GENOMICS **37,** 183–186 (1996)
ARTICLE NO. 0540

Associations of UBE2I with RAD52, UBL1, p53, and RAD51 Proteins in a Yeast Two-Hybrid System

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*

Received March 15, 1996; accepted August 6, 1996

The yeast RAD52-dependent pathway is involved in DNA recombination and double-strand break repair. Yeast ubiquitin-conjugating enzyme UBC9 participates in S- and M-phase cyclin degradation and mitotic control. Using the human RAD52 protein as the "bait" in a yeast two-hybrid system, we have identified a human homolog of yeast UBC9, designated UBE2I, that interacts with RAD52, RAD51, p53, and a ubiquitin-like protein UBL1. These interactions are UBE2I-specific, since another DNA repair-related ubiquitin-conjugating enzyme, RAD6 (UBC2), does not interact with these proteins. The interaction of UBE2I with RAD52 is mediated by RAD52's self-association region. These results suggest that the RAD52-dependent processes, cell cycle control, p53-mediated pathway(s), and ubiquitination interact through human UBE2I. © 1996 Academic Press, Inc.

INTRODUCTION

Cellular exposure to DNA damaging agents induces many cellular processes, including DNA damage repair. The RAD52 epistasis gene group is required for DNA double-strand break (DSB)[2] repair and mitotic/meiotic recombination. Many yeast proteins that participate in the RAD52-dependent DSB repair pathway have been identified. However, only two human counterparts of these proteins have been reported, i.e., RAD51 and RAD52 (Muris *et al.*, 1994; Shen *et al.*, 1995; Shinohara *et al.*, 1993). The human RAD51 protein shows many similarities to yeast RAD51, including DNA binding activity and interaction with the human RAD52 protein (Benson *et al.*, 1994; Shen *et al.*, 1996a). Overexpression of human RAD52 in monkey cells enhances their resistance to radiation (Park, 1995). Human RAD52 has at least two independent functional domains that mediate self-association (Shen *et al.*, 1996b) and interaction with human RAD51 (Shen *et al.*, 1996a).

Ubiquitin-conjugating enzymes constitute a family of proteins, including RAD6 (UBC2) and cdc34 (UBC3), that specifically conjugate ubiquitin to selected proteins (Hershko, 1991; Jentsch, 1992; Jentsch *et al.*, 1990; Koken *et al.*, 1991). Many crucial biochemical processes, including selective protein degradation, apoptosis, cell cycle control, ribosome biogenesis, and DNA repair are regulated by ubiquitination (for review, see Finley and Chau, 1991; Jentsch, 1992). RAD6 participates in DNA repair presumably by ubiquitinating histones and associating with RAD18 proteins (Finley and Chau, 1991; Jentsch, 1992).

In an effort to identify a novel protein(s) in this repair pathway, we used a yeast two-hybrid system to screen for proteins that may associate with the human RAD52 protein. We identified the human homolog of yeast ubiquitin-conjugating enzyme UBC9 through its interaction with the human RAD52 protein. This human gene has been assigned the symbol UBE2I by the Human Gene Nomenclature Committee.

MATERIALS AND METHODS

Yeast two-hybrid system. Materials and methods for yeast two-hybrid systems used in this study have been extensively described in our previous reports (Shen *et al.*, 1996a,b,c); therefore, they will be discussed only briefly here. The yeast strains SFY526 (Clontech Laboratories, Palo Alto, CA) were used to examine the interaction of two known proteins. β-galactosidase (*LacZ*) activity, representing the interaction of two fusion proteins in the yeast two-hybrid system, was measured by color development in filter assays according to the Matchmaker Kit manual (Clontech Laboratories) and previous reports (Shen *et al.*, 1996a,b,c). Yeast HF7c and a pACT vector-based cDNA library were used to screen a cDNA for RAD52-interacting proteins using procedures described in the two-hybrid system manual (Clontech Laboratories Inc.) and a previous report (Shen *et al.*, 1996c).

Cloning of RAD6 cDNA. The RAD6 two-hybrid construct was cloned by two consecutive PCR amplifications. For the primary PCR analysis, human RAD6 cDNA was amplified from a cDNA pool isolated from the pACT cDNA library (Clontech) by using primers

[1] To whom correspondence should be addressed at LS-6, MS M888, Life Sciences Division, Los Alamos National Laboratory, 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; UBE2I, a human homolog of the yeast ubiquitin-conjugating enzyme UBC9; UBL1, a 12-kDa ubiquitin-like protein; Gal4-DA, the Gal-4 DNA activation domain (amino acids 768–881); Gal4-DB, the Gal-4 DNA binding domain (amino acids 1–147); *LacZ*, β-galactosidase gene.

0888-7543/96 $18.00

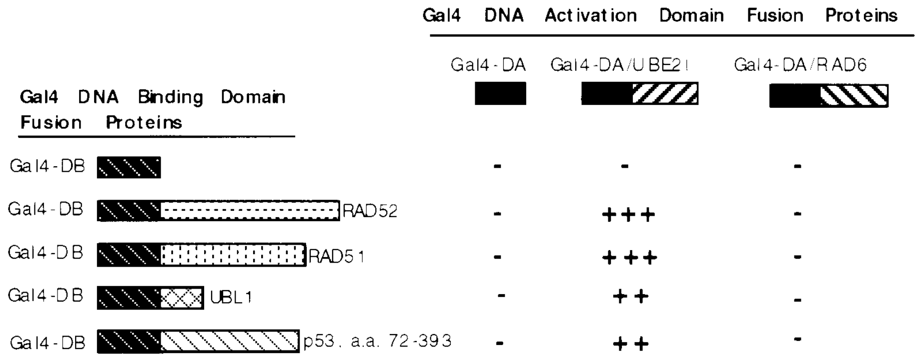

FIG. 1. Yeast two-hybrid interaction analysis of UBE2I and RAD6 with other proteins by filter *LacZ* assay results. See references (Shen *et al.,* 1996a,b,c) and manufacturer's manual (Clontech) for experimental details. (+++) denotes visible blue color development (representing *LacZ* activity in yeast) within 1 h; (++) denotes blue color development within 1–2 h; (−) denotes no visible blue color after 16 h. Abbreviations: Gal4-DA, Gal4 DNA activation domain; Gal4-DB, Gal4 DNA-binding domain; UBE2I, human UBE2I protein; RAD6, human RAD6 protein; UBL1, 12-kDa human RAD51/RAD52-associated ubiquitin-like protein (please see Shen *et al.,* 1996c, for details).

5′AACCCGAGACCCCAGTGTAT and 5′GACAGGAGTAGGGAG-GTGAC. For the secondary PCR, a *Bgl*II-tagged primer CTATAG-ATCTGCGACATGTCCACCCCGGC and a *Sal*I-tagged primer 5′TGCAGGTCGACGCTGTACCCGGGGTCAAC were used for amplification. This purified secondary PCR product was cut with *Bgl*II and *Sal*I and inserted into two two-hybrid vectors (pGBT9 and pGAD424) predigested with *Bam*HI and *Sal*I. Four independent RAD6 cDNA clones were sequenced. Comparison of these cDNA sequences to the published RAD6 cDNA sequence (GenBank Accession No. M74524) reveals only one mismatch, i.e., GGG (Gly) > GAG (Glu) at codon 49. Because this sequence was found in all four independent clones, Glu being a conserved Gly substitution, and because GAG (Glu) is normally present in another RAD6 cDNA clone (GenBank Accession No. M74525), we concluded that our RAD6 is a normal copy of human RAD6 cDNA.

Other procedures. DNA sequencing, Northern hybridization, and construction of yeast expression vectors have been described (Shen *et al.,* 1995, 1996a,b,c) and will be briefly discussed in the figure legends.

RESULTS

cDNA Cloning of UBE2I, a Homolog of the Yeast Ubiquitin-Conjugating Enzyme UBC9 or Hus5

A 1093-bp cDNA clone (see GenBank Accession No. U38785) was isolated from 0.3×10^6 independent clones via interaction with human RAD52 in a yeast two-hybrid system. The cDNA codes for an 18-kDa protein of 158 amino acids (data not shown). The predicted amino acid sequence showed 81% similarity (66% identity) with the *Schizosaccharomyces pombe* mitosis ubiquitin-conjugating enzyme Hus5 (Al-Khodairy *et al.,* 1995), 75% similarity (56% identity) with the *Saccharomyces cerevisiae* ubiquitin-conjugating enzyme UBC9 (Seufert *et al.,* 1995), and 59% similarity (42% identity) with the human RAD6 (UBC2) protein (Koken *et al.,* 1991). A signature sequence for all ubiquitin-conjugating enzymes (Hershko, 1991; Jentsch, 1992; Jentsch *et al.,* 1990) was found in this protein by searching a protein motif database. Based on these analyses, we conclude that the gene codes for an ubiquitin-conjugating enzyme, i.e., the human homolog of *S. cerevisiae* UBC9 or *S. pombe* Hus5. This human gene is named UBE2I according to the recommendation of the Human Gene Nomenclature Committee.

UBE2I Specifically Associates with RAD52, RAD51, p53, and UBL1 in the Yeast Two-Hybrid System

We further characterized the interaction of UBE2I with RAD52 and RAD51 using a different two-hybrid system than the one used in the original screening. Figure 1 demonstrates an interaction between RAD52/RAD51 and UBE2I. In contrast, there is no association between human RAD51/RAD52 and human RAD6. Human RAD6 (UBC2) protein was compared with UBE2I because it is the human protein with the closest amino acid sequence to UBE2I and because it is a DNA repair enzyme. This result therefore suggests that the interaction between UBE2I and the human RAD51/RAD52 complex is UBE2I-specific.

Since the tumor suppresser protein p53 and a newly identified ubiquitin-like protein (UBL1) are implicated in the RAD51/RAD52 complex (Gibson *et al.,* 1996; Sturzbecher *et al.,* 1996; Shen *et al.,* 1996c), we further tested their associations with UBE2I (Fig. 1). Our data show that human UBE2I, and not RAD6, associates with p53 and UBL1. Truncated p53 protein (amino acids 72–393) lacking the N-terminal transactivation region was used to eliminate any false positives that could have resulted from the p53 transactivation domain in the two-hybrid system.

UBE2I Interacts with RAD52 through the Self-Association Domain of the RAD52 Protein

The human RAD52 protein shares homology with yeast RAD52 only in the N-terminus 1/3 region (Shen *et al.,* 1995). To map the RAD52 region that interacts with UBE2I, a series of RAD52 constructs was used. Figure 2 shows that region 65–165 interacts with UBE2I. This region has been assigned as the RAD52 self-interaction domain (Shen *et al.,* 1996b). The RAD52 self-interaction region is highly conserved in many organisms (Muris *et al.,* 1994; Shen *et al.,* 1995), inferring that this self-interaction is likely to be very important for RAD52's function. It is possible that the interaction of UBE2I with the RAD52 protein may target RAD52 for degradation or modulate its function by competing with RAD52 self-association.

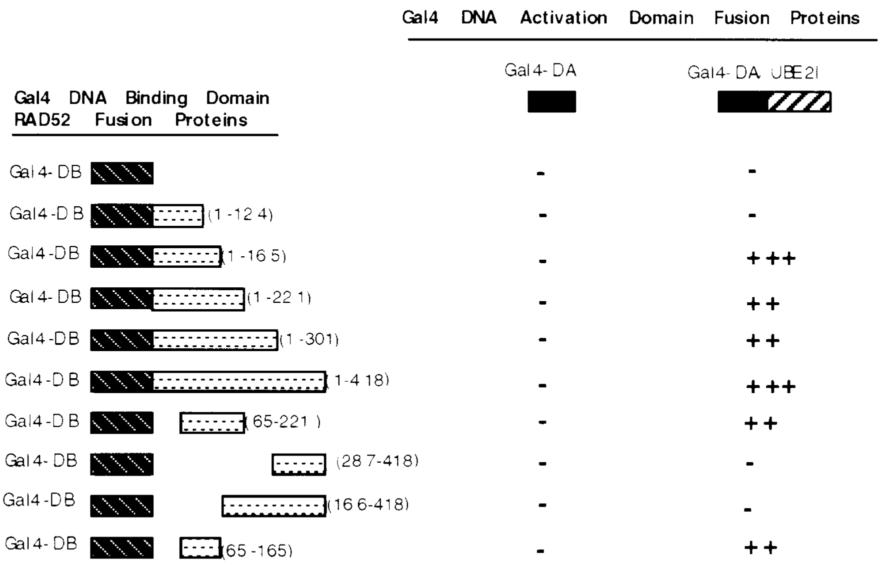

FIG. 2. The UBE2I-interacting region of the human RAD52 protein is mapped to amino acids 65–165. For details of truncated RAD52 constructs, see Shen *et al.* (1996b). Other symbols are the same as in Fig. 1.

UBE2I mRNA Is Highly Expressed in Testis and Localized at Chromosome 16p13.3

Northern blotting (Fig. 3) shows that the UBE2I gene is expressed in many tissues, with the highest mRNA level in testis. Mouse and human RAD51 (Shinohara *et al.*, 1993) and chicken RAD52 mRNA (Bezzubova *et al.*, 1993) levels are also most elevated in testis. In addition to the 1.4-kb mRNA band in the Northern blot, a minor band of ~3.4 kb was detected. This implies that a closely related mRNA species may be expressed in these human tissues. By using the cDNA as a FISH probe, UBE2I was mapped to chromosome 16p13.3 (data not shown; available upon request).

DISCUSSION

Recently, Kovalenko *et al.* (1996) cloned the human homolog of the yeast UBC9 gene using RAD51 as the bait in a yeast two-hybrid system. In this report, we cloned the same cDNA by using RAD52 as the bait. In addition, we have shown that UBE2I associates with RAD52, p53, and UBL1 as well as RAD51. Our data also show that UBE2I is specific in its interactions with these proteins compared with the RAD6 DNA repair protein. Another significant finding is that the interaction between UBE2I and RAD52 is mediated by RAD52's self-interaction domain, which is highly conserved among all RAD52 proteins from different species (Shen *et al.*, 1995, 1996b).

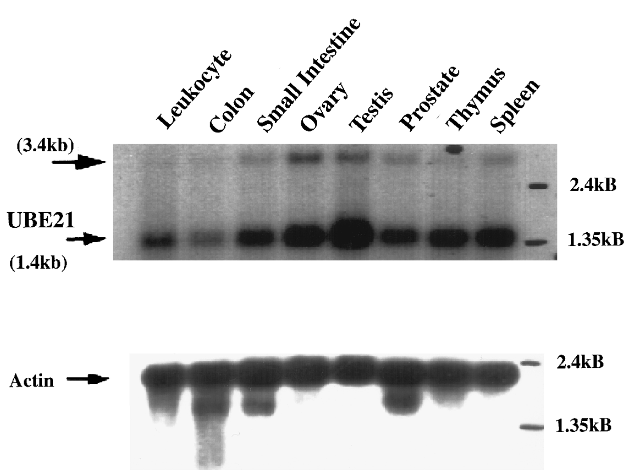

FIG. 3. A multiple-tissue mRNA blot (Clontech Laboratories) with 2 μg of mRNA/lane that was sequentially hybridized to UBE2I cDNA probe (top) or β-actin probe (bottom) according to the manufacturer's protocol. Stripping was performed between hybridizations.

The tumor suppresser protein p53 has been found to be degraded by a ubiquitination pathway (Blumenfeld *et al.*, 1994; Ciechanover *et al.*, 1994), an 18-kDa rabbit ubiquitin-conjugating enzyme (E2-F1) responsible for p53 degradation has been purified, and 46 amino acids in three E2-F1 peptide fragments have been obtained by peptide sequencing (Blumenfeld *et al.*, 1994). It is worth mentioning that in the most favorable alignment, only 14 of these 46 amino acids in E2-F1 are identical to UBE2I. In the same regions, there are 34 amino acids in the *S. pombe* Hus5 protein and 26 amino acids in *S. cerevisiae* UBC9 identical to human UBE2I. UBE2I is probably not the homolog of rabbit E2-F1, considering that the evolutionary relationship of human to rabbit is closer than that of human to yeast.

In *S. cerevisiae*, the UBC9 protein participates in S- and M-phase cyclin degradation (Seufert *et al.*, 1995). In *S. pombe*, the Hus5 protein is required for normal mitosis control, and *Hus5* mutants show reduced radiation resistance (Al-Khodairy *et al.*, 1995). We have observed that the human RAD52 and RAD51 proteins are highly expressed in S and G2/M phases, but relatively poorly expressed in other phases of the cell cycle (Chen *et al.*, 1996). These observations and the coexpression of RAD51/RAD52/UBE2I mRNA in testis (Fig. 3) indicate that the association of UBE2I with RAD52/RAD51 is likely to be functionally relevant. However, it is not clear whether this association results in degradation of individual proteins, results in the disassembly of the protein complex by the ubiquitination pathway, or is involved in biogenesis of a repair complex through ubiquitin's chaperone-like function.

UBE2I's association with so many proteins in the yeast two-hybrid system justifies commentary. The yeast two-hybrid system may detect a protein complex formation mediated by yeast proteins. It is understood that RAD52 and RAD51 directly interact and that RAD51 binds to p53 (Gibson *et al.*, 1996; Sturzbecher *et al.*, 1996). Therefore, these interactions among UBE2I, RAD51, RAD52, p53, and UBL1 in the yeast two-hybrid system may be mediated by a complex formation among human proteins and some yeast homologs of these proteins. Currently, purified protein is being used to determine if direct protein–protein binding between UBE2I and each one of these proteins is involved.

To conclude, the interactions of UBE2I with RAD51, RAD52, p53, and UBL1 in yeast two-hybrid systems (this report), interaction between RAD51 and p53 (Gibson *et al.*, 1996; Sturzbecher *et al.*, 1996), and the yeast UBC9/Hus5 data (Al-Khodairy *et al.*, 1995; Seufert *et al.*, 1995) suggest that RAD52/RAD51-dependent DSB repair, UBL1- and cyclin-mediated cell cycle control, and p53-regulated processes may interact with each other through their association with the UBE2I protein.

ACKNOWLEDGMENTS

This research was supported by the US Department of Energy and NIH Grant CA50519 to D.J.C. and by a director's postdoctoral fellowship from Los Alamos National Laboratory to Z.S. We thank Drs. E. Morton Bradbury, Scott Peterson, and Robert Cary for helpful discussions and a critical reading of the manuscript.

REFERENCES

Al-Khodairy, F., Enoch, T., Hagan, I. M., and Carr, A. M. (1995). The *Schizosaccharomyces pombe* hus5 gene encodes a ubiquitin conjugating enzyme required for normal mitosis. *J. Cell Sci.* **108:** 475–486.

Benson, F. E., Stasiak, A., and West, S. C. (1994). Purification and characterization of the human Rad51 protein, an analogue of *E. coli* RecA. *EMBO J.* **13:** 5764–5771.

Bezzubova, O. Y., Schmidt, H., Ostermann, K., Heyer, W. D., and Buerstedde, J. M. (1993). Identification of a chicken RAD52 homologue suggests conservation of the RAD52 recombination pathway throughout the evolution of higher eukaryotes. *Nucleic Acids Res.* **21:** 5945–5949.

Blumenfeld, N., Gonen, H., Mayer, A., Smith, C. E., Siegel, N. R., Schwartz, A. L., and Ciechanover, A. (1994). Purification and characterization of a novel species of ubiquitin-carrier protein, E2, that is involved in degradation of non-"N-end rule" protein substrates. *J. Biol. Chem.* **269:** 9574–9581.

Chen, F., Nastasi, A., Shen, Z., Crissman, H., and Chen, D. J. (1996). Cell cycle specific expression of the human homologs of the yeast DSB repair proteins RAD51 and RAD52, submitted for publication.

Ciechanover, A., Shkedy, D., Oren, M., and Bercovich, B. (1994). Degradation of the tumor suppressor protein p53 by the ubiquitin-mediated proteolytic system requires a novel species of ubiquitin-carrier protein, E2. *J. Biol. Chem.* **269:** 9582–9589.

Finley, D., and Chau, V. (1991). Ubiquitination. *Annu. Rev. Cell Biol.* **7:** 25–69.

Gibson, M., Wang, X., Sturzbecher, W., and Harris, C. (1996). Interaction of p53 with the human RAD51 protein. Proceedings of Keystone Symposia, "Molecular Mechanisms in DNA Replication and Recombination," p. 92.

Hershko, A. (1991). The ubiquitin pathway of protein degradation and proteolysis of ubiquitin–protein conjugates. *Biochem. Soc. Trans.* **19:** 726–729.

Jentsch, S. (1992). The ubiquitin-conjugation system. *Annu. Rev. Genet.* **26:** 179–207.

Jentsch, S., Seufert, W., Sommer, T., and Reins, H. A. (1990). Ubiquitin-conjugating enzymes: Novel regulators of eukaryotic cells. *Trends Biochem. Sci.* **15:** 195–198.

Koken, M. H., Reynolds, P., Jaspers-Dekker, I., Prakash, L., Prakash, S., Bootsma, D., and Hoeijmakers, J. H. (1991). Structural and functional conservation of two human homologs of the yeast DNA repair gene RAD6. *Proc. Natl. Acad. Sci. USA* **88:** 8865–8869.

Kovalenko, O. V., Plug, A. W., Haaf, T., Gonda, D. K., Ashley, T., Ward, D. C., Radding, C. M., and Golub, E. I. (1996). Mammalian ubiquitin-conjugatting enzyme Ubc9 interacts with Rad51 recombination protein and localizes in synaptonemal complex. *Proc. Natl. Acad. Sci. USA* **93:** 2958–2963.

Muris, D. F., Bezzubova, O., Buerstedde, J. M., Vreeken, K., Balajee, A. S., Osgood, C. J., Troelstra, C., Hoeijmakers, J. H., Ostermann, K., Schmidt, H., *et al.* (1994). Cloning of human and mouse genes homologous to RAD52, a yeast gene involved in DNA repair and recombination. *Mutat. Res.* **315:** 295–305.

Park, M. S. (1995). Expression of human RAD52 confers resistance to ionizing radiation in mammalian cells. *J. Biol. Chem.* **270:** 15467–15470.

Seufert, W., Futcher, B., and Jentsch, S. (1995). Role of a ubiquitin-conjugating enzyme in degradation of S- and M-phase cyclins. *Nature* **373:** 78–81.

Shen, Z., Denison, K., Lobb, R., Gatewood, J. M., and Chen, D. J. (1995). The human and mouse homologs of the yeast RAD52 gene: cDNA cloning, sequence analysis, assignment to human chromosome 12p12.2–p13, and mRNA expression in mouse tissues. *Genomics* **25:** 199–206.

Shen, Z., Cloud, G., Chen, D., and Park, M. (1996a). Specific interaction between human RAD51 and RAD52 proteins. *J. Biol. Chem.* **271:** 148–152.

Shen, Z., Peterson, S., Comeaux, J., Zastraw, D., Moyzis, R., Bradbury, E., and Chen, D. (1996b). Self-association of human RAD52 protein. *Mutat. Res. DNA Repair*, in press.

Shen, Z., Pardington-Purtymun, P. E., Comeaux, J. C., Moyzis, R. K., and Chen, D. J. (1996c). UBL1, a human ubiquitin-like protein associating with human RAD51/RAD52 proteins. *Genomics* **36:** 271–279.

Shinohara, A., Ogawa, H., Matsuda, Y., Ushio, N., Ikeo, K., and Ogawa, T. (1993). Cloning of human, mouse and fission yeast recombination genes homologous to RAD51 and recA. *Nature Genet.* **4:** 239–243.

Sturzbecher, H. W., Donzelmann, B., Henning, W., Knippschild, U., and Buchhop, S. (1996). p53 is linked directly to homologous recombination processes via RAD51/RecA protein interaction. *EMBO J.* **15**(8): 1992–2002.