The *Bacillus subtilis* *yabQ* gene is essential for formation of the spore cortex

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An extensive screening for transcripts with probes specific for the genes in a 108 kb region from *rrnO* to *spo0H* of the *Bacillus subtilis* chromosome led to identification of an operon, *yabP-yabQ-divIC-yabR*, the expression of which was initiated at the second hour of sporulation and in a σ^E -dependent manner. Among three *y* genes in the operon, deletion of the *yabQ* gene, which is predicted to encode a protein product of 468 residues with five membrane-spanning domains, resulted in a large decrease in numbers of chloroform-, lysozyme- and heat-resistant spores, compared to findings with the wild-type strain. Electron microscopy revealed that development of the spore cortex was blocked in the *yabQ* mutant. In addition, although the spore coat was visible, the inner coat layer of the mutant seemed partially detached from the outer coat. In sporangia of the strains harbouring an in-frame fusion of the green fluorescent protein gene to *yabQ*, fluorescence was detected around the forespore. This localization did not depend on *SpoIVA* or on *CotE* functions, both of which determine proper localization of coat proteins and cortex formation. The *yabQ* deletion did not affect expression of genes involved in cortex synthesis. These results suggest that the YabQ protein localizes in the membrane of the forespore and plays an important role in cortex formation.

Keywords: Gram-positive bacterium, sporulation, cell differentiation, green fluorescent protein

INTRODUCTION

The Gram-positive soil bacterium *Bacillus subtilis* produces dormant spores which can survive for long periods and under extreme conditions when environmental nutrition is exhausted (reviewed by Piggot & Coote, 1976; Losick *et al.*, 1986; Errington, 1993). *B. subtilis* initiates spore formation by dividing asymmetrically, the result being formation of a larger mother cell and a smaller forespore cell. The mother cell engulfs the forespore so that it is surrounded by a double membrane. Peptidoglycan accumulates in the inter-space of the two membranes and forms a thick spore cortex that provides resistance against heat and organic solvents. In addition, the proteinaceous spore coat is layered on the outer

surface of the forespore double membrane and protects the spore from actions of all large molecules, including lytic enzymes such as lysozyme. More than 100 genes involved in these developmental stages have been identified and their biological functions analysed. RNA polymerases containing developmentally specific sigma factors, $E\sigma^H$, $E\sigma^F$, $E\sigma^E$, $E\sigma^G$ and $E\sigma^K$, temporally and spatially activated during sporulation, regulate expression of the sporulation genes in a stage-specific and compartment-specific manner (Igo & Losick, 1986; Stragier *et al.*, 1988; Stragier & Losick, 1996).

With completion of the sequencing of the *B. subtilis* genome, we might be able to identify all the genes involved in the sporulation process described above. In a 108 kb segment of the *B. subtilis* genome that covers between *rrnO* and *spo0H*, 90 ORFs were found, 52 of which had unknown function (Ogasawara *et al.*, 1994). To identify novel genes involved in sporulation, Northern analysis was done using probes encompassing each gene in this region. We searched for genes that were transcribed after the onset of sporulation and whose

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Abbreviation: GFP, green fluorescent protein.

inactivation caused some effects on spore formation and germination. We characterized two genes, *yaaH* and *yabG*, which were specifically induced during sporulation and involved in germination of spores and assembly of the spore coat, respectively (Kodama *et al.*, 1999; Takamatsu *et al.*, 2000). We now report the function of the *yabQ* gene expressed during sporulation by $E\sigma^E$ and which is involved in formation of the spore cortex.

METHODS

Bacterial strains and plasmids. *B. subtilis* strains and plasmids used in this study are listed in Table 1.

An in-frame deletion mutation of *yabP* or *yabQ* was obtained by two-step allele replacement, as described by Asai *et al.* (2000). Genomic regions upstream and downstream of *yabP* were PCR-amplified using a primer pair of bPUF (5'-GGTTTCTGAAGCTCCTCACG-3') and bPUR (5'-CCCTTTAGCTTTATCCCCATTCATCTATTTCAGCCCCC-3'), and a primer pair of bPDF (5'-GGGGGCTGAATAGATGAATGGGGATAAAGCTAAAGGG-3') and bPDR (5'-CCGTGTGATGGTCGTTCT-3'), respectively, then used to obtain a *B. subtilis* strain, ASK220, in which codons 3–87 of the *yabP* structural gene (100 aa) were deleted. Genomic regions upstream and downstream of *yabQ* were PCR-amplified using a primer pair of bQUF (5'-GCGAAGGAAGTAGCTGACC-3') and bQUR (5'-GGAGAAAACCTATCAGGCTCAGCGTCATTTAAACAACCTTGC-3'), and a primer pair of bQDF (5'-GCAAGTTGTTTAAATGACGCTGAGCCTGATAGGTTTTCTCC-3') and bQDR (5'-TACTTGCTCTTCTTC-3'), respectively, then used to obtain a *B. subtilis*

strain, ASK221, in which codons 4–140 of the *yabQ* structural gene (211 aa) were deleted.

Oligonucleotide primers 8158RTF (5'-CGGCCAAAAGCT-TGTAAC-3') and 8158RTR (5'-GGAGGATCCATAATA-TGAATTCATCTATTTCAG-3') were used to amplify a 208 bp segment of the *yabP* upstream region. The PCR product, restricted at *Hind*III and *Bam*HI sites introduced by the primers, was inserted into the *Hind*III/*Bam*HI-restricted pMUTINT3 (Vagner *et al.*, 1998; Moriya *et al.*, 1998) to obtain plasmid pMU158RT. The resulting plasmid was transformed into *B. subtilis* 168 by a single cross-over recombination for erythromycin resistance (0.5 µg erythromycin ml⁻¹), to yield strain ASK222.

An integration vector for green fluorescent protein (GFP) fusion was constructed as follows. A fragment encoding the C-terminal portion of YabQ and a fragment encoding the C-terminal portion of CotE were PCR-amplified using primers QGFPP (5'-GTCGTCGACATGCGGAGCGATCATC-3') and QGFPR (5'-GAAGAATTCTCTCTTCAAAAACCGTGTG-3'), and COTEGFPF (5'-GTCGTCGACCATTTCCGCCAATGG-3') and COTEGFPR (5'-GAAGAATTCTTCAGGATCTCCCAC-3'), respectively. The PCR products of 280 and 208 bp were digested by *Sal*I and *Eco*RI and introduced between the *Sal*I and *Eco*RI sites of pMm2 (Takamatsu *et al.*, 2000) to generate plasmids pMmyabq and pMmcote, respectively. The resultant plasmids were transformed into *B. subtilis* 168 by a single cross-over for erythromycin resistance to yield strain ASK223 or ASK226 in which the *yabQ* or *cotE* genes were switched with the *yabQ*– or *cotE*–*gfp* genes, respectively. The *yabQ*–*gfp* fusion was transferred to the chromosome of *B. subtilis* P20 (Coot, 1972) and *B. subtilis* RL48 (Zheng *et al.*, 1988) and the resulting strains were named ASK224 and ASK225, respectively. The

Table 1. *B. subtilis* strains and plasmids used in this study

Strain or plasmid	Genotype or description	Source or reference
Strains		
168	<i>trpC2</i>	Laboratory stock
ASK203	<i>trpC2 spoIIIGAB::kan</i>	Kodama <i>et al.</i> (1999)
ASK204	<i>trpC2 spoIIIG::kan</i>	Kodama <i>et al.</i> (1999)
ASK205	<i>trpC2 spoIVCB::erm</i>	Kodama <i>et al.</i> (1999)
ASK220	<i>trpC2 yabPΔ1</i>	This work
ASK221	<i>trpC2 yabQΔ1</i>	This work
ASK222	<i>trpC2 yabPΩpMU158RT (erm)</i>	This work
ASK223	<i>trpC2 yabQ–gfp (erm)</i>	This work
P20	<i>pheA12 spoIVA178</i>	Coot (1972)
RL48	<i>trpC2 cotE::cat</i>	Zheng <i>et al.</i> (1988)
ASK224	<i>pheA12 yabQ–gfp (erm) spoIVA178</i>	This work
ASK225	<i>trpC2 yabQ–gfp (erm) cotE::cat</i>	This work
ASK226	<i>trpC2 cotE–gfp (erm)</i>	This work
KP429	<i>amyE::gfp–spoIVA</i>	Price & Losick (1999)
ASK227	<i>trpC2 yabQΔ1 amyE::gfp–spoIVA</i>	This work
ASK228	<i>trpC2 yabQΔ1 cotE–gfp (erm)</i>	This work
Plasmids		
pMUTINT3	<i>erm bla lacZ lacI Pspac</i>	Vagner <i>et al.</i> (1998); Moriya <i>et al.</i> (1998)
pMU158RT	<i>erm bla yabP'–lacZ lacI Pspac–yabP</i>	This work
pMm2	<i>erm bla gfp lacI Pspac</i>	Takamatsu <i>et al.</i> (2000)
pMmyabq	<i>erm bla yabQ–gfp lacI Pspac–yabQ</i>	This work
pMmcote	<i>erm bla cotE–gfp lacI Pspac–cotE</i>	This work

Fig. 1. (a) Arrangement of genes around the *yabQ* locus on the *B. subtilis* genome. Transcripts detected by a probe encompassing the 5'-portion of *yabR* (indicated by a thick bar) are shown by arrows below the ORF map, and are designated T1, T2, T3, T4 and T5, respectively. The genes covered by these transcripts were determined by Northern analysis experiments using probes covering each gene indicated in the figure. The positions of the $E\sigma^E$ -dependent promoter (P_{T5}) and promoter for the *divIC*-*yabR* genes (P_{T3}) are also shown on the map. The primer-binding sites used for two-step deletion mutants are indicated by open arrows. (b) Northern blot using the *yabR* specific probe. Total RNA was prepared from a *B. subtilis* 168 culture in DS medium at the times shown on the top of the blot (hours before and after the end of the vegetative growth phase). The approximate positions of transcripts are indicated. The size of RNA is shown on the left of the blot. (c) Northern blots using RNA from the wild-type (lanes 1 and 2), *spoIIIGAB* (lanes 3 and 4), *spoIIIG* (lanes 5 and 6) or *spoIVCB* (lanes 7 and 8) mutants and the *yabR* specific probe. Total RNA was prepared from the cells at 2 h (lanes 1, 3, 5 and 7) and 4 h (lanes 2, 4, 6 and 8) after the end of vegetative growth. The approximate positions of transcripts and the size of RNA are shown as in (b).

gfp-spoIVA (Price & Losick, 1999) and *cotE-gfp* fusions were transferred to the chromosome of ASK221 and the resulting strains were named ASK227 and ASK228, respectively. Transformation of *B. subtilis* was done as described by Kawamura *et al.* (1980).

Sporulation medium and measurement of spore resistance. Difco sporulation (DS) medium (Schaeffer *et al.*, 1965) was used to produce *B. subtilis* spores. Cells were grown in DS medium at 37 °C for 18 h after the end of exponential growth, and the spore resistance was assayed as follows. The culture was heated at 80 °C for 30 min, and treated with lysozyme (final concentration, 0.25 mg lysozyme ml⁻¹) at 37 °C for 10 min, or with 10% (v/v) chloroform at room temperature for 10 min, as described by Nicholson & Setlow (1990). A portion of the sample was diluted in distilled water, plated on Luria-Bertani agar, and incubated overnight at 37 °C. The number of survivors was determined by counting colonies.

Northern and primer extension analysis. Total RNA was extracted from *B. subtilis* cells as described by Igo & Losick (1986). In Northern analysis, hybridization and detection were performed using digoxigenin-labelled RNA probes (Asai *et al.*, 2000). Primer extension analysis was carried out using digoxigenin-end-labelled primers (Takamatsu *et al.*, 2000).

DNA ladders for size markers were obtained using the same digoxigenin-end-labelled primers and a DIG *Taq* DNA sequencing kit (Boehringer Mannheim).

Phase-contrast and fluorescence microscopy. An aliquot of the culture of *B. subtilis* cells in DS medium was transferred onto a microscope slide coated with poly-L-lysine. Images of phase-contrast and fluorescence from GFP were observed under an Olympus fluorescence microscope (AX70) with a U-MNIBA mirror cube unit. The images were captured with a cooled charge-coupled device camera (PXL-1400; Photometrics) and analysed using image processing software, IPLAB SPECTRUM (Signal Analytic Corporation).

Electron microscopy. Glutaraldehyde was added to a final concentration of 0.3% (w/v) to the culture of *B. subtilis* cells allowed to sporulate for 8, 10 and 18 h after the onset of sporulation. The cells were collected by centrifugation and prefixed with 3% (w/v) glutaraldehyde in 50 mM phosphate buffer (pH 6.5) for 2 h at room temperature, followed by washing five times with the same buffer. Cells were then collected by centrifugation and fixed with a final concentration of 1% (w/v) osmium tetroxide in 50 mM phosphate buffer (pH 6.5) for 2 h at room temperature. After washing five times with distilled water, they were prestained for 2 h with 0.5%

(w/v) uranyl acetate and embedded in 2% (w/v) agar. The agar block was dehydrated with ethanol and acetone, followed by embedding in Spurr's resin (Spurr, 1969) prior to thin sectioning. Thin sections were obtained using a Diatome diamond knife on a Leica ULTRACUT UCT, stained with 3% (w/v) uranyl acetate and then with lead citrate (Reynolds, 1963), and examined under a Hitachi H7100 electron microscope.

RESULTS AND DISCUSSION

An operon containing the *yabP*, *yabQ*, *divIC* and *yabR* genes is induced at the middle stage of sporulation by RNA polymerase containing σ^E

Through systematic Northern analysis of transcripts in a chromosomal region between *rrnO* and *spo0H*, a 1.9 kb transcript which appeared 2 h after the onset of

sporulation was detected using a probe specific for the *yabR* gene (Fig. 1b). This transcript also hybridized with probes specific for the *yabP*, *yabQ* and *divIC* genes (data not shown). The size of the transcript corresponded exactly to the nucleotide length covering the start of *yabP* to the end of *yabR*, indicating that the four genes were co-transcribed at the middle stage of sporulation (T5 in Fig. 1a). To determine which sigma factor was concerned with the transcription, Northern analysis was done using RNA prepared from sigma-factor-deficient mutants. The results in Fig. 1(c) show that while the transcript was detected in *spoIIIG* (σ^G) and *spoIVCB* (σ^K) mutants, it disappeared in the *spoIIGAB* (σ^E) mutant. In addition, we determined the 5'-terminus of the transcript in a primer extension experiment and found possible -10 and -35 sequences of the $E\sigma^E$ promoter (Fig. 2).

Fig. 2. Determination of the start site of the T5 transcript by primer extension. Total RNA was prepared from strain ASK222 at the end of the vegetative phase of growth (lane 1) and 2 h later (lane 2), and primer extension experiments were done, as described in Methods, using primers RT1 (a) or RT2 (b). Sequencing ladders were generated using the primers used for the reverse transcriptase reaction (lanes labelled by T, G, C and A). The extension product is marked with an arrowhead. The sequence and the transcription start sites (indicated by asterisks) are shown. (c) Nucleotide sequence of the promoter region of the T5 transcript. The deduced amino acid sequences of the C-terminal portion of YabO and the N-terminal portion of YabP are shown. A putative ribosome-binding site of the *yabP* gene is doubly underlined. The experimentally determined transcription start site (+1) and the deduced -10 and -35 regions are underlined. Consensus sequences recognized by $E\sigma^E$ are shown below.

Table 2. Sporulation frequencies in a strain carrying a *yabQ* deletion

Values in parentheses refer to percentage of spores.

Strain	Viable cells ml ⁻¹	Heat-resistant spores ml ⁻¹	Lysozyme-resistant spores ml ⁻¹	Chloroform-resistant spores ml ⁻¹
168	5.6 × 10 ⁸	3.9 × 10 ⁸ (69.6)	3.7 × 10 ⁸ (58.9)	4.0 × 10 ⁸ (71.4)
ASK221	3.1 × 10 ⁸	3.7 × 10 ⁴ (0.012)	7.4 × 10 ⁶ (2.4)	1.1 × 10 ⁶ (0.4)

The *divIC* gene is essential for cell division in vegetative growth and is required for asymmetric septation during sporulation (Sadaie & Kada, 1983; Levin & Losick, 1994). Consistent with this, additional transcripts covering *divIC* were detected, as schematically summarized in Fig. 1(a). Two transcripts, 5.3 kb (T1) and 3.7 kb (T2), were weak during vegetative growth, and a 1.0 kb transcript (T3) was detected during both vegetative and sporulation phases (Fig. 1b). Furthermore, a 0.5 kb transcript (T4) covering the *yabR* gene was strongly induced during the vegetative phase.

Effects of inactivation of the *yabP*, *yabQ* or *yabR* gene on spore formation

Next, we examined the effects of inactivation of each of the three *y* genes in the *yabP*–*yabR* operon on sporulation. The insertion of exogenous plasmid DNA, pMUTINT3 (Vagner *et al.*, 1998; Moriya *et al.*, 1998), into the *yabR* gene by a single cross-over integration resulted in about a 10-fold reduction of sporulation frequency (data not shown); however, this was not further studied. Because inactivation of the *yabP* or *yabQ* gene by inserting the plasmid DNA leads to a polar effect on downstream *divIC* and *yabR* expression, we introduced an in-frame deletion within the *yabP* or *yabQ* gene by the two-step allele-replacement method (Asai *et al.*, 2000). A promoter for *divIC* that is active from the vegetative phase (T3 in Fig. 1) was found within the *yabQ* gene by primer extension analysis (data not shown). Therefore, we avoided deleting the *divIC* promoter to obtain the *yabQ* deletion mutation. A strain carrying a deletion from codons 3 to 87 of the *yabP* structural gene (100 aa), ASK220, could sporulate and germinate, similar to the parental strain (data not shown). In contrast, a *yabQ* deletion mutant, ASK221, in which codons 4–140 of the structural gene (211 aa) were deleted, produced abnormal spores sensitive to lysozyme, chloroform and, especially, heat treatments (Table 2). Although transcription of the *yabQ* gene was weak during the vegetative phase in the wild-type cell (T1 and T2 in Fig. 1), growth of the *yabQ* mutant was comparable to that of the parental strain. Thus we postulated that the defective phenotype of the *yabQ* mutant in sporulation was due to the loss of the functional *yabQ* product at the phase of sporulation.

At the vegetative phase, *yabQ* is cotranscribed with *yabM*, which is a paralogue of *spoVB*, a gene required for maturation of the cortex (Popham & Stragier, 1991). However, the inactivation of *yabM* did not affect the sporulation (data not shown).

Synthesis of the spore cortex was blocked considerably in the *yabQ* mutant

Normal spores become phase-bright under the phase-contrast microscope after spore coat synthesis followed by core dehydration (Piggot & Coote, 1976; Errington, 1993). However, large amounts of spores produced in the *yabQ* mutant were phase-dark and did not become

Fig. 3. Phase-contrast microscopy of sporulating cells of the wild-type 168 (a) and the *yabQ* mutant (b) in DS medium. Bar, 2 μ m.

phase-bright, even after further incubation (Fig. 3). In wild-type cells, cortex synthesis was clearly observed under the electron microscope 8 h after the initiation of sporulation (Fig. 4a–d). In contrast, examination of the *yabQ* cells during sporulation revealed that synthesis of the spore cortex was blocked in them, resulting in production of defective sporangia that had almost entirely lost the spore cortex. In addition, the inner spore coat layer of the mutant spores seemed partially detached from the outer coat layer, suggesting that the YabQ deletion also affected the coat protein assembly (Fig. 4e–h). The spore cortex provides a heat-resistance property to the dormant spore. On the other hand, coat layers yield resistance to toxic solvents and lytic enzymes (Zheng *et al.*, 1988; Naclerio *et al.*, 1996; Takamatsu *et al.*, 1999). Thus the characteristics of the morphology of the *yabQ* mutant spore were compatible with the sensitivity of the spore to lysozyme, chloroform and heat treatments.

The YabQ protein localized on the surface of the forespore, independently of CotE and SpoIVA function

The results described above indicated that YabQ protein is synthesized in the mother cell by $E\sigma^E$ and plays an important role in cortex and coat formation. To determine the precise role of YabQ in spore formation, we next examined localization. We introduced an in-frame fusion of *gfp* to *yabQ* into the *B. subtilis* chromosome and observed the sporulating cells under a

Fig. 4. Electron microscopy of spores of the wild-type 168 (a, b, c and d) and the *yabQ* mutant (e, f, g and h) strains. Samples were prepared from cells allowed to sporulate for 8 (a, e), 10 (b, f) and 18 (c, d, g, h) h after the onset of sporulation. Small arrowheads indicate the boundary between the spore coat and the cortex. Large arrowheads indicate spore coat structure. Bar, 0.5 μ m.

fluorescence microscope. Fluorescence in the vegetatively growing cell was nil. After expression of *yabQ* was initiated during sporulation, fluorescence appeared around the forespore (Fig. 5a, b), as observed for fluorescence from *gfp-spoIVA* (Fig. 5e). This observation indicated that the YabQ proteins were localized around the forespore. YabQ fluorescence was observed as an arch shape as well as a ring shape. We speculate that this difference was caused by asynchronous development of the sporulating cells under our conditions. A similar arch shape was observed for SpoVIA, while the SpoIVA proteins were thought to finally surround the forespore (Price & Losick, 1999). Five putative transmembrane domains were predicted from the amino acid sequence of the YabQ protein by the Kyte–Doolittle plot (Kyte & Doolittle, 1982), and a putative signal peptide sequence was found at the N-terminus region, suggesting that it is a membrane protein (see information

described in the website at <http://bacillus.genome.ad.jp>). Thus the localization of the YabQ–GFP fluorescence would suggest that the YabQ protein is associated with the forespore membrane.

CotE and SpoIVA proteins, the expression of which is also dependent on $E\sigma^E$, localize around the forespore and guide the proper assembly of spore coat proteins. The SpoIVA protein is also essential for cortex formation (Driks, 1999). We next examined the effects of their mutation on localization of the YabQ protein. The behaviour of the YabQ fluorescence was not affected by introducing the *spoIVA* or *cotE* mutation into the *B. subtilis* cells harbouring the *yabQ-gfp* fusion (Fig. 5c, d), supporting the assumption of localization of YabQ on the forespore membrane. Conversely, the fluorescence directed by *spoIVA*– and *cotE*–*gfp* fusion was not affected by the *yabQ* mutation (Fig. 5f, h).

Fig. 5. Detection of YabQ-GFP, GFP-SpoIVA and CotE-GFP fusions in sporulating *B. subtilis* cells. (a, b) Wild-type 168, (c) *spoIVA178* or (d) *cotE* mutant carrying the *yabQ-gfp* fusion. (e) Wild-type 168 or (f) *yabQ* mutant carrying the *gfp-spoIVA* fusion. (g) Wild-type or (h) *yabQ* mutant carrying the *cotE-gfp* fusion. Cells were allowed to sporulate in DS medium. Samples were withdrawn 6 h after the onset of sporulation. Phase-contrast and fluorescence microscope images were overlaid. Bar, 2 μ m.

Fig. 6. Transcription of genes involved in cortex formation in the *yabQ* mutant. Northern hybridization was done to detect transcripts of *dacB* (lanes 1–4), *spoVR* (lanes 5–8), *spoVD* (lanes 9–12) and *spoVE* (lanes 13–16). The predicted transcripts of each gene are approximately 2.5, 1.5, 2.0 and 4.9 kb, respectively. Total RNA was prepared from cells of *B. subtilis* 168 (lanes 1, 2, 5, 6, 9, 10, 13 and 14) and the *yabQ* mutant (lanes 3, 4, 7, 8, 11, 12, 15 and 16), which were harvested at 4 (lanes 1, 3, 5, 7, 9, 11, 13 and 15) and 6 (lanes 2, 4, 6, 8, 10, 12, 14 and 16) h after the end of vegetative growth. Arrowheads represent the positions of transcripts.

Effect of the *yabQ* deletion on the expression of genes involved in cortex synthesis

We also asked if the YabQ protein might regulate the expression of genes involved in spore cortex synthesis. Several $E\sigma^E$ -dependent genes, mostly designated *spoV*, have been reported to participate in cortex synthesis. A family of so-called penicillin-binding proteins (PBPs) catalyses development of the peptidoglycan layer of the cortex (DacB and SpoVD) (Daniel *et al.*, 1994; Popham *et al.*, 1999). SpoVE protein, which is highly homologous with cell elongation protein (RodA), is also required for synthesis of the cortex peptidoglycan (Daniel & Errington, 1993; Henriques *et al.*, 1998). Mutation in the *spoVR* gene causes a defect in cortex synthesis, although the precise function was not obvious (Beall & Moran, 1994). Northern analysis of the expression of the *dacB*, *spoVD*, *spoVE* and *spoVR* genes in the *yabQ* mutant revealed that they were normally expressed and were independent of YabQ function (Fig. 6).

When *lacZ* was fused to *spoIID* (Rong *et al.*, 1986), *sspE* (Sun *et al.*, 1989) and *cotA* (Zheng & Losick, 1990) (kindly provided by Dr P. Stragier, Institut de Biologie Physico-Chimique, Paris, France), transcribed by $E\sigma^E$, $E\sigma^G$ and $E\sigma^K$, respectively, and introduced into the *amyE* locus on the chromosome of the *yabQ* mutant, expression of the fusions was similar to that seen in the wild-type strain, suggesting that those sporulation sigma factors were normally activated in the *yabQ* mutant (data not shown).

Role of the YabQ protein in spore formation

Extensive Northern analysis revealed $E\sigma^E$ -dependent transcription from the *yabP-yabQ-divIC-yabR* operon during sporulation. Deletion of the first ORF, the *yabP* gene, had no apparent effect on either sporulation or germination. However, deletion of the second ORF, *yabQ*, made spores sensitive to lysozyme, chloroform and, especially, heat treatment. Microscopic analysis showed that *yabQ* mutant spores possessed no obvious spore cortex layer and had an impaired spore coat, suggesting that *yabQ* plays an important role in synthesis of the spore cortex and coat. The YabQ protein was predicted to have five transmembrane domains and a signal sequence at the N-terminus region. YabQ fluorescence signals were observed surrounding the forespore independently of CotE and SpoVIA functions, suggesting that YabQ is associated with the forespore membrane. Expression of the genes participating in cortex synthesis and the sporulation sigma factors σ^E , σ^G and σ^K occurred normally in the *yabQ* deletion mutant. These findings suggest that the YabQ protein plays a direct role in cortex formation. An interesting possibility might be that YabQ is a transporter for components required for synthesis of the spore cortex and the defect affects coat assembly.

During preparation of this manuscript, Fawcett *et al.* (2000) reported analysis of genes expressed during sporulation by transcriptional profile analysis with a macro-array. They began building null mutations of

possible sporulation genes thus identified, and deletion of *yabP* or *yabQ* was found to block sporulation at a late stage.

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