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DNA Polymerases Required for Repair of UV-Induced Damage in *Saccharomyces cerevisiae*

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The ability of yeast DNA polymerase mutant strains to carry out repair synthesis after UV irradiation was studied by analysis of postirradiation molecular weight changes in cellular DNA. Neither DNA polymerase α, δ, ε, nor Rev3 single mutants evidenced a defect in repair. A mutant defective in all four of these DNA polymerases, however, showed accumulation of single-strand breaks, indicating defective repair. Pairwise combination of polymerase mutations revealed a repair defect only in DNA polymerase δ and ε double mutants. The extent of repair in the double mutant was no greater than that in the quadruple mutant, suggesting that DNA polymerases α and Rev3p play very minor, if any, roles. Taken together, the data suggest that DNA polymerases δ and ε are both potentially able to perform repair synthesis and that in the absence of one, the other can efficiently substitute. Thus, two of the DNA polymerases involved in DNA replication are also involved in DNA repair, adding to the accumulating evidence that the two processes are coupled.

Genetic and biochemical studies have identified four nuclear DNA polymerases from *Saccharomyces cerevisiae*: α, β, δ, and ε (9). The *REV3* gene encodes a polypeptide with sequence similarity to DNA polymerases, although an in vitro DNA polymerase activity has not been demonstrated (18). DNA polymerases α, δ, and ε are essential, and DNA polymerases β and Rev3 are nonessential. Shifting *pol1-17* (polymerase α), *pol3-1* (polymerase δ), *pol2-11*, or *pol2-12* (polymerase ε) mutant strains from the permissive to the restrictive temperature results in an immediate block in the synthesis of chromosomal size DNA, providing evidence that DNA polymerases α, δ, and ε are at the replication fork (4, 5, 7, 26). Reconstitution of in vitro replication systems suggests that DNA polymerase α is involved exclusively in the initiation of synthesis of Okazaki fragments and that DNA polymerase δ efficiently extends the Okazaki fragments (30). The specific role of DNA polymerase ε has not been defined in in vitro replication systems, but DNA polymerase ε can substitute for DNA polymerase δ in some reactions. Nonessential polymerase Rev3p is required for induced mutagenesis by DNA-damaging agents and is not required for chromosomal replication (18). The DNA polymerase β protein is not required for either mitotic DNA replication or DNA repair (5a, 16, 21, 24). Transcript analysis and meiotic dissection studies suggest a role in meiosis (5a).

Besides chromosomal synthesis, DNA polymerases perform another important role in DNA metabolism: DNA repair. Repair of DNA damage involves several different pathways, including nucleotide excision repair (*rad1*, *rad2*, *rad3*, *rad4*, *rad10*, *rad14*, *rad25* [*SSL2*], and *SSL1*), postreplication repair (*rad6* and *rad18*), and recombinational repair (1, 10). In our studies, we have focused on repair of UV light-induced damage, the primary lesion being pyrimidine dimers. After UV treatment, nucleotide excision repair is the primary pathway for repair of pyrimidine dimers. According to recent models, after recognition of the damage, which is thought to be mediated by a multiprotein assembly, perhaps including the *RAD14* gene product, a helicase (*RAD3* or *RAD25*) unwinds the DNA and dual incisions by the *RAD2* and *RAD1,10* endonucleases, 3′ and 5′ to the damage, respectively, release the damaged DNA. The size of the resulting gap is only 30 nucleotides (for a review, see reference 29). Following excision of the damage, repair synthesis fills in the gap around the site of damage and the continuity of the DNA is restored by ligase.

Several *Escherichia coli* DNA polymerases, including replicative DNA polymerase III, have been shown to carry out repair synthesis under various conditions (14). While less is known about the role of the eukaryotic DNA polymerases in repair synthesis, the recent introduction of in vitro repair systems in mammalian cells, *S. cerevisiae*, and *Xenopus* oocytes, combined with the availability of a wide range of well-characterized yeast polymerase mutants, is beginning to clarify their contributions. We have shown that DNA polymerase α is not required for repair of X-ray-induced single-strand breaks and, since X-radiation induces many different kinds of lesions, have proposed that DNA polymerase α is dedicated to DNA replication and that one or more of the other polymerases must be involved in repair (7). A role for DNA polymerase δ or ε in repair was first suggested indirectly by demonstration of a requirement for proliferating cell nuclear antigen (PCNA) in an in vitro DNA repair assay (25). PCNA is an accessory protein of DNA polymerase δ and, in the presence of 100 mM NaCl, has also been shown to stimulate DNA polymerase ε. Direct evidence of a role for DNA polymerase δ in nucleotide excision repair is provided by the fact that DNA polymerase δ antibodies inhibit repair of UV-irradiated plasmids in this in vitro system (33). Similarly, reconstitution of base excision repair with purified proteins in *Xenopus* oocytes implicates DNA polymerase δ in PCNA-dependent base excision repair (17). Genetic evidence of a repair role for DNA polymerase δ is that some yeast *pol3* mutants show reduced survival at high doses of methyl methanesulfonate (MMS) and others show defects in repair synthesis after MMS treatment at the restrictive temperature, suggesting that they are deficient in base excision repair (3, 28). By contrast, however, yeast DNA polymerase δ mutants were not UV sensitive (3). It was suggested that another cellular DNA polymerase, such as DNA polymerase ε, whose biochemical properties are very similar to those of DNA polymerase δ, might be able to compensate for the absence of DNA polymerase δ in nucleotide excision re-

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pair. Mammalian DNA polymerase ε was initially purified as a repair polymerase from human fibroblasts, and the use of differential polymerase inhibitors has also pointed to a role for DNA polymerase ε in base excision repair in permeabilized cell systems (15, 19). Wang et al. (31) have analyzed the roles of DNA polymerases α, δ, ε, and Rev3p by using a soluble yeast in vitro repair system and found that DNA polymerase ε mutants were deficient in repair of base-damaged DNA. However, *pol2* mutants have not been shown to have repair defects in vivo (2).

DNA polymerase β may also be able to participate in repair, although in *S. cerevisiae* it is not required. Mammalian DNA polymerase β expressed in *S. cerevisiae* can suppress the MMS sensitivity of a yeast DNA polymerase δ mutant (3). In addition, DNA polymerase β can substitute for DNA polymerase δ in *Xenopus* in vitro repair, but repair by polymerase β differs from that by polymerase δ in being PCNA independent (17). As in the early stages of the investigation of any biological process, it is not clear whether repair synthesis is a highly organized and integrated process like replication or one that has much looser specificity with respect to the DNA polymerase required.

One method for demonstrating a requirement for a gene in repair involves exposing a strain with a mutation in the gene to a DNA-damaging agent and measuring survival at different doses. If the gene is not essential, survival data can yield unequivocal information about the requirement for a gene in repair; but if the gene is essential, survival data are less informative. An approach to measuring DNA repair that does not involve survival assays is analysis of postirradiation molecular weight changes in the DNA. For instance, when X rays are used to produce damage, the DNA is fragmented immediately after irradiation and repair is observed when the molecular weight of the DNA increases during postirradiation incubation (10). A strain defective in a repair polymerase should accumulate single-strand breaks during postirradiation incubation. By demonstrating the ability of yeast *pol1-17* mutants to carry out such repair at the restrictive temperature, we deduced that DNA polymerase α is not required for repair synthesis (7). We have extended the analysis to mutants affecting DNA polymerases δ, ε, and Rev3p and have used UV irradiation as the DNA-damaging agent instead of X rays. Single mutants are capable of repair synthesis, but a DNA polymerase δ-ε double mutant showed an accumulation of single-strand breaks similar to that seen in a ligase mutant. The results suggest that DNA polymerases δ and ε are both potentially able to perform repair synthesis. This is interesting when compared to DNA replication, for which both polymerases are essential and one cannot compensate for a lack of the other.

MATERIALS AND METHODS

DNA repair protocol. Cells (1 ml) were labeled overnight with [^{3}H]uracil (10 μCi/ml) at 23°C. Cells were harvested in mid-log phase, washed with water, and resuspended in 1 ml of water. The washed cells were transferred to 9.6-cm^2 petri dishes and irradiated with UV light (15 to 90 J/m^2) in the dark with shaking every 15 s. All succeeding steps were carried out in the dark. After irradiation, cells were collected and resuspended in YEPD medium (2% glucose, 2% peptone, 1% yeast extract) and incubated for 1 h at 38.5°C. After incubation, cells were collected, placed on ice, microcentrifuged, resuspended in 0.1 M Tris HCl, (pH 8.5)–0.01 M EDTA–2% 2-mercaptoethanol, and incubated on ice for 15 min. Cells were washed with ice-cold 0.01 M KPO_4 (pH 7.2)–0.01 M EDTA, resuspended in 150 μl of 0.01 M KPO_4–0.01 M EDTA, and placed on ice. Cells were transferred to the bottom of 5-ml polyallomer tubes. Twenty microliters of 10-mg/ml Zymolyase (in 50% glycerol–0.01 M KPO_4–0.01 M EDTA) and 20 μl of 10% Nonidet P-40 were added to cells on the bottom of the centrifuge tube, and the tube was placed at 37°C for 10 min. If cells were assayed for the presence of pyrimidine dimers, 20 μl of 10× T4 endonuclease V buffer (1 M NaCl, 0.1 Tris HCl [pH 8.0], 0.01 M EDTA) was added and the mixture was incubated with 1 μl of T4 endonuclease for 10 min at 22°C. T4 endonuclease V was a gift of Kevin Sweder and Lori Lommel, Stanford University, Stanford, Calif. Sarkosyl was then added to 2%. Fifteen to thirty percent sucrose gradients with 0.3 M NaOH–0.7 M NaCl–0.03 M EDTA were pumped into the bottom of the tube. After 1 h, gradients were spun at 12,000 rpm for 22 h at 17°C in an SW50.1 rotor. Samples were collected from the bottom of the tubes in 0.2-ml fractions. Three milliliters of 1 M HCl–0.1 M sodium PP_i plus carrier DNA was added to the fractions. RNA was quantitatively hydrolyzed during the centrifugation. Verification that all of the RNA was hydrolyzed during centrifugation was obtained by measuring [^{3}H]uracil in DNA replication mutants and analyzing the data with alkaline sucrose sedimentation. Samples were precipitated and counted on glass fiber filters. Fraction numbers are normalized to 100 in the various gradients presented (see Fig. 1 to 4).

Molecular size (M_i) was computed by using the formula $M_i/M_{T4} = (d_i/d_{T4})^{2.63}$. d_i is the distance of the ith fraction, and d_{T4} is the distance sedimented by T4 DNA; d_{T4} was at position 33 (as measured from the top) of the gradient; molecular size (M_{T4}) was 166,000 bp (11). A number average molecular size (M_n) can be computed with the formula $M_n = \Sigma C_i/\Sigma(C_i/M_i)$. C_i is the percentage of counts in the ith fraction, and M_i is computed with the previous formula (11). The latter formula is used to compute the M_n of peaks (12).

Strains. Strains used are shown in Table 1. A364a, **a**370 (*pol3-1*), and α370 (*pol3-1*) were from L. Hartwell, University of Washington, Seattle. TC102 was from Merl Hoekstra. All *pol1* and *pol2* mutants were from this laboratory (4, 5, 7). *rev3*Δ was from C. Lawrence, University of Rochester, Rochester, N.Y. All multiple mutants were constructed in this laboratory. Other mutants were from the Yeast Genetic Stock Center, University of California, Berkeley.

TABLE 1. Strains used in this study

Strain	Genotype
TC102	α *leu2 ura3-52*
TC102-2-11	α *pol2-11 leu2 ura3-52*
TC102-2-12	α *pol2-12 leu2 ura3-52*
A364A	**a** *ade1 ade2 ura1 his7 lys2 tyr1 gal1*
ts370	**a** *pol3-1 ade1 ade2 his7 lys2 tyr1 ura1 gal1*
ts280	**a** *cdc9-2 ade1 ade2 his7 lys2 tyr1 gal1 ura1*
488-1-17	α *pol1-17 trp1 leu2 ura3-52 his1-7*
X12-6B	**a** *rad1-1 ade2-1 gal2*
CLK	α *ura3-52 trp1 ade2 gal2*
CLK-3-1	α *pol3-1 ura3-52 trp1 ade2 gal2*
CLK-2-11	α *pol2-11 ura3-52 trp1 ade2 gal2*
CLK2-12	α *pol2-12 ura3-52 trp1 ade2 gal2*
PCR-0	**a** *pol1-17 pol3-1 rev3*Δ::*LEU2 leu2 trp1 ura3*
PCR-2-12-21B	α *pol2-17 pol2-13 pol3-1 rev3*Δ::*LEU2 leu2 trp1 ura3*
PCR-2-12-13D	**a** *pol2-12 pol3-1 rev3*Δ::*LEU2 leu2 trp1 ura3*
PCR-2-12-B	α *pol1-17 pol2-12 rev3*Δ::*LEU2 leu2 trp1 ura3*

RESULTS

Rationale. It was important to have DNA polymerase mutants with very severe defects, since it is likely that only a small amount of polymerase activity is required for repair. First, the repaired patches are only 30 nucleotides long, and second, DNA repair synthesis after UV treatment occurs in the presence of concentrations of hydroxyurea that block chromosomal synthesis, although this could also reflect a lesser requirement during repair for nucleotide precursor pools (10, 13). We have previously demonstrated that the temperature-sensitive *pol1-17*, *pol2-12*, *pol2-11*, and *pol3-1* strains synthesize little chromosomal size DNA at the restrictive temperature. (4–6, 26). Also, the mutant polymerases made in strains carrying alleles *pol1-17*, *pol2-12*, and *pol3-1* are defective in in vitro DNA polymerase assays (4–6, 26). Thus, strains with these mutations were chosen for repair studies. Although Rev3p has not been shown to exhibit DNA polymerase activity in vitro, *rev3* mutant strains are moderately sensitive to UV irradiation (18). A strain with a deletion of the gene almost certainly lacks the in vivo Rev3 function and was chosen for these studies. Yeast strains with deletions of the catalytic region of DNA polymerase β_{70} are neither UV sensitive, X-ray sensitive, nor MMS sensitive (5a, 16, 21, 24). A 2.2-kb DNA polymerase β message

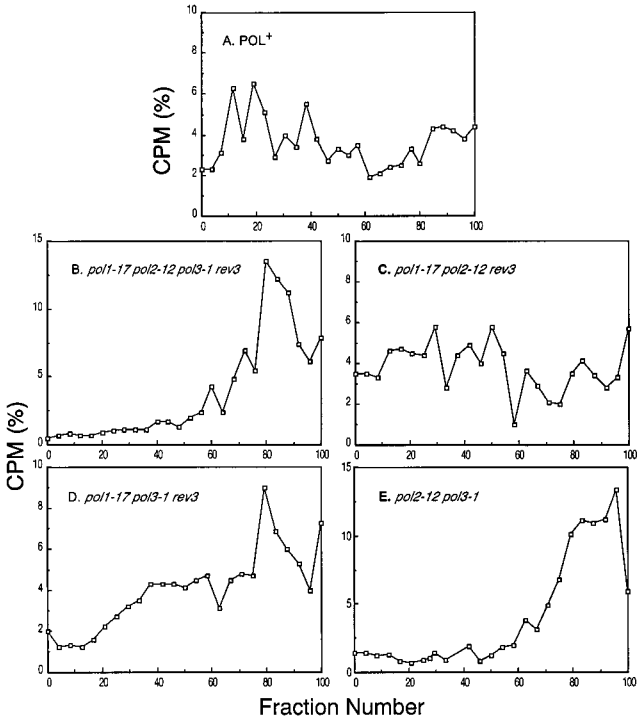

FIG. 1. Repair of single-strand breaks in multiple DNA polymerase mutant strains. The strains used to construct these mutants are described in Materials and Methods. All temperature-sensitive alleles were generated and characterized previously in this laboratory (4, 7, 8). The *rev3*Δ strain was from C. Lawrence, University of Rochester, Rochester, N.Y. Cells were irradiated with UV light at 60 J/m^2, repair was allowed to occur for 1 h at 38.5°C, and centrifugation was done for 2 h at 12,000 rpm as described in Materials and Methods. Sedimentation is from right to left. (A) Wild-type TC102; (B) *pol1-17 pol2-12 pol3-1 rev3*Δ; (C) *pol1-17 pol2-12 rev3*Δ; (D) *pol1-17 pol3-1 rev3*Δ; (E) *pol2-12 pol3-1*.

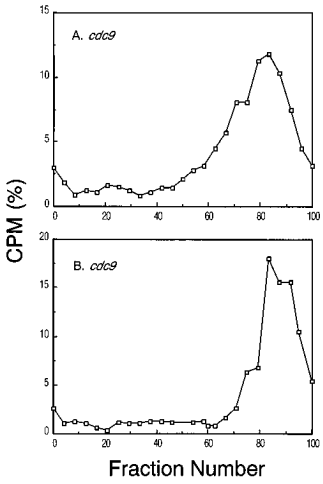

FIG. 2. Repair of single-strand breaks in a DNA ligase, *cdc9*, mutant. Cells were irradiated with UV light at 15 (A) or 30 (B) J/m^2, incubated in YPD medium for 1 h at 37°C, and analyzed by alkaline sucrose gradient centrifugation.

is induced in meiosis and is either very rare or not expressed in mitotically dividing cells (5a, 16). The DNA polymerase β mutant was not included in the repair studies reported here.

The analysis of repair synthesis is based on the assumption that DNA polymerase mutants defective in a UV repair function will be proficient for excision of damaged bases but deficient in filling in and thus in closing the resulting gap. Thus, a polymerase mutant would be expected to accumulate single-strand breaks in its chromosomes following UV irradiation in a fashion similar to that of a DNA ligase mutant (32). DNA is labeled by growth of cells in [^{3}H]uracil overnight. The cells are harvested and treated with UV doses ranging from 15 to 90 J/m^2. Because cells are grown at the permissive temperature, prior to irradiation, gaps resulting from defective replication are minimized. Repair is allowed to occur in YEPD medium for 1 h at the restrictive temperature. The cells are then harvested, and the size of the DNA is determined on an alkaline sucrose gradient. Chromosomal breakage is analyzed by velocity sedimentation in alkaline sucrose gradients. When centrifugation is carried out at a low speed (12,000 rpm) for a long time, e.g., 22 h in an SW50.1 rotor, the molecular weight of chromosomal size DNA is measured (20). T4 DNA (166 kb) sediments at position 67 (100 is the top and 0 is the bottom of gradient). High molecular weight indicates that repair has occurred; low molecular weight indicates that there is a defect.

Quadruple DNA polymerase mutants are deficient in repair. To ascertain whether any of the four known polymerases is required for repair, a quadruple mutant carrying conditional mutations in the three essential polymerases and a deletion of the nonessential *REV3* gene was constructed. Although synthetic lethality might have been expected, the mutant was viable at 23°C, despite having lesions in all three essential DNA polymerases.

A wild-type strain and the *pol1-17 pol2-12 pol3-1 rev3*Δ quadruple mutant were compared for the ability to repair UV-induced single-strand breaks. Cells were treated with UV light at 60 J/m^2, a dose which results in about 10% survival of the wild-type strain (data not shown). Repair was allowed to occur at 38.5°C for 1 h. The Pol$^+$ DNA sedimented in the lower half of the gradient (Fig. 1A), similar to non-UV-treated DNA (see Fig. 4). If position 66.7 corresponds to 166 kb, then according to the formula for M_i in Materials and Methods, position 60 corresponds to about 300 kb, the size of the smallest yeast chromosome. Position 50 corresponds to 500 kb, and position 25 corresponds to 1,500 kb. Fractions 60 to 0 contained chromosomal size DNA. Chromosomal size DNA was observed in the gradient of *POL1* POL2 POL3 REV3 cells which were UV treated and incubated. The UV dose used in this experiment, 60 J/m^2, resulted in 6,700 dimers per genome or about 400 dimers per chromosome (22). One assumes that a significant fraction of the 400 dimers per chromosome are incised on the basis of estimates from the data in Fig. 2, which yielded 160 dimers incised per chromosome. Therefore, the observation of chromosomal size DNA after UV treatment and incubation indicates extensive repair synthesis in the *POL1 POL2 POL3 REV3* strain. In three different *POL*1 *POL*2 *POL*3 *REV*3 strains, no significant variation in repair was found. In the quadruple mutant, however, most of the DNA after repair sedimented between positions 80 and 90, corresponding to an M_n of 20 kb (Fig. 1B). Little DNA was found at positions 0 to 60, the part of the gradient that corresponds to chromosomal size DNA. The low molecular weight is due to a defect in repair of radiation-induced damage and not a result of lesions due to the polymerase mutations themselves, since most of the DNA from non-UV-treated mutants sedimented at a position similar to that of the wild type (data not shown but pattern similar to Fig. 1C in several experiments). Thus, the extent of repair is drastically reduced compared to the wild type and one or more of these four polymerases must play a major role in gap filling during UV repair.

Pairwise combinations of polymerase mutants. To define which of the four DNA polymerases was responsible for the

repair deficiency, the UV repair ability of mutants carrying pairwise combinations of mutations in the essential DNA polymerases was next investigated. As demonstrated in Fig. 1C and D, almost all of the UV light-induced gaps were repaired in the *pol1-17 pol2-12 rev3*Δ and *pol1-17 pol3-1 rev3*Δ mutant strains. Chromosomal size DNA was observed after UV treatment and incubation, which indicates efficient repair of incised gaps. In a strain defective in both polymerases δ and ε, the *pol3-1 pol2-12* double mutant strain, however, there was a significant defect in repair with almost all of the DNA sedimenting between positions 80 and 100, corresponding to an M_n of approximately 20 kb. Chromosomal size DNA was not observed in the gradient (positions 0 to 60), indicating a significant defect in repair (Fig. 1E). In four separate experiments, the sedimentation profile of the double mutant was almost identical to that of the *pol1-17 pol2-12 pol3-1 rev3*Δ quadruple mutant, suggesting that the double mutant is just as defective as the quadruple mutant. Thus, DNA polymerases δ and ε appear to be the major polymerases contributing to repair and polymerases α and Rev3p do not appear to contribute. Furthermore, if one polymerase does not repair the gap the other one does, since only when both are knocked out is repair significantly affected.

To calibrate the extent of the repair defect observed in the polymerase mutants, we compared the response of the polymerase mutants to that of *cdc9* mutants given two different doses of UV light. As demonstrated by Wilcox and Prakash (32), when a *cdc9* mutant is treated with UV light and incubated at the restrictive temperature, single-strand breaks accumulate in UV incision-proficient strains but not in incision-deficient strains. Figure 2 illustrates sedimentation profiles of repaired DNA from *cdc9-2* mutant cells treated with UV light at either 15 or 30 J/m^2 and incubated at 37°C. Little chromosomal size DNA was observed at 15 J/m^2, with the DNA sedimenting at a position corresponding to an M_n of 20 kb. After exposure to 30 J/m^2, no chromosomal size DNA was observed and DNA sedimented at a peak corresponding to about 14 kb. The sedimentation profile of *pol2-12 pol3-1* strains after exposure to 60 J/m^2 was almost identical to those of *cdc9* strains receiving 15 to 30 J/m^2. Thus, both the *pol1-17 pol2-12 pol3-1 rev3*Δ and *pol2-12 pol3-1* strains may retain a slightly greater capacity for repair than the *cdc9* mutant but significantly less than the *POL*$^+$, *pol1-17 pol3-1*, and *pol1-17 pol2-12* mutant strains. The slightly increased capacity for repair over the *cdc9* mutant may be due either to residual activity of either mutant polymerase δ or ε at the restrictive temperature or to compensation by other, possibly unidentified, polymerases.

Single polymerase mutations do not give rise to a repair defect. Single DNA polymerase mutants were also tested for a repair defect, and these results were included as further controls for the experiments with multiple mutants. We had previously shown that *pol1-17* strains are proficient at single-strand break repair after exposure to ionizing radiation and decided to extend these results to UV irradiation. Figure 3 illustrates data comparing repair of UV-induced breaks in *POL*$^+$, *pol1-17*, *pol2-11*, *pol2-12*, and *pol3-1* strains. Panel A illustrates a sedimentation profile of DNA from a Pol$^+$ strain treated with UV light at 90 J/m^2 and incubated for 1 h at 38.5°C. In the single-mutant experiments, higher UV light doses were chosen to maximize differences between Pol$^+$ and Pol$^-$ mutant strains. After exposure to 90 J/m^2, the DNA was shifted to a significantly lower molecular weight than after exposure to 60 J/m^2, suggesting that the repair apparatus is saturated at these doses. Panel B illustrates DNA from a *pol1-17* strain treated identically. The DNA profile of *pol1-17* cells was shifted to a slightly lower molecular weight relative to

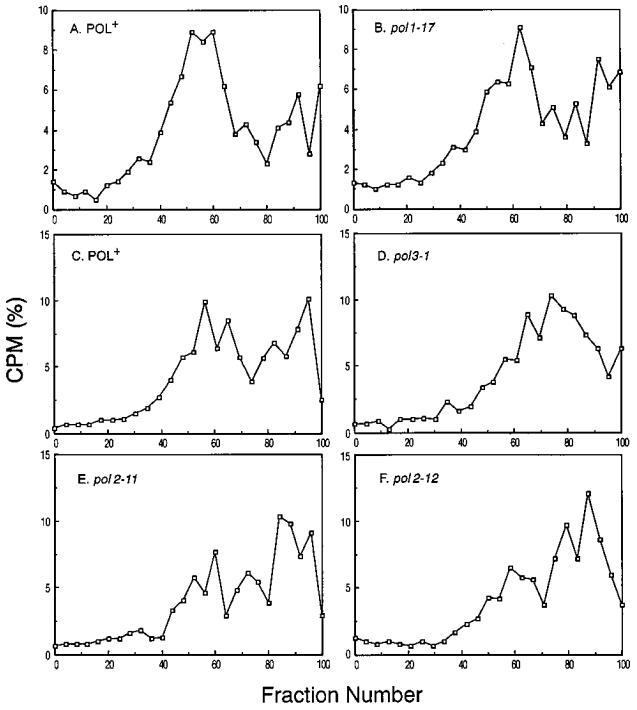

FIG. 3. Repair of single-strand breaks in mutants containing single mutations in the essential DNA polymerases. Irradiation was with UV light at 90 J/m^2, repair was for 1 h at 39°C, and centrifugation was for 22 h. (A) TC102 (*POL*$^+$); (B) *pol1-17*; (C) CLK (*POL*$^+$); (D) *pol3-1*; (E) *pol2-11*; (F) *pol2-12*. The wild-type strain in panel A is isogenic with *pol1-17*, and the strain in panel C is the parent of the *pol2-12* and *pol3-1* mutant strains.

that of Pol$^+$ cells. However, it is unlikely that the small change in profile between the two strains represents a significant difference in repair capacity between the Pol$^+$ and *pol1* mutant strains.

Panels C to F illustrate that there was also no significant difference in repair between the *POL*$^+$ strain and the *pol2-11*, *pol2-12*, and *pol3-1* strains. Since DNA was observed in all of the gradients at positions 40 to 60, which corresponds to chromosomal size DNA (800 to 275 kb). The gradients suggest that extensive repair synthesis occurred in the *POL*$^+$, *pol1-17*, *pol2-11*, *pol2-12*, and *pol3-1* strains. There was less chromosome size DNA in the *pol2* and *pol3* gradients than in the *POL*$^+$ and *pol1* gradients, suggesting a marginal defect in repair that appears at a dose of 90 J/m^2. However, the presence of chromosomal size DNA suggests that extensive repair synthesis occurred in the *POL*$^+$, *pol2-11*, *pol2-12*, and *pol3-1* strains.

None of the mutants is defective in incision. Since the repair assay employed measures the outcome of an incision and gap repair sequence, a formal possibility remained that the high-molecular-weight DNA observed in the mutants was not the result of efficient gap filling but rather a result of failure to incise the lesions in the mutants. If the latter were true, then the high-molecular-weight DNA in the mutant cells after UV treatment and repair should be susceptible to the phage T4 UV endonuclease, which specifically incises UV-induced pyrimidine dimers. To see if the DNA in the mutants contained residual, unincised lesions, cells were treated with UV endonuclease after UV treatment and repair but before sedimentation. Figure 4 illustrates an experiment analyzing the residual presence of pyrimidine dimers after UV treatment and incubation in a wild-type strain and a *rad1* strain known to be

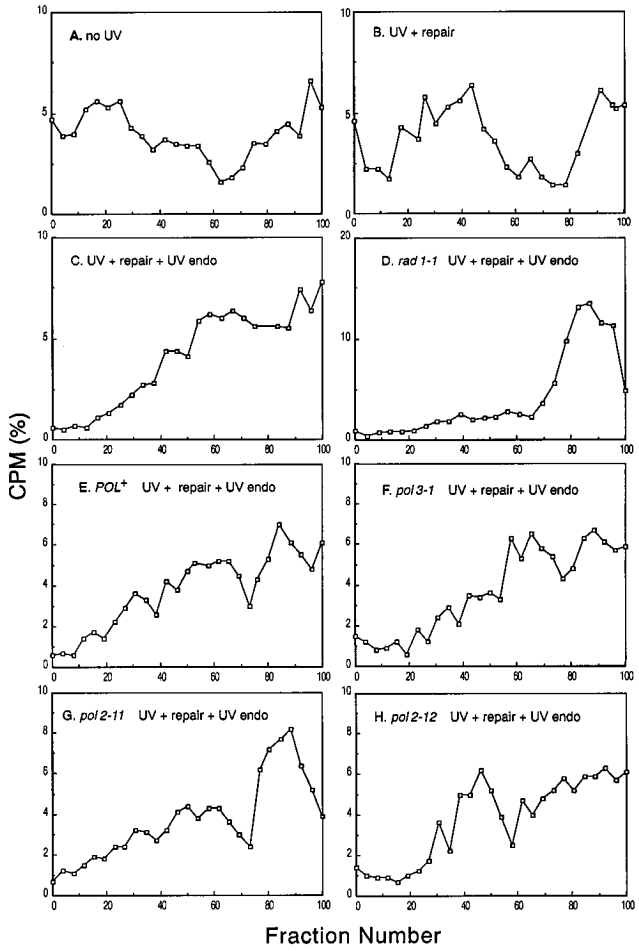

FIG. 4. Analysis of pyrimidine dimers remaining after 1 h of postirradiation incubation. To test for the presence of pyrimidine dimers, cells were lysed on the bottom of centrifuge tubes and then incubated with T4 endonuclease (endo) for 10 min before the 15 to 30% gradient was pumped in. (A) Pol^+ strain, no UV light; (B) Pol^+ strain, UV light at 30 J/m^2 plus 1 h of repair; (C) Pol^+ strain, 1 h of repair plus UV endonuclease; (D) *rad1* mutant, 1 h of repair plus UV endonuclease; (E, F, G, and H) *POL*$^+$, *pol3-1*, *pol2-11*, and *pol2-12* strains, respectively, 1 h of repair plus UV endonuclease.

deficient in the incision stage of repair (13). Treating unirradiated DNA with the UV endonuclease caused no chromosomal breakage, showing that the endonuclease does not have nonspecific nuclease activity (data not shown). Figure 4A illustrates a sedimentation profile of non-UV-treated DNA, and panel B shows the profile of UV-treated and repaired DNA from a Pol^+ Rad^+ strain. Figure 4C illustrates the sedimentation profile of the same cells treated with UV light at 30 J/m^2, incubated for 60 min in YEPD medium, and then treated with UV endonuclease. The endonuclease did cause some degradation of the repaired DNA (compare Fig. 4B and C), suggesting that at this dose some dimers fail to be incised, as expected, since such a dose causes 34% survival. Nevertheless, the experiment demonstrates that most of the pyrimidine dimers were being both incised and repaired since there were significant numbers of counts in the fraction between positions 20 and 60. This conclusion is reinforced by the DNA sedimentation profile of *rad1* cells treated with UV light, incubated in YPD medium, lysed, and then treated with UV endonuclease (Fig. 4D). DNA was significantly degraded, since the dimers were not removed from the DNA. When Rad^+ cells were UV treated without incubation and immediately converted to spheroplasts, the DNA was degraded by the UV endonuclease, similar to that of *rad1* cells (data not shown). We then tested the polymerase mutants for the ability to incise, and the data are illustrated in Fig. 4E to H. Cells were UV treated at 60 J/m^2, incubated in YPD medium to allow repair, and lysed, and the DNA was treated with UV endonuclease. As shown in panels A to D, the sedimentation pattern of repaired DNA treated with UV endonuclease from the *cdc2*, *pol2-11*, and *pol2-12* mutants was indistinguishable for the Pol^+ pattern and chromosomal size DNA was present in all of the gradients. Thus, there did not appear to be any significant difference in the ability to incise at UV lesions between the wild-type and polymerase mutant strains at the restrictive temperature under these experimental conditions. The same number of UV dimers was removed in both, suggesting that most of the UV dimers were incised and the resulting gap was repaired under the conditions used here.

DISCUSSION

The persistence of transient breaks was used as an assay for repair after UV irradiation of yeast polymerase mutants. In Pol^+ strains, the gaps created at sites of damage by the excision repair pathway are transient, and single-strand breaks persist only if repair synthesis and/or ligation is prevented, as in a DNA ligase mutant (Fig. 2). We have shown that single-strand breaks also persist in a *pol2-12 pol3-1* strain but not in strains having a mutation in only one of the polymerases, such as *pol1-17*, *pol2-11*, *pol2-12*, *pol3-1*, or *rev3*Δ. Addition of the *pol1-17* and *rev3*Δ mutations to the *pol2 pol3* double mutant did not further decrease the repair efficiency. Thus, no single DNA polymerase is required to repair gaps after pyrimidine dimer removal. Of the four DNA polymerases investigated, DNA polymerases α and Rev3p did not make a detectable contribution to repair synthesis. Replicative DNA polymerases δ and ε do participate in repair synthesis, and both are potentially capable of filling the gap.

Genetic analysis of *S. cerevisiae* suggests that both DNA polymerases δ and ε are essential for cellular viability (8). Biochemical studies of the simian virus 40 in vitro replication system have delineated roles for DNA polymerase δ in leading- and lagging-strand DNA synthesis; however, no specific role for DNA polymerase ε has been identified (30). It has therefore been suggested that DNA polymerase ε is involved in an essential DNA replication-linked DNA repair pathway. Our data suggest the UV repair function performed by DNA polymerase ε is not its essential function, however, because DNA polymerase δ can repair UV light-induced gaps in the absence of DNA polymerase ε. Additional types of repair would have to be measured for a firm conclusion, but an essential role for polymerase ε in replication itself is also suggested more directly by the fact that polymerase ε mutants do not carry out extensive DNA synthesis at restrictive temperatures (5).

Since the two polymerases compensate for one another efficiently, it is not possible to evaluate their individual contributions. However, recent evidence suggests that different types of damage may be repaired more efficiently by different polymerases. Base excision repair, for instance, unlike excision of pyrimidine dimers, is defective in yeast polymerase δ mutants, even in the presence of DNA polymerase ε, suggesting that polymerase ε cannot compensate as efficiently in methyl excision repair as in UV repair (3). Furthermore, overexpression of mammalian DNA polymerase β can compensate for the loss of yeast polymerase δ (3), which is interesting and surprising for several reasons. First, DNA polymerase β belongs to a

synthetic class of DNA polymerases completely different from that of polymerases δ and ε. DNA polymerases δ and ε, by contrast, are very similar to each other biochemically. Not only are their synthetic abilities similar, but they even share identical cofactors, PCNA and replication factor C (RF-C), which are not used by polymerase β. Second, *S. cerevisiae* contains a 70-kDa homolog of mammalian polymerase β, yeast polymerase β_{70}, yet this enzyme does not compensate for the loss of polymerase δ. Genetic analysis suggests no role for polymerase β in mitotic repair since neither polymerase β deletion mutants nor polymerase β-δ or β-ε double mutants are sensitive to UV light, X-rays, or low levels of MMS (5a, 16, 21, 24). The lack of a defect in polymerase β mutants, however, may stem from the fact that yeast polymerase β is only expressed at significant levels in meiosis (5a). Repair during meiosis may involve an alternative DNA polymerase not available in mitosis. The recent findings of Blank et al. (3) make it interesting to speculate as to whether yeast polymerase β expressed from a mitotically active promoter can restore UV repair to the yeast polymerase δ-ε double mutant, just as mammalian polymerase β can restore base excision repair to the polymerase δ mutant.

The interchangeable capacities of the various similar and different DNA polymerases for repair become even more intriguing with the recent demonstration that *Xenopus* DNA polymerase β can accomplish base excision repair in a reconstituted *Xenopus* repair system (17). However, DNA polymerase δ repair is PCNA dependent while polymerase β repair is PCNA independent. The cold-sensitive yeast *cdc44* mutant, with a defect affecting another DNA polymerase δ-ε cofactor, RF-C, is UV sensitive and could be used to test whether yeast repair requires these polymerase accessory proteins in the presence of polymerase β.

The *cdc9* mutant strain has a greater repair defect than the *pol2-12 pol3-1* strain since the DNA sedimentation profile of *pol1-17 pol2-12 pol3-1 rev3*Δ strains after exposure to UV light at 60 J/m^2 is similar to that of a *cdc9* strain after exposure to only 15 to 20 J/m^2. Possible explanations are that the *pol2-12* and *pol3-1* mutants are leaky or that another polymerase provides some repair synthesis. Since the sedimentation profiles of *pol2-12 pol3-1* and *pol1-17 pol2-12 pol3-1 rev3*Δ were the same after UV treatment and incubation, neither polymerase α nor Rev3p appears to repair gaps in the *pol2-12 pol3-1* double mutant strain. The *pol1-17* mutation itself may be leaky, or yeast polymerase β_{70} may be involved. This is unlikely since, as mentioned above, yeast polymerase β_{70} is expressed at nearly undetectable levels in normal mitotic cells, although it may be induced by UV. Although double polymerase β-δ and β-ε mutants are not repair defective (21), triple mutants may show defects.

Single-strand DNA binding protein RP-A, DNA polymerase accessory protein PCNA, DNA polymerase δ itself, and DNA polymerase β have been implicated in UV repair in in vitro metazoan repair systems. DNA polymerase ε has been shown to participate in base excision (thymine glycol) repair in yeast extracts (31). These requirements are consistent with the conclusions presented here, especially since PCNA is a processivity factor for DNA polymerase δ and DNA polymerase ε. One also expects a requirement for DNA polymerase δ accessory protein RF-C in repair of UV light-induced gaps, and there is some evidence of its involvement in *S. cerevisiae*, as mentioned above. The model for repair synthesis that we favor is that after UV treatment and excision of the resulting dimer, RF-C and PCNA bind to the 3′ termini of the gaps. These accessory proteins allow either DNA polymerase δ or ε to bind to the primer terminus and repair the gap. In the absence of PCNA, DNA polymerase β can find the gaps in some cases. The results presented here and previously do not identify a repair function for DNA polymerase α or Rev3p. Rev3p is likely involved in *trans*-dimer synthesis and thus is not involved in repair of UV dimers. DNA polymerase α forms a complex with primase (9). If the only in vivo substrates of DNA polymerase α are primers synthesized by primase, then one would not expect DNA polymerase α to repair damaged DNA. Thus, DNA polymerase α seems to be reserved strictly for DNA replication.

The *rad18* postreplication repair and *rad52* recombination repair pathways presumably repair DNA damage which has escaped the excision repair pathway. Wilcox and Prakash (32) have reported that a significant percentage of UV dimers are not incised by the excision repair pathway. Also, *rad18* strains are very sensitive to UV treatment and are presumably unable to repair damage which has not been repaired by the excision repair pathway (13). By using an excision-deficient strain, Resnick et al. (23) found that DNA replication can bypass pyrimidine dimers. The Rev3 protein may play a role in the bypass, along with DNA polymerases δ and ε. The assay used in our studies does not measure this type of repair directly. However, the similarity between the ligase and the polymerase mutants might suggest that we measured the sum of all of the pathways and that the same polymerases have the potential to participate in all of them.

An important outcome of this study is that while DNA polymerase α appears to be reserved for replication, the two other replicative DNA polymerases are also used for repair. Given the exquisite specificity of the replication apparatus itself, as evidenced by the fact that polymerase δ and ε are each essential and cannot perform each other's roles (8), the lack of specificity for repair versus replication is somewhat surprising. The use of the same polymerases in repair and replication may suggest that, at least in the repair pathways analyzed to date, there is a more direct coupling between DNA replication and DNA repair than previously appreciated. The results may suggest that repair can occur during replication and that there is some benefit to coordination of progress of the replication fork and removal of lesions. On the other hand, there may be an entirely separate pool of polymerases δ and ε for repair, perhaps associated with the incision enzymes. Biochemical characterization of these complex polymerase enzyme assemblies and quantitation of the abundance of polymerase species with specific subunit assemblies has not progressed far enough to decide this. Indeed, it is not even known if different pools of polymerases exist and if they reflect different protein assemblies. The recently implied role of PCNA in sensing the environment that the replication fork is traversing and the participation of PCNA-dependent polymerases in repair may argue for the more direct link. Kinetic studies with *E. coli* DNA polymerase III and phage T4 polymerase indicate that DNA polymerases dissociate from the replication apparatus when stalled at DNA secondary structure, leaving their PCNA-like subunits behind. They might also stall at damage and leave PCNA behind, marking the damage for repair by another molecule of the respective polymerase (27). Additional studies are required to clarify the mechanisms involved.

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