fined. We postulate that VirB2 and VirB5 are the structural components of the pilus because of sequence homology with the pilin of the *Escherichia coli* F plasmid and a putative pilin subunit encoded by the IncN plasmid pKM101 (*22*). The requirement for VirD4 in pilus assembly is surprising, because its homolog in the IncP system, TraG, is not essential for the synthesis of P pili (*23*). However, construction of P pili requires TraF for which a homolog does not exist on the Ti plasmid (*24*). The requirement for VirD4 in pilus assembly may suggest that regions of VirD4 replace TraF in pilus assembly.

Once assembled, these Ti plasmid-encoded pili probably function in a manner similar to that of the classic sex pilus encoded by the F plasmid (*14*). The *Agrobacterium* pili probably attach to the recipient plant cell to establish a stable mating pair. The pili may then retract to create a channel for movement of the T-DNA strand and VirE2 protein directly into the plant cell. Therefore, further studies of *Agrobacterium*-mediated DNA transfer should lead to a better understanding of not only interkingdom but also interbacterial DNA transfer.

REFERENCES AND NOTES

1. J. R. Zupan and P. Zambryski, *Plant Physiol.* **107**, 1041 (1995).
2. P. Bundock, A. den Dulk-Ras, A. Beijersbergen, P. Hooykaas, *EMBO J.* **14**, 3206 (1995).
3. J. D. Heath, T. C. Charles, E. W. Nester, in *Two-Component Signal Transduction*, J. A. Hoch and T. J. Silhavy, Eds. (American Society for Microbiology, Washington, DC, 1995), pp. 367–385.
4. A. N. Binns, C. E. Beaupré, E. M. Dale, *J. Bacteriol.* **177**, 4890 (1995).
5. K. J. Fullner and E. W. Nester, in preparation.
6. M. Lessl and E. Lanka, *Cell* **77**, 321 (1994); S. C. Winans, D. L. Burns, P. J. Christie, *Trends Microbiol.* **4**, 64 (1996).
7. M. Tummuru, S. A. Sharma, M. J. Blaser, *Mol. Microbiol.* **18**, 867 (1995).
8. K. J. Fullner and E. W. Nester, *J. Bacteriol.* **178**, 1498 (1996).
9. D. Maher, R. Sherburne, D. E. Taylor, *ibid.* **175**, 2175 (1993).
10. Bacterial strains were grown on mannitol glutamate/Luria agar [G. A. Cangelosi, E. A. Best, G. Martinetti, E. W. Nester, *Methods Enzymol.* **204**, 384 (1991)] and after 2 to 3 days were inoculated into mannitol glutamate/Luria broth. Cells grown overnight were collected and resuspended to an optical density at 600 nm (OD_{600}) equal to 0.25 in CIB broth (*8*) with antibiotics. Cultures were incubated at 28°C with shaking for 6 hours, then were diluted with CIB broth to OD_{600} = 0.22. Two aliquots of 5 μl each were spotted onto a 4-ml CIB agar plate (*8*). Two plates, a total of four spots, were prepared for each strain. All plates were inverted and incubated at 19°C for 2 to 3 days.
11. We washed cells off plates with 50 μl of 10 mM $MgCl_2$ and negatively stained cells by mixing them with an equal volume of a 2% aqueous solution of phosphotungstic acid (pH 7.4). Cells were spotted onto Formvar-coated grids. After 15 s, excess liquid was removed. Samples were viewed with a JEOL1200 EXII transmission electron microscope operated at 80 kV.
12. A. G. Matthysse, *J. Bacteriol.* **154**, 906 (1983).
13. A. J. Riker, *J. Agric. Res.* **32**, 83 (1926).
14. P. Zambryski, in *Mobile DNA*, D. Berg and M. Howe, Eds. (American Society for Microbiology, Washington, DC, 1989), pp. 309–333.
15. P. J. Christie, J. E. Ward, S. C. Winans, E. W. Nester, *J. Bacteriol.* **170**, 2659 (1988); N. Grimsley, B. Hohn, C. Ramos, C. Kado, P. Rogowsky, *Mol. Gen. Genet.* **217**, 309 (1989); A. Beijersbergen, A. Den Dulk-Ras, R. Schilperoort, P. J. J. Hooykaas, *Science* **256**, 1324 (1992).
16. Nonpolar *virB* mutants, as described in (*25*), were as follows: A348ΔB1, A348ΔB2, A348ΔB3, A348ΔB5, A348ΔB6, A348ΔB7, A348ΔB8, A348ΔB10, At12501, Ax42, and At10011.
17. K. J. Fullner, thesis, University of Washington, Seattle (1996). The *virB* promoter (PvirB), the *virB* operon, and the *virD4* gene were cloned into high copy vectors to create pKJF91, pKJF72a, and pWD102, respectively. The *nptII* gene was first cloned adjacent to PvirB on pKJF91 and was carried through all cloning steps to provide selection of kanamycin resistance. The *virD4* gene was placed under control of the *virB* promoter by insertion of PvirB upstream of *virD4* on pWD102. PvirB-*virD4* was moved onto pKJF72a adjacent to the *virB* operon. Both loci were moved as a single fragment onto pGP159 (*26*).
18. C. Sundberg, L. Meek, K. Carroll, A. Das, W. Ream, *J. Bacteriol.* **178**, 1207 (1996).
19. D. E. Bradley, *Plasmid* **4**, 155 (1980); L. S. Frost, in *Bacterial Conjugation*, D. B. Clewell, Ed. (Plenum, New York, 1993), pp. 189–221.
20. D. E. Bradley, *Plasmid* **1**, 376 (1978).
21. N. Willetts and C. Crowther, *Genet. Res.* **37**, 311 (1981).
22. K. Shirasu and C. I. Kado, *FEMS Microbiol. Lett.* **111**, 287 (1993); R. F. Pohlman, H. D. Genetti, S. C. Winans, *Mol. Microbiol.* **14**, 655 (1994).
23. J. Haase, R. Lurz, A. M. Grahn, D. H. Bamford, E. Lanka, *J. Bacteriol.* **177**, 4779 (1995).
24. V. L. Waters, B. Strack, W. Pansegrau, E. Lanka, D. G. Guiney, *ibid.* **174**, 6666 (1992).
25. K. J. Fullner, K. M. Stephens, E. W. Nester, *Mol. Gen. Genet.* **245**, 705 (1994); E. Dale, A. Binns, J. Ward, *J. Bacteriol.* **175**, 887 (1993); B. R. Berger and P. J. Christie, *ibid.* **176**, 3646 (1994); K. M. Stephens, C. Roush, E. Nester, *ibid.* **177**, 27 (1994).
26. G. J. Pazour, C. N. Ta, A. Das, *J. Bacteriol.* **174**, 4169 (1992).
27. R. DeFeyter, C. I. Kado, D. W. Gabriel, *Gene* **88**, 65 (1990).
28. D. Sciaky, A. L. Montoya, M. D. Chilton, *Plasmid* **1**, 238 (1978).
29. B. Watson, T. C. Currier, M. P. Gordon, M. D. Chilton, E. W. Nester, *J. Bacteriol.* **123**, 255 (1975).
30. The 3.2-kb Bam HI fragment of pSW108 [S. C. Winans, P. Allenza, S. E. Stachel, K. E. McBride, E. W. Nester, *Nucleic Acids Res.* **15**, 825 (1987)], which contains the *virE* operon of pTiA6, was cloned into the Bam HI site of the IncW vector pUFR047 (*27*) to create pWD200. The plasmid pWD200 complemented various *virE* mutants for tumorigenesis and production of the VirE2 protein.
31. K. L. Piers, J. D. Heath, X. Liang, K. M. Stephens, E. W. Nester, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 1613 (1996).

32. We thank W. Deng for constructing pWD200 and pWD102; P. Christie and A. Binns for providing strains; and C. Manoil, B. Traxler, and K. Hughes for reading the manuscript. Supported by NIH grant GM32618 and by a predoctoral fellowship to K.J.F. from the University of Washington Committee for Plant-Molecular Integration and Function.

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Role of Postreplicative DNA Mismatch Repair in the Cytotoxic Action of Thioguanine

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It is proposed here that the delayed cytotoxicity of thioguanine involves the postreplicative DNA mismatch repair system. After incorporation into DNA, the thioguanine is chemically methylated by *S*-adenosylmethionine to form S^6-methylthioguanine. During DNA replication, the S^6-methylthioguanine directs incorporation of either thymine or cytosine into the growing DNA strand, and the resultant S^6-methylthioguanine–thymine pairs are recognized by the postreplicative mismatch repair system. Azathioprine, an immunosuppressant used in organ transplantation, is partly converted to thioguanine. Because the carcinogenicity of *N*-nitrosamines depends on formation of O^6-alkylguanine in DNA, the formation of the analog S^6-methylthioguanine during azathioprine treatment may partly explain the high incidence of cancer after transplantation.

Mercaptopurine and thioguanine are cytotoxic drugs used in the treatment of acute leukemia, and azathioprine, a pro-drug that is converted in vivo to mercaptopurine, is used for immune suppression in transplant surgery (*1*). The cytotoxicity of thioguanine and mercaptopurine involves changes in purine metabolism and, in the case of both drugs, the formation of 2′-deoxy-6-thioguanosine triphosphate and the incorporation of thioguanine into DNA (*1*). The delayed cytotoxicity and chromosome damage that are characteristic of these drugs are associated with this incorporation (*2*).

N-methyl-*N*-nitrosourea produces similar delayed cytotoxicity (*3*) and chromosome damage (*4*). An indication that the superficial resemblance between these poisons may have a deeper mechanistic basis has come from the observation that certain eukaryotic cells that are resistant to *N*-methyl-*N*-nitrosourea are also resistant to thioguanine (*5*, *6*). Both drugs produce few-

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er sister chromatid exchanges in the resistant than in normal cells (*7*). Investigation showed that these resistant cells lack a component of the postreplicative mismatch repair pathway (*8*) and has supported the earlier suggestion (*9*) that the toxicity of *N*-methyl-*N*-nitrosourea depends on (i) methylation of O^6 of guanine in DNA, (ii) miscoding of this methylated base during DNA replication to give O^6-methylguanine–T base pairs in the new DNA, and (iii) recognition of these pairs by the mismatch binding proteins hMSH2 and GTBP (hMutSα) (*10*).

Our investigation of the role of postreplicative mismatch repair in the toxicity of thioguanine began with the observation that the sulfur of thioguanine residues in DNA reacts readily with alkylating agents, including those, such as methyl iodide, that react by an S_N2 mechanism and do not react with O^6 of guanine (*11*). This raised the possibility that in vivo thioguanine in DNA might be methylated by *S*-adenosylmethionine (SAM), because SAM is known to act as a weak methylating agent (*12, 13*). An experiment in vitro (*14*) showed that SAM methylates DNA containing thioguanine (Fig. 1). The methylation was predominantly of the S^6 of thioguanine residues (Fig. 1), and the extent of methylation was about 38 times greater than the reported methylation of *N*7 of guanine in natural DNA under the same conditions (*13*).

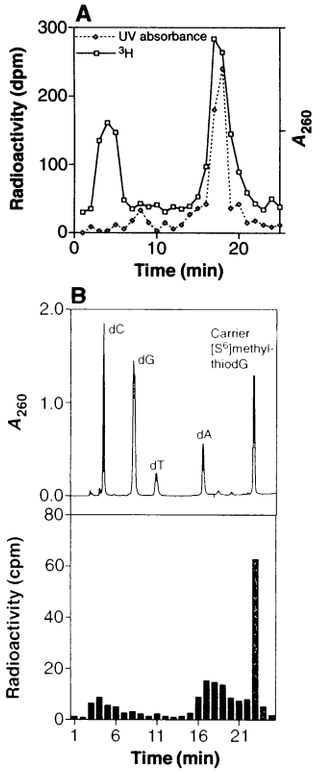

Fig. 1. Methylation in vitro of thioguanine in DNA by [^{3}H-methyl]SAM to form S^6-methylthioguanine (*14*). After the reaction, marker DNA containing S^6-methylthioguanine was added as carrier. This carrier DNA was separated from the original thioguanine-containing DNA by chromatography. Half the recovered carrier was rechromatographed. (**A**) shows that reaction between SAM and the thioguanine-containing DNA had produced a radioactive product that eluted from the column with the marker DNA containing S^6-methylthioguanine. The other half of the recovered carrier was hydrolyzed to nucleosides, and these were separated by HPLC. UV, ultraviolet; dpm, disintegrations per minute; A_{260}, absorbance at 260 nm. (**B**) shows that the radioactivity was in a nucleoside that eluted with the marker S^6-methylthiodeoxyguanosine ([S^6]methylthiodG); cpm, counts per minute.

Next, we monitored the incorporation of thioguanine and the formation of S^6-methylthioguanine in the DNA of cells in culture (*15*). A CHO cell line that is resistant to both *N*-methyl-*N*-nitrosourea and thioguanine (clone B) (*5*) was grown for 3 days in medium containing thioguanine. The medium was then replaced with fresh medium with L-[^{3}H-methyl]methionine but no thioguanine. After 26 hours, the cells were harvested. Analysis of the cellular DNA revealed that 3% of the guanine had been replaced by thioguanine, and 1.6 in 10^4 of these thioguanine residues had been methylated during the 26-hour incubation with [^{3}H]methionine. This is equivalent to 1.2 S^6-methylthioguanine residues per 10^6 nucleotides of DNA, a figure comparable to the amount of O^6-methylguanine (eight O^6-methylguanine residues per 10^6 nucleotides) produced by exposure of the same cell line to *N*-methyl-*N*-nitrosourea (375 μM for 30 min) (*7*). Comparison of the amount of O^6-methylguanine and S^6-methylthioguanine must take into account the fact that removal of the methyl group of S^6-methylthioguanine by the human DNA repair protein, O^6-alkylguanine–DNA–alkyltransferase, is 10^6 times slower (k = 79 M^{-1} s^{-1}) than the removal of the methyl group of O^6-methylguanine (k = 5.7 × 10^7 M^{-1} s^{-1}) (*16*).

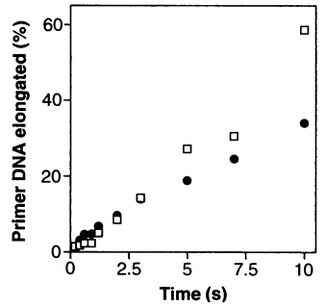

Fig. 2. Coding properties of S^6-methylthioguanine during DNA synthesis. The graph shows the incorporation by the Klenow fragment of *E. coli* DNA polymerase (*17*) of dCTP (□) or thymidine triphosphate (●) into the growing DNA strand directed by an S^6-methylthioguanine in the template DNA strand.

To determine the coding properties of S^6-methylthioguanine, DNA containing S^6-methylthioguanine was used as a template for DNA synthesis in vitro (*17*). Thymine and cytosine were incorporated opposite S^6-methylthioguanine at similar rates (Fig. 2). This miscoding is similar to that of O^6-methylguanine, except that under similar conditions thymine is incorporated opposite O^6-methylguanine four times faster than cytosine (*18*).

We then tested whether the mismatch binding protein complex hMutSα would bind to the S^6-methylthioguanine–T base pairs produced by this miscoding. When a HeLa cell extract was incubated with DNA containing one S^6-methylthioguanine–T pair (*19*) the mismatch was bound by a protein or proteins in the extract (Fig. 3). This appears to be recognition of the S^6-methylthioguanine–T base pairs by the hMutSα proteins of the postreplicative mismatch repair system because (i) the complex had the same mobility as the complex with DNA containing a G-T mismatch, (ii) DNA containing a G-T mismatch competed for the protein or proteins that bound an S^6-methylthioguanine–T mismatch, and (iii) DNA containing the S^6-methylthioguanine–T mismatch competed for the protein or proteins that bound a G-T mismatch (Fig. 3). Although there was generally little binding to DNA with an S^6-methylthiogua-

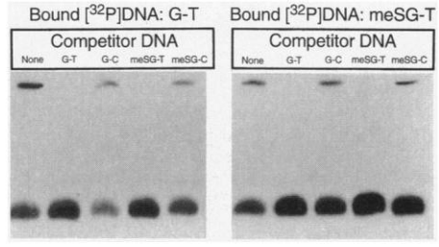

Fig. 3. Binding of HeLa cell proteins to DNA containing either a G-T (left panel) or an S^6-methylthioguanine–T (meSG-T) base pair (right panel) (*19*). The upper bands in the autoradiograph correspond to protein-bound DNA and the lower bands correspond to free DNA. The left panel shows that the protein binding of the G-T mismatch can be competed out with excess DNA containing either a G-T or meSG-T base pair but not by DNA containing a G-C or meSG-C base pair. The right panel shows that the binding of an meSG-T base pair can be competed out by excess DNA containing a meSG-T or G-T base pair but not by DNA containing a G-C or meSG-C base pair.

nine–C base pair, the proteins did bind to one DNA in which there was a cytosine 5′ to the S^6-methylthioguanine–C pair (the remaining sequence was the same as shown in the legend to Fig. 3). This raises the possibility that miscoding during DNA replication may not be necessary for recognition of the S^6-methylthioguanine in some sequence contexts.

These results and the fact that thioguanine produces sister chromatid exchanges (*20*), a type of chromosome damage associated with postreplicative mismatch repair (*21*), suggest that the methylation of thioguanine residues in DNA by *S*-adenosylmethionine, miscoding by this methylated base during DNA synthesis, and the recognition of the resultant S^6-methylthioguanine–T pairs by the postreplicative mismatch repair system are the basic steps in the delayed cytotoxicity of thioguanine and mercaptopurine. This would be an interesting example in which a DNA repair system mediates the cytotoxic action of a clinically important drug rather than defending against it. Griffin *et al.* (*22*) have shown that hMutSα binds thioguanine-T base pairs in DNA, but studies of the replication of DNA containing thioguanine (*23*) suggest that it does not miscode with sufficient frequency for the toxicity to be ascribed to thioguanine-T base pairs.

These results may help to explain the unusual prevalence of certain cancers among long-term survivors of organ transplantation (*24*). This has previously been ascribed to immune suppression. For some cancers, such as Kaposi's sarcoma, which are also common in immunodeficiency diseases such as acquired immunodeficiency syndrome (AIDS), this hypothesis is probably correct. However, most of these transplant patients had been treated with azathioprine and it has been suggested that some types of cancers that appear commonly after transplantation, but not in AIDS patients, might be the result of chemical carcinogenesis by azathioprine (*25*). Azathioprine is converted in vivo to thioguanine nucleotides (*25*), which could result in the formation of S^6-methylthioguanine in DNA. This is the analog of O^6-alkylguanine, the most important DNA modification in the carcinogenic action of the methylating *N*-nitroso compounds. Conceivably, then, the formation of S^6-methylthioguanine in DNA might induce these cancers either by mutagenesis or by the mechanism proposed by Karran and Bignami (*10*). These authors have suggested that because cells can avoid the cytotoxicity of methylating nitroso compounds by losing mismatch repair, chronic exposure to these compounds would select for cells with a mutator phenotype and thus predispose the animal to cancer. Because cells can also protect themselves against the cytotoxicity of thioguanine by losing mismatch repair, one might speculate that cancer in transplant patients may reflect loss of mismatch repair.

REFERENCES AND NOTES

1. G. B. Elion, *Science* **244**, 41 (1989).
2. D. M. Tidd and A. R. Paterson, *Cancer Res.* **34**, 738 (1974).
3. J. E. Plant and J. J. Roberts, *Chem. Biol. Interact.* **3**, 337 (1971).
4. A. Rasouli-Nia, Sibghat-Ullah, R. Mirzayans, M. C. Paterson, R. S. Day III, *Mutat. Res.* **314**, 99 (1994).
5. G. Aquilina, A. Zijno, N. Moscufo, E. Dogliotti, M. Bignami, *Carcinogenesis* **10**, 1219 (1989).
6. M. H. L. Green *et al.*, *ibid.*, p. 893.
7. G. Aquilina *et al.*, *Cancer Res.* **50**, 4248 (1990).
8. P. Branch, G. Aquilina, M. Bignami, P. Karran, *Nature* **362**, 652 (1993); A. Kat *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6424 (1993); G. Aquilina, P. Hess, S. Fiumicino, S. Ceccotti, M. Bignami, *Cancer Res.* **55**, 2569 (1995); P. Branch, R. Hampson, P. Karran, *ibid.*, p. 2304; M. T. Hawn *et al.*, *ibid.*, p. 3721.
9. M. Jones and R. Wagner, *Mol. Gen. Genet.* **184**, 562 (1981); P. Karran and M. G. Marinus, *Nature* **296**, 868 (1982).
10. P. Karran and M. Bignami, *Bioessays* **16**, 833 (1994).
11. Y.-Z. Xu, Q. Zheng, P. F. Swann, *Nucleosides Nucleotides* **14**, 929 (1995).
12. L. R. Barrows and P. N. Magee, *Carcinogenesis* **3**, 349 (1982).
13. B. Rydberg and T. Lindahl, *EMBO J.* **1**, 211 (1982).
14. A self-complementary dodecadeoxynucleotide CGCthioGAGCTCGCG (thioG indicates thioguanine) (4.6 units of absorbance at 260 nm), synthesized as described [Y.-Z. Xu, Q. Zheng, P. F. Swann, *Tetrahedron* **48**, 1729 (1992)], was incubated for 4 hours at 37°C in 550 μl of [^{3}H-methyl] SAM (1.2×10^{-5} M) in 0.15 M potassium cacodylate and 1 mM EDTA (pH 7). The solution was desalted on Bio-Gel P4 (Bio-Rad, Hercules, CA) to separate the DNA from the remaining *S*-adenosylmethionine. A dodecamer containing S^6-methylthioguanine was added as carrier and the DNA was chromatographed on MonoQ HR5/5 (Pharmacia) (*11*). Fractions of eluant containing the carrier S^6-methylthioguanine–DNA were desalted (with NAP 25, Pharmacia; then with Bio-Gel P4) and divided into two samples. The first sample was chromatographed again on MonoQ and yielded two peaks of radioactivity, the second of which eluted with the marker DNA containing S^6-methylthioguanine (the first peak was not identified but was believed to be either [^{3}H]SAM or a breakdown product of it). The other sample was hydrolyzed enzymically to nucleosides that were then separated by high-performance liquid chromatography (HPLC). A discrete peak of radioactivity was associated with the S^6-methylthiodeoxyguanosine marker; this represented 2.25 μmol of S^6-methylthiodeoxyguanosine per mole of thioguanine in the oligomer. In experiments with unmodified DNA under identical conditions, SAM has been found to produce 0.06 μmol of *N*7-methylguanine per mole of guanine (*13*).
15. The CHO cells were grown in Hams F12 (Gibco BRL, Uxbridge, UK) containing thioguanine (0.5 μg/ml), L-methionine (4.5 μg/ml), and 10% fetal calf serum. The medium was changed each day. After 3 days, the medium was replaced with fresh medium without thioguanine but with L-[^{3}H-methyl]methionine (24 μCi/ml of medium). After 26 hours, the cells were harvested and DNA was prepared from them (with a 100G tip; Qiagen, Dorking, UK). The amount of thioguanine in the DNA was estimated by the absorbance at 340 nm. The DNA was hydrolyzed enzymically to nucleosides, S^6-methylthiodeoxyguanosine was added as carrier, and the nucleosides were separated by HPLC as above. The amount of S^6-[^{3}H]methylthiodeoxyguanosine was estimated with the assumption that the specific radioactivity of the SAM pool would be the same as that of the methionine in the medium.
16. DNA duplexes [5′-^{32}P]GGCGCTXGAGGCGTG containing at position X either S^6-methylthioguanine (DNA concentration 250 nM) or O^6-methylguanine (DNA concentration 0.04 nM), both base-paired to C, were incubated with recombinant human O^6-alkylguanine–DNA–alkyltransferase (420 nM for S^6-methylthioguanine and 0.04 nM for O^6-methylguanine) in 100 μl of 50 mM tris-HCl (pH 7.6) buffer containing 10 mM dithiothreitol and bovine serum albumin (200 μg/ml). At intervals, samples were removed and the repaired demethylated DNA was separated from the substrate DNA by chromatography on MonoQ (*11*). The amount of repaired and remaining unrepaired DNA was determined by scintillation counting.
17. The DNA sequence 5′-CGCTACTTAXGCTATCGGATCT, where X is S^6-methylthioguanine, was synthesized and purified (*11*), and annealed to the primer strand 5′-[^{32}P]AGATCCGATAGC. A solution containing this duplex and the Klenow fragment of *Escherichia coli* DNA polymerase [0.2 μM DNA and 0.24 μM Klenow fragment (Pharmacia) in 40 μl of 2.5 mM EDTA and 50 mM tris-HCl (pH 7.4)] was mixed in a rapid reaction apparatus with an equal volume of 80 μM deoxycytidine triphosphate (dCTP) or 80 μM deoxythymidine triphosphate (dTTP) (Pharmacia) in 12.5 mM $MgCl_2$ and 50 mM tris-HCl (pH 7.4). At the desired time, the reaction was quenched in 60 μl of 0.3 M EDTA. The elongated primer strand was separated from the original primer by electrophoresis through a 20% denaturing polyacrylamide gel. The positions of the original and of the elongated [^{32}P]-labeled DNA were found by autoradiography and the corresponding bands were excised and quantified by scintillation counting.
18. H. B. Tan, P. F. Swann, E. M. Chance, *Biochemistry* **33**, 5335 (1994).
19. HeLa cell extracts were prepared as described [J. Jiricny, M. Hughes, N. Corman, B. B. Rudkin, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8860 (1988)]. Extract [0.39 mg of protein per milliliter of 20 mM Hepes (pH 7.5), 50 mM KCl, and 5 mM $MgCl_2$, containing 4% Ficoll 400] was incubated at 0°C with poly dI-dC, a DNA copolymer of alternating inosine and cytosine (31 μg/ml). After 15 min, a 34–base pair double-stranded DNA of sequence 5′-[^{32}P]AGCTTGGCTGCAGGGXGACGGATCCCCGGGAATT, where the nucleotide at X was either G–base-paired to T in the other strand or meSG–base-paired to T, was added to a final concentration of 0.5 nM together with a 40-fold excess of nonradioactive oligomer containing a G-C, G-T, meSG-C, or meSG-T base pair at position X. The incubation continued at room temperature for 20 min. Samples were then loaded directly onto a 6% polyacrylamide nondenaturing gel for electrophoresis (at 10°C at 8 V/cm for 2 hours). The gel was dried and then autoradiographed.
20. W. J. Bodell, *Mutagenesis* **6**, 175 (1991).
21. M. Radman, in *Genetic Recombination*, R. Kucherlapati and G. R. Smith, Eds. (American Society for Microbiology, Washington, DC, 1988), pp. 169–189.
22. S. Griffin, P. Branch, Y.-Z. Xu, P. Karran, *Biochemistry* **33**, 4787 (1994).
23. H. P. Rappaport, *ibid.*, **32**, 3047 (1993).
24. I. Penn, *Cancer Detect. Prev.* **18**, 241 (1994).
25. L. Lennard, S. Thomas, C. I. Harrington, J. L. Maddocks, *Br. J. Dermatol.* **113**, 723 (1985).

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