The Major Replicative Histone Chaperone CAF-1 Suppresses the Activity of the DNA Mismatch Repair System in the Cytotoxic Response to a DNA-methylating Agent*

Lyudmila Y. Kadyrova, Basanta K. Dahal, and Farid A. Kadyrov

From the Department of Biochemistry and Molecular Biology, Southern Illinois University, School of Medicine, Carbondale, Illinois 62901

Edited by Patrick Sung

The DNA mismatch repair (MMR) system corrects DNA mismatches in the genome. It is also required for the cytotoxic response of 6\textsuperscript{-}methylguanine-DNA methyltransferase (MGMT)-deficient mammalian cells and yeast nag1\Delta rad52\Delta cells to treatment with S\textsubscript{n}1-type methylating agents, which produce cytotoxic 6\textsuperscript{-}methylguanine (6\textsuperscript{-}mG) DNA lesions. Specifically, an activity of the MMR system causes degradation of irreparable 6\textsuperscript{-}mG-T mispair-containing DNA, triggering cell death; this process forms the basis of treatments of MGMT-deficient cancers with S\textsubscript{n}1-type methylating drugs. Recent research supports the view that degradation of irreparable 6\textsuperscript{-}mG-T mispair-containing DNA by the MMR system and CAF-1-dependent packaging of the newly replicated DNA into nucleosomes are two concomitant processes that interact with each other. Here, we studied whether CAF-1 modulates the activity of the MMR system in the cytotoxic response to S\textsubscript{n}1-type methylating agents. We found that CAF-1 suppresses the activity of the MMR system in the cytotoxic response of yeast nag1\Delta rad52\Delta cells to the prototypic S\textsubscript{n}1-type methylating agent N\textsuperscript{-}methyl-N\textsuperscript{-}nitro-N\textsuperscript{-}
nitrosoguanidine. We also report evidence that in human MGMT-deficient cell-free extracts, CAF-1-dependent packaging of irreparable 6\textsuperscript{-}mG-T mispair-containing DNA into nucleosomes suppresses its degradation by the MMR system. Taken together, these findings suggest that CAF-1-dependent incorporation of irreparable 6\textsuperscript{-}mG-T mispair-containing DNA into nucleosomes suppresses its degradation by the MMR system, thereby defending the cell against killing by the S\textsubscript{n}1-type methylating agent.

The DNA mismatch repair (MMR) system has several activities that are involved in genome metabolism (1–3). The correction of DNA mismatches is the best understood activity of the MMR system. Eukaryotic MMR leads to the correction of errors caused by the replication fork DNA polymerases α, δ, and ε, and it also changes the outcome of homologous recombination (11–17). A considerable number of proteins have been suggested to contribute to or regulate eukaryotic MMR (6, 18–20). Strong evidence indicates the primary mismatch recognition factor MutSα (MSH2-MSH6 heterodimer), MutLα (MLH1-PM2 heterodimer in humans and Mlh1-Pms1 heterodimer in budding yeast) endonuclease, the replicative clamp proliferating cell nuclear antigen (PCNA), the clamp loader replication factor C (RFC), the exonuclease EXO1, the secondary mismatch recognition factor MutSβ (MSH2-MSH3 heterodimer), and the replicative DNA polymerase δ (Pol δ) play major roles in eukaryotic MMR (21–39).

Several eukaryotic MMR reactions have been described (10, 36, 40–43). One of these reactions removes mismatches on both the 3' and 5'-nicked DNA molecules in an excision-dependent manner and is probably necessary for the majority of MMR events in wild-type cells (36). This MMR reaction depends on the activities of MutSα, MutLα, PCNA, RFC, EXO1, RPA, and Pol δ (32, 33, 35–37, 41) and is initiated by recognition of the mismatch by MutSα (21, 22). After recognizing the mismatch, MutSα cooperates with RFC-loaded PCNA to activate MutLα endonuclease (37, 41, 44). The activated MutLα endonuclease incises the discontinuous daughter strand. An incision that is generated by MutLα endonuclease 5' to the mismatch is used by EXO1 to enter the DNA and excise the mismatch in a 5' → 3'-directed reaction modulated by MutSα and RPA (33, 35, 37, 45). A gap generated by EXO1 action is filled in by Pol ε holoenzyme. Although Pol ε holoenzyme can also perform gap filling in a reconstituted excision-dependent MMR reaction, the specific activity of this enzyme is much lower than that of Pol δ holoenzyme (43). The mutator phenotype of an exo1Δ mutant is weaker compared with the mutator phenotype of an msh2Δ or mih1Δ mutant, indicating that the MMR system can remove DNA polymerase errors in EXO1-deficient cells (14, 28, 34). In agreement with this idea, an excision-independent MMR reaction that involves MutSα, MutLα, PCNA, RFC, RPA, and Pol δ has been described (41). Like the excision-dependent MMR reaction, the excision-independent MMR reaction requires the MutLα endonuclease activity for inci-
sion of the discontinuous daughter strand. After MutLα endonuclease incises the discontinuous daughter strand, the Pol δ holoenzyme uses a MutLα-generated 3’ end that is 5’ to the mismatch to perform strand displacement DNA synthesis that removes the mismatch. The role of the Pol δ holoenzyme in the excision-independent MMR reaction may be unique because the replacement of the Pol δ holoenzyme with the Pol ε holoenzyme abolishes the excision-independent MMR reaction (43).

One of the activities of the MMR system is required for the cytotoxic response of MGMT (O^6-methyl guanine methyl transferase)-deficient mammalian cells and yeast mgt1Δ rad52Δ cells to S_{1,1}-type methylating agents (46, 47). This activity of the MMR system depends on MutSα and MutLα and is necessary for several therapies against MGMT-deficient cancers (3). The treatment involves dacarbazine, procarbazone, or temozolomide, each of which is an S_{1,1}-type methylating drug that triggers death of MGMT-deficient cancer cells by activating the MMR system. O^6-Methylguanine (O^6-mG) is the cytotoxic product of treatment of the cell with the S_{1,1}-type methylating agent (48). Normally, O^6-mG lesions are removed by MGMT that protects the cell from killing by the S_{1,1}-type methylating agent (49). However, a significant number of cancers are deficient in MGMT due to methylation of the MGMT promoter (50). MGMT-deficient cancer cells treated with the S_{1,1}-type methylating agent accumulate O^6-mG-T mispairs that are recognized by MutSα (51). Upon recognition of an O^6-mG-T mispair, MutSα initiates its repair. If the O^6-mG is on the discontinuous strand, it gets repaired (52). In contrast, if the O^6-mG is on the continuous strand, it triggers futile cycles of MMR (52). The futile cycles of MMR lead to the formation of persistent strand breaks (53), which are converted in the next S phase into double strand breaks that cause cell cycle arrest followed by cell death (54, 55). Consistent with this, double strand break repair defects sensitize eukaryotic cells to the killing effects of S_{1,1}-type methylating agents (47, 56).

The heterotrimeric CAF-1 is the major histone chaperone for the assembly of nucleosomes onto the newly replicated DNA (57–61). CAF-1 loads histone (H3-H4)_2 tetramers onto DNA, producing tetrasomes (62, 63). Each tetrasome is then converted into a nucleosome by the addition of two histone H2A-H2B dimers (57, 64). CAF-1 interacts physically with PCNA, and this interaction is necessary for the action of CAF-1 on the newly replicated DNA (65, 66). Recent research has indicated that postreplicative MMR coincides with CAF-1-dependent nucleosome assembly and that the two processes interact with each other (43, 63, 67). Furthermore, recent findings are consistent with the idea that the eukaryotic MMR system degrades irreparable O^6-mG-T mispair-containing DNA when it is being packaged into nucleosomes by the CAF-1-dependent mechanism (53, 63, 67). We show here that CAF-1 suppresses the activity of the MMR system in the cytotoxic response to S_{1,1}-type methylating agents.

Results

**CAF-1 Suppressed the Activity of the MMR System in the Cytotoxic Response of Yeast mgt1Δ rad52Δ Cells to MNNG**—The cytotoxic response to S_{1,1}-type methylating agents occurs in the chromatin environment (46, 52, 53, 68). However, it has remained unknown whether the chromatin environment affects the cytotoxic response to S_{1,1}-type methylating agents. It has also been unknown whether histone chaperones, proteins that are involved in the control of chromatin environment, influence the cytotoxic response to S_{1,1}-type methylating agents. We wanted to study whether the major replicative histone chaperone CAF-1 impacts the cytotoxic response to an S_{1,1}-type methylating agent. Previous research showed that MGMT-deficient mammalian cells and Saccharomyces cerevisiae mgt1Δ rad52Δ that lack Mgt (the yeast ortholog of MGMT (69)) and the recombination mediator Rad52 (70) are efficiently killed by the prototypic S_{1,1}-type methylating agent MNNG in a manner that involves the MMR system (46, 47, 53). Thus, we utilized the yeast mgt1Δ rad52Δ cells to determine whether CAF-1 impacts the cytotoxic response to MNNG. In budding yeast, CAC1 encodes the largest subunit of CAF-1 (60). Accordingly, we investigated whether loss of the CAC1 gene increased the sensitivity of the yeast mgt1Δ rad52Δ cells to killing by MNNG. The use of an MNNG cytotoxicity assay permitted us to establish that the cac1Δ mgt1Δ rad52Δ cells were more sensitive to treatment with MNNG than the mgt1Δ rad52Δ cells (Fig. 1A). A more detailed analysis revealed that ~1.7% of the mgt1Δ rad52Δ cells and only ~0.2% of the cac1Δ mgt1Δ rad52Δ cells survived the treatment with MNNG (Fig. 1C).

The survival fraction of the MNNG-treated cac1Δ mgt1Δ rad52Δ cells was ~9 times smaller than that of the MNNG-treated mgt1Δ rad52Δ cells. We then conducted experiments to determine whether MNNG killed the cac1Δ mgt1Δ rad52Δ cells via an MMR system-dependent mechanism (Fig. 1C). The results showed that the deletion of the MMR system gene MLH1 rescued the sensitivity of the cac1Δ mgt1Δ rad52Δ cells to the cytotoxic effect of MNNG. The experiments also demonstrated that the mlh1Δ cac1Δ mgt1Δ rad52Δ cells were as resistant to the MNNG treatment as the mlh1Δ mgt1Δ rad52Δ cells. Based on these findings, we concluded that loss of CAC1 sensitizes the yeast mgt1Δ rad52Δ cells to MMR system-dependent killing by MNNG.

The cytotoxicity of S_{1,1}-type methylators is mediated by replication- and MMR system-dependent double strand breaks that these agents form. A number of other DNA-damaging agents generate strand breaks that kill cells. Among them are camptothecin, hydroxyurea, and bleomycin. Camptothecin induces replication-dependent double strand breaks by stabilizing topoisomerase I-DNA covalent complexes (71), hydroxyurea causes double strand breaks by depleting the dNTP pools (72), and bleomycin creates double strand and single strand breaks by attacking DNA (73). Unlike the S_{1,1}-type methylating agent, camptothecin, hydroxyurea, and bleomycin kill cells via MMR system-independent mechanisms. Nevertheless, there is a significant similarity between one of these drugs, camptothecin, and MNNG in that double strand breaks caused by these two agents are formed during DNA replication. The results of our genetic experiments (Fig. 1, A and C) raised the possibility that loss of CAC1 sensitized the yeast mgt1Δ rad52Δ cells to the cytotoxic effects of DNA-damaging agents that generate DNA breaks. To address this possibility, we studied the effect of CAC1 absence on the sensitivity of the mgt1Δ rad52Δ cells to camptothecin, hydroxyurea, and bleomycin (Fig. 1, D.
An analysis of the data showed that the \( cac1 \Delta \) \( mgt1 \Delta \) \( rad52 \Delta \) cells and the \( mgt1 \Delta \) \( rad52 \Delta \) cells had the same sensitivities to bleomycin, camptothecin, and hydroxyurea. Thus, \( CAC1 \) absence did not affect the sensitivity of the yeast \( mgt1 \Delta \) \( rad52 \Delta \) cells to bleomycin, camptothecin, and hydroxyurea, drugs that kill cells via MMR system-independent mechanisms. Because MNNG kills the yeast \( mgt1 \Delta \) \( rad52 \Delta \) cells by activating an MMR system-dependent mechanism and bleomycin, camptothecin, and hydroxyurea kill the cells via other mechanisms, the results of our genetic experiments (Fig.

**FIGURE 1.** \( CAF-1 \) suppresses the activity of the MMR system in the cytotoxic response of yeast \( mgt1 \Delta \) \( rad52 \Delta \) cells to MNNG. Cytotoxicity assays were carried out as detailed under “Experimental Procedures.” \( A \) and \( B \), cytotoxic responses of \( cac1 \Delta \) \( mgt1 \Delta \) \( rad52 \Delta \) and \( cac2 \Delta \) \( mgt1 \Delta \) \( rad52 \Delta \) cells to treatment with 1 \( \mu M \) MNNG. 10-Fold serial dilutions of yeast cultures that were treated or not treated with 1 \( \mu M \) MNNG were spotted on the YPDAU plates. \( C \), quantitative analysis of cytotoxic responses of \( cac1 \Delta \) \( mgt1 \Delta \) \( rad52 \Delta \) to treatment with 30 \( \mu g/ml \) bleomycin (BLE). The data in \( C \) and \( D \) are averages \( \pm \) 1 S.D. (error bars) \( (n \geq 3) \). \( E \) and \( F \), cytotoxic responses of \( cac1 \Delta \) \( mgt1 \Delta \) \( rad52 \Delta \) and \( cac2 \Delta \) \( mgt1 \Delta \) \( rad52 \Delta \) cells to treatment with 0.5 \( \mu g/ml \) camptothecin and 10 mM hydroxyurea. 10-Fold serial dilutions of yeast cultures that were treated or not with 1 \( \mu M \) MNNG were spotted on the YPDAU plates.
FIGURE 2. Nucleosome assembly reactions reconstituted with an extract and purified CAF-1. The experiments were conducted and analyzed as described under “Experimental Procedures.” A, analysis of CAF-1 in 293T cytosolic extract (293T CE) and 293T nuclear extract (293T NE) by Western blotting with antibodies against the CAF-1 p150 subunit. Quantification of the data showed that 293T cytosolic extract (150 μg) contains 0.077 ± 0.009 pmol of CAF-1. B, analysis of nucleosome assembly in reaction mixtures containing the indicated components. The nucleosome assembly products were visualized with the 32P-labeled probe c154, which is complementary to the continuous strand. A diagram on the left indicates the relative positions of the 32P-labeled probe (a bar with an asterisk), the strand break, and a mismatch.

1, A and C–E) indicated that Cac1 increases the survival of the MNNG-treated mgt1Δ rad52Δ cells by being involved in a process that suppresses the cytotoxic activity of the MMR system.

CAC2 codes for the second subunit of yeast CAF-1 (60). We studied whether deletion of CAC2 sensitized the mgt1Δ rad52Δ cells to MNNG (Fig. 1B). The data showed that the cac2Δ mgt1Δ rad52Δ cells were more sensitive to MNNG than the mgt1Δ rad52Δ cells (Fig. 1, B and C). A comparison of the sensitivities of the cac2Δ mgt1Δ rad52Δ, msh2Δ mgt1Δ rad52Δ, cac2Δ mgt1Δ rad52Δ, and mgt1Δ rad52Δ cells (Fig. 1C) revealed that MNNG killed the cac2Δ mgt1Δ rad52Δ cells via an MMR system-dependent mechanism. As expected, the cac2Δ mgt1Δ rad52Δ cells were as sensitive to camptothecin and hydroxyurea as the mgt1Δ rad52Δ cells (Fig. 1F). Thus, the results of these and previous experiments (Fig. 1, A–F) demonstrated that CAF-1 increases the survival of MNNG-treated mgt1Δ rad52Δ cells by suppressing the cytotoxic activity of the MMR system.

Loss of HIR2, RTT106, or HHT2-HHF2 Did Not Change the Sensitivity of Yeast mgt1Δ rad52Δ Cells to MNNG—Histone H3-H4 chaperone Hir (Hir1-Hir2-Hir3-Hpc2 complex) plays a major role in replication-independent nucleosome assembly (74), and histone H3-H4 chaperone Rtt106 participates in replication-coupled nucleosome assembly (75). We analyzed whether the absence of HIR2 or RTT106 had an effect on the sensitivity of the mgt1Δ rad52Δ cells to MNNG. The experiments showed that lack of HIR2 or RTT106 did not change the sensitivity of the mgt1Δ rad52Δ cells to MNNG (Fig. 1G).

The molecular activity of CAF-1 is to load histone H3-H4 tetramers onto newly replicated DNA (59, 62, 64). We wanted to explore whether decreasing histone H3-H4 gene dosage affected the survival of MNNG-treated mgt1Δ rad52Δ cells. The yeast genome contains two histone H3-H4 gene loci, HHT1-HHF1 and HHT2-HHF2. Compared with HHT1-HHF1, HHT2-HHF2 produces about 5-7 times more mRNA (76). Either locus is sufficient to maintain the existence of the yeast cell. We measured the sensitivity of hht2-hhf2Δ mgt1Δ rad52Δ cells to MNNG and found it to be no different from that of the mgt1Δ rad52Δ cells (Fig. 1G).

CAF-1-dependent Packaging of Irreparable O6-mG-T Mispair-containing DNA into Nucleosomes Suppressed Its Degradation by the MMR System—MMR system-dependent degradation of irreparable O6-mG-T mispair-containing DNA is involved in the cytotoxic response to the S,1-type methylating drug (52, 53, 68). Our genetic experiments indicated that CAF-1 activity suppresses degradation of irreparable O6-mG-T mispair-containing DNA by the MMR system (Fig. 1). To find evidence that CAF-1-dependent incorporation of irreparable O6-mG-T mispair-containing DNA into nucleosomes suppresses its degradation by the MMR system, we performed biochemical experiments that are summarized in Figs. 2–7; DNA substrates that we utilized in these experiments were 3’-nicked O6-mG-T (3’ O6-mG-T), 3’-nicked G-T (3’ G-T), and 3’-nicked A-T (3’ A-T) DNAs. The substrates were made using a plasmid, pAH1A, as a starting material and differed from each other by 1–2 bases (52). The 3’-nicked O6-mG-T DNA contained a single O6-mG-T mispair, the 3’-nicked G-T DNA carried a single G-T mispair, and the 3’-nicked A-T DNA lacked a mispair. The 3’-nicked O6-mG-T DNA was irreparable by the MMR system because the O6-mG was on the continuous strand. In these biochemical experiments, we used a cytosolic extract prepared from human embryonic kidney cell line 293T that lacked MutLo and MGMT (77, 78) and had a reduced level of CAF-1 (Fig. 2A) (58). Although the 293T cytosolic extract lacks the MMR system due to the absence of MutLo (77), supplementation of the extract with purified MutLo reconstitutes the MMR system (78). In agreement with previous studies (37, 52, 63, 77–79), our control experiments showed that 1) the reconstituted MMR system failed to repair an O6-mG-T mispair on a nicked DNA (3’-nicked O6-mG-T DNA) but repaired a G-T mispair on a similar nicked DNA (the 3’-nicked G-T DNA) (Fig. 3), 2) supplementation of the 293T cytosolic extract with purified MutLo-E705K endonuclease mutant (37) did not lead to reconstitution of the MMR system (Fig. 3), 3) the omission of
A product of degradation of the discontinuous strand of the 3’- nicked O6-mG-T DNA was observed in the reaction mixture that was incubated for 10–120 min (Fig. 5B). This observation indicated that the reconstituted MMR system caused persistent degradation of the discontinuous strand of the 3’- nicked O6-mG-T DNA. In contrast, the products of degradation of the discontinuous strand of the 3’- nicked G-T DNA that were present in the reaction mixture incubated for 10 min (Fig. 4A, lane 3) were not detected in the same reaction mixture that was incubated for 60 min (Fig. 5A, lane 3). This finding is consistent with the view that the product of degradation of the discontinuous strand of the 3’- nicked G-T DNA is an intermediate of the MMR reaction (37).

The ~130-nt product was identified by Southern hybridization with a 32P-labeled probe that was complementary to a discontinuous strand sequence located 2 nt downstream from the mismatched T (Figs. 4A and 5A). However, Southern hybridization with a 32P-labeled probe that was complementary to a discontinuous strand sequence located 2 nt upstream from the mismatched T did not detect the ~130-nt product (Fig. 5C). This finding indicated that the 5’ end of the ~130-nt product was located at or near the mismatch. To determine whether a different part of the 3’- nicked O6-mG-T DNA contained a MutLα endonuclease-dependent strand break, we carried out Southern hybridizations with two other 32P-labeled probes (Fig. 5, D and E). One of the probes was complementary to a discontinuous strand sequence that was downstream from the preexisting strand break (Fig. 5D) and the other to a continuous strand that was upstream from the preexisting strand break (Fig. 5E).

exogenous dNTPs and the addition of the DNA polymerase inhibitor aphidicolin led to a nearly complete inhibition of the mismatch correction activity of the reconstituted MMR system (Fig. 3, 4) the 293T cytosolic extract had a weak nucleosome assembly activity (Fig. 2B) due to the reduced level of CAF-1 (Fig. 2A).

We next utilized Southern hybridization to detect MMR system-dependent degradation of the irreparable O6-mG-T mispair-containing DNA that was reconstituted with the 293T cytosolic extract in the absence or presence of the purified CAF-1 (Figs. 4–7). The initial experiments in this series analyzed degradation products that were separated on denaturing agarose gels (Figs. 4–6). The data showed that the reconstituted MMR system degraded the discontinuous strand of an irreparable O6-mG-T mispair-containing DNA (the 3’- nicked O6-mG-T DNA) leading to the formation of a ~130-nt product (Figs. 4A and 5A, lane 10). Importantly, the ~130-nt product was not observed in the reaction mixture that contained endonuclease-deficient MutLα-E705K instead of MutLα (Figs. 4A and 5A, lane 13) demonstrating that the endonuclease activity of MutLα is required for the degradation of the 3’- nicked O6-mG-T DNA. Additional experiments revealed that the ~130-nt product or a similar product was not formed from the 3’- nicked A-T DNA in the reaction mixture that contained the reconstituted MMR system (Figs. 4A and 5A, lane 17). The results of a time course analysis demonstrated that the ~130-nt product of degradation of the discontinuous strand of the 3’- nicked O6-mG-T DNA is required for the degradation of the 3’- nicked O6-mG-T DNA leading to the formation of a ~130-nt product (Fig. 5A) demonstrating that the endonuclease activity of MutLα is required for the degradation of the 3’- nicked O6-mG-T DNA. The 3’- nicked O6-mG-T DNA was observed in the reaction mixture that was incubated for 10–120 min (Fig. 5B). This observation indicated that the reconstituted MMR system caused persistent degradation of the discontinuous strand of the 3’- nicked O6-mG-T DNA. In contrast, the products of degradation of the discontinuous strand of the 3’- nicked G-T DNA that were present in the reaction mixture incubated for 10 min (Fig. 4A, lane 3) were not detected in the same reaction mixture that was incubated for 60 min (Fig. 5A, lane 3). This finding is consistent with the view that the product of degradation of the discontinuous strand of the 3’- nicked G-T DNA is an intermediate of the MMR reaction (37).
The MMR System and CAF-1

The yield of the discontinuous strand of irreparable O6-mG-T mispair-containing DNA was decreased 4 times (Fig. 8). Supplementation of the 293T extract- and MutLα-containing reaction mixture with 0.6 or 2.4 pmol of purified CAF-1 caused a significant reduction in the yield of the gapped product of the 3’-nicked O6-mG-T DNA (Fig. 7, B–E). This finding supports the view that CAF-1-dependent packaging of irreparable O6-mG-T mispair-containing DNA into nucleosomes suppresses its degradation by the MMR system.

Degradation of an Irreparable O6-mG-T Mispair-containing DNA by the Activated MutLα Endonuclease in a Defined System—Our biochemical experiments (Figs. 4–7) have implicated MutLα endonuclease activity in MMR system-dependent degradation of the discontinuous strand of irreparable O6-mG-T mispair-containing DNA. However, these experiments did not provide a clear view of how MutLα endonuclease activity is involved in this process. To better understand the involvement of MutLα endonuclease activity in MMR system-dependent degradation of the discontinuous strand of irreparable O6-mG-T mispair-containing DNA, we carried out additional experiments summarized in Fig. 8. In these experiments, we studied degradation of the discontinuous strand of the 3’-nicked O6-mG-T DNA in a defined system (37). Purified proteins that were included in the defined system were MutLα endonuclease, the mismatch recognition factor MutSα, the PCNA clamp, the RFC clamp loader, and the single-stranded DNA-binding protein RPA. It can be seen that the discontinuous strand of the 3’-nicked O6-mG-T DNA was degraded in the defined system in a MutLα endonuclease concentration-dependent manner (Fig. 8, A (lanes 13–17) and B). The level of degradation of the discontinuous strand of the 3’-nicked O6-mG-T DNA was 5–6 times higher than the degradation level of the discontinuous strand of the control 3’-nicked A-T DNA (Fig. 8B). No degradation of the discontinuous strand of the 3’-nicked O6-mG-T DNA was observed in the defined system in which the endonuclease-deficient MutLα-E705K substituted for MutLα (Fig. 8, A (lanes 6, 12, and 18) and C). This informa-
tion indicated that MutLα provided the endonuclease activity that degraded the discontinuous strand of an irreparable O^6-mG-T mispair-containing DNA in the presence of MutSα, PCNA, RFC, and RPA. Importantly, the ~130-nt fragment that was detected in the cell extract system (Fig. 5A, lane 10) was not a preferred product of the endonuclease reaction in the defined system (Fig. 8A, lanes 15–17). This is an indication that the defined system lacks one or more factors that are involved in the formation of the ~130-nt fragment in the extract system. While degrading the discontinuous strand, MutLα endonu-

![Image of gel and graphs with bands and lanes](image-url)
The MMR System and CAF-1

Mammalian MGMT-deficient and yeast mgt1Δ rad52Δ cells are efficiently killed by low doses of Sα1-type methylating agents (46, 47, 78, 80–82). Several anticancer therapies exploit the marked sensitivity of MGMT-deficient cells to Sα1-type methylating agents. The marked sensitivity of mammalian MGMT-deficient and yeast mgt1Δ rad52Δ cells to Sα1-type methylating agents is a result of the MMR system-dependent cytotoxic response (46, 47, 78, 80–82). Previous research showed that the cytotoxic response to the Sα1-type methylating drug involves MMR system-dependent degradation of irreparable O6-mG-containing DNA that leads to the formation of lethal double strand breaks (47, 53, 56). The MMR system starts to degrade irreparable O6-mG-containing nascent DNA behind the replication fork (53). At the same time, this DNA is incorporated into nucleosomes in the CAF-1-orchestrated process (57–61). It was previously unknown whether concomitant CAF-1-dependent nucleosome assembly affects degradation of irreparable O6-mG-containing DNA by the MMR system. We have found that CAF-1 suppresses the activity of the MMR system in the cytotoxic response of yeast mgt1Δ rad52Δ cells to MNNG (Fig. 1). We have also found that in an MGMT-deficient extract system, CAF-1-dependent incorporation of an irreparable O6-mG-T mispair-containing DNA into nucleosomes (Fig. 2B) correlates with a substantial decrease in degradation of this DNA by a MutLα endonuclease-dependent mechanism (Figs. 5–7). These findings imply that CAF-1-dependent packaging of irreparable O6-mG-T mispair-containing DNA into nucleosomes suppresses its degradation by the MMR system, therefore defending the cell against killing by the Sα1-type methylating drug. Consistent with this, we have established that loss of CAF-1 does not affect the sensitivity of yeast mgt1Δ rad52Δ cells to bleomycin, camptothecin, and hydroxyurea, DNA-damaging drugs that kill cells in an MMR system-independent manner (Fig. 1). It is known that 1–2 dou-

The MMR System and CAF-1

Mammalian MGMT-deficient and yeast mgt1Δ rad52Δ cells are efficiently killed by low doses of Sα1-type methylating agents (46, 47, 78, 80–82). Several anticancer therapies exploit the marked sensitivity of MGMT-deficient cells to Sα1-type methylating agents. The marked sensitivity of mammalian MGMT-deficient and yeast mgt1Δ rad52Δ cells to Sα1-type methylating agents is a result of the MMR system-dependent cytotoxic response (46, 47, 78, 80–82). Previous research showed that the cytotoxic response to the Sα1-type methylating drug involves MMR system-dependent degradation of irreparable O6-mG-containing DNA that leads to the formation of lethal double strand breaks (47, 53, 56). The MMR system starts to degrade irreparable O6-mG-containing nascent DNA behind the replication fork (53). At the same time, this DNA is incorporated into nucleosomes in the CAF-1-orchestrated process (57–61). It was previously unknown whether concomitant CAF-1-dependent nucleosome assembly affects degradation of irreparable O6-mG-containing DNA by the MMR system. We have found that CAF-1 suppresses the activity of the MMR system in the cytotoxic response of yeast mgt1Δ rad52Δ cells to MNNG (Fig. 1). We have also found that in an MGMT-deficient extract system, CAF-1-dependent incorporation of an irreparable O6-mG-T mispair-containing DNA into nucleosomes (Fig. 2B) correlates with a substantial decrease in degradation of this DNA by a MutLα endonuclease-dependent mechanism (Figs. 5–7). These findings imply that CAF-1-dependent packaging of irreparable O6-mG-T mispair-containing DNA into nucleosomes suppresses its degradation by the MMR system, therefore defending the cell against killing by the Sα1-type methylating drug. Consistent with this, we have established that loss of CAF-1 does not affect the sensitivity of yeast mgt1Δ rad52Δ cells to bleomycin, camptothecin, and hydroxyurea, DNA-damaging drugs that kill cells in an MMR system-independent manner (Fig. 1). It is known that 1–2 dou-

Discussion

Mammalian MGMT-deficient and yeast mgt1Δ rad52Δ cells are efficiently killed by low doses of Sα1-type methylating agents (46, 47, 78, 80–82). Several anticancer therapies exploit the marked sensitivity of MGMT-deficient cells to Sα1-type methylating agents. The marked sensitivity of mammalian MGMT-deficient and yeast mgt1Δ rad52Δ cells to Sα1-type methylating agents is a result of the MMR system-dependent cytotoxic response (46, 47, 78, 80–82). Previous research showed that the cytotoxic response to the Sα1-type methylating drug involves MMR system-dependent degradation of irreparable O6-mG-containing DNA that leads to the formation of lethal double strand breaks (47, 53, 56). The MMR system starts to degrade irreparable O6-mG-containing nascent DNA behind the replication fork (53). At the same time, this DNA is incorporated into nucleosomes in the CAF-1-orchestrated process (57–61). It was previously unknown whether concomitant CAF-1-dependent nucleosome assembly affects degradation of irreparable O6-mG-containing DNA by the MMR system. We have found that CAF-1 suppresses the activity of the MMR system in the cytotoxic response of yeast mgt1Δ rad52Δ cells to MNNG (Fig. 1). We have also found that in an MGMT-deficient extract system, CAF-1-dependent incorporation of an irreparable O6-mG-T mispair-containing DNA into nucleosomes (Fig. 2B) correlates with a substantial decrease in degradation of this DNA by a MutLα endonuclease-dependent mechanism (Figs. 5–7). These findings imply that CAF-1-dependent packaging of irreparable O6-mG-T mispair-containing DNA into nucleosomes suppresses its degradation by the MMR system, therefore defending the cell against killing by the Sα1-type methylating drug. Consistent with this, we have established that loss of CAF-1 does not affect the sensitivity of yeast mgt1Δ rad52Δ cells to bleomycin, camptothecin, and hydroxyurea, DNA-damaging drugs that kill cells in an MMR system-independent manner (Fig. 1). It is known that 1–2 dou-

FIGURE 5. CAF-1-dependent suppression of persistent MutLα endonuclease-dependent degradation of an irreparable O6-mG-T mispair-containing DNA in an extract system. The experiments were carried out and analyzed as described under “Experimental Procedures.” The reaction mixtures included the indicated components and were incubated for 60 min (A and C–F) or 10–120 min (B) at 37 °C. Each reaction mixture analyzed in A–E contained 162 fmol of a DNA substrate, and each reaction mixture analyzed in F contained 41 fmol of a DNA substrate. Exogenous dNTPs were not included in the reaction mixtures that contained aphidicolin (0.1 mM). The recovered DNAs were digested with Clal, separated on alkaline agarose gels, transferred onto a nylon membrane, and hybridized with the 32P-labeled probe ts154, which is complementary to the discontinuous strand. A, DNA species visualized in one of the experiments. The diagram outlines relative positions of the probe (a bar with an asterisk), unique Clal site, strand break, and mismatch. B, formation of the ~130-nt product of degradation of the 3'-nicked O6-mG-T DNA as a function of the amount of purified CAF-1. The data were obtained by quantification of images, including those shown in A and Fig. 4A and are averages ± 1 S.D. (error bars) (n = 3).

FIGURE 6. CAF-1 suppresses persistent degradation of an irreparable O6-mG-T mispair-containing DNA in an extract system in a concentration-dependent manner. The experiments were carried out and analyzed as detailed under “Experimental Procedures.” Each reaction mixture contained a DNA substrate (41 fmol) and the other indicated components and was incubated for 60 min at 37 °C. The recovered DNAs were digested with Clal, separated on an alkaline agarose gel, transferred onto a nylon membrane, and hybridized with the 32P-labeled probe ts154, which is complementary to the discontinuous strand. A, DNA species visualized in one of the experiments. The diagram outlines relative positions of the probe (a bar with an asterisk), unique Clal site, strand break, and mismatch. B, formation of the ~130-nt product of degradation of the 3'-nicked O6-mG-T DNA as a function of the amount of purified CAF-1. The data were obtained by quantification of images, including those shown in A and Fig. 4A and are averages ± 1 S.D. (error bars) (n = 3).
ble strand breaks are sufficient to kill the yeast rad52 cell (83). Therefore, the fact that the MNNG treatment kills the cac1Δ mgt1Δ rad52Δ and cac2Δ mgt1Δ rad52Δ cells more efficiently than the mgt1Δ rad52Δ cells indicates that CAF-1 loss increases the fraction of MNNG-treated cells that experience at least 1–2 double strand breaks.

In addition to CAF-1, S. cerevisiae cells contain several other histone chaperones, including HIR and Rtt106 (75). Our results indicated that loss of HIR2 or Rtt106 does not increase the sensitivity of mgt1Δ rad52Δ cells to MNNG (Fig. 1G). Thus, these results imply that neither of these histone chaperones has a non-redundant function that provides a protection for mgt1Δ rad52Δ cells from the cytotoxic activity of the MMR system. We determined that decreasing histone H3-H4 gene dosage by deletion of the HHT2-HHF2 locus in the mgt1Δ rad52Δ cells does not change their sensitivity to MNNG (Fig. 1G). A previous report described that hht2-hhf2Δ does not affect the level of the chromatin H3-H4 histones but decreases the level of the soluble H3-H4 histones 2-fold (84). Thus, it appears that a small decrease in the level of the soluble histones H3-H4 does not affect the cytotoxic activity of the MMR system.

An earlier study has implicated MutLα endonuclease activity in the cytotoxic response to the Sα1-type methylating drug in the yeast and mammalian cells (68). We have now shown that activated MutLα endonuclease degrades the discontinuous strand of an irreparable O6-mG-T mispair-containing DNA in an extract system and in a purified system (Figs. 4–8). This information implies that MutLα endonuclease-dependent deg-
radation of the discontinuous strand of irreparable $O^6\text{-mG-T}$ mispair-containing nuclear DNA is involved in the cytotoxic response to the $S_n1$-type methylating drug. MutL$\alpha$ endonuclease degrades the $3'$-nicked $O^6\text{-mG-T}$ and G-T DNA substrates in the presence of MutS$\alpha$, PCNA, RFC, and RPA in a very similar way (Fig. 8A, lanes 3–5 and lanes 15–17), suggesting that the same mechanism activates MutL$\alpha$ endonuclease in MMR and in the cytotoxic response to the $S_n1$-type methylating drug. The mechanism of activation of MutL$\alpha$ endonuclease in MMR requires the presence of MutS$\alpha$ and a mismatch (37, 39, 42) (Fig. 8). The $O^6\text{-mG-T}$ mispair is one of many mispairs recognized by MutS$\alpha$ (21). A crystallographic study determined that MutS$\alpha$ has the same structure in the MutS$\alpha$-G-T DNA and MutS$\alpha$-$O^6\text{-mG-T}$ DNA crystals (85). This information and the results of our analysis of the defined reactions (Fig. 8) suggest that adoption of the same structure by MutS$\alpha$ on the G-T mispair and on the irreparable $O^6\text{-mG-T}$ mispair permits the protein to convey the same activating signal to MutL$\alpha$.

**FIGURE 8.** MutL$\alpha$ endonuclease-dependent degradation of an irreparable $O^6\text{-mG-T}$ mispair-containing DNA in a defined system. The experiments were performed and analyzed as described under “Experimental Procedures.” Each reaction mixture contained a DNA substrate (81 fmol) and the indicated proteins and was incubated for 10 min at 37 °C. When MutS$\alpha$, PCNA, RFC, and RPA were present in the reaction mixtures, their concentrations were 40, 24, 4, and 40 nM, respectively. The recovered DNAs that either were not digested (A and C) or were digested (E) with ClaI were resolved on alkaline agarose gels and hybridized with the $^{32}$P-labeled probes ts154, ts1, and c29. The ts154 and ts1 probes are complementary to the discontinuous strands, and the c29 probe is complementary to the continuous strands. A and C, DNA species visualized with the $^{32}$P-labeled probes ts154 and ts1, respectively. B, degradation of the discontinuous strands of the $3'$-nicked DNA substrates as a function of MutL$\alpha$ endonuclease concentration. The data were obtained by quantification of images generated with the $^{32}$P-labeled probes ts154 and ts1, respectively. D, degradation of the discontinuous strands of the $3'$-nicked DNA substrates in the presence of the MutL$\alpha$-E705K mutant. The data were obtained by quantification of images generated with the $^{32}$P-labeled probe ts1. One of the images is shown in C. The data in B and D are averages ± 1 S.D. (error bars) (n = 3). E, DNA species visualized with the $^{32}$P-labeled probe c29. Each of the diagrams depicts the relative positions of the $^{32}$P-labeled probe (a bar with an asterisk), the strand break, and a mismatch.
The MMR System and CAF-1

endonuclease during MMR and the cytotoxic response to the S_{1}-type methylating drug. Although the major product of persistent MutLα endonuclease-dependent degradation of the 3'-nicked O^6-mG-T DNA in the extract system has a size of ~130 nt (Figs. 4A and 5A (lane 10) and 6A (lane 8)), the products of MutLα endonuclease-dependent degradation of the 3'-nicked O^6-mG-T DNA in the extract system that was supplemented with aphidicolin have sizes in the range of 100–2,000 nt (Fig. 4, A and B, lane 14). Because aphidicolin is an inhibitor of the biosynthetic activities of Pol δ and Pol ε, these findings suggest that DNA synthesis and ligation that occur on the MutLα-degraded DNA in the extract system in the absence of aphidicolin are necessary to remove the majority of the 100–2,000-nt products.

It is important to note that a previous work described that MutLα-dependent processing of the 3'-nicked O^6-mG-T DNA in the HCT116BR nuclear extract-containing system leads to the formation of a ~130-nt product (52) that does not appear to be different from the one that we have detected in our extract system (Figs. 4–7). This observation implies that reactions that occur in different extract systems generate the same product of degradation of the 3'-nicked O^6-mG-T DNA. We have observed that MutLα endonuclease-dependent degradation of the 3'-nicked O^6-mG-T DNA in our extract system leads to the formation of a gapped product (Fig. 7). The gap was generated in the discontinuous strand in the presence of dNTPs and was consistent MutL/H11032 system-dependent processing of the 3' located downstream from the mispaired T. Thus, the MMR in the discontinuous strand in the presence of dNTPs and was formation of a gapped product (Fig. 7). The gap was generated downstream from the 3'-nicked O^6-mG-T DNA. We have observed that MutLα endonuclease-dependent degradation of the 3'-nicked O^6-mG-T DNA in our extract system leads to the formation of a gapped product (Fig. 7). The gap was generated in the discontinuous strand in the presence of dNTPs and was located downstream from the mispaired T. Thus, the MMR system-dependent processing of the 3'-nicked O^6-mG-T DNA in the cell extract generates two kinds of DNA products; one of them carries the gap (Fig. 7), and the other contains the ~130-nt fragment (Figs. 5A and 6A). It is likely that the gap is formed when the excision step is not blocked by the O^6-mG, whereas the ~130-nt fragment is formed when the excision step is blocked by the lesion. The presence of a gap in the irreparable DNA is in good agreement with a previous study that documented that gaps are formed behind replication forks in response to treatment of both mammalian MGMT-deficient cells and yeast mgt1Δ rad52Δ cells with MNNG (53). The fact that the gap was generated downstream from the mismatched T (Fig. 7) suggests that O^6-mG is a stronger block for the DNA polymerization reaction than for the excision reaction.

The S_{1}-type methylating drug temozolomide is used for treatment of glioblastoma patients. However, recurrent glioblastomas often arise during post-treatment period (86). This indicates that an approach that increases the sensitivity of MGMT-deficient tumors to treatment with the S_{1}-type methylating drug might suppress recurrent cancers. More research is needed to determine whether defective replication-coupled nucleosome assembly increases the sensitivity of MGMT-deficient cancer cells to treatment with the S_{1}-type methylating drug.

Experimental Procedures

Yeast Strains—S. cerevisiae strains that were used in this study were derivatives of the wild-type haploid strain E134 (MATα ade5-1 lys2::InsE-A_{14} trp1-289 his7-2 leu2-3,112 ura3-52) (13). The lithium acetate/PEG4000/DMSO transformation method and PCR-amplified disruption cassettes were used to generate the strains.

MNNG and Bleomycin Cytotoxicity Assays—LiquidYPD medium (1% yeast extract, 2% Bacto-peptone, 2% dextrose, 60 mg/liter adenine, 60 mg/liter uracil), YPD plates, 1 mM stock solutions of MNNG (Wako Chemicals USA) in DMSO, a 2 mg/ml stock solution of bleomycin (Santa Cruz Biotechnology) in DMSO, and DMSO were used in the assays. Yeast cultures were grown to saturation in liquid YPD medium for ~20 h at 30 °C. The saturated cultures were diluted 10-fold in fresh medium and grown for 4 h at 30 °C. The cultures were then diluted with fresh medium to A_{600} = 1.3, and aliquots of the diluted cultures were treated with 1 μM MNNG, 0.1% DMSO (vehicle control in the MNNG toxicity assay), 30 μg/ml bleomycin, and 1.5% DMSO (vehicle control in the bleomycin cytotoxicity assay) for 2 h at 30 °C. After treatment, the cultures were diluted, and appropriate dilutions of the cultures were spread on YPD plates. The plates were incubated for 3–4 days at 30 °C, and colonies were counted. A somewhat different MNNG cytotoxicity assay was also used in this study. In this assay, 10-fold serial dilutions of the treated cultures were made and spotted on YPD plates. The plates were incubated for 2 days at 30 °C and photographed.

Camptothecin and Hydroxyurea Cytotoxicity Assays—Yeast cultures were grown to saturation as described above and diluted to A_{600} = 1.4 with sterile water. 10-fold serial dilutions of the cultures were prepared and spotted on YPD plates, YPD plates containing 0.5 μg/ml camptothecin (Enzo Life Science), and YPD plates containing 10 mM hydroxyurea (US Biological). After incubation for 2 days at 30 °C, the plates were photographed.

Cell Extract and Recombinant Cells—293T cells were grown as an attached culture in DMEM/high glucose medium that was supplemented with 10% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.29 μg/ml l-glutamine. Cytosolic and nuclear extracts from proliferating 293T cells were prepared according to a described procedure (63). Recombinant human CAF-1, MutSα, MutLα, MutLα-E705K, PCNA, RFC, and RPA were isolated in nearly homogeneous forms as described previously (37, 63).

Western Blotting—Samples of the purified CAF-1, 293T cytosolic extract (15 μg), and 293T nuclear extract (15 μg) were separated on a denaturing SDS gel and transferred onto a PVDF membrane. After the protein transfer step, the membrane was incubated with α-CAF-1 p150 antibodies (catalog no. sc-10772, lot E2004, rabbit polyclonal IgG, Santa Cruz Biotechnology, Inc.) and then with ECL HRP-conjugated secondary antibodies (catalog no. NA934V, lot 389592, donkey antibody, GE Healthcare). Immune complexes were visualized utilizing ECL2 Western blotting substrate (Thermo Fisher Scientific) and a CCD camera. Amounts of CAF-1 in the samples of the 293T cytosolic and nuclear extracts were measured by quantification of the data with the ImageJ software.

DNA Substrates, Oligonucleotides, and 32P-Labeled Hybridization Probes—3'-Nicked O^6-mG-T, 3'-nicked G-T, and 3'-nicked A-T DNAs were essentially prepared as described previously (52), except that after DNA ligation, unligated material was degraded by exonuclease III (New England Biolabs),
and the remaining DNA was purified by chromatography on BND-cellulose (Sigma). The Oβ-mg-containing oligonucleotide that was used to prepare the 3’- nicked Oβ-mg-T DNA was synthesized by Midland Certified Reagent Co. All other oligonucleotides used in this study were synthesized by IDT. To prepare a 32P-labeled hybridization probe, the oligonucleotide was labeled at the 5’ end with 32P by T4 polynucleotide kinase in the presence of [γ-32P]ATP. The sequences of oligonucleotides used to construct the DNA substrates and prepare 32P-labeled hybridization probes are shown in Table 1.

### Nucleosome Assembly Reactions in 293T Cytoplasmic Extract

The nucleosome assembly reactions were carried out at 37 °C in 40-µl mixtures that contained 20 mM HEPES-NaOH (pH 7.4), 100 mM KCl, 8 mM MgCl2, 2 mM DTT, 0.2 mg/ml BSA, 0.1 mM each of the four dNTPs, 3 mM ATP, 20 mM creatine phosphate, 0.02 mg/ml creatine phosphokinase, 1% glycerol (v/v), 74 µg of 293T cytoplasmic extract, purified CAF-1 (0 or 1.2 pmol), MutLa (0 or 1.6 pmol), and 81 fmol (0.1 µg) of a 3’- nicked DNA (the 3’- nicked Oβ-mg-T DNA, the 3’- nicked G-T DNA, or the 3’- nicked A-T DNA). The reaction mixtures were incubated for 10 min at 37 °C, and each reaction was terminated by the addition of a 30-µl mixture containing 0.35% SDS, 0.4 M NaCl, 13 mM EDTA, 0.33 mg/ml Proteinase K, and 2 mg/ml glycogen, followed by incubation of the mixtures for 15 min at 50 °C. The mixtures were then extracted by phenol/chloroform, and the DNAs were recovered from the precipitates by ethanol precipitation. To detect whether a 3’- nicked DNA was degraded in the reaction, a fraction of the recovered DNA was cleaved with ClaI, separated on an alkaline 1.4% agarose gel, and stained with ethidium bromide and quantified by ImageJ software. To detect whether the repair of Oβ-mg-T or G-T mispairs occurred in a reaction mixture, a fraction of the recovered DNA was cleaved with BamHI and XhoI and separated on a 1.2% agarose gel, followed by staining of the gel with ethidium bromide and quantification of DNA species with the Image software. To detect whether a 3’- nicked DNA was degraded in the reaction, a fraction of the recovered DNA was cleaved with ClaI, separated on an alkaline 1.4% agarose gel, and stained with ethidium bromide and quantified by ImageJ software. To detect whether the repair of Oβ-mg-T or G-T mispairs occurred in a reaction mixture, a fraction of the recovered DNA was cleaved with ClaI, resolved on a native 1.4% agarose gel, transferred onto a nylon membrane, and hybridized with a 32P-labeled probe. Indirectly labeled DNA species were visualized and quantified as described above.

### Cleavage of DNA by Activated MutLa Endonuclease in a Defined System

The reactions were performed in 40-µl mixtures that contained 20 mM HEPES-NaOH (pH 7.4), 120 mM KCl, 5 mM MgCl2, 3 mM ATP, 2 mM DTT, 0.2 mg/ml BSA, 2% glycerol (v/v), and 2 µl (81 fmol) of a 3’- nicked DNA (the 3’- nicked Oβ-mg-T DNA, the 3’- nicked G-T DNA, or the 3’- nicked A-T DNA). When incubated, MutLa (2, 6, or 20 nm), MutLa-E705K (20 nm), PCNA (24 nm), RFC (4 nm), and RPA (40 nm) were included in the reaction mixtures. The reaction mixtures were incubated for 10 min at 37 °C, and each reaction was terminated by the addition of a 30-µl mixture containing 0.35% SDS, 0.4 M NaCl, 13 mM EDTA, 0.33 mg/ml Proteinase K, and 2 mg/ml glycogen, followed by incuba-
The MMR System and CAF-1

3. Iyer, R. R., Pluciennik, A., Burdett, V., and Modrich, P. L. (2006) DNA mismatch repair: functions and mechanisms. Chem. Rev. 106, 302–323

4. Jiricny, J. (2006) The multifaceted mismatch-repair system. Nat. Rev. Mol. Cell Biol. 7, 335–346

5. Pen˜a-Diaz, J., and Jiricny, J. (2012) Mammalian mismatch repair: error-free or error-prone? Trends Biochem. Sci. 37, 206–214

6. Umar, A., Buermeyer, A. B., Simon, J. A., Thomas, D. C., Clark, A. B., Liskay, R. M., and Kunkel, T. A. (1996) Requirement for PCNA in DNA mismatch repair at a step preceding DNA resynthesis. Cell 87, 65–73

7. Johnson, R. E., Kovvali, G. K., Guzder, S. N., Amin, N. S., Holm, C., Habraken, Y., Sung, P., Prakash, L., and Prakash, S. (1996) Evidence for involvement of yeast proliferating cell nuclear antigen in DNA mismatch repair. J. Biol. Chem. 271, 27987–27990

8. Modrich, P. (2009) DNA polymerase δ is required for human mismatch repair in vitro. J. Biol. Chem. 272, 10917–10921

9. Smart, J., and Kolodner, R. D. (2015) Activation of Saccharomyces cerevisiae Mlh1-Pms1 endonuclease in a reconstituted mismatch repair system. J. Biol. Chem. 290, 21580–21590

10. Kadyrova, L. Y., Dahal, B. K., and Kadyrov, F. A. (2015) Evidences that the hMSH2-PMS1 endonuclease in a reconstituted mismatch repair system removes 1-nt Okazaki fragment flaps. Mol. Gen. Genet. 289, 39752–39761
43. Rodrigues Blanko, E., Kadyrova, L. Y., and Kadyrov, F. A. (2016) DNA mismatch repair interacts with CAF-1- and ASF1A-H3-H4-dependent histone (H3-H4) tetramer deposition. *J. Biol. Chem.* **291**, 9203–9217.

44. Pluciennik, A., Dzantiev, L., Iyer, R. R., Constantini, N., Kadyrov, F. A., and Modrich, P. (2010) PCNA function in the activation and strand direction of MutLε endonuclease in mismatch repair. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 16066–16071.

45. Shao, H., Baitinger, C., Soderblom, E. J., Burdett, V., and Modrich, P. (2014) Hydrolytic function of Exo1 in mammalian mismatch repair. *Nucleic Acids Res.* **42**, 7104–7112.

46. Branch, P., Aquilina, G., Bignami, M., and Karran, P. (1993) Defective mismatch binding and a mutator phenotype in cells tolerant to DNA damage. *Nature* **362**, 652–654.

47. Cejka, P., Mojas, N., Gillet, L., Schär, P., and Jiricny, J. (2005) Homologous recombination rescues mismatch-repair-dependent cytotoxicity of S(N)1-type methylating agents in *S. cerevisiae*. *Curr. Biol.* **15**, 1395–1400.

48. Loveless, A. (1969) Possible relevance of O-6 alklylation of deoxyguanosine to the mutagenic activity and carcinogenicity of nitrosamines and nitroamides. *Nature* **223**, 206–207.

49. Glassner, B. J., Weeda, G., Allan, J. M., Broekhof, J. L., Carls, N. H., Donker, I., Engelward, B. P., Hampson, R. J., Hoesm, R., Hickman, M. J., Roth, R. B., Warren, H. B., Wu, M. M., Hoeijmakers, J. H., and Samson, L. D. (1999) DNA repair in mammalian cells. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 339–347.

50. Esteller, M., Garcia-Foncillas, J., Andion, E., Goodman, S. N., Hidalgo, O. F., Vanaclocha, V., Baylin, S. B., and Herman, J. G. (2000) Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to O6-alkylating agents. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 1105–1114.

51. York, S. J., and Modrich, P. (2006) Mismatch repair-deficient iterative excision at irreparable O6-methylguanine lesions in human nuclear extracts. *J. Biol. Chem.* **281**, 22674–22683.

52. Zhukovskaya, N., Branch, P., Aquilina, G., and Karran, P. (1994) DNA replication arrest and tolerance to DNA methylation damage. *Carcinogenesis* **15**, 2189–2194.

53. Zbijowskaya, N., Branch, P., Aquilina, G., and Karran, P. (1994) DNA replication arrest and tolerance to DNA methylation damage. *Carcinogenesis* **15**, 2189–2194.

54. Stojic, L., Mojas, N., Cejka, P., Di Pietro, M., Ferrari, S., Marra, G., and Jiricny, J. (2004) Mismatch repair-deficient G1 checkpoint induced by low doses of SN1 type methylating agents requires the ATR kinase. *Genes Dev.* **18**, 1331–1344.

55. Tsaryk, R., Fabian, K., Thacker, J., and Kaina, B. (2006) Xrscc2 deficiency sensitizes cells to apoptosis by MNNG and the alkylating anticancer drugs temozolomide, fotemustine and mafosfamide. *Cancer Lett.* **239**, 305–313.

56. Smith, S., and Stillman, B. (1989) Purification and characterization of CAF-1, a human cell factor required for chromatin assembly during DNA replication in vitro. *Cell* **58**, 15–25.

57. Kaufman, P. D., Kobayashi, R., Kessler, N., and Stillman, B. (1995) The p150 and p60 subunits of chromatin assembly factor I: a molecular link between newly synthesized histones and DNA replication. *Cell* **81**, 1105–1114.

58. Verreault, A., Kaufman, P. D., Kobayashi, R., and Stillman, B. (1996) Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. *Cell* **87**, 95–104.

59. Kaufman, P. D., Kobayashi, R., and Stillman, B. (1997) Ultraviolet radiation sensitivity and reduction of telomeric silencing in Saccharomyces cerevisiae cells lacking chromatin assembly factor I. *Genes Dev.* **11**, 345–357.

60. Hoek, M., and Stillman, B. (2003) Chromatin assembly factor I is essential and couples chromatin assembly to DNA replication in *in vivo*. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 12183–12188.

61. Smith, S., and Stillman, B. (1991) Stepwise assembly of chromatin during DNA replication in *in vitro*. *EMBO J.* **10**, 971–980.

62. Kadyrova, L. Y., Blanko, E. R., and Kadyrov, F. A. (2011) CAF-1-dependent control of degradation of the discontinuous strands during mismatch repair. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 2753–2758.

63. Kadyrova, L. Y., Rodrigues Blanko, E., and Kadyrov, F. A. (2013) Human CAF-1-dependent nucleosome assembly in a defined system. *Cell Cycle* **12**, 3286–3297.

64. Schöpf, B., Bregenhorn, S., Quivy, J. P., Kadyrov, F. A., Almouzni, G., and Jiricny, J. (2012) Interplay between mismatch repair and chromatin assembly. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 1895–1900.

65. Erdemir, N., Nguyen, M., Deschênes, S. M., and Liskay, R. M. (2007) Mutations affecting a putative MutLε endonuclease motif impact multiple mismatch repair functions. *DNA Repair* **6**, 1463–1470.

66. Xiao, W., Derfler, B., Chen, J., and Samson, L. (2003) Primary sequence and biological functions of a Saccharomyces cerevisiae O6-methylguanine O4-methylthymine DNA repair methyltransferase gene. *EMBO J.* **10**, 2179–2186.

67. Sung, P. (1997) Function of yeast Rad52 protein as a mediator between replication protein A and the Rad51 recombination. *J. Biol. Chem.* **272**, 28194–28197.

68. Hsiang, Y. H., Liou, M. G., and Liu, L. F. (1989) Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complexes as a mechanism of cell killing by camptothecin. *Cancer Res.* **49**, 5077–5082.

69. Krackof, I. H., Brown, N. C., and Reichard, P. (1968) Inhibition of ribonucleoside diphosphate reductase by hydroxyurea. *Cancer Res.* **28**, 1559–1565.

70. Povirk, L. F. (1996) DNA damage and mutagenesis by radiodinetic DNA cleaving agents: bleomycin, necarozinostatin and other endonucleases. *Mutat. Res.* **355**, 71–89.

71. Green, E. M., Antczak, A. J., Bailey, A. O., Franco, A. A., Wu, K. J., Yates, J. R. 3rd, and Kaufman, P. D. (2005) Replication-independent histone deposition by the HiR complex and Asf1. *Curr. Biol.* **15**, 2044–2049.

72. Burgess, R. J., and Zhang, Z. (2013) Histone chaperones in nucleosome assembly and human disease. *Nat. Struct. Mol. Biol.* **20**, 14–22.

73. Cross, S. L., and Smith, M. M. (1988) Comparison of the structure and cell cycle expression of mRNAs encoded by two histone H3-H4 loci in Saccharomyces cerevisiae. *Mol. Cell. Biol.* **8**, 945–954.

74. Trojan, J., Zeuzem, S., Randolph, A., Hemmerle, C., Brieger, A., Raedle, J., Plotz, G., Jiricny, J., and Marra, G. (2002) Functional analysis of hMLH1 variants and HNPPC-related mutations using a human expression system. *Gastroenterology* **122**, 211–219.

75. Cejka, P., Stojic, L., Mojas, N., Russell, A. M., Heinimann, K., Cannavò, E., di Pietro, M., Marra, G., and Jiricny, J. (2003) Methylation-induced G1/M arrest requires a full complement of the mismatch repair protein hMLH1. *EMBO J.* **22**, 2245–2254.

76. Holmes, J., Jr., Clark, S., and Modrich, P. (1990) Strand-specific mismatch correction in nuclear extracts of human and Drosophila melanogaster cell lines. *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5837–5841.

77. Kat, A., Thilly, W. G., Fang, W. H., Longley, M. J., Li, G. M., and Modrich, P. (1993) An alkylating-tolerant, mutator human cell line is deficient in strand-specific mismatch repair. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6424–6428.

78. de Wind, N., Dekker, M., Berns, A., Radman, M., and te Riele, H. (1995) Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell* **82**, 321–330.
The MMR System and CAF-1

82. Umar, A., Koi, M., Risinger, J. I., Glaab, W. E., Tindall, K. R., Kolodner, R. D., Boland, C. R., Barrett, J. C., and Kunkel, T. A. (1997) Correction of hypermutability, N-methyl-N'-nitro-N-nitrosoguanidine resistance, and defective DNA mismatch repair by introducing chromosome 2 into human tumor cells with mutations in MSH2 and MSH6. *Cancer Res.* **57**, 3949–3955

83. Resnick, M. A., and Martin, P. (1976) The repair of double-strand breaks in the nuclear DNA of *Saccharomyces cerevisiae* and its genetic control. *Mol. Gen. Genet.* **143**, 119–129

84. Liang, D., Burkhart, S. L., Singh, R. K., Kabbaj, M. H., and Gunjan, A. (2012) Histone dosage regulates DNA damage sensitivity in a checkpoint-independent manner by the homologous recombination pathway. *Nucleic Acids Res.* **40**, 9604–9620

85. Warren, J. J., Pohlhaus, T. J., Changela, A., Iyer, R. R., Modrich, P. L., and Beese, L. S. (2007) Structure of the human MutSα DNA lesion recognition complex. *Mol. Cell* **26**, 579–592

86. Cahill, D. P., Levine, K. K., Betensky, R. A., Codd, P. J., Romany, C. A., Reavie, L. B., Batchelor, T. T., Futreal, P. A., Stratton, M. R., Curry, W. T., Iafrate, A. J., and Louis, D. N. (2007) Loss of the mismatch repair protein MSH6 in human glioblastomas is associated with tumor progression during temozolomide treatment. *Clin. Cancer Res.* **13**, 2038–2045