Regulation of Stage I1 of Sporulation in *Bacillus subtilis*

By SANDRA CLARKE* AND JOEL MANDELSTAM

Microbiology Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK

(Received 17 December 1986; revised 19 March 1987)

A mutation, spo-87, in the spoOJ locus of *Bacillus subtilis* allows appreciable transcription of spoIIA, spoIID and spoIIG and later operons, even though most of the cells are morphologically blocked at stage 0 and the incidence of heat-resistant spores is about 1 per 10^4 cells. This mutation therefore appears to disengage the genetic control of sporulation from the morphological changes to which it should be connected. spoIIA and spoIIG are transcribed independently of one another. However, the products of both operons are needed for the activation of spoIID which occurs later. This indicates a convergence of parallel pathways of operon expression. We have also shown that nonsense mutations in spoZZAC (which codes for a sigma factor of RNA polymerase) prevent transcription of spoIID; by contrast, missense mutations in the same gene allow transcription of spoIID.

INTRODUCTION

Sporulation in bacilli is conventionally divided into six stages based on the appearance of the cells in thin section in the electron microscope (Ryter, 1965; Kay & Warren, 1968). The whole developmental process is controlled by a minimum of 50 genetic loci (Losick et al., 1986); the total number may exceed 60 (Hranueli et al., 1974).

Stage 0 and stage I represent the vegetative cell, and mutations in a number of genetic loci will prevent further development. These are designated spoOA, spoOB, etc., and it has recently become apparent that most of them, possibly all, are expressed during vegetative growth (Losick et al., 1986; Yamashita et al., 1986) and therefore can be disregarded for our purposes.

Sporulation from the developmental point of view can therefore be considered as beginning at stage II. This begins with the laying down of an asymmetric division septum consisting of a double membrane without appreciable cell wall material. The membrane grows so that the larger of the compartments in the cell engulfs the smaller, which then develops further to form the spore. The series of events between septation and engulfment is regulated by about six known genetic loci (designated spoIIA, spoIIB, etc.) and it includes some associated biochemical 'markers' of which the most easily measured is the formation of extracellular alkaline phosphatase (Waites et al., 1970; Glenn & Mandelstam, 1971). Mutations in any of the spoIIloci may either block development after septation or they may cause the formation of a variety of morphological malformations such as multiple septa, premature deposition of cell wall material, etc. These aberrant cell forms have been illustrated in a number of papers (see e.g. Ryter et al., 1966; Coote, 1972a; Young, 1976).

One of the loci, spoIIG, contains a gene coding for a protein of M, 31000 (Stragier et al., 1984) which is presumably processed by the protein product of another locus, spoIIE, to give a sigma factor for RNA polymerase, σ^29, specifically associated with sporulation (Trempy et al., 1985a,b).

Another of the loci associated with this stage is spoIIA. This has been cloned and sequenced (Fort & Piggot, 1984) and shown to consist of three genes, spoIIAA, spoIIAB and spoIIAC. From measurements of the size of the mRNA produced during sporulation, the three genes have been
shown to be expressed as an operon (Savva & Mandelstam, 1986). Earlier work (Errington & Mandelstam, 1983) had shown that mutations in the A and C genes of this operon led to different phenotypes. Some of them, lying in either of the genes, completely prevented sporulation (asporogenous) and also alkaline phosphatase formation, while others blocked spore formation incompletely (oligosporogenous) and allowed formation of phosphatase at about half the normal rate. Quite closely linked mutations within the same genes could produce either phenotype. In all, 17 mutations have been found which all lie in the A and C genes whereas no sporulation phenotype has so far been associated with the B gene (J. Errington, personal communication).

From the nucleotide sequence of the spoIIA operon it appeared that the C gene codes for a protein with a high degree of homology to a variety of sigma factors including the σ^{25} already referred to (Errington et al., 1985; Stragier et al., 1984). In view of a report by Trempy et al. (1985a) that expression of spoIIG depended on spoIIA26, and since we knew that both spoIIA and spoIIG were needed for expression of spoIID, the following provisional order of dependence of sporulation operons was proposed (Clarke et al., 1986):

\[ \text{spoIIA} \rightarrow [\text{spoIIG}, \text{spoIIE}] \rightarrow \text{spoIID} \rightarrow \]

The work described in the present paper was undertaken because we found that some mutations in spoIIA, as we have already mentioned, permitted significant expression of alkaline phosphatase. These mutations also allowed partial expression of the spoIID operon. Because of these results we decided to examine mutant spoIIA26 which Trempy et al. (1985a, b) had obtained from the Bacillus Stock Center as a strain carrying a mutation in spoIIA. On checking the mutant supplied both by the Center and, kindly, by Dr W. G. Haldenwang, we found that it behaved in all respects as a stage 0 mutant. This clearly cast doubt on our earlier conclusion that spoIIA preceded spoIIG in a linear sequence.

Our present results show that: (a) spoIIA and spoIIG are expressed independently of one another and that expression of both operons is necessary for the expression of spoIID; (b) a mutation in the spoIJ locus, spo-87, disengages the sequential induction of operons from morphological development.

**METHODS**

**Bacterial strains, phages and plasmids.** These are listed in Table 1.

**Induction of sporulation and measurement of marker events.** Cells were grown and induced to sporulate at 37°C by the method of Sterlini & Mandelstam (1969). Alkaline phosphatase was measured as described by Glenn & Mandelstam (1971) and modified by Errington & Mandelstam (1983). One unit of enzyme hydrolyses 1 nmol p-nitrophenyl phosphate in 1 min at 30°C. β-Galactosidase was assayed as described by Errington & Mandelstam (1986) or as modified by Clarke et al. (1986). One unit hydrolyses 1 nmol MUG (4-methylumbelliferyl β-D-galactoside) min^{-1}. Methods for electron microscopy were those of Kay & Warren (1968).

**DNA preparation.** Plasmid DNA was extracted from *Escherichia coli* by the alkaline lysis method (Maniatis et al., 1982). Phage φ105 DNA was prepared as described by Jenkinson & Mandelstam (1983). *Bacillus subtilis* chromosomal DNA for transformations was prepared according to Ward & Zahler (1973).

**DNA-RNA ‘Northern’ hybridizations.** The choice of probe and its construction were as follows. Plasmid pGSIIG11 (a gift from J. Szulmajster) contains a 1133 bp PstI fragment which codes for a 239 amino acid polypeptide and is able to transform spoIIG recipients to Spo^+ (Stragier et al., 1984). We constructed the plasmid pUC7IIG by subcloning this PstI fragment into the PstI site in plasmid pUC7 (Messing et al., 1981). Ligation, transformation and recombinant screening were as described previously (Clarke et al., 1986). The PstI fragment was then removed from plasmid pUC7IIG with EcoRI, which cleaved sites in the polylinker of pUC7, and cloned into phage D1:1t (Flock, 1977). Selection of phage φ105IIG from the bank of recombinant phages was essentially as described elsewhere (Jenkinson & Mandelstam, 1983; Errington, 1984). The resulting recombinant phage φ105IIG was able to transduce to Spo^+ strains containing mutations spoIIG41, spoIIG55 and spoIIG66. These results indicated that plasmid pUC7IIG would be an adequate probe for the detection of spoIIG mRNA.

RNA was isolated from spo mutants (Table 1) 2.5 h after resuspension in sporulation medium. Trempy et al. (1985b) had determined that this would be a time of maximum abundance of spoIIG message. Cultures (100 ml) were centrifuged for 3 min and the pellets placed on ice. Cells blocked at stage III or later were washed with 1 ml ice-cold lysozyme solution (10 mg ml^{-1} in 50 mm-Tris/HCl, pH 8), spun for 30 s in a microfuge, quickly resuspended in 0.5 ml lysozyme solution, frozen in liquid nitrogen and stored at -70°C. Stage 0 and stage II
### Table 1. Bacterial strains, phages and plasmids

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Genotype</th>
<th>Source/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis*</td>
<td>lys-l metC3 tal-1 spoIID::lacZ cat (φ105LD2)</td>
<td>Clarke et al. (1986)</td>
</tr>
<tr>
<td>SG46†</td>
<td>1.5 spoI1A1 trpC2</td>
<td>E1, Piggot (1973)</td>
</tr>
<tr>
<td></td>
<td>6.2 spoI1G6 metC3</td>
<td>E10, Piggot (1973)</td>
</tr>
<tr>
<td></td>
<td>69.1 spoI1A69 trpC2</td>
<td>NG18.6, Piggot (1973)</td>
</tr>
<tr>
<td></td>
<td>168‡ trpC2</td>
<td>Laboratory stock</td>
</tr>
<tr>
<td></td>
<td>560 spoI1A560 hisH2</td>
<td>Errington &amp; Mandelstam (1983)</td>
</tr>
<tr>
<td></td>
<td>562.5 spoI1A562 trpC2</td>
<td>Errington &amp; Mandelstam (1983)</td>
</tr>
<tr>
<td></td>
<td>563.3 spoI1A563 metC3</td>
<td>Errington &amp; Mandelstam (1983)</td>
</tr>
<tr>
<td></td>
<td>564.3 spoI1A564 metC3</td>
<td>Errington &amp; Mandelstam (1983)</td>
</tr>
<tr>
<td>MY2000.578</td>
<td>spoI1A578 hisH2 pyrD1</td>
<td>M. Yudkin†</td>
</tr>
<tr>
<td>609</td>
<td>spoI1A42 lys-l</td>
<td>J. Errington†</td>
</tr>
<tr>
<td>IS31</td>
<td>spoI1A312 spoI1A312 ade spo</td>
<td>BGSC§ strain 26U of Ionesco et al. (1970)</td>
</tr>
<tr>
<td>E. coli JM103</td>
<td>Δ(lac-pro) thi strA supE endA sbeB15 F' traD36 proAB lac* ZAM15</td>
<td>Messing et al. (1981)</td>
</tr>
<tr>
<td>Phages</td>
<td>Relevant properties</td>
<td>Reference</td>
</tr>
<tr>
<td>φ105LD2</td>
<td>spoIID*, 2 kbp MboI insert in φ105J9</td>
<td>Lopez-Diaz et al. (1986)</td>
</tr>
<tr>
<td>φ105D1:1t</td>
<td></td>
<td>Flock (1977)</td>
</tr>
<tr>
<td>φ105DS1</td>
<td>1-1 kbp FstI fragment in φ105D1:1t</td>
<td>This paper</td>
</tr>
<tr>
<td>φ105IG</td>
<td></td>
<td>Savva &amp; Mandelstam (1984)</td>
</tr>
<tr>
<td>Plasmids</td>
<td>Relevant properties</td>
<td>Reference</td>
</tr>
<tr>
<td>pUC7</td>
<td>Ap®</td>
<td>Messing et al. (1981)</td>
</tr>
<tr>
<td>pSG11G11</td>
<td>spoI1G clone</td>
<td>Stragier et al. (1984)</td>
</tr>
<tr>
<td>pUC711G</td>
<td>1-1 kbp FstI fragment in pUC7</td>
<td>This paper</td>
</tr>
</tbody>
</table>

* The other spo mutants used are listed in Errington & Mandelstam (1986).
† Strains 168 and SG46 are Spo+. The latter carries an intact copy of spoIID on the φ105LD2 prophage.
‡ Microbiology Unit, Department of Biochemistry, University of Oxford.
§ Bacillus Genetic Stock Center, The Ohio State University.

Mutants were washed with cold Tris/HCl only, since cells blocked early in sporulation lyse easily. Stored cells were thawed at room temperature and added to 3 ml guanidinium solution (6.0 M). Cells at stage III or later were refrozen in liquid nitrogen and thawed, before the addition of guanidinium, to facilitate breakage of the cells. The RNA was extracted by the guanidinium/caesium chloride method described by Maniatis et al. (1982) and electrophoresed through gels containing formaldehyde, also as described by Maniatis et al. (1982). The denatured RNA was transferred from the gels to nitrocellulose, the filters prepared and the hybridizations and autoradiography were carried out as described in ‘Membrane Transfer and Detection Methods’ (Amersham). The DNA probe was nick-translated and labelled with [32P]dCTP using a kit from Amersham.

Transduction and infection with phage φ105. This was done as described previously (Clarke et al., 1986).

Transfer of spo::lacZ fusions into spo mutants. Two methods were used.

(a) spoIID. Competent cells were transformed with DNA from strain SG46, which has a spoIID::lacZ transcriptional gene fusion on the chromosome. The resulting transformants would be double mutants since, in addition to the original spo mutation, the spoIID gene would now be disrupted by the insertion of lacZ. The mutant was then complemented by transduction with phage φ105LD2, which carries the complete spoIID transcription unit (Clarke et al., 1986), i.e. an intact spoIID gene is contained in the prophage and the phenotype is Spo+. The transformations, selection of transformants and complementation with phage φ105LD2 were done as described previously (Clarke et al., 1986).
(b) spoIIA and spoIIIC. Both gene fusions had been cloned into phage φ105 and were transferred by transduction as described by Errington & Mandelstam (1986) and Turner et al. (1986).

RESULTS

Effects of mutations in spo0J on morphological changes and on the expression of operons controlling stage II

The locus spo0J, which maps near the replication origin in B. subtilis (Trowsdale et al., 1979; Moriya et al., 1985), is defined by two mutations spo-87 and spo-93 (Hranueli et al., 1974). The recombination index (RI) between these is 0.39 (Hranueli et al., 1974). On the basis of work done in this laboratory and elsewhere (see e.g. Carlton, 1966; Coote, 1972; Errington & Mandelstam, 1984; Lamont & Mandelstam, 1984) this almost certainly indicates that the mutations lie in separate genes and possibly even in different operons. Certainly, the phenotypes of strains carrying these mutations are distinctly different.

Strain 93.2 is morphologically blocked at stage 0 (Hranueli et al., 1974) and it fails to ‘turn on’ spoIIA (Errington & Mandelstam, 1986), spoIID (Fig. 1) or alkaline phosphatase (Fig. 2). Because mutants carrying spo-93 thus appeared to be like other stage 0 mutants and also because we had difficulty in extracting mRNA from the cells, no serious attempt was made to investigate them more extensively.

Strain 87.2 is also morphologically a stage 0 mutant. Electron microscopic examination shows that, at t4, 3% of the cells have reached stage II and 6% have reached a malformed stage III, viz. the engulfed ‘prespore’ appears to be surrounded by a single instead of a double membrane; the remaining cells are blocked at stage 0. In both mutants, about 1 in 10000 cells makes a heat-resistant spore (J. Errington, personal communication). Although in strain 87.2 the overwhelming majority of the cells remain at stage 0, lacZ fusion experiments show that spoIIC and spoIIA are both turned on (results not shown) and so is spoIID (Fig. 1). Furthermore, ‘Northern’ hybridization gives a positive signal for spoIIG (Table 2). It is also of interest that the formation of alkaline phosphatase, a stage II marker event, begins at about t1 (Fig. 2), as it does in the wild-type, although the rate of formation is reduced slightly. Nevertheless, both in amount and in the ‘take-off point’ at about t1, the curve for strain 87.2 is clearly distinguishable from the negative result obtained with strain 93.2.

The finding that the expression of sporulation operons is strikingly different in strains carrying two different mutations, apparently in spo0J, will be considered later (Discussion).

Effects of mutations in spoIIA on spore formation, sporulation phenotype, the formation of alkaline phosphatase and the expression of spoIID

From the earlier work already referred to (Errington & Mandelstam, 1983) we knew that even closely linked mutations in the A and C genes of the spoIIA operon could vary in their effects on spore formation and on the occurrence of biochemical marker events linked to stage II. Since then, the nature of some of these mutations has been determined by sequencing experiments (Yudkin, 1987) and shown to be either missense or nonsense. This made it possible to attempt to make a more rational correlation between the mutations and their phenotypic effects.

We accordingly measured the formation of alkaline phosphatase and the expression of spoIID in spoIIA mutants carrying the spoIID::lac2 transcriptional gene fusion (see Methods).

The more interesting and more interpretable results were those obtained with strains carrying mutations in the C gene, which codes for a sigma factor (Errington et al., 1985). Three of the mutants (strains 1.5, 63.2 and MY2000.578; mutations 1, 63 and 578 in Table 3) carry nonsense mutations (Yudkin, 1987) and in each of them there was no production of alkaline phosphatase (Table 3) and no significant expression of mRNA for spoIID. A typical result (Fig. 3) showed that strain 63.2 exhibits the same slow hydrolysis of a β-galactosidase substrate in the control and in the constructed strain carrying a lacZ fusion. By contrast, strains (numbers 560, 563.3 and 564.3) carrying missense mutations (Yudkin, 1987) were oligosporogenous (Errington & Mandelstam, 1983) and produced alkaline phosphatase (Table 3). Thus far the results merely confirm those previously reported by Errington & Mandelstam (1983) but, more interestingly,
Stage II of B. subtilis sporulation

**Fig. 1.** Production of β-galactosidase when a spoIID::lacZ transcriptional fusion was transferred to the chromosomes of sporulation mutants 93.2 and 87.2 by transformation with DNA from strain SG46. This was followed by transduction with phage φ105LD2 to insert an intact copy of the spoIID gene. Cells (1 ml) in sporulation medium were assayed for β-galactosidase at hourly intervals from t₀. ▲, SG46, spoIID::lacZ control; △, 87.2 with spoIID::lacZ fusion; ○, 93.2 with spoIID::lacZ fusion.

**Fig. 2.** Production of alkaline phosphatase. Cell suspensions (1 ml) in sporulation medium were assayed at hourly intervals from t₀. ●, 168, wild-type control; △, 87.2; ○, 93.2.

**Table 2.** Synthesis of spoIIG mRNA in mutants carrying defects in different spo loci

The mRNA was detected by 'Northern' hybridization. RNA was isolated 2.5 h after the cells were transferred to sporulation medium and probed for spoIIG mRNA as described in Methods. Some of the hybridizations are shown in Fig. 4. +, mRNA present; −, mRNA absent; ND, not determined.

<table>
<thead>
<tr>
<th>Letter designation of mutated locus</th>
<th>Stage of block:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>−</td>
</tr>
<tr>
<td>B</td>
<td>−</td>
</tr>
<tr>
<td>C</td>
<td>−</td>
</tr>
<tr>
<td>D</td>
<td>−</td>
</tr>
<tr>
<td>E</td>
<td>−</td>
</tr>
<tr>
<td>F</td>
<td>−</td>
</tr>
<tr>
<td>G</td>
<td>−</td>
</tr>
<tr>
<td>H</td>
<td>−</td>
</tr>
<tr>
<td>J (spo-87)</td>
<td>+</td>
</tr>
<tr>
<td>K (spo-93)</td>
<td>ND</td>
</tr>
</tbody>
</table>
Fig. 3. β-Galactosidase production when a spoIID::lacZ transcriptional fusion was transferred to the chromosomes of strains carrying spoIIA mutations. This was followed by transduction with phage φ105LD2 to insert an intact copy of the spoIID gene. Cells (1 ml) in sporulation medium were assayed at hourly intervals from t₀. ▲, SG46, spoIID::lacZ control; ○, 63.2 control; △, 63.2 with spoIID::lacZ; ●, 562.5 with spoIID::lacZ; □, 563.3 with spoIID::lacZ.

Table 3. Effects of mutations in the spoIIA operon

Alkaline phosphatase and the expression of spoIID (see also Fig. 3) were measured in spoIIA mutants carrying a spoIID::lacZ transcriptional gene fusion and an intact copy of the spoIID gene in the prophage of φ105LD2.

<table>
<thead>
<tr>
<th>Mutation (and type*)</th>
<th>Alkaline phosphatase</th>
<th>Expression of spoIID</th>
<th>Sporulation phenotype†</th>
</tr>
</thead>
<tbody>
<tr>
<td>spoIIA42 (missense)</td>
<td>—</td>
<td>—</td>
<td>Osp</td>
</tr>
<tr>
<td>spoIIA69 (missense)</td>
<td>—</td>
<td>—</td>
<td>Spo⁻</td>
</tr>
<tr>
<td>spoIIA562 (unknown)</td>
<td>+</td>
<td>+</td>
<td>Osp</td>
</tr>
<tr>
<td>spoIIA563 (nonsense)</td>
<td>—</td>
<td>—</td>
<td>Spo⁻</td>
</tr>
<tr>
<td>spoIIA560 (missense)</td>
<td>+</td>
<td>+</td>
<td>Osp</td>
</tr>
<tr>
<td>spoIIA563 (missense)</td>
<td>+</td>
<td>+</td>
<td>Osp</td>
</tr>
<tr>
<td>spoIIA564 (missense)</td>
<td>+</td>
<td>+</td>
<td>Osp</td>
</tr>
<tr>
<td>spoIIA578 (missense)</td>
<td>—</td>
<td>—</td>
<td>Spo⁻</td>
</tr>
</tbody>
</table>

* See Yudkin et al. (1985); Yudkin (1987).
† See Errington & Mandelstam (1983).

the missense mutations also allow expression of spoIID. The result for one of the mutants (563.3, Fig. 3) resembles that obtained with the Spo⁺ control in that there is a sharp rise in the production of mRNA for spoIID at about t₁ in contrast to what is found in the nonsense mutant, strain 63.2.

Our observations on the effects of mutations in the A gene of spoIIA have been less extensive, and results have so far been obtained with only three mutants (Table 3). These show that interpretation may prove to be more difficult. Thus, strain 562.5 is oligosporogenous and it is positive for the expression of both alkaline phosphatase (Table 3) and spoIID (Fig. 3, Table 3). However, strains 42.2 and 69.1, which both carry missense mutations (Yudkin et al., 1985), differ in phenotype. Neither strain produces alkaline phosphatase or mRNA for spoIID but one of them is oligosporogenous while the other is asporogenous. Even with this limited analysis it is
clear that the phenotypes of strains carrying missense and nonsense mutations in the A gene will cut across the fairly clear distinction between phenotypes resulting from corresponding changes in the C gene.

**Effect of mutation spoIIA26 on stage II events**

The strain containing this mutation, originally designated 26U by Ionesco *et al.* (1970), and linked by transduction with phage PBS1 to *lys-1*, was classified as a spoIIA mutant and deposited as such in the Bacillus Stock Center (strain IS31). It was then used by Trempy *et al.* (1985a, b), who found that it prevented the expression of spoIIG. It thus appeared that spoIIA was necessary for the expression of spoIIG and the provisional order of expression of stage II operons deduced by us and given in the Introduction was based on this finding.

It was obviously of interest to know more of the phenotypic characteristics of strain IS31 and to compare them with those of the spoIIA mutants described in Table 3. However, examination of thin sections of the mutant in the electron microscope showed that it is, in fact, blocked at stage 0. In addition, phage 4105DS1, which contains the entire spoIIA locus (Savva & Mandelstam, 1984), was unable to transduce strain IS31 to Spo+. Thus, morphologically strain IS31 is clearly a stage 0 mutant and we have not attempted to look at its properties more closely. This result alters the order of spore gene expression proposed by Turner *et al.* (1986) and Clarke *et al.* (1986) (see Discussion).

**Dependence pattern of spoIIG**

Once our experiments had shown that the apparent dependence of spoIIG on spoIIA was an invalid conclusion based upon the mis-classification of strain IS31 it became necessary to establish the dependence relationships of spoIIG. This was done as follows by DNA–RNA hybridization ('Northern' blotting).

RNA was isolated from several strains (numbers MY2000.578, 69.1, 42.2, 562.5 and 563.3) carrying spoIIA mutations. All these strains gave positive signals in 'Northern' hybridization with plasmid pUC7IIG as a probe.

Since the spoIIG gene (Stragier *et al.*, 1984) and the spoIAC gene (Errington *et al.*, 1985) code for sigma factors with extensive regions of homology we had to check the possibility that a spoIIG probe might hybridize to the mRNA for spoIAC. The experiments included two control strains: one of these, strain 63.2, has a nonsense mutation at the beginning of the spoIAC gene (Yudkin, 1987) while strain 609 has a deletion covering the entire spoIIA locus (J. Errington, personal communication). RNA from both strains gave positive signals when hybridized to the spoIIG probe (Fig. 4). It was thus clear that mRNA for spoIIG was indeed synthesized in mutant strains that were unable to generate a functional mRNA corresponding to spoIAC.

Some examples of the results of 'Northern' hybridization experiments are shown in Fig. 4. Strains carrying mutations spoOA43 and spoIIG55 were used as negative and positive controls. Although there was some smearing of the signals, presumably as a result of degradation of mRNA, the distinction between positive and negative strains was easily made.

The expression of spoIIG is a variety of other sporulation mutants was then investigated (see Table 2). Expression of the message is dependent upon all the stage 0 loci with the exception, already mentioned, of one of the spoOJ mutations (spo-87). It is apparently not dependent on any other sporulation operon and it is even expressed in strains carrying deletions of spoIIA (see previous paragraph) as well as in other strains carrying missense mutations in spoIIA.

**DISCUSSION**

**Dissociation of morphological changes from the expression of sporulation operons by a mutation in spoOJ**

Most of the stage 0 operons are not only expressed during vegetative growth, but mutations in them affect a number of apparently unconnected physiological properties of the cells such as competence in genetic transformation (Spizizen, 1965) and susceptibility to infection by bacteriophages (Ito & Spizizen, 1972), etc., and cause a total block which affects sporulation
Fig. 4. 'Northern' hybridization analysis showing the presence of spoIIG mRNA in spoIIA mutants. Each lane contains about 8 µg RNA, extracted 2.5 h after cells were transferred to sporulation medium, and probed with plasmid pUC7IIG. RNA from strains 55.2 and 43.2 was used as positive and negative control, respectively. Lanes: 1, 63.2 spoIAC63; 2, 562.5 spoIIA562; 3, 609 AspoIIA; 4, 43.2 spoOA43; 5, 55.2 spoIIG55; 6, 563.3 spoIAC563; 7, DNA size markers. Plasmid pUC7IIG was cut with PstI. The bottom band is the 1.13 kbp insert which contains the spoIIG gene.

both morphologically and in the expression of sporulation-specific operons (see for example Errington & Mandelstam, 1986; Clarke et al., 1986; Turner et al., 1986; Savva & Mandelstam, 1986). It was therefore proposed (Clarke et al., 1986) that, in general, the stage 0 operons could be excluded from a consideration of the developmental stages leading to spore formation. Instead, they could be regarded as affecting the general make-up of the cell so that it is changed in a number of diverse properties which include, among other things, the ability to form an asymmetric septum and the ability to initiate the dependent sequence of operon expression that controls the development of the spore. The exception appears to be spoO1, which is defined by two mutations, spo-87 and spo-93. These, by genetic mapping, are so far apart that they are almost certainly in separate genes (see above).

Mutation spo-87 is of interest because in the majority of cells carrying it (>90%) sporulation is blocked morphologically at stage 0. It was therefore curious that Trempy et al. (1985a) and Rong et al. (1986) should respectively have found that spoIIG and spoIID were transcribed in strains carrying spo-87.

The findings presented here show that this mutation also allows the expression of another operon specifically concerned with stage II, viz. spoIIA. It also allows the formation of alkaline phosphatase, which is characteristically associated with stage II (Fig. 2), the expression of spoIIIC, and also the synthesis of dipicolinic acid (J. Errington, personal communication), which in normal development is a stage V event. It is thus apparent that in strain 87.2 the expression of the dependent sequence of sporulation operons has been set in train but that it is dissociated from the morphological changes that should accompany it.

Independent expression of two operons, spoIIA and spoIIG, coding for sigma factors

The analysis of mutants carrying partial or total deletions of spoIAC (see Results) shows that they can nevertheless express spoIIG. Conversely, mutations in spoIIG do not prevent expression
Fig. 5. Tentative scheme illustrating the order of expression of operons concerned in stage II of sporulation. In general, mutations in any of the stage 0 operons prevent morphological changes and the transcription of stage II operons. The exception is a mutation in the spo0J locus which blocks septum formation, but allows transcription of sporulation operons to be set in train (see text). spoIIA codes for a σ factor; spoIIG codes for a 'pre-σ factor' which is presumed to be converted to the active protein, σ^{28}, by the intervention of the protein product of spoIIE. The genes coding for σ factors thus seem to be turned on independently and the products of both are needed for the transcription of spoIID and of 'later' operons (see text). Brackets indicate that the order of operon expression has not yet been resolved and question marks indicate the possible intervention of undiscovered stage II operons.

of spoIIA (Savva & Mandelstam, 1986; Errington & Mandelstam, 1986). We are therefore left with the curious finding that two genes coding for very closely homologous σ factors (both with M_{r} values of about 29000) are expressed independently at the very outset of induction of sporulation.

The two genes, or their protein products, have further properties in common. (a) Their expression is dependent on an intact set of stage 0 genes, but (b) the expression of both – and that of 'later' operons in the dependent sequence – occurs in a strain carrying spo-87. (c) The protein products of both genes are needed for the expression of spoIID (Clarke et al., 1986).

In molecular terms it is difficult to conceive that the transcription of spoIID depends on the simultaneous presence of two RNA polymerases containing slightly different σ factors. Instead, it is more likely that one or both of these loci promotes the expression of stage II operons which are as yet unrecognized and that the products of these play a part in the transcription of spoIID. The latter has the characteristic properties of a DNA-binding protein and presumably it plays a regulatory role (Holland et al., 1987). This would in turn lead to the transcription of operons that regulate the later stages of sporulation (Turner et al., 1986).

In summary, we offer a tentative scheme for the expression of the operons regulating stage II of sporulation (Fig. 5).

This work was supported by the Science and Engineering Research Council.

REFERENCES


