JOURNAL OF BACTERIOLOGY, Jan. 1995, p. 364–371 Vol. 177, No. 2
0021-9193/95/$04.00+0

Characterization of a Mutant Strain of *Saccharomyces cerevisiae* with a Deletion of the *RAD27* Gene, a Structural Homolog of the *RAD2* Nucleotide Excision Repair Gene

MICHAEL S. REAGAN, CHRISTOPHER PITTENGER, WOLFRAM SIEDE, AND ERROL C. FRIEDBERG*

Laboratory of Molecular Pathology, Department of Pathology, University of Texas Southwestern Medical Center, Dallas, Texas 75235

Received 2 September 1994/Accepted 26 October 1994

We have constructed a strain of *Saccharomyces cerevisiae* with a deletion of the YKL510 open reading frame, which was initially identified in chromosome XI as a homolog of the *RAD2* nucleotide excision repair gene (A. Jacquier, P. Legrain, and B. Dujon, Yeast 8:121–132, 1992). The mutant strain exhibits increased sensitivity to UV light and to the alkylating agent methylmethane sulfonate but not to ionizing radiation. We have renamed the YKL510 open reading frame the *RAD27* gene, in keeping with the accepted nomenclature for radiation-sensitive yeast mutants. Epistasis analysis indicates that the gene is in the *RAD6* group of genes, which are involved in DNA damage tolerance. The mutant strain also exhibits increased plasmid loss, increased spontaneous mutagenesis, and a temperature-sensitive lethality whose phenotype suggests a defect in DNA replication. Levels of the *RAD27* gene transcript are cell cycle regulated in a manner similar to those for several other genes whose products are known to be involved in DNA replication. We discuss the possible role of Rad27 protein in DNA repair and replication.

Mutations in a number of genes from the yeast *Saccharomyces cerevisiae* render this organism sensitive to UV and/or ionizing radiation (6, 7, 27). Strains bearing such mutations have been designated *rad* (for radiation sensitivity) mutants (8). The corresponding wild-type alleles participate in several distinct DNA repair and DNA damage tolerance pathways (7, 27). It is likely that some of these genes encode proteins that also participate in DNA replication and/or recombination, since the processes of repair, replication, and recombination may share the need for common enzymes such as DNA polymerases, ligases, and nucleases. Hence, defects in such genes would be expected to affect the efficiency and fidelity of more than one of these aspects of DNA metabolism.

Among the many genes that are indispensable for nucleotide excision repair of DNA is one designated *RAD2* (30, 39). Biochemical characterization of purified Rad2 protein has demonstrated that it is an endonuclease which is believed to participate in damage-specific incision of DNA (10). The Rad2 protein of *S. cerevisiae* comprises 1,031 amino acids, and homologs have been identified in humans, *Xenopus* organisms, and *Schizosaccharomyces pombe* (4, 32). All of these proteins share two highly conserved regions (Fig. 1). One of these is located at the N terminus and is conserved over the first 105 amino acids. A second conserved stretch of ~140 amino acids is located more internally toward the C terminus of the linear polypeptide (4, 32).

During a recent systematic effort to sequence the entire chromosome XI of *S. cerevisiae*, an open reading frame (ORF) was identified and was given the designation YKL510 (16). Examination of the translated amino acid sequence revealed a potential polypeptide of 383 amino acids with remarkable homology to that of the translated *RAD2* gene (16). In the predicted Rad2 protein the conserved N-terminal and internal motifs are separated by over 600 amino acids, whereas in the YKL510 ORF they are almost immediately adjacent (Fig. 1). Recently the human (25), murine (12), and *S. pombe* (25) homologs of the YKL510 gene were cloned. All are highly conserved throughout their lengths and share the N-terminal and internal regions of homology with the *S. cerevisiae RAD2* gene.

The amino acid sequence homology between the protein predicted from the YKL510 ORF and the Rad2 endonuclease of *S. cerevisiae* (as well as the human, *Xenopus*, and *S. pombe* homologs) is intriguing. *S. pombe* strains carrying mutations in the YKL510 homolog exhibit increased UV sensitivity as well as chromosome instability (25). In the present study we have cloned the YKL510 locus and constructed a mutant strain with a deletion of the entire ORF. This strain manifests temperature-sensitive growth defects, including cell cycle arrest when grown at 37°C. Additionally, the mutant displays profound chromosomal instability and a marked sensitivity to the alkylating agent methylmethane sulfonate (MMS). The mutant also displays a modest sensitivity to UV radiation. Consonant with the accepted nomenclature for radiation-sensitive yeast mutants (8), we have renamed this gene *RAD27*. We show here that transcription of the *RAD27* gene is also cell cycle regulated.

Recent biochemical experiments have shown that the YKL510 (*RAD27*) gene product and its murine homolog are endowed with DNA endonuclease and 5′→3′ exonuclease activities (11, 12). Additionally, in vitro replication studies indicate a role for the probable mammalian homolog of Rad27 protein in lagging-strand DNA synthesis during DNA replication (9, 15, 37). Our phenotypic characterization of the mutant strain from *S. cerevisiae* is consistent with a role for the *RAD27* gene product in DNA replication and suggests that the *RAD27* gene also plays some role(s) in the repair of various forms of DNA damage.

MATERIALS AND METHODS

Yeast strains. The yeast strains used are listed in Table 1.

Cloning the *RAD27* ORF. In order to amplify the yeast *RAD27* gene from

* Corresponding author. Phone: (214) 648-4020. Fax: (214) 648-4067.

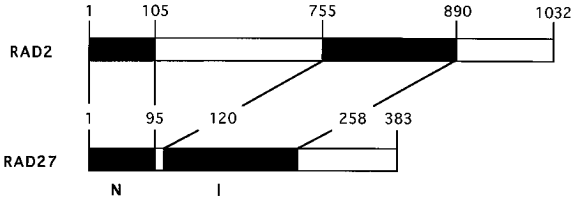

FIG. 1. Comparison of the Rad2 and Rad27 proteins. The amino-terminal (N) and internal (I) regions of homology are filled in. The numbers refer to amino acid positions in the protein.

strain SX46A genomic DNA, we used the following primers for PCR. In primer 179 (5′-CCCCTGCAGTGCAAATATGGTGATTTG-3′), the underlined bases are complementary to positions −682 to −666 relative to the *RAD27* translational start site and the 5′ extension contains a *Pst*I site for cloning. In primer 180 (5′-CCCCCCGGGTTTCAAAGTAGAACCTGGATGT-3′), the underlined bases are complementary to positions +1807 to +1785 relative to the *RAD27* translational start site and the 5′ extension contains an *Ava*I site for cloning. We employed 25 cycles of PCR at 94°C for 1 min, 50°C for 2 min, and 72°C for 3 min, followed by a 7-min 72°C soaking period. The PCR product was digested with *Ava*I and *Pst*II and cloned into pUC19 to generate plasmid pMR92393.

Deletion of the *RAD27* ORF. We generated two independent deletions of the *RAD27* ORF by transplacement with DNA fragments carrying either the *URA3* or *TRP1* gene. Plasmid pMR92393 was digested with *Sty*I and *Mlu*I, the appropriate fragment was isolated, and the ends were filled in. A blunt-ended *Hin*dIII fragment from plasmid YEp24 containing the *URA3* gene was ligated into the plasmid, generating plasmid pMRrad27Δ::URA3. To delete the *RAD27* gene, the plasmid was digested with *Eco*RI and *Sph*I and used to transform strain SX46A to uracil prototrophy. The resulting colonies were monitored for deletion by Southern analysis. A blunt-ended *Eco*RI-*Bgl*II fragment containing the *TRP1* gene was isolated from plasmid YRp7 and ligated into *Sty*I-*Mlu*I-digested plasmid pMR92393, generating plasmid pMRrad27Δ::TRP1. To delete the *RAD27* ORF, the plasmid was digested with *Kpn*I and *Sph*I and used to transform strain SX46A to tryptophan prototrophy. The resulting colonies were again checked for proper deletion of the *RAD27* ORF by Southern analysis.

Construction of a plasmid carrying the *RAD27* ORF. Plasmid pMR92393 was digested with *Ava*I and *Sty*I, the ends were filled in, and the plasmid was recircularized. The resulting product was digested with *Pst*I and *Sac*I, and the *RAD27* fragment was purified and ligated into *Pst*I-*Sac*I-digested pRS314 (35).

Microscopy. For studies at permissive temperatures, cells were grown in YPD at 30°C to an optical density at 600 nm of 0.5 to 1, fixed in ethanol, and suspended in 0.2 mg of DAPI (4′,6-diamidino-2-phenylindole) per ml for 5 min. Cells were then washed and resuspended in water. SX46Arad27Δ::URA3 cells were grown in YPD at 30°C to an optical density at 600 nm of 0.1, switched to 37°C, and incubated overnight before DAPI staining.

Sensitivity to DNA-damaging agents. All strains except those containing plasmid pRS314 or pRS314RAD27 were grown in YPD and plated on YPD agar for survival measurements. Cells containing these plasmids were grown in yeast minimal medium supplemented with Ade, His, and Ura and were plated on the same medium. For quantitation of UV radiation or gamma ray sensitivity, cells were grown to stationary phase at 30°C and various dilutions were plated. The plates were exposed to the indicated amount of UV radiation (254-nm-radiation germicidal lamp) or gamma rays (^{137}Cs source) and incubated in the dark for 3 to 4 days at 30°C before colonies were counted. Survival curves represent average values from at least two independent experiments.

For quantitation of MMS sensitivity, cells were grown to stationary phase at 30°C, harvested by centrifugation, washed with water, and resuspended in 0.1 M potassium phosphate buffer (pH 7) at 2×10^7 cells per ml. The amounts of MMS indicated in Fig. 3C were added, and cells were incubated at 30°C with shaking for 30 min. The cells were then collected by centrifugation and washed four times with water, and various dilutions were plated. Plates were incubated at 30°C for 3 to 4 days before colonies were counted. Survival curves represent average values from at least two independent experiments.

Flow cytometry. Synchronization of early-log-phase cells with α-factor was performed as described previously (33) except that synchronization was at 23°C for 3.5 h. At various times after release from arrest, aliquots (10^7 cells) were washed with water and fixed in 80% ethanol. Fixed cells were kept overnight at 4°C, washed with water, resuspended in 200 ml of 50 mM sodium citrate, and analyzed on a Becton-Dickinson FACScan flow cytometer, with use of the CELLFIT program (version 2.0) for analysis of DNA content.

Measurement of spontaneous and induced mutagenesis. The rate of spontaneous mutagenesis was measured by reversion to Ade$^+$. At least 20 parallel 2-ml YPD cultures were inoculated with ~40 cells and grown for 3 or 4 days to stationary phase. Cells were then plated on yeast minimal agar supplemented with Trp, His, and Ura, and the plates were incubated at 30°C. The data were evaluated according to the method of the median (22, 24). The frequency of UV radiation-induced mutations was also measured by reversion to Ade$^+$. A total of 10^7 cells were plated on yeast minimal agar supplemented with Trp, His, and Ura, and the plates were exposed to UV radiation (254-nm-radiation germicidal lamp) at various doses. The plates were incubated in the dark at 30°C for 4 days, and Ade$^+$ colonies were counted.

Chromosome loss assay. The centromeric plasmid YRp14/CEN4/ARS1/SUP11 (14) was introduced into wild-type and mutant cells, and Ura$^+$ transformants were selected. Four independent transformants from each strain were restreaked for single colonies. These were picked from the selective plates into water, and an appropriate dilution was plated directly onto YPD plates. The plates were incubated at 30°C for 6 to 8 days to allow red color to develop.

Northern (RNA) analysis. Cells were synchronized with α-factor as described above. RNA was isolated by the hot phenol technique (2). The RNA was fractionated on a 1% formaldehyde gel and blotted onto GeneScreen Plus by standard methods (1). The filters were probed with *RAD27* and *URA3* riboprobes and a histone H2A PCR product labeled by the random primer method. Hybridization and washing conditions were as suggested by the manufacturer. Quantitation of mRNA was performed with a Molecular Dynamics Phosphoimager with ImageQuant software.

TABLE 1. Yeast strains used

Strain	Genotype
SX46A	**a** *RAD ade2 his3-532 trp1-289 ura3-52*
SX46Arad27Δ::URA3	**a** *rad27*Δ::*URA3 ade2 his3-532 trp1-289 ura3-52*
SX46Arad27Δ::TRP1	**a** *rad27*Δ::*TRP1 ade2 his3-352 trp1-289 ura3-52*
SX46Arad2Δ	**a** *rad2*Δ::*TRP1 ade2 his3-352 trp1-289 ura3-52*
MR2510	**a** *rad2*Δ::*TRP1 rad27*Δ::*URA3 ade2 his3-352 trp1-289 ura3-52*
YH20	**a** *rad10*Δ::*HIS3 ade2 his3-532 trp1-289 ura3-52*
MR10510	**a** *rad10*Δ::*HIS3 rad27*Δ::*URA3 ade2 his3-352 trp1-289 ura3-52*
WS9102-1C	*rad18-2 ade2 ura3-52 arg4-17*
MR18510	*rad18-2 rad27*Δ::*URA3 ade2 ura3-52 arg4-17*

RESULTS

Sensitivity of the *rad27* mutant to DNA-damaging agents. To establish whether the YKL510 ORF initially established by DNA sequencing is functional, we constructed a mutant strain with a deletion of the entire ORF by gene transplacement (31) and investigated this strain for specific phenotypes. Several independent haploid isolates were examined by Southern analysis and shown to carry the *URA3* transplacement diagrammatically represented in Fig. 2. In view of the observation that the translated sequence of this ORF is homologous with that of the *S. cerevisiae RAD2* gene, which is involved in nucleotide excision repair (16), we initially examined the sensitivity of the mutant strain to several types of DNA-damaging agents. As shown in Fig. 3A, the mutant strain was moderately sensitive to UV radiation compared with an otherwise isogenic parent. However, the extent of this sensitivity was considerably less than that of an isogenic strain with a deletion of the *RAD2* gene (Fig. 3A). Historically, *S. cerevisiae* mutants that are abnormally sensitive to UV radiation, ionizing radiation, or radiomimetic chemicals have been called *rad* mutants (8). We have therefore designated this mutant strain the *rad27*Δ::*URA3* mutant. The sensitivity of the *rad27*Δ::*URA3* mutant to UV radiation was fully complemented by transformation of the mutant with a plasmid carrying the cloned *RAD27* ORF but not by transformation with the plasmid vector alone (Fig. 3A). Since the plasmid-borne gene carries just the *RAD27* ORF with no flanking genes, this result indicates that the UV radiation-sensitive phenotype is not due to interference with the integrity of adjacent genes during the construction of the mutant by transplacement. The *rad27*Δ::*URA3* mutant strain was

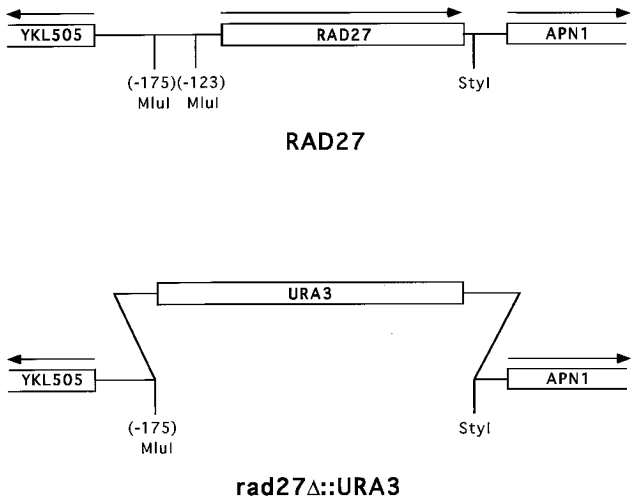

FIG. 2. Schematic representation of the *RAD27* locus in the wild-type and deletion strains. The boxes represent the ORFs, with the arrows above indicating the direction of transcription. The *Mlu*I and *Sty*I restriction enzyme cutting sites used to delete the *RAD27* gene are shown.

also moderately sensitive to the UV-mimetic chemical 4-nitroquinoline-1-oxide (data not shown).

The *rad27*Δ::*URA3* mutant is not abnormally sensitive to killing by ionizing radiation (Fig. 3B). However, exposure to the alkylating agent MMS resulted in extensive killing of the mutant (Fig. 3C). At a concentration of MMS that yielded 100% survival of the isogenic parent strain, survival of the mutant strain was reduced by 2 to 3 orders of magnitude. This phenotype was also fully corrected by a plasmid containing the cloned *RAD27* gene but not by the plasmid vector alone (Fig. 3C).

S. cerevisiae rad mutants have been organized into three largely nonoverlapping epistasis groups referred to as the *rad3*, *rad52*, and *rad6* groups for one of the members of each (6, 27). The wild-type members of the *rad3* epistasis group are nucleotide excision repair genes. The *rad6* group of genes is involved with DNA damage tolerance mechanisms, and the *rad52* group of genes functions in the repair of double-strand breaks via a recombinational pathway(s). We performed an analysis of epistasis by examining the sensitivity of the *rad27*Δ::*URA3* mutant in combination with mutants in the *rad3* and *rad6* epistasis groups. As shown in Fig. 4A, a strain with a deletion of both the *RAD27* and *RAD2* genes or of both the *RAD27* and *RAD10* genes (*RAD10* is also indispensable for nucleotide excision repair [30, 39]) was more sensitive to UV radiation than the respective *rad2* or *rad10* single mutant. This additive UV radiation sensitivity suggests that *RAD27* does not participate in the *RAD2*- and *RAD10*-dependent nucleotide excision repair pathway. In contrast, a combination of the *rad27*Δ::*URA3* mutation and a *rad18* mutation (a member of the *rad6* epistasis group) did not increase the sensitivity of the *rad18* mutant to UV radiation (Fig. 4B). These results place the *rad27* mutant in the *rad6* epistasis group and suggest that the *RAD27* gene product is required for a cellular response(s) to DNA damage associated with some form of DNA damage tolerance. We were unable to generate viable *rad27 rad52* haploid strains by gene disruption. This combination of mutations may be lethal, or, alternatively, this finding may reflect a reduced recombination proficiency in strains carrying mutations in these genes.

Growth characteristics and morphology of the mutant strain. At 37°C the *rad27*Δ::*URA3* mutant strain manifested growth

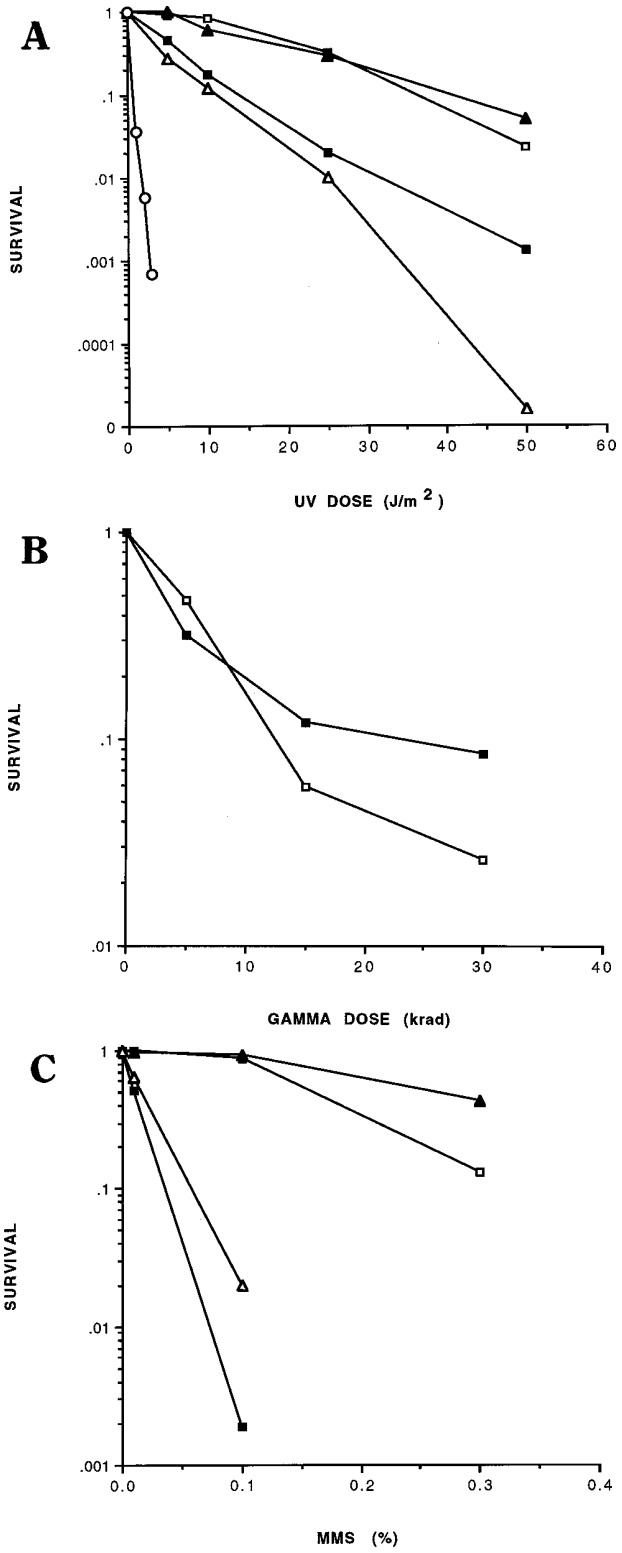

FIG. 3. Sensitivity of cells to DNA-damaging agents. (A) Sensitivity to UV radiation of wild-type (□), *rad27*Δ::*URA3* (■), *rad27*Δ::*URA3*(pRS314RAD27) (▲), *rad27*Δ::*URA3*(pRS314) (△), and *rad2*Δ (○) strains. pRS314RAD27 contains the *RAD27* gene; pRS314 is the vector lacking the insert. The *rad2*Δ strain is shown for comparison. (B) Sensitivity to gamma radiation of wild-type and *rad27*Δ::*URA3* strains. Symbols are as for panel A. (C) Sensitivity to MMS of wild-type, *rad27*Δ::*URA3*, *rad27*Δ(pRAD27), and *rad27*Δ::*URA3*(pRS314) strains. Symbols are as for panel A. At 0.3% MMS no survivors were seen in the *rad27*Δ::*URA3* or *rad27*Δ::*URA3*(pRS314) culture even when 10% of the cells were plated.

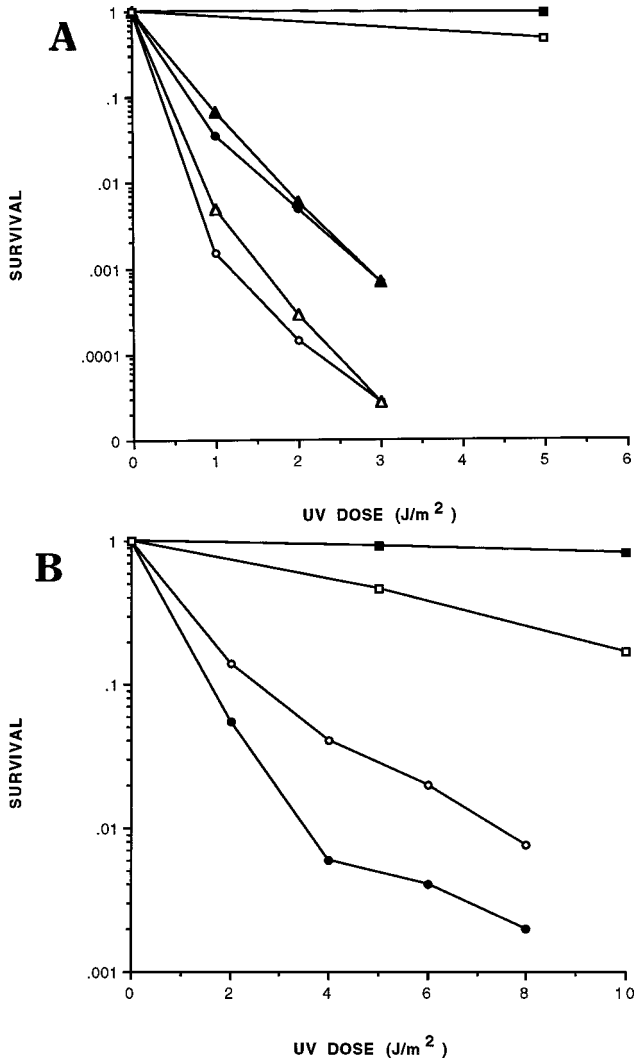

FIG. 4. Epistasis analysis of the *rad27*Δ::*URA3* mutation. (A) UV sensitivity of wild-type (■), *rad27*Δ::*URA3* (□), *rad10*Δ (●), *rad10*Δ *rad27*Δ::*URA3* (○), *rad2*Δ (▲), and *rad2*Δ *rad27*Δ::*URA3* (△) strains. (B) UV sensitivity of wild-type (■), *rad27*Δ::*URA3* (□), *rad18-2* (●), and *rad18-2 rad27*Δ::*URA3* (○) strains.

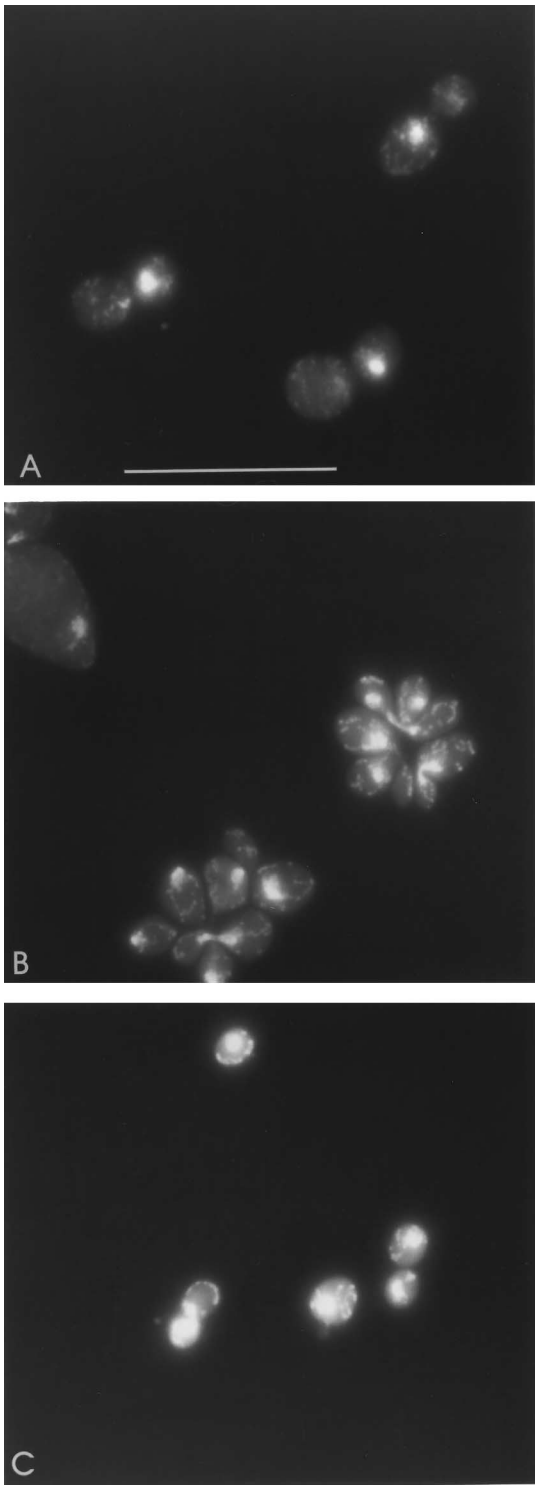

FIG. 5. Morphologies of wild-type and *rad27*Δ::*URA3* cells. Cells were stained with DAPI as described in Materials and Methods and photographed with UV illumination. (A) *rad27*Δ::*URA3* cells arrested at 37°C. (B) Mid-log-phase *rad27*Δ::*URA3* cells growing at 30°C. (C) Mid-log-phase wild-type cells growing at 30°C. Magnification, ×850; bar, 5 μm.

arrest and the formation of large, budded, dumbbell-shaped cells (Fig. 5A). Examination of these cells after staining with DAPI showed the presence of a single nucleus in the mother cell or in the neck between mother and daughter cells (Fig. 5A). These morphological observations suggest that at the restrictive temperature the deletion mutant undergoes cell cycle arrest. To examine this parameter more precisely, we monitored the DNA content of cells by flow cytometry. Wild-type and mutant cells were grown at 23°C to mid-log phase, arrested with the mating pheromone α-factor, and then released from arrest at 36°C. Both the arrested wild-type and mutant cells showed a single peak of fluorescence corresponding to a 1N DNA content, as expected from α-factor arrest at START (Fig. 6). Upon release from arrest, wild-type cells were observed to traverse the cell cycle normally. In contrast, mutant cells accumulated a 2N DNA content, consistent with arrest in the late S or G_2 phase of the cell cycle (Fig. 6).

At 30°C several independent isolates of haploid *rad27*Δ::*URA3* cells were observed to have a doubling time ~50% slower than that of isogenic wild-type cells (data not shown). Growth was also significantly slower than in the wild-type parent at 16 and 23°C. Microscopic examination of asynchronous cultures of mutant cells from a single haploid isolate demonstrated a variety of morphologic abnormalities when cells were

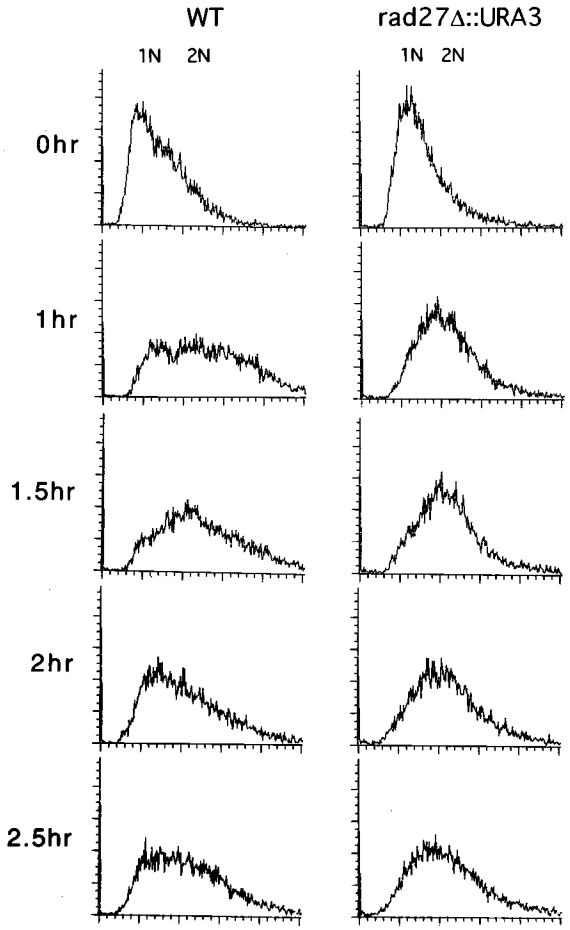

FIG. 6. Flow cytometric analysis of DNA content in wild-type (WT) and *rad27*Δ::*URA3* cells at the indicated times after release at 36°C from α-factor arrest. The 1N and 2N peaks are labeled. The cells were grown and arrested as described in Materials and Methods.

grown at 30°C (Fig. 5B and C). The mutant cells were considerably larger than wild-type cells, and many of them showed grossly abnormal morphology, including giant cells, elongated cells, linear chains of several cells connected end to end, and groups of 6 to 12 cells clumped together in a manner distinct from the established "clumpy" phenotype.

Collectively, the results described above suggest that cells with a deletion of the *RAD27* gene are able to replicate much if not all their DNA. However, an event(s) that is aberrant at permissive temperatures and is totally defective at 37°C leads to cell cycle arrest in the late S or G_2 phase.

Mutagenesis and chromosome stability in the *rad27*Δ::*URA3* mutant. Many genes in the *rad6* epistasis group are known to be involved in both spontaneous and DNA damage-induced mutagenesis (6), although their precise contributions to these phenomena are poorly understood. Defects in some genes in this group result in an increase in the rate of spontaneous mutagenesis. Mutations in other genes result in a reduction or abolition of DNA damage-induced mutagenesis, while yet other genes can influence both spontaneous and DNA damage-induced mutagenesis (21, 23). We investigated both of these phenomena in the *rad27*Δ::*URA3* mutant. The wild-type and mutant strains are phenotypically Ade^- because of a nonsense mutation in the *ADE2* gene. We therefore measured spontaneous reversion to Ade^+ (which can occur by reversion at the *ade2* locus or by generation of a suppressor tRNA) in wild-type and *rad27*Δ::*URA3* mutant strains. We observed a sixfold-higher level of spontaneous mutation per generation in the mutant compared with that in the wild-type strain (Table 2). In contrast, when we measured UV radiation-induced reversion to Ade^+, we found no significant differences between the two strains (Table 2).

TABLE 2. Spontaneous and UV radiation-induced mutagenesis

Strain	Spontaneous mutation rate/generation (10^{-8})	UV radiation-induced mutagenesis[a]		
		UV dose (J/m^2)	Survivors (%)	Mutants/10^6 survivors
SX46A	1	5	92	4.6
		10	75	9.9
SX46Arad27Δ::URA3	6.25	5	33	4.6
		10	19	8.7

[a] In both experiments, reversion of the cells to Ade^+ was measured as described in Materials and Methods.

Mutations in proteins involved in either DNA replication or mitosis can result in chromosomal instability and hence an increased rate of chromosome loss (13, 26). Indeed, such a phenotype has been independently demonstrated in studies of the *S. pombe* homolog of the *RAD27* gene (designated *rad2*$^+$; not to be confused with the *RAD2* gene of *S. cerevisiae* [25]). To investigate this phenotype, we monitored the stability of a centromeric plasmid in a strain in which the *RAD27* gene was replaced with the *TRP1* gene (14, 20). These cells were transformed with a centromeric autonomously replicating plasmid carrying a tRNA suppressor gene. Suppression of the Ade^- phenotype results in white colony color which is retained during stable plasmid segregation. However, loss of the plasmid results in white colonies with red sectors. The plasmid loss per generation can be calculated by determining the percentage of half red, half white colonies that derive from cells that suffered a plasmid loss event in the first cell division. This plasmid is typically lost at a rate of $\sim 10^{-2}$ per generation (14). The wild-type strain showed the expected pattern of predominantly white colonies (Fig. 7). In contrast, four independent *rad27*Δ::*TRP1* mutant transformants yielded mainly solid red colonies, indicating that these cells lost the plasmid with a very high frequency. Indeed, we were unable to quantitate the loss rate, since we almost never observed half-sectored colonies.

Expression of the *RAD27* gene is cell cycle regulated. The promoter region of the *RAD27* gene contains two *Mlu*I motifs (ACGCGT) at nucleotide positions −123 and −175 with respect to the ATG translational start site (Fig. 2). This sequence has been shown to be a DNA-binding site for a transcription factor that confers cell cycle-dependent expression of several yeast genes (17, 18). In order to determine whether transcription of the *RAD27* gene is regulated during the cell cycle, we performed Northern analysis of the transcript in wild-type cells synchronized in G_1 with α-factor. Upon release from the synchronized state, yeast cells typically undergo several well-synchronized divisions. As shown in Fig. 8, the *RAD27* gene encodes a 1.35-kb transcript which is cell cycle regulated. Induction of *RAD27* occurs prior to the maximal expression of histone H2A, which is induced in early S phase (38). This timing places the induction of *RAD27* concomitant with that of a number of other cell cycle-regulated genes whose products are known to be involved in DNA replication (see Discussion). When normalized to the expression of the non-cell-cycle-regulated *URA3* gene, the induction of *RAD27* was determined to

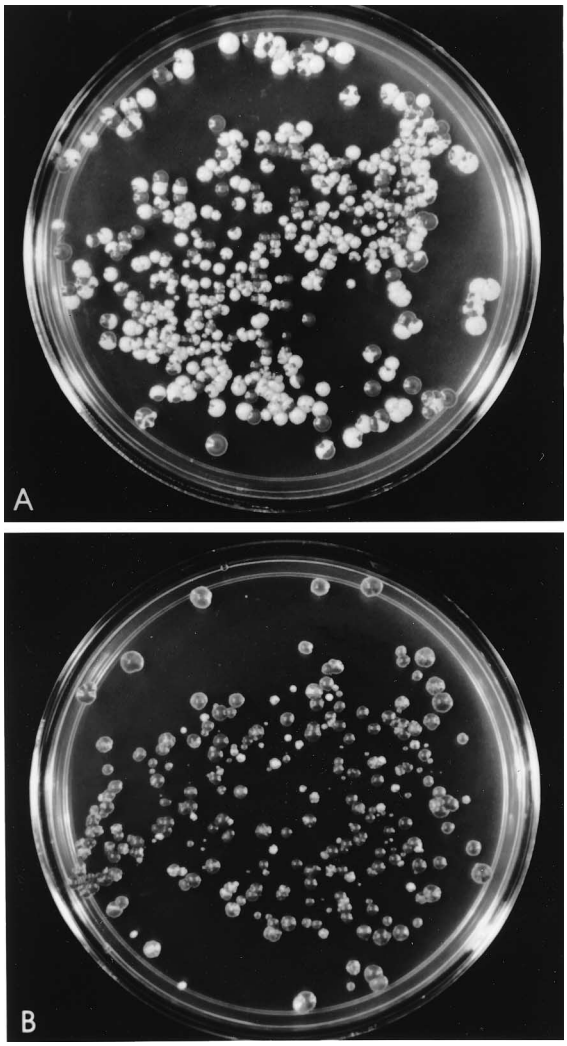

FIG. 7. Plasmid loss in the wild-type (A) and *rad27*Δ::*URA3* (B) strains. Colonies growing on YPD plates after 6 to 8 days at 30°C are shown.

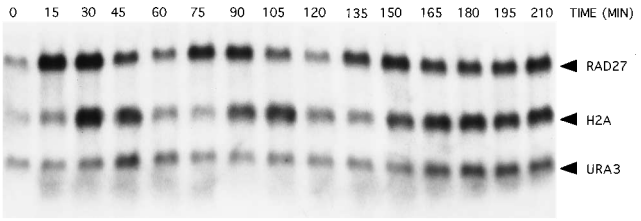

FIG. 8. Northern blot analysis of cell cycle-regulated transcription of the *RAD27* gene. Wild-type cells were arrested with α-factor and then released, and aliquots of cells were collected at the indicated times for RNA preparation. Zero time represents cells collected immediately after release from arrest. The *URA3* transcript serves as a non-cell-cycle-regulated control. The histone H2A transcript serves as a marker for S phase, since this gene is known to be induced during S phase.

be ca. fourfold. The possibility that *RAD27* may also be induced following the exposure of cells to DNA-damaging agents is currently under investigation.

DISCUSSION

We have initiated an analysis of the function of an ORF, originally identified as YKL510, located on the long arm of chromosome XI of *S. cerevisiae*. On the basis of the sensitivity of a deletion mutant to UV radiation, we have adopted the systematic nomenclature for radiation-sensitive yeast mutants and have renamed the wild-type allele *RAD27*. The modest sensitivity of a *rad27* deletion mutant to UV radiation and its resistance to ionizing radiation possibly explain why the *RAD27* gene was not previously identified in genetic screens for radiation-sensitive yeast mutants. The ability of a plasmid carrying just the *RAD27* gene to fully complement both the UV radiation and MMS sensitivities of the mutant indicates that all mutant phenotypes arise exclusively from a defect in the *RAD27* gene. This is an important consideration since the gene immediately downstream of *RAD27* is *APN1*, which encodes the major apurinic/apyrimidinic endonuclease in *S. cerevisiae*. Deletion of the *APN1* gene also results in increased sensitivity to MMS (29).

Deletion of the *RAD27* gene results in a severe growth defect. At 37°C growth is completely arrested, and it is also significantly reduced at all permissive temperatures tested. The morphology of growth-arrested cells at 37°C combined with an analysis of DNA content indicates that their cell cycle is arrested in the late S or G_2 phase. It is well established that yeast cells that are unable to complete normal DNA replication arrest as large budded cells with a single nucleus (28). The presence of this characteristic morphology in the *rad27*Δ::*URA3* mutant at the restrictive temperature suggests that in the absence of *RAD27* function, DNA replication is incomplete or results in a diploid genome which is unable to undergo normal segregation.

It is curious that a strain with a complete deletion of a gene manifests temperature-sensitive lethality. However, this phenomenon is not unprecedented in the literature (3, 5). This observation suggests that another protein(s) can substitute for the essential function of the *RAD27* gene product at 30°C but not at 37°C. Alternatively, Rad27 protein may contribute to the stability of a multiprotein complex which completely loses its integrity at the restrictive temperature.

Many of the other phenotypes of a *rad27*Δ mutant are consistent with a role of the *RAD27* gene in stable DNA replication. The mutant can maintain a plasmid minichromosome under selective conditions, but when selection is removed, the plasmid is rapidly lost. Chromosome loss has been associated with mutations in other proteins known to be involved in DNA replication (13, 26). In addition to temperature-sensitive growth arrest and chromosome loss, the *rad27*Δ strain exhibits a significant increase in the frequency of spontaneous mutation ($Ade^- \rightarrow Ade^+$) but no increase in frequencies of mutations induced by exposure to UV radiation. Increased spontaneous mutagenesis is also consistent with a defect in DNA replication. However, this phenotype may alternatively reflect defective repair or tolerance of spontaneous DNA damage.

The *RAD27* gene encodes a 1.35-kb transcript whose abundance in asynchronous cultures is approximately the same as that of *URA3* mRNA. The gene is cell cycle regulated, resulting in about a fourfold increase in the level of *RAD27* mRNA in early G_1 phase. A similar pattern of transcriptional regulation has been observed for more than 20 genes which are involved in DNA replication in *S. cerevisiae* (17), including those that encode DNA polymerases α (*POL1*) and δ (*POL3*) and DNA ligase (*CDC9*) and genes involved in the biosynthesis of nucleotide precursors for DNA synthesis, such as thymidylate kinase (*CDC8*), ribonucleotide reductase (*RNR1* and *RNR2*), and thymidylate synthase (*CDC21*). These observations, to-

gether with the mutant phenotypes of cell cycle stage-specific growth arrest, minichromosome instability, and increased spontaneous mutagenesis, provide strong circumstantial evidence that the product of the *RAD27* gene has some role(s) in DNA replication in *S. cerevisiae*.

Several observations suggest that the primary role of Rad27 protein and its mammalian homologs is to allow completion of lagging-strand DNA synthesis during DNA replication. First, Rad27 protein is endowed with both DNA endonuclease and 5′→3′ exonuclease activities (12). Second, studies with human and murine in vitro DNA replication systems have led to the purification of a protein which is almost certainly the mammalian homolog of Rad27 protein (9, 15, 37). This protein, which is also endowed with nuclease activity, is required for the maturation and ligation of Okazaki fragments generated during discontinuous synthesis of the lagging strand in DNA replication. The purified protein also exhibits 5′→3′ RNA exonuclease activity on RNA-DNA hybrids (9, 15) and has been postulated to be required for the degradation of RNA at the 5′ ends of Okazaki fragments. In the absence of this protein, nicks in the lagging strand are not sealed and lagging-strand DNA synthesis cannot be completed. RNase H cannot substitute for this protein in vitro (9, 15, 37). Interestingly, the probable bovine analog of Rad27 protein has been detected in partially purified fractions of DNA polymerase ε and is required for complete DNA synthesis with a synthetic DNA substrate designed to mimic the lagging-strand template (34, 36). RNase activity has not been demonstrated with the yeast Rad27 protein. However, given the high level of sequence conservation between this protein and its mammalian homologs, it seems likely that such an activity exists.

The putative role of the Rad27 protein in the stable completion of DNA replication is supported by the remarkably similar phenotypes of the *rad27*Δ::*URA3* mutant strain and an *Escherichia coli* strain designated *polAex1* (*polA480ex*), which carries a mutation that abolishes the 5′→3′ exonuclease activity of this polymerase (19). This mutant strain is sensitive to temperature, sensitive to MMS (but not to ionizing radiation), and moderately sensitive to UV radiation. At the nonpermissive temperature the *E. coli* mutant is unable to seal Okazaki fragments and incorporate them into high-molecular-weight DNA. This defect is thought to be a consequence of the inability of the enzyme to degrade RNA in Okazaki fragments. The amino acid sequences of the yeast *RAD27* gene and the N-terminal 323 amino acids of the region of the *E. coli polA* gene, which encodes the 5′→3′ exonuclease activity, are 22.4% identical and 45% similar.

Our original interest in the YKL510 ORF was sparked by the observation of a striking similarity in the amino acid sequence of the putative polypeptide encoded by this ORF and regions of the endonuclease encoded by the yeast *RAD2* gene, which is known to be involved in nucleotide excision repair (6, 27). An analysis of epistasis indicates that the *RAD27* gene is not a member of the nucleotide excision repair epistasis group but rather falls into the *rad6* epistasis group, genes of which are involved in DNA damage tolerance and mutagenesis. This observation is consistent with the moderate sensitivity to UV radiation and with the spontaneous mutator phenotype. Hence, the amino acid sequence homology likely reflects the fact that both the yeast *RAD2* and *RAD27* gene products are endowed with nuclease activities.

It is not evident why deletion of the *RAD27* gene confers such profound sensitivity to the alkylating agent MMS, particularly in light of normal levels of resistance to ionizing radiation. Similar hypersensitivity has been observed after treatment of *rad27*Δ::*URA3* cells with other methylating agents (data not shown). As mentioned above, this phenotype has been observed in the *E. coli polAex1* mutant, and it may reflect a special role of this class of nucleases in the repair of alkylation damage to DNA.

ACKNOWLEDGMENTS

These studies were supported by research grant CA-12428 from the U.S. Public Health Service.

We thank our laboratory colleagues for helpful discussions and for critical review of the manuscript. We are also grateful to Michael Lieber and Felicity Watts for communicating results prior to publication and to Tomas Lindahl for bringing to our attention the similar phenotypes of the *rad27* mutant and the *E. coli* 5′→3′ exonuclease mutant of DNA polymerase I.

REFERENCES

1. **Ausubel, F. M., R. E. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1989. Current protocols in molecular biology. John Wiley and Sons, New York.
2. **Aves, S. J., B. W. Durkacz, A. Carr, and P. Nurse.** 1985. Cloning, sequencing and transcriptional control of the *Schizosaccharomyces pombe* cdc10 'start' gene. EMBO J. **4:**457–463.
3. **Cannon, J. F., and K. Tatchell.** 1987. Characterization of *Saccharomyces cerevisiae* genes encoding subunits of cyclic AMP-dependent protein kinase. Mol. Cell. Biol. **7:**2653–2663.
4. **Carr, A. M., K. S. Sheldrick, J. M. Murray, R. Al-Harithy, F. Z. Watts, and A. R. Lehmann.** 1993. Evolutionary conservation of excision repair in *Schizosaccharomyces pombe*: evidence for a family of sequences related to the *Saccharomyces cerevisiae RAD2* gene. Nucleic Acids Res. **21:**1345–1349.
5. **Feldmann, H., and E. L. Winnekar.** 1993. A putative homologue of the human autoantigen Ku from *Saccharomyces cerevisiae*. J. Biol. Chem. **268:**12895–12900.
6. **Friedberg, E. C.** 1988. Deoxyribonucleic acid repair in the yeast *Saccharomyces cerevisiae*. Microbiol. Rev. **52:**70–102.
7. **Friedberg, E. C.** 1991. Yeast genes involved in DNA-repair processes: new looks on old faces. Mol. Microbiol. **5:**2303–2310.
8. **Game, J. C., and B. S. Cox.** 1971. Allelism tests of mutants affecting sensitivity to radiation in yeast and a proposed nomenclature. Mutat. Res. **12:**328–331.
9. **Goulian, M., S. H. Richards, C. J. Heard, and B. M. Bigsby.** 1990. Discontinuous DNA synthesis by purified mammalian proteins. J. Biol. Chem. **265:**18461–18471.
10. **Habraken, Y., P. Sung, L. Prakash, and S. Prakash.** 1993. Yeast excision repair gene *RAD2* encodes a single-stranded DNA nuclease. Nature (London) **366:**365–368.
11. **Harrington, J. J., and M. Lieber.** 1994. The characterization of a mammalian DNA structure-specific endonuclease. EMBO J. **13:**1235–1246.
12. **Harrington, J. J., and M. Lieber.** 1994. Functional domains within *FEN-1* and *RAD2* define a family of structure-specific endonucleases: implications for nucleotide excision repair. Genes Dev. **8:**1344–1355.
13. **Hartwell, L. H., and D. Smith.** 1985. Altered fidelity of mitotic chromosome transmission in cell cycle mutants of *Saccharomyces cerevisiae*. Genetics **110:**381–395.
14. **Hieter, P., C. Mann, M. Snyder, and R. W. Davis.** 1985. Mitotic stability of yeast chromosomes: a colony color assay that measures nondisjunction and chromosome loss. Cell **40:**381–392.
15. **Ishimi, Y., A. Claude, P. Bullock, and J. Hurwitz.** 1988. Complete enzymatic synthesis of DNA containing the SV40 origin of replication. J. Biol. Chem. **263:**19723–19733.
16. **Jacquier, A., P. Legrain, and B. Dujon.** 1992. Sequence of a 10.7kb segment of yeast chromosome XI identifies the *APN1* and the *BAF1* loci and reveals one tRNA gene and several new open reading frames including homologues to *RAD2* and kinases. Yeast **8:**121–132.
17. **Johnston, L. H., and N. F. Lowndes.** 1992. Cell cycle control of DNA synthesis in budding yeast. Nucleic Acids Res. **20:**2403–2410.
18. **Koch, C., and K. Nasmyth.** 1994. Cell cycle regulated transcription in yeast. Curr. Opin. Cell Biol. **6:**451–459.
19. **Konrad, E. B., and I. R. Lehman.** 1974. A conditional lethal mutant of *Escherichia coli* K12 defective in the 5′→3′ exonuclease associated with DNA polymerase I. Proc. Natl. Acad. Sci. USA **71:**2048–2051.
20. **Koshland, D., C. Mann, M. Snyder, and R. W. Davis.** 1985. Genetic analysis of the mitotic transmission of minichromosomes. Cell **40:**393–403.
21. **Lawrence, C. W.** 1982. Mutagenesis in *Saccharomyces cerevisiae*. Adv. Genet. **21:**173–254.
22. **Lea, D. E., and C. A. Coulson.** 1949. The distribution of the numbers of mutants in bacterial populations. J. Genet. **49:**264–284.
23. **Lemontt, J. F.** 1979. Genetic and physiological factors affecting repair and mutagenesis in yeast, p. 85–120. *In* W. M. Generoso, M. D. Shelby, and F. J.

de Serres (ed.), DNA repair and mutagenesis in eukaryotes. Plenum Publishing Corp., New York.
24. **Luria, S. F., and M. Delbruck.** 1943. Mutations of bacteria from virus sensitivity to virus resistance. Genetics **28:**491–511.
25. **Murray, J. M., M. Tavassoli, R. Al-Harithy, K. S. Sheldrick, A. R. Lehmann, A. M. Carr, and F. Z. Watts.** 1994. Structural and functional conservation of the human homolog of the *Schizosaccharomyces pombe rad2* gene, which is required for chromosome segregation and recovery from DNA damage. Mol. Cell. Biol. **14:**4878–4888.
26. **Palmer, R. E., E. Hogan, and D. Koshland.** 1990. Mitotic transmission of artificial chromosomes in cdc mutants of the yeast, *Saccharomyces cerevisiae*. Genetics **125:**763–774.
27. **Prakash, S., P. Sung, and L. Prakash.** 1993. DNA repair genes and proteins of *Saccharomyces cerevisiae*. Annu. Rev. Genet. **27:**33–70.
28. **Pringle, J. R., and L. H. Hartwell.** 1981. The *Saccharomyces cerevisiae* cell cycle, p. 97–142. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast Saccharomyces. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Ramotar, D., S. C. Popoff, E. B. Gralla, and B. Demple.** 1991. Cellular role of yeast Apn1 apurinic endonuclease/3′-diesterase: repair of oxidative and alkylation DNA damage and control of spontaneous mutation. Mol. Cell. Biol. **11:**4537–4544.
30. **Reynolds, R. J., and E. C. Friedberg.** 1981. Molecular mechanism of pyrimidine dimer excision in *Saccharomyces cerevisiae*: incision of ultraviolet-irradiated deoxyribonucleic acid in vivo. J. Bacteriol. **146:**692–704.
31. **Rothstein, R.** 1984. Targeting, disruption, replacement, and allelic rescue: integrative DNA transformation in yeast. Methods Enzymol. **194:**281–301.
32. **Scherly, D., T. Nouspikel, J. Corlet, C. Ucla, A. Bairoch, and S. G. Clarkson.** 1993. Complementation of the DNA repair defect in xeroderma pigmentosum group G cells by a human cDNA related to yeast *RAD2*. Nature (London) **363:**182–185.
33. **Siede, W. S., A. S. Friedberg, and E. C. Friedberg.** 1993. *RAD9*-dependent G1 arrest defines a second checkpoint for damaged DNA in the cell cycle of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **90:**7985–7989.
34. **Siegal, G., J. J. Turchi, T. W. Myers, and R. A. Bambera.** 1992. A 5′ to 3′ exonuclease functionally interacts with calf DNA polymerase ε. Proc. Natl. Acad. Sci. USA **89:**9377–9381.
35. **Sikorsky, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
36. **Turchi, J. J., and R. A. Bambera.** 1993. Completion of mammalian lagging strand DNA replication using purified proteins. J. Biol. Chem. **268:**15136–15141.
37. **Waga, S., G. Bauer, and B. Stillman.** 1994. Reconstitution of complete SV40 replication with purified replication factors. J. Biol. Chem. **269:**10923–10934.
38. **White, J. H. M., S. R. Green, D. G. Barker, L. B. Dumas, and L. H. Johnston.** 1987. The *CDC8* transcript is cell cycle regulated in yeast and is expressed coordinately with *CDC9* and *CDC21* at a point preceding histone transcription. Exp. Cell Res. **171:**223–231.
39. **Wilcox, D. R., and L. Prakash.** 1981. Incision and postincision steps of pyrimidine dimer removal in excision-defective mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **148:**618–623.