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Nonsense Suppression in a Multiauxotrophic Derivative of *Escherichia coli* 15T⁻: Identification and Consequences of an Amber Triplet in the Deoxyribomutase Gene

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Previously, arginine revertants of *Escherichia coli* WWU, a derivative of *E. coli* 15T⁻, have been subdivided by two independent methods: (i) the streak morphology on nutrient agar, and (ii) the pattern of phage growth using amber and ochre mutants of bacteriophage T4. In the first assay, revertants were subdivided into two classes according to the appearance of streaks after incubation on nutrient agar, a thick, even line of growth defining *normal* revertants and a thin, irregular line defining *aberrant* revertants. In the second assay, revertants were classified by the suppressors they contained. The present work demonstrates that revertants containing an amber suppressor show the aberrant morphology and are also able to catabolize thymidine for energy and carbon. This is in contrast to the parent WWU containing no suppressor, which shows a normal morphology and cannot utilize thymidine as an energy source. Revertants containing no suppressor, isolated specifically for their ability to catabolize thymidine, show an aberrant morphology. Together, these results indicate that the aberrant morphology results from suppression of an amber triplet in a gene of the thymidine catabolic pathway. Enzyme assays show the amber triplet to be in the gene specifying deoxyribomutase. It is suggested that the aberrant arginine revertants are analogous to high thymine-requiring mutants and that, in general, high and low thymine-requiring mutants differ from one another in their ability to catabolize deoxyribose-1-phosphate.

Arginine revertants of *Escherichia coli* WWU may be divided into two easily separable types, *normal* and *aberrant*, on the basis of their streak morphology when grown on nutrient agar (1). Some revertants give rise to a thick, even line of growth (normal arginine revertants); others give rise to a thin, irregular line of growth after 18 to 22 hr of incubation (aberrant arginine revertants). Revertants may also be divided into classes according to the particular suppressors that they contain. Arginine auxotrophy in WWU is due to an amber triplet in a structural gene for the arginine biosynthetic pathway, and prototrophy can result from mutational events that produce amber or ochre suppressors (12). Revertants containing different suppressors are distinguishable, since they give rise to different patterns of phage growth when used as hosts for a collection of amber mutants and an ochre mutant of bacteriophage T4.

The first objective of the present study was to see if a correlation existed between the aberrant arginine revertants and one or more of the classes of arginine revertants containing a suppressor. Since it was soon found that all revertants containing amber suppressors and only these revertants were aberrant, the second goal was to explain the correlation between amber suppression and the aberrant phenotype.

Harrison (7) has noted that thymine-requiring strains of *E. coli* may be regarded as "high thymine requiring," in which case large concentrations of thymine supplement are required to achieve normal growth in defined medium, or as "low thymine requiring," in which case thymine

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supplements need not exceed by any large margin the concentration required for deoxyribonucleic acid (DNA) synthesis. Breitman and others (2, 7) have also observed that high thymine-requiring strains may be converted to low thymine-requiring strains by a single mutational event and that, in general, newly isolated thymine-requiring strains are first high thymine requiring, becoming low thymine requiring after a second mutational event.

Consequently, it seemed possible that the presence of an amber suppressor in WWU could affect both arginine reversion and the aberrant phenotype if the aberrant phenotype was in fact the phenotype of a high-thymine requirer and the transition from low (WWU is a low-thymine requirer) to high thymine requiring resulted from the suppression of an amber triplet. By using the observation that high thymine-requiring strains catabolize thymidine for energy and low thymine-requiring strains do not (Stacey, *personal communication*), it has been possible to demonstrate the existence of an amber triplet in the structural gene for deoxyribomutase and to accredit the aberrant phenotype to inefficient thymine utilization which occurs whenever deoxyribomutase is functional.

MATERIALS AND METHODS

Strains. E. coli WWU, a multiauxotroph of *E. coli* strain 15, requiring thymine, uracil, tryptophan, arginine, methionine, and proline, has been described previously (13). [In some laboratories this strain is called TAU-bar. Since this strain was not derived from strain TAU, we prefer to emphasize its origin by using the initials WWU (Weatherwax-Wax-Uracil).] The collection of arginine revertants used for the data of Table 1 were obtained by using a variety of mutagens and were characterized by using amber mutants and an ochre mutant of bacteriophage T4 as described in an earlier report (12). *E. coli* strain $15T^-$ was supplied by K. Stacey (The University of Sussex, England).

Media. Two types of nutrient media were used, Oxoid and Difco, because of their respective availabilities at the two laboratories in which this work was conducted. Oxoid nutrient medium contained Oxoid Nutrient Broth no. 2 at 25 g/liter of distilled water. This medium was hardened with "Davis" agar (New Zealand) at 12.5 g/liter of broth, when solid medium was required. Difco nutrient medium contained 8 g of Difco Nutrient Broth per liter of distilled water, hardened with 15 g of Difco agar per liter of broth when a solid medium was required. The aberrant streak morphology could be observed on either of these nutrient media.

Supplemented minimal agar medium contained A-1 salts-glucose solution (13) supplemented with L-tryptophan, L-arginine, L-methionine, and L-proline, each at 100 mg/liter, uracil at 60 mg/liter, thymidine at 20 mg/liter, and Difco agar at 15 g/liter of solution. Specific alterations of this general medium were required in certain experiments (*see below*).

Assay for normal or aberrant mutants. Samples of strains were streaked onto nutrient agar and were incubated at 37 C for 18 to 22 hr. Some yielded a conventional, solid line (normal) of growth and others yielded a thin, irregular line (aberrant) of growth (1).

Assay for arginine independence and thymidine catabolism. Samples of mutant strains were streaked onto supplemented minimal agar medium lacking arginine, to determine arginine independence, or onto supplemented minimal agar medium containing thymidine at 1 g/liter in place of glucose, to determine thymidine-catabolizing ability. A substantial bacterial growth, after approximately 16 hr of incubation, defined an individual sample as arginine-independent or capable of thymidine catabolism, depending on the assay agar. No growth, under similar circumstances, caused the sample to be classified as arginine-dependent or incapable of thymidine catabolism, respectively.

Thymidine-catabolizing revertants of WWU were selected on supplemented minimal agar medium containing thymidine, at 1 g/liter, in place of glucose as an energy source and Difco Nutrient Broth at 0.2 g/liter (to increase the number of spontaneous revertants).

Assay for suppressor mutation. The different classes of revertants derived from WWU were defined by the patterns of phage growth, by use of a variety of amber mutants and one ochre mutant of bacteriophage T4, as described previously (12).

In vitro enzyme assay. Extracts were prepared from cultures of an arginine revertant with a normal phenotype (class 6) and an arginine revertant with an aberrant phenotype (class 2 amber) grown in Nutrient Broth (Oxoid) supplemented with thymidine at a concentration of 50 mg/liter to prevent viability decline in the aberrant culture (*see* Fig. 3). One-liter cultures were grown with aeration at 37 C to a cell concentration of approximately 2×10^8 bacteria per ml and harvested by centrifugation.

The extraction and enzyme incubation procedures employed were those of Hoffman and Lampen (8) and Racker (14), with slight modifications. The bacteria were washed twice and suspended in 10 ml of 0.9% NaCl solution. They were sonically treated over ice for 4 min by use of a Soniprobe type 1130A (Dawe Instruments Ltd., London; power setting at six) in intervals of 20 sec on, 10 sec off. The cell extracts were cleared of large debris by low-speed centrifugation and were stored overnight at 4 C.

When assaying for thymidine phosphorylase activity, the incubation tubes each contained 1.5 ml of cell extract, 2.5 ml of $NaHCO_3$ solution (0.1 M), 0.05 ml of thymidine solution (50 mg/ml), and 0.2 ml of potassium phosphate solution (0.1 M at *p*H 7.3). When assaying for deoxyriboaldolase activity, the incubation tubes each contained 1.0 ml of cell extract, 1.0 ml of $NaHCO_3$ solution (0.1 M), 26 mg of fructose-1,6-diphosphate, and 0.1 ml of acetaldehyde solution

(5.65%). The fructose-1,6-diphosphate provided a ready source of glyceraldehyde-3-phosphate (14).

The production of deoxyribose or deoxyribomonophosphate was followed by removing 0.2-ml samples during incubation (37 C) and assaying these with the Dische diphenylamine assay (5). Samples were diluted with water to a volume of 1 ml and were mixed with 2 ml of diphenylamine reagent (1 g of diphenylamine in 100 ml of glacial acetic acid and 2.75 ml of concentrated H_2SO_4). Samples were heated at boiling-water temperature for 10 min and cooled to room temperature. The optical density, at 595 mμ, was determined relative to a blank prepared by processing a sample in which the cell extract was replaced by saline solution.

RESULTS

Correlation between arginine-revertant classes, aberrant growth, and the ability to catabolize thymidine. To test the possibility that a specific suppressor was responsible for the aberrant arginine revertant, stock cultures of individually isolated arginine revertants were used. These arginine revertants contained amber suppressors (class 1, 2, or 3), ochre suppressors (class 4 or 5), or no suppressor (class 6). At least 30 revertants of each type were tested for normal or aberrant growth and for the ability to catabolize thymidine (*see* Table 1). All revertants containing a class 1, 2, or 3 amber suppressor gave an aberrant streak morphology; all other revertants (classes 4, 5, and 6) produced normal growth. Hence, of a total of 284 revertants tested, all revertants containing amber suppressors gave an aberrant streak morphology, and all revertants which gave aberrant growth contained an amber suppressor. In addition, the aberrant phenotype and the ability to catabolize thymidine were concomitant traits.

TABLE 1. *Correlation of suppressor mutation, aberrant streak morphology, and the ability to catabolize thymidine among arginine-independent revertants from E. coli WWU*[a]

Revertant class	No. tested	Streak morphology		Thymidine catabolism	
		No. normal	No. aberrant	No. capable	No. incapable
1 (amber)	51	0	51	51	0
2 (amber)	47	0	47	47	0
3 (amber)	46	0	46	46	0
4[b] (ochre)	35[c]	35	0	0[d]	35
5 (ochre)	53[c]	53	0	0	53
6 (back-mutation)	52	52	0	0	52

[a] In the parent *E. coli* WWU, streak morphology is normal, and it is incapable of thymidine catabolism.

[b] These revertants are defined by phage growth (12) and have since been found to comprise a multiple class. Recently, we were able to infer amino acid insertions for some ochre suppressors (Person and Osborn, Proc. Natl. Acad. Sci. U.S., *in press*), and the revertants referred to as class 4 contain ochre suppressors that are thought to insert glutamine or tyrosine.

[c] These arginine revertants grew slowly in the absence of arginine.

[d] Most of these revertants gave a slight growth after 3 days of incubation.

Pleiotropic consequences of single genetic alterations. Arginine revertants containing a class 1, 2, or 3 amber suppressor differed from the parent *E. coli* WWU in three aspects of their phenotype: (i) reversion of the arginine biosynthetic defect; (ii) reversion of the thymidine catabolism defect; and (iii) the establishment of an aberrant growth potential. If these three aspects of the phenotype were similarly and specifically dependent on the amber suppressor, any of the phenotypic characteristics should serve equally well to select revertants derived from WWU that contained amber suppressors. Hence, thymidine-catabolizing revertants derived from WWU were isolated and examined.

Altogether, 160 spontaneous thymidine-catabolizing revertants were isolated and classified according to suppression, arginine requirement, and growth morphology on nutrient agar (Table 2). Revertants containing the class 1, 2, or 3 amber suppressor were found, and these thymidine-catabolizing revertants were also arginine-independent and aberrant. Thus, revertants containing an amber suppressor, selected as thymidine-catabolizing revertants, were identical to those containing the same amber suppressor but selected as arginine-independent revertants.

There was also a large number of thymidine-catabolizing revertants that contained no suppressor (Table 2, class 6). These apparently represented true back-mutations of the defective structural gene. Since these revertants were not arginine-independent, just as true back-mutation arginine revertants (Table 1, class 6) were not capable of catabolizing thymidine, two distinct genetic loci are required to mediate arginine biosynthesis and thymidine catabolism. However, since all the thymidine-catabolizing revertants without suppressors did have the aberrant phenotype, a distinct genetic locus for the aberrant growth characteristic is not required and, in fact, would seem to be identical to that mediating thymidine catabolism.

It was not possible to specifically select revertants on the basis of aberrant growth; therefore selection according to this phenotypic aspect

TABLE 2. *Correlation of suppressor mutation, aberrant streak morphology, and arginine independence among thymidine-catabolizing revertants from E. coli WWU*

Revertant class	No. tested	Streak morphology		No. arginine-independent
		No. normal	No. aberrant	
1 (amber)	10	0	10	10
2 (amber)	24	0	24	24
3 (amber)	9	0	9	9
–[a] (ochre)	18	18	0	0[b]
6 (back-mutation)	99	0	99	0

[a] These revertants are similar to, but probably not identical with, class 5 revertants, as defined previously (12).

[b] After incubation for 2 to 3 days, a few discrete colonies appeared in a streak that was otherwise devoid of growth.

was not done. Nevertheless, it was possible to do the reverse: to select mutants that gave normal growth from revertants that gave aberrant growth. Individual arginine revertants containing a class 1, 2, or 3 amber suppressor (aberrant arginine revertants) were individually grown. Approximately 10^6 bacteria of each type were spread onto nutrient agar plates. Solid growing colonies that appeared in the thin lawns after 24 hr of incubation were isolated and tested to see if they supported the growth of the phage mutant B17 (an amber mutant of T4 phage that grows well on revertants containing a class 1 or class 2 amber suppressor and to a limited extent on a revertant containing a class 3 amber suppressor), if they were dependent on arginine for growth, and if they could catabolize thymidine. Of 150 mutants with normal morphology (50 derived from each of the 3 revertant types), 113 were arginine-dependent, incapable of thymidine catabolism, and no longer supported the growth of phage mutant B17. Thus, the amber suppressor seemed to have been altered by a single mutational event that removed all three phenotypic aspects originally associated with the presence of an amber suppressor.

Site of the defect in the thymidine catabolic pathway. Other deoxyribosides will serve as an energy source for the thymidine-catabolizing, aberrant revertants. Thus, deoxyuridine, deoxycytidine, and deoxyadenosine supported the growth of revertants containing a class 1, 2, or 3 amber suppressor just as well as did thymidine. These compounds did not support growth of revertants from other classes. Deoxyribose did not support growth of any of the six revertant types. In general, catabolism of deoxyribosides appears to be restricted to the arginine revertants with an aberrant phenotype (class 1, 2, and 3 amber suppressors).

Lampen (11) showed that *E. coli* could catabolize thymidine for energy via three enzymatic steps: thymidine phosphorylase, yielding thymine and deoxyribose-1-phosphate; deoxyribomutase, yielding deoxyribose-5-phosphate; and deoxyriboaldolase, yielding glyceraldehyde-3-phosphate and acetaldehyde. In vitro assays were used to determine which step in this sequence was functional in the aberrant arginine revertants but nonfunctional in the normal arginine revertants.

Cell extracts were prepared from cultures of an aberrant arginine revertant (containing a class 2 amber suppressor) and from a normal arginine revertant (class 6) as described above. In the first assay procedure, the extracts were incubated with thymidine and phosphate (Fig. 1). Both extracts mediated the production of a diphenylamine-reactive material, presumably deoxyribomonophosphate. Since this activity could not be observed in the absence of phosphate (data not given here), it was assumed to be thymidine phosphorylase activity. Upon continued incubation, the extract from aberrant arginine revertants caused almost complete degradation of the

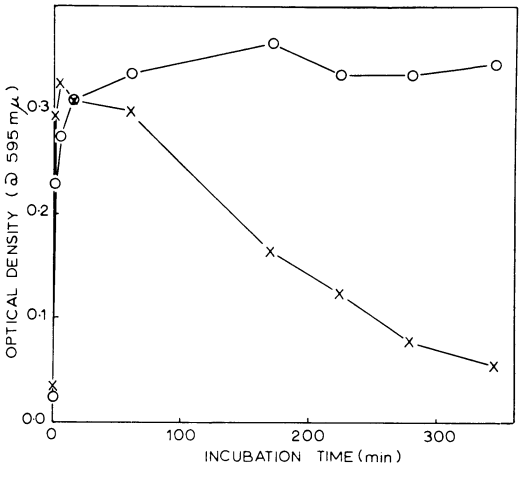

FIG. 1. *In vitro assay for thymidine catabolism. Cell extracts were prepared and assayed as described. Extract from arginine revertants giving an aberrant streak morphology caused diphenylamine-reactive material to be produced from thymidine and subsequently caused the degradation of this material* (×). *Extract from arginine revertants giving a normal streak morphology caused diphenylamine-reactive material to be produced from thymidine but did not cause degradation* (○).

diphenylamine-reactive material. On the other hand, no similar degradation occurred with extracts from normal revertant bacteria. In numerous experiments involving fresh extract preparations and very long incubation periods, extracts from the normal revertant bacteria never produced the degradation that could be demonstrated in the presence of extracts from aberrant revertant bacteria. Thus, the first assay procedure showed thymidine phosphorylase activity to be present in both revertant types. Moreover, it showed deoxyribomutase and deoxyriboaldolase activity to be present in aberrant arginine revertants, since deoxyribose-1-phosphate and deoxyribose-5-phosphate are diphenylamine-reactive, and suggested that the inactive enzyme in the normal arginine revertant was deoxyribomutase or deoxyriboaldolase.

Another assay procedure was used to assay for deoxyriboaldolase activity, the last step in the catabolic pathway. Because the deoxyriboaldolase-mediated reaction is known to be reversible and because it is more convenient to measure it in the direction of deoxyribose-5-phosphate synthesis (14), the production of a diphenylamine-reactive material was again used as a measure of enzyme activity. Extracts from both the aberrant and the normal arginine revertants were found to mediate the production of a diphenylamine-reactive material (Fig. 2). This suggested that deoxyriboaldolase activity was present in both extracts, and consequently, that deoxyribomutase was the inactive enzyme in normal arginine revertants.

Aberrant phenotype. An arginine revertant containing a class 2 amber suppressor that gave rise to an aberrant streak morphology when incubated on nutrient agar also gave rise to an abnormal mode of growth in liquid nutrient culture. The bacteria grew exponentially to about 5×10^7 viable bacteria per ml, and the viable titer then declined (Fig. 3, A). Thymidine added to a concentration of 20 mg/liter prevented the viability decline (Fig. 3, B). Similarly, thymidine added to nutrient agar caused an aberrant revertant to produce a thick lawn after incubation, whereas only a thin lawn of growth resulted when thymidine was not added to the nutrient agar.

By use of the latter procedure, a variety of compounds were tested to see which might prevent the aberrant growth morphology. Approximately 10-mg samples of the compounds to be tested were placed in the center of nutrient agar plates, after each had been spread with about 10^6 aberrant arginine revertants. Thymine, deoxyadenosine, deoxycytidine, and thymidine produced thick growth where the compounds had been placed. Uracil, cytosine, adenine, uridine, cytidine, ribose, and deoxyribose had no positive effect on the aberrant growth morphology. Hence, thymine and deoxyribosides in general

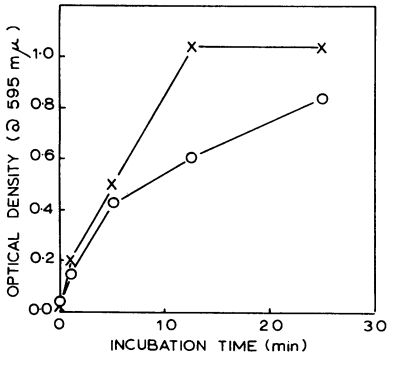

FIG. 2. *In vitro assay for deoxyriboaldolase. Cell extracts were the same as those described in Fig. 1. Extracts from arginine revertants giving an aberrant streak morphology* (X) *and from arginine revertants giving a normal streak morphology* (O) *both caused the production of a diphenylamine-reactive material. One optical density unit represents a deoxyribose concentration of approximately 1.8 mg/ml.*

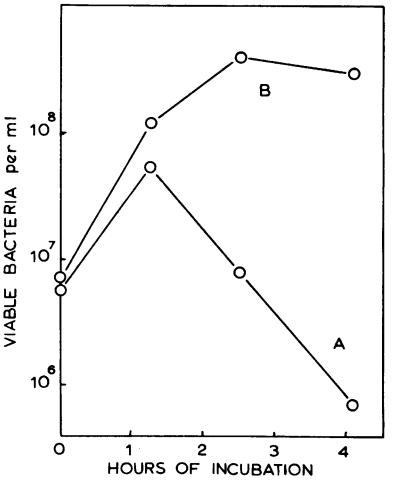

FIG. 3. *Growth of an aberrant arginine revertant in nutrient broth. Two 10-ml nutrient broth cultures (Oxoid) were inoculated simultaneously with equal concentrations of aberrant revertant bacteria (containing a class 2 suppressor). Thymidine had previously been added to one of the cultures to a concentration of 20 μg/ml. During incubation at 37 C with aeration, samples were removed from each culture and titered for viable bacteria on nutrient agar. The culture without thymidine added showed a viability decline after limited growth (A); the culture with thymidine added did not show a viability decline (B).*

were found to prevent the aberrant growth morphology.

The response of revertants with an aberrant phenotype to various deoxyribosides may depend somewhat on the particular amber suppressor contained by the revertant. For example, the amount of thymidine that must be added to revertant cultures in defined media to give growth kinetics in each which are indistinguishable from those of WWU depends on the amber suppressor contained in the revertant. A revertant containing a class 3 amber suppressor requires the greatest concentration of thymidine and a revertant containing a class 2 amber suppressor requires the least.

Concentration of thymine residues in nutrient broth. Since aberrant growth was observed in a nutrient environment and it seemed possible that the aberrant revertant was similar to a high thymine-requiring mutant, it was of interest to determine the concentration of thymine residues in nutrient broth. Cultures of *E. coli* 15T$^-$ were grown in nutrient broth supplemented with increasing amounts of ^{3}H-thymidine. All cultures grew similarly. By measuring the specific activities of the bacteria grown in these cultures (removed by filtration during exponential growth) and assuming no discrimination in utilization between the thymine residue in broth and the added ^{3}H-thymidine, the concentration of thymine residues in nutrient broth before the addition of the ^{3}H-thymidine could be calculated. The results indicated a concentration of 12 mμmoles per ml or 1.5 μg of thymine per ml.

It was not determined whether the thymine residues in nutrient broth were present as thymine or thymidine. However, it was observed that a tracer amount of ^{3}H-thymidine added to a culture similar to that described in Fig. 3 was converted to ^{3}H-thymine. During growth, samples of the supernatant fluid were removed and subjected to descending paper chromatography by use of a water solvent. At a time when the viability stopped increasing in the growing culture, about one-half of the radioactive thymidine in the supernatant fluid had been converted to radioactive thymine. Hence, even if all of the thymine residues normally present in nutrient broth happened to be in the form of thymidine, the phosphorylase activity in growing cultures rather quickly converted these residues to free thymine. In consequence, the organisms found themselves in a low concentration of thymine.

DISCUSSION

The data of Tables 1 and 2 show all *E. coli* WWU revertants containing amber suppressors to be arginine-independent, capable of thymidine catabolism, and aberrant in phenotype. Since the same threefold alteration in phenotype is obtained whether cells are selected for reversion of the arginine biosynthetic defect or for reversion of the thymidine catabolic defect, and the threefold alteration is readily reversed by spontaneous mutations that make the amber suppressor nonfunctional, one may conclude that the three alterations in the phenotype are the pleiotropic consequence of a single genetic alteration yielding an amber suppressor. Table 1 shows arginine revertants arising by back-mutation (class 6) to be arginine-independent, but normal in terms of streak morphology and still incapable of thymidine catabolism. Table 2 shows that thymidine-catabolizing revertants arising by back-mutation (class 6) also give rise to an aberrant streak morphology but are not arginine-independent. Taken together, these results suggest the following conclusions: (i) the defect in thymidine catabolism is the result of an amber triplet; (ii) in WWU there are, in fact, two genetically distinct amber triplets, one in the pathway for arginine synthesis and one in the pathway for thymidine catabolism; and (iii) the genetic defect in thymidine catabolism is the same as that controlling the potential for aberrant growth.

The in vitro studies showed that extracts from an arginine revertant with an aberrant phenotype (containing a class 2 amber suppressor) produced deoxyribomonophosphate from thymidine by a phosphorylase reaction and degraded the product upon continued incubation. In these respects, the in vitro data concurred with the in vivo finding that aberrant revertants could catabolize thymidine for energy. Extracts from a revertant characterized by a normal streak morphology (class 6) also produced deoxyribomonophosphate, indicating the presence of thymidine phosphorylase activity, but these extracts did not degrade the deoxyribomonophosphate produced (which could accumulate as 1-position or 5-position deoxyribomonophosphate). This finding showed that the defective enzyme in normal arginine revertants is not thymidine phosphorylase. Such a conclusion might have been expected, since deoxyadenosine was also not catabolized by normal arginine revertants, and it is known that the phosphorylase for deoxyadenosine differs from that for thymidine and deoxyuridine (15).

Since the extracts from aberrant arginine revertants and from normal arginine revertants both showed an activity which was probably that of deoxyriboaldolase, mediating the final step in thymidine catabolism, the enzyme present in the former revertant type but absent in the latter was probably deoxyribomutase. The idea that

deoxyribomutase was defective unless corrected by mutation is substantiated by the work of T. R. Breitman and R. M. Bradford (*in preparation*), where *E. coli* strain 15T$^-$ is shown to have lost this enzyme activity. Consequently, it may be concluded that the amber triplet defect, which causes normal arginine revertants as well as WWU itself to be incapable of thymidine catabolism, is in the structural gene specifying deoxyribomutase.

A variety of deoxyribosides prevent aberrant growth. Therefore, deoxyribose-1-phosphate, a common product of phosphorylase activity, may be the single metabolite critical in the metabolism of the aberrant revertant. Since the concentration of thymine residues in nutrient broth is low and there is enough phosphorylase activity in growing cultures of aberrant revertants to convert a large proportion of any exogenous thymidine to thymine, inefficient thymine incorporation could cause the viability decline of aberrant revertants. If the thymine required for DNA synthesis were incorporated via thymidine phosphorylase in a manner that has been suggested (9; B. Dale, S. Rooman, and G. R. Greenberg, Bacteriol. Proc., p. 179, 1967), a pool of deoxyribose-1-phosphate would be essential. The function or nonfunction of deoxyribomutase could decrease or increase, respectively, the size of this pool and thereby alter the endogenous supply of thymidine, an essential DNA precursor. When the mutase is functional, as in the case of the aberrant revertant whether restored by amber suppression or back-mutation, the deoxyribose-1-phosphate pool would be small (unless augmented by phosphorylase activities acting on exogenous deoxyriboside supplements) and the supply of thymidine for DNA synthesis would be limited. The resulting unbalanced growth could destroy colony-forming ability in a manner similar to thymineless death (4). Thus, the aberrant streak morphology is probably due to the events that produce thymineless death.

The aberrant revertants very likely correspond to high thymine-requiring mutants. Stacey (*personal communication*) has found a complete correlation between the high thymine-requiring character found in many strains and the ability to catabolize thymidine; Roepke has recently reported "very light growth" of a high thymine-requiring mutant in nutrient broth (16); and Kammen has found the presence of deoxynucleosides to help thymine incorporation into strains which would be high-thymine requiring (9). Finally, Phillips (*personal communication*) has observed that thymine-independent mutants derived from WWU never show an aberrant streak morphology, regardless of the suppressor they may contain.

The exact concentration of thymine required by aberrant revertants for normal growth may depend rather critically on the pool size of deoxyribose-1-phosphate, or more specifically, on the quantity and specific activity of the mutase. Class 2 amber suppressors have been found to be less efficient in overcoming polypeptide chain termination than class 1 and class 3 amber suppressors (6, 10) and ochre suppressors less efficient than any of the amber suppressors (3). We find slight differences among the various mutants, consistent with these unequal efficiencies of suppression. Thus, revertants containing a class 1 or class 3 amber suppressor require more thymidine to give normal growth in defined medium than do revertants containing a class 2 amber suppressor; after prolonged incubation (72 hr) on nutrient agar, revertants containing ochre suppressors give an altered streak morphology which is similar in some respects to that of aberrant revertants (Osborn, *unpublished data*). In general, it seems that the more efficient the suppressor, the more "aberrant" is the phenotype.

Breitman and Bradford have suggested that high thymine-requiring strains differ from low thymine-requiring strains in their ability to catabolize deoxyribose-5-phosphate (2). They cite a low thymine-requiring strain, 70 V3-462, which lacks deoxyriboaldolase. [Strain 70 V3-462 was derived from strain 70-462, which has deoxyriboaldolase activity. *E. coli* 15T$^-$ was also derived from 70-462 but need not be identical to 70 V3-462 (16).] Our data show WWU, which is derived from the low thymine-requiring strain 15T$^-$, to be deficient in deoxyribomutase activity. Consequently, we suggest that, in general, high and low thymine-requiring strains differ from one another in their ability to catabolize deoxyribose-1-phosphate, with low thymine-requiring strains being incapable of catabolism because of blocks at either the mutase (e.g., strain 15T$^-$) or the aldolase (e.g., strain 70 V3-462) site.

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