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# A Role for Yeast and Human Translesion Synthesis DNA Polymerases in Promoting Replication through 3-Methyl Adenine[▽]

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**3-Methyl adenine (3meA), a minor-groove DNA lesion, presents a strong block to synthesis by replicative DNA polymerases (Pols). To elucidate the means by which replication through this DNA lesion is mediated in eukaryotic cells, here we carry out genetic studies in the yeast *Saccharomyces cerevisiae* treated with the alkylating agent methyl methanesulfonate. From the studies presented here, we infer that replication through the 3meA lesion in yeast cells can be mediated by the action of three Rad6-Rad18-dependent pathways that include translesion synthesis (TLS) by Polη or -ζ and an Mms2-Ubc13-Rad5-dependent pathway which presumably operates via template switching. We also express human Pols ι and κ in yeast cells and show that they too can mediate replication through the 3meA lesion in yeast cells, indicating a high degree of evolutionary conservation of the mechanisms that control TLS in yeast and human cells. We discuss these results in the context of previous observations that have been made for the roles of Pols η, ι, and κ in promoting replication through the minor-groove $N^2$-dG adducts.**

Replicative DNA polymerase (Pols) are highly sensitive to geometric distortions in DNA; consequently, they are inhibited by the presence of DNA lesions in the template strand. A number of Pols that promote replication through DNA lesions exist in eukaryotes, and they are highly specialized for the roles they play in translesion synthesis (TLS) (40). For example, both yeast and human Polηs are highly adept at promoting error-free replication through UV-induced cyclobutane pyrimidine dimers (CPDs) (15, 20, 50, 52), and inactivation of Polη in humans causes the cancer-prone syndrome of the variant form of xeroderma pigmentosum (14, 30).

Although proficient replication through a DNA lesion such as a CPD can be accomplished by Polη alone, replication through many of the different lesions present in DNA requires the sequential action of two Pols, in which one polymerase inserts the nucleotide opposite the DNA lesion and another polymerase performs the subsequent extension reaction (40, 41). Yeast Polζ, comprised of the Rev3 catalytic and Rev7 accessory subunits (39), is highly specialized for performing the extension step of TLS (8, 19, 21, 35). Although humans also have the Rev3 and Rev7 proteins, there is no biochemical information available on the role of human Polζ in TLS.

In addition to Polη, humans have Pols ι and κ, which belong to the Y family of Pols (40). Polι differs strikingly from Pols η and κ and almost all other Pols in that it incorporates nucleotides opposite template purines with much higher efficiency and fidelity than opposite template pyrimidines (6, 19, 44, 49). Moreover, even opposite template purines, Polι exhibits higher catalytic efficiency and fidelity opposite template A than opposite template G. The ternary crystal structures of Polι bound to template A or G and the correct incoming deoxynucleoside triphosphate have shown that the purine template adopts a *syn* conformation in the Polι active site and forms a Hoogsteen base pair with the incoming nucleotide, which remains in the *anti* conformation (36–38). Hoogsteen base pairing explains the basis for the higher efficiency of correct nucleotide incorporation opposite template purines than opposite template pyrimidines, as only the purine bases have the Hoogsteen edge via which they can hydrogen bond with the correct incoming nucleotide.

The ability of the Polι active site to push the template purine into the *syn* conformation provides an elegant mechanism by which this polymerase can incorporate nucleotides opposite minor-groove DNA lesions. The minor-groove $N^2$ group of guanine is highly reactive and can conjugate with a large variety of endogenously formed products. For example, the reaction of acrolein, an α,β-unsaturated aldehyde formed in cells as a product of lipid peroxidation, with the $N^2$ group of guanine in DNA followed by ring closure at N-1, leads to the formation of the cyclic adduct γ-hydroxy-1,$N^2$-propano-2′-deoxyguanosine (γ-HOPdG). Polι incorporates a C opposite this adduct with the same catalytic efficiency ($k_{cat}/K_m$) as it does opposite the nonadducted template G residue (51). Polι, however, is unable to carry out the subsequent extension reaction, and this step is modulated by Polκ (51). The sequential actions of Pols ι and κ can also promote replication through the structurally more complex aldehyde products of lipid peroxidation, such as *trans*-4-hydroxy-2-nonenal (HNE) (55).

γ-HOPdG adopts a ring-closed 1,$N^2$-exo cyclic form when present as a templating residue, but upon pairing with a C, it changes from the closed cyclic form to a ring-opened conformation able to form a normal Watson-Crick base pair (3, 23, 24). Studies with the permanently ring-opened or ring-closed structural analogs of γ-HOPdG have yielded useful information about the relative abilities of Pols η, ι, and κ to replicate through such $N^2$-dG adducts, and the combined results of these and other studies have indicated that these Pols vary in

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their response to different $N^2$-dG adducts. For example, the ring-closed form of γ-HOPdG is a strong block for nucleotide incorporation by Pols η and κ, with only Polι being capable of inserting a C opposite from it (33, 54). And, even though Polκ can extend from a C opposite γ-HOPdG, which can adopt both the ring-opened and ring-closed forms (51), it is unable to extend from the permanently ring-closed analog of γ-HOPdG (54), suggesting that Polκ can extend only if γ-HOPdG is in the ring-opened conformation. The ability of Polι to push the ring-closed analog of γ-HOPdG into a *syn* conformation would allow for its Hoogsteen base pairing with dCTP, but since Polκ cannot accommodate such a structure in its active site (29), it will not carry out the subsequent extension reaction. Polκ can, however, proficiently extend from the C opposite γ-HOPdG, because the pairing with a C will trigger the change from the ring-closed to the ring-opened form of γ-HOPdG capable of forming a normal Watson-Crick base pair from which Polκ can extend. The proficient ability of Polκ to extend from a C opposite from the γ-HOPdG or HNE lesions could derive from the fact that the minor-groove edge of the templating residue at the template-primer junction is open to solvent and not obstructed by the Polκ active site (29).

In contrast to the inhibitory effects of the ring-closed analog of γ-HOPdG on DNA synthesis by the various TLS Pols, the permanently ring-opened form of γ-HOPdG does not present a significant block to synthesis by Pols η, ι, or κ, as they can each carry out both nucleotide incorporation and the extension reactions opposite from it (33, 54). Thus, we assume that an $N^2$-dG minor-groove adduct, such as the ring-open form of γ-HOPdG, can be accommodated in the active site of these Pols at both the nucleotide insertion and subsequent extension steps.

To further investigate the ability of various polymerases to mediate TLS through minor-groove DNA adducts, we have carried out genetic experiments in the yeast *Saccharomyces cerevisiae* to examine the roles of yeast and human TLS Pols in promoting replication through an N-3 minor-groove adduct of adenine. Treatment of cells with methyl methanesulfonate (MMS) methylates the bases in DNA, particularly adenine at the N-3 position (3meA) and guanine at the N-7 position (7meG) (1, 26). In vitro studies have indicated that 3meA, which projects into the minor groove, is a strong inhibitor of DNA polymerases such as *Escherichia coli* Pol I or avian myeloblastosis virus reverse transcriptase, and DNA synthesis terminates one nucleotide before the lesion. 7meG projects into the major groove and does not block synthesis by these polymerases (25). Genetic studies in yeast have corroborated the blocking action of 3meA on synthesis by the replicative polymerases (see Results, below, for elaboration of this point). 3meA could be blocking replicative polymerases for a number of reasons. First, since the high-fidelity DNA polymerases use a minor-groove-sensing mechanism, in which certain residues from the polymerase form hydrogen bonds with the N-3 of a purine or the $O^2$ of a pyrimidine to detect the correct Watson-Crick geometry of the postinsertion template-primer base pair (22, 53), 3meA would be inhibitory to these polymerases at the extension step. Second, the presence of a methyl group at N-3 could block synthesis by replicative polymerases at both the insertion and extension steps because of the steric constraints imposed in the active site adjacent to the minor-groove edge of the templating nucleotide as well as the template nucleotide at the template-primer junction.

Here we provide evidence for the role of yeast Pols η and ζ and of human Pols ι and κ in promoting replication through the 3meA adduct. We discuss the implications of these observations in the context of previous biochemical studies that have examined the role of different TLS Pols in promoting replication through the variety of minor-groove DNA lesions.

## MATERIALS AND METHODS

**Yeast strains.** All the deletion strains used in these studies were derived from the wild-type yeast strain EMY74.7, *MAT***a** *his3-*Δ*1 leu2-3,112 trp1*Δ *ura3-52*, by the one-step gene replacement method (42).

**MMS survival and mutagenesis.** Cells grown overnight in yeast extract-peptone-dextrose (YPD) medium were washed with distilled water, sonicated to disperse clumps, and resuspended in 0.05 M $KPO_4$ buffer, pH 7.0, at a density of $3 \times 10^8$ cells/ml. After treatment of cell suspensions with various concentrations of MMS for 20 min at 30°C with vigorous shaking, an equal volume of 10% sodium thiosulfate was added to inactivate the MMS. Appropriate dilutions were plated on YPD for viability determinations and on synthetic complete medium containing canavanine but lacking arginine for determinations of the frequency of canavanine-resistant colonies.

**Expression of human Pols ι and κ in yeast cells.** The genes encoding human Polι (19) and Polκ (previously designated Polθ [16]) were cloned into the expression vector pMA91 (32), in which expression is under the control of the efficiently expressed yeast *PGK1* promoter. The plasmids generated, pBJ1129 for expression of Polι and pPOL15 for expression of Polκ, were introduced into various yeast strains, such as *mag1*Δ and other mutant strains, and tested for their ability to restore resistance to MMS by the serial dilution spot test. For these studies, cells were grown to mid-logarithmic phase in synthetic complete medium lacking leucine to maintain selection of the plasmid, washed, and resuspended in water to a density of $2 \times 10^8$/ml. Aliquots (200 μl) of serial 10-fold dilutions were pipetted into a 96-well microtiter dish, followed by transfer to YPD plates containing different concentrations of MMS, and plates were incubated at 30°C for 2 days before photographing.

## RESULTS

**Involvement of yeast DNA Pols η and ζ in promoting replication through the 3meA lesion.** Genetic studies in yeast have indicated that replication through UV-induced DNA lesions can be handled via at least three different Rad6-Rad18-dependent pathways (45) that include TLS by Pols η and ζ and a postreplicational repair pathway, which presumably involves template switching and a copy choice type of DNA synthesis and requires the Mms2-Ubc13 ubiquitin-conjugating enzyme (12), together with the Rad5 protein, which harbors a DNA-dependent ATPase and a ubiquitin ligase activity (17, 48). To determine the contribution that these three pathways make to 3meA bypass during replication, we examined the MMS sensitivity of yeast strains deleted for the genes that function in the Rad6-Rad18-dependent lesion bypass process.

We found that deletion of the *REV3* gene, which encodes the catalytic subunit of Polζ, confers a higher level of sensitivity to MMS than does deletion of *RAD30*, encoding Polη, whereas the *mms2*Δ mutation confers an intermediate level of MMS sensitivity between that resulting from the *rad30*Δ and *rev3*Δ mutations, and the *rad5*Δ mutation elicits a somewhat higher level of MMS sensitivity than the *rev3*Δ mutation (Fig. 1A).

The methylated bases 3meA and 7meG, formed in DNA upon treatment with MMS, are removed by an N-methyl purine DNA glycosylase encoded in yeast by the *MAG1* gene (2), and deletion of *MAG1* confers a high level of MMS sensitivity upon yeast cells (Fig. 1A). Since 3meA would be a block to

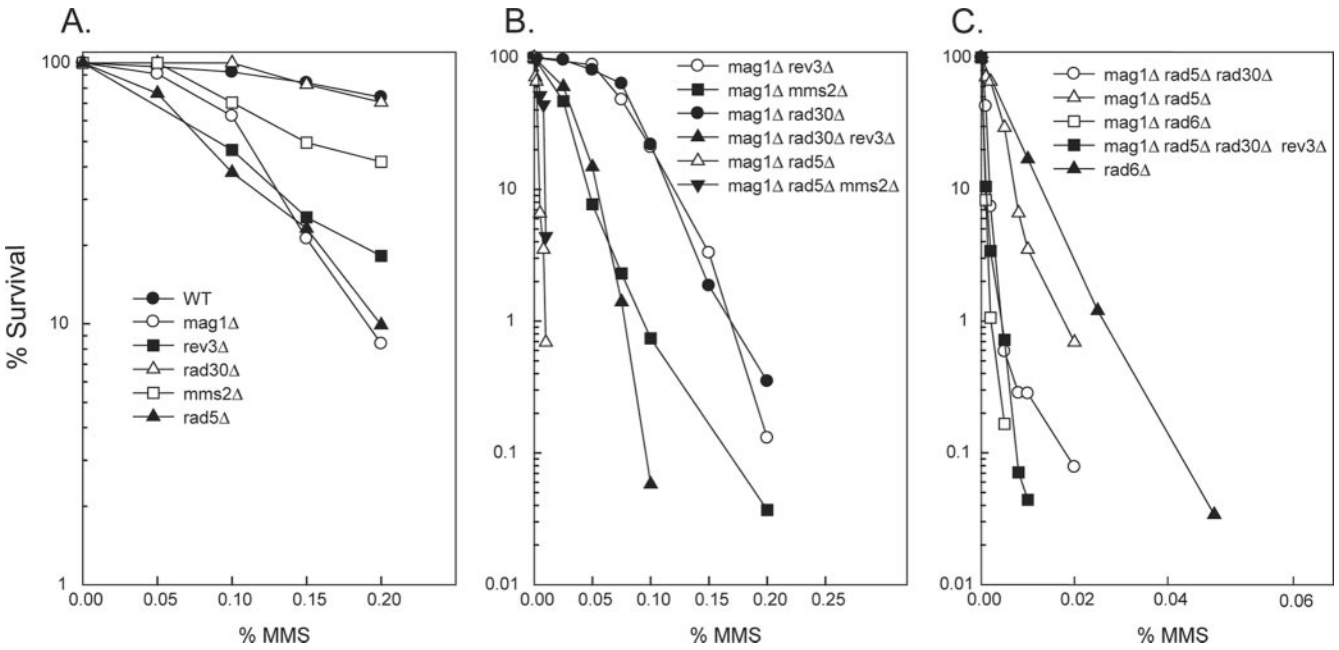


FIG. 1. MMS sensitivity of yeast strains carrying deletions of genes belonging to the *RAD6* epistasis group. Cells were treated with the indicated concentrations of MMS (percent [vol/vol]) at 30°C for 20 min, followed by inactivation of MMS with sodium thiosulfate. Appropriate dilutions of cells were plated on YPD for viability determinations. The survival curves represent an average of at least three experiments for each strain. WT, wild type. (A) MMS sensitivity of strains deleted for the TLS polymerases η and ζ and for the *MMS2* and *RAD5* genes, involved in postreplicational repair. (B) MMS sensitivity of the *mag1*Δ strain in combination with deletions of genes encoding the TLS polymerase η or ζ or genes that function in PRR. (C) MMS sensitivity of the *mag1*Δ strain in combination with deletions of genes, so that all three pathways of Rad6-Rad18-dependent lesion bypass have been inactivated.

synthesis by the replicative polymerases, the enhanced MMS sensitivity of the *mag1*Δ strain must result from the blocking effects of 3meA on replication. The experiments indicating that the treatment of yeast or human cells with Me-lex, a compound which selectively generates 3meA, confers a high level of cytotoxicity and that this cytotoxicity is further enhanced in the *mag1*Δ yeast strain have provided corroborative evidence that if not repaired, 3meA is inhibitory to normal DNA synthesis during replication (4, 34). The introduction of the *rad30*Δ or the *rev3*Δ mutation into the *mag1*Δ strain led to a synergistic enhancement of MMS sensitivity in the *mag1*Δ *rad30*Δ or *mag1*Δ *rev3*Δ double mutants compared to that in the respective single mutants (compare Fig. 1A and B); furthermore, a synergistic enhancement of MMS sensitivity occurred in the *mag1*Δ *rad30*Δ *rev3*Δ triple mutant strain compared to the MMS sensitivity of the *mag1*Δ *rad30*Δ or *mag1*Δ *rev3*Δ strains (Fig. 1B). We infer from these observations that Pols η and ζ provide alternate means by which replication through the 3meA lesion can be accomplished.

**Role for the Mms2-Ubc13-Rad5-dependent postreplication repair pathway in 3meA bypass.** The Mms2-Ubc13-Rad5-dependent pathway promotes the repair of discontinuities that form in the newly synthesized DNA from UV-damaged templates (45). This postreplicational repair (PRR) pathway presumably occurs by template switching and involves a copy choice type of synthesis in which replication through the DNA lesion is mediated by using the newly synthesized strand of the undamaged sister duplex as the template (10). The operation of this pathway requires Mms2-Ubc13-Rad5-dependent lysine 63-linked polyubiquitylation of PCNA, in which the ubiquitin ligase function of Rad5 promotes the polyubiquitylation of PCNA by Mms2-Ubc13 (7, 11, 43). In addition to its role as a ubiquitin ligase, Rad5 is likely to also function in postreplication repair in a more direct manner wherein its ATPase activity would modulate the template switching process (5).

We found that a synergistic enhancement in MMS sensitivity occurs in the *mag1*Δ *mms2*Δ and *mag1*Δ *rad5*Δ double mutant strains compared to that in the respective single mutant strains (compare Fig. 1A and B). The *mag1*Δ *rad5*Δ strain, however, displays a much higher level of MMS sensitivity than the *mag1*Δ *mms2*Δ strain, whereas the MMS sensitivity of the *mag1*Δ *rad5*Δ *mms2*Δ strain remained the same as that of the *mag1*Δ *rad5*Δ strain, which is in keeping with the epistasis of the *rad5*Δ mutation over the *mms2*Δ mutation (Fig. 1B). For UV damage also, although Rad5 functions in Mms2-Ubc13-dependent PRR, the *rad5*Δ mutation causes a much higher level of UV sensitivity than the *mms2*Δ and *ubc13*Δ mutations, and we have previously ascribed the increased UV sensitivity of the *rad5*Δ mutation to the additional role of Rad5 in affecting the efficiency of TLS mediated by Pols η and ζ (5). However, how Rad5 contributes to TLS by these Pols is not understood.

To verify that the TLS mediated by Pols η and ζ and that lesion bypass mediated by the Rad5-dependent pathway provide three different means for promoting replication through the 3meA lesion, we compared the MMS sensitivity of the triple mutant strain, in which all three pathways have been inactivated, with that of the double mutant strains, in which only two of the pathways have been inactivated. As expected, the MMS sensitivity of the *mag1*Δ *rad5*Δ strain was greatly enhanced upon the introduction of the *rad30*Δ mutation, and

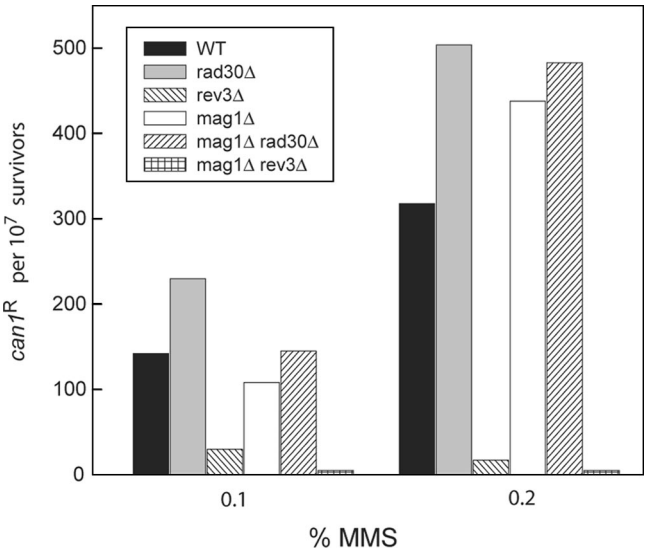


FIG. 2. MMS-induced *can1*$^r$ mutations in various mutant strains. Cells were treated for 20 min at 30°C with the indicated MMS concentrations. Following inactivation of MMS, cells were spread onto the surface of YPD plates for viability determinations and onto synthetic complete medium plates containing canavanine but lacking arginine for determining the frequency of *can1*$^r$ mutations. Each histogram represents the average of two to three experiments. WT, wild type.

introduction of the *rev3*Δ mutation into the *mag1*Δ *rad5*Δ *rad30*Δ strain led to a further increase in MMS sensitivity (Fig. 1C). Since the MMS sensitivity of the *mag1*Δ *rad5*Δ *rad30*Δ *rev3*Δ strain was nearly the same as that of the *mag1*Δ *rad6*Δ strain (Fig. 1C), Polη- and -ζ-dependent TLS and Rad5-dependent lesion bypass provide three different means of Rad6-Rad18-dependent bypass of 3meA during replication.

**TLS mediated by Pols η and ζ through the 3meA lesion is predominantly error free.** In yeast cells, inactivation of Polη confers a large enhancement in the incidence of mutagenesis induced by UV light (31, 57), consistent with the role of Polη in the error-free bypass of CPDs, and inactivation of Polζ confers a large decrease in the frequency of UV-induced mutations (27, 28), which accords with its role in promoting the mutagenic bypass of UV lesions.

To determine if replication through the 3meA lesion involves a mutagenic process, we first examined the frequency of MMS-induced mutations in the *mag1*Δ strain. Our observation that MMS induced *can1*$^r$ mutations occur at about the same rate in the *mag1*Δ strain as in the wild-type strain (Fig. 2), however, suggested that replication through the 3meA lesion by Pols η and ζ was mediated in a predominantly error-free way.

Although 3meA is the blocking lesion for synthesis by the replicative polymerases, 7meG is the predominant lesion formed in DNA upon MMS treatment (1, 26). Because spontaneous depurination of 7meG would lead to the formation of abasic sites, we presume that the increase in the *can1*$^r$ mutation frequency obtained in MMS-treated wild-type cells results from the mutagenic TLS that occurs opposite the abasic sites that happened to have escaped repair by the action of AP endonucleases or by nucleotide excision repair (46). In fact, the frequency of MMS-induced *can1*$^r$ mutations rises greatly in the *apn1*Δ *apn2*Δ strain, which lacks both the AP endonucleases, and it rises even further in the *apn1*Δ *apn2*Δ *rad14*Δ strain, which additionally lacks the nucleotide excision repair pathway (46). Also, since the elevated incidence of MMS-induced *can1*$^r$ mutations in the *apn1*Δ *apn2*Δ strain is not affected by the *rad30*Δ mutation, Polη does not contribute to TLS through the abasic sites in any significant way (8). By contrast, the *rev3*Δ mutation confers a large reduction in the frequency of MMS-induced *can1*$^r$ mutations in the *apn1*Δ *apn2*Δ strain, consistent with the role of Polζ in extending from the nucleotide inserted opposite the abasic site by another polymerase (8, 18). Since we observed that the frequency of *can1*$^r$ mutations is not significantly affected by the *rad30*Δ mutation in MMS-treated wild-type or *mag1*Δ cells, whereas the *rev3*Δ mutation confers a much-reduced level of mutagenesis in both these genetic backgrounds (Fig. 2), we infer that MMS-induced mutations in the wild-type or *mag1*Δ yeast cells emanate from the TLS mediated by Polζ opposite the abasic sites and, as expected from biochemical experiments indicating a highly inefficient bypass of abasic lesions by Polη (9), this Pol has no significant impact on the TLS through this lesion.

**Human Pols ι and κ promote TLS through 3meA in yeast cells.** The genetic studies with the yeast *rad30*Δ and *rev3*Δ mutations reported here indicated that both Pols η and ζ can promote TLS through the 3meA lesion. In addition to these Pols, humans contain Pols ι and κ, able to promote replication through the DNA lesions. To examine if human Pols ι and κ can also support replication through the 3meA lesion, we expressed these Pols in yeast cells and tested whether they could restore MMS resistance to *mag1*Δ and *mag1*Δ *rad30*Δ yeast strains. As shown in Fig. 3, the expression of Pol ι or Pol κ in *mag1*Δ or *mag1*Δ *rad30*Δ yeast cells led to a large increase in the MMS resistance of these cells; thus, both these human Pols are able to function in yeast cells in promoting TLS through the 3meA lesions.

## DISCUSSION

Although MMS treatment causes the formation of 3meA and 7meG in DNA, 3meA presents a strong block to synthesis by the replicative polymerases; hence, the cytotoxicity of MMS results from 3meA and not from the 7meG lesion. Accordingly, the MMS sensitivity of yeast cells is greatly enhanced in the absence of Mag1, which is required for 3meA removal. Since the 3meA lesions would persist in *mag1*Δ cells, for replication to proceed through the lesion site would require the action of lesion bypass processes.

Here we provide genetic evidence that replication through the 3meA lesion in yeast cells can be effected by three Rad6-Rad18-dependent pathways that include TLS mediated by Pols η and ζ and an Mms2-Ubc13-Rad5-dependent pathway which presumably operates by template switching and involves a copy choice type of DNA synthesis. In addition to the inferred role of yeast Pols η and ζ in promoting TLS through the 3meA lesion, we show here that the expression of human Polι or Polκ in yeast cells confers a large increase in MMS resistance to *mag1*Δ and *mag1*Δ *rad30*Δ cells, indicating that both these human Pols are also able to support replication through the 3meA lesion. Our observations for the role of yeast Pols η and ζ and human Pols ι and κ in promoting TLS through the 3meA

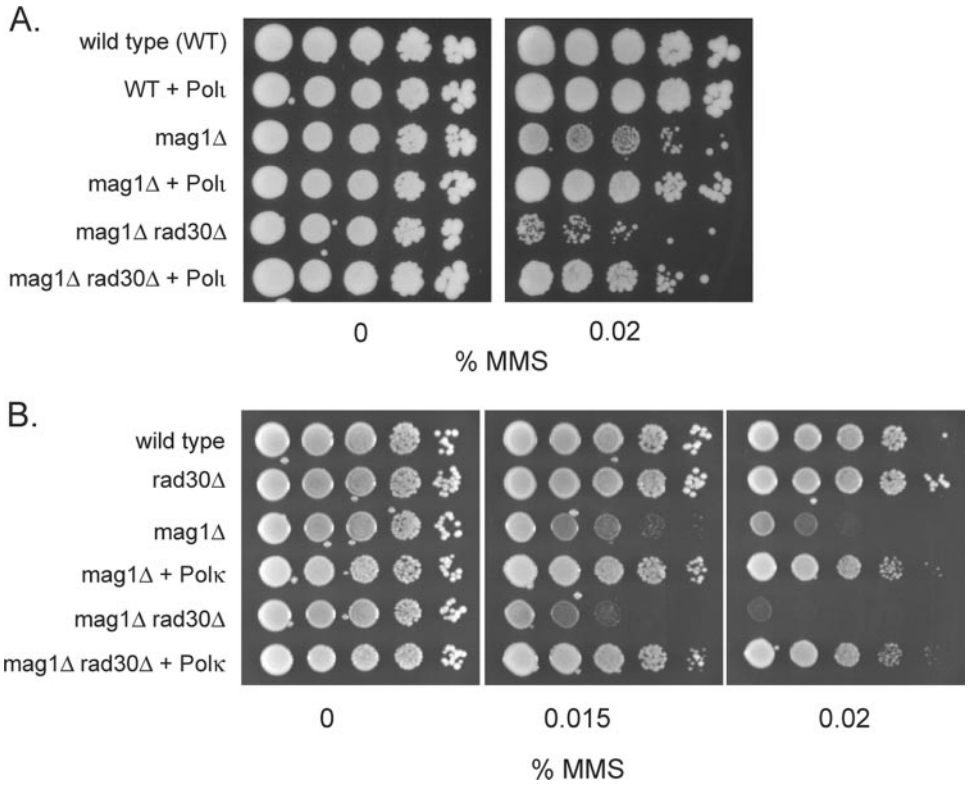


FIG. 3. Enhanced MMS resistance conferred upon *mag1*Δ and *mag1*Δ *rad30*Δ strains by human Pols ι and κ. For each strain, a sample of cells containing a given 10-fold serial dilution was pipetted into a well of a 96-well microtiter dish as described in Materials and Methods. Cells were transferred to YPD plates containing different MMS concentrations and photographed after 2 days of incubation at 30°C.

lesion when put into the context of the available information on their role in the bypass of various $N^2$-dG adducts lead us to draw conclusions that are relevant to the variety of reactions these Pols can support opposite the minor-groove lesions of differing structural complexity.

Previously, we showed that yeast and human Polη and human Pols ι and κ can all replicate through the $N^2$-dG adduct, the ring-opened analog of γ-HOPdG. Thus, in spite of the fact that this adduct would project into the minor groove, it presents no significant block to any of these polymerases at either the nucleotide insertion step or at the subsequent extension step. By contrast, the HNE adduct, which also conjugates at the $N^2$-dG and which because of its increased size would be more blocking to replication than the γ-HOPdG adduct, is handled very differently by human Pols η, ι, and κ. Whereas Polη is inhibited by the HNE-dG adduct at both the insertion and extension steps, Polι can efficiently insert a C opposite from it but cannot carry out the subsequent extension reaction. Polκ, on the other hand, is unable to insert a nucleotide opposite from HNE-dG, but it can extend from a C inserted opposite it by Polι. Whereas the proficiency of Polι to insert a C opposite the HNE-dG adduct would derive from its ability to push the adduct into a *syn* conformation, where there would be no steric hindrance from the Polι active site, Polι will be unable to extend from the C inserted opposite the HNE-dG adduct because of the structural constraints in Polι at the templating side of the template-primer junction. Polκ, however, is able to extend from a C opposite HNE-dG, because the ring-open form of HNE-dG at the template-primer junction can be easily accommodated in the Polκ active site (29). Overall, then, it appears that whereas lesions such as the ring-open form of γ-HOPdG can be accommodated in the active sites of Pols η, ι, and κ at both the insertion and extension steps, for a structurally more complex lesion, such as HNE-dG, only the combined action of Pols ι and κ can promote replication through it. We presume this reflects the abilities of Polι to accommodate the lesion in its active site at the insertion step and of Polκ to accommodate the lesion in its active site for mediating the extension reaction.

The $N^2$-dG adducts, such as γ-HOPdG and HNE, can block synthesis by replicative DNA polymerases, presumably because their presence at the site of either the templating residue or in the template at the template-primer junction introduces a steric hindrance into the active site. A minor-groove adduct such as 3meA would be additionally blocking to replicative polymerases because of the involvement of N-3 in hydrogen bonding with the residues in the polymerase. Since high-fidelity polymerases use this minor-groove hydrogen bonding to detect the correct Watson-Crick geometry of the postinsertion template primer base pair, a 3meA lesion would be particularly susceptible to detection by this mechanism at the extension step of lesion bypass. 3meA could additionally block replicative polymerases at both the insertion and extension steps because of the steric constraints in the active site.

The involvement of yeast Pols η and ζ and of human Pols κ and ι in 3meA bypass suggest that these Pols do not form

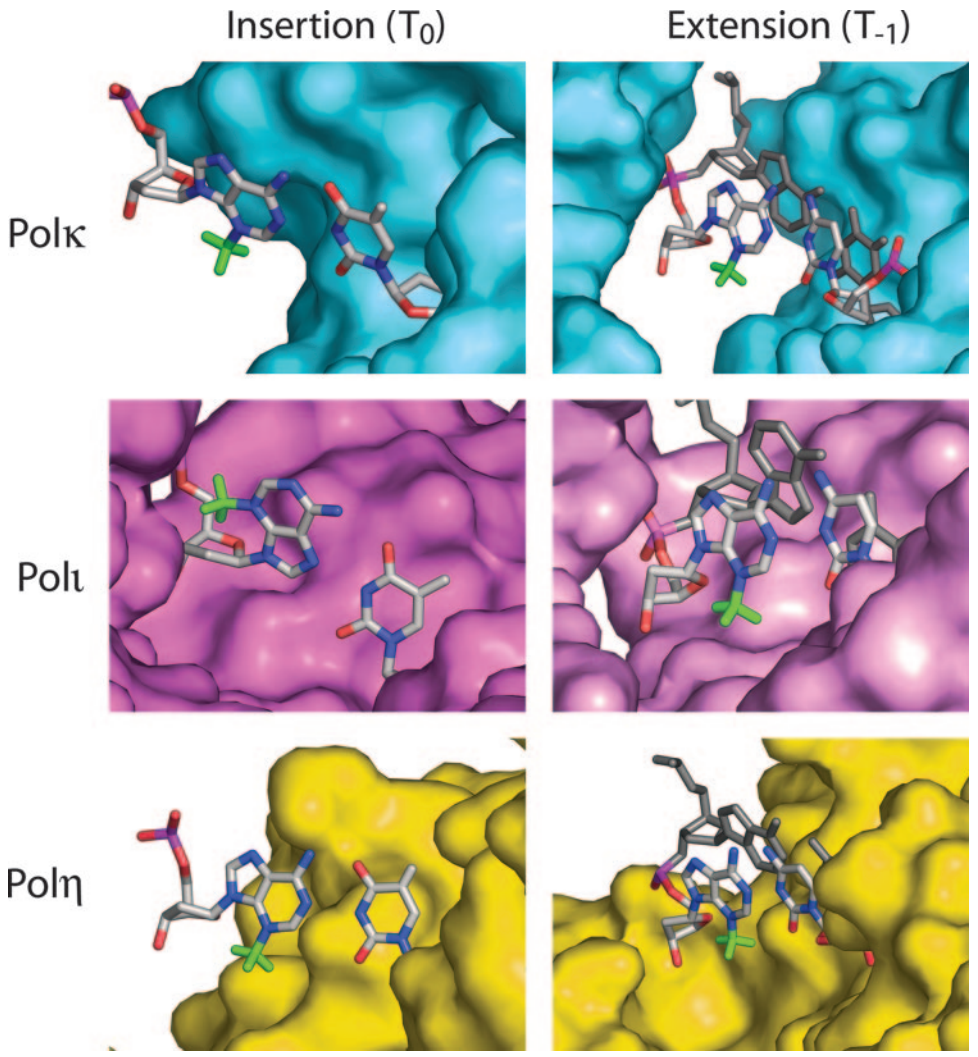


FIG. 4. Modeling of 3meA in the active sites of Polκ, Polι, and Polη. 3meA was modeled in place of A at the templating base (insertion) or in place of G at the postinsertion template residue (extension) for each polymerase. Surface representations of Polκ (upper panels; PDB accession no. 2OH2), Polι (middle panels; PDB accession no. 2FLL), and Polη (lower panels; PDB accession no. 1JIH) are shown in cyan, magenta, and yellow, respectively. The methyl group of 3meA is shown in green. The DNA from the Polκ structure was modeled into Polη based on the alignment of the Polκ and Polη catalytic residues. The incoming dTTP is shown opposite the 3meA at position $T_0$. For modeling 3meA at the $T_{-1}$ position, the G residue present in the structures was replaced by 3meA; the original cognate C residue is shown to provide a reference point for the base pair; the next template residue and incoming dTTP are shown in gray. The thumb region of each polymerase was removed to provide a clear view of the active sites.

hydrogen bonds with the N-3 of an A present at the templating position or at the template-primer junction and, therefore, they lack the sensing mechanism for the correct Watson-Crick base-pairing geometry at either of these positions. In keeping with this inference, we have shown previously that the replacement of a guanine with 3-deazaguanine at the templating position or at the postinsertion template site has no adverse effect on synthesis by yeast Polη or human Polκ (53, 56; unpublished observations).

To examine whether a 3meA present on the templating residue or on the template residue at the template-primer junction can be accommodated into the active sites of Pols η, ι, and κ, we modeled the 3meA lesion into the active sites of these Pols at either the templating base ($T_0$) or the preceding template residue ($T_{-1}$), at the postinsertion site. The ternary structures of Polκ and Polι with DNA and incoming deoxynucleoside triphosphate have been solved (29, 35–38, 47). Modeling of 3meA in Polκ shows that there is ample room in the active site to accommodate the methyl group when positioned at either the templating base or the postinsertion site (Fig. 4). Thus, 3meA is not expected to be a block at either the insertion step or the subsequent extension from 3meA by Polκ. In fact, the 3meA would not impart any steric impediment to Polκ as the DNA passes through the protein during replication. This scenario also holds for Polη. We modeled the DNA from Polκ into the active site of Polη, and again, there is no contact between the protein and the minor-groove perturbation of 3meA when present at either the templating base or opposite the primer terminus (Fig. 4). Although Polι is able to rescue the MMS sensitivity of the yeast *mag1*Δ and *mag1*Δ

*rad30*Δ strains, and thus is predicted to bypass 3meA, the structural modeling suggests steric constraints. Polι inserts nucleotides opposite purine templates by rotating the purine residue into the *syn* conformation and forming a Hoogsteen base pair with the incoming pyrimidine. When 3meA is modeled into the Polι active site, we find that the methyl group clashes with the 5′ oxygen in the DNA backbone, which would inhibit the *syn* conformation and thus make Hoogsteen base pair formation unlikely. However, it may be that the complete rotation of the 3meA residue into the *syn* conformation is somehow prevented in the Polι active site, and a single hydrogen bond can still form between the N-7 of 3meA and the N-3 of the incoming T. As we have shown previously, for proficient T incorporation opposite template A, only N-7 hydrogen bonding is needed (13). Polι is also less open to minor-groove disturbances at the template-primer junction than is Polκ or Polη. When 3meA is modeled into Polι at the $T_{-1}$ position, there is substantially less room, and the methyl group comes in close proximity to the active site floor. However, there is no severe clash, suggesting that Polι would be able to extend from 3meA paired with T.

Finally, the observation that human Pols ι and κ are able to promote TLS opposite the 3meA lesion in yeast cells points to a high degree of evolutionary conservation of the mechanisms that control TLS in yeast and human cells. The ability of Pols ι and κ to function in TLS in yeast cells indicates that they can access the replication ensemble stalled at the lesion site, which we presume involves their binding to PCNA since that is a necessary precondition for TLS to occur in both yeast and human cells (40). Furthermore, we consider it quite likely that the TLS Pols are additionally involved in physical interactions with many of the other components of the replication ensemble and suspect that they too have been conserved between yeast and humans.

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