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Evidence for Unique DNA Repair Activity Encoded by a Cloned *Serratia marcescens* Gene: Suppression of *Escherichia coli* Mutations That Reduce Repair of Alkylated DNA

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A recombinant plasmid containing a *Serratia marcescens* DNA repair gene has been analyzed biochemically and genetically in *Escherichia coli* mutants deficient for repair of alkylated DNA. The cloned gene suppressed sensitivity to methyl methanesulfonate of an *E. coli* strain deficient in 3-methyladenine DNA glycosylases I and II (i.e., *E. coli tag alkA*) and two different *E. coli recA* mutants. Attempts to suppress the methyl methanesulfonate sensitivity of the *E. coli recA* mutant by using the cloned *E. coli tag* and *alkA* genes were not successful. Southern blot analysis did not reveal any homology between the *S. marcescens* gene and various known *E. coli* DNA repair genes. Biochemical analysis with the *S. marcescens* gene showed that the encoded DNA repair protein liberated 3-methyladenine from alkylated DNA, indicating that the DNA repair molecule is an *S. marcescens* 3-methyladenine DNA glycosylase. The ability to suppress both types of *E. coli* DNA repair mutations, however, suggests that the *S. marcescens* gene is a unique bacterial DNA repair gene.

When DNA is exposed to the monofunctional alkylating agent methyl methanesulfonate (MMS), N^3-methyladenine is one of the major products formed (2). Excision of this base is accomplished by 3-methyladenine DNA glycosylases, which exist in both procaryotic and eucaryotic organisms (6). Base excision by this type of enzyme results in liberation of the modified base in its free form (11, 13, 15, 25).

It has long been known that procaryotic organisms lacking the capacity to repair N^3-methyladenine are more sensitive to alkylation damage than are organisms which have such repair mechanisms (10). For example, it has been shown that *Escherichia coli* mutants deficient in 3-methyladenine DNA glycosylases I and II (i.e., *E. coli tag alkA*) are highly sensitive to DNA alkylation, resulting in the formation of 3-methyladenine (3, 5). This observation provided strong evidence that 3-methyladenine has cytotoxic effects if not removed from DNA. Furthermore, such results stimulated extensive genetic and biochemical studies, which have culminated in the cloning of 3-methyladenine DNA glycosylase genes from *E. coli* and in the elucidation of the biochemical function of two such enzymes, TagI (3-methyladenine glycosylase I) and TagII (3-methyladenine glycosylase II) (3, 8, 9, 18, 19, 21, 23, 27).

In addition to functional DNA glycosylases, *E. coli* is dependent on other cellular responses for resistance to alkylating agents such as MMS. The generalized DNA repair cascade known as the SOS response is also required (29). This response is initiated by cleavage of the Lex repressor protein by the *recA* gene product (29). Thus, *E. coli recA* mutants exhibit extreme sensitivity to alkylating agents (e.g., MMS) since the SOS response cannot be elicited (6, 28). While it is required for resistance to alkylation damage, the SOS repair pathway does not regulate the activity of TagI or TagII in *E. coli*. In fact, induction of *alkA* was shown to be independent of *recA* (4, 18), and *tag* is expressed constitutively (21).

We have been studying DNA repair in gram-negative bacteria other than *E. coli* in an effort to determine whether certain DNA repair mechanisms have been conserved through evolution. Our efforts have focused on the isolation and characterization of a DNA glycosylase gene(s) from *Serratia marcescens*. The initial paper in this study described the suppression of MMS sensitivity exhibited by *E. coli tag*, *alkA*, and *recA* mutants by a cloned *S. marcescens* DNA repair gene (17), which was preliminarily characterized as a functional analog of *E. coli tag*.

In the current report we present evidence that the *S. marcescens* DNA repair gene is unique in comparison to the *E. coli tag*, *alkA*, and *recA* genes. More specifically, only the *S. marcescens* gene was capable of suppressing *E. coli tag alkA* and *E. coli recA* mutations. The active protein encoded by the cloned gene, a 42-kilodalton (kDa) molecule (17), releases 3-methyladenine from alkylated substrate DNA. Finally, Southern blot analysis failed to reveal any homology between the *S. marcescens* gene and various *E. coli* genes known to be involved in DNA repair. Our results suggest that *S. marcescens* possesses a novel DNA repair mechanism which is functional in *E. coli* and can effectively suppress distinct mutations which result in deficiency for the repair of alkylated DNA.

Suppression of *E. coli tag alkA* and *E. coli recA* mutations. In our previous report, it was speculated that a possible explanation for suppression of the *E. coli recA* mutation by the *S. marcescens* gene (*rpr*) was that the cloned gene encoded a 3-methyladenine DNA glycosylase that restored, in part, resistance to MMS. It was reasoned that if this were true, the cloned *E. coli tag* and *alkA* genes might also be able to suppress an *E. coli recA* mutation. To determine the validity of this hypothesis, the comparative abilities of the *rpr* and the *E. coli tag* and *alkA* genes to suppress an *E. coli recA* mutation and an *E. coli tag alkA* mutation were determined.

The recombinant plasmids used in this experiment are listed in Table 1. Plasmid pSM9 harbors the *rpr* gene on a 1.5-kilobase (kb) *Sma*I-*Hin*dIII fragment. This plasmid is a deletion derivative of pSM4, which has been described

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant genotype or phenotype[a]	Source or reference
S. marcescens ATCC 25419	Wild type	Laboratory stock
E. coli K-12		
χ2813	*recA56*	NEB[b]
MV1932	*tag-1 alkA1*	M. Volkert
DH5α	*recA1*	Laboratory stock
PF1018	*alkA*$^+$ *recA*$^+$ *tag*$^+$	P. Foster
Plasmids		
pCY5	*tag*$^+$ Amp^r	M. Sekiguchi
pYN1000	*alkA*$^+$ Amp^r Tet^r	M. Sekiguchi
pJC859	*recA*$^+$ Amp^r	A. J. Clark
pSM9	*rpr*$^+$ Amp^r	This work
pUC18	Amp^r	Laboratory stock
pGW2607	*ada*$^+$ alkB$^+$ Amp^r	G. Walker

[a] *rpr* is the *S. marcescens* DNA repair gene.
[b] NEB, New England BioLabs.

before (17). Plasmid pSM9 is as effective at restoring MMS resistance as pSM4. Both plasmids produce the same *S. marcescens* proteins (i.e., polypeptides of 42 and 16 kDa). Furthermore, analysis of deletion derivatives of pSM9 revealed that it was not possible for the cloned segment to contain two distinct, nonoverlapping genes (data not shown). Therefore, the 16-kDa protein is either a breakdown product of the 42-kDa protein or the product of a gene having a reading frame overlapping that of the 42-kDa-protein gene.

Plasmids pSM9, pCY5, (*tag*$^+$ in pUC8 [23]), pYN1000 (*alkA*$^+$ in pBR322 [19]), pJC859 (*recA*$^+$ in pBR322), and pUC18 were introduced into *E. coli* strains MV1932 (*tag alkA*) and χ2813 (*recA*). Table 2 shows the plating efficiencies of these two strains harboring the various plasmids. This experiment was performed as described previously (5). pCY5 (*tag*$^+$) and pYN1000 (alkA$^+$) did not restore MMS resistance to the *E. coli recA* mutant (χ2813). Also, pJC859 (*recA*$^+$) did not restore resistance to MV1932 (*E. coli tag alkA*). Plasmid pSM9, however, significantly increased the MMS resistance of the *E. coli tag alkA* mutant (MV1932) and the *E. coli recA* mutant (χ2813). Although pSM9 was not completely effective in either the *tag alkA* or *recA* mutant, it is clear that the cloned *S. marcescens* gene was capable of suppressing the MMS sensitivity caused by the two distinct *E. coli* DNA repair mutations.

There existed the possibility that the effect of pSM9 on *recA* (χ2813) was allele specific. To alleviate this concern, a second *E. coli recA* mutant was tested. Strain DH5α carries a *recA1* point mutation, which is distinct from the *recA56* allele of χ2813. As was seen with χ2813, only pJC859 and pSM9 were able to restore MMS resistance to DH5α, proving that the effect of pSM9 was specific for the *recA* mutations and not the individual allele (data not shown). These data indicated that the ability of the *S. marcescens rpr* gene to suppress the *E. coli recA* and *tag alkA* mutations is novel, since none of the *E. coli* DNA repair genes showed such heterologous suppression abilities.

TABLE 2. Effect of various plasmids on the MMS sensitivity of *E. coli* mutants deficient in alkylation repair

Strain[a] and plasmid	Plating efficiency (%) on Luria agar containing ampicillin (50 μg/ml) and 0.013% MMS
MV1932(pYN1000)	97.5
MV1932(pCY5)	81.7
MV1932(pSM9)	15.5
MV1932(pJC859)	0.1
MV1932(pUC18)	0.15
χ2813(pYN1000)	0.02
χ2813(pCY5)	0.1
χ2813(pSM9)	17.6
χ2813(pJC859)	86
χ2813(pUC18)	0.17

[a] MV1932 is an *E. coli tag alkA* mutant. χ2813 is an *E. coli recA* mutant.

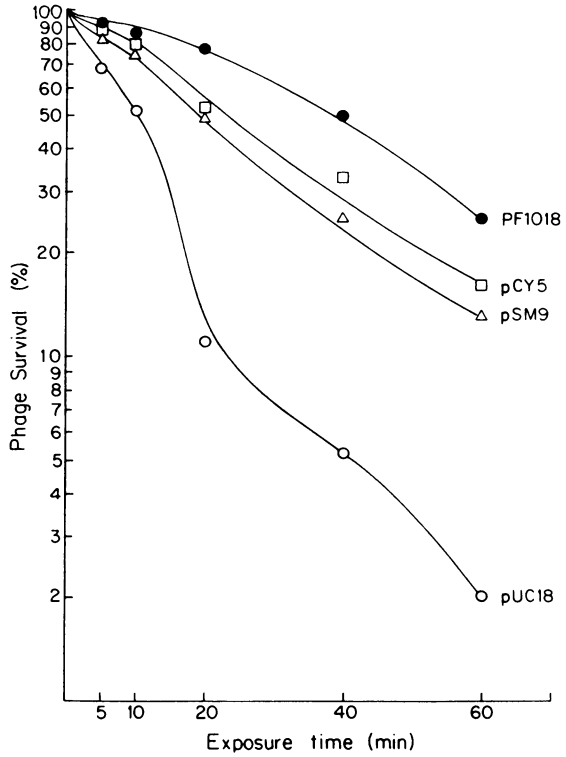

FIG. 1. Effects of pSM9 on the host cell reactivation of MMS-treated bacteriophage lambda cI857 in strain MV1932 (*E. coli tag alkA*). Symbols: ○, pUC18; △, pSM9; □, pCY5; ●, host cell reactivation in strain PF1018 (*tag*$^+$ *alkA*$^+$ *recA*$^+$) without plasmid. MV1932 and PF1018 were derived from parental strain AB1157 (1). MMS was used at a concentration of 50 mM.

The above data, however, do not offer a definitive explanation as to the mechanism of *recA* suppression by *rpr*. It is evident, though, that merely supplementing a *recA* mutant strain with exogenous 3-methyladenine DNA glycosylase genes does not compensate for the absence of the SOS response (Table 2). Thus, our initial hypothesis (17) (see above) for restoration of MMS resistance to an *E. coli recA* mutant by *rpr* is no longer plausible. Nevertheless, Rpr must be multifunctional since it suppresses the *tag alkA* and *recA* mutations. Such a characteristic must be unique from those of TagI and TagII in order to permit suppression of a *recA* mutation, thus restoring, in part, resistance to MMS.

Host cell reactivation of MMS-treated bacteriophage. *E. coli* MV1932 is deficient for 3-methyladenine DNA glycosylases I and II. Because it lacks these two enzymes, MV1932 is unable to reactivate bacteriophage lambda which has been exposed to MMS (5, 10). We examined whether pSM9 could restore viability to MMS-treated lambda phage. Figure 1 shows the results of an experiment designed to test this possibility. As expected, lambda was not reactivated by

TABLE 3. Summary of Southern blot hybridization experiments

Sample DNA	Hybridization[a] with gene probes			
	E. coli tag	*E. coli alkA*	*E. coli recA*	*S. marcescens rpr*
S. marcescens	−	−	+	+
E. coli K-12	+	+	+	±
E. coli tag	+	−	ND	−
E. coli alkA	−	+	ND	−
E. coli recA	ND	ND	+	−
E. coli ada-alkB	ND	ND	ND	−
S. marcescens rpr	−	−	−	+

[a] A plus sign indicates significant hybridization between probe and sample. A minus sign indicates no hybridization even after extensive exposure of the film. A plus-minus sign indicates weak hybridization. ND, Not done. All blots were washed under mild stringency conditions.

TABLE 4. 3-Methyladenine DNA glycosylase activity in an *E. coli tag alkA* mutant (MV1932) transformed with various plasmids

Strain and plasmid	N^3-Methyladenine released[a] (fmol)
MV1932	6.0
MV1932(pUC18)	6.0
MV1932(pCY5) (*tag*)	25.0
MV1932(pSM9) (*rpr*)	23.0
Blank	6.0

[a] 95 fmol of 3-methyladenine were present in the alkylated substrate DNA. The assay for DNA glycosylase activity was as described in Materials and Methods. Equal amounts (32 μg) of cell extract protein were reacted with alkylated substrate DNA. The reaction mixture blank contained an equivalent amount of acetylated bovine serum albumin.

pUC18-containing MV1932. There was a dramatic increase in lambda survival when either pSM9 or pCY5 (*tag*$^+$) was present in MV1932. Neither plasmid, however, restored lambda survival levels to that of *E. coli* PF1018 (*tag*$^+$ *alkA*$^+$).

This assay (22) provided a useful mechanism for evaluating DNA repair activity. The *rpr* gene product was capable of reactivating lambda (Fig. 1). Interestingly, *rpr* was as effective as *tag* in this assay. This result would not be predicted from the data shown in Table 2. Why *rpr* was more efficient at host cell reactivation than at restoration of MMS resistance to the *E. coli tag alkA* mutant is unclear. Regardless, this experiment proved that Rpr repairs DNA directly and does not function by preventing damage to DNA.

Sequence homology between the *S. marcescens* DNA repair gene and *E. coli* DNA repair genes. To further the molecular analysis of *rpr*, we tested for the possibility of DNA homology between the *S. marcescens* gene and some known *E. coli* DNA repair genes. The cloned *E. coli* genes used in this experiment included the *recA* gene (pJC859), *tag* gene (pCY5), *alkA* gene (pYN1000), and the *ada-alkB* operon (pGW2607, a pBR322 derivative containing the *ada-alkB* genes [12]). Also, chromosomal DNA from *E. coli* K-12 was examined. In a Southern blot analysis (26), the above samples were digested with the appropriate restriction endonucleases and probed with the 1.5-kb *Sma*I-*Hin*dIII insert fragment of pSM9. Hybridizations and washings (mild stringency conditions) were carried out as described before (16). Even after extensive exposure of the film, no hybridization of the *S. marcescens* probe to the cloned *E. coli* genes was detected. There was, however, a weak band visible with the *E. coli* chromosomal DNA sample. More specifically, an 8- to 9-kb *Bam*HI *E. coli* chromosomal fragment showed slight hybridization to the probe (data not shown). In a separate experiment, it was shown that an 8-kb *Bam*HI *S. marcescens* fragment hybridized strongly to an *E. coli recA* probe, implying that *S. marcescens* has a *recA* analog (unpublished observations). Thus, Southern blot analysis showed that the cloned gene had no detectable homology with the *E. coli recA*, *tag*, or *alkA* genes even though it suppressed the MMS sensitivity of *E. coli* mutants defective for those genes. Finally, the *alkA* and *tag* genes were used to probe a *Bam*HI digest of *S. marcescens* chromosomal DNA. No hybridization was detected. The results of Southern blot experiments are summarized in Table 3.

Assay for enzyme activity of pSM9. It was important to characterize the biochemical activity of pSM9 since we had shown its unique capabilities. Therefore, an experiment to determine the ability of the *S. marcescens* DNA repair protein to excise modified bases from alkylated substrate DNA was performed. Extracts from strain MV1932 (*E. coli tag alkA*) harboring the various plasmids were prepared as described before (24). These crude extracts were examined for the ability to remove 3-methyladenine from alkylated substrate DNA. The assay for 3-methyladenine DNA glycosylase activity was done as described before (27). Extracts of cells containing either pCY5 or pSM9 released comparable amounts of 3-methyladenine from alkylated substrate DNA (Table 4). Cell extracts harboring the cloning vector pUC18 as well as extracts from host strain MV1932 did not liberate 3-methyladenine from substrate DNA.

The possibility that pSM9 might have activity towards other DNA base modifications was also examined. From this analysis, it was determined that pSM9 did not encode a protein which possessed the capacity to remove N^1-methyladenine, O^6-methylguanine, N^3-methylguanine, or N^6-methyladenine (data not shown). N^7-methylguanine was released as <5% of the total N^7-methylguanine in the substrate DNA in all samples and thus did not affect the interpretation of the results. These data indicate that suppression of the *E. coli tag alkA* mutation was due to the activity of *S. marcescens* 3-methyladenine DNA glycosylase, i.e., Rpr. Furthermore, Rpr had the same narrow activity spectrum as *E. coli* TagI with respect to removal of alkylated purines (Table 4 and text). Finally, the data in Table 4 are consistent with data shown in Fig. 1. That is, *tag* and *rpr* exhibited similar profiles in host cell reactivation and 3-methyladenine DNA glycosylase activity.

Obviously, the data presented here are somewhat paradoxical. In Table 4, data indicate that the *rpr* gene encodes a protein with 3-methyladenine DNA glycosylase activity, which explains *rpr* suppression of the *E. coli tag alkA* mutation (Table 2 and Fig. 1). One of the most interesting aspects of this work, however, is that *rpr* suppresses the *recA* mutation as efficiently as it does the *tag alkA* mutation. The fact that *rpr* suppresses the *recA* mutation suggests that *rpr* must encode a unique DNA repair activity. One possibility is that Rpr has a lambda *gam*-like function and thus inhibits DNA degradation by RecBCD. Such activity might permit growth of the *E. coli recA* mutants on MMS. Finally, it is known that N^7-methylguanine and N^3-methyladenine are removed by DNA glycosylase activity, with the result being apurinic/apyrimidinic (AP) sites left in the DNA (14). Such sites are potentially mutagenic. It is possible that one mechanism to repair AP sites generated subsequent to the action of DNA glycosylase activity involves encoding proteins which have DNA glycosylase activity and AP endonuclease activity residing in the same protein. This type of activity has been found in the T4 bacteriophage (20) and

Micrococcus luteus (7) pyrimidine dimer DNA glycosylases. Perhaps *S. marcescens* has evolved a similar system for the repair of alkylated DNA. It is not possible to determine this at present. The repair protein will have to be purified before we can determine whether or not *rpr* does encode such dual activity.

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LITERATURE CITED

1. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
2. **Beranek, D. T., C. C. Weis, and D. H. Swensen.** 1980. A comprehensive quantitative analysis of methylated and ethylated DNA using high pressure liquid chromatography. Carcinogenesis **1**:595–606.
3. **Clarke, N. D., M. Kvaal, and E. Seeberg.** 1984. Cloning of *Escherichia coli* genes encoding 3-methyladenine DNA glycosylases I and II. Mol. Gen. Genet. **197**:368–372.
4. **Evensen, G.** 1985. Induction of 3-methyladenine DNA glycosylase II is *recA*$^+$-independent. Mutat. Res. **146**:143–147.
5. **Evensen, G., and E. Seeberg.** 1982. Adaptation to alkylation resistance involves the induction of a DNA glycosylase. Nature (London) **296**:773–775.
6. **Hanawalt, P. C., P. K. Cooper, A. K. Ganesan, and C. A. Smith.** 1979. DNA repair in bacteria and mammalian cells. Annu. Rev. Biochem. **48**:783–836.
7. **Haseltine, W. A., L. K. Gordon, C. P. Lindan, R. H. Grafstrom, N. L. Shaper, and L. Grossman.** 1980. Cleavage of pyrimidine dimers in specific DNA sequences by a pyrimidine dimer DNA glycosylase of *M. luteus*. Nature (London) **285**:634–641.
8. **Kaasen, I., G. B. Evensen, and E. Seeberg.** 1986. Amplified expression of the *tag*$^+$ and *alkA*$^+$ genes in *Escherichia coli*: identification of gene products and effects on alkylation resistance. J. Bacteriol. **168**:642–647.
9. **Karran, P., T. Hjelmgren, and T. Lindahl.** 1982. Induction of a DNA glycosylase is part of the adaptive response to alkylating agents. Nature (London) **296**:770–773.
10. **Karran, P., T. Lindahl, I. Ofsteng, G. B. Evensen, and E. Seeberg.** 1980. *Escherichia coli* mutants deficient in 3-methyladenine DNA glycosylase. J. Mol. Biol. **140**:101–127.
11. **Laval, J.** 1977. Two enzymes are required for strand incision in repair of alkylated DNA. Nature (London) **269**:829–833.
12. **Lemotte, P. K., and G. C. Walker.** 1985. Induction and autoregulation of *ada*, a positively acting element regulating the response of *Escherichia coli* K-12 to methylating agents. J. Bacteriol. **161**:888–895.
13. **Lindahl, T.** 1976. New class of enzymes acting on damaged DNA. Nature (London) **259**:64–66.
14. **Lindahl, T.** 1979. DNA glycosylases, endonucleases for apurinic/apyrimidinic sites, and base excision repair. Prog. Nucleic Acids Res. Mol. Biol. **22**:135–144.
15. **Male, R., D. E. Helland, and K. Kleppe.** 1985. Purification and characterization of 3-methyladenine glycosylase from calf thymus. J. Biol. Chem. **260**:1623–1629.
16. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Murphy, K. E., and H. D. Braymer.** 1989. Molecular cloning and characterization of a genetic region from *Serratia marcescens* involved in DNA repair. Mol. Microb. **3**:249–255.
18. **Nakabeppu, Y., H. Kondo, and M. Sekiguchi.** 1984. Cloning and characterization of the *alkA* gene of *Escherichia coli* that encodes 3-methyladenine DNA glycosylase II. J. Biol. Chem. **259**:13723–13729.
19. **Nakabeppu, Y., T. Miyata, H. Kondo, S. Iwanaga, and M. Sekiguchi.** 1984. Structure and expression of the *alkA* gene of *Escherichia coli* involved in the adaptive response to alkylating agents. J. Biol. Chem. **259**:13730–13736.
20. **Radany, E. H., and E. C. Friedberg.** 1980. A pyrimidine dimer DNA glycosylase activity associated with the *v* gene product of bacteriophage T4. Nature (London) **286**:182–185.
21. **Riazuddin, S., and T. Lindahl.** 1978. Properties of 3-methyladenine glycosylase from *Escherichia coli*. Biochemistry **17**:2110–2118.
22. **Rupert, C. S., and W. Harm.** 1966. Reactivation after photobiological damage. Adv. Radiat. Biol. **2**:1–9.
23. **Sakumi, K., Y. Nakabeppu, Y. Yamomoto, S. Kawabata, S. Iwanaga, and M. Sekiguchi.** 1986. Purification and structure of 3-methyladenine DNA glycosylase I of *Escherichia coli*. J. Biol. Chem. **261**:15761–15766.
24. **Seeberg, E., J. Nissen-Meyer, and P. Strike.** 1976. Incision of ultraviolet irradiated DNA requires three different gene products. Nature (London) **263**:524–526.
25. **Singer, B., and T. P. Brent.** 1981. Human lymphoblasts contain DNA glycosylase activity excising N-3 and N-7 methyl and ethyl purines but not O^6-alkylguanine and alkyladenines. Proc. Natl. Acad. Sci. USA **78**:856–860.
26. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
27. **Thomas, L., Y. Chul-Hak, and D. A. Goldthwait.** 1982. Two DNA glycosylases in *Escherichia coli* which release primarily 3-methyladenine. Biochemistry **21**:1162–1169.
28. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
29. **Walker, G. C.** 1987. The SOS response of *Escherichia coli*, p. 1346–1357. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.