Proc. Nat. Acad. Sci. USA
Vol. 69, No. 6, pp. 1366–1370, June 1972

Indirect Suppression of *recB* and *recC* Mutations by Exonuclease I Deficiency

(*Escherichia coli*/UV sensitivity/mitomycin C/recombination deficiency)

SIDNEY R. KUSHNER*, HARUKO NAGAISHI, AND ALVIN J. CLARK†

Department of Molecular Biology, University of California, Berkeley, Calif. 94720

Communicated by Allan Campbell, March 16, 1972

ABSTRACT ***recB*⁻ and *recC*⁻ strains of *Escherichia coli* K12 are recombination deficient and sensitive to ultraviolet light and the drug, mitomycin C. We have reported that *sbcB* mutations indirectly suppress all three phenotypes and result in the loss of exonuclease I. In this publication we report the occurrence of other mutations that lead to loss of exonuclease I and indirectly suppress mitomycin and UV sensitivity but not recombination deficiency. These mutations (called *xonA*) are cotransducible with *his* and closely linked with *sbcB*. Both *sbcB* and *xonA* mutant strains appear to have identical residual amounts of nuclease activity on single-stranded DNA. It is hypothesized that exonuclease I possesses a second activity other than its known ability to degrade single-stranded DNA from a 3′-OH terminus. Accordingly, *sbcB* mutations would alter the second activity while *xonA* mutations would not.**

The presence of *recB* and *recC* mutations‡ in *Escherichia coli* leads to a 100- to 200-fold decrease in genetic recombination as well as to an increased sensitivity to ultraviolet light (UV) and the drug mitomycin C (1, 2). Barbour *et al.* (3) showed that such mutations could be indirectly suppressed by *sbcA* mutations. Kushner *et al.* (4) found *recB* and *recC* mutations could also be indirectly suppressed by *sbcB* mutations, distinguishable from the unmapped *sbcA* mutations by their cotransducibility with *his*. In all *sbcB*⁻ mutants studied the return of wild-type levels of genetic recombination and UV and mitomycin resistance was accompanied by the loss of exonuclease I (4). It was suggested that *sbcB* was, in fact, the structural gene for exonuclease I. Genetic and enzymatic studies on strains carrying deletions in the *his* region tended to confirm this hypothesis (Templin, A., Kushner, S. R. & Clark, A. J., in preparation).

In this communication a new class of mutants is reported that indirectly suppresses the UV and mitomycin sensitivity resulting from the presence of *recB* and *recC* mutations; however, the inability to perform genetic recombination remains unaffected. Such mutations are called *xonA*⁻ because they also result in the loss of exonuclease I activity. The *xonA* locus cotransduces with *his* and is closely linked with *sbcB*. It is not clear at present whether *xonA*⁻ and *sbcB*⁻ represent mutations in independent, but closely linked, genes or mutations in the same gene. Regardless, it may be that exonuclease I has an activity other than its known (5) 3′→5′ action on single-stranded DNA.

MATERIALS AND METHODS

The experiments described were mostly performed with revertants and transductants of derivatives of AB1157, a multiply marked auxotrophic strain of *E. coli* K12. BW7 and BW46 were of a different genetic background and were generously provided by Dr. Bernard Weiss. Both were prototrophs and were sensitive to mitomycin C due to a *mtc*⁻ mutation similar to that described by Otsuji (6). In addition, BW46 is a derivative of BW7, deficient in exonuclease I, isolated by the method of Milcarek and Weiss (7).

Reagents were obtained from the following sources: carrier free (^{32}P) H_3PO_4, Schwarz Biochemical Corp.; yeast nucleic acid, Calbiochem Corp.; Brij-58, Atlas Chemical Industries; bovine serum albumin, Pentex, Inc.; and mitomycin C, Sigma Chemical Corp.

^{32}P-labeled DNA from *E. coli* B was prepared by the method of Lehman (10). Scintillation fluid was made according to a formula listed by Clark *et al.* (11).

The procedures and media for conjugational and transductional crosses and for the discrimination of Rec⁻ from Rec⁺ strains have been described (12–14). All revertant strains were checked for the *recB-recC* ATP-dependent nuclease by the assay of Barbour *et al.* (15). Mitomycin-resistant revertants of Rec⁻ mutants were selected by the method of Barbour *et al.* (3), except that the mitomycin concentration was 1 μg/ml.

Ultraviolet irradiation was done with a calibrated 15 W G.E. germicidal lamp (12). We determined resistance to mitomycin by spreading cells from an L broth culture in stationary phase directly onto L-agar plates containing the concentrations of the drug indicated and then incubating to allow survivors to form colonies.

For quantitative enzymatic determinations strains were grown in EM9 as described (4). Exonuclease I activity was determined by growth of the strains in 10 ml of L broth in an overnight standing culture. The cells were collected by cen-

* Present address: Department of Biochemistry, Stanford Medical School, Palo Alto, California 94305.
† To whom reprint requests should be sent.
‡ Nomenclature conforms in most ways to the recommendations of Demerec *et al.* (8) except that the minus sign is used with the gene symbol to indicate a general mutant allele when the specific allele number is not required. The gene symbols are those noted by Taylor (9) with the exception of *sbc*, which stands for a set of genes involved in the indirect suppression of *recB21* or *recC22*, and *xonA*, which stands for a gene involved in the production of exonuclease I. Phenotypic abbreviations are as follows: Rec, recombination; UV, ultraviolet irradiation; Mit, mitomycin; − (minus), requiring when used with abbreviations of amino acids, and deficient when used with Rec; + (plus), independence when used with abbreviations of amino acids and proficient when used with Rec; R, resistance; S, sensitivity.

TABLE 1. *Exonuclease I activity of xonA1 hemizygote and xonA⁺/xonA1 heterozygote*

Strain	Genotype	Exonuclease I (units/mg protein)
BW7	*his⁺ xonA⁺*	40.5
BW46	*his⁺ xonA1*	11.2
JC8211*	*Fhis-323 xonA⁺/his⁺ xonA1*	39.8
JC7623	*his⁻ sbcB15*	10.2

* Donor derivative of BW46, which contains F*his-323 xonA⁺* [i.e., F30 (17)].

trifugation and lysed immediately by the Brij-lysis procedure as outlined by Barbour *et al.* (3). Exonuclease I was assayed as described (4). One unit of exonuclease I is defined as that amount of protein that catalyzes the release of 1.0 nmol of nucleotide from heat-denatured DNA in 30 min at 37°. Protein concentrations were determined by the method of Lowry *et al.* (16).

RESULTS

Mutant strains lacking exonuclease I had heretofore been obtained as mitomycin-resistant revertants from *recB⁻ recC⁻* and *recB⁺ recC⁻* strains of *E. coli* (4). In addition to becoming Mit^R, these strains had also become resistant to UV (UV^R) and recombination proficient (Rec⁺). They retained their original *recB* and *recC* mutations and, in addition, acquired a suppressor mutation termed *sbcB⁻*. Since all of these *sbcB* mutants had lost exonuclease I, it was hypothesized that any exonuclease I deficiency mutation would suppress *recB* and *recC* mutations and lead to a Rec⁺, Mit^R, and UV^R phenotype in a *recB⁻ recC⁻* strain. A strain deficient in exonuclease I which was isolated by screening several thousand survivors of a heavily mutagenized population of wild-type *E. coli* cells for the loss of enzymatic activity, was graciously provided by Dr. Bernard Weiss. The mutation in this strain was termed *xonA1* until its identity with *sbcB* mutations in genetic location and phenotypic properties could be demonstrated. Because the *sbcB* mutations were cotransducible with *his⁺*, the Weiss mutant, BW46, was used as a donor of *his⁺* in P1 transduction of the *his⁻xonA⁺* strain AB1157. Forty His⁺ transductants were isolated and tested for exonuclease I activity. Twenty-three were ExoI⁻, indicating that *xonA1* was 58% cotransducible with *his⁺*. Furthermore, as shown in Table 1, *xonA1* is recessive and lies in the region spanned by the episome F30 (17), which carries *sbcB⁺*.

In order to test the ability of *xonA1* to suppress *recB* and *recC* mutations, we lysed BW46 (*xonA1*) and BW7 (*xonA⁺*) with P1 phage. 59% of 4962 His⁺ transductants of JC7623 (*his⁻recB⁻recC⁻sbcB⁻*) produced by P1 grown on the *xonA⁺* strain became Rec⁻, UV^s, and Mit^s and regained normal amounts of exonuclease I. Presumably they inherited *sbcB⁺* from the donor. The characteristics of two representative transductants, JC8270 and JC8257, are shown in Table 2. On the other hand, nearly 100% (1395/1396) of the His⁺ transductants obtained from a lysate grown on the *xonA1* mutant remained Mit^R and UV^R. The one Mit^s UV^s strain, JC8256, had regained exonuclease I activity (Table 2), and was presumably formed by recombination between *xonA1* and *sbcB15*. Forty of the Mit^R UV^R transductants were tested, and all were deficient in exonuclease I. Of the 1395 Mit^R UV^R transductants, 66.5% were Rec⁻, presumably through inheritance of *xonA1 sbcB⁺*. The remaining 33.5% were Mit^R UV^R Rec⁺, presumably by retention of *xonA⁺ sbcB15*, although a few of these may have inherited *xonA1 sbcB15*. The properties of two Rec⁻ Mit^R UV^R transductants, JC8218 and JC8219, and one Rec⁺ Mit^R UV^R transductant, JC8213, are indicated in Table 2 and Fig. 1. The two Rec⁻ transductants were quantitatively as UV^R and Mit^R as the Rec⁺ trans-

TABLE 2. *Properties of two recB⁻ recC⁻ xonA1 transductants and related strains*

	Strain	Phenotype	Recombination frequency (%)*	Exonuclease I (units/mg protein)	Presumptive genotype
a.	Parental strains for comparison				
	JC5519	Rec⁻Mit^{s}UV^s	0.05	39	*recB21 recC22 his⁻ sbcB⁺ xonA⁺*
	JC7623	Rec⁺Mit^{R}UV^R	24.0	10	*recB21 recC22 his⁻ sbcB15 xonA⁺*
b.	Representative transductants from cross P1.BW7 x JC7623				
	JC8270	Rec⁺Mit^{R}UV^R	27.0	10	*recB21 recC22 his⁺ sbcB15 xonA⁺*
	JC8257	Rec⁻Mit^{s}UV^s	0.04	38	*recB21 recC22 his⁺ sbcB⁺ xonA⁺*
c.	Representative transductants from cross P1.BW46 x JC7623				
	JC8213	Rec⁺Mit^{R}UV^R	30.0	10	*recB21 recC22 his⁺ sbcB⁻ xonA⁺* or *recB2 recC22 his⁺ sbcB⁻ xonA1*
	JC8218	Rec⁻Mit^{R}UV^R	0.13	10	*recB21 recC22 his⁺ sbcB⁺ xonA1*
	JC8219	Rec⁻Mit^{R}UV^R	0.14	9	*recB21 recC22 his⁺ sbcB⁺ xonA1*
	JC8256	Rec⁻Mit^{s}UV^s	0.05	53	*recB21 recC22 his⁺ sbcB⁺ xonA⁺*

* Each strain crossed with Hfr JC158; Thr⁺Leu[Sm^{R}Ser⁺] recombinants selected.

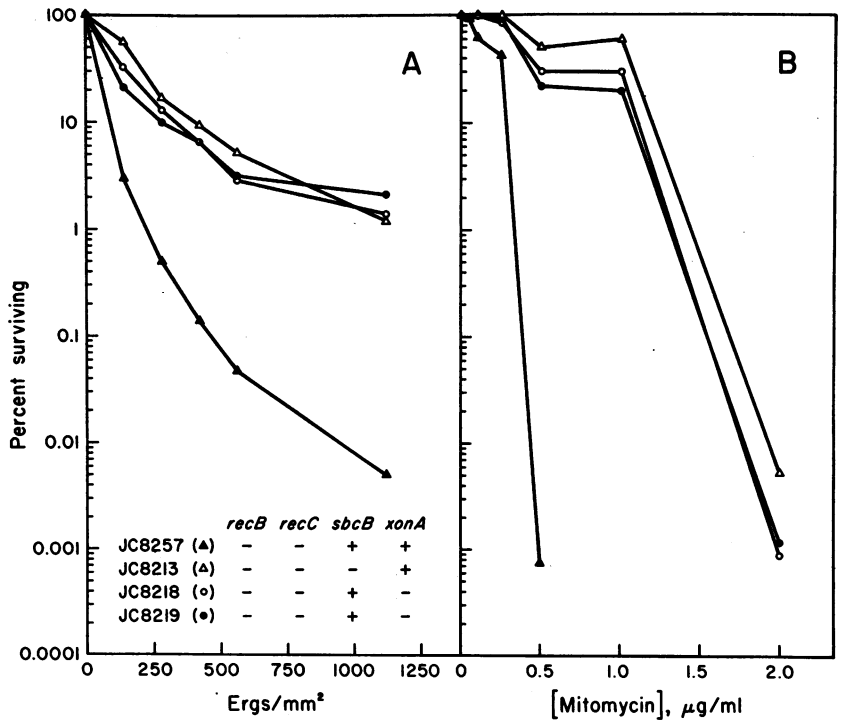

FIG. 1. Response of indirectly suppressed *recB⁻ recC⁻* strains to UV irradiation (*A*) and exposure to mitomycin C (*B*). A nonsuppressed *recB⁻ recC⁻* strain was used for comparison against one *sbcB* mutant and two *xonA* mutants, each of which is also *recB⁻ recC⁻*.

ductant (Fig. 1*A* and *B*), and none of the three had regained normal levels of exonuclease I (Table 2). Hence, in the presence of *xonA1* the mitomycin and UV sensitivity conferred by the *recB⁻* and *recC⁻* mutations was indirectly suppressed, apparently through the loss of exonuclease I activity; however, the loss of this activity (i.e., the ability to degrade single-stranded DNA in the 3′→5′ direction) did not suppress the Rec⁻ phenotype of *recB⁻* and *recC⁻* cells.

Attempts were made to isolate directly strains similar to JC8218 by treatment of JC7645, a *his⁺* derivative of a *recB21 recC22* double mutant, with nitrosoguanidine and plating the survivors on agar plates containing mitomycin. 5% of the colonies were still Mit^s, 15% exhibited the *xonA⁻* phenotype (i.e., were Mit^{R}UV^{R}Rec⁻), and 80% were of the *sbcB⁻* variety (i.e., were Mit^{R}UV^{R}Rec⁺). In Table 3 are listed the characteristics of several of the independently isolated Mit^R-UV^R*xonA⁻* strains. All these mutants had lost exonuclease I activity but remained phenotypically Rec⁻, although recombination frequencies 5- to 13-fold higher than that of the parent, JC7645, were noted. When two of these mutants, JC8262 and JC8265, were used as P1 transductional donors and JC7623 was used as recipient, the *xonA* mutations were found to be 71.9 and 77.3% cotransducible with *his⁺*, respectively. Derivatives of JC8262 and JC8265 inheriting F30 (F*his-323 xonA⁺ sbcB⁺*) became UV^s and Mit^s and regained exonuclease I. The properties of two such heterozygotes, JC8289 and JC8292, are listed in Table 4.

These findings indicated that there might be two genes in-

TABLE 3. *Characteristics of xonA⁻ mutants*

Strain	Genotype	Fraction surviving: UV 200 ergs/mm²	Fraction surviving: Mitomycin 1 μg/ml	% Recombination*	Recombination deficiency index†	Exonuclease I (units/mg protein)
JC7645	*recB21 recC22 sbcB⁺*	0.01	3.1×10^{-6}	0.04	750	41.7
JC8213	*recB21 recC22 sbcB15*	0.35	0.60	30.0	1	10.3
JC8260	*recB21 recC22 xonA2*	0.48	0.97	0.33	91	8.3
JC8262	*recB21 recC22 xonA4*	0.37	0.73	0.37	81	10.8
JC8264	*recB21 recC22 xonA6*	0.76	0.67	0.21	143	14.4
JC8265	*recB21 recC22 xonA7*	0.35	0.62	0.25	120	9.9
JC8266	*recB21 recC22 xonA8*	0.79	0.54	0.27	111	10.6
JC8267	*recB21 recC22 xonA9*	0.37	0.67	0.54	56	15.0

* Each strain crossed with Hfr JC158; Thr⁺Leu⁺[Sm^{R}Ser⁺] recombinants were selected.

† Percent recombination of JC8213 was divided by the percent recombination of each of the strains.

volved in the production of exonuclease I: *xonA* and *sbcB*. In addition, they indicated that the *sbcB* mutant characterized enzymatically in this laboratory (4) might, in fact, be a double mutant carrying both a *xonA* mutation to suppress the Mit^s and UV^s phenotypes and an *sbcB* mutation to suppress the Rec$^-$ phenotype of the *recB*$^-$ *recC*$^-$ mutations. To test this hypothesis, a P1 transductional cross was performed in which the *sbcB15* mutant, JC7623, was the recipient and the *his*$^+$-*xonA*$^+$*sbcB*$^+$ strain, BW7, was the donor. His$^+$ transductants were screened for the *xonA*$^-$ *sbcB*$^+$ phenotype (i.e., Mit^{R}UV^{R}Rec$^-$) and the hypothetical true *xonA*$^+$ *sbcB*$^-$ phenotype (i.e., Mit^{s}UV^{s}Rec$^+$). If JC7623 were in fact a double mutant, these two types of transductants should occur at a low frequency, which would be dependent on the linkage of the two alleles and their location relative to *his*. On the other hand, if JC7623 carried only a single mutation affecting exonuclease I, neither of these types of transductants would be expected. Out of 4962 His$^+$ transductants, none was obtained that was Mit^{R}UV^{R}Rec$^-$ or Mit^{s}UV^{s}Rec$^+$. With the *sbcB9* mutant JC7617, as a recipient, 2970 His$^+$ transductants were screened and no Mit^{R}UV^{R}Rec$^-$ or Mit^{s}UV^{s}Rec$^+$ transductants were found. These results indicate that in both cases the *sbcB* mutants carried no undetected *xonA* mutation.

DISCUSSION

We previously have shown that all *sbcB*$^-$ mutations result in loss of exonuclease I activity as measured by the degradation denatured DNA, presumably preferentially from a 3′OH end. We therefore concluded that the absence of this activity was directly responsible for the suppression of all the phenotypes associated with *recB* and *recC* mutations (4). In other words, we hypothesized that the presence of exonuclease I activity prevented both recombination and repair of UV and mitomycin damage in the absence of the *recB-recC* enzyme. The findings presented here indicate that *xonA* mutations, which also lead to a disappearance of exonuclease I activity, can only effectively suppress the UV^s and Mit^s phenotypes. There are several alternatives that explain the difference between the effects of *xonA* and *sbcB* mutations.

The first explanation assumes that *sbcB* and *xonA* are synonyms for the same gene. Accordingly, *sbcB* mutations might destroy all exonuclease I activity while *xonA* mutations might leave a small amount of residual activity that is sufficient to prevent recombination, but low enough to permit repair of UV- and mitomycin-induced damage. The slight recovery of recombination proficiency by *xonA* mutants shown in Table 3 and the large proportion of Mit^R revertants from JC7645, which are the *sbcB*$^-$ variety, would fit this hypothesis. A direct enzymatic test is difficult because of the high residual levels of exonuclease activity on denatured DNA found in crude extracts of both *sbcB* and *xonA* mutants. As shown in Tables 2 and 3, residual activity is 20–30% of wild-type levels. Preliminary studies on the purified residual activity from the *sbc15* strain, JC7689, with anti-exonuclease I serum indicate that this strain contains less than 1.5% of wild-type exonuclease I. A similar experiment must be done with a *xonA* mutant to determine if a higher residual amount of exonuclease I is present in such a strain.

A second, although, we feel, unlikely alternative holds that *sbcB* mutations are recessive control mutations preventing the synthesis of two products. One of these is exonuclease I and the other is an unknown product that is responsible for inhibition of recombination in a *recB*$^-$ *recC*$^-$ strain. Accordingly, only *xonA* mutations would affect the structure of exonuclease I.

TABLE 4. *Characteristics of F30 derivatives and transductional progeny of two xonA mutants*

Strain	Genotype	% Surviving 200 ergs/mm^2	% Recombination*	Exonuclease I (units/mg protein)
JC8262	*recB21 recC22 xonA4*	37	0.37	10.8
JC8292†	*recB21 recC22 xonA4*/F*xonA*$^+$	3.9	—	52.1
JC8287‡	*recB21 recC22 xonA4*	40	0.10	7.7
JC8265	*recB21 recC22 xonA7*	35	0.25	9.9
JC8289†	*recB21 recC22 xonA7*/ F*xonA*$^+$	3.8	—	48.0
JC8284§	*recB21 recC22 xonA7*	41	0.12	8.8

* Each strain was crossed with Hfr JC158; Thr$^+$Leu$^+$[Sm^R-Ser$^+$] recombinants were selected.

† Contains F*his-323 xonA*$^+$.

‡ Transductant of JC7623 made with P1 lysate from JC8262.

§ Transductant of JC7623 made with P1 lysate from JC8265.

A third explanation is that *xonA* and *sbcB* are two genes, both of which are essential for the 3′→5′ exonucleolytic activity of exonuclease I on single-stranded DNA, but one of which, *sbcB*, is required for an as yet unknown activity. According to this hypothesis it is the 3′→5′ exonucleolytic activity on single-stranded DNA that prevents repair and the unknown activity that prevents recombination in the *recB*$^-$ *recC*$^-$ strain. If the *sbcB* subunit is mutant, both activities would be lost, and the *recB*$^-$ *recC*$^-$ strain would become UV^R Rec$^+$. A mutant *xonA* subunit, on the other hand, would eliminate only the 3′→5′ exonucleolytic degradation, leaving the second activity unaltered; hence the *recB*$^-$ *recC*$^-$ strain would become UV^R Rec$^-$. A known enzyme that has properties somewhat analogous to these proposed for exonuclease I is tryptophan synthetase. The *trpA* subunit catalyzes the conversion of indole glycerol phosphate to indole, while the *trpA* and *trpB* subunits together catalyze the conversion of indole glycerol phosphate to tryptophan. It might also be possible that *sbcB* and *xonA* represent the same gene and that their polypeptide product possesses two types of activities. Complementation studies are now in progress to determine if *xonA* and *sbcB* are separate cistrons.

The appearance of phenotypically Rec$^-$ UV^R Mit^R revertants from a Rec$^-$ UV^{s}Mit^s parent suggests an enzymatic difference in the pathways for recombination and for repair of UV- and mitomycin-induced damage in the absence of the *recB-recC* enzyme combination. It appears that, in a *recB*$^-$ *recC*$^-$ strain, the presence of 3′ -OH terminated single-stranded regions may be required for survival after UV irradiation and mitomycin treatment but not for recombination.

This investigation was supported by Public Health Service Research Grant AI 05371 from the National Institute of Allergy and Infectious Diseases, a Postdoctoral Fellowship Grant GM 47038-01 from the National Institute of General Medical Sci-

ences, Grant No. 576 from the California Division of the American Cancer Society, and Biomedical Sciences Support Grant FR-7006 from the General Research Support Branch, Division of Research Resources, Bureau of Health Professions Education and Manpower Training, National Institutes of Health.

1. Willetts, N. S. & Mount, D. W. (1969) *J. Bacteriol.* **100**, 923–934.
2. Low, B. (1968) *Proc. Nat. Acad. Sci. USA* **60**, 160–167.
3. Barbour, S. D., Nagaishi, H., Templin, A. & Clark, A. J. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 128–135.
4. Kushner, S. R., Nagaishi, H., Templin, A. & Clark, A. J. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 824–827.
5. Lehman, I. R. & Nussbaum, A. L. (1964) *J. Biol. Chem.* **239**, 2628–2636.
6. Otsuji, N. (1968) *J. Bacteriol.* **95**, 540–545.
7. Milcarek, C. & Weiss, B. (1971) *Fed. Proc.* **30**, 1156 Abstr.
8. Demerec, M., Adelberg, E. A., Clark, A. J. & Hartman, P. E. (1966) *Genetics* **54**, 61–76.
9. Taylor, A. L. (1970) *Bacteriol. Rev.* **34**, 155–175.
10. Lehman, I. R. (1960) *J. Biol. Chem.* **235**, 1479–1487.
11. Clark, A. J., Chamberlain, M., Boyce, R. P. & Howard-Flanders, P. (1966) *J. Mol. Biol.* **19**, 442–454.
12. Clark, A. J. (1967) *J. Cell. Physiol.* **70**, Suppl. 1, 165–180.
13. Clark, A. J. & Margulies, A. D. (1965) *Proc. Nat. Acad. Sci. USA* **53**, 451–459.
14. Willets, N. S., Clark, A. J. & Low, B. (1969) *J. Bacteriol.* **97**, 244–249.
15. Barbour, S. D. & Clark, A. J. (1970) *Proc. Nat. Acad. Sci. USA* **65**, 955–961.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
17. Bastarrachea, F. & Clark, A. J. (1968) *Genetics* **60**, 641–660.