JOURNAL OF BACTERIOLOGY, Jan. 1976, p. 382-384
Vol. 125, No. 1

Increased Loss of Duplicated Genes in Streptomycin-Resistant (*strA*) Mutants of *Escherichia coli* K-12

MICHAEL H. SIMONIAN AND RAYMOND D. MOSTELLER*

Department of Biochemistry, University of Southern California, School of Medicine, Los Angeles, California 90033

Received for publication 6 October 1975

The recombination-dependent loss of a duplicated portion of the *Escherichia coli* chromosome is five- to tenfold greater in strains containing streptomycin resistance (*strA*) mutations than in the *strA*$^+$ parental strain. Streptomycin (500 μg/ml) partially reverses the increase. These results suggest an interaction between *strA* mutations and recombination.

Mutants of *Escherichia coli* that are resistant to a high concentration (500 μg/ml) of streptomycin contain an altered 30*S* ribosomal protein that is specified by the *strA* gene (4, 16). This protein, S12, is believed to participate in the ribosomal selection of aminoacyl transfer ribonucleic acids during translation (8) and perhaps in the initiation of translation (13). Recently, Chakrabarti and Gorini (5, 6) found that *strA* mutations enhance transcription of the bacteriophage T7 genome in male strains of *E. coli* and that certain rifampin-resistant (*rif*) mutations reduce or abolish this transcription. These findings suggest a possible interaction between ribonucleic acid polymerase and the S12 protein. In the present studies, we have found a correlation between mutations in the *strA* locus and the recombination (*recA*)-dependent loss of duplicated chromosomal material.

Strain RM106 of *E. coli* K-12 (W3110) contains two strongly polar mutations ($E9829_{amber}$ and $E9851_{ochre}$) in the operator-proximal gene (*trpE*) of the *trp* operon. Due to the polar mutations growth of strain RM106 is inhibited by a high concentration (30 μg/ml) of anthranilic acid (21), although the tryptophan growth requirement of this strain may be satisfied by a low concentration (2 μg/ml) of anthranilic acid or indole. Among mutants of strain RM106 selected for relief of polarity (resistance to 30 μg of anthranilic acid per ml), one strain (RM213) was isolated that contains a duplication of the operator-distal portion of the *trp* operon. Conjugation experiments with an Hfr derivative of strain RM213 indicate that the duplicated region, designated (*trpDCBA*), is an integral part of the bacterial chromosome and that it is located close to the normal site of the *trp* operon; however, (*trpDCBA*) and the *trp* operon are not co-transducible during bacteriophage P1-mediated transduction but are transferred separately at approximately equal frequencies (Simonian and Mosteller, unpublished observations). Comparison of the levels of *trp* operon enzymes in derepressed and repressed cultures of strain RM213, the parental strain RM106, and a segregant strain MS279 which has lost (*trpDCBA*) indicates that (*trpDCBA*) is probably linked to a transcription promoter which is not subject to tryptophan repression (Table 1).

The duplicated region in strain RM213 is lost spontaneously by a recombination (*recA*)-dependent process (Table 2) at a frequency characteristic of genetic duplications in prokaryotes (1, 3, 7, 10-12). Stationary-phase, L-broth cultures of strain RM213 contain a fairly constant fraction (3%) of anthranilic acid-sensitive segregants that have lost (*trpDCBA*). While studying the loss of (*trpDCBA*) in several derivatives of strain RM213, we observed that a streptomycin-resistant strain, MS330 (*strA* introduced by transduction), exhibited a sixfold greater loss of (*trpDCBA*) than strain RM213 (Table 2). To determine whether this was a general effect of *strA* mutations, we also tested the loss of (*trpDCBA*) in seven independently isolated *strA* mutants of strain RM213. All seven isolates exhibited a similar increase (five- to tenfold) in the loss of (*trpDCBA*) (Table 2). All segregants from the eight *strA* strains remained streptomycin resistant. Since streptomycin is known to reverse completely or partially the phenotypic expression of *strA* mutations under some conditions (2, 4, 9, 14, 17, 18), we also tested the loss of (*trpDCBA*) in cultures containing streptomycin. The results indicate that streptomycin decreases the loss of (*trpDCBA*) in *strA* mutants, but not to the level observed in the streptomycin-sensitive strain RM213 (Table 2).

One possible trivial explanation of our find-

TABLE 1. *Effect of (trpDCBA) on levels of trp operon enzymes*

Strain no.	Genotype[a]	PRTase (units/mg)[b]		TSase α (units/mg)[b]	
		Derepressed	Repressed	Derepressed	Repressed
RM106 (parent)	*trpE9829 trpE9851*	0.07	<0.01	1.58	0.50
RM213	*trpE9829 trpE9851 (trpDCBA)*	0.20	0.21	5.41	4.12
MS279 (segregant)	*trpE9829 trpE9851*	0.06	<0.01	2.12	0.75
RM3	*trpE5972*	2.74	<0.01	18.32	0.51

[a] All strains are derivatives of *E. coli* K-12 (W3110). *trpE9829 trpE9851* and (*trpDCBA*) are explained in the text. Strain MS279 is an anthranilic acid-sensitive segregant obtained from strain RM213 (Table 2). Data for strain RM3, which contains the nonpolar *trpE5972* mutation, are included for comparison.

[b] Phosphoribosyl transferase (PRTase) and tryptophan synthetase α polypeptide (TSase α) were determined in sonified cell extracts as described previously (15). PRTase and TSase α activities are specified by the *trpD* and *trpA* genes, respectively. Derepressed cultures were grown to stationary phase (4×10^8 cells/ml) in minimal-salts medium (20) supplemented with 0.4% glucose and 1.2 μg of indole per ml. Repressed cultures were harvested in logarithmic phase (4×10^8 cells/ml) in mimimal-salts medium supplemented with 0.4% glucose and 40 μg of L-tryptophan per ml.

TABLE 2. *Loss of (trpDCBA) in strain RM213 and its streptomycin-resistant derivatives*

Strain no.	Relevant genotype[a]	Segregation frequency[b]			
		L-broth medium		L-broth medium + streptomycin	
		No. of colonies tested	% Segregants	No. of colonies tested	% Segregants
RM213	(*trpDCBA*)	916	3.5		
MS401	(*trpDCBA*) *strA1 recA1*	805	0		
MS330	(*trpDCBA*) *strA1*	1,090	18	747	10
MS330-1	(*trpDCBA*) *strA2*	180	32	309	17
MS330-2	(*trpDCBA*) *strA3*	247	19	133	10
MS330-3	(*trpDCBA*) *strA4*	135	18	311	10
MS330-4	(*trpDCBA*) *strA5*	90	28	134	8
MS330-5	(*trpDCBA*) *strA6*	138	25	154	14
MS330-6	(*trpDCBA*) *strA7*	184	14	135	4
MS330-7	(*trpDCBA*) *strA8*	154	27	81	10

[a] All strains are derivatives of *E. coli* K-12 (W3110) *trpE9829 trpE9851*. (*trpDCBA*) is explained in the text. The *recA1* allele was introduced by conjugation with Hfr KL16-99. The *strA1* allele was introduced by P1-mediated transduction, and all other *strA* strains were isolated from independent clones of RM213 on L-broth agar medium containing 500 μg of streptomycin sulfate per ml.

[b] Five-milliliter cultures started from single-colony isolates of each strain were grown to stationary phase at 37 C in L-broth medium (10 g of tryptone [Difco]per liter, 5 g of yeast extract per liter, 5 g of NaCl per liter, 1 g of glucose per liter) with or without 500 μg of streptomycin sulfate per ml. Portions of each culture were spread on minimal-agar medium (20) containing 20 μg of indole per ml and incubated at 37 C for 48 h. The resulting colonies were tested for loss of (*trpDCBA*) by replica plating onto minimal-agar medium containing 30 μg of anthranilic acid per ml.

ings could be that streptomycin-resistant segregants grow better than streptomycin-sensitive segregants relative to their respective (*trpDCBA*)-containing parents and thus accumulate to a greater extent in L-broth cultures. However, we have found that each of the segregants tested grows at the same rate as its respective parent (Table 3) and attains approximately the same final cell density (3×10^9 to 4×10^9 cells/ml) when grown in L-broth medium. We conclude that the increased loss of (*trpDCBA*) in *strA* strains is not due to large

TABLE 3. *Growth rates of (trpDCBA)-containing strains and their segregants in L-broth medium*

Bacterial strains	Growth rates (doublings/h)[a]	
	(*trpDCBA*)-containing strains	Anthranilic acid-sensitive segregants
Streptomycin sensitive		
RM213	2.3	2.4
Streptomycin resistant		
MS330	2.2	2.2
MS330-1	2.0	2.1
MS330-2	2.1	NT[b]
MS330-3	2.1	2.0
MS330-4	1.9	2.1
MS330-5	2.0	2.1
MS330-6	2.1	NT[b]
MS330-7	2.1	2.1

[a] Growth rates of (*trpDCBA*)-containing strains and their respective segregants were determined by absorbance at 600 nm in L-broth at 37 C.

[b] NT, Not tested.

differences in growth rates or survival. It is not likely that there is a direct interaction between the *strA* gene and (*trpDCBA*) or between the *strA* and *recA* genes (or *recB* or *recC*), since these genetic loci are known to be widely separated on the *E. coli* chromosome (19; Simonian and Mosteller, unpublished observations). A more plausible explanation is that the recombination process is affected by translational restriction resulting from the *strA* mutations. This idea is supported by the fact that streptomycin, which is known to relieve restriction by *strA* mutations (2, 4, 9, 14, 17, 18), partially reverses the increased loss of the duplication. For example, altered synthesis of a protein involved in replication or repair of deoxyribonucleic acid may affect the extent of exposed single-stranded regions in the chromosome and thereby enhance the frequency of recombination. Alternatively, the mutant form of the S12 protein, which is known to interact with ribonucleic acids during translation, may affect recombination directly by binding to deoxyribonucleic acid. Further experiments are in progress to investigate these possibilities.

We are grateful to Linda Benjamin for typing the manuscript with her usual efficiency.

This work was supported by Public Health Service research grant RR05356 from the Division of Research Resources and grant CA14089 from the National Cancer Institute, and by a grant from the National Science Foundation (GB29478X). M.H.S. was supported by a fellowship from the Calbiochem Foundation.

LITERATURE CITED

1. Ames, B. N., P. E. Hartman, and F. Jacob. 1963. Chromosomal alterations affecting the regulation of histidine biosynthetic enzymes in *Salmonella*. J. Mol. Biol. **7**:23-42.
2. Anderson, W. F., L. Gorini, and L. Breckenridge. 1965. Role of ribosomes in streptomycin-activated suppression. Proc. Natl. Acad. Sci. U.S.A. **54**:1076-1083.
3. Beeftinck, F., R. Cunin, and N. Glansdorff. 1974. Arginine gene duplications in recombination proficient and deficient strains of *Escherichia coli* K-12. Mol. Gen. Genet. **132**:241-253.
4. Breckenridge, L., and L. Gorini. 1970. Genetic analysis of streptomycin resistance in *Escherichia coli*. Genetics **65**:9-25.
5. Chakrabarti, S., and L. Gorini. 1975. A link between streptomycin and rifampicin mutation. Proc. Natl. Acad. Sci. U.S.A. **72**:2084-2087.
6. Chakrabarti, S., and L. Gorini. 1975. Growth of bacteriophages MS2 and T7 on streptomycin-resistant mutants of *Escherichia coli*. J. Bacteriol. **121**:670-674.
7. Folk, W. R., and P. Berg. 1971. Duplication of the structural gene for glycyl-transfer RNA synthesis in *Escherichia coli*. J. Mol. Biol. **58**:595-610.
8. Gorini, L. 1971. Ribosomal discrimination of tRNAs. Nature (London) New Biol. **234**:261-264.
9. Gorini, L., and E. Kataja. 1964. Phenotypic repair by streptomycin of defective genotypes in *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **51**:487-493.
10. Hill, C. W., F. Foulds, L. Soll, and P. Berg. 1969. Instability of a missense suppressor resulting from a duplication of genetic material. J. Mol. Biol. **39**:563-581.
11. Horiuchi, T., S. Horiuchi, and A. Novick. 1963. The genetic basis of hyper-synthesis of β-galactoside. Genetics **48**:157-169.
12. Jackson, E. N., and C. Yanofsky. 1973. Duplication-translocations of tryptophan operon genes in *Escherichia coli*. J. Bacteriol. **116**:33-40.
13. Kang, S.-S. 1970. A mutant of *Escherichia coli* with temperature-sensitive streptomycin protein. Proc. Natl. Acad. Sci. U.S.A. **65**:544-550.
14. Lederberg, E. M., L. Cavalli-Sforza, and J. Lederberg. 1964. Interaction of streptomycin and a suppressor for galactose fermentation in *E. coli* K-12. Proc. Natl. Acad. Sci. U.S.A. **51**:678-682.
15. Mosteller, R. D., and B. B. Mandula. 1973. Kinetics of derepression of the tryptophan operon of *Escherichia coli* and *Salmonella typhimurium* under different culture conditions. J. Mol. Biol. **80**:801-823.
16. Ozaki, M., S. Mizushima, and M. Nomura. 1969. Identification and functional characterization of the protein controlled by the streptomycin-resistant locus in *E. coli*. Nature (London) **222**:333-339.
17. Rosset, R., and L. Gorini. 1969. A ribosomal ambiguity mutation. J. Mol. Biol. **39**:95-112.
18. Strigini, P., and L. Gorini. 1970. Ribosomal mutations affecting efficiency of amber suppression. J. Mol. Biol. **47**:517-530.
19. Taylor, A. L., and C. H. Trotter. 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. **36**:504-524.
20. Vogel, H., and D. M. Bonner. 1956. Acetylornithase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97-106.
21. Yanofsky, C., V. Horn, M. Bonner, and S. Stasiowski. 1971. Polarity and enzyme functions in mutants of the first three genes of the tryptophan operon of *Escherichia coli*. Genetics **69**:409-433.