JOURNAL OF VIROLOGY, Apr. 1982, p. 131–134
0022-538X/82/040131-04$02.00/0

Vol. 42, No. 1

Correlation of the Genetic Map and the Endonuclease Site Map of *Bacillus subtilis* Bacteriophage SP02

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By marker rescue of bacteriophage SP02 *sus* mutants with purified bacteriophage SP02 DNA fragments, 11 of the 17 known bacteriophage SP02 *sus* loci were assigned to discrete DNA fragments. The left-most genetic locus, *susA*, was found to reside near one bacteriophage SP02 terminus (*Eco*RI-C1 fragment), whereas the right-most genetic locus, *susP*, was found to reside near the other bacteriophage SP02 terminus (*Eco*RI-C2 fragment). The physical locations of the intervening genetic loci were found to be consistent with the previously determined genetic order. Evidence was also obtained which suggested that at least one end of a transforming DNA fragment is degraded during DNA uptake by the competent bacterium.

SP02 is a temperate bacteriophage whose normal host is *Bacillus subtilis* 168. The *Bgl*I, *Bgl*II, *Eco*RI, *Sac*I, *Sal*I, *Sma*I, and *Xba*I endonuclease sites have been located within the circular 23-megadalton (Md) SP02 genome (9; Yoneda et al., Gene, in press). There is also a genetic map of bacteriophage SP02 based on the analysis of various *sus* loci (8). Yasunaka et al. (8) identified 17 essential genes by complementation and ordered them in a unique linear sequence by two-factor crosses. Of these genes, only *susL* has an identified function, that of a bacteriophage SP02-specific DNA polymerase (4). In this communication we report the correlation of the bacteriophage SP02 genetic map to the bacteriophage SP02 endonuclease site map by determination of the locations of specific *sus* loci within discrete bacteriophage SP02 DNA fragments.

(A preliminary report of these experiments has appeared in abstract form [S. Graham and Y. Yoneda, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, S42, p. 247].)

MATERIALS AND METHODS

Bacteria and bacteriophage strains. The strains of *B. subtilis* and bacteriophage SP02 used in this study are described in Table 1. *B. subtilis* strain BR151 is a highly competent strain which is nonpermissive for growth of the bacteriophage SP02 *sus* mutants. *B. subtilis* TV173, which carries a suppressor originally described by Georgopoulous (1), is permissive for growth of the bacteriophage SP02 *sus* mutants. All of the bacteriophage SP02 *sus* strains used were those originally described by Yasunaka et al. (8) and were generously provided for this study by S. Okubo.

Growth of bacteria and bacteriophage. The *B. subtilis* strains were grown, lysogens were made and induced, and stocks were stored as previously described (7, 9).

Isolation of DNA fragments. Bacteriophage SP02 DNA was isolated and treated with endonuclease, and the resulting DNA fragments were separated by agarose gel electrophoresis (9). DNA bands were excised from the agarose gel, and the DNA fragments were extracted from the agarose either by "squeezing" (9) or by electroelution into a dialysis membrane (6). Although both methods yielded biologically active DNA, the latter method was more rapid and gave a higher yield of DNA. DNA fragments were religated with T4 DNA ligase by the procedure of Tanaka and Weisblum (5).

Marker rescue. *B. subtilis* strain BR151 was grown to competence (5). Bacteriophage SP02 DNA (1 μg) was added to 2×10^8 colony-forming units of the competent cells along with 10^9 PFU of one of the bacteriophage SP02 *sus* mutants. The mixture was incubated for 60 min at 37°C. Infectious centers were assayed by using BR151 as an indicator (6).

RESULTS

Marker rescue of conditionally lethal mutations. (i) *Eco*RI fragments. The four unique bacteriophage SP02 DNA *Eco*RI cleavage fragments, *Eco*RI-A, *Eco*RI-B, *Eco*RI-C1C2, and *Eco*RI-D, were isolated from the gels, and each was tested for its ability to rescue various genetic markers present in 11 of the 17 mapped phage complementation groups (Table 2). The *Eco*RI-D fragment rescued the bacteriophage SP02 *sus*B and *sus*C mutants. The large *Eco*RI-A fragment, equal to ~70% of the bacteriophage SP02 genome, rescued bacteriophage SP02 mutants *susD*, *susG*, *susH*, *susI*, and *susK*. The *Eco*RI-B fragment rescued bacteriophage SP02 mutants *susL*, *susN*, and *susP*. The *Eco*RI-C1C2

TABLE 1. Bacterial strains and bacteriophage used

Strain	Genotype
BR151	*trpC2 lys3 metB10*
TV173	*trpC2 metB10 sup3*
SP02 43	*susA*
SP02 299	*susB*
SP02 293	*susD*
SP02 237	*susG*
SP02 214	*susH*
SP02 338	*susI*
SP02 39	*susK*
SP02 244	*susL*
SP02 208	*susN*
SP02 121	*susP*

fragment did not rescue any of the tested mutants. The SP02 *susA* mutant was not rescued by any of the *Eco*RI fragments (singly or in combination). However, ligation of an additional fragment of DNA to the *Eco*RI-C1 fragment, regardless of the source of the additional DNA (e.g., *Escherichia coli* plasmid pBR322), gave rescue of bacteriophage SP02 *susA*. The results presented in Table 2 suggested a DNA fragment order of C1C2-D-A-B with *susA* located in *Eco*RI-C1 and *susP* located in *Eco*RI-B. This order was in perfect agreement with the established order of the bacteriophage SP02 *sus* markers and with the bacteriophage SP02 *Eco*RI fragments (Fig. 1).

(ii) *Bgl*I fragments. To correlate more precisely the bacteriophage SP02 genetic map with the bacteriophage SP02 endonuclease site map, the six unique bacteriophage SP02 *Bgl*I fragments A, B, C, F, G, and I, as well as the composite DE fragment, were used to rescue the bacteriophage SP02 *sus* mutants (Table 3). The *Bgl*I-C fragment rescued bacteriophage SP02 *susA*, *susB*, and *susC*. The small *Bgl*I-G fragment (0.58 Md) rescued the *susG* mutant. The *Bgl*I-B fragment rescued bacteriophage SP02 *susH* and *susI*, and the *Bgl*I-A fragment rescued bacteriophage SP02 *susK* and *susL*. The composite *Bgl*I-DE fragment rescued the bacteriophage SP02 *susN* and *susP* markers. The bacteriophage SP02 *susD* marker was not rescued by any of the *Bgl*I fragments. The *Bgl*I fragments I and F did not contain any of the tested bacteriophage SP02 *sus* loci. These results suggested a *Bgl*I fragment order of G-C-B-A-(DE), with *susA* located in the *Bgl*I-C fragment and *susP* located in the *Bgl*I-DE fragment. This order was in perfect agreement with the established order of the bacteriophage SP02 *Bgl*I fragments and with the relationship of *Bgl*I fragments to *Eco*RI fragments (Fig. 1). Both the *Eco*RI-B fragment and the *Bgl*I-A fragment rescued the *susL* mutant. Therefore, *susL* was located within the 0.75-Md overlap of fragments *Eco*RI-B and *Bgl*I-A (Fig. 1).

(iii) *Sac*I fragments—location of *susH*, *susI*, and *susK*. Both the *susH* and *susI* mutants were rescued by the large *Eco*RI-A fragment and the *Bgl*I-B fragment. Thus, *susH* and *susI* were located within the 4.11-Md overlap region of fragments *Eco*RI-A and *Bgl*I-B (Fig. 1). Both *susH* and *susI* were more precisely located by rescuing the mutants with bacteriophage SP02 *Sac*I fragments which overlapped the *Bgl*I-B fragment (Table 4). The *Sac*I-A fragment rescued both *susH* and *susI*. Therefore, *susH* and *susI* were located within the 2.55-Md overlap of fragments *Bgl*I-B and *Sac*I-A (Fig. 1).

The bacteriophage SP02 *susK* mutant was rescued both by the *Eco*RI-A fragment and by the *Bgl*I-A fragment. Therefore, *susK* was located within the 3.36-Md overlap of fragments *Eco*RI-A, *Bgl*I-A, and *Sac*I-C.

DISCUSSION

The common junction between bacteriophage SP02 DNA fragments *Eco*RI-C1 and *Eco*RI-C2,

TABLE 2. Marker rescue of conditionally lethal mutations with isolated *Eco*RI-generated fragments of bacteriophage SP02 DNA[a]

*Eco*RI fragment (Md)	No. of infectious centers (plaques/ml) in the following *sus* mutant:										
	A	*B*	*C*	*D*	*G*	*H*	*I*	*K*	*L*	*N*	*P*
C1C2 (1.62)	0	0	0	70	0	10	2,000	0	0	0	0
D (0.66)	0	**2,000**	**2,200**	40	0				0		0
A (16.04)	0	100	0	**1,900**	**550,000**	**200,000**	**520,000**	**390**	0	380	450
B (4.74)	0	560	0	130	68,000	12,000	22,000	10	**180**	**17,000**	**20,000**
C1C2 + A	0										
C1C2 + D	0										
C1C2D	600										
C1D	170										
C1 pBR322	1,000										
C1C2 + pBR322	0										

[a] These values have been corrected for reversion rates obtained from control infections with no added bacteriophage SP02 DNA fragments.

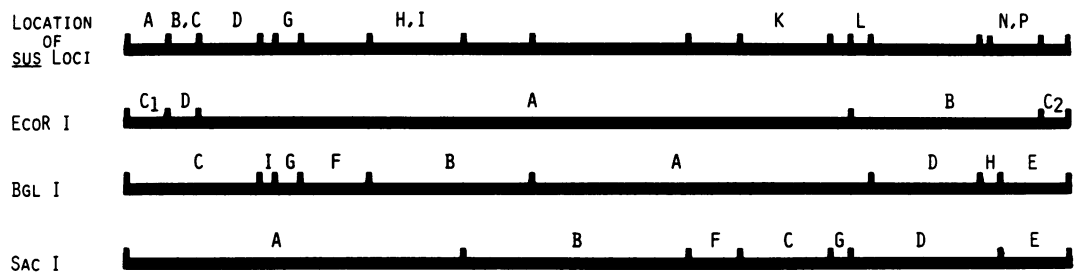

FIG. 1. Physical location of the SP02 *sus* loci. The various SP02 loci were located with specific SP02 DNA fragments (top line, the letters refer to the SP02 *sus* loci) as described in the text. The other three lines locate the *Eco*RI, *Bgl*I, and *Sac*I endonuclease sites within the SP02 genome (the letters refer to the appropriate endonuclease-generated DNA fragment). The SP02 map has been linearized for ease of comparison.

*Bgl*I-C and *Bgl*I-E, and *Sac*I-A and *Sac*I-E is heat labile (8; Yoneda et al., in press). The physical data suggested that this shared heat-labile junction was the point at which the bacteriophage SP02 chromosome circularized (Yoneda et al., in press). The locations of the bacteriophage SP02 *susA* and *susP* loci on the bacteriophage SP02 endonuclease site maps (Fig. 1) also suggested that the bacteriophage SP02 chromosome circularized at the junction of *Eco*RI-C1 and *Eco*RI-C2 and of *Bgl*I-C and *Bgl*I-DE. The *Eco*RI-C1 fragment contained one end of the genetic map (*susA*), whereas the *Eco*RI-B fragment contained the other end (*susP*). The intervening genetic loci (*susB*, *susC*, etc.) were aligned within the intervening *Eco*RI-D and *Eco*RI-A fragments. Thus, both physical and genetic analysis suggested that the heat-labile junction is the point of bacteriophage SP02 chromosome circularization.

Although the *Eco*RI-C1 fragment contained the *susA* locus, the *Eco*RI-C1 fragment did not rescue *susA*. However, ligation of an additional segment of DNA from any source to the *Eco*RI-C1 fragment resulted in efficient rescue of *susA*. It has been postulated that during DNA uptake by competent *B. subtilis* cells, a stretch of nucleotides is removed from the end(s) of the DNA molecule (3). Apparently, the additional DNA ligated to the *Eco*RI-C1 fragment prevented the degradation from reaching the *susA* locus. This interpretation implied that the *susA* locus was located quite close to the end of the *Eco*RI-C1 fragment.

There are regions of the bacteriophage SP02 chromosome which contain no essential genetic information (2). The *susH* and *susI* loci were located in the *Sac*I-A fragment. The *susK* locus was located in the *Sac*I-C fragment. A deletion containing 0.54 Md of *Sac*I-F and 1.5 Md of *Sac*I-C had no effect on *susK* (data not shown). Therefore, *susI* and *susK* were separated by a minimum of 7.1 Md of DNA. Yasunaka et al. (8) found only two genetic loci, *susJ* and a locus designed C_1, between *susI* and *susK*. Their mapping data suggested that *susJ* and C_1 were well separated from each other and from *susI* and *susK*. Unfortunately, both *susJ* and C_1 were too unstable in our hands for their positions to be fixed by the marker rescue procedure. Whereas the scarcity of genetic markers suggests that a large portion of this region may be genetically silent, deletion mutants have been isolated only in the area of the *Sac*I-F and *Sac*I-C junction.

The correlation of the SP02 *sus* loci map to the

TABLE 3. Marker rescue of conditionally lethal bacteriophage SP02 mutations with isolated *Bgl*I-generated fragments of bacteriophage SP02 DNA[a]

*Bgl*I fragment (Md)	No. of infectious centers (plaques/ml) in the following *sus* mutants:										
	A	*B*	*C*	*D*	*G*	*H*	*I*	*K*	*L*	*N*	*P*
C (3.12)	**9,000**	**83,000**	**50,000**	0	0						
I (0.45)	0	80	70	0	40						
G (0.58)		70	10	30	1,200	40	770				
F (1.62)		380	60	10	0	40	140				
B (4.11)						**39,000**	**360,000**	550	140	120	1,000
A (8.31)					0	1,000	7,000	**6,400**	**1,200**	0	200
DE (4.86)	120								0	**7,700**	**10,000**

[a] These values have been corrected for reversion rates obtained from control infections with no added bacteriophage SP02 DNA fragments.

TABLE 4. Marker rescue of conditionally lethal mutations with isolated *Sac*I-generated fragments of bacteriophage SP02 DNA[a]

*Sac*I fragment (Md)	No. of infectious centers (plaques/ml) in the following *sus* mutants:					
	H	*I*	*K*	*L*	*N*	*P*
A (8.23)	335,000	7,700	0	0		
B (4.05)	1,650	65	0	0		
F (1.55)						
C (3.36)			1,800	0		
D (3.12)				420	140	0
E (2.81)			0	0	1,440	1,140

[a] These values have been corrected for reversion rates obtained from control infections with no added bacteriophage SP02 DNA fragments.

circular bacteriophage SP02 endonuclease site map located several *sus* loci within DNA fragments of suitable size for DNA cloning and DNA sequencing. For example, the *susA* locus was within the 0.78-Md *Eco*RI-C1 fragment, the *susB* and *susC* loci were within the 0.58-Md *Bgl*I-G fragment, and the *susL* locus was within the 0.75-Md overlap of fragments *Bgl*I-A and *Eco*I-B. A comparison of the wild-type DNA sequence to the DNA sequence of the corresponding *sus* loci might reveal the molecular mechanism of suppression by the *B. subtilis sus* locus.

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