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# Biochemical and Genetic Studies of Recombination Proficiency in *Escherichia coli*, II. Rec+ Revertants Caused by Indirect Suppression of Rec– Mutations

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**Abstract.** All *recB*$^-$ and *recC*$^-$ mutants of *E. coli* carry out significant residual genetic recombination, whereas all *recA*$^-$ mutants form no recombinants. This observation suggests that an alternative minor pathway of recombination, independent of *recB*$^+$ and *recC*$^+$ products, may be operative in *Escherichia coli*. Rec$^+$ revertants of *recB*$^-$ *recC*$^+$, *recB*$^+$ *recC*$^-$, and *recB*$^-$ *recC*$^-$ strains of *E. coli* have been isolated and are shown to fall into at least two major genotypic classes. One class carries revertant mutations which map in or very near the *recB* and *recC* genes. In this class an ATP-dependent DNase characteristic of wild type *E. coli* is restored. The reversions in this class are probably back-mutations or intragenic suppressor mutations. A second class carries revertant mutations which are located far from the *recB* and *recC* genes. In this class there is a high level of DNase activity which does *not* require ATP and is inactive on T4 DNA. Indirect and not informational suppression appears to be responsible for the second class of revertants. The suggestion is made that restoration of recombination by indirect suppression involves an activation or derepression of one or a series of enzymes, which participate in a pathway of recombination, alternative to the *recB* and *recC* pathway, but normally of minor importance. The ATP-independent DNase may be one of these enzymes.

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Mutations affecting recombination proficiency in *E. coli* K12 have been found to occur in at least three genes: *recA*, *recB*, and *recC*.[1,2] An ATP-dependent DNase activity has been found in lysates of wild-type strains but is missing in lysates of *recB* or *recC* mutant strains.[3–5] Tests of a series of merodiploid strains heterozygous or homozygous for *recB21* and *recC22* indicate that these mutations are responsible for the loss of the ATP-dependent nuclease activity.[5] Revertant studies are also desirable to establish this point. Oishi[4] has examined one ultraviolet resistant (UV$^R$) revertant which has regained nuclease activity.

Our studies of Rec$^+$ revertants are reported here. A preliminary report of our work has been presented.[6]

Two types of reversion are possible: back-mutation at the original site and suppression. One possibility for suppression is suggested by the occurrence of

significant residual recombination in all *recB*$^-$ and *recC*$^-$ mutants tested.[2,7] This residual recombination may be due to a functional analog of the ATP-dependent nuclease, or to a series of enzymes involved in an alternative pathway of recombination, independent of the *recB* and *recC* genes. A mutation causing activation or derepression of the analog or the alternative pathway would restore recombination proficiency by indirect suppression.[8] We have obtained Rec$^+$ revertants by back-mutation and also by indirect suppression.

**Materials and Methods. Strains:** Most of the Rec$^-$ strains used are derived from two distantly related *E. coli* K12 F$^-$ strains. One of the F$^-$ parent strains is characterized by a mutation abolishing endonuclease I activity[9] and the other by its multiple mutations to auxotrophy. The distinction is made because these characteristics represent a convenient reference to the different genetic backgrounds of the strains. Two Hfr strains JC5412 and JC158 have been described previously.[1,10] Amber mutant T4 phages B22, E51, E219, HL627, N57, N116, and N122 were obtained from Dr. P. Scotti. Ochre mutant T4 phage 427 was obtained from W. Brammer. Appropriate standard Su$^+$ and Su$^-$ tester strains were obtained from Drs. P. Scotti, R. Bruner, and W. Brammer.

Nomenclature conforms in most ways to the recommendations of Demerec *et al.*[11] However, 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 recorded by Taylor and Trotter[12] with the exception of a new symbol (*sbc*) introduced in this paper. *sbc* stands for the set of genes which are involved with suppression of *recB21* or *recC22*. Phenotypic abbreviations are as follows: Rec, recombination; UV, ultraviolet irradiation; Gal, galactose; His, histidine; Sm, streptomycin; Ser, serine; Mit, mitomycin; EMS, ethyl methane sulfonate; Arg, arginine "$-$", requiring when used with abbreviations of amino acids and deficient when used with Rec; "$+$", independence when used with abbreviations of amino acids and proficient when used with Rec; S, sensitivity; R, resistance.

**Methods:** The procedures and media for conjugational crosses and the discrimination of Rec$^-$ from Rec$^+$, and UV$^S$ from UV$^R$ cells have been described previously.[1,13] Interruption of mating was performed with a vibrating mixer by vigorous agitation for 90 sec of an aliquot of mating mixture. The procedures and media for transduction with phage P1 have been described previously.[14] The growth of cells, lysis procedure, and procedure for assay of ATP-dependent nuclease have been described by Barbour and Clark.[5] Assay of the ATP-independent DNase reported in this paper is the same, but no ATP or nucleotide derivative is added. Determining the *rec* genotype of Rec$^-$ transductants was performed by crossing with appropriate *recC*$^-$, *recB*$^-$, and *recB*$^-$ *recC*$^-$ Hfr strains.[15]

T4 DNA was prepared by phenol extraction of purified T4 phage labeled with [$^3$H]-thymidine.[16]

Mitomycin resistant revertants of Rec$^-$ mutants were isolated by plating cultures on L medium[14] supplemented with 0.5 $\mu$g/ml mitomycin C. This concentration of the antibiotic permitted 50% survival of Rec$^+$ cells and less than 1% survival of Rec$^-$ cells. All operations with mitomycin C were carried out in dim light; the mitomycin was dissolved just prior to its addition to the medium; and the plates were stored and incubated in the dark.

**Results. Isolation and characterization of Rec$^+$ revertants:** Three Rec$^-$ mutants derived from an endonuclease I deficient mutant[5] were treated with ethyl methane sulfonate (EMS) or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) as previously described[1] except that the time of treatment with EMS was 30 min. After time for expression of mutant phenotypes, each culture was plated on complex medium with and without mitomycin. The NTG-treated cultures of the *recB21 recC*$^+$ and *recB*$^+$ *recC22* single mutants showed about $10^{-3}$ per cent survival, and the EMS-treated cultures about $10^{-1}$ per cent survival, in the

presence of mitomycin. The *recB21 recC22* double mutant showed similar survival frequencies: $10^{-2}$ per cent after NTG and $10^{-1}$ per cent following EMS treatment. Between 8 and 34% of the survivors were $UV^R$; there were as many $UV^R$ derivatives of the double *rec*$^-$ strain as from either of the two single *rec*$^-$ strains. Two independent $UV^R$ survivors of mitomycin treatment from each *rec* mutant were further characterized; their independence was assured by choosing one induced by each mutagen.

The six $UV^R$ strains were first characterized by determining quantitatively their survival following a given dose of irradiation. As seen in Table 1, the revertant strains were fully as resistant as their *rec*$^+$ ancestor, JC6724, while their immediate *rec*$^-$ ancestors were more sensitive, showing about one-fiftieth the level of survival. To test their recombination proficiency, the revertants were crossed with an Hfr strain that transfers the *rec* genes distal to the selected *gal*$^+$ gene. In Table 1 it is shown that the revertants are as proficient in forming Gal$^+$ [Ser$^+$] recombinants as JC6724 and about 50–300 times more proficient than their Rec$^-$ ancestors. Thus it is clear that the revertants had become both $UV^R$ and Rec$^+$.

The revertants were then tested for their *recB* and *recC* genotype. Both of these genes are closely linked to *argA*.[2,17] P1 transduction using the revertants as *argA*$^+$ donors can be used to reveal the presence of *recB* or *recC* mutations. An *argA*$^-$ *recB*$^+$ *recC*$^+$ strain is used as a recipient. Fig. 1 shows that if back-mutation or intragenic suppression has occurred in the revertants, essentially no Rec$^-$ Arg$^+$ transductants are expected; if other types of suppression have occurred then Rec$^-$ Arg$^+$ transductants will appear as frequently as when a Rec$^-$ strains is used as donor. The results indicate that the revertants of

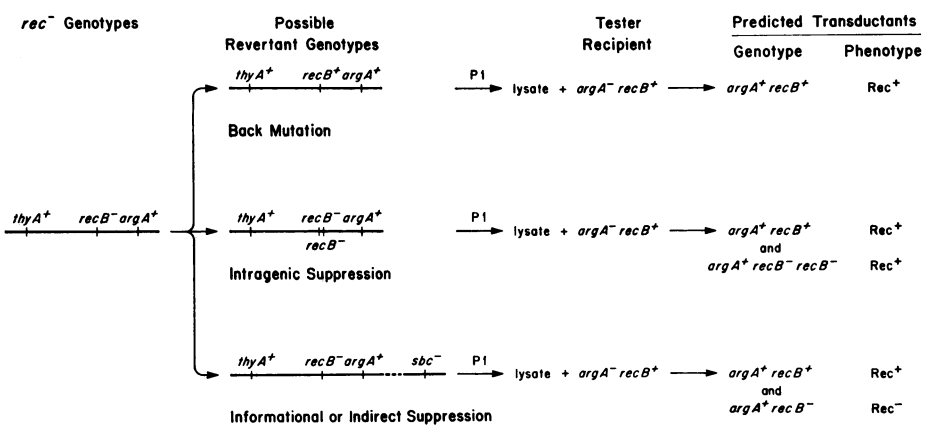


FIG. 1. Representation of the hypotheses involved in the use of P1 transduction to establish the genetic nature of Rec$^+$ revertant mutations. The test is based on the known P1-cotransduction frequencies of *recB* and *recC* with *argA* (60–80%).[2] The representation of intragenic suppression indicates the presence of two *recB* mutations, the original one and an additional suppressing one. In the case that intragenic suppression occurs a very low frequency of Arg$^+$ Rec$^-$ transductants would result due to recombination between the *recB* mutations. In practice we did not test enough Arg$^+$ transductants to detect this.

TABLE 1. *Phenotype and genotype of revertant strains derived from recB and recC mutants whose genetic background is characterized by their endA mutation.*

| Strain no. | Genotype | Recombination frequency %[a] | Per cent survival after UV irrad. of 270 ergs/mm²/sec | Arg⁺ transductants[b] No. tested | No. of following genotype: *recB*⁻ | *recC*⁻ | *recB*⁻ *recC*⁻ | Units of nuclease activity/mg protein[e]: ATP-dependent | ATP-independent |
|---|---|---|---|---|---|---|---|---|---|
| JC6724 | *recB*⁺ *recC*⁺ *sbc*⁺ | 15, 19, 38 | 55 | 68 | 0 | 0 | 0 | 7 | 1 |
| JC6722 | *recB21 recC*⁺ *sbc*⁺ | 0.1 | 0.4 | 91 | 75 | 0 | 0 | nd | 1 |
| JC5174[c] | *recB21 recC*⁺ *sbc-1* | 11 | 40 | 56 | 30 | 0 | 0 | nd | 10 |
| JC5172[d] | *recB21 recC*⁺ *sbc-2* | 14 | 75 | 7 | 4 | 0 | 0 | nd | 11 |
| JC6721 | *recB*⁺ *recC22 sbc*⁺ | 0.5 | 0.8 | 100 | 0 | 74 | 0 | nd | 2 |
| JC5175[c] | *recB*⁺ *recC22 sbc-3* | 20 | 94 | 27 | 0 | 18 | 0 | nd | 12 |
| JC5170[d] | *recB*⁺ *recC22 sbc-4* | 11 | 58 | 64 | 0 | 42 | 0 | nd | 7 |
| JC6720 | *recB21 recC22 sbc*⁺ | 0.09 | 2.0 | 26 | 2 | 3 | 16 | nd | 2 |
| JC5183[c] | *recB21 recC22 sbc-5* | 30 | ...[f] | 27 | 2 | 1 | 11 | nd | 17 |
| JC5176[d] | *recB21 recC22 sbc-6* | 20 | 59 | 71 | 8 | 10 | 38 | nd | 8 |

[a] Number of Gal⁺ [Ser⁺] recombinants per 100 donor cells. Donor JC158, which transfers 0...*thr*⁺...*lac*⁺...*gal*⁺ and is *serA*⁻. The matings were interrupted after 60 min.

[b] P1 phage grown on each strain and the lysates used to transduce *argA*⁺ into JC5467, which is *argA*⁻ *recB*⁺ *recC*⁺. Multiplicity of infection (m.o.i.) was about 0.05.

[c] Revertant obtained following NTG (nitrosoguanidine) mutagenesis.

[d] Revertant obtained following EMS (ethylmethanesulfonate) mutagenesis.

[e] DNA degradation is measured in the presence and absence of added ATP. The latter is considered a measure of ATP-independent activity, while the difference between the former and the latter is considered a measure of the ATP-dependent activity. In lysates of the revertant strains generally more activity is measured in the absence of ATP than in its presence. In these cases, the ATP-dependent activity is recorded as nd (not detected). Activities are measured both in crude lysate and supernatant fraction as explained by Barbour and Clark.[5]

[f] Not determined.

TABLE 2. *Comparison of nuclease activities of* Rec⁻ *and two kinds of* Rec⁺ *strains.*

| Strain desig. | *rec* genotype | DNase specific activity (units/mg protein) Substrate (native): *E. coli* DNA −ATP | *E. coli* DNA +ATP | T4 DNA −ATP | T4 DNA +ATP |
|---|---|---|---|---|---|
| 1. JC6724 | *recC*⁺ *sbc*⁺ | 1 | 29 | 0.5 | 48 |
| 2. JC6721 | *recC22 sbc*⁺ | 1.5 | 1 | 0.5 | ... |
| 3. JC5170 | *recC22 sbc-4* | 11 | 9 | 0.3 | 0.2 |

Brij 58 supernatants were assayed on either purified radioactive native *E. coli* or T4 DNA (30 nmol) in 0.5 ml reaction volume (Barbour and Clark, 1970). ATP present at a final concentration of $2 \times 10^{-4}$ M.

the *recB21 recC22*, doubly mutant, strain still carry both *rec* mutations (Table 1) and hence are suppressed revertants. The revertants of the single *rec*$^-$ strains *also* still carried the original *rec* mutations (Table 1) and hence were also suppressed revertants. We have named the genes which, when mutant, suppress *recB* and *recC* mutations, the *sbc* genes. For simplicity we hypothesize that a single mutation is responsible for the suppression in each revertant and have numbered the six suppressive alleles accordingly (Table 1).

**Nature of the suppression of *recB* and *recC* mutations:** Three types of suppression have been distinguished: indirect, intragenic, and informational.[9] The latter two types are characterized by action directly on the mutant product. Intragenic suppression occurs by a second mutation within the gene carrying the first mutation but at a different site (e.g., frameshift reversion). The original mutation and its suppressor are tightly linked and would be cotransduced frequently. If this type of suppression were operative in any of the six revertants described here, then we would expect them to have yielded few if any Rec$^-$ transductants (among the small number tested), owing to the close linkage between the original mutation and its suppressor. In fact, all the Rec$^+$ revertants yielded just as high a percentage of Rec$^-$ Arg$^+$ transductant as their ancestors. On this basis we rule out intragenic suppression in these cases.

Informational suppression involves an effect of the suppressor on the translation system; e.g., the suppressor modifies transfer RNA, the ribosome, or an activating enzyme. One of the most common manifestations of this type of suppression is nonsense codon suppression, or more specifically, amber codon suppression. We tested the ancestral Rec$^+$ and Rec$^-$ strains. Of seven phage strains used, six (B22, E51, E219, H6627, N116, N122) produced normal plaques on the ancestral strains and on the revertants, indicating that all of these strains carry at least one potent amber suppressing gene (which does not affect the *recB21* and *recC22* alleles). The seventh phage, mutant N57, showed only a slight degree of suppression in the ancestral Rec$^+$ strain JC6724, i.e., it formed very faint plaques. The Rec$^-$ strains and their Rec$^+$ revertants were no more permissive as hosts than JC6724 for N57. Thus we were unable to find any evidence for additional amber suppression in any of the six revertants to Rec$^+$. In addition we have tested the strains for ochre suppression activity using T4 phage ochre mutant 427. All ten bacterial strains in Table 1 were nonpermissive for this phage. We have yet to test for UGA codon suppression. These data alone can not rule out informational suppression as responsible for the regaining of Rec$^+$ and UV$^R$ properties in the *rec*$^-$ strains, but it does not appear likely that nonsense codon suppression is involved.

**An ATP-independent DNase restored in suppressed Rec$^+$ revertants:** We did obtain some evidence which makes informational suppression very unlikely. An ATP-dependent DNase activity is present in *recB*$^+$ *recC*$^+$ strains but is absent from the *recB*$^-$ and *recC*$^-$ strains used here.[5,6] We examined lysates of the revertant Rec$^+$ strains for their level of ATP-dependent nuclease activity. In these strains, we have found that high levels of DNase are restored, but ATP is not required for activity (Table 1). In fact ATP partially inhibits this DNase (Table 2). This DNase is also strikingly different from the ATP-dependent

DNase in its activity on native T4 DNA (Table 2). The ATP-dependent DNase is more active on T4 than *E. coli* DNA, but the revertant DNase is virtually inactive on T4 DNA. Several other criteria (thermal lability; pH and magnesium dependence) also indicate that *recB*$^+$ *recC*$^+$ ATP-dependent DNase and this revertant DNase are different enzymes[5,6] (S. D. Barbour, manuscript in preparation). These results are more consistent with the hypothesis of indirect suppression than with the hypothesis of informational suppression.

**Additional revertants of *recB* and *recC* mutant strains:** The use of the endonuclease I-deficient series of *rec* mutants to obtain revertants presented difficulties in mapping the suppressing alleles, since these strains have only one reference marker. We therefore began to study the revertants obtained from a multiply auxotrophic series of *rec* mutants. The results obtained from *recB21 recC*$^+$ and *recB*$^+$ *recC22* mutant strains are reported in Table 3. Three independent Mit$^R$ revertants of each strain were chosen: one EMS-induced, one NTG-induced, and one spontaneous. As seen from Table 3 all six revertants have regained UV$^R$ and Rec$^+$ properties characteristic of the wild type. Only one out of these six revertants appears to have retained the original *rec* mutation. The other five are either true back-mutants or else they carry a closely linked, probably intragenic, suppressor. These latter revertants have all regained ATP-dependent DNAse ac-

TABLE 3. *Phenotype and genotype of revertant strains derived from recB and recC mutants whose genetic background is characterized by their multiply auxotrophic nature.*

| Strain No. | Genotype | Recombination frequency (%)[a] | Per cent survival after UV irrad. of 270 ergs/mm$^2$/sec | Arg$^+$ Transductants[b] | | | Units of Nuclease | |
|---|---|---|---|---|---|---|---|---|
| | | | | No. tested | No. of Genotype | | Activity/mg Protein[f] | |
| | | | | | *recB*$^-$ *recC*$^-$ | *recB*$^-$ *recC*$^-$ | ATP-dependent | ATP-independent |
| AB1157 | *recB*$^+$ *recC*$^+$ *sbc*$^+$ | 20 | 50 | 100 | 0 | 0 | 10 | 2 |
| JC5743 | *recB21 recC*$^+$ *sbc*$^+$ | 0.085 | 3.8 | 100 | 36 | 0 | nd | 2 |
| JC4679[c] | *recB*$^+$ *recC*$^+$ *sbc*$^+$ | 36 | 112 | 100 | 0 | 0 | 6 | 1 |
| JC4680[d] | *recB*$^+$ *recC*$^+$ *sbc*$^+$ | 19 | 112 | 100 | 0 | 0 | 7 | 1 |
| JC4681[e] | *recB21 recC*$^+$ *sbc-7* | 14 | 22 | 100 | 65 | 0 | nd | 1 |
| JC5489 | *recB*$^+$ *recC22 sbc*$^+$ | 0.27 | 0.94 | 100 | 0 | 53 | nd | 2 |
| JC4676[c] | *recB*$^+$ *recC*$^+$ *sbc*$^+$ | 44 | 56 | 100 | 0 | 0 | 8 | 2 |
| JC4677[d] | *recB*$^+$ *recC*$^+$ *sbc*$^+$ | 3.6 | 111 | 100 | 0 | 0 | 4 | 2 |
| JC4678[e] | *recB*$^+$ *recC*$^+$ *sbc*$^+$ | 26 | 91 | 100 | 0 | 0 | 7 | 1 |

[a] Number of Thr$^+$Leu$^+$ [Ser$^+$Str$^R$] recombinants per 100 donor cells. Donor JC158, which transfers 0...*thr*$^+$...*leu*$^+$...*gal*$^+$... and is *serA*$^-$. The matings were interrupted after 60 min.

[b] P1 phage grown on each strain and the lysates used to transduce *argA*$^+$ into JC5467, which is *argA*$^-$*recB*$^+$*recC*$^+$. Multiplicity of infection (m.o.i.) was about 0.05.

[c] Revertants obtained spontaneously.

[d] Revertants obtained following EMS treatment.

[e] Revertants obtained following NTG treatment.

[f] See footnote *e* to Table 1 for explanation; nd = not detectable.

tivity consistent with their being back-mutants or intragenically suppressed mutants. The one identifiably suppressed revertant shows no increase in ATP-dependent or ATP-independent nuclease activities. A different mode of indirect suppression appears to be a plausible working hypothesis to explain the $Rec^+$ property of this strain.

From the single *rec* mutants of the multiple auxotrophic line, five of six revertants were true back-mutants, while from the endonuclease I-deficient line none of the four revertants of the single $rec^-$ mutant strains were true back-mutants. This difference, if borne out by further test, implies a significant difference in genetic background of the two strains. Precisely what this difference is must await mapping studies on the *sbc* mutations.

There are three reasons to believe that endonuclease I itself is not a factor in the present studies: First, endonuclease I-deficient strains carry out recombination normally.[5,9] Second, $recB^-$ and $recC^-$ strains that contain endonuclease I are $Rec^-$ (reference 4; Barbour and Clark, unpublished; and this paper). Finally, endonuclease I activity is not detected and does not interfere with the DNase assay used[4] (Barbour, unpublished; and this paper).

**Characteristics of a spontaneously occurring suppressed $recB^-$ Hfr strain:** Three years ago, a *recB21* Hfr strain was constructed by Willetts (see reference number 1). The strain was $UV^S$ and when used as a recipient, it showed poor recombination ability in keeping with its genotype. Later it was noted in our laboratory and in others (e.g., B. Low, personal communication) that the phenotype of this strain was $UV^R$.[14] We have found that this Hfr strain still carries the original *recB21* mutation and consequently is a suppressed revertant carrying an *sbc-8* mutation. High levels of ATP-independent nuclease activity are present in this strain.

**Discussion.** At least two classes of revertants of *recB* and *recC* mutants have been found. One class does not show the original *rec* mutation by transduction. This class may contain both true back-mutants and intragenically suppressed revertants since the number of transductants tested was too low to have detected a closely linked, intragenic, suppressor mutation. The five revertants in this class have recovered the wild type recombination proficiency and resistance to UV. In addition they have recovered the ATP-dependent nuclease activity characteristic of the wild type.

The second class of revertants carry *recB* or *recC* mutations which are suppressed. Indirect suppression is indicated (1) by the absence of detectable ochre codon suppression, or additional amber codon suppression, and, more importantly, (2) by the high level of an ATP-*independent* nuclease activity which differs in substrate specificity and several other parameters (S. D. Barbour, unpublished) from the ATP-dependent nuclease determined by the *recB* and *recC* genes. The appearance of this ATP-independent nuclease activity may be explained as an increase in the amount of a preexisting enzyme or as the removal of an inhibitor of a preexisting enzyme, among many alternatives. We favor the hypothesis of an increase in the amount of a preexisting enzyme, i.e., derepression. However, we do not know at present that the *sbc* mutations have affected any regulatory function. Mapping studies and an understanding of the different

genetic behavior of the endonuclease I-deficient and multiply auxotrophic strains will help to clarify this point.

We believe that the increase in DNase activity may explain the recombination proficiency and UV resistance of the second class of revertants we have described. It is still to be determined, however, whether the ATP-independent nuclease is functionally the same as the ATP-dependent nuclease, or whether the ATP-independent nuclease participates with other enzymes in a shunt or second recombination pathway. At present we favor the second pathway hypothesis for reasons which will be developed in a subsequent paper. This hypothetical second pathway would account for the residual recombination observed in *recB* and *recC* mutants. Furthermore *recA* would then be predicted to participate in both pathways to account for the complete absence of recombination in *recA* mutants.[7]

An additional complexity is revealed by the existence of the revertant JC4681 which belongs to neither the first nor second classes just mentioned. This strain shows no detectably higher nuclease activity than is present in the Rec$^-$ parent, although it could contain a labile nuclease not detected by our standard procedure. JC4681 belongs to a third class of revertants characteristically found only in the multiply auxotrophic AB1157 line of *recB*$^-$ and *recC*$^-$ strains (A. J. Clark, A. Templin, and H. Nagaishi, unpublished results). The implication may be drawn that AB1157 is genetically unable to produce the ATP-independent nuclease because of a prior mutation. In this strain we consequently discovered a different kind of suppression, which explains the Rec$^+$ nature of the revertants like JC4681.

Abbreviations: *sbc*, genes for the suppression of *recB* and *recC;* EMS, ethyl methane sulfonate; NTG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

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