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Control of the cell-specificity of σ^F activity in *Bacillus subtilis*

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SUMMARY

Sporulation in *Bacillus subtilis* is a simple developmental system involving the differentiation of two cell types that are formed by an asymmetric cell division. Major changes in the pattern of transcription during sporulation are brought about by the synthesis of new sigma factors (σ), which are subunits of RNA polymerase that determine promoter specificity. Transcription in the smaller prespore cell type is initiated by a sigma factor called σ^F, the activity of which is subject to tight spatial and temporal control. It is negatively regulated by an anti-sigma factor, SpoIIAB, which is in turn controlled by an anti-anti-sigma factor, SpoIIAA. SpoIIAA and SpoIIAB participate in two contrasting reactions *in vitro*. In the presence of ATP, the proteins interact transiently and SpoIIAA is inactivated by phosphorylation on a specific serine residue; SpoIIAA then remains free to inhibit σ^F. In the presence of ADP, SpoIIAA binds tightly to SpoIIAB, and σ^F is set free. Release of σ^F activity *in vivo* might thus be effected by a prespore-specific reduction in the ATP/ADP ratio.

Genetic experiments have implicated a fourth protein, called SpoIIE, in this system. It now appears that SpoIIE has two important and independent functions in the establishment of the prespore-specific transcription by σ^F. First it regulates σ^F activity, probably acting as a phosphatase to regenerate the active, non-phosphorylated form of SpoIIAA. Second it controls the formation of the septum that generates the prespore compartment. Combination of these two functions in a single polypeptide may provide a means of coupling gene expression with morphogenesis.

1. INTRODUCTION

The promoter specficity of bacterial RNA poymerase is dictated by a subunit called sigma. Unlike eukaryotic polymerases the sigma subunit generally binds to promoters only when it is associated with the catalytic core (one subfamily of sigma factors, typified by σ^{54}, are exceptional in being able to bind directly to DNA; Buck & Cannon 1992). Sigma factors behave as potent positive regulators of transcriptional initiation. With the exception of the σ^{54} family, all recognize sequences centred around -10 and -35 with respect to the initiation site for mRNA synthesis. Synthesis, or activation, of a new sigma factor allows RNA polymerase to recognize and initiate transcription of a set of previously inactive genes. *E. coli* and *Bacillus subtilis* both encode about ten different sigma factors. The 'major' sigma factor (σ^{70} or σ^A) is responsible for transcription of most housekeeping genes. 'Minor' sigma factors generally control collections of genes that allow the cell to respond to specific stresses or stimuli, such as heat shock, or to coordinate the formation of complex structures, such as flagella or spores.

2. SPORULATION SIGMA FACTORS

Five of the nine known minor sigma factors of *B. subtilis* are involved in the regulation of sporulation (reviewed by Losick & Stragier 1992; Errington 1993; Haldenwang 1995). Their general functions and time of appearance during sporulation are summarized in table 1.

σ^H differs from the others in being active in vegetative (non-sporulating) cells. In addition to controlling several genes needed for the initiation and early stages of sporulation, it is also involved in the regulation of other stationary phase phenomena, such as competence. The other four sigma factors are all made during sporulation only, and all control gene expression in one or other of the two differentiating cell types: either in the prespore (σ^F and σ^G), or in the mother cell (σ^E and σ^K). In both cells, one sigma factor acts early in development (σ^F and σ^E) and the other becomes active later (σ^G and σ^K). The late ones are restricted to a specific cell type primarily because their genes are transcribed by RNA polymerase containing the sigma factor that is active in the same cell type earlier in sporulation. For example, the gene encoding σ^G is transcribed, at least initially, by RNA polymerase containing σ^F.

How the early sigma factors become active in only

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Table 1. *General properties of the sigma factors involved in the regulation of sporulation in* B. subtilis (*from Haldenwang 1995*)

sigma factor (other name)	gene	function	promoter consensus[a]
σ^A (σ^{43}, σ^{55})	*rpoD*	housekeeping/early spoulation	TTGACA-17-TATAAT
σ^H (σ^{30})	*spo0H*	early stationary phase; competence and sporulation	RWAGGAXXT-14-HGAAT
σ^F	*spoIIAC*	prespore, early	GCATR-15-GGHRARHTX
σ^E (σ^{29})	*spoIIGB*	mother cell, early	ZHATAXX-14-CATACAHT
σ^G	*spoIIIG*	prespore, late	GHATR-18-CATXHTA
σ^K (σ^{27})	*spoIIIC-spoIVC or sigK*	mother cell, late	AC-17-CATANNNTA

[a] H, A or C; N, A, C, G, or T; R, A or G; W, A, G or C; X, A or T; Z, T or G.

one of the two cell types is a particularly important question. In the case of σ^E, the primary protein product is made as an inactive precursor, pro-σ^E, which is activated by proteolytic processing (Trempy *et al.* 1985; LaBell *et al.* 1987). pro- σ^E is probably present in both of the differentiating cells, but its activity is unleashed only in the mother cell (Driks & Losick 1991; Errington & Illing 1992). This localization is achieved, probably at the level of processing, by an intercellular signal transduction pathway emanating from a gene, *spoIIR*, that is transcribed only in the prespore by RNA polymerase containing σ^F (Karow *et al.* 1995; Londoño-Vallejo & Stragier 1995). Although details of this mechanism remain to be resolved, its dependence on the localized activity of a gene in the prespore means that the mechanism whereby σ^F activity is restricted to the prespore is of pivotal importance in the differentiation system. Progress in understanding the biochemical basis for the temporal and spatial regulation of σ^F activity is discussed below.

3. CELL SPECIFICITY OF σ^F ACTIVITY

Although σ^F drives transcription of genes only in the prespore compartment (Margolis *et al.* 1991; Partridge *et al.* 1991), synthesis of the protein is strongly induced early in sporulation, *before* formation of the spore septum (Gholamhoseinian & Piggot 1989; Min *et al.* 1993; Partridge & Errington 1993). It is even made at low levels in vegetative cells (Partridge *et al.* 1991; Min *et al.* 1993). The protein must therefore be held in a latent state until after septation. Thereafter, its activity is released only in the prespore cell type. Control over σ^F activity is thought to be coupled to recognition of some special physiological feature of the prespore compartment (Margolis *et al.* 1991). Thus σ^F activation does not occur if formation of the prespore septum is prevented by withholding any one of a number of cell division proteins (Beall & Lutkenhaus 1991; Levin & Losick 1994; R. A. Daniel & J. Errington, unpublished results). Moreover, in cells with a mutation in the *spoIIG* locus of *B. subtilis*, in which two prespore-like cells are formed rather than a prespore and a mother cell (Piggot & Coote 1976; Illing & Errington 1991), σ^F is activated in both of the polar compartments (Lewis *et al.* 1994; see figure 1). The nature of the physiological signal that the system responds to is still unclear, though biochemical experiments indicate that changes in the levels of adenine nucleotides may be important (see below). These biochemical studies have focused on the products of three genes identified by mutations that specifically affect σ^F activation.

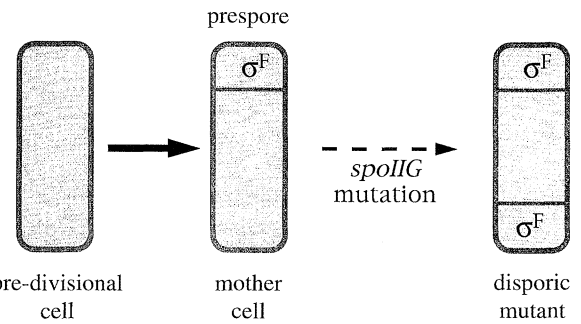

Figure 1. Cell-specificity of σ^F activation. The σ^F protein is made in the predivisional cell but it is not active at that time, as indicated by the light type face. After asymmetric septation the protein presumably segregates into both the small prespore and larger mother cell compartments. It becomes active specifically in the mother cell, as indicated by the black type face. The mechanism responsible for the release of σ^F activity may respond to some distinguishing physiological feature of the prespore (Margolis *et al.* 1991), because in *spoIIG* mutants, in which a second prespore-like cell is made (the disporic phenotype), σ^F becomes active in both polar compartments (Lewis *et al.* 1994).

4. BIOCHEMISTRY OF σ^F REGULATION

(a) *The SpoIIAB anti-sigma factor*

The problem of how σ^F is prevented from becoming active, both before septation and later on in the mother cell, was solved by the identification of a gene, *spoIIAB*, apparently encoding an inhibitor (Schmidt *et al.* 1990; Partridge *et al.* 1991). Because the *spoIIAB* gene lies in the same operon as σ^F (Fort & Piggot 1984), the two genes are expressed coordinately, so that SpoIIAB protein is present whenever σ^F is made. The nature of the interaction between SpoIIAB and σ^F has been studied *in vitro*. Two groups independently showed that purified SpoIIAB can inhibit σ^F activity in a run-off transcription assay (Duncan & Losick 1993; Min *et al.* 1993). SpoIIAB is therefore an anti-sigma factor. Inhibition of transcription was more or less complete on addition of a twofold molar excess of SpoIIAB over σ^F (Diederich *et al.* 1994). This stoichiometry was in accordance with several lines of evidence indicating that SpoIIAB is predominantly a homodimer in solution (Duncan & Losick 1993; Magnin *et al.* 1995). That the interaction between AB and σ^F is direct was

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demonstrated by the detection of a complex, both by protein-protein cross-linking (Duncan & Losick 1993; Alper *et al.* 1994) and by non-denaturing polyacrylamide gel electrophoresis (J. F Wilkinson & J. Errington, unpublished results). The cross-linking assay provided a means of testing the effects of various nucleotides and possible cofactors on binding of AB to σ^F. ATP and its non-hydrolysable analogues strongly stimulated the binding of SpoIIAB to σ^F. Weak binding was also detected with ADP, but other nucleotides, such as AMP, CTP, GTP etc, had little or no effect (Alper *et al.* 1994).

(*b*) *The SpoIIAA anti-anti-sigma factor*

The first of the three genes in the *spoIIA* operon, *spoIIAA*, was known from genetic analysis also to be involved in the regulation of σ^F. The phenotypes of classically isolated mutations in this gene indicated that the SpoIIAA protein was a positive regulator of σ^F activation, which probably acted by antagonizing the action of the SpoIIAB anti-sigma factor (Schmidt *et al.* 1990; Partridge *et al.* 1991). Initial experiments with purified SpoIIAA were disappointing, in that addition of this protein to reactions containing σ^F and SpoIIAB did not result in the stimulation of transcription (Min *et al.* 1993). However, two interactions between SpoIIAA and SpoIIAB were subsequently discovered which play crucial roles in the regulation of σ^F.

(*c*) *SpoIIAB as a protein kinase*

Experiments to test SpoIIAB protein for kinase activity were prompted by the finding that it has significant amino acid sequence similarity to the C-terminal domains of several bacterial histidine protein kinases (HPKs). HPKs belong to a family of proteins sometimes called sensor proteins that control a variety of bacterial adaptive responses (Parkinson 1993). Typically, a membrane-bound HPK recognizes some form of environmental stimulus and autophosphorylates a conserved histidine residue. The phosphate group is then transferred to an aspartic acid residue of a cognate 'response regulator' protein. The phosphorylated form of the response regulator initiates an adaptive response, often by binding to DNA and stimulating, or inhibiting, the transcription of one or more genes. Studies of SpoIIAB showed that it did indeed have kinase activity; it rapidly and quantitatively transfers the gamma phosphate from ATP to SpoIIAA (Min *et al.* 1993; Diederich *et al.* 1994). Unexpectedly, the product, SpoIIAA-phosphate (IIAA-P) was shown by both genetic and biochemical methods to be phosphorylated on a single serine residue, serine-58 (Diederich *et al.* 1994; Najafi *et al.* 1995). So although SpoIIAB resembles, in part, the HPKs, and although it catalyses the transfer of the γ-phosphate group from ATP to a protein substrate, it differs from all other known members of this family in that phosphate transfer does not seem to involve histidine autophosphorylation (at least no stable intermediate has been detected) and the final destination of the phosphate group is a serine residue, rather than aspartate.

Phosphorylation of SpoIIAA is crucial in regulating σ^F activation. Replacement of S58 by an acidic residue, aspartate, blocks σ^F activity *in vivo*, whereas insertion of alanine, to render the protein non-phosphorylatable, gives a phenotype in which σ^F activity is released prematurely (Diederich *et al.* 1994). Phosphorylation of SpoIIAA results in a large conformational change in the protein, which prevents it from binding to SpoIIAB (Magnin *et al.* 1995).

(*d*) *Binding of SpoIIAA and SpoIIAB in the presence of ADP*

Alper *et al.* (1994) used protein–protein cross-linking to study the interactions between SpoIIAA and SpoIIAB and discovered that the proteins form a stable complex in the presence of ADP. This complex has also been studied in detail by native gel electrophoresis and chromatography (Diederich *et al.* 1994; Magnin *et al.* 1995). Surprisingly, an interaction was not detected by cross-linking in the presence of ATP, despite the fact that the two proteins are known to come together during the phosphorylation reaction. Most likely this is because the phosphorylation and release of SpoIIAA by SpoIIAB is rapid, and SpoIIAA-P does not bind to SpoIIAB, even in the presence of ADP (Alper *et al.* 1994; Diederich *et al.* 1994; Magnin *et al.* 1995).

In summary, ATP and ADP were found to have strongly contrasting effects on the interactions between SpoIIAA, SpoIIAB and σ^F. In the presence of ATP, SpoIIAB rapidly and quantitatively phosphorylates SpoIIAA, thereby inactivating it, and binding of SpoIIAB to σ^F is strongly stimulated. Both effects serve to promote the inhibition of σ^F activity. In the presence of ADP, however, SpoIIAA binds tightly to SpoIIAB, and the interaction between SpoIIAB and σ^F is weak. Both of these effects tend to lead to the release of σ^F activity.

5. THE BIFUNCTIONAL SPOIIE PROTEIN

Discovery of the contrasting effects of ATP and ADP on σ^F activation *in vitro* led to the idea that a change in the ratio of ATP to ADP might be the physiological signal triggering the release of σ^F in the prespore (Alper *et al.* 1994; Diederich *et al.* 1994). Support for this idea came from experiments in which the *spoIIA* operon was artificially induced in vegetative cells of *B. subtilis* and the cell's energy charge was depressed by treatment with sublethal concentrations of an uncoupler of oxidative phosphorylation (Alper *et al.* 1994). However, three lines of evidence suggest that an additional factor might be needed for σ^F activation during sporulation. First, in the uncoupler experiments the induction of σ^F activity was weak and occurred only after a delay of several hours. This could be due to the absence of an important regulatory factor from vegetative cells. Second, the S58D mutation in SpoIIAA, which inserts an acidic residue at the site of the phosphorylatable serine residue in SpoIIAA, was found to behave as a dominant negative mutation, suggesting that this 'permenantly phosphorylated' form of SpoIIAA interacts with a factor needed to release σ^F activity (J. F. Wilkinson & J. Errington,

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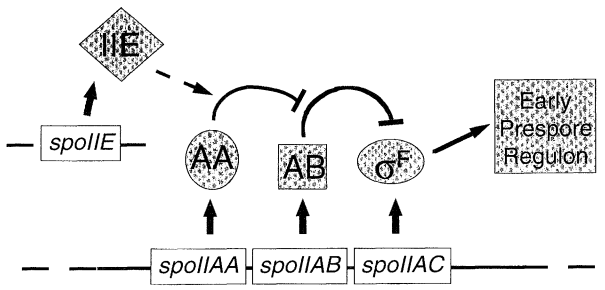

Figure 2. Genes involved in the regulation of σ^F activity, and their inter-relations. Four genes have been implicated in the regulation of σ^F activity, as indicated by the italic names in boxes. Three of them are coordinately regulated in an operon, called the *spoIIA* operon. The fourth gene, *spoIIE* is in an unlinked monocistronic locus. The SpoIIAB protein acts as an inhibitor, or anti-sigma factor of σ^F, as indicated by the curved line ending in a bar. SpoIIAA behaves as an antagonist of SpoIIAB, as indicated similarly. *spoIIE* mutations behave similarly to *spoIIAA*, so this gene product could either positively regulate SpoIIAA (as shown) or it might act separately as an antagonist of SpoIIAB.

unpublished results). Third, the phosphorylation of SpoIIAA by SpoIIAB in the presence of ATP is a very rapid and efficient reaction, so even with a substantial rise in ADP levels in the cell, a means of obtaining non-phosphorylated SpoIIAA might be needed (Diederich *et al.* 1994).

The *spoIIE* gene was the third location of mutations affecting σ^F activation (Margolis *et al.* 1991; Partridge *et al.* 1991).These mutations behave like the classical *spoIIAA* mutations, execpt that they seem to produce an additional morphological defect; their septa, though positioned correctly, are much thicker than normal spore septa, being more reminiscent of the septa of vegetative cells (Illing & Errington 1991). We have recently discovered, by the application of newly developed light microscopic methods, that some *spoIIE* mutants actually have a substantially reduced rate of asymmetric septation, with a delay of at least 20 min in septum completion (A. Feucht & J. Errington, unpublished results). The initial block in septation is eventually overcome in many of the cells but only thick, ultrastructurally abnormal septa are formed. The SpoIIE protein thus has an important role in the initiation of septation and is required for correct separation of the prespore and mother cell compartments. Because the number of septa eventually formed is substantial (about 50 % of the number formed by the wild type), the fact that the block in σ^F activity is complete could be due to SpoIIE having a second, more direct role in the regulation of σ^F.

Evidence suggesting a direct role for SpoIIE has come from the finding of protein sequence similarity between the C-terminal half of SpoIIE and proteins implicated in the regulation of another *B. subtilis* σ factor, σ^B. σ^B is closely related in sequence and promoter recognition to σ^F, and it lies in an operon preceded by homologues of SpoIIAB (RsbW) and SpoIIAA (RsbV) (Kalman *et al.* 1990). Genetic and biochemical experiments indicate that σ^B is regulated in a manner very similar to that of σ^F (Benson & Haldenwang 1992, 1993; Boylan *et al.* 1992; Dufour & Haldenwang 1994). However, the σ^B operon is more complex, with a total of eight coding sequences (Wise

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          1                                                          60
SpoIIEBs  ILVKAEQHSP HPNGYSHVAF GSTKSYRVST GAAHAAKGGG LVSGDSYSMM ELGARKYAAA
SpoIIEBm  IVVKNEECAK YPQGYCHVSF GCTKAYVVDT GVAHAAKGGG FVSGDSYSMI ELNAGKYALA
RsbU      EKSEIEIAAN VQQTLLGTKV PQEEALDIGA ISVPAKQ... .MSGDYYH.F VKDKESINIA
Icfg      ELEQGRRVQQ IQQNFLPISL PDLQQWQINA VFEPARS... .VSGDFYDAF LLGDDYLAMS
RsbX      .......... ........MI QVEENEHIQT LVYQLNKEGK SICGDSF.FM KADDKELICA

          61                                                        120
SpoIIEBs  ISDGMGNGAR AHFESNETIK LLEKILESGI DEKIAIKTIN .......... ..........
SpoIIEBm  ISDGMGNGER AHYESSETLQ LLKQILQTGI EETIAIKSIN .......... ..........
RsbU      IADVIGKGIP AALCMSMIKY AMDSLPETGI HPSQVLKNLN .......... ..RVVEQNVD
Icfg      IGDVCDKGVG AAMFMGLFRS LLRVFSGETM PGDTCIRDVN YKCSANDGNG KKKVIVQFLN
RsbX      VADGLGSGSL ANESSAAIKD LVENYASEDV ESIIERCNQA MKNKRG.... ..........

          121                                                       180
SpoIIEBs  .......SIL SLRTTDEIYS TLDLSIIDLQ DASCKFLKVG STPSFI..KR GDQVMKVQAS
SpoIIEBm  .......SIL SLRTNDEIFS TLDLAMIDLQ DANVNFLKIG STPSFI..KR GDKVIKIQAS
RsbU      A......... ......SMFI TMFYANYNMD KHQFTYASAG HEPGFYYSQK DNTFYDLEAK
Icfg      AVRLTNDYIA TEHGDMAMFA TLFFGVIDIS NGNLSYINAG HEPVFIL.NS EGIKHRLKST
RsbX      .......... .........A TASILKINFE QRQFTYCSVG NVRFILHSPS GESFYPLPIS

          181                                                       240
SpoIIEBs  NLPIGIINEF DVEVVSEQLK AGDLLIMMSD G..IFEGPKH VENHDLW..M KRKMKGLKTN
SpoIIEBm  NLPMGIIEEF EVDVVNEQMK AEDLLIMMSD G..VFEGPKH VENYEMW..M KRKIGELQTN
RsbU      GLVLGISQDY DYKQFDQHLE KGDMIVLFSD G..VTECR.T ENGFLERPDL QKLIEEHMCS
Icfg      GPAVGMMPNS TFTIDSLKID PGEMLIGYTD G..VIDARSP TKEFFGRQRL METLTANF.S
RsbX      GYLSGKPQKY ..KTHTATYE KGSKFIIHTD GLNVPDIRSH LKKGQSVEEI SNSLKMYTTS

          241                                                       296
SpoIIEBs  DPQEIADLLM EEVIRTRSGQ IE.DDMTVVV VRIDHNTPKW ASIPVPAIFQ NKQEIS
SpoIIEBm  DPQEIADLIM EEVIRTKVGL IE.DDMTVVV AKLQHNTPKW SSIPSYAYRN LAQ...
RsbU      SAQEMVKNIY DSLLKLQDFQ LQ.DDFTLIV LRRKV..... .......... ......
Icfg      AKTEILDIIK QELIGHIDGS IQFDDITMIA VYRN...... .......... ......
RsbX      RKDDLTYILG QLS..... .......... .......... .......... ......
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Figure 3. Similarities between the C-terminal domain of SpoIIE and proteins from sequence databases. The sequence alignments were generated by the programme PILEUP (Wisconsin Package, version 8.0, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin 53711, U.S.A.). Sequence accession numbers were as follows: SpoIIEBs, *B. subtilis* SpoIIE protein, Swiss Prot SP2E BACSU, residues 566–827; SpoIIEBm, *B. megaterium* SpoIIE protein, NCBI gi 857491, residues 326–585; RsbU, *B. subtilis* RsbU protein, Swiss Prot RSBU BACSU residues 97–335; RsbX, *B. subtilis* RsbX protein, Swiss Prot SIBX BACSO residues 1–199; Icfg, *Synechoccus* ICFG protein, Swiss Prot ICFG SYNY3, residues 373–638.

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& Price 1995). Two of these appear to be further homologues of SpoIIAA (RsbS) and SpoIIAB (RsbT). RsbR is also similar to SpoIIAA, though it is longer, with a non-homologous N-terminal domain. The remaining two coding sequences, RsbU and RsbX, are also related to each other, and genetic experiments implicate both in the complex regulatory network controlling σ^B activity (Boylan *et al.* 1992; Voelker *et al.* 1995; Wise & Price 1995).

We were surprised to find that RsbU and to a lesser extent, RsbX, show highly significant similarity to the C-terminal part of SpoIIE (figure 3). As RsbU appears to be a regulator of σ^B activity but is not likely to be responsive to a morphological event such as septation, it seemed possible that the C-terminal domain of SpoIIE, related to RsbU, might have a direct regulatory role in σ^F activation. We have recently isolated two new phenotypic classes of *spoIIE* mutant, which reinforce this view (A. Feucht & J. Errington, unpublished results). In the first class there is a defect in septation, characteristic of *spoIIE* mutants, but no significant reduction in σ^F activity. In the second class, σ^F activity is blocked but septation is almost normal. The isolation of mutations conferring these contrasting phenotypic effects strongly supports the view that SpoIIE is a bifunctional protein: one function is morphogenic, required for proper formation of the prespore compartment; the other is regulatory, involved in the compartmentalized release of σ^F activity. Although there are several possible ways in which the regulatory function of SpoIIE might be mediated, the possibility that it is a phosphatase acting to generate non-phosphorylated SpoIIAA would be compatible with the *in vitro* and *in vivo* experiments described above. Consistent with this notion, preliminary experiments indicate that the *spoIIE* mutations that block σ^F activation do so by preventing the accumulation of non-phosphorylated SpoIIAA (T. Magnin, A. Feucht, M. D. Yudkin & J. Errington, unpublished results).

6. A MODEL FOR REGULATION OF σ^F ACTIVITY

Figure 4 shows a model for the release of σ^F activity during sporulation, taking into account our current understanding of the functions of the various proteins operating in the hierarchy.

In the metabolically active vegetative cell and mother cell types, where σ^F is inactive, ATP is relatively abundant. Under such conditions, the phosphorylation and inactivation of SpoIIAA by SpoIIAB is probably rapid and efficient. SpoIIAB would then be free to bind to and inhibit σ^F. In the prespore, two factors might control the release of σ^F: first, activation of the SpoIIE phosphatase would increase the supply of non-phosphorylated SpoIIAA; second, a rise in the ADP concentration would allow the non-phosphorylated SpoIIAA to bind SpoIIAB, thereby freeing σ^F from the anti-sigma factor. Detection of a change in the energy charge of the prespore, relative to that of the mother cell, has proved to be technically difficult. Fortunately, it may be more straightforward to dissect the molecular basis for the postulated localization of SpoIIE activity.

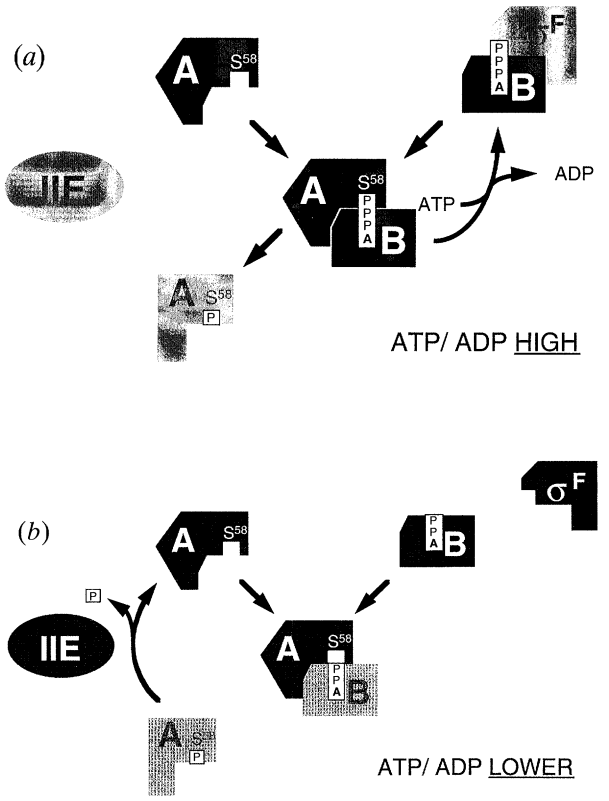

Figure 4. Model for the regulation of σ^F activity. (*a*) The inhibitory state operating in the predivisional cell and the mother cell. SpoIIE (IIE), SpoIIAA (A), SpoIIAB (B) and σ^F are shown as boxes, which are dark with white lettering if the protein is active and pale with grey lettering if inactive. In the presence of abundant ATP, SpoIIAA and SpoIIAB associate and the γ-phosphate of ATP is transferred to serine 58 (S58) of SpoIIAA. The complex dissociates to release SpoIIAA-P, which is inactive and has undergone a conformational change. The SpoIIAB is now free to bind to and inhibit σ^F. Presumably, this involves exchange of ADP for ATP, though this has not been demonstrated. (*b*) The release of ^{F} activity in the prespore. It is postulated that the release of σ^F activity involves a reduction in the energy charge of the cell and/or activation of the SpoIIE phosphatase. SpoIIE drives the dephosphorylation of SpoIIAA to provide a source of non-phosphorylated SpoIIAA, which, in the presence of ADP, binds tightly to SpoIIAB, thus freeing σ^F.

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Note added in proof (*February 1996*):
Since this paper was submitted, several papers have shed new light on the role of the SpoIIE protein. Duncan *et al.* (*Science, Wash.* **270**, 641–644) have shown that the C-terminal domain of SpoIIE is a specific phosphatase that can dephosphorylate SpoIIAA-P *in vitro*. Two groups have provided evidence that SpoIIE acts to dephosphorylate SpoIIAA-P *in vivo* (Feucht *et al. Genes Dev.* 1996; Duncan *et al. Proc. natn. Acad. Sci. U.S.A.* 1996, both in press). Arigoni *et al.* (*Science, Wash.* **270**, 637–640) have shown that SpoIIE is targetted to the sites of asymmetric septation, in accordance with its postulated role in septation.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES