Genetic Suppressors of Bacteriophage T5 Amber Mutants

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Nonsense mutations in structural genes appear to abort polypeptide chain synthesis at the amino acid site governed by the mutant codon. The two presently recognized types of nonsense mutant, amber and ochre, have been assigned the codons UAG and UAA, respectively. Certain bacteria harbor one or more genetic suppressors that counteract the effect of appropriate nonsense mutations in their own structural genes as well as in those of infecting viruses. The suppressors, which may become active (su^+) or inactive (su^-) by mutation, permit completion of the affected polypeptide chains by inserting an amino acid at the nonsense site. The type of amino acid inserted depends on the suppressor. At the phenotypic level, a given amber suppressor works with most amber mutants but no ochre mutants. A given ochre suppressor works with most ochre mutants and also with some amber mutants. When a mutant fails to respond to a suppressor that works with other mutants of the same class, the current theory suggests that the polypeptide chain is completed, but is defective owing to the nature of the inserted amino acid. Recent papers, which give additional references, are those by R. Hill and G. S. Stent (Biochem. Biophys. Res. Commun. **18**:757, 1965), S. Kaplan, A. O. W. Stretton, and S. Brenner (J. Mol. Biol. **14**:528, 1965), E. Gallucci and A. Garen (J. Mol. Biol. **15**:193, 1966), T. K. Gartner and E. Orias (J. Bacteriol. **91**:1021, 1966), and R. E. Webster, D. L. Engelhardt, and N. D. Zinder (Proc. Natl. Acad. Sci. U.S. **55**:155, 1966).

To extend our genetic analysis of phage T5 (W. D. Fattig and F. Lanni, Genetics **51**:157, 1965; Y. T. Lanni, F. Lanni, and M. J. Tevethia, Science **152**:208, 1966), we needed an *Escherichia coli* strain that would both allow rapid phage adsorption and suppress amber (am) mutants. The mutants, obtained by mutagenesis with 5-bromodeoxyuridine, were identified as amber mutants by their ability to grow in strain CR63, which has a classical amber suppressor, but not in an su^- derivative of CR63 or, in the many cases tested, strain B, a classical su^- strain. Strain F, the only available strain allowing fast adsorption

of T5, is su^- for all our am mutants. We report here the isolation and some properties of su^+ derivatives.

The source was a histidine auxotroph, which we derived from strain F by mutagenesis with 2-aminopurine. If the auxotroph happened to have arisen by nonsense mutation, phenotypic reversion could occur by mutation to su^+ . Such suppressed mutants might permit growth of our T5 am mutants.

Five cultures of the auxotroph, started from separate colonies, were grown overnight in nutrient broth at 37 C, washed thoroughly with non-nutrient buffer, and plated on minimal agar. All of the 154 revertant colonies, from a total of about 2×10^9 plated cells, were picked to broth and spot-tested with one T5 am mutant. The 21 su^+ isolates were easily sorted into four well-defined groups (Table 1) with a set of 13 representative am mutants. Tests with one isolate from each su^+ group were extended to a total of 48 am mutants, which represent 21 cistrons according to current enumeration. To increase the discrimination, we used 50 to 100 plaque formers per spot instead of the much higher number often employed. Within the class of positive responses, we were therefore able to note differences in plaque size, which generally may be expected to correlate with the more tediously estimated average burst size.

In spot tests at 30 C, 42 am mutants responded to all four su^+ testers, and the remainder responded at least to two. Thus, among 192 combinations of 48 mutants and four suppressors, 184 gave a clearly positive result. Judged from plaque sizes, the responses were, however, variable. With rare exception, and then only when plaques were very tiny, the plaque number was similar on all testers; and plaques on a given tester were generally fairly uniform, regardless of their average size. The heterogeneity of positive responses can be seen in Table 2, which was assembled from a larger body of concurrent tests.

The following comments, often referring to Table 2 for examples, are based on our whole ex-

TABLE 1. Classification of *su*⁺ revertants recovered from independent cultures of a histidine auxotroph of *Escherichia coli* strain F

Culture	Revertants tested	Total <i>su</i> ⁺	No. in group			
			A	B	C	D
1	112	2	0	1	0	1
2	10	8	2	5	0	1
3	10	6	0	6	0	0
4	12	3	0	0	3	0
5	10	2	0	1	0	1
Total	154	21	2	13	3	3

perience, which confirms in several respects the experience of other workers.

Certain individual mutants, such as *am16*, distinguish all four *su*⁺ types. This would not be true if we recorded only positive and negative responses and neglected the plaque sizes.

Although one cannot be sure without explicit study, the sharp differences in appropriate comparisons suggest that each of the four suppressors inserts a different amino acid at the nonsense site. If so, the high frequency of positive responses indicates that many amino acids can be interchanged with only secondary effects on polypeptide function. The plaque sizes presumably reflect these secondary effects and perhaps also the relative efficiencies of the several suppressors (A. Garen, S. Garen, and R. C. Wilhelm, *J. Mol.*

Biol. **14**:167, 1965; S. Kaplan, A. O. W. Stretton, and S. Brenner, *J. Mol. Biol.* **14**:528, 1965).

As expected, the pattern of responses to the suppressors is mutant-specific, not cistron-specific. For example, *am7* and *am11*, at different sites in the same cistron, give different patterns, whereas *am1*, in a different cistron, resembles *am7*. However, mutants at different sites in the same cistron, for example, *am7* and *am30*, may give similar patterns. Contrasts between mutants afford preliminary evidence that they occupy different sites.

Mutants *am2* and *am5*, in the same cistron, give the same, highly distinctive pattern. Since these mutants resemble each other in other comparisons and have so far given no evidence of recombination, we tested their patterns with the specific purpose of obtaining additional evidence of identity. The results support this inference. Note that *am9-2*, at a separate site in the same cistron, gives a different pattern.

Mutants *am2* and *am5* exemplify occasional *am* mutants that are temperature-sensitive (*ts*) in some *su*⁺ hosts and not others. This feature (M. L. Dirksen, J. C. Hutson, and J. M. Buchanan, *Proc. Natl. Acad. Sci. U.S.A.* **50**:507, 1963; G. Eggertsson and E. A. Adelberg, *Genetics* **52**:319, 1965) suggests that the *ts* and *am* characters are different manifestations of the same mutation. If two separate mutations were involved, we might generally expect to see the *ts* character in all *su*⁺ hosts. Actually, *am2* and *am5* fail to complement not only as ambers but

TABLE 2. Suppression of selected T5 *am* mutants by *su*⁺ tester strains^a

Amber	Strain F (<i>su</i> ⁻)		Tester strain (<i>su</i> ⁺ group)							
			HR18 (A)		HR23 (B)		HR34 (C)		HR41 (D)	
	30 C	37 C	30 C	37 C	30 C	37 C	30 C	37 C	30 C	37 C
1	0	0	b	b	m/b	b	m/b	b	b	b
2	0	0	b	b	m	0	m/b	0	b	b
5	0	0	b	b	m	0	m/b	0	b	b
9-2	0	0	b	b	m/b	m	m/b	m	b	b
7	0	0	b	b	m/b	m/b	m/b	b	b	b
11	0	0	b	b	0	0	s/m	s	b	b
30	0	0	m/b	m/b	m/b	b	m/b	b	b	m/b
13	0	0	b	b	s	m/b	m/b	m/b	b	b
16	0	0	0	0	vt	vt	m/b	m	s/m	s
26	0	0	t	vt	s/m	s	m/b	m	t	s
29	0	0	m/b	m/b	t	0	m/b	m	b	b
Wild	b	b	b	b	b	b	b	b	b	b

^a Nutrient agar plates were seeded with about 10⁸ cells from an overnight culture, spotted with 0.01 ml of each phage (50 to 100 plaque formers), and incubated at the temperatures shown. Plaque sizes: b, big (diameter about 1 mm); m, medium; s, small; t, tiny; vt, very tiny; intermediate sizes are shown by m/b and s/m; 0 = no plaques. Mutants in the same cistron (2, 5, and 9-2; 7, 11, and 30) were isolated independently.

also as *ts* mutants. Interpretation of the negative responses in HR23 and HR34 at 37 C would ordinarily be ambiguous without additional information. The positive responses at 30 C suggest that the suppressors are working also at 37 C, in keeping with the current belief that a suppressor that works (at the molecular level) with one amber mutant should work with all. The diagnostic value of testing suppression at several temperatures seems evident.

Taking plaque sizes and temperature effects into account, the suppression spectrum of group C strains, typified by HR34, resembles that of CR63, suggesting that the group C suppressor is an amber suppressor and, accordingly, that the histidine auxotroph carries an amber, not ochre, mutation (S. Brenner and J. R. Beckwith, *J. Mol. Biol.* 13:629, 1965; E. Gallucci and A. Garen, *J. Mol. Biol.* 15:193, 1966). HR34 resembles strain F with respect to growth parameters and also the usual parameters of T5 infection, in-

cluding adsorption rate constant. We have adopted HR34 as a host for genetic crosses.

In conclusion, it appears that the isolation and differentiation of nonsense suppressors in a new experimental system can be an impressively simple matter, if appropriate use is made of suitable phage mutants. Conversely, a set of suppressors is valuable in the genetic analysis of the phage mutants. Our results are readily interpreted by, and therefore support in several ways, the current theory of amber mutants and their suppression.

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