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Suppressors of Mutations in the Bacteriophage T4 Gene Coding for Both RNA Ligase and Tail Fiber Attachment Activities

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The protein product of T4 gene *63* catalyzes both the attachment of tail fibers to fiberless phage particles and the ligation of single-stranded RNA (Snopek et al., Proc. Natl. Acad. Sci. U.S.A. **74**:3355-3359, 1977). To investigate whether the gene *63* product has a role in nucleotide metabolism, we isolated false revertants of *am*M69 in gene *63*. We screened for revertants that could grow at 30°C but not at 43°C on *Escherichia coli* OK305 when nucleotides were limiting. These false revertants contained the original mutation in gene *63* and new suppressor mutations. Some of these suppressor mutations caused temperature sensitivity by themselves, allowing single mutants carrying the suppressor to be recognized and isolated. The results of mapping and complementation studies indicated that most of these *ts* suppressors were in the *t* gene (lysis), one was in gene *5* (baseplate), and one was in gene *18* (sheath). The mutation in gene *18*, *ts*DH638, suppressed three different amber mutations in gene *63* but did not suppress amber mutations in several other genes. None of the suppressors that were characterized were in genes with known functions in nucleotide metabolism. However, an intriguing property of these false revertants was that they were very sensitive to hydroxyurea, an inhibitor of nucleotide metabolism.

The protein product of T4 gene *63* catalyzes the last step in phage morphogenesis, the attachment of tail fibers to otherwise complete phage particles (18, 19). As many as six tail fibers are attached to each tail baseplate by a noncovalent association, and the gene *63* protein does not become part of the structure (16, 18). Phage with as few as three or four fibers are infectious. This attachment of tail fibers occurs spontaneously (i.e., in the absence of the gene *63* protein) in infected cells at a rate sufficient to produce about 10 infectious phage per cell in a normal infection (18, 19). Snopek et al. (12) have reported that the same gene *63* protein is also an RNA ligase and that the two different activities of this protein differ in requirements and in response to some inhibitors.

Several observations are consistent with the suggestion that the gene *63* product might have a role in nucleotide metabolism: (i) the gene *63* product begins to be synthesized early after infection of *Escherichia coli* (19); (ii) on the genetic map of T4, gene *63* is close to several genes which code for enzymes involved in nucleotide metabolism such as ribonucleotide reductase, thymidylate synthetase, and dihydrofolate reductase (8, 20); and (iii) although gene *63* is nonessential for plaque formation on many strains of *E. coli*, mutants defective in gene *63* fail to make plaques on *E. coli* OK305, which is defective in nucleotide metabolism.

To investigate whether the gene *63* product has a role in nucleotide metabolism, we isolated false revertants of a gene *63* amber mutant under conditions of nucleotide limitation. These false revertants contain the original mutation in gene *63* and new suppressor mutations that allow bypass of gene *63* function. Characterization of these suppressor mutations has revealed which gene products can be altered to make the gene *63* product unnecessary for plaque formation on *E. coli* OK305. None of these suppressor mutations are in genes with functions that are related to nucleotide metabolism or to RNA ligation. Our results are consistent with the suggestion that the attachment of tail fibers is the major physiological role of the gene *63* product.

MATERIALS AND METHODS

Bacteriophage strains. T4Do, an osmotic shock-resistant derivative of T4D, was the standard bacteriophage type with which all mutants were compared. T4 amber and temperature-sensitive (*ts*) mutants (5) were isolated at the California Institute of Technology

and were obtained from W. B. Wood. Mutants *ts*B49, hD7, and h49 were obtained from E. B. Goldberg at the Tufts University School of Medicine. Double mutants were constructed by crossing appropriate single mutants and isolating a recombinant that failed to complement either parent.

Bacterial strains. *E. coli* strains B and CR63 were used for preparing phage stocks and phage crosses. *E. coli* CR63 was used whenever amber mutants were involved because it is the permissive host for such mutants. *E. coli* OK305 is a derivative of *E. coli* B that requires pyrimidine for growth and is deficient in cytidine deaminase activity (9). *E. coli* C32 was also obtained from E. B. Goldberg and was used to recognize T4 mutants hD7 and h49 (1).

Media. Broth medium, synthetic (S) medium, and agar plates containing about 30 ml of broth or synthetic medium were prepared as described by Goscin and Hall (7). *E. coli* OK305 was grown on synthetic medium supplemented with uracil or cytidine (20 μg/ml). Chemicals were purchased from the sources given by Goscin and Hall (7).

Phage crosses. Crosses were performed as described by Hall et al. (9).

Burst size. Measurements of burst size were performed as described by Goscin and Hall (7). Exponentially growing cells were collected by centrifugation, suspended in fresh medium at 5×10^8/ml, and infected with 0.1 phage per bacterium.

Complementation tests. Complementation tests were performed as described by Goscin and Hall (7). These were done at 43°C with OK305 cells on plates made with synthetic medium containing 20 μg of cytidine per ml as the only pyrimidine source (S+CR plates) and containing hydroxyurea (20 mg per plate).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Electrophoresis of extracts of infected cells was performed as described by Chace and Hall (3) except that sodium dodecyl sulfate and acrylamide were from Bio-Rad, the cells were not subjected to UV radiation, and the gels (12.5%) were run at 15 mA for about 6 h. Autoradiograms of the dried gels were prepared using Kodak XRP-5 film.

RESULTS

Isolation of false revertants. We have found that amber mutants defective in gene *63*, such as *am*M69, do not form plaques on *E. coli* OK305 on S+CR plates. The cells grow slowly because they synthesize uridine nucleotides from cytidine at a slow rate. Nonmutagenized stocks of *am*M69 contain phage, at a frequency of about 10^{-4}, which can form plaques under these conditions. These phage are false revertants which still contain the original mutation in gene *63*, as shown by crossing it out, and new suppressor mutations. We screened for revertants of *am*M69 that could form plaques at 30°C but not at 43°C on *E. coli* OK305 on S+CR plates. About 7% of the revertants picked at 30°C were unable to grow at 43°C. Some of these temperature-sensitive revertants (about one-third) contained new suppressor mutations which cause temperature sensitivity by themselves, allowing single mutants (*ts*) carrying the suppressor to be recognized and isolated.

Characterization of the revertants. Crosses of these false revertants to wild-type T4 yielded *am*M69 single mutants at a frequency of about 15% in the progeny, indicating that the revertants do still contain the original mutation and that the new suppressor mutations are not close to gene *63* on the genetic map.

The *ts* single mutants were in three complementation groups (see below). One mutant in each group was chosen for further analysis. Two of the mutants, *ts*DH638 and *ts*DH634, were crossed to *am*M69 to reconstruct the *ts-am*M69 double mutants. These double mutants formed plaques on *E. coli* OK305 on S+CR medium, showing that the *ts* mutations were responsible for the suppression.

The false revertants (*ts-am*M69 double mutants) could produce the gene *63* product if the *ts* mutation resulted in the suppression of amber mutations. That this is not the case was indicated by the following: (i) the false revertants occurred at a frequency much higher than amber suppressors occur (14); (ii) none of the *ts* suppressors mapped (see mapping below) in the tRNA genes where mutations causing suppression of ambers map (15); (iii) *ts*DH638 and *ts*DH6318 were used to construct double mutants with several amber mutants, and the *ts-am* doubles did not grow any better than the *am* single mutants on cells that did not contain an amber suppressor (data not shown; the double mutants constructed were *ts*DH638-*am*A452 [gene *28*], *ts*DH638-*am*E198 [gene *57*], *ts*DH638-*am*NG333 [gene *23*], *ts*DH638-*am*B25 [gene *34*], *ts*DH6318-*am*A452 [gene *28*], and *ts*DH6318-*am*E198 [gene *57*]); (iv) all the false revertants failed to complement the *am*M69 (gene *63*) single mutant; and (v) we were unable to detect normal-size gene *63* product in cells infected by these false revertants by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography (Fig. 1).

Although *ts*DH638 does not suppress amber mutations in genes other than *63*, it does suppress two other amber mutations (*am*mn13 and *am*mn15.6) in gene *63*. The *ts-am* doubles were constructed, and they formed plaques with a high efficiency on OK305 on S+CR medium. Therefore *ts*DH638 and, probably, the other suppressors are not mutation-specific but gene-specific suppressors that allow T4 growth in *E. coli* OK305 in the absence of the gene *63* product.

Growth studies. Table 1 shows the growth of *ts*DH638 and *ts*DH6318 as single mutants and

as double mutants with *am*M69 (gene *63*) under different conditions. Although the double mutants could form plaques on *E. coli* OK305 on synthetic plates with cytidine as the only pyrimidine source, they could not form plaques if the cytidine was replaced with uracil. This is not because of poorer phage growth, because the double mutants have a larger burst size on OK305 cells with uracil than with cytidine. We have previously reported (7) that a smaller burst size than normal is required to form plaques on *E. coli* OK305 growing slowly on cytidine. It is noteworthy that the growth of the double mutants on cytidine could be eliminated by hydroxyurea, which inhibits nucleotide metabolism by inhibiting ribonucleotide reductase (13). Table 1 also shows that these two *ts* suppressors each increased the burst size of *am*M69 growing on *E. coli* OK305 in S+CR medium about sixfold and thereby allowed plaque formation. The single *ts* mutants grew quite well on different cells and media at 30°C, but did not produce any phage if the infection was at 41°C.

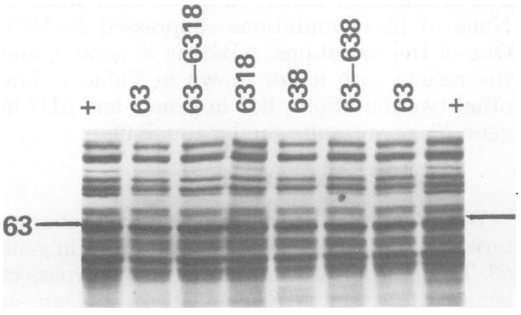

FIG. 1. *Autoradiogram of a sodium dodecyl sulfate-polyacrylamide slab gel after infection of E. coli B by T4Do (+), amM69 in gene 63 (63), amM69-tsDH6318 double mutant (63-6318), tsDH6318 (6318), tsDH638 (638), or amM69-tsDH638 double mutant (63-638). Proteins were pulse-labeled with a mixture of ^{14}C-labeled amino acids from 6 to 9 min after infection at 37°C. The lines at the edges of the figure indicate the position of the protein coded by gene 63.*

The growth of the *ts*DH634 single mutant and the *am*M69-*ts*DH634 double mutant under different conditions (Table 1) was similar to that for *ts*DH638 and *ts*DH6318 but showed some differences. The *ts*DH634 single mutant was sensitive to hydroxyurea and had a burst size on *E. coli* OK305 in synthetic medium that was about two times that of wild-type T4. The *am*M69-*ts*DH634 double had a burst size several times that of the other two double mutants. Cells infected by either the *ts*DH634 single or the double mutant had delayed lysis (data not shown).

Genetic mapping and complementation studies. Spot complementation tests were done with the *ts* suppressors and other mutants defective in many genes (Table 2). Among many mutants tested, *ts*DH634 only failed to complement mutants defective in the *t* gene, *ts*DH638 only failed to complement mutants defective in gene *18*, and *ts*DH6318 only failed to complement mutants defective in gene *5*. Eleven other *ts* suppressors were characterized, and all of them failed to complement *ts*DH634 and other mutants defective in the *t* gene, but did complement all other mutants tested.

Crosses were done between the *ts* suppressors and other mutants defective in many genes (Table 3). As expected from the complementation results, *ts*DH634 mapped near mutations in the *t* gene, *ts*DH638 mapped near mutations in gene *18*, and *ts*DH6318 mapped near mutations in gene *5*. Maps for the *t* gene and gene *5* are given

TABLE 1. *Growth of T4 mutants on different E. coli strains and media*

Phage	EOP[a] on:				Burst size[b] on:				
	CR63 (Br)[c]	OK305 (S+CR)	OK305 (S+CR+HU)[d]	OK305 (S+U)[e]	B (Br)	CR63 (Br)	OK305 (S+CR)	OK305 (S+U)[f]	CR63 (Br[41°C])
T4D*o*	1	1	0.9	1	290	230	100	130	42
*am*M69 (*63*)	1	10^{-4}	10^{-6}	10^{-6}	4.5	140	0.6	0.8	77
*am*M69-*ts*DH638	1	0.9	10^{-5}	10^{-5}	15	200	3.8	8.0	0.03
*am*M69-*ts*DH6318	1	0.9	10^{-6}	10^{-6}	15	30	3.7	7.1	0.1
*ts*DH638	1	0.9	0.9	1	310	280	100	150	0.05
*ts*DH6318	1	0.9	0.9	0.9	240	270	97	120	0.1
*ts*DH634	1	0.8	10^{-3}	0.1	—[g]	—	250	200	—
*am*M69-*ts*DH634	1	0.5	10^{-6}	10^{-6}	—	—	30	20	—

[a] Efficiency of plating at 30°C.
[b] Phage produced per infected cell in 90 min at 30°C unless indicated otherwise.
[c] Br, Broth medium.
[d] HU, Hydroxyurea, 20 mg per plate.
[e] U, Uracil, 250 μg per plate.
[f] U, Uracil, 20 μg/ml.
[g] —, Not done.

in Fig. 2 and 3. In both cases the *ts* suppressor appeared to map between other mutations in the gene. Several *ts* suppressors defective in the *t* gene were crossed to each other, and in all cases recombinants were observed. In gene *18*, *ts*DH638 was mapped with respect to two other *ts* mutations, *ts*A38 and *ts*B39. No wild-type recombinants above background (10^{-6}) were produced in crosses between *ts*DH638 and *ts*A38, and both of these mutants showed about 0.4% recombination with *ts*B39.

TABLE 2. *Complementation tests*[a]

Phage	Complementation with phage (defective gene):			
	*am*M69 (*63*)	*am*A3 (*t*)	*ts*A38 (*18*)	*am*B256 (*5*)
*ts*DH634	+	0	+	+
*ts*DH638	+	−	0	+
*ts*DH6318	+	−	+	0

[a] These tests were performed by adding phage of one type to the top agar with cells, pouring this onto an agar plate, and spotting the other phage onto the hardened top agar as described in the text. Production of wild-type plaques (+) indicates complementation followed by recombination.

TABLE 3. *Recombination percentages*[a]

Phage	Phage (defective gene)			
	*am*M69 (*63*)	*am*A3 (*t*)	*ts*A38 (*18*)	*am*B256 (*5*)
*ts*DH634	30	1.0	36	—[b]
*ts*DH638	35	—	<0.001	—
*ts*DH6318	37	—	27	1.3

[a] Twice the percentage of wild-type recombinants produced in crosses between the two mutants indicated.

[b] —, Not done.

Suppression studies. The results above suggest that any mutation in the *t* gene will suppress gene *63* mutants, but this was not clear for genes *5* and *18*. Therefore several double mutants were constructed between *ts* mutations in genes *5* and *18* and *am*M69 and tested for the ability to form plaques on *E. coli* OK305 (Table 4). Only one of the mutations tested, *ts*A38 in gene *18*, suppressed *am*M69.

These *ts* suppressors could act by altering the phage particle such that it was infectious with fewer tail fibers than normal. Mutants with these properties have been reported by Crawford (J. T. Crawford, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, S155, p. 305). We obtained three mutants of this type from E. B. Goldberg and constructed double mutants with *am*M69. None of these mutations suppressed *am*M69. One of the mutations, *ts*B49, is in gene *5*, and the results with it are shown in Table 4. The other two mutations, h49 in gene *8* and hD7 in gene *10*, gave results similar to *ts*B49.

DISCUSSION

We have described the isolation and characterization of *ts* suppressors of mutations in gene *63*. These suppressor mutations allow bypass of gene *63* function in *E. coli* OK305 and are in genes *t* (lysis), *5* (baseplate), and *18* (sheath). The suppressors were studied in hopes of finding evidence for a role of the gene *63* product in nucleotide metabolism. However, none of the suppressor mutations are in genes with known functions in nucleotide metabolism. Also, the genes that the suppressors do affect do not have functions with any obvious relation to the RNA

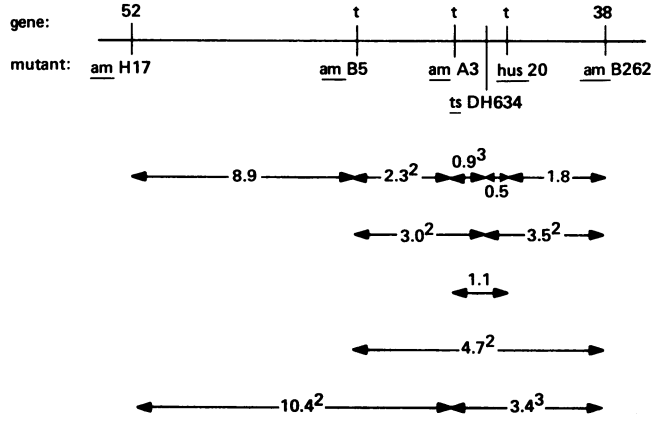

FIG. 2. *Genetic map showing the location of tsDH634 relative to the positions of mutations in the t gene and average frequencies of recombinants obtained in two-factor crosses. The superscript indicates the number of times a particular cross was done.*

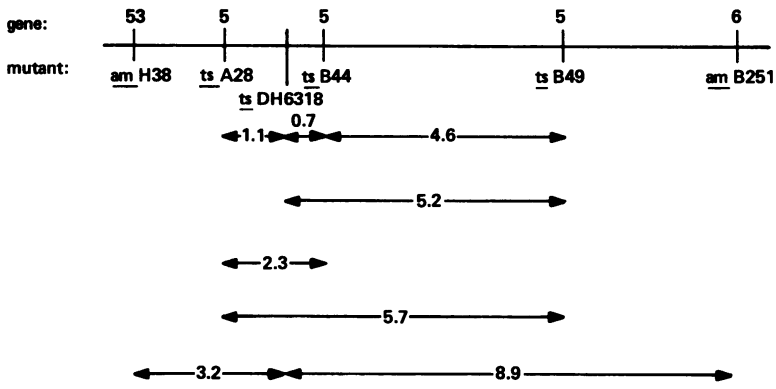

FIG. 3. *Genetic map showing the location of tsDH6318 relative to the positions of mutations in gene 5 and average frequencies of recombinants obtained in two-factor crosses. Each value is the average of two crosses.*

TABLE 4. *Efficiency of plating of T4 mutants on different E. coli strains and media at 30°C*

Phage (defective gene)	CR63 (Br)[a]	OK305 (S+CR)[b]	OK305 (S+U)[c]
T4D*o*	1	1	1
*am*M69 (*63*)	1	10^{-4}	10^{-6}
*am*M69-*ts*DH638 (*18*)	1	0.9	10^{-5}
*am*M69-*ts*A38 (*18*)	1	0.8	10^{-5}
*am*M69-*ts*B39 (*18*)	1	10^{-4}	10^{-5}
*am*M69-*ts*DH6318 (*5*)	1	0.9	10^{-6}
*am*M69-*ts*A28 (*5*)	1	10^{-4}	10^{-5}
*am*M69-*ts*B44 (*5*)	1	10^{-5}	10^{-5}
*am*M69-*ts*B49 (*5*)	1	10^{-4}	10^{-4}

[a] Br, Broth medium.
[b] CR, Cytidine, 20 μg/ml.
[c] U, Uracil, 250 μg per plate.

ligase activity of the gene *63* product. It appears that the major physiological role of the gene *63* product is the attachment of tail fibers. An intriguing property of the false revertants is that they are very sensitive to hydroxyurea, an inhibitor of nucleotide metabolism, leaving the possibility that the gene *63* product may have some role in nucleotide metabolism. Recently, David et al. (4) have suggested that the RNA ligase activity of the gene *63* product may be involved in the processing of host tRNA's into novel tRNA species during infection.

It is not clear why mutants in gene *63* do not grow on *E. coli* OK305, since the only known defects in this strain are in nucleotide metabolism. We have found that addition of divalent cations to the medium will partially suppress a gene *63* mutant (K. Trofatter and D. Hall, unpublished data). Thus, the ionic composition of the medium may affect the spontaneous rate of tail fiber attachment in infected OK305 cells.

Most of the suppressor mutations, 12 of 14, are in the *t* gene described by Josslin (10). The mechanism of suppression for these suppressors would appear to be somewhat trivial. Mutants carrying mutations at different sites in the *t* gene show delayed lysis and in some cases much higher than normal burst sizes when lysis does occur (10). Given more time and possibly a higher concentration of tail fibers or fiberless phage particles or both, it seems likely that the normal spontaneous fiber attachment would produce more infectious phage per cell. This mechanism of suppression would be consistent with the finding that the suppressors in the *t* gene are at different sites and occur at a relatively high frequency.

The mechanism of suppression for the other two mutations, one in gene *5* and one in gene *18*, is more complex. One possible mechanism is that fewer phage particles are made per cell and therefore each has a higher probability of having enough fibers attached spontaneously to be infectious. This is the "balance of assembly processes" mechanism suggested by Floor (6). This seems unlikely because both the *ts*DH6318 (gene *5*) and the *ts*DH638 (gene *18*) single mutants have a burst size similar to that for wild-type T4. A second possibility is that altered phage are made which are infectious with fewer tail fibers than normal. This seems reasonable because the gene *5* product is in the tail baseplate and the gene *18* product is the major tail sheath protein (11). However, this does not seem to be the mechanism, because we have tested three mutants, one of which is defective in gene *5*, known to be infectious with fewer fibers, and none of these suppresses *am*M69 in gene *63*. A third possible mechanism is that altered phage are made which have a higher than normal rate of spontaneous addition of tail fibers. This also seems reasonable because genes *5* and *18* do affect the structure of the tail and an altered tail structure could affect the rate of tail fiber attachment. The observation that, of the muta-

tions tested, only those at one site in each gene will bypass the need for the gene *63* product indicates that only specific changes in the gene *5* product or the gene *18* product can produce this result. The alterations in tail structure produced by these changes are probably in the baseplate where tail fibers become attached, but they also could involve the phage whiskers, which are required for efficient tail fiber attachment (17). The whiskers are filaments that extend outward from the top of the tail and that appear to help position the tail fibers for attachment (17).

The third mechanism of suppression seems the most likely by a process of elimination, but there is no direct evidence for it. In a related study by Bishop and Wood (2), it was found that a mutation in gene *37* allows bypass of the need for the gene *38* product. They suggest that the protein coded by gene *38* normally promotes the dimerization of the protein coded by gene *37* in tail fiber assembly and that specific mutations in gene *37* which promote spontaneous dimerization would eliminate the need for the gene *38* protein. This mechanism would be quite analogous to the one we have suggested above.

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