR reactions were prepared accordingly to the manufacturer's protocol (Qiagen).

ing of bacterial cells (Levdikov *et al.*, 2004, Cladiere *et al.*, 2006). The products of *def* (*yloK*) (Huntington *et al.*, 2000) and *fnt* (*yloL*) participate in post-translational modification of proteins (Haas *et al.*, 2001).

The level of transcription of individual genes differs across the *priA-cpgA* region as measured by real-time PCR

Providing that the region *priA-yloN* forms a single operon, a real-time PCR scan of the level of mRNAs corresponding to the ORFs throughout the whole region should ideally give a relatively constant level of signal. In order to investigate the expression of genes identified in the transcription unit *priA-yloN* pairs of primers corresponding to different ORFs were first tested in PCR reactions with *B. subtilis* chromosomal DNA as a template to confirm that they were correctly designed. In each case the primers gave similar levels of products in control experiments (not shown). RNA samples were prepared from late exponential phase bacteria cultivated in LB medium. The results presented in Fig. 2 reveal differences in the levels of transcript of genes across the *priA-yloN* region and this variation is difficult to reconcile with the presence of a single transcript. The presence of a weak signal in the case of the internal part of the *priA* gene (PCR probe 8-7) in Fig. 2, in contrast to the signal from the *def* probe (PCR probe 10-9) may indicate the presence of a promoter inside the *priA* gene, in addition to a presumptive promoter upstream of *priA*. The transcript detected using probes 10-9, 12-11, 15-14 and 17-16 shows a relatively high, constant level, although the probe 18-17 shows a much lower one. On the basis of these results we postulate that the analysed transcript starts at the distal part of the *priA* gene and terminates in the proximal part of the *yloN* gene (Fig. 2).

A possible promoter located in the *priA-fnt* fragment

The real-time PCR analysis of the *priA-cpgA* region (Fig. 2) enabled press on the possible location of a promoter(s) upstream of *def*. On this basis we PCR-amplified fragment B from the *B. subtilis* chromosome (1250 bp, starting from 1645585 to 1646833) and cloned it into the pDG1728 vector upstream of the *lacZ* gene with translational signals of the *B. subtilis spoVG* gene. The resulting plasmid pB-*lacZ* was then integrated into the *B. subtilis* chromosome in the non-essential locus *amyE*. This approach provides the possibility for monitoring the expression of a gene, a promoter or a genome fragment of interest in the host chromosome. An integrant strain was then tested for β -galactosidase activity on LB solid medium containing X-gal. The strain contain-

ing the chromosomal B-*lacZ* fusion showed detectable activity of β -galactosidase after an overnight incubation of the plates at 37°C.

The promoter within the distal part of *priA* becomes activated in the late exponential phase

The real-time PCR analysis of the *priA-yloN* unit together with the preliminary β -galactosidase plate test suggested the presence of one or more putative promoters functioning in the *priA-fnt* region. To confirm this, an integrant strain harbouring the B-*lacZ* fusion (*priA-fnt* region) in the *amyE* locus was screened for β -galactosidase activity during growth in rich liquid medium. The results demonstrated a significant increase in the β -galactosidase activity with

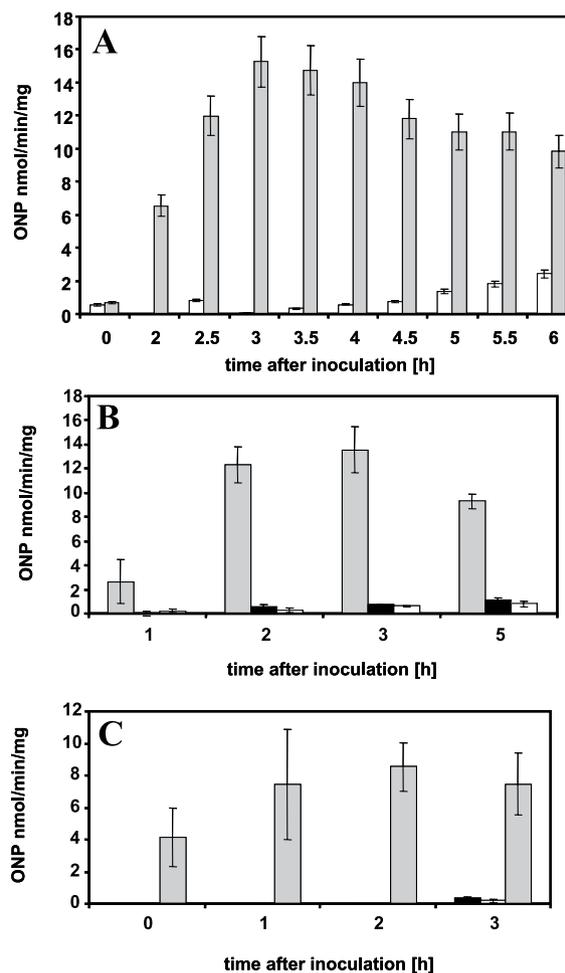


Figure 3. Transcriptional activity of the *priA-fnt* region (fragment B) as assessed by β -galactosidase activity. Bacterial cultures were grown in rich medium at 37°C. Time 0 indicates the point of dilution of overnight cultures. Panel A. β -Galactosidase activity of fusion strains: white bars, MM1105 (*lacZ*); grey bars, MM1107 (fragment B-*lacZ*). Panel B. β -Galactosidase activity of fusion strains: MM1108 (B1-*lacZ*, white bars), MM1109 (B2-*lacZ*, grey bars) and MM1110 (B3-*lacZ*, black bars). Panel C. β -Galactosidase activity of strains: MM1111 (B1a-*lacZ*, white bars), MM1112 (B1b-*lacZ*, grey bars) and MM1113 (B1c-*lacZ*, black bars).

The insertion of pKH30 plasmid into the *yloM* gene results only in an approximately 5-fold decrease in level of the transcripts, downstream of the insertion site as measured by real-time PCR (not shown). The ratio of the β -galactosidase activity measured with the use of the *lacZ* fusions with *prpC*, *prkC* or *cpgA* genes in strains 168 and MM1630 remains consistent with the real-time PCR analysis.

DISCUSSION

The *priA-cpgA* region of the *B. subtilis* chromosome was originally postulated to be transcribed as a single, polycistronic mRNA extending from *yloI* located immediately upstream of *priA* to the *yloS* gene (see Fig. 1). This assumption was made solely on the basis of DNA sequence analysis (Foulger & Errington, 1998). However, the ORFs in this region correspond to a wide variety of possible functions. Thus, for example, whilst the product of the *priA* gene was identified as a primosomal factor Y involved in DNA replication restart (Marians, 1999), *prpC* and *prkC* encode proteins apparently important in a signalling pathway involved in development (Obuchowski *et al.*, 2000, Madec *et al.*, 2002), and the products of *def* and *fnt* are involved in post-translational modifications (Haas *et al.*, 2001). Such a variety of functions might be difficult to reconcile with a single transcription unit.

The transcription in that region was originally postulated to start upstream of the *priA* and *yloI* genes (Foulger & Errington, 1998). In this work we provide evidence for the presence of an internal promoter functioning within the *priA* gene. First, the transcription start site inside the *priA* gene was postulated on the basis of real-time PCR analysis of transcript levels in bacterial cells in the late exponential growth phase in rich medium (LB). The observed increase of the real-time PCR signal in the case of the distal part of this gene coincided with the activity of an internal promoter mapped in this region of the genome (designated the *pdef* promoter). Activity of this promoter was detected both by the *lacZ* fusion and by primer extension.

Previous work revealed that at least two promoters function within the unit (Iwanicki *et al.*, 2005; see Fig. 1). However, both identified promoters are weak or become activated only in specific conditions, i.e. the pO promoter in late exponential phase and pQ after ethanol shock. The results presented here clearly show that the promoter located inside the *priA* gene may be responsible for the basal level of expression of the *prpC-prkC-cpgA* genes. This hypothesis is supported by the fact that the identified promoter has a sequence which is recognized by

the *B. subtilis* "housekeeping" σ subunit (σ A) (Haldenwang, 1995; Sonenshein *et al.*, 2002).

In conclusion, our analysis of the transcription in the *priA-cpgA* region of the *B. subtilis* genome indicated that regulation of the genes in this region may be complex. Although most of the genes in the *def-cpgA* region appear to be transcribed as a single transcript, at least under conditions tested, the presence of three transcription start sites suggests a much more complicated pattern of transcription. However, from this analysis we cannot exclude the possibility that other promoters and terminators might operate in this region of the *B. subtilis* genome (Yoshida *et al.*, 2000).

Acknowledgements

These studies were supported by grants from the Ministry of Science and Higher Education, project No. 2 P04A 008 27 and P04A 039 30.

REFERENCES

- Cladiere L, Hamze K, Madec E, Levdivkov VM, Wilkinson AJ, Holland BI, Seror SJ (2006) The GTPase, CpgA (YloQ), a putative translation factor, is implicated in morphogenesis in *Bacillus subtilis*. *Mol Gen Genomics* **275**: 409–420.
- de Hoon MJL, Makita Y, Nakai K, Myiano S (2005) Prediction of transcriptional terminators in *Bacillus subtilis* and related species. *PLoS Comp Biol* **1**: 212–221.
- Foulger D, Errington J (1998) A 28 kbp segment from the spoVM region of the *Bacillus subtilis* 168 genome. *Microbiology* **144**: 801–805.
- Guerout-Fleury AM, Frandsen N, Stragier P (1996) Plasmids for ectopic integration in *Bacillus subtilis*. *Gene* **180**: 57–61.
- Haas M, Beyer D, Gahlmann R, Freiberg C (2001) YrkB is the main peptide deformylase in *Bacillus subtilis*, a eubacterium containing two functional peptide deformylases. *Microbiology* **147**: 1783–1791.
- Haldenwang WG (1995) The sigma factors of *Bacillus subtilis*. *Microbiol Rev* **59**: 1–30.
- Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. *J Mol Biol* **166**: 557–580.
- Huntington KM, Yi T, Wei Y, Pei D (2000) Synthesis and antibacterial activity of peptide deformylase inhibitors. *Biochemistry* **39**: 4543–4551.
- Iwanicki A, Hinc K, Seror S, Wegrzyn G, Obuchowski M (2005) Transcription in the *prpC-yloQ* region in *Bacillus subtilis*. *Arch Microbiol* **183**: 421–430.
- Kobayashi K, Ehrlich SD, Ogasawara N *et al.* (2003) Essential *Bacillus subtilis* genes. *Proc Natl Acad Sci USA* **100**: 4678–4683.
- Kunst, F, Ogasawara N, Moszer I *et al.* (1997) The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. *Nature* **390**: 249–256.
- Levdivkov VM, Blagova E, Brannigan JA, Cladiere L, Antonson AA, Isupov MN, Seror SJ, Wilkinson AJ (2004) The crystal structure of YloQ, a circularly permuted GT-Pase essential for *Bacillus subtilis* viability. *J Mol Biol* **340**: 767–782.

- Madec E, Laszkiewicz A, Iwanicki A, Obuchowski M, Seror S (2002) Characterisation of a membrane-linked Ser/Thr protein kinase in *Bacillus subtilis*, implicated in developmental processes *Mol Microbiol* **46**: 571–586.
- Madec E, Stensballe A, Kjellström SJ, Cladière L, Obuchowski M, Jensen ON, Seror SJ (2003) Mass spectrometry and site directed mutagenesis identify several autophosphorylated residues required for the activity of PrkC, a Ser/Thr kinase from *Bacillus subtilis*. *J Mol Biol* **330**: 459–472.
- Marians KJ (1999) PriA: at the crossroads of DNA replication and recombination. *Prog Nucleic Acid Res Mol Biol* **63**: 39–67.
- Miller JH (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Obuchowski M, Madec E, Delattre D, Boel G, Iwanicki A, Foulger D, Seror SJ (2000) Characterization of PrpC from *Bacillus subtilis*, a member of the PPM phosphatase family. *J Bacteriol* **182**: 5634–5638.
- Sonenshein AL, Hoch JA, Losick R (2002) *Bacillus subtilis and its Closest Relatives: from Genes to Cells*. ASM Press, Washington.
- Vagner V, Dervyn E, Ehrlich D (1998) A vector for epitopic integration in *Bacillus subtilis*. *Microbiology* **144**: 3097–3104.
- Volker U, Engelmann S, Maul B, Riethdorf S, Volker A, Schmid R, Mach H, Hecker M (1994) Analysis of the induction of general stress proteins of *Bacillus subtilis*. *Microbiology* **140**: 741–752.
- Yoshida K, Ishio I, Nagakawa E, Yamamoto Y, Yamamoto M, Fujita Y (2000) Systematic study of gene expression and transcription organization in the *gntZ-ywaA* region of the *Bacillus subtilis* genome. *Microbiology* **146**: 573–579.