Human MutSα Specifically Binds to DNA Containing Aminofluorene and Acetylaminofluorene Adducts*

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Defects in mismatch repair are associated with several types of cancer. It is also generally believed that environmental carcinogens are responsible for the initiation of cancers by the induction of mutations in critical genes. Prior genetic studies have suggested that the mismatch repair system can also recognize certain forms of DNA damage such as O6-methylguanine and UV photoproducts, and, therefore, mismatch repair may play a role in environmental agent-induced carcinogenesis. To examine this hypothesis, hMutSα, a heterodimer which consists of hMSH2 and GTBP and participates in strand-specific mismatch repair, was tested for its ability to recognize DNA containing a site-specific C8-guanine adduct of aminofluorene (AF) or N-acetyl-2-aminofluorene (AAF). We show here that hMutSα specifically binds to both AF and AAF adducts. This binding requires both hMSH2 and GTBP. Results from competition and titration experiments indicate that the binding efficiency of hMutSα to AF and AAF is about 60% of that to a G-T mismatch, but is at least 10-fold that to an otherwise identical homoduplex DNA without the chemical modification. The specific binding of AF and AAF adducts by hMutSα suggests that strand-specific mismatch repair is involved in processing DNA damage induced by environmental carcinogens.

It is generally accepted that mutations are the primary cause of human cancer and that these can result from errors in DNA metabolism or from DNA damage induced by environmental carcinogens. To safeguard the fidelity of genetic information, both prokaryotes and eukaryotes possess mutation avoidance systems to remove these premutagenic lesions before a somatic mutation is induced. Methyl-directed, MutHLS-dependent mismatch repair in Escherichia coli is believed to be responsible for the fidelity of DNA replication or recombination (1). Recently, human mismatch repair has been demonstrated to play a crucial role in cancer avoidance (extensively reviewed in Refs. 2 and 3).

A major breakthrough in linking mismatch repair to human cancer was the observation of frequent alteration of simple repeat sequences (microsatellite) in tumors derived from patients with history of hereditary nonpolyposis colorectal carcinoma (HNPCC)3 (Refs. 2 and 3 and references therein). With microsatellite alteration as a clue, HNPCC susceptibility genes hMSH2 (4, 5), hMLH1 (6, 7), hPMS1, and hPMS2 (8) were rapidly identified. In agreement with the genetic analyses, in vitro biochemical assays have shown that tumor cells with microsatellite instability are defective in strand-specific mismatch repair (9–14). Further evidence that a mismatch repair defect contributes to genetic instability has been provided recently by the studies of PMS2 or MSH2 knockout mice in which the knockout animals acquire a hypermutable phenotype in microsatellite repeat sequences and are predisposed to cancer (15–17). In addition to its original description in colorectal cancer, microsatellite instability has also been documented in a substantial fraction of about 20 types of sporadic tumors (reviewed in Refs. 3 and 18). These hypermutable tumors may also be defective in mismatch repair.

Most chemical carcinogens are known to covalently react with DNA to form carcinogen-DNA adducts (19). Presumably, these adducts play an active role in human tumorigenesis by the induction of mutations in genes critical for control of cellular growth. The association of numerous human cancers with environmental carcinogen exposure and microsatellite instability suggests a role of mismatch repair in the processing of DNA damage caused by carcinogens. In fact, several lines of evidence indicate that mismatch repair proteins may be involved in repair of UV-induced pyrimidine dimers (20–22) and O6-methylguanine (O6-meG) adducts caused by alkylating agents (reviewed in Refs. 23 and 24).

To explore the possibility that environmental carcinogen adducts are processed by mismatch repair, we tested hMutSα, a human mismatch recognition protein (12), for its ability to bind to oligonucleotides containing a site-specific dG adduct of either 2-aminofluorene (AF) or N-acetyl-2-aminofluorene (AAF). AF and AAF are members of tumorigenic aromatic amines that widely exist in the environment as products of tobacco smoke, fuel combustion, dyes, and cooked meat. Therefore, these compounds have become model molecules for mechanistic studies of cancer induction by aromatic amines. Activated AF and AAF react with DNA in a high degree of specificity at the C8 position of guanine, forming major adducts of N-(2′-deoxyguanosine-8-yl)-2-aminofluorene (dG-AF) and N-(2′-deoxyguanosine-8-yl)-N-acetyl-2-aminofluorene (dG-AAF) (see Fig. 1), respectively (reviewed in Ref. 25). Extensive mutagenesis studies in vitro and in vivo have demonstrated that both AF and AAF adducts induce base substitution and frameshift mutations (Refs. 25 and 26 and references therein). We report here that similar to the interactions observed for mispairs, hMutSα specifically binds to DNA containing AF and AAF adducts, suggesting that mismatch repair may be involved in the processing of DNA damage caused by these carcinogens.

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§ The abbreviations used are: HNPCC, hereditary nonpolyposis colorectal carcinoma; AF, 2-aminofluorene; AAF, N-acetyl-2-aminofluorene; dG-AF, N-(2′-deoxyguanosine-8-yl)-2-aminofluorene; dG-AAF, N-(2′-deoxyguanosine-8-yl)-N-acetyl-2-aminofluorene; GTBP, G-T-binding protein; O6-meG, O6-methylguanine.
Binding of hMutSα to AF and AAF Adducts

MATERIALS AND METHODS

Cell Lines and Nuclear Extracts—HeLa S<sub>3</sub> cells were purchased from the National Cell Culture Center, Minneapolis, MN. Lymphoblastoid line MT1 (a gift from P. Modrich, Duke University, Durham, NC) was grown in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% horse serum (HyClone) as described (24). LoVo cells were grown in Dulbecco’s modified Eagle’s medium/Ham’s F-12 medium with 10% fetal bovine serum. Nuclear extracts from all cell lines were prepared as described (9, 27).

Construction of Oligonucleotide Duplexes Containing AF and AAF Adducts—The dodecamer 5'-d(TGATGAAACATG)-3' containing a site-specific dG-AAF adduct at the sixth G was synthesized as described (28). The oligonucleotides containing AF adduct were derived for hMutS<sub>0–4</sub> as described (12) with some modifications. In brief, the samples mixtures were incubated on ice for 20 min followed by addition of 5 µl of 50% sucrose and 10% (w/v) sucrose, frozen in liquid N<sub>2</sub>, and stored at −80 °C. Purified hMutSα retained full activity under these storage conditions for at least 3 months. SDS-polyacrylamide gel electrophoresis indicated that the purified protein is near homogeneity (>99% purity) and contained only two polypeptides, 160-kDa GTBP and 99% purity) and contained only two polypeptides, 160-kDa GTBP and 105-kDa hMSH2, based on Coomasie Brilliant Blue staining (data not shown).

Band Shift Assay—Unless otherwise specified, band shift assays were performed in 25-µl reactions containing 0.5 pmol of 32P-labeled oligonucleotide duplexes, 66 ng (about 0.25 pmol) of purified hMutSα, or 10 µg of crude nuclear extracts, 10 µl Hepes-KOH (pH 7.5), 110 µM KCl, 1 mM EDTA, 1 mM dithiothreitol, and 0.4% glycerol. Reaction mixtures were incubated on ice for 20 min followed by addition of 5 µl of 50% sucrose (w/v). The samples were then fractionated at room temperature through a 6% nondenaturing polyacrylamide gel in 6.7 mM Tris acetate (pH 7.5) and 1 mM EDTA with buffer recirculation. Bands were detected by autoradiography.

RESULTS

Human MutSα Binds to Oligonucleotides Containing Either an AAF or AF Adduct—Human MutSα is a heterodimer comprised of hMSH2 and GTBP polypeptides. This heterodimer is capable of binding to both base-base mismatches and small insertion/deletion mispairs and participates in human strand-specific mismatch repair (12, 30). To explore the role of mismatch repair in chemical carcinogenesis, purified hMutSα was examined for its ability to recognize a 31-mer oligonucleotide duplex containing either a site-specific AF or AAF adduct using a band shift assay. Most spectral and theoretical studies suggest that even though the AF and AAF adducts (Fig. 1) produce distortion in the DNA helix, oligonucleotide duplexes containing these adducts form a stable structure in solution (31–33). Since hMutSα binds efficiently to 31-mer oligonucleotide duplexes containing mispairs (12), the adduct-containing 12-mers were elongated to 31-mers by primer extension (see “Materials and Methods”). The AF- and AAF-modified 31-mer mispairs were identified in the bulky adduct (28). As shown in Fig. 2, purified hMutSα binds to oligonucleotide duplexes containing either AF adduct or AAF adduct (duplex I in Fig. 1) to a similar extent as it binds a oligonucleotide heteroduplex containing a G-T mismatch (duplex III in Fig. 1). This binding is at least 10-fold more than the nonspecific binding observed with an otherwise identical G-C homoduplex not containing an adduct (duplex II in Fig. 1). The binding of hMutSα to AF and AAF adducts was inhibited when 1 mM ATP was present in the reaction. ATP was also found to block the interactions of hMutSα with the G-T mismatch (Fig. 2) and other mispairs (12). The findings suggest that mismatches and the chemical adducts are recognized by hMutSα through a similar mechanism.

Human MutSα Binds to G-T Mismatch More Efficiently Than to an AAF or AF Adduct—To determine the binding efficiency of hMutSα to the modified DNA, increasing amounts of purified hMutSα were allowed to bind to the G-T mismatch and AAF-modified heteroduplexes. As expected, the bound fractions in each case increase proportionally as the concentration of hMutSα increases (Fig. 3, A and B). However, the amount bound for the AAF-modified duplex is approximately half that observed for the G-T mismatch (Fig. 3B), indicating that the binding affinity of hMutSα to a G-T mispair is higher than to an AAF adduct. In the case of G-T mismatch, 0.5 pmol and 1.0 pmol of hMutSα was required to shift 0.25 and 0.5 pmol of substrate (Fig. 3B), respectively, suggesting a molar ratio of hMutSα:G-T mismatch at 2:1.

Interestingly, when high concentrations of hMutSα were used to interact with AAF, a second band (complex II, Fig. 3A)
was observed which was smaller than the standard hMutSα-heteroduplex complex (complex I). Although this complex was observed for the G-T mismatch, it is much stronger for the AAF substrate (Fig. 3A). Since the similar bands were also observed in the interaction of recombinant hMSH2 with insertion/deletion mispairs (34), we performed a Western analysis to determine if the hMSH2 subunit of hMutSα is responsible for the activity. As shown in Fig. 3C, antibodies against hMSH2 recognize only complex I but not complex II, and, therefore, hMSH2 is not the protein causing this faster moving band. Whether or not this band shift is due to the binding of the GTBP subunit is unknown. Finally, results similar to that obtained for the AAF adduct were obtained when AAF substrate was titrated with hMutSα (data not shown).

To further test the specificity of hMutSα to AF and AAF adducts, we performed competition studies in which either an unlabeled G-T heteroduplex or an unlabeled A-T homoduplex was included in the band shift assays. This unlabeled G-T heteroduplex contained a mismatch located in the middle of the sequence (duplex IV in Fig. 1), which has previously been shown to be bound by hMutSα with a high efficiency (12). As expected, the level of bound DNA for all the substrates decreased as the concentration of the cold G-T mispair was increased. When an equimole (0.5 pmol) of the competitor was present, approximately 40% of the complex remained while a 2-fold excess resulted in a 6-fold (for G-T substrate) or more (for AF and AAF substrates) reduction (Fig. 4A). Theoretically, such a 2:1 ratio should give a 67% reduction if both heteroduplexes possess identical affinities for hMutSα. These results indicate that a mismatch in the middle of the sequence is a better substrate for hMutSα than a heteroduplex which contains a mismatch near the terminus.

Fig. 4B shows competition using an A-T homoduplex (duplex V in Fig. 1). As expected, in the presence of 0.5 pmol of cold A-T homoduplex, the bound complex of 32P-labeled G-C homodu-
Binding of hMutSα to AF and AAF Adducts

**Fig. 5.** Human MutSα holoenzyme-dependent binding of AF and AAF heteroduplexes. Purified hMutSα (66 ng), or crude nuclear extracts (10 μg) derived from GTBP-defective MT1 cells, hMSH2-defective LoVo cells, and mismatch repair-proficient HeLa cells were used in band shift assays (see “Materials and Methods”) as indicated.

Tracts was reduced by 50%, but there was no significant change in labeled AAF, AF, or G-T heteroduplex complexes. A 50% inhibition of the bound 32P-labeled heteroduplexes required the addition of an approximately 20-fold excess of cold homoduplex (Fig. 4B). Finally, addition of a 20-fold (10 pmol) excess of cold G-T mismatch almost eliminates the interaction of hMutSα with all 32P-labeled substrates (Fig. 4A), whereas even a 100-fold excess of A-T homoduplex could not completely eliminate binding to the AAF, AF, or G-T heteroduplexes (data not shown). Taken together, these results show that the AAF and AF adducts are specifically recognized by hMutSα as heteroduplexes.

Binding of hMutSα to AAF or AF Adducts and a Mismatch Requires Two Subunits—Recombinant hMutSα and hMutSα (12, 30) have been shown to bind to both base-base mismatches and insertion/deletion mispairs. However, nuclear extracts derived from GTBP-deficient MT1 (36) are proficient in repair of 2–4 nucleotide insertion/deletion mispairs, but are deficient in processing base-base and single nucleotide insertion/deletion mispairs (12). Nuclear extracts of LoVo cells, harboring defects in both alleles of the hMSH2 gene, are defective in repair of all heteroduplexes tested (10, 12). These findings suggest that hMSH2 plays a crucial role in recognition of all mismatches while GTBP is largely responsible for identifying base-base mismatches.

To test if duplexes containing the AAF or AF adduct can be recognized by individual subunits of hMutSα, band shift assays were performed using nuclear extracts derived from mismatch repair mutant lines LoVo and MT1 as well as the repair-proficient HeLa line. As shown in Fig. 5, several common bands are observed in all three nuclear extracts following reaction with AAF and AF adducts and with a G-T mismatch. Unlike the binding of hMutSα to mismatches, the binding of these proteins to the heteroduplex is not influenced by the presence of ATP. Western blots indicate that none of the bands detected in nuclear extracts of MT1 and LoVo are due to binding by hMSH2 (data not shown). In fact, the common bands shown in all three extracts are nonspecific because these proteins also bind to a G-C homoduplex (data not shown). However, the band corresponding to the complex of heteroduplex and hMutSα (right two lanes in Fig. 5) was observed in the HeLa nuclear extract, but apparently not observed in the two mutant extracts (Fig. 5). Also, in the HeLa extract, this band disappears when 1 mM ATP was added to the reaction (Fig. 5). Finally, Western analysis indicates that hMSH2 antibodies recognize this band (data not shown), confirming that it is the complex of hMutSα and adduct-containing oligonucleotides. These results suggest that recognition of AF and AAF adducts requires both subunits of the hMutSα.

**DISCUSSION**

Environmental carcinogen exposure has been known for decades to be associated with a variety of types of human cancer. It is generally believed that these carcinogens exert their carcinogenic effects by covalently modifying genomic DNA to form DNA adducts that induce mutations to initiate carcinogenesis. Fortunately, cells possess DNA repair systems to remove these premutagenic lesions.

One of the first systems identified to process bulky DNA adduct is the nucleotide excision repair pathway that removes a variety of DNA adducts including UV-induced pyrimidine dimers and chemical carcinogen-induced DNA lesions (37, 38). Since carcinogen adducts play an active role in carcinogenesis, one would expect that defects in nucleotide excision repair would result in cancer predisposition. Among cancer syndromes, at present only xeroderma pigmentosum has been found to be associated with a defect in nucleotide excision repair (37, 38), suggesting that genetic defects in other repair pathways may also be involved in tumorigenesis.

Defects in mismatch repair have clearly been demonstrated to be associated with cancer syndromes (39). Although direct evidence has been lacking, implication of mismatch repair in the processing of certain types of DNA damage has been previously documented. Mellon and co-workers (21, 22) have recently demonstrated that cells defective in MutS or MutL function are also defective in transcription-coupled nucleotide excision repair, where UV-induced cyclobutane pyrimidine dimers are preferentially removed from transcribed strands of active genes. Feng et al. (20) showed that UV-stimulated recombinant frequencies were about twice as high in mismatch repair mutant cells as observed in wild type cells. Since MutS and MutL proteins block RecA-mediated strand transfer between DNAs with divergent sequences (40), the low frequency of UV-stimulated recombination in wild-type cells may be attributed to the inhibition of the process by mismatch repair components. Finally, mismatch repair has been postulated to play a role in the removal of alkylated DNA adducts such as O6-methylguanine (reviewed in Ref. 23). Taken together, these data suggest that mismatch repair is not only responsible for correcting mismatches derived from errors in DNA metabolism, but is also involved in the processing of DNA adducts induced by environmental carcinogens.

Our results here show that hMutSα is capable of recognizing deoxyguanosine adducts of AAF and AF in duplex DNA. The interaction between hMutSα and the DNA adducts is inhibited by ATP, which is similar to the interaction of hMutSα with mispairs (12). The ATP inhibition suggests that the protein binds to mispairs and to the carcinogen adducts through a similar mechanism. It is generally believed that all mispairs (including base-base mismatches and small insertion/deletion mispairs) adopt polymorphic (at least two) conformations which more or less destabilize DNA structure depending on different mispairs and their neighboring bases (reviewed in Ref. 41). Similar to the mismatches, the dG-AF and dG-AAF adducts have been suggested to exist in at least two conformations, with the fluoresence ring either inserted into the helix (base displacement) or located on the exterior of the helix although the AF structure (see Fig. 1A) produces less distortion in the DNA helix than the closely related AAF adduct (reviewed in...
The extent of DNA distortion by these adducts is also dependent on the sequence within which the adduct is located. Therefore, it is possible that one or all of these structures may mimic structures caused by base-base mismatch or an insertion/deletion mispair and that it is these common features in these structures that are recognized by the MutS homologs.

It is known that mismatch recognition by MutS or its homologs is the initiation step of the mismatch repair pathway, which leads to the correction of base-base mismatches or small mispairs. Therefore, it is possible that one or all of these structures may mimic structures caused by a base-base mismatch or an insertion/deletion mispair by the system in both prokaryotes and eukaryotes. Therefore, the binding of hMutSα to AF and AAF adducts in duplex DNA supports direct participation of human mismatch repair in the processing of DNA damage caused by chemical carcinogens, although further biochemical as well as biological studies are required to address this question.

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REFERENCES

1. Modrich, P. (1991) Annu. Rev. Genet. 25, 229–253
2. Kolodner, R. D. (1995) Trends Biochem. Sci. 20, 397–401
3. Modrich, P., and Lahue, R. (1996) Annu. Rev. Biochem. 65, 101–133
4. Fishel, R., Lescoe, M. K., Rao, M. R., and Lescoe, M. K. (1995) Cell 79, 1027–1038
5. Leach, F. S., Nicolaides, N. C., Padopouloos, N., Liu, B., Jen, J., Parsons, R., Peltonaki, P., Sistonen, P., Ashtonen, L. A., Nyström-Lahni, M., Guan, X.-Y., Zhang, J., Melzer, P. S., Yu, J.-W., Kao, F.-T., Chen, D. J., Cersoaletti, K. M., Fournier, R. E. K., Todd, S., Lewis, T., Leach, R. J., Naylor, S. L., Weissenhach, J., Mecklin, J. P., Jørgensen, H., Petersen, G. M., Hamilton, S. R., Green, J., Janss, J., Watson, P., Lynch, H. T., Trent, J. M., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. (1993) Cell 75, 1215–1225
6. Brunner, C. E., Baker, S., Morrison, P. T., Warren, G., Smith, L. G., Lescoe, M. K., Kane, M., Karsabin, C., Liford, J., Lindblom, A., Tannergard, P., Hamilton, S. R., Petersen, G. M., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. (1994) Science 260, 528–526
7. Padopouloos, N., Nicolaides, N. C., Wei, Y.-F., Ruben, S. M., Carter, K. C., Rosen, C. A., Hasseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D., Venter, J. C., Jun, J., H. T., Peltonaki, P., Mecklin, J. P., de la Chapelle, A., Kinzler, K. W., and Vogelstein, B. (1994) Science 263, 1625–1629
8. Nicolaides, N. C., Padopouloos, N., Liu, B., Wei, Y.-F., Carter, K. C., Ruben, S. M., Rosen, C. A., Hasseltine, W. A., Fleischmann, R. D., Fraser, C. M., Adams, M. D., Venter, J. C., Unipol, M. G., Hamilton, S. R., Petersen, G. M., de la Chapelle, A., Vogelstein, B., and Kinzler, K. W. (1994) Nature 371, 78–80
9. Parsons, R., Li, G. M., Longley, M. J., Fang, W. H., Padopouloos, N., Jen, J., de la Chapelle, A., Kinzler, K. W., Vogelstein, B., and Modrich, P. (1993) Cell 75, 1227–1236
10. Umar, A., Boyer, J. C., Thomas, D. C., Nguyen, D. C., Risinger, J. I., Boyd, J., Ionov, Y., Peruchio, M., and Kunkel, T. A. (1994) J. Biol. Chem. 269, 14367–14370
11. Risinger, J. I., Umar, A., Barrett, J. C., and Kunkel, T. A. (1995) J. Biol. Chem. 269, 15183–15186
12. Drummond, J. T., Li, G.-M., Longley, M. J., and Modrich, P. (1995) Science 268, 1099–1212
13. Boyer, J. C., Umar, A., Risinger, J. I., Lipford, J. R., K. M., Yan, S., Barrett, J. C., Kolodner, R. D., and Kunkel, T. A. (1995) Cancer Res. 55, 6603–6607
14. Risinger, J. I., Umar, A., Boyer, J. C., Evans, A. C., Berchuck, A., Kunkel, T. A., and Barrett, J. (1995) Cancer Res. 55, 5664–5669
15. Baker, S. M., Brunner, C. E., Zhang, L., Plug, A. W., Rohnback, Z., Warren, G., Elliott, E. A., Yu, J., Ashley, T., Arnheim, N., Flavell, R. A., and Liskay, R. M. (1995) Cell 82, 309–319
16. de la Chapelle, A., Dekker, M., Beres, A., Radman, M., and Riele, H. (1995) Cell 82, 321–330
17. Reitmair, A. H., Schmitz, R., Ewel, A., Bapat, B., Redston, M., Mitri, A., Waterhouse, P., Mittrucker, H. W., Wakeham, A., Liu, B., Thomas, A., Grieser, H., Gasser, S., Ballhausen, W. G., Fishel, R., and Mak, T. W. (1995) Nat. Genet. 11, 64–70
18. Essleman, J. R., and Markowitz, S. D. (1995) Curr. Opin. Oncol. 7, 83–89
19. Friedberg, E. C., Walker, G. C., and Siede, W. (1995) DNA Repair and Mutagenesis, pp. 19–47, ASM Press, Washington, D. C.
20. Feng, W. Y., Lee, E. H., and Hays, J. B. (1991) Genetics 129, 1071–1020
21. Mellon, I., and Champe, G. N. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 1292–1297
22. Mellon, I., Rajapal, D., Koi, M., Boland, R., and Champe, G. (1996) Science 272, 557–560
23. Karran, P., and Bignami, M. (1992) Methods Enzymol. 206, 465–498
24. Thomas, D. C., Veaute, X., Kunkel, T. A., and Fuchs, R. P. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 7752–7756
25. Holmes, J., Clark, S., and Modrich, P. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 5837–5841
26. Thomas, D. C., Veaute, X., Kunkel, T. A., and Fuchs, R. P. (1994) Nat. Genet. 21, 851–855
27. Zhou, Y., and Romano, L. J. (1993) Biochemistry 32, 14043–14052
28. Shiibutani, A., Gentles, R., Johnson, F., and Grollman, A. P. (1991) Carcinogenesis 12, 813–818
29. Palombo, F., Gallinari, P., Jaccarino, L., Lettieri, T., Hughes, M. D’Arrigo, A., Truong, O., Husan, J. J., and Jiricny, J. (1995) Science 268, 1912–1914
30. O’Wendler, S., Sanford, D., Yu, S., Lasker, R. A., Broyde, S., and Krugh, T. (1993) Biochemistry 32, 2481–2487
31. Kumar, A., Haji, M., K txt, J. J., and Jones, J. (1994) Biochemistry 33, 7462–7476
32. Whelan, C., Dhalluin, C., Puch, P. F., and Levente, J. F. (1994) Nucleic Acids Res. 22, 4646–4652
33. Fishel, R., Ewel, A., Lee, S., Lescoe, M. K., and Griffith, J. (1994) Science 266, 3238–3241
34. Fishel, R., Ewel, A., and Lescoe, M. K. (1994) Cancer Res. 54, 5539–5542
35. Padopouloos, N., Nicolaides, N. C., Liu, B., Parsons, R. E., Palombo, F., D’Arrigo, A., Markowitz, S., Wilson, J. K. V., Kinzler, K., Jiricny, J., and Vogelstein, B. (1995) Science 268, 1914–1917
36. Hanawalt, P. (1994) Science 266, 1957–1958
37. Sancar, A. (1994) Science 266, 1954–1956
38. Modrich, P. (1994) Science 266, 1959–1960
39. Worth, L., Jr., Clark, S., Radman, M., and Modrich, P. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 3238–3241
40. Fazakerley, G. V., and Bouard, Y. (1995) Methods Enzymol. 261, 145–163
