*THE CONVERSION OF AMBER SUPPRESSORS TO OCHRE SUPPRESSORS**

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Communicated by Charles Yanofsky, May 7, 1968

When the amber codon (UAG)[1, 2] and the ochre codon (UAA)[1] occur in a particular bacterium or bacteriophage messenger RNA, the corresponding polypeptide chain is terminated at the site of the nonsense codon.[3] The presence of a suppressor may cause amino acid insertion resulting in completion of the peptide chain with an efficiency dependent on the suppressor.[4, 5] Functional protein may result, depending on the amino acid inserted. Amber suppressors, su_1^+, su_2^+, and su_3^+, which cause the effective translation of only the amber codon,[6] have been shown to produce altered transfer RNA's[7–9] which cause the insertion of serine,[10–12] glutamine, and tyrosine,[5, 13] respectively, at the site of the amber codon. Amber suppressor tRNA's may arise by single base changes in the DNA information specifying an anticodon in a species of seryl-, glutaminyl-, or tyrosyl-tRNA, thereby transforming an anticodon one base different from the complement of UAG to an anticodon that is complementary to UAG.[5, 7, 14–16] Similarly, at least some ochre suppressors could also be tRNA's with altered anticodons that are complementary to UAA.[16, 17]

According to the anticodon model for suppressor mutations, it should be possible to convert an amber suppressor to an ochre suppressor. Just as the amber and ochre codons differ by a single base, so would the anticodons of tRNA's that can recognize these two codons, i.e.,

nonsense codon:	$5'^{UAG}3'$,	$5'^{UAA}3'$;
tRNA anticodon:	$5'^{CUA}3'$,	$5'^{UUA}3'$.

Evidence will be presented here to show that ochre suppressors have been derived from each of the three known amber suppressors by means of a specific mutagen, uracil-$5H^3$ decay, that is thought to cause C → T changes.[16, 18] In *E. coli*, uracil and cytosine are rapidly interconverted[19] so that the radioactive decays that produce a majority of mutations when a cell is labeled with uracil-$5H^3$ or cytosine-$5H^3$ originate as labeled cytosine residues incorporated into DNA.[20]

Materials and Methods.—Isolation of revertants: (a) *Uracil-$5H^3$ decay:* A given bacterial strain (see Table 1) was labeled with uracil-$5H^3$ in A-1 minimal medium[21] containing leucine and tyrosine (20 mg/liter) for strains WU36-18, WU36-10 class 1 am, and WU36-10 class 2 am, or with the addition of tryptophan (20 mg/liter) for strain A2. Labeled cells were filtered, resuspended in A-1 medium, and held at 4°C to accumulate 100–200 decays/cell. Tyrosine-independent revertants were isolated from WU36-10 class 1 am and WU36-10 class 2 am, and from WU36-18 by plating 0.2-ml aliquots of stored cells on plates containing A-1 medium, agar (15 gm/liter), nutrient broth (0.2 gm/liter), and leucine (20 mg/liter). Leucine-independent revertants of WU36-18 were isolated on similar plates except that leucine was omitted and tyrosine added (20 mg/liter). Tryptophan-independent revertants of A2 were isolated on minimal plates containing nutrient broth (0.2 gm/liter).

(b) *2 Amino-purine (2AP):* A log phase culture of WU36-10 class 2 am at 6×10^8 cells/ml growing at 37°C in supplemented A-1 medium was grown an additional 50 min

TABLE 1. *Characteristics of the bacterial strains of E. coli used.*

Bacterial strain	Source	Type of amber suppressor*	Mutant gene and type of nonsense triplet†	Nutritional dependence
WU36-10	Dr. E. Witkin	None	leu_{am}^-, tyr_{oc}^-	leu^-tyr^-
WU36-10	As in ref. 23	Class 1 am	leu_{am}^-, tyr_{oc}^-	leu^+tyr^-
WU36-10	As in ref. 23	Class 2 am	leu_{am}^-, tyr_{oc}^-	leu^+tyr^-
WU36-18	Dr. E. Witkin	Class 2 am	leu_{oc}^-, tyr_{oc}^-	leu^-tyr^-
A2	Dr. C. Yanofsky	Class 3 am	*try* syn_{oc}^- (*A* gene)	try^-
WWU	See refs. 24 and 25	None	arg_{am}^-, $dRPM_{am}^-$, *thy* syn_{oc}^-	arg^-thy^-‡
WWU	As in ref. 16	Class 3 am	arg_{am}^-, $dRPM_{am}^-$, *thy* syn_{oc}^-	arg^+thy^-

* Each strain was tested with a set of nonsense mutants of bacteriophage T4 previously shown to differentiate the three amber suppressors su_1^+, su_2^+, and su_3^+ (see ref. 16).

† Revertants for the auxotrophic requirement were isolated. If revertants containing an amber suppressor are found, the gene contains an amber triplet, whereas if revertants containing an ochre suppressor are found, the gene may contain an ochre triplet.

‡ In this case, other mutant genes exist, but they do not appear to contain nonsense codons.

in the presence of 2 mg/ml of 2AP. Isolation of tyrosine-independent revertants was as above.

(c) *Ultraviolet light (UV):* WWU class 3 am was grown and irradiated as described previously[16] except that thymidine was added at 40 mg/ml. Thymidine-independent revertants of WWU class 3 am were isolated on minimal plates containing nutrient broth (0.2 gm/liter); thymidine (1.5 mg/liter); arginine, proline, methionine, and tryptophan (100 mg/liter each); and uracil (60 mg/liter).

Assay of revertant bacteria: Revertant colonies that appeared after 48 hr at 37°C were streaked, incubated 12–36 hr at 37°C, and stored at 4°C. Cells from each streak were grown in nutrient broth (8 gm/liter), containing thymidine (40 mg/liter) and used as hosts to test, as in reference 16, for the growth of amber mutants and an ochre mutant of bacteriophage T4. Revertants of WU36-10 and WU36-18 were also tested for their nutritional dependence by streaking on minimal agar.

Transduction tests: The transducing phage, P1kc, was obtained from Dr. Stephen Phillips (Washington University, St. Louis) and donor lysates were prepared on A2, revertants of A2, H12R8a and H12R7a (the latter two strains supplied by Dr. Alan Garen, Yale University), by procedures as described in reference 22. The recipient was a K12 strain that is his_{am}^- *cys* B^- su^-.

Results.—Conversion of class 1 and class 2 amber suppressors to the corresponding ochre suppressors: Although ochre suppressors can recognize amber codons,[6] the leucine requirement (amber codon) in WU36-10 cannot be overcome by an ochre suppressor that can overcome the tyrosine requirement (ochre codon). These two loci revert independently, and in addition the leucine requirement can only be overcome by a class 1 or class 2 amber suppressor.[23] While this mutation to leucine dependence may be a special case, it provides a convenient assay system for detecting the conversion of an amber suppressor to an ochre suppressor. For example, three types of tyrosine-independent revertants could result from treatment of WU36-10 class 1 am or WU36-10 class 2 am with a mutagen: (1) revertants due to structural gene changes converting the ochre triplet from nonsense to sense; these revertants are expected to be leu^+ tyr^+; (2) revertants due to the *de novo* formation of an ochre suppressor; these revertants should also be leu^+ tyr^+; (3) revertants due to conversion of an amber suppressor to an ochre suppressor. These cells could be leu^- tyr^+ and if so would be distinguishable by their nutritional dependence. Using uracil-5H[3] decay as a mutagen, we find revertants of all three types (Table 2). Of 700 tyrosine-

independent revertants examined in six independent isolations from cells containing a class 1 amber suppressor, 174 contained a double change in nutritional dependence and are thought to contain a converted suppressor. All of the remaining tyrosine-independent revertants were also leucine-independent. Almost all of these (95 of 100 tested) gave a pattern of phage growth similar to that of the amber suppressor parent cell except for a positive spot print on ochre phage 427 suggesting the presence of an ochre suppressor in addition to the original amber suppressor (*de novo* formation of an ochre suppressor). The remainder gave rise to an unaltered pattern of phage growth (structural gene revertants). Similar results were obtained with WU36-10 class 2 am (line 2, Table 2). In this case,

TABLE 2. *Conversion of amber suppressors to ochre suppressors; classification of revertants by nutritional requirement.*

Leucine-independent derivatives of WU36-10	Mutagen used	Total number of revertants examined	Number of Tyrosine Revertants That Are: Leucine-independent	Leucine-dependent
WU36-10, class 1 am	Uracil-5H^3	700	526	174
WU36-10, class 2 am	Uracil-5H^3	360	263	97
WU36-10, class 2 am	2AP	199	196	3

Tyrosine-independent revertants were isolated and tested by streaking on minimal agar for their leucine requirement. Leucine-dependent cells (column 5) are cells thought to contain an amber suppressor that has been converted to an ochre suppressor which inserts the same amino acid. No correction has been made for revertants occurring spontaneously (about 5% of the total).

of 360 tyrosine-independent revertants, 97 were leucine-dependent; this indicates that these revertants contain a converted suppressor. Results obtained with *E coli* WU36-18, which contains a class 2 amber suppressor and ochre triplets in tyrosine and leucine genes, are shown in the final two lines of Table 3. The patterns of phage growth of the three types of revertants isolated were essentially indistinguishable from those for revertants isolated from WU36-10, class 2 am. For cells thought to contain either a converted class 1 amber suppressor or a converted class 2 amber suppressor, as determined by their nutritional dependence, striking differences in the pattern of phage growth were observed (see Table 5).

TABLE 3. *Conversion of amber suppressors to ochre suppressors; classification of revertants by the pattern of phage growth.*

Strains containing amber suppressors from which revertants were isolated	Phenotype for which revertants were selected	Net no. of revertants examined	Net No. of Revertants Containing: Amber suppressor only	Amber and an ochre suppressor	Ochre suppressor only
A2, class 3 am	*try*$^+$	148.8	21.4	86.4	41.0
WWU, class 3 am	*thy*$^+$	13*	4	1	8
WU36-18, class 2 am	*tyr*$^+$	37*	0	27	10
"	*leu*$^+$	149.3	2.3	102.6	44.4

The strains listed in column 1 were treated with uracil-5H^3 decay (UV for WWU), and resulting revertants (column 3) were classified by their patterns of phage growth (see Table 5). A type thought to correspond to revertants containing an ochre suppressor derived from an amber suppressor was observed for each mutant strain and the number of these is indicated in the last column (see Table 5). The number of revertants of each type has been corrected for revertants of spontaneous origin except where indicated (*). Nonintegral numbers result from this correction.

Conversion of amber to ochre suppressors has also been demonstrated with UV light and ethyl methane sulfonate (EMS), which produce the same transitions as uracil-5H[3] decay,[16] and conversion occurs at a similar frequency (data not shown). However, when 2AP is used as the mutagen, only 3 of 199 tyrosine-independent revertants contain a converted suppressor (Table 2).

Conversion of class 3 amber suppressor to the corresponding ochre suppressor: A strain that is mutant in the *A* gene of tryptophan synthetase, A2, contains a class 3 amber suppressor and an ochre triplet in the *A* gene. With this strain, the conversion of an amber suppressor to an ochre suppressor was inferred by means of the patterns of phage growth (Table 3). Tryptophan-independent revertants produced by uracil-5H[3] decay were divided by spot printing into three types (structural gene revertants, *de novo* formation of ochre suppressor, converted suppressor). Of the 106 revertants, 41 had a pattern of phage growth corresponding to a converted suppressor. The conversion of the class 3 amber suppressor was also observed after ultraviolet irradiation of *E. coli* WWU, class 3 am (Table 3). Since this cell contains an ochre triplet in the thymidylate synthetase gene,[24, 25] thymidine-independent revertants were isolated.

Transduction tests: The suppressors in A2 and revertants isolated from A2 were transduced into recipient cells (*cys* B$^-$, *his*$_{am}^-$, su$^-$). *Cys* B was used as the selected marker, and cysteine-independent transductant colonies were streaked onto plates lacking histidine to infer the presence of a suppressor. Streaks giving rise to growth that were spot printed contained a suppressor (Table 4), which was identified by the pattern of phage growth. The cotransduction frequency of *cys* B and the indicated suppressor is shown in the fourth column of Table 4. Cotransductants isolated from cells designated as containing a converted suppressor gave a pattern of phage growth characteristic of a cell containing a class 3 ochre suppressor, while all of the cotransductants isolated from cells designated as containing a class 3 amber suppressor plus an ochre suppressor contained the class 3 amber suppressor. Data for H12R8a and H12R7a are shown for comparison.

TABLE 4. *The cotransduction of the cys B and the class 3 suppressor genes with phage P1kc.*

Strain	Total number of transductant colonies	Number of *his*$^+$ transductant colonies	Cotransduction frequency	Suppressor identification
A2	1371	440	0.32	Class 3 am
A2 revertant (class 3 am → class 3 oc)	1583	466	0.29	Class 3 oc
A2 revertant (class 3 am→ class 3 am + oc)	1601	503	0.31	Class 3 am
H12R8a	643	192	0.30	Class 3 am
H12R7a	1080	288	0.27	Class 3 oc

Donor lysates of P1kc were prepared with su$^+$ strains as hosts (column 1). A su$^-$, *cys* B recipient cell was used since the genes specifying *cys* B and su$_3^+$ are located near each other and can be cotransduced.[22] The recipient cell also contained an amber triplet in a distant gene (*his*) that cannot be cotransduced.[22] *Cys* B was the selected marker. Tryptophan was also used as an unselected marker since A2 contains an ochre triplet in the *A* gene of tryptophan synthetase. At least two different donor lysates were used in a total of at least four independent experiments for each strain. In the case of revertants of A2 at least two independently isolated revertants were used. Approximately 100 cotransductant colonies were spot printed for each strain listed in the table. All colonies that were spot printed contained the suppressor indicated in column 5.

Comparison of patterns of phage growth for revertants containing ochre suppressors derived from amber suppressors and from su⁻ cells: Table 5 shows patterns of phage growth for each of the cases in which ochre suppressors have been isolated from amber suppressors. The pattern of phage growth for cells containing the original amber suppressor is shown on the first line and that for cells containing the ochre suppressor derived from the amber suppressor on the next line. The remaining line, for derivatives of WU36-10 and WWU, shows a pattern of phage growth found for some revertants isolated directly from a su⁻ cell. The pattern of phage growth for H12R7a, which contains an ochre suppressor that inserts tyrosine,[17] is also shown in Table 5.

Discussion.—For each bacterial strain used, three different types of revertants were obtained as determined by the patterns of phage growth (see *Results*). It is clear that ochre suppressors have been derived from each of the three characterized amber suppressors. One has only to decide which pattern of phage growth corresponds to revertants containing a converted suppressor (class 3 am → class 3 oc) and which corresponds to revertants containing an ochre suppressor in addition to the pre-existing class 3 amber suppressor (class 3 am → class 3 am + oc). Since ochre suppressors cannot overcome the leucine requirement in WU36-10, it would seem very likely that converted suppressors in this strain correspond to cells that have become leucine-dependent as indicated in Table 2. Thus, for tyrosine revertants derived from WU36-10, class 1 am, and WU36-10, class 2 am, suppressor conversion is presumed to occur in the revertants that have a changed nutritional requirement from *leu*⁺ *tyr*⁻ to *leu*⁻ *tyr*⁺. Relative to the parent cell there is a marked change in the pattern of phage growth for revertants that are *leu*⁻ *tyr*⁺ so that the spot print assay can be used independently as an assay for the conversion of an amber suppressor to an ochre suppressor. On the other hand, tyrosine-independent revertants that are also leucine-independent and contain an ochre suppressor are presumed to be of the second type (class 3 am → class 3 am + oc) and have a pattern of phage growth that appears to be that of an ochre suppressor added to that of the original amber suppressor.

In the case of tryptophan-independent revertants of A2, the pattern of phage growth alone was initially used to identify revertants containing an ochre suppressor derived from an amber suppressor (Tables 3 and 5). The transduction data in Table 4 support this interpretation. All cotransductants isolated from revertants designated by spot printing to contain a converted suppressor contain a class 3 oc suppressor at what appears to be the same site as the class 3 amber suppressor in A2. On the other hand, the class 3 amber suppressor was recovered from all cotransductants that were designated as containing the original class 3 amber suppressor plus an ochre suppressor. The class 3 amber suppressor in A2, the class 3 ochre suppressor derived from the class 3 amber suppressor in A2, and the su_3^+ amber suppressor in H12R8a give similar cotransduction frequencies.[26] The cotransduction frequency for the su_4^+ ochre suppressor in H12R7a is slightly smaller.[22, 27]

The conversion of an amber suppressor to an ochre suppressor occurs efficiently when uracil-$5H^3$ decay is used as a mutagen since this agent specifically causes C → T changes. The *de novo* production of ochre suppressors by this mutagen is also expected.[23] Since UV and EMS produce GC → AT transitions,[16] they should

TABLE 5. *The patterns of phage growth for the bacterial strains used.*

Bacterial Strains: Type of suppressor	Bacterial Strains: Derived from	T4	Amber and Ochre Mutants of T4 Bacteriophages: B22 (43)	OC427	55	N122 (42)	N82 (44)	120	NG80 (7)	NG101 (7)	NG217 (7)	NG218 (7)	NG370 (43)	N102 (6)	N132 (8)	B255 (10)	E609 (13)	NG322 (5)	NG329 (13)	NG54 (15)	NG319 (15)	NG19 (34)	NG273 (43)	N69 (12)	N133 (15)	NG168 (5)	A453 (32)	B17 (23)	NG75 (12)	NG237 (15)
Class 1 am	WU36-10	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Class 1 oc	WU36-10, class 1 am	+	+	+	+	+	+	+	+	+	+	+	+	+	W	+	+	+	+	+	P	+	+	W	W	+	P	−	P	P
Class 1 oc	WU36-10	+	+	+	+	+	+	+	+	+	+	+	P	W	W	+	+	+	+	+	+	+	+	−	W	W	P	−	P	−
Class 2 am	WU36-10	+	+	−	+	+	+	+	+	+	+	+	+	W	W	W	W	+	W	+	+	−	P	+	P	+	W	+	+	+
Class 2 oc	WU36-10, class 2 am	+	+	+	W	+	P	+	W	P	W	W	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Class 2 oc	WU36-10	+	+	+	W	+	P	+	W	−	W	W	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Class 2 am	WU36-18*	+	+	−	+	+	+	+	+	+	+	+	+	W	W	W	W	+	W	+	+	−	P	+	P	+	+	+	+	+
Class 2 oc	WU36-18	+	+	+	W	+	P	+	W	P	W	W	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Class 3 am	A2*	+	+	−	+	+	+	+	+	+	+	+	W	+	+	+	+	+	+	+	+	+	+	W	+	+	+	P	P	−
Class 3 oc	A2	+	+	+	W	+	P	+	+	+	+	+	W	P	W	W	W	+	W	W	+	W	+	−	−	P	−	−	−	−
Class 3 am	WWU	+	+	−	+	+	+	+	+	+	+	+	P	+	+	+	+	+	+	+	+	+	+	P	+	+	+	P	P	−
Class 3 oc	WWU, class 3 am	+	+	+	W	+	+	+	+	+	+	+	−	W	W	W	W	+	W	W	+	W	+	−	−	−	−	−	−	−
Class 3 oc	WWU	+	+	+	W	+	+	+	+	+	+	+	−	W	W	W	W	+	W	W	+	W	+	−	−	−	−	−	−	−
Su_4^+	H12R7a	+	+	+	W	+	P	+	+	+	+	+	P	−	−	W	W	+	P	P	+	W	+	−	−	−	−	−	−	−

This table characterizes the patterns of phage growth on T4, an ochre mutant of T4, and 27 amber mutants of T4 for the strains used in Tables 2 and 3. The number in parentheses for the mutant phage refers to the gene containing the nonsense triplet. The first line for each strain shows the pattern of phage growth for the parent cell, which in this case contains an amber suppressor. The class 1 am, class 2 am, and class 3 am suppressors have been shown to be similar by their patterns of phage growth to su_1^+, su_2^+, and su_3^+. The second line in each set shows the pattern of phage growth for an ochre suppressor derived from an amber suppressor. The third line for WU36-10 and for WWU shows the pattern of phage growth for a particular class of revertants isolated from a su^- parent cell. The striking feature of the patterns of phage growth shown is that the six types of suppressors listed are separable from each other and form reasonably distinct categories despite the range of bacteria used. The suppressor designated as a class 1 ochre suppressor appears among revertants of a locus containing either an amber or an ochre codon with a very low frequency in the mutagens we have used, which makes detection difficult. This is in contrast to the class 2 or class 3 ochre suppressors for the same strains and the same mutagens.

Plus sign signifies cloudy or clear lysis in the area of the prong point; W, incomplete lysis corresponding to a lower plating efficiency; P, test is mostly but not always negative by spot printing and generally corresponds to a very low efficiency of phage growth by plating (all plaques that develop contain amber phage). These categories are broad and there could be considerable differences in the burst size of amber mutants within one of these categories.

* These are the patterns of phage growth for WU36-18 and A2 and not for cells derived from A2 and WU36-18 as the column heading implies

also produce converted suppressors, and they do. The lack of conversion by 2AP is expected since it is thought to produce the opposite transitions, AT → GC.[16]

The patterns of phage growth for revertants containing a class 1, 2, or 3 amber suppressor and for revertants containing an ochre suppressor derived from the three amber suppressors are clearly distinguishable (Table 5). The characteristic features of the patterns of phage growth for cells containing converted suppressors are as follows: (1) they support the growth of the ochre mutant phage, and the corresponding amber suppressors do not; and (2) they support the growth of a smaller number of amber mutants than the corresponding amber suppressor. These two differences are observed beginning with a class 1, 2, or 3 amber suppressor in all of the strains used. The patterns of phage growth for ochre suppressors derived from amber suppressors that have been characterized as to amino acid insertion may be used to infer the amino acid insertion for ochre suppressors derived from su^- cells. Cells with ochre suppressors that give essentially these same patterns of phage growth can be isolated from su^- cells and we have also designated these suppressors as class 1 oc, class 2 oc, and class 3 oc (Table 5). We assume that converted suppressors insert the same amino acids as the original suppressors, and therefore we conclude that cells that contain an ochre suppressor and are isolated from a su^- cell with the class 1 oc pattern insert serine, those with the class 2 oc pattern insert glutamine, and those with the class 3 oc pattern insert tyrosine.

An ochre suppressor originating from a su^- cell and a converted suppressor thought to insert the same amino acid do show small differences in the ability to support the growth of the amber mutants and the ochre mutant of T4, but the striking aspect of the two suppressors is that the patterns of phage growth are so similar. The similarities probably reflect a common mechanism of formation whereas the small differences may reflect the difference in the tRNA species altered. This could be the case if the mechanism resulting in the formation of ochre suppressors *de novo* and from the conversion of amber suppressors inserting serine, glutamine, or tyrosine is due to altered anticodons.

Preliminary data show that cells containing an ochre suppressor are smaller, grow slower even in enriched media, support the growth of $T4^+$ at a reduced level, and contain a decreased amount of T4 lysozyme during phage infection than cells containing an amber suppressor thought to insert the same amino acid (unpublished observations, this laboratory). Thus, it appears that the decrease in the number of amber mutant phages giving a positive spot print reflects the general abnormal nature of cells containing ochre suppressors, and is probably not due to a difference in recognition of UAG codons by CUA and UUA tRNA anticodons. It would seem more likely that UAA exists normally as a codon in *E. coli* and that the cell is abnormal because of the presence of a suppressor that interferes with the UAA function. The apparent low efficiency with which ochre suppressors insert amino acids[27, 28] may result from competition among UAA codons for ochre suppressor tRNA.

Summary.—Amber suppressors, equivalent to su_1^+, su_2^+, and su_3^+ by their pattern of phage growth, have been converted to ochre suppressors which are presumed to insert the same amino acids. The ochre suppressors obtained in

each case may be differentiated from the original amber suppressors and from each other by their pattern of phage growth on a set of amber mutants and an ochre mutant of bacteriophage T4. Cells containing ochre suppressors with essentially the same three patterns of phage growth have been isolated directly from su^- cells, and we have used the patterns of phage growth to infer amino acid insertion for these ochre suppressors. The data suggest that ochre suppressors leading to the amino acid insertion of serine, glutamine, and tyrosine can occur by single base substitution in anticodons of tRNA's and that UAA codons have a normal function in *E. coli*.

We thank Mrs. Helen Newton for help with the experiments, and our colleagues for help with the various aspects of the manuscript. We also thank Dr. Charles Yanofsky for his comments on the manuscript as well as for sending us the recipient cells for transduction experiments.

* This research was supported by grant NsG-324 from the National Aeronautics and Space Administration and by grant GB-4485 from the National Science Foundation.

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