Expression of the Bacillus subtilis spoIVB gene is under dual $\sigma^F/\sigma^G$ control

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The expression of the Bacillus subtilis spoIVB gene, which encodes a developmental cell–cell signalling molecule, has been characterized. In some conditions, this gene can be transcribed by RNA polymerase associated with either $\sigma^F$ or $\sigma^G$, in contrast to previous studies implying exclusive control by $\sigma^G$. However, during sporulation, only $\sigma^G$ directs significant levels of spoIVB expression.

Keywords: Bacillus subtilis, sporulation, spoIVB gene, sigma factors

INTRODUCTION

The Bacillus subtilis spoIVB gene encodes a 46 kDa polypeptide that is required for intercompartmental activation of the transcription factor $\sigma^K$ in the ‘$\sigma^K$-checkpoint’ (Cutting et al., 1991a). The $\sigma^K$-checkpoint provides a mechanism for coupling gene expression between the two compartments (forespore and mother cell) of the sporulating cell (Cutting et al., 1990). spoIVB has been shown to be the only gene transcribed by RNA polymerase associated with $\sigma^K$ (EaG) in the forespore compartment that is required to activate $\sigma^K$ in the opposed mother cell chamber (Gomez et al., 1995). Activation of $\sigma^K$ is brought about by proteolytic processing of an inactive, pro-$\sigma^K$ protein to its mature form, $\sigma^K$ (Lu et al., 1990). Proteolytic cleavage of pro-$\sigma^K$ is mediated by the combined action of three proteins, BofA, SpoIVFA and SpoIVB, which are thought to reside in the outermost membrane layer of the forespore (Cutting et al., 1991b; Ricca et al., 1992). Thus, SpoIVB must act through at least one membrane bilayer (the inner forespore membrane) to stimulate the processing complex. Processing of pro-$\sigma^K$ in the mother cell occurs only upon receipt of the forespore (SpoIVB) signal and this provides a timing mechanism for ensuring that late gene expression (controlled by $\sigma^K$) is coordinated with earlier developmental events.

In this work we have re-examined spoIVB expression. We show that spoIVB transcription can be driven not only by EaG but also by EaF.

 METHODS

General methods. Sporulation was induced by the resuspension method (Nicholson & Setlow, 1990). Two methods were used for determination of $\beta$-galactosidase activity as described by Nicholson & Setlow (1990). For standard detection we used the substrate o-nitrophenyl $\beta$-D-galactopyranoside (ONPG), while for greater sensitivity, the fluorescent substrate 4-methylumbelliferyl $\beta$-D-galactopyranoside (MUG) was used.

Bacterial strains. Strains used in this work were congenic with the prototrophic wild-type strain PY79 (Youngman et al., 1984). Strains containing the spoIIGA1 (SC500) and spoIIA1C1 (SC1159) mutations have been described elsewhere (Cutting et al., 1990, 1991a).

Construction of spoIVB-lacZ. The spoIVB-lacZ transcriptional fusion was constructed by cloning an EooR1–EooRV fragment from pJB2026 (Van Hoy & Hoch, 1990) into the EooR1–EooRV sites of pTKlac (Kenney & Moran, 1987), creating plasmid pEL2. pJB2026 contains a 450 bp StyI–StuI fragment carrying the 5′-region of spoIVB fused to the spoVG ribosome-binding site and lacZ (Van Hoy & Hoch, 1990). This fragment, with a flanking segment of lacZ, was released by EcoRI–EcoRV digestion. The spoIVB-lacZ fusion obtained by this procedure was identical to the one contained in pJB2026. DNA-mediated transformation of spo' cells (PY79) with pEL2 allowed isolation of strain MG68 (spoIVB::spoIVB-lacZ) containing pEL2 integrated at the spoIVB locus by a single ‘Campbell-type’ recombination. Following integration, a partial gene duplication generates one intact spoIVB gene and spoIVB-lacZ containing the lacZ gene under the control of the spoIVB regulatory elements. To construct strains containing the spoIVB-lacZ transcriptional fusion, chromosomal DNA prepared from MG68 cells was introduced into appropriate recipient cells by transformation.

Primer extensions. RNA was prepared from vegetative cultures expressing either $\sigma^G$ or $\sigma^G$, or from sporulating cultures. Primer extension reactions were performed as described previously (Cutting et al., 1989, 1991b).
RESULTS AND DISCUSSION

Induction of σt in vegetative cells leads to efficient expression of spoIVB-lacZ

A new routine method to determine whether a gene can be recognized by a particular sigma factor is to artificially induce the transcription factor in vegetatively growing cells and determine whether expression of the developmental gene results. We induced synthesis of an active form of either σF or σG during vegetative growth and measured spoIVB-directed β-galactosidase synthesis at appropriate times thereafter. In these experiments spoIIIGΔ1 cells (strain SC500) were used, containing the spoIVB-lacZ fusion and either a σF (pSDA4; Shazand et al., 1995) or σG (pDG298; Sun et al., 1989) overproducing plasmid. These autonomously replicating vectors contain the structural genes for σF (spoIIAC; pSDA4) and σG (spoIIIG; pDG298) fused downstream, and under the control of, the IPTG-inducible spac promoter. We used spoIIIGΔ1 cells in this experiment for two reasons: first, to confirm that upon σF induction the observed spoIVB-lacZ expression was due only to synthesis of σF (since EoF is able to transcribe the spoIIIIG gene which encodes σF); second, spoIIIIG is autoregulatory so we wanted to ensure that no transcription from the chromosomal gene occurred. Fig. 1(a) shows that addition of IPTG to vegetatively growing spoIIIGΔ1 cells containing pSDA4 or pDG298 resulted in significant induction of spoIVB-directed β-galactosidase synthesis, demonstrating that both σF and σG were capable of directing the transcription of spoIVB in vivo, at least in vegetative cells overproducing σF or σG. This result is in disagreement with a previous report (Cutting et al., 1991a), in which spoIVB was suggested to be transcribed exclusively by EoG. However, in that report, the ability of EoF to direct the transcription of spoIVB in σF-overproducing vegetative cells was not addressed.

Primer extension analysis

To test whether EoF and EoG recognize the same promoter sequences in the spoIVB regulatory region we performed primer extension analysis using total RNA prepared from (1) exponentially growing spoIIIGΔ1 cells containing pSDA4 or pDG298 (allowing IPTG-induction of σF and σG respectively), or (2) sporulating cells (spo+) harvested at 0, 2.5 and 5 h after the initiation of sporulation. Using two primers for cDNA synthesis, OMG17 and OMG22 (Fig. 3a), we detected a transcript from vegetative cultures in which either σF or σG was induced (Fig. 2 shows OMG17). The same transcript was present in RNA harvested from sporulating cells at 2.5 and 5 h after the initiation of sporulation but not at 0 h. The putative transcription start site was 56 bp upstream of the spoIVB ORF (see Fig. 3a). Preceding this site were sequences, AAAAtTAA and gtTATAA, that could correspond to the −10 and −35 regions respectively, of σF- and σG-controlled promoters (Fig. 3b). These results confirm that spoIVB is indeed transcribed by both EoF and EoG, at least in vegetative cells overproducing σF or σG, using the same transcription start site. In previous work (Van Hoy & Hoch, 1990) two possible transcription start sites have been identified (P1 and P2 in Fig. 3a) but these do not correspond to the start site identified here. Fig. 3(b) shows an alignment of the 5’ regions of all forespore-expressed genes whose promoters have been mapped, incorporating those transcribed exclusively by EoF (i.e. spoIIR), σF and σG (e.g. gpr), or only σG (e.g. spoA). The −10 and −35 sequences of these genes, including spoIVB, are well conserved. It has been reported that genes transcribed by EoF contain additional sequence conservation at positions −15 and −16 preceding the transcription start site (Sun et al., 1991b). Specifically, one
or, better, two Gs at these positions seem(s) to be important for $\sigma^F$ recognition and indeed this is clear in Fig. 3(b). The spoIVB promoter contains a G at each of these positions, which would support spoIVB being recognized by $\sigma^F$ in addition to $\sigma^G$.

Expression of spoIVB–lacZ

Finally, we examined the expression of spoIVB during sporulation in wild-type cells and in cells containing a null mutation in the structural gene for $\sigma^G$ (spoIIIGA1) or $\sigma^F$ (spoILAC1). When wild-type cells containing a spoIVB–
lacZ fusion (strain MG68) were induced to sporulate, spoIVB expression initiated at approximately 3 h ($t_2$) after the onset of spore formation (Fig. 1b). The profile of gene expression was in good agreement with that of previous studies (Cutting et al., 1991a). However, when we examined expression of spoIVB–lacZ in spoIIIGA1 cells we could detect residual levels of spoIVB expression that were, approximately, tenfold lower than in wild-type cells containing a spoIVB–lacZ fusion and tenfold higher than in wild-type cells containing no fusion (Fig. 1b). spoIVB–
directed $\beta$-galactosidase synthesis in the spoIIIGA1 mutant
initiated at about $t_{1.5}$ approximately 1 h before that found in wild-type cells and at a time when $Eo^F$ is present and functional in the forespore chamber (Stragier, 1992).

Since $Eo^F$ is present and active in the forespore prior to $Eo^G$, and we had found that $Eo^F$ can direct the transcription of $spaIVB$ in vegetative cells overproducing $Eo^F$, an obvious explanation for the residual levels of $spaIVB$ expression in the $spolIIG$ mutant was that in the absence of $Eo^G$ transcription of $spaIVB$ was directed by $Eo^F$. Accordingly, we found no measurable levels of $spaIVB$ expression in $spolIAC1$ cells containing a $spaIVB$--lacz fusion (Fig. 1b). This experiment has been repeated numerous times with comparable results. (Note that in Fig. 1(b) the more sensitive MUG assay was used for detection of $\beta$-galactosidase activity.)

**Conclusions**

In summary, we provide evidence that $spaIVB$ can be transcribed by $Eo^F$ and by $Eo^G$, as has been shown for some other forespore-expressed genes (Partridge & Errington, 1993). Two of them, $gpr$ and $dacF$, produce a distinctive 'biphasic' pattern of expression during sporulation, where first $Eo^F$ (phase 1) and then $Eo^G$ (phase 2) directs gene expression (Partridge & Errington, 1993). We do not observe this 'biphasic' pattern of expression for $spaIVB$ and we conclude that $spaIVB$ is not normally transcribed by $Eo^G$ during sporulation in wild-type cells. However, a null mutation in the gene encoding $Eo^G$ allows expression of $spaIVB$ early during sporulation ($t_{1.5}$) and this expression requires $Eo^F$. Since this shows that $Eo^F$ can, under some conditions, transcribe $spaIVB$ we speculate that in wild-type cells a $Eo^G$-dependent mechanism would repress $Eo^F$-dependent transcription of $spaIVB$. Such a mechanism could occur since low levels of $Eo^G$ are produced constitutively. Indeed, two mechanisms have been reported that prevent the inappropriate expression of $Eo^G$-dependent genes during vegetative growth or at the early stages of sporulation (Rother et al., 1990; Schmidt et al., 1994), but we cannot exclude that a certain leakage in the control of $Eo^G$ activity could result in the expression of a putative $Eo^F$ repressor. A $Eo^G$-dependent mechanism for repressing $Eo^F$-dependent transcription of $spaIVB$ could be an important mechanism for preventing early expression of $spaIVB$ and premature triggering of pro-$Eo^K$ processing in the mother cell compartment.

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