New Suppressor Mutation *sur0B* of *spo0B* and *spo0F* Mutations in *Bacillus subtilis*

By KAZUAKI SHOJI,¹ SETSUKO HIRATSUKA,¹ FUJIO KAWAMURA²
AND YASUO KOBAYASHI^{1*}

¹Department of Applied Biochemistry, Faculty of Applied Biological Sciences, Hiroshima University, Higashi Hiroshima 724, Japan

²Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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Two extragenic suppressor mutations, *sur0B20* and *sur0F1*, which restore the sporulation of *spo0B* or *spo0F* mutants of *Bacillus subtilis* to the wild-type level, were obtained. These suppressor mutations were located in the *spo0A* gene. Their location is close to that of the *sof-1* mutation, which suppresses *spo0B*, *spo0E* and *spo0F* mutations. However, *spo0* strains bearing the *sur0B20* mutation differed in several phenotypic characteristics from *spo0* mutants bearing the *sof-1* suppressor. Nucleotide sequence analysis revealed that the *sur0B20* and *sur0F1* mutations resulted in Glu₁₄ to Val and Asn₁₂ to Lys conversion, respectively, in the *spo0A* gene. This result indicates that *sur0B20* is a new suppressor of *spo0B* and *spo0F* mutations, whereas *sur0F1* is identical to *sof-1*.

INTRODUCTION

The initiation of sporulation in *Bacillus subtilis* is blocked by *spo0* mutations, the sites of which define eight loci (*spo0A*, *0B*, *0E*, *0F*, *0H*, *0J*, *0K* and *0L*) on the bacterial chromosome. Despite their crucial role in the initiation of spore development, little is known about the *spo0* genes and their function in the initial events of spore formation. However, nucleotide sequence analysis has recently revealed that the *spo0A*, *spo0B* and *spo0F* products share significant homology (Kudoh *et al.*, 1985; Ikeuchi *et al.*, 1985, 1986). The *spo0A* and *spo0F* products also share homology with the *Escherichia coli* genes *ompR*, *sfrA* and *ntrC*, which play an important role in the cellular response to various environmental signals, such as changes in osmolarity, presence of toxic compounds, and nitrogen limitation (Trach *et al.*, 1985; Yoshikawa *et al.*, 1986). Also, several data suggest that the *spo0H* gene product is a new sigma-factor (σ^H) of RNA polymerase required for the sporulation process (Dubnau *et al.*, 1988).

To analyse the interactions between the *spo0* genes and their products, isolation and characterization of extragenic suppressors is a powerful approach (Hoch *et al.*, 1985). Two groups have isolated extragenic suppressors, *sof-1* and *rvtA11*, that restore the sporulation of *spo0F* mutants to the wild-type level (Kawamura & Saito, 1983; Sharrock *et al.*, 1984). These suppressor mutations lie between *lys-1* and *aroD* on the *B. subtilis* chromosome. Recently, *sof-1* was shown to be a missense mutation in the *spo0A* gene that can suppress defects not only in *spo0F*, but also in *spo0B* and *spo0E* (Hoch *et al.*, 1985). These results indicate that the *spo0A* gene product may interact with *spo0B*, *spo0E* and *spo0F* gene products and that the *sof-1* mutation bypasses the requirement for *spo0B*, *spo0E* and *spo0F* gene products. Besides these suppressors, the *rev-4* mutation suppresses various sporulation-defective phenotypes caused by mutations in the RNA polymerase β subunit, ribosomal proteins and elongation factor G (Sharrock & Leighton, 1981, 1982). The *abrB703* mutation suppresses the dependence of *spoVG* transcription on *spo0A* and *spo0B* (Zuber & Losick, 1987) and also a variety of other pleiotropic phenotypes

Table 1. *Bacterial strains*

Strain	Genotype	Origin*
<i>B. subtilis</i>		
168		University of Tokyo
1A96 (JH642)	<i>trpC2 pheA1</i>	<i>Bacillus</i> Genetic Stock Center (BGSC)
1S17 (JH647)	<i>trpC2 pheA1 spo0E11</i>	BGSC
1S19 (JH649)	<i>trpC2 pheA1 spo0F221</i>	BGSC
1S22	<i>trpC2 rpoB2 spo0H17</i>	BGSC
1S27	<i>metC3 tal-1 spo0J87</i>	BGSC
1S28	<i>trpC2 spo0K141</i>	BGSC
1A8 (QB935)	<i>trpC2 lys-1 aroD120</i>	BGSC
1A11 (QB123)	<i>trpC2 sacA321 ctrA1</i>	BGSC
UOT0531	<i>trpC2 leuA8 metB51 nonB1</i>	University of Tokyo
UOT0550	<i>trpC2 leuA8 metB51 nonB1 spo0FΔS sof-1</i>	University of Tokyo
HUSB1	<i>trpC2 leuA8 metB51 nonB1 spo0BΔB</i>	pUBSBΔB — ^{tf} → UOT0531
BR20	<i>trpC2 leuA8 metB51 nonB1 spo0BΔB sur0B20</i>	Spontaneous Spo ⁺ revertant of HUSB1 (this paper)
FR1	<i>trpC2 pheA1 spo0F221 sur0F1</i>	Spontaneous Spo ⁺ revertant of 1S19 (this paper)
KB8	<i>trpC2 lys-1 spo0BΔB</i>	HUSB1 — ^c → 1A8
KE8	<i>trpC2 lys-1 spo0E11</i>	1S17 — ^c → 1A8
KF8	<i>trpC2 lys-1 spo0F221</i>	1S19 — ^c → 1A8
KH8	<i>trpC2 lys-1 spo0H17</i>	1S22 — ^c → 1A8
KJ8	<i>trpC2 lys-1 spo0J87</i>	1S27 — ^c → 1A8
KK8	<i>trpC2 lys-1 spo0K141</i>	1S28 — ^c → 1A8
HU4160†	<i>trpC2 spo0E11 sur0B20</i>	BR20 — ^{td} → KE8
HU4170†	<i>trpC2 spo0F221 sur0B20</i>	BR20 — ^{td} → KF8
HU4180†	<i>trpC2 spo0H17 sur0B20</i>	BR20 — ^{td} → KH8
HU4190†	<i>trpC2 spo0J87 sur0B20</i>	BR20 — ^{td} → KJ8
HU4200†	<i>trpC2 spo0K141 sur0B20</i>	BR20 — ^{td} → KK8
HU5130†	<i>trpC2 spo0BΔB sur0F1</i>	FR1 — ^{td} → KB8
HU5160†	<i>trpC2 spo0E11 sur0F1</i>	FR1 — ^{td} → KE8
HU5180†	<i>trpC2 spo0H17 sur0F1</i>	FR1 — ^{td} → KH8
HU5190†	<i>trpC2 spo0J87 sur0F1</i>	FR1 — ^{td} → KJ8
HU5200†	<i>trpC2 spo0K141 sur0F1</i>	FR1 — ^{td} → KK8
HU6100†	<i>trpC2 aroD120 sur0B20</i>	BR20 — ^{td} → 1A8
HU6200†	<i>trpC2 aroD120 sur0F1</i>	FR1 — ^{td} → 1A8
BR201	<i>leuA8 metB51 nonB1 spo0BΔB sur0B20</i>	168 — ^{tf} → BR20
FR101	<i>pheA1 spo0F221 sur0F1</i>	168 — ^{tf} → FR1
<i>E. coli</i>		
JM105	$\Delta(lac-pro)$ <i>thi strA sbcB15 hsdR4 F'traD36 proAB lacPΔZΔM15</i>	J. Messing (University of Minnesota)

* tf, transformation; c, congression; td, transduction. For plasmid pUBSBΔB, see Fig. 1.

† *sur* traits (Spo⁺) were confirmed by back cross transduction to KB8. About half of the Lys⁺ transductants could sporulate on Schaeffer sporulation plates at 37 °C, indicating that the donor strains had the *sur* mutations.

associated with *spo0A* and *spo0B* mutations. However, mutations in *abrB* do not suppress the sporulation defect of *spo0A* strains (Trowsdale *et al.*, 1978, 1979).

Here we report attempts to isolate new suppressors of *spo0B* and *spo0F* mutations.

METHODS

Bacterial strains. The *Bacillus subtilis* and *Escherichia coli* strains used are listed in Table 1.

Plasmids and bacteriophages. The plasmids shown in Fig. 1(a), i.e. pCA0A-HB9, pCA0A-Xba and pCA0A-BE9, were derivatives of an integration vector pCA191 (K. Yamada, unpublished) which is a derivative of pUC19 containing the *cat* gene from pC194. This plasmid can replicate in *E. coli*, but not in *B. subtilis*. pCA0A-BE9 carries a truncated *spo0A* gene with its promoter. pCA0A-HB9 carries the complete coding region of the *spo0A* gene but does not have the *spo0A* promoter, and pCA0A-Xba lacks not only the *spo0A* promoter, but also an upstream part of the coding sequence. Another plasmid, pUBSBΔB, was constructed as shown in Fig. 1(b) from

Fig. 1. Schematic outlines of the construction of plasmids: (a) *pCA0A-BE9*, *pCA0A-HB9* and *pCA0A-Xba*, and (b) *pUBSBAB*. Symbols for the restriction endonucleases are: A, *AvaI*; B, *BclI*; Bg, *BglII*; Bm, *BamHI*; C, *Clal*; E, *EcoRI*; H, *HindIII*; Hc, *HincII*; X, *XbaI*. The open box indicates the *spo0A* gene. P_{OA} represents the promoter region of the *spo0A* gene. Arrows indicate open reading frames and their respective 5' to 3' orientations. Ap^r , Cm^r and Km^r indicate the genes of ampicillin, chloramphenicol and kanamycin resistance, respectively. Origins of DNA replication in *E. coli* or *B. subtilis* are shown as ori (○) or (●), respectively.

pUBSBAAval125 (Oda *et al.*, 1985). M13mp10 and M13mp11 phages were obtained from Amersham Japan.

Isolation of revertants from *spo0BΔB* and *spo0F221* mutants. *B. subtilis* strains HUSB1(*spo0BΔB*) and JH649(*spo0F221*) were inoculated into Schaeffer sporulation medium (Schaeffer *et al.*, 1963) to an initial density of 10 Klett units (no. 66 filter), and incubated for 24 h at 37 °C with shaking. The cells were then plated on NB (Nutrient Broth, Difco) plates after heating at 80 °C for 10 min. Spontaneous Spo^+ revertants were obtained after 2 d incubation at 37 °C.

Preparation of DNA. Chromosomal DNA was prepared by the method of Bott & Wilson (1967), and plasmid DNA was isolated by the alkaline extraction method of Birnboim & Doly (1979).

Transformation and transduction experiments. Transformation experiments were performed according to Bott & Wilson (1967), and PBS1 transduction was done by the method of Yamagishi & Takahashi (1968).

Fig. 2. Schematic diagram of the procedure for cloning of *sur* mutations. Plasmid pCA0A-BE9 was integrated into the chromosome of strains BR20 and FR1 (*Spo*⁺) and *Spo*⁺/Cm^R integrants were isolated. Chromosomal DNA was extracted, digested with *Eco*RI endonuclease, and ligated with T4 DNA ligase. *E. coli* JM105 was transformed with this DNA mixture and Cm^R transformants were selected. By this method, pSB205 and pSF111 were obtained from chromosomal DNA of BR20 and FR1 integrants, respectively.

Enzymes. All restriction enzymes were purchased from Toyobo Co. T4 DNA ligase was obtained from Nippon Gene Co. Enzymes were used according to the supplier's recommended assay procedures.

Cloning of *sur* mutations. Strains BR20 and FR1 carrying *sur* mutations were transformed to chloramphenicol resistance (Cm^R) with pCA0A-BE9. A mixture of Cm^R/*Spo*⁺ and Cm^R/*Spo*⁻ integrants was obtained. Chromosomal DNA (2 µg) from the Cm^R/*Spo*⁺ integrants was restricted with 10 units of *Eco*RI endonuclease for 7 h at 37 °C. After phenol extraction and ethanol precipitation, the digested DNA was religated with T4 DNA ligase and used to transform competent cells of *E. coli* JM105 to Cm^R. The Cm^R transformants harboured plasmids similar to pCA0A-BE9, except that the truncated portion of the *spo0A* gene now carried a *sur* mutation (see Fig. 2).

Nucleotide sequencing of *sur* mutations. Nucleotide sequencing was done by the dideoxy chain-termination method (Sanger *et al.*, 1977) with a sequencing kit obtained from Takara Shuzo Co. Isolated restriction fragments were cloned into the single-stranded DNA-cloning vectors M13mp10 and M13mp11; *E. coli* JM105 was used as host strain.

RESULTS

Isolation of suppressor mutations from *spo0B* and *spo0F* mutants

To facilitate the isolation of extragenic rather than intragenic suppressor mutations, we constructed a *spo0B* deletion mutant. First, we constructed a plasmid, pUBSBΔB, carrying the *spo0B* gene with a 41 bp deletion (Fig. 1b). Strain UOT0531, a highly competent strain constructed by Kawamura & Saito (1983), was then transformed with *Eco*RI-digested pUBSBΔB (1 µg) and incubated on Schaeffer sporulation plates. After 2 d incubation, seven

Table 2. Three-factor transduction crosses to determine the map position of the *sur0B20* and *sur0F1* mutations

Donor	Recipient	Selection	Recombinant classes*			No. of recombinants
			<i>trp</i>	<i>lys</i>	<i>sur</i> †	
BR201 (<i>sur0B20 spo0BΔB</i>)	KB8 (<i>trpC2 lys-1 spo0BΔB</i>)	Trp ⁺	1	1	1	10
			1	1	0	37
			1	0	1	0
			1	0	0	24
		Lys ⁺	1	1	1	13
			1	1	0	49
			0	1	1	15
			0	1	0	23
FR101 (<i>sur0F1 spo0F221</i>)	KF8 (<i>trpC2 lys-1 spo0F221</i>)	Trp ⁺	1	1	1	18
			1	1	0	33
			1	0	1	3
			1	0	0	20
		Lys ⁺	1	1	1	18
			1	1	0	33
			0	1	1	22
			0	1	0	26

* Donor and recipient markers are denoted by 1 and 0, respectively.

† *sur* traits (Spo⁺) were tested by incubating Trp⁺ or Lys⁺ transformants on Schaeffer sporulation plates at 37 °C for 2 d.

Spo⁻ colonies were obtained, without any selection, at a frequency of about 2×10^{-3} . The presence of the *spo0B* deletion mutation in one of these Spo⁻ colonies (HUSB1) was confirmed by its linkage to the *pheA1* marker using PBS1 transduction. This strain was used for the isolation of Spo⁺ revertants.

Spontaneous suppressor mutants were isolated at a frequency of 10^{-8} from strains HUSB1 (*spo0BΔB*) and JH649 (*spo0F221*). The sporulation frequency of various Spo⁺ revertants ranged from 10^4 to 10^8 spores ml⁻¹. Among these revertants carrying putative suppressor mutations (*sur*), BR20 (*spo0BΔB sur0B20*) and FR1 (*spo0F221 sur0F1*) were used for genetic analysis because of their high sporulation frequency (see Table 4).

To confirm whether the revertants still possessed the original *spo0* mutations, PBS1 transduction was done using strains BR20 and FR1 as donors. The *spo0B* and *spo0F* loci are about 98% cotransduced with *pheA1* and *ctrA1*, respectively (Piggot & Coote, 1976). Accordingly, JH642 (*trpC2 pheA1*) and QB123 (*sacA321 ctrA1 trpC2*) were used as recipients and *pheA*⁺ and *ctrA*⁺ transductants were selected. About 98% of these transductants were Spo⁻, indicating that BR20 and FR1 still possess their respective *spo0B* and *spo0F* mutations.

Mapping of the *sur* mutations of BR20 and FR1

To identify the chromosomal location of the newly isolated suppressor mutations, *sur0B20* and *sur0F1*, we performed PBS1 transduction crosses with BR20 and FR1 as donors and various auxotrophs carrying the *spo0BΔB* or *spo0F221* mutation as recipients. These were constructed from the kit of strains designed for genetic mapping by Dedonder *et al.* (1977). Among various markers tested, only *lys-1* was cotransduced with *sur* (Spo⁺). Table 2 shows the results of three-factor crosses with BR201 or FR101 as donor and KB8 or KF8 as recipient. The results indicate that both *sur0B20* and *sur0F1* are located on the side of *lys-1* distal to *trpC2*. The cotransduction frequencies between *sur0B20* (*sur0F1*) and *lys-1* and *sur0B20* (*sur0F1*) and *trpC2* were 28% (40%) and 14% (28%), respectively.

These results suggest that the *sur* mutations may be similar to *sof-1* or *rvtA11*, which were previously isolated using *spo0F* mutants (Kawamura & Saito, 1983; Sharrock *et al.*, 1984).

Table 3. Identification of the *sur* mutations of BR20 and FR1 as *spo0A* alleles by using an integrational plasmid

Donor	Recipient	No. of integrants*	
		Cm ^R Spo ⁺	Cm ^R Spo ⁻
pCA0A-HB9	BR20 (<i>sur0B20 spo0BΔB</i>)	373	130
	FR1 (<i>sur0F1 spo0F221</i>)	440	32
	UOT0550 (<i>sof-1 spo0FΔS</i>)	288	126
	UOT0531 (<i>spo</i> ⁺)	550	0

* Integrants were selected on Schaeffer sporulation plates containing chloramphenicol (5 µg ml⁻¹).

Table 4. Effect of *sur0B20* and *sur0F1* suppressor mutations on the sporulation frequency of various *spo0* mutants

All strains were grown in Schaeffer medium and sporulation frequency was determined after 24 h incubation at 37 °C. The wild-type strain gave 5.0×10^8 spores ml⁻¹.

Mutant	Relevant genotype	No. of spores ml ⁻¹
HU6100	<i>sur0B20</i>	1.9×10^8
HU6200	<i>sur0F1</i>	1.2×10^8
HUSB1	<i>spo0BΔB</i>	<10
BR20	<i>spo0BΔB sur0B20</i>	2.2×10^8
HU5130	<i>spo0BΔB sur0F1</i>	1.5×10^8
1S17	<i>spo0E11</i>	1.3×10^5
HU4160	<i>spo0E11 sur0B20</i>	9.1×10^8
HU5160	<i>spo0E11 sur0F1</i>	1.4×10^7
1S19	<i>spo0F221</i>	<10
HU4170	<i>spo0F221 sur0B20</i>	4.1×10^4
FR1	<i>spo0F221 sur0F1</i>	7.4×10^7
1S22	<i>spo0H17</i>	<10
HU4180	<i>spo0H17 sur0B20</i>	<10
HU5180	<i>spo0H17 sur0F1</i>	<10
1S27	<i>spo0J87</i>	1.2×10^6
HU4190	<i>spo0J87 sur0B20</i>	2.4×10^6
HU5190	<i>spo0J87 sur0F1</i>	2.8×10^6
1S28	<i>spo0K141</i>	3.0×10^5
HU4200	<i>spo0K141 sur0B20</i>	1.7×10^6
HU5200	<i>spo0K141 sur0F1</i>	1.4×10^8

Identification of *sur* mutations as *spo0A* alleles

Since the *sof-1* mutation is a missense mutation in the *spo0A* coding sequence (Hoch *et al.*, 1985), we constructed an integrational plasmid pCA0A-HB9 (Fig. 1) to test whether the *sur* mutations are also *spo0A* alleles. This plasmid, which carries the entire *spo0A* coding sequence but lacks the *spo0A* promoter, was used as donor to transform strains BR20 and FR1. Plasmid integrants of each revertant were selected on Schaeffer sporulation medium containing chloramphenicol (5 µg ml⁻¹). If the *sur* mutations are *spo0A* alleles, a mixture of Spo⁺ and Spo⁻ transformants will be obtained, depending upon whether the *sur* mutation is incorporated into the intact, or into the promoter-less, copy of *spo0A*, respectively. As expected, a portion of the integrants gave Spo⁻ colonies (Table 3). This result indicated that both *sur0B20* and *sur0F1* are *spo0A* alleles. Furthermore, when plasmid pCA0A-Xba was used as donor, no Spo⁻ integrants were obtained (data not shown). This result indicates that the *sur* mutations are located upstream from the *Xba*I site in the *spo0A* gene (see Fig. 1).

Effect of suppressor mutations on the sporulation of *spo0* mutants

Table 4 shows the suppressor activity of *sur0B20* and *sur0F1* on the sporulation of various *spo0* mutants. The phenotype of strains harbouring either *sur* mutation in the absence of *spo0BΔB* or

Fig. 3. Autoradiograph of a portion of the sequencing gel for *surOB20* and *surOF1*. Arrows indicate the base alterations in the *surOB20* and *surOF1* mutation sites.

Fig. 4. Base alterations and amino acid changes associated with the *surOB20* and *surOF1* mutations in the amino-terminal region of the *spoOA* gene. The deduced Shine-Dalgarno sequence is underlined.

spo0F221 is *Spo*⁺. However, these *sur* mutations exhibit various suppressor activities not only on the *spo0B* and *spo0F* mutations, but also on other *spo0* mutations. *sur0B20* restored sporulation of *spo0B* and *spo0E* mutants almost completely and to a lesser extent that of a *spo0F* mutant, but it did not restore sporulation of *spo0H*, *spo0J* or *spo0K* mutants. In contrast, *sur0F1* restored sporulation of *spo0B*, *spo0F* and *spo0K* mutants almost completely, but not that of *spo0H* or *spo0J* mutants. These results indicate that *sur0B20* and *sur0F1* are different suppressors, although both are *spo0A* alleles.

Cloning and nucleotide sequencing of sur mutations

To determine the nucleotide sequence of *sur* mutations, plasmids pSB205 and pSF111, carrying *surOB20* and *surOF1*, respectively, were constructed as described in Methods (Fig. 2). These plasmids could transform strain HUSB1 (*spo0BΔB*) to Spo⁺, indicating that they carry *sur* mutations.

Fig. 3 shows the results of the nucleotide sequencing of the *sur* mutations. These results indicate that, as suggested by the results in Table 4, *surOB20* and *surOF1* are different missense mutations in the 14th codon [GAG (Glu) to GTG (Val)] and the 12th codon [AAT (Asn) to AAG (Lys)] of the *spo0A* gene, respectively (Fig. 4). The *surOB20* mutation is a new suppressor

mutation whereas *surOF1* is identical to the *sof-1* mutation isolated previously (Kawamura & Saito, 1983).

DISCUSSION

In studying the sporulation process in *B. subtilis*, cloning and characterization of sporulation genes has proved to be very important. In addition, isolation and characterization of second-site suppressors of sporulation mutations is an excellent means of grouping sporulation genes into functionally related classes (Hoch *et al.*, 1985). So far, several suppressors have been found and they have revealed unexpected interactions among *spo0* genes. Analysis of the *sof-1* and *rvtA11* suppressors, which are *spo0A* alleles, has raised the possibility that the *spo0A* gene product may interact with *spo0B*, *spo0E* and *spo0F* gene products at the initiation of sporulation. Other mutations, such as *crsA* (Takahashi, 1979; Sun & Takahashi, 1982), which confers catabolite-resistant sporulation and is an allele of *rpoD*, restore the defective sporulation of *spo0K* and *spo0J* mutants to the wild-type level and partially restore the defective sporulation of *spo0B* and *spo0F* mutants (Kawamura *et al.*, 1985; Leung *et al.*, 1985). These results suggest that several *spo0* gene products interact with RNA polymerase sigma-factor (σ^A).

In our experiments, suppressor mutations of *spo0BΔB* and *spo0F221* were isolated and mapped in the *spo0A* region by PBS1 transduction (Table 2) and they were confirmed as *spo0A* alleles using pCA0A-HB9 (Table 3). Nucleotide sequencing of the *sur* mutations revealed missense changes in the 12th or 14th codon of the *spo0A* gene. The *surOB20* mutation led to a glutamic acid to valine substitution at the 14th codon, whereas the *surOF1* mutation led to an asparagine to lysine substitution at the 12th codon. The difference in mutational sites between *surOB20* and *surOF1* may account for their different suppressor activities on various *spo0* mutations (Table 4). The change of asparagine to lysine at the 12th codon is the change of an uncharged amino acid to a positively charged amino acid, and the change of glutamic acid to valine at the 14th codon is the change of a negatively charged amino acid to an uncharged hydrophobic amino acid. These alterations of amino acids may cause conformational changes in the N-terminal region of the Spo0A protein and result in the alteration of interactions between this protein and other Spo0 proteins. On the other hand, *surOF1* and *sof-1* are identical mutations, but their suppressor activities on *spo0F* mutations are slightly different: the *sof-1* mutation restores sporulation of the *spo0FΔS* mutant to the wild-type level (2.4×10^8 spores ml⁻¹), whereas *surOF1* only partially restores sporulation of the *spo0F221* mutant (7.4×10^7 spores ml⁻¹). This may be due to differences in genetic backgrounds, since strains UOT0550 (*sof-1*) and FR1 (*surOF1*) show different colony morphology. It is also possible that *spo0F221* is a missense mutation, and that the resulting abnormal Spo0F protein may inhibit the sporulation of strain FR1.

According to the model of two-component signal transduction systems, sensor proteins that are sensitive to environmental stimuli interact with regulator proteins (Nixon *et al.*, 1986). In *E. coli*, such two-component systems are known for osmolarity control (EnvZ/OmpR), nitrogen limitation (NtrB/NtrC), toxic compounds (CpxA/SfrA) and phosphate limitation (PhoR/PhoB). Regulator proteins such as OmpR, NtrC, SfrA or PhoB are homologous with the Spo0A protein in the N-terminal region. The sensor proteins, EnvZ, NtrB, CpxA and PhoR, interact with the N-terminal domains of the regulators (Ronson *et al.*, 1987). This situation resembles the Spo0B, Spo0F/Spo0A system. However, the two-component model may not be directly applicable to the Spo0B, Spo0F/Spo0A system, because sensor proteins are transmembrane proteins (except for NtrB protein in *E. coli*) but Spo0B and Spo0F proteins are localized in the cytoplasm, and there is no homology in amino acid sequence between Spo0B (Spo0F) and sensor proteins. We are now purifying the Spo0A, Spo0B and Spo0F proteins, to elucidate the interactions among them.

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