UAG SUPPRESSOR CODED BY BACTERIOPHAGE T4

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1. Introduction

In *Escherichia coli* the amber triplet, UAG, causes premature termination of the growing polypeptide chain [1,2]. Strains which carry amber suppressors reverse this effect through an altered tRNA which permits the reading of UAG as an amino acid [3,4]. Although numerous amber mutations have been isolated in both bacteria and bateriophages, amber suppressor tRNA's have been demonstrated only in bacteria [2]. In view of the recent reports that bacteriophage T4 brings about the *de novo* synthesis of several tRNA's [5–7], the question arises: can one of the tRNA's be converted to an amber suppressor? The answer appears to be yes and this communication documents the evidence.

2. Results and discussion

2.1. *Spontaneous reversion of a double amber mutant*

To look for a phage T4 coded suppressor, revertants of a double amber mutant were examined. This strategy was adopted to avoid the background of normal reversion by single amber mutants. The double mutant used here is B20, NO22, which is defective in genes 14 and 48, respectively [8]. Among a total of 5×10^9 double mutant particles on a series of plates seeded with the su_I^- strain *E. coli* B, several small, faint plaques and a single wild type plaque were found. When picked and replated, using the su_I^+ strain *E. coli* CR63 [1] as indicator, both classes of revertants retained the phenotypes exhibited on strain B. Since the partial revertants form small plaques on the su^- and su^+ bacterial strains, it has not been possible to prepare suitable lysates for their analysis and they have been set aside temporarily. However, the wild-type revertant, designated as B20, NO22R1, has been analysed in detail. When crossed with T4, three types of amber mutants were segregated: the original double mutant and each of the two single mutants. These results show that there must have been an additional set of segregants which escaped detection, namely the suppressor, designated as psu_I^+, responsible for conferring the am^+ phenotype to B20, NO22R1. It is conceivable that psu_I^+ could operate as a gene-specific suppressor, somehow obviating the requirement for gene 14 and 48 products, or as a site-specific suppressor which recognises the amber triplet. In the latter case, psu_I^+ would be analogous to the classical *E. coli* suppressor genes.

2.2. *psu_I^+ is an amber suppressor*

Suppression of other amber mutations by psu_I^+ would immediately identify the nature of the new suppressor. A rapid spot test cross [9] was devised to test the acceptability of psu_I^+ with various rII amber mutants. The tester phage with the psu_I^+ gene also carried a deletion mutation (r 1272) of the entire rII region [9]. When this strain is crossed with an rII amber mutant, no authentic r^+ recombinants can be generated; however, apparent r^+ phage will appear if the recombinant rII amber, psu_I^+ is suppressed. The results given in table 1 show that psu_I^+ suppresses many rII amber mutations, while ochre and UGA mutations are not suppressed. In several cases, the three kinds of mutations at the same site were examined for suppression by psu_I^+; only the amber was suppressed (table 1). Furthermore, it has been found that a T4 lysozyme ochre mutation is not suppressed by psu_I^+ whereas the

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Table 1
Qualitative suppression pattern of some rII mutants by psu^+_I

A. *Mutants which are suppressed*

Amber mutants:

A cistron: HD120, HF103, S172, HB129, S99, N34, X20/a*, X170/a, X319/a.

B cistron: HE122, EM84, HB74, NT332, X237, AP164, HB232, X417, HD231, 360/a, X511/a, X27/a, N24/a, N7/a, SD160/a, N29/a.

Ochre and UGA mutants:

none.

B. *Mutants which are not suppressed*

Amber mutants:

A cistron: S116, N97, N11, S24, N19.

B cistron: 2074.

Ochre mutants:

A cistron: X20, X170, X319.

B cistron: 360, X511, X27, N24, N7, SD160, N29.

UGA mutants:

A cistron: X20UGA.**

B citsron: 360UGA.

rII mutants are from the Cambridge Collection and have been described previously [11]. Spot test crosses [9] between r1272, psu^+_I and various rII mutants were performed by adsorbing the parental phages to strain B and spotting the infected complexes on a plate seeded with strain CA244 (Hfr Hλ, su⁻) [11]. The responses were scored after overnight incubation at 32°C. r1272, psu^+_I was isolated as a recombinant from the cross B20, NO22 R1 by r1272 ans was made isogenic to r1272 by extensive backcrossing.

* An amber mutants derived from the ochre mutant at the same site [10].

** A UGA mutant derived from the ochre mutant at the same site [10].

Table 2
Burst sizes of mutants with the psu^+_I gene

T4D	100.0
H36psu^+_I	69.3
H11psu^+_I	86.0
E18psu^+_I	31.6
N58psu^+_I	73.5
8–82psu^+_I	87.5
H36	0.0
H11	0.0
E18	0.0
N58	0.0
8–82	0.2

Burst size measurements were performed on strain B at 37°C as previously described [11]. Burst sizes are expressed as percentage of the wild type, which was several hundred phages per cell. Amber, psu^+_I double mutants were isolated as recombinants from a cross with B20, NO22R1. Prior to the cross, B20, NO22R1 was inactivated with approximately 20 T4-lethal hits of UV light [10] so as to increase the frequency of the desired recombinant among progeny which formed plaques on strain B. The composition of double mutants was verified by segregating the associated amber mutant and identifying it by spot test crosses [9]. H36, psu^+_I also has been isolated from a cross of H36 by *r*1272, psu^+_I. The two H36, psu^+_I strains cannot be distinguished by burst size measurements.

amber at the same site is suppressed (results to be published elsewhere). This asymmetry in suppression of the three classes of mutations shows that psu^+_I is an amber suppressor [11].

2.3. *The efficiency of suppression*

Amber mutations in other phage genes are also suppressed. These include mutations in genes coding for the head protein (gene 23, sites H11 and H36), the sheath protein (gene 18, site E18), a tail fiber protein (gene 34, site N58) and the phage lysozyme (gene e, site 8–82) [8]. In each case the suppressed mutant forms plaques with about the same efficiency on the su^+_I and su⁻ bacterial strains, implying good suppression. Measuring the burst sizes on the su⁻ strain (table 2) shows more directly that the suppressor is efficient. Preliminary results of an independent experiment support these conclusions. By use of a previously described technique, which measures the transmission coefficient beyond an amber mutation in the gene coding for the phage head protein [12], approximately equal amounts of suppressed and non-suppressed ^{14}C-labelled polypeptides were found in H36, psu^+_I infected cells. This result sets the efficiency of suppression at approximately 50%.

In contrast to the efficient suppression described above, amber mutations in the rII genes give weak responses with the suppressor (data not given). In searching for an explanation for this finding, it is instructive to point out that the rII functions are expressed and required earlier in the lytic cycle than are genes for structural proteins [13]. Successful rescue of rII ambers thus requires that the psu_I^+ gene product be made available early; therefore, the apparent anomaly could depend on the time course for synthesis of the suppressor.

2.4. *The amino acid inserted*

Unfortunately, it is not possible to identify the amino acid from the suppression spectrum with rII amber mutants by analogy with the bacterial suppressors. Since rII mutants are weakly suppressed, it would be difficult to decide whether a negative response resulted from a bad amino acid replacement or whether the amount of suppressed product was critically below that required for activity.

Some progress in identifying the amino acid had been made using the direct approach which proved successful with the bacterial suppressors [12]. By examining ^{14}C-labelled head protein synthesised by H36, psu_I^+, we learned that the amino acid is neutral and that tryptophan, tyrosine, phenylalanine, leucine, asparagine and glutamine are not likely candidates. Recent results suggest that the amino acid is serine or threonine, but a decision between the two is not yet possible.

3. Summary

It is shown that bacteriophage T4 is genetically competent to code for an amber suppressor. Suppression of amber mutations by this new phage derivative can be efficient and results in the insertion of a neutral amino acid. Although no conclusive evidence is presently available, it seems almost certain that the suppressor is a phage-coded tRNA.

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