Effects of new mutations in the *spoIIAB* gene of *Bacillus subtilis* on the regulation of σ^F and σ^G activities

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The *spoIIAB* gene of *Bacillus subtilis* encodes an inhibitor of σ^F , a transcription factor that plays a crucial role in the establishment of prespore-specific gene expression during sporulation. The SpoIIAB protein can probably also inhibit a closely related sigma factor σ^G , which determines the later phase of prespore-specific transcription. We have isolated two new missense mutations in the *spoIIAB* gene. *spoIIAB28* behaves like the previously described *spoIIAB1* mutation, in that it mainly affects the activity of σ^G . In contrast, the *spoIIAB22* mutation seems to be impaired mainly in its ability to inhibit σ^F . All three missense mutations are clustered in the N-terminal coding region of *spoIIAB*, suggesting that this region of the protein interacts with the sigma factors. The extreme N-terminal part of SpoIIAB may be specifically concerned with the regulation of σ^G activity.

Introduction

Soon after the onset of sporulation in *Bacillus subtilis*, the cell divides asymmetrically to produce a smaller prespore and larger mother cell. The two cells immediately embark on separate programmes of gene expression, which are set in motion by the segregation of two sigma factor activities, σ^F and σ^E . The two sigma factors are both encoded by genes that are transcribed and translated in the pre-septational cell, so the important problem of the establishment of cell-specific transcription can be reduced to that of the cell-specific regulation of the two sigma factor activities (Errington, 1993; Errington & Illing, 1992; Losick & Stragier, 1992). The activity of σ^F is thought to be regulated by the action of two co-translated proteins encoded by the *spoIIAA* and *spoIIAB* genes. The *spoIIAB* gene encodes an inhibitor of σ^F (the *spoIIAC* product); inactivation of *spoIIAB* causes hyper-expression of σ^F -dependent genes (Coppolecchia *et al.*, 1991; Schmidt *et al.*, 1990), and purified SpoIIAB protein inhibits transcription by $E\sigma^F$ *in vitro* (Duncan & Losick, 1993; Min *et al.*, 1993).

SpoIIAA is probably an antagonist of SpoIIAB; mutations in *spoIIAA* abolish σ^F activity, but only in the presence of SpoIIAB (Schmidt *et al.*, 1990; Margolis *et al.*, 1991). The cell-specificity of σ^F activity could thus be mediated by a regulatory mechanism acting through SpoIIAB. A similar arrangement of regulatory genes has

been found in the *sigB* operon of *B. subtilis* (Benson & Haldenwang, 1992; Boylan *et al.*, 1992).

Although deletion of *spoIIAB* primarily affects σ^F -dependent gene expression, and prevents spore formation (Coppolecchia *et al.*, 1991; Margolis *et al.*, 1991), the first *spoIIAB* mutation described, *spoIIAB1* (Rather *et al.*, 1990), was isolated on the basis of causing overexpression of σ^G -dependent genes. Curiously, the mutation had little or no effect on spore formation, and its effect on σ^G -dependent gene expression was manifested only under conditions in which sporulation did not occur (Rather *et al.*, 1990).

Here we describe the isolation and characterization of two new alleles of *spoIIAB*, which lie close to the previously isolated *spoIIAB1* mutation of Rather *et al.* (1990). The new *spoIIAB* alleles have more severe phenotypic effects than *spoIIAB1*. Surprisingly, one of the mutations seems to specifically impair the regulation of σ^G activity, whereas the other affects both σ^G and σ^F . The results suggest that the N-terminal region of SpoIIAB is important in the recognition of σ^F and σ^G .

Methods

Bacterial strains and plasmids. These are listed in Table 1. The *spoIIAB22* and *spoIIAB28* mutations were made isogenic by transforming chromosomal DNA from the original mutants into strain MB75, selecting Lys⁺ on LGM medium (lactate glutamate minimal agar; Piggot, 1973) containing methionine (200 µg ml⁻¹), and isolating Spo⁻ Cm^S congressants (i.e. transformants that had simultaneously acquired the *spoIIAB* mutation). The *spoIIAB1* mutation was introduced into an isogenic background by transforming chromosomal DNA from strain EU6001 into strain 802, with selection for Lys⁺, and

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Table 1. *Bacterial strains and plasmids*

Bacterial strains	Relevant genotype	Source/reference
<i>B. subtilis</i>		
EU6001	<i>spoIAB1</i> Ω (<i>gdh-lacZ ermC</i>) <i>aroD120 trpC2</i>	Rather <i>et al.</i> (1990)
MB75	<i>metC3 lys-1</i>	Laboratory stock
698	<i>spoIIGB55</i> Ω (<i>spoIIG'-lacZ ermC</i>)687 <i>trpC2</i>	Partridge <i>et al.</i> (1991)
801	<i>spoIIGB55 spoIIG::pSG139 (spoIIG'-lacZ) trpC2 lacR1 lacA17</i>	This study
802	<i>metC3 lys-1 gpr::pPS1395 (gpr'-lacZ)</i>	This study
803	<i>gpr::pPS1395 (gpr'-lacZ) metC3</i>	This study
804	<i>gpr::pPS1395 (gpr'-lacZ) metC3</i> Ω (<i>spoIIG::ermC</i>)731	This study
805	<i>metC3 sspA::pPS300 (sspA'-lacZ)</i>	This study
806	<i>spoIAB22 spoIIGB55</i> Ω (<i>spoIIG'-lacZ ermC</i>)687 <i>trpC2</i>	This study
807	<i>spoIAB22 spoIIA::pSG3 spoIIGB55</i> Ω (<i>spoIIG'-lacZ ermC</i>)687 <i>trpC2</i>	This study
808	<i>spoIAB22 spoIIG::pSG139 (spoIIG'-lacZ) trpC2</i>	This study
809	<i>metC3 spoIAB22</i> Ω (<i>spoIIG'-lacZ ermC</i>)687	This study
810	<i>gpr::pPS1395 (gpr'-lacZ) metC3 spoIAB22</i>	This study
811	<i>gpr::pPS1395 (gpr'-lacZ) metC3 spoIAB22</i> Ω (<i>spoIIG::ermC</i>)731	This study
823	<i>metC3 spoIAB22 sspA::pPS300 (sspA'-lacZ)</i>	This study
824	<i>metC3 spoIAB28</i>	This study
825	<i>metC3 spoIAB28 spoIIA::pSG3</i>	This study
826	<i>gpr::pPS1395 (gpr'-lacZ) spoIAB28 trpC2</i>	This study
827	<i>gpr::pPS1395 (gpr'-lacZ) metC3 spoIAB28</i>	This study
828	<i>gpr::pPS1395 (gpr'-lacZ) metC3 spoIAB28</i> Ω (<i>spoIIG::ermC</i>)731	This study
829	<i>metC3 spoIAB28 sspA::pPS300 (sspA'-lacZ)</i>	This study
830	<i>gpr::pPS1395 (gpr'-lacZ) metC3 spoIAB1</i>	This study
831	<i>gpr::pPS1395 (gpr'-lacZ) metC3 spoIAB1</i> Ω (<i>spoIIG::ermC</i>)731	This study
832	<i>metC3 spoIAB1 sspA::pPS300 (sspA'-lacZ)</i>	This study
<i>E. coli</i>		
DH5 α	F ⁻ ϕ 80dlacZ Δ M15 <i>endA1 recA1 hsdR17(r_K m_K) supE44 thi-1 λ^- gyrA96 relA1 thi-1 Δ(lacZYA-argF)U169</i>	Gibco BRL, New York
Plasmids		
pIC350	<i>bla cat spoIIAABC*</i>	I. Challoner-Courtney (unpublished)
pPF2	<i>bla spoIIAAB†</i>	Fort & Piggot (1984)
pPS300	<i>bla cat sspA'-lacZ</i>	Mason <i>et al.</i> (1988)
pPS1395	<i>bla cat gpr'-lacZ</i>	P. Setlow‡
pSG2	<i>bla cat</i> (integrational vector)	Fort & Errington (1985)
pSG9	<i>bla cat 'spoIIAC spoVAA'</i>	Fort & Errington (1985)
pSG139	<i>bla cat spoIIG'-lacZ</i>	Foulger & Errington (1989)
pSG632	<i>bla cat</i> 1.5 kb of DNA from upstream of <i>spoIIAA</i> gene	Partridge <i>et al.</i> (1991)
pSG624	<i>bla cat spoIIAABC</i>	Partridge <i>et al.</i> (1991)
Constructed plasmids		
p2A(4)	<i>bla spoIIAAB</i>	pPF2 deleted with unidirectional deletion removing DNA
pSG3	<i>bla cat spoIIAABC</i>	<i>SaII-SstI</i> insert from p2A(4) cloned into <i>SaII-SstI</i> -digested pSG2
pSG36	<i>bla cat spoIIAABC</i>	<i>HindIII-SaII</i> insert from pPF2 cloned into <i>EcoRI-SaII</i> digested pSG2
pSG278	<i>bla cat spoIIAAB22</i>	<i>AccI-ClaI</i> -digested chromosomal DNA from strain 807 ligated and transformed into <i>E. coli</i> DH5 α
pSG279	<i>bla cat spoIIAC</i>	<i>EcoRI-ClaI</i> -digested pIC350 self-ligated
pSG280	<i>bla cat spoIIAAB28</i>	<i>AccI-ClaI</i> -digested chromosomal DNA from strain 825 ligated and transformed into <i>E. coli</i> DH5 α

* Contains 1.6 kb *EcoRI-PstI* fragment cloned in pTZ19R (Mead *et al.*, 1986).

† Contains the 2.2 kb *HindIII-SaII* fragment from pHM2 cloned into pUC13 (see Fort & Piggot, 1984).

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§ Restriction site filled in with the Klenow fragment of DNA polymerase.

isolating dark-blue, Em^s congressants (on medium containing X-Gal; see below). The linkage of these mutations to *lys-1* was determined by counting the percentage of Spo⁻ or dark-blue transformants. As in previous experiments with *spoIIAB* mutations (e.g. Coppolecchia *et al.*, 1991; Schmidt *et al.*, 1990), we noticed that suppressor mutations arose relatively frequently. As in previous reports, these suppressor mutations predominantly occurred in the *spoIIAC* gene (D. Foulger, unpublished results). We avoided the problem by routinely working from frozen stock cultures, prepared from transformant colonies allowed only a minimal period of growth after isolation. The phenotypic consequences of the mutations described were reproduced in colonies obtained immediately after transformation of *B. subtilis* strains with integrative plasmids, shown by DNA sequencing to carry only a single base substitution in the *spoIIAB* gene.

General methods. *B. subtilis* strains were grown on plates containing nutrient agar (Oxoid) or LB agar (Rather *et al.*, 1990). Chloramphenicol (5 µg ml⁻¹), kanamycin (5 µg ml⁻¹), or erythromycin (1 µg ml⁻¹) and lincomycin (25 µg ml⁻¹) were added as required. β-Galactosidase activity was detected in plates by addition of 0.01% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). *B. subtilis* strains were made competent for transformation with DNA by the method of Anagnostopoulos & Spizizen (1961), as modified by Jenkinson (1983). Chromosomal DNA was prepared from *B. subtilis* by the rapid method of Ward & Zahler (1973) for mapping purposes, and by the method of Errington (1984) for cloning. Transformation of *Escherichia coli* and DNA manipulations were carried out using standard procedures (e.g. Sambrook *et al.*, 1989).

NTG mutagenesis. Strain 801 was used for the isolation of mutations bypassing the effects of *spoIIGB55* on expression of *spoIIIG-lacZ*. NTG mutagenesis was carried out as described by Errington & Vogt (1990), and the mutagenized cells were plated on nutrient agar containing X-Gal.

Assay of β-galactosidase. Cells were harvested from nutrient agar plates as described by Rather *et al.* (1990). β-Galactosidase activity was determined from a 0.5 ml sample of cell suspension. β-Galactosidase activity (defined according to Miller, 1972) was determined by an assay based on hydrolysis of *o*-nitrophenol-β-D-galactoside, as described by Nicholson & Setlow (1990).

Cloning mutant alleles of *spoIIAB*. To clone the mutant allele *spoIIAB22*, strain 806 was transformed with plasmid pSG3, with selection for chloramphenicol resistance. Among the transformants were a small proportion of blue colonies (on plates containing X-Gal), presumably arising by gene conversion (see Results), and thus a state in which both copies of the *spoIIAB* gene carried the mutation. Chromosomal DNA from a blue isolate was digested with *AccI* and *Clal*, then diluted and religated, which would be expected to generate a plasmid similar to pSG3, but carrying a copy of the mutant *spoIIAB22* gene truncated at the *Clal* site. Transformation into *E. coli* DH5α, and selection on ampicillin plates (100 mg ml⁻¹), resulted in the isolation of a plasmid (pSG278) showing the expected restriction pattern. The plasmid was then checked by transformation back into *B. subtilis*. The chloramphenicol-resistant transformants showed the expected segregation of Spo⁺ and Spo⁻ phenotypes.

The mutant allele *spoIIAB28* was cloned from a Spo⁻ chloramphenicol-resistant colony isolated following transformation of strain 824 with plasmid pSG3. Again, the Spo⁻ colonies presumably arose by gene conversion. The mutation was isolated on the plasmid as described above, again generating a plasmid (pSG280) with the expected restriction pattern, and giving segregation of Spo⁺ and Spo⁻ transformants of *B. subtilis*.

DNA sequencing. Plasmid DNA was denatured using sodium hydroxide, then neutralized by the addition of ammonium acetate, as

described by Murphy & Ward (1989). DNA sequencing reactions were done with a Sequenase kit as described by the manufacturers (USB). The following synthetic oligonucleotides complementary to sequences in *spoIIA* DNA (kindly provided by I. Challoner-Courtney and M. D. Yudkin, Microbiology Unit, Department of Biochemistry, University of Oxford, UK), or to the vector, were used as primers (all 5' to 3'): AAGGCTCATGCTCATT, GGTCTCCAAGTTCAGC, GAGTGCCTGCTGTTTCAG, GCATTCGTGACAGCCTC, CAAGCTCAGGCTTAGTC, CAGGAAACAGCTATGAC. The products of the sequencing reactions were separated on 6% polyacrylamide gels containing 7 M-urea, prepared as described in the Sequagel Kit (National Diagnostics).

Results

Isolation and sequencing of new spoIIAB mutations

We have recently isolated two new NTG-induced mutations in the *spoIIAB* gene of *B. subtilis*. One of them, designated *spoIIAB22*, was obtained during attempts to obtain mutations bypassing the effects of mutations in the *spoIIGB* gene on expression of *spoIIIG-lacZ* (Partridge & Errington, 1993). The second, designated *spoIIAB28*, was obtained as a mutation causing increased expression of *gpr-lacZ* in wild-type cells (L. Wu, unpublished results).

The two mutants were mapped to the *spoIIA* region on the basis of transformation linkage (about 21%) to *lys-1*⁺ (see Piggot & Coote, 1976). To localize mutation *spoIIAB22* more specifically, integration plasmids containing various cloned parts of the *spoIIA* operon (Fig. 1) were transformed into strain 809 with selection for chloramphenicol resistance (in the presence of X-Gal), and the colony-colour phenotypes were scored. White (wild-type) and blue (mutant) segregants were obtained following transformation with plasmids pSG3, pSG36 and pSG624, but the other plasmids tested (pSG9, pSG279 and pSG632) gave only mutant transformants. This showed that the mutation lay in the promoter region of the *spoIIA* operon, or in the coding portions of the *spoIIAA* or *spoIIAB* genes. Plasmids pSG3 and pSG36 gave a relatively low proportion of mutant transformants (< 10%, compared with > 80% for pSG624), suggesting that the mutation lay in the *spoIIAB* gene, and was complemented by the second wild-type copy of *spoIIAB* produced on integration of these plasmids. The *spoIIAB28* mutation in strain 824 was localized to the *spoIIAB* gene in a similar manner, except that the presence of the mutation was scored on the basis of Spo phenotype (both new mutations reduce the incidence of sporulation on nutrient agar; D. Foulger, unpublished results).

The two new mutant alleles of *spoIIAB*, in strains 808 (*spoIIAB22*) and 826 (*spoIIAB28*), were cloned as derivatives of plasmid pSG3 (see Methods). The derivative plasmids were shown to carry the appropriate

Fig. 1. Map of the *spoIIA* integration plasmids. The open boxes indicate the locations of the *spoIIA* open reading frames. The *spo* prefix has been omitted from the gene designations. The abbreviations for restriction endonuclease cleavage sites are as follows: B, *Bgl*II; C, *Cl*AI; E, *Eco*RI; H, *Hind*III; P, *Pst*I; S, *Sca*I; V, *Pvu*II.

mutant alleles by transformation into strains containing a wild-type *spoIIAB* gene (strain 698) or the appropriate *spoIIAB* mutation (strains 806 and 824). As expected, both plasmids gave a mixture of wild-type and mutant transformants of strain 698, and only mutant transformants of their respective mutant recipient strain. The mutant phenotype was scored either by overexpression of *spoIIIG'*-*lacZ* on nutrient agar containing X-Gal, or by a translucent oligosporogenous phenotype, depending on the recipient strain used.

The inserts in the two plasmids were sequenced. Both contained single base substitutions within the *spoIIAB* coding region. Mutation *spoIIAB22* was found to be an A to G transition at base 827 (as defined by Fort and Piggot, 1984), which would result in the replacement of aspartate by glycine at residue 31 in the N-terminal region of SpoIIAB. Mutation *spoIIAB28* was found to be a C to T transition at base 793, which would result in

the replacement of arginine by cysteine at residue 20 of SpoIIAB (Fig. 2).

Effects of the new *spoIIAB* mutations on the activities of σ^F and σ^G

Previous work has shown that deletion mutations in the *spoIIAB* gene can cause great increases in the activities of both σ^F and σ^G (Schmidt *et al.*, 1990; Coppolecchia *et al.*, 1991; I. Challoner-Courtney & M. D. Yudkin, unpublished results). The idea that SpoIIAB might regulate both sigma factors is not surprising, in view of the especially close sequence similarity between σ^F and σ^G (Errington, 1991). In contrast to the broad effects of deletions of *spoIIAB*, the only previously characterized missense mutation, *spoIIAB1*, seemed to affect only σ^G activity (Rather *et al.*, 1990). Recent work indicates that the SpoIIAB protein inhibits σ^F (and presumably also σ^G) by a direct protein-protein interaction (Duncan & Losick, 1993; Min *et al.*, 1993), which raised the possibility that the *spoIIAB1* mutation might specifically affect the interaction of the SpoIIAB protein with σ^G . We therefore wanted to determine whether the *spoIIAB1* allele does act specifically on the regulation of σ^G activity, and also whether the new mutations, shown above to lie close to *spoIIAB1*, also show specificity for one or other of the σ factors.

To measure the effects of the mutations on σ^F activity, we introduced a *gpr-lacZ* fusion into each of the *spoIIAB* mutants. Because the *gpr* promoter is also used, albeit less efficiently, by $E\sigma^G$ (Sun *et al.*, 1991), we measured expression in the presence and absence of a *spoIIIG* mutant eliminating σ^G , reasoning that the level of expression in the *spoIIIG*⁻ background would give an indication of specific transcription by $E\sigma^F$. Like the results described elsewhere for the *spoIIAB1* mutation (Rather *et al.*, 1990), we found that the phenotypic effects of the new mutations were more severe on solid media than in liquid sporulation medium. For this reason, and

Fig. 2. Alignments of SpoIIAB with RsbW and KinA, and locations of point mutations. The alignments are based on the output from the program FASTA run as part of the GCG package, version 7. Identical residues are shaded. The lines above the KinA sequence indicate those regions of the protein which are, according to Antoniewski *et al.* (1990), highly conserved in the transmitter domains of the sensor proteins. The amino acid substitutions caused by the three known *spoIIAB* missense mutations are indicated below the SpoIIAB sequence. The KinA and RsbW sequences were taken from the EMBL data library, accession numbers M29450 and M34995, respectively. The EMBL accession number for the SpoIIAB sequence is M17643 (Fort & Piggot, 1984).

Table 2. Effect of *spoIIAB* mutations on *gpr-lacZ* expression on LB agar plates

Cells were harvested at different times from LB agar plates and assayed for β -galactosidase activity (Miller units). Strains either carried *gpr-lacZ* and the *spoIIIG731* mutation (strains 831, 811, 828, 804), or *gpr-lacZ* in a *spoIIIG*⁺ background (strains 830, 810, 827, 803). Each value is the mean of four samples taken from separate plates, with standard deviations given in parentheses.

Time (h)	<i>spoIIIG</i>	β -Galactosidase activity in strains containing <i>gpr-lacZ</i> and a <i>spoIIAB</i> mutation							
		<i>spoIIAB</i> ⁺	<i>spoIIAB1</i>	<i>spoIIAB28</i>	<i>spoIIAB22</i>				
21	+	5.3 (0.2)	63 (6.2)	156 (4.3)	183 (12)				
	–	3.5 (0.2)	3.0 (0.2)	7.3 (0.3)	46 (1.1)				
24	+	5.5 (0.1)	74 (7.5)	178 (7.9)	212 (8.3)				
	–	3.7 (0.1)	3.3 (0.1)	9.9 (0.3)	88 (6.4)				
27	+	6.3 (0.2)	81 (0.3)	206 (9.9)	237 (4.6)				
	–	4.1 (1.1)	6.3 (0.8)	16 (0.1)	170 (8.8)				

Table 3. Effects of *spoIIAB* mutations on *sspA-lacZ* expression on LB agar

Cells were harvested at the times shown from LB agar plate cultures and assayed for β -galactosidase activity. Results were obtained with derivatives of strains 832, 829, 823 and 805, carrying plasmid pUB-A (*sspA-lacZ*). Each value is the mean of six samples taken from three different plates, with standard deviations given in parentheses.

Time (h)	β -Galactosidase activity in strains containing <i>sspA-lacZ</i> and a <i>spoIIAB</i> mutation			
	<i>spoIIAB</i> ⁺	<i>spoIIAB1</i>	<i>spoIIAB28</i>	<i>spoIIAB22</i>
21	11.9 (0.95)	326 (41)	905 (34)	988 (49)
24	15.4 (2.5)	469 (68)	1222 (9)	1522 (19)
26	16.3 (0.95)	440 (54)	1348 (37)	1725 (96)

to allow comparison of our results with those of Rather *et al.* (1990), we describe here enzyme activities from cultures grown on solid medium. All of the mutations led to greatly enhanced expression of the *gpr-lacZ* fusion in the *spoIIIG*⁺ background, as expected of mutations relieving the effects of an inhibitor of σ^F and/or σ^G (Table 2). The *spoIIAB1* mutation had little, if any, effect on the expression of *gpr-lacZ* in the *spoIIIG*[–] background, indicating that the mutant protein exerts an essentially normal level of regulation on σ^F . The *spoIIAB28* mutation caused a slight increase in *gpr-lacZ* expression in this background (2–4 times that of the wild type), indicating a slightly diminished inhibition of σ^F . By contrast, the *spoIIAB22* mutation resulted in greatly increased *gpr-lacZ* expression (which we interpret as σ^F activity), amounting to 10–50 times that of the wild-type.

The effects of the various mutations on the regulation of σ^G activity are much harder to quantify, because the gene which encodes it, *spoIIIG*, can be transcribed by

both σ^F and σ^G itself (Sun *et al.*, 1991). Thus, σ^G synthesis is influenced by the level of σ^F activity, and the resulting σ^G can have a positive autoregulatory effect (Karmazyn-Campelli *et al.*, 1989; Rather *et al.*, 1990). However, a tentative indication of the relative effects of the mutations on σ^G activity could be obtained by subtracting the levels of expression of *gpr-lacZ* in the *spoIIIG*[–] background from those obtained in the *spoIIIG*⁺ background (Table 2). This seemed to confirm that all three mutations cause increased σ^G activity, compared to the wild-type. However, it also appeared that *spoIIAB28* caused a larger increase in σ^G activity than *spoIIAB22*. Similar results were obtained in experiments measuring the effects of *spoIIAB22* and *spoIIAB28* on expression of a different σ^F -dependent reporter gene, namely *spoIIIG-lacZ* (results not shown). To measure σ^G activity more specifically, we tested the effects of the three *spoIIAB* mutations on expression of a promoter that is recognized only by σ^G (at least *in vivo*; Sun *et al.*, 1989), that of the *sspA* gene. The results (Table 3) confirmed that all three mutations caused a substantial (25–100-fold) increase in σ^G activity on LB agar. The new mutations gave rise to almost equal amounts of *sspA-lacZ* expression, with the *spoIIAB1* mutation giving about threefold less.

Discussion

We have isolated two new missense mutations in *spoIIAB*. They lie, like *spoIIAB1*, in the N-terminal coding region of the gene (Fig. 2). Both new mutations have more severe phenotypic effects than *spoIIAB1*: in addition to the effects we have described on gene expression, they also cause severely impaired sporulation on nutrient agar (D. Foulger, unpublished observations). Our results confirm that *spoIIAB1* seems to affect mainly σ^G activity (Rather *et al.*, 1990). The effects of one of the new mutations, *spoIIAB28*, are again almost specific for σ^G . In the absence of σ^G (*spoIIIG*[–] background), there is little more expression of *gpr-lacZ* or *spoIIIG-lacZ* than in the equivalent *spoIIAB*⁺ cells (Table 2 and data not shown). Thus, regulation of σ^F activity, which probably occurs by a direct protein–protein interaction (Duncan & Losick, 1993; Min *et al.*, 1993), appears to be near normal in these mutants. In contrast, the *spoIIAB22* mutant is impaired in the regulation of σ^F activity: a substantial amount of transcription of both *gpr-lacZ* and *spoIIIG-lacZ* occurs in the absence of σ^G . We conclude that missense mutations in the N-terminal coding region of the *spoIIAB* gene can have different effects with respect to the regulation of σ^F and σ^G .

Unfortunately, it is difficult to assess the effects of the *spoIIAB22* mutation on σ^G activity because σ^F can direct synthesis of σ^G (the product of the *spoIIIG* gene). Although *sspA-lacZ* provides a measure of σ^G activity in

the cell, in the *spoIIAB22* mutant the increased activity could represent two components: one due to the enhanced level of σ^G protein resulting from increased transcription by $E\sigma^F$, the other due to a direct effect of the mutation on the regulation of σ^G activity. However, since the levels of *sspA-lacZ* expression in the *spoIIAB22* and *spoIIAB28* mutants are fairly similar, it seems likely that *spoIIAB28* is more severely affected than *spoIIAB22* in the regulation of σ^G activity.

There are two obvious ways of explaining the finding that mutations in the N-terminal coding region of *spoIIAB* can differentially affect the regulation of σ^F and σ^G . The mutations could lie in a region of SpoIIAB that interacts directly with σ^F and σ^G , where some amino acid substitutions might be expected to impair the interaction with both sigma factors and others might be relatively specific. Alternatively, it is possible that SpoIIAB interacts with σ^F and σ^G in different ways. On this assumption, the extreme N-terminal region of SpoIIAB, containing *spoIIAB1* and *spoIIAB28* but not *spoIIAB22*, would be involved specifically in the interaction with σ^G .

A likely functional analogue of *spoIIAB* is to be found in the *sigB* operon of *B. subtilis*, which encodes another 'minor' σ factor, σ^B . The products of *spoIIAB* and *rsbW* (from the *sigB* operon) exhibit 27% amino acid identity (Kalman *et al.*, 1990; Fig. 2), and genetic evidence indicates that RsbW is probably an inhibitor of σ^B (Benson & Haldenwang, 1992; Boylan *et al.*, 1992). Interestingly, the *spoIIAB1* and *spoIIAB28* mutations cause substitutions in residues of SpoIIAB that are conserved in RsbW, whereas the *spoIIAB22* mutation makes a reciprocal kind of change: the previously divergent residue becomes identical to that of RsbW (Fig. 2).

We have recently noted that certain motifs characteristic of a family of bacterial proteins called environmental sensors, which seem to be involved in phosphotransferase reactions (Stock *et al.*, 1989), lie in various parts of the SpoIIAB sequence but notably not in this N-terminal region (Fig. 2). Biochemical studies indicate that SpoIIAB does indeed have kinase activity (Min *et al.*, 1993), and it is thus tempting to speculate that the reason why the N-terminal region of SpoIIAB is relatively dissimilar to the other kinases is that it is involved specifically in sigma factor recognition. Biochemical analysis of the new *spoIIAB* mutants in relation to their interactions with σ^F and σ^G should help to clarify the role of SpoIIAB in the regulation of cell-specific transcription during sporulation.

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