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Identification of the Transcriptional Suppressor *sof-1* as an Alteration in the *spo0A* Protein†

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The mutation *sof-1* suppresses the sporulation defect of mutations in either the *spo0B*, *spo0E*, or *spo0F* stage 0 sporulation genes. Through the use of integrative plasmids carrying the portion of the chromosome including the *spo0A* locus and flanking regions, the *sof-1* mutation was localized near the *spo0A* locus. A plasmid carrying a fragment of DNA with *sof* genetic activity was constructed. Nucleic acid sequence analysis of this fragment revealed a single base change that resulted in a substitution of lysine for asparagine in the 12th codon of the *spo0A* gene. The results indicate that certain missense mutations in the *spo0A* gene bypass the necessity for the *spo0B*, *spo0E*, and *spo0F* gene products in sporulation. Several models for the interaction of these gene products may be imagined.

The initiation of sporulation in *Bacillus subtilis* may be blocked by mutations at several genes called *spo0* genes (9). In addition to preventing the formation of the asymmetric septum, the initial event in sporulation, mutants carrying mutations in these genes have a wide variety of pleiotropic phenotypes (4). It has recently been discovered that transcription from several promoters controlled by sigma-37 or sigma-28 RNA polymerases is blocked by *spo0* mutants. Thus the transcription of the *spoVG* gene, a gene transcribed with sigma-32- or sigma-37-containing RNA polymerases, is blocked by *spo0A*, *spo0B*, and *spo0E*, *spo0F*, and *spo0H* mutations (14). Similarly, transcription of two sigma-28 RNA polymerase-dependent genes is blocked by *spo0A*, *spo0B*, *spo0E*, and *spo0F*, but not by *spo0H*, mutations (7). The mechanism by which *spo0* gene products intervene in transcription from these genes is not known. One approach to the study of the functions of *spo0* genes has been the isolation of suppressors of their action. One of the most common partial suppressors of *spo0A* is the so-called *cpsX* or *abr* suppressors (8, 20). These suppressors map in at least three locations, with the most plentiful mutations of this group in the *abrB* locus located near the origin of the chromosome (20). Mutations in the *abrB* locus result in ribosomes with various protein alterations and may reflect a change in the maturation of the ribosome (18). The *abrB* mutations suppress mutations in *spo0A* or *spo0B* genes in an allele-nonspecific manner. Similar mutations such as *rev-4* have been identified that suppress many of the pleiotropic sporulation phenotypes caused by mutations in sporulation genes as well as those in RNA polymerase, ribosomal proteins, and protein synthesis factor EF-G (16). These types of suppressors have a great number of phenotypes that they alter within the cell, and it has been difficult to pinpoint their mechanism of action. Two groups have recently isolated suppressors of *spo0F* mutations that allow sporulation to occur in the presence of the defective *spo0F* allele. Two such mutations, *sof-1* (12) and *rvtA11* (17), have been found to map in the region of the chromosome between *lys-1* and *aroD* and close to the *spo0A* locus. In this communication we show that the *sof-1* mutation is an allele of the *spo0A* gene.

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MATERIALS AND METHODS

Bacterial strain and plasmids. *Escherichia coli* 294 (*endoI thy hsdR*) cells were made competent and transformed by a procedure described by Dagert and Ehrlich (5). *B. subtilis* UOT0550 (*trpC2 metB51 leuA8 nonB1 ΔspoOF sof-1*) was obtained from Fujio Kawamura and transformed by the procedure of Anagnostopoulos and Spizizen (1). Stage 0 sporulation mutant *B. subtilis* strains used were JH649 (*trpC2 phe-1 spo0F221*), JH648 (*trpC2 phe-1 spo0B136*), and JH647 (*trpC2 phe-1 spo0E1*). Four strains carrying different alleles at the *spo0H* locus were also tested, as well as a variety of later-stage sporulation mutants. The plasmids indicated in Fig. 1 were derivatives of the integrative vector pJH101 (6).

Enzymes. All restriction enzymes were purchased from New England Biolabs or Bethesda Research Laboratories and used according to the recommended assay procedures of the suppliers. T4 DNA ligase was obtained from New England Biolabs. Polynucleotide kinase and calf intestinal alkaline phosphatase were obtained from Boehringer-Mannheim Biochemicals. Proteinase K was obtained from E-M Biochemicals.

DNA isolation. UOT0550::pJF1361 chromosomal DNA was prepared by a procedure described by Piwnicka et al. (15), with the following changes: sarkosyl NL and pronase were replaced with 1% Triton X-100 and proteinase K, respectively. The DNA was ethanol precipitated twice after the dialysis step.

Plasmid DNA was isolated in a modification of the alkaline extraction of Birnboim and Doly (2). Minipreparations of plasmid DNA were obtained by the boiling method of Holmes and Quigley (10).

Cloning *sof-1*. *B. subtilis* UOT0550 was transformed for chloramphenicol resistance with pJF1361, yielding UTO550::pJF1361. Chromosomal DNA (50 μg) from a chloramphenicol-resistant, sporulation-positive transformant was restricted with 30 U of *Eco*RI for 2 h at 37°C. The DNA was extracted with phenol, phenol-chloroform (1:1, vol/vol), and chloroform, collected by precipitation with ethanol, and dried under vacuum. The chromosomal *Eco*RI digest was religated in a volume of 13 μl with T4 DNA ligase and

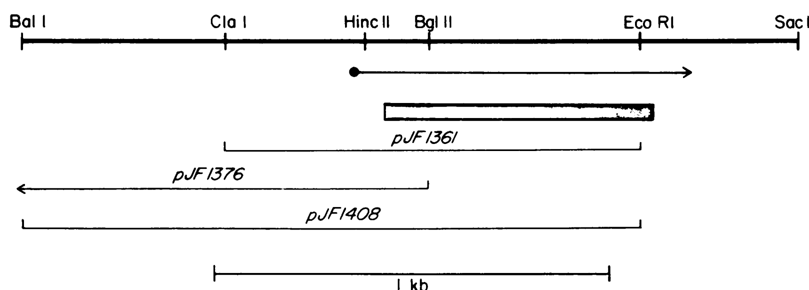

FIG. 1. Restriction map of the *spo0A* locus and plasmids covering the region. The arrow indicates the direction and extent of transcription of the *spo0A* locus. The shaded box shows the location of the *spo0A* protein. The restriction fragments contained within the plasmids used are indicated. Plasmid pJF1376 contains approximately 4.8 kilobase pairs of additional DNA to the left of the region shown.

transformed into competent *E. coli* 294 cells. Minipreparations of plasmid DNA from three ampicillin- and chloramphenicol-resistant transformants were prepared and restricted with *Eco*RI and *Cla*I and electrophoresed on a 1% agarose gel containing 50 μg of ethidium bromide (electrode buffer, 1× TBE: 50 mM Tris, 57 mM borate, 2 mM EDTA, [pH 8.1]) per ml. One of the three plasmids was found to contain an *Eco*RI-*Cla*I insert of approximately 1 kilobase and was designated pJH2074.

DNA-sequence analysis of sof-1. DNA sequence data for pJH2074 were obtained by the method of Maxam and Gilbert (13), with a reagent kit obtained from New England Nuclear Corp. 5′-end-labeled fragments were obtained by the following method. Plasmid DNA (30 μg) was restricted and the 5′ termini were treated with 10 U of calf intestinal alkaline phosphatase for 30 min at 37°C. After two extractions with phenol and one with chloroform, the dephosphorylated DNA was collected by ethanol precipitation and dried under vacuum. The 5′ ends were labeled for 90 min at 37°C in a volume of 15 μl containing 50 mM Tris-hydrochloride (pH 7.6), 10 mM $MgCl_2$, 10 mM dithiothreitol, 10 mM spermidine, 300 μCi of [λ-^{32}P]ATP, and 5.5 U of polynucleotide kinase. The labeled DNA was collected by ethanol precipitation, dried, and restricted at a second site. End-labeled fragments were detected by autoradiography after electrophoresis in 3.5 or 4% polyacrylamide gels (cross-link, 19:1; electrobe buffer, 1× TBE) and electroeluted into dialysis membranes in 0.25× TBE.

Sequencing gels were cast with double-thick spacers at the anode end, so that the gels varied continuously in thickness from 0.35 to 0.7 mm. Gels were typically 90 cm long and 6% polyacylamide (39:1 cross-link) in 8.3 M urea. After electrophoresis, the gels were fixed in a solution of 10% glacial acetic acid–10% methanol for 15 to 20 min and then rinsed with several changes of deionized water. The fixed gels were then dried under vacuum and autoradiographed with a single sheet of Kodak XRP.

RESULTS

Plasmid mapping of sof-1 mutation. Plasmids containing various chromosomal fragments corresponding to the region around and including the *spo0A* locus were used to identify the location of the *sof-1* mutation. The extent of this region carried in the plasmids is indicated in Fig. 1. All of these inserts are in the integrative vector pJH101, which is unable to replicate in *B. subtilis* but can integrate if a region of homology is present on the plasmid (6). The plasmids shown were used to transform the strain UOT0550 (*spo0F*ΔS *sof-1*) for chloramphenicol resistance. It was expected that if the incoming plasmid carried the wild-type allele for *sof-1*, a certain proportion of the transformants would become Spo$^-$, depending on the site of integration of the plasmid in a Campbell-type recombination. Spo$^-$ chloramphenicol-resistant transformants were observed among the chloramphenicol-resistant transformants for all of the plasmids tested. Their proportions were 23, 31, and 64% for plasmids pJF1361, pJF1408, and pJF1376, respectively. Control crosses with a chloramphenicol-resistant replicating plasmid not carrying any of this region of the chromosome did not give Spo$^-$ transformants among the chloramphenicol-resistant transformants. The results therefore indicated that all of the plasmids carried a portion of DNA that could correct the *sof-1* allele. This narrows the location of the *sof-1* allele to the area between the *Cla*I and *Bgl*II restriction sites, since this is the only region of the chromosome that is common to all three plasmids. This region of the chromosome contains the promoter for the *spo0A* gene and the first 20% of the coding sequence for the gene. There is, however, an open reading frame that extends from approximately the *Bal*I site past the *Cla*I site that has not been shown to code for any known protein.

Cloning of the sof-1 mutation. To isolate the *sof-1* mutation on a cloned fragment, a Spo$^+$ chloramphenicol-resistant transformant from the transformation with plasmid pJF1361 was grown and chromosomal DNA was extracted. This DNA was digested with endonuclease *Eco*RI and ligated with T4 ligase. The ligation mixture was used to transform an *E. coli* strain for chloramphenicol and ampicillin resistance. Three transformants were found, and one of them was further characterized. The plasmid pJH2074 found to reside within this transformant was the same size and had the same restriction map as the original pJF1361. When pJH2074 was used to transform the strain JH649 (*trpC2 phe-1 spo0F221*), Spo$^+$ chloramphenicol-resistant transformants were observed among the chloramphenicol-resistant transformants.

Reversion of spo mutations with sof-1. Plasmid pJH2074 was used to transform a series of sporulation mutants to determine the specificity of suppression of the *sof-1* mutation. With this plasmid, chloramphenicol-resistant transformants of Spo$^-$ strains were selected, and the proportion of Spo$^+$ transformants among the chloramphenicol-resistant transformants was determined. In this type of analysis, the pJH2074 was found to revert both *spo0E* and *spo0B* strains but not the other *spo0* mutants tested. It was unable to revert any of the alleles of the *spo0H* locus. Among the late-spore mutants tested, including defects in the *spoIIB*, *spoIIC*, *spoIIG*, and *spoIIA* genes, none were revertable by the *sof-1* mutation.

Sequencing plasmid pJH2074. The entire insert within pJH2074 was subjected to sequence analysis by the Maxam-Gilbert technique. When this sequence was analyzed, only one base change was observed (Fig. 2), and this change

results in a transversion in the sequence ATAATC of T to a G, resulting in the sequence ATAAGC. This sequence codes for part of the amino terminal end of the *spo0A* gene product (Fig. 3), and the mutation results in a substitution of a lysine for an asparagine in the 12th codon of the *spo0A* gene product. No other differences were observed in the entire sequence from that of the wild type.

DISCUSSION

The *sof-1* mutation maps in the same general region as the previously described *rvtA* mutation. Both *sof-1* and *rvtA11* revert the sporulation defect of mutations in the stage 0 genes *spo0F*, *spo0B*, and *spo0E*. In addition, *rvtA11* has been to shown confer resistance to aliphatic alcohols on sporulation of *B. subtilis*. Thus, *rvtA* and *ssa* confer an identical phenotype, i.e., resistance to sporulation and resistance to aliphatic alcohols; they map in the same general region of the chromosome (3). Genetic studies indicate that *rvtA11* is almost certainly an allele of the *spo0A* locus (T. Leighton, personal communication). Mutations giving rise to a catabolite resistance sporulation phenotype have also been mapped in this region of the chromosome (19), and the *crsC* mutations have been shown by Sharrock et al. (17) to revert the sporulation phenotype of *spo0E* strains but not of *spo0B* or *spo0F* strains. Although it is unknown at this time whether *crsC* is another allele of the *spo0A* gene, it seems likely that this might be the case on the basis of map position of *crsC* mutants and the observation that strains bearing the *sof-1* allele are resistant to catabolite repression of sporulation (F. Kawamura, unpublished results). Therefore, independently isolated mutations that give rise to a variety of sporulation-associated phenotypes and map in this region appear to be the consequence of mutation in a single gene, the *spo0A* gene.

The most surprising result from these studies is the observation that *sof-1* and *rvtA11* mutations are capable of suppressing the sporulation defect in *spo0B*, *spo0E*, and *spo0F* mutants. Neither *spo0B* nor *spo0F* mutants has ever been observed to sporulate above reversion levels. The fact that a mutation in the *spo0A* gene giving rise to an altered *spo0A* gene product is capable of overcoming mutations in these genes suggests to us that the *spo0E*, *spo0B*, and *spo0F* gene products interact in a concerted or sequential fashion to effect the activity of the *spo0A* gene product. We therefore imagined the *spo0A* gene product to exist in two possible forms, inactive and active, and the *spo0* genes to somehow effect the conversion of *spo0A* from an inactive to an active form. Although this concept is not unlike an inducer-repressor interaction, the interaction may indeed be at the macromolecular level. The *sof-1* mutation is therefore envisioned as an altered *spo0A* gene product that no longer requires the interaction of these *spo0* genes to be active.

```
        +1
        AACATGTAGCAAGGGTGAATCCTGTTAACTACATTTGGG

        GAGGAAGAAAC GTG GAG AAA AAT AAA GTT TGT
                    fMet Glu Lys Ile Lys Val Cys

wild    GTT GCT GAT GAT|AAT|CGA GAG CTG GTA AGC
        Val Ala Asp Asp|Asn|Arg Glu Leu Val Ser

sof-1   GTT GCT GAT GAT|AAG|CGA GAG CTG GTA AGC
        Val Ala Asp Asp|Lys|Arg Glu Leu Val Ser
```

FIG. 3. Amino terminal portion of the *spo0A* gene.

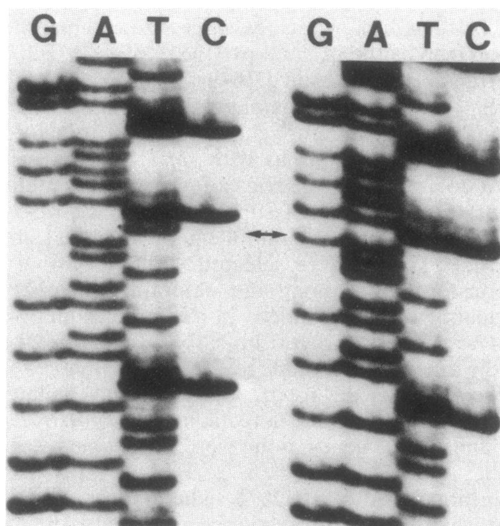

FIG. 2. Autoradiograph of a portion of the sequencing gel for the *Cla*I-*Eco*RI fragment isolated from wild-type and *sof-1* strains.

The recent results of Gilman and Chamberlin (7) on transcription from promoters controlled by the sigma-28 form of RNA polymerase may shed some light on the role of the *spo0A* protein. These investigators found that transcription from several of the sigma-28 controlled promoters was blocked in *spo0A*, *spo0B*, *spo0F*, and *spo0E* mutants. Furthermore, transcription could be restored in the *spo0F*, *spo0B*, and *spo0E* strains by introduction of the *rvtA11* allele into these strains. Since we now know that an altered *spo0A* gene product such as that brought about by the *rvtA11* or the *sof-1* allele is sufficient to allow transcription of these promoters to occur, it is tempting to suggest that *spo0A* gene product acts as a cotranscriptional factor that interacts with RNA polymerase molecules containing minor forms of sigma factors. Such a model might help to explain the observation that mutations in the β subunit of RNA polymerase giving rise to rifampin resistance are able to make some of the less pleiotropic missense mutations of *spo0A*, such as the *spo0A9V* allele, resemble the more pleiotropic null alleles at this locus (11). Such results are suggestive of an interaction between RNA polymerase and the *spo0A* protein, although other explanations are possible.

None of the present data allow us to distinguish between a direct interaction model in which the *spo0A* gene product is a transcription factor and an indirect model such as the possibility that *spo0A* is an activator of some important protein required for transcription. The purification of the *spo0A* gene product and a determination of its properties in vitro would certainly shed some light on this problem.

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