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Nucleotide Sequence and Transcriptional Regulation of the Yeast Recombinational Repair Gene *RAD51*

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The *RAD51* gene of *Saccharomyces cerevisiae* is required both for recombination and for the repair of DNA damage caused by X rays. Here we report the sequence and transcriptional regulation of this gene. The RAD51 protein shares significant homology (~50%) over a 70-amino-acid stretch with the RAD57 protein (J. A. Kans and R. K. Mortimer, Gene 105:139–140, 1991), the product of another yeast recombinational repair gene, and also moderate (~27%), but potentially significant, homology with the bacterial RecA protein. The homologies cover a region that encodes a putative nucleotide binding site of the RAD51 protein. Sequences upstream of the coding region for RAD51 protein share homology with the damage response sequence element of *RAD54*, an upstream activating sequence required for damage regulation of the *RAD54* transcript, and also contain two sites for restriction enzyme *Mlu*I; the presence of *Mlu*I restriction sites has been associated with cell cycle regulation. A 1.6-kb transcript corresponding to *RAD51* was observed, and levels of this transcript increased rapidly after exposure to relatively low doses of X rays. Additionally, *RAD51* transcript levels were found to vary throughout the cell cycle, peaking in late G_1 to early S. The observed cell cycle regulation is consistent with that of a group of genes involved primarily in DNA synthesis and replication which are thought to be coordinately cell cycle regulated. Cells arrested in early G_1 were still capable of increasing levels of *RAD51* transcript after irradiation, indicating that increased *RAD51* transcript levels after X-ray exposure are not solely due to an X-ray-induced cessation of the cell cycle at a period when the level of *RAD51* expression is normally high.

Organisms are able to sense and respond to DNA damage caused by a variety of environmental agents; mutations which block these abilities can lead to a loss of growth capacity and eventually to cell death. In the yeast *Saccharomyces cerevisiae*, genetic analyses of mutations conferring an increase in sensitivity to irradiation have defined three epistasis groups, the *RAD3*, *RAD6*, and *RAD52* groups, which are thought to reflect three repair pathways (18; for reviews, see references 15, 16, and 20). The *RAD3* group, which is characterized by mutations that confer a high degree of sensitivity to UV radiation, are known to act in excision repair of damaged DNA. The *RAD6* group is required for resistance to both UV and X rays; a subset of these genes is believed to act in a pathway of postreplication repair. The *RAD52* group, made up of the genes *RAD50* to *RAD57*, is required for resistance to X-ray radiation.

Three genes in the *RAD50*-to-*RAD57* group, *RAD51*, *RAD52*, and *RAD54*, form a subset displaying a number of similar phenotypes which indicate roles in general recombination and in DNA repair that involves recombinational activity (16). Mutations in any one of these three loci confer the most extreme sensitivity to X rays of any of the yeast *rad* mutations but only slight sensitivity to UV (16, 18, 19). Damage arising from X-ray irradiation includes DNA double-strand breaks, the repair of which is believed to involve a recombinational mechanism (19, 50). Defects in *RAD51*, *RAD52*, or *RAD54* block both the repair of double-strand breaks (9, 45) and radiation-induced mitotic recombination (16, 40, 50). The genes are also required for homothallic mating-type switching (17, 37), a recombinogenic process initiated by a site-specific double-strand break and characterized by an exchange of genetic information at the *MAT* locus (22). Indeed, abortive mating-type switching in strains carrying mutations in *rad51*, *rad52*, or *rad54* constitutes a lethal event (17, 37). In addition, diploid strains homozygous for *rad51* or *rad52* mutations have decreased levels of meiotic recombination, poor sporulation efficiency, and extremely low spore viability (19, 40). Of the three genes, only *RAD52* and *RAD54* have been previously characterized at the molecular level (1, 7, 8, 13). The fact that *RAD51* is required for both repair of damaged DNA and general recombination is of interest and has led us to undertake a detailed study of this gene and its expression.

In eucaryotes, less is known of the cellular response to DNA-damaging agents than in procaryotes. Work on *Escherichia coli* has elucidated a cellular response to DNA-damaging agents that has been called the SOS response (for a review, see reference 60). The cornerstone of the response is a coordinately regulated increase in the transcript levels of a group of DNA repair genes as a result of exposure of cells to DNA-damaging agents. The RecA protein plays a central role, being required both for the coordinate derepression of transcript levels of genes involved in the SOS response and for recombinational repair of DNA. The SOS response is very flexible, as indicated by variations in the timing and magnitude of changes in transcript levels of the individual genes that are being regulated.

Similarly, an inducible repair response also exists in eucaryotes. In both mammalian and yeast cells, prior treatment with low doses of X rays induces a transient increase in

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TABLE 1. Yeast strains used in these experiments

Strain	Genotype
X2180	*MAT***a**/*MAT*α *mal*/*mal mel*/*mel gal2*/*gal2 SUC2*/*SUC2 CUP1*/*CUP1*
X2180-1A	*MAT***a** *mal mel gal2 SUC2 CUP1*
CG378	*MAT***a** *ade5 can1 leu2-3,112 trp1-289 ura3-52 gal2* [KIL-o]
CG7879	*MAT***a**/*MAT*α *leu2-3,112*/*leu2-3,112 trp1-289*/*trp1-289 ura3-52*/*ura3-5 ade5*/*ade5 gal2*/*gal2*
CG378Δ51	Same as CG378, except with *rad51*::LEU2 deletion
XGB2-4C	*MAT***a** *rad51-1 leu2-3,112*
XGB2-4C-pGB315bp51	Same as XGB2-4C, but carrying the *CEN* plasmid pGB315bp51
XGB7	*MAT***a**/*MAT*α *rad51*::*LEU2*/*rad51*::*LEU2 leu2-3,112*/*leu2-3,112 ura3-52*/*ura3-52*
XGB4	*MAT***a**/*MAT*α *rad51*::*LEU2*/*rad51*::*LEU2 leu2-3,112*/*leu2-3,112 trp1-289*/*trp1-289 ura3-52*/*ura3-52 ade5*/*ade5 gal2*/*gal2*
XGB5	Isogenic to XGB4, except *RAD*$^{+}$

resistance to a subsequent higher dose (4, 5, 64). The protective effect is blocked by agents that inhibit protein or RNA synthesis (4). As in *E. coli*, one aspect appears to be the regulation of many genes at the transcriptional level in response to environmental stress. An increase in the message levels of a variety of genes following exposure to DNA-damaging agents has been observed in mammalian and yeast cells (14, 38, 49). In *S. cerevisiae*, of the *RAD* genes assayed, *RAD2*, *RAD6*, *RAD7*, *RAD18*, *RAD23*, and *RAD54* are known to have increased transcript levels in response to DNA-damaging agents (7, 13, 28, 29, 34–36, 46). However, transcript levels of many other genes in the three repair epistasis groups do not appear to be regulated in response to DNA damage (8, 15). Detailed deletion analyses of the upstream regions of two of the genes, *RAD2* (56) and *RAD54* (7), have identified various sequence elements which are required for enhanced expression after exposure to DNA-damaging agents. In the case of *RAD54*, a 29-bp segment, called the damage response sequence (DRS), has also been shown to be sufficient to impart X-ray-inducible expression on a heterologous reporter gene (7). Unlike *E. coli*, direct comparisons of the regulatory sequences have not uncovered a common regulatory element for the damage-inducible genes in *S. cerevisiae* (28, 55, 56), although the possibility of conserved regulatory sequences within each of the different radiation epistasis groups has been proposed (28).

In contrast, cell cycle regulation of transcript levels of a number of *S. cerevisiae* genes, which are primarily required for DNA synthesis and replication but which are also involved in repair, appears to be dependent in part on conserved regulatory sequences (for a review, see reference 24). Transcript levels of these genes appear to be coordinately regulated during the cell cycle and in some instances, such as *CDC9* (DNA ligase) and *POL1* (DNA polymerase), to respond to exposure to DNA-damaging agents (25, 44, 63). The conserved cell cycle regulatory sequences are identifiable by their similarity to the recognition site of the *Mlu*I restriction endonuclease. Specific factors that bind to these sequences in a cell cycle-dependent fashion have been reported (33, 39, 59).

Here we report the molecular characterization and transcriptional regulation of *RAD51*. We have determined the nucleotide sequence of *RAD51* and the transcript levels in response to X rays and throughout the cell cycle; we found that the transcript level varies in both instances. Two different sequences upstream of *RAD51* indicate that its transcription may be under common control both with *RAD54* and with coordinately regulated genes involved in DNA replication. In addition, the predicted sequence of RAD51 protein shares strong homology in one region to the protein encoded by another yeast X-ray repair gene, *RAD57* (30), as well as moderate homology to the RecA protein.

MATERIALS AND METHODS

Strains and media. All yeast strains used in these experiments are listed in Table 1. Strains X2180, X2180-1A, and CG378 were obtained from the Yeast Genetic Stock Center (University of California, Berkeley). *E. coli* HB101, JM105, and DH5α were used in plasmid construction. All yeast cultures were grown in either YEPD medium (1% yeast extract [Difco], 2% Bacto Peptone [Difco], 2% glucose) or YNB medium (0.67% yeast nitrogen base [Difco], 0.5% glucose, and appropriate nutritional supplements).

Plasmid and disruption constructions. Constructed plasmids used in subcloning experiments (Fig. 1B) were derived from the *rad51-1*-complementing genomic fragment originally isolated, which was shown by integration and genetic analysis to contain the sequences encoding the *RAD51* gene (6). A series of subclones from the genomic fragment were created by digestion of YEp13-RAD51-23 (6) with the appropriate restriction enzymes and ligation of gel-isolated fragments into the multiple cloning site of pUC12. The yeast *URA3* gene was also placed in these vectors as a selectable marker. Subclone 1 is a *Hin*dIII fragment containing both *RAD51* sequences and the 2μm origin of replication; subclone 2 was constructed by digesting YEp13-RAD51-23 initially with *Xba*I and religating (creating YEp13-51ΔX), then digesting with *Xba*I and *Pst*I, and ligating the *RAD51*-containing fragment into pUC12; subclone 3 was constructed by *Bam*HI digestion of YEp13-RAD51-23 and ligating the *RAD51*-containing fragment both in YRp7 (creating YRp7bb51) and back into YEp13 (creating YEp13bb51); subclone 4 was created by a partial digestion of subclone 2 with both *Spe*I and *Xba*I and religation; subclone 5 was constructed by digesting YEp13-51ΔX with *Eco*RI and cloning the fragment containing both the *RAD51* sequence and 2μm origin of replication into YIp5; subclone 6 was derived by digesting subclone 2 with *Bam*HI and *Pst*I and ligating the *RAD51*-containing fragment in the *CEN* plasmid pRS315 (57), creating pGB315bp51.

RAD51 was deleted by the method of gene replacement (48). pAM10, a plasmid containing *RAD51* and neighboring sequences, was digested with both *Stu*I and *Nru*I, and the *RAD51*-containing fragment was replaced by a 1.6-kb *Hpa*I-*Xmn*I fragment containing *LEU2*. A linear fragment in which

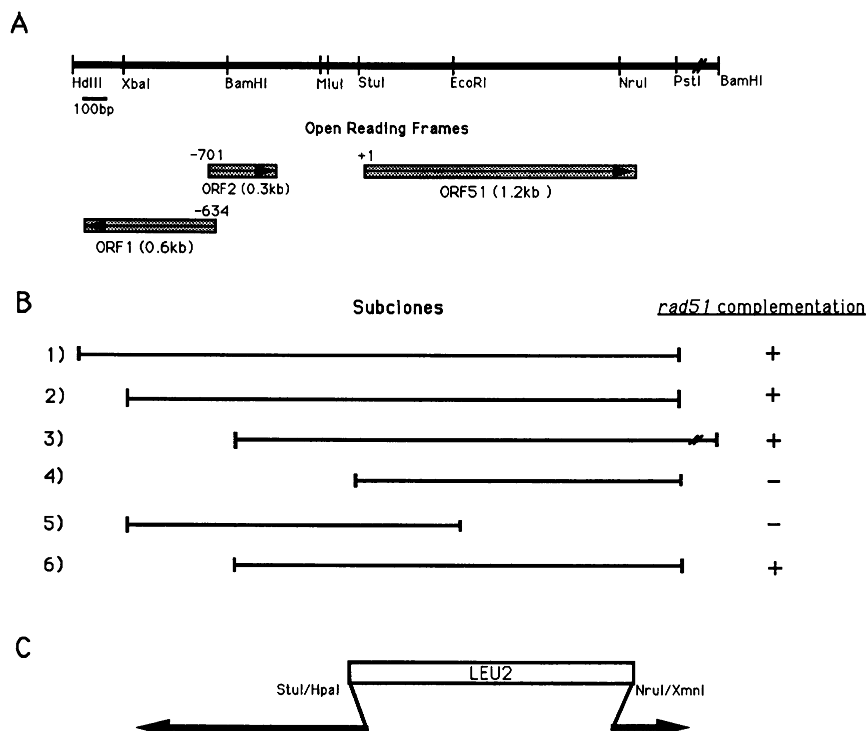

FIG. 1. (A) Restriction map of a 2.8-kb genomic fragment, which complements *rad51-1* and has been sequenced (see text). The regions corresponding to three ORFs predicted from the nucleotide sequence are depicted as stippled boxes. Arrows within each box show the predicted direction of transcription for each ORF. The first methionine of ORF51 is labeled +1; the number of base pairs upstream of ORF51 where the first methionine residues occur in ORF1 and ORF2 are shown (the size of each ORF is shown in parentheses). (B) Subclones of the genomic fragment (solid lines) are shown. All subclones were placed in multicopy plasmids and assayed for their ability to complement the X-ray sensitivity of the *rad51-1* mutation. Subclone 6 was additionally tested in a single-copy (*CEN*) plasmid. Subclones: 1, *Hin*dIII-*Pst*I fragment containing all three ORFs; subclone 2, *Xba*I-*Pst*I fragment with ORF1 partially deleted; subclone 3, *Bam*HI-*Bam*HI fragment with ORF1 deleted and ORF2 partially deleted; subclone 4, *Stu*I-*Pst*I fragment containing all of ORF51 but no upstream sequence; subclone 5, *Xba*I to *Eco*RI, containing ORF2 and partial deletions of ORF51 and ORF1; subclone 6, *Bam*HI-*Pst*I fragment containing an intact copy of ORF51 and 645 bp of upstream sequence. Symbols: +, complementation of X-ray sensitivity of the *rad51-1* mutation; −, failure to complement. (C) A constructed genomic deletion of *RAD51* is shown. The chromosomal region extending from *Stu*I to *Nru*I, containing most of ORF51, was replaced with a restriction fragment containing the *LEU2* sequence.

RAD51 had been deleted was isolated and used to transform yeast strain CG378, creating strain CG378Δ51.

Survival curves. The X-ray source was a Machlett OEG 60 tube with a beryllium window, operated at 50 kV and 20 mA. The dose rate was ~200 rads/s. Cells were irradiated on the surface of solid YEPD medium, and surviving colonies were counted 3 to 4 days later.

Sequencing and sequence analysis. All nucleotide sequencing was done by the method of Sanger et al. (52). Subclones containing *RAD51* and neighboring sequences were placed in pUC12 or pUC13, and further constructs were made as new restriction sites were determined. Specific oligonucleotide primers were constructed as required to obtain overlapping sequence from both strands. Sequence comparisons against sequences in nucleotide and protein data bases were run by using FASTA (43) (including TFASTA and LFASTA) and BLAST (2) sequence comparison software packages.

RNA preparation and Northern (RNA) analysis. Preparation of total yeast RNA was done as described by Schmitt et al. (54). Briefly, samples were centrifuged and resuspended in 350 μl of AE buffer (50 mM Na acetate [pH 5.2], 10 mM EDTA). Forty microliters of 10% sodium dodecyl sulfate (SDS) and 400 μl of phenol (phenol equilibrated previously in AE buffer) were added, and samples were heated for 4 min at 65°C. The samples were then rapidly frozen in a dry ice-methanol bath and centrifuged. The aqueous layer was extracted with phenol-chloroform for 5 min at room temperature. Forty microliters of 3 M Na acetate and 2.5 volumes of ethanol were added to the aqueous phase to precipitate the RNA. Samples were frozen for 1 h at −70°C and then centrifuged for 30 min. RNA pellets obtained in this manner were routinely resuspended in 20 to 40 μl of sterile water. All solutions, with the exception of phenol and chloroform, were treated overnight with 0.1% diethylpyrocarbonate (Sigma Chemical) and autoclaved to remove RNase activity. A 4.5-μl portion of each resuspended sample was loaded per lane and run in gels containing morpholinepropanesulfonic acid (MOPS), formaldehyde, and 1% agarose as described previously (51). Gels were blotted onto either nitrocellulose (Schleicher and Schuell) or nylon (Magna Graph). Hybridizations with ^{32}P-labeled DNA probes were carried out for 16 h at 42°C in a solution consisting of 50% formamide, 5× Denhardt's reagent, 0.1% SDS, 5× SSPE (1× SSPE is 0.15 M NaCl plus 0.015 M sodium citrate) (Sigma), and 10 μg of denatured salmon sperm DNA per ml. DNA probes were prepared by random primer labeling using a premixed kit (Amersham) in the presence of [α-^{32}P]dCTP (3,000 Ci/mmol). Hybridized signal was visualized both by autoradiography on Kodak X-OMAT film and by using a Molecular Dynamics Storage Phosphor Imager apparatus.

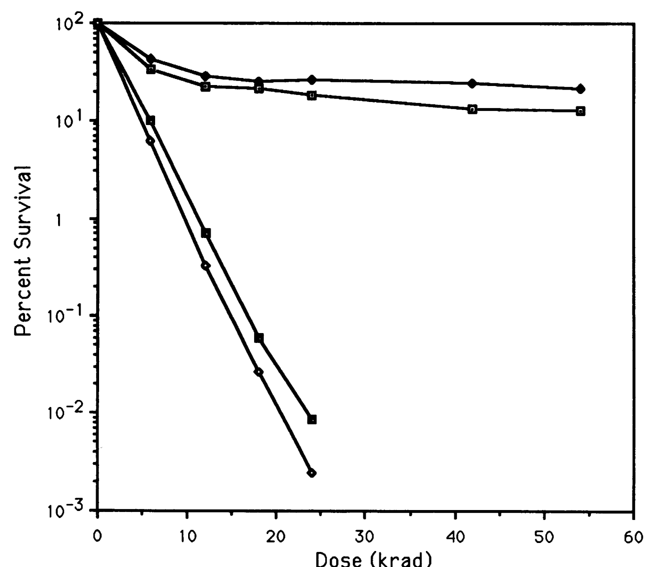

FIG. 2. Survival of various strains after exposure to X rays. Symbols: ⊡, strain CG378 (*RAD*$^+$); ■, strain XGB2-4C (*rad51-1*); ◇, CG378Δ51 (*RAD51* deleted); ◆, XGB2-4C-pGB315bp51 (*rad51-1*, but carries the single-copy *CEN* plasmid pGB315bp51 containing *RAD51* sequences).

^{32}P-labeled single-stranded DNA probes used for identification of transcripts corresponding to ORF51, ORF1, or ORF2 were constructed by isolating restriction fragments overlapping various regions of *RAD51* and neighboring sequences, cloning in M13mp18 or M13mp19, and isolating single-stranded DNA as previously described (51). The single-stranded isolates were then used as templates for DNA replication in the presence of radionucleotides. After denaturation, these probes were hybridized to Northern blots of total yeast RNA.

***RAD51* transcript regulation in response to X rays.** Cells were grown to early to mid-log phase (optical density at 660 nm, 0.1 to 0.5). Identical samples were filtered onto sterile filters (pore size, 0.45 μm) (Whatman) and irradiated. Control samples were treated the same way but were not irradiated. X-ray doses were given as described above for survival curves. Filtered samples were then resuspended in fresh medium and placed at 30°C, after which total RNA was isolated. For experiments determining the dependence of *RAD51* transcript levels on X-ray dose, otherwise identical samples were irradiated with doses ranging from 0 to 60 krads and incubated for 40 min. Samples were then pelleted by centrifugation, resuspended in AE buffer, and quickly frozen in a dry ice-methanol bath until RNA could be extracted. Experiments determining the time dependence of *RAD51* transcript levels after irradiation were carried out similarly to dose-response experiments described above, except that a uniform dose of 40 krads was given and the time of incubation was varied from 0 to 5.5 h.

RNA samples were run on MOPS–formaldehyde–1% agarose gels, transferred to nitrocellulose, and then probed with radiolabeled DNA probes specific for *RAD51*, *DED1*, and *HIS3* transcripts. The probe for *RAD51* transcript was made from a 1.3-kb *Mlu*I-*Nru*I fragment containing only *RAD51* sequences (Fig. 1A). The probe for *DED1* and *HIS3* was made from a 1.25-kb *Msp*I-*Bam*HI fragment containing all of *DED1* and 0.4 kb of *HIS3* coding sequence (58). Quantification of hybridized signal was performed by both densitometry of autoradiograms using a densitometer (E-C Apparatus Corp.) and by direct imaging and quantification using a Molecular Dynamics Storage Phosphor Imager and ImageQuant image analysis software (27). Levels of *DED1* and *HIS3* transcripts were used in lane-to-lane comparisons as loading controls.

Synchronization and cell cycle arrest experiments. Strain CG378 was grown in YNB medium to mid- to late log phase. Cultures were diluted 15- to 20-fold and allowed to grow to early log phase (optical density at 660 nm, 0.3 to 0.4). After samples were taken, α-factor (Sigma) was added to a final concentration of 2.5 μg/ml. Further samples were taken at 60, 95, and 165 min. α-factor was removed by rapid filtration and washing of the cells. Cells were then resuspended in an equal amount of fresh medium, and samples were taken at 15-min intervals. For experiments in which cells were held in prolonged arrest, 3 to 4 μg of α-factor per ml was used. Cell morphology and percentage of budded cells were monitored throughout the experiments.

After the samples were run on MOPS–formaldehyde–1% agarose gels and transfer to nylon, RNA samples were probed for *RAD51*, *HTA1*, and *Protein1* transcript. The probe for *RAD51* transcript is described above. The probe for *HTA1* and *Protein1* transcripts was made from a 2.3-kb *Sac*I fragment from pDC26A (a gift of M. Osley and S. Tsang). Northern blots of synchronous samples were probed with either ^{32}P- or digoxigenin-labeled probes. Labeling of digoxigenin-labeled DNA probes was carried out as described above for radiolabeled probes except that digoxigenin-labeled deoxynucleoside triphosphates were used. Visualization of hybridized probe was accomplished using a Boehringer Mannheim Genius Nonradioactive detection kit in combination with Lumiphos 530 (Boehringer Mannheim). Autoradiography of chemiluminescent hybridization signals was performed at room temperature for 1 to 30 min. Quantification of data was performed both by densitometry of autoradiograms and by direct visualization and quantification of chemiluminescent hybridization signals using a computerized CCD camera-based imaging apparatus (developed at Human Genome Project at Lawrence Berkeley Laboratory).

Nucleotide sequence accession number. The nucleotide sequence presented in this article is listed in GenBank under accession number M88470.

RESULTS

Subcloning and deletion analysis defining *RAD51*. *RAD51* was originally cloned as a 5.6-kb genomic fragment that complemented the *rad51-1* mutation (6). In order to determine the region within this fragment that contains *RAD51*, subclones were constructed, transformed into *S. cerevisiae*, and assayed for the ability to complement X-ray sensitivity conferred by the *rad51-1* mutation (Fig. 1). A 2.8-kb subclone, which complemented *rad51* mutations, has been sequenced (see below) and was found to contain three open reading frames (ORFs). The ORFs have been named ORF1, ORF2, and ORF51 for ease of discussion and are shown in Fig. 1A. Subclones in multicopy plasmids that contain all of ORF1 or ORF2 but only part of ORF51 did not complement the *rad51-1* mutation, while those containing all of ORF51 did complement the mutation (Fig. 1B). Furthermore, a fragment containing ORF51 and approximately 600 bp of upstream sequence cloned into a single-copy centromeric plasmid also complemented *rad51-1* (Fig. 1B and Fig. 2).

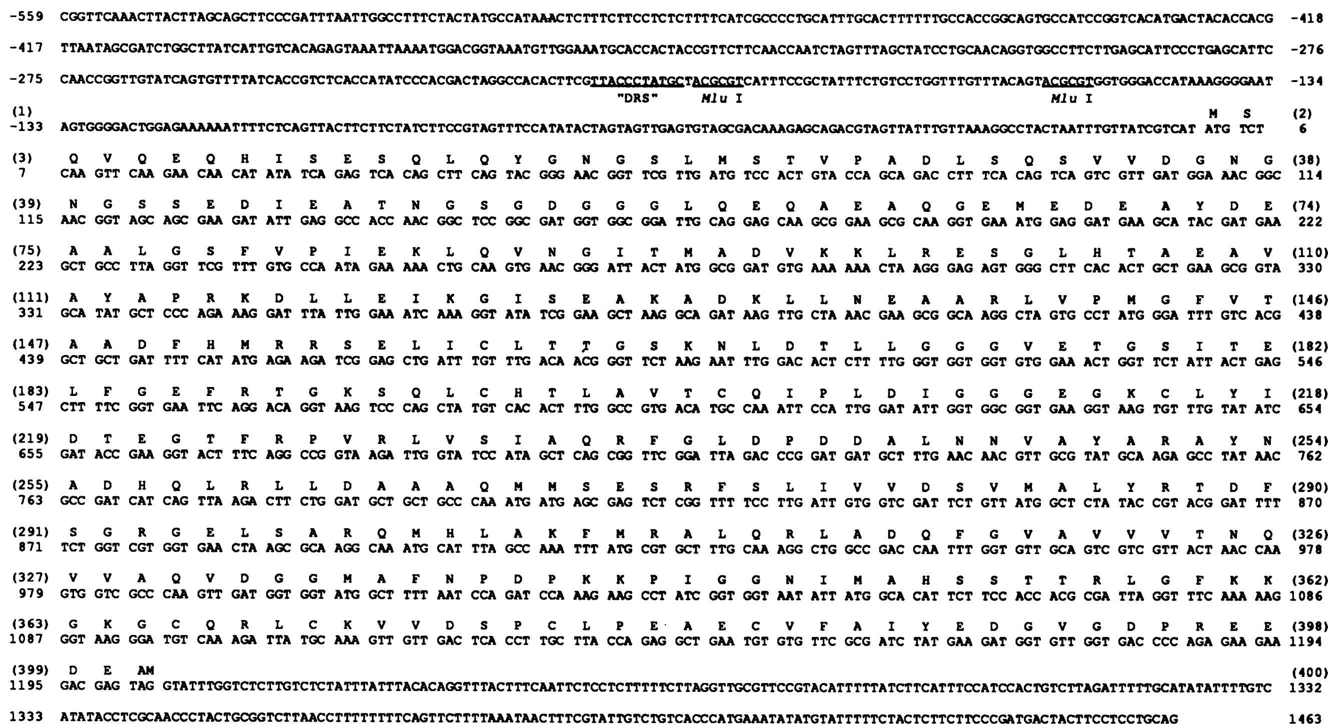

FIG. 3. Nucleotide sequence of the *RAD51* coding and upstream region. Nucleotide sequence positions are shown at the sides, with the first nucleotide of the first methionine codon of RAD51 protein designated +1. The putative amino acid sequence for RAD51 protein is listed above the nucleotide sequence. Amino acid sequence positions are shown in parentheses, with the first methionine labeled 1. Nucleotide sequences upstream of the *RAD51* coding region which are homologous to an internal section of the *RAD54* DRS element or the recognition site of restriction endonuclease *Mlu*I are underlined and labeled.

Since only ORF51 is contained entirely within this fragment, this result indicates that ORF51 is the coding region of *RAD51*.

A chromosomal deletion removing ORF51 and replacing it with the *LEU2* gene was isolated (Fig. 1C) in order to compare deletions of *RAD51* to previously characterized *rad51* mutations. The construct was verified by Southern (data not shown) and Northern hybridization (see below). Strain CG378Δ51 in which *RAD51* had been deleted showed no obvious growth defects at temperatures of 23, 30, or 37°C, indicating that *RAD51* is not an essential gene. Survival curves showed that a strain in which *RAD51* had been deleted was slightly more sensitive to X-ray irradiation than a closely related strain carrying the *rad51-1* mutation (Fig. 2). The increased sensitivity could indicate some slight leakiness in the *rad51-1* mutation or could be the result of a modifier present in the *rad51-1* strain. The meiotic phenotype of strain XGB4, a diploid with both copies of *RAD51* deleted, was similar to those of diploids homozygous for the *rad51* mutation (19, 40). It showed both decreased sporulation efficiency and inviable spores. No viable spores were observed upon dissection of 20 four-spored tetrads arising from XGB4. An isogenic wild-type strain, XGB5, yielded 100% spore viability in 15 tetrads.

Attempts at constructing a deletion of ORF1, which is located upstream of *RAD51*, were successful only in diploid strains. Strain CG378ΔORF1 in which one chromosomal copy of ORF1 was replaced by the *LEU2* sequence was constructed. Tetrad analysis of this strain yielded two viable spores and two dead spores, with the mutant *leu2* allele segregating consistently with the live spores, indicating that spores in which ORF1 had been deleted were inviable. Thus, ORF1 appears to be an essential gene of unknown function.

Nucleotide sequencing and sequence analysis of *RAD51*. The nucleotide sequence of the *RAD51* gene and approximately 1.4 kb of neighboring sequence were determined by the method of Sanger et al. (52). The nucleotide sequence and the deduced amino acid sequence of RAD51 are presented in Fig. 3. The sequence is 100% confirmed for the region containing all of ORF51 and 300 bp of the upstream region. Of the three ORFs defined by nucleotide sequence analysis, ORF2 and ORF51 are predicted to be transcribed in the same direction, while ORF1 would be transcribed divergently. A protein 400 residues long and with a molecular weight of 42,968 is predicted to arise from translation of ORF51. Preliminary sequence data on ORF1 and ORF2 are available from GenBank.

Comparisons of the putative RAD51 protein sequence with previously defined functional motifs and comparisons of the nucleotide sequence upstream of the coding region of *RAD51* with known transcriptional regulatory elements revealed some conserved features. At residues 185 to 194 is the GEFRT**GKS**QL sequence, which matches the conserved sequence motif found in many ATP/GTP-binding proteins (61). No motifs consistent with DNA binding, such as a leucine zipper or zinc finger, were readily apparent, although residues from 40 to 80 and 95 to 135 could form helix-turn-helix DNA binding domains (42). The sequence upstream of ORF51 includes two *Mlu*I restriction sites (underlined in Fig. 3). These sequences have been shown to be sufficient to regulate message levels during the cell cycle (33, 39, 59). Additionally, upstream of the two *Mlu*I sites, a short region

FIG. 4. Sequence comparisons with RAD51 protein. Identical amino acids are boxed in black. Gaps required to attain the best fit are shown as dashes. Conserved but not identical residues are not demarcated. Numbers in parentheses indicate the regions of each protein which are involved in the homology. Position 1 for RAD51 protein is the first methionine residue. (A) Amino acid sequence comparison between RAD51 and RAD57 proteins. (B) Sequence comparison between RAD51 protein and the *E. coli* RecA protein. The first amino acid of RecA is the first alanine residue.

(underlined in Fig. 3) was found to match with 11 bases at the 5′ end of the 29-bp *RAD54* DRS (7). The *RAD54* DRS is required for the X-ray induction of *RAD54* transcript levels.

Amino acid sequence comparison has previously uncovered a region of homology between the RAD51 and RAD57 proteins (30) (Fig. 4A). There is approximately 50% identity and 80% conservative homology over a continuous region between residues 159 to 231 of the RAD51 protein and residues 100 to 172 of the RAD57 protein. Overall, the two proteins share homology of 26% identity over 64% of RAD51 and 44% of RAD57. The highly conserved region overlaps the putative nucleotide binding site discussed above. No other significant sequence homology was found between the predicted amino acid sequence of RAD51 and those of other reported RAD proteins in *S. cerevisiae*.

To determine whether *RAD51* shares homology with other eucaryotic or procaryotic repair genes, a computer analysis was performed comparing the RAD51 amino acid sequence against known sequences in data bases. The results indicated that RAD51 protein shares a moderate amount of homology with the bacterial strand exchange protein RecA (47). A sequence alignment of the two proteins is shown in Fig. 4B. A region extending from residues 3 to 227 in RecA and 126 to 362 in RAD51 has 26.5% identity and 67% conservative homology. This is over 59 and 64% of RAD51 and RecA, respectively. The homology is in two regions: surrounding the putative nucleoside triphosphate-binding sequence of RAD51 and sequences closer to the carboxy terminus of RAD51.

Identification of the *RAD51* transcript. Northern hybridization analysis of total yeast RNA was undertaken in order to determine whether *RAD51* transcript was observable in a log-phase population of cells. A double-stranded probe that overlapped all three ORFs (ORF1, ORF2, and ORF51) hybridized to two transcripts: one approximately 1.6 kb long and the other approximately 0.9 kb long (Fig. 5). In order to determine which ORFs were being transcribed, both single-stranded and double-stranded DNA probes overlapping the three ORFs were utilized. The 1.6-kb transcript was observed only with probes which overlapped ORF51 and which hybridized specifically to messages transcribed in the direction predicted for transcription of ORF51 (data not shown). Additionally, this transcript was absent in the RNA isolated from strains CG378Δ51 (haploid) and XGB7 (diploid) (Fig. 5) in which ORF51 had been deleted. This shows that the 1.6-kb message originated from *RAD51*. Probes overlapping ORF1 and ORF2 detected only the single 0.9-kb message. This transcript was observed only when the probes which overlapped ORF1 and hybridized to messages transcribed

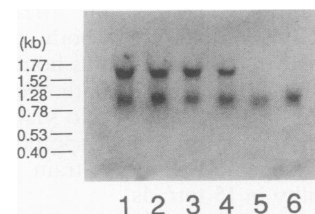

FIG. 5. Northern blot of total yeast RNA probed with a DNA fragment extending from *Xba*I to *Pst*I (shown in Fig. 1B as subclone 1) which overlaps ORF1, ORF2, and *RAD51* (ORF51). The position and size of simultaneously run RNA size standards are shown to the left. Lane 1, X2180; lane 2, X2180-1A; lane 3, CG7879; lane 4, CG378; lane 5, XGB7 (*rad51*::*LEU2*/*rad51*::*LEU2*); lane 6, CG378Δ51 (*rad51*::*LEU2*).

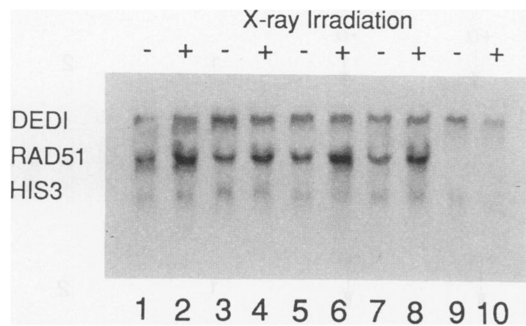

FIG. 6. Effect on the level of *RAD51* transcript after X-ray irradiation of various haploid or diploid strains. Cells were X-ray irradiated with a dose of 40 krads or were not irradiated. Total RNA was analyzed by Northern hybridization utilizing probes specific for *RAD51*, *HIS3*, and *DED1* transcripts. *DED1* transcript levels were not seen to vary after X-ray exposure in multiple experiments and were used as in-lane loading controls. The transcripts are indicated at left. Strains X2180 (diploid) (lanes 1 and 2), X2180-1A (haploid) (lanes 3 and 4), CG7879 (diploid) (lanes 5 and 6), CG378 (haploid) (lanes 7 and 8), and XGB7 (*rad51::LEU2/rad51::LEU2*) were irradiated with 40 krads (+) or were not irradiated (−).

divergently from ORF51 were utilized (data not shown). This is consistent with transcription of ORF1 but not ORF2. No transcript corresponding to ORF2 was detected.

***RAD51* transcript levels increase after exposure to X rays.** We examined whether levels of *RAD51* transcript increased in cells after exposure to X-ray radiation. Wild-type diploid yeast cells are much more resistant to X-ray irradiation than haploid cells (3). While it is known that a majority of the increased radioresistance observed in **a**/α diploids is attributable to the redundant nature of a diploid genome and their capacity for recombinational repair, it is also possible that these cells may have higher activities of repair processes than haploids. To examine this question, we determined the response of *RAD51* transcript levels in both haploids and diploids. Haploid and diploid strains were given a dose of 40 krads of X-ray radiation and incubated for approximately 60 min. Steady-state levels of *RAD51* transcript were approximately 3 times higher in irradiated haploids and 6 times higher in irradiated diploids than in unirradiated control samples (Fig. 6). A probe homologous only to ORF51 was used to detect *RAD51* transcripts, while *DED1* and *HIS3* transcript levels (58) were used as internal controls in each lane (*DED1* and *HIS3* transcript levels were not seen to vary after X-ray exposure in repeated experiments). *DED1* levels were used to normalize *RAD51* signal levels in lane-to-lane comparisons. *LEU2* and *ARG4* transcript levels have also been used as controls with similar results (data not shown). Quantities of hybridized signal were determined both by densitometer measurements of autoradiograms and using a Molecular Dynamics Storage Phosphor Imager (27).

Levels of *RAD51* transcript increase rapidly after low doses of X rays. To determine the dose-dependent response of *RAD51* transcript levels, cells were exposed to increasing doses of X rays ranging from 0 to 60 krads. A near-maximal response after exposure to doses as low as 5 krads was seen in both haploid (Fig. 7) and diploid strains (data not shown). Doses from 0 to 5 krads led to a progressive increase in the level of *RAD51* transcript (Fig. 7B). Here we have defined a low dose as one that yields low levels of lethality in repair-competent (RAD^+) cells but substantial lethality in populations that are defective in repair. Doses below 5 krads in

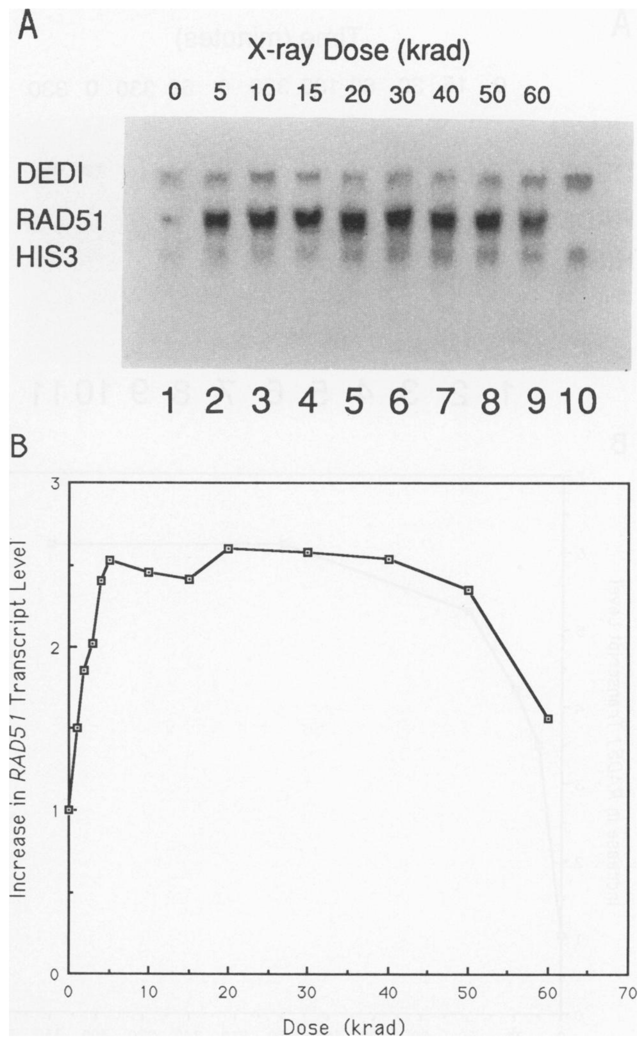

FIG. 7. Dose-dependent response of *RAD51* transcript levels. Samples were exposed to doses of X rays ranging from 0 to 60 krads. (A) Northern blot of irradiated samples. Transcripts corresponding to *RAD51*, *DED1*, and *HIS3* are indicated to the left. Lane 1, CG378, unexposed; lanes 2 to 9, CG378 exposed to various doses of X rays as indicated; lane 10, XGB7 (*rad51::LEU2/rad51::LEU2*) as the control. (B) Graphic representation of *RAD51* transcript levels from two separate experiments is shown. *RAD51* transcript levels were normalized to *DED1* transcript levels in each lane and then compared with each other. The induction ratio was derived by normalizing the transcript level of the unexposed sample (lane 1) to 1.

haploid cells and up to 40 krads in diploid cells typically cause only moderate amounts of lethality in repair-competent cells. The time dependence of the increase in *RAD51* transcript levels was determined in cells given a dose of 40 krads of X rays. *RAD51* transcript levels increased rapidly, with the increase readily detectable 15 min after exposure, and reaching maximal levels within 30 to 60 min (Fig. 8). These levels were maintained for at least 5 h. These data show that *RAD51* transcript levels increase rapidly after relatively low doses of irradiation.

***RAD51* is cell cycle regulated.** Sequences upstream of *RAD51* are identical to those known at other loci to be involved in regulation of transcript levels throughout the cell

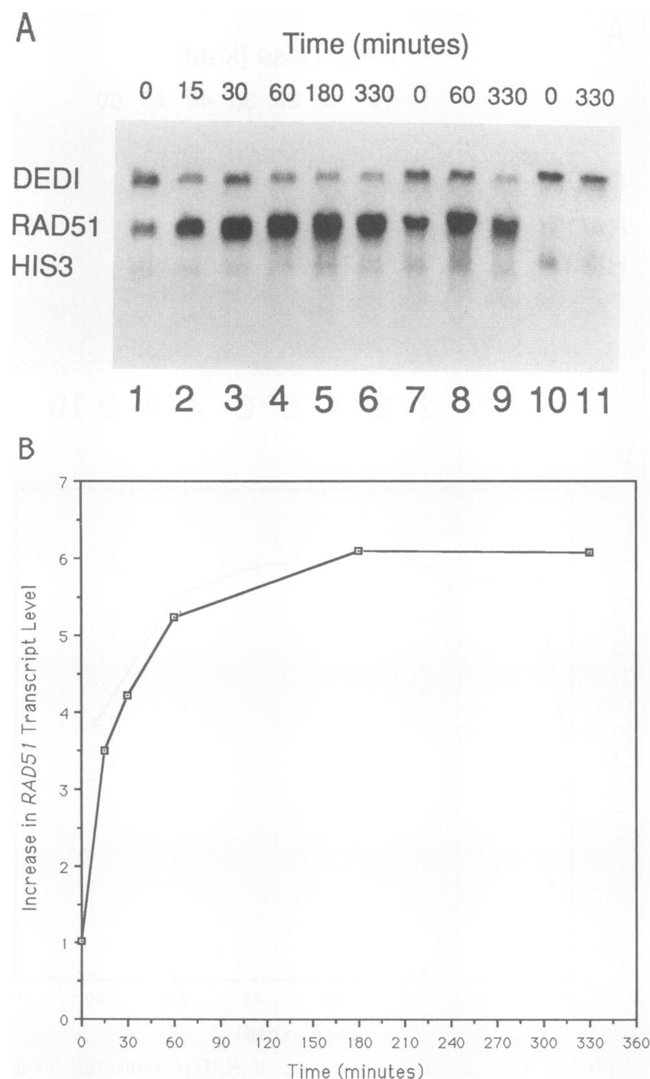

FIG. 8. Time course of X-ray induction of *RAD51* transcript levels. (A) Northern hybridization of samples which were allowed increasing times of incubation following X-ray exposure. *RAD51*, *DED1*, and *HIS3* transcripts are indicated to the left. Strains X2180 (lanes 1 to 6), X2180-1A (lanes 7 to 10), and XGB7 (*rad51*::*LEU2*/*rad51*::*LEU2*) (lanes 10 and 11) were unexposed (lanes 1, 7, and 10) or were exposed (remaining lanes) to 40 krads of X rays and incubated for the length of time indicated above each lane. (B) Graphic representation of *RAD51* transcript levels shown in panel A (lanes 1 to 6) with respect to time following X-ray exposure. Quantification was done as described in the legend to Fig. 7B.

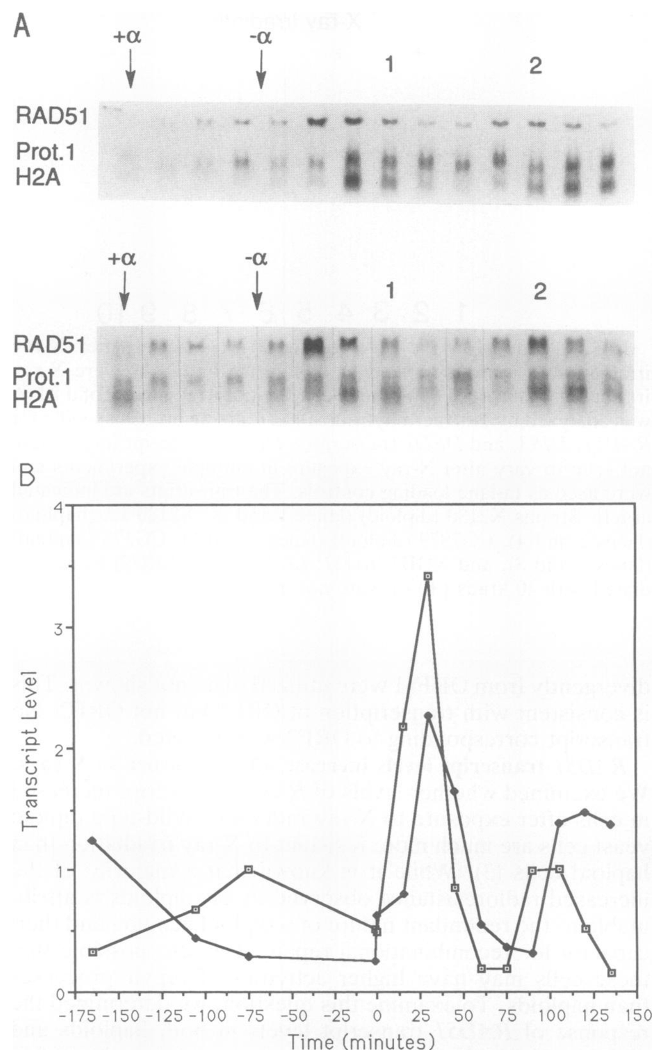

FIG. 9. Cell cycle dependence of *RAD51* transcript level is shown. Yeast cultures were synchronized in the G_1 phase of the cell cycle by exposure to α-factor and then released. (A) Northern hybridizations showing two separate experiments. Exposure to α-factor (+α) and release (−α) are indicated by arrows at the top of each blot. RNA samples were taken at 15-min intervals after release; 1 and 2 indicate 1 and 2 h after release, respectively. Northern blots were probed for *RAD51* transcript, the histone *HTA1* (H2A) transcript, and *Protein1* transcript (Prot.1). Transcripts are indicated to the left. Levels of the cell cycle-independent *Protein1* transcript were used as exposure controls on autoradiograms. (B) Graphic representation of *RAD51* and *HTA1* (H2A) transcript levels throughout the cell cycle. *RAD51* and *HTA1* (H2A) transcript levels from the lower Northern blot depicted in panel A were normalized by using levels of *Protein1* transcript in each lane. This ratio was plotted with respect to time; α-factor was added at −165 min and removed at 0 min. Symbols: ⊡, *RAD51* transcript level; ◆, H2A transcript level.

cycle (*Mlu*I sites underlined in Fig. 3). This finding, combined with the observations that cells exposed to DNA-damaging agents undergo cell cycle arrest (62) and that *RAD51* transcript levels increase after exposure to X rays, led us to examine the question of whether *RAD51* was cell cycle regulated. In order to determine whether transcript levels varied during the cell cycle, yeast cultures were synchronized in G_1 with the α-mating pheromone and then released by removal of the pheromone. Two separate experiments that show that the levels of *RAD51* transcript vary in a cell cycle-dependent fashion are presented (Fig. 9A). Cycling of transcript levels through two cell cycles can be seen, with the second cycle showing less synchrony, as expected. *RAD51* transcript levels peaked before those of the yeast histone H2A transcript, indicating that *RAD51* transcript levels increase near the G_1-to-S transition; H2A transcript levels were used as an internal control for the quality of cell cycle synchrony and as a timing mark for cell cycle stage (21). Levels of *RAD51* transcript were quantified and normalized using levels of the *Protein1* transcript as in-lane controls. *Protein1* is a gene adjacent to the histone

gene encoding H2A but is expressed independently of the cell cycle (21). Levels of *RAD51* transcript were found to increase approximately sevenfold during the first of the two observed cell cycles (Fig. 9B). A small, but reproducible, increase in *RAD51* transcript levels after extended incubation in α-factor was also observed (Fig. 9) (2a). This may be due to direct action of α-factor or be in response to an indirect effect, such as α-factor-induced inhibition of DNA synthesis (22) and/or any related degradation of genomic DNA.

***RAD51* transcript levels respond to X rays outside S/G_2.** The fact that *RAD51* is cell cycle regulated suggested the possibility that increases in its transcript level after X-ray irradiation could be the result of partial synchronization of irradiated cultures at a stage when *RAD51* expression is normally high, rather than a direct result of X-ray exposure. In order to establish whether X-ray induction and cell cycle regulation of *RAD51* transcript levels could be uncoupled, we attempted to determine whether an increase in *RAD51* transcript levels could be induced outside the period of the cell cycle when it is normally high. Levels of *RAD51* transcript following X-ray irradiation were assayed in cells that were held in G_1 with α-factor. The α-factor cell cycle block is prior to the point in the cell cycle when *RAD51* transcript peaked (see above). G_1-arrested cells exposed to 40 krads of X-ray radiation had increased *RAD51* transcript levels, compared with that of unexposed arrested control samples, similar to that seen in irradiated log-phase populations (data not shown). G_1 arrest was monitored by both cell morphology and transcript levels of both the cell cycle-regulated yeast histone H2A and the cell cycle-independent transcript from the *Protein1* gene. Thus, damage induction and cell cycle regulation of *RAD51* transcript levels appear to be at least partially uncoupled.

DISCUSSION

Although the genetic phenotype associated with mutations at the *RAD51* locus has been well characterized with respect to deficiencies in recombinational repair and meiosis (16, 40, 41), little molecular analysis on this gene has previously been done. In this communication, we have presented the nucleotide sequence and transcriptional regulation of the *RAD51* gene. The deduced amino acid sequence of RAD51 predicts a 400-residue, ~43,000-molecular-weight protein that contains a nucleotide cofactor binding motif. Northern hybridization analysis has shown that *RAD51* transcripts are detectable in a log-phase population of cells and that steady-state transcript levels increase three- to sixfold after cells are exposed to X rays. Additionally, *RAD51* transcript levels were found to be cell cycle regulated, peaking around the G_1-to-S transition.

The subcloning data presented here vary from those in the original report on *RAD51* (6). Calderon et al. (6) found that a subclone of their *rad51-1*-complementing genomic fragment containing all of ORF51 (Fig. 1, subclone 3) in YRp7 did not complement the *rad51-1* mutation, implying that *RAD51* activity required sequences outside this region. While we have verified the failure of subclone 3 to complement *rad51-1* in YRp7, we found that the same fragment in the plasmid originally used to clone *RAD51* (YEp13) does complement the *rad51-1* mutation. Because we have also found that other subclones require only ORF51 to complement defects in *RAD51*, we believe that the previously published result involving subclone 3 is the result of a cloning artifact linked to the use of the YRp7 plasmid.

Analysis of the nucleotide sequence upstream of the coding region of *RAD51* uncovered sequence elements which have been previously characterized as being sufficient for regulation of transcript levels at other loci. An 11-bp match to an internal section of the 29-bp DRS of the yeast *RAD54* gene (7) was found. *RAD54* is required for recombinational repair and, like *RAD51*, levels of *RAD54* transcript have been shown to increase three- to fivefold in response to X rays (8, 13). The increase is dependent on the presence of the DRS (7). While a match for the entire *RAD54* DRS was not found, it is not currently known whether all or only a part of the 29-bp sequence defined as the *RAD54* DRS is required for X-ray-dependent regulation of the *RAD54* transcript (7). Experiments are in progress to determine whether the conserved sequence between *RAD51* and *RAD54* plays a role in the regulation of *RAD51*.

The transcript levels of both *RAD51* and *RAD54* increase three- to fivefold after exposure to X-rays independent of cell cycle progression (7, 8), and while no clear phenotype was seen for a transcriptionally uninducible *RAD54* construct (7), it may be significant that *RAD51* transcript levels respond rapidly to low doses of a DNA-damaging agent. In *S. cerevisiae*, Budd and Mortimer (4) have shown that X-ray doses as low as 1.5 krads increase cell survival to a subsequent larger exposure and that the increased survival is dependent on new protein synthesis. This result was taken to indicate that a damage-inducible response exists in *S. cerevisiae* which requires relatively low doses for activation. Consistent with this, increases in *RAD51* transcript levels were readily detectable in this dose range over a time period of 15 to 30 min. It is not clear from the data presented whether the progressive increase in *RAD51* transcript levels with increasing doses observed at doses below 5 krads (shown in Fig. 7B) represents a uniform response of the entire cell population or is the response of a subset of the population that has accumulated a critical level or some specific type of damage. The data do indicate that exposure to relatively low doses of X rays is sufficient to increase levels of *RAD51* transcript.

In addition to the homology to the *RAD54* DRS, two cleavage sites of restriction enzyme *Mlu*I are present upstream of the coding region for *RAD51*. A number of genes which are required in DNA metabolism, including *CDC9*, *POL1*, *CDC8* (thymidylate kinase), *CDC21* (thymidylate synthase), and *PRI1* (DNA primase), are believed to be transcriptionally regulated in a coordinate fashion during the cell cycle (24, 26). Transcript levels of these genes increase at the same time, shortly before the expression of histone genes, but after the cessation in cell cycling caused by mutations in *CDC28* (25) or exposure to α-factor (33, 39). *Mlu*I or *Mlu*I-like sequences are present in the upstream regions of all of these genes, and in the case of *CDC9* (33), *POL1* (59), and *CDC21* (39), these sequences have been shown to be required for cell cycle regulation of expression. In addition, these sequences are sufficient for cell cycle regulation of transcript levels when placed upstream of a reporter gene. The distances of the *Mlu*I sequences from the putative coding region of *RAD51* and the spacing between the two *Mlu*I sites (−150 bp away from the first methionine of the RAD51 protein, with 35 bp between the two sites) are similar to those found at *POL1* (−202 bp, 29 bp) and *CDC21* (−117 bp, 31 bp). However, there is no specific sequence homology in the intervening region. Our findings that *RAD51* is transcriptionally regulated during the cell cycle slightly before increases occur in H2A expression and concurrently with increases in *POL1* transcript levels (data not shown)

indicate that *RAD51* may be another member of this coordinately regulated group of genes. It should be noted that while many cell cycle-regulated DNA synthesis genes containing *Mlu*I sequences are also inducible by DNA-damaging agents, the presence of *Mlu*I sequences alone is not sufficient for damage regulation (25, 26). Similarly, the presence of *Mlu*I sequences is not required for regulation of many damage-inducible genes in *S. cerevisiae* (11, 23, 56). Further experiments will be required to determine what role, if any, *Mlu*I sequences play in the regulation of *RAD51*.

The period of the cell cycle during which *RAD51* transcript levels were observed to increase, late G_1 to S, was earlier than the stage (G_2) at which X-ray-induced cessation of the cell cycle is known to occur (62). However, S makes up a small fraction of the normal yeast cell cycle, and the timing of the cell cycle is affected by irradiation, so it is possible that increased levels of *RAD51* transcript after exposure to X rays may be due to partial synchronization of the irradiated cells at a stage when *RAD51* is normally highly expressed. The fact that irradiated cells held in G_1 with α-factor showed increases in the levels of *RAD51* transcript similar to those observed for X-irradiated log-phase cells is evidence that the X-ray-dependent transcriptional regulation is not solely due to the observed cell cycle regulation. Conversely, it is unlikely that the observed cycling of *RAD51* transcript levels was due to an α-factor-induced artifact, since regulation through two separate cycles was readily apparent. This is similar to the results obtained for other yeast loci involved in repair, such as *RNR1* (ribonucleotide reductase subunit), *POL1*, and *CDC9* where transcript levels are known to be regulated during the cell cycle and also inducible by DNA-damaging agents in a cell cycle-independent fashion (12, 25, 44).

The fact that *RAD51* is cell cycle regulated, in addition to responding to DNA-damaging agents, is somewhat surprising. While the presence of the *Mlu*I sites indicated that *RAD51* was likely to be cell cycle regulated, among other damage-inducible genes whose primary mitotic function appears to be DNA repair, such as *RAD2*, *RAD6*, *RAD7*, *RAD18*, *RAD23*, *RAD54*, *PHR1* (photolyase required for photoreactivation of DNA damage), and *RNR3* (large subunit of ribonucleotide reductase), *RAD51* represents the first example of a gene that is cell cycle regulated. This shows that some aspect of the regulation of *RAD51* differs from these other repair genes. Furthermore, the cell cycle regulation of *RAD51* implies some cell cycle-dependent activity; increased recombinational repair capacity may be required around the period of DNA synthesis. The induction of expression of genes required for DNA synthesis and of genes involved in DNA repair would seem to share at least one common goal: maintenance of the integrity of genomic DNA, either during replication or after damage. Thus, both the cell cycle regulation and X-ray regulation of *RAD51* transcript could fit with its known role in repair.

What do the observed homologies between RAD51 and RAD57 proteins and RAD51 and RecA proteins reveal about *RAD51*? *rad57* mutations fall into the same radiation epistasis group as those in *RAD51* (18, 19). However, strains in which the *RAD57* locus has been deleted have a cold-sensitive phenotype with respect to X-ray sensitivity (53). The region of the homology between RAD51 and RAD57 includes the putative nucleotide-binding sequence of RAD51 and extends in both directions. When placed in a multicopy vector, *RAD51* does not complement the X-ray sensitivity of yeast strains in which *RAD57* has been deleted (data not shown), implying that overexpression of *RAD51* does not compensate for the loss of *RAD57* activity. The region of shared homology may represent conservation of the nucleotide-binding sequences coupled with a common ancestry of this region. Weaker homology was found between the bacterial strand exchange protein RecA and the RAD51 protein. Searches with both FASTA (43) and BLAST (2) detected RecA protein from a variety of bacteria as being ~30% homologous to RAD51 over an 240-residue stretch with a *z* value (32), derived from comparisons with randomly shuffled sequences, of ~8. A *z* value above 6 combined with greater than 25% homology over a significant portion of the protein is thought to indicate the likelihood of a common ancestor and the possibility that the homology is of biological significance (10, 32). The most extensive homology is seen in portions of RecA that are thought to be involved in the binding and hydrolysis of ATP (31, 47). Biochemical and functional complementation studies will be required to assess the significance of these homologies.

In *S. cerevisiae*, the response to damage is complex, involving multiple pathways of regulation and numerous overlapping activities that play various roles in DNA repair and DNA metabolism. Previous work with *RAD51* has shown that it is required for the repair of DNA damaged by X-irradiation. Here we have shown it to be a gene that responds to both irradiation- and cell cycle-related signals with increased transcript levels. Further work will be necessary to determine which sequences are required for the transcriptional regulation of *RAD51* and to answer questions concerning their roles in cell cycle regulation and DNA repair.

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