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Lysogenization by phage P22 carrying amber mutations in Genes *12* and *18*

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SUMMARY

Temperature-sensitive mutants in genes *12* and *18* of phage P22 show recessive DNA-negative phenotypes, but dominant integration-negative phenotypes. In contrast, *am* mutants in genes *12* and *18* are recessive for both DNA synthesis and lysogeny. The data are interpreted to indicate that the active forms of these two gene products are multimers.

1. INTRODUCTION

Temperature-sensitive mutants in genes *18* and *12* of bacteriophage P22 were found by Levine & Schott (1971) to have pleiotropic effects. Upon infection of the host, *S. typhimurium*, at high temperatures, these mutants fail to synthesize phage DNA and to establish the lysogenic condition. In mixed infections, P22 *12⁻ts1* and P22 *18⁻ts1* mutants complement each other for DNA synthesis and phage production but *not* for the ability to lysogenize. Furthermore, in mixed infections between either of these *ts* mutants and wild-type phage, the mutants interfere with lysogenization by wild type. That is, these mutants show recessive DNA-negative phenotypes, but dominant integration-negative phenotypes.

Levine & Schott (1971) suggested that each of the two gene products has, in addition to its enzymic site, two additional sites, one specific for DNA replication and the other for lysogeny. The active form of each product is multimeric. The defective monomers produced at high temperature are, in each case, recessive to the normal ones in DNA-synthesizing activity, but are dominant in lysogenizing activity. It was further suggested that chain-terminating mutations in either locus might not be expected to be dominant over the wild-type allele, since in this case no complete peptides are made. The multimers might then contain only normal peptides and complementation for both DNA synthesis and lysogeny would occur. The availability of *amber* mutants in genes *12* and *18* (Bode, Dopatka & Prell, 1973) makes it possible to test parts of this hypothesis.

2. MATERIALS AND METHODS

Log phase cells of strain 18 *Su*$^-$ and strain 192 *Su*$^+$ grown in nutrient broth were infected with various combinations of P22*c*$^+$, P22*c*$^+$ *12*$^-$*amH145* and P22*c*$^+$ *18*$^-$*amH100* at a multiplicity of infection of 10 of each phage. After 10 min adsorption at 37 °C, the infected cells were diluted in prewarmed broth to about 1×10^3 cells/ml and incubated for 200 min at 37 °C. By this time the surviving infected cells (85–100 % of the input bacteria) have undergone approximately ten divisions and segregation of lysogenic and non-lysogenic progeny has stabilized (Levine & Schott, 1971). Samples of each infection were then plated on nutrient agar for isolated colonies. After overnight incubation at 37 °C, 60–180 colonies from each combination were stabbed and tested for lysogeny. Each colony was cross-streaked against P22 *c2–5* phage to test for the immune state of the colonies.

3. RESULTS

Typical results are given in Table 1. All infections of the *Su*$^+$ bacteria led to successful lysogenization. In contrast, the amber mutants in either gene *12* or gene *18* were unable to lysogenize *Su*$^-$ cells in single infection. However, coinfection of *Su*$^-$ cells with *am*$^-$ and wild-type phage resulted in an efficiency of lysogenization comparable to that observed with wild-type alone and coinfection with the two *am*$^-$ mutants gave lysogenization at levels comparable to that of the mixed infection in the permissive host.

Table 1. *Lysogenization of* Su$^+$ *and* Su$^-$ *bacteria by P22* 12$^-$ *and P22* 18$^-$ *amber mutants*

	% lysogenic progeny in:	
Infecting phage	*Su*$^+$ host	*Su*$^-$ host
P22*c*$^+$	24	50
P22*c*$^+$ *18*$^-$*amH-102*	22	0
P22*c*$^+$ *12*$^-$*amH-145*	9	0
P22*c*$^+$ + P22*c*$^+$ *18*$^-$*amH-100*	28	49
P22*c*$^+$ + P22*c*$^+$ *12*$^-$*amH-145*	21	57
P22*c*$^+$ *18*$^-$*amH-100* + P22*c*$^+$ *12*$^-$*amH-145*	15	13

Log-phase cells of strain 18 *Su*$^-$ and strain 192 *Su*$^+$ were infected at 37° with multiplicities of infection of 10 of each of the above phages and incubated as described in the text for 200 min to permit segregation of lysogenic and non-lysogenic progeny. Samples were plated on nutrient agar and isolated colonies tested for immunity, as described in the text.

4. DISCUSSION

These results with amber mutants confirm the earlier findings (Levine & Schott, 1971) that non-permissive conditions of infection interfere with the ability of P22 *12*$^-$ and *18*$^-$ phage to integrate as prophage. Both *ts* and *am* mutants showed dramatically decreased abilities to undergo lysogenization. The data support the requirement for common functions for genome replication and prophage integration.

In contrast to the *ts* mutants, the *am* mutants in genes *12* and *18* were recessive

for both genome replication and lysogenization. This lends credence to the suggestion that the active forms of these two gene products are multimers and that the defective monomers produced at high temperature by *ts* alleles of these loci play positive roles in the dominant integration-negative phenotypes of the *ts* mutants.

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