Nuclear Translocation of Mismatch Repair Proteins MSH2 and MSH6 as a Response of Cells to Alkylating Agents

Received for publication, June 20, 2000, and in revised form, August 16, 2000
Published, JBC Papers in Press, August 22, 2000, DOI 10.1074/jbc.M005377200

Markus Christmann and Bernd Kaina

From the Division of Applied Toxicology, Institute of Toxicology, University of Mainz, Obere Zahlbacher Strasse 67, D-55131 Mainz, Germany

Mammalian mismatch repair has been implicated in mismatch correction, the prevention of mutagenesis and cancer, and the induction of genotoxicity and apoptosis. Here, we show that treatment of cells specifically with agents inducing O\textsuperscript{6}-methylguanine in DNA, such as N\textsuperscript{-}methyl-N\textsuperscript{-}nitro-N\textsuperscript{-}nitrosoguanidine and N\textsuperscript{-}methyl-N\textsuperscript{-}nitroso-derivatives, elevates the level of MSH2 and MSH6 and increases GT mismatch binding activity in the nucleus. This inducible response occurs immediately after alkyla-
tion, is long-lasting and dose-dependent, and results from translocation of the preformed MutS\textalpha complex (composed of MSH2 and MSH6) from the cytoplasm into the nucleus. It is not caused by an increase in MSH2 gene activity. Cells expressing the DNA repair protein O\textsuperscript{6}-methylguanine-DNA methyltransferase (MGMT), thus having the ability to repair O\textsuperscript{6}-methylguanine, showed no translocation of MutS\textalpha, whereas inhibition of MGMT by O\textsuperscript{6}-benzylguanine provoked the transloca-
tion. The results demonstrate that O\textsuperscript{6}-methylguanine les-
sions are involved in triggering nuclear accumulation of MSH2 and MSH6. The finding that treatment of cells with O\textsuperscript{6}-methylguanine-generating mutagens results in an increase of MutS\textalpha and GT binding activity in the nucleus indicates a novel type of genotoxic stress response.

Mammalian DNA mismatch repair is implicated in the repair of DNA base mismatches arising from spontaneous base instability and during replication (1). Mismatch repair is associated with the prevention of mutagenesis and cancer (2, 3) and the induction of genotoxicity and apoptosis (4–6). The mismatch repair system is able not only to detect and repair mismatches derived from spontaneous base modification and replication but can also process chemically induced DNA damage. An important group of chemicals associated with mismatch repair are alkylating mutagens. These agents, such as N\textsuperscript{-}methyl-N\textsuperscript{-}nitro-N\textsuperscript{-}nitrosoguanidine (MNNG)\textsuperscript{1} and N\textsuperscript{-}meth-
yl-N\textsuperscript{-}nitrosourea (MNU), cause methylation in the O\textsuperscript{6} position of guanine. The resulting O\textsuperscript{6}-methylguanine (O\textsuperscript{6}-MeG) pairs with thymine instead of cytosine, thus leading to GC \rightarrow AT transition mutations (7–9). O\textsuperscript{6}-MeG paired with thymine, as well as various other mutagen-induced lesions such as 1,2-
 intrastrand d(GpG) cross-links generated by cisplatin, are subject of repair by the mismatch repair system (10, 11). In the case of O\textsuperscript{6}-MeG-generating agents, which are most powerful mutagens and carcinogens, a lack of mismatch repair confers resistance to cytotoxicity and thus increases the mutagenic response of cells (12–14); this implies that genotoxic and cyto-
toxic effects of O\textsuperscript{6}-MeG are mediated by mismatch repair. This could occur by erroneous mismatch repair cycles because of repeated misincorporation of thymine opposite O\textsuperscript{6}-MeG or by direct signaling of apoptotic functions due to faulty mismatch repair activity. Since O\textsuperscript{6}-MeG is a highly mutagenic and geno-
toxic lesion, its repair by the repair protein O\textsuperscript{6}-methylguanine-
DNA methyltransferase (MGMT) prior to replication is highly important for avoiding mutagenic, carcinogenic, and genotoxic effects. MGMT activity is highly variable among different tissues and cell types, and numerous cases (including cell lines, tumors, and knockout mice) have been reported unable to re-
pair O\textsuperscript{6}-MeG (15, 16). In these cases, O\textsuperscript{6}-MeG-triggered cell death via mismatch repair might be advantageous for the organ-
ism as it lowers alkylation-induced mutation pressure.

Mismatch repair of O\textsuperscript{6}-methylguanine-thymine base pairs is initiated by the binding of a protein complex (designated as MutS\textalpha) to the mismatch (17). This complex is composed of the bacterial MutS homologous proteins MSH2 and MSH6 (18), which is also known as GT-binding protein. In addition, MSH2 can also form a complex with the mismatch repair protein MSH3, designated MutS\textbeta (19, 20). Depending on the binding partner, the heterodimers have different substrate specifici-
ties and therefore play different roles in mismatch repair. Thus, the MutS\textalpha complex (MSH2 and MSH6) is able to bind to base-base mismatches and to insertion/deletion mismatches (21–23) in contrast to the MutS\textbeta complex (MSH2 and MSH3), which is capable of binding to insertion/deletion mismatches. Upon binding to the mismatch, MutS\textalpha associates with another heterodimeric complex (MutL\textalpha), consisting of the MutL homol-
ogous mismatch repair proteins MLH1 and PMS2 (24).

Whereas the function of the individual mismatch repair protein

\textsuperscript{1} This work was supported by the Deutsche Forschungsgemeinschaft (SFB 519/B4). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\textsuperscript{‡} To whom correspondence should be addressed. Tel.: 0049-6131-393-3246; Fax: 0049-6131-393-3421; E-mail: Kaina@mail.uni-mainz.de.

\textsuperscript{1} The abbreviations used are: MNNG, N\textsuperscript{-}methyl-N\textsuperscript{-}nitro-N\textsuperscript{-}nitrosoguanidine; MNU, N\textsuperscript{-}methyl-N\textsuperscript{-}nitrosourea; ENU, N\textsuperscript{-}ethyl-N\textsuperscript{-}nitroso-
rauccurine; MMS, methyl methanesulfonate; O\textsuperscript{6}-MeG, O\textsuperscript{6}-methylguanine; O6-BG, O\textsuperscript{6}-benzy-
guanine; MGMT, O\textsuperscript{6}-methylguanine-DNA methyltrans-
ferase; MMR, mismatch repair; PBS, phosphate-buffered saline; DTT, dithiothreitol; PMSP, phenylmethylsulfonfluoride; EMSA, electrophoretic mobility shift assay; CAT, chloramphenicol acetyltrans-
ferase; NLS, nuclear localization signal; MAPK, mitogen-activated pro-
tein kinase; h, human (e.g. hMSH2, hERK2).
Nuclear Translocation of MSH2/MSH6 as Cellular Response to Alkylation

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—The cell lines used (H4IIE, H5, BK4, E, HeLa MR, HeLaS3, and HeLa MR-adsC3) were grown at 6% CO2, 37 °C in F12/Dulbecco’s minimal essential medium containing 5% fetal calf serum. DLD1 cells were grown at 6% CO2, 37 °C in F12/Dulbecco’s minimal essential medium containing 10% fetal calf serum.

**Protein Extracts**—For preparing whole cell extracts, cells were washed twice with ice-cold phosphate-buffered saline (PBS), harvested, resuspended in buffer (20 mM Tris-HCL, pH 8.5, 1 mM EDTA, 2 mM DTT, 0.5 mM PMSF, 5% glycerol) and sonicated on ice with a Branson sonifier (Branson, Dunbury, CT) at 30 kHz, three times for 15 s. The homogenates were centrifuged (10,000 × g for 10 min at 4 °C), and the clear supernatants were stored frozen at −80 °C. For preparation of nuclear extracts, cells were suspended in 1 ml of lysis buffer I (10 mM Tris-HCL, pH 7.4, 40 mM NaCl, 3 mM MgCl2, 0.5 mM PMSF, 2 mM DTT) and incubated for 10 min on ice. After the addition of Nonidet P-40 (final concentration 0.5%) the solution was vortexed, incubated on ice for 5 min, and centrifuged (400 × g, 5 min). Thereafter, the pellet was washed again with lysis buffer I. To isolate nuclear extracts suitable for EMSA, pellets were resuspended in 2 volumes of lysis buffer II (20 mM HEPES-KOH, pH 7.4, 600 mM KCl, 0.2 mM EDTA, 0.5 mM PMSF, 2 mM DTT) and incubated for 30 min on ice. After centrifugation, the supernatant was diluted by the addition of 1 volume of lysis buffer III (20 mM HEPES-KOH, pH 7.4, 0.2 mM EDTA, 0.5 mM PMSF, 2 mM DTT). Glycerol was added to a final concentration of 20%, and aliquots were shock-frozen in liquid nitrogen and stored at −80 °C. The amount of protein was determined as described (28). To isolate nuclear extracts for Western blot analysis, pellets were resuspended in 150 μl of lysis buffer IV (20 mM Tris-HCL, pH 7.4, 40 mM Na3PO4, 5 mM MgCl2, 50 mM NaF, 100 μM Na3VO4, 10 mM EDTA, 1% Triton X-100, 1% SDS) and sonicated. After centrifugation, the amount of protein in the supernatant was determined. Extracts were stored at −80 °C. For preparing cytoplasmic protein extracts, cells were harvested as described above. The cell pellets were resuspended in 1 ml of RIPA buffer and incubated for 10 min on ice. After the addition of Nonidet P-40 (final concentration 0.5%), the solution was vortexed, incubated on ice for 2 min, and centrifuged (10,000 × g, 2 min). The supernatant was centrifuged again to remove traces of nuclei. The amount of protein in the supernatant was determined as described, and extracts were stored at −80 °C.

**Mismatch Repair Assays**—For gel retardation assays, 29-nucleotide oligomers with the general sequence 5′-GGGGATCGAGGCGACTGTCAGTGAATCT-3′ were annealed to oligomers with the general sequence 5′-GGGGATCTACTAGNAGCTGCAGCTCGAG-3′ (n = C or T) and labeled with [γ-32P]ATP using polynucleotide kinase. An aliquot of 4 μg of nuclear proteins was incubated in 10% glycerol, 10 mM HEPES-KOH, pH 7.9, 50 mM KCl, 4 mM MgCl2, 4 mM Tris-HCL, 0.5 mM DTT, 0.5 mM EDTA, 1 μg of bovine serum albumin, 1 μg polyclonal antibody as described under “Experimental Procedures” and subjected to EMSA analysis.

**CAT Assays**—The human MSH2 promoter was cloned from HeLa S3 DNA as described (29). For transient transfection, 105 cells were seeded 16-18 h earlier on 10-cm dishes. Cells were transfected 1 day later with 10 μg of hMSH2 promoter-CAT construct, using the calcium phosphate co-precipitation method (30). Cells were incubated 36 h after transfection with mutagens, and 6 h later, cell extracts were prepared and the amount of CAT protein per cell extract protein was determined by the CAT ELISA kit from Roche Molecular Biochemicals.

**Immunoprecipitation**—For preparing nuclear protein extracts, cells were harvested as described above. The cell pellets were resuspended in 1 ml of RIPA buffer (10 mM Tris-HCL, pH 8.0, 140 mM NaCl, 1 mM PMSF, 2 mM DTT) and incubated for 10 min on ice. After the addition of Nonidet P-40 (final concentration 0.5%), the solution was vortexed, incubated on ice for 5 min, and centrifuged (400 × g, 5 min). The pellet was washed and resuspended in 1 ml of RIPA buffer. Thereafter, Triton X-100 (final concentration 1%) and SDS (final concentration 0.1%) were added, and samples were incubated for 10 min on ice. For co-immunoprecipitation experiments, instead of Triton X-100 and SDS, Nonidet P-40 was added (0.5%) and incubated for 20 min on ice and centrifuged (10,000 × g, 10 min). The supernatant was incubated on ice for 30 min together with 20 μl of protein G and centrifuged (400 × g, 2 min) to remove proteins that unspecifically bind protein G. The supernatant was incubated on ice together with 15 μl of the specific antibody. 1 h later, 25 μl of protein G was added and incubated for an additional 2 h. The protein-antibody complex was isolated by centrifuging it five times (400 × g, 2 min), washing it in 1 ml of RIPA buffer, and subjecting it to Western blot analysis. For preparation of cytoplasmic protein extracts, cells were harvested as described above. The cell pellets were resuspended in 1 ml of RIPA buffer and incubated for 10 min on ice. After the addition of Nonidet P-40 (final concentration 0.5%), the solution was vortexed, incubated on ice for 2 min, and centrifuged (10,000 × g, 2 min). The supernatant was centrifuged to remove traces of nuclei and incubated with protein G together with the specific antibody as described above.

**Immunofluorescence**—Cells were grown on coated glass slides in 10-cm dishes. 2 h after treatment with MNNG, cells were fixed for 30 min in PBS containing 4% paraformaldehyde and 0.2% Triton X-100. After several rinses in cold PBS, cells were preincubated for 30 min in PBS containing 1% bovine serum albumin. After additional rinses in cold PBS, cells were incubated for 2 h at 4 °C with the primary antibody anti-MSH2 or anti-MSH6 (1:500) and thereafter rinsed again in PBS. After incubation with a rhodamine-conjugated anti-mouse IgG (Dako) and washing in PBS, cells were analyzed by fluorescence microscopy.
were treated with 25 μM MMS, which induces only very low amounts of O6-alkylguanine in DNA. The MSH2 protein level was not enhanced, however, after exposure to Western blot analysis. H4IIE and H5 are rat hepatoma cells expressing wild-type p53, and E cells are p53-deficient (knockout) cells. HeLa MR cells were included for comparison.

RESULTS

Alkylating Agents Induce Increase in Nuclear Level of MSH2—Initially, we observed that treatment of HeLa MR cells with a methylating agent such as MMS induces an increase in the level of GT binding activity in nuclear extracts (Fig. 1A). A major component of the GT binding complex is MSH2. To determine whether induction of GT binding activity is caused by an increase in the level of MSH2, we quantified this mismatch repair protein in nontreated and methylated-exposed cells. As shown in Fig. 1B, treatment of HeLa MR cells with MMS significantly enhanced the level of MSH2 in nuclear extracts. This was also found to be the case for other highly potent alkylating mutagens, such as MNU and ENU, having in common the capacity to induce O6-alkylguanine in DNA. The MSH2 protein level was not enhanced, however, after exposure of cells to MMS, which induces only very low amounts of O6-MeG in DNA, nor was the amount enhanced following ultraviolet light and x-ray treatment (data not shown). The dose-response curve of induction revealed that MSH2 increased in amount linearly with doses up to 25 μM MMS, where the maximum of increase was observed (~8-fold above the control level). With higher doses of MMS (~25 μM), the expression of MSH2 again declined, approaching the control level (Fig. 2A, left panel). We should note that even very low, nontoxic doses of MNNG (0.5 and 1 μM) caused an increase in the level of MSH2 (data not shown). Time course experiments revealed an immediate-early increase of MSH2 protein detectable already 30 min after the addition of MNNG to the medium, with a maximum of expression 4 h later. The increased MSH2 level stayed above the control level for at least 24 h after methylating treatment (Fig. 2A, right panel).

Increase in Nuclear MSH2 Level Is Caused by Translocation—Transcriptional activation of the msh2 gene or mRNA stabilization would be a reasonable explanation for an observed increase in the MSH2 protein level. This, however, was not the case, since MNNG treatment did not increase MSH2 mRNA expression (Fig. 2B). Also, the human MSH2 promoter, which was cloned into an expression plasmid and transiently transfected into HeLa MR cells, was found not to be inducible upon treatment with MNNG or with other alkylating agents (MNU, ENU, MMS) (Fig. 2C). Obviously, the observed increase in MSH2 protein level is due neither to transcriptional activation of MSH2 nor to stabilization of MSH2 mRNA. The MNNG-induced increase of the amount of nuclear MSH2 protein was independent of de novo protein synthesis, since it occurred in the presence of cycloheximide and anisomycin (data not shown). An increase in the MSH2 protein level upon MNNG treatment was observed in different cell lines from human and rodent origin, an example of which is given in Fig. 2D. Remarkably, the increase was not dependent on the p53 status, since it was found in p53 wild-type, p53 mutant, and p53 null (knockout) cells (Fig. 2D and data not shown).

In the experiments performed so far, nuclear extracts have been used to analyze the level of MSH2 protein upon alkyla-

Role of MSH6 in MSH2 Translocation—In view of the fact
that MSH2 forms the GT binding complex MutSα together with MSH6, the question arises whether the expression level of MSH6 is also altered upon alkylation. Similar to MSH2, MSH6 was found to be present at a high level in the cytoplasm (see Fig. 4A). Upon treatment of cells with MNNG, the overall MSH6 level (determined in total cell extracts, data not shown) did not change, whereas it increased in the nuclear and decreased in the cytoplasmic fraction (Fig. 4, A and B (for quantification)). This indicates that MSH6, like MSH2, is subject to nuclear translocation in response to alkylation. We also observed an increase in the amount of two other MMR proteins, PMS2 and MLH1, within the nuclear fraction after treatment with MNNG. It is noted that we were unable to detect any MLH1 protein in the cytoplasmic fraction, which may indicate that for this protein a mechanism other than nuclear translocation is responsible for the increase in nuclear level (Fig. 4A).

Nuclear translocation of MSH2 and MSH6 in MNNG-treated cells was confirmed by immunoprecipitation experiments (data not shown) and by immunofluorescence. As shown in Fig. 4C, both MSH2 and MSH6 were detectable in cells in situ in the cytoplasm, whereas upon MNNG treatment the proteins were no longer detectable there, but significant levels were found in the nucleus.

The complex composed of MSH2 and MSH6 is known to be preformed in total and nuclear extracts, which have been used for investigation in most experiments reported thus far (19, 31). To find out whether MutSα is cytoplasmically preformed and thus subject to nuclear translocation, we performed co-immunoprecipitation experiments using nuclear and cytoplasmic extracts of HeLa MR cells. In nuclear and cytoplasmic extracts treated with an anti-hMSH6 antibody, the MSH2 protein was found to be co-precipitated. On the other hand, the addition of an anti-hMSH2 antibody to the extracts resulted in co-precipitation of MSH6 (Fig. 5). This shows that formation of the complex of MSH2 and MSH6 had occurred already in the cytoplasm. It also indicates that the preformed MutSα complex is translocated from the cytoplasm into the nucleus upon MNNG treatment. If true, this would suggest that lack of either one of the proteins prevents nuclear translocation of the second protein. This is indeed the case, because in DLD1 cells deficient for MSH6 (32), translocation of MSH2 into the nucleus was not found (Fig. 6A). Also, as one would expect, GT binding activity of nuclear extracts did not increase in these cells upon MNNG treatment, in contrast to HeLa MR control cells (Fig. 6B). At the same time, this experiment confirmed the MSH6 deficiency of the cells we used.) Obviously, nuclear translocation of MSH2 requires MSH6. In this context, it should be noted that computer analysis revealed that MSH6 contains nuclear localization signal (NLS) sequences not found in MSH2 protein (data not shown). It thus appears that the NLS of MSH6 are utilized for transportation of the preformed cytoplasmic MutSα complex into the nucleus upon DNA methylation.

**Role of O6-MeG in Nuclear Translocation of MutSα—**

Increases in GT binding activity and nuclear accumulation of MSH2 and MSH6 were observed upon treatment of cells with agents that induce O6-MeG in DNA (see Fig. 1). O6-MeG is removed from DNA by the repair protein MGMT in a relatively fast methyl group transfer reaction (33). The HeLa cells (strain HeLa MR) utilized for the experiments described above are deficient for MGMT and thus unable to remove O6-MeG from DNA (34). If O6-MeG provided the signal triggering the response observed, one would expect cells deficient in MGMT to be more sensitive than MGMT proficient cells as regards to MSH2 translocation and the induction of GT binding activity. This indeed was the case. As shown in Fig. 7A, an increase of MSH2 protein also occurred in cells expressing MGMT (HeLa S3 cells, 750 fmol/mg protein MGMT) although at a much higher dosage level of the mutagen (25 μM MNNG), at which point MGMT repair capacity appears to be saturated. When HeLa S3 cells were preincubated with O6-benzylguanine (O6-
Mammalian cells respond on exposure to genotoxic agents with the activation of a variety of functions, because of post-translational modification of pre-existing proteins and gene activation (for review see Ref. 27). Here, we report an increase in the nuclear level of mismatch repair proteins upon alkyla-

The increase of O6-BG to HeLa S3 cells prior to MNNG treatment not only led to an increase in the nuclear MSH2 protein level but also evoked the appearance of the GT binding complex, which was not seen without the inhibitor (Fig. 8). In the control experiment with HeLa MR cells, O6-BG was unable to enhance further GT binding (Fig. 8), indicating that drug treatment on its own was not responsible for the effect observed. In HeLa MR cells stably transfected with the ada gene of Escherichia coli (36), which is as effective as MGMT in repairing O6-MeG in the transgenic cell line (37, 38), the increase of GT binding upon MNNG treatment was abrogated; thus, the cells behaved like HeLa S3 cells. In contrast to MGMT, the Ada protein is not subject to inhibition by O6-BG (39). As expected, treatment of HeLa MR ada-transfected cells with O6-BG did not lead to an increase of GT binding as compared with HeLa S3 cells (Fig. 8). Collectively, the data indicate that O6-MeG lesions are involved primarily in triggering nuclear translocation of MSH2 and MSH6.

**Discussion**

Mammalian cells respond on exposure to genotoxic agents with the activation of a variety of functions, because of post-translational modification of pre-existing proteins and gene activation (for review see Ref. 27). Here, we report an increase in the nuclear level of mismatch repair proteins upon alkyla-

The addition of O6-BG to HeLa S3 cells prior to MNNG treatment not only led to an increase in the nuclear MSH2 protein level but also evoked the appearance of the GT binding complex, which was not seen without the inhibitor (Fig. 8). In the control experiment with HeLa MR cells, O6-BG was unable to enhance further GT binding (Fig. 8), indicating that drug treatment on its own was not responsible for the effect observed. In HeLa MR cells stably transfected with the ada gene of Escherichia coli (36), which is as effective as MGMT in repairing O6-MeG in the transgenic cell line (37, 38), the increase of GT binding upon MNNG treatment was abrogated; thus, the cells behaved like HeLa S3 cells. In contrast to MGMT, the Ada protein is not subject to inhibition by O6-BG (39). As expected, treatment of HeLa MR ada-transfected cells with O6-BG did not lead to an increase of GT binding as compared with HeLa S3 cells (Fig. 8). Collectively, the data indicate that O6-MeG lesions are involved primarily in triggering nuclear translocation of MSH2 and MSH6.

**Discussion**

Mammalian cells respond on exposure to genotoxic agents with the activation of a variety of functions, because of post-translational modification of pre-existing proteins and gene activation (for review see Ref. 27). Here, we report an increase in the nuclear level of mismatch repair proteins upon alkyla-

The addition of O6-BG to HeLa S3 cells prior to MNNG treatment not only led to an increase in the nuclear MSH2 protein level but also evoked the appearance of the GT binding complex, which was not seen without the inhibitor (Fig. 8). In the control experiment with HeLa MR cells, O6-BG was unable to enhance further GT binding (Fig. 8), indicating that drug treatment on its own was not responsible for the effect observed. In HeLa MR cells stably transfected with the ada gene of Escherichia coli (36), which is as effective as MGMT in repairing O6-MeG in the transgenic cell line (37, 38), the increase of GT binding upon MNNG treatment was abrogated; thus, the cells behaved like HeLa S3 cells. In contrast to MGMT, the Ada protein is not subject to inhibition by O6-BG (39). As expected, treatment of HeLa MR ada-transfected cells with O6-BG did not lead to an increase of GT binding as compared with HeLa S3 cells (Fig. 8). Collectively, the data indicate that O6-MeG lesions are involved primarily in triggering nuclear translocation of MSH2 and MSH6.
a single O6-MeG/C pair; this binding is elevated upon MNNG treatment of cells (data not shown). Because nuclear translocation of MSH2 and MSH6 occurs immediately after exposure of cells to O6-MeG-generating agents detectable already 30 min after the beginning of treatment, where only insignificant numbers of O6-MeG/T mismatches will be formed in replicating cells, we hypothesize that O6-MeG/C base pairs are the major early trigger for nuclear accumulation of MSH2 and MSH6 and the induction of nuclear GT binding activity.

How do O6-MeG lesions trigger nuclear translocation of MutSβ? The presence of O6-MeG in DNA could provide in itself the signal leading to translocation of MutSβ. Another possibility is that binding of MutSβ to O6-MeG lesions leads to sequestration of free MutSβ in the nucleus, which finally may provide

the signal for transportation of MSH2 and MSH6 from the cytoplasm into the nucleus. Nuclear translocation of MutSβ is unlikely to be the result of a passive dislocation of the complex into the nucleus because MutSβ and even the single component MSH2 (102 kDa) and MSH6 (160 kDa) are too big to enter the nucleus through the nuclear pore simply by diffusion. Only proteins up to 40 kDa are able to get through the nuclear pore complex in a passive way (for review see Ref. 44). Therefore, MSH2 and MSH6 must enter the nucleus actively by NLS-mediated transport. Analysis of the amino acid sequence of MSH2 and MSH6 reveals a lack of NLS in MSH2, whereas MSH6 contains several polyoma large T- and SV40 large T-antigen-like NLS (45) as well as several bipartite sequences (46). The presence of NLS sequences within the MSH6 protein may explain the finding that nuclear translocation of MSH2 is related to the translocation of MSH6. Thus, MSH2 alone was not effective in entering the nucleus upon alkylation, which was proven by using DLD1 cells that are deficient in MSH6. Based on these data, we conclude that MSH6 is utilized for the nuclear transportation of MSH2, hooking MSH2 into the nucleus.

The involvement of an active transport would imply that signaling triggered by O6-MeG lesions leads to post-translational modification of MMR components that stimulate nuclear translocation. Post-translational protein modification can lead to changes in the NLS. Thus, the nuclear translocation of SV40 T-antigen is regulated by phosphorylation of the NLS or NLS flanking sequences within the protein (47). Another possibility is that post-translational modification alters the assembly of the MutSβ complex. Differences in nuclear transportation between monomeric proteins and dimer complexes have been shown for MAPK (48). This protein is bound to MAPK kinase in the cytoplasm, is released after phosphorylation, and enters
the nucleus by passive diffusion. Upon treatment with serum, MAPK forms homodimers that can enter the nucleus by the more efficient active transport. Currently, we are analyzing whether MSH2 and MSH6 are subject to post-translational modification. Our preliminary data show that MSH2 and MSH6 can be phosphorylated in vitro and under in vivo conditions.2

The data reported here indicate that regulation of MMR upon DNA damage occurs largely at the level of post-translational modification (including nuclear transportation) rather than at the level of gene activation. The immediate-early translocation of MMR proteins into the nucleus, triggered very likely by O6-MeG/C and O6-MeG/T lesions, is supposed to provoke an increase in MMR capacity in the nucleus; this would be important, in view of O6-MeG/C lesions that form during replication and presumably occur as a result of mutations at or near the transition point. At least at the level of gene expression or is expressed at a low level (7, 8, 14, 29, 50). MMR defects, therefore, have a strong impact on the mutagenic and carcinogenic response of cells exposed to alkylating agents. The data presented here imply that not only a deficiency of MMR proteins but also defects in nuclear translocation could alter the mutagenic and carcinogenic response of cells to endogenously formed methylating species, environmental carcinogens, and methylating drugs used in tumor therapy.

Acknowledgments—We gratefully acknowledge Dr. Jörg Dosch for initial experiments and Uta Eichhorn-Grombacher for technical assistance.

REFERENCES

1. Modrich, P. (1997) J. Biol. Chem. 272, 24727–24730
2. Aaltonen, L. A., Poeltmäki, P., Leach, F. S., Siitonen, P., Pylkkanen, L., Mecklin, J. P., Jarvinen, H., Powell, S. M., Jen, J., Hamilton, S. R., Petersen, O. M., Kinzler, K. W., Vogelstein, B., and Chapelle, A. (1993) Science 260, 812–816
3. Kolodner, R. (1996) Genes Dev. 10, 1433–1442
4. Kaina, B., Ziouta, A., Ochs, K., and Coquerelle, T. (1997) Mutat. Res. 381, 227–241
5. Branch, P., Hampson, R., and Karran, P. (1995) Cancer Res. 55, 2304–2309
6. Hickman, M. J., and Samson, L. D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 10764–10769
7. Karran, P., and Bignami, M. (1992) Nucleic Acids Res. 20, 2933–2940
8. Hampson, R., Humbert, P., Macpherson, P., Aquilina, G., and Karran, P. (1997) J. Biol. Chem. 272, 28596–28606
9. Loveless, A. (1989) Nature 338, 644–644
10. Yamasaki, D., O’Regan, E., Brown, R., and Karran, P. (1997) Nucleic Acids Res. 25, 491–495
11. Branch, P., Aquilina, G., Bignami, M., and Karran, P. (1993) Nature 362, 652–654
12. Karran, P., and Bignami, M. (1994) Bioassays 16, 833–839

2 M. Christmann and B. Kaina, unpublished observations.