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Cloning, Comparative Mapping, and RNA Expression of the Mouse Homologues of the *Saccharomyces cerevisiae* Nucleotide Excision Repair Gene *RAD23*

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The *Saccharomyces cerevisiae RAD23* gene is involved in nucleotide excision repair (NER). Two human homologs of *RAD23*, *HHR23A* and *HHR23B* (HGMW-approved symbols RAD23A and RAD23B), were previously isolated. The HHR23B protein is complexed with the protein defective in the cancer-prone repair syndrome xeroderma pigmentosum, complementation group C, and is specifically involved in the global genome NER subpathway. The cloning of both mouse homologs (designated MHR23A and MHR23B) and detailed sequence comparison permitted the deduction of the following overall structure for all RAD23 homologs: an ubiquitin-like N-terminus followed by a strongly conserved 50-amino-acid domain that is repeated at the C-terminus. We also found this domain as a specific C-terminal extension of one of the ubiquitin-conjugating enzymes, providing a second link with the ubiquitin pathway. By means of *in situ* hybridization, *MHR23A* was assigned to mouse chromosome 8C3 and *MHR23B* to 4B3. Because of the close chromosomal proximity of human *XPC* and *HHR23B*, the mouse *XPC* chromosomal location was determined (6D). Physical disconnection of the genes in mouse argues against a functional significance of the colocalization of these genes in human. Northern blot analysis revealed constitutive expression of both *MHR23* genes in all tissues examined. Elevated RNA expression of both *MHR23* genes was observed in testis. Although the RAD23 equivalents are well conserved during evolution, the mammalian genes did not express the UV-inducible phenotype of their yeast counterpart. This may point to a fundamental difference between the UV responses of yeast and human. No stage-specific mRNA expression during the cell cycle was observed for the mammalian *RAD23* homologs.

INTRODUCTION

The fundamental importance of DNA repair systems is illustrated by a number of cancer-prone human genetic disorders that are thought to be due to defects in DNA surveillance mechanisms like xeroderma pigmentosum (XP), Fanconi anemia, and Bloom syndrome (for a comprehensive review, see Friedberg *et al.*, 1995). XP and two other conditions, Cockayne syndrome (CS) and trichothiodystrophy (TTD), are associated with defects in the nucleotide excision repair (NER) pathway. This system deals with the elimination of a diverse array of structurally unrelated lesions, including various UV-induced photoproducts (cyclobutane pyrimidine dimers and 6-4 photoproducts), chemical adducts, and crosslinks (Van Houten, 1990). A defect in one of at least seven genes, XP-A to XP-G, can cause the genetic recessive disease XP. XP patients show extreme sun sensitivity, pronounced pigmentation abnormalities in UV-exposed areas of the skin, and frequently, accelerated neurodegeneration. Importantly, the disease is associated with a >1000-fold increased risk of skin cancer (see Cleaver and Kraemer, 1995, for a review). In contrast, CS patients are not cancer-prone and display a less severe sun sensitivity. Instead, this disorder is characterized by developmental impairment, including severe neurological abnormalities due to dysmyelination of the nervous system (Nance and Berry, 1992). Two CS complementation groups have been identified: CS-A and CS-B (Lehmann, 1987). TTD patients share many clinical features with CS, but have, in addition, brittle hair and nails (due to a reduced content of a class of ultra-high cysteine matrix proteins) and ichthyosis (Itin and Pittelkow, 1990). Three TTD genes are known, two of which are identical to XPB and XPD (Hoeijmakers, 1994). A striking selective correlation is found among all three TTD genes and mutations in subunits of basal transcription factor TFIIH, which has a dual involvement in transcription initiation and NER. Therefore, we have proposed that the "non-XP" features of TTD are due to a partial impairment of the transcription function of the complex, in addition to a defect in the NER function. Thus, TTD can be regarded as a combined repair–transcription syndrome (Vermeulen *et al.*, 1994; Bootsma and Hoeijmakers, 1993).

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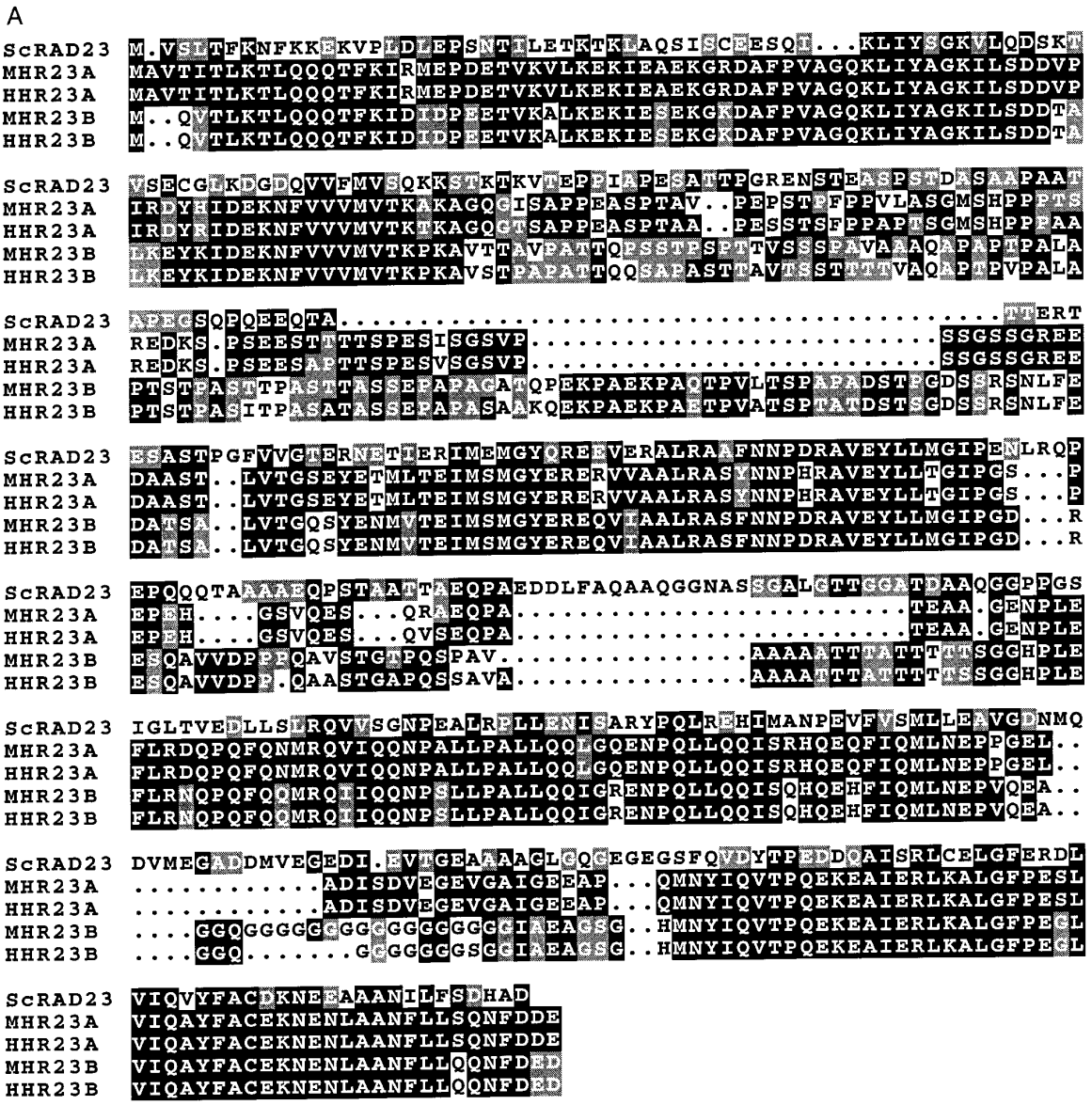

FIG. 1. (**A**) Sequence alignment of the yeast, mouse, and human homologues of RAD23 with each other. Conserved sequences between yeast RAD23, MHR23A, HHR23A, MHR23B, and HHR23B. The amino acid sequence of the mouse and human proteins are compared with that of yeast RAD23. Identical amino acids are presented in black boxes, whereas similar residues (A, S, T, P, and G; D, E, N, and Q; R, K, and H; I, L, V, and M; F, Y, and W) are given in gray boxes. (**B**) Schematic representation of the RAD23 protein homologs. Schematical presentation of conserved (short bar) and nonconserved (long bar) amino acid changes in mouse and human RAD23 proteins. The different domains discussed in the text are indicated. The EMBL accession number for the MHR23A gene is X92410 and for the MHR23B gene is X92411.

Recently, we reported the identification and cloning of two human homologs of the yeast NER gene *RAD23: HHR23A* and *HHR23B* (HGMW-approved symbols RAD23A and RAD23B) (Masutani *et al.*, 1994). Yeast *rad23* null mutants display an intermediate UV-sensitive phenotype, suggesting that the affected product is not indispensable for NER. The RAD23 protein contains an N-terminal ubiquitin-like domain (Watkins *et al.*, 1993). The HHR23B gene product was found in a tight complex with the XPC protein. This complex has a very high ssDNA binding activity. XPC cells harbor a specific defect in the repair of the nontranscribed sequences of the genome, including the nontranscribed strand of active genes, whereas the NER subpathway that accomplishes the preferential repair of the transcribed strand of active genes (transcription-coupled repair) is still operational (Venema *et al.*, 1989). This implies a selective role of the XPC complex in the global genome NER system (Van Hoffen *et al.*, 1995). Here, we report the cloning and characterization of the mouse homologs of RAD23, the chromosomal localization, the expression profile, and the identification of novel domains in the primary amino acid sequence.

MATERIALS AND METHODS

Cloning and nucleotide sequence analysis. General molecular biological procedures for nucleic acid isolation, (sub)cloning, DNA se-

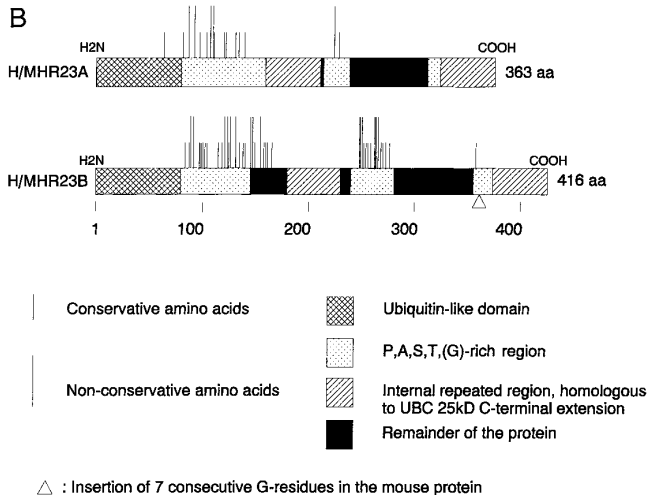

FIG. 1—*Continued*

quencing, PCR, and hybridization were as detailed in Sambrook *et al.* (1989). PCR-derived probes of both *HHR23* cDNAs were used to screen a mouse testis cDNA library (Stratagene). Clones hybridizing with the human probes were picked, rescreened for purification, and examined by restriction enzyme analysis. Hybridization of human probes to mouse DNA was at 62°C in a hybridization mixture containing 10× Denhardt's solution, 10% dextransulfate, 0.1% SDS, 3× SSC, 50 mg of sonicated salmon sperm DNA per liter. Washing was performed twice for 20 min each in 3× SSC, twice for 20 min each in 1× SSC, and twice for 20 min each in 0.3× SSC at 62°C. Hybridization was detected by autoradiography on Fuji RX medical X-ray film with intensifying screens at −80°C. After two rounds of rescreens, EMBL-3 phages were converted into pBluescript II KS (Stratagene) or pTZ19R (Pharmacia) vectors and transformed to competent DH5αF′ cells. Sequence analysis on double-stranded DNA was performed with the T7 DNA polymerase modification (Pharmacia) of the dideoxynucleotide chain termination method (Sanger *et al.*, 1977), using sequence-derived oligonucleotides and exonuclease clones for sequencing both strands. For separation of the fragments, 6% acrylamide sequencing gels were used. The 5′ end of *MHR23B* was derived from a mouse 17.5-day embryo 5′ stretch cDNA library (ML1029a; Clontech). Genomic clones were derived by screening a mouse CCE library with PCR fragments covering the mouse cDNAs of both *MHR23* genes.

Chromosomal localization. *In situ* hybridization experiments using biotin-labeled or digoxigenin-labeled genomic fragments of *MHR23A* and *MHR23B* were performed as described elsewhere (Pinkel *et al.*, 1986). After incubation with avidin D–FITC (Vector, USA), the biotin-labeled probes were visualized by FISH. The fluorescent signal was amplified with biotinylated goat anti-avidin D. Probes labeled with digoxigenin were visualized using Texas red-conjugated antidigoxigenin antibody.

Northern blot analysis. RNA samples were separated on an 1% agarose gel and transferred to Zeta probe membrane (Bio-Rad) as described by the manufacturer. Total RNA was isolated from adult (Balb/c) mice using the LiCl/urea method (Auffray and Rougeon, 1980). Filters were hybridized using mouse *MHR23A* and *MHR23B* ^{32}P-labeled cDNA probes. Labeling of DNA probes was carried out using the random priming protocol (Sambrook *et al.*, 1989).

Cell culture. HeLa cells were grown on F10/DMEM medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. HeLa cells were synchronized using double thymidine block (Bootsma *et al.*, 1964; Galavazi *et al.*, 1966) to study stage-specific expression during the cell cycle. A primary culture of epidermal keratinocytes derived from human foreskin was established as described elsewhere (Rheinwald and Green, 1975). Prior to RNA isolation, keratinocytes were UV-irradiated with 30 J/m^2, and HeLa cells were irradiated with 1 J/m^2 (UV-C).

Computer analysis. Sequences were analyzed and compared using the BLAST algorithm (Altschul *et al.*, 1990) and the GenBank and EMBL databases.

RESULTS

Cloning and Sequence Analysis of the cDNAs Encoding the Mouse RAD23 Equivalents

To obtain cDNAs encoding the murine homologs of *HHR23A* and *HHR23B*, a mouse testis library was screened with both human cDNA probes. Analysis of several cDNA clones indicated the presence of two distinct *RAD23* genes, tentatively designated *MHR23A* and *MHR23B* (mouse homologs of *RAD23*). Additionally, for *MHR23B*, a 17.5-day mouse embryo library was screened to obtain the missing 5′ end of the cDNA. The *MHR23A* ORF encodes an acidic protein (p*I* 4.4) of 363 amino acids, with a calculated molecular mass of 39,772 Da. The *MHR23B* ORF encodes an acidic protein (p*I* 4.6) of 416 amino acids with a calculated molecular mass of 43,520 Da. Both the mouse A and B polypeptides share, respectively, 96 and 88% sequence identity and 99 and 95% sequence similarity to their human counterparts. The human and mouse 23A proteins are equal in size. A notable difference between the human and the mouse 23B proteins is the insertion of a stretch of seven glycine residues in the latter at position 339. The MHR23A and MHR23B gene products exhibit 58% amino acid sequence identity and 77% homology to each other. Both proteins share about 62% homology to their yeast counterpart (Fig. 1A).

Detailed comparison of the mouse, human, and yeast RAD23 homologs permitted the identification of strongly conserved and more variable parts of the proteins that were not apparent from the human–yeast comparison and the identification of several domains in the RAD23 amino acid sequence that were not previously noted (Fig. 1). Together, these sequences account for the major part of the primary protein sequence. Figure 1B shows schematically the deduced overall structure of the RAD23 A and B proteins. At the N-terminus, a previously identified 80-amino-acid ubiquitin-like domain is present. It is followed by a region of approximately 65 amino acids predominantly (86%) composed of the physicochemically related amino acids proline, alanine, serine, and threonine (termed here "PAST" domain). This segment shows many sequence changes between human and mouse, suggesting that the precise primary sequence is probably not critical. Computer analysis revealed within the remainder of the protein two internal repetitive 50-amino-acid elements with significant homology to each other. The first domain is followed by a variable PAST-rich region, and the second is preceded by such a segment.

The internal repeated sequence is fully conserved between mouse and human, presumably a reflection of its functional importance. The significance of this domain is further underlined by the finding of clear sequence similarity to a C-terminal extension present

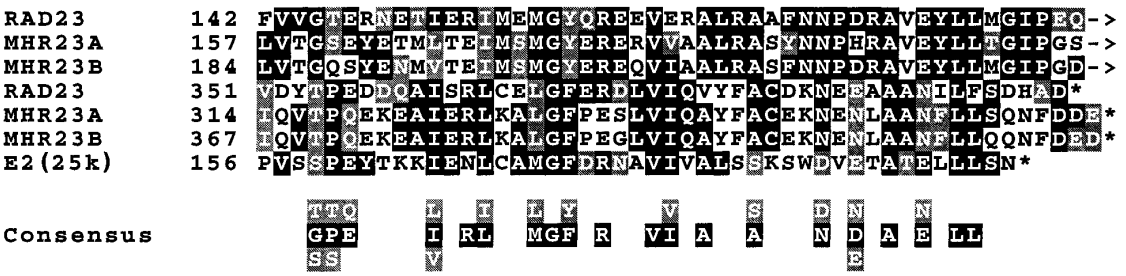

FIG. 2. Sequence alignment of the internally duplicated regions of RAD23 and homologues. Alignment of the internally duplicated regions of yeast, mouse RAD23, and the C-terminus of a bovine ubiquitin-conjugating enzyme E2(25K). The human sequences are not included in this figure, since these are exactly identical to the mouse sequences. Identical amino acids are presented in black boxes, whereas similar residues (A, S, T, P, and G; D, E, N, and Q; R, K, and H; I, L, V, and M; F, Y, and W) are given in gray boxes.

in a bovine ubiquitin-conjugating enzyme E2(25K) (Chen *et al.*, 1991), shown in Fig. 2. Comparison with various databases also identified homology to two genome project clones: one of *Arabidopsis thalia* (Accession No. Z26691) and one of *Plasmodium falciparum* (Accession No. T09564). These partial clones represent the RAD23 homologs in the corresponding species. Based on the presence of multiple conserved residues including a C-terminal cysteine residue, these short EST sequences are very likely the RAD23 homologs of these species.

Chromosomal Localization, Comparative Mapping

Both human *RAD23* homologs are located on different autosomal chromosomes. Interestingly, the *HHR23B* and *XPC* genes, the products of which form a tight complex, were found to colocalize to human chromosome 3p25.1 (Legerski *et al.*, 1994; van der Spek *et al.*, 1994). We have mapped the mouse equivalents to determine whether this colocalization is preserved during evolution. Genomic clones isolated from a mouse CCE genomic λ library were utilized for mapping purposes. Biotinylated probes hybridized with mouse metaphase spreads assigned the *MHR23A* gene to chromosome 8C3 (Fig. 3A) and the *MHR23B* to chromosome 4B3 (Fig. 3B). To confirm the *MHR23B* localization, a mouse leukemia cell line (Red8) that contains three copies of chromosome 4 was used. Furthermore, we determined that the mouse *XPC* gene maps to the 6D locus (shown in Fig. 3C), being in complete agreement with the localization of the human equivalent to the syntenic locus 3p25 (Legerski *et al.*, 1994; van der Spek *et al.*, 1994). Therefore, in contrast to the human situation, *XPC* and *MHR23B* do not colocalize in mouse.

Northern Blot Analysis of Mouse Tissues

To obtain clues about the potential function of the mammalian homologs of RAD23, we studied the expression properties of the genes. Northern blot analysis was performed using RNA from different mouse tissues. As shown in Fig. 4, both genes are expressed at the RNA level in all tissues and organs examined. When corrected for slight differences in the amount of RNA loaded in each lane (see 18S rRNA intensity from the ethidium bromide-stained gel), it is apparent that most tissues have roughly similar levels of *MHR23* transcripts. Clearly elevated expression was repeatedly observed for both *MHR23A* and *MHR23B* mRNAs in testis tissue compared to that in other tissues examined. The *MHR23A* probe visualized a transcript of 1.7 kb. The *MHR23B* gene was found to specify transcripts of 3.2 and 2.7 kb, both migrating just below the 28S ribosomal band. This size difference is most likely due to alternative polyadenylation of this gene, which was also observed in certain human tissues (data not shown). Additionally, the *MHR23A* probe detected enhanced expression in muscle tissue, whereas *MHR23B* showed enhanced RNA levels in brain, although in both cases expression was lower than that in testis (Fig. 4). We also examined whether expression of any of the *HHR23* transcripts changed during the mitotic cell cycle. RNA isolated from different stages (G1, S, G2, and mitosis). HeLa cells were synchronized by double thymidine block as described by Bootsma *et al.* (1964) and did not display significant differences in RNA levels of either *HHR23* gene (data not shown). In this regard, it is worth noting that no periodic fluctuations in *RAD23* mRNA levels were observed during the cell cycle in yeast (Madura and Prakash, 1990).

UV Inducibility of HHR23A and HHR23B mRNA Expression

RAD23 belongs to a small subset of yeast repair genes, the expression of which is induced at the mRNA level upon UV exposure and during meiotic prophase (Madura and Prakash, 1990). Although the significance of this phenomenon is unknown, it is thought that it constitutes part of a mechanism similar to the well-studied SOS response in *Escherichia coli* (Walker, 1985). Whether such a system also exists in higher organisms is still an open question. To see whether the feature of UV inducibility is conserved, we analyzed the levels of *HHR23* mRNA in response to UV irradiation in a cell type that is most relevant in this respect: human cultured keratinocytes. RNA isolated at various timepoints after UV exposure (30 J/m^2) was analyzed by Northern blot hybridization. The *HHR23A* probe

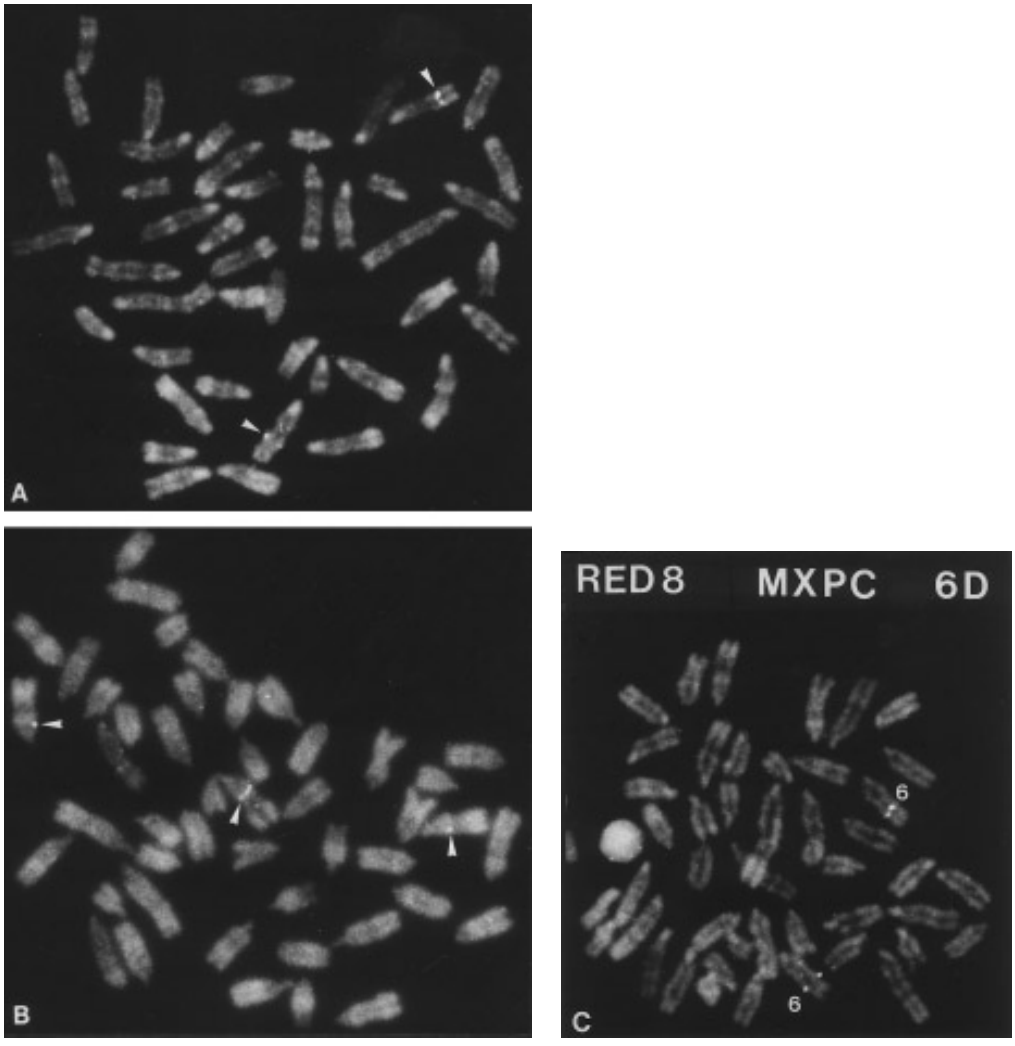

FIG. 3. (**A** and **B**) *In situ* hybridization of metaphase chromosomes with labeled *MHR23A* and *MHR23B* probes. The arrowheads indicate the hybridization signals on the mouse chromosomes visualized with FITC. (**A**) The signals for *MHR23A* (8C3); (**B**) the *MHR23B* gene (4B3). The cell line used for mapping of the *MHR23B* gene is trisomic for chromosome 4, as indicated by the arrowheads. (**C**) *In situ* hybridization of metaphase chromosomes with a biotinylated genomic *XPC* probe.

visualizes a mRNA of 1.7 kb, whereas the *HHR23B* probe detects a transcript of 2.8 kb. Figure 5 shows that the levels of *HHR23A* and *HHR23B* transcripts actually decline upon UV irradiation in contrast to expression of the *SPRR2* gene, a known UV-inducible gene included as a positive control (Gibbs *et al.*, 1993). Similarly, we did not find any evidence for UV induction in HeLa cells exposed to a lower UV dose (1 J/m^2) that were analyzed at time points much shorter after UV challenge (data not shown). We conclude that *HHR23A* and *HHR23B* do not express the UV-inducible phenotype of their yeast counterpart.

DISCUSSION

Relationship between RAD23 and Nucleotide Excision Repair

The phenotype of the yeast *rad23* mutant suggests that the protein is not indispensable for yeast NER, as gene disruption only induces a partial UV sensitivity (Miller *et al.*, 1982). However, the *rad23* mutants were found to be almost totally defective in repair of 6-4 photoproducts and cyclobutane pyrimidine dimers after a UV dose of 50 J/m^2 (McCready, 1994; J. Brouwer, Leiden, pers. comm., 1994). One of the human homologs of RAD23, HHR23B, is complexed with the XPC protein. Cells from XP-C individuals harbor a specific defect in the global genome repair subpathway of NER, with transcription-coupled repair being unaffected (Venema *et al.*, 1991). Assuming that this property of RAD23 is conserved, one predicts that the protein in yeast is also selectively implicated in the global genome repair process and that it is complexed with RAD4, the presumed yeast XPC homolog. However, curiously, *rad4* mutants display a total NER deficiency (Verhage *et al.*, 1994).

Unfortunately, no mammalian mutants of any of the RAD23 homologs have been identified (Masutani *et al.*, 1994; van der Spek, unpublished observation), possibly due to functional redundancy of the two homologous

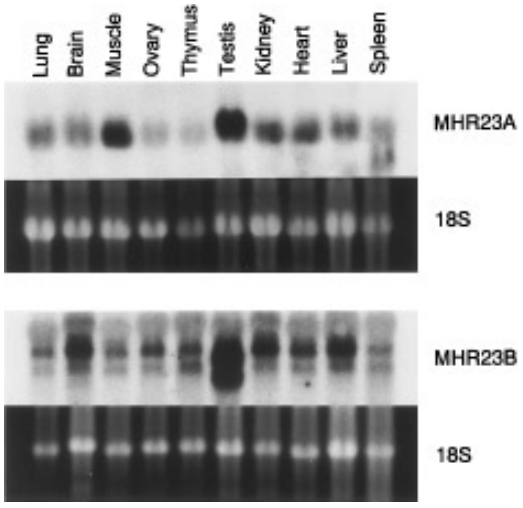

FIG. 4. Northern blot analysis of different mouse tissues for mRNA expression of MHR23A and MHR23B. RNA (15 μg total cell RNA) was size-fractionated on a 0.8% agarose gel and, after blotting to Bio-Rad zetaprobe-GT filters, was hybridized with *MHR23A* and *MHR23B* probes. The intensity of the ethidium bromide-stained 18S rRNA band reflects the amount of RNA loaded in each lane for all the tissues examined.

gene products. The only biochemical activity assigned to the HHR23B–XPC complex is a very high affinity for ssDNA. The latter is likely to be due to the XPC component, as the purified HHR23B polypeptide does not exhibit strong DNA binding activity (Van der Spek, manuscript in preparation).

Links with the Ubiquitin Pathway

Concerning the function of RAD23 and its mammalian homologs, the findings reported here point to a specific connection with the ubiquitin pathway. The protein carries a strongly conserved ubiquitin-like N-terminus. First, the N-terminal 80 amino acids of RAD23 and its mammalian homologs contain a ubiquitin-like moiety, and deletion analysis has provided evidence that it is indispensable for the NER function of RAD23 in yeast (Watkins *et al.*, 1993). Consistent with this finding, the entire amino acid sequence of this region is strictly conserved between mouse and human, including lysine 48, which in ubiquitin is required for polyubiquitination (Chau *et al.*, 1989). Conservation of this residue suggests the possibility of covalent attachment of ubiquitin to RAD23.

A second independent link with the ubiquitin pathway was disclosed by the finding that the internally duplicated region (Fig. 2) displays sequence homology with the C-terminus of the bovine E2(25K) member of the family of ubiquitin-conjugating (UBC) enzymes. The significance of this homology is potentially multiple. First, both 50-amino-acid regions are strictly conserved between human and mouse and are the most strongly conserved segments of the protein from yeast to human. This strongly suggests that they have an important function. Second, the idea that they represent distinct domains is supported by the observation that both domains are preceded by regions with the same general characteristics: a high content of PAST(G) residues and a rapid evolution. Furthermore, the homology to the C-terminus of the E2(25K) ubiquitin-conjugating enzyme starts exactly behind the core domain shared with all UBC enzymes and constitutes the entire part of the protein that remains. Notably, in RAD23, the second element also contains the last 50 amino acids of the polypeptide. Third, the level of homology is highly significant. Using the sequence of one of the RAD23 elements in a computer search, the E2(25K) C-terminal extension stands out from all sequences in the database. The above considerations strongly support the idea that the RAD23 internal repeat constitutes a highly conserved, distinct domain with a specific function similar to that carried out by one of the ubiquitin-conjugating enzymes.

Ubiquitin, one of the most highly conserved and ubiquitous polypeptides known, marks proteins for nonlysosomal proteolytic degradation or for proper folding and has a regulatory role in cellular homeostasis, the stress response, organelle biosynthesis, protein translocation across membranes, and DNA repair (see Ciechanover, 1994, for a recent review). Covalent attachment of one or multiple ubiquitin moieties to a target protein is the result of a number of activation and conjugation steps. Recently, Spence *et al.* (1995) described a ubiquitin mutant with specific defects in DNA repair and multiubiquitination.

The members of the UBC family (in yeast, at least 12 members) share a highly homologous 150-amino-acid domain containing the Cys residue (C^{88}) required for the ubiquitin-E2 thiol ester intermediate (Jentsch, 1992). A number of UBC members have in addition a unique C-terminal extension, presumably related to their specific function. Since each of the UBC enzymes is believed to target a selective set of proteins, it is possible that at least one of the functions of the unique C-terminal domain is to provide the specificity of interaction with target proteins or ubiquitin–protein ligase. For instance, the very acidic C-terminal domain of the

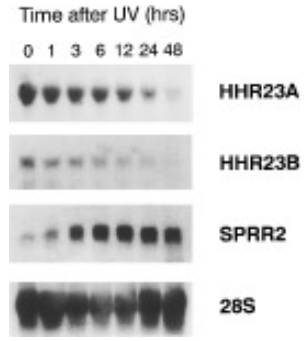

FIG. 5. Effect of UV irradiation on HHR23 transcription. RNA (15 μg total cell RNA) derived from UV-irradiated keratinocytes was size-fractionated on a 0.8% agarose gel. RNAs were transferred to Bio-Rad zetaprobe-GT filters and hybridized with *MHR23A* and *MHR23B* probes. Various timepoints after UV irradiation (30 J/m^2) were analyzed. The 18S ribosomal band is visualized by autoradiography to indicate the amounts of RNA loaded.

yeast UBC repair enzyme RAD6 is thought to mediate interaction with the basic histones. Ubiquitination of histones may be part of the chromatin transactions required for the postreplication repair, mutagenesis, and sporulation events in which this enzyme is implicated (Jentsch *et al.*, 1987).

Unfortunately, the specific function of the bovine E2(25K) UBC protein that could provide clues to the role of the equivalent duplicated domain in RAD23 is unknown, and any yeast homologs have not yet been identified. Consequently, no mutants are available to assess the biological function of the protein. However, in light of the affiliation of the ubiquitin system with the postreplication repair pathway, one might speculate that RAD23 is implicated in modulation of chromatin structure in the context of the global genome repair subpathway. RAD23 thereby provides the first indication for a possible link between NER and the ubiquitin system.

Gene Duplication

The *in situ* hybridization results obtained for mouse *XPC* and *MHR23A* are in complete agreement with the localization of their human counterparts on chromosome 3p25 and 19p13 (Legerski *et al.*, 1994; van der Spek *et al.*, 1994). The *MHR23B* gene provides a new reference anchor locus on mouse chromosome 4B3, which could be useful for comparative gene mapping and linkage analysis in mammals (Copeland *et al.*, 1993). The colocalization of *XPC* and *HHR23B* on human chromosome 3p25.1 is not observed in mouse. This argues against the possibility that the colocalization in human has an important function, e.g., in gene regulation or in complex formation.

Since the evolutionary distance of budding yeast to human is about 1200×10^6 years, one can roughly estimate from the degree of divergence of the human, mouse, and yeast genes that the *RAD23* gene duplicated approximately $700-800 \times 10^6$ years ago. This calculation assumes that the rate of evolution for the different homologs has remained constant. The reason for this duplication of *RAD23* is not known. Because of the high level of sequence homology, both gene products might possess a redundant function. The finding that they are both expressed simultaneously in all tissues examined supports this presumption.

Expression Properties

Yeast *RAD23* mRNA is induced fivefold in meiotic prophase, coinciding with recombination (Madura and Prakash, 1990). Although *rad23* mutants undergo sporulation and produce viable spores, it is not known whether meiotic recombination is affected. Enhanced expression of both *MHR23* equivalents was found in testis tissue. Thus, the feature of meiosis-specific induction of RAD23 expression may be a preserved property of the gene, perhaps pointing to its involvement in meiotic recombination.

RAD23 represents the second example of evolutionary duplication of a repair gene, with the first being RAD6 (Koken *et al.*, 1991). A number of additional parallels between these genes can be noted. In addition to the links with the ubiquitin pathway mentioned above, both genes display elevated levels of expression during mammalian spermatogenesis and yeast meiosis and sporulation. Both are—for repair genes—abundantly expressed at the protein level based on a calculation of molecules per cell (Van der Spek, manuscript in preparation). Finally, in yeast, both belong to the small subset of repair genes of which the expression is UV-inducible (Koken *et al.*, submitted for publication; this report). Notably, in both cases this feature does not appear to be conserved in mammals. This may point to a principle difference in the UV response between lower organisms that are fully subject to sudden changes in their environment and higher species in which the cellular milieu is kept relatively constant.

Cloning of the mouse homologs of repair genes can give valuable insight into the clinical consequences of molecular defects in the relevant gene. In particular, the predisposition to cancer and other clinical hallmarks of human NER disorders can be investigated by means of targeted gene replacement in MHR23-defective cell lines and mice. These experiments are in progress.

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