Isolation and Properties of Suppressor-Sensitive Mutants of *Bacillus subtilis* Bacteriophage SPO2

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Suppressor-sensitive mutants of *Bacillus subtilis* phage SPO2 were isolated and classified into 18 cistrons. A linear genetic map was constructed. The genes controlling early functions of phage development were clustered near the CI gene which may produce phage repressor.

The attachment sites of SPO2 and $\phi 105$, temperate bacteriophages of *Bacillus subtilis*, on the bacterial chromosome were recently reported (4, 9). By using a suppressor mutant of *B. subtilis* recently found by Okubo and Yanagida (6), we isolated suppressor-sensitive (*sus*) mutants (1a) of SPO2. This report presents the tentative mapping and describes some properties of the mutants.

The strains used were HA101 as the non-permissive host (sup^-) and HA101B as the permissive host (sup^+ ; reference 6). Sixty-two *sus* mutants were isolated from phages grown in the presence of *N*-methyl-*N'*-nitro-*N*-nitroso-guanidine. One of these *sus* mutants (c100) forms clear plaques on HA101 but forms turbid plaques on HA101B. Presumably, this mutational site resides in the gene producing a repressor substance of SPO2. We shall refer to this gene by the tentative designation, CI. No clear-plaque mutant which complements the CI mutant for lysogenization has thus far been obtained; however, CI^{ts} mutants which may produce temperature-sensitive repressor, as in the case of coliphage λ (3, 5), were isolated (Takano, Yasunaka, and Horiuchi, *unpublished data*).

The remaining sixty-one *sus* mutants were classified by complementation testing into 18 cistrons, A through R. Two-factor crosses among *sus* mutants belonging to cistrons A through P were performed. No ultraviolet irradiation was used in these crosses. These mutants were arranged by increasing recombination frequencies. Tentative mapping is shown in Table 1.

The CI gene appears to be located in the central portion of the map; 10 cistrons fell in the left half of the map and 6 cistrons in the right half. There were approximately 15% wild-type recombinants in the cross between *sus*A43 and *sus* P121. This result suggests the genetic map of SPO2 to be linear, although the deoxyribonucleic (DNA) molecule of SPO2 could be circularized by heating and annealing (8). We have not yet mapped two cistrons, Q and R, because of difficulty in the preparation of high-titer lysate.

Next, the genes relating to phage-specific DNA synthesis were studied. Ultraviolet-irradiated *B. subtilis* $\text{thy}^- \text{uvr}^- \text{sup}^-$ (7) was infected with each *sus* mutant, and ³H-thymidine was simultaneously added to the culture. The radioactivity incorporated into DNA during 120 min of incubation was measured. No detectable DNA synthesis was observed upon infection by *sus*K, *sus*L, or *sus*M, and bacteria infected with *sus*J synthesized less than 10% of the DNA made by cells infected with wild-type phage (Table 1). Other *sus* mutants synthesized nearly normal amounts of DNA as compared with wild-type phage. Phage heads or tails cannot be detected in the lysate of nonpermissive host infected by *sus*J, *sus*K, *sus*L, and *sus*M by electron microscopy (Yasunaka, Amako, Takeya, and Horiuchi, *unpublished data*). The cistrons J through M, and clustered near the CI gene, control early functions.

Our data show that *B. subtilis* phage SPO2 is quite similar to coliphage λ in its genetical arrangement (2). In this connection, it is interesting that a phage SPO2 is so much like phage λ in the relative size, shape, and the amount of DNA (1, 2a, 2b).

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TABLE 1. Genetic map of the *sus* mutants of SPO2

Determination	Cistron															
	A	B	C	D	E	F	G	H	I	J	CI	K	L	M	N	P
	1.6 ^a	0.7	1.1	0.1	0.4	0.7	1.3	2.7	2.5	0.4	2.0	2.4	1.0	4.0	0.6	3.1
Mutational site.....	43	299	318	293	250	404	237	10	365	385	c100	39	222	12	208	252
No. of <i>sus</i> mutants isolated.	1	3	1	1	1	1	2	13	4	2	1	3	8	1	2	3
DNA synthesis in nonpermissive host ^b	1.5	1.9	0.9	0.9	2.2	1.1	0.8	1.4	1.2	0.08	1.5	-0.03	-0.04	-0.1	2.5	2.0

^a Recombination frequency between the mutational site of the adjacent cistrons, expressed as twice the percentage of *sus*⁺ recombinants in the total number of plaques.

^b DNA synthesis: the radioactivity incorporated by noninfected cells was about 250 counts per min per ml and the radioactivity incorporated by wild-type phage-infected cells was about 1,500 counts per min per ml in the several experiments. The subtracted value in each experiment was set at 1, and the other values were expressed as relative to this value.

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Index to Date of Issue

Month	Date of Issue	Pages
January	1/25/70	1-98
February	2/17/70	99-274
March	3/26/70	275-412
April	4/17/70	413-540
May	5/15/70	541-662
June	6/19/70	663-822