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UBL1, a Human Ubiquitin-like Protein Associating with Human RAD51/RAD52 Proteins

ZHIYUAN SHEN,* PAIGE E. PARDINGTON-PURTYMUN,* JARMON C. COMEAUX,* ROBERT K. MOYZIS,† AND DAVID J. CHEN*,[1]

*DNA Damage and Repair Group, Life Sciences Division, and †Center for Human Genome Studies, Los Alamos National Laboratories, Los Alamos, New Mexico 87545

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The RAD51/RAD52-dependent DNA repair pathway is involved in DNA recombination and DNA double-strand break repair in yeast. Although many proteins in the RAD51/RAD52-dependent DNA repair pathway have been identified in yeast, a novel protein(s) that functions with RAD51/RAD52 may also exist in humans. Using a yeast two-hybrid system, we have identified a 12-kDa protein that associates with the human RAD51 and RAD52 proteins. This protein shares significant amino acid homology with the yeast protein SMT3, which functionally associates with the yeast mitosis fidelity protein MIF2. It also shares moderate homology with ubiquitin and several other proteins, including the N-terminus of the RAD23 protein and a ubiquitin cross reacting protein. Therefore, the gene is tentatively designated *UBL1* for ubiquitin-like 1. The *UBL1* mRNA is expressed in many human tissues, most highly in testis. The *UBL1* gene is mapped to chromosome 2q32.2–q33, and a related sequence may be located on chromosome 1q23–q25.

INTRODUCTION

DNA double-strand break (DSB)[2] is one of the most important forms of DNA damage caused by ionizing radiation. Efficient repair of DSB is essential for the cell to recover from radiation damage. In yeast, the *RAD52* epistasis group genes encode proteins involved in DSB repair and recombination (Friedberg *et al.*, 1991). Several yeast genes in the RAD52-dependent repair pathway have been cloned. These include *RAD50*, *RAD51*, *RAD52*, *RAD53* (*SPK1*), *RAD54*, *RAD55*, and *RAD57* (Adzuma *et al.*, 1984; Alani *et al.*, 1989; Basile *et al.*, 1992; Emery *et al.*, 1991; Kans and Mortimer, 1991; Lovett, 1994; Schild *et al.*, 1983; Shinohara *et al.*, 1992; Zheng *et al.*, 1993). It is known that RAD51 is a RecA-like protein that has DNA-binding, ATP-binding, and DNA strand-exchange activities, while RAD54 contains regions homologous to DNA helicase. Interactions among RAD52, RAD51, RAD55, and RAD57 have been identified (Firmenich *et al.*, 1995; Hays *et al.*, 1995; Shinohara *et al.*, 1992; Smith and Rothstein, 1995). RAD52 and RAD54 are highly expressed during yeast meiosis (Cole *et al.*, 1989). Yeast RAD51 and RAD53 also have cell-cycle-dependent expression (Basile *et al.*, 1992; Zheng *et al.*, 1993), and RAD53 also participates in cell cycle control (Sanchez *et al.*, 1996).

In humans, only RAD51 and RAD52 have been reported (Muris *et al.*, 1994; Shen *et al.*, 1995; Shinohara *et al.*, 1993). The human RAD51 protein has activities similar to those of yeast RAD51, including DNA binding and a specific interaction with RAD52 protein (Benson *et al.*, 1994; Shen *et al.*, 1996a). Also, we have found that human RAD52 protein self-associates (Shen *et al.*, 1996b). More recently, RAD51 has been shown to accumulate in the synaptonemal complex, indicating involvement of RAD51 in meiosis and chromosome recombination (Haaf *et al.*, 1995). Human RAD52 overexpression in monkey cells enhances their resistance to radiation and increases the frequency of homologous recombination (Park, 1995). However, little is known about how these interactions might fit into the context of DSB repair.

The importance of DSB repair factors is not limited to their roles in modulating radiation sensitivity, but includes their roles in DNA recombination, in repair of alkylating and cross-linking agent-induced DNA damage, and in some physiological processes. For example, mutations in the yeast *RAD52* group genes result in increased sensitivity to the alkylating agent methyl methanesulfonate (MMS). Indeed, the yeast *RAD52* gene was cloned by its ability to complement MMS sensitivity (Adzuma *et al.*, 1984; Schild *et al.*, 1983). The *RAD52* gene has been found to function in plasmid

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[1] To whom correspondence should be addressed at LS-6, MS M888, Life Sciences Division, Los Alamos National Lab, Los Alamos, NM 87545. Telephone: (505) 667-2789. Fax: (505) 665-3024. E-mail: dchen@telomere.lanl.gov.

[2] Abbreviations used: DSB, DNA double-strand break; UBL1, a ubiquitin-like protein 1, 12 kDa; Gal4-DA, the DNA activation domain (amino acids 768–881) of Gal-4 protein; Gal4-DB, the DNA binding domain (amino acids 1–147) of Gal-4 protein; *LacZ*, β-galactosidase gene; MMS, methyl methanesulfonate.

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recombination induced by the cross-linking agent psoralen (Han and Saffran, 1992; Saffran *et al.*, 1992). In mammalian immune systems, various types of antibody genes or antigen receptor genes are generated by V(D)J rejoining, where a DNA strand-break is introduced, and the DSB repair machinery complies. In other cases, integration of viral DNA into the host genome may require DSB repair-associated mechanisms. During meiosis, mechanisms of chromosome exchange (recombination) may overlap with DSB repair. However, the mechanism of the RAD52-associated repair pathway is poorly understood, especially in human cells.

To elucidate the DSB repair mechanism in humans, one of the first steps is to identify human proteins involved in DSB repair. It is assumed that some proteins involved in the same repair pathway may associate with each other in the cells. Therefore, one strategy to identify novel proteins participating in the RAD51/RAD52-dependent repair pathway is to identify proteins that actually interact with known proteins, such as RAD51 and RAD52. We have initiated experiments to identify a RAD51/RAD52 interacting protein(s) by using the yeast two-hybrid approach. This approach would also identify a gene(s) involved in meiosis. In this article, we report the cDNA cloning of a ubiquitin-like gene by using the two-hybrid approach. This gene is designated *UBL1* for ubiquitin-like 1, as recommended by the Human Gene Nomenclature Committee. Tissue-specific mRNA expression shows that *UBL1* expresses highest in testis. By FISH analysis using a cDNA probe and PCR analysis using a panel of mouse hybrid cells that contain a single human chromosome, the *UBL1* gene was mapped to chromosome 2q32.2–q33; a related sequence may be located on chromosome 1q23–q25.

MATERIALS AND METHODS

Materials. The yeast strains SFY526 (MATa; *ura3-52; his3-200; ade 2-101; lys 2-801; trp 1-901; leu2-3,112; can^r; gal4-542; gal80-538; URA3::GAL1-LacZ*) and HF7c (MATa, *ura3-52; his3-200; lys2-801; ade2-101; trp1-901; leu2-3,112; gal4-542; gal80-538; LYS2::GAL1-HIS3; URA3::(GAL4 17mers)$_3$-CYC1-LacZ*) were purchased from Clontech Laboratories (Palo Alto, CA). SFY526 has a *LacZ* reporter gene fused downstream of the Gal-1 promoter. HF7c has a *His* reporter gene controlled by a Gal-1 promoter and a *LacZ* reporter gene controlled by a CYC1 promoter. The Gal-4 DNA activation domain (Gal4-DA) fused cDNA library in pACT vector was also purchased from Clontech. Two hybrid vectors for RAD51 and RAD52 proteins have been previously reported (Shen *et al.*, 1996a,b). SD and YPD media/plates were prepared as described in the two-hybrid system manual (Clontech Laboratories Inc.).

Library screen using the yeast two-hybrid system. The library screening for this yeast two-hybrid system was performed according to the Matchmaker Kit manual (Clontech Laboratory Inc.). Briefly, HF7c yeast was first transfected with pGBT9/RAD51 expressing the Gal4-DB/RAD51 fusion protein using the polyethylene glycol/lithium acetate method. Yeast HF7c with pGBT9/RAD51 was subsequently transfected with cDNAs isolated from the pACT library. These co-transfected yeast were grown in SD/Try$^-$/Leu$^-$/His$^-$ agar plates. A positive clone in SD/Trp$^-$/Leu$^-$/His$^-$ should contain a pACT plasmid coding for a Gal4-DB/RAD51 interacting protein or a protein that is able to activate the reporter gene (*His*) without the RAD51 protein.

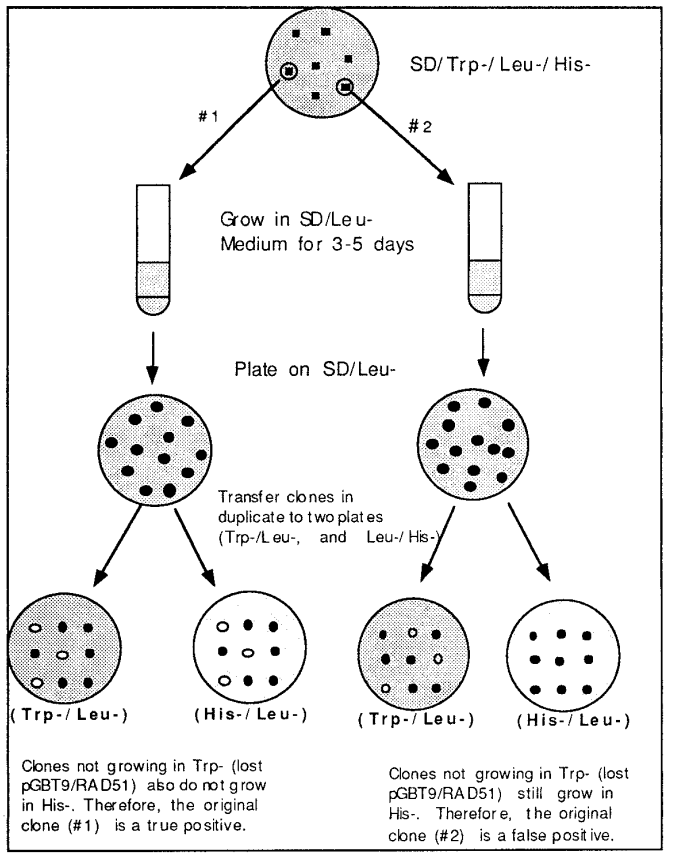

FIG. 1. Procedures to eliminate false positives in a yeast two-hybrid screen. See text for details.

The second case is a false positive and was to be eliminated. To do this, the procedure illustrated in Fig. 1 was used. A clone grown on SD/Trp$^-$/Leu$^-$/His$^-$ was grown in SD/Leu$^-$ medium for 5 days and then plated onto SD/Leu$^-$ agar plates. Due to Try auxotrophs, some of the yeast grown on SD/Leu$^-$ agar plates may have lost plasmid pGBT9/RAD51(Trp$^+$). Then, 25 individual colonies from SD/Leu$^-$ plates were duplicately transferred onto SD/Try$^-$/Leu$^-$ and SD/Leu$^-$/His$^-$ agar plates in an organized manner. When the original clone on SD/Trp$^-$/Leu$^-$/His$^-$ is a true positive, these clones from SD/Leu$^-$ plates, which do not grow in SD/Trp$^-$/Leu$^-$ (i.e., have lost pGBT9/RAD51), will also not grow in SD/Leu$^-$/His$^-$. If the original clone on SD/Trp$^-$/Leu$^-$/His$^-$ plate is a false positive, a colony will grow in SD/Leu$^-$/His$^-$ even if it has lost pGBT9/RAD51 (i.e., not grown in SD/Trp$^-$/Leu$^-$). Therefore, true positive clones were identified. The pACT plasmids from positive yeast clones were isolated and electroporated into HB101 cells, from which a large-scale purification of pACT plasmids was performed. The isolated plasmids were then subjected to automated DNA sequencing, as will be described later.

In vivo assay of protein interaction using the yeast two-hybrid system. A description of the protein interaction assay using the yeast two-hybrid system can be found in the manufacturer's Matchmaker manual (Clontech Laboratories) and previous publications (Shen *et al.*, 1996a,b). Briefly, plasmids for the two fusion constructs (one fused with the Gal4-DB, the other fused with Gal4-DA) were cotransfected into the genetically constructed yeast cells SFY526. Transformed yeast cells were grown on Trp$^-$/Leu$^-$ synthetic agar plates for 3 days to select yeast clones bearing both fusion vectors. To measure the expression of the β-galactosidase (*LacZ*) reporter gene, which correlates with the interaction of two fusion proteins expressed from these two vectors, *LacZ* activity in three independent transformants was measured by filter assay (see Matchmaker Manual; and Shen *et al.*, 1996a,b). Quantitative *LacZ* activity in Miller's unit (Miller, 1972) was assayed according to the Matchmaker kit manual (Clontech Laboratories). Briefly, yeast from a single clone were grown

overnight in synthetic media lacking Trp/Leu. The density of yeast was determined by measuring the absorbance at 600 nm. Then, 0.1 ml of culture was mixed with 0.7 ml of Z-buffer (16.1 g/liter $Na_2HPO_4 \cdot 7H_2O$; 5.5 g/liter $NaH_2PO_4 \cdot H_2O$; 0.75 g/liter KCl; 0.246 g/liter $MgSO_4 \cdot 7H_2O$; pH 7.0), 50 μl of $CHCl_3$, and 50 μl of 0.1% SDS. *O*-Nitrophenylgalactoside (4 mg/ml) was used as substrate for *LacZ*. After 2 h of 30°C incubation, the reaction mix was centrifuged, and the absorbance of the supernatant was read at 420 nm. The *LacZ* in Miller's unit was calculated as $1000 \times [\text{OD } 420/(t \times V \times \text{OD } 600]$, where t is time of incubation, V is volume of yeast culture, and OD 600 is the absorbance of yeast culture at 600 nm.

Chromosome localization by cDNA FISH mapping. To map the chromosome, the protocol described by Heng *et al.* was used (Heng *et al.*, 1992; Heng and Tsui, 1993, 1994). Briefly, human lymphocytes were synchronized with phytohemagglutinin, and metaphase spreads were made by standard procedures. cDNA probe was biotinylated with dATP using the BRL BioNick labeling kit and hybridized to the chromosome slides. The FISH and DAPI signals were photographed separately. The DAPI chromosome pictures were superimposed on the FISH signal pictures to localize the region better.

DNA sequencing. A primer-walking strategy was used to sequence cloned plasmid cDNA inserts. Sequencing was performed with the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit and the ABI 373A automatic DNA sequencer (Applied Biosystems Inc., Foster City, CA) according to the manufacturer's protocols. Both cDNA strands were sequenced at least once. Sequence editing was performed with the SeqEd software (Applied Biosystems) on a Macintosh computer. Further sequence analysis was performed using the GCG sequence analysis software package (University of Wisconsin, Madison, WI).

Northern hybridization. A multiple-tissue Northern blot containing mRNA from leukocyte, colon, small intestine, ovary, testis, prostate, thymus, and spleen was purchased from Clontech Laboratories. This membrane was sequentially hybridized with *UBL1* cDNA, human *RAD51* cDNA, and human β-actin cDNA (provided with the blot), with stripping between each hybridization according to the protocol provided with the membrane. Hybridization probes were labeled with a random labeling kit (Amersham Corp., Arlington Heights, IL).

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   1   GGAGCGAGGTTCTGCTTACCCGAGGCCTCTGCTGTGCGGAGACCCCCGGGTGAAGCCACC
  61   GTCATCATGTCTGACCAGGAGGCCAAACCTTCAACTGAGGACTTGGGGGATAAGAAGGAA
   1         MetSerAspGlnGluAlaLysProSerThrGluAspLeuGlyAspLysLysGlu

 121   GGTGAATATATTAAACTCAAAGTCATTGGACAGGATAGCAGTGAGATTCACTTCAAAGTG
  19   GlyGluTyrIleLysLeuLysValIleGlyGlnAspSerSerGluIleHisPheLysVal

 181   AAAATGACAACACATCTCAAGAAACTCAAAGAATCATACTGTCAAAGACAGGGTGTTCCA
  39   LysMetThrThrHisLeuLysLysLeuLysGluSerTyrCysGlnArgGlnGlyValPro

 241   ATGAATTCACTCAGGTTTCTCTTTGAGGGTCAGAGAATTGCTGATAATCATACTCCAAAA
  59   MetAsnSerLeuArgPheLeuPheGluGlyGlnArgIleAlaAspAsnHisThrProLys

 301   GAACTGGGAATGGAGGAAGAAGATGTGATTGAAGTTTATCAGGAACAAACGGGGGGTCAT
  79   GluLeuGlyMetGluGluGluAspValIleGluValTyrGlnGluGlnThrGlyGlyHis

 361   TCAACAGTTTAGATATTCTTTTTATTTTTTTTCTTTTCCCTCAATCCTTTTTTATTTTTA
  99   SerThrVal***

 421   AAAATAGTTCTTTTGTAATGTGGTGTTCAAAACGGAATTGAAAACTGGCACCCCATCTCT
 481   TTGAAACATCTGGTAATTTGAATTCTAGTGCTCATTATTCATTATTGTTTGTTTTCATTG
 541   TGCTGATTTTTGGTGATCAAGCCTCAGTCCCCTTCATATTACCCTCTCCTTTTTAAAAAT
 601   TACGTGTGCACAGAGAGGTCACCTTTTTCAGGACATTGCATTTTCAGGCTTGTGGTGATA
 661   AATAAGATCGACCAATGCAAGTGTTCATAATGACTTTCCAATTGGCCCTGATGTTCTAGC
 721   ATGTGATTACTTCACTCCTGGACTGTGACTTTCAGTGGGAGATGGAAGTTTTTCAGAGAA
 781   CTGAACTGTGGAAAAATGACCTTTCCTTAACTTGAAGCTACTTTTAAAATTTGAGGGTCT
 841   GGACCAAAAGAAGAGGAATATCAGGTTGAAGTCAAGATGACAGATAAGGTGAGAGTAATG
 901   ACTAACTCCAAAGATGGCTTCACTGAAGAAAAGGCATTTTAAGATTTTTTAAAAATCTTG
 961   TCAGAAGATCCCAGAAAAGTTCTAATTTTCATTAGCAATTAATAAAGCTATACATGC
```

FIG. 2. cDNA and predicted amino acid sequences. The cDNA clone was isolated by screening a pACT cDNA library (Clontech Laboratories) with pGBT9/RAD51 according to the manufacturer's instructions and previously described procedures (see Materials and Methods). *His*$^-$-selected positive clones were *LacZ* assayed in HF7c cells using a different promoter. Yeast clone plasmids were isolated and electroporated into HB101 cells. Growth in M9 (Leu$^-$) minimal media resulted in selection of bacteria containing only the pACT vector. Purified pACT plasmids were sequenced with an ABI 373A automatic DNA sequencer (ABI Biosystems) by using a primer-walking strategy. Bidirectional sequencing and at least two independent sequencing reactions were performed to obtain the cDNA sequence. A potential poly(A) addition signal in the cDNA is underlined. The sequence has been deposited with GenBank under Accession No. U38784.

```
UBL1     1 MSD....QEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKK 46
ScSMT3   1 MSDSEVNQEAKP..EVKPEVKPETHINLKV.SDGSSEIFFKIKKTTPLRR 47

UBL1    47 LKESYCQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVYQEQTG 96
ScSMT   48 LMEAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIG 97

UBL1    97 GHSTV 101
ScSMT3  98 GATY  101

UBL1    22 IKLKVIGQDSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRI 71
Ub.      1 MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQL 50

UBL1    72 ADNHTPKELGMEEEDVIEVYQEQTGGHSTV 101
Ub.     51 EDGRTLSDYNIQKESTLHLVLRLRGG 7

UBL1    19 GEYIKLKVIGQDSSEIHFKVKMTTHLKKLK.ESYCQRQGVPMNSLRFLFE 67
HHR23A   2 AVTITLKTLQQQTFKIRMEPDETVKVLKEKIEAEKGRDAFPVAGQKLIYA 51

UBL1    68 GQRIADNHTPKELGMEEEDVIEVYQEQT.GGHST 100
HHR23A  52 GKILSDDVPIRDYRIDEKNFVVVMVTKTKAGQGT 85

UBL1    22 IKLKVIGQDSSEIHFKVKMTTH.LKKLKESYCQRQGVPMNSLRFLFEGQR 70
HHR23B   3 VTLKTLQQQTFKIDIDPEETVKALKEKIESEKGKDAFPVAGQKLIYAGKI 52

UBL1    71 IADNHTPKELGMEEEDVIEVYQEQTGGHSTV 101
HHR23B  53 LNDDTALKEYKIDEKNFVVVMVTKPKAVSTP 83

UBL1     2 SDQEAKPSTEDLGDKKEGEYIKLKVIGQDSSEIHFKVKMTTHLKKLKESY 51
UCRP    62 SQQLGPGSTVLLVVDKCDEPLSILVRNNKGRSSTYEVRLTGTVAHLKQQV 111

UBL1    52 CQRQGVPMNSLRFLFEGQRIADNHTPKELGMEEEDVIEVYQEQTG 96
UCRP   112 SGLEGVQDDLFWLTFEGKPLEDQLPLGEYGLKPLSTVMNLRLRGG 156
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FIG. 3. Alignment of the UBL1 amino acid sequence with those of the yeast SMT3 protein (GenBank Accession No. U33057), ubiquitin, human RAD23A (HHR23A), human RAD23B (HHR23B), and a ubiquitin cross reacting protein (UCRP). The alignment was performed with the BESTFIT program in the GCG sequence analysis software package (University of Wisconsin, Madison).

Other molecular methods. Primers were synthesized with an Applied Biosystems Model 394 synthesizer. pGAD424/UBL1 was constructed by fusing bases 67–375 (Fig. 2) to the Gal4 DNA activation domain in vector pGAD424, and pGBT424/hRAD12 was constructed by fusing bases 67–375 (Fig. 2) to the Gal4 DNA binding domain in pGBT9 vector by PCR using *Bam*HI- and *Sal*I-tagged primers. Detailed procedures and construction of other two-hybrid vectors have been described previously (Shen *et al.*, 1996a).

TABLE 1

Homology between UBL1 and SMT3, Ubiquitin, and Other Ubiquitin-like Proteins

	UBL1	SMT3	Ubiquitin	HHR23A	HHR23B	UCRP
UBL1	100%					
SMT3	72%	100%				
Ubiquitin	45%	43%	100%			
HHR23A	52%	38%	67%	100%		
HHR23B	48%	44%	70%	82%	100%	
UCRP	43%	43%	60%	41%	41%	100%

Note. The BESTFIT program in the GCG package was used for sequence analysis. Only the N-terminal 85 amino acids were compared for HHR23A and HHR23B.

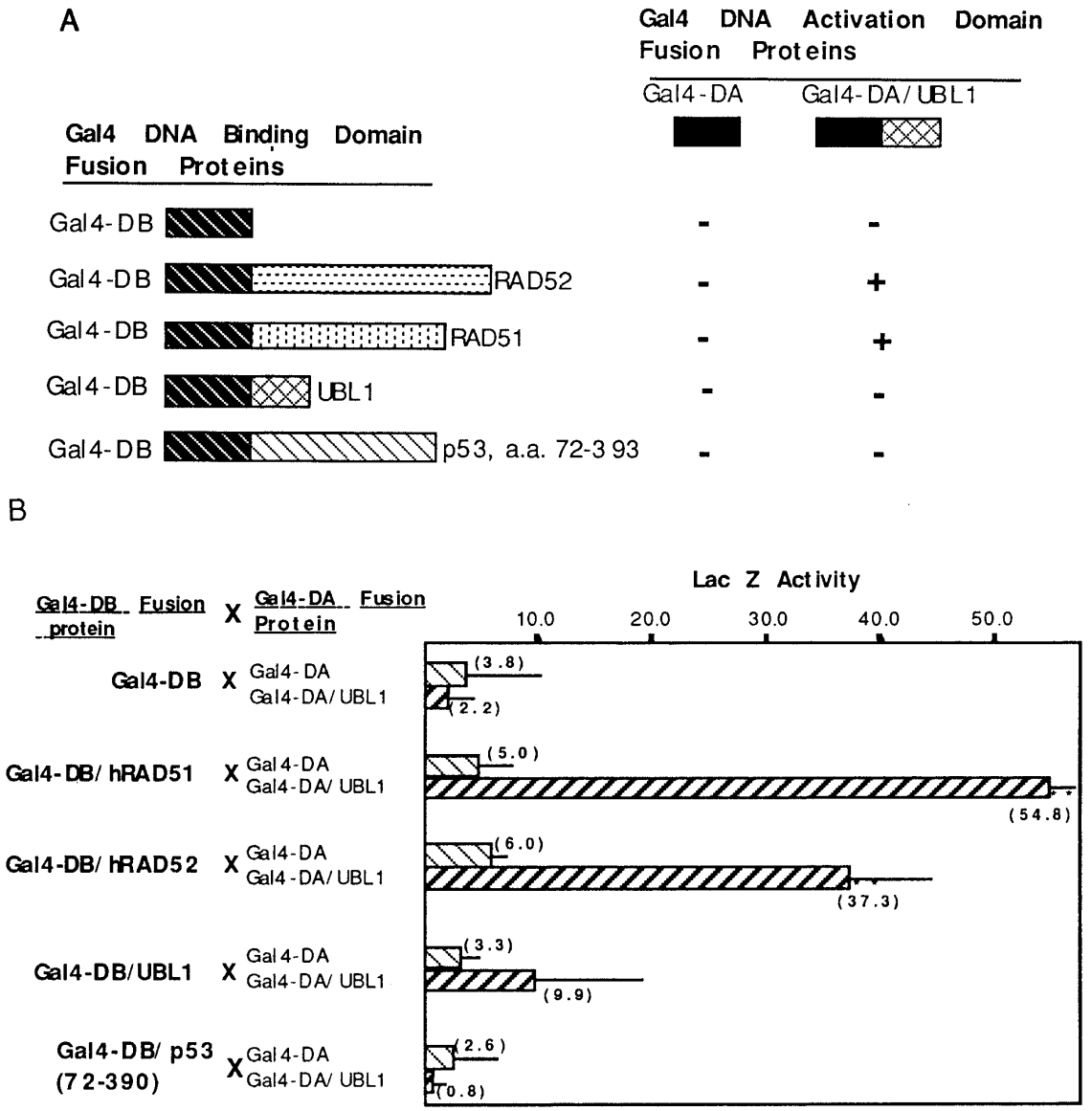

FIG. 4. Interaction of UBL1 with RAD51 and RAD52 in a yeast two-hybrid system. (A) Interaction of UBL1 with other proteins by *LacZ* filter assay. (B) Quantitative *LacZ* assay for UBL1's interaction with other proteins. The numbers in parentheses are the average *LacZ* activity of at least three independent colonies. Error bars indicate the standard errors. Experimental procedures for two-hybrid liquid assay and *LacZ* unit definition can be found under Materials and Methods. ***LacZ* activity is statistically higher than that in the controls as tested by Student's *t* test ($P_1 < 0.001$).

RESULTS

cDNA Cloning and Sequence Analysis

UBL1 was identified from a pACT vector-based library through its interaction with the human RAD51 (hRAD51) protein in a two-hybrid system utilizing *His* and *LacZ* as selection genes in the HF7c yeast strain (Clontech Laboratories). Subsequently, a 1017-bp cDNA was isolated from 0.3×10^6 independent clones (Fig. 2). The cDNA contains an open reading frame starting at base 67 and ending at base 369 (Fig. 2). Because of the characteristic base A at position 64 (Kozak, 1984), bases 67–69 were assigned as the translation start codon. This open reading frame codes for a protein of 101 amino acids with a molecular mass of 12 kDa.

Comparison of the amino acid sequence with nonredundant protein databases (including GenBank, SwissPort, PIR, etc.) showed no direct match. However, a yeast protein, SMT3 (GenBank Accession No. U33057), has 72% similarity and 52% identity with UBL1 (Fig. 3). Also, many proteins in the ubiquitin family showed moderate homology (40–55% similarity, ~20% identity) to UBL1. Figure 3 shows the alignment of the UBL1 protein sequence to human ubiquitin (Callis *et al.*, 1989) and several ubiquitin-like proteins, including the N-terminal 85 amino acids of the human RAD23 proteins (Masutani *et al.*, 1994; van der Spek *et al.*, 1994) and an interferon-inducible protein (UCRP) (Loeb and Haas, 1992, 1994). Table 1 summarizes the homology between the above-mentioned ubiquitin-like proteins.

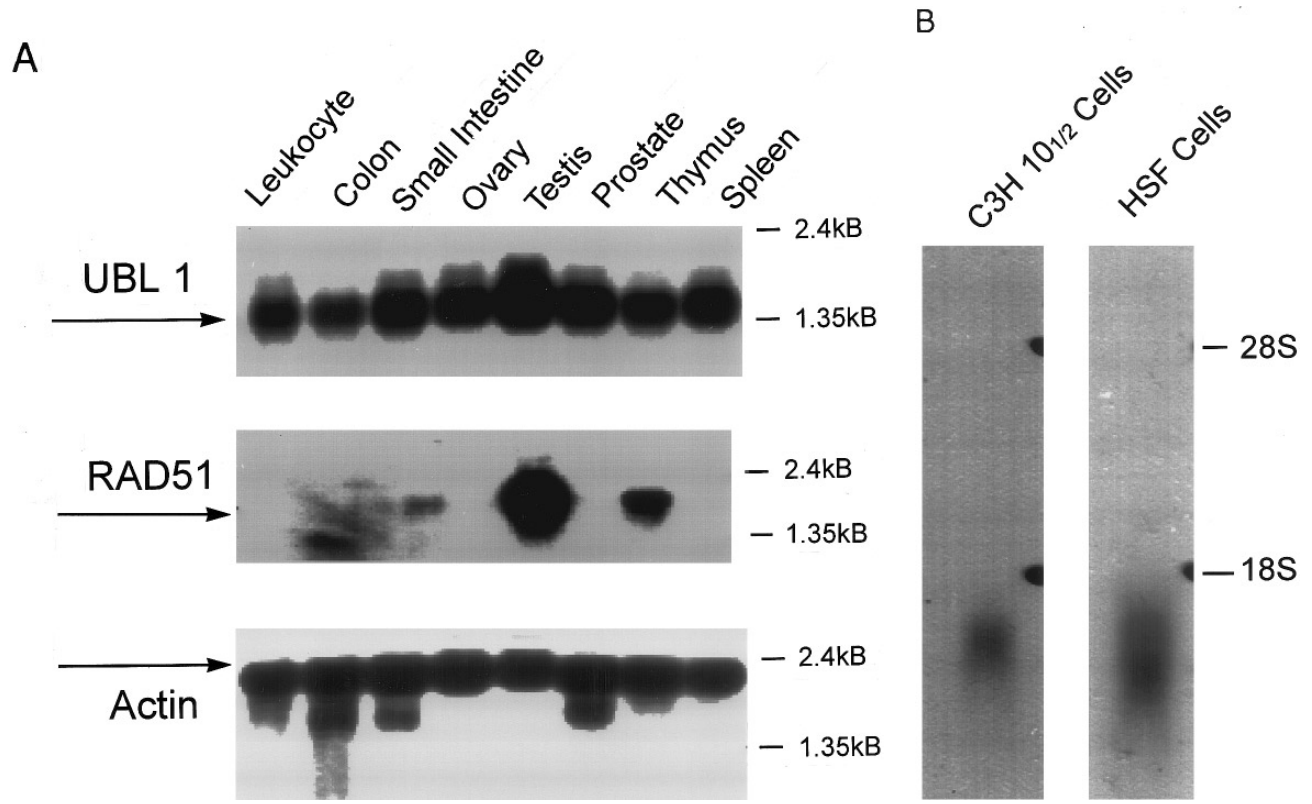

FIG. 5. mRNA analysis of UBL1. (A) Multiple tissue mRNA blot (Clontech Laboratories) with 2 μg of mRNA on each lane was sequentially hybridized with *UBL1* cDNA probe, human *RAD51* cDNA probe, and β-actin probe according to the manufacturer's protocol, with stripping between each hybridization. (B) Northern analysis of UBL1 in human skin fibroblast (HSF) and C3H mouse $10\frac{1}{2}$ embryo fibroblast (C3H $10\frac{1}{2}$ cells). The procedure is as described previously (Shen *et al.*, 1995).

UBL1 Interacts with Human RAD52 as well as with RAD51

To confirm its interaction with hRAD51, only the coding region of UBL1 was fused to the Gal4 DNA activation domain in vector pGAD424, which contains a weaker promoter than the pACT vector (Clontech Laboratories). This fusion also eliminated the noncoding region at the 5′-end of *UBL1* cDNA. UBL1's interaction with hRAD51 was further tested in another yeast strain, SFY526 (Clontech Laboratories), using *LacZ* as the reporter gene. As shown in Fig. 4, neither the vector alone nor UBL1 itself activated the expression of *LacZ*. Cotransfection of pVD3 (amino acids 72–390 of p53 fused to the Gal4 DNA binding domain in the pGBT9 vector) or pGBT9/UBL1 with pGAD424/UBL1 did not activate the *LacZ* gene, indicating no association of UBL1 with the truncated p53 nor UBL1 itself. However, when pGBT9/RAD51 or pGBT9/hRAD52 was cotransfected with pGAD424/UBL1, the *LacZ* gene was activated, indicating an association of UBL1 with hRAD51 as well as hRAD52.

Expression of UBL1 and Human RAD51 mRNA in Testis

Since it has been shown that the human *RAD51* mRNA is highly expressed in testis where meiosis and mitosis are active (Shinohara *et al.*, 1993), we further examined the *UBL1* mRNA level in several tissues, including testis, by Northern blot (Fig. 5A). It is evident that, although *UBL1* mRNA is expressed in all the tissues tested, testis exhibits the highest level of mRNA, which is consistent with human *RAD51*.

Northern hybridization to human and mouse total RNA showed a single mRNA species of 1.3–1.4 kb (Fig. 5B), indicating that transcripts of this gene exist in human and mouse cells. Since the average poly(A) tail in an mRNA is about 250 bases (Birnstiel *et al.*, 1985) and a putative poly(A) addition site (AATAAA) is identified in the 1017-bp cDNA clone (Fig. 2), this cDNA clone (Fig. 2) is at least close to full length.

Chromosome Localization of UBL1

The chromosome localization of *UBL1* was determined by FISH analysis using the full-length cDNA as a probe (Heng *et al.*, 1992; Heng and Tsui, 1993, 1994). Among 100 cells examined, 50 showed paired chromatid signals from chromosome 2q only (Figs. 6A and 6B), 7 showed paired chromatid signals from chromosome 1q only, 32 showed signals from both chromosomes 1q and 2q (Figs. 6C and 6D), and 4 showed paired chromatid signals from chromosome 5 (data not shown). Therefore, 82% of the cells showed signals from chromosome 2q, and 39% of the cells showed signals from chromosome 1q. To define the regional localization further, 10

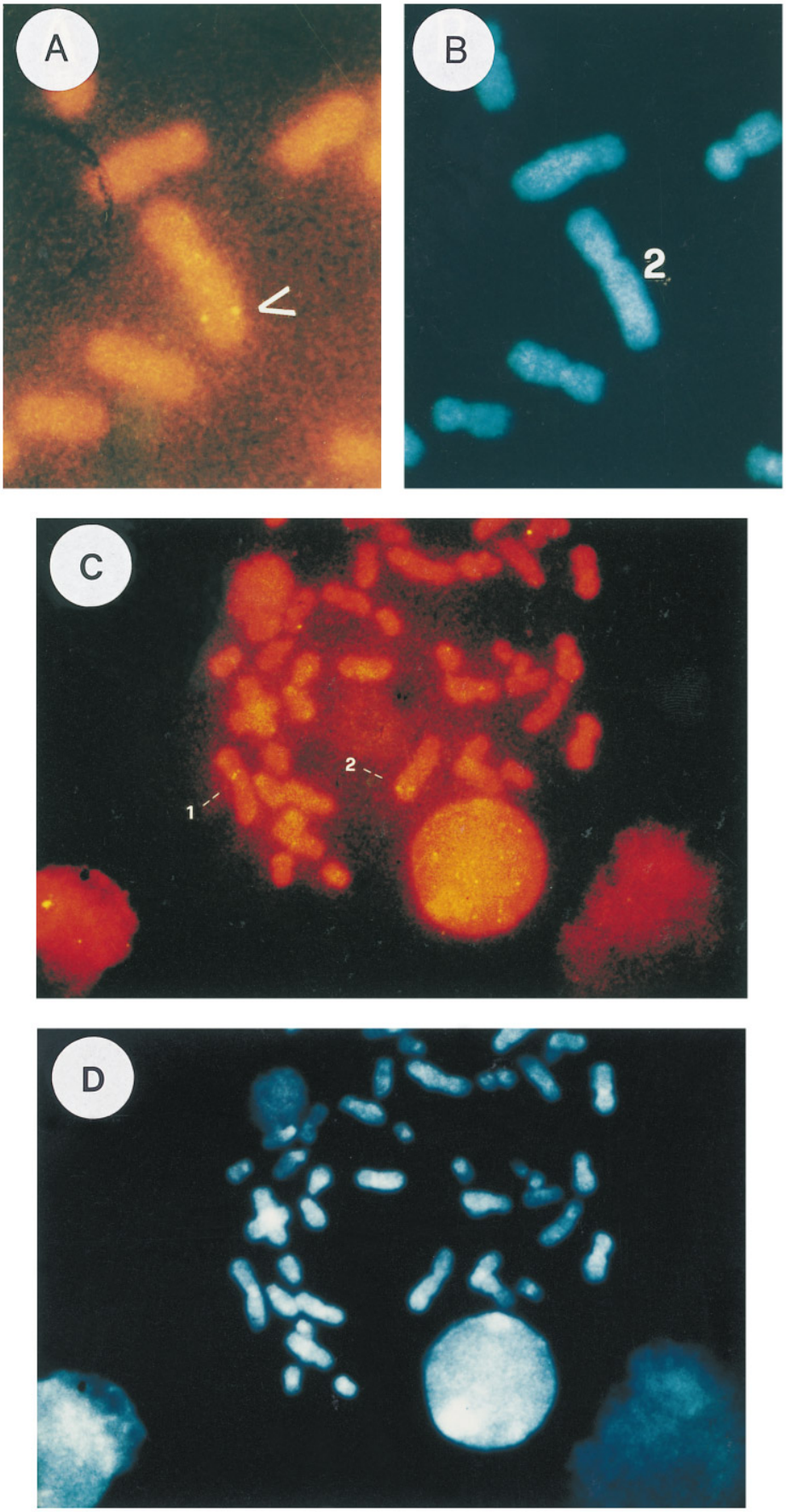

FIG. 6. Chromosome localization of the *UBL1* gene. (A and B) cDNA FISH (A) and DAPI banding (B) analysis of *UBL1* showing the localization on chromosome 2q. (C and D) Signals can be seen from both chromosome 1q and chromosome 2q. See Materials and Methods for technical details.

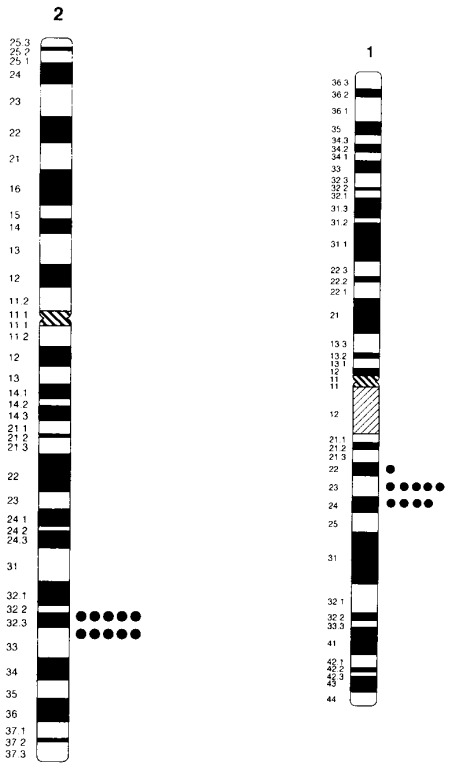

FIG. 7. Detailed analysis of 10 individual cells showing the signal localization at 2q32.3–q33 and 1q23–q24 (drawing not to scale).

individual cells were analyzed by comparing the locations of FISH signals with chromosome banding (Fig. 7). Based on these data, we concluded that *UBL1* is located on 2q32.3–q33 and that a closely related sequence is located on 1q23–q24.

To confirm the chromosome localization, DNAs from a panel of rodent hybrids, each containing only a single human chromosome, were used for PCR analysis as described previously (Shen *et al.*, 1995). Figure 8A shows that a signal identical to total human DNA was amplified from human chromosomes 1, 2, and 5 and a weaker signal from chromosome 13, indicating the presence of sequences closely related to *UBL1* on these chromosomes.

We have also searched GenBank for homologous DNA sequences and found that several expressed sequence tags (ESTs) in GenBank have significant nucleic acid identity with *UBL1* cDNA. Two of them (Accession Nos. R17443 and T16960) have 99% identity with *UBL1* in a region of ~400 bp, presumably because they are ESTs from *UBL1* directly. One (GenBank Accession No. T08096) has 62% identity with *UBL1* cDNA in a region of 234 bp. Another (GenBank Accession No. T08856) has 57% identity in a region of 184 bp. The last two entries have 81% of the nucleic acids identical in a region of 212 bp. Therefore, a family of *UBL1* homologous genes may exist in the mammalian genome. This may explain why signals from other chromosomes are detectable.

To confirm the regional localization on chromosome 2 further, a panel of human radiation hybrids containing partial human chromosome 2 (Chen *et al.*, 1994) were used for regional mapping by PCR analysis (Fig. 8B). As shown in Fig. 8B, while DNA from cell lines containing the region 2q32.3–q33 (cell lines 6CS-5, XHB-78, 6x(neo2)-18, and XRV15b(neo2)-11) showed a positive PCR result, cell lines not containing this region (cell lines 6CS-7 and XHB-104) showed negative PCR results. These data confirmed the localization to 2q32.3–q33.

DISCUSSION

Similar to ubiquitin, ubiquitin-like proteins seem to be involved in many cellular processes. For example, a UCRP has been shown to be conjugated to many cellular proteins that are distributed in a cytoskeleton pattern. The N-terminal amino acids homologous to ubiquitin in RAD23 proteins are also essential for RAD23's DNA repair function (Pejovic, 1995).

The location of *UBL1* to 2q32.3–q33 and a related gene on 1q23–q24 is worthy of discussion. The 2q33 region has been identified as an aphidicolin-inducible fragile site (Tedeschi *et al.*, 1992), and several cancer cells, such as human small-cell lung carcinoma (Kohno *et al.*, 1994) and ependymomas (Rogatto *et al.*, 1993), have chromosome changes within the region 2q32–q33. A potential tumor suppressor gene has also been mapped to 1q23–q24 (Horikawa *et al.*, 1995). Genetic changes on 1q23–q25 have been observed in ovarian cancers and in Burkitt lymphoma-derived cell lines (Polito *et al.*, 1995).

RAD51 shares a moderate sequence homology and some functional similarity (such as DNA binding and filament formation along DNA strands) with the bacterial recombination protein RecA (Benson *et al.*, 1994; Ogawa *et al.*, 1993; Shinohara *et al.*, 1992; 1993). The human RAD52 protein shares significant homology with the yeast RAD52 protein only at the N-terminus (Muris *et al.*, 1994; Shen *et al.*, 1995). Two independent functional domains involved in self-interaction and interaction with RAD51 have been identified (Shen *et al.*, 1996a,b). In addition, expression of human RAD52 in monkey cells enhances radiation resistance (Park, 1995). *UBL1*'s yeast homolog, *SMT3*, is a suppressor of the yeast gene *MIF2* mutation (see GenBank Accession No. U33057). MIF2 protein is a yeast centromere protein with homology to the mammalian centromere protein CENP-C (Brown, 1995; Meluh and Koshland, 1995). MIF2 is also involved in yeast chromosome segregation (Brown *et al.*, 1993). These results may therefore suggest that UBL1 is also involved in mitosis. Coincidentally, human *RAD51* is highly expressed during the S/G2/M phase of the cell cycle in mammalian cells, and human *RAD52* in the G2/M phase (unpublished data). We have cloned a human homolog of yeast ubiquitin-conjugation enzyme UBC9, which is involved in S- and M-phase cyclin degradation (Seufert *et al.*, 1995) and mitosis control (Al-Khodairy *et al.*, 1995). This hUBC9-like protein also interacts with UBL1, hRAD52, hRAD51, and p53 proteins (Shen *et al.*,

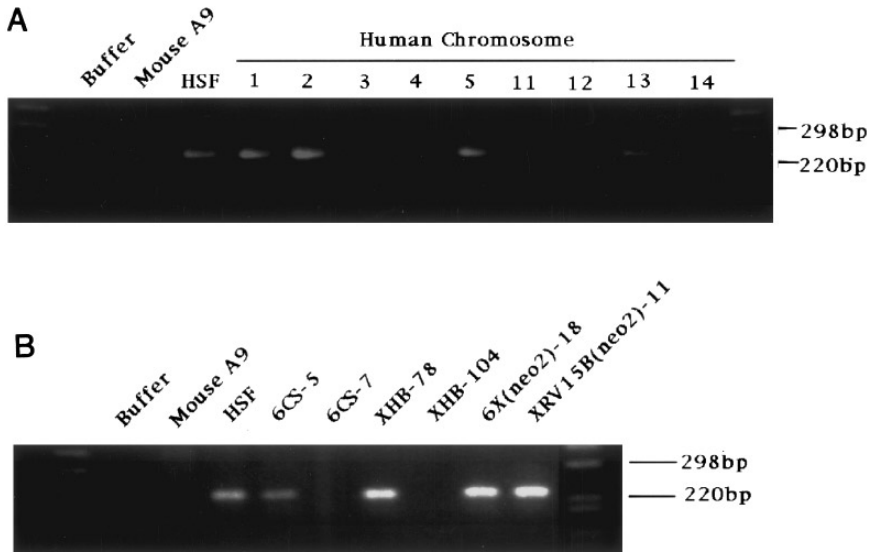

FIG. 8. PCR analysis of human chromosome hybrids in mouse cells as described before. (A) PCR from chromosomes 1, 2, 3, 4, 5, 11, 12, 13, and 14 are shown. "Buffer" denotes negative control using buffer alone as the PCR template, "mouse A9" indicates DNA from mouse A9 cells from which the hybrid panel was constructed, and "HSF" denotes total DNA from human skin fibroblast. Other lanes are molecular size markers. PCR results from other human chromosomes are negative and not shown. The primers used are 5′GGTGATCAAGCCTCAGTC (positions 552–569 in Fig. 2) and 5′CCACAGTTCAGTTCTCTG (positions 791–774 in Fig. 2). (B) A few X-ray hybrids, containing partial human chromosome 2, were further analyzed by PCR. Labels at the top denote the cell lines used; other labels are the same as in A. While DNA from cell lines containing the region 2q32.3–q33 (cell lines 6CS-5, XHB-78, 6x(neo2)-18, and XRV15b(neo2)-11) showed a positive PCR result, cell lines not containing this region (cell lines 6CS-7 and XHB-104) showed negative PCR results. For detailed information about these cell lines, please refer to Chen *et al.* (1994).

1996c). Therefore, we believe the association of UBL1 with RAD51/RAD52 to be functionally relevant. The association of UBL1 and hRAD51/hRAD52 also suggests that DNA repair/recombination pathways and cell cycle/mitosis control pathways may interface with each other.

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