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The *Saccharomyces cerevisiae RAD30* Gene, a Homologue of *Escherichia coli dinB* and *umuC*, Is DNA Damage Inducible and Functions in a Novel Error-Free Postreplication Repair Mechanism

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ABSTRACT

Damage-inducible mutagenesis in prokaryotes is largely dependent upon the activity of the UmuD'C-like proteins. Since many DNA repair processes are structurally and/or functionally conserved between prokaryotes and eukaryotes, we investigated the role of *RAD30*, a previously uncharacterized *Saccharomyces cerevisiae* DNA repair gene related to the *Escherichia coli dinB*, *umuC* and *S. cerevisiae REV1* genes, in UV resistance and UV-induced mutagenesis. Similar to its prokaryotic homologues, *RAD30* was found to be damage inducible. Like many *S. cerevisiae* genes involved in error-prone DNA repair, epistasis analysis clearly places *RAD30* in the *RAD6* group and *rad30* mutants display moderate UV sensitivity reminiscent of *rev* mutants. However, unlike *rev* mutants, no defect in UV-induced reversion was seen in *rad30* strains. While *rad6* and *rad18* are both epistatic to *rad30*, no epistasis was observed with *rev1*, *rev3*, *rev7* or *rad5*, all of which are members of the *RAD6* epistasis group. These findings suggest that *RAD30* participates in a novel error-free repair pathway dependent on *RAD6* and *RAD18*, but independent of *REV1*, *REV3*, *REV7* and *RAD5*.

VARIOUS mechanisms exist for the recognition and repair of damaged DNA in order to maintain the integrity of the cell's genetic information. Unrepaired DNA lesions can, however, block the progression of DNA polymerase at a replication fork and as a consequence lead to single-stranded gaps in DNA (reviewed in FRIEDBERG *et al.* 1991, 1995). While cell division is transiently inhibited by DNA damage, eventually the cell needs to replicate the entire genome to complete division. At least two mechanisms have been described that allow the cell to make duplex DNA from a damaged template. The first is translesion DNA synthesis. In *Escherichia coli*, for example, translesion DNA synthesis is mediated by the UmuD'C proteins in conjunction with RecA. As part of the SOS response, the *umuDC* operon is derepressed (BAGG *et al.* 1981) and the UmuD protein is posttranslationally processed to its mutagenically active form, UmuD' (BURCKHARDT *et al.* 1988; NOHMI *et al.* 1988; SHINAGAWA *et al.* 1988). A dimer of UmuD' and a monomer of UmuC interact to form a $UmuD'_2C$ complex (WOODGATE *et al.* 1989; BRUCK *et al.* 1996) that appears to be targeted to sites of DNA damage by associating with a RecA nucleoprotein filament (BAILONE *et al.* 1991; FRANK *et al.* 1993). It is believed that the $UmuD'_2C$ complex together with RecA enable DNA polymerase III to replicate past an otherwise replication blocking lesion in DNA (RAJAGOPALAN *et al.* 1992; WOODGATE and LEVINE 1996). While this translesion DNA synthesis is generally quite accurate, misincorporation of bases opposite the site of damage can occur (LAWRENCE *et al.* 1996; SZEKERES *et al.* 1996). As a result, this phenomenon is often referred to as damage-induced mutagenesis or SOS mutagenesis.

While error-prone translesion synthesis provides one mechanism to tolerate a lesion, alternative mechanisms can facilitate replication past a lesion (ECHOLS and GOODMAN 1991; FRIEDBERG *et al.* 1995; KOFFEL-SCHWARTZ *et al.* 1996). These mechanisms are generally considered damage avoidance, error-free pathways. One model of such a mechanism, termed daughter-strand gap repair, postulates that replication reinitiates downstream of a site of DNA damage, leaving a gap in the daughter strand. RecA-mediated homologous pairing with the sister DNA molecule and subsequent strand exchange past the site of the lesion eventually fills in the gap (RUPP and HOWARD-FLANDERS 1968; RUPP *et al.* 1971). Another model, termed strand switching, postulates that a DNA polymerase molecule, blocked at a site of damage on one DNA strand, would associate with the replicated duplex DNA from the complementary strand. The polymerase bypasses the lesion by using the newly synthesized daughter strand as a template. Once the inhibitory lesion has been passed, replication switches back to the original template (HIGGINS *et al.* 1976). In *E. coli*, this process is presumably mediated by RecA and perhaps other protein factors (ECHOLS and GOODMAN 1991).

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In eukaryotes, it is likely that mechanisms analogous to translesion DNA synthesis, daughter-strand gap repair and strand switching exist to ensure that DNA synthesis can eventually proceed past sites of unrepaired DNA damage. In the yeast *Saccharomyces cerevisiae*, genes assigned to the *RAD6* epistasis group are thought to mediate postreplication repair of single-stranded regions of DNA (reviewed in FRIEDBERG 1988; FRIEDBERG *et al.* 1991, 1995). Evidence exists for both error-free and error-prone mechanisms of postreplication repair. With regard to the latter, both *RAD6* and *RAD18* are necessary for postreplication repair and damage-induced mutagenesis in yeast (reviewed in FRIEDBERG 1988; FRIEDBERG *et al.* 1991, 1995), although there is a marked disparity in the UV sensitivity of *rad6* and *rad18* mutants. Further, mutations in the *REV1*, *REV3* and *REV7* genes do not exhibit a gross defect in postreplication repair but do exhibit a marked reduction in damage-induced mutagenesis (reviewed in FRIEDBERG *et al.* 1991, 1995 and see below). These observations have been interpreted as indicating that postreplication in yeast consists of both error-free and error-prone mechanisms.

The *rev* mutants of *S. cerevisiae* were identified on the basis of their reduced reversion frequency and are characterized by a modest sensitivity to UV light (LEMONTT 1971; LAWRENCE *et al.* 1985). The Rev1p, Rev3p and Rev7p proteins are required for damage-induced mutagenesis and presumably function in a mechanism comparable to translesion DNA synthesis in prokaryotes (reviewed in FRIEDBERG *et al.* 1991, 1995). Rev3p shows high homology to DNA polymerases (MORRISON *et al.* 1989) and it has been recently demonstrated that the Rev3p and Rev7p proteins associate to form a complex capable of replication past a thymine-thymine dimer in DNA (NELSON *et al.* 1996). Furthermore, a region of the Rev1p protein shares homology with the *E. coli* UmuC protein, hinting at the possibility that the functions of UmuC in translesion DNA synthesis may also be conserved in Rev1p (LARIMER *et al.* 1989).

Recently, the damage-inducible *dinB* gene from *E. coli* has been cloned and sequenced, revealing that DinB, like yeast Rev1p, shares a region of significant homology with UmuC (OHMORI *et al.* 1995; H. OHMORI personal communication). While the role of *dinB* in DNA repair and mutagenesis has yet to be fully elucidated, DinB protein has previously been shown to play a role in untargeted mutagenesis of bacteriophage lambda (BROTCORNE-LANNOYE and MAENHAUT-MICHEL 1986; MAENHAUT-MICHEL and CAILLET-FAUQUET 1990). DinB appears to be ubiquitous since homologues have been identified in archaea (KULAEVA *et al.* 1996) and many prokaryotic genomes, as well as in eukaryotes such as *Caenorhabditis elegans* (WILSON *et al.* 1994). Based upon these observations, we have previously suggested that the prokaryotic-specific UmuC-like proteins, the eukaryotic-specific Rev1p-like proteins and the DinB-like proteins together comprise a superfamily of proteins involved in mutagenic DNA repair (KULAEVA *et al.* 1996). Given their ubiquitous nature, it seems likely that DinB-like proteins play an important role in the cellular response to DNA damage. Indeed, amino acid homology searches of the complete *S. cerevisiae* genome revealed a gene (SCD9461.8; YDR419W) that is related to the UmuC-, DinB- and Rev1p-like proteins (KULAEVA *et al.* 1996). The homology of this previously uncharacterized *S. cerevisiae* gene, which we have designated *RAD30*, to *dinB*, *umuC* and *REV1*, prompted us to investigate the role of *RAD30* in repair of UV damage and in UV-induced mutagenesis.

MATERIALS AND METHODS

Media: All *S. cerevisiae* strains were maintained on YPD (2% peptone, 2% dextrose, 1% yeast extract). Synthetic complete medium (SC) and single omission media used for selection of diploids and for tetrad analysis were made as described previously (SHERMAN *et al.* 1986) and modified by the addition of 100 μg/ml L-leucine.

Strains: All yeast strains used in this investigation were derived from strains W303-1A or W303-1B (THOMAS and ROTHSTEIN 1989) and are listed in Table 1. Methods for mating, diploid selection, sporulation and tetrad dissection have been described previously (SHERMAN *et al.* 1986). *E. coli* strain DH5α (GIBCO BRL/Life Technologies) was used as host for all plasmids.

Yeast transformations were performed as previously described (ITO *et al.* 1983; GIETZ *et al.* 1992). Transformants were streaked and purified on appropriate omission medium and single colonies were picked for further analysis. Genomic blots were performed to confirm the structure of all transformants described below (SOUTHERN 1975).

Based on sequence information provided by the Stanford Yeast Genomic Database (CHERRY *et al.* 1995; DIETRICH *et al.* 1995), the *RAD30* open reading frame (SCD9461.8; YDR419W) was PCR amplified using primer SDP1 5′ggactag tACGCTACCTAATCCTGCCGATCATAGGATA3′ [the 5′ end of which maps 261 base pairs (bp) upstream of the initiation ATG], and primer SDP2 5′ccactagtGGTATGTAATATTCTG TGAGTCATGTCTAC3′ (which maps 305 bp downstream of the TGA stop codon) (see Figure 2). Both PCR primers were designed with *Spe*I recognition sequences at their ends (lowercase lettering) to facilitate the cloning of the entire 2464-bp *RAD30* PCR product into plasmid pRS404 (Stratagene) to create plasmid pJM80 (Table 2). The PCR reaction was performed using the manufacturer's recommended reaction conditions and 200 ng yeast genomic DNA from strain S288c and 5 units Pfu DNA polymerase (Stratagene) (40 cycles of 94° for 1 min, 48° for 2 min and 72° for 3 min). The cloned PCR product was subsequently sequenced to confirm that it indeed encoded *RAD30* and that no mutations were generated during the PCR process (data not shown). The 2.46-kilobase (kb) *Spe*I fragment from pJM80 was subsequently cloned into the unique *Spe*I site of pRS415 to create plasmid pJM96, which was used for complementation analysis (Table 2). A 1.7-kb *Sma*I to *Cla*I *HIS3* fragment from pUC18-*HIS3* (ROTHSTEIN 1991) was cloned into the unique *Stu*I and *Nar*I sites to generate pJM82 (see Figure 2; Table 2). In this plasmid, the *RAD30* open reading frame is completely replaced by the *S. cerevisiae* *HIS3* gene (see Figure 2). The *rad30::HIS3* fragment from pJM82 was subsequently released by digestion with *Spe*I and was used to create a genomic disruption of *RAD30* in a W303 isogenic strain (T145; Table 1).

TABLE 1

***Saccharomyces cerevisiae* strains**

Strain	Genotype	Origin
W303-1A	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad5-G535R*	R. ROTHSTEIN
W303-1B	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad5-G535R*	R. ROTHSTEIN
W1588-4A	*MATα RAD5*	R. ROTHSTEIN
W1588-4C	*MAT***a** *RAD5*	R. ROTHSTEIN
T145	*MATα rad30::HIS3*	This study
C10	*RAD30 RAD5*	This study
C10	*rad30::HIS3 RAD5*	This study
T153	*MATα rad30::HIS3 RAD5 pJM96-CENARS-LEU2-RAD30*	This study
T159	*MATα rad1::LEU2 RAD5*	This study
T160	*MATα rad1::LEU2 RAD5*	This study
T161	*MATα rad30::HIS3 rad1::LEU2 RAD5*	This study
T162	*MAT***a** *rad30::HIS3 rad1::LEU2 RAD5*	This study
T167	*MAT***a** *RAD5 pJM134-CENARS-URA3-trp1-1*	This study
M30	*MATα rad6::LEU2 RAD5*	This study
M31	*MAT***a** *rad6::LEU2 RAD5*	This study
M32	*MATα rad30::HIS3 rad6::LEU2 RAD5*	This study
M33	*MATα rad30::HIS3 rad6::LEU2 RAD5*	This study
M34	*MATα rad18::LEU2 RAD5*	This study
M35	*MAT***a** *rad18::LEU2 RAD5*	This study
M36	*MAT***a** *rad30::HIS3 rad18::LEU2 RAD5*	This study
M37	*MAT***a** *rad30::HIS3 rad18::LEU2 RAD5*	This study
M56	*MATα rad52-8::TRP1 RAD5*	This study
M57	*MAT***a** *rad52-8::TRP1 RAD5*	This study
M58	*MAT***a** *rad30::HIS3 rad52-8::TRP1 RAD5*	This study
M59	*MATα rad30::HIS3 rad52-8::TRP1 RAD5*	This study
T149	*MATα rev1::HIS3 RAD5*	This study
C15	*rev1::HIS3 RAD5*	This study
C15	*rad30::HIS3 rev1::HIS3 RAD5*	This study
T147	*MATα rev3::HisG-URA3*	This study
C13	*rev3::HisG-URA3 RAD5*	This study
C17	*rad30::HIS3 rev3::HisG-URA3 RAD5*	This study
T148	*MATα rev7::HisG-URA3*	This study
C18	*rev7::HisG-URA3 RAD5*	This study
C18	*rad30::HIS3 rev7::HisG-URA3 RAD5*	This study
T176	*MATα rad5::HIS3*	This study
C22	*rad5::HIS3*	This study
C22	*rad30::HIS3 rad5::HIS3*	This study

All strains are isogenic to W303 and the genotypes are identical to W303 except where noted. Strains designated with a C represent haploid segregants from genetic crosses. In most instances multiple haploid segregants with identical genotypes (except mating type) were used from each cross. C number and relevant genotype, excluding mating type, are listed only once to designate all segregants used.

In a similar manner, the *RAD5* gene was PCR amplified (~4.4 kb) using primers REV21 5′tccccgcggCTGCAGAA GAGCAAGGCTTTGTAA3′ and REV22 5′ACGCGTCGAC TAATTGGTAGTTTCTTGT3′ and ligated into the *Sac*II and *Sal*I sites of pRS404 (Stratagene), thereby creating plasmid pJM111 (Table 2). The entire *RAD5* open reading frame from *Stu*I to *Spe*I was replaced with a 1.7-kb *Sma*I to *Xba*I *HIS3* fragment from pUC18-*HIS3* to generate plasmid pJM112 (Table 2). The *rad5::HIS3* fragment from pJM112 was subsequently released by digestion with *Sac*II and *Sal*I and used to create a genomic disruption of *RAD5* in a W303 isogenic strain (T176; Table 1).

Disruptions of *REV1*, *REV3* and *REV7* were created in W303 isogenic strains using plasmids kindly provided by CHRISTOPHER LAWRENCE (Table 1). Plasmid pFL240 carries a *rev1::HIS3* disruption (LARIMER *et al.* 1989) (Table 2). Plasmid pYPG101 carries a *rev3::HisG-URA3* disruption and plasmid pYPG102 carries a *rev7::HisG-URA3* disruption (P. E. GIBBS and C. W. LAWRENCE, unpublished results) (Table 2).

W303 isogenic strains contain a glycine to arginine missense mutation in *RAD5* (G535[GGG] to R[AGG]) (FAN *et al.* 1996). Strains W1588-4A or W1588-4C (Table 1) are W303 isogenic strains in which the *rad5-G535R* allele has been replaced by the wild-type *RAD5* gene (R. ROTHSTEIN personal communication) and were used to backcross all strains used in this study. Since the *rad5-G535R* missense allele in W303 strains creates a new *Mnl*I site, the allele of *RAD5* in spore colonies can be determined by colony PCR and subsequent digestion of the PCR product with *Mnl*I. PCR was performed on spore colonies using primers RD5F 5′TGATAAACCCATTATGGAAGC3′ and RD5R 5′AGGACAAGATAAAACTAAAGA, which map to either side of the *rad5-G535R* allele and amplify a 239-bp fragment. After amplification, *Mnl*I was added directly to the PCR reaction mixture and incubated at 37° for 1 hour. Reaction

TABLE 2

Plasmids used in this study

Plasmid	Relevant gene	Description/comments
pJM80	*RAD30*	pRS404 derivative containing a PCR-amplified 2.5-kb *RAD30* fragment cloned into the *Spe*I site
pJM82	*rad30::HIS3*	pRS404 derivative containing the *rad30::HIS3* disruption; used to create chromosomal *rad30::HIS3* disruption
pJM96	*RAD30*	pRS415 derivative containing *RAD30* from pJM80; used in complementation experiments
pJM111	*RAD5*	pRS404 derivative containing a PCR-amplified 4.4-kb *RAD5* fragment cloned from *Sac*II to *Sal*I
pJM112	*rad::HIS3*	pRS404 derivative containing the *rad5::HIS3* disruption; used to create chromosomal *rad5::HIS3* disruption
pFL240	*rev1::HIS3*	Larimer *et al.* (1989); used to create a chromosomal *rev1::HIS3* disruption
pYPG101	*rev3::HisG-URA3*	P. E. Gibbs and C. W. Lawrence (unpublished results); used to create a chromosomal *rev3::HisGLURA3* disruption
pYPG102	*rev7::HisG-URA3*	P. E. Gibbs and C. W. Lawrence (unpublished results); used to create a chromosomal *rev7::HisG-URA3* disruption
pJM124	*TRP1* and *URA3*	pRS414 derivative used to clone *trp1-1* via gap repair
pJM134	*trp1-1* and *URA3*	pRS414 derivative containing *trp1-1;* used to sequence the *trp1-1* allele

products were then separated by electrophoresis in a 1% agarose gel.

Cloning of the *trp1-1* allele was accomplished by the plasmid gap repair procedure (Orr-Weaver *et al.* 1983). A 1.1-kb *Sal*I to *Nsi*I *URA3* fragment from pUC18-URA3 was cloned into the *Sal*I and *Pst*I sites of pRS414 (Stratagene) to create pJM124 (Table 2). The majority of the *TRP1* open reading frame was removed from plasmid pJM124 by digestion with *Sna*BI and partial digestion with *Hin*dIII. The 5.2-kb linear gapped plasmid was gel purified and transformed into a wild-type W303 isogenic strain (C10-2A; Table 1). Genomic DNA was isolated from a Ura^+ Trp^- transformant (T167; Table 1) and transformed into *E. coli* to recover the gap-repaired plasmid. This plasmid, designated pJM134 (Table 2), was transformed into C10-2A to confirm the Trp^- phenotype. The *trp1* gene carried on pJM134 was sequenced to identify the *trp1-1* mutation. We found that the *trp1-1* allele is an amber stop codon at codon 83 (E83[GAG]) of the *TRP1* gene, verifying previous reports that *trp1-1* is an amber suppressible allele (R. Rothstein, personal communication).

Northern analysis of *RAD30*: Starter cultures were grown overnight at 30° with shaking. Prewarmed 90 ml cultures (30°) were inoculated with 10 ml of the overnight culture and grown ~3 hours until they reached midlog phase. The cultures were then centrifuged at 2200 RPM for 5 min and resuspended in the same volume (100 ml) of sterile water. A 10-ml aliquot was centrifuged, resuspended with 80 μl of a 50 mM NaAoc, 10 mM EDTA solution, transferred to a 1.5-ml microfuge tube, frozen in dry ice and designated as the untreated control RNA preparation. Of the remaining 90 ml, 45 ml at a time was placed into a sterile 14-cm glass petri dish, gently stirred with a stirbar and irradiated with 80 J/m^2 UV light (peak wavelength of 254 nm). Such treatment resulted in ~66% cell survival. A 10-ml aliquot was taken and handled identically to the untreated control and was designated as the zero time control. The remaining 80 ml was centrifuged, resuspended in 80 ml of prewarmed YPD (30°) and cultured in the dark at 30° with shaking. Aliquots (10 ml) were taken every 20 min, until 60 min, and then subsequent aliquots were taken every 30 min. Each aliquot was treated as previously described. All of the preceding manipulations were carried out in rooms with subdued lighting or yellow light to avoid any unwanted photoreactivation.

RNA was prepared as previously described (Schmitt *et al.* 1990). Briefly, after thawing the NaAoc-EDTA cell suspension on ice, 8 μl of 10% SDS was added and the suspension was vortexed. Eighty microliters of phenol was then added and the suspension was vortexed and incubated at 65° for 4 min. The suspension was then frozen in dry ice, centrifuged in a microfuge for 6 min and the upper aqueous phase was transferred to a fresh microfuge tube. The aqueous phase was then extracted again with 80 μl of phenol/chloroform. RNA was precipitated by addition of 8 μl of 3 M NaAoc pH 5.3 and 2.5 vol of ethanol. The pellet was resuspended in 20 μl of sterile DEPC-treated water and stored at −80° until required.

RNA samples were prepared for electrophoresis by addition of 5× RNA loading buffer (Qiagen) followed by incubation at 65° for 10 min. The RNA sample was then loaded onto a 1% agarose 375 mM formaldehyde/1× MOPS gel and run at 50 mA for 3–4 hours. RNA was transferred to a hybond-N+ nylon membrane (Hybond) using 20× SSC. Next, the membrane was baked at 80° for 2 hours. The membrane was probed using a 1.9-kb *Xma*I to *Pst*I *RAD30* fragment and subsequently using a 1.1-kb *Hin*dIII to *Bam*HI *ACT1* probe. Membranes were exposed to Kodak Bio-Max MR or DupontReflections NEF film for various times. Films were subjected to densitometric analysis using the program IMAGE version 1.59 (available from the National Technical Information Service), on a Apple Macintosh PowerPC 8100/100AV equipped with a Sierra Scientific MS-4030 high resolution video camera and Data Translation Quick Capture DT2255 Frame Grabber Board.

UV survival and *trp1-1* reversion assay: Twenty milliliters SC or omission medium (−leu) liquid cultures were started from 2–3-day-old colonies and grown at 30° with shaking until stationary phase. To determine UV survival, appropriate dilutions of the cultures were plated in duplicate on SC plates and were either untreated (0 UV control) or treated with specified doses of UV. Reversion assays were performed in a similar manner except that cells were plated in duplicate on tryptophan omission plates (−Trp). Two or three independent cultures were used for both UV survival and UV-induced mutagenesis experiments. In addition, two isogenic strains of each genotype, wild-type or mutant, were used. All procedures were carried out in rooms with subdued lighting or yellow light to avoid photoreactivation. Plates were incubated at 30°

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Sc Rad30p    28 HIDMNAFFAQ VEQMRCGLSK EDPVVCVQW- ---NSIIAVS YAARKYGISR    73
Ec DinB       6 HVDMDCFFAA VEMRDNPALR DIPIAIGGSR ERRGVISTAN YPARKFGVRS    55
Ce F22B7.6   87 CIDMDAYFAA VEMRDNPALR TVPMAVGSS- ---AMLSTSN YLARRFGVRA   132
Consensus     1 HIDMDAFFAA VEMRDNPALR ..P.A.G.S- ---..IST.N Y.ARKFGVR.    50

Sc Rad30p       MDTIQEALKK CSNLIPIHTA VFKKGEDFWQ YHDGCGSWVQ DPAKQISVED   123
Ec DinB         AMPTGMALKL CPHL-TLLPG RFDA------ Y--------K EASNHIR---    87
Ce F22B7.6      GMPGFISNKL CPSL-TIVPG NYPK------ Y--------T KVSRQFS---   164
Consensus       .MP...ALKL CP.L-TI.PG .F.K------ Y--------. ..S.QIS---   100

Sc Rad30p       HKVSLEPYRR ESRKALKIFK SACDLVERAS IDEVFLDLGR ICFNM-----   168
Ec DinB         -----EIFSR --------YT SR---IEPLS LDEAYLDVTD SVHCH-----   116
Ce F22B7.6      -----QIFME --------YD SD---VGMMS LDEAFIDLTD YVASNTEKKT   198
Consensus       -----EIF.R --------Y. S.---VE..S LDEAFLDLTD .V...-----   150

Sc Rad30p       ---LMFDNEY ---------- -ELTGDLKLK DAL-----SN IREAFIGGNY   199
Ec DinB         ------GS-- ---------- -ATL----IA QEI------- RQTIFNELQL   136
Ce F22B7.6      FKRHRFGGDC PCWLPRFDEN ENTLEDLKIE ESICPKCEKS RKIYYDHVEF   248
Consensus       ---..FG... ---------- -.TL.DLKI. ..I-----.. R...F.....   200

Sc Rad30p       DIN--SHLPL IP-------- ---------- ---EKI-KSL -KFEGD-VF-   222
Ec DinB         TAS--AGVAP VKF------- ---------- --LAKIASDM NKPNGQFVI-   164
Ce F22B7.6      GTGREEAVRE IRFRVEQLTG LTCSAGIASN FMLAKICSDL NKPNGQYVLE   298
Consensus       ..--..V.. I.F------- ---------- --LAKI.SDL NKPNGQ.V.-   250

Sc Rad30p       -NPEG-RDLI TDWDDVILAL GSQVC----- -KGIRD                 250
Ec DinB         -TPAEVPAFL QTLPLAKIPG VGKVSAAKLE AMGLRT                 199
Ce F22B7.6      NDKNAIMEFL KDLPIRKVGG IGRVCEAQLK AMDIQT                 334
Consensus       -.P.....FL .DLP..K..G .G.VC.A.L. AMGIRT                 286
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FIGURE 1.—Homology of the amino terminal region of *S. cerevisiae* Rad30p to *E. coli* DinB and *C. elegans* Rad30p-like proteins. *S. cerevisiae* Rad30p from amino acid residue 28 to 250 is aligned with *E. coli* DinB from residue 6 to 199 and *C. elegans* Rad30p-like protein (F22B7.6) from residue 87 to 334. Amino acid residues that are identical or are highly conserved in all three proteins are shaded. Alignments were performed using the program GeneWorks, version 2.5 (Intelligenetics Inc.).

in the dark for 3 days (SC plates) or 6 days (−Trp plates), after which time colonies were counted. The percent survival reported represents an arithmetic mean from at least two independent cultures for each UV dose. Trp^+ revertants per 10^8 survivors were calculated by dividing 1×10^8 by the number of cells surviving and multiplying by the mean number of Trp^+ revertants at each UV dose. The Trp^+ revertants per 10^8 survivors reported represent an arithmetic mean from at least two independent cultures for each UV dose.

RESULTS

Identification, cloning and disruption of *RAD30*: The Saccharomyces Genome Database (SGD; CHERRY *et al.* 1995) was screened using programs based on the BLAST algorithm (ALTSCHUL *et al.* 1990, 1994). The TBLASTN program was used to screen the entire nucleotide sequence of the yeast genome translated in six frames with the *E. coli* DinB protein. Via this analysis, we identified an open reading frame, SCD9461.8; YDR419W, located on chromosome IV (DIETRICH *et al.* 1995), that encodes a protein of 632 amino acids and that shares significant homology with the *E. coli* DinB protein (OHMORI *et al.* 1995; KULAEVA *et al.* 1996) and the *C. elegans* protein encoded by the F22B7.6 open reading frame (WILSON *et al.* 1994; KULAEVA *et al.* 1996) (Figure 1). In addition, this protein shares homology with prokaryotic UmuC-like proteins and the eukaryotic Rev1p-like proteins (KULAEVA *et al.* 1996). We and others find that disruption of this open reading frame results in modest UV sensitivity (see below and W. SIEDE, personal communication) and have therefore designated this new gene *RAD30*.

To subclone the entire *RAD30* open reading frame, oligonucleotide primers were designed 261 bp upstream of the ATG initiation codon and 305 bp downstream of the stop codon and used to PCR amplify a 2.5-kb chromosomal fragment (see MATERIALS AND METHODS). This fragment was cloned into pRS404 to create pJM80 (Table 2). A *rad30::HIS3* disruption was subsequently created in plasmid pJM82 and was transformed into W303-1B (FIGURE 2; TABLES 1 and 2; THOMAS and ROTHSTEIN 1989) to create a *rad30::HIS3* genomic disruption (see MATERIALS AND METHODS). Transformants prototrophic for histidine were isolated and their genomic DNA was probed by Southern blot analysis to confirm that the disruption of *RAD30* with *HIS3* had occurred (data not shown). One transformant, designated T145 (Table 1), was used for further analyses.

During the course of these studies, it was determined that the W303 background contains a glycine to arginine missense mutation in the *RAD5* gene at codon 535 (FAN *et al.* 1996), so strains carrying the *rad30::HIS3* disruption were subsequently back crossed into W1588-4A or W1588-4C, W303 isogenic strains in which the *rad5-G535R* allele was replaced by the wild-type *RAD5* gene (R. ROTHSTEIN, personal communication). The

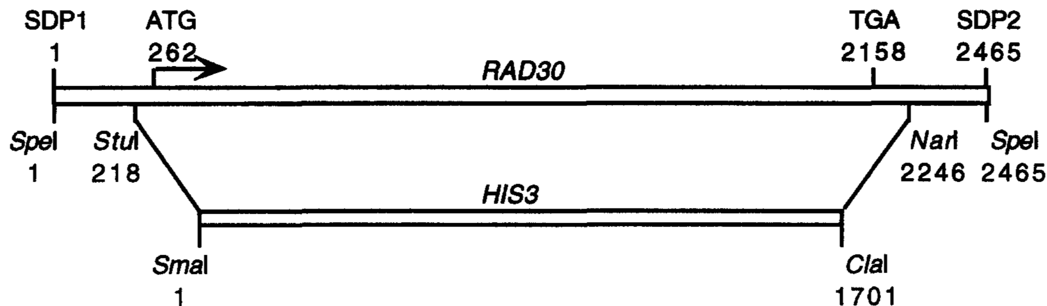

FIGURE 2.—Disruption of the *RAD30* open reading frame. Positions of the two PCR primers (SDP1 and SDP2) used to amplify the *RAD30* fragment are shown. The *SpeI* restriction sites designed at the ends of the PCR primers facilitated the cloning of this fragment to create plasmid pJM80 (Table 2). The ATG initiation codon and TGA stop codon of *RAD30* are shown. The *StuI* and *NarI* restriction sites were used to remove the entire *RAD30* open reading frame. A 1.7-kb *SmaI* to *ClaI HIS3* fragment from pUC18-*HIS3* replaces the *RAD30* gene in plasmid pJM82 (Table 2).

wild-type *RAD5* and *rad5-G535R* alleles are distinguishable by colony PCR and restriction analysis (see MATERIALS AND METHODS). *RAD5 rad30::HIS3* segregants were then used for further analyses (Table 1; see below).

UV sensitivity and UV-induced reversion in *rad30* disruption strains: Since the Rad30p shares homology with DinB-like, UmuC-like and Rev1p-like proteins (KULAEVA *et al.* 1996), all of which are thought to play some role in UV-induced mutagenesis, we examined the UV sensitivity and UV-induced reversion frequency of the *trp1-1* allele in haploid *rad30* strains. Our analysis shows that *rad30* disruption strains are indeed more sensitive to UV light than wild-type strains (Figure 3A). Furthermore, the UV sensitivity of *rad30::HIS3* strains is complemented by plasmid pJM96 containing the *RAD30* open reading frame (Figure 3A; Table 2). Although plasmid pJM96 does not contain the putative DRE element (see below), expression of *RAD30* from this plasmid appears to be sufficient to fully complement the *rad30* deletion. The modest level of UV sensitivity of *rad30* strains is reminiscent of *rev* mutant strains. However, in contrast to *rev* mutant strains, no obvious defect in UV-induced reversion of *trp1-1* was found in *rad30* strains at the various doses of UV light used here (Figure 3B; see below).

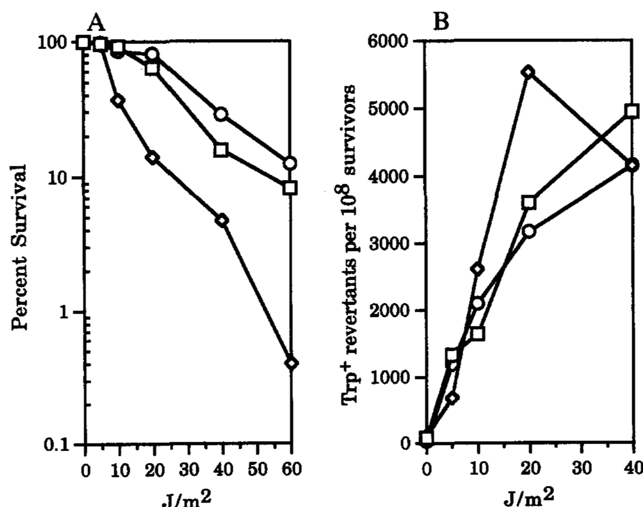

FIGURE 3.—UV sensitivity and UV-induced reversion in *rad30* strains. (A) UV survival curve of wild-type (C10 segregants) (□), *rad30* (C10 segregants) (◇) and *rad30* pJM96 (T153) (○). (B) Mean Trp^+ reversion frequencies of wild-type (C10 segregants) (□), *rad30* (C10 segregants) (◇) and *rad30* pJM96 (T153) (○). UV survival and reversion is reported as a mean value at each UV fluence from at least two independent experiments.

***RAD30* is a member of the *RAD6* epistasis group:** Despite the fact that *rad30* mutants do not show any observable effect on reversion of *trp1-1*, the structural similarity of Rad30p to Rev1p and the modest UV sensitivity of strains carrying the *rad30* disruption suggested that *RAD30* may belong to the *RAD6* DNA repair epistasis group rather than either the *RAD3* or the *RAD52* group. The homology between *RAD30* and *REV1* hinted that *RAD30*, like *REV1*, might play some role in postreplication repair. To test this hypothesis, *rad30 rad6* and *rad30 rad18* double mutant strains were constructed and examined for UV survival. UV survival curves of both the *rad30 rad6* and the *rad30 rad18* double mutant strains are identical to that of the single *rad6* and *rad18* single mutant strains, respectively, indicating that both *RAD6* and *RAD18* are epistatic to *RAD30* (Figure 4A). The epistasis observed for UV sensitivity in *rad30 rad6* and *rad30 rad18* double mutant strains is not due to the disparity in UV sensitivity between *rad30* mutants and either *rad6* or *rad18* mutants, since a *rad1* mutation (which like *rad6* and *rad18* mutations results in extreme UV sensitivity) and *rad30* show no epistatic relationship (Figure 4B). In addition, since *rad1* and *rad30* show no epistasis, *RAD30* is not a member of the *RAD3* epistasis group. Further, *rad30 rad52* double mutant strains are much more sensitive than are either of the single mutant strains, indicating that *RAD30* does not function in the *RAD52* recombination pathway (Figure 4C). We conclude that *RAD30* participates in a DNA repair mechanism that is dependent on both *RAD6* and *RAD18*.

UV induction of *RAD30* transcripts: Analysis of the promoter region of the *RAD30* open reading frame re-

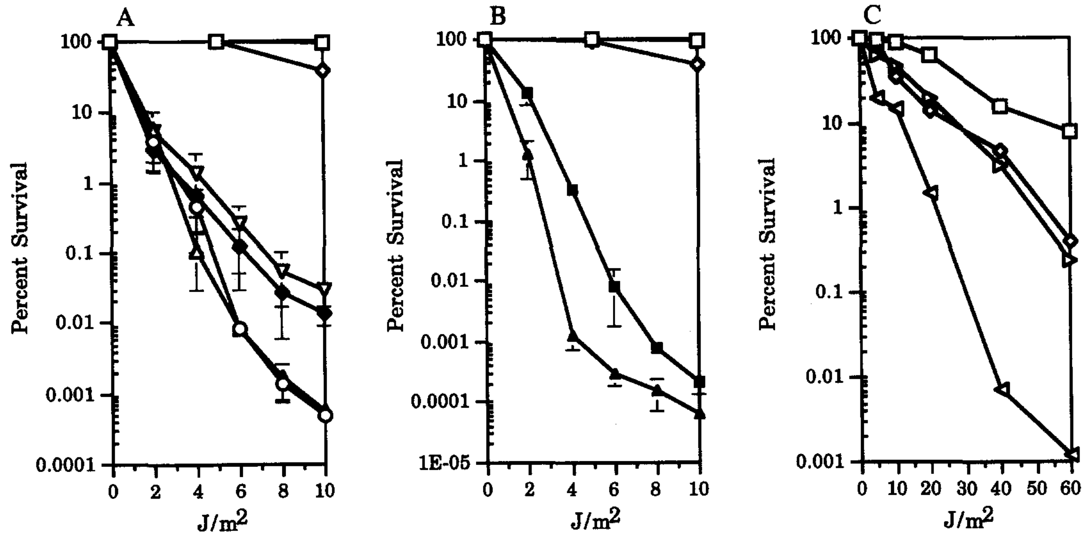

FIGURE 4.—UV sensitivity of *rad30, rad6, rad18, rad1* and *rad52* strains. (A) UV survival curve of wild-type (C10 segregants) (□), *rad30* (C10 segregants) (◇), *rad6* (M30 and M31) (O), *rad30 rad6* (M32 and M33) (△), *rad18* (M34 and M35) (▽) and *rad30 rad18* (M36 and M37) (♦). (B) UV survival curve of wild-type (C10 segregants) (□), *rad30* (C10 segregants) (◇), *rad1* (T159 and T160) (■) and *rad30 rad1* (T161 and T162) (▲). For A and B, UV survival is reported as a mean value at each UV fluence from three independent experiments. Error bars indicate the standard deviation ($\sigma n - 1$) at each UV fluence. (C) UV survival curve of wild-type (C10 segregants) (□), *rad30* (C10 segregants) (◇), *rad52* (M56 and M57) (▷), *rad30 rad52* (M58 and M59) (◁). UV survival is reported as a mean value at each UV fluence from two independent experiments. For each panel, UV survival data for wild-type and *rad30* strains is taken from Figure 3A and is represented for ease of comparison.

vealed two sequences indicative of DNA damage-inducible genes. First, a DRE (damage recognition element) containing the sequence 5′CATGGTTGCC3′ is located at position −312 relative to the putative initiation ATG codon of *RAD30* (Table 3). This sequence is identical to the DRE sequence of *RAD16*, a damage-inducible nucleotide excision repair gene, and highly similar to the DRE sequences of *RAD6* and *RAD18*, both postreplication DNA repair genes (Table 3). In addition, another upstream sequence, 5′GGCCTTCTTTTCTA3′ at position −382, is highly similar to sequences upstream of the *RAD5, RAD6* and *RAD18* postreplication repair genes (Table 3). *RAD5, RAD6* and *RAD18* have all been shown to be damage inducible (MADURA *et al.* 1990; JONES and PRAKASH 1991; FRIEDBERG *et al.* 1995). Based on these findings, it seemed likely that *RAD30* would also be damage inducible. To test this hypothesis, aliquots were taken from a culture of wild-type yeast at periodic intervals after exposure to UV light and RNA was isolated for Northern blot analysis. An *ACT1* (actin) probe was used as a noninducible control to determine relative amounts of RNA from the various time points taken. Quantification of the *RAD30* transcripts revealed that *RAD30* is induced ~3.5-fold after treatment with

TABLE 3

Comparison of possible DRE and other *RAD30* promoter sequences

Gene	Position[a]	Sequence[b]	References
RAD30[c]	−312	CATGGTTGCC	This study
RAD16[c]	−309	CATGGTTGCC	XIAO *et al.* (1993)
RAD6[c]	−181	CGGGGTAGCC	REYNOLDS *et al.* (1985)
RAD18[c]	−416	GTTGGATGAG	JONES *et al.* (1988)
Consensus		CGWGGWNGMM	XIAO *et al.* (1993) JANG *et al.* (1996)
RAD30[c]	−383	GGCCTTCTTTTCTA	This study
RAD5[c]	−260	TGAATTCTATTCTA	JOHNSON *et al.* (1992)
RAD6[c]	−228	TGACTACATTTCCC	JONES *et al.* (1991)
RAD18[c]	−335	TAACTTCTTTTCCC	JONES *et al.* (1991)
Consensus		TGACTTCTTTTCYM	

[a] Relative to the first nucleotide of the ATG start codon of each gene.
[b] The top half of the table shows DRE sequences while the lower half shows upstream promoter sequences of postreplication repair genes. Nucleotides that differ from the consensus are in boldface.
[c] DNA damage inducible.

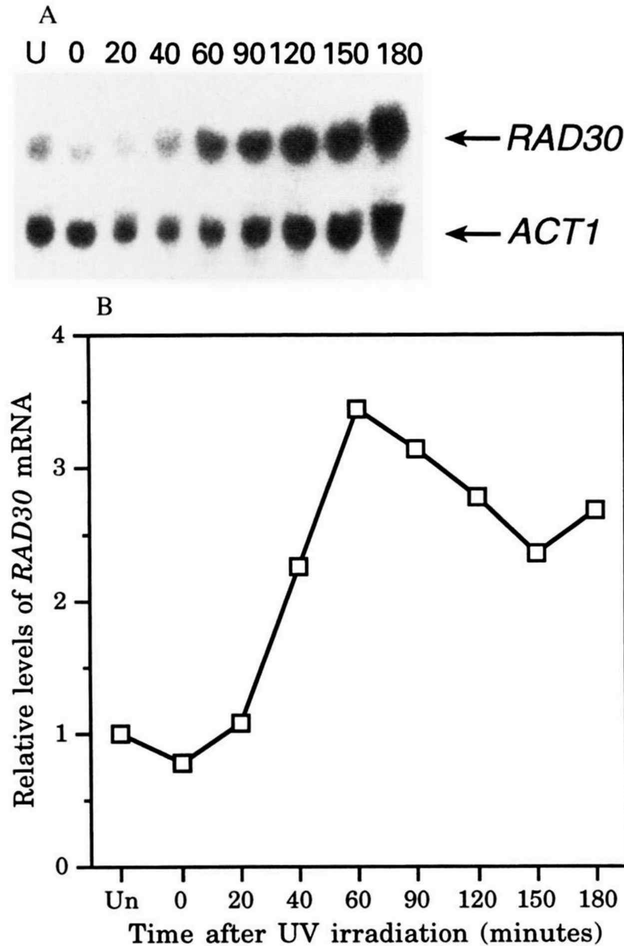

FIGURE 5.—UV induction of *RAD30* transcription. Wild-type yeast strain C10-2A was exposed to UV radiation (80 J/m^2) and total RNA was isolated at specified intervals after the treatment. Northern blots were first probed with random primed radiolabeled (Lofstrand) *RAD30* and subsequently probed with random primed radiolabeled *ACT1* (see MATERIALS AND METHODS). (A) A composite of different exposure times of the Northern filter after hybridization with *RAD30* and subsequently with *ACT1* is shown. Lane 1, RNA isolated from untreated cells. Lanes 2–9, RNA isolated from cells at 0, 20, 40, 60, 90, 120, 150 and 180 min, respectively, after UV exposure. (B) Multiple autoradiograms of different exposure times were subjected to densitometric analysis using the IMAGE program, version 1.59 (see MATERIALS AND METHODS), and relative levels of *RAD30* and *ACT1* mRNA were determined. The ratio of the level of *RAD30* mRNA to that of the *ACT1* mRNA was calculated for each timepoint and the values were plotted relative to the value for untreated cells (UN).

UV light (Figure 5, A and B) and, in this respect, is similar to the *E. coli umuC* and *dinB* genes, both of which are damage inducible.

Analysis of *rad30 rev* and *rad30 rad5* double mutant strains: The favorable comparison of *RAD30* to the *E. coli umuC, dinB* and *REV1* genes suggested to us that *RAD30* might be involved in a form of mutagenic DNA repair, yet, as noted above (see Figure 3B), *rad30* mutants did not exhibit any mutagenic repair phenotype. As a consequence, we considered the alternative possibility that *RAD30* functions in such a manner as to prevent error-prone DNA repair rather than promote it. To test this hypothesis, we examined single *rev* and *rad30 rev* double mutant strains for an effect on UV sensitivity and UV-induced reversion. As previously reported, *rev1, rev3* and *rev7* (LEMONTT 1971; LAWRENCE 1985) and *rad30* strains are all slightly sensitive to UV light (Figures 3A and 6, A–C). However, *rad30 rev1, rad30 rev3* and *rad30 rev7* double mutant strains are all more sensitive to UV light than are any of the single mutant strains. Unlike *rad30* strains, *rev1, rev3* and *rev7* strains are all deficient for UV-induced reversion of *trp1-1* (data not shown). In addition, the corresponding *rad30 rev* double mutants are also deficient for UV-induced mutagenesis (data not shown). Taken together these results indicate that *RAD30* functions in a DNA repair pathway that appears to be independent of the *REV1, REV3* and *REV7* genes.

RAD5 mutants exhibit increased spontaneous mutagenesis and locus-specific effects on UV-induced mutation and it has therefore been suggested that *RAD5* most likely functions in an error-free postreplication repair mechanism (reviewed in FRIEDBERG *et al.* 1991, 1995). The Rad5p protein shares homology with a group of helicase-like proteins including Rad16p, Rad54p, Snf2p, Mot1p, *Drosophila melanogaster* BRM and human ERCC6 (reviewed in FRIEDBERG *et al.* 1995). Strains mutant for *rad5* show a greater sensitivity to UV radiation than *rev1* or *rev3* mutants and no epistatic relationship with *rev1* or *rev3* (JOHNSON *et al.* 1992). In addition, *rad5* mutants show enhanced stability of simple repetitive sequences, suggesting that Rad5p functions in a DNA replication complex affecting template slippage (JOHNSON *et al.* 1992).

We examined the epistatic relationships of *rad30* and *rad5* for both UV sensitivity and UV-induced reversion. Figure 7A shows that *rad30 rad5* strains are more sensitive to UV light than are either of the single mutant strains, indicating that *RAD30* also operates in a repair pathway independent from *RAD5*. In contrast to the nonmutable phenotype of *rev1, rev3* and *rev7* mutants, *rad5* mutants were able to promote significant levels of UV-induced mutagenesis at the *trp1-1* locus (Figure 7B). Interestingly, UV-induced reversion in *rad30 rad5* strains is dramatically enhanced over *rad5* strains (Figure 7B). For example, at 2 J/m^2, *rad5* strains give rise to 644 revertants per 10^8 survivors while *rad30 rad5* strains give rise to 6108 revertants per 10^8 survivors. This 10-fold increase in mutagenesis in the *rad30 rad5* double mutant strain compared to the *rad5* strain suggests that under normal conditions, *RAD30* may participate in an error-free mechanism of DNA repair rather than an error-prone mechanism.

DISCUSSION

Many types of DNA lesions constitute a block to DNA replication and, if left unrepaired, can result in single-

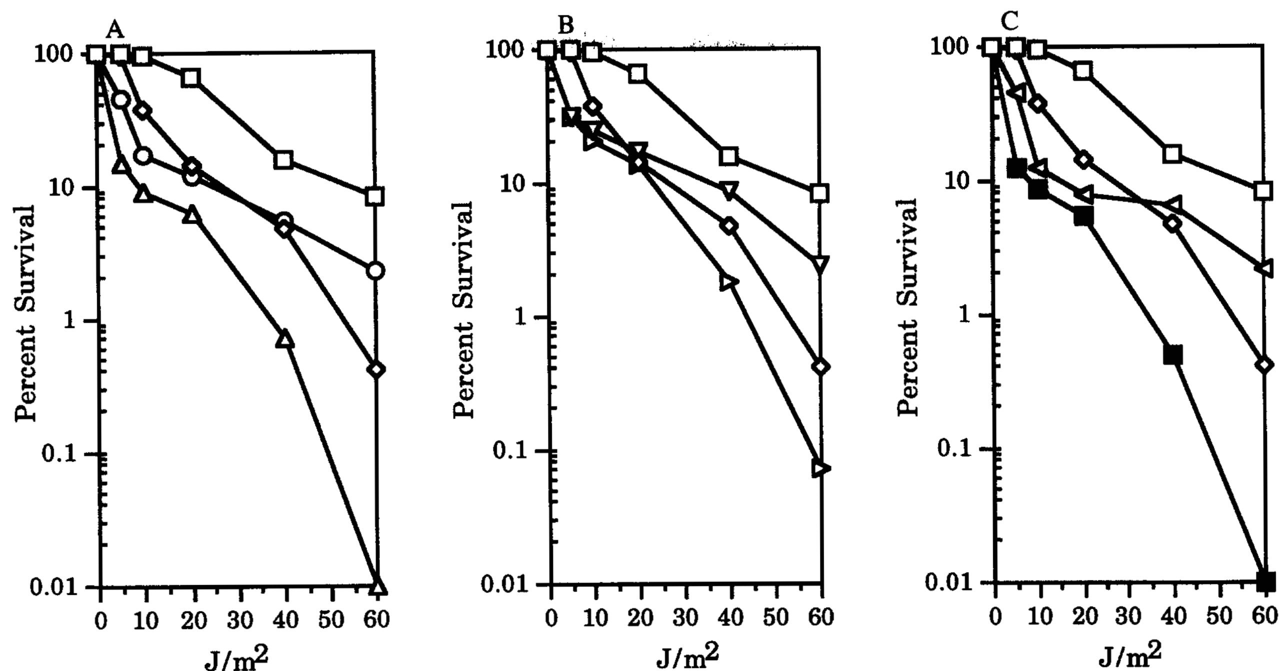

FIGURE 6.—UV sensitivity of *rad30*, *rev1*, *rev3* and *rev7* strains. (A) UV survival curve of wild-type (C10 segregants) (□), *rad30* (C10 segregants) (◇), *rev1* (C15 segregants) (○) and *rad30 rev1* (C15 segregants) (△). (B) UV survival curve of wild-type (C10 segregants) (□), *rad30* (C10 segregants) (◇), *rev3* (C13 segregants) (▽) and *rad30 rev3* (C17 segregants) (▷). (C) UV survival curve of wild-type (C10 segregants) (□), *rad30* (C10 segregants) (◇), *rev7* (C18 segregants) (◁) and *rad30 rev7* (C18 segregants) (■). UV survival is reported as a mean value at each UV fluence from two independent experiments. UV survival data for wild-type and *rad30* strains is taken from Figure 3A and is represented for ease of comparison.

stranded gaps in newly replicated DNA. Inducible repair mechanisms such as translesion DNA synthesis and strand-switching facilitate the replicative filling-in of these gaps. Translesion DNA synthesis has been extensively studied in *E. coli* and is known to require the RecA, UmuD′ and UmuC proteins. Yet the actual mechanism of translesion synthesis remains to be elucidated. It is, however, becoming clear that, when available, damage avoidance error-free pathways are predominantly used rather than tranlesion DNA synthesis (KOFFEL-SCHWARTZ *et al.* 1996).

In *S. cerevisiae*, mutations included in the *RAD6* epistasis group, such as *rad6*, *rad18*, *rev1*, *rev3* and *rev7*, are defective for error-prone repair or damage-induced mutagenesis (reviewed in FRIEDBERG *et al.* 1991, 1995), while other genes, such as *rad5*, are thought to be involved in an error-free repair mechanism. Unlike *E. coli umuD* and *umuC*, which function during replication, many of the genes in the the *RAD6* group are thought to be involved in postreplication repair of single-stranded gaps. However, only two mutations in this group, *rad6* and *rad18*, are known to be completely defective for postreplication repair (DI CAPRIO and COX 1981; PRAKASH 1981). Recently, it has been shown that PCNA (*POL30*) plays a role in postreplication repair in yeast (TORRES-RAMOS *et al.* 1996). This study shows that both *rad6* and *rad18* are epistatic to the *pol30-46* mutation for UV sensitivity. Furthermore, *pol30-46 rev3* double mutant strains are nearly as sensitive to UV as are *rad6Δ* strains, indicating that PCNA may be required for all error-free (nonmutagenic) postreplication repair in yeast.

We have identified a gene in *S. cerevisiae*, designated *RAD30*, that shares homology with *E. coli umuC* and *dinB* and with *S. cerevisiae REV1*. As discussed above, *umuC* (along with its partner UmuD′) is necessary for damage-induced mutagenesis. Similarly, *REV1* in yeast is necessary for damage-induced mutagenesis. *E. coli dinB*, in contrast, is thought to be specifically involved in untargeted mutagenesis of lambda phage and plays no role in damage induced mutagenesis (BROTCARNE-LANNOYE and MAENHAUT-MICHEL 1986; MAENHAUT-MICHEL and CAILLET-FAUQUET 1990). Sequence comparisons between the UmuC-like, DinB-like and Rev1p-like proteins suggest that they comprise a superfamily of DNA repair proteins (OHMORI *et al.* 1995; KULAEVA *et al.* 1996). Based on its similarity to other genes involved in error-prone DNA repair, we decided to explore the effects of a *rad30* mutation on UV sensitivity and UV-induced mutagenesis in yeast.

Examination of UV survival of *rad30* disrupted strains revealed that, like *rev* mutants, *rad30* strains show only a mild sensitivity to UV radiation. However, unlike *rev* mutants, *rad30* strains are not defective for either spontaneous (Figure 3B; for the 0 UV dose, wild-type strains gave an average of 82 revertants/10^8 survivors, while *rad30* strains gave 110 revertants/10^8 survivors) nor UV-induced mutagenesis. It is not yet known what effects

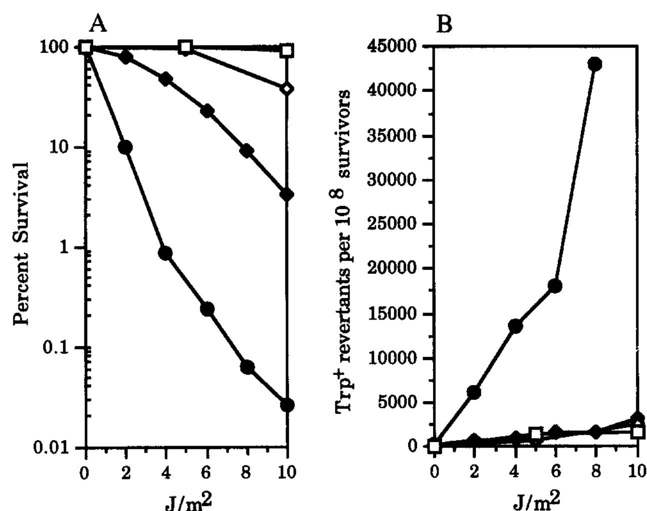

FIGURE 7.—UV sensitivity and UV-induced reversion in *rad30* and *rad5* strains. (A) UV survival curve of wild-type (C10 segregants) (□), *rad30* (C10 segregants) (◇), *rad5* (C22 segregants) (●) and *rad30 rad5* (C22 segregants) (●). (B) Mean Trp$^+$ reversion frequencies of wild-type (C10 segregants) (□), *rad30* (C10 segregants) (◇), *rad5* (C22 segregants) (●) and *rad30 rad5* (C22 segregants) (●). UV survival and reversion is reported as a mean value at each UV fluence from two independent experiments. UV survival and reversion data for wild-type and *rad30* strains is taken from Figure 3A and B and is represented for ease of comparison.

mutations in *RAD30* will have on untargeted mutagenesis in yeast. However, since spontaneous mutagenesis in *rad30* strains occurs at wild-type levels, it would appear that, unlike the *E. coli* DinB protein, Rad30p does not play a major role in untargeted mutagenesis in yeast. More detailed experimentation should, however, clarify this point.

Epistasis analysis of *rad30 rad6* and *rad30 rad18* strains indicated that both *rad6* and *rad18* are epistatic to *rad30*. Therefore, it would appear that *RAD30* functions via a *RAD6*- and *RAD18*-dependent postreplication repair mechanism. In contrast, *rad30 rev1*, *rad30 rev3* and *rad30 rev7* double mutant strains are all more sensitive to UV radiation than the single mutant strains, demonstrating that *RAD30* functions via a *REV*-independent pathway that is most likely error free. However, loss of the presumptive error-free *RAD30*-dependent mechanism does not result in a significant increase in damage-induced mutagenesis. This can be explained if an alternate error-free pathway exists that can compensate for the loss of the *RAD30*-dependent mechanism. We found that *rad30 rev* double mutant strains are less sensitive to UV radiation than are *rad6* strains, suggesting that there are indeed several error-free repair pathways (see *RAD5* discussion below). However, it remains possible that the *rad30Δ* mutation does not completely block the pathway or another gene product may partially substitute for Rad30p in the same pathway.

Since, similar to *RAD30*, *RAD5* also functions in a pathway distinct from the *REV* genes, we investigated UV sensitivity and UV-induced mutagenesis in *rad30 rad5* double mutants. We found that *rad30 rad5* double mutant strains exhibit a synergystic increase in UV sensitivity and show enhanced UV-induced mutagenesis when compared to either of the single mutant strains. The simplest interpretation of these results is that *RAD30* functions in a mechanism distinct from *RAD5* but that there is some overlap of these two error-free postreplication repair pathways. Alternatively, it is possible that *RAD5* and *RAD30* function in the same error-free pathway, assuming that neither the *rad5Δ* nor the *rad30Δ* mutations completely block this pathway. When both *RAD5* and *RAD30* are deleted the pathway would be completely blocked and UV sensitivity and UV-induced mutagenesis would be enhanced. This is a reasonable assumption for *rad5Δ*, since the Rad5p protein shares homology with two other repair proteins, Rad16p and Rad54p, and synergystic interactions between these three genes have been observed (GLASSNER and MORTIMER 1994). However, it was found that *rad5Δ rev3Δ* double mutant strains are nearly as sensitive to UV light as are *rad6Δ*, suggesting that *RAD5* may mediate most error-free postreplication repair (JOHNSON *et al.* 1992). Further, this result also suggests that a *rad5Δ* mutation represents a complete block in the pathway. If this is indeed the case, then our finding that *rad30 rad5* double mutant strains exhibit synergystically enhanced UV sensitivity over the single mutant strains would indicate two different pathways. In either case, our findings suggest that *RAD30* plays a role in a *RAD6/RAD18*-dependent *REV*-independent error-free postreplication repair mechanism that appears to be independent of *RAD5*. As discussed above, *POL30* may be necessary for most if not all error-free postreplication repair in yeast. Further experimentation with *rad5 pol30-46* and *rad30 pol30-46* double mutant strains should, therefore, help to elucidate the pathways of error-free postreplication repair in yeast.

It is intriguing to speculate on the mechanism of action of Rad30p. One attractive hypothesis is that Rad30p functions in a template-switching mechanism (HIGGINS *et al.* 1976) to facilitate avoidance of DNA damage. In this type of mechanism, the fidelity of replication remains high, reducing the likelihood of mutations and leading to error-free damage avoidance. Another possible mechanism that Rad30p could mediate is daughter-strand-gap repair (RUPP and HOWARD-FLANDERS 1968; RUPP *et al.* 1971). In this mechanism, a single-strand gap, left in the daughter strand opposite a UV dimer, is filled in by a recombinational exchange. During recombination the dimer can be transferred to the newly replicated DNA. In *E. coli*, ~50% of UV dimers are transferred (GANESAN 1974). In yeast, transfer of the dimer to the newly synthesized DNA is, however, rare (RESNICK *et al.* 1981), suggesting that daughter-strand-gap repair is not a major mechanism in yeast.

Sequence analysis of the promoter region of *RAD30*

revealed a damage recognition element (DRE) identical to a DRE in the promoter region of the damage-inducible *RAD16* gene involved in nucleotide excision repair. In addition, another sequence in the *RAD30* promoter is very similar to promoter sequences from the postreplication repair genes *RAD5*, *RAD6* and *RAD18*. Northern analysis of UV-treated yeast demonstrated that transcription of *RAD30* is induced ~3.5-fold 60 min after UV treatment. Such observations imply a role for *RAD30* in the cellular response to DNA damage. Further, since *RAD30* is epistatic to both *RAD6* and *RAD18*, it should be assigned to the *RAD6* epistasis group. Of the 24 genes currently assigned to the *RAD6* group, only five other genes have been shown to be damage inducible; these include *RAD5*, *RAD6*, *RAD18*, *CDC8* (thymidylate kinase) and *CDC9* (DNA ligase) (both *CDC8* and *CDC9* are also assigned to the nucleotide excision repair *RAD3* group) (reviewed in FRIEDBERG *et al.* 1995). Interestingly, none of the *REV* genes involved in damage-induced mutagenesis has been shown to be damage inducible. Our findings that *RAD30* and *RAD5* may function in different error-free repair pathways suggests that there are multiple mechanisms for postreplication repair in yeast and that two presumably independent error-free pathways are damage inducible.

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