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Genetics

DNA polymerase δ is required for base excision repair of DNA methylation damage in *Saccharomyces cerevisiae*

[*CDC2(POL3)*/DNA polymerase β/DNA polymerase ε/DNA replication/functional complementation]

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ABSTRACT We present evidence that DNA polymerase δ of *Saccharomyces cerevisiae*, an enzyme that is essential for viability and chromosomal replication, is also required for base excision repair of exogenous DNA methylation damage. The large catalytic subunit of DNA polymerase δ is encoded by the *CDC2(POL3)* gene. We find that the mutant allele *cdc2-2* confers sensitivity to killing by methyl methanesulfonate (MMS) but allows wild-type levels of UV survival. MMS survival of haploid *cdc2-2* strains is lower than wild type at the permissive growth temperature of 20°C. Survival is further decreased relative to wild type by treatment with MMS at 36°C, a nonpermissive temperature for growth of mutant cells. A second DNA polymerase δ allele, *cdc2-1*, also confers a temperature-sensitive defect in MMS survival while allowing nearly wild-type levels of UV survival. These observations provide an *in vivo* genetic demonstration that a specific eukaryotic DNA polymerase is required for survival of exogenous methylation damage. MMS sensitivity of a *cdc2-2* mutant at 20°C is complemented by expression of mammalian DNA polymerase β, an enzyme that fills single-strand gaps in duplex DNA *in vitro* and whose only known catalytic activity is polymerization of deoxyribonucleotides. We conclude, therefore, that the MMS survival deficit in *cdc2-2* cells is caused by failure of mutant DNA polymerase δ to fill single-strand gaps arising in base excision repair of methylation damage. We discuss our results in light of current concepts of the physiologic roles of DNA polymerases δ and ε in DNA replication and repair.

DNA polymerase activity is required in DNA replication and DNA repair. The function of DNA polymerase activity in repair is to fill single-strand gaps, such as those arising from removal of damaged nucleotides. In *Escherichia coli*, chromosomal replication and DNA repair synthesis are carried out, for the most part, by two different DNA polymerases. DNA polymerase III holoenzyme, which catalyzes highly processive synthesis, copies the leading strand and most of the lagging strand during chromosomal replication. DNA polymerase I catalyzes gap-filling synthesis, joining Okazaki fragments on the lagging strand and filling single-strand gaps arising in repair and recombination (1).

A more complex and incompletely understood situation prevails in eukaryotes (2, 3). Three DNA polymerases—DNA polymerases α, δ, and ε—are essential for viability in *Saccharomyces cerevisiae*. Each polymerase is apparently required for chromosomal replication (4) and, importantly, each has a homologue in mammals. It is widely accepted that DNA polymerase α–primase catalyzes initiation at origins of replication and begins Okazaki fragment synthesis on the lagging strand. However there is insufficient information to assign specific roles to DNA polymerases δ and ε *in vivo*. Both of these catalytically similar enzymes have properties expected of a replicative polymerase, both have been shown to support rates of synthesis consistent with fork progression in yeast cells (5), and both have been proposed to catalyze leading strand synthesis in various models of chromosomal replication (5–7). In addition, both DNA polymerases δ and ε isolated from calf thymus have been shown to synthesize ligatable products in a gap-filling assay (8, 9), as would be expected of a lagging strand polymerase. In an intensively studied *in vitro* system, replication of simian virus 40 viral DNA can be reconstituted with only DNA polymerases α and δ (6), prompting the idea that DNA polymerase ε may catalyze an essential, as yet unidentified, repair function at the replication fork. DNA polymerase ε has recently been implicated in other DNA repair functions—namely, nucleotide excision repair in UV-irradiated permeabilized human fibroblasts (10) and base excision repair (BER) of thymine glycol lesions in yeast nuclear extracts (11). In this context of ongoing uncertainty regarding the physiologic roles of DNA polymerases δ and ε, we present here *in vivo* evidence that mutants of *S. cerevisiae* DNA polymerase δ are defective in survival and repair of methylation damage. Apparently, DNA polymerase δ, a favored candidate for highly processive leading strand replication, functions in repair of a ubiquitous and important form of DNA damage.

Methylating agents, which are common in the environment and are generated by endogenous metabolism, are among the most biologically significant molecules that damage DNA. Methylation of DNA is cytotoxic, mutagenic, clastogenic, and carcinogenic (12). Survival of methylation damage depends on removal of adducts by BER (13). For example, disruption of the *S. cerevisiae* gene for N^3-methyladenine-DNA glycosylase, which catalyzes the initial step in BER of N^3-methyladenine, substantially increases the cytotoxicity of methylating agents (14).

One well-documented route of BER involves the action of a DNA glycosylase, which cleaves the glycosidic bond in the damaged nucleotide to release the altered base, apurinic/apyrimidinic endonuclease, which cleaves the DNA backbone 5′ to the abasic site, and DNA deoxyribophosphodiesterase, which excises the 5′-terminal sugar phosphate to generate a single nucleotide gap (13, 15). In some instances, an exonuclease rather than the phosphodiesterase may remove the terminal sugar phosphate and several adjacent nucleotides (16). The resulting single-strand gap is a template-primer for DNA polymerase, which copies the undamaged DNA strand. DNA ligase forms the final phosphodiester bond to restore an intact, double-strand structure. This pathway is established in prokaryotes, yeast, and mammals (13, 15). A notable unresolved issue, which we address here, is the identity of the eukaryotic DNA polymerase(s) that catalyzes the requisite repair synthesis.

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Abbreviations: MMS, methyl methanesulfonate; BER, base excision repair; MER, methyl excision repair.

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EXPERIMENTAL PROCEDURES

Strains and Media. The following *S. cerevisiae* strains were obtained from L. Hartwell (University of Washington, Seattle, WA): 9049-14-3 (**MATa** *ura3-52 his7 canl pep4-3 prbl-1122*) and 9048-13-2 (same but *cdc2-2*); 4076-27 (**MATa** *ural his7*), H2C2A1 (same but *cdc2-2*); 6607-6a (**MATa** *ura3-52 his-3Δ200 leu2-3,112 hom3 gall canl*) and 6613-53a (same but *cdc2-2*); 9101-9-4 (**MATa** *cdc2-1 ural his7* and/or *his3 lys2 tyrl*). Rich medium (YPD) contained 1% yeast extract/2% peptone/2% glucose.

Methyl Methanesulfonate (MMS) Survival. Procedures were modified from those of Kupiec and Simchen (17). Cells were grown to stationary phase in YPD medium at 20°C, washed twice with 0.05 M potassium phosphate buffer, pH 7.0, and resuspended in buffer at 2×10^7 cells per ml. Aliquots from the same culture were used for treatment of cells at either permissive temperature (20°C or 21°C) or at nonpermissive temperature (36°C or 37°C) as specified in the figure legends. Cell suspensions were preincubated for 30 min at the specified temperature, and MMS (Sigma) was then added to final concentrations up to 1%. After 40 min, an equal volume of freshly prepared 10% (wt/vol) NaS_2O_3 was added, and cells were diluted in buffer and spread on YPD plates. For cells treated at nonpermissive temperature, cells were diluted in prewarmed buffer, spread on prewarmed plates, held at nonpermissive temperature for 2 hr, and then transferred to 20°C to allow growth of survivors. Cells treated at permissive temperature were plated at that temperature and incubated thereafter at 20°C. Colonies were counted after 3–4 days.

UV Survival. Cells were grown, washed, and resuspended in buffer as described above. For treatment at permissive temperature, cells were preincubated in buffer for 30 or 60 min and diluted and spread on YPD plates at 20°C–21°C. After irradiation, plates were incubated in the dark at 20°C. For treatment at nonpermissive temperature, cells were preincubated in buffer for 30 min (Fig. 1*D*) or 60 min (Fig. 2*D*) at 36°C–37°C, diluted in prewarmed buffer, and spread on prewarmed YPD plates. After irradiation, plates were held for 2 hr in the dark at 36°C–37°C and then transferred to 20°C to allow growth of survivors. Plates were incubated in the dark at 20°C for 3–4 days before counting colonies.

RESULTS

The DNA Polymerase δ Allele *cdc2-2* Causes a Temperature-Sensitive Defect in Survival of MMS Damage. *cdc2-2* (18) is a classic mutant allele of the essential *CDC2* gene (also known as *POL3*) that encodes the large catalytic subunit of DNA polymerase δ (2, 3). *cdc2-2* mutants display temperature-sensitive growth (18) and DNA replication (19, 20). Moreover, DNA polymerase δ purified from *cdc2-2* cells is temperature sensitive *in vitro* (21). While these and other phenotypic effects of the *cdc2-2* mutation are known (e.g., ref. 22), the consequences for DNA repair remain to be determined.

Fig. 1 shows the effect on survival of treating *cdc2-2* cells with the methylating agent MMS at permissive growth temperature (21°C, Fig. 1*A*) and nonpermissive growth temperature (36°C, Fig. 1*B*). Three main observations are readily apparent. (*i*) Comparison of Figs. 1 *A* and *B* shows that MMS treatment of wild-type *CDC2* cells at elevated temperature increases killing. Greater lethality at higher temperature is due to increased yield of methyl adducts and possibly to decreased levels of heat-labile DNA repair activities such as O^6-methylguanine-DNA methyltransferase and N^3-methyladenine-DNA glycosylase (23). These sources of lethality apply to wild-type *CDC2* and *cdc2-2* strains alike. (*ii*) Survival of mutant cells treated at 21°C is less than wild type (Fig. 1*A*). Both mutant and wild-type cells display similar shoulders on the survival curves at low MMS concentrations, where potentially lethal lesions are either repaired or toler-

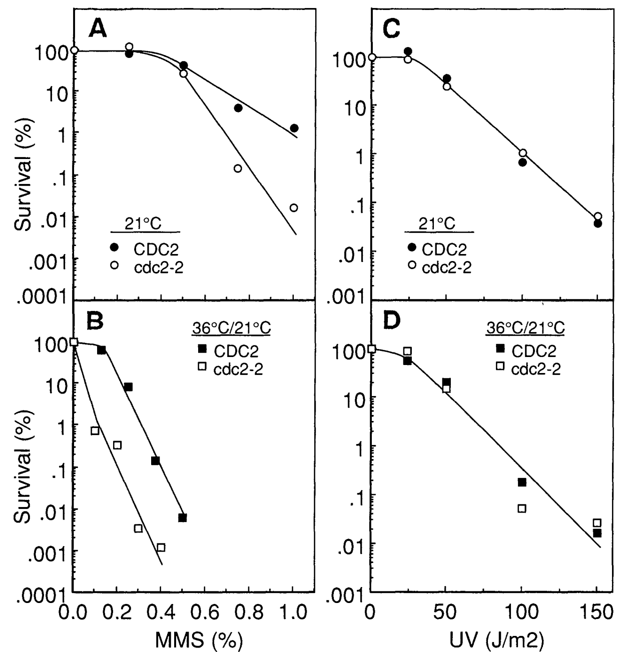

FIG. 1. MMS and UV survival of a *cdc2-2* mutant. Strains 9049-14-3 (*CDC2*) and 9048-13-2 (*cdc2-2*) were grown to stationary phase in YPD medium at 20°C and were treated with MMS or UV at permissive growth temperature (21°C) or at nonpermissive growth temperature (36°C), as described in *Experimental Procedures:* (*A*) MMS, 21°C; (*B*) MMS, 36°C; (*C*) UV, 21°C; (*D*) UV, 36°C. The viability of cells incubated at 36°C but not treated with MMS or UV was ≈100% (*CDC2*) and 75% (*cdc2-2*).

ated to the same extent by both strains, without killing. However the mutant is more susceptible to killing at higher MMS concentrations. (*iii*) Treatment at 36°C (Fig. 1*B*) further reduces survival of the mutant relative to wild type. As shown, the shoulder of resistance in the wild-type curve is lacking in the mutant curve. Therefore the ability to repair or tolerate potentially lethal lesions without killing, which the mutant possesses to the same extent as the wild-type strain at 21°C, is abolished. The MMS survival defect is thus temperature sensitive in the formal genetic sense, as are the growth (18) and replication defects (19, 20) and the catalytic activity of the *cdc2-2* polymerase *in vitro* (21).

In contrast to their MMS sensitivity, *cdc2-2* cells display wild-type resistance to UV, even when treated at nonpermissive temperature (Fig. 1 *C* and *D*). Thus the *cdc2-2* allele does not detectably alter nucleotide excision repair of pyrimidine dimers, the predominant lethal lesion. In addition to defining the repair capabilities of mutant cells, the UV data serve as a procedural control. The UV results show that the MMS sensitivity observed at 21°C (Fig. 1*A*) does not arise from a subtle defect in DNA replication that secondarily reduces survival. The UV data also show that treatment of mutant cells at 36°C (Fig. 1*B*) does not exert a nonspecific, deleterious effect on DNA repair that diminishes survival in response to all DNA-damaging agents.

The *cdc2-1* Allele Also Causes a Temperature-Sensitive Defect in MMS Survival. The *cdc2-1* allele causes a more profound growth impairment than does *cdc2-2*. For example, *cdc2-1* cells do not grow on YPD agar at 30°C, as do *cdc2-2* cells (22), and form smaller colonies at 25°C and 20°C than do *cdc2-2* and wild-type cells (our unpublished observations). MMS and UV survival data for a *cdc2-1* mutant are shown in Fig. 2. At 20°C (Fig. 2*A*), *cdc2-1* cells are only slightly more sensitive to killing by MMS than are *CDC2* cells. They show a comparable shoulder of resistance at low MMS concentrations and up to 3-fold reduction in survival at higher MMS

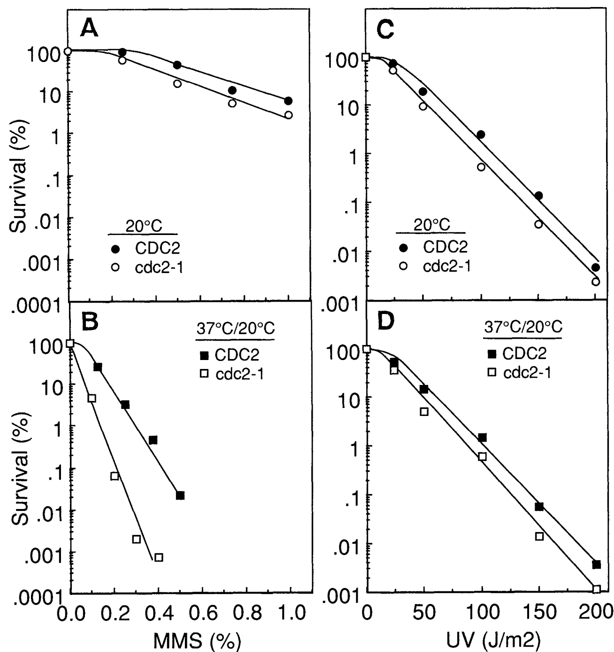

FIG. 2. MMS and UV survival of a *cdc2-1* mutant. Strains 4076-27 (*CDC2*) and 9101-9-4 (*cdc2-1*) were grown to stationary phase in YPD medium at 20°C and were treated with MMS or UV at permissive growth temperature (20°C) or at nonpermissive growth temperature (37°C), as described in *Experimental Procedures:* (*A*) MMS, 20°C; (*B*) MMS, 37°C; (*C*) UV, 20°C; (*D*) UV, 37°C. The viability of cells incubated at 37°C but not treated with MMS or UV was ≈95% (*CDC2*) and 75% (*cdc2-1*).

concentrations. The small diminution in survival may reflect an added, endogenous repair burden imposed by defective DNA replication (see *Discussion*). As in the case of the *cdc2-2* mutant (Fig. 1*B*), treatment at nonpermissive temperature reduces the MMS survival of both *CDC2* and *cdc2-1* cells, but the reduction in survival is greater for the mutant than for the wild type (Fig. 2*B*). As shown in Fig. 2 *C* and *D*, *cdc2-1* cells are slightly more sensitive to UV-irradiation than wild type, but the sensitivity is not increased by treatment at nonpermissive temperature. The up to 5-fold decrease in UV survival at higher doses is comparable to the reduction in MMS survival at 20°C and could likewise reflect faulty DNA replication. These results indicate that the *cdc2-1* allele also confers a temperature-sensitive defect in MMS survival.

The MMS Sensitivity and Growth Phenotypes of *cdc2-2* and *cdc2-1* Mutants Are Separable. As described above, the *cdc2-1* allele causes greater temperature sensitivity of growth than does the *cdc2-2* allele. Conversely, the results in Figs. 1*A* and 2*A* indicate that the *cdc2-2* allele causes greater sensitivity to MMS at 20°C–21°C. We have confirmed that the *cdc2-2* allele confers a larger MMS survival deficit at permissive growth temperatures in numerous additional experiments, including a side-by-side comparison of cells grown to stationary phase at 20°C in synthetic complete medium (data not shown).

Mammalian DNA Polymerase β Complements the MMS Sensitivity of a *cdc2-2* Mutant. The *cdc2-2* and *cdc2-1* mutants resemble mutants of *E. coli* DNA polymerase I in their sensitivity to killing by MMS (24). The sensitivity of the *E. coli* mutants is due to failure to fill repair gaps (1). Recently, it has been shown that the MMS sensitivity of an *E. coli* DNA polymerase I mutant can be complemented by mammalian DNA polymerase β (25), a well-characterized, 39-kDa DNA polymerase whose only known enzymatic activity is the polymerization of deoxyribonucleotides (26). DNA polymerase β prefers duplex DNA containing short single-strand gaps as a template-primer *in vitro* (27) and likely catalyzes DNA repair synthesis in mammalian cells (15, 26–28).

As is the case for *E. coli* DNA polymerase I mutants, the most direct explanation for the MMS sensitivity of yeast DNA polymerase δ mutants is failure to fill repair gaps. To confirm this explanation, we sought to complement the sensitivity of the *cdc2-2* mutant by expression of mammalian DNA polymerase β. Accordingly, we transfected wild-type and mutant cells with an expression vector containing the cDNA for rat DNA polymerase β under control of the *GAL1* promoter. Exposure of transfected cells to galactose resulted in expression of DNA polymerase β, as evidenced by immunoblotting (Fig. 3*A*) and activity gel (Fig. 3*B*) analysis. Similar amounts of protein migrating at 39 kDa and staining with anti-DNA polymerase β antiserum were observed in wild-type and mutant cells (Fig. 3*A*). Likewise, the amounts of DNA polymerase activity comigrating with the DNA polymerase β standard were similar (Fig. 3*B*). The effect of expressing DNA polymerase β on MMS sensitivity of *CDC2* and *cdc2-2* cells is illustrated in Fig. 3*C*. *CDC2* cells carrying either the vector alone or the vector with the cDNA insert display wild-type sensitivity to MMS (compare with Fig. 1*A*). *cdc2-2* cells carrying the vector alone likewise have sensitivity comparable to that shown for this allele (Fig. 1*A*). In contrast, *cdc2-2* cells carrying the vector with the DNA polymerase β insert display wild-type survival. These results show that rat DNA polymerase β can functionally complement the MMS sensitivity of *cdc2-2* cells, reducing lethality up to 350-fold to restore wild-type levels of survival. The data strongly support the conclusion that the lethal defect in *cdc2-2* cells is failure of the mutant DNA polymerase δ to fill single-strand gaps arising in methyl excision repair (MER).

DISCUSSION

We present here strong *in vivo* evidence that, in *S. cerevisiae*, DNA polymerase δ is required for survival and repair of exogenous methylation damage. Our conclusions are consistent with the finding of Aguilera and Klein (31) that the *CDC2* allele designated *hpr6* (for hyper-recombination) causes inability to grow on MMS, and with the sucrose density gradient analysis of Suszek *et al.* (ref. 32, see below). Our work is based on the particularly informative phenotype of the *cdc2-2* mutation in the 125-kDa catalytic subunit of DNA polymerase δ. This mutation causes a substantial MMS survival deficit at a temperature where the generation time is unaffected. *cdc2-2* mutants display a wild-type doubling time at the permissive growth temperature of 21°C (i.e., 2.9 hr in YPD medium, our unpublished data), providing a background of normal or near-normal DNA replication in which to assess DNA repair capacity. This is important because faulty replication is believed to cause endogenous DNA damage (22), such as single-strand gaps, thus generating an intrinsic DNA repair burden. Endogenous damage combines additively or synergistically with exogenous damage to amplify the total repair burden of cells exposed to DNA-damaging agents. Thus, meaningful comparison of survival curves for wild-type and abnormally replicating mutant cells can be problematic when assessing the function of a particular DNA polymerase. This ambiguity is minimized by the *cdc2-2* mutation. Moreover, the UV survival of *cdc2-2* cells is indistinguishable from wild type, showing that neither the mutation nor the experimental protocol imposes a general, deleterious effect on survival in response to DNA damage. We therefore conclude that the *cdc2-2* allele causes a defect in survival of methylation damage that is manifest at permissive growth temperature and is increased by treatment at nonpermissive growth temperature.

The deficit in MMS survival caused by the *cdc2-2* allele can be complemented by mammalian DNA polymerase β, providing compelling evidence that the lethal defect in the mutant is

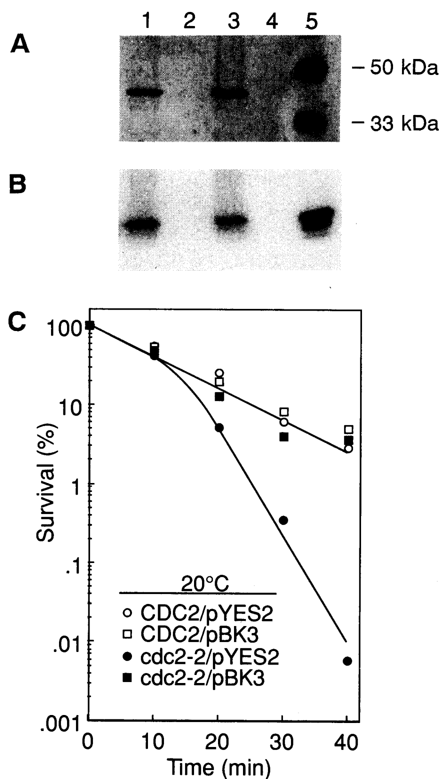

FIG. 3. Complementation of the MMS sensitivity of a *cdc2-2* mutant by mammalian DNA polymerase β. The cDNA for rat DNA polymerase β was amplified from pβL (25) with primers that were complementary to the 5′ and 3′ ends of the cDNA and contained *Kpn* I and *Xba* I sites, respectively, at their 5′ termini. The amplified cDNA was cloned into the expression vector pYES2 (Invitrogen) at the *Kpn* I and *Xba* I sites, yielding pBK3 and placing the DNA polymerase β cDNA under control of the *GAL1* promoter. Plasmids pYES2 and pBK3 were transformed into strains 6607-6a (*CDC2*) and 6613-53a (*cdc2-2*) by a modified lithium acetate procedure (29), and Ura^+ transformants were selected on synthetic minimal plates lacking uracil. Transformants were grown to a density of 1–2 × 10^7 per ml at 20°C in synthetic complete medium containing 2% raffinose and lacking uracil. Galactose was added to 0.5%, and cells were incubated an additional 3 hr at 20°C to allow expression of DNA polymerase β. Cells were then harvested by centrifugation. (*A*) Immunoblot. Freshly harvested cells were heated in sample buffer for 4 min at 100°C and frozen at −70°C. Aliquots equivalent to 10^6 cells were subjected to electrophoresis in a SDS/7.5% polyacrylamide gel, and immunopositive bands were visualized using affinity-purified DNA polymerase β rabbit antiserum as described in ref. 25. Lanes: 1, *CDC2*/pBK3; 2, *CDC2*/pYES2; 3, *cdc2-2*/pBK3; 4, *cdc2-2*/pYES2; 5, molecular weight standards (Bio-Rad). (*B*) DNA polymerase activity gel. Freshly harvested cells were heated in sample buffer without reducing agent (62.5 mM Tris · HCl, pH 6.8/2 mM EDTA/2% SDS/trace bromophenol blue) for 4 min at 100°C and frozen at −70°C. Aliquots equivalent to 10^6 cells were subjected to electrophoresis in a SDS/7.5% polyacrylamide gel cast with gapped DNA in the matrix, and DNA polymerase activity was visualized essentially as described (30). Lanes: 1, *CDC2*/pBK3; 2, *CDC2*/pYES2; 3, *cdc2-2*/pBK3; 4, *cdc2-2* pYES2; 5, 26 pg of rat DNA polymerase β (from S. Wilson, University of Texas, Galveston). Visual comparison with the standard in lane 5 indicates that roughly 10 pg of DNA polymerase β was visible in lanes 1 and 3, corresponding to 100 molecules per cell. Lanes 2 and 4, loaded with cells carrying the vector without insert, were entirely devoid of activity bands. (*C*) MMS sensitivity. Survival of freshly harvested cells was determined at 20°C as described in *Experimental Procedures*, except that preincubation in buffer was omitted and cells were treated with 1.0% MMS for increased time intervals.

an inability to repair single-strand gaps arising in MER. Because DNA polymerase β possesses neither exonuclease domains (33–35) nor exonuclease activity *in vitro* (26), the 3′-5′ exonuclease activity of yeast DNA polymerase δ is either inessential for MMS survival or executes its required function in the *cdc2-2* mutant. It is telling that the MMS repair defect is manifest at a temperature (20°C) that permits doubling at the wild-type rate. This observation indicates that at 20°C the ability to catalyze formation of phosphodiester bonds and to sustain processivity of DNA replication is sufficient to maintain a wild-type generation time and suggests that the mutant polymerase is defective in using MER gaps as a template-primer. Perhaps the *cdc2-2* polymerase cannot interact normally with the 3′ terminus of the gaps arising in MER—i.e., with the DNA itself or with a protein(s) at the terminus—or cannot interact normally with a MER complex.

Examination of a second DNA polymerase δ allele, *cdc2-1*, supports the conclusion drawn from the *cdc2-2* allele that DNA polymerase δ is required for MER. Moreover, comparison of these two alleles indicates that the MMS survival and DNA replication defects associated with mutation in the *CDC2* gene are dissociable. Our data are consistent with the suggestion of Suszek *et al.* (32), based on alkaline sucrose density gradient centrifugation, that repair of MMS-induced single-strand breaks in *cdc2-1* diploids is defective at 36°C but is not defective at 21°C. The sedimentation data in this work are inconclusive because the effect of exposure to 36°C on the DNA of undamaged control cells was not examined. Therefore, the observed anomalies in DNA could be due to defective repair or to defective replication or to both.

Our results do not indicate whether *S. cerevisiae* DNA polymerases other than DNA polymerase δ participate in MER. They do show, however, that, under our experimental conditions, DNA polymerases α and ε cannot support wild-type levels of MMS survival when DNA polymerase δ is inactive. Notably, a DNA polymerase β-like enzyme has recently been found in *S. cerevisiae*. The newly discovered enzyme is not essential for viability (36, 37), nor do strains carrying a deletion mutation in the polymerase gene exhibit increased sensitivity to a variety of DNA-damaging agents, including MMS (37). The β-like polymerase, too, assuming it is present in our strains, is unable to replace the MMS survival function of DNA polymerase δ. It should be emphasized that even though wild-type levels of DNA polymerases α and ε and (presumably) the β-like polymerase cannot fully compensate for the MMS survival deficit, one or more of these enzymes may, in fact, function in MER. An illustrative case is that of *E. coli* DNA polymerase II, which has been shown to catalyze repair synthesis in cells deficient in DNA polymerases I and III but cannot restore wild-type survival (1, 38).

The requirement for DNA polymerase δ in MER in *S. cerevisiae* raises the question of the role of the enzyme in repair of methylation damage in other eukaryotes. Hammond *et al.* (28) have shown that aphidicolin-sensitive, (*p*-butylphenyl)deoxyguanosine triphosphate-resistant polymerase activity is involved in repair of *N*-methyl-*N′*-nitro-*N*-nitrosoguanidine damage in human fibroblasts. This activity could be that of DNA polymerases δ and/or ε.

With respect to other types of lesions repaired in base excision pathways, Wang *et al.* (11) have shown that BER of OsO_4- and UV-damaged DNA is defective in nuclear extracts of a DNA polymerase ε mutant but is not defective in extracts of DNA polymerase δ (*cdc2-1*) or DNA polymerase α mutants. A major lesion caused by OsO_4 and a minor lesion caused by UV is thymine glycol, which is subject to BER (13). The observations of Wang *et al.* (11) are consistent with the possibility that BER of different DNA lesions preferentially involves different DNA polymerases. Another possibility is that the *in vitro* system does not reflect the capacity of DNA polymerases ε and α to fill repair gaps *in vivo*.

With respect to nucleotide excision repair (39), the survival characteristics of the *cdc2-2* (Fig. 1 *C* and *D*) and *cdc2-1* mutants (Fig. 2 *C* and *D*) do not reveal the role of DNA

polymerase δ in repair of UV damage. The mutant polymerases may, in fact, be functioning at wild-type levels in repair of UV damage. Or, they may be defective in repair of UV damage, in which case the defect is masked by the compensatory function of another DNA polymerase(s). Or, DNA polymerase δ may have no role in repair of the major lethal UV lesion. Notably, repair of UV-damaged plasmids in extracts of human cells requires proliferating cell nuclear antigen, an accessory protein affecting DNA polymerases δ and ε (40). DNA polymerase ε restores repair synthesis to UV-irradiated, permeabilized human fibroblasts (10), underscoring the possibility that DNA polymerase ε is involved in nucleotide excision repair.

Our work is relevant to current concepts of the roles of eukaryotic DNA polymerases δ and ε in DNA replication and repair. The *E. coli* paradigm has contributed to a tendency to regard DNA polymerases as either replicative (DNA polymerase III) or reparative (DNA polymerase I). Yet, while DNA polymerase III holoenzyme is the principal catalyst for chromosomal replication, wild-type cells contain an equal or greater amount of the core enzyme that may perform functions other than replication. Indeed, studies of mutants defective in DNA polymerases I and/or III indicate that DNA polymerase III catalyzes closure of repair gaps. Conversely, DNA polymerase I can support DNA replication, as is the case in cells bearing a conditional or nonsense mutation in DNA polymerase III and a mutation in DNA gyrase B (1). In *S. cerevisiae,* DNA polymerase δ is an excellent candidate for leading strand replication (5), yet our results indicate that this polymerase fills repair gaps *in vivo*. DNA polymerase ε, the other candidate for leading strand (7), and also for lagging strand (5), replication is also implicated in repair of exogenous damage (11). Therefore the *S. cerevisiae* polymerase(s) that catalyzes highly processive synthesis at the replication fork may have an important function(s) in DNA repair as well. A comparable situation may exist in mammalian cells. This duality of DNA polymerase function would seem to be adaptive, permitting rapid accommodation to changing DNA synthetic demand.

The capacity to perform various DNA synthetic functions reflects both the inherent catalytic properties of a DNA polymerase and its capacity to interact with other proteins involved in replication and repair. Versatility of function and surprising interchangeability of DNA synthetic catalysts is illustrated by recent examples of functional complementation, beginning with the work of Sweasy and Loeb (25, 41). Thus, mammalian DNA polymerase β has been shown to substitute for the replication function of DNA polymerase I in *E. coli* (41) and in *S. typhimurium* (42). DNA polymerase β can also replace the MMS repair function of DNA polymerase I in *E. coli* (25) and of DNA polymerase δ in yeast. Even more startling, human immunodeficiency virus reverse transcriptase can substitute for the replication function of DNA polymerase I in *E. coli* (B.K. and L.A.L., unpublished work). The compensatory function by endogenous or exogenous DNA polymerases in various bacterial and yeast mutants indicates that, in some circumstances, the total DNA polymerase concentration in a cell, rather than the availability of a particular polymerase, determines whether DNA synthetic demand is met with efficiency permissive of survival (42).

Note Added in Proof. A recent report (43) describes involvement of DNA polymerase δ in repair of UV-damaged plasmid DNA in extracts of HeLa cell nuclei.

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