INTRODUCTION OF A MUTATOR GENE FROM *ESCHERICHIA COLI* K12 INTO *ESCHERICHIA COLI* C

Patrice J. Zamenhof

Department of Biological Chemistry, UCLA Medical School

and Molecular Biology Institute,

University of California, Los Angeles 90024

Received January 6, 1969

SUMMARY: The bacterial mutator gene *ast-1*, which arose in *E. coli* A.T.C.C. #11887 and which was transferred by conjugation to *E. coli* K12, has been transferred from K12 to *E. coli* C and its expression in the latter strain observed. Mutation rates were elevated in *E. coli* C *ast-1* up to 380-fold over those in control populations. Bacteriophage ΦX174 plated normally on the ast^+ controls, but gave rise to turbid plaques much reduced in size at an efficiency of plating of 0.4 when plated on *E. coli* C *ast-1*. It was tentatively concluded that ast--induced mutations and/or interference with replicative processes in ΦX174 may be responsible for these observations.

The bacterial mutator gene *ast-1* deemed responsible for the high spontaneous mutation rate suddenly noticed in *E. coli* strain ATCC #11887 has been transferred to *E. coli* K12 and its location on the genetic map determined (Zamenhof, 1966). This report concerns the transfer of *ast-1* from *E. coli* K12 to *E. coli* C ---- the preparation of *ast-1* and ast^+ *E. coli* C siblings, the expression of *ast-1* in *E. coli* C, and preliminary observations of its effects on efficiencies of plating and plaque morphology of ΦX174.

The rules of genetic nomenclature proposed by Demerec et al (1966) will be applied herein. Definitions of gene symbols follow Taylor and Trotter (1967). Media and standard conditions of culture and mating have been described elsewhere (Adelberg and Burns, 1960).

Escherichia coli C, strain 416 (C416) (arg⁻ tonB try⁻ met⁻ pur⁻) was crossed with AB 311 (thr⁻ leu⁻; O-his-pro-met), Try⁺ (Thr⁺ Leu⁺) recombinants issuing at a frequency of 0.001 per cent. All of 200 recombinants tested were TonB⁺, 15% Arg⁺, 9% Pur⁺, and 3% Met⁺. One recombinant, DZ144 (arg⁻ met⁻ pur⁻), was then crossed with DZ117 (tonA ast-1 his⁻ met⁻ F-lac; O-pro-ast-leu), Arg⁺ (His⁺) and Pur⁺ (His⁺) recombinants arising at frequencies of 0.36% and 0.007%, respectively. Purified tonA clones from each recombinant type were screened for high mutability (Zamenhof, 1966), which appeared to be present in 87% and 72% of the respective recombinant classes. To ensure that presumtive tonA ast-1 recombinants were derivatives of E. coli C, wet mounts of several and of DZ117, C416, and DZ144 were examined microscopically and respective lawns of growth spot-tested with phage ϕX174 (prepared on C416). Cells of DZ117 were seen to be typical coliform bacilli, approximately 1 x 3 microns in size, and gave no lytic response with ϕX174. Cells of C416, DZ144, and the presumed E. coli C ast-1 clones were all short rods, approximately 1 x 1.5 microns in size, all showing clear lysis with ϕX174. The presence of ast-1 in the E. coli C derivatives was verified, and one such, DZ176 (tonA ast-1 met⁻), was selected for additional experiments. A sibling recombinant giving no evidence of high mutability was checked for microscopic appearance and ϕX174 - response was designated DZ177 (tonA ast⁺ met⁻).

The mutation rates of several loci in DZ176 (ast-1) and DZ177 (ast⁺) were estimated. Results are summarized in Table 1. The extensive elevation of various mutation rates of DZ176 over those of DZ177 confirms the presence and expression of ast-1 in the former.

A stock of ϕX174 (prepared on C416) was titered on C416, DZ176, and DZ177. Plaque morphology and the efficiency of plating (EOP)

Table 1. Mutation rates of E. coli C ast-1 and ast$^+$ siblings*

Mutation	DZ176	DZ177	ast-1/ast$^+$
Azi^s-Azi^r	(8.9± 2.6$^\Delta$) x 10^{-6}	(1.2± 0.2) x 10^{-7}	74
Met$^-$-Met$^+$	(5.6± 1.4) x 10^{-7}	(5.8± 2.1) x 10^{-9}	97
ΦX174^{s-r}	(5.3± 1.2) x 10^{-4}	(1.4± 0.3) x 10^{-6}	378
T3^s - T3^r	(5.6± 2.1) x 10^{-8}	(7.4± 1.6) x 10^{-10}	76
T7^s - T7^r	(1.0± 0.2) x 10^{-7}	(7.4± 2.8) x 10^{-10}	135

* Values based on 3 determinations, performed as described previously (Zamenhof, 1966).

$^\Delta$ Standard deviation

appeared normal on DZ177, but on DZ176 the plaques were turbid, much reduced in size, and the EOP ranged from 0.3 to 0.5. ΦX174 prepared on DZ176 plated normally on DZ177, but gave small, turbid plaques on DZ176 with an EOP of 0.4. The aberrant plaque morphology and lowered EOP of ΦX174 plated on DZ176 as an indicator strain may reflect ast--induced mutations and/or interference with replicative processes in ΦX174 during early cycles of multiplication that prevent or alter further lysis and plaque formation.

The preparation of the siblings DZ176 (ast-1) and DZ177 (ast$^+$) makes possible investigation of the effects of this mutator gene on intracellularly multiplying ΦX174. Such studies may help to reveal the nature of the gene product of ast.

Acknowledgements

This investigation was supported by Public Health Service Grant HD-01825 from the National Institute of Child Health and Human Development. The author is the recipient of Public Health Service Career Development Award 5K03GM35238 from the National Institute of General

Medical Sciences. Appreciation is due to Diane Durkos Smith for excellent technical assistance.

References

Adelberg, E. A., and S. N. Burns. 1960. Genetic variation in the sex factor of Escherichia coli. J. Bacteriol. 79:321-330.

Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics 54:61-76.

Taylor, A. J. and C. D. Trotter. 1967. Revised linkage map of Escherichia coli. Bacteriol. Revs. 31:332-353.

Zamenhof, P. J. 1966. A genetic locus responsible for generalized high mutability in Escherichia coli. Proc. Natl. Acad. Sci. U.S. 56:845-852.