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

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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) 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 histones and associating with RAD18 proteins (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 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 pool isolated from the pACT cDNA library (Clontech) by using primers 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).

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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 Gal4-DNA activation domain (amino acids 768–881); Gal4-DB, the Gal4-DNA binding domain (amino acids 1–147); LacZ, β-galactosidase gene.

hances 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).

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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 suppressor 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 RAD25 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).

The tumor suppressor 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 (Seuffert 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.

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