DIRECTED MUTATION IN A SYNCHRONIZED BACTERIAL POPULATION*

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The DNA of E. Coli Hfr has been shown to replicate in a sequential, polarized fashion from the F locus forward (Nagata, 1963). Since mutagenic base analogues act only after incorporation into bacterial DNA (Rudner, 1961), it can be surmised that a given gene should be induced to mutate upon exposure to the analogue for only that fraction of the total replication during which that gene is replicating.

To check this prediction, a synchronously dividing population of E. Coli Hfr P4X-6 thy^- met^- nic^- gua^- T_1^s Str^s was prepared by fractional filtration; DNA replication in such populations is also synchronous (Nagata, 1963). The mutation studied was a reversion to prototrophy (gua^+) from a condition of guanine requirement (gua^-) which had been induced by 2-aminopurine (AP). The post-filtration culture was allowed one complete replication to establish synchrony, as determined by observing the increase in turbidity. Immediately before the onset of the second generation, the culture was split into two parts; one received a ten-minute pulse of AP (400μg/ml) in the very first part of the next

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generation; the other, an identical pulse later in the cycle. At the completion of each ten-minute exposure, the AP was washed out by a chase of guanosine and adenosine (2mg of each/ml), diluting the culture to 70%, and centrifugation and resuspension in supplemented medium. At least two doublings subsequent to the replication cycle in which the pulse was given were allowed before aliquots were plated for mutant count. Samples for viable count and DNA determination (diphenylamine reaction) were taken at fifteen-minute intervals throughout the experiment.

The results indicate that incorporation of the mutagenic analogue, the first step toward mutation, occurs during the replication of the mutated locus. The gua$^-$ locus was reverted by a ten-minute pulse at the very beginning of the replication cycle (mutant frequency 1.3×10^{-7}). Incubation with AP for an entire generation resulted in a similar reversion frequency of 2.0×10^{-7}. An identical pulse in a later period of the replication cycle resulted in a reversion frequency of only 1.3×10^{-8}; the spontaneous reversion frequency of this locus was also 1.3×10^{-8}.

These observations indicate that the base analogue is effective only when available during the replication of that part of the chromosome which houses the locus under study. This conclusion accords with the fact that the mutagenic efficiency of a pulse given during the replication of the locus is identical with the efficiency of exposure during an entire replication. An observation that leads us to believe that the mutagen is available to the replicating DNA only during the pulse itself, i. e. that it is effectively washed out, is that the frequency of reversion among the cells which received a pulse and a chase at times other than when the gua locus was replicating was no higher than spontaneous. If the muta-

gen had not been effectively removed, it would have been able to act during the next replication of the locus.

Preliminary results obtained with another Hfr, CS101 *met*$^-$ *Str*^{s}, indicate that mutation to streptomycin resistance may also be effected by employing the pulse technique. The effective analogue exposure time (second half of the replication cycle) agrees with the location of the *str* locus (64% from the *F* locus) observed in mapping by recombination analysis (Jacob and Wollman, 1961). In F$^-$ strains where the DNA does not begin to replicate from a single site (Nagata, 1963) exposure to a mutagenic base analogue at any time during a synchronized replication will mutate a given locus (Strelzoff, 1962).

With important modification, such as extended generation time, directed mutation could conceivably be employed as an accurate mapping technique. The observations described above may also be taken as further proof of the sequential and polarized replication of the bacterial chromosome.

References

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