Medium-dependent sporulation resulting from a mutation in the *spoIIAB* gene of *Bacillus subtilis*

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The SpoIIAB protein of *Bacillus subtilis* is an anti-sigma factor that controls the release of σ^F in the prespore during sporulation. A missense mutation, *spoIIAB22*, in the N-terminal coding portion of *spoIIAB* was isolated previously on the basis of conferring increased σ^F activity on agar plates. We present the results of experiments further characterizing the phenotypic effects of the *spoIIAB22* mutation. The mutation severely impairs spore formation on nutrient agar (NA) plates. Surprisingly, however, cultures induced to sporulate in liquid medium by the resuspension method showed little or no reduction in spore formation, and gene expression was more or less normal. The effects on sporulation on NA can be suppressed by mutations in the *spoIIAC* gene encoding σ^F . The implications for our understanding of the regulation of σ^F and σ^G activities, and the nature of the apparent medium dependence of sporulation in the mutant, are discussed.

Keywords: sporulation, *Bacillus subtilis*, anti-sigma factor, suppressor mutations, σ^F

INTRODUCTION

Classically, sporulation mutants of *Bacillus subtilis* were generally isolated as translucent unpigmented colonies on rich medium. The detailed phenotypic properties of such mutants were then usually characterized in liquid medium either by nutrient exhaustion or by resuspension (for a review see Piggot & Coote, 1976). Mutations causing defective sporulation on some media but more or less normal sporulation on others have been described. In many cases the latter class of mutants could be assigned to a specific nutritional defect (reviewed by Freese & Heinze, 1984). Here we describe the phenotypic properties of a mutation in a well-characterized regulatory gene, *spoIIAB*, which unexpectedly causes medium-dependent sporulation.

The *spoIIAB* gene of *B. subtilis* encodes an anti-sigma factor, which inhibits the activities of σ^F and probably σ^G during sporulation (Duncan & Losick, 1993; Min *et al.*, 1993; Partridge *et al.*, 1991; Rather *et al.*, 1990; Schmidt *et al.*, 1990). These sigma factors are responsible for transcription in the forespore compartment during sporulation. The action of SpoIIAB probably serves to prevent

σ^F from becoming active, both before septation, and in the mother cell compartment after septation (Margolis *et al.*, 1991). *spoIIAB* is the middle gene of a three gene operon (Fort & Piggot, 1984). The third gene encodes σ^F itself (Errington *et al.*, 1985; Sun *et al.*, 1989). The first gene, *spoIIAA*, encodes an antagonist of SpoIIAB, or anti-anti-sigma factor (Alper *et al.*, 1994; Diederich *et al.*, 1994; Schmidt *et al.*, 1990). Presumably, SpoIIAB and possibly SpoIIAA can sense some special feature of the prespore microenvironment and regulate σ^F activity on the basis of this information (Lewis *et al.*, 1994; Margolis *et al.*, 1991). Although the biochemical basis for the interactions between these proteins is beginning to be elucidated (Alper *et al.*, 1994; Duncan & Losick, 1993; Min *et al.*, 1993; Diederich *et al.*, 1994), little is known about their regulation *in vivo*. An understanding of the molecular basis of the regulation of σ^F activity is important because σ^F plays a key role in triggering the complex programmes of gene expression that determine prespore and mother cell differentiation (reviewed recently: Errington, 1993; Losick & Stragier, 1992).

Previous work on strains containing the *spoIIAB22* mutation has shown that the mutant protein is impaired in the inhibition of σ^F activity, at least during growth on nutrient agar (NA) plates (Foulger & Errington, 1993). Under these conditions *spoIIAB22* mutants also display abnormally high levels of σ^G activity. However, because

This paper is dedicated to the memory of Iain Challoner-Courtney.

Abbreviations: APase, alkaline phosphatase; MUG, 4-methylumbelliferyl- β -D-galactosidase.

σ^G synthesis can be increased by transcription directed by σ^F , it was not clear whether the mutant protein was impaired in the regulation of σ^G activity or whether the effect on σ^G was indirect. This paper describes further investigations of the phenotypic consequences of the *spoIIAB22* mutation and the isolation and characterization of suppressor mutations that relieve its effects on sporulation.

METHODS

Bacterial strains, plasmids and bacteriophages. The strains used are listed in Table 1. Phage $\phi 105J114$, used to complement the *spoIIGB55* mutation in strain 839, was isolated by East & Errington (1989). Plasmid pSG279 is a derivative of pSGMU2 (Fort & Errington, 1985), containing a truncated derivative of the *spoIIAC* gene, stretching from an *EcoRI** site 61 bp from the upstream end of the gene, to the *PstI* site just after the termination codon. Plasmid pPS300 (Mason *et al.*, 1988) was a kind gift from P. Setlow (University of Connecticut Health Center, Farmington, CT, USA).

General methods. *B. subtilis* strains were grown on plates containing NA (Oxoid) or LB agar (Rather *et al.*, 1990). Chloramphenicol ($5 \mu\text{g ml}^{-1}$), kanamycin ($5 \mu\text{g ml}^{-1}$), or erythromycin ($1 \mu\text{g ml}^{-1}$) and lincomycin ($25 \mu\text{g ml}^{-1}$) were added as required. β -Galactosidase activity was detected in plates by addition of 0.01% X-Gal. *B. subtilis* strains were made

competent for transformation with DNA by the method of Anagnostopoulos & Spizizen (1961) as modified by Jenkinson (1983). Transduction with bacteriophage $\phi 105$ was as described by Errington & Mandelstam (1986). Chromosomal DNA for transformation was prepared from *B. subtilis* by the rapid method of Ward & Zahler (1973). Chromosomal DNA for cloning experiments was prepared as described by Errington (1984). Transformation of *Escherichia coli* and DNA manipulations were carried out using standard procedures (e.g. Sambrook *et al.*, 1989).

Sporulation of *B. subtilis*. *B. subtilis* cultures grown in hydrolysed casein growth media at 37°C were induced to sporulate by resuspension in sporulation medium (SM) (Sterlini & Mandelstam, 1969), as specified by Partridge & Errington (1993). Time (h) after resuspension was denoted t_0 , t_1 etc.

Measurement of heat resistance. Heat resistance in liquid cultures was measured as described by Foulger & Errington (1991). Samples (0.5 ml), taken at t_0 , were heated at 80°C for 15 min, diluted and plates on Oxoid NA. To measure heat resistance on plates, a fresh single colony was used to heavily inoculate a NA plate. The cells from three separate plate cultures were harvested in about 5 ml SM. The cell suspension was diluted in SM to an OD_{600} of about 2.0 (near to the value obtained with cells sporulating in liquid medium). Heat resistance was determined similarly to that of the liquid cultures by heating a 1 ml sample of culture at 80°C for 15 min.

Enzyme assays. β -Galactosidase activity was measured by assays

Table 1. Bacterial strains

Strain	Markers	Construction/reference*
55.3	<i>trpC2 spoIIGB55</i>	Errington & Mandelstam (1986)
168	<i>trpC2</i> (Spo ⁺)	Laboratory stock
713	<i>trpC2</i> Ω (<i>spoIV A'-lacZ cat</i>)713	Stevens <i>et al.</i> (1992)
731	<i>trpC2</i> Ω (<i>spoIIIG::ermC</i>)731	Partridge & Errington (1993)
800	<i>metC3</i>	806 DNA $\rightarrow$ MB75, Lys ⁺ selected, screened for Spo ⁺
801	<i>trpC2 lacA17 lacR1 spoIIGB55 spoIIIG⁺::pSG139</i> (<i>spoIIIG'-lacZ</i>)	Foulger & Errington (1993)
803	<i>metC3 chr::pPS1395 (gpr'-lacZ cat)</i>	Foulger & Errington (1993)
804	<i>metC3</i> Ω (<i>spoIIIG::ermC</i>)731 <i>gpr⁺::pPS1395 (gpr'-lacZ cat)</i>	Foulger & Errington (1993)
805	<i>metC3 sspA⁺::pPS300 (sspA'-lacZ cat)</i>	Foulger & Errington (1993)
806	<i>trpC2 spoIIAB22 spoIIGB55</i> Ω (<i>spoIIIG'-lacZ ermC</i>)687	Foulger & Errington (1993)
810	<i>metC3 spoIIAB22 gpr⁺::pPS1395 (gpr'-lacZ cat)</i>	Foulger & Errington (1993)
811	<i>metC3 spoIIAB22</i> Ω (<i>spoIIIG::ermC</i>)731 <i>gpr⁺::pPS1395</i> (<i>gpr'-lacZ cat</i>)	Foulger & Errington (1993)
812	<i>metC3 spoIIAB22</i> (Spo ⁺) <i>sup-10</i> (<i>spoIIACK247T</i>)	Spo ⁺ revertant of 833
813	<i>metC3 spoIIAB22</i> (Spo ⁺) <i>sup-11</i>	Spo ⁺ revertant of 833
814	<i>metC3 spoIIAB22</i> (Spo ⁺) <i>sup-12</i> (<i>spoIIACS222P</i>)	Spo ⁺ revertant of 833
815	<i>metC3 spoIIAB22</i> (Spo ⁺) <i>sup-13</i> (<i>spoIIACW190G</i>)	Spo ⁺ revertant of 833
823	<i>metC3 spoIIAB22 sspA⁺::pPS300 (sspA'-lacZ cat)</i>	pPS300 DNA $\rightarrow$ 800, Cm ^R selected
833	<i>metC3 spoIIAB22</i>	806 DNA $\rightarrow$ MB75. Lys ⁺ selected, screened for Spo ⁺
838	<i>trpC2 spoIIIG⁺::pSG139 (spoIIIG'-lacZ cat)</i>	pSG139 DNA $\rightarrow$ 168, Cm ^R selected
839	<i>trpC2 spoIIAB22 spoIIIG⁺::pSG139 (spoIIIG'-lacZ cat)</i> [<i>spoIIGB55</i>] ($\phi 105J114$) <i>spoIIIGAB⁺</i>	843 infected with $\phi 105J114$
840	<i>metC3 spoIIAB22</i> Ω (<i>spoIV A'-lacZ cat</i>)713	713 DNA $\rightarrow$ 833, Cm ^R selected
841	<i>metC3</i> Ω (<i>spoIV A'-lacZ cat</i>)713	713 DNA $\rightarrow$ 800, Cm ^R selected
843	<i>trpC2 spoIIGB55 spoIIIG⁺::pSG139 (spoIIIG'-lacZ)</i>	801 DNA $\rightarrow$ 55.3, Cm ^R selected, screened for blue colony on plate containing X-Gal
MB75	<i>lys-1 metC3</i> (Spo ⁺)	Laboratory stock

* Arrows indicate that the DNA was transformed into the strain with the number following; Cm^R, resistance to chloramphenicol.

based on the hydrolysis of either 4-methylumbelliferyl- β -D-galactosidase (MUG assay), or *o*-nitrophenol- β -D-galactoside (ONPG assay). The MUG assay was done as described by Errington & Mandelstam (1986), except that the cells were permeabilized by treatment with lysozyme (Mason *et al.*, 1988). One MUG unit of β -galactosidase activity is the amount of enzyme that produces 1 nmol 4-methylumbelliferone min⁻¹ at 30 °C. The ONPG assay was done as described by Nicholson & Setlow (1990). The units were as defined by Miller (1972). For assay of β -galactosidase in cells grown on agar plates, the cells were harvested as described by Rather *et al.* (1990) and the enzyme activity was determined from a 0.5 ml sample of cell suspension. Alkaline phosphatase (APase) activity was assayed as described by Errington & Mandelstam (1983). One unit of APase activity is the amount of enzyme that hydrolyses 1 nmol *p*-nitrophenyl phosphate in 1 min at 30 °C.

Cloning *spoIIAC* mutations suppressing the effects of *spoIIAB22*. Four Spo⁺ revertants of a *spoIIAB22* mutant were isolated (strains 812, 813, 814 and 815). Each of the revertants was transformed with an integration plasmid, pSG279, containing a 5'-deleted copy of the *spoIIAC* gene (see earlier). A Spo⁻ colony was isolated from each transformation, on the assumption that the suppressor mutation would be present in the non-functional copy of *spoIIAC* generated during plasmid integration. Chromosomal DNA from each isolate was digested with *Pst*I. The digests were diluted, religated, and transformed into *E. coli* DH5 α with selection for ampicillin resistance. The plasmids recovered were checked for the expected restriction pattern (indistinguishable from that of pSG279).

DNA sequencing. Double-stranded plasmid DNA was sequenced as described previously (Foulger & Errington, 1993), with a Sequenase kit (USB) and synthetic oligonucleotide primers complementary to the *spoIIA* sequence (Challoner-Courtney & Yudkin, 1993).

RESULTS

Effects of the *spoIIAB22* mutation on spore formation

Some of the effects of the *spoIIAB22* mutation on expression of σ^F - and σ^G -dependent genes have been described previously (Foulger & Errington, 1993). In the course of characterizing the phenotype of the mutant further, we noted that it showed severely impaired spore formation on NA plates. Table 2 shows that the spore frequency on NA plates was less than 1% of that of the isogenic *spoIIAB*⁺ strain, when measured after 26 h.

Although the spore frequencies were somewhat higher in later samples, these estimations are probably less accurate because microscopic examination of the cultures suggested, not surprisingly, that the number of viable cells (but not of spores) fell as the cultures aged. When we tried to quantify the sporulation frequency under the more readily defined conditions of liquid resuspension (Sterlini & Mandelstam, 1969), we were surprised to find that the sporulation efficiency was near normal (Table 2). It thus seemed that the effect of the *spoIIAB22* mutation on sporulation was strongly medium-dependent.

Effects of the *spoIIAB22* mutation on gene expression under sporulating and non-sporulating conditions

We have shown that the activities of both σ^F and σ^G on NA are increased in the presence of the *spoIIAB22* mutation (Foulger & Errington, 1993). Detailed quantitative analysis of gene expression in plate cultures is problematic because individual cells are exposed to different conditions depending on their location, giving a very heterogeneous population. Rather *et al.* (1990) reported that the *spoIIAB1* mutation caused an increased level of σ^G -dependent gene expression in liquid YT medium, even though this medium does not induce a significant degree of sporulation in either wild-type or mutant strains. We therefore tested the effects of *spoIIAB22* under similar conditions. As shown in Fig. 1(a), like the *spoIIAB1* mutation, *spoIIAB22* increased the expression of a σ^G -dependent reporter (*sspA-lacZ*) in liquid YT medium, compared with isogenic *spoIIAB*⁺ cells. Unlike *spoIIAB1*, *spoIIAB22* also showed an increase in σ^F activity (as measured by expression of *gpr'-lacZ* in a *spoIIIG* background; Fig. 1b) under these non-sporulating conditions. Interestingly, little expression of either *lacZ* fusion occurred during vegetative growth but strong induction of expression occurred some hours after the end of exponential growth. Thus, although σ^F and σ^G are unleashed abnormally in the *spoIIAB22* mutant, their release is still associated with the stationary phase of the growth cycle. The results also showed that the *spoIIAB22* mutation can cause substantial inappropriate release of both σ^F and σ^G activities in liquid culture as well as on solid media.

Table 2. Sporulation efficiencies of wild-type and mutant under different conditions

Strain	Heat resistant c.f.u. (percentage of viable c.f.u.)*					
	Plate culture†			Resuspension‡		
	26 h	48 h	71 h	5 h	7 h	9 h
803 (<i>spoIIAB</i> ⁺)	30 (8.6 × 10 ⁷)	52 (6.5 × 10 ⁷)	75 (7.1 × 10 ⁷)	7.7 (8.9 × 10 ⁷)	59 (7.2 × 10 ⁷)	55 (9.3 × 10 ⁷)
810 (<i>spoIIAB22</i>)	0.02 (1.6 × 10 ⁸)	0.9 (5.2 × 10 ⁷)	6.6 (4.6 × 10 ⁷)	1.8 (7.4 × 10 ⁷)	76 (7.5 × 10 ⁷)	91 (7.2 × 10 ⁷)

* Viable c.f.u. ml⁻¹ OD₆₀₀⁻¹ given in parentheses.

† Time after plating on NA.

‡ Time after resuspension.

Fig. 1. Effects of the *spoIIAB22* mutation on expression of *lacZ* fusions to sporulation genes *sspA* (a) and *gpr* (b) in liquid YT medium. Each strain was grown overnight in YT medium, then diluted to an OD₆₀₀ of about 0.1. The growth of the culture was followed and, soon after the end of exponential growth, samples were taken for assay of β -galactosidase. Each panel shows a comparison of the *spoIIAB22* mutant ($\circ$) with an isogenic *spoIIAB*⁺ strain ($\triangle$). The strains in (b) had a mutation in the *spoIIIG* gene so that expression due only to the σ^F form of RNA polymerase was measured. The strains used were 804, 805, 811 and 823.

Table 3. Effect of the *spoIIAB22* mutation on expression of *spoIVA*'-*lacZ* on NA plates

Mean OD₆₀₀ values of the cell suspensions are given in parentheses. The times shown indicate hours after plating out on NA.

Strain	β -Galactosidase activity (Miller units)		
	20 h	46 h	70 h
840 (<i>spoIIAB22</i>)	7.2 (0.44)	12 (0.65)	15 (0.71)
841 (<i>spoIIAB</i> ⁺)	16 (0.62)	36 (0.89)	35 (0.63)

It seemed unlikely that the increased synthesis of σ^F - or σ^G -dependent gene products was entirely responsible for the impairment in sporulation on NA plates. We noted that when a *lacZ* fusion to the σ^E -dependent (mother-cell specific) *spoIVA* gene was introduced into the *spoIIAB* mutant, the level of β -galactosidase activity appeared to be reduced compared with *spoIIAB*⁺ derivatives, as judged by colony colour on NA plates containing X-Gal. Quantitative measurement of β -galactosidase in such cultures confirmed that expression of *spoIVA*'-*lacZ* was reduced by two- to threefold by the *spoIIAB22* mutation (Table 3). The reduction in σ^E activity may thus

contribute to the poor sporulation of this mutant on NA plates.

The greater spore yield following induction of sporulation of the *spoIIAB22* mutant by the resuspension method suggested that the expression of σ^F - and σ^E -dependent genes would be nearer normal under these conditions than on solid media. Isogenic *spoIIAB*⁺ and *spoIIAB22* strains containing various reporter genes were induced to sporulate by resuspension in SM, and β -galactosidase activity was measured. In contrast to the results obtained with plate cultures and liquid YT medium, following resuspension strains carrying the *spoIIAB22* mutation showed, if anything, a reduction in prespore-specific gene expression (Fig. 2a, b). Transcription of both *gpr*'-*lacZ* and *spoIIIG*'-*lacZ* increased sharply at about the normal time, but the levels of expression were about twofold less in the *spoIIAB22* mutants than in the isogenic wild-type strains. Similar results were obtained for *gpr*-*lacZ* when the contribution made by σ^G (Sussman & Setlow, 1991) was eliminated by introducing a *spoIIIG* mutation, except that the overall levels of expression were reduced about two- to fourfold (Fig. 2a). There was a low but reproducible level of β -galactosidase activity at the onset of sporulation in the *spoIIAB22* mutant, when measured either by *gpr*'-*lacZ* or by *spoIIIG*'-*lacZ* (Fig. 2a, b). This early phase of expression was presumably due mainly to σ^G activity because it was abolished by a *spoIIIG* mutation (Fig. 2a). It was also detected with the *sspA*-*lacZ* fusion (Fig. 2c), the promoter of which is thought to be recognized specifically by σ^G (i.e. not by σ^F ; Sun *et al.*, 1991). The presence of a low level of σ^G activity at the time of resuspension in starvation medium probably reflects the fact that the regulation of σ^G or, more likely, σ^F activity is perturbed in non-sporulating cells carrying the *spoIIAB22* mutation (see above).

To test the effects of *spoIIAB22* on σ^E activity in cells induced to sporulate by the resuspension procedure, we measured APase. The results of two independent experiments are shown in Fig. 2(d). No consistent differences were observed between the time courses for *spoIIAB22* and *spoIIAB*⁺ strains. Similar results were obtained for a *lacZ* fusion to the σ^E -dependent *spoIVA* gene (data not shown).

In summary, these results confirmed that the phenotypic penetrance of the *spoIIAB22* mutation is much less in liquid sporulation medium than in liquid YT medium or on NA plates.

Isolation and characterization of mutations suppressing the effects of the *spoIIAB22* mutation on sporulation on NA

Plate cultures of *spoIIAB* deletion mutants readily acquire secondary mutations that reduce forespore-specific gene expression (Schmidt *et al.*, 1990; Coppolecchia *et al.*, 1991). These secondary mutations tend to lie in the *spoIIAC* gene (Schmidt *et al.*, 1990; Coppolecchia *et al.*, 1991). Unlike the previously described secondary mutations, some of the ones arising in cultures of the *spoIIAB22* mutant appeared to restore spore formation.

Fig. 2. Effects of the *spoIIAB22* mutation on gene expression during sporulation induced by the resuspension method. In each panel the results obtained with a strain bearing the *spoIIAB22* mutation (triangles) are compared with isogenic *spoIIAB*⁺ strains (circles). (a) Effects of *spoIIAB22* on expression of *gpr*'-*lacZ*, in the presence (open symbols) or absence (filled symbols) of a functional *spoIIIG* gene, using strains 803, 804, 810 and 811; (b) effects on expression of *spoIIIG*'-*lacZ* in strains 838 and 839; (c) effects on expression of *sspA*'-*lacZ* in strains 805 and 823; (d) effects on synthesis of APase in strains 803 and 810 – data from two independent experiments are shown.

To determine the nature of these reversion events, four revertants showing increased spore formation were isolated (strains 812, 813, 814 and 815). Each of the revertants was transformed with an integration plasmid, pSG279, containing a deleted copy of *spoIIAC* missing 61 bp from the upstream end. Integration of this plasmid resulted in

segregation of Spo⁺ and Spo⁻ colonies, demonstrating that the second mutation was in *spoIIAC*, and that the *spoIIAB22* mutation was still present in the four strains examined. Three of the *spoIIAC* suppressor alleles were recovered by plasmid excision (see Methods). Transformation of the *spoIIAB22* mutant with each of these plasmids resulted in segregation of Spo⁺ and Spo⁻ colonies, demonstrating that the plasmids did carry suppressor alleles of *spoIIAC*. The inserts in the plasmids were sequenced. All three plasmids contained single base substitutions in the *spoIIAC* gene, which would give rise to single amino acid changes in the C-terminal region of σ^F (Fig. 3).

To determine the effects of these suppressor mutations on σ^F -dependent gene expression in the *spoIIAB22* mutant, we introduced a *spoIIIG-lacZ* fusion (which also inactivated the *spoIIIG* gene) into each of the *spoIIAC spoIIAB22* double mutant strains. In all four cases, the suppressor mutation reduced expression of *spoIIIG-lacZ* in plate culture to near that of the wild-type (data not shown). The suppressors thus appeared to act by reducing σ^F -dependent transcription.

DISCUSSION

Some of the properties of the *spoIIAB22* mutation are similar to those of the *spoIIAB1* mutation of Rather *et al.* (1990). It exhibited impaired regulation of σ^G activity, and the effects were particularly apparent under conditions in which sporulation does not occur (e.g. YT medium; Fig. 1). However, the new mutation differs from *spoIIAB1* in two important respects. Firstly, it causes a defect not only in the regulation of σ^G but also of σ^F (Foulger & Errington, 1993). Secondly, it severely impairs sporulation under certain conditions (Table 2). It is not yet possible to tell whether the increase in σ^G is the result of an impairment in the interaction between SpoIIAB and σ^G or an indirect consequence of the increased σ^F activity (see Foulger & Errington, 1993). However, the fact that the effects on sporulation and gene expression can be bypassed by mutations in the *spoIIAC* gene encoding σ^F (Fig. 3) suggests that the regulation of σ^G activity may be near normal in *spoIIAB22* mutants (the premature release of a low level of σ^G activity in the experiments shown in Fig. 2 could in principle be due to σ^G synthesized as a result of a slight release of σ^F activity). It has not been possible to bypass the effects of either the *spoIIAB28* missense mutation (Foulger & Errington, 1993) or *spoIIAB* null mutations by mutating *spoIIAC* in this way (Coppolecchia *et al.*, 1991; D. Foulger and J. Errington, unpublished results). This further indication that the *spoIIAB22* mutation specifically affects the interaction with σ^F and not σ^G is consistent with our previous suggestion that the N-terminal region of SpoIIAB is involved in sigma-factor recognition (Foulger & Errington, 1993).

The *spoIIAC* mutations that suppressed the effects of *spoIIAB22* were scattered throughout the C-terminal coding region of the gene. Their properties gave no specific clues as to the nature of suppression. However,

1 MDVEVKKNGK NAQLDHEVK ELIKQSQNGD QQARDLLIEK NMRLVWSVVQ RFLNRGYEPP

Core binding -10

61 DLFGICIGL LKSVDFDLT YDVRFTYAV PMITGEIQR FIRDGTVKVS RSLKELGNKI

121 RRAKDELSKT LGRVPTVQET ADHLEIEAED VVLAQEAURA PSSIHETVYE NDGDPITLLD

-35

181 QIADNSEEKW FDKIALKEAI SDLEEREKLI VYLRYYKDQT QSEVAERLGI SQVQVSRLEK

G P G A

815 814 224 236

241 KILKQIKVQM DHTDG*

T L

812 249

Fig. 3. Amino acid sequence of σ^F of *B. subtilis* and the substitutions generated by mutations suppressing the effects of the *spoIIAB22* mutation. The amino acids shown in bold face type are those that are conserved among the *B. subtilis* sigma-factor family as shown in the alignment of Stragier *et al.* (1989). The assignments of the core-binding region and promoter recognition regions are also from Stragier *et al.* (1989). The substituted amino acid is shown below the residue that it replaces and the allele number of the mutation is given. Alleles 224, 236 and 249 are the suppressors of a *spoIIAB* null mutation reported by Coppolecchia *et al.* (1991).

they lie in the same general region of *spoIIAC* as mutations that relieve the toxicity of the increased σ^F activity in a *spoIIAB* deletion allele (Coppolecchia *et al.*, 1991). Conceivably, the new suppressor mutations could act simply by reducing the activity of σ^F . If so, the reduction is probably slight because none of them conferred a Spo^- phenotype in a *spoIIAB*⁺ background (D. Foulger and J. Errington, unpublished results).

Why are the effects of the *spoIIAB22* mutation medium-dependent? One possibility is that the stability of one or more proteins may vary according to environmental conditions. Interestingly, one other class of mutations that can affect the level of σ^G activity under various conditions lies in the *B. subtilis* homologue of the *lon* gene of *E. coli* (Schmidt *et al.*, 1994; D. Foulger and J. Errington, unpublished results). *lon* encodes a protease that controls the stability of various proteins, including a number of important regulators (Gottesman, 1987). A second possible explanation for the medium dependence is related to the recent finding that the regulation of σ^F activity may be controlled by the ATP/ADP ratio, which is postulated to fall in the prespore (Alper *et al.*, 1994; Diederich *et al.*, 1994). If this model is correct, SpoIIAB plays a key role in sensing a threshold level for the ATP/ADP ratio. If the *spoIIAB22* mutation changed the threshold value, the result would be abnormal release of σ^F activity. The likely strong medium-dependence of the ATP/ADP ratio could then explain the effects we have observed.

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REFERENCES

- Alper, S., Duncan, L. & Losick, R. (1994). An adenosine nucleotide switch controlling the activity of a cell type-specific transcription factor in *B. subtilis*. *Cell* **77**, 195–205.
- Anagnostopoulos, C. & Spizizen, J. (1961). Requirements for transformation in *Bacillus subtilis*. *J Bacteriol* **81**, 741–746.
- Challoner-Courtney, I. J. & Yudkin, M. D. (1993). Molecular and phenotypic characterization of promoter-proximal mutations in the *spoIIA* locus of *Bacillus subtilis*. *J Bacteriol* **175**, 5636–5641.
- Coppolecchia, R., DeGrazia, H. & Moran, C. P., Jr (1991). Deletion of *spoIIAB* blocks endospore formation in *Bacillus subtilis* at an early stage. *J Bacteriol* **173**, 6678–6685.
- Diederich, B., Wilkinson, J. F., Magnin, T., Najafi, S. M. A., Errington, J. & Yudkin, M. D. (1994). Role of interactions between SpoIIAA and SpoIIAB in regulating cell-specific transcription factor σ^F of *Bacillus subtilis*. *Genes & Dev* **8**, 2653–2663.
- Duncan, L. & Losick, R. (1993). SpoIIAB is an anti- σ factor that binds to and inhibits transcription by regulatory protein σ^F from *Bacillus subtilis*. *Proc Natl Acad Sci USA* **90**, 2325–2329.
- East, A. K. & Errington, J. (1989). A new bacteriophage vector for cloning in *Bacillus subtilis* and the use of ϕ 105 for protein synthesis in maxicells. *Gene* **81**, 35–43.
- Errington, J. (1984). Efficient *Bacillus subtilis* cloning system using bacteriophage vector ϕ 105J9. *J Gen Microbiol* **130**, 2615–2628.
- Errington, J. (1993). *Bacillus subtilis* sporulation: regulation of gene expression and control of morphogenesis. *Microbiol Rev* **57**, 1–33.
- Errington, J. & Mandelstam, J. (1983). Variety of sporulation phenotypes resulting from mutations in a single regulatory locus, *spoIIA*, in *Bacillus subtilis*. *J Gen Microbiol* **129**, 2091–2101.
- Errington, J. & Mandelstam, J. (1986). Use of a *lacZ* gene fusion to determine the dependence pattern of sporulation operon *spoIIA* in *spo* mutants of *Bacillus subtilis*. *J Gen Microbiol* **132**, 2967–2976.
- Errington, J., Fort, P. & Mandelstam, J. (1985). Duplicated sporulation genes in bacteria: implications for simple developmental systems. *FEBS Lett* **188**, 184–188.
- Fort, P. & Errington, J. (1985). Nucleotide sequence and complementation analysis of a polycistronic sporulation operon, *spoVA*, in *Bacillus subtilis*. *J Gen Microbiol* **131**, 1091–1105.
- Fort, P. & Piggot, P. J. (1984). Nucleotide sequence of sporulation locus *spoIIA* in *Bacillus subtilis*. *J Gen Microbiol* **130**, 2147–2153.
- Foulger, D. & Errington, J. (1991). Sequential activation of dual promoters by different sigma factors maintains *spoVJ* expression during successive developmental stages of *Bacillus subtilis*. *Mol Microbiol* **5**, 1363–1373.
- Foulger, D. & Errington, J. (1993). Effects of new mutations in the *spoIIAB* gene of *Bacillus subtilis* on the regulation of σ^F and σ^G activities. *J Gen Microbiol* **139**, 3197–3203.
- Freese, E. & Heinze, J. (1984). Metabolic and genetic control of bacterial sporulation. In *The Bacterial Spore*, vol. 2, pp. 101–172. Edited by A. Hurst & G. W. Gould. London: Academic Press.

- Gottesman, S. (1987).** Regulation by proteolysis. In *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, pp. 1308–1312. Edited by F. C. Neidhardt, J. L. Ingraham, K. Brooks Low, B. Magasanik, M. Schaechter & H. E. Umbarger. Washington, DC: American Society for Microbiology.
- Jenkinson, H. F. (1983).** Altered arrangement of proteins in the spore coat of a germination mutant of *Bacillus subtilis*. *J Gen Microbiol* **129**, 1945–1958.
- Lewis, P. J., Partridge, S. R. & Errington, J. (1994).** σ factors, asymmetry, and the determination of cell fate in *Bacillus subtilis*. *Proc Natl Acad Sci USA* **91**, 3849–3853.
- Losick, R. & Stragier, P. (1992).** Crisscross regulation of cell-type-specific gene expression during development in *B. subtilis*. *Nature* **355**, 601–604.
- Margolis, P., Driks, A. & Losick, R. (1991).** Establishment of cell type by compartmentalized activation of a transcription factor. *Science* **254**, 562–565.
- Mason, J. M., Hackett, R. H. & Setlow, P. (1988).** Regulation of expression of genes coding for small, acid-soluble proteins of *Bacillus subtilis* spores: studies using *lacZ* gene fusions. *J Bacteriol* **170**, 239–244.
- Miller, J. H. (1972).** *Experiments in Molecular Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Min, K.-T., Hilditch, C. M., Diederich, B., Errington, J. & Yudkin, M. D. (1993).** σ^F , the first compartment-specific transcription factor of *B. subtilis* is regulated by an anti- σ factor that is also a protein kinase. *Cell* **74**, 735–742.
- Nicholson, W. L. & Setlow, P. (1990).** Sporulation, germination and outgrowth. In *Molecular Biological Methods for Bacillus*, pp. 391–450. Edited by C. R. Harwood & S. M. Cutting. Chichester: Wiley.
- Partridge, S. R. & Errington, J. (1993).** The importance of morphological events and intercellular interactions in the regulation of prespore-specific gene expression during sporulation in *Bacillus subtilis*. *Mol Microbiol* **8**, 945–955.
- Partridge, S. R., Foulger, D. & Errington, J. (1991).** The role of σ^F in prespore-specific transcription in *Bacillus subtilis*. *Mol Microbiol* **5**, 757–767.
- Piggot, P. J. & Coote, J. G. (1976).** Genetic aspects of bacterial endospore formation. *Bacteriol Rev* **40**, 908–962.
- Rather, P. N., Coppolecchia, R., DeGrazia, H. & Moran, C. P., Jr (1990).** Negative regulator of σ^G -controlled gene expression in stationary-phase *Bacillus subtilis*. *J Bacteriol* **172**, 709–715.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989).** *Molecular Cloning: a Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Schmidt, R., Margolis, P., Duncan, L., Coppolecchia, R., Moran, C. P., Jr & Losick, R. (1990).** Control of developmental transcription factor σ^F by sporulation regulatory proteins SpoIIAA and SpoIIAB in *Bacillus subtilis*. *Proc Natl Acad Sci USA* **87**, 9221–9225.
- Schmidt, R., Decatur, A. L., Rather, P. N., Moran, C. P., Jr & Losick, R. (1994).** *Bacillus subtilis* Lon protease prevents inappropriate transcription of genes under the control of the sporulation transcription factor σ^G . *J Bacteriol* **176**, 6528–6537.
- Sterlini, J. M. & Mandelstam, J. (1969).** Commitment to sporulation in *Bacillus subtilis* and its relationship to the development of actinomycin resistance. *Biochem J* **113**, 29–37.
- Stevens, C. M., Daniel, R., Illing, N. & Errington, J. (1992).** Characterization of a sporulation gene, *spoIV_A*, involved in spore coat morphogenesis in *Bacillus subtilis*. *J Bacteriol* **174**, 586–594.
- Stragier, P., Kunkel, B., Kroos, L. & Losick, R. (1989).** Chromosomal rearrangement generating a composite gene for a developmental transcription factor. *Science* **243**, 507–512.
- Sun, D., Stragier, P. & Setlow, P. (1989).** Identification of a new σ -factor involved in compartmentalized gene expression during sporulation of *Bacillus subtilis*. *Genes & Dev* **3**, 141–149.
- Sun, D., Fajardo-Cavazos, P., Sussman, M. D., Tovar-Rojo, F., Cabrera-Martinez, R.-M. & Setlow, P. (1991).** Effect of chromosome location of *Bacillus subtilis* forespore genes on their *spo* gene dependence and transcription by $E\sigma^F$: identification of features of good $E\sigma^F$ -dependent promoters. *J Bacteriol* **173**, 7867–7874.
- Sussman, M. D. & Setlow, P. (1991).** Cloning, nucleotide sequence, and regulation of the *Bacillus subtilis* *gpr* gene, which codes for the protease that initiates degradation of small, acid-soluble proteins during spore germination. *J Bacteriol* **173**, 291–300.
- Ward, J. B., Jr & Zahler, S. A. (1973).** Genetic studies of leucine biosynthesis in *Bacillus subtilis*. *J Bacteriol* **116**, 719–726.

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