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Escherichia coli recBC Deletion Mutants

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Mutants of *Escherichia coli* with deletions of the *recB* and *recC* genes were obtained by two methods using transposable DNA elements. The phenotypes of these mutants are similar to those of mutants with *recBC* point mutations. These results indicate that the RecBC gene products, exonuclease V, is not essential for the growth of *E. coli* but is important for DNA repair and recombination.

The *recB* and *recC* genes of *Escherichia coli* code for the RecBC enzyme (exonuclease V), which promotes homologous recombination in the RecA-RecBC pathway (2, 5, 10, 21). *recB* and *recC* mutants have been isolated by either their deficiency in homologous recombination or their sensitivity to DNA-damaging agents (5–7). These mutants also lack the ATP-dependent double-stranded DNA exonuclease activity and the ATP-stimulated single-stranded DNA endonuclease activity associated with the RecBC enzyme (2, 9).

One phenotype of the *recB* and *recC* mutants raises the question of whether a low level of RecBC function may be essential for the growth of *E. coli*. *recB* and *recC* mutants show a remarkably higher segregation of nonviable progeny than do wild-type cells: whereas more than 90% of the microscopically visible cells of a *recBC*$^+$ culture form visible colonies, only 10 to 20% of those of a *recB* or *recC* mutant culture do so (3). The decreased viability of these mutants is apparently not due only to their deficiency in recombination and repair since *recA* mutants, despite being more recombination deficient, are more viable (3). A possible explanation of the decreased viability of *recBC* mutants is that a low level of RecBC activity, enough to reduce but not to abolish the viability of the cells, remains in these mutants. The missense *recB* or *recC* alleles might encode an impaired but residually active protein, and the nonsence alleles might encode a truncated but partially active protein. In this view, a deletion of the *recB* and *recC* genes would make the cell completely inviable. In this paper we report the isolation and characterization of *recBC* deletion mutants; their viability indicates that RecBC functions are not essential for *E. coli* growth.

We used two different methods to generate deletions in the *recB* and *recC* genes; these methods involved the transposon Tn*10* and a derivative of phage Mu, both of which generate deletions near the site of their insertion (12, 13). In one method we isolated tetracycline-sensitive derivatives of a strain with an insertion of Tn*10* in *argA*, a gene adjacent to the *recB* gene (see Fig. 1) (1). Some of the tetracycline-sensitive derivatives are likely to contain deletions in the adjacent *recB* and *recC* genes. The other method involved an insertion of a derivative of Mu called Mu d(Ap *lac*) (4) in the *thyA* gene, a gene adjacent to the *recC* gene (1). Mu d(Ap *lac*) contains a thermolabile Mu repressor (4); some of the temperature-resistant survivors of this strain are likely to contain deletions in the adjacent *recB* and *recC* genes.

We used several properties of *recBC* mutants to screen the putative *recBC* deletion mutations. Phage T4 gene *2* mutants (hereafter called T4 *2*$^-$) form plaques on *recBC* mutant cells but not on *recBC*$^+$ cells. Presumably the gene *2* product of phage T4 protects the injected T4 DNA from nucleolytic degradation of RecBC enzyme (17). Phage P2 plates on *recBC* mutant hosts at a much lower efficiency than on *recBC*$^+$ hosts (20). *recBC* mutants are more sensitive to UV light than are *recBC*$^+$ cells (8).

The bacterial strains used are described in Table 1. Chlorotetracycline, ampicillin, and fusaric acid were purchased from Sigma Chemical Co., St. Louis, Mo. Mu d(Ap *lac*) was obtained from David Hagen (University of Oregon, Eugene). Strain AC38 containing *thyA*::Mu d(Ap *lac*) was constructed by mutagenizing a culture of strain 594 with Mu d(Ap *lac*) as described previously (4) and by selecting for thymine auxotrophy (trimethoprim resistance) (16). One of the Thy$^-$ candidates, strain AC38, was transduced to Thy$^+$ with P1 grown on strain 594, a *thyA*$^+$ *recBC*$^+$ strain. All 90 Thy$^+$ transductants of *E. coli* AC38 were ampicillin and Mu sensitive; this indicated that *E. coli* AC38 contained a single Mu d(Ap *lac*) insertion in the *thyA* gene. Selection for tetracycline-sensitive bacteria was done as described previously (14), as were P1-mediated transduction and measurement of UV sensitivity (19). For complementation analysis, strains S928, S930, and V79 (19) carrying the F'15 factor with the *recB21*, *recC22*, or *recC73* mutation were mated with recipients containing putative deletions in the *recB* and *recC* genes. Although the donor bacteria were multiply auxotrophic, recipients were *thyA* or *argA* or *thyA argA*; merodiploids were therefore selected on minimal medium on which neither the donors nor the recipients could grow. After purification, merodiploids were tested for their resistance to UV irradiation and for their ability to allow the growth of phages T4 *2*$^-$ and P2 by spotting the phages onto lawns of the merodiploids. Merodiploids that showed *recBC* mutant phenotypes were tested for their ability to form *recBC*$^+$ recombinants. Each of these merodiploids was grown overnight, and 5×10^4 cells of each were spotted onto L plates containing 1.2 μg of nitrofurantoin per ml. Nitrofurantoin is a DNA-damaging agent which at this concentration inhibits the growth of *recBC* mutants but allows the growth of *recBC*$^+$ recombinants (15). The appearance of colonies able to grow in the presence of nitrofurantoin thus indicates that the merodiploids can form *recBC*$^+$ recombinants.

Isolation of mutants. To generate tetracycline-sensitive, *recBC* mutant derivatives from a strain with the *argA*::Tn*10* insertion, cultures of strain AFT228 were grown to late

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TABLE 1. Bacterial strains[a]

Strain	Genotype	Reference or source[b]
AFT228	*argA81*::Tn*10*	19
594	*lac gal rpsL*	Our collection
V66	*recF143 his-4 met rpsL31 gal*(?) *xyl*(?) *ara*(?) *argA*	19
AC285	As V66, plus *thyA*	Our collection
AC290	As V66, plus Δ(*thyA-recC*)*238*	AC42 × V66
AC291	As V66, plus Δ(*argA-thyA*)*235* Ap^r	AC30 × V66
V68	As V66, plus *recC73*	19
AC279	As V68, plus *thyA*	Our collection
AC38	As 594, plus *thyA*::Mu d(Ap *lac*)	This paper
AC44	As 594, plus Δ(*argA-thyA*)*236*	S of AC38
AC2	As 594, plus Δ(*recC-thyA*)*237*	S of AC38
AC42	As 594, plus Δ(*recC-thyA*)*238*	S of AC38
AC30	As 594, plus Δ(*argA-thyA*)*235* Ap^r	S of AC38
AC113	Δ(*argA-thyA*)*232*	S of AFT228
AC111	Δ(*argA-recC*)*234*	S of AFT228
AC110	Δ(*argA-recB*)*231*	S of AFT228
AC114	Δ(*argA-recB*)*241*	S of AFT228
AC292	*argA*	S of AFT228
AC302	*argA thyA*	S of AC292
AC319	*argA thyA recC73*	Our collection
AC333	*thyA recC73*	Our collection
AC300	*thyA* $^+$	S of 594

[a] Additional strains used in this paper have been described in reference 19. This table describes one of each class of *recBC* deletion strains. The rest of the deletions are shown in Fig. 1.

[b] S, Spontaneous derivative; A × B, result of P1 transduction where strain A was the donor and strain B was the recipient.

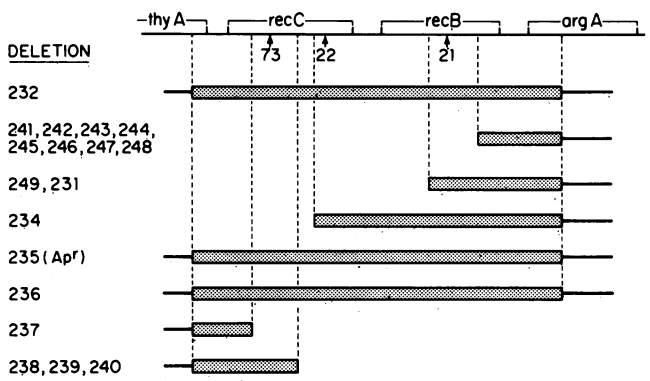

FIG. 1. *recBC* deletion mutants. The extent shown for each deletion was deduced from its ability to complement the *recB* and *recC* point mutations and on the ability of the RecBC$^-$ merodiploids containing a *recBC* deletion on the chromosome and a *recB* or *recC* point mutation on an F′ factor to form Rec$^+$ recombinants (see the text). Rec$^+$ recombinants were scored as colonies on nitrofurantoin plates as described in the text; 10^4 merodiploid cells were then spotted onto plates containing nitrofurantoin. Merodiploids able to recombine formed 10 to 100 nitrofurantoin-resistant colonies per 10^4 cells spotted. The mutants contained either deletions that did not span the point mutation or inversions that may or may not span the point mutations. Merodiploids producing no nitrofurantoin-resistant colonies from 10^4 cells were deduced to contain deletions failing to recombine with the point mutation carried in that particular merodiploid. The *thyA* and the *argA* ends of the deletions may go beyond the points shown here. It is not known whether deletions 234 and 238 overlap. The figure is not drawn to scale.

exponential phase in L broth, and 5×10^5 bacteria were plated on tetracycline-sensitive selection plates (14) and incubated overnight at 37°C. The frequency of tetracycline-sensitive mutants was ca. 10^{-3}. After purification, the tetracycline-sensitive clones were screened for the loss of RecBC function by cross-streaking them against lines of T4 *2*$^-$ and P2 (2×10^8 phage per ml) on Trypticase (BBL Microbiology Systems, Cockeysville, Md.) plates (19). Of ca. 3,000 tetracycline-sensitive derivatives, 12 were sensitive to T4 *2*$^-$ but not to P2 and were therefore candidates for containing deletions in the *recB* and *recC* genes.

To generate temperature-resistant *recBC* mutant derivatives from a strain with the *thyA*::Mu d(Ap *lac*) insertion, ca. 10^5 cells of strain AC38 were streaked onto L plates with 50 μg of thymine per ml and incubated overnight at 43°C. Temperature-resistant colonies were purified on the same medium at 43°C and then cross-streaked against P2 and T4 *2*$^-$. Of 80 temperature-resistant derivatives, 6 were sensitive to T4 *2*$^-$ but not to P2, indicating that they too had lost RecBC functions and were candidates for containing deletions in the *recB* and *recC* genes.

TABLE 2. Effect of *recBC* deletion on cell viability and recombination proficiency

Strain	Relevant genotype	Cell viability[a]	Relative frequency of recombinants per viable recipient by:		
			P1 transduction		Conjugation
			zcb-222::Tn*10*[b]	*his*[c]	*his*[d]
AC302	*argA thyA recBC*$^+$	1.00	1.0		
AC319	*argA thyA recC73*	0.15	0.045		
AC113	Δ(*argA-thyA*)*232*	0.12	0.062		
AC30	Δ(*argA-thyA*)*235* Ap^r	0.17	0.033		
AC110	Δ(*argA-recB*)*231*	0.11	0.024		
AC42	Δ(*thyA-recC*)*238*	0.11	0.024		
AC300	*thyA recBC*$^+$	0.95	1.0		
AC333	*thyA recC22*	012	0.019		
AC285	*argA thyA recBC*$^+$	0.90	1.0	1.0	1.0
AC279	*argA thyA recC73*	0.13	0.018	0.11	<0.002
AC291	Δ(*argA-thyA*)*235* Ap^r	0.12	0.023	0.13	<0.002

[a] Viability is expressed as the ratio of the number of CFU to microscopically observed cells. Cells were grown exponentially in LB broth and plated on L plates supplemented with thymine for CFU determinations.

[b] The donor was strain S1000 (19). The recombinant frequencies for strains AC302, AC300, and AC285 were 7×10^{-6}, 5.2×10^{-6}, and 9×10^{-6} per P1 PFU, respectively.

[c] The donor strain was AFT228. The recombinant frequency for strain AC285 was 9.2×10^{-6} per P1 PFU.

[d] The donor was Hfr strain S727 (19). Selection was for His$^+$ (Str^r) exconjugants. The recombinant frequency for *recBC*$^+$ was 1.21×10^{-2} per Hfr donor cell. For each recipient, the frequency of transfer of F′ *his*$^+$ from strain V156 (19) varied from 0.15 to 0.32 per F′ donor cell.

Mapping and complementation analyses. The genetic characteristics of each of these 18 candidates were determined in a number of ways. To test whether the mutations in the candidates were deletions, we determined the genetic linkage between the Arg^- (or Thy^-) and $RecBC^-$ characters. If the mutations were deletions, we would expect 100% linkage between these characters. For comparison, point mutations in *thyA* are ca. 65, 41, and 23% linked to *recC*, *recB*, and *argA* point mutations, respectively, in P1-mediated transduction (22). Each of the $RecBC^-$ mutants generated from *argA*::Tn*10* was transduced to Arg^+ by P1 transduction. All of the Arg^+ transductants tested (at least 50 for each candidate) also had $RecBC^+$ phenotypes; these results indicate that the lesions in the *recB* and *recC* genes are 100% linked to *argA* and suggest that they are deletions extending from *argA* into *recB* or *recC*. Similarly, each of the $RecBC^-$ mutants generated from *thyA*::Mu d(Ap *lac*) was transduced to Thy^+ by P1 transduction. All of the Thy^+ transductants tested (at least 50 for each candidate) also had the $RecBC^+$ phenotype. The 100% linkage of *thyA* and *recBC* in these mutants suggests that they contain deletions extending from the *thyA* gene to the *recC* and *recB* genes.

To determine which gene(s) the mutations disrupt, we carried out complementation analyses by introducing F'15 factors containing *recB* or *recC* point mutations. Among the 12 $RecBC^-$ candidates generated from *argA*::Tn*10*, 10 were *recB* and 2 were *recB recC*. Among the six candidates generated from *thyA*::Mu d(Ap *lac*), four were *recC* and two were *recC recB*. The results of these complementation analyses are summarized in Fig. 1.

As a further test of whether these mutants contained deletions, we examined their ability to recombine with *recBC* mutants carrying point mutations *recB21*, *recC22*, and *recC73* to produce $recBC^+$ derivatives. Three $RecBC^-$ mutants, AC113, and AC44, and AC30 (derived from independent cultures), failed to recombine with any of the *recB* or *recC* alleles (Fig. 1). Furthermore, these three mutants were Thy^- $RecC^-$ $RecB^-$ Arg^-, and these characters were 100% cotransducible. These results strongly suggest that the lesions in these mutants are deletions that removed the entire region between *thyA* and *argA*. The rest of the mutants recombined with mutants containing certain but not other *recB* and *recC* point mutations (Fig. 1), and we assume that they contain deletions of part of the *recBC* region.

However, it is possible that some of these mutants contain inversions rather than deletions of the *recB* and *recC* genes. Only two classes of mutants may contain inversions, the *thyA recC* class and the *argA recB* class. Since the *recB* gene has its own promoter between itself and the *recC* gene (18), neither the *argA recC* nor the *argA thyA* inversion would inactivate the *recB* gene. These two classes of mutants are therefore unlikely to contain inversions.

Comparison of *recBC* point and deletion mutants. To determine whether the *recBC* deletion mutants differed from the previously isolated *recBC* point mutants, the viability and the UV sensitivity of representative mutants were compared. By both criteria, the *recBC* deletion mutants were very similar to the *recC73* point mutant (Table 2; Fig. 2). To show that the viability of the strains deleted for the *recB* and the *recC* genes was not due to the presence of an unlinked second site mutation, the Δ(*argA-thyA*)*235* deletion from strain AC30 was transferred by P1 transduction to strain V66. Since the Ap^r determinant in strain AC30 was 100% linked to the *recBC* deletion, the deletion could be transferred by selecting for ampicillin resistance. The frequency of Ap^r transductants of *E. coli* V66 was ca. 10^{-6} transductants per P1 phage; all of the Ap^r transductants were also $RecBC^-$ as judged by their sensitivity to phage T4 2^-. The high frequency of Ap^r $RecBC^-$ transductants argues against the possibility that the viable $RecBC^-$ transductants contain a second site suppressor mutation. Strain AC291, the strain V66 derivative with the Δ(*argA-thyA*)*235* deletion, segregated as high a fraction of nonviable progeny as did an isogenic strain, AC279, containing the presumed missense allele *recC73* (Table 2). The recombination proficiencies of strains AC279 and AC291 were also comparable, as measured by P1 transduction and conjugation (Table 2).

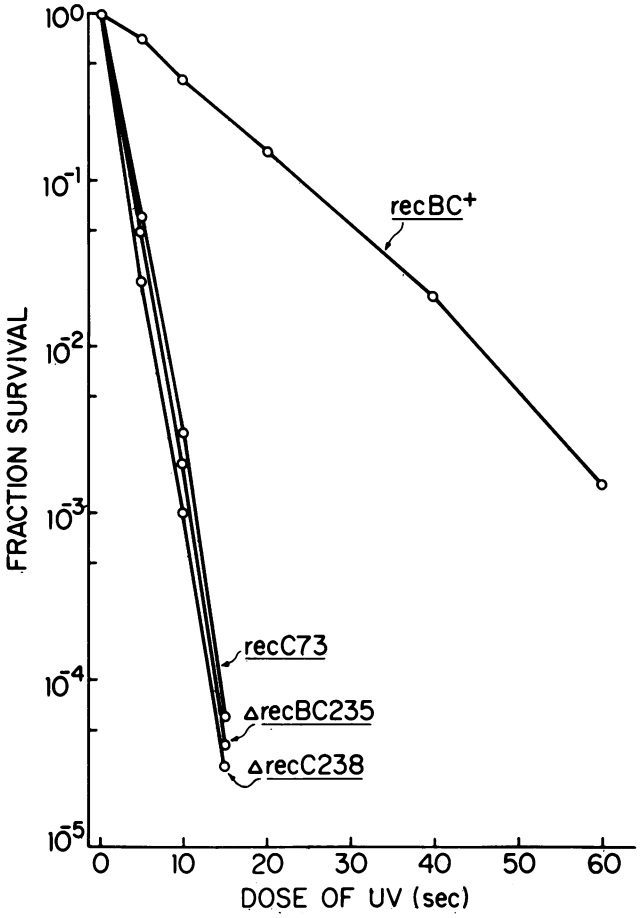

FIG. 2. UV sensitivity of *recBC* deletion mutants. Bacteria with the indicated *recB* and *recC* alleles were grown exponentially in L broth, irradiated with UV light (20 ergs mm^{-2} s^{-1}), and plated on L plates supplemented with thymine for the determination of surviving CFU. Data are the fraction of the CFU surviving after the indicated exposure to UV irradiation. The strain with the *recBC* deletion is AC291, that with the *recC* deletion is AC290, that with the $recBC^+$ allele is AC285, and that with the *recC73* allele is AC279. All strains contained the *recF143* mutation (Table 1) to increase the degree of sensitivity to UV light conferred by *recBC* mutations (11). Semiquantitative tests in $recF^+$ backgrounds indicated that the UV sensitivities of *recBC* point and deletion mutants are similar (data not shown).

This report shows that a complete deletion of the *recB* and *recC* genes does not decrease the viability of the cell any more than does a *recC* point mutation. We therefore conclude that RecBC functions are not essential for the growth of *E. coli*. The reason for the low viability of *recBC* mutants remains unknown. One view postulates that the inviability is

caused by decreased DNA repair in the *recBC* mutants and the consequent accumulation of lethal DNA lesions (3). We have isolated a new class of *recBC* mutants that are as proficient in recombination, repair, and viability as *recBC*$^+$ cells but that lack the ATP-dependent double-stranded DNA exonuclease activity of the RecBC enzyme (A. Chaudhury and G. Smith, Proc. Natl. Acad. Sci. U.S.A., in press). The phenotype of these mutants indicates that this nuclease activity is not required for high viability. It is likely that some other RecBC activity, such as DNA unwinding, required for recombination and repair is also required for high viability, but its role in the maintenance of cell viability remains to be determined.

The deletions decribed in this paper should be useful in the mapping of the genes between the *argA* and *thyA* loci. Furthermore, the *recBC* deletions should be useful in experiments where reversion of *recB* and *recC* point mutations is a problem. The strains deleted in the *recBC* genes may also be useful in phenotypic analysis of mutations generated in vitro from the cloned *recB* and *recC* genes, by preventing recombination between the cloned sequences and the chromosomal sequences and by decreasing the frequency of integration of the cloned DNA into the chromosome.

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LITERATURE CITED

1. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
2. **Buttin, G., and M. Wright.** 1968. Enzymatic DNA degradation in *E. coli*: its relationship to synthetic processes at the chromosomal level. Cold Spring Harbor Symp. Quant. Biol. **33**:259–269.
3. **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.
4. **Casadaban, M. J., and S. N. Cohen.** 1979. Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: in vivo probe for transcriptional control sequences. Proc. Natl. Acad. Sci. U.S.A. **76**:4530–4533.
5. **Clark, A. J.** 1973. Recombination deficient mutants of *Escherichia coli* and other bacteria. Annu. Rev. Genet. **7**:67–86.
6. **Clark, A. J., and A. D. Margulies.** 1965. Isolation and characterization of recombination-deficient mutants of *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **53**:451–459.
7. **Emmerson, P. T.** 1968. Recombination-deficient mutants of *Escherichia coli* K12 that map between *thyA* and *argA*. Genetics **60**:19–30.
8. **Emmerson, P. T., and P. Howard-Flanders.** 1967. Cotransduction with *thy* of a gene required for genetic recombination in *Escherichia coli*. J. Bacteriol. **93**:1729–1731.
9. **Goldmark, P. J., and S. Linn.** 1970. An endonuclease activity form *Escherichia coli* absent from certain *rec*$^-$ strains. Proc. Natl. Acad. Sci. U.S.A. **67**:434–441.
10. **Hickson, I. D., and P. T. Emmerson.** 1981. Identification of the *Escherichia coli recB* and *recC* gene products. Nature (London) **294**:578–580.
11. **Horii, Z.-I., and A. J. Clark.** 1973. Genetic analysis of the RecF pathway to genetic recombination in *Escherichia coli* K12: isolation and characterization of mutants. J. Mol. Biol. **80**:327–344.
12. **Khatoon, H., and A. I. Bukhari.** 1981. DNA rearrangments associated with reversion of bacteriophage Mu-induced mutations. Genetics **98**:1–24.
13. **Kleckner, N., K. Reichardt, and D. Botstein.** 1979. Inversions and deletions of the *Salmonella* chromosome generated by the translocatable tetracycline resistance element Tn*10*. J. Mol. Biol. **127**:89–115.
14. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. **145**:1110–1112.
15. **McCalla, D. R.** 1965. Nitrofuran derivatives as radiometric agents: cross-resistance studies with *Escherichia coli*. Can. J. Microbiol. **11**:185–191.
16. **Miller, J. H.** 1972. Experiments in bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Oliver, D. B., and E. B. Goldberg.** 1977. Protection of parental T4 DNA from a restriction exonuclease by the product of gene 2. J. Mol. Biol. **116**:877–881.
18. **Sasaki, M., T. Fujiyoshi, K. Shimada, and Y. Takagi.** 1982. Fine structure of the *recB* and the *recC* gene region of *Escherichia coli*. Biochem. Biophys. Res. Commun. **109**:414–421.
19. **Schultz, D. W., A. F. Taylor, and G. R. Smith.** 1983. *Escherichia coli* RecBC pseudorevertants lacking chi recombinational hotspot activity. J. Bacteriol. **155**:664–680.
20. **Sironi, G.** 1969. Mutants of *E. coli* unable to be lysogenized by the temperate bacteriophage P2. Virology **37**:163–176.
21. **Tomizawa, J. I., and H. Ogawa.** 1972. Structural gene of ATP-dependent deoxyribonuclease of *Escherichia coli*. Nature (London) New Biol. **239**:14–16.
22. **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.