MOLECULAR BIOLOGY OF MICROBIAL DIFFERENTIATION

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Molecular Biology of Microbial Differentiation

Proceedings of the Ninth International Spore Conference, Asilomar, California, 3-6 September 1984

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American Society for Microbiology
Washington, D.C. 1985

Library of Congress Cataloging in Publication Data

International Spores Conference (9th: 1984: Asilomar, Calif.)
Molecular biology of microbial differentiation.

Includes indexes.
1. Microbial differentiation—Congresses. 2. Bacillus subtilis—Congresses. 3. Bacteria, Sporeforming—Congresses. 4. Molecular biology—Congresses. 5. Bacteria—Physiology—Congresses. I. Hoch, James A. II. Setlow, Peter. III. Title.
QR73.5.I58 1984 576'.13 85-1413

ISBN 0-914826-75-1

Preface

This book contains contributions from the participants at the Ninth International Spores Conference, held at the Asilomar Conference Center in Pacific Grove, Calif., 3–6 September 1984. The proceedings were organized by a program committee consisting of Roy Doi and Richard Losick, Co-Chairmen, with James Hoch, Peter Setlow, Issar Smith, and Linc Sonenshein. This meeting differed from several of those in the past in that the lectures were mainly concentrated in the area of the molecular biology of the sporulation and germination processes. The decision to focus the contents of the lectures was based upon the very rapid progress being made in this area of study of bacterial development. This book reflects the excellence of the presentations in this interesting and exciting area of microbial differentiation.

The meeting was made possible through the generous support of the National Institutes of Health, The National Science Foundation, and the U.S. Army Office of Research. Generous support from the following corporations was important to the success of this meeting: Abbott Laboratories of North Chicago, Bayer AG/Miles, Campbell's Soup, Cetus Corp., Dow Chemical Co., E. I. Du Pont de Nemours & Co., Ethicon, Gist-Brocades nv, Hoffmann-La Roche Inc., Merck Sharp & Dohme Research Laboratories, Monsanto, R. J. Reynolds, and Syntro Corp. We are especially grateful for this industrial support as it permitted many scientists with little federal support to participate and also made possible publication of this book.

Since the last meeting of two of our colleagues have passed away. Elizabeth B. Freese was a dedicated worker in the sporulation field and published on many aspects of the biochemistry of sporulation. Hans J. Rhaese was a provocative investigator in the area of nucleotide control of sporulation. We mourn the passing of these two friends.

The editors of this volume would like to express their gratitude to the authors submitting manuscripts to this book. Not only were the manuscripts of excellent quality and submitted (mostly) on time, but also they required very little editing on our part. This is the easiest editorial job either of us has ever had.

We also thank, on behalf of the meeting participants and spectators, the unselfish efforts of Roy H. Doi in the preparation for and execution of the meeting. His hard work and organizational talents were reflected in the excellence of the meeting.

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Contents

CONTROL OF DEVELOPMENTAL TRANSCRIPTION

METABOLIC CONTROL OF GENE EXPRESSION AND SPORULATION

ENDOTOXIN FORMATION IN SPORULATING ORGANISMS

MICROBIAL DIFFERENTIATION SYSTEMS

Molecular Cloning and Genetics of Sporulation and Germination Genes

Genes Controlling Development in *Bacillus subtilis*†

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It is clear that one of the driving forces behind the study of the genetics of *Bacillus subtilis* is the quest to understand the simple differentiation cycle of sporulation and germination that this organism undergoes. The study of this process has resulted in the accumulation of vast amounts of knowledge of the physiology and genetics of postexponential-phase bacteria. It is this knowledge that will certainly be applicable to an understanding of microbial differentiation in various forms of procaryotes and eucaryotes and, it is hoped, will lend insights into the unique genetic mechanisms occurring during this phase of the life cycle.

Although it has only been a few years since the last compilation of a genetic map of this sort (25, 26), there have been substantial additions of new markers to the genetic map. This has resulted not only from a continuation of classical genetic studies using DNA-mediated transformation and transduction methods, but also from a minor explosion of molecular cloning of genes of interest in this organism. Several libraries of *B. subtilis* DNA have been constructed in bacteriophage λ (14), cosmid (4), and other vectors (32, 62), and these libraries have been a rich source of fragments of the chromosome carrying genes of interest. One of the more useful genetic tools used to study the genetic location of cloned genes is the so-called integrative vector (15). These vectors lack an origin of replication for *B. subtilis* and therefore cannot replicate in this organism but can integrate into the chromosome of the organism if a suitable region of homology, e.g., a cloned region of the genome, is cloned within the vector. In most cases such vectors include a chloramphenicol resistance gene which can be expressed in *B. subtilis* when integrated into the chromosome. In addition, all constructions in the vector are done in a permissive host such as *Escherichia coli*. Mapping of the location of the integrated plasmid allows one to determine the genetic location of the cloned gene of interest. Such methods have been particularly useful for genes that would have no obvious phenotype if mutated and for those genes whose genetics is not readily determined. In any case, the existence of integrative vectors provides a link between cloning and genetics of this organism.

Another powerful tool which will result in a rapid expansion of knowledge about the genetics of this organism is the transposon Tn*917*. The methodology for the use of this transposon to insert into the chromosome of *B. subtilis* has been painstakingly worked out by P. Youngman and associates. This system is described in detail in another paper (Youngman et al., this volume) and will not be described here. It is clear that transposon-mediated mutagenesis and transcriptional coupling will play a powerful role in our understanding of the mechanisms of gene expression in *B. subtilis*. Thus, it seems likely that within several years the density of genetic markers on the map as we presently know it should at least double or perhaps triple. Much of this will be the result of the discovery, in cloned DNA, of open reading frames of interest that can now be identified easily and manipulated. One may also speculate that within this time frame a significant portion of the genome will have been identified in cloned form and we should know substantially more about the molecular genetics of this organism.

In this article we have attempted to compile the known sporulation mutations, their genetic map position, information as to whether they have been cloned, and the number of complementation groups or open reading frames within each cloned fragment. A genetic map of *B. subtilis* showing both auxotrophic and developmental markers is presented in Fig. 1. A number of the markers on the map have not been compiled previously, and only those related to development are documented here. All of the developmental loci are inside the circle; the auxotrophic and other loci are outside the circle. The location of the origin is most likely to be very close to the *rrn0* operon, although its exact

† Publication no. 3784-BCR from the Research Institute of Scripps Clinic.

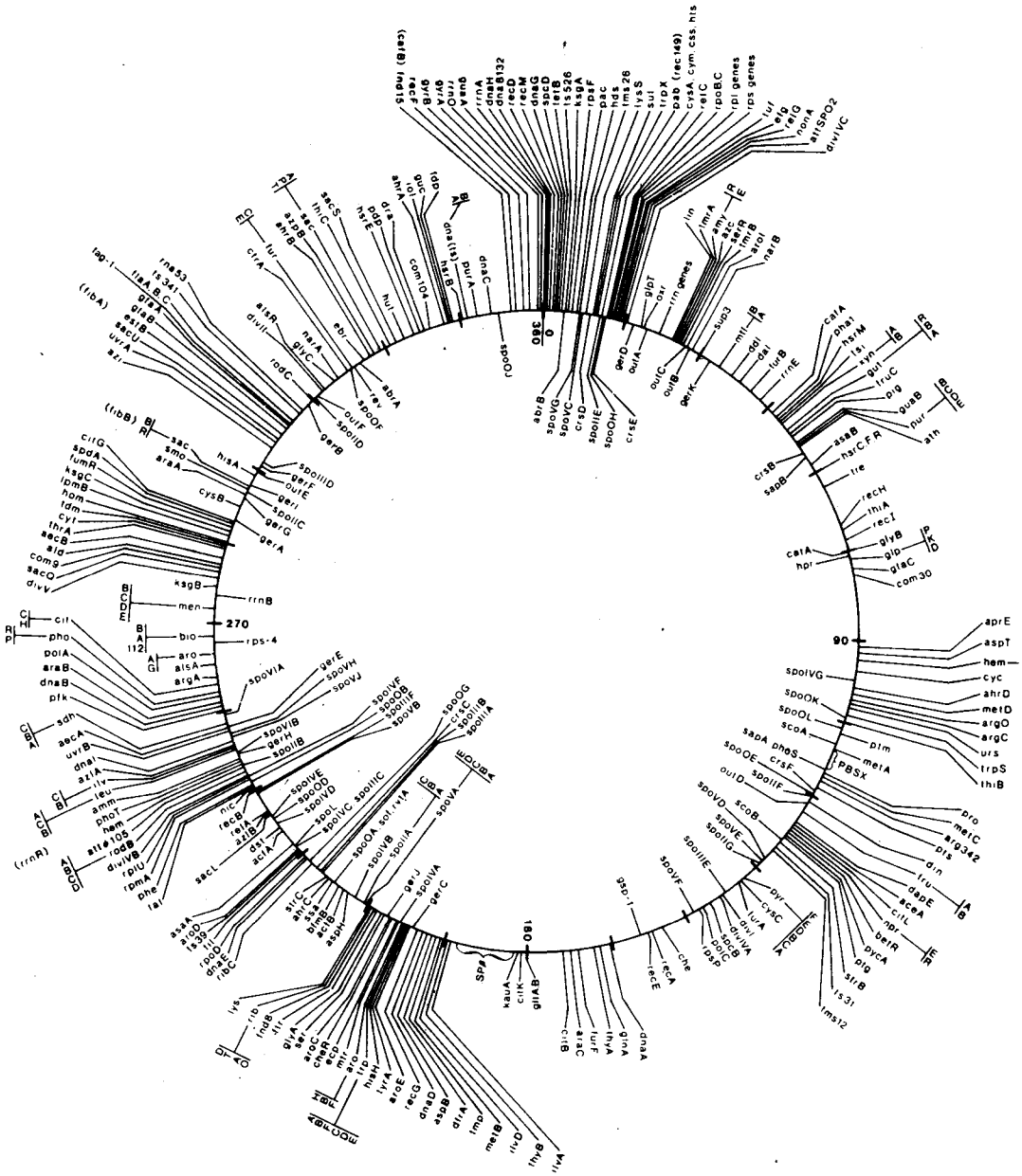

FIG. 1. Genetic map of the *B. subtilis* chromosome.

location has not been unequivocally determined (H. Yoshikawa, personal communication). The terminus of the map is located very close to the *gltA* and *gltB* loci (according to the data of Weiss and Wake [82] and Monteiro et al. [M. J. Monteiro, M. G. Sargent, and P. J. Piggott, J. Gen. Microbiol., in press]). In general, the order of markers is also shown; however, this order should not be taken as definitive. The markers were placed according to our best estimate from the published data. In some rare cases, three-factor crosses have been done for all of the markers within a region, but in the majority of the cases this is not true.

Table 1 lists the loci presently known to be associated with development in *B. subtilis*. Several of these loci have been cloned, and this information is also included. Of those cloned loci examined in detail by nucleic acid sequencing, some have been shown to be polycistronic.

TABLE 1. Sporulation and germination markers

Locus	Map position (°)	Comments
abrA	325	Antibiotic resistant. Mutation suppresses some of the pleiotropic phenotypes, but not Spo$^-$, of *spc0A* mutations (76). May be the same as *rev-4*.
abrB	3	Antibiotic resistant. Major locus for mutations suppressing pleiotropic phenotypes of *spo0* mutations without locus or allele specificity. Ribosome alterations have been observed in these mutants (77, 78). Same locus as *cpsX* (22, 23) and probably as *absA*, *absB* (36), and *tolA* (35).
abrC		Weak intragenic suppressors of *spo0A* (76).
catA	89	Overproduces extracellular protease; can sporulate in presence of glucose (37). Probably the same as *scoC* (47) and possibly the same as *hpr* (27).
cpsX		See *abrB*.
crsA	222	Carbon source-resistant sporulation; resistant to novobiocin and acridine orange during sporulation (74). Mutation in *rpoD* (R. H. Doi, personal communication); has similar phenotype to *rvt* mutations (69).
crsB	55	Requires high glucose for sporulation (74).
crsC	221	Carbon source-resistant sporulation (74).
crsD	8	As *crsC*.
crsE	11	Carbon source-resistant sporulation (74); maps in *rpoBC* operon; *rfm-11* suppresses *crsE*; *stv std* mutants show partial resistance to catabolites in sporulation (75).
crsF	118	As *crsC* (74).
gdh		Structural gene for glucose dehydrogenase. Cloned on a phage vector (80).
gerA	290	Defective in germination response to alanine and related amino acids (50, 64, 79). Isolated from λ clone banks (49); contains several genes (A. Moir, personal communication).
gerB	314	Defective in germination response to the combinations of glucose, fructose, asparagine, and KCl (50).
gerC	201	Temperature-sensitive germination in alanine (50, 79). Has not been separated from linked mutations in the original isolate.
gerD	16	Defective germination in a range of germinants (50, 58).
gerE	253	Defective germination in a range of germinants. May be a spore coat defect. Cloned in SPβ (48, 50).
gerF	301	Defective germination in a range of germinants (50, 58).
gerG	294	Mutant lacks phosphoglycerate kinase activity (17). Germinates poorly in alanine; sporulates poorly (60).
gerH	247	Defective germination in a range of germinants (58).
gerI	297	Defective germination in a range of germinants (58).
gerJ	207	Defective germination in a range of germinants; map order *gerJ-aroC-mtr-spoVIA-aroB2-trpC2*. Allele *gerJ51* (also called *tzm*) is present in many laboratory strains (81).
gerK	32	Defective germination response to glucose (34).
outA	21	Blocked in outgrowth after RNA, protein, and DNA synthesis has started; previous designation *gspIV* (2, 20, 58).
outB	28	Blocked in outgrowth before most macromolecular synthesis has started; previous designation *gsp-81* (1, 18, 58).
outC	27	Blocked in outgrowth after RNA and protein synthesis has started, but before synthesis of DNA; previous designation *gsp-25* (2, 58).
outD	122	Blocked in outgrowth; protein and DNA synthesis reduced; previous designation *gsp-1* (19, 58).
outE	300	Blocked in outgrowth; RNA synthesis normal, protein synthesis reduced, and DNA synthesis prevented; previous designation *gsp-42* (2, 58).
outF	316	Blocked in outgrowth; RNA and protein synthesis reduced and DNA synthesis prevented; previous designation *gsp-4* (2, 58).
rev-4	324	Suppressor of some of the pleiotropic effects (but not asporogeny) of *spo0* mutations; suppresses effect on sporulation of various drug resistance mutations (67, 68). May be the same as *abrA*.
rvtA	218	Suppressor of sporulation defect in *spo0B*, *spo0E*, and *spo0F* mutants (69). May be the same as *sof-1*.
rvt		Mutations causing the same phenotype as *rvtA* mutations but not mapping in the *rvtA* region (69).
sapA	114	Mutations overcome sporulation phosphatase-negative phenotype of early blocked *spo* mutants. Not definitely separate from *phoS* locus defined by mutations that cause constitutive phosphatase production (59). Deletions of region have PhoS phenotype (54).

Continued

TABLE 1—*Continued*

Locus	Map position (°)	Comments
sapB	56	Mutations overcome sporulation phosphatase-negative phenotype of early blocked *spo* mutations (59).
sas	211	Weak intragenic suppressor mutations of *spoIIA* (85).
scoA	109	Protease and phosphatase overproduction; delayed spore formation (47).
scoB	129	Protease and phosphatase overproduction; delayed spore formation (13, 38).
sof-1	218	Suppressor of sporulation defects in *spo0B*, *spo0E*, and *spo0F* mutants (30a, 42). The *sof-1* mutation is an alteration in codon 12 of the *spo0A* gene. Probably the same as *rvtA*.
spo0A	218	Mutants are blocked at stage 0 (30, 46). Locus codes for a protein of 29,700 daltons as determined from nucleic acid sequence (F. A. Ferrari et al., this volume). Mutants exhibit a wide variety of pleiotropic phenotypes, possibly as a result of transcription defects from promoters under control of minor forms of RNA polymerase (21, 86). Transcribed during vegetative growth.
spo0B	241	Mutants are blocked at stage 0 (30). Locus codes for a protein of 22,500 daltons as well as another protein of unknown function on a polycistronic transcript (15a; J. Bouvier, P. Stragier, C. Bonamy, and J. Szulmajster, Proc. Natl. Acad. Sci. U.S.A., in press). Mutants have most of the phenotypes of mutants bearing *spo0A* mutations. Transcribed during vegetative growth.
spo0C	218	Mutations with less pleiotropic phenotypes now known to be missense alterations in the *spo0A* gene product (F. A. Ferrari et al., this volume).
spo0D	234	Single allele resulting in a stage 0 block of sporulation. Mapped but not further characterized (33).
spo0E	120	Oligosporogenous mutations giving a stage 0 block. Possibly more than one gene. Phenotypes are less pleiotropic than *spo0A*, *spo0B*, or *spo0F* mutations (11, 30).
spo0F	323	Cloned on phage and plasmid vectors (43). DNA sequence contains a single open reading frame for a protein of 19,055 daltons (70). Inhibits sporulation when present in multiple copies (43): five copies inhibit; two do not (P. J. Piggot et al., this volume).
spo0G	223	Single allele resulting in stage 0 block of sporulation. Maps in the region of *spo0A* locus but genetically distinct from *spo0A* (33).
spo0H	11	Stage 0 locus that codes for a protein of 22,000 daltons as determined from the sequence of the *B. licheniformis* cloned gene. This gene complements *spo0H* mutations of *B. subtilis*. Transcript contains long 5′ and 3′ untranslated regions (61). Least pleiotropic of *spo0* mutations (29, 53).
spo0J	352	A stage 0 locus consisting of two alleles that yield a phenotype similar to *spo0H* mutations (31). Map close to the *spo-CM1* mutation which may be complemented by certain phage infections (10, 77). Closely linked by transformation to the gyrase gene.
spo0K	101	Stage 0 mutation mapping very close to the tryptophanyl tRNA synthetase gene (11; J. A. Hoch, unpublished data).
spo0L	106	Uncharacterized allele giving a *spo0* phenotype and mapping near to *spo0K* but genetically distinct from it (Hoch, unpublished data; listed in references 25 and 26).
spoIIA	211	Mutant blocked at stage II of sporulation. Region cloned on plasmid (45, 57) and phage (65) vectors. Transcribed as a polycistronic unit (57). DNA sequence has three adjacent open reading frames coding for proteins of 13, 16, and 22 kilodaltons (16). Located very close to, but in a separate transcriptional unit from, *spoVA* (55). Transformation starts 1 to 1.5 h after the start of sporulation (Piggot et al., this volume).
spoIIB	244	(11)
spoIIC	296	Cloned on phage and plasmid vectors (3).
spoIID	316	(53)
spoIIE	10	Cloned on plasmid vector (M. Young, personal communication).
spoIIF	120	(31)
spoIIG	135	Analysis of temperature-sensitive mutant indicates that expression must be tightly controlled (84). Cloned in plasmid and phage vector (5, 9). DNA sequence has homology with *rpoD* gene (73).
spoIIIA	220	Blocked at stage III of sporulation (53).
spoIIIB	221	(53). Cloned on phage vector (41).
spoIIIC	227	Possibly the same as *spoIVC* (33, 56).
spoIIID	302	(33)

Continued

TABLE 1—*Continued*

Locus	Map position (°)	Comments
spoIIIE	142	(33)
spoIIIF	239	Map order *hemA-spoIIB-attϕ105-rodB-divIVB-spo0B-pheA-nic-recB-spoIIIF-spoVB* (44).
spoIVA	204	Blocked at stage IV of sporulation. Linked to *trpC* by transformation (45, 54).
spoIVB	213	May be an allele of *spo0A* (11; Hoch, unpublished data).
spoIVC	227	Contains at least two cistrons (12). Linked to *aroD* by transformation (58).
spoIVD	233	(31)
spoIVE	234	(33)
spoIVF	242	Linked to *spo0B* by transformation (11, 44).
spoIVG	97	(53)
spoVA	211	Blocked at stage V of sporulation. Map order *spoIIA-spoVA-lys* (55). Cloned on a plasmid and on a phage (57, 65). Transcribed as a polycistronic unit with open reading frames for proteins of 23, 15, 16, 36 and 34 kilodaltons (P. Fort and J. Errington, J. Gen. Microbiol., in press).
spoVB	239	(31, 43)
spoVC	7	Cloned on plasmid vectors (51).
spoVD	133	Linked to *spoVE* by transformation (11, 31).
spoVE	133	(11, 31). Isolated in λ clone bank and subcloned on a plasmid (P. J. Piggot and K.-F. Chak, unpublished data).
spoVF	148	Probably the same as *dpa* (6, 58). Mutants form octanol- and chloroform-resistant, heat-sensitive spores (6, 58). Form heat-resistant spores in the presence of dipicolinic acid.
spoVG	6	Previously called 0.4-kb gene (24, 63). Transcription turned on within 30 min of the start of sporulation (66).
spoVH	251	(28)
spoVJ	250	(28)
spoVIA	255	Blocked at stage VI of sporulation. Map order *argA-spoVIA-gerE-leuA* (39).
spoVIB	247	Map order *citF-gerE-spoVH-spoVJ-ilvB-leuA-spoVIB-pheA* (40).
spoL	227	"Decadent" sporulation (7).
sprE	91	Structural gene for subtilisin E. Map order (*hpr*, *glyB*)-*sprE-metD* (71, 83).
ssa	218	Alcohol-resistant sporulation. Maps very close to *spo0A* (8). *rvt* mutations have same phenotype (69). Probably *rvtA* = *sof-1* = *ssa* = *spo0A*.
"0.3-kb gene"		Turned on at a late stage (T_3 to T_4) of sporulation. Requires correct functioning of at least *spo0B*, *spoIIA*, *spoIIE*, and *spoIIIE*. Codes for a protein of 6,750 daltons (52, 72).

This is the case for *spo0B*, *spoIIA*, and *spoVA* and probably will hold for other loci as well. Thus, one should not equate loci with single genes. One of the surprising findings in the sequencing of sporulation genes is the relatively low molecular weight of their putative gene products. None of these gene products exceed 30,000 daltons for the *spo0A*, *spo0B*, *spo0F*, *spo0H*, and *spoIIA* loci. This is considerably less than the size of the majority of vegetative proteins or proteins made in sporulating cells. The significance of this observation is unclear.

The promise that cloning of sporulation genes will increase our knowledge of the regulation of such genes and give insights into the functions of our gene products is now being fulfilled. It is hoped that such studies will allow us to paint a comprehensive picture of the mechanisms controlling development at the cellular level.

This research was supported, in part, by Public Health Service grant GM19416 from the National Institute of General Medical Sciences.

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Molecular Cloning and Nucleotide Sequence of the *spo0A* Locus and Its Mutations

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The initiation of sporulation appears to be controlled by at least nine genes called *spo0* genes. The majority of the alleles of these *spo0* genes map in five loci that are unlinked on the *Bacillus subtilis* chromosome; the other four loci are defined by one or two alleles at each locus (6, 12). If we assume that sporulation is triggered by some internal metabolic signal that is transmitted to the transcription machinery to initiate the transcription of sporulation-specific genes, then *spo0* mutants may be envisioned as being blocked in any one of these processes, including the formation of the metabolic signal or the mechanism by which this signal is transmitted to the transcription machinery. Mutations in the transcription machinery itself also might be specific for sporulation. In addition to being defective in sporulation, stage 0 mutants have large pleiotropic effects on the synthesis of a wide variety of gene products that appear concomitantly with the sporulation process (2). The most pleiotropic phenotypes are exhibited by the mutations in the *spo0A* locus. In this report we examine the sequence of the *spo0A* locus and of some of its mutations that have profound effects on these phenotypes.

RESULTS

The *spo0A* locus was identified and isolated from a library of *B. subtilis* DNA cloned in the λ vector Charon 4A (3). The insert within one of these clones was subjected to extensive restriction enzyme analysis, and subfragments of the inserts were cloned within the integrative vector pJH101 (4). Using these subcloned fragments, we were able to identify those regions of the original cloned insert that contained transforming activity for *spo0A* mutations. Figure 1 shows the location of alleles in the *spo0A* locus, as defined by the subfragments cloned in the plasmids indicated at the bottom of the figure. All of the alleles except two were contained within the *Hpa*I to *Eco*RI fragment of 840 base pairs (bp). None of the mutations was found to the left of the *Hpa*I site. Two mutations were found in the *Eco*RI to *Hin*cII fragment of 660 bp to the right of the majority of the alleles. These alleles to the right include the *spo0A9V* mutation, which is the classically less pleiotropic mutation in the *spo0A* locus (2). It should be noted that integration of the plasmids pJF1377 and pJF2042 by Campbell-type recombination in Spo^+ strains results in a Cm^r Spo^- phenotype. This result suggests that the fragments contained within these plasmids are included within the *spo0A* transcription unit. None of the other plasmids gives this phenotype when integrated as described.

To verify the location of the deletion 204, which covers many of the *spo0A* alleles, chromosomal DNAs from the wild-type strains and a strain bearing this deletion were digested with restriction endonucleases *Eco*RI and *Bgl*II, electrophoresed on agarose gels, transferred to nitrocellulose, and probed with a radioactive fragment DNA extending from the *Eco*RI site leftward to a *Bal*I site just left of the indicated *Cla*I site in Fig. 1. The results of this analysis, shown in Fig. 2, indicate that the *Eco*RI to *Bgl*II fragment is 0.53 kilobase in the Spo^+ strain and 0.35 kilobase in the deletion strain. Thus, the deletion is approximately 180 bp long and is verified to be between the *Eco*RI and *Bgl*II sites.

Sequence of the *spo0A* locus. The entire *spo0A* locus and its flanking regions were sequenced by use of the Maxam and Gilbert technique (9). The potential open reading frames derived from this sequence are shown in Fig. 3. A large open reading frame extending from the *Hpa*I site through the *Eco*RI site, from left to right as shown in Fig. 3, was present. Since the majority of the *spo0A* mutations were located between the *Hpa*I and *Eco*RI restriction sites, we concluded that this major open reading frame must include the *spo0A* locus. In the region between the *Eco*RI site and the *Hin*cII site, no open reading frame of any substantial size was evident. This region contains both the *spo0A9V* and *spo0A153* alleles. One sizable open reading frame with the potential to code for a protein of about 17,000 molecular weight was uncovered in the region immediately after the *Bal*I restriction site and it stopped after the *Cla*I site.

Transcription of the *spo0A* locus. High-resolution S1 mapping (1) was used to determine the sites of initiation and termination of transcription for the *spo0A* locus in this region. mRNA

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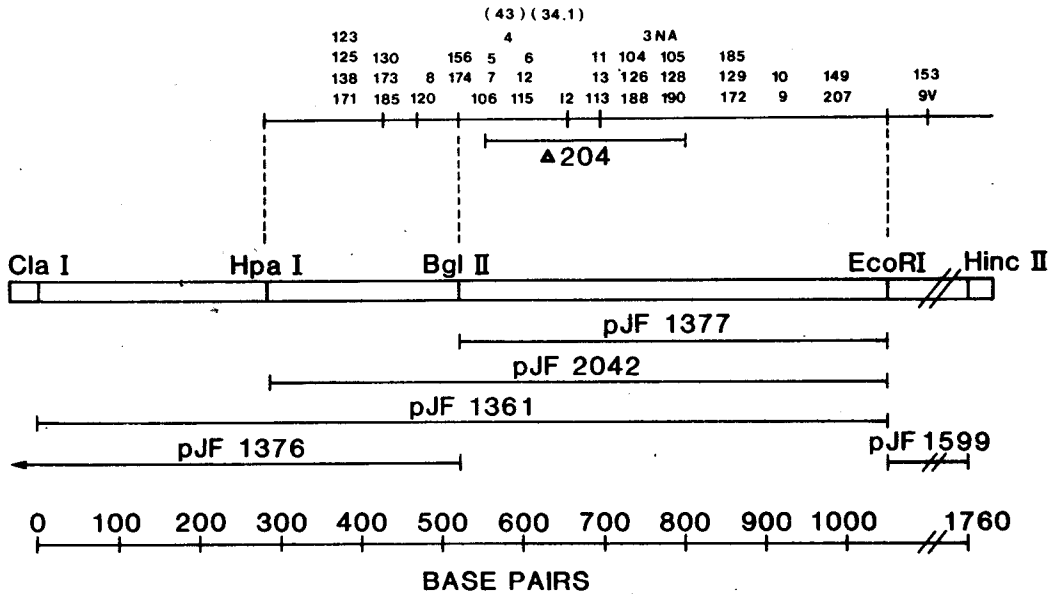

FIG. 1. Genetic and restriction maps of the *spo0A* locus. The top line is the genetic map of the region showing the restriction fragments in which the alleles fall. All the alleles above the deletion 204 bar are contained within the deletion. The plasmids have the indicated amount of DNA cloned in pJH101. Plasmid pJF1376 extends to the next *Eco*RI site, which is approximately 5.3 kilobases to the left of the *Bgl*II site.

fractions from logarithmically growing cells were hybridized to restriction fragments end labeled at the *Eco*RI and *Bgl*II sites. The site of transcription initiation was found to be approximately 270 bp upstream to the left of the *Bgl*II site. Termination of transcription was approximately 130 bp downstream to the right of the *Eco*RI site. This termination site (Fig. 4) has a typical terminatorlike structure preceding it that is capable of forming a hairpin loop with a calculated ΔG (25°C) = −19.2 kcal (ca. −80.4 kJ)/mol (15).

A putative promoter sequence ahead of the transcription start site has been found that resembles closely those observed for sigma-37-dependent promoters of *spoVC* (11), *spoVG* (10), and *sprE* (16) (Fig. 5). The −10 region of *spo0A* is identical to the −10 region of *spoVC* in seven of nine positions. The −35 region is identical to the −35 region of *spoVC* in four of six positions. In addition, an 11-bp sequence overlapping the putative −35 region of *spo0A* was found in the promoter region upstream from the *spo0F* coding sequence (14). If one aligns this sequence with known sigma-37 promoters, a good homology is found, suggesting that this may indicate a sigma-37-dependent promoter for the *spo0F* locus. We believe that the translation start for the *spo0A* gene is the GTG codon at position +51 (Fig. 4). This codon is commonly used for translation starts in *B. subtilis*, and it is preceded by a strong ribosome binding site, GGAGG, located an optimal distance upstream. Earlier, we thought that the ATG codon downstream from this in the same frame at position +135 was the start of translation (4a). However, the location of the *sof-1* allele described below at position +86 suggests that the GTG codon is the actual start of translation. The protein specified by this sequence is 29,691 daltons.

Identification of the *spo0C* alleles. It has been a question for some time whether the *spo0A9V* allele was a less pleiotropic mutation of the *spo0A* locus or whether it actually was a mutation in a second locus linked to *spo0A* that has a different phenotype from *spo0A* mutations. To resolve this question, we cloned both the *spo0A9V* and *spo0A153* mutations and determined their sequence by the Maxam and Gilbert technique (9). The results of these analyses showed that both alleles were in the same base of the 11th codon preceding the translation stop codon of the *spo0A* gene (Fig. 6). Although these mutations reside in the same base of the same codon, they result in different substitutions of amino acids in the protein. Thus, both mutations give rise to a missense protein that is partially active, resulting in some of the phenotypes of the complete *spo0A* alleles.

Genetic location of the *sof-1* mutation. Two groups of investigators have recently isolated suppressors of *spo0F* mutations that allow sporulation to occur in the presence of a defective *spo0F* gene product (8, 13). In addition, such

suppressors are active on strains carrying *spo0B* or *spo0E* mutations. Both of these mutations, *sof-1* and *rvt11*, were found to map in the region of the chromosome between *lys-1* and *aroD* and close to the *spo0A* locus. To map the *sof-1* mutation with respect to the *spo0A* locus, we used the plasmids described in Fig. 1 to determine the location of the *sof-1* allele. A strain, UOT0550 (*spo0FΔS sof-1*), was transformed by each of these integrative vectors, and among the chloramphenicol-resistant transformants the presence of the wild-type allele of *sof-1* was assayed by determining whether any of the Cm^r transformants were now Spo^-. Plasmids pJF1376 and pJF1361 were capable of transforming the strain to Spo^-. This locates the *sof-1* allele in the overlapping region in these plasmids, that is, the region between the *Cla*I and *Bgl*II restriction sites.

Cloning of the *sof-1* mutation. To clone the *sof-1* mutation, a transformant from a cross with plasmid pJF1361 was extracted and its chromosomal DNA was subjected to digestion with restriction endonuclease *Eco*RI. This *Eco*RI digest was then self-ligated with T4 ligase, and the ligation mixture was used to transform *Escherichia coli*. Among the chloramphenicol-resistant *E. coli* strains that arose from this transformation, one plasmid was found that was identical to plasmid pJF1361 in restriction map and was capable of transforming the *sof* phenotype to strains bearing mutations in the *spo0F* gene. Thus, this plasmid contains the *sof-1* allele rather than the wild-type allele in the DNA between the *Cla*I and *Bgl*II sites. Sequence analysis of the insert in this plasmid, pJH2074, revealed only a single base change (Fig. 7), and this change results in a transversion of a T to a G in the sequence ATAATC, resulting in the ATAAGC. This sequence codes for part of the amino-terminal end of the *spo0A* gene (Fig. 8), and the mutation results in a substitution of lysine for asparagine in the 12th position of the *spo0A* gene product. No other differences from the wild type were observed in this sequence.

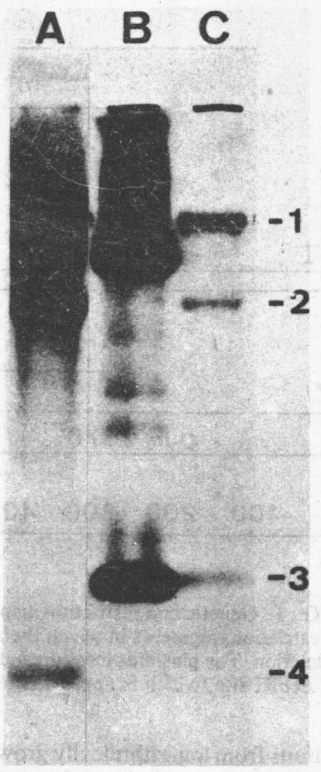

FIG. 2. Southern analysis of the deletion 204-carrying strain. Chromosomal DNAs from the wild-type strain (lane C) and a strain carrying the Δ204 mutation (lane A) or the plasmid pJF1377 (lane B) were digested with *Bgl*II and *Eco*RI restriction nucleases. The digests were electrophoresed in agarose, transferred to nitrocellulose membranes, and hybridized to nick-translated plasmid pJF1361. Band 1 is a 5.3-kilobase *Eco*RI-*Bgl*II fragment starting at the *Bgl*II site in the *spo0A* gene and ending at an *Eco*RI site to the left (see Fig. 1) of the gene. Band 2 is the *Eco*RI-*Eco*RI fragment containing the 9V allele to the right of the *spo0A* gene. This fragment appears because the probe is contaminated with labeled DNA homologous to this region. Bands 3 and 4 are the *Bgl*II-*Eco*RI fragments from the *spo0A* gene.

DISCUSSION

The *spo0A* locus is believed to code for a protein of 29,691 daltons, as determined from the nucleic acid sequence of the locus. Because we have been unable to obtain an intact *spo0A* gene in either *E. coli* or *B. subtilis* on a plasmid, we have been unable to confirm this by minicell-type experiments. By use of gene fusion experiments we have been able to show that the copy number of the *spo0A* gene product is approximately 10 to 100 copies per cell, and this gene product is maximally expressed during the vegetative phase of growth, declining as the cells enter sporulation (unpublished data). This is consistent with our previous observations that the *spo0A* gene products are vegetative products and lends support to our working hypothesis of many years that these gene products are dispensable vegetative functions that sense the metabolic state of the cell and regulate the decision as to whether to divide or to begin sporulation.

Perhaps one of the most interesting results of this study is the finding that the *sof-1* suppressor is a missense mutation in the amino-terminal portion of the *spo0A* protein. Since the *sof-1* suppressor is capable of suppressing mutations in the *spo0B*, *spo0E*, and *spo0F* genes, it seems clear that the *spo0B*, *spo0E*, and *spo0F* gene