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Nonrandom Mutagenesis of the *Escherichia coli* Genome by Nitrosoguanidine

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The distribution on the genetic map of mutations induced with nitrosoguanidine in stationary-phase cultures of *Escherichia coli* is nonrandom.

As part of an undergraduate laboratory course in genetics and microbiology at the Massachusetts Institute of Technology, we isolated and mapped a number of temperature-sensitive mutants induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) treatment of cultures of an F^- strain (DB1032, *ara⁻gal⁻glpT⁻xyl⁻*) of *Escherichia coli* K-12.

The map distribution of mutations induced in stationary cultures is nonrandom. We interpret this result by assuming that, upon dilution into the mutagenic solution, stationary-phase cells initiate deoxyribonuclic acid (DNA) replication at a unique point on the chromosome, that replication proceeds in one direction, and that NG acts at the replication point.

Bacteria were grown in broth. Stationary cultures had a final density of 2×10^9 to 3×10^9 cells/ml; exponential cultures had a density of 2×10^8 to 5×10^8 cells/ml. Mutagenesis was carried out by 20-fold dilution of cells into 0.1 M citrate buffer (*p*H 5.5) containing 100 to 500 μg of NG per ml (2), followed by incubation at 37 C without aeration for 30 min. Mutagenized cultures were diluted 1,000-fold into broth, allowed to grow overnight at 30 C, diluted, and plated on minimal agar. After incubation at 30 C for 22 to 26 hr, the plates were incubated at 42 C for 16 to 24 additional hr. Small colonies were tested for temperature sensitivity. Mapping was accomplished with a modified interrupted-conjugation technique (4), by using ability to grow at 42 C as the selective character. Many standard Hfr strains were used. Time of entry was calibrated by using sugar-nonutilizing markers in the F^- strain.

The loci of all the known genetic lesions resulting in certain phenotypes (e.g., requirement for proline) are restricted to limited regions of the genetic map of *E. coli*, whereas certain other phenotypes (e.g., requirement for purines) can result from mutations in genes located in several different places on the map (3). Mutants exhibiting phenotypes of the former class can be assigned by phenotype alone to the right (0 to 45 min) or to the left (45 to 90 min) half of the map. The phenotypes and assignments of the mutants we obtained are given in Table 1. Mutagenesis of stationary cultures yielded no mutants assignable to the right half of the map, but 33 could be assigned to the left half. Mutagenesis of exponential cultures, on the other hand, yielded 17 mutants assignable to the left half, and 6 mutants assignable to the right half.

This result was further substantiated by genetic analysis of the mutants. Thirty-six mutants (including those with assignable and unassignable phenotypes) were mapped (Fig. 1). All assignments (16/16) by phenotype were confirmed in genetic mapping experiments. Mutants derived from stationary cultures carry mutations which mapped only in the left half of the map, whereas mutagenesis of exponential cultures produced lesions which are distributed over the entire map. Our results resemble those of Cerda-Olmedo et al. (1; Cold Spring Harbor Symp. Quant. Biol., *in*

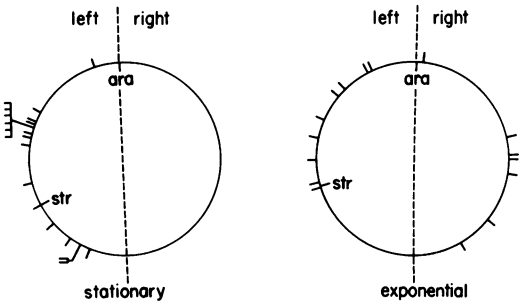

FIG. 1. *Genetic map positions of mutations derived from NG treatment of stationary and exponential cultures. The total map length is 90 min, with ara at 0 min.*

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TABLE 1. *Mutant assignment by phenotype*

Phenotype	Growth on	No. of mutants	
		Stationary culture	Exponential culture
Ascribable to known genes mapping in left half only	Arginine only[a]	0	1[b] (1)
	Ornithine	1 (1)	0
	Isoleucine[a]	3 (3)	0
	Isoleucine + valine[c]	5	9 (1)
	Isoleucine, threonine[a,c]	10 (1)	3
	Lysine	1	0
	Methionine	9 (2)	4
	Serine	1 (1)	0
	Glycine[a]	1 (1)	0
	Cysteine, not $S_2O_3^=$ or SO_3^-	2 (2)	0
Ascribable to known genes mapping in right half only	Histidine	0	1 (1)
	Leucine	0	1
	Threonine	0	2 (1)
	Tryptophan	0	1 (1)
	Proline	0	1 (1)
	Cysteine, $S_2O_3^=$, not SO_3^-	0	0
Ascribable to known genes mapping in left as well as right half	Adenine[a]	0	4 (3)
	Phenylalanine + tyrosine + tryptophan + *p*-aminobenzoic acid[a]	1 (1)	3 (1)
	Adenine, guanine	0	1
Not ascribable to known genes	Other nutritionals	9 (5)	17 (1)
	No growth in broth at 42 C	6 (2)	9 (6)

[a] Indicates that at least one mutant in this category has been characterized with respect to the enzymatic lesion responsible for this phenotype.

[b] Indicates the number of mutants isolated showing this phenotype. Numbers in parentheses indicate the number of mutants in this category which were mapped.

[c] A comma signifies that either compound will suffice; + indicates that all components are required.

press). They are readily accounted for given the following assumptions. (i) NG mutagenesis occurs primarily at the replication fork. (ii) The only cells in the stationary cultures which can be mutagenized are those which have recently initiated replication at a unique origin. This initiation might take place upon dilution into the mutagenesis buffer. (iii) Either the unique origin of replication is near 0 min on the standard (90 min) genetic map (3) and replication proceeds counterclockwise, or the origin is near 50 min and replication proceeds clockwise around the map (Fig. 1). In view of the results of others, we much prefer the latter alternative (1, 5).

We stress that all of our mutants represent forward mutations and that there was no selection with respect to exact phenotype. Our results show that the use of NG in mutant searches requires careful consideration of the state of the culture to be treated.

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