**Requirement of Yeast DNA Polymerase δ in Post-replicative Repair of UV-damaged DNA**

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DNA lesions in the template strand pose a block to the replication machinery. Replication across such lesions may occur by a mutagenic bypass process in which a wrong base is inserted opposite the lesion or may involve processes that are relatively error-free. Genetic studies in the yeast *Saccharomyces cerevisiae* have indicated the requirement of REV3-encoded DNA polymerase in mutagenic bypass. The DNA polymerase responsible for error-free bypass, however, has not been identified, but genetic studies implicating proliferating cell nuclear antigen in this process have suggested that either DNA polymerase δ or DNA polymerase ε may be involved. Here, we use temperature-sensitive (ts) conditional lethal mutations of the *S. cerevisiae* POL2 and POL3 genes, which encode DNA polymerase ε and δ, respectively, and show that post-replication bypass of UV-damaged DNA is severely inhibited in the *pol3-3* mutant at the restrictive temperature. By contrast, the *pol2-18* mutation has no adverse effect on this process at the restrictive temperature. From these observations, we infer a requirement of DNA polymerase δ in post-replication bypass of UV-damaged DNA.

Lesions in DNA formed by UV light and many other DNA damaging agents are removed by nucleotide excision repair. However, if UV damage is not removed and replication is initiated from templates containing such lesions, because of the inability of the replicative machinery to bypass damage, a gap is formed in the newly synthesized strand across from the damage site. In *Escherichia coli*, this gap is filled in by error-free recombinational or mutagenic bypass processes (for a review see Ref. 1). Mutagenic bypass has been reconstituted in *E. coli* with purified RecA, UmuC, UmuD, and PolIII (2).

Genetic studies in the yeast *Saccharomyces cerevisiae* have indicated the requirement of RAD6 and RAD18 genes in post-replication bypass. Mutations in both genes cause extreme sensitivity to UV light and confer a defect in post-replicative bypass of UV-damaged DNA (3). UV-induced mutagenesis is also abolished in the *rad6* and *rad18* mutants (4–6). Rad6, a ubiquitin-conjugating enzyme, exists in *vitro* in a tight complex with Rad18, a DNA binding protein (7). The manner by which the Rad6-Rad18 complex functions in post-replicative bypass is not known.

In *S. cerevisiae*, the REV1, REV3, and REV7 genes function in mutagenic bypass. Mutations in these REV genes confer only a modest increase in UV sensitivity, but they cause a drastic reduction in UV mutagenesis (4, 8, 9). Recently, it has been shown that Rev3, together with Rev7, has a DNA polymerase activity (Polε) that can bypass a thymine-thymine *cis-syn* cyclobutane dimer (10). Rev3 is the catalytic subunit of Polε (11). Because, by comparison with mutations of the *RAD6* and *RAD18* genes, mutations of REV3 affect UV sensitivity marginally (12) and have little effect on the efficiency of post-replicative gap filling (3), it has been surmised that error-free REV3-independent processes play a major role in post-replicative bypass of UV-damaged DNA. Recently, we reported genetic studies implicating a role of PCNA in error-free bypass of UV-damaged DNA (13). PCNA is an essential processivity factor for DNA polymerase δ, and it stimulates the activity of DNA polymerase ε (14), suggesting that either or both of these DNA polymerases may function with PCNA in post-replicative bypass. Even though Polδ and Pol ε are both essential for viability of yeast cells (15, 16), only Polδ performs efficient elongation of both leading and lagging strands in the reconstituted SV40 system (17), and recent cross-linking experiments have provided further support for the requirement of Polδ in SV40 replication, as well as in nuclear chromosomal replication (18).

Because of the lack of evidence for a direct involvement of Polε in DNA replication, it has been suggested that Polε functions in replication-linked repair processes that are essential for cell viability (19).

Here, we examine whether temperature-sensitive (ts) mutations in yeast DNA polymerase δ and DNA polymerase ε inactive post-replicative bypass of UV-damaged DNA. Studies such as those reported here are essential for assessing the biological role of a gene under normal physiological conditions. Interestingly, our results indicate the involvement of Polδ, but not of Polε, in post-replicative bypass.

**MATERIALS AND METHODS**

**Strains**—Ethidium bromide mutagenesis was used to obtain *ρε* derivatives lacking mitochondrial DNA, resulting in the following strains used in these experiments: YPO-32, *MATa ade2/15 lys2-801 leu2-3, 112 ura3-52 rad1Δ ρε*; YPO-36, *MATa ade2/15 leu2-3, 112 trp1Δ ura3-52 rad1Δ pol3-3 ρε*; and YPO-31, *MATa ade2/15 his3Δ1 lys2-801 leu2-3, 112 trp1Δ ura3-52 rad1Δ pol2-18 ρε*.

**Sedimentation in Alkaline Sucrose Gradients**—Strains grown overnight at 30 °C in synthetic complete medium lacking uracil but supplemented with uridine were irradiated at a fluence rate of 0.1 J/m²/s and pulse labeled for 15 min with [3H]uracil. The pulse labeling period was followed by a 45- (for YPO-32 and YPO-31) or 75-min (for YPO-36) chase period with or without an additional 4-h incubation at the permissive or restrictive temperature in synthetic complete medium supplemented with a high concentration of uracil as described (3). Conversion of cells to spheroplasts, alkaline sucrose gradient sedimentation, and processing of samples were as described (13), except that acid precipitation of alkali-hydrolyzed samples was carried out with HCl rather than tri-chloroacetic acid.
and then subjected to a 15-min pulse with \[^3H\]uracil followed by a chase gradients.

The \( \text{rad}1\Delta \) strain YPO-32 was UV irradiated at 2.5 J/m\(^2\) and then subjected to a 15-min pulse with \[^3H\]uracil followed by a chase period in high uracil medium for 45 min at 30 °C (\( \bullet \)), or, following this treatment, cells were incubated in high uracil medium for 4 h at 37 °C (\( \bigcirc \)) prior to conversion to spheroplasts. Sedimentation pattern of DNA from uniformly labeled unirradiated cells grown overnight at 30 °C (\( \bigcirc \)).

**RESULTS**

The DNA Polymerase Mutants—The POL2 and POL3 genes of \( S.\ cervisiae \) encode DNA polymerase \( \epsilon \) and DNA polymerase \( \delta \), respectively. To examine the involvement of these DNA polymerases in post-replicative bypass, we utilized the temperature-sensitive (ts) conditional lethal mutations \( \text{pol}2-18 \) and \( \text{pol}3-3 \). The \( \text{pol}2-18 \) mutation is described in Ref. 20, and the \( \text{pol}3-3 \) mutation, originally referred to as \( \text{cdc}2-3 \), is described in Ref. 21. Although both mutations permit growth at 30 °C, growth of the \( \text{pol}2-18 \) mutant is inhibited at 39 °C, and the \( \text{pol}3-3 \) mutant does not grow at 37 °C. The rationale of the experiments is that if the function of a particular DNA polymerase is required in DNA replication or in some other essential process but not in post-replicative damage bypass, then the latter process will not be affected upon transfer of mutant cells to the restrictive temperature. If, however, proficient post-replicative bypass occurs in a mutant at the permissive temperature but not at the restrictive temperature, that would indicate the involvement of that DNA polymerase in damage bypass.

**Analysis of Post-replication Repair in a Mutant Defective in Nucleotide Excision Repair**—Because of a defect in the incision step of nucleotide excision repair, UV damage is not removed from the \( \text{rad}1\Delta \) strain, and survival of UV-irradiated \( \text{rad}1\Delta \) cells, therefore, depends upon post-replicative bypass processes (3). When \( \text{rad}1\Delta \) cells are UV irradiated at 2.5 J/m\(^2\) and the size of newly synthesized DNA is examined by pulse labeling of DNA with \[^3H\]uracil for 15 min followed by a chase for an additional 45 min at 30 °C, DNA sediments toward the top in alkaline sucrose gradients (Fig. 1), indicative of discontinuities. By contrast, in unirradiated \( \text{rad}1\Delta \) cells, the size of DNA synthesized following the 15-min pulse and 45-min chase periods is the same as in uniformly labeled cells (data not shown), indicating that this time interval is sufficient to reconstitute normal size DNA in unirradiated cells. The size of the newly synthesized DNA in the \( \text{rad}1\Delta \) mutant decreases with increasing UV dose and correlates with the average distance between photoproducts present in parental DNA (3). The discontinuities in the newly synthesized DNA thus presumably represent gaps that occur across from the damage sites in the template strand.

If, following UV irradiation and a 15-min pulse with a 45-min chase at 30 °C, \( \text{rad}1\Delta \) cells are incubated for 4 h at 30 °C (data not shown) or at 37 °C (Fig. 1), the size of daughter strands becomes the same as in unirradiated control cells, indicating that post-replicative gap filling processes have restored normal parental size to daughter strands.

**Impaired Post-replication Repair in a Pol\( \delta \) Mutant**—To investigate if Pol\( \delta \) was involved in post-replicative bypass, we coupled the \( \text{pol}3-3 \) mutation to \( \text{rad}1\Delta \) and examined the ability of the \( \text{pol}3-3 \) \( \text{rad}1\Delta \) mutant to perform damage bypass (Fig. 2). The low molecular size DNA synthesized in unirradiated \( \text{pol}3-3 \) \( \text{rad}1\Delta \) cells following a 15-min pulse with \[^3H\]uracil attains normal size in cells incubated in high uracil medium for a further 75 min at 30 °C (Fig. 2A). The \( \text{pol}3-3 \) \( \text{rad}1\Delta \) cells were UV-irradiated at 2.5 J/m\(^2\) and pulse labeled with \[^3H\]uracil for 15 min, and cells were incubated for an additional 75 min at 30 °C. DNA from these cells sediments toward the top of the gradient, indicating the presence of discontinuities in the newly synthesized strand (Fig. 2A). In \( \text{pol}3-3 \) \( \text{rad}1\Delta \) cells that were incubated for an additional 4 h period at 30 °C, DNA became longer, reaching the same size as DNA from unirradiated cells (Fig. 2A). Thus, \( \text{pol}3-3 \) performs efficient damage bypass at the permissive temperature.

To determine the effect of inactivation of Pol\( \delta \) activity on damage bypass, UV-irradiated \( \text{pol}3-3 \) \( \text{rad}1\Delta \) cells were pulse labeled with \[^3H\]uracil for 15 min, incubated for an additional 75 min at 30 °C, and then transferred to the restrictive temperature (37 °C) for 4 h. Alkaline sucrose gradient sedimentation of DNA from these cells indicates that \( \text{pol}3-3 \) \( \text{rad}1\Delta \) cells do not reconstitute normal size DNA at the restrictive temperature (Fig. 2B). To exclude the possibility of DNA degradation at the restrictive temperature, unirradiated \( \text{pol}3-3 \) \( \text{rad}1\Delta \) cells were pulse labeled for 15 min, incubated for 75 min at 30 °C, and then transferred to 37 °C for 4 h. However, no evidence of DNA breakage was seen (Fig. 2B). Thus, our results indicate that inactivation of Pol\( \delta \) activity engenders a defect in post-replication repair, thus implicating a role of Pol\( \delta \) in this process.

**Proficient Post-replication Repair in the Pol\( \delta \) Mutant**—To determine if Pol\( \delta \) functions in post-replication repair, we examined the ability of the \( \text{pol}2-18 \) \( \text{rad}1\Delta \) mutant to convert low molecular size DNA made from UV-damaged templates to normal size DNA (Fig. 3). DNA from cells of the \( \text{pol}2-18 \) \( \text{rad}1\Delta \) mutant pulse labeled with \[^3H\]uracil for 15 min without prior UV irradiation attains parental size during a 45-min chase at 30 °C (data not shown). DNA from \( \text{pol}2-18 \) \( \text{rad}1\Delta \) cells exposed to 2.5 J/m\(^2\) of UV irradiation and then given a 15-min pulse and a 45-min chase at 30 °C sediments toward the top of the gradient; however, parental size DNA is reconstituted upon further incubation of these cells for 4 h at the restrictive temperature (39 °C). These results suggest that Pol\( \delta \) may not be required for post-replicative bypass of UV-damaged DNA.

**DISCUSSION**

Because no definitive role has been assigned to Pol\( \delta \) in DNA replication, it has been thought that the primary function of this DNA polymerase is in replication-linked repair processes that are essential for cell viability (19). However, our studies provide no evidence for a role of Pol\( \delta \) in post-replication repair of UV-damaged DNA. Rather, we find that Pol\( \delta \), the major
replicative polymerase, is required for post-replicative repair. Recent studies have also indicated the requirement of Pol\textsubscript{d} in DNA mismatch repair, and Pol\textsubscript{e} is apparently dispensable in this process as well (22). Thus, in addition to its essential role in the elongation of both the leading and lagging DNA strands, Pol\textsubscript{d} function is utilized in different post-replicative repair processes as well.

Our previous studies with the pol30-46 mutation have indicated an involvement of PCNA in error-free post-replicational repair (13). By contrast to the rev3\textsuperscript{D} mutation, which drastically reduces the formation of UV-induced mutations, pol30-46 has no effect on UV mutagenesis, and the rev3\textsuperscript{D} pol30-46 double mutant exhibits a synergistic increase in UV sensitivity over either of the single mutants (13). Based upon these and other results, we have suggested that PCNA and Rev3 function, respectively, in the error-free and mutagenic modes of damage bypass (13). Because PCNA is an essential processivity factor for Pol\textsubscript{d} and because by contrast to rev3, which has little effect on post-replication repair (3), the pol30-46 and pol3-3 mutations greatly reduce the efficiency of post-replication repair, we suggest that Pol\textsubscript{d} and PCNA function together in the error-free mode of damage bypass, and this mechanism is responsible for the majority of damage bypass.

How might Pol\textsubscript{d} and PCNA effect damage bypass? One possibility is that these proteins carry out error-free translesion synthesis, whereas the Rev3 pathway functions in mutagenic bypass of those lesions that cannot be handled by Pol\textsubscript{d}. Interestingly, by contrast to E. coli, DNA lesions are bypassed with much greater accuracy in S. cerevisiae (23, 24). For example, studies with single stranded shuttle vectors that carry a defined and uniquely placed mutagenic lesion have indicated that in SOS-induced E. coli, the cis-syn cyclobutane pyrimidine dimer (CPD) is bypassed in \textasciitilde 16\% of the DNA molecules, and about 8\% of the bypass products have targeted mutations. By contrast, in S. cerevisiae, bypass occurs in 80\% of CPD containing DNA molecules, and bypass is \textasciitilde 20-fold more accurate than...
in *E. coli*. Moreover, in yeast, (6-4) photoproducts can be bypassed in an error-free way in a large proportion of single stranded DNA molecules (~70%) compared with *E. coli*, where accurate replication of (6-4) photoproducts occurs only in ~9% of DNA molecules (23, 24). Translesion synthesis by Polδ may account for the efficient and accurate bypass of DNA lesions in *S. cerevisiae*. Studies with purified calf thymus Polδ and PCNA indicating that in the presence of PCNA Polδ can bypass cis-syn and trans-syn-I cyclobutane dimers are consistent with this idea (25).

Alternatively, or in addition, Polδ and PCNA could effect damage bypass by mechanisms other than translesion synthesis. One such mechanism could be a "copy choice" type of DNA synthesis, in which upon encountering the lesion, the DNA polymerase switches from the template strand to the undamaged sister chromatid and switches back after the undamaged template has been copied past the point of DNA damage. Such a model has been proposed to explain replication across damage sites in mammalian cells (26), and such a mechanism has been reconstituted in bacteriophage T4 using purified proteins (27). Recombinational mechanisms may also be utilized in Polδ/PCNA-mediated damage bypass.

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