Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 7173–7177, November 1985
Biochemistry

uvrA and *recA* mutations inhibit a site-specific transition produced by a single O^6-methylguanine in gene *G* of bacteriophage ϕX174

(site-specific mutagenesis/O^6-*n*-butylguanine/DNA repair)

ROBERT W. CHAMBERS, EWA SLEDZIEWSKA-GOJSKA†, SAMIM HIRANI-HOJATTI‡, AND HENRYK BOROWY-BOROWSKI

Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia, B3H 4H7, Canada

Communicated by Severo Ochoa, June 18, 1985

ABSTRACT **Using site-specific mutagenesis, we have examined the mutagenic activity *in vivo* of O^6-methylguanine or O^6-*n*-butylguanine located at a preselected site in gene *G* of bacteriophage ϕX174. The experiments were designed so that the phage mutant produced by a targeted transition from either of these alkylated derivatives would be recognizable by a simple plaque assay. Spheroplasts derived from normal and repair-deficient cells were transfected, and the lysates were screened for mutant virus. In cells with normal repair, DNA carrying the methylguanine produced the expected transition in 15% of the total phage; DNA carrying the butylguanine produced the same mutation in 0.3% of the phage. In cells deficient in excision repair (*uvrA*) the transition frequency went up by a factor of 8 for O^6-butylguanine and down by a factor of 40 for O^6-methylguanine. In cells deficient in recombination (*recA*), the transition frequency increased 1.5-fold for butylguanine and decreased by a factor of 8 for methylguanine. The data show that (*i*) both methyl- and butylguanine produce site-directed transitions in ϕX174; (*ii*) the transition occurs in *recA* cells; (*iii*) the frequency of the transition is influenced by both *recA* and *uvrA* mutations; (*iv*) the *recA* and *uvrA* mutations alter the transition frequency for methylguanine and butylguanine in opposite directions.**

The gene products of *uvrA*, *-B*, and *-C* play a key role in removing a variety of damaged purine or pyrimidine nucleotide residues that deform the DNA helix (1–7). The *ada* gene product, on the other hand, appears to operate only on O^6-methyl- or O^6-ethylguanine in DNA, in an alkyl-transfer reaction that regenerates the guanine (8–12). In this paper we report our initial studies dealing with effects of DNA repair on the frequency of site-directed mutations produced *in vivo* by alkylated purine or pyrimidine residues located at preselected positions in an essential gene of bacteriophage ϕX174. The results show an unexpected effect of *uvrA* and *recA* mutations on the production of a specific transition from an O^6-methylguanine or O^6-*n*-butylguanine in the first position of the third codon in gene *G* of ϕX174.

MATERIALS AND METHODS

Bacterial Strains. *Escherichia coli* AB1157 and its isogeneic derivatives AB1886 (*uvrA6*), AB2463 (*recA13*), and AB2480 (*uvrA6 recA13*) were obtained from B. J. Bachmann (*E. coli* Genetic Stock Center, Department of Microbiology, Yale University, New Haven, CT). These bacteria were transformed with the plasmid pϕXG105 as described (13). HF4738 (*recA*/pϕXG), a *recA* strain carrying pϕXG105, has been described (14). *E. coli* C was from the American Type Culture Collection.

Phage Stocks. The mutants ϕXGms3 $(CCG)^{Pro}$ and ϕXGms3 $(TCG)^{Ser}$ carry the codons indicated as the third codon of gene *G*. They were obtained by site-specific mutagenesis by the procedure described in ref. 15. Details of their preparation and characterization will be published elsewhere.

Form I′ DNA. A series of oligonucleotide primers, AAAAGTCGG*AAACAT (G* = G, m^6G, or bu^6G) were synthesized by the method of Tanaka and Letsinger (16) with one important modification: The blocking groups were removed by treatment with concentrated ammonium hydroxide at 65°C for 3 days. Each 15-mer was purified by TLC and HPLC. Particular care was taken to separate the oligomer containing a 2,6-diaminopurine residue, produced by ammonolysis of the O^6-alkyl group in *N*-isobutyryl-O^6-alkyl-2′-deoxyguanylate residues (17, 18), from the desired product. After enzymatic phosphorylation with polynucleotide kinase in the presence of [^{32}P]ATP, the labeled product was purified further by electrophoresis in a 20% acrylamide gel in TBE buffer (89 mM Tris borate, pH 8/1 mM EDTA) in the presence of 8 M urea. The final product was characterized by nucleoside analysis and gel electrophoresis. *N*-isobutyryl-O^6-butyl-5′-dimethoxytrityl-2′-deoxyguanosine used for synthesis of the oligonucleotide primer was shown to be >98% *n*-butyl isomer by HPLC analysis using authentic samples of the *iso*- and *sec*-butyl isomers synthesized by the method of Gaffney and Jones (18); attempts to synthesize the *tert*-butyl derivative by this method failed, so we have no standard for this isomer, but no peak at the expected position of this isomer was detected. The details of these syntheses and characterizations will be published elsewhere.

Modified DNA was synthesized essentially as described (15). Form I′ DNA was isolated by electrophoresis in the dark in a 1% low-melting-agarose gel in the presence of ethidium bromide at 0.5 μg/ml. Under these conditions the form I′ supercoils and separates cleanly from all other types of DNA present. The band containing the DNA was cut out and most of the ethidium bromide was removed in the dark by soaking the gel piece in Tris/EDTA buffer (20 mM Tris Cl, pH 8.0/1 mM EDTA). The DNA was recovered by melting the gel in the above buffer at 60°C and then extracting at room temperature first with phenol, followed by phenol/$CHCl_3$ (1:1 vol/vol), and then with $CHCl_3$. The aqueous solution was concentrated by equilibrating it with 1-butanol. The DNA

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MeNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; ts, temperature-sensitive.

†Current address: Polish Academy of Sciences, Institute of Biochemistry and Biophysics, ul Rakowiecka 36, 02-532 Warsaw, Poland.

‡Current address: The Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario, Canada M4X 1K9.

was precipitated with ethanol and redissolved in Tris/EDTA buffer. The DNA gave a single radioactive band by agarose gel electrophoresis (as above) with the correct mobility for form I′ DNA.

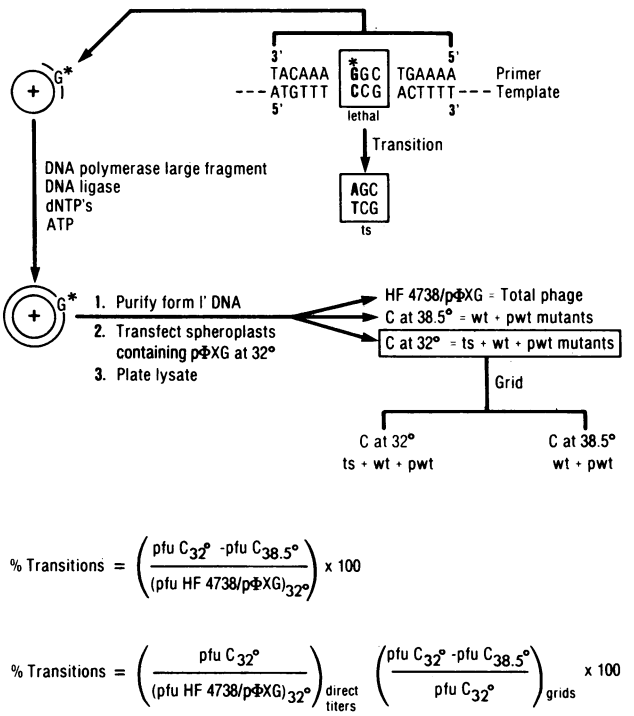
Production of Mutant Phage. Spheroplasts were prepared as described by Guthrie and Sinsheimer (19). Because the protamine sulfate suggested by Benzinger *et al.* (20) was found to cause clumping when added to the fresh preparation, the spheroplasts were stored in the presence of Mg^{2+} at 4°C without protamine sulfate. Just before use, 2.5 μl of 1% (wt/vol) protamine sulfate was added per ml of spheroplast preparation used, and the preparation was checked for competence by transfection with wild-type φX form I DNA. Unless specified otherwise, transfection was carried out with 10^8 molecules of wild-type DNA or 2.5×10^8 molecules of modified DNA and 4×10^8 spheroplasts in 4 ml of Tris buffer, using a standard protocol (15, 19). Only spheroplast preparations that gave $>10^{-3}$ phage per molecule of input DNA were used. The transfections with modified DNA were carried out within 12 hr of checking spheroplast competence. Further details are given in the legends to the appropriate figures and tables.

RESULTS

The experiments described in this paper are based on a site-specific mutagenesis system that focuses on the third codon of gene *G* in bacteriophage φX174. The general properties of this system and the experimental design for studying the molecular mechanisms of mutation by single, well-characterized adducts that are known to form when carcinogens react with DNA *in vivo* have been discussed (15, 21). We have synthesized and characterized two new φX gene *G* mutants (unpublished experiments). One of these, φXGms3 $(TCG)^{Ser}$, carries a serine codon, TCG, instead of the wild-type glutamine codon, CAG, as the third codon of gene *G*. This phage grows on *E. coli* C at 32°C but not at 38.5°C. The other mutant phage, φXGms3 $(CCG)^{Pro}$, carries a proline codon as the third codon of gene *G*. This mutation is lethal; the mutant will grow only on a host carrying a functional copy of gene *G* on the plasmid pφXG. The DNA from these mutants has been isolated and characterized; nucleotide sequence analysis showed the presence of the expected codon. These DNAs play a key role in the experiments described here.

The protocol for the experiments described in this paper is shown in Fig. 1. DNA from the mutant φXGms3 $(CCG)^{Pro}$ provided the template for the synthesis of site-modified DNA. O^6-methyl- or O^6-butyl-2′-deoxyguanylate residues were introduced into the appropriate oligonucleotide primers by stepwise synthesis, and these 15-mers were incorporated into form I′ DNA by elongation and ligation in the usual manner (15). The modified form I′ DNA was purified and then used to transfect suitable spheroplasts at 32°C. Spheroplasts were prepared from a parental strain of *E. coli* with normal repair pathways as well as from isogeneic strains carrying *uvrA*, *recA*, and *uvrA recA* mutations. In all experiments, the spheroplasts carried pφXG so that normal gene *G* product was always present in the cells, making them permissive for virus production even when the DNA carried a lethal mutation in both strands, as would be the case after replication of the parental template (+) strand or after repair of the O^6-alkylguanine in the parental (−) strand.

Potentially, the spheroplast lysates can contain several different types of phage, but it is possible to screen for the desired transition mutant, as shown in Fig. 1. Phage derived from the template strand or from the complementary strand after error-free repair of the O^6-alkylguanine carry the lethal proline codon. These phage will grow on *E. coli* strain HF4738/pφXG, but not on *E. coli* C. A transition produced



$$\% \text{ Transitions} = \left(\frac{\text{pfu } C_{32^\circ} - \text{pfu } C_{38.5^\circ}}{(\text{pfu HF 4738/p}\Phi\text{XG})_{32^\circ}} \right) \times 100$$

$$\% \text{ Transitions} = \left(\frac{\text{pfu } C_{32^\circ}}{(\text{pfu HF 4738/p}\Phi\text{XG})_{32^\circ}} \right)_{\text{direct titers}} \left(\frac{\text{pfu } C_{32^\circ} - \text{pfu } C_{38.5^\circ}}{\text{pfu } C_{32^\circ}} \right)_{\text{grids}} \times 100$$

FIG. 1. Experimental protocol. Enzymatic synthesis of the form I′ DNA was carried out by elongation and ligation of a synthetic oligonucleotide primer on a single-stranded, circular DNA isolated from a well-characterized mutant of φX174. This mutant carries the lethal codon, $(CCG)^{Pro}$, as the third codon of gene *G* and requires a host carrying a functional copy of gene *G* on a plasmid, pφXG, for growth. The base-pairing created by hybridization of the primer to the template is shown; the third codon, where the modification is located, is boxed. The synthetic form I′ DNA was purified before use in transfection assays. All phage produced by transfections must contain either the original template DNA, which carries a lethal mutation, or progeny DNA produced by replication. A transition, during replication, of the O^6-methyl- or O^6-butylguanine (G*) changes the proline codon to TCG, which codes for serine. This produces a temperature-sensitive phage that will grow on *E. coli* C at 32°C but not at 38.5°C (15). By use of the assay shown in the figure, this mutant can be distinguished from phage produced from the template DNA or from the complementary DNA after repair of the O^6-alkylguanine residue. "Direct titers" were obtained by mixing duplicate aliquots (usually 0.1 ml) of the spheroplast lysate with 10^8 cells in soft agar and pouring the mixture onto nutrient agar plates. The plates were incubated at the temperatures indicated and read after 5–6 hr. The plaque count was checked again after 16-hr incubation. "Grids" were made by picking plaques produced on *E. coli* C after 5–6 hr incubation at 32°C and stabbing sequentially onto two plates of prepoured soft agar containing 10^8 cells (*E. coli* C) each. "Grid" plates were incubated at the indicated temperatures and read after 5–6 hr. pfu, Plaque-forming units; ts, temperature-sensitive; wt, wild type; pwt, pseudo-wild type.

by either an O^6-methylguanine or O^6-butylguanine converts the proline codon, CCG, to a serine codon, TCG. This produces the temperature-sensitive mutant φXGms3 $(TCG)^{Ser}$, which will grow on *E. coli* C at 32°C but not at 38.5°C. Two other types of phage, which can grow on *E. coli* C at 38.5°C, might be produced. One of these is the wild-type revertant resulting either from a spontaneous transversion at the second position of the third codon or from recombination of the mutant gene with the wild-type gene in pφXG. The second is a pseudo-wild-type phage arising either from a second-site mutation that overcomes the lethal effect of the proline codon or, conceivably, from a transversion at the O^6-alkylguanine to give a threonine or alanine codon (if these amino acid substitutions happen to give the pseudo-wild-type phenotype). We have no information about the effect of these

particular transversions on the phenotype, but we expect the frequency of these mutations to be low compared to the transition (22–24). Therefore, only wild-type and, possibly, pseudo-wild-type phage should present a potential problem, and these phage can be distinguished from those carrying the targeted mutation by use of the plaque assays illustrated in Fig. 1.

Our first experiment was designed to see whether the expected transition mutation was produced from the O^6-methylguanine located at position 2400. Since this transition converts the phage phenotype from lethal to temperature-sensitive (ts), we examined production of ts mutants before and after treatment of cells with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MeNNG) (Table 1). Twelve percent of the total phage produced by normal spheroplasts had the phenotype (ts) expected for a site-specific transition that converts the proline codon to the serine codon. To demonstrate that these mutants arise from the O^6-methylguanine, we pretreated the cells with MeNNG to induce the alkyl-transfer repair system and repeated the experiment. The mutation frequency went down by a factor of 3. This experiment indicated that the expected transition was occurring and that its frequency could be measured by a plaque assay even though this particular assay (the difference between plaque counts on *E. coli* C at 32°C and 38.5°C) is subject to a number of small errors (including statistical errors and differences in plating efficiencies at the two temperatures).

To compare the repair of an O^6-methylguanine with that of a similiar but more bulky base, we addressed the question: Do both methylated and butylated guanines produce the targeted transition and, if so, at what frequencies? Our first set of experiments was done in spheroplasts derived from a well-characterized strain of *E. coli* K-12, AB1157/pφXG. These cells have normal DNA repair and carry a functional copy of φX gene *G* on the plasmid pφXG. The results are shown in the top part of Table 2. We consider any phage that grows on *E. coli* C a mutant, since we are starting with DNA from a phage, φXGms3 $(CCG)^{Pro}$, that will not grow on this host. Inspection of the data reveals several important points. First, unmodified synthetic DNA prepared in an identical manner to the modified molecules gave a significant number of phage that grew at 32°C on *E. coli* C ("ts + wt" in the table), but when 200 of these plaques were examined further by gridding (see Fig. 1), they all grew on *E. coli* C at both 32°C and 38.5°C. Thus, none of these phage have the properties of the desired transition mutant, and it seems likely that they are wild-type phage produced by recombination with the plasmid pφXG in the spheroplasts (for further evidence see below). Second, with DNA carrying the O^6-methylguanine, 63% of the mutant fraction grew on *E. coli* C at 32°C but not at 38.5°C. Thus, almost 16% of the total phage produced in these spheroplasts had the properties of the expected transition mutant. Third, with DNA carrying the O^6-butylguanine, phage with the properties of the expected transition mutant represented 5% of the total mutants and 0.3% of the total phage.

Table 1. A transition produced from O^6-methylguanine located in the first position of the third codon in gene *G* of φX174

	Plaque-forming units/ml of lysate				
		Mutant phage		Transition mutants	
Induction	Total phage	ts + wt	wt	No.	% of phage
None	570	100	30	70	12
MeNNG	3800	320	180	140	4

Spheroplasts were prepared from *E. coli* AB1157/pφXG. These cells are Ada^+, $UvrA^+$, and $RecA^+$. They were grown to a density of 2.5×10^8 per ml. The culture was split and half was treated with MeNNG (1 μg/ml) at room temperature for 1 hr. Both treated and untreated cells were recovered by centrifugation and converted to spheroplasts. Transfection was carried out 15 min after spheroplast formation, using form I' DNA prepared as illustrated in Fig. 1 and described in *Materials and Methods*. The input of DNA was two molecules per spheroplast. The incubation was at 30°C for 2 hr. Cells were lysed by dilution and vigorous agitation. The lysates were titered on *E. coli* HF4738 (*recA*/pφXG) at 30°C (total phage), *E. coli* C at 32°C (ts + wt, temperature-sensitive plus wild-type plus pseudo-wild-type), and *E. coli* C at 38.5°C (wt, wild-type plus pseudo-wild-type). All titers were determined in duplicate. The average of these two determinations is recorded in the table. It was assumed that all the ts mutants were produced by a specific transition.

Table 2. Effect of recombination on the targeted transition frequency of O^6-alkylguanine

		Phage/ml of lysate			
Cells	Base*	Total phage	ts + wt	ts/(ts + wt)	% mutants
wt	Gua	14,000	1,000	0	0
	O^6-MeGua	40,000	10,000	0.63	16
	O^6-BuGua	55,000	3,200	0.05	0.3
recA	Gua	930	None†		
	O^6-MeGua	6,200	130	0.94	2
	O^6-BuGua	1,600	7	0.96	0.4

Spheroplasts were prepared from *E. coli* AB1157/pφXG (wt, wild-type) and AB2463(*recA*/pφXG). Both strains are Ada^+. Before using cells for preparing spheroplasts, the *recA* locus was checked by measuring the sensitivity of the cells to killing by ultraviolet light. Transfection was carried out as described in *Materials and Methods*. Incubation and lysis were as described in the legend to Table 1 (noninduced cells). The DNA in each case was form I' prepared as shown in Fig. 1 and purified as described in *Materials and Methods*. The DNA input was ≈0.6 molecules per spheroplast. Titers, expressed in phage/ml, were determined as described for Table 1. Approximately 200 plaques were picked from *E. coli* C grown at 32°C and "gridded" as described in the legend to Fig. 1. From these grids, the fraction ts/(ts + wt), where wt includes both wild-type and pseudo-wild-type phage, was obtained. It was assumed that all the ts phage arose from a site-specific transition.

*At the first position of codon 3 in gene *G* of φX174.

†No plaques with 0.1 ml of undiluted lysate per plate.

We were concerned by the large number of phage that grew at both 32°C and 38.5°C on *E. coli* C. Humayun and Chambers (13) had shown that growth of φX gene *G* mutants on $RecA^+$ host cells carrying pφXG gives plaques with a very high wild-type phage content (13). These arose by recombination with the plasmid pφXG, to produce the wild-type phage. Once this occurs, the wild-type phage "take over" because they enjoy a significant growth advantage (as measured by burst size) over most gene *G* mutants (25, 26). It seemed likely that this was occurring in the experiments described here and that this was responsible for the large number of phage that grew at both temperatures. To examine this possibility, we repeated the transfection using spheroplasts isogeneic with AB1157/pφXG but carrying a *recA* mutation. These cells, AB2463 (*recA*/pφXG), are defective in recombination. The results of the transfection are shown in the bottom half of Table 2. Now 94–96% of the mutants were the expected transition mutant. These results agree well with previous data showing that recombination with pφXG to produce wild-type phage from a gene *G* amber mutant occurred in $RecA^-$ hosts at only 2–5% the level seen in $RecA^+$ hosts (13).

From these experiments, we conclude that the transition does occur at the expected site from either O^6-methyl- or O^6-*n*-butylguanine located in the first position of the third codon of gene *G* in φX174 DNA. Furthermore, the mutation frequency resulting from O^6-methylguanine is about 50 times greater than for O^6-butylguanine in cells with normal repair and about 4.5 times greater in *recA* cells. The data also show

a 1.5-fold increase in mutation frequency from the O^6-butylguanine in *recA* cells. This *recA* effect probably reflects repair by recombination with pφXG. The *recA* effect with the O^6-methylguanine is in the opposite direction; i.e., the mutation frequency goes down by a factor of 7. The results suggest that the RecA protein inhibits repair of O^6-methylguanine but not of O^6-butylguanine. The mechanism responsible for this effect is unknown.

Classical mutagenesis experiments reported by Todd and Schendel (27) indicate that a mutation in the *uvrA* gene increases mutations that arise by treating cells (*E. coli* AB1157 and AB1886) with *N*-butyl-*N'*-nitro-*N*-nitrosoguanidine but has no effect on mutations resulting from treatment with *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine. These authors assumed that O^6-alkylguanine was responsible for mutations leading to the observed phenotypic reversion (Arg^- to Arg^+), but there is no assurance in an experiment of this kind that an O^6-alkylguanine *per se* is responsible for the effect seen. Therefore, it was of interest to see what effect a *uvrA* mutation might have on the mutation frequencies produced from a single O^6-alkylguanine located at a known site in the DNA. It was also of interest to examine further the effect of a defect in the *recA* gene on the mutation frequency. The results are shown in Fig. 2.

No site-specific-transition mutants were found in the control (transfection with unmodified φX form I' DNA carrying the proline codon in gene *G*, data not shown). Fig. 2*A* shows that site-specific transitions from O^6-butylguanine increased 8-fold in *uvrA* cells compared to spheroplasts with normal excision repair. This result is as expected; it reflects repair of the butylguanine by excision. The transition frequency from the butylguanine again showed a 1.5-fold increase in *uvrA recA* cells (compare $UvrA^+RecA^+$ with $UvrA^+RecA^-$ in Fig. 2*A* and in Table 2; also compare $UvrA^-RecA^+$ with $UvrA^-RecA^-$ in Fig. 2*A*). Thus, even though the effect is small, it seems to be reproducible. The combined effect seen in *uvrA recA* cells is large (13-fold) compared to cells with normal repair and provides further evidence that both the *uvrA* and the *recA* mutations increase the transition frequency from O^6-butylguanine. This combined effect probably represents reduction of both excision and recombination repair.

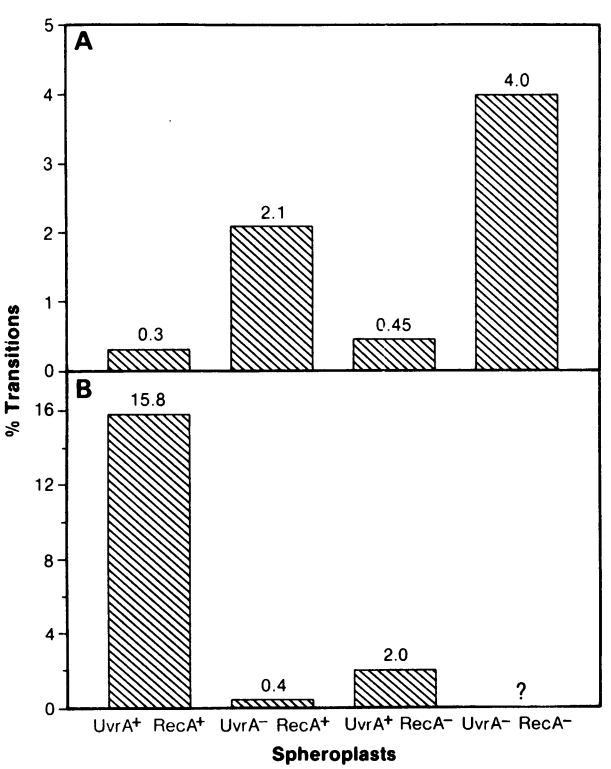


FIG. 2. The effect of *uvrA* and *recA* mutations on the frequency of transitions produced from O^6-butylguanine (*A*) or O^6-methylguanine (*B*) located in the first position of the third codon in gene *G* of φX174 form I' DNA. Note the different scales in *A* and *B*.

The results with O^6-methylguanine are quite different from those with the butyl derivative. As shown in Fig. 2*B*, the mutation frequency decreased by a factor of 40 in cells with normal repair compared to *uvrA recA* cells. The *recA* mutation also decreased the transitions (compare $UvrA^+$ $RecA^+$ and $UvrA^+RecA^-$ in Fig. 2*B*). The *recA* effect is smaller than the *uvrA* effect (factor of 8 compared to factor of 40) but much larger than the effect seen with O^6-butylguanine, and it operates in the opposite direction. The data obtained with the double mutant (*uvrA recA*) were inconclusive (no plaques were found on *E. coli* C). Regardless of the uncertainty with *uvrA recA*, the effects produced by the *uvrA* and *recA* mutations on the transition frequency produced from an O^6-methylguanine located in the first position of the third codon in φX gene *G* are clear. Both the *uvrA* and *recA* mutations inhibit specific transitions directed by O^6-methylguanine.

All the experiments reported here were done with a well-characterized *E. coli* K-12 cell line (AB1157 and isogeneic mutants derived from it). The parent cells, and presumably the isogeneic mutants, have an inducible methyltransfer repair system (8), and we have verified that the cells we used showed the inducible response to MeNNG (Table 1). Under nonadaptive conditions, AB1157 has a measurable level of methyl-transfer repair (28, 29), yet we did not find it necessary to saturate this repair system in order to observe a high mutation frequency. These results cannot be extrapolated *a priori* to other strains because different strains may contain different constitutive levels of methyl-transfer repair activity (refs. 28 and 29; unpublished data).

DISCUSSION

We have examined the mutagenic activity of an O^6-methylguanine and an O^6-*n*-butylguanine located at residue 2400 in the (−)-strand of φX174 form I' DNA (first position of the third codon in gene *G*) (30). In a normal DNA repair background, the O^6-butylguanine produced the expected transition in 0.3% of the total phage. The methyl derivative produced the transition in 15% of the phage. These results show that an O^6-methylguanine *per se* produces a transition *in vivo* and that an O^6-butylguanine produces the same mutation, but at a frequency lower by a factor of 50.

In a $UvrA^-$ background, the mutation frequency from the O^6-butylguanine went up 7-fold. In a $RecA^-$ background, the frequency went up 1.5-fold. These results are consistent with results from classical mutagenesis studies (27) and with widely held views concerning the repair of bulky residues in DNA (31). Our results indicate that O^6-butylguanine is repaired primarily by excision. This lesion is also repaired to a lesser extent by recombination, but this may be an artifact of the particular system we are using, in which spheroplasts carry a copy of the normal φX gene *G* on the plasmid pφXG. The results also show that O^6-butylguanine produces mutations in a *recA* mutant.

The results with O^6-methylguanine were quite different and unexpected. It has been shown by site-specific mutagenesis using bacteriophage M13 mp8 that the mutation frequency from O^6-methylguanine is only 0.4% in cells [*E. coli* MM294A (Ada^+)] with normal repair capability (32). Using a different cell line (*E. coli* AB1157), we found the expected transition in 15% of the total phage produced in Ada^+ spheroplasts. This number is a lower limit because not all the modified primer molecules are retained during the enzymatic synthesis

of form I' DNA; some are replaced by nick-translation activity that seems to be present in all commercial samples of *E. coli* DNA polymerase I large fragment that we have tried.

Our results show that a single lesion in an extrachromosomal DNA can escape repair and produce mutations in a significant number of cells even when they have a normal repair capability.

Surprisingly, in a UvrA$^-$ background, the transitions produced from the methylated guanine went down by a factor of 40. In a RecA$^-$ background, they went down by a factor of 8. In both cases, however, mutations were found at a significant frequency. We have tentative evidence (not shown) that *uvrB* and probably *uvrC* mutants produce the same effect. If, as is widely believed, O^6-methyl- and O^6-ethylguanine are repaired exclusively by the alkyltransferase system, then one would not expect a *uvrA* mutation to have any effect on repair of these lesions. In agreement with this expectation, Todd and Schendel (27) have reported that there is no difference in the revertant frequency (Arg$^-$ to Arg$^+$) when the same UvrA$^+$ and UvrA$^-$ cells used here (AB1157 and AB1886) are treated with *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine. In contrast, we see a large effect of the *uvrA* mutation on mutations produced from O^6-methylguanine located at a specific position in ϕX DNA. It is not clear whether the difference between our results using site-specific mutagenesis and the results obtained by classical mutagenesis is due to differences in the systems or to a fundamental difference in the way O^6-methyl- and O^6-ethylguanine are handled *in vivo*.

Two important observations need to be explained. (*i*) In AB1157, which has normal repair systems, O^6-methylguanine is 50 times more mutagenic than O^6-butylguanine. (*ii*) Both *uvrA* and *recA* mutations increase the mutation frequency from butylguanine but decrease the frequency from methylguanine. Two possibilities must be considered. (*a*) Both methyl and butyl derivatives are excised by the Uvr system, but the cutting is strand-specific for butylguanine and random for methylguanine. (*b*) The butyl derivative is excised but the methyl derivative is not. These alternatives can be examined *in vitro* by use of purified proteins (3–7). Whichever is correct, our data indicate that important structural differences that influence the action of the repair proteins exist between these two modified DNAs. There are two lines of evidence indicating that the region around the methylguanine is not in the classical B-DNA conformation: One is based on theoretical considerations that predict that the presence of O^6-methylguanine bases stabilizes the Z conformation (33). The second is experimental and comes from physical measurements on small self-complementary oligonucleotides that form O^6-methylguanine·cytosine base pairs upon annealing (17, 34). Melting curves show that the methylguanine destabilizes the helix. CD spectra show important differences from that of normal B-DNA. There is no information of this kind for O^6-butylguanine.

In vitro studies with DNA polymerase I indicate that 2'-deoxy-O^6-methylguanosine 5'-triphosphate pairs with a thymine base in the template strand 95–98% of the time during incorporation (24). If the same mispairing frequency occurs *in vivo* during replication of modified ϕX form I' DNA, then there is a high probability that a transition will occur in each round of replication where an O^6-methylguanine is encountered. This, combined with inhibition of methyl-transfer repair by the *uvr* and *recA* mutations, could be responsible for the high mutation frequency we have observed with the methylated guanine compared to the butyl derivative.

Regardless of the actual mechanism that is responsible for our results, it appears that the 50-fold difference in transition frequencies produced by O^6-methylguanine and O^6-butylguanine is due to differences in repair rather than differences in mispairing, though we cannot rule out a contribution by the latter.

There is little doubt that site-specific mutagenesis is a valuable approach for studying the molecular mechanisms of mutation by a variety of chemicals that are known to be both mutagenic and carcinogenic. It also appears that the ϕX system we have developed may be an important tool for examining certain aspects of DNA repair, both *in vitro* and *in vivo*, in a way that has not been possible before.

We thank Dr. Raymond G. Fenwick for several critical discussions and Dr. Christopher W. Helleiner for editorial assistance. We are indebted to Carolyn Stampeen and Gregory Stuart for technical assistance. This work was supported by a Terry Fox Special Initiatives Award from the National Cancer Institute of Canada.

1. Howard-Flanders, P., Boyce, R. P. & Theriot, L. (1966) *Genetics* **53,** 1119–1136.
2. Grossman, L. (1975) in *Molecular Mechanisms for DNA Repair*, eds. Hanawalt, P. C. & Setlow, R. B. (Plenum, New York), Part A, pp. 175–182.
3. Seeberg, E. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2569–2573.
4. Seeberg, E. (1981) *Prog. Nucleic Acid Res. Mol. Biol.* **26,** 217–226.
5. Rupp, W. D., Sancar, A. & Sancar, G. B. (1982) *Biochimie* **64,** 595–598.
6. Sancar, A. & Rupp, W. D. (1983) *Cell* **33,** 249–260.
7. Sancar, A., Franklin, K. A. & Sancar, G. B. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7397–7401.
8. Samson, L. & Cairns, J. (1977) *Nature (London)* **267,** 281–283.
9. Schendel, P. F. & Robins, P. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 6017–6020.
10. Foote, R. S., Mitra, S. & Pal, B. C. (1980) *Biochem. Biophys. Res. Commun.* **97,** 654–659.
11. Defais, M., Jeggo, P., Samson, L., Cairns, J., Robins, P., Sedgwick, B. & Dodson, L. A. (1981) *Prog. Nucleic Acid Res. Mol. Biol.* **26,** 237–244.
12. Teo, I., Sedgwick, B., Demple, B., Li, B. & Lindahl, T. (1984) *EMBO J.* **3,** 2151–2157.
13. Humayun, M. Z. & Chambers, R. W. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 774–778.
14. Humayun, M. Z. & Chambers, R. W. (1978) in *The Single-Stranded DNA Phages*, eds. Denhardt, D. T., Dressler, D. & Ray, D. S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 477–481.
15. Bhanot, O. S., Khan, S. A. & Chambers, R. W. (1979) *J. Biol. Chem.* **254,** 12684–12693.
16. Tanaka, T. & Letsinger, R. L. (1982) *Nucleic Acids Res.* **10,** 3249–3260.
17. Kuzmich, S., Marky, L. A. & Jones, R. A. (1983) *Nucleic Acids Res.* **11,** 3393–3404.
18. Gaffney, B. L. & Jones, R. A. (1982) *Tetrahedron Lett.* **23,** 2253–2256.
19. Guthrie, G. D. & Sinsheimer, R. L. (1963) *Biochim. Biophys. Acta* **72,** 290–297.
20. Benzinger, R., Kleber, I. & Huskey, R. (1971) *J. Virol.* **7,** 646–650.
21. Chambers, R. W. (1982) in *Molecular and Cellular Mechanisms of Mutagenesis*, eds. Lemontt, J. F. & Generoso, W. M. (Plenum, New York), pp. 121–145.
22. Topal, M. D. & Fresco, J. R. (1976) *Nature (London)* **263,** 285–289.
23. Sinha, N. K. & Haimes, M. D. (1981) *J. Biol. Chem.* **256,** 10671–10683.
24. Toorchen, D. & Topal, M. D. (1983) *Carcinogenesis* **4,** 1591–1597.
25. Humayun, M. Z. & Chambers, R. W. (1979) *Nature (London)* **278,** 524–529.
26. Chambers, R. W., Kucan, I. & Kucan, Z. (1982) *Nucleic Acids Res.* **10,** 6465–6473.
27. Todd, M. L. & Schendel, P. F. (1983) *J. Bact.* **156,** 6–12.
28. Robins, P. & Cairns, J. (1979) *Nature (London)* **280,** 74–76.
29. Karran, P., Lindahl, T. & Griffin, B. (1979) *Nature (London)* **280,** 76–77.
30. Sanger, F., Coulson, A. R., Friedman, T., Air, G. M., Barrell, B. G., Brown, N. L., Fiddes, J. C., Hutchison, C. A., III, Slocombe, P. M. & Smith, M. (1978) *J. Mol. Biol.* **125,** 225–246.
31. Friedberg, E. C., Ehmann, U. F. & Williams, J. I. (1979) *Adv. Radiat. Biol.* **8,** 85–174.
32. Loechler, E. L., Green, C. L. & Essigmann, J. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6271–6275.
33. Van Lier, J. J. C., Smits, M. T. & Buck, H. M. (1983) *Eur. J. Biochem.* **132,** 55–62.
34. Gaffney, B. L., Marky, L. A. & Jones, R. A. (1984) *Biochemistry* **23,** 5686–5691.