Spontaneous Temperature-Sensitive Mutations in Bacteriophage T7

JAMES C. STONE†* AND R. C. MILLER, JR.

Department of Microbiology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5

Attempts to recover temperature-sensitive mutations affecting genes 13 and 14 (virion proteins) in bacteriophage T7 by analysis of amber revertants were confounded by the frequent occurrence of spontaneous temperature-sensitive mutations in other genes. These incidental temperature-sensitive mutations are physically distinct from but may be functionally related to genes 13 and 14, as shown by complementation and recombination studies. The possibility that these incidental temperature-sensitive mutations represent secondary-site suppressors of the pseudonormal suppressed amber products is discussed.

A convenient method for isolating temperature-sensitive mutations in a particular gene involves the recovery and subsequent testing of spontaneous amber mutation pseudo-revertants. According to this scheme, some of the single base changes converting an amber codon to a sense codon do not restore the original wild-type sequence and, thus, represent missense mutations. Temperature-sensitive mutations obtained by this stratgem tend to fall into the following two categories: (i) mutations arising frequently and encoding temperature-sensitive gene functions by virtue of appropriate residue changes at the desired site and (ii) mutations arising spuriously elsewhere in the genome, in genes unrelated to the gene under study. This approach has been used successfully to isolate bacteriophage T7 gene 4 (DNA primase) temperature-sensitive mutations (6).

In the course of studies on plasmid-phage recombination in bacteriophage T7-infected *Escherichia coli* (2), temperature-sensitive mutations in genes 13 and 14 (virion proteins) were sought to serve as recombination markers. Phage mutants that were temperature sensitive for growth were obtained among amber 13 and amber 14 revertants, but these mutants defined a third category. The temperature-sensitive mutations arose frequently and were located in genes that are physically distinct from but may be functionally related to the amber mutant genes under study. An account of this puzzling observation and a plausible explanation for it are presented here.

Phage strains were obtained from F. W. Studier. The methods used to grow phage, prepare high-titer stocks, and perform spot tests and recombination and complementation assays have been described elsewhere (2, 3). *E. coli* strain 011' (SupE) was used as the permissive strain in the preparation of all amber mutant phage stocks; for other purposes strain C600 (SupE) was often used as the permissive strain (1). Strain B23 was used as the nonpermissive host.

Phage mutants temperature sensitive for growth are readily recovered among gene 13 and 14 amber revertants. To isolate temperature-sensitive mutations in genes 13 and 14, am13-89 and am14-140 phage stocks were first plated onto the amber-permissive strain, strain 011' (SupE). Three plaques of phage am13-89 and one plaque of phage am14-40 were picked into 1.0 ml of broth. Such phage suspensions contained approximately 10^6 PFU when they were plated onto strain, 011' and approximately 10^2 PFU (amber revertants) when they were

plated onto strain B23. Between 45 and 137 amber revertant plaques from each suspension (total, 298) were tested for temperature-sensitive growth by spotting on replica plates with strain B23-seeded top agar media at 30 and 42°C.

Three temperature-sensitive mutants, mutants 13-4, 13-6, and 13-9, were recovered from the revertants of am13-89. Each arose from a separate phage suspension. One temperature-sensitive mutant, mutant 14-1, was recovered from the am14-140 revertants. Each of the temperature-sensitive mutants derived from am13-89 must have occurred after the original stocks were plated onto strain 011', since each was in a minority in the phage suspension. Thus, the mutations arose independently and spontaneously. They were not induced by the mutagenesis treatment used to generate the original amber mutations. The frequency of temperature-sensitive mutants among the amber revertants was 1.3% (4 of 298).

The mutant 13-9 temperature-sensitive mutation apparently reverted to wild type (i.e., nonamber, non-temperature sensitive) at a high frequency. This mutation was not studied further. Stocks of the other three temperature-sensitive mutants contained wild-type revertants at frequencies typical of T7 single-base mutations (10^{-4}).

Temperature-sensitive mutations are not in genes 13 and 14. Three types of experiments demonstrated that the temperature-sensitive mutations did not affect the genes harboring the original amber mutations. First, infection at 30°C of cells containing pRS142, a plasmid containing most or all of wild-type genes 13 and 14, with phage 13-4 or 14-1 reproducibly failed to yield wild-type recombinant progeny. Wild-type recombinants constituted approximately 5% of the progeny after infection of pRS142-containing cells with either am13-89 or am14-140 (2). Second, co-infection of strain C600 cells with mutant 13-4 and am13-89 resulted in 1 to 2% wild-type recombinant progeny, as determined by plating under permissive and doubly restrictive conditions. Finally, complementation spot tests and complementation tests done in solution demonstrated that mutant 13-4 and am13-89 are in different functional groups. Likewise mutant 13-6 complemented am13-149, and mutant 14-1 complemented am14-140. Thus, the temperature-sensitive mutations of mutants 13-4, 13-6, and 14-1 affect genes other than those that experienced the amber reversion.

Temperature-sensitive mutations define a common complementation group. During the experiments described above, we discovered that, although the temperature-sensitive mutations were complementary to the amber mutations existing in the parental amber phage stocks, they defined a common

* Corresponding author.
† Present address: Department of Genetics, Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8.
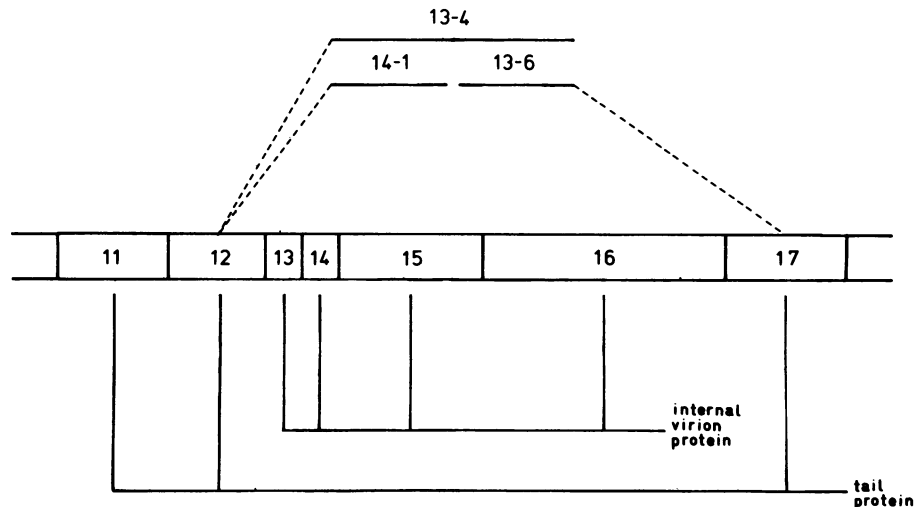
FIG. 1. Complementation pattern obtained by inter se matings with temperature-sensitive mutants 13-4, 14-1, and 13-6. The dashed lines indicate the approximate map positions of each temperature-sensitive mutation on a segment of the standard T-7 map, drawn to scale. The product of each gene is also indicated.

complementation group themselves. Specifically, phage 13-4 was not complemented by either phage 13-6 or phage 14-1, whereas the two latter phages were mutually complementary. This pattern was observed consistently in 10 complementation spot tests (Fig. 1).

The pattern of complementation precluded the possibility that the mutations blocked adsorption to host cells. Furthermore, the temperature-sensitive mutations apparently did not affect the thermolability of the phage particles, as determined by examining the effect on titers of incubating wild-type and mutant 13-4 phage suspensions at 42°C for up to 5 h. Our results are consistent with the hypothesis that temperature-sensitive mutational blocks affect the synthesis or assembly of functional phage components.

Temperature-sensitive mutations arise in am13-89 phage stocks independent of amber mutation reversion, but do not define a mutational hotspot. The demonstration that the temperature-sensitive mutations affect genes other than genes 13 and 14 raised the possibility that the occurrence of these mutations was not related to the amber reversion, reflecting spontaneous events in all T7 phage populations. Accordingly, wild-type T7 and am13-89 stocks were plated onto strain C600, and individual plaques were tested for temperature-sensitive growth by replica-spotting on strain C600-seeded top agar media at 30 and 42°C. No temperature-sensitive mutants were found among 511 plaques from the wild-type stock. From 152 am13-89 plaques, 3 temperature-sensitive mutants were recovered. One of these produced tiny plaques when it was plated on strain C600 at 42°C and presumably arose independently of the other two, which were virtually defective at 42°C. Note that these mutations were clonally distinct from those described above. These results demonstrate that temperature-sensitive mutations frequently arise in am13-89 phage populations but apparently do not arise as frequently in wild-type phage populations.

Temperature-sensitive mutations isolated among amber revertants of am13-89 and am14-140 map to T7 tail structure genes. To localize the mutations of temperature-sensitive mutants 13-4, 13-6, and 14-1 on the T-7 genetic map, each mutant was assayed for growth at the restrictive temperature on *E. coli* containing defined segments of the T7 genome

molecularly cloned in pBR322. (The mapping of the mutations of mutants 13-4, 13-6, and 14-1 was kindly performed by F. W. Studier and was confirmed by us by using plasmids made available by F. W. Studier.) This method of genetic mapping is reproducible, sensitive, and less ambigous than conventional techniques (5). Our results demonstrated that the mutations of mutants 13-4 and 14-1 were in gene 12, whereas the mutation of mutant 13-6 mapped to gene 17. Both of these genes encode tail components (4). Our results are shown in Fig. 1. Note that the mapping information was unexpected, given the evidence that the mutations affected a common complementation group.

The temperature-sensitive mutations described above are perplexing in a number of respects. The fact that the mutations of mutants 13-4, 13-6, and 14-1 map to genes other than those experiencing amber reversion and the demonstration that mutants with similar temperature-sensitive mutations exist in an am13-89 phage stock negate the possibility that these mutants arose as pseudorevertants that encoded thermolabile as opposed to truncated polypeptides. However, the frequency of temperature-sensitive mutants is remarkably high to be explained by random, spontaneous events. Furthermore, the finding that the mutations of these mutants map to genes having a common function and the failure to find similar mutants in a wild-type population argue against a random, spontaneous origin.

Finally, the pattern of complementation between the temperature-sensitive mutations is highly unusual, since it implies that mutations affecting two separate tail genes fail, in *trans*, to produce a functional product.

This latter result may offer the only clue to what processes generated these baffling observations. It seems likely that the products of genes 12, 13, 14 and 17 fit together in the mature phage particle and that slight structural perturbations in this macromolecular association are deleterious to phage reproduction. The am13-89 and am14-140 products, when suppressed in strain O11', encode proteins that have glutamine inserted at the mutant site. If the wild-type residue is other than glutamine, we propose that the translationally suppressed amber products distort the phage structure. Mutations affecting neighboring subunits in the complex and resulting in compensatory changes in the macromolecular

association would then be selected, and some of these could encode thermolabile products. Although these compensatory mutations would be rare, their frequency in the phage populations would rapidly increase as a result of their normalizing the slightly deleterious effect of the suppressed amber. Thus, the failure of the mutations of mutants 13-4 and 13-6 to complement can be viewed as the synergistic and unbalanced effect of two mutations effecting the proposed compensatory changes to the phage structure in the absence of suppressed amber products. If each virion normally contains several copies of the proteins encoded by genes 12 and 17, the presence of mutually incompatible mutant products in cells co-infected by mutant 13-4 and 13-6 phages might virtually preclude assembly of viable progeny.

This explanation demands a number of assumptions, but it accounts for all of our results and leads to several testable predictions. For example, the occurrence of temperature-sensitive tail structure mutations in am*13* and am*14* stocks should be strictly dependent upon the suppressor gene present during permissive growth of the amber phage population, and no temperature-sensitive mutations should occur when translational suppression results in a wild-type polypeptide sequence. This concept may have broader implications for the study of protein-protein interactions.

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