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

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Received for publication 23 April 1971

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, JCS219 (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+ strR, in which His+ [StrR] 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-strR, in which Thr+ Leu+ [StrR] 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 obtained with the data presented in Table 1. Complementation analysis of recA mutants

<table>
<thead>
<tr>
<th>Donor strains</th>
<th>Recombination deficiency index* with recipient strain</th>
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<tbody>
<tr>
<td>JCS209 rec+</td>
<td>JC5219 rec+</td>
</tr>
<tr>
<td>JCS088 recA56</td>
<td>AB2463 recA13</td>
</tr>
<tr>
<td></td>
<td>T28 recA99</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>10*</td>
</tr>
<tr>
<td></td>
<td>&gt;3 × 10*</td>
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</tbody>
</table>

* The donors (3, 11) have the same origin and direction of transfer (Fig. 1) and are His+ thr-. The recipients are F- thr- leu- pro- arg- his- strR. 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+ [StrR] recombinants.

* 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. JCS029 and JCS088 yield approximately the same numbers of His+ [StrR] 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 Plvira (12) grown on JC2915 (11) and a thyA+cysC- transductant (DM20) selected. DM20 was then transduced with phage Plvira 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 (1a, 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.

This research was supported by a grant from the American Cancer Society.

I thank A. J. Clark, J. G. Gross, and Harris Bernstein for providing phage and bacterial strains.

LITERATURE CITED


