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Isolation and Genetic Analysis of a Strain of *Escherichia coli* K-12 with an Amber *recA* Mutation

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The isolation, properties, and genetic analysis of a strain of *Escherichia coli* K-12 with an amber *recA* mutation are described. The experiments demonstrate that the *recA* product is a protein that is probably not essential for growth.

Previous studies (2, 7, 12) have shown that mutants of *Escherichia coli* K-12 which carry a *recA* mutation are extremely defective in genetic recombination and sensitive to ultraviolet light (UV). To assist in biochemical isolation of the *recA* product, a strain has been constructed which is believed to carry a *recA* amber mutation. This strain is expected to produce very low levels of the *recA* protein because of the known behavior of an amber mutation in efficiently terminating the polypeptide chain (8).

To obtain a *recA* amber mutation, it was necessary to start with a strain of *E. coli* K-12 in which *recA* mutations could be detected and characterized and did not carry an ochre or an amber suppressor. A strain meeting these requirements, JC5219 (A. J. Clark, *personal communication*), was mutagenized with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and the survivors were plated on L agar (12) containing 0.08% methyl-methane-sulfonate (MMS). After 18 hr of incubation at 37 C on this medium, *recA* mutants (along with other classes of mutants) form small colonies and *recA*$^+$ cells form large colonies. The small colonies were picked and cultures were tested for their sensitivity to UV and their ability to undergo genetic recombination. From 150,000 survivors of the mutagenic treatment, approximately 50 mutants sensitive to UV and MMS were obtained. Four of these showed the extreme sensitivities and recombination deficiencies characteristic of *recA* mutants. One of the four, designated T28, was shown to carry a mutation which did not complement another *recA* mutation (Table 1) and was suppressed by amber suppressors, as described below.

When T28 was mated with Hfr strains which transfer an amber suppressor mutation, the yield of colonies on selective medium was 10- to 50-fold greater than that obtained in matings with the same Hfr strains which do not carry and transfer the amber suppressor. In a cross between KL96 *supD*$^-$ (4) and T28 *his*$^-$ *str*^{R}, in which His$^+$ [Str^R] recombinants were selected, 177 of 198 recombinants analyzed had inherited the unselected *supD*$^-$ marker and had also acquired the Rec$^+$ phenotype. The presence of *supD*$^-$ was detected by ability to plate T4amE18. In a second cross between HfrH *supE*$^-$ (4) and T28 *thr*$^-$*leu*$^-$*str*^{R}, in which Thr$^+$Leu$^+$[Str^R] colonies were selected, 72 of 195 analyzed had inherited the unselected *supE*$^-$ marker, and all these were Rec$^+$. The presence of *supE*$^-$ was detected by ability to plate T4amE727.

The above crosses demonstrated that *recA99* is an amber mutation. Additional proof was ob-

TABLE 1. *Complementation analysis of recA mutants*[a]

Donor strains	Recombination deficiency index[b] with recipient strain		
	JC5219 *rec*$^+$	AB2463 *recA13*	T28 *recA99*
JC5029 *rec*$^+$	1	5	4
JC5088 *recA56*	1	10^4	$>3 \times 10^4$

[a] The donors (3, 11) have the same origin and direction of transfer (Fig. 1) and are *ilv*$^-$*thr*$^-$. The recipients are F$^-$*thr*$^-$*leu*$^-$*pro*$^-$*arg*$^-$*his*$^-$*str*^{R}. Matings were carried out at 37 C for 60 min, as described by Willetts et al. (12) and were plated out without interruption on medium selective for His$^+$[Str^R] recombinants.

[b] The recombination deficiency index (3) is the number of recombinants obtained with the *rec*$^+$ recipient divided by the number obtained with the *rec*$^-$ recipient in crosses with the same donor culture. A large value of the deficiency index represents absence of complementation in the temporary zygote formed by conjugation. JC5029 and JC5088 yielded approximately the same numbers of His$^+$[Str]^R recombinants when mated with JC5219.

tained by the observation that two of five independent UV-resistant revertants of T28 had acquired an amber suppressor which permitted them to plate amber mutants of T4 which would not plate on T28 itself.

Strain T28 and its parent, JC5219, were found in the above analysis to support the growth of a small proportion of amber mutants of T4. This may reflect the presence of a weak amber or ochre suppressor mutation that might allow synthesis of some *recA* protein in the strain bearing the amber *recA* mutation. The lowest possible levels were desirable, so that the product of *recA* gene could be identified biochemically by its absence in extracts of the mutant strain. Consequently, *recA99* was transduced into strain 594 (1), which is unable to plate any one of a large number of T4 amber mutants tested, and the resulting RecA$^-$ strain is not expected to produce detectable levels of the *recA* product. Strain 594 was made Thy$^-$ by trimethoprim selection (9) and then transduced with phage P1*vira* (12) grown on JC2915 (11) and a *thyA*$^+$*cysC*$^-$ transductant (DM20) selected. DM20 was then transduced with phage P1*vira* grown on T28, and both a *cysC*$^+$*recA99*$^-$ (DM455) and a *cysC*$^+$*recA*$^+$ (DM456) transductant were obtained (12). The latter strains were unable to plate amber mutants of T4, and are probably isogenic, differing only in the *recA99* and *recA*$^+$ alleles. The presence of *recA99* in DM455 resulted in properties characteristic of RecA$^-$ strains, i.e., extreme sensitivity to UV, extreme deficiency in recombination, and slow growth (1*a*, 2, 5–7, 12).

The results of this study demonstrate that the *recA* product is a protein and that cell growth is possible when the protein is not produced as the result of an amber mutation.

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LITERATURE CITED

1. Campbell, A. 1961. Sensitive mutants of bacteriophage λ. Virology **14**:22–23.
1*a*. Capaldo-Kimball, F., and S. D. Barbour. 1971. Involvement of recombination genes in growth and viability of *Escherichia coli* K-12. J. Bacteriol. **106**:204–212.
2. Clark, A. J., and A. D. Margulies. 1965. Isolation and characterization of recombination-deficient mutants of *Escherichia coli* K12. Proc. Nat. Acad. Sc. U.S.A. **53**: 451–459.

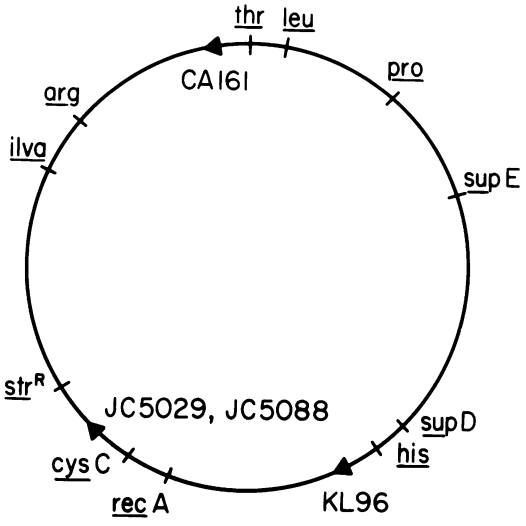

FIG. 1. *Linkage map of Escherichia coli K-12 (10) showing position of markers and origin and direction of transfer of the Hfr strains.*

3. Clark, A. J. 1967. The beginning of a genetic analysis of recombination proficiency. J. Cell. Physiol. **70** (Suppl. 1):165–180.
4. Gross, J., and M. Gross. 1969. Genetic analysis of an *E. coli* strain with a mutation affecting DNA polymerase. Nature (London) **224**:1166–1168.
5. Haefner, K. 1968. Spontaneous lethal sectoring, a further feature of *Escherichia coli* K-12 strains deficient in the function of *rec* and *uvr* genes. J. Bacteriol. **96**:652–659.
6. Hertman, I. 1969. Survival, DNA breakdown and induction of prophage λ in *Escherichia coli* K-12 *recA uvrB* double mutants. Genet. Res. **14**:291–307.
7. Howard-Flanders, P., and L. Theriot. 1966. Mutants of *E. coli* defective in DNA repair and in genetic recombination. Genetics **53**:1137–1150.
8. Sarabhai, A. S., A. O. W. Stretton, S. Brenner, and A. Bolle. 1964. Co-linearity of the gene with the polypeptide chain. Nature (London) **201**:13–17.
9. Stacey, K. A., and E. Simson. 1965. Improved method for the isolation of thymine-requiring mutants of *Escherichia coli*. J. Bacteriol. **90**:554–555.
10. Taylor, A. L. 1970. Current linkage map of *Escherichia coli*. Bacteriol. Rev. **34**:155–175.
11. Willetts, N. S., and A. J. Clark. 1969. Characteristics of some multiply recombination-deficient strains of *Escherichia coli*. J. Bacteriol. **100**:231–239.
12. Willetts, N. S., A. J. Clark, and B. Low. 1969. Genetic location of certain mutations conferring recombination-deficiency in *Escherichia coli*. J. Bacteriol. **97**:244–249.
13. Willetts, N. S., and D. W. Mount. 1969. Genetic analysis of recombination-deficient mutants of *Escherichia coli* K-12 carrying *rec* mutations cotransducible with *thyA*. J. Bacteriol. **100**:923–934.