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In Vivo Studies of Temperature-Sensitive *recB* and *recC* Mutants

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Some in vivo properties of *Escherichia coli* K-12 strains carrying *recB270* (formerly *recBts1*) and *recC271* (formerly *recCts1*) mutations have been determined. Single *recB270* and *recC271* mutants appear normal at 30 C with regard to ultraviolet and mitomycin C sensitivity, recombination proficiency, and viability. At 43 C these strains become sensitive to ultraviolet and mitomycin C, while showing only a slight decrease in recombination proficiency. The viable titers of the single mutants are somewhat reduced at 43 C. Double mutant strains carrying *polA1* and *recB270* or *recC271* are inviable at 43 C. The double mutant strain (*recB270 recC271*) is sensitive to both UV and mitomycin C at 30 C, but shows only slightly reduced recombination proficiency. At 43 C the strain resembles absolute *recB* and *recC* mutants in all respects. In addition, the double mutant strain exhibits a temperature-induced drop in viable titer. The triple mutant *polA1 recB270 recC271* is viable at 30 C. Two hypotheses are advanced to explain these results.

Mutations in the *recB* and/or *recC* loci of *Escherichia coli* result in reduced proficiency of recombination. In addition, *recB* and *recC* mutants show an increased sensitivity to ultraviolet (UV) light, to the cross-linking agent mitomycin C, and to X-irradiation (6, 17). Growing cultures of cells with *recB* and *recC* mutations also contain a large fraction of inviable cells (3, 9). Monk and Kinross (11) have further shown that a *recB21* mutation in conjunction with a temperature-sensitive mutation in DNA polymerase I (*polA*) renders the cell inviable at high temperature.

Several groups have identified the product of the *recB* and *recC* genes as an ATP-dependent exonuclease active on both single- and double-stranded deoxyribonucleic acid (1, 2, 7, 8, 12). The purified enzyme also degrades single-stranded closed circular DNA endonucleolytically. This latter activity is stimulated by, but is not absolutely dependent upon, adenosine 5'-triphosphate. Tomizawa and Ogawa (15) demonstrated that *recB* and *recC* are the structural genes for this enzyme by isolating temperature-sensitive mutants and showing that the *recB-recC* deoxyribonuclease was abnormally thermolabile in crude extracts.

By making use of the temperature-sensitive *recB* and *recC* mutants isolated by Tomizawa and Ogawa (15), we have attempted to determine whether the pleiotrophic effects observed in vivo have their counterpart in the multiple enzymatic activities associated with the *recB-recC* products in vitro. The consequences of a restrictive temperature in vivo on viability, recombination proficiency, and sensitivity to mitomycin C and to UV irradiation was correlated with alterations in the catalytic properties of the partially purified enzymes.

In this paper are described the properties of the *recB270* (formerly *recBts1*) and *recC271* (formerly *recCts1*) temperature-sensitive mutants in vivo. The following paper presents an analysis of the partially purified enzymes isolated from *recB270*, *rec271* and *recB270 recC271* mutants (10).

MATERIALS AND METHODS

Bacterial strains and media. The genotypes of the *E. coli* K-12 strains used in this work are listed in Table 1. Nomenclature conforms to that of Demerec et al. (5) 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 given by Taylor and Trotter (13).

The complex medium used was Luria (L) broth, which contained 10 g of tryptone, 5 g of yeast extract (Difco), and 10 g of NaCl per liter, adjusted to pH 7. L broth minus NaCl (LMN) was identical to the above except that the NaCl was omitted. For solid media, 2% agar was added. The minimal medium used (M56/2) is described by Willetts et al. (16). All media were prewarmed to either 30 or 43 C.

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TABLE 1. *Bacterial strains used in the work*

Strain no.	Sex	*recB*	*recC*	*str*	*arg*	*his*	*thr*	*leu*	*pro*	*met*	*thi*	*thy*	*ser*	*ilv*	Other markers	Source
AB1157	F⁻	+	+	31	E3	4	1	6	A2	+	1	+	+	+		A. J. Clark
JC5422	F⁻	+	+	31	E3	4	1	6	A2	+	1	A325	+	+		A. J. Clark
JC5743	F⁻	21	+	31	E3	4	1	6	A2	+	1	+	+	+		A. J. Clark
JC5489	F⁻	+	22	31	E3	4	1	6	A2	+	1	+	+	+		A. J. Clark
JC5519	F⁻	21	22	31	E3	4	1	6	A2	+	1	+	+	+		A. J. Clark
JC1557	F⁻	+	+	309	66	1	+	7	+	B1	+	+	+	+		A. J. Clark
N108	F⁻	+	271	309	66	1	+	7	+	B1	+	+	+	+		J. Tomizawa
N138	F⁻	270	+	309	66	1	+	7	+	B1	+	+	+	+		J. Tomizawa
N221	F⁻	270	271	309	66	1	+	7	+	B1	+	+	+	+		J. Tomizawa
SK116	F⁻	+	271	31	E3	4	1	6	A2	+	1	+	+	+		This paper
SK119	F⁻	270	+	31	E3	4	1	6	A2	+	1	+	+	+		This paper
SK129	F⁻	270	271	31	E3	4	1	6	A2	+	1	+	+	+		This paper
JG112	F⁻	+	+	R	+	+	+	+	+	−	+	−	+	+	*polA1*	B. Konrad
SK202	F⁻	+	271	R	+	+	+	+	+	+	+	+	+	+	*polA1*	This paper
SK204	F⁻	+	+	R	+	+	+	+	+	+	+	+	+	+	*polA1*	This paper
SK206	F⁻	270	+	R	+	+	+	+	+	+	+	+	+	+	*polA1*	This paper
SK211	F⁻	270	271	R	+	+	+	+	+	+	+	+	+	+	*polA1*	This paper
JC158	Hfr	+	+	S	+	+	+	+	+	+	+	+	A⁻	+	HfrH (87')[a]	A. J. Clark
JC5029	Hfr	+	+	S	+	+	300	+	+	+	+	+	+	318	KL16 (56')	A. J. Clark

[a] Point of origin.

Procedures for conjugation and transduction. The procedures for conjugational and transductional crosses were those described by Willetts et al. (16). For conjugational crosses the recipient strains were grown at 30 or 43 C, mated for 1 h at 30 or 43 C, and then plated on prewarmed selective media. Thy⁺ transductants were selected for their ability to grow on minimal plates in the absence of added thymine. The *recB270* or *recC271* mutations were identified by their UV sensitivity using the replica-plating techniques described by Clark and Margulies (4). Plates received approximately 1,000 ergs/mm² from a GE germicidal lamp.

UV survival analysis. Overnight standing cultures grown in L broth at the indicated temperatures were diluted 20-fold in fresh broth and grown with aeration to a cell density of 10^8 per ml. The cells were concentrated by centrifugation at room temperature and suspended in twice the volume of M56/2 buffer. The suspension was placed in a large petri dish at a thickness of 2 mm and exposed to a GE germicidal lamp with continuous agitation. Doses were determined with a calibrated Laterjet meter. At the indicated times, samples were removed, diluted in M56/2 buffer, and spread directly on either L or LMN agar plates and incubated at 30 or 43 C.

Mitomycin C resistance. Overnight standing cultures grown in L broth at 30 or 43 C were diluted in M56/2 buffer and spread on L or LMN agar plates containing the indicated concentrations of mitomycin C (Sigma Chemical Co.).

Measurements of viable titer. Overnight standing cultures grown at 30 or 43 C were diluted 10-fold into fresh L broth prewarmed to the designated temperature. At the times indicated, Klett readings were taken using a Klett-Summerson Colorimeter with a no. 42 green filter. Samples were also removed and plated on L agar plates to determine the viable cell count. For temperature shift experiments, cultures were grown into early log phase at 30 C, and then switched to 43 C. The first reading at 43 C was taken 15 min after temperature shift.

RESULTS

Transfer of temperature-sensitive mutations. The *recB270* and *recC271* mutations were obtained by treatment of wild-type bacteria with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, a mutagen which yields multiple effects (15). The *recB270* and *recC271* alleles were therefore transferred into the genetically defined multiple auxotrophic strain JC5422 (a *thy*⁻ derivative of AB1157) by P1 transduction. *recC* mutations cotransduce with *thyA* at frequencies between 74 to 86%, and *recB* mutations between 34 to 50% (17). Thy⁺ transductants made using donor P1 grown on N108 (*recC271*), N138 (*recB270*), and N221 (*recB270 recC271*) were screened for their sensitivity to UV irradiation. With N138 as a donor 42% (76/181) of the Thy⁺ transductants were abnormally UV sensitive at 42 C indicating the presence of the *recB270* allele. In the case of N108, 81% (95/118) of the transductants were temperature sensitive in their response to UV irradiation. The double mutation, *recB270 recC271*, was scored by measuring UV sensitivity at 30. Inheritance occurred at a frequency of 38% (60/157) with N221 as donor.

UV sensitivity of recB270 and recC271. The sensitivity of SK116 (*recC271*), SK119 (*recB270*), and SK129 (*recB270 recC271*) to UV

irradiation is summarized in Table 2. The UV sensitivity of the two single mutants was not as great as that of strains carrying absolute *recB21* and *recC22* alleles. The addition of 1% sodium chloride to the plating medium resulted in partial suppression of the UV sensitivity at 43 C. Although the double mutant was UV sensitive at 30 C, it was even more sensitive when shifted to 43 C. Cells grown and irradiated at 43 C but plated at 30 C showed the same survival as when they were grown at 30 C.

Resistance to mitomycin C. In the absence of NaCl the mutant strains were sensitive to mitomycin C at 43 C but not at 30 C (Table 3). The double mutant was sensitive at 30 C as well. In the presence of NaCl, however, the single mutants were almost as resistant to the drug at 43 C as the wild-type strain. While there appeared to be some effect of NaCl on the absolute *recB21* and *recC22* mutations, the degree of suppression observed with the temperature-sensitive mutants was several orders of magnitude greater. The double mutant (SK129) was resistant to mitomycin C at 30 C in the presence of NaCl but at 43 C was as sensitive to the agent as the absolute mutants.

Recombination proficiency of strains carrying recB270 and recC271. The ability of *recB270* and *recC271* strains to carry out genetic recombination was tested in conjugational crosses with two Hfr strains. The first, JC5029, has its origin at 56 min and transfers the *thyA-argA* region early. Since it transfers the wild-type alleles of *recB* and *recC*, proximal to the selected *his*$^+$ marker, all the strains tested appeared recombination proficient (Table 4). On the other hand, crosses with JC158 (point of origin = 87 min) clearly distinguished the recombination deficient strains. In contrast to their UV and mitomycin sensitivity, strains carrying *recB270* and *recC271* showed only a slightly reduced recombination proficiency at 43 C (Table 4). The double mutant exhibited a slightly increased deficiency index at 30 C; however, at 43 C it was as recombination deficient as a *recB21 recC22* control strain.

Cell growth in the presence and absence of sodium chloride. In the absence of NaCl the *recB270* and *recC271* mutants showed a considerable lag before the onset of exponential growth. However, once this lag was overcome, their growth rates were comparable to that of the wild-type parent. In the presence of NaCl the three strains appeared identical (Fig. 1).

Conditional lethality of polA1recB270 and polA1 recC271 mutant strains. Monk and Kinross (11) showed that a strain carrying *recB21* and a temperature-sensitive mutation in deoxyribonucleic acid polymerase I, *polA12*, was conditionally lethal. The multiple mutants, SK202 (*polA1 recB270*) and SK206 (*polA1 recC271*), were constructed by P1 transduction of strain J6112 (*thyA*$^-$ *polA1*). Both SK202 and SK206 are conditionally lethal (Table 5). The triple mutant, SK211 (*polA1 recB270 recC271*), though viable at 30 C, grows more slowly than the double mutants.

Approximately 70% of the colonies that appeared at 43 C were temperature-resistant revertants. One out of 92 of these revertants appeared to arise from a back mutation in the

TABLE 2. *UV sensitivity of recB270 and recC271 strains*

Strain	Relevant genotype	Fraction surviving after 750 ergs/mm^2			
		30 C		43 C	
		+NaCl	-NaCl	+NaCl	-NaCl
AB1157	*recB*$^+$ *recC*$^+$	0.68	0.62	0.68	0.62
JC5743	*recB21 recC*$^+$	0.0007	0.0009	0.0007	0.0009
SK119	*recB270 recC*$^+$	0.60	0.70	0.04	0.007
JC5489	*recB*$^+$ *recC22*	0.002	0.0009	0.002	0.0009
SK116	*recB*$^+$ *recC271*	0.74	0.70	0.05	0.007
JC5519	*recB21 recC22*	0.0005	0.0008	0.0005	0.0008
SK129	*recB270 recC271*	0.03	0.02	0.003	0.001

TABLE 3. *Mitomycin C sensitivity of recB270 and recC271 strains*

Strain	Genotype	Fraction surviving mitomycin C (0.5 mg/ml)			
		30 C		43 C	
		+NaCl	-NaCl	+NaCl	-NaCl
AB1157	*recB*$^+$ *recC*$^+$		0.84	0.54	0.58
JC5743	*recB21 recC*$^+$		0.002	0.00001	<0.00001
SK119	*recB270 recC*$^+$	0.78	0.68	0.22	<0.00001
JC5489	*recB*$^+$ *recC22*		0.0002	0.0001	<0.00001
SK116	*recB*$^+$ *recC271*	0.78	0.72	0.16	<0.00001
JC5519	*recB21 recC22*	0.0002	0.002	0.00008	<0.00001
SK129	*recB270 recC271*	0.32	0.09	0.00003	<0.00001

TABLE 4. *Recombination proficiency of recB270 and recC271 strains*

Strain	Genotype	Deficiency Indexes[a]		
		JC158 (30 C) TL⁺ [Ser⁺ Sm^R]	JC158 (43 C) TL⁻ [Ser⁻ Sm^R]	JC5029 (43 C) His⁺ [Ilv⁺ Sm^R]
AB1157	*recB⁺ recC⁺*	1.0	1.1	1.0
JC5743	*recB21 recC⁺*	2.4×10^2	4.2×10^2	5.4
SK119	*recB270 recC⁺*	1.8	4.6	3.6
JC5489	*recB⁺* recC22	7.3×10^1	1.0×10^2	4.5
SK116	*recB⁺ recC271*	2.5	7.4	3.6
JC5519	*recB21 recC22*	1.6×10^2	1.8×10^2	6.4
SK129	*recB270 recC271*	6.3	5.3×10^2	5.6

[a] Deficiency indices are calculated by dividing the frequency at which progeny are produced in a cross of an Hfr with AB1157 at 30 C by the frequency at which progeny are produced in a cross with the same donor with either a Rec⁺ or Rec⁻ recipient at 30 and 43 C. The recombination frequency of JC158 × AB1157 was 18.4%. The recombination frequency of JC5029 × AB1157 was 1.1%. One-hour matings were used in all experiments.

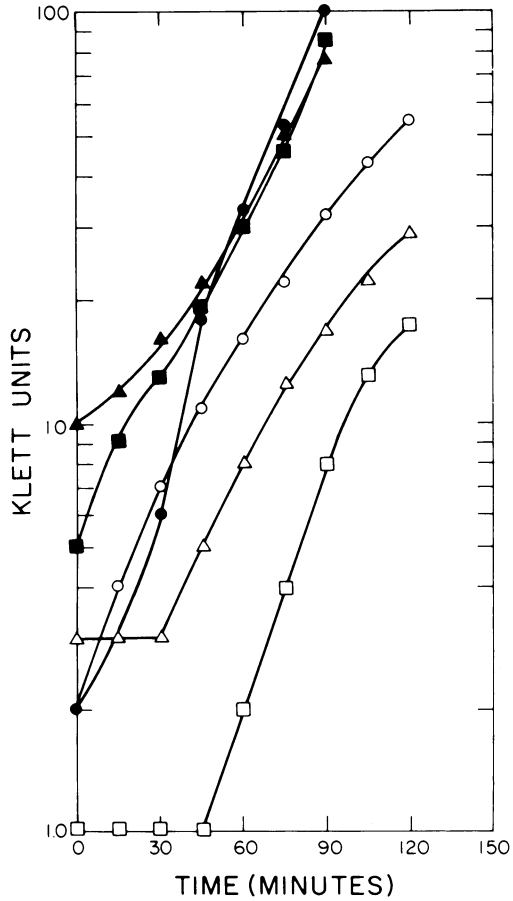

FIG. 1. *Growth curves of recB270 and recC271 strains in the presence and absence of NaCl. Overnight standing cultures grown at 43 C in Luria broth ± NaCl were diluted 1:10 into fresh Luria broth ± NaCl and grown with shaking at 43 C. At indicated time intervals Klett readings were taken using a Klett-Summerson Colorimeter with a green filter.* (●) *AB1157 (wild type) in L broth;* (○) *AB1157 in LMN broth;* (■) *SK119 (recB270) in L broth;* (□) *SK119 in LMN broth;* (▲) *SK116 (recC27) in L broth;* (△) *SK116 in LMN broth.*

polA gene as judged by its resistance to methylmethane sulfonate. The others were either the result of indirect suppression of the *recB270* and/or *recC271* alleles (14) or reversion of the mutations to the wild type.

Cell viability as a function of temperature. Changes in optical density and cell viability in growing cultures of *recB270*, *recC271*, and *recB270recC271* strains are shown in Table 6. Strains carrying absolute mutations in *recB* and *recC* show a three- to fourfold drop in the number of colony-forming cells per Klett unit as compared to their wild-type parent. *recB270* and *recC271* mutants grown at 30 C gave values comparable to wild-type controls but the viable titers were significantly reduced at 43 C. In a temperature shift experiment, there was some decrease in the colony-forming ability but the effect was not as great as when the cells were only grown at 43 C.

On the other hand, the viable titer of the double mutant was the same as the wild type at 30 even though this strain was UV and mitomycin C sensitive at this temperature. When grown at 43 C, SK129 showed a large drop in viability (Table 6). In a temperature shift experiment there was a rapid drop (15 min after the shift) in the colony-forming ability per Klett unit. The values obtained were again not as low as when the cells were exposed only to 43 C.

DISCUSSION

A summary of the phenotypic properties of *recB270*, *recC271*, and *recB270 recC271* strains is presented in Table 7. The two single mutants

show a clearcut temperature-sensitive response to UV irradiation and to mitomycin C but are only partially impaired in recombination. The enzymatic defect is sufficient, however, to produce conditional lethality in conjunction with a *polA1* mutation. On the other hand, the double mutant at 30 C resembles the single mutants at 43 C with regard to UV and mitomycin sensitivity, but shows normal colony-forming ability and is viable in the presence of *polA1*. At 43 C, the double mutant is in every respect as deficient as the absolute *recB* and *recC* mutants.

Assuming that the multiple activities of the *recB-recC* nuclease measured in vitro are functional in vivo, one explanation for the observed results is that in a single *recB270* or *recC271* mutant one or more activities are thermosensitive but the remaining ones are not. In the double *recB270 recC271* mutant one or more additional activities would be thermosensitive. This hypothesis would suggest that the repair and recombination functions of the enzyme are essentially independent of each other.

A second possibility simply involves residual levels of activity in the mutants. Partial inactivation at 43 of one or more of the activities associated with the *recB-recC* nuclease could result in increased sensitivity to UV and mitomycin C but would be sufficient to allow almost normal levels of genetic recombination and cell viability.

One way of distinguishing between these two possibilities is to examine the multiple enzymatic functions in the partially purified enzymes specified by the *recB270*, *recC271*, and *recB270 recC271* mutants. The results of such

TABLE 5. *Conditional lethality of polA recB and recC multiple mutant strains*

Strain	Genotype	Fraction surviving [a]	
		30 C	43 C
SK202	*recB⁺ recC271 polA1*	1	2.0×10^{-3}
SK204	*recB⁺ recC⁺ polA1*	1	0.91
SK206	*recB270 recC⁺ polA1*	1	3.2×10^{-4}
SK211	*recB270 recC271 polA1*	1	7.2×10^{-5}

[a] Overnight standing cultures grown in L broth at 30 C were plated on L agar and grown at 30 or 43 C. The number of colony-forming cells at 30 C for each strain was taken as 1.0.

TABLE 6. *Viability of recB270 and recC271 strains*

Strain	Genotype	Viable cells/Klett unit (10^{-6})[a]		
		30 C	43 C	30-43 C
AB1157	*recB⁺ recC⁺*	2.40	2.31	
SK119	*recB270 recC⁺*	2.27 (2.74)[b]	1.24	(2.01)
JC5743	*recB21 recC⁺*	0.79	0.62	
SK116	*recB⁺ recC271*	2.60 (2.40)	1.38	(2.06)
JC5489	*recB⁺ recC22*	0.69	0.72	
SK129	*recB270 recC271*	2.22 (2.15)	0.87	(1.52)
JC5519	*recB21 recC22*	0.66	0.58	

[a] Viable cells per Klett unit is an average of at least eight values.

[b] Numbers in parentheses represent values obtained from the temperature shift experiment. Cells were grown for 2 h at 30 C after which they were shifted to 43 C. Viable cells per Klett unit values were determined every 15 min.

TABLE 7. *Summary of phenotypic properties associated with recB270 and recC271 mutations*

Property	Allele	Temp	
		30 C	43 C
Ultraviolet sensitivity	*recB270*	Resistant	Sensitive
	recC271	Resistant	Sensitive
	recB270 recC271	Sensitive	Very sensitive
Mitomycin C sensitivity	*recB270*	Resistant	Sensitive
	recC271	Resistant	Sensitive
	recB270 recC271	Sensitive	Very sensitive
Recombination	*recB270*	Normal	Slightly reduced
	recC271	Normal	Slightly reduced
	recB270 recC271	Slightly reduced	Deficient
Effects of a *polA* mutation	*recB270*	Viable	Lethal
	recC271	Viable	Lethal
	recB270 recC271	Viable	Lethal
Viability	*recB270*	Normal	Reduced
	recC271	Normal	Reduced
	recB270 recC271	Normal	Sharply reduced

an analysis is presented in the following paper (10).

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LITERATURE CITED

1. Barbour, S. D., and A. J. Clark. 1970. Biochemical and genetic studies of recombination proficiency in *Escherichia coli*. I. Enzymatic activity associated with *recB*$^+$ and *recC*$^+$ genes. Proc. Nat. Acad. Sci. U.S.A. **65**:955-961.
2. Buttin, G., and M. Wright. 1968. Enzymatic DNA degradation in *E. coli*: its relationship to synthetic processes at the chromosome 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. Clark, A. J., and A. D. Margulies. 1965. Isolation and characterization of recombination-deficiency mutants of *Escherichia coli*. Proc. Nat. Acad. Sci. U. S. A. **53**:451-459.
5. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61-74.
6. Emmerson, P. T. 1968. Recombination deficient mutants of *Escherichia coli* K-12 that map between *thyA* and *argA*. Genetics **60**:19-30.
7. Goldmark, P. J., and S. Linn. 1970. An endonuclease activity from *Escherichia coli* absent from certain *rec*$^-$ strains. Proc. Nat. Acad. Sci. U. S. A. **67**:434-441.
8. Goldmark, P. J., and S. Linn. 1972. Purification and properties of the *recBC* DNase of *Escherichia coli* K-12. J. Biol. Chem. **247**:1849-1860.
9. Haefner, K. 1968. Spontaneous lethal sectoring, a further feature of *Escherichia coli* strains deficient in the function of *rec* and *uvr* genes. J. Bacteriol. **96**:652-659.
10. Kushner, S. R. 1974. Differential thermolability of exonuclease and endonuclease activities of the *recBC* nuclease isolated from thermosensitive *recB* and *recC* mutants. J. Bacteriol. **120**:1219-1222.
11. Monk, M., and J. Kinross. 1972. Conditional lethality of *recA* and *recB* derivatives of a strain of *Escherichia coli* K-12 with a temperature-sensitive deoxyribonucleic acid polymerase I. J. Bacteriol. **109**:971-978.
12. Oishi, M. 1969. An ATP-dependent deoxyribonuclease from *Escherichia coli* with a possible role in genetic recombination. Proc. Nat. Acad. Sci. U. S. A. **64**:1292-1299.
13. Taylor, A. L., and C. D. Trotter. 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. **36**:504-524.
14. Templin, A., S. R. Kushner, and A. J. Clark. 1972. Genetic analysis of mutations indirectly suppressing *recB* and *recC* mutations. Genetics **72**:205-215.
15. Tomizawa, J., and H. Ogawa. 1972. Structural genes of ATP-dependent deoxyribonuclease of *Escherichia coli*. Nature N. Biol. **239**:14-16.
16. 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.
17. Willetts, N. S., and D. W. Mount. 1969. Genetic analysis of recombination-deficient mutants of *Escherichia coli* K-12 carrying *rec* mutations co-transducible with *thyA*. J. Bacteriol. **100**:923-934.