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Dominant Negative Rat DNA Polymerase β Mutants Interfere with Base Excision Repair in *Saccharomyces cerevisiae*

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DNA polymerase β is one of the smallest known eukaryotic DNA polymerases. This polymerase has been very well characterized in vitro, but its functional role in vivo has yet to be determined. Using a novel competition assay in *Escherichia coli*, we isolated two DNA polymerase β dominant negative mutants. When we overexpressed the dominant negative mutant proteins in *Saccharomyces cerevisiae*, the cells became sensitive to methyl methanesulfonate. Interestingly, overexpression of the same polymerase β mutant proteins did not confer sensitivity to UV damage, strongly suggesting that the mutant proteins interfere with the process of base excision repair but not nucleotide excision repair in *S. cerevisiae*. Our data implicate a role for polymerase IV, the *S. cerevisiae* polymerase β homolog, in base excision repair in *S. cerevisiae*.

Mammalian DNA polymerase β is a 39-kDa monomeric protein that is believed to function in DNA repair. Evidence from many laboratories has shown that chemical inhibitors of DNA polymerase β inhibit DNA repair in cells treated with DNA-damaging agents such as methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), methyl nitrosourea, bleomycin, and gamma irradiation (6, 10, 17). In addition, DNA polymerase β mRNA levels are increased in Chinese hamster ovary (CHO) cells exposed to MNNG or methyl methanesulfonate (MMS) (7). However, data from in vitro studies suggest that other cellular polymerases, in particular δ (2) and α and ε (29), may also play a role in DNA repair (6).

DNA polymerase β most likely functions in the process of base excision repair. Base excision repair is a mechanism used by cells to remove harmful DNA adducts caused by alkylation with MMS or MNNG. In base excision repair, the DNA lesion is first recognized and removed from the sugar by a DNA glycosylase, creating an abasic site. The abasic site is then processed sequentially by an apurinic/apyrimidinic endonuclease and an exonuclease. The small gap created by these enzymes is filled by a DNA polymerase, and the nick at the end of this patch is sealed by a DNA ligase (8). DNA polymerase β may function in the role of filling the small gaps that occur during this process because it has a high affinity for duplex DNA containing short gaps (23). In addition, it has been demonstrated that DNA polymerase β acts as a processive polymerase when filling gaps up to 6 nucleotides in length (23). In vitro studies with purified DNA polymerase β have also implicated polymerase β in base excision repair (1, 5, 22, 28). While these results show that DNA polymerase β has the ability to participate in base excision repair in vitro, very little evidence has demonstrated the role of polymerase β in vivo.

Genetic analysis of DNA polymerase β has been unable to define its physiological role. Mice that are homozygous for a disrupted polymerase β allele die during embryogenesis, suggesting that polymerase β might be necessary for this process (9). However, when the polymerase IV gene, which encodes a protein that has 26% identity and 50% homology with human and rat polymerase β homologs (3, 16, 18, 21) is disrupted in *Saccharomyces cerevisiae*, no unique phenotype is detected (16, 18). One possible explanation for a lack of phenotype in these cells is functional redundancy. The yeast cells may be able to compensate for the loss of polymerase IV by utilizing other endogenous polymerases normally involved in processes such as DNA repair or growth. Therefore, our approach to investigate the role of polymerase β in vivo utilizes dominant negative polymerase β mutants which are capable of functionally disrupting a cellular process. We demonstrate that these polymerase β mutants interfere with the yeast cells' ability to perform base excision repair but not nucleotide excision repair, suggesting that the polymerase β homolog in *S. cerevisiae*, polymerase IV, functions in base excision repair in vivo.

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MATERIALS AND METHODS

Bacterial strains and media. All strains were derived from SC18 (*E. coli* B/r), with the genotype *recA718 polA12 uvrA115 fad751*::Tn*10 trpE65 lon-11 sulA1 mal* (30). Minimal medium was E medium (27) supplemented with 0.4% glucose and tryptophan (20 μg/ml). For the efficiency-of-plating assay, cells were plated on CAA medium, which is E medium with 1.5% Bacto agar (Difco) and 0.4% vitamin-free casamino acids (CAA; Difco). Transformants were selected on LB agar supplemented with either chloramphenicol (30 μg/ml) alone or chloramphenicol (30 μg/ml) plus ampicillin (50 μg/ml). Nutrient broth (NB) was prepared according to the directions from Difco.

Yeast strains and media. Strain 6607-6a is *MAT***a** *ura3-52 his-3Δ200 leu2-3, 112 hom3 gal1 can1* (2). Rich medium (YPD) was prepared as described before (20). Synthetic complete (SC) medium (20) was supplemented with 2% raffinose instead of 2% glucose and appropriate amino acids. Transformants of strain 6607-6a were selected on SC medium with 2% raffinose in the absence of uracil.

Construction of polβ-TR. Plasmid pβL (25) was digested with the restriction enzyme *Bam*HI. The small fragment containing the nucleotide sequence encoding amino acids 171 to 340 of rat DNA polymerase β was removed, and the plasmid was religated to yield polβ-TR. The truncated protein (polβ-TR) expressed by this plasmid has been shown to bind to DNA and to have no catalytic activity with regard to DNA synthesis (4). Polβ-14 was identified in a screen for rat polymerase β mutants that has been described previously (26). The expression of both polβ-TR and polβ-14 is under the control of the *lac* promoter, rendering them inducible by isopropyl-β-D-thiogalactopyranoside (IPTG). The plasmid harboring the wild-type polymerase β sequence contains a ColE1 origin of replication, while the plasmid harboring the cDNA sequences for expression of polβ-TR and polβ-14 contain a pSC101 origin of replication. These are compatible origins that allow the coexpression of both wild-type and mutant polymerase β proteins.

To construct the cDNA sequence encoding polβ-TR in the pYes2 yeast expression vector, plasmid pBK3 was obtained from Ann Blank and Baek Kim. It consists of the cDNA for wild-type polymerase β cloned into the plasmid vector pYes 2 (2). pBK3 was digested with the restriction enzymes *Xba*I and *Bam*HI, the small fragment consisting of the DNA sequence coding for amino acids 171 to 340 of DNA polymerase β was removed, and the vector was religated to yield pTR-Y. To subclone polβ-14 in the pYes2 expression vector, a plasmid containing the polβ-14 cDNA was digested with *Bam*HI and *Xba*I restriction enzymes.

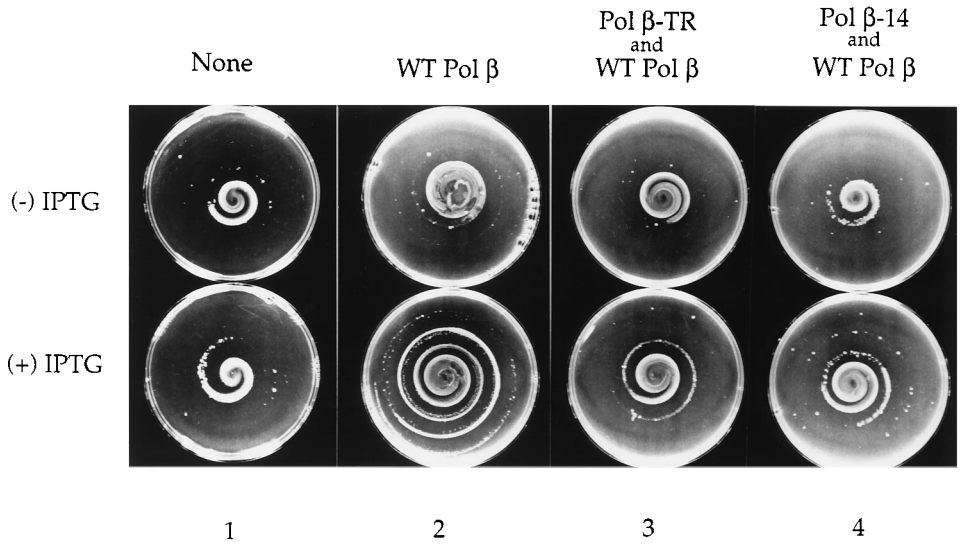

FIG. 1. Rotary streak competition assay. Log-phase cultures of the control *recA718 polA12*^{ts} cells (panel 1) or the same cells harboring plasmids encoding wild-type (WT) polymerase (Polβ) (panel 2), polβ-TR plus WT Polβ (panel 3), or polβ-14 plus WT Polβ (panel 4) were grown in the absence (top) or presence (bottom) of 1 mM IPTG. The cells were streaked onto 0.4% CAA plates by drawing an inoculating loop slowly across the radius of the plate spun on a turntable to create a density gradient of cells as described in the text. Results shown are representative of three independent determinations.

The 561-bp fragment encoding amino acids 170 to 340 of the polβ-14 allele was gel purified and ligated into pBK3 digested with the same enzymes, replacing the wild-type sequence coding for amino acids 170 to 340 to yield p14-Y.

Rotary streak (*E. coli*). Individual *E. coli* colonies were grown overnight at 30°C in NB with or without 1 mM IPTG. With a sterile inoculating loop, cells were streaked onto E medium (0.4% CAA) plates with or without 1 mM IPTG in a rotary fashion, resulting in a gradient of cell densities. Duplicate plates were incubated at 30 and 42°C for two nights (30).

Plating efficiency (*E. coli*). The plating efficiency assay has been described previously (25). Briefly, individual colonies were grown to logarithmic phase at 30°C in NB in the presence or absence of 1 mM IPTG. Cells were diluted in saline and plated onto CAA plates with or without 1 mM IPTG. Duplicate plates were incubated at 30 and 42°C for 2 days, and colonies were counted. Plating efficiency was calculated as [(CFU at 42°C)/(CFU at 30°C)]×100.

MMS survival (*E. coli*). The methods for determining MMS survival have been described previously (25). Briefly, individual colonies were grown to logarithmic phase at 30°C in E broth with 1 mM IPTG and treated with various concentrations of MMS at 42°C for 1 h. Cells were resuspended in saline, diluted, plated on E medium containing 1 mM IPTG, and incubated at 42°C for 2 days. Colonies were counted, and percent survival was calculated.

MMS survival (*S. cerevisiae*). Survival data were obtained by a modification of methods described previously (2, 14). Briefly, individual colonies were grown to stationary phase at 22°C in SC broth with 2% raffinose. Cells were washed twice with 0.05 M potassium phosphate buffer, pH 7.0, and resuspended at 1×10^7 to 2×10^7 cells per ml in 0.05 M potassium phosphate buffer. MMS was added to a final concentration of 1%. Aliquots were removed at various times, diluted in 0.05 M potassium phosphate buffer, and plated on YPD medium. Cells were incubated for 3 days at 22°C, and colonies were counted. For expression of polymerase β constructs, cells were incubated with 0.5% galactose for 3 h and then treated as described above except that cells were diluted for plating into 0.05 M potassium phosphate buffer containing 0.5% galactose.

UV survival (*S. cerevisiae*). Cells were grown, washed, and resuspended in 0.05 M potassium phosphate buffer as described above. Cells were placed in a 60-mm plastic tissue culture dish and exposed to 254 nm light at a dose rate of 2 J/m^2/s, with agitation without visible light. Aliquots were removed at various times, diluted in potassium phosphate buffer, and plated on YPD. The plates were incubated for 3 days in the dark, and colonies were counted. Protein expression was induced with 0.5% galactose, and survival was calculated as described above.

Growth curve (*S. cerevisiae*). Growth curves were obtained for the 6607-6a yeast strain at 22°C in liquid SC medium in the absence of uracil. Log-phase cultures were diluted to an optical density at 600 nm (OD_{600}) of 0.05 in SC liquid medium (without uracil), protein expression was induced with 0.5% galactose, and densities were monitored until the cells reached the stationary phase (OD_{600} of 1 to 2).

RESULTS

A very powerful approach to investigating the function of a protein is through the use of dominant negative mutations (11). These types of mutations encode a mutant gene product capable of interfering with the function of the wild-type gene product in the cell. Thus, the cell becomes deficient in the targeted cellular function. This phenotype is referred to as dominant because the deficient phenotype occurs in the presence of the wild-type gene product. See the review by Herskowitz (11) for a more comprehensive review of dominant negative mutations. We decided to use this approach to identify the cellular role of DNA polymerase β in vivo. To identify dominant negative polymerase β mutants, we used the *E. coli* complementation system described below.

Expression of the polβ-TR and polβ-14 proteins inhibits complementation of the *recA718 polA12*^{ts} strain by wild-type DNA polymerase β. We and others have previously shown that the conditional lethal *recA718 polA12*^{ts} mutant is unable to form single colonies on rich medium at 42°C and is sensitive to MMS (25, 30). However, when wild-type DNA polymerase β is expressed in the *recA718 polA12*^{ts} mutant, it is able to restore the growth defect on rich medium at 42°C (24, 25) and renders cells resistant to MMS. This is because DNA polymerase β can substitute for DNA polymerase I during DNA replication and base excision repair (24). We used this assay to determine whether specific polymerase β mutants could compete with wild-type DNA polymerase β and interfere with the ability of polymerase β to complement the growth defect of the *recA718 polA12*^{ts} strain on rich medium.

Plasmids harboring the polβ-TR and polβ-14 mutant cDNAs were transformed into *recA718 polA12*^{ts} mutant cells alone and into *recA718 polA12*^{ts} cells containing wild-type polymerase β. Figure 1 shows the results from a representative rotary streak assay. The top row shows growth on 0.4% CAA medium in the absence of IPTG (i.e., no protein expression), and the bottom row shows growth on 0.4% CAA medium in the presence of IPTG (protein expression). In the presence of IPTG, wild-type polymerase β complements the growth defect of the *recA718 polA12*^{ts} strain, resulting in growth of discrete colonies at lower cell densities. However, when wild-type polymerase β is expressed together with polβ-TR (panel 3) or polβ-14 (panel 4), the growth resembles that of the *recA718*

TABLE 1. Polymerase β mutant proteins interfere with the activity of wild-type polymerase β in *E. coli*[a]

Experiment	Plasmid	CFU/ml		Plating efficiency (%)
		30°C	42°C	
1	None	1.1×10^6	4.0×10^3	0.5
	Polβ	5.2×10^6	4.5×10^6	90
	Polβ-TR	1.4×10^6	1.4×10^4	1.0
	Polβ-14	1.3×10^6	1.0×10^4	1.3
2	None	1.6×10^9	$\leq 1.0 \times 10^5$	<0.1
	Polβ	2.7×10^9	2.6×10^9	96
	Polβ and polβ-TR	4.1×10^9	5.8×10^8	14
	Polβ and polβ-14	3.1×10^9	4.3×10^8	14

[a] In experiment 1, individual colonies of the control *recA718 polA12*^{ts} mutant strain or of this strain harboring plasmids encoding wild-type polymerase β (Polβ), polβ-TR, or polβ-14 were grown to logarithmic phase in the presence of 1 mM IPTG (see text). In experiment 2, individual colonies of the control *recA718 polA12*^{ts} strain or of this strain harboring plasmids encoding wild-type polymerase β, polβ-TR plus wild-type polymerase β, or polβ-14 plus wild-type polymerase β were grown to logarithmic phase in the presence of 1 mM IPTG (see text). Results are representative of three independent determinations.

polA12^{ts} mutant (compare panel 1 with panels 3 and 4). This strongly suggests that these mutants interfere with the ability of wild-type polymerase β to complement the growth defect.

To further characterize this phenotype, quantitative plating efficiency assays were performed. Table 1 shows the plating efficiency of the *recA718 polA12*^{ts} mutant alone and the *recA718 polA12*^{ts} mutant expressing wild-type polymerase β, polβ-TR, or polβ-14 protein. The plating efficiency of the *recA718 polA12*^{ts} strain alone is very poor (0.5%); however, expression of the wild-type polymerase β protein was able to complement fully the growth defect of the *recA718 polA12*^{ts} mutant (plating efficiency, 90%), whereas neither the polβ-TR (1.0%) nor the polβ-14 (1.3%) mutant protein had the ability to complement the growth defect. This demonstrates that neither the polβ-TR nor polβ-14 mutant protein is able to substitute for *E. coli* polymerase I in DNA replication. Table 1 also shows the plating efficiency of the *recA718 polA12*^{ts} mutant strain and the *recA718 polA12*^{ts} strains expressing polβ-TR or polβ-14 in the presence of wild-type polymerase β. While the *recA718 polA12*^{ts} strain has very poor plating efficiency (<0.1%), expression of wild-type polymerase β protein is able to complement the growth defect (96%); however, wild-type polymerase β is unable to complement the growth defect to the same extent when it is expressed together with polβ-TR (14%) or polβ-14 (14%). These data demonstrate that polβ-TR and polβ-14 interfere with the ability of wild-type polymerase to substitute for *E. coli* polymerase I in DNA replication. The dominant negative phenotype is not a universal feature of polymerase β mutants. Several other mutants of polymerase β that also lack catalytic activity (26) have no effect on wild-type polymerase β's ability to complement the growth defect of the *recA718 polA12*^{ts} strain (data not shown). Thus, polβ-TR and polβ-14 appear to be specific dominant negative mutants of polymerase β.

Another phenotype of the *recA718 polA12*^{ts} mutant strain is that it is sensitive to MMS. Previous work has demonstrated that expression of wild-type polymerase β can render the *recA718 polA12*^{ts} mutant resistant to MMS (25). To address whether our dominant negative mutants would also interfere with polymerase β's ability to complement the MMS sensitivity, *recA718 polA12*^{ts} cells and cells expressing polβ-TR or polβ-14 in the presence of wild-type polymerase β were exposed to various concentrations of MMS. Figure 2 shows the results from these experiments. The *recA718 polA12*^{ts} strain alone is very sensitive to MMS, but expression of wild-type polymerase β protein renders the *recA718 polA12*^{ts} cells resistant to MMS. However, when the polβ-TR or polβ-14 protein was expressed in the presence of wild-type polymerase β, the cells remained sensitive to MMS. This demonstrates that these mutant proteins interfere with the ability of wild-type polymerase β to substitute for *E. coli* polymerase I in base excision repair in *E. coli.*

Expression of polβ-TR and polβ-14 mutant proteins in *S. cerevisiae.* The results presented above suggest that the polβ-TR and polβ-14 mutant proteins are dominant to wild-type polymerase β in *E. coli*. However, polymerase β is an enzyme found in eukaryotic cells ranging from *S. cerevisiae* to *Homo sapiens*. In order to investigate the physiological role of DNA polymerase β in eukaryotes, we transformed *S. cerevisiae* 6607-6a yeast cells with our dominant negative polymerase β mutants. We reasoned that these mutants would also possess a dominant negative phenotype in cells which contain the polymerase IV protein, which is the polymerase β homolog in *S. cerevisiae*. The 6607-6a strain was transformed with plasmid pBK3, pTR-Y, or p14-Y under the control of the *GAL1* promoter. Exposure of the yeast to galactose results in expression of polymerase β, polβ-TR, or polβ-14, respectively, as determined by immunoblotting (data not shown). Expression of the wild-type, polβ-TR, or polβ-14 protein had no effect on growth of the yeast cells (Fig. 3).

Dominant negative polymerase β mutants render cells sensitive to MMS. Yeast cells containing the pYes2 plasmid alone or with plasmid pBK3, pTR-Y, or p14-Y were induced to express wild-type, polβ-TR, polβ-14 mutant protein and exposed to 1% MMS for various times. Figure 4 shows the results of these experiments. Cells expressing the polβ-TR or polβ-14 protein showed increased MMS sensitivity, as much as 10-fold greater than that of cells expressing wild-type polymerase β or the plasmid alone (Fig. 4A). This is a specific effect resulting from the expression of the mutant proteins because it is dependent on the presence of galactose. In the absence of galactose (i.e., no protein expression), all the yeast strains showed

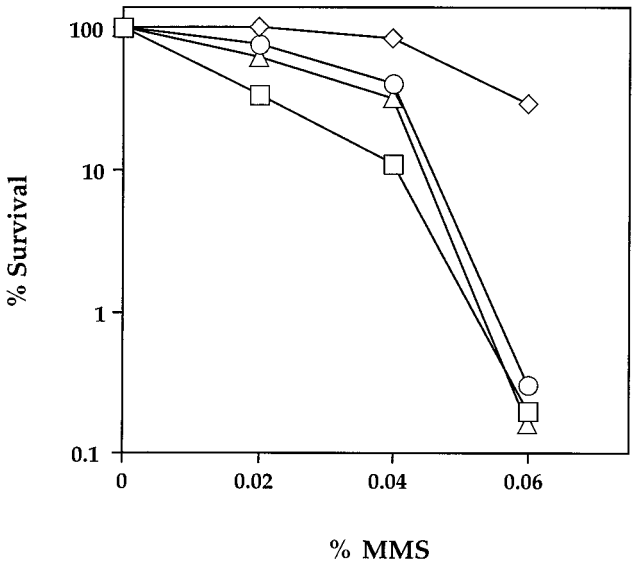

FIG. 2. MMS sensitivity in *E. coli*. Log-phase cultures of the control *recA718 polA12*^{ts} strain (□) or of this strain harboring plasmids for the expression of wild-type (◇), polβ-TR plus wild-type (○), or polβ-14 plus wild-type (△) proteins were grown in the presence of 1 mM IPTG (see text). Results shown are representative of two independent determinations.

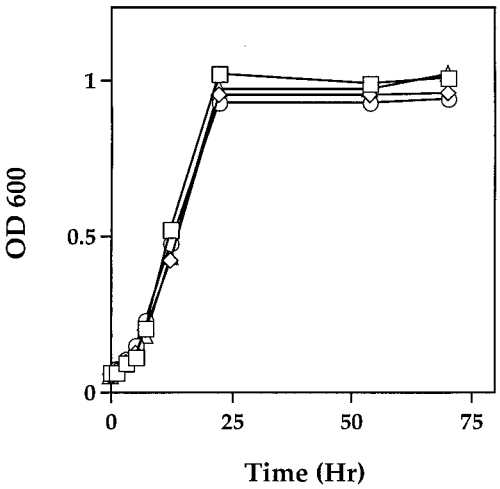

FIG. 3. Yeast growth curve. Log-phase cultures of 6607-6a yeast cells harboring plasmids for expression of the pYes2 vector (□) or wild-type (◇), polβ-14 (○), or polβ-TR (△) protein were diluted to an OD_{600} of 0.05 and incubated in the presence of 0.5% galactose and densities were assayed as described in the text. Results shown are representative of two independent determinations.

similar MMS resistance (Fig. 4B). These data show that the dominant negative polymerase β mutants interfere with the ability of *S. cerevisiae* to survive MMS damage, most likely by interfering with the process of base excision repair (19).

Expression of DNA polymerase β mutants does not result in UV sensitivity. To investigate the role of DNA polymerase β in repair of UV-induced damage, cells were exposed to various UV doses; the results are shown in Fig. 5. The results demonstrate that expression of the polβ-TR or polβ-14 mutant protein does not alter the UV resistance of the 6607-6a strain compared with expression of the wild-type protein or the vector alone. This suggests that expression of the dominant negative mutant proteins does not interfere with the process of nucleotide excision repair in *S. cerevisiae*. The data also demonstrate the specificity of the dominant negative polβ-TR and polβ-14 mutant proteins in that they have the ability to discriminate between two different types of repair processes.

DISCUSSION

Dominant negative mutants identified in *E. coli*. We identified two DNA polymerase β mutants that are dominant to wild-type polymerase β by using a competition assay in *E. coli*. This assay relies on the fact that the *recA718 polA12^{ts} E. coli* strain is unable to grow on rich medium at 42°C and is sensitive to MMS. Expression of wild-type polymerase β protein is able to complement this growth defect by substituting for *E. coli* polymerase I in DNA replication and base excision repair. When the mutant proteins polβ-TR and polβ-14 are expressed in *recA718 polA12^{ts}* cells in the presence of wild-type polymerase β, the cells are unable to grow at 42°C and are sensitive to MMS (Table 1 and Fig. 2). These results strongly suggest that polβ-TR and polβ-14 interfere with the ability of wild-type polymerase β to substitute for polymerase I in DNA replication and repair in *E. coli.*

Mutant proteins interfere with base excision repair in *S. cerevisiae*. When the dominant negative mutant proteins polβ-TR and polβ-14 were expressed in *S. cerevisiae* 6607-6a, it became up to 10-fold more sensitive to MMS than cells expressing wild-type polymerase β or the vector alone (Fig. 4A). These data demonstrate that the dominant negative polymerase β mutant proteins interfere with the repair of alkylation damage in *S. cerevisiae* 6607-6a. Because alkylation damage caused by agents such as MMS is usually processed by the base excision repair pathway, the most likely explanation for our results is that the polymerase β mutant proteins interfere with base excision repair in the 6607-6a strain.

Dominant negative mutant proteins do not block nucleotide excision repair. The dominant negative polymerase β proteins do not sensitize *S. cerevisiae* 6607-6a to UV light (Fig. 5). Since UV damage is removed by the process of nucleotide excision repair, our data strongly suggest that the polymerase β mutant proteins do not interfere with nucleotide excision repair. Fur-

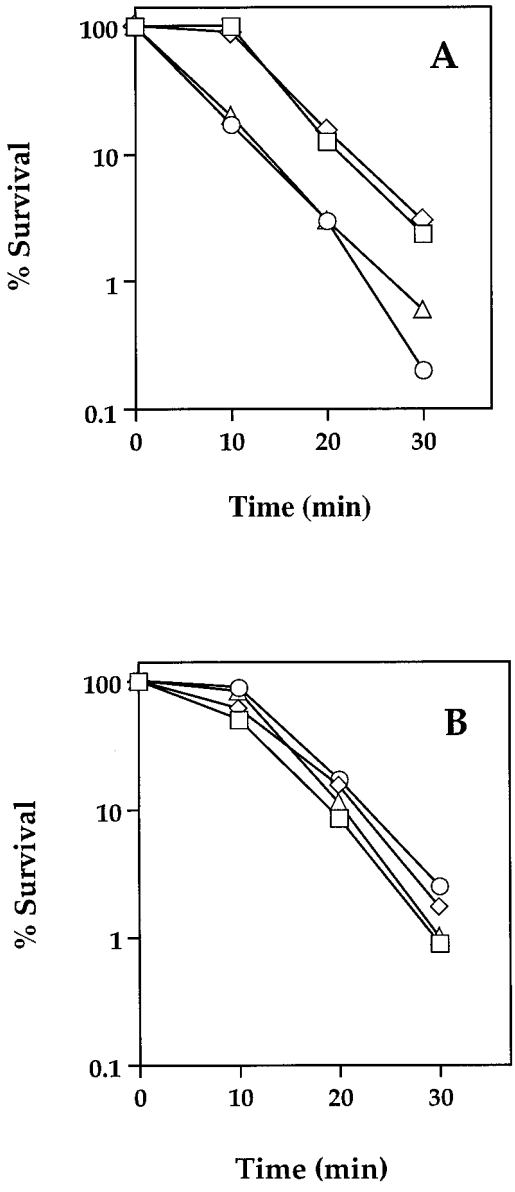

FIG. 4. MMS sensitivity in *S. cerevisiae*. (A) Strain 6607-6a yeast cells harboring plasmids for the expression of pYes vector alone (□) or wild-type (◇), polβ-14 (○), or polβ-TR (△) protein were incubated in the presence of 0.5% galactose as described in the text. Results shown are representative of three independent determinations. (B) Strain 6607-6a yeast cells harboring plasmids for the expression of pYes vector alone (□) or wild-type (◇), polβ-14 (○), or polβ-TR (△) protein were incubated in the absence of galactose as described in the text. Results shown are representative of three independent determinations.

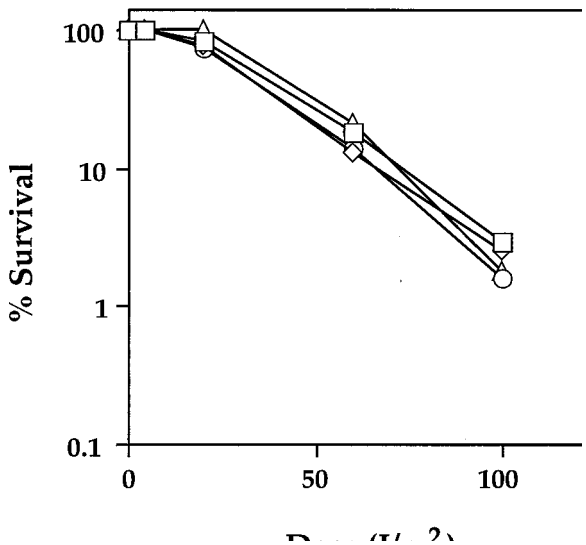

FIG. 5. UV sensitivity in *S. cerevisiae*. Strain 6607-6a yeast cells harboring plasmids for the expression of pYes vector alone (□) or wild-type (◇), polβ-14 (○), or polβ-TR (△) protein were incubated in the presence of 0.5% galactose as described in the text. Results shown are representative of two independent determinations.

thermore, these data demonstrate the specificity of the polβ-TR and polβ-14 proteins. The large gaps produced during nucleotide excision repair are not the preferred substrates for DNA polymerase β in in vitro assays, and they are also not good substrates for polβ-TR and polβ-14 in vivo. In addition, expression of the dominant negative proteins in *S. cerevisiae* did not result in the inhibition of growth (Fig. 3), suggesting that these proteins do not interfere with DNA replication.

Polymerase β mutant proteins may interact nonproductively with DNA. Dominant negative proteins are believed to act by two possible mechanisms (11). Under the first mechanism, the protein is part of a protein complex or acts as a multimer; the dominant negative protein could become part of the protein complex and thereby inactivate the complex. In our case, in both bacteria and yeast cells, the polymerase β dominant negative proteins could interact in a nonproductive way with other proteins involved in base excision repair, inhibiting this process. Because protein-protein interactions are usually highly specific, we believe that it is unlikely that rat polymerase β would interact with proteins that function in base excision repair in either *E. coli* or *S. cerevisiae*. It is also not likely that rat polymerase β would form a complex with yeast polymerase IV, because polymerase β is not known to act as a multimer. However, we cannot rule out at this time the possibility that the dominant negative polymerase β proteins interact with other factors involved in base excision repair in either bacteria or yeast cells. In the second mechanism, a dominant negative protein would interact with a critical substrate in a nonproductive manner and thereby prevent other proteins from having access to the substrate. We believe that this is a more likely explanation for the phenotypes that we observed when we expressed our dominant negative proteins in *E. coli* and in *S. cerevisiae*. We suggest that the dominant negative polymerase β proteins interfere with DNA repair by competing with the wild-type polymerase β in *E. coli* or an endogenous DNA polymerase in *S. cerevisiae*, most likely polymerase IV, for binding to DNA at the gap resulting from the excision of the damaged base. This hypothesis is supported by the biochemical characteristics of the polβ-TR and polβ-14 proteins. The polβ-TR protein is merely a DNA-binding protein and is unable to function in DNA synthesis (4); therefore, binding to the gap would be nonproductive. The measured K_m for polβ-14 with respect to gapped DNA is four times lower than the K_m of wild-type polymerase β on the same template (23a). This strongly suggests that polβ-14 binds more tightly to the base excision repair gap than does wild-type polymerase β. In addition, the V_{max} of the polβ-14 enzyme is approximately 10 times lower than that of the wild-type enzyme (23a), suggesting that polβ-14 catalyzes DNA synthesis at a much slower rate than the wild-type enzyme. Lastly, polymerase β prefers to bind to the types of small gaps that are formed during base excision repair and functions in a processive manner to fill them in. Taken together, these data are consistent with the possibility that the dominant negative polymerase β proteins act in yeast cells by competing with an endogenous DNA polymerase for access to the repair gap.

Polymerase IV may function in base excision repair in yeast cells. Expression of our dominant negative polymerase β proteins most likely interferes specifically with the process of base excision repair in *S. cerevisiae*. Because the yeast homolog to polymerase β is polymerase IV, our data strongly suggest that polymerase IV functions in base excision repair in yeast cells. This is substantiated by work from other laboratories (1, 5, 22, 28) demonstrating that polymerase IV possess biochemical characteristics in vitro similar to those of rat polymerase β (1, 5, 16, 18, 21). Furthermore, work by Singhal et al. (22) demonstrated that polymerase β is able to function in base excision repair in an in vitro system. In addition, recent work by Husain et al. (12) demonstrated that a 14-kDa domain of DNA polymerase β could inhibit the ability of wild-type polymerase β to incorporate [α-^{32}P]dTTP into a template primer in vitro. This fragment, like our polβ-TR, contains only the DNA-binding domain of polymerase β.

It is interesting that disruption of the polymerase IV gene in *S. cerevisiae* results either in no phenotype (18) or in only slight sensitivity to MMS upon chronic exposure to the agent (16). One possibility for the absence of MMS sensitivity in the *polIV*-disrupted *S. cerevisiae* strains is redundancy, meaning that other endogenous *S. cerevisiae* DNA polymerases may be able to substitute for polymerase IV in gap filling during base excision repair. This redundancy would have been eliminated by our approach of expressing dominant negative polymerase β proteins in *S. cerevisiae.*

Other studies have suggested roles for polymerase δ or ε in base excision repair. Blank et al. (2) showed that a *cdc2* (which encodes the large catalytic subunit of polymerase δ) mutant of *S. cerevisiae* is sensitive to MMS, demonstrating a role for polymerase δ in base excision repair. Interestingly, these workers also showed that overexpression of mammalian polymerase β in the *cdc2* mutant restored MMS resistance to this strain, which is consistent with our data suggesting that polymerase β can participate in base excision repair. Wang and coworkers (29) have implicated a role for polymerase ε in base excision repair by showing that it functions in this process in vitro.

In summary, we have demonstrated through the use of dominant negative polymerase β mutants that polymerase β mutant proteins interfere specifically with base excision repair in *S. cerevisiae*, suggesting that the endogenous yeast polymerase β homolog functions in base excision repair.

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