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Nonsense Suppressor Mutants of Bacteriophage T5

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Mutants of bacteriophage T5 carrying amber suppressors have been isolated. These amber suppressors are located in a region of the genome defined by the st(0) deletion and are able to suppress amber mutations in all regions of the genome except the FST segment.

The genome of bacteriophage T5 codes for multiple tRNA species clustered in a nonessential region of the genome (*1*). Deletion mutants lacking many of these tRNAs are apparently normal in their growth (*1*). This raises the question of whether these tRNAs are able to function *in vivo*. One approach to this question is to determine whether mutant forms of these nonessential tRNAs can function as nonsense suppressors, able to suppress amber mutations in phage genes, as have been found in the related phage BF23 (*2*) and in the unrelated phage T4 (*3*).

A unique feature of phage T5 is its mode of injection. After adsorption, about 8% of the chromosome (the FST or first step transfer segment) enters the cell; then the functions of at least two gene products (from genes A1 and A2) are required for entry of the remaining 92% of the chromosome and for productive infection (*5, 6*). Assignment of genes to the FST is a tedious process. If, however, none of the possible suppressor mutations in T5 is located on the FST, then it follows that amber mutations on the FST should not be suppressed by a T5 suppressor, allowing a relatively simple screen for essential genes on the FST. Okada *et al.* (*2*) have described nonsense suppressor mutants of the closely related phage BF23. The results presented here are entirely consistent with their results.

The T5 amber mutants used in this study are listed (along with other phage and bacterial strains) in Table 1. The reversion frequencies of the amber mutants were found to vary between 0.1×10^{-6} and 7×10^{-6}. Phage strains carrying pairs of these mutations were isolated as recombinants of crosses between strains carrying single amber mutations. The reversion frequencies of these double mutants were found to fall into two classes (Table 2). One class reverted much less frequently than single amber mutants (fewer than 2×10^{-8}) as would be expected for double mutants with independently reverting mutations; the other class reverted at frequencies similar to those of single amber mutants (0.5×10^{-6} to 7×10^{-6}). Several independent revertants of the double amber mutant T5 *am*B2, *am*C1 were backcrossed with wild type and the progeny were plated on the permissive host strain Fsu^+. These backcross progeny were then tested by complementation against strains T5 *am*B2 and T5 *am*C1 for the presence of either or both of the original mutations. In each reverent, both *am*B2 and *am*C1 were shown to be present (Table 3). Both amber alleles were likewise present in a similar revertant of the double mutant T5 *am*D19, *am*D21 (Table 3). Thus the apparent reversion of the double mutants is due to suppression of the mutant phenotype rather than to true reversion of the amber

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TABLE 1

STRAIN LIST

Bacterial strains	Relevant characteristics	Reference
Fsu^-	*E. coli F*, nonpermissive to amber	(*11*)
Fsu_5^+	*E. coli F*, permissive to amber	(*11*)
EG47	*E. coli K12*, su^-, *rpsL594*[a]	(*12*)

Phage strains	Reversion frequency on $Fsu^- \times 10^{-6}$	Relevant characteristics
T5		Wild type
T5st(0)		Heat-stable deletion mutant of T5
T5 *am*A1[b]	2.5	
T5 *am*B1	0.1	
T5 *am*B2	6.5	
T5 *am*C1	0.8	
T5 *am*C2	0.2	
T5 *am*D2	0.1	
T5 *am*D3	NT[c]	
T5 *am*D4	5.0	
T5 *am*D19	5.0	
T5 *am*D21	0.2	
T5 *am*-103	2.5	
T5 *am*-401	0.9	
T5 *am*-501	1.0	
T5 *am*-601	1.3	Same cistron as *am*-602
T5 *am*-602	2.5	Same cistron as *am*-601

[a] The *rpsL594* allele is a streptomycin resistance mutation which seems to make strains carrying it less phenotypically leaky for amber mutations (*13*).

[b] Strains with a letter following the *am* designation are from the collection of D. J. McCorquodale. The letter and number (e.g., A1, D21) signify the gene mutated. The strains with a dash following the *am* designation have not been assigned to genes on the standard map of T5; the number represents the allele isolation number.

[c] Not tested.

alleles. These suppressor-bearing mutants grow equally well at 30° and 40° and appear to make slightly larger plaques on strain Fsu^+ than on the nonsuppressing strains Fsu^- or EG47.

Deletion mutants of T5 can be isolated as survivors of treatment with chelating agents at high temperature (*7*, *8*). We isolated such deletion mutants to determine whether these suppressors lie in a nonessential region of the genome. Putative deletion derivatives were isolated from each of five (nonindependent) revertants of T5 *am*B2, *am*C1, plated under permissive conditions, and then tested for the amber phenotype. Those that scored as amber were then tested by complementation against T5 *am*B2, T5 *am*C1, and T5 *am*D19. All the putative deletions that scored as amber complemented T5 *am*D19, but none complemented either T5 *am*B2 or T5 *am*C1. This suggested that the suppressor had been lost by the isolation of the deletion derivatives (or alternatively that some function required for the expression of the suppressor had been lost). To confirm loss of the suppressor, two of these deletion mutants that scored as amber were backcrossed with the double amber mutant T5 *am*B2, *am*C1. No phenotypically am^+ progeny were recovered (less than 0.07% recombination) from the cross, showing that it was the suppressor itself that had been lost in the deletion derivatives.

The derivatives stable to heat and chelating agents presented here had not been shown to be deletions except by the criterion of inheritable heat stability in the presence of chelating agents. Therefore, a well-characterized deletion mutant, T5st(O), was examined (*7*, *9*, *10*). Strains

TABLE 2

REVERSION FREQUENCY OF DOUBLE AMBER MUTANTS

Mutant strain	Reversion frequency ($\times 10^6$) Fsu^-	EG47
T5 *am*A1, *am*B1	<0.006	—
T5 *am*A1, *am*C2	<0.009	—
T5 *am*A1, *am*D2	<0.07	—
T5 *am*A1, *am*D3	<0.005	—
T5 *am*A1, *am*D21	<0.03	—
T5 *am*B1, *am*D3	—	<0.02
T5 *am*B2, *am*C1	2.2	0.93
T5 *am*C2, *am*D21	2.4	—
T5 *am*D3, *am*D4	0.12	—
T5 *am*D3, *am*D21	—	7.2
T5 *am*D4, *am*D21	—	4.7
T5 *am*D19, *am*D21	0.55	0.05
T5 *am*-103, *am*-501	33	—
T5 *am*-401, *am*-501	2.7	—
T5 *am*-501, *am*-601	0.6	—

Note. Stocks of double mutants were grown on Fsu^+ as permissive host and the lysates were titered on Fsu^+ as permissive and either Fsu^- or EG47 as nonpermissive hosts. Reversion frequency is the ratio of the titer on a nonpermissive host to that on a permissive host.

TABLE 3

RECOVERY OF AMBERS IN BACKCROSSES OF REVERTANTS WITH $T5^+$

Strain crossed with wild-type T5	Number of progeny tested	Number of *am* progeny found	Distribution of *am* recovery: *amB2*	*amC1*	*amB2, amC1*
T5, *am*B2, *am*C1, *sup*-1	100	7	1	1	5
T5 *am*B2, *am*C1, *sup*-2	100	7	4	2	1
T5 *am*B2, *am*C1, *sup*-3	100	6	2	2	2
T5 *am*B2, *am*C1, *sup*-4	100	7	4	0	3
T5 *am*B2, *am*C1, *sup*-5	100	5	0	3	2
T5 *am*B2, *am*C1, *sup*-6	25	2	0	1	1
T5 *am*B2, *am*C1, *sup*-7	25	1	0	0	1
T5 *am*B2, *am*C1, *sup*-8	25	4	0	1	3
T5 *am*B2, *am*C1, *sup*-9	25	4	2	1	1
T5 *am*B2, *am*C2, *sup*-10	50	4	2	1	1
T5 *am*D19, *am*D21, *sup*-11	146	30	—	—	—

Note. Suppressor-carrying strains were crossed with wild-type T5 by infecting Fsu^+ with a multiplicity of 5 of each phage, diluting after adsorption, and allowing phage growth to proceed for 2 hr at 37°. The output of the cross was sterilized with chloroform and was heated at 45° for 15 min to prevent clumping of particles (which leads to artifactual recombination frequencies). The progeny were plated on Fsu^+ and plaques were picked and tested for complementation as described elsewhere (*14*). In the last cross (with *sup*-11) 5 of the 30 *am* progeny were tested against single amber mutants. Four carried both *am*D19 and *am*D21, while one carried only *am*D21.

carrying amber mutations and the st(O) deletion were isolated and the reversion frequencies of strains carrying one or two amber mutations, with and without the st(O) deletion, are given in Table 4. There was no significant difference in the reversion of single mutants with or without the st(O) deletion, but there was no measurable reversion of the double amber mutants in strains carrying the st(O) deletion. Since reversion did not yield suppressors in strains carrying the st(O) deletion, we attempted to cross a suppressor into an st(O)-carrying strain by recombination. When two such suppressor-bearing strains, T5 *am*B2, *am*C1, *sup*-1 and T5 *am*B2, *am*C1, *sup*-2, were crossed with either T5 *am*B2, st(O) or T5 *am*C1, st(O), no phenotypically $amber^+$ recombinants were detected among the genetically heat and chelation stable progeny (recombination frequency less than 10^{-6}) (Table 5). Given the high recombination frequency for T5 crosses, this suggests that the suppressor lies within the region covered by the st(O) deletion.

Finally, one suppressor-bearing strain, T5 *am*B2, *am*C1, *sup*-1, was crossed with five other independently isolated suppressors of the same double amber mutant and with one suppressor of the double amber mutant T5 *am*D19, *am*D21 (viz. T5 *am*D19, *am*D21, *sup*-11). Of the 200 progeny tested from each of the six crosses, none recovered the amber phenotype, so we have been unable to demonstrate recombination between the independently derived suppressors described here.

Two observations suggest that we have isolated and mapped a nonsense suppressor in phage T5. Several pairs of amber mutations revert to growth on su^- hosts at about the same frequency as single amber mutants. This reversion is the result of an unlinked mutation in a nonessential region of the genome which is covered by the st(O) deletion and is probably deleted in the deletion, am^- derivatives of the suppressed double ambers described here.

No suppressors could be isolated when one of the pair of amber mutations was in gene A1, on the FST segment of the chromosome. The st(O) deletion does not overlap the FST (*1*) so the suppressor is

TABLE 4

REVERSION FREQUENCIES OF AMBER MUTANTS WITH AND WITHOUT DELETIONS

Mutant strain	Reversion frequency ($\times 10^6$)[a]	
	Fsu$^-$	EG47
T5 *am*B2, *am*C1	2.2	0.93
T5 *am*B2, *am*C1, st(O)	<0.02	<0.01
T5 *am*B1, *am*D3	—[b]	<0.02
T5 *am*B1, *am*D3, st(O)	—	<0.07
T5 *am*C2, *am*D21	2.4	—
T5 *am*C2, *am*D21, st(O)	<0.02	—
T5 *am*D19, *am*D21	0.55	0.05
T5 *am*D19, *am*D21, st(O)	<0.07	<0.07
T5 *am*B2, *am*C1, *del*[*sup*-1]-1[c]	—	<0.005
T5 *am*B2, *am*C1, *del*[*sup*-2]-2	—	<0.02
T5 *am*B2, *am*C1, *del*[*sup*-3]-3	—	<0.005
T5 *am*B2, *am*C1, *del*[*sup*-4]-4	—	<0.001
T5 *am*B2, *am*C1, *del*[*sup*-5]-5	—	<0.001
T5 *am*B1	0.1	—
T5 *am*B1, st(O)	—	1.7
T5 *am*B2	6.5	—
T5 *am*B2, st(O)	—	0.14
T5 *am*C1	0.8	—
T5 *am*C1, st(O)	3.2	2.7
T5 *am*D19	5.0	—
T5 *am*D19, st(O)	3.6	2.9
T5 *am*D21	0.2	—
T5 *am*D21, st(O)	0.2	0.06

[a] Reversion frequency is expressed as the ratio of the titer on the nonpermissive strain (either Fsu$^-$ or EG47) to the titer on the permissive strain (Fsu$^+$).

[b] A dash (—) indicates that the frequency was not measured.

[c] *del*-1, *del*-2, *del*-3, *del*-4, and *del*-5 are independent deletions (as defined by inheritable heat stability) of the suppressors. *sup*-1 through *sup*-5 were isolated from the same lysate and are thus not necessarily independent mutations.

not in the FST. Hence the suppressor would not be available to suppress an *am*A1 mutation until after the rest of the genome had entered the cell. Since a functional A1 gene product is essential for this entry (*6, 11*), mutations on the FST should not be suppressible by T5 suppressors. The fact that no strains carrying the *am*A1 mutation could be suppressed thus supports the mapping of the suppressor. The strain carrying the *am*B1, *am*D3 pair of mutations also seemed nonsuppressible. Both *am*B1 and *am*D3 are suppressible by bacterial suppressors *su*I, *su*II, and *su*III (data not shown) and neither of these genes is thougnt to lie on the FST segment (*10, 11*). Results from other pairs of amber mutations show that at least *am*D3 can be suppressed by the T5 suppressor. A possible explanation might be that gene B1 lies on the FST and is therefore nonsuppressible by the T5 suppressor. It is also possible that gene B1 is required for tRNA expression or that the *am*B1 mutation cannot be efficiently suppressed by the T5 suppressor, though the latter argument seems unlikely. A few of the amber mutations in T5 (including *am*D21) are suppressed by bacterial suppressor *su*I but not by *su*II or *su*III and *am*D21 is suppressed by the T5 suppressor, suggesting that *su*I-specific amber mutations can be suppressed by the T5 suppressor. The *am*B1 allele is suppressed by bacterial suppressor *su*I (as well as *su*II and *su*III) and so ought to be suppressible by the T5 suppressor.

The T5 genome codes for a wide variety of tRNA species, most or all of which are dispensible (*1*). From the genetic code, there are several species of tRNA encoded by T5 which would be able to mutate to suppressors in a single step and some of these seem to lie outside the region defined by the st(O) deletion. Thus we are unable to explain our inability to detect suppressors of double amber mutants in st(O)-bearing strains and we are unable to explain our inability to separate independent suppressors by recombination. The suppressors of phage BF23 were readily separated by recombination (*2*), suggesting that our selection procedure resulted in the repeated isolation of the same suppressor (perhaps by requiring particular specificity) or that there is a recombinational abnormality in this region of the T5 genome.

Finally, all of the amber mutants we have used were isolated using strain Fsu$_\beta^+$ as the permissive host. Most of the mutants were suppressible by all three of the common bacterial suppressors when tested in *E. coli* K12 derivatives, but a few were suppressed by only *su*I in *E. coli* K12 derivatives. Thus we confirm the observation of Hendrickson and McCorquodale (*11*) that the amber suppressor in strain Fsu$_\beta^+$ is analogous to *su*I of *E. coli* K12.

TABLE 5

CROSS OF AN ST(O)-CARRYING STRAIN WITH A SUPPRESSOR-CARRYING STRAIN

Parental strains crossed	Total progeny (on Fsu$^+$)	Progeny on EG47 (*su*$^-$) after heat treatment	Fraction genetically heat stable among survivors
T5 *am*B2, *am*C1, *sup*-1 × T5 *am*B2, st(O)	3.7×10^{10}	8.0×10^5	0/10
T5 *am*B2, *am*C1, *sup*-1 × T5 *am*C1, st(O)	1.0×10^{10}	9.8×10^5	0/10
T5 *am*B2, *am*C1, *sup*-2 × T5 *am*B2, st(O)	0.97×10^{10}	5.2×10^5	NT
T5 *am*B2, *am*C1, *sup*-2 × T5 *am*C1, st(O)	1.2×10^{10}	15.2×10^5	1/10

Note. Crosses were performed as described in the note to Table 3. In each cross, 10 of the progeny that grew on EG47 after one round of heat treatment were subjected to a second round of heat treatment. Only one of the 30 survivors tested remained heat stable, the other 29 were *phenotypically* heat resistant (*7*) and thus did not carry the st(O) deletion. The one st(O)-carrying, *am*$^+$ survivor was found to be a revertant of the *am*C1 allele since the *am*C1 allele could not be recovered when this survivor was backcrossed with wild-type T5 (no *amber* recombinants out of 200 progeny tested). These data indicate that the recombination frequency between st(O) and the suppressor is less than 5×10^{-6}. NT, not tested.

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