Proc. Nat. Acad. Sci. USA
Vol. 68, No. 12, pp. 3158–3162, December 1971

Localized Mutagenesis of Any Specific Small Region of the Bacterial Chromosome

(phage P22/*Salmonella typhimurium*/transduction/hydroxylamine/temperature-sensitive mutants)

JEN-SHIANG HONG AND BRUCE N. AMES

Department of Biochemistry, University of California, Berkeley, Calif. 94720

Communicated by Daniel E. Koshland, Jr., October 1, 1971

ABSTRACT **A method, which we call *localized mutagenesis*, is described for the isolation of temperature-sensitive and other types of mutations in any specific small region (about 1%) of the bacterial chromosome. The principle of this method is to mutate the transducing DNA rather than the bacterial DNA. One can select for the introduction of this mutated DNA into any particular region of the bacterial chromosome by transducing an auxotrophic marker in that region to prototrophy, thereby introducing new mutations in the neighborhood. We have used this method to isolate many different temperature-sensitive mutations in genes of unknown function in particular regions of the chromosome. Since the method is very simple, it can be used to saturate any region of the map with mutations in essential genes, or for various types of genetic manipulations. Although we have used hydroxylamine-mutagenized phage P22 and *Salmonella typhimurium*, the method should be applicable to other mutagens and bacteria and transducing phage.**

Temperature-sensitive (ts) mutations in bacteria are extremely useful because they can be obtained in genes that code for proteins that are essential for the growth of the cell. Since the pioneering work of Horowitz and Leupold (1), temperature-sensitive mutants in bacteria have been used to study activating enzymes (2), ribosomes (3–5), DNA synthesis (6), and other components and aspects of macromolecular syntheses.

A number of workers have isolated ts mutants by mutagenizing *Escherichia coli* or *Salmonella typhimurium*. Although the isolation of these mutants is easy, the mapping of them involves a considerable amount of work since they may map anywhere on the chromosome. We have developed a simple method, which we call *localized mutagenesis*, for the easy isolation of ts and other mutants in any specific small region of the chromosome.

MATERIALS AND METHODS

Bacterial Strains. All strains were derived from *S. typhimurium* strain LT-2.

Phage. Phage used for mutagenesis and transduction was P22-*int*-4, a nonintegrating mutant of P22 (7). The phage sensitivity test was done with P22-H5, a clear (c_2) mutant of P22. Phage were stored in T2 buffer (8).

Media. Nutrient broth (Difco) was used as maximally supplemented medium. E medium (9) (made 0.4% in glucose) was used as minimal-salts medium. Solid medium contained 1.5% agar.

Transducing Phage Mutagenesis. Phage (P22-*int*-4) was grown on wild-type strain LT-2 at 37°C with shaking in a medium composed of 100 parts of nutrient broth, 3.5 parts of 40% glucose, and 1.3 parts of concentrated (50X) medium E. The phage, a mixture of phage and bacterial DNA in phage heads, usually had a titer of 1–3 × 10^{11}/ml. Concentrated phage (10^{12}/ml) in 0.9% saline was mutagenized at 37°C with hydroxylamine (10). To 1 part of phage in saline were added 5 parts of 0.1 M sodium phosphate buffer (pH 6.0) containing 1 mM EDTA, and 4 parts of 1 M hydroxylamine·HCl (adjusted to pH 6.0 with NaOH) containing 1 mM EDTA. At the desired time the phage were collected by centrifugation (40 min, 38,000 × *g*) in the SS-34 head of the Sorvall RC-2 centrifuge and washed twice with T2 buffer. The mutagenesis was monitored by examination of the fraction of surviving phage, which is used as an index of the mutagenesis of transducing DNA. Under these conditions the inactivation is exponential and the time required was about 8 hr for 10% survival and about 24 hr for 0.1% survival. Phage were stored in T2 buffer at 4°C with a few drops of chloroform added.

Isolation of ts Mutants. The method for isolation of ts mutants is to transduce auxotrophic bacteria to prototrophy with mutagenized transducing phage at room temperature (25°C) on minimal medium plates. When colonies of transductants appear and are still small in size the plates are shifted to 40°C. Under these conditions ts mutants stop growing and remain small, while normal transductant colonies continue to grow. After a further incubation, small colonies are picked and tested for temperature sensitivity.

Recipient bacteria used for transduction were grown to stationary phase in 12 ml of nutrient broth at 37°C. The culture was centrifuged at 1500 × *g* for 10 min and resuspended in about 6 ml of T2 buffer (about 2 × 10^9 bacteria/ml). To this fresh suspension was added 0.1 ml of mutagenized phage, which had an initial titer of 1–2 × 10^{12} plaque-forming particles per ml before mutagenesis. After 10 min of adsorption at 37°C, a 0.2-ml aliquot was spread uniformly on each minimal-glucose agar plate. The plates were incubated at room temperature (about 25°C) for about 40–50 hr, then at 40°C for 12–24 hr. Each small colony was transferred in a pattern, with a sterile wooden applicator stick, to a pair of identical minimal-glucose agar plates. One plate was incubated at 40°C and the other at room temperature for 1–2 days. About 1% of the small colonies failed to grow at 40°C.

Abbreviation: ts, temperature-sensitive (i.e., heat-sensitive).

In order to obtain a phage-sensitive clone, the mutants were promptly picked from the plates incubated at room temperature, streaked out on nutrient-broth plates, and incubated overnight at room temperature. About 10 single colonies from each mutant were then tested for phage sensitivity by cross-streaking against the clear (c_2) mutant phage P22-H5 (10^6/ml) on nutrient-broth plates. A phage-sensitive clone was isolated from each mutant and retested for temperature sensitivity.

RESULTS

Frequency of ts mutants among small and total transductants

Table 1 summarizes the results of the isolation of temperature-sensitive mutants by localized mutagenesis with transduction in the *hisG*, the *thi*, and the *purF* regions of the *S. typhimurium* chromosome. As seen in Table 1, about 1/100 of the small transductants are ts mutants. By use of a temperature shift and the picking of only the small transductants, it is necessary to test only this small fraction (2.4–7%) of the total transductants. About 1/800 to 1/10,000 of the total transductants are ts mutants, depending on the recipient mutant used. It is also clear from Table 1 that in the isolation with *hisG2804* as the recipient marker, the frequency of occurrence of ts mutants is similar whether phage used for transduction was mutagenized to 5 or 0.03% survival.

ts mutations are cotransducible with recipient marker

The 61 mutations listed in Table 1 have been mapped. In every case the mutation is cotransducible with the recipient marker used for its isolation.

ts Mutants in the Histidine Region. 29 ts mutants were isolated by transducing out the *hisG2804* mutation (this strain has a negligible reversion rate and a histidine-requirement at both 25°C and 40°C) on a minimal-medium plate with mutagenized phage (Table 1). These ts mutants were tested to see whether they were able to grow at the nonpermissive temperature (40°C) on minimal medium supplemented with histidine. All 29 mutants were found to be ts histidine-requiring mutants. By complementation test (11), the sites were found to be located in various genes within the histidine operon and, therefore, linked to *hisG2804* (Table 2). Mutations in *hisE* and *hisG* are about 30% cotransducible by P22-mediated transduction (12). No irremediable ts mutants were expected, as the region on either side of the histidine operon can be deleted with no lethality (12).

ts Mutants in the Thiamine Region. 24 ts mutants (which can grow on minimal medium at 25°C, but not at 40°C) were isolated by the use of *thi-502* (which can not grow on minimal medium at either temperature) as the recipient in the transduction (Table 1). For 23 of the 24 mutants, this inability to grow at 40°C was remediable by a component of nutrient broth: 21 by thiamine (*thi-524* and 20 others), one by purines (*pur-824*), and one by methionine (*met-820*). The prevalence of ts *thi* mutants can be accounted for by the presence in this region of the cluster of the genes involved in the long pathway of thiamine biosynthesis (13). Our other two remediable mutants appear to be in the purine cluster (*purD,H,J*) and the methionine cluster (*metA,H*) that are known to be cotransducible with *thi*. The irremediable mutant, *xmi-1**, and the 23 remediable mutants are all cotransducible with *thi-502* and have been mapped relative to *argA*, *rif* (RNA polymerase), and *thi-502* by two- and three-factor crosses (Table 3). The map of the region we have constructed from these data is shown in Fig. 1. The linkage relations determined from the three-point crosses and the two-point crosses agree quite closely and unambiguously establish the gene order. The map of *arg*, *thi*, *pur*, and *met* agrees with work in both *E. coli* and *S. typhimurium* (13, 15). We have established (16) that *rif* lies between *arg* and *thi;* the results presented here confirm this.

Table 1. *Summary of the isolation of ts mutants by the localized mutagenesis method with a temperature-shift enrichment*

Recipient	Small/total transductants (%)	Small colonies tested	ts Mutants isolated
*hisG2804**	2.4	1200	17
hisG2804†	2.4	1000	12
*thi-502**	7.0	1400	24
*purF145**	3.1	2800	8

Transductions were done with a phage preparation that was mutagenized with hydroxylamine to a survival of 0.03%(*) or 5%(†).

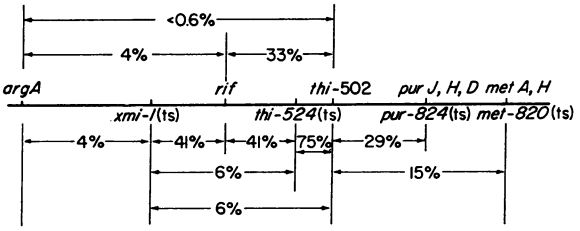

FIG. 1. A genetic map of the ts mutations relative to known markers in the *arg-thi* region.

Linked auxotrophic markers can be isolated from a ts mutant, and this property could be useful in mapping unmapped ts mutants. We have transduced the mutant *xmi-1* on a nutrient-broth plate at 40°C with mutagenized phage (0.03% survival) and replicated all the transductants. Of 1600 transductants examined, two were arginine-requiring and one was thiamine-requiring, the mutants being auxotrophic at both 25°C and 40°C.

It is not difficult to bracket a gene between mutations using the localized mutagenesis method, and we have done this with the *rif* gene for studies on the RNA polymerase. A series of

* The isolation of conditional (ts) mutations in various unknown essential genes raises the question of how to name the genes by the standard (14) nomenclature. As we do not have a phenotype to name the various genes by, we will use the map position, which we do know, to name the genes by the following convention. Each gene name will contain the conventional three letters, but the first letter will always be *x* to set apart the class of conditional mutants. The second and third letters will indicate the position on the 138-min *S. typhimurium* map (13) by the following scheme: *xaa*, *xab*, . . . *xaj* will indicate minutes 1–9; *xba*, *xbb*, . . . *xbj* minutes 10–19, etc. Thus, unidentified ts mutants isolated from *thi*, which is at minute 128, will all be *xmi*. If mutations in several genes in different positions within the *xmi* region are isolated, they can be given the standard capital letters in the fourth position e.g., *xmiA*, *xmiB*. If, in the future, the gene product is identified the name can be changed. Blocks of isolation numbers can be obtained from Sanderson (13), and used as for any other gene designation. The one current name beginning with *x*, *xyl* = xylose, will not interfere.

TABLE 2. *Results of the complementation test of ts mutants isolated with hisG2804 as the recipient*

Experiment*	No. of ts mutants in histidine operon									
	O	*G*	*D*	*C*	*B*	*H*	*A*	*F*	*I*	*E*
I	—	6	5	4	1	0	1	0	0	0
II	—	3	4	3	2	0	0	0	0	0

* Mutants were isolated after transduction with phage mutagenized to a survival of either 0.03% (I) or 5% (II).

arg thi-502 double mutants were constructed by the use of diethylsulfate mutagenesis (17) and penicillin selection (17). One of the strains, *arg-519 thi-502* (TA 2319), had an arginine mutation that was cotransducible with *xmi-1* (and *rif-43*), and this transduction, on a thiamine plate at 25°C, yielded the desired strain *xmi-1 thi 502* (TA 2320).

ts Mutants in the Purine-F Region. 8 ts mutants were isolated by the use of *purF145* as the recipient in the transduction (Table 1). All were found to be cotransducible with *purF145* (Table 4).

Three of the ts mutants have been identified as *fabB* mutants. The *fabB* gene (fatty acid biosynthesis) has been described in *E. coli* in the homologous position between *purF* and *aroC* (*aroC* of *E. coli* = *aroD* of *S. typhimurium*) and is required for the synthesis of unsaturated fatty acids (18, 19). Our mutants (*fabB1*, *fabB2*, and *fabB3*) lyse when they are shifted from 25 to 40°C, but will grow and not lyse (at 40°C, the nonpermissive temperature) if oleic acid is added to the medium. They are about 28% linked to *purF*, between *hisT* and *aroD*. The remaining five mutants (*xhc-1** through 5) are not reparable by nutrient broth at 40°C and are all about 85% cotransducible with *purF145*. In addition to these two classes of mutants, we have also mapped a pyridoxine-requiring mutant, *pdx-518*, which was isolated by B. Guirard using this method: transducing out *purF145 aroD5* with mutagenized phage (0.03% survival) on a pyridoxine-supplemented plate gave five *pdx* mutants out of 5831 transductants replicated. A class of pyridoxine mutants, *pdxB*, is known to map in this region in *E. coli* (15).

The order of genes in this region, *hisP-purF-hisT-aroD* was established and the linkages were determined (20). One minor complication of mapping in this region is that *purF145* appears to be a deletion, which would account for anomalous results on reciprocal crosses between *hisP*, or *xhc-5*, and *purF145* (Ames, G. F., and Govons, S. personal communication).

We have located the *xhc* gene between *purF145* and *hisT*, and the *fabB* gene between *hisT* and *aroD*, on the basis of the crosses shown in Table 4 and Fig. 2. The *pdx-518* mutation is clearly between *purF* and *fabB*, close to *hisT*, but it is not clear on which side of *hisT* it is.

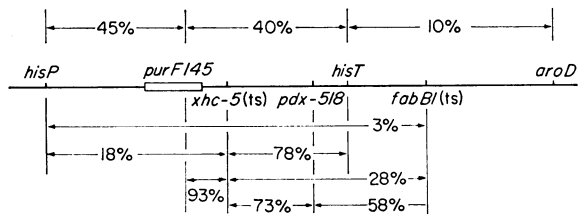

FIG. 2. A genetic map of the ts mutations relative to known markers in the *purF-aroD* region.

Isolation of Mutants Other than ts Types. In addition to ts mutants, the method can also be used for the isolation of cold-sensitive mutants in essential genes. We have isolated such mutants, which grow at 37°C, but will not grow at 23°C, in several areas of the chromosome. They appear to occur somewhat less frequently than do ts mutants.

Auxotrophic mutants can also be isolated, such as the *pdx* mutants described in the preceding section, and the method furnishes a simple way to replace any auxotrophic marker with a closely linked marker.

Mutants that can be identified on a plate can also be isolated easily. Various classes of histidine regulatory mutants, which can be recognized by their wrinkled colony morphology on glucose plates, can be isolated by transducing out a neighboring marker: *hisR* (using *cya*), *hisO* (using *hisG*), *hisT* (using *purF*, e.g., *hisT6360*, Table 4), and *hisS* (using an auxotrophic *hisS* mutant). In addition, we have observed that about 1/1000 of the transductants of *hisG2804* had haloes of feeding; these are presumably feedback-resistant mutants in the *hisG* gene (12).

Mutants with a lethal hit in a gene appear to be much more common than ts mutants. S. Govons and N. K. Hooper in this laboratory have used localized mutagenesis (0.03% survival) to obtain glycogen biosynthetic mutants by transducing out the closely linked (67%:84/126) *asd* (aspartic semialdehyde dehydrogenase) marker. By staining the asd^+ transductants with iodine vapor, under conditions where the bacteria make some glycogen, they find that 2.1% (31/1474) of the transductants do not make glycogen (stain white) and 2.4% (35/1474) have some abnormality (stain dark or blue).

DISCUSSION

The principle of the localized mutagenesis method is to mutate the transducing DNA rather than the bacterial DNA. One can select for the introduction of this mutated DNA into any particular region of the bacterial chromosome by selecting prototrophic transductants from an auxotrophic recipient. Since transduction is a rare event (10^{-5}–10^{-6}), mutations are introduced only in that 1% or less of the chromosome that has been replaced by the transducing DNA. The method developed is quite simple and the data on the isolation of various ts and other mutants in three areas of the *S. typhimurium* chromosome demonstrate its convenience and utility. Several investigators had used a similar approach, but the method was not perfected or exploited. Adye (21) showed that histidine-requiring mutants could be obtained by transduction of a histidine deletion with phage mutagenized with nitrous acid. He pointed out that the method was useful for the isolation of leaky mutants that usually escaped a pencillin selection. A

similar approach with phage P1 and *E. coli* by the use of ultraviolet and nitrous-acid mutagenesis had been tried, but was unsuccessful (22). The general principle has also been used in bacterial transformation (23, 24).

We have found the localized mutagenesis method quite convenient in various projects in bacterial biochemical genetics. (*a*) When there are no genetic markers near a particular gene of interest it is possible to isolate ts mutations as nearby markers. For example, we started this work with an interest in the gene for RNA polymerase (*rif*) and we have been able to isolate close markers on both sides of this gene. (*b*) The method can be used for mapping an unmapped irremediable ts mutation of auxotrophic marker. For example, the isolation of arginine- and thiamine-requiring mutants from *xmi-1* would have positioned it on the map if we had not known its position to start with. Similarly the isolation of the ts purine and methionine mutants would have positioned the thiamine gene of *thi-502*. (*c*) The method can also be used to replace a mutation by a temperature-sensitive mutation (or other type) in the same gene. We have isolated a large number of ts histidine-requiring mutations in this way. Various mutants with altered enzymes have been isolated in the *hisS* gene (histidyl-tRNA synthetase) and in the glycogen genes. (*d*) We also have found it useful for the isolation of particular classes of mutants that are adjacent to known auxotrophic markers that can be transduced to prototrophy. Histidine regulatory mutations in particular regions can be isolated directly without a positive selection because the phenotype can be recognized (the histidine regulatory mutants have a wrinkled colony morphology). For example, strain *hisT6360* was isolated from the *purF* transduction. Feedback mutants can also be easily seen because of zones of feeding. One class of pyridoxine mutants, which is normally a rare class, was isolated specifically.

TABLE 3. *Mapping of ts mutants in the arg-thi region*

Donor	Recipient	Selective temp. (°C)	Recombinants donor phenotype/total
thi-524 (ts)	*thi-502*	25	75% (61/81)
met-820 (ts)	*thi-502*	25	15% (9/59)
xmi-1 (ts)	*thi-502*	25	6% (6/100)
pur-824 (ts)	*thi-502*	25	29% (16/56)
20 *thi* (ts)	*thi-502*	25	79%–95%
thi-524 (ts)	*xmi-1* (ts)	40	6% (6/100)
xmi-1 (ts)	*argA94*	25	4% (8/200)
argA94	*thi-502*	37	<0.6% (0/175)
*rif-43**	*xmi-1* (ts)	40	41% (63/153)
rif-43	*thi-502*	37	33% (32/96)
rif-43	*thi-524* (ts)	40	41% (38/93)
rif-43	*argA94*	37	4% (8/221)
			phenotype/total
met-820 (ts)	*rif-31 thi-502*	25	Rif$^+$Ts$^+$ 30% (29/98)
			Rif$^+$Ts 1% (1/98)
			Rif Ts$^+$ 59% (58/98)
			Rif Ts 10% (10/98)
thi-524 (ts)	*rif-31 thi-502*	25	Rif$^+$Ts$^+$ <1% (0/83)
			Rif$^+$Ts 29% (24/83)
			Rif Ts$^+$ 24% (20/83)
			Rif Ts 47% (39/83)

All selections on minimal medium except *thi-524* × *xmi-1* on thiamine medium and *argA94* × *thi-502* on arginine medium.

* The *rif* marker was scored for by testing rifampicin resistance by the radial-streak method (17), with 0.04 mg of rifampicin on a filter-paper disc.

$^+$ = wild-type phenotype.

TABLE 4. *Mapping of ts mutants in the purF-aroD region*

Donor	Recipient	Recombinants donor phenotype/total
xhc-1 (ts)	*purF145*	93% (82/88)
xhc-2 (ts)	*purF145*	88% (69/78)
xhc-3 (ts)	*purF145*	78% (66/85)
xhc-4 (ts)	*purF145*	81% (30/37)
xhc-5 (ts)	*purF145*	93% (43/46)
fabB1 (ts)	*purF145*	28% (22/79)
fabB2 (ts)	*purF145*	23% (25/108)
fabB3 (ts)	*purF145*	29% (31/106)
xhc-5 (ts)	*purF145 aroD5*	100% (52/52)
fabB1 (ts)	*purF145 aroD5*	94% (29/31)
hisP1661	*fabB1* (ts)	3% (2/78)*
hisP1661	*xhc-5* (ts)	18% (13/71)*
hisT6360	*xhc-5* (ts)	78% (297/383)*
fabB1 (ts)	*pdx-518*	52% (30/57)
xhc-5 (ts)	*pdx-518*	73% (19/26)

All selections in minimal medium at 25°C, except as noted.
* Selected at 40°C.

The method can presumably be adapted to any transducing system in bacteria and should prove to be useful in the large number of organisms where transducing phage is available, but where the genetics is not as advanced as *E. coli* or *S. typhimurium*. It should be possible to establish linkage relationships by isolation of ts mutants that are remediable by nutrient broth by the use of various auxotrophs as recipients, and then to identify the growth-requirement by the usual method of using synthetic pools (17). This method could also be used to isolate ts mutations in transformable systems in bacteria by mutation of transforming DNA and selection for the transformation of an auxotrophic marker to prototrophy. The principle of the method should also be applicable to eukaryotes, if suitable systems are developed.

A considerable number of essential genes are involved in the synthesis of the cell membrane or cell wall, and ts mutations in these genes result in the mutant cell lysing and being killed when the temperature is shifted from 25 to 40°C (19, 25). The enrichment method used appears to recover this class, however, as indicated by the isolation of the ts mutants in the *fabB* gene. Presumably some cells of the mutant colony survive in spite of the temperature shift.

The genome of *S. typhimurium* is about 4.5×10^6 base pairs (26), or about 4500 genes if we assume a standard gene of about 1000 base pairs. A rough estimate (B.N.A., unpublished) of the proportions indicates that there are about 1500 essential irremediable genes, 1000 essential remediable genes (by a component of nutrient broth), and 2000 silent genes, whose mutation is not immediately apparent in terms of aerobic growth on a minimal glucose plate. Thus, the number of irremediable genes in a 1% length of the chromosome is small enough so that it should be possible to saturate the *S. typhimurium* (or *E. coli*) map with ts mutations by this method. One difficulty in doing this is that when a group of mutants are isolated, e.g., *xhc-1* through *5*, it is not clear whether they are in the same gene or in a cluster of genes, as the linkage only indicates that they are in about the same place. Perhaps abortive complementation tests would help in this analysis. Nevertheless, if different laboratories work in different regions of the map with this method the map should fill in quite rapidly.

The length of bacterial DNA carried by a transducing phage is fixed by the size of the phage head and determines the maximum length of a region that can be analyzed by localized mutagenesis. P22 phage carries about 42,000 base pairs of DNA (27), which is about 1% of the bacterial chromosome length. If we define a standard gene as 1000 base pairs, then about 42 bacterial genes can fit inside a P22 transducing particle.

The approximate level of mutagenesis in the transducing particles can be calculated from the level of killing of the phage particles. If we assume that inactivation of one gene can inactivate a phage particle, then treatment with hydroxylamine so that there is a 10% survival of plaque-forming units should result in 90% of the transducing pieces containing at least one inactivated gene, and most with double hits. The probability that any particular gene is mutated, out of the 42 genes in the transducing particle, is clearly quite high (e.g., the 2.1% for glycogen) and should be on the order of a few percent, though some of these mutations will be eliminated in the crossing-over during transduction. The frequency of ts mutants is much lower than this. This may be because it is much easier to obtain a mutation that causes the complete inactivation of a protein, rather than one that causes heat inactivation over a 15°C range. We would also not detect any temperature-sensitive mutants that have a high frequency of reversion. Despite the low frequency of ts mutants among the total transductants, it is relatively little work to isolate them if one uses temperature-shift enrichment. The use of phage inactivated to 0.03% survival gave no better results than phage inactivated to 5% survival; because of the problem of double mutants, we recommend that 10% survival be used. If there are many noninactivating mutations for every inactivating mutation (12, 28), then there may be considerable numbers of relatively neutral mutations in the mutants isolated. This is a general problem in mutagenesis. To minimize the probability of isolating a double mutant, we routinely reisolate our ts mutants from a backcross to the original recipient, a cross that is done in any case in the determination of linkage.

Hydroxylamine is a highly specific mutagen. It reacts with cytosine and causes the specific replacement of a G by an A, thus causing the GC to AT transition (29). Thus, all of the mutants isolated by our method are due to a GC to AT base-pair change and represent a highly restricted set of amino-acid changes. As the hydroxylamine mutagenesis is well worked out and is so satisfactory, we have not explored other mutagens known to react with DNA. The procedures developed by Freese (29) and Tessman (10) for treating phage with hydroxylamine minimize phage inactivation other than that due to the mutagenic action on DNA.

NOTE ADDED IN PROOF

In the experiments we have described, no time was allowed for expression of mutations. We have found, to our surprise, that allowing expression (a 6-hr growth period in nutrient broth before plating; phage killed to 10% survival) results in no marked increase in the number of mutants detected. The theoretical aspects of this are somewhat complicated, involving repair, mechanism of transductional recombination, and the mechanism of hydroxylamine mutagenesis. It may be desirable to allow time for segregation in other systems, however.

We thank J. R. Roth for suggesting hydroxylamine mutagenesis and for many helpful discussions and R. Dahlquist for his calculations on the theory of probability of mutations. J.S.H. was the recipient of a USPHS Postdoctoral Fellowship. This work was supported by an AEC gramt AT(04-3)-34, P. A. 156 to B.N.A.

1. Horowitz, N. H., and U. Leupold, *Cold Spring Harbor Symp. Quant. Biol.*, **16**, 65 (1951).
2. Neidhardt, F. C., *Bacteriol. Rev.*, **30**, 701 (1966).
3. Guthrie, C., H. Nashimoto, and M. Nomura, *Proc. Nat. Acad. Sci. USA*, **63**, 384 (1969).
4. Philips, S. L., D. Schlessinger, and D. Apirion, *Proc. Nat. Acad. Sci. USA*, **62**, 772 (1969).
5. Tai, P. C., D. P. Kessler, and J. Ingraham, *J. Bacteriol.*, **97**, 1298 (1969).
6. Kohiyama, M., D. Cousin, A. Ryter, and F. Jacob, *Ann. Inst. Pasteur, Paris*, **110**, 465 (1966).
7. Smith, H. O., and M. Levine, *Virology*, **31**, 207 (1967).
8. Hershey, A. D., and M. Chase, *J. Gen. Physiol.*, **36**, 39 (1952).
9. Vogel, H. J., and D. M. Bonner, *J. Biol. Chem.*, **218**, 97 (1956).
10. Tessman, I., *Virology*, **35**, 330 (1968).
11. Garrick-Silversmith, L., and P. E. Hartman, *Genetics*, **66**, 231 (1970).
12. Hartman, P. E., Z. Hartman, R. C. Stahl, and B. N. Ames, *Advan. Genet.*, **16**, (1971), in press.
13. Sanderson, K. E., *Bacteriol Rev.*, **34**, 176 (1970).
14. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman, *Genetics*, **54**, 61 (1966).
15. Taylor, A. L., *Bacteriol. Rev.*, **34**, 155 (1970).
16. Hong, J. S., G. R. Smith, and B. N. Ames, *Proc. Nat. Acad. Sci. USA*, **68**, 2258 (1971).
17. Roth, J. R., *Methods Enzymol.*, ed. H. Tabor and C. Tabor (Academic Press, New York, 1970), Vol. 17A, p. 3.
18. Epstein, W., and C. F. Fox, *J. Bacteriol.*, **103**, 273 (1970).
19. Cronan, J. E., C. H. Birge, and P. R. Vagelos, *J. Bacteriol.*, **100**, 601 (1969).
20. Ames, G. F., and J. R. Roth, *J. Bacteriol.*, **96**, 1742 (1968).
21. Adye, J., *Virology*, **18**, 627 (1962).
22. Helling, R. B., *J. Bacteriol.*, **100**, 224 (1969).
23. Anagnostopoulis, C., and I. P. Crawford, *Proc. Nat. Acad. Sci. USA*, **47**, 378 (1961).
24. Kelley, M. S., and R. H. Pritchard, *J. Bacteriol.*, **89**, 1314 (1965).
25. Matsuzawa, H., M. Matsuhashi, A. Oka, and Y. Sugino, *Biochem. Biophys. Res. Commun.*, **36**, 682 (1969).
26. Maaloe, O., and N. Kjeldgaard, *Control of Macromolecular Synthesis* (W. A. Benjamin, New York, 1966).
27. Rhoades, M., L. A. MacHattie, and C. A. Thomas, *J. Mol. Biol.*, **37**, 21 (1968).
28. Whitfield, H. J., R. G. Martin, and B. N. Ames, *J. Mol. Biol.*, **21**, 335 (1966).
29. Freese, E., in *Chemical Mutagens*, ed. A. Hollaender (Plenum Press, New York, 1971), Vol. 1, p. 1.