nuclear and three mitochondrial) for which a tuatara sequence was available was analyzed separately. An additional 11 nuclear genes were used to estimate divergence times. Genes used for time estimation were selected based on the presence of at least two major groups of reptiles (including birds), at least one mammal sequence for calibration, and a more distant outgroup (*Xenopus* in most cases) for testing rate constancy with respect to the calibration lineage. The mitochondrial data were partitioned into protein-coding (cytochrome b; *cytb*) and non–protein-coding (*mt-npc*; tRNA genes for alanine, asparagine, cysteine, tryptophan, tyrosine, and valine, and the two rRNA genes); these were treated as two separate genes. The 24 nuclear genes are 18S ribosomal RNA (*18S rRNA*), 28S rRNA, alcohol dehydrogenase (*adh-1*), alpha crystallin A (*a-cryst*), alpha enolase (*a-eno*), alpha globin A chain (*a-glob*), aromatase, beta globin (*b-glob*), calcitonin (*calcit*), calcium-activated potassium channel (*capc*), c-mos proto-oncogene (*c-mos*), cytochrome c (*cytc*), insulin, lactate dehydrogenase A (*ldha*), lactate dehydrogenase B (*ldhb*), lysozyme, myoglobin, pancreatic ribonuclease (*p-ribo*), prolactin, prothrombin (*proth*), rhodopsin, somatotropin (*somato*), superoxide dismutase (*sod*), and tyrosinase (*tyros*). Taxon names and accession numbers are available at www.sciencemag.org/feature/data/986617.shl.

18. Sequence alignments were made with ClustalW [J. D. Thompson, D. G. Higgins, T. J. Gibson, *Nucleic Acids Res.* **22**, 4673 (1994)] and visually refined. Maximum likelihood, maximum parsimony, and neighbor-joining trees were constructed with MOLPHY [J. Adachi and M. Hasegawa, *MOLPHY Version 2.3: Programs for Molecular Phylogenetics Based on Maximum Likelihood* (*Comp. Sci. Monogr. 28*, Institute of Statistical Mathematics, Tokyo, 1996)], PAUP [D. L. Swofford, *PAUP*. Phylogenetic Analysis using Parsimony (*and Other Methods). Version 4.* (Sinauer Associates, Sunderland, MA, 1998)], and MEGA [S. Kumar, K. Tamura, M. Nei, *MEGA: Molecular Evolutionary Genetic Analysis* (Pennsylvania State University, University Park, PA, 1993)], respectively. Amino acid sequence data were analyzed with the JTT-F model for maximum likelihood and a gamma correction for neighbor-joining. Gene-specific gamma shape parameters were estimated from the amino acid sequence data [X. Gu and J. Zhang, *Mol. Biol. Evol.* **14**, 1106 (1997)]: *A-cryst* (0.33), *A-eno* (0.16), *A-glob* (0.84), *B-glob* (1.05), *Calcit* (>5), *C-mos* (0.46), *Cytb* (0.67), *Cytc* (0.21), *Insulin* (0.82), *Ldha* (0.33), *Ldhb* (0.34), *Myoglobin* (0.82), and combined nuclear proteins (0.38). DNA sequence analyses (neighbor-joining) were performed with a Kimura 2-parameter distance correction (nuclear genes) or Kimura transversion distance correction (mitochondrial genes). Statistical confidence estimates of topologies and branches were obtained with the RELL bootstrap method for maximum likelihood, the standard bootstrap method (5000 replications) for maximum parsimony and neighbor-joining, and with the four-cluster and interior-branch tests [S. Kumar, *Phyltest: A Program for Testing Phylogenetic Hypotheses* (Institute of Molecular Evolutionary Genetics, Pennsylvania State University, University Park, PA, ed. 2.0, 1996)]. Alignments are available from the corresponding author.
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20. Eleven of the original 13 nuclear genes, the two mitochondrial data partitions, and 11 additional nuclear genes were used for estimating pairwise divergence times [S. B. Hedges, P. H. Parker, C. G. Sibley, S. Kumar, *Nature* **381**, 226 (1996); S. Kumar and S. B. Hedges, *ibid.* **392**, 917 (1998)] among the major groups of reptiles. Two genes, calcitonin and insulin, were omitted because of their short (<100 amino acids) sequence lengths. The fossil divergence between mammals and reptiles (including birds) at 310 Ma was used as the calibration. For each divergence time estimate, three rate constancy tests [N. Takezaki, A. Rzhetsky, M. Nei, *Mol. Biol. Evol.* **12**, 823 (1995)] were performed: the two lineages in question and each lineage versus the calibration lineage. Divergence times were used only if rate constancy was not rejected in all three tests. These constant-rate divergence times were then averaged between groups defined in the phylogenetic analysis of combined data (Fig. 1B), and their standard errors were estimated. The upper and lower 5% (or at least the highest and lowest) time estimates were excluded to minimize the effect of outliers caused by gene paralogy or other biases. Additional details are available at www.sciencemag.org/feature/data/986617.shl.
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27. We thank S. Pyott and M. van Tuinen for laboratory assistance; X. Gu and J. Zhang for the program to estimate the gamma parameter; C. Hass, S. Kumar, M. van Tuinen, and R. Thomas for comments and discussion; and C. Daugherty and L. Maxson for materials. This research was supported in part by a grant from the Howard Hughes Medical Institute (Undergraduate Biological Sciences Education Program) to Penn State and by grants from the NSF (DEB-9615643) and NASA (NCC2-1057) to S.B.H.

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# Efficient Bypass of a Thymine-Thymine Dimer by Yeast DNA Polymerase, Polη

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The *RAD30* gene of the yeast *Saccharomyces cerevisiae* is required for the error-free postreplicational repair of DNA that has been damaged by ultraviolet irradiation. Here, *RAD30* is shown to encode a DNA polymerase that can replicate efficiently past a thymine-thymine *cis-syn* cyclobutane dimer, a lesion that normally blocks DNA polymerases. When incubated in vitro with all four nucleotides, Rad30 incorporates two adenines opposite the thymine-thymine dimer. Rad30 is the seventh eukaryotic DNA polymerase to be described and hence is named DNA polymerase η.

Cells possess a variety of mechanisms to repair damaged DNA. If left unrepaired, DNA lesions in the template strand can block the replication machinery (*1*). In *S. cerevisiae*, genes in the *RAD6* epistasis group function in the replication of DNA that has been damaged by ultraviolet (UV) light and other agents (*2*). Of the genes in this group, *REV1*, *REV3*, and *REV7* are required for UV mutagenesis, whereas *RAD5* and *RAD30* function in alternate pathways of error-free bypass of UV-induced DNA damage (*3, 4*). Rev3 and Rev7 associate to form a DNA polymerase, Polζ, that can weakly bypass *cis-syn* thymine-thymine (T-T) dimers (*5*). Rev1 has a deoxycytidyl transferase activity that transfers a deoxycytidine 5′-monophosphate residue to the 3′ end of a DNA primer in a template-dependent reaction (*6*). The addition of Rev1 has no effect on the Polζ bypass of *cis-syn* T-T dimers (*6*). Rad5 is a DNA-dependent adenosine triphosphatase (*7*) and Rad30 is homologous with *Escherichia coli* DinB and UmuC and with *S. cerevisiae* Rev1 (*4*). Here, we elucidate the function of yeast Rad30.

The *RAD30* gene was fused in-frame downstream of the glutathione S-transferase (*GST*) gene, and the resulting fusion protein (~95 kD) was purified to near homogeneity (*8*) from a protease-deficient yeast strain harboring the GST-Rad30 expression plasmid pBJ590. Plasmid pBJ590 fully complements the UV sensitivity of the *rad30Δ* mutation, indicating that the Rad30 fusion protein functions normally in vivo. We first examined whether Rad30 has deoxynucleotidyl transferase activity (*9*). DNA substrates containing a different template nucleotide at the primer-template junction were incubated with Rad30 in the presence of just one deoxynucleoside triphosphate (dNTP). Rad30 predominantly incorporated the correct nucleotide across from each of the four template nucleotides, but incorrect nucleotides were also inserted across from some template residues (Fig. 1A). For instance, a C residue and a T residue were weakly incorporated opposite template C (Fig. 1A, lane 6) and opposite template G (Fig. 1A, lane 11), respectively. Rad30 exhibited the lowest specificity when the template nucleotide was T, as in substrate S-3. Whereas the A residue was incorporated with the highest efficiency (Fig. 1A, lane 16), G, T, and C residues were also incorporated with varying efficiencies (Fig. 1A, lanes 15, 17, and 18, respectively). The pattern of misincorporations seemed to depend on the sequence context of the template. For example,

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in substrate S-4, which has A as the template nucleotide, a T residue was incorporated opposite the A template, and a T residue was inserted across from the subsequent C residue in the template (Fig. 1A, lane 23). However, a T residue was not incorporated across from the C template in substrate S-1 (Fig. 1A, lane 5). Oligo deoxyriboadenylate or oligo deoxyribothymidylate was not extended by Rad30 in the absence of template DNA, and no incorporation was observed when ribonucleoside triphosphates replaced dNTPs.

Because Rad30 added each of the four nucleotides onto the primer in a template-specific manner in the presence of a single dNTP, we next examined whether the enzyme synthesized DNA in the presence of all four nucleotides (*9*). Extensive synthesis was observed for all four DNA substrates examined, and polymerization proceeded to the end of the template (Fig. 1B, lanes 1 through 4). Under the conditions used, the DNA polymerase activity was proportional to enzyme concentration and was linear with time.

We examined the sensitivity of Rad30 activity to known inhibitors of DNA polymerases. Rad30 had 90% activity at 150 nM aphidicolin [50 μg/ml (Fig. 1B, lane 5), which is a concentration that completely inhibits yeast polymerases α, δ, and ε (*10*)]. Yeast DNA polymerases α, δ, ε, and γ are 50% inhibited when preincubated with 1 mM *N*-ethylmaleimide (NEM) for 4.7, 3.6, 2.8, and 0.5 min, respectively (*10*). Rad30 retained 80% activity when preincubated for 10 min in 10 mM NEM (Fig. 1B, lane 9). DNA polymerase β is sensitive to dideoxyribonucleoside triphosphates (ddNTPs), being completely inhibited at a ratio of 5:1 of ddNTP:dNTP (*11*). Rad30 exhibits a modest sensitivity to ddNTPs, showing 30% activity at a ddNTP:dNTP ratio of 10:1 (Fig. 1B, lane 12). The insensitivity of Rad30 to inhibitors of yeast DNA polymerases α, δ, ε, and γ indicated that Rad30 was distinct from these other enzymes. To exclude the possibility that Polζ and Polβ contaminated our Rad30 preparation, we showed that GST-Rad30 purified from a *rev3*Δ yeast strain, deleted for the Polζ catalytic subunit, and purified from a *pol*βΔ yeast strain had the same activity as GST-Rad30 from the wild-type strain.

To confirm that the DNA polymerase activity was intrinsic to Rad30, we showed that the activity copurified with the GST-Rad30 protein (Fig. 2A) and that a mutant Rad30, which was missing the carboxyl terminal 404 amino acids (*12*), had no activity with any of the four DNA substrates (Fig. 2B). The corresponding *rad30* mutant gene did not complement the UV sensitivity of the *rad30*Δ strain. The SIDEVF domain (*13*) present in Rad30 at residues 153 to 158 is highly conserved among Rad30, DinB, UmuC, and Rev1. A *rad30* mutation in which $Asp^{155}$ and $Glu^{156}$ have been changed to Ala did not complement the UV sensitivity of the *rad30*Δ strain, and the mutant protein lacked DNA polymerase activity (*14*). From these observations, we conclude that Rad30 is a DNA polymerase.

To test the possibility that Rad30 performs error-free translesion synthesis, we examined whether the enzyme incorporated a nucleotide opposite a *cis-syn* T-T dimer (*9*) by carrying out assays in the presence of only one of the four dNTPs. Similar to previous results with template T (Fig. 1A, lanes 13 through 18), Rad30 incorporated nucleotides across from the nondamaged TT template (Fig. 3A, lanes 3 through 5). Again, an A residue was preferentially incorporated, but G and T residues were also incorporated. Two A residues were also incorporated opposite the dimer, and this was followed by the addition of two more A residues (Fig. 3A, lane 10). As on the nondamaged template, G and T residues were also incorporated across from the dimer (Fig. 3A, lanes 9 and 11).

We next examined the ability of Rad30 to replicate the damaged DNA template in the

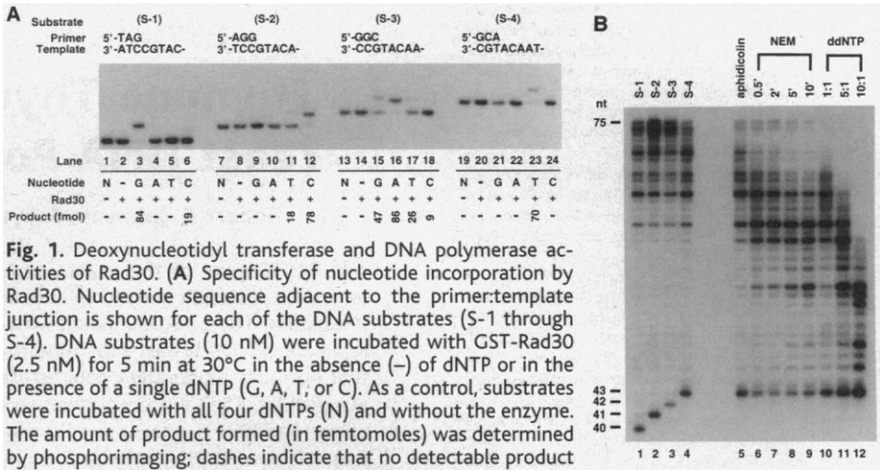


**Fig. 1.** Deoxynucleotidyl transferase and DNA polymerase activities of Rad30. (**A**) Specificity of nucleotide incorporation by Rad30. Nucleotide sequence adjacent to the primer:template junction is shown for each of the DNA substrates (S-1 through S-4). DNA substrates (10 nM) were incubated with GST-Rad30 (2.5 nM) for 5 min at 30°C in the absence (–) of dNTP or in the presence of a single dNTP (G, A, T, or C). As a control, substrates were incubated with all four dNTPs (N) and without the enzyme. The amount of product formed (in femtomoles) was determined by phosphorimaging; dashes indicate that no detectable product was formed. (**B**) DNA polymerase activity of GST-Rad30. GST-Rad30 (2.5 nM) was incubated for 5 min at 30°C with DNA substrates (10 nM) S-1, S-2, S-3, and S-4 (lanes 1 through 4, respectively) in the presence of all four dNTPs. Lanes 5 through 12 show the effect of inhibitors on Rad30 DNA polymerase activity. GST-Rad30 (2.5 nM) was incubated with DNA substrate S-4 (10 nM) for 5 min at 30°C in the presence of all four dNTPs, and inhibitors were added as indicated. In lane 5, aphidicolin (50 μg/ml) was added in the regular assay, and deoxycytidine 5′-triphosphate concentration was lowered to 10 μM (*10*). Lanes 6 through 9 show 0.5-, 2-, 5-, and 10-min preincubation, respectively, with 10 mM NEM (*10*). Lanes 10 through 12 show 1:1, 5:1, and 10:1 ddNTP:dNTP, respectively. The reactions contained all four ddNTPs. The positions of the 40-, 41-, 42-, and 43-nt primers and the 75-nt full-length product are indicated.

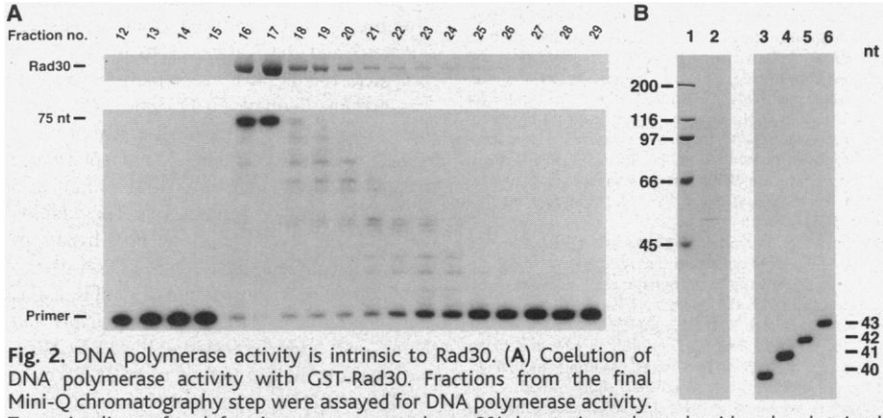


**Fig. 2.** DNA polymerase activity is intrinsic to Rad30. (**A**) Coelution of DNA polymerase activity with GST-Rad30. Fractions from the final Mini-Q chromatography step were assayed for DNA polymerase activity. Two microliters of each fraction were separated on a 9% denaturing polyacrylamide gel and stained with Coomassie blue (top). Fractions were diluted 50-fold and assayed for DNA polymerase activity with substrate S-3 (bottom). (**B**) Carboxyl-terminal truncation of Rad30 abolishes DNA polymerase activity. Approximately 200 ng of carboxyl-terminally truncated GST-Rad30 (lane 2) was subjected to electrophoresis on a 9% denaturing polyacrylamide gel. Lane 1 contains size markers (in kilodaltons). Lanes 3 through 6 show the activity of 10 nM of carboxyl-terminus deleted GST-Rad30 with 10 nM of substrates S-1, S-2, S-3, and S-4, respectively. Reactions were carried out in the presence of all four dNTPs for 5 min at 30°C.

presence of all four dNTPs. For this, we performed both "standing start" and "running start" experiments. In the standing start substrate, the primer is annealed to the template DNA so that the 3′ hydroxyl of the primer is located just before the T-T dimer (*9*). In the running start substrate, the 3′ hydroxyl of the primer is located 15 nucleotides upstream of the T-T dimer (*9*). In contrast to yeast DNA polymerase δ, which stopped just before the *cis-syn* T-T dimer and did not carry out any translesion synthesis (Fig. 3B, lane 2), Rad30 efficiently inserted nucleotides across from the T-T dimer and carried out efficient synthesis past the dimer (Fig. 3B, lanes 3 through 6). In the running start assay, 82% of the primers were extended by Rad30 past the TT residues in the nondamaged template (Fig. 3B, lane 3), and remarkably, 71% were extended past the dimer on the damaged template (Fig. 3B, lane 4). In the standing start assay, 87% of the primers were extended past the TT residues in the nondamaged template (Fig. 3B, lane 5), and 85% were extended past the T-T dimer on the damaged template (Fig. 3B, lane 6). Sequence analysis of the nascent DNA (*15*) revealed that two A residues were inserted opposite the undamaged TT site and also opposite the T-T dimer. There were no misincorporations elsewhere in the DNhhA.

Rad30 is homologous to *E. coli* UmuC and *S. cerevisiae* Rev1 (*4*). The *umuC* and *rev1* mutants are largely nonmutable by UV irradiation and by a variety of chemical agents. Recent reconstitution studies with *E. coli* proteins (*16*) suggest that UmuD′ and UmuC can promote misincorporation opposite an abasic site in the DNA template and can promote bypass of this lesion by DNA polymerase III. In yeast, Rev1 inserts a C residue opposite an abasic site, but does not insert a C residue or any other nucleotide opposite the T-T dimer, and in vitro, it has no effect on T-T dimer bypass by Polζ (*6*). The carboxyl terminus of Rad30 (residues 290 through 632) bears no homology to UmuC and Rev1 and may contribute to the unique DNA polymerase activity of Rad30. Rad30 differs from the other known prokaryotic and eukaryotic DNA polymerases in both primary structure and biochemical properties; thus, it represents a new class of DNA polymerases.

Xeroderma pigmentosum variant (XP-V) patients exhibit an increased incidence of sunlight-induced skin cancers. Unlike classical XP cells, XP-V cells have normal nucleotide excision repair but are defective in the replication of UV-damaged DNA (*1*). XP-V cell-free extracts are deficient in bypass replication of a *cis-syn* T-T dimer (*17*). XP-V cells are hypermutable with UV light, and they exhibit an unusual mutational spectrum (*18*). From these studies, it has been inferred that XP-V cells are less likely than normal cells to incorporate deoxyadenosine 5′-monophosphate opposite photoproducts involving thymine (*18*). Our observations raise the possibility that XP-V cells harbor a defect in the human counterpart of yeast Rad30.

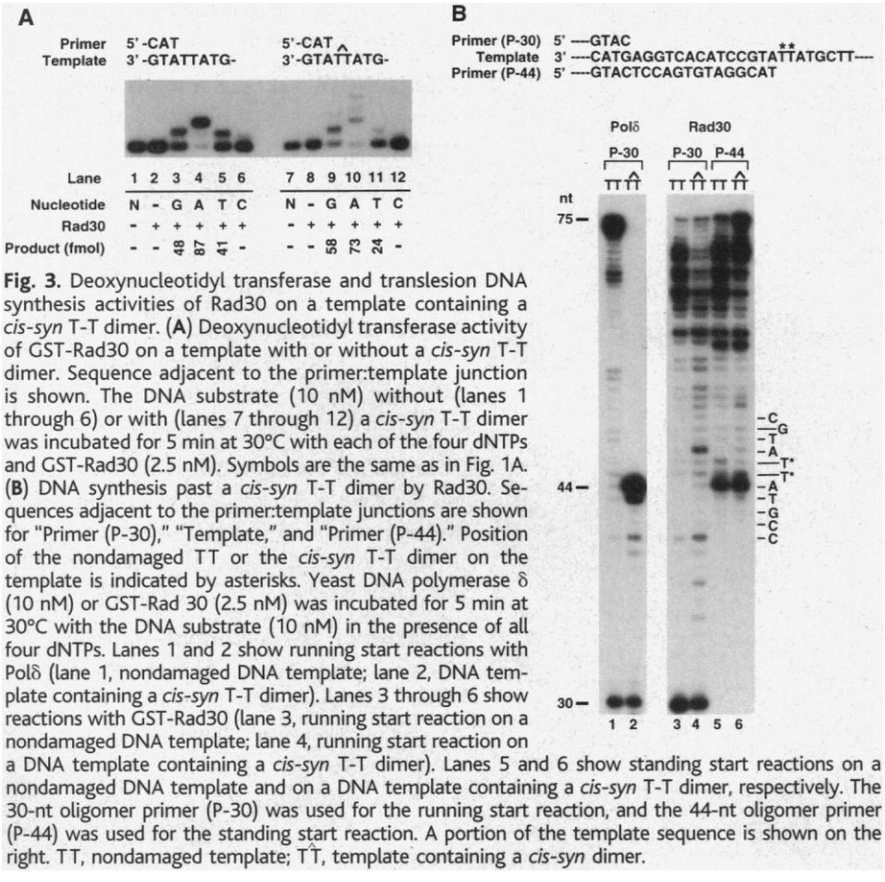


**Fig. 3.** Deoxynucleotidyl transferase and translesion DNA synthesis activities of Rad30 on a template containing a *cis-syn* T-T dimer. (**A**) Deoxynucleotidyl transferase activity of GST-Rad30 on a template with or without a *cis-syn* T-T dimer. Sequence adjacent to the primer:template junction is shown. The DNA substrate (10 nM) without (lanes 1 through 6) or with (lanes 7 through 12) a *cis-syn* T-T dimer was incubated for 5 min at 30°C with each of the four dNTPs and GST-Rad30 (2.5 nM). Symbols are the same as in Fig. 1A. (**B**) DNA synthesis past a *cis-syn* T-T dimer by Rad30. Sequences adjacent to the primer:template junctions are shown for "Primer (P-30)," "Template," and "Primer (P-44)." Position of the nondamaged TT or the *cis-syn* T-T dimer on the template is indicated by asterisks. Yeast DNA polymerase δ (10 nM) or GST-Rad 30 (2.5 nM) was incubated for 5 min at 30°C with the DNA substrate (10 nM) in the presence of all four dNTPs. Lanes 1 and 2 show running start reactions with Polδ (lane 1, nondamaged DNA template; lane 2, DNA template containing a *cis-syn* T-T dimer). Lanes 3 through 6 show reactions with GST-Rad30 (lane 3, running start reaction on a nondamaged DNA template; lane 4, running start reaction on a DNA template containing a *cis-syn* T-T dimer). Lanes 5 and 6 show standing start reactions on a nondamaged DNA template and on a DNA template containing a *cis-syn* T-T dimer, respectively. The 30-nt oligomer primer (P-30) was used for the running start reaction, and the 44-nt oligomer primer (P-44) was used for the standing start reaction. A portion of the template sequence is shown on the right. TT, nondamaged template; T^T, template containing a *cis-syn* dimer.

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8. Yeast strain BJ5464 harboring plasmid pBJ590 (*GAL-PGK:GST-RAD30*) was grown in medium containing 1% yeast extract, 2% peptone, 3% glycerol, and 2.5% lactate and lacking dextrose for 16 hours before the addition of 2% galactose. To purify GST-Rad30, we resuspended cells in two volumes of cell breakage buffer [50 mM tris-HCl (pH 7.5), 10% sucrose, 300 mM NaCl, 0.5 mM EDTA, 10 mM β-mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride, and 0.5 mM benzamidine] and lysed them in a French press before centrifugation at 100,000*g*. The extract was passed over a 100-μl glutathione Sepharose 4B column (Pharmacia) at 4°C. The column was washed with 10 volumes of K buffer [10 mM $KPO_4$ (pH 7.4), 10% glycerol, and 10 mM β-mercaptoethanol] containing 1 M NaCl followed by a wash with 10 volumes of K buffer containing 50 mM KCl. GST-Rad30 was batch eluted twice with one column volume of 100 mM tris-HCl (pH 7.5) containing 100 mM NaCl, 0.01% NP-40, and 20 mM glutathione. The eluate was then pooled and loaded directly onto a Mini-Q column (Pharmacia), washed with 10 column volumes of K buffer containing 50 mM KCl, and eluted with 10 column volumes of 50 to 500 mM KCl gradient. The GST-Rad30 fractions were equilibrated in K buffer containing 50 mM KCl by microconcentration in a Microcon-30 microconcentrator (Amicon, Beverly, MA). Aliquots were frozen at −70°C.
9. Deoxynucleotidyl transferase and DNA polymerase reactions (10 μl) contained 25 mM $KPO_4$ (pH 7.0), 5 mM $MgCl_2$, 5 mM dithiothreitol, bovine serum albumin (100 μg/ml), 10% glycerol, 100 μM dNTP (as indicated), 10 nM of 5′ $^{32}$P-labeled oligonucleotide primer annealed to an oligonucleotide template, and 2.5 nM GST-Rad30. After incubation for 5 min at 30°C, reactions were terminated by the addition of 50 mM EDTA, 1% SDS, and proteinase K (200 ng/ml) and were placed at 55°C for 30 min. DNA was precipitated by the addition of 10 μg of herring sperm DNA, 300 mM sodium acetate (NaOAc), and three volumes of 95% ethyl alcohol. Samples were dried under vacuum before being resuspended in 15-μl tris-borate-EDTA buffer containing 90% formamide, 0.3% bromphenol blue, and 0.3% cyanol blue. The reaction products were resolved on 10% polyacrylamide gels containing 8 M urea and were dried before autoradiography at −70°C with intensifying screens. DNA substrates S-1, S-2, S-3, and S-4 were generated by annealing the 75-nucleotide (nt) oligomer template 5′-AGCTACCATGCCTGCCTCAAGAGTTCG-TAACATGCCTACACTGGAGTACCGGAGCATCGTCGTG-ACTGGGAAAAC-3′ to the 40-, 41-, 42-, and 43-nt 5′ $^{32}$P-labeled oligomer primers N4264: 5′-GTTTTCCCA-GTCACGACGATGCTCCGGTACTCCAGTGTAG-3′, N4265: 5′-GTTTTCCCAGTCACGACGATGCTCCG-GTACTCCAGTGTAGG-3′, N4266: 5′-GTTTTCC-CAGTCACGACGATGCTCCGGTACTCCAGTGTAGGC-3′, and N4267: 5′-GTTTTCCCAGTCACGACGATGCTC-CGGTACTCCAGTGTAGGCA-3′, respectively. For assays

on a DNA substrate containing a *cis-syn* T-T dimer, the 5′ $^{32}$P-labeled 44-nt oligomer primer N4309: 5′-GTTTTCCCAGTCACGACGATGCTCCGGTACTCCA-GTGTAGGCAT-3′ or the 30-nt oligomer primer that contains the first 30 nt of N4309 was annealed to the 75-nt oligomer template 5′-AGCTACCATGCCTGCACG-AAGAGTTCGTATTATGCCTACACTGGAGTACCGGAG-CATCGTCGTGACTGGGAAAAC-3′, which contained or did not contain a *cis-syn* T-T dimer at the underlined position. A Molecular Dynamics STORM phosphoimager and the ImageQuant software were used for quantitation. Percent activity was determined from the number of nucleotides incorporated.


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12. To generate the carboxyl-terminal truncation of Rad30, we introduced a +1 frameshift mutation into the *RAD30* gene at the unique Bgl II site at position +681 in relation to +1 A of the ATG start codon. This mutation interrupts the *RAD30* reading frame at amino acid position 228 and adds 23 amino acids from an alternate reading frame, yielding a 55-kD GST-Rad30 carboxyl-terminus deleted protein.
13. Single-letter abbreviations for amino acids are as follows: D, Asp; E, Glu; F, Phe; I, Ile; S, Ser; and V, Val.
14. R. E. Johnson, S. Prakash, L. Prakash, unpublished results.
15. For sequencing, reactions were carried out as described (*9*) except that oligonucleotide primers were not labeled, the DNA substrate concentration was increased to 100 nM, and Rad30 concentration was increased to 40 nM. Reactions were carried out for 30 min and then stopped by the addition of 50 mM EDTA. DNA products were extracted with phenol: chloroform and precipitated with ethanol and 300 mM NaOAc. The DNA was resuspended in 10 mM tris-HCl (pH 7.5) and sequenced by the dideoxynucleotide method with sequenase (U.S. Biochemical Corporation, Cleveland, OH) and the radiolabeled oligonucleotide N4494: 5′-AGCTACCATGCCTGC-3′ that would anneal to the DNA strand synthesized by Rad30. This analysis allows for the detection of mutations with ~95% accuracy.
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19. We thank J. Stephen-Taylor for the *cis-syn* T-T dimer and P. Burgers for yeast Polδ and accessory proteins. Supported by NIH grant GM19261.

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