MUTAGENS WHICH CAUSE DELETIONS IN *ESCHERICHIA COLI*¹

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WE have undertaken a study of several mutagens to determine their usefulness in the isolation of deletion mutations in *Escherichia coli*. No systematic investigation of deletion inducing agents in *E. coli* has been described. A related study is that of TESSMAN (1962) who has shown that a high proportion of T4rII mutations induced by *in vitro* treatment with nitrous acid are deletions. In addition, the nature of mutants of the histidine operon induced by various mutagens has been presented by LOPER, GRABNAR, STAHL, HARTMAN and HARTMAN (1964).

To screen for deletions we have analyzed reversion of a polar mutation of the lactose (*lac*) operon of *E. coli* (NEWTON, BECKWITH, ZIPSER and BRENNER 1965). A strain carrying *lac*₂⁻, an ochre mutation mapping near the operator end of the *z* gene, (the structural gene for β -galactosidase), makes no β -galactosidase or, as a result of the polarity effect, galactoside permease, (specified by the γ gene). Since this permease is necessary for melibiose utilization at 42°C (PARDEE 1957; BECKWITH 1963; PRESTIDGE and PARDEE 1965), in addition to being Lac⁻, this strain is also melibiose⁻ (Mel⁻) at 42°C. Revertants of such a strain can be detected on lactose or melibiose synthetic medium. Mel⁺ revertants fall into three general classes. 1) Mel⁺ revertants which are also *lac*⁺ (*z*⁺ γ ⁺) occur as a result either of back mutation within the altered codon or of unlinked ochre suppressor mutations (BECKWITH 1963; BRENNER and BECKWITH 1965). 2) Mel⁺ revertants which are still *z*⁻ can also result from unlinked suppressor mutations. These suppressors are either polarity suppressors such as *suA*, or mutations allowing accumulation of melibiose by a different transport system, (BECKWITH 1964a; SCAIFE and BECKWITH 1966; ARDITTI, SCAIFE and BECKWITH, unpublished results).

3) Finally, deletions in the *lac* region which remove segments of the *z* gene can restore permease activity resulting in a *z*⁻ γ ⁺ phenotype. So far, three different types (Figure 1) of deletions have been found to restore permease activity to polar mutants. Deletions internal to the *z* gene which remove the polar mutation (I) will reduce or abolish the polarity effects (BECKWITH 1964b). Deletions which remove the polar mutation and, in addition, extend outside the *lac* region fusing the operon to some other functioning gene (II) will also allow γ gene expression (BECKWITH 1964b). Deletions which do not cover the original polar mutant, but which cover much of the region between the mutation and the γ gene (III) reduce

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FIGURE 1.—Deletions which suppress polarity.

polarity effects (NEWTON *et al.*, 1965; ZIPSER and NEWTON 1967). Thus, a certain fraction of Mel^+ revertants of such polar mutants are deletions.

In this paper, we use this system to show that nitrous acid, X rays and ultra-violet (UV) irradiation markedly increase the frequency of deletions in the bacterial population. We have also characterized the types of deletions induced by these mutagens and the types found amongst spontaneous mutants.

MATERIALS AND METHODS

Bacterial strains: The strain X-7167 carrying lac^-_2 is an *E. coli* K12 female strain requiring vitamin B1 and is also i^- (constitutive). The lac^- mutants used for mapping have been described before (NEWTON *et al.* 1965). The $\phi 80$ HFT lysate for suC^+ was described by SIGNER (1966).

Media: Melibiose—MacConkey agar is Difco MacConkey medium with 1% melibiose added. LB broth consists of 10 g Bacto-Tryptone, 5 g yeast extract and 10 g NaCl in 1 l of water. Synthetic medium is M63 (PARDEE, JACOB and MONOD 1959).

Isolation and characterization of revertants: Revertants were selected on melibiose synthetic agar at 42°C after two days incubation and were purified twice on melibiose MacConkey agar. Each revertant was grown up in LB broth, streaked on lactose-minimal agar using thin paper strips saturated with the culture, and a drop of a $\phi 80$ suC^+ HFT lysate spotted onto the streaks. Those Mel^+ revertants which were also Lac^+ grew confluent along the length of the streak; those mel^+ Lac^- revertants which were suppressible by suC^+ grew only at the spot where the lysate had been placed.

Spot tests to map the non-suppressible Lac^- revertants were done by mixing a drop of a culture of a revertant with a drop of a culture of an Hfr or F' donor for the different z^- mutants on lactose-synthetic agar. Point mutants were chosen for mapping from each of 16 segments of the z gene. After incubation at 37°C for two days, the plates were scored for Lac^+ recombinants.

Mutagenesis: Spontaneous: Spontaneous revertants were isolated by plating out an overnight culture of X-7167.

Nitrous acid (NA): An 0.05 M sodium nitrite (Mallinckrodt Lot #7824) solution in 0.1 M acetate buffer, pH 4.6, was prepared no more than 1 hr before its use. Five ml of a stationary phase broth culture (1 or 2×10^9) of X-7167 was centrifuged, washed in the same volume of acetate buffer and then resuspended in 0.3 ml of the nitrous acid solution. After 10 min at 37°C, 5 ml of M63 salts-buffer was added to stop the reaction. The bacteria were centrifuged and resuspended in 10 ml of LB broth and grown overnight. Portions of this culture were plated for revertants. The 10 min treatment resulted in 99% killing. There is no alteration of reversion frequency with increased levels of killing obtained by longer periods of treatment. Control cell lines treated in the same way, but without nitrous acid, showed no increase in reversion over the spontaneous frequency.

UV: The bacteria (X-7167) were grown to $2-4 \times 10^8$ cells/ml in 5 ml of LB broth, centrifuged and resuspended in 5 ml of M63 salts-buffer. The suspension was irradiated for 100 sec with a General Electric 15 watt germicidal lamp, G 15T8, at a distance of 27 inches, resulting in 95% killing. The irradiated suspension was centrifuged the pellet resuspended in 10 ml of LB broth and this culture grown overnight. Again, increased levels of killing do not change the reversion frequency.

X-rays: X-ray irradiation of overnight cultures of X-7167 was performed under a Norelco X-ray machine at 100 kv, 10 mA to give about 30,000 rads. At this dose, the killing was from 95–99%. The irradiated cultures were then handled in the same way as after nitrous acid treatment. As with UV and nitrous acid, the reversion frequency is the same over a wide range of doses.

2-amino purine (2AP): A culture of X-7167 was diluted into 5 ml of LB broth containing 600 μ gm/ml of 2 AP (Mann Research Laboratories) so as to give approximately 100 cells per dilution tube. The cultures were grown overnight and aliquots spread on melibiose-synthetic agar. One revertant colony was picked from each culture.

Reversion of deletions to Lac⁺: To test for reversion of deletions with N-methyl-N'-nitro-N-nitrosoguanidine, (Aldrich Chemical Co.) a crystal of the compound was placed in the center of a plate spread with the appropriate culture. A control with the strain X-7167 showed a heavy circle of revertants around the mutagen spot.

RESULTS AND DISCUSSION

We have screened for deletions amongst Mel⁺ revertants by the following technique. Cultures of the revertants are streaked onto lactose synthetic agar. A drop of ϕ 80 HFT lysate for *suC⁺* is spotted onto these streaks (see MATERIALS AND METHODS for details). *SuC* is an ochre suppressor which suppresses to Lac⁺ the original mutant, *lac₂*. Revertants which are Lac⁺ will show confluent growth on these plates. If a revertant is *z⁻ γ ⁺*, but is still suppressed to Lac⁺ by the ϕ 80 *suC⁺* lysate, the *z* gene and its *lac₂⁻* site must be unaltered. However, if the *z⁻ γ ⁺* revertant is not suppressed by *suC⁺*, either the *lac₂⁻* site, or other segments of the *z* gene, or both must have been altered in the mutational event that caused reversion.

As a first approximation, it can be considered that *z⁻ γ ⁺* revertants not suppressible by *suC⁺* are deletions. It is possible, of course, that some of the non-suppressible *z⁻ γ ⁺* revertants are due to mutations from nonsense to missense within the ochre codon. Such revertants should be further revertible to *lac⁺* by the base-substitution mutagen N-methyl-N'-nitro-N-nitrosoguanidine. We have yet to find any revertant with such properties. Therefore, we believe that none of the reversions classified by this test as deletions is due to a secondary base substitution within the altered codon.

The proportion of deletions, characterized in this way, found amongst spontaneous Mel⁺ revertants of *lac₂⁻* and amongst revertants using the mutagens 2AP, nitrous acid, X rays and UV is shown in Table 1.

All mutagens tested, with the possible exception of 2AP, significantly increase the frequency of deletions in the bacterial population. The single deletion found after 2AP mutagenesis may either be a spontaneous revertant or it may be that 2AP does induce deletion formation with a low probability. The most effective mutagenic treatments in inducing deletions are ultraviolet irradiation and nitrous acid. TESSMAN (1962) has already reported that nitrous acid causes deletions with *in vitro* treated *E. coli* bacteriophage T4. X rays do not appear to be very effective in mutagenizing *E. coli* K12 nor do they induce a very high proportion of deletions in this system.

In order to characterize the types of deletions found under the different conditions, we have mapped all of the deletion revertants (Figure 2). The spectrum

TABLE 1
Proportion of deletions among Mel^+ revertants of lac^-_2

	Median frequency of reversion	Total number of revertants analyzed	Lac ⁺	z^- suppressible	z gene deletions	z gene deletions as percent of total	Approximate absolute frequency of deletions
Spontaneous	9×10^{-8}	826	427	374	25	3	2.7×10^{-9}
HNO ₂	9×10^{-6}	807	233	517	57	7	6.3×10^{-7}
UV	9×10^{-5}	856	592	251	13	1.5	1.4×10^{-6}
X rays	3.5×10^{-6}	487	181	292	14	3	1.0×10^{-7}
2AP	2.5×10^{-5}	310	62	247	1	0.3	8×10^{-8}

For spontaneous mutants, 35 cultures of independent colonies were examined; for nitrous acid-induced revertants, 34 such cultures were examined; for ultraviolet induced revertants, 35 cultures and for X rays, 21 cultures. Each of the 310 revertants isolated after AP treatment comes from an independent culture. Deletions were classified as such as indicated in the text.

FIGURE 2.—Mapping of deletions

The numbers 1 through 16 correspond to the segments of the z gene described by NEWTON *et al* (1965). Deletions were mapped according to the technique in MATERIALS AND METHODS. Since the strain X-7167 is i^- , we could not easily determine whether deletions which cover all early point mutants, extend beyond the i^- gene. Some of these deletions may be fully within the z gene. Except for a few instances, repeats of a particular deletion were not found in the same culture. Thus, nearly all deletions are due to independent events. One X-ray induced deletion, which only covers lac^-_{U118} is difficult to explain. Peculiar reversion properties of this revertant indicate that it may not be a simple deletion. Abbreviations are S for spontaneous and X for X rays.

of deletions found differs with the mutagen. Over half of the nitrous acid-induced deletions" do not map as deletions; that is, they recombine with all z^- point mutants tested. Another 28% cover all early z^- mutations and end in the very small region between lac_2^- and lac_{U131}^- . This region may represent a "hot spot" for ends of nitrous acid-induced deletions. In contrast to nitrous acid-induced deletions, there is a wider variety of deletion types amongst the spontaneous revertants. We have not isolated enough deletions with UV and X rays to determine if there are "hot spots".

The "deletion" revertants which do not map as deletions were also found among spontaneous, X-ray- and UV-induced revertants. We have already concluded that these revertants cannot be due to the conversion of nonsense to missense; two other explanations come to mind. Either they are microdeletions which eliminate lac_2^- and a small region around it, or they are frameshift mutations mapping before lac_2^- . The latter could reverse the polarity effects by shifting the ochre mutant codon out of phase. Some of these frame-shifts would be still Mel^+ , since the analogous frame-shift mutants ω and x-84 do make sufficient levels of permease for growth on melibiose (NEWTON *et al.* 1965; SCAIFE and BECKWITH 1966). WHITFIELD, MARTIN and AMES (1966) have shown that nitrous acid induces frame-shifts in *Salmonella typhimurium*.

Although these microdeletions cannot be induced to back-mutate further to lac^+ by nitrosoguanidine, they do revert spontaneously to lac^+ at very low frequencies (5×10^{-10}). In addition, the small mappable deletions in this region covering either lac_{x-84}^- or lac_{ω}^- and lac_{x-84}^- can also revert to lac^+ at low frequency. Larger deletions do not so revert. These second-step revertants may be due either to the introduction of a second mutation restoring the correct reading frame, or extension of the deletion. At any rate, it appears from these reversion studies that this region of the z gene is not essential to β -galactosidase function. It is possible that even some of the Lac^+ revertants are microdeletions.

One of the deletion revertants covers only the YA 482 (segment 4) site in the z gene. This deletion does not fit into any of the classes described in the introduction which are known to reverse polarity effects. One possibility is that the mutation in this revertant has created a new initiation point for operon expression. This point might act either at the transcription or the translation level (GRODZICKER and ZIPSER 1969).

It should be pointed out that there is one major qualification in attempting to extend the data presented here to mutagenesis in general in *E. coli*. In this case, we are selecting for relatively specific sorts of deletions which restore activity to a mutationally altered system. This aspect of the system may affect results if, for instance, one of the mutagens causes a high proportion of very large deletions. In this system, where at least one deletion end must remain in the z gene, most very large deletions would not have been detected. Despite this qualification, it is obvious that these results have relevance to questions of what mutagens cause deletions and what sorts of deletions these are.

SUMMARY

We find that nitrous acid and ultraviolet light are very effective in inducing deletion mutations in *E. coli*. X rays do not appear to be as useful for this purpose. In the system studied, a substantial proportion of nitrous acid-induced deletions appear to be repeats of a single type. In addition, a majority of nitrous acid induced deletions are microdeletions.

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