NOTE

Bacillus subtilis Bacteriophage SP01, SP82, and ϕ e Require Host Lysyl- and Tryptophanyl-tRNA Synthetases for Phage Development

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Infection of *Bacillus subtilis* mutants having temperature-sensitive lysyl- or tryptophanyl-tRNA synthetases with bacteriophage SP01, SP82, or ϕ e indicates that both host enzymes are essential for phage development. No apparent modification of the temperature-sensitive phenotype of the mutant host enzymes occurs during phage infection.

The aminoacyl-tRNA synthetases are integral components of a cell's protein-synthesizing machinery (8). Modification or take-over of any of these host enzymes during bacteriophage infection, or the phage-directed synthesis of a new aminoacyl-tRNA synthetase, would offer an extremely useful device for phage control of the host's protein-synthesizing system. Alternatively, it is of interest to know the extent to which bacteriophage depend upon this class of host enzymes for their own development. T₄ phage infection of *Escherichia coli* is accompanied by a phage-directed modification in the host valyl-tRNA synthetase (3, 5). This observation initially suggested a regulatory role for the modified aminoacyl-tRNA synthetase. However, the absence of marked changes in enzymological properties of the modified enzyme (5), coupled with the isolation of phage mutants which could develop normally despite a failure to carry out the conversion (7), indicated that the enzyme modification had little significance for phage development. A study of several *E. coli* aminoacyl-tRNA synthetases (4, 6) revealed that the host enzymes were essential for phage development but that only the valyl-tRNA synthetase was altered during infection.

We investigated the roles of the tryptophanyl- and lysyl-tRNA synthetases during SP01, SP82, and ϕ e bacteriophage infection of *Bacillus subtilis* (2). To determine whether these enzymes were essential for phage development, and if new or modified host aminoacyl-tRNA synthetase activity appeared during phage in-

fection, we employed mutants of *B. subtilis* which have altered, temperature-sensitive, lysyl- and tryptophanyl-tRNA synthetase activities (F. Racine and W. Steinberg, unpublished data; 10). The experiments were based on the assumption that phage modification of an existing aminoacyl-tRNA synthetase, or phage specification of a new enzyme, would result in the loss of the host's temperature-sensitive phenotype (inhibition of protein synthesis at 42 C); phage development could then proceed at what was previously a restrictive temperature. Failure to produce the change would block maturation and hence indicate the obligatory requirement of the phage for the host enzyme.

Phage maturation during one-step growth (1) at the permissive (30 C) and restrictive (42 C) temperatures was assayed by testing for intracellular and total (released) phage. The bacterial strains used and their genotypes are listed in Table 1. Phage stocks were prepared from infected host cells grown at 37 C in Difco antibiotic medium no. 3. After cell lysis, the debris was removed by centrifugation at $10,000 \times g$ for 10 min, and the supernatant fluid was centrifuged at $17,000 \times g$ for 1.5 h. The resulting phage pellet was suspended overnight (at 4 C) without agitation, in 20 ml of TM buffer [0.1 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.2, containing 5×10^{-3} M $MgCl_2$]. The centrifugation steps were repeated, and the phage pellet was suspended in 5 ml of TM buffer for every 500 ml of lysate.

Host cells for one-step growth curves were grown in Spizizen minimal glucose (MG) medium (9). Phage and cells were titrated immedi-

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TABLE 1. *Effect of temperature and host strain on the burst size and latent period of B. subtilis phages SP01, ϕ e, and SP82^a*

Host	Host genotype	Phage	Latent period (min)		Burst size ^b	
			30 C	42 C	30 C	42 C
168M	<i>trpC2</i>	SP01 ϕ e	49	40	59	12
			72	47	25	18
VB167	<i>trpC2</i> , <i>lys-1PR13</i>	SP82	77	45	48	13
GSY1307	<i>trpC2</i> , <i>trpS1</i>	SP01	67	np ^c	170	0
		ϕ e	80	np	20	0
		SP82	70	np	20	0
VB122	<i>lys-1PR13</i> , <i>lysS1</i>	SP01	66	np	50	0
		ϕ e	80	np	11	0
		SP82	90	np	25	0

^a Data are derived in part from Fig. 1 and 2.

^b The burst size was the phage titer after the burst divided by the number of infected cells. The number of infected cells was calculated from a Poisson distribution since the multiplicity of infection was known.

^c Nonpermissive.

ately before adsorption. Unadsorbed phage titer was determined either by filtering a 1-ml sample through a nitrocellulose membrane filter (0.45- μ m pore size, Millipore Corp., Bedford, Mass.), or by vigorously mixing 1.0 ml with one drop of CHCl_3 . Titrations employed the soft-agar overlay method (1) with SPNA medium which contained the following (per liter): Difco nutrient broth, 8 g; KCl, 1 g; and agar, either 15 g for the basal layer or 7 g for the overlay. Intracellular phage was assayed by mixing 1.0-ml samples with 100 μ g lysozyme in the presence of 0.3 M NaN_3 . After 5 min at 37 C, sodium dodecyl sulfate was added to a concentration of 0.01%, and an appropriately diluted volume was mixed with soft agar for titration. The procedure had no effect on mature phage.

L-Amino acids (A grade) and chloramphenicol were obtained from Calbiochem (Los Angeles, Calif.). Lysozyme (eggwhite, three times crystallized, 18,000 U/mg) was obtained from Sigma Chemical Co. (St. Louis, Mo.). L-[¹⁴C]lysine (312 mCi/mmol) was purchased from Schwarz-Mann (Orangeburg, N.Y.), and L-[¹⁴C]tryptophan (side chain label, 17.1 mCi/mmol) was a product of New England Nuclear (Boston, Mass.).

The phages SP01, SP82, and ϕ e complete the lytic cycle at 42 C in a wild-type host, although the burst size is lower in comparison to that obtained at 30 C (Table 1). When the *lysS1* and *trpS1* mutants were infected at 42 C with any of the three phages, there was no burst of phage progeny (not shown), whereas infection pro-

ceeded normally at 30 C (Fig. 1 and 2). Since it was possible that a period of macromolecular synthesis at 30 C might be required before phage could develop at 42 C, infected cultures were incubated for 25 min before transfer to 42 C. This time period was chosen because, at 30 C, intracellular phage began to rise 35 to 40 min after infection (Fig. 1 and 2). But, even with this extended incubation period, there was no significant increase in phage titer when the cultures were transferred to 42 C (Fig. 1 and 2). The decrease in phage titer apparently results from the completion of early eclipse events at 30 C and the subsequent failure to develop into infective virions at 42 C. Strain VB122 (*lysS1*) infected by phage ϕ e and SP01 produced a measurable increase in intracellular phage titer (Fig. 2), but the level of intracellular phage represented at most only a few percent of that produced at 30 C. No lysyl-tRNA synthetase attachment activity was detected in the ϕ e- or SP01-infected mutant at several time points (Table 2).

The effect of phage infection of the *lysS1* and *trpS1* mutants on protein synthesis was also examined (Fig. 3). As measured by the incorporation of ¹⁴C-labeled amino acids, infected cells did not synthesize more protein at the restrictive temperature than uninfected cells.

The results reported here indicate that for the three *B. subtilis* phage tested, SP01, SP82, and ϕ e, the lysyl- and tryptophanyl-tRNA synthetase activities of their host are essential for phage development. During infection, no appar-

FIG. 1. Effect of temperature on the one-step growth curve of phage SP01, SP82, and ϕe infecting strain GSY1307 (*trpS1*). Strain GSY1307 was grown at 30 C in MG medium supplemented with 2 μ g of L-tryptophan per ml. Phage was added (MOI = 0.004 to 0.042) when the OD (525 nm) reached 0.4 (7×10^7 CFU/ml). After 5 min of adsorption, the culture was diluted 10⁴-fold in the same medium. At 20 min, one-half of the culture was shifted to 42 C. Samples

FIG. 2. Effect of temperature on the one-step growth curve of phage SP01, SP82, and ϕe infecting strain VB122 (*lysS1*). The procedures were the same as described for Fig. 1 except that L-tryptophan was omitted from the medium (MOI = 0.007 to 0.036). Total phage: (○) 30 C; (●) 42 C. Intracellular phage: (×) 30 C; (▲) 42 C.

(0.1 ml) were assayed at intervals for either the total or intracellular phage titer. Total phage: (○) 30 C; (●) 42 C. Intracellular phage: (×) 30 C; (▲) 42 C.

FIG. 3. Effect of temperature on protein synthesis in *trpS1* and *lysS1* mutants infected with phage SP01, SP82, or $\phi\phi$. Exponential-phase cultures of strains GSY1307 (*trpS1*) and VB122 (*lysS1*) were grown at 30 C in MG medium supplemented with L-tryptophan (1.5 $\mu\text{g}/\text{ml}$, strain GSY1307) or L-lysine (1 $\mu\text{g}/\text{ml}$, strain VB122). When the OD (525 nm) reached 0.2 to 0.5 (4×10^7 to 9×10^7 CFU/ml), phage was added (MOI = 10). At 20 min postinfection, samples (10 ml) of the infected cultures were added to flasks incubating at 30 C and 42 C containing either L- ^{14}C tryptophan (final concentration, 0.025 μCi per nmol per ml, strain GSY1307) or L- ^{14}C lysine (final concentration, 0.025 μCi per 5 nmol per ml, strain VB122). At the indicated intervals, samples (0.5 ml) were removed and the amount of radioactivity incorporated into hot trichloroacetic acid-precipitable material was determined (11). The top figures show the optical density of the cultures. The bottom figures show the incorporation into hot trichloroacetic acid-precipitable material. (A and B) Strain GSY1307. (C and D) Strain VB122. Symbols (top figures): (O) uninfected; (X) phage $\phi\phi$; (●) phage SP82; (Δ) phage SP01. Symbols (bottom figures): (X) 30 C, uninfected; (●) 30 C, phage SP01; (□) 30 C, phage $\phi\phi$; (Δ) 42 C, phage SP01 or SP82; (■) 42 C, phage $\phi\phi$; (O) 42 C, uninfected.

TABLE 2. Effect of phage infection on the *lysyl-tRNA* synthetase attachment activity of a *lysS*⁺ strain and the *lysS1* mutant^a

Host	Time post infection (min)	Phage	Specific activity ^b
VB167 (<i>lysS</i> ⁺)	0	None	101
	10	SP01	100
	30	SP01	103
	10	φe	115
	30	φe	107
VB122 (<i>lysS1</i>)	0	None	0.010
	20	SP01	0.009
	30	SP01	0.008
	10	φe	0.012
	20	φe	0.010
	30	φe	0.010

^a One liter of each of the host strains was grown in MG medium at 30 C. When the OD (525 nm) reached 0.3 (5×10^7 CFU/ml), the cultures were infected with either SP01 or φe (MOI = 10). At the indicated times at 30 C, 200-ml samples were removed, and chloramphenicol (100 μg/ml) was added. The cells were washed twice by centrifugation ($8,000 \times g$ for 10 min), suspended in 5 ml of buffer A (9), and disrupted by ultrasonic vibration for 2 min. Unbroken cells and debris were removed by centrifugation at $27,000 \times g$ for 30 min, and the supernatant fluid was assayed for the adenosine triphosphate (ATP)-dependent attachment of L-[¹⁴C]lysine to tRNA using 20 μg of protein per assay (10).

^b Values are expressed as nanomoles of L-[¹⁴C]lysine attached to tRNA per milligram of protein per hour. Strain VB122 lacks lysyl-tRNA synthetase attachment activity but has the L-lysine-dependent pyrophosphate-ATP exchange activity.

ent modification occurs which affects the temperature-sensitive phenotype of the mutant host enzymes. However, this does not rule out

the possibility that the phage may modify the normal host enzymes. If this does occur, the phage-induced modification apparently is not extensive enough to alter the temperature-sensitive phenotype.

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