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XAB2, a Novel Tetratricopeptide Repeat Protein Involved in Transcription-coupled DNA Repair and Transcription*

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Nucleotide excision repair is a highly versatile DNA repair system responsible for elimination of a wide variety of lesions from the genome. It is comprised of two subpathways: transcription-coupled repair that accomplishes efficient removal of damage blocking transcription and global genome repair. Recently, the basic mechanism of global genome repair has emerged from biochemical studies. However, little is known about transcription-coupled repair in eukaryotes. Here we report the identification of a novel protein designated XAB2 (XPA-binding protein 2) that was identified by virtue of its ability to interact with XPA, a factor central to both nucleotide excision repair subpathways. The XAB2 protein of 855 amino acids consists mainly of 15 tetratricopeptide repeats. In addition to interacting with XPA, immunoprecipitation experiments demonstrated that a fraction of XAB2 is able to interact with the transcription-coupled repair-specific proteins CSA and CSB as well as RNA polymerase II. Furthermore, antibodies against XAB2 inhibited both transcription-coupled repair and transcription *in vivo* but not global genome repair when microinjected into living fibroblasts. These results indicate that XAB2 is a novel component involved in transcription-coupled repair and transcription.

NER[1] is a highly versatile and strongly conserved DNA damage repair pathway. It maintains the genetic information by removing a wide variety of lesions from DNA including UV-induced cyclobutane pyrimidine dimers and 6/4 photoproducts as well as numerous chemical adducts (1). Two subpathways can be discerned in NER: global genome repair (GGR) and transcription-coupled repair (TCR) (2). Lesions that actually block transcription, such as cyclobutane pyrimidine dimers (which are inefficiently removed by GGR), are preferentially removed from the transcribed strand of active genes by TCR to allow rapid recovery of RNA synthesis (3, 4).

The importance of NER is highlighted by the clinical features of rare human hereditary conditions caused by a deficiency in NER, such as xeroderma pigmentosum (XP) and Cockayne syndrome (CS). XP patients show striking hypersensitivity to sunlight and an extremely high incidence of skin cancer in sun-exposed areas and frequently progressive neurological degeneration. Seven genetic complementation groups of XP are known, XP-A through XP-G. In addition, an XP variant group (XP-V) is defective in postreplication repair (5). Cells from XP-C patients are deficient only in GGR but not in TCR (6, 7). CS patients show photohypersensitivity, cachectic dwarfism, and severe mental retardation but, unlike XP patients, no predisposition to skin cancer (8). Two genetic complementation groups exist: CS-A and CS-B. In contrast to XP-C, the defect within CS is restricted to TCR (9, 10). To date, all of the known genes responsible for XP and CS (the *XPA-XPG*, *XPV*, *CSA*, and *CSB* genes) have been cloned (5).

Recently, the core reaction of NER in humans has been reconstituted *in vitro* with purified proteins (11–13), and the outlines of the mechanism of GGR have been elucidated (reviewed in Ref. 14). The XPC-HR23B complex is the main factor to initiate global genome repair by sensing and binding to various types of lesion (15). The UV-DDB protein complex that is affected in XP-E patients is required for recognition of a specific subset of damage, such as cyclobutane pyrimidine dimers (16). The binding of XPC-HR23B complex to a lesion presumably induces a conformational change in the DNA around the injury. TFIIH, a general transcription initiation factor containing the XPB and XPD DNA helicases, is recruited to the recognized injury and locally unwinds the DNA duplex by its bidirectional DNA helicase activities to form an open reaction intermediate. XPA in a complex with replication protein A is likely to be involved in verification of the damage, proper orientation of the NER machinery around the injury, and stabilization of the opened intermediate. At the same time, replication protein A positions the structure-specific endonucleases at the appropriate sites for dual incision: XPG 2–8 bases at the 3′ side and the ERCC1-XPF complex 15–24 nucleotides 5′ of the lesion. After removal of the damage-containing 24–32-mer oligonucleotide, the resulting gap in the DNA is filled by general replication factors, and the final nick is sealed

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The nucleotide sequence reported in this paper has been submitted to the DDBJ/GenBank™/EBI Data Bank with accession number AB026111.

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[1] The abbreviations used are: NER, nucleotide excision repair; GGR, global genome repair; TCR, transcription-coupled repair; XP, xeroderma pigmentosum; CS, Cockayne syndrome; TPR, tetratricopeptide repeat; GST, glutathione *S*-transferase; HA, haemagglutinin; WCE, whole cell extract; UDS, unscheduled DNA synthesis; RRS, recovery of RNA synthesis; ORF, open reading frame.

by DNA ligase (see Refs. 14 and 17 for recent reviews and specific references therein).

The molecular mechanism of TCR is only resolved for *Escherichia coli* (18). The *Mfd* gene product (containing helicase motifs but without helicase activity) has been identified as a transcription-repair coupling factor that displaces an elongating RNA polymerase blocked in front of a lesion and then recruits the UvrABC *E. coli* excinuclease, which accomplishes removal of the lesion. In humans, genetic and cell biological evidence indicates that CSA and CSB play a key role in TCR (9, 10), but their functions remain to be elucidated. CSA is a 44-kDa protein with WD-40 repeats, which appears to have a potential for interaction with other proteins. It has been reported that CSA interacts with CSB and the p44 subunit of TFIIH *in vitro* (19). CSB is a 168-kDa protein with helicase motifs that belongs to the SWI/SNF family (20). We have previously shown that CSB is associated with RNA polymerase II *in vivo* (21), and we and others have shown that CSB has a DNA-dependent ATPase activity but no detectable classical helicase activity (22, 23). Since both CSB and Mfd contain helicase motifs, CSB may play a role equivalent to Mfd in mammalian cells. However, unlike Mfd, CSB has no detectable activity to dissociate RNA polymerase II stalled at a lesion from the DNA (24). It has been shown that CSB interacts with RNA polymerase II in a complex containing DNA and nascent RNA *in vitro* (25). The resulting quaternary complex has been shown to have an ability to recruit TFIIH, suggesting that CSB would recruit the NER proteins *in vivo* when RNA polymerase II encounters the lesion on the transcribed strand (26).

In the present study, we isolated a cDNA encoding a novel tetratricopeptide repeat (TPR) protein, designated XAB2 (XPA-binding protein 2). We found that XAB2 associates with both TCR-specific factors CSA and CSB and with RNA polymerase II. Furthermore, microinjection of anti-XAB2 antibodies specifically inhibited transcription as well as TCR but not GGR, suggesting that XAB2 is a novel factor participating in TCR and transcription itself.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid System—Screening of a HeLa cDNA library for isolating cDNAs encoding XPA binding proteins was performed using the yeast two-hybrid system as described (27). Positive transformants were classified into several groups based on cross-hybridization. Out of 281 positive clones, 54 belonged to the group of XAB2. To obtain full-length cDNA of XAB2, we screened a HeLa cDNA library in λ ZAP II (provided by Dr. H. Nojima, Osaka University) using the 939-base pair *Sma*I fragment of XAB2 cDNA as a probe. In addition, 5′-rapid amplification of cDNA ends was performed with 5′-AmpliFINDER RACE KIT (CLONTECH) using the P1 primer (5′-TTCATAGGCAGGGTCGGTCACACAG-3′) and P2 primer (5′-TGTGCCCGACGCGCCTTCAGGTATC-3′) according to the protocol supplied by the manufacturer. The full-length cDNA of XAB2 was reconstructed in pBluescript SK(−) by insertion of the *Eco*RI–*Kpn*I fragment from the rapid amplification of cDNA ends product into the *Eco*RI and *Kpn*I sites of the cDNA from the HeLa λ Zap library screenings.

In Vitro Pull-down Assay—Glutathione *S*-transferase (GST)-XPA fusion protein was prepared as described previously (27). GST-XAB2 fusion protein was obtained by in-frame cloning the full-length XAB2 cDNA into pGEX-5X-2 (Amersham Pharmacia Biotech). Referring to the published data (19), the CSA cDNA was isolated from WI38 VA13 cells with RT-PCR using an upper primer (5′-CGAATTCTCGAGGATATGCTGGGGTTTTTGTC-3′) with an *Eco*RI site and a lower primer (5′-TTGGTCGACTCTGTTTTAGGATTTTATGCAAA-3′) with a *Sal*I site. The amplified product was digested with *Eco*RI and *Sal*I and inserted into pBluescript SK(−) and pGEX-5X-2 for *in vitro* translation and GST fusion protein, respectively. *In vitro* translation of proteins and pull-down assays using GST, GST-XAB2, or GST-CSA fusion protein were performed as described (27).

Anti-XAB2 Antisera—Two antisera were prepared. Anti-XAB2FL was raised against the full-length recombinant XAB2, obtained using BAC-to-BAC Baculovirus Expression System (Life Technologies, Inc.). The *Eco*RI–*Xho*I fragment containing the full-length cDNA of XAB2 was inserted into pFASTBAC1 (Life Technologies, Inc.) plasmid, and then the recombinant baculoviruses were obtained by following the instruction manual. Sf9 cells (1×10^8 cells) were infected with the recombinant baculoviruses at 27 °C at a multiplicity of infection of 0.5. After 3 days of incubation, cells were harvested and washed twice with ice-cold PBS. The cell pellet was resuspended in NETN buffer (150 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl (pH 7.8), 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 0.5 μg/ml leupeptin, and 0.5 μg/ml pepstatin), and the suspension was centrifuged at 12,000 × *g* for 20 min. Almost all of the recombinant XAB2 were recovered in the pellet. After washed with NETN buffer, the pellet was dissolved in SDS sample buffer and then applied to SDS-polyacrylamide gel electrophoresis on 7% polyacrylamide gel. To visualize the recombinant XAB2, the gel was stained with CBB-R250 and destained in water. The gel stripe containing the recombinant XAB2 was cut out and used for immunization. Anti-XAB2C was raised against the C-terminal part (amino acid residues 694–855) of XAB2, overproduced as a GST fusion product in *E. coli*. The *Bgl*II–*Xho*I fragment of XAB2 cDNA was inserted into *Bam*HI and *Xho*I sites of pGEX-5X-2. The GST fusion C-terminal 162 amino acids of XAB2 were purified with glutathione-Sepharose beads (Amersham Pharmacia Biotech) in NETN buffer.

Immunoprecipitation—To examine the interactions of XAB2 with CSA, we used the SV40-transformed CS-A fibroblast line CS3BE-SV and CS3BE-SV(dtCSA) cells. CS3BE-SV expressed no endogenous CSA, while CS3BE-SV(dtCSA) stably expressing hemagglutinin (HA)-/His_6-double-tagged CSA at physiological levels showed a normal UV sensitivity.[2] Whole cell extracts (WCE) of these cells were prepared with NETN buffer as described previously (27). The WCE (4 mg) was incubated with 5 μg of anti-HA mouse monoclonal antibody (12CA5) at 4 °C for 12 h. The immunocomplexes were subsequently precipitated with 40 μl (bed volume) of Protein G-Sepharose beads (Amersham Pharmacia Biotech). After extensive washing with NETN buffer, bound proteins were eluted by boiling in SDS sample buffer. To examine the interactions of XAB2 with CSB, the SV40-transformed CS1AN-SV (2tCSB) cell line (stably expressing functional and physiological levels of HA-/His_6-double-tagged CSB) and HeLa cells were used for preparing Manley's WCE as described previously (21). The WCE (4 mg) was incubated with 5 μg of anti-HA mouse monoclonal antibody (12CA5) at 4 °C for 6 h. The protein-antibody complexes were subsequently bound to Protein G-Sepharose beads (Amersham Pharmacia Biotech). After extensive washing with buffer A (25 mM HEPES-KOH (pH 7.9), 100 mM KCl, 10% glycerol, 0.1% Nonidet P-40, 1 mM 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride), bound proteins were eluted by incubation with a synthetic HA-peptide (YPYDVPDYA) at 1 mg/ml in buffer A. For co-immunoprecipitation of XAB2 with RNA polymerase II, HeLa WCE (4 mg), prepared with NETN, was incubated with anti-RNA polymerase II mouse monoclonal antibody (8WG16; a kind gift from Dr. J-M. Egly, CNRS/INSERM/Université Louis Pasteur) or anti-XAB2FL at 4 °C for 12 h. The immunocomplexes were purified in NETN buffer as described above, and bound proteins were eluted by boiling in SDS sample buffer. The proteins in the eluates (immunoprecipitated fractions) were separated on SDS-polyacrylamide gel electrophoresis and subjected to immunoblot analyses. The WCEs (20 μg) were also subjected to immunoblot analyses with the immunoprecipitated fractions. Anti-HA rat monoclonal antibody (3F10) was used to detect HA-tagged CSA and CSB.

Microinjection—Microinjections were performed into homopolykaryons of DNA repair-proficient control primary fibroblasts (C5RO) and XP21RO (XP-C) cells obtained after cell fusion as described (21). NER activity (unscheduled DNA synthesis (UDS)) was measured 24 h after injection. After microinjection, cells were UV-irradiated at 15 J/m^2 and then subjected to a 2-h incubation in culture medium containing [^{3}H]thymidine (20 μCi/ml; specific activity, 120 Ci/mmol) washed with PBS, fixed, and processed for autoradiography. Recovery of RNA synthesis (RRS) after UV irradiation was determined as follows. 24 h after injection, cells were exposed to 15 J/m^2 of UV light, incubated for an additional 24 h in normal culture medium, washed with PBS, and subsequently incubated for another 1 h in culture medium containing [^{3}H]uridine (10 μCi/ml; specific activity, 50 Ci/mmol), fixed, and processed for autoradiography. Overall normal RNA synthesis (transcription) was determined, 48 h after injection as described above for RRS but without prior UV irradiation. UDS, RRS, and transcription levels were quantified by counting the autoradiographically induced silver grains above the nuclei (at least 100 nuclei). The relative levels of repair and transcription in the injected cells were obtained by dividing the

[2] E. Citterio, unpublished results.

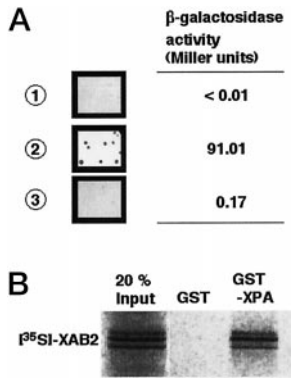

FIG. 1. **A novel protein, XAB2, interacts with XPA.** *A*, yeast two-hybrid assay showing a specific interaction of XPA and XAB2. A yeast strain expressing both XPA fused to the Gal4 DNA-binding domain and XAB2 fused to the Gal4 activation domain showed clear β-galactosidase activity (numerous blue colonies apparent in *2*). No β-galactosidase activity was induced in yeast strains expressing XPA fused to the Gal4 DNA-binding domain and the Gal4 activation domain (without XAB2) (*2*) or the Gal4 DNA-binding domain (without XPA) and XAB2 fused to the Gal4 activation domain (*3*). The enzyme activities measured by a quantitative liquid assay are shown in the table on the *right*. *B*, *in vitro* pull-down assays using *in vitro* translated, [^{35}S]Met-labeled XAB2 with GST or GST-XPA. 5× amounts of [^{35}S]Met-labeled XAB2 in the *left lane* (*20% Input*) were used for pull-down assays.

mean grain count number by the number of grains above the nuclei of surrounding noninjected cells.

RESULTS

XAB2 Is an XPA-interacting Protein—To identify protein interactions within NER and/or with other nuclear constituents, we performed a yeast two-hybrid screen with XPA as a bait (27) (see "Experimental Procedures"). In addition to previously identified XPA-interacting NER proteins (such as ERCC1 (28, 29) and the p34 subunit of replication protein A (27, 30)), we isolated a cDNA encoding a novel protein, designated XAB2 (XPA-binding protein 2). The specific interaction of XPA and XAB2 in yeast (Fig. 1*A*) was confirmed by *in vitro* pull-down assays using GST-XPA fusion protein and *in vitro* translated XAB2 (Fig. 1*B*).

XAB2 Is a Novel Tetratricopeptide Protein—Sequencing of the complete cDNA (reconstructed after 5′-rapid amplification of cDNA ends; see "Experimental Procedures") revealed a predicted acidic protein (pI 5.8) of 855 amino acids containing three stretches of acidic residues (Figs. 2 and 3*A*). Sequence homology searches using NCBI PSI-BLAST to match the XAB2 sequence against sequences in nonredundant protein data bases revealed three apparent homologs of *Drosophila melanogaster* (ORF of CG6197, accession number AAF58348; 60.6% identical), *Caenorhabditis elegans* (ORF of C50F2.3, accession number AAB37794; 47.5% identical), and *Schizosaccharomyces pombe* (ORF of SPBC211.02c, accession number CAB75410; 40.5% identical) (31). The searches also revealed that SYF1 is a most homologous protein in *Saccharomyces cerevisiae*, although the overall homology is not as high as in the other species (23.8% identical). The alignment of these proteins with XAB2 is shown in Fig. 2. The function of these proteins has not been identified. However, the strong conservation of XAB2 observed between lower and higher eukaryotes suggests the importance of this protein. The *SYF1* gene had been identified as a synthetic lethal mutant with the *CDC40*/*PRP17* gene, which is involved in S phase progression of the cell cycle and pre-mRNA splicing in *S. cerevisiae* (47). The homology observed between XAB2 and SYF1 gives rise to a possibility that in addition to NER, XAB2 may be involved in cell cycle control and pre-mRNA splicing (see "Discussion"). The alignment of these proteins also revealed that the carboxyl-terminal portion (29 amino acid residues at positions 825–853) of XAB2 is conserved in the *D. melanogaster* and *C. elegans* proteins, but absent in the *S. pombe* and *S. cerevisiae* proteins (Fig. 2). This region may have a specific role only in multicellular organisms.

The apparent homologs of XAB2 listed above contain TPRs that are degenerate repeats composed of 34-amino acid motifs (33). Three classes of TPR (classes I–III) are categorized based on the conserved sequences (34). TPRs are found in proteins of different organisms ranging from bacteria to humans implicated in protein complexes with diverged functions such as cell cycle control, transcriptional regulation, RNA processing, and mitochondrial and peroxisomal protein transport (35, 36). Mutational and structural analyses suggest that TPR domains play a role in intra- and intermolecular protein interactions (37–39). Sequence analysis revealed that XAB2 has 15 motifs of the class I TPR covering most of the protein (Figs. 2 and 3), suggesting that XAB2 may function as an important factor for protein-complex formations in NER.

XAB2 Interacts with CSA, CSB, and RNA Polymerase II—Since TPR proteins have been frequently found in complexes with WD-40 repeat-containing polypeptides (40, 41), we focused on the CSA protein, the known NER factor containing WD-40 repeats (19). As shown in Fig. 4*A*, *in vitro* translated XAB2 was indeed able to bind to GST-CSA fusion protein, and inversely *in vitro* translated CSA interacted with GST-XAB2 fusion protein. To verify the interaction *in vivo*, immunoprecipitations were performed using WCE of CS-A cells stably expressing functional HA-tagged CSA (see "Experimental Procedures"). Anti-HA monoclonal antibodies co-immunoprecipitated a small but significant fraction of XAB2 together with HA-tagged CSA (Fig. 4*B*), suggesting that at least part of XAB2 is associated with CSA *in vivo*.

The interaction with CSA prompted us to examine whether XAB2 interacts with CSB as well, since both CS proteins are specifically involved in the TCR pathway. Using WCE of CS-B cells stably expressing physiological levels of functional HA-/His_6-double-tagged CSB (2tCSB) (see Ref. 21 for documentation of these cells), immunoprecipitations with anti-HA monoclonal antibodies revealed an association of significant quantities of XAB2 with CSB (Fig. 5*A*, *upper part*). This immunoprecipitated fraction also contained a significant proportion of RNA polymerase II, as we have previously shown (21). The XAB2-CSB interaction is specific, since neither endogenous CSB nor XAB2 was precipitated with the anti-HA antibody when a WCE of HeLa without expression of 2tCSB was used (Fig. 5*A*, *lower part*).

Previously, we have shown that CSB together with RNA polymerase II is a part of a large protein complex (>700 kDa) (21). Immunoblot analysis of HeLa WCE, fractionated under physiological salt conditions by Superdex-200, revealed that XAB2 is present in fractions with an estimated molecular mass of >700 kDa (Fig. 5*B*), whereas a monomer of XAB2 is approximately 100 kDa. The migration pattern of XAB2 largely coincides with that of RNA polymerase II and CSB and differed from other NER and transcription factors assayed in the same fractions, such as ERCC1 (which forms a complex with XPF) and the XPB subunit of TFIIH, both migrating with a lower apparent molecular mass. The association of XAB2 with RNA polymerase II is further supported by an identical co-migration of the two proteins in the presence of 1 M KCl (Fig. 5*B*, *lower panel*), suggesting that the interaction is highly salt-resistant. In agreement with these observations, RNA polymerase II large subunit and XAB2 co-immunoprecipitated from HeLa WCE (Fig. 5*C*). RNA polymerase II large subunit was detected in the immunoprecipitated fraction with anti-XAB2 antiserum but absent in the fraction with control serum (Fig. 5*C*, *a*).

FIG. 2. **XAB2 amino acid sequence aligned with related proteins from four different species.** The sequences are XAB2 (*Homo sapiens*), ORF of CG6197 (*D. melanogaster*; AAF58348), ORF of C50F2.3 (*C. elegans*; AAB37794), ORF of SPBC211.02c (*S. pombe*; CAB75410), and SYF1 (*S. cerevisiae*; NP_010704). Identical amino acid residues are shown in *darkly shaded boxes*, and conservative substitutions are shown in *lightly shaded boxes*. *Underlines* with *Roman numerals* indicate the regions of TPR motifs.

Conversely, anti-RNA polymerase II (large subunit) antibodies but not anti-HA control antibodies precipitated part of XAB2 from HeLa WCE (Fig. 5*C*, *lower panel*). These findings provide evidence that XAB2 interacts with the CSB-RNA polymerase II complex *in vivo*.

In Vivo Function of XAB2—The interactions of XAB2 with XPA, CSA, and CSB-RNA polymerase II complex suggest a possible role for XAB2 in the TCR subpathway of NER. To further analyze the XAB2 function in living cells, we examined the effect of microinjected anti-XAB2 antisera on various NER parameters. Two antisera were used, one raised against the full-length XAB2 and the other against the C-terminal part (amino acid residues 694–855), designated anti-XAB2FL and anti-XAB2C, respectively. Microinjection of both anti-XAB2FL and anti-XAB2C did not significantly inhibit UV-induced UDS of normal human fibroblasts, which is mainly derived from

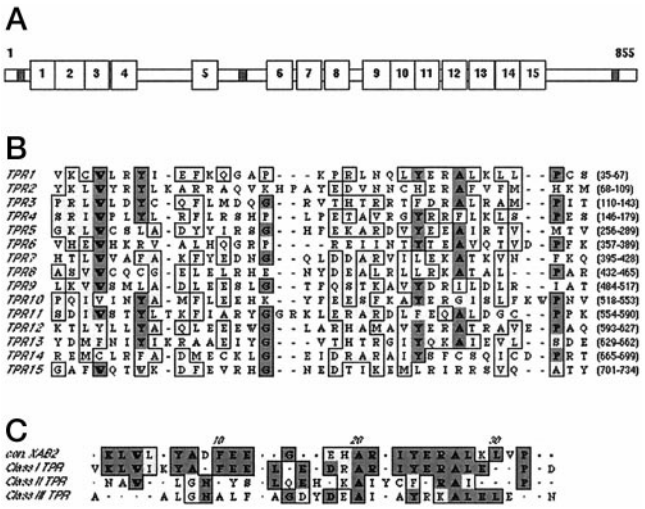

FIG. 3. **XAB2 has 15 TPR motifs.** *A*, schematic representation of the XAB2 protein. *Open boxes* with *numbers* and *hatched boxes* indicate TPR motifs and acidic regions, respectively. *B*, TPR sequence alignment. The 15 repeats in the predicted XAB2 protein were aligned. *Dark shading*, amino acid residues found in more than eight out of the 15 repeats; *light shading*, conservative substitutions found in more than seven of the 15 repeats. The substitutions were based on the following groupings: Phe, Tyr, and Trp; Ser, Thr, Ala, Gly, and Pro; Ile, Leu, Val, and Met; Asp, Glu, Asn, and Gln; and Arg, His, and Lys. Gaps in the sequence alignments are indicated by *dashes*. *Numbering* to the *right* corresponds to the amino acid positions of the TPRs in the XAB2 amino acid sequence. Five amino acid residues (RCVTD; 85–89) were deleted from TPR2 in the alignment. *C*, consensus sequence of TPRs in XAB2. The XAB2 consensus sequence is aligned with the consensus of the three classes of TPRs (34). The residues found most frequently at each position in the TPRs of XAB2 were used in the XAB2 consensus sequence. More variable positions are represented by *dots*.

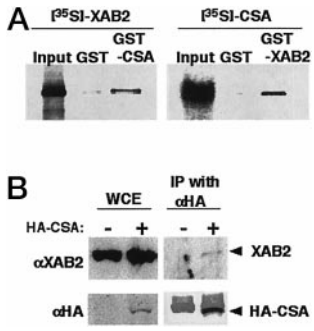

FIG. 4. **Association of XAB2 with CSA.** *A*, *in vitro* binding of XAB2 with CSA. *In vitro* pull-down assays were performed using *in vitro* translated, [^{35}S]Met-labeled XAB2 or [^{35}S]Met-labeled CSA with GST, GST-CSA, or GST-XAB2. *B*, co-immunoprecipitation of XAB2 with CSA. WCEs and immunoprecipitated fractions of WCE from CS3BE-SV cells with (+) or without (−) the expression of HA-CSA were analyzed by immunoblotting using anti-XAB2FL antiserum (*upper panels*) or anti-HA rat monoclonal antibody (3F10; *lower panels*; the lighter signals in both *lanes* are the heavy chain of mouse monoclonal antibody 12CA5 used for the immunoprecipitation).

GGR (42) (Fig. 6*A* and Table I). In contrast, injection of anti-XAB2 antisera in fibroblasts of XP-C patients, carrying a specific defect in GGR, induced a significant reduction of the residual UDS (Table I). Since UDS in XP-C cells is derived only from TCR (6), these results suggest that anti-XAB2 antisera directly interfere with the TCR rather than the GGR pathway. The finding that both anti-XAB2 antisera also inhibited the recovery of RNA synthesis after UV irradiation (RRS) in normal human cells (Fig. 6*B* and Table I) is consistent with the above observations, since the failure of RRS is ascribed to the defect of TCR (20, 43). Anti-CSB antiserum induced a similar effect on the above NER parameters (Table I), whereas injected

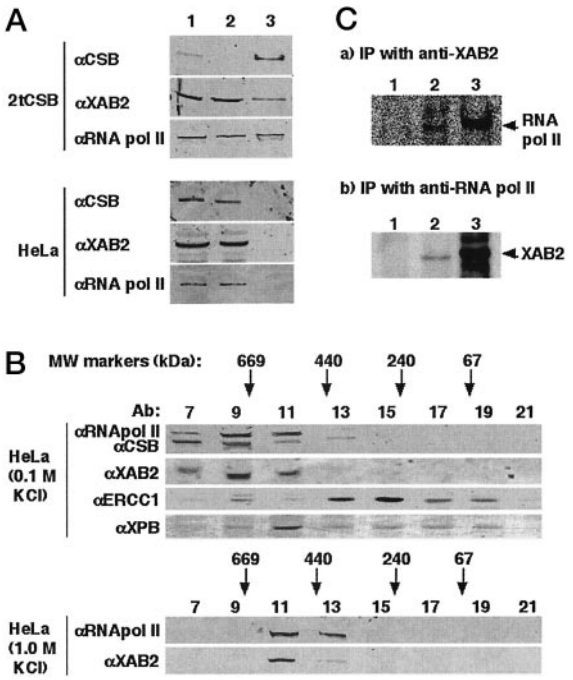

FIG. 5. **Interaction of XAB2 with CSB and RNA polymerase II.** *A*, co-immunoprecipitation of XAB2 with CSB. Immunoprecipitations with anti-HA mouse monoclonal antibody 12CA5 were performed using WCE of CS1AN-SV (2tCSB) cells (*top*) or HeLa cells (*bottom*). WCE (*lane 1*), nonbound fraction (*lane 2*), and the fraction eluted with HA peptide (*lane 3*) were analyzed with the indicated antibody. *B*, immunoblot analyses of size-fractionated WCE. HeLa WCE was separated on a Superdex-200 column at 0.1 M KCl (*top*) or 1.0 M KCl (*bottom*). Immunoblot analysis of the collected fractions was performed with the indicated antisera. The sizes and positions of molecular weight markers are shown at the *top* of the blots. *C*, co-immunoprecipitation of RNA polymerase II and XAB2. Immunoprecipitated fractions from HeLa WCE with preimmune serum (negative control; *lane 1*), anti-XAB2 antiserum (*lane 2*), and anti-RNA polymerase II monoclonal antibody 8WG16 (positive control; *lane 3*) were analyzed by 8WG16 (*upper panel*). Immunoprecipitated fractions from HeLa WCE with anti-HA mouse monoclonal antibody 12CA5 (negative control; *lane 1*), 8WG16 (*lane 2*), and anti-XAB2FL antiserum (positive control; *lane 3*) were analyzed by anti-XAB2FL antiserum (*lower panel*). 5% of the immunoprecipitated fractions were loaded in positive control lanes.

anti-ERCC1 antiserum affected both subpathways of NER, consistent with its essential function both in TCR and GGR. Injection of preimmune serum (Table I) or a number of other nonimmune sera and antibodies against various non-NER proteins (data not shown) did not induce any effect on DNA repair in normal human fibroblasts, indicating that the effect of XAB2 antisera is highly specific. The inhibitory effect of anti-XAB2 antisera on the process of TCR indicates that this protein plays a role in the same pathway as the CS proteins. However, in contrast to anti-CSB antiserum, anti-XAB2FL also induced a significant inhibition of normal RNA synthesis (Fig. 6*C* and Table I). This inhibitory effect was not observed using anti-XAB2C (Table I), suggesting that the C-terminal region (amino acid residues 694–855) of XAB2 may play an important role in TCR but not in transcription itself.

As previously shown, injection of nonimmune sera as well as antibodies against other factors only involved in NER failed to exert inhibition of transcription, in contrast to antisera against various proteins implicated in both NER and transcription initiation (21, 44). In conclusion, the results of the antiserum microinjection experiments suggest that XAB2 functions both in TCR and in normal transcription but has no role in GGR.

DISCUSSION

We found a novel protein, XAB2, which interacts with TCR-specific CSA, CSB proteins, and RNA polymerase II as well as

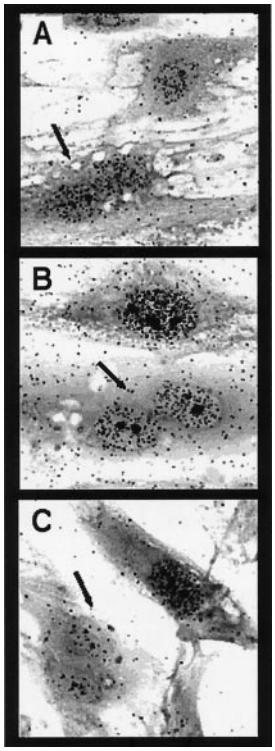

FIG. 6. **Inhibitions of recovery of RNA synthesis and transcription but not global genome repair by anti-XAB2 antiserum *in vivo*.** Anti-XAB2FL antiserum was injected into the cytoplasm of binuclear cells (indicated by an *arrow*) obtained after fusion of normal human fibroblasts. Subsequently the effect on DNA repair synthesis after UV-irradiation (UDS) predominantly derived from global genome repair (*A*), recovery of RNA synthesis after UV irradiation (RRS) (*B*), and transcription (normal RNA synthesis without UV- irradiation) (*C*) was assessed.

TABLE I
Effect of anti-XAB2 antiserum microinjection on DNA repair and transcription

Antiserum	UDS[a]		RRS[c]	Transcription[d]
	Wild type	XPC[b]		
	%	%	%	%
Preimmune	100	100	100	100
Anti-XAB2FL[e]	94–98	30–55	25–35	41–64
Anti-XAB2C[e]	96–98	74	40–55	95–106
Anti-CSB	100	20	37	100
Anti-ERCC1	3–10	ND[f]	21	100

[a] Unscheduled DNA synthesis (DNA repair synthesis) levels expressed as a percentage of UDS compared with noninjected neighboring cells.

[b] Percentage of the residual UDS in XP-C cells was 25 ± 5% of normal level.

[c] Percentage of RNA synthesis recovery after UV exposure in injected cells compared with noninjected neighboring cells.

[d] Percentage of overall RNA synthesis in nonirradiated injected cells.

[e] All experiments were repeated at least three times with the following exceptions. Anti-XAB2FL and anti-XAB2C injections into XP-C cells were performed twice and once, respectively. Given percentages, expressed as the observed (maximum) variation between different experiments, are derived from autoradiographic grain countings of at least 100 nuclei.

[f] ND, not done.

with the core NER factor XPA. Our microinjection experiments revealed that anti-XAB2 antisera caused specific inhibition of UV-induced UDS in XP-C cells (which only have functional TCR) and had no significant effect on UV-induced UDS in normal human cells (predominantly derived from GGR). We also observed inhibitory effects of anti-XAB2 antisera on recovery of RNA synthesis after UV irradiation in normal human cells. Together these results indicate that XAB2 is involved in TCR but not in GGR. In addition, antiserum against the entire XAB2 (anti-XAB2FL) inhibited transcription in non-UV-irradiated normal cells, strongly suggesting that XAB2 could be a novel factor involved in the transcription process itself. Since transcription is essential for TCR, it is likely that the observed inhibition of TCR is a consequence of the inhibitory effect of anti-XAB2FL on transcription. However, the anti-XAB2C (the antiserum against the carboxyl-terminal portion of XAB2) inhibited the recovery of RNA synthesis after UV irradiation without apparent inhibitory effects on transcription. These observations suggest that besides being involved in transcription, XAB2 could work as a TCR-specific factor, possibly through the carboxyl-terminal portion.

The molecular mechanism for the coupling of transcription and NER in eukaryotes is unknown. Presumably, a lesion on the transcribed strand is first encountered and marked by an RNA polymerase II elongation complex (thus bypassing the need for the XPC-HR23B complex). Then core NER factors are recruited by TCR-specific proteins such as CSA and CSB (2). CSB was found *in vitro* and *in vivo* to reside in an RNA polymerase II complex, probably in an elongation mode (21, 25). A quaternary complex consisting of CSB, RNA polymerase II, template DNA, and nascent RNA has been shown to be able to recruit TFIIH *in vitro* (26). The function of CSA is more obscure. *In vitro* associations of CSA with various NER factors have been reported (19), but no stable *in vivo* association to either the transcription machinery or to NER factors have been identified (21). In the present study, we found a dual interaction of part of XAB2 with a fraction of both CSA and CSB as well as the interaction with XPA. This raises the possibility that XAB2 links these TCR-specific proteins to assure recruitment and/or access of core NER factors to the lesion identified by the stalled RNA polymerase II in the elongation complex. The notion that these interactions are transient may explain our observation that only a small proportion of XAB2 is bound to CSA and RNA polymerase II (Figs. 4*B* and 5*C*). This is consistent with the fact that CSA and CSB appear to reside in different protein complexes (21).

Sequence homology searches using NCBI PSI-BLAST identified the apparent homologs of XAB2 in *D. melanogaster*, *C. elegans*, and *S. pombe*. XAB2 also showed a homology, albeit to a lower degree, to SYF1 of *S. cerevisiae* (Fig. 2). The sequence conservation extended over the entire length of XAB2, suggesting an important role of the protein that does not tolerate many evolutionary changes. An intriguing finding is the presence of XAB2 in the complete *Drosophila* genome sequence, in view of the notion that this organism appears to lack CSA and CSB homologs (45). This is consistent with an early report that *Drosophila* embryonal cells lack detectable TCR (46). The absence of a TCR pathway in *Drosophila* supports the idea that XAB2 has additional functions beyond TCR and is consistent with a role in the transcription process itself as suggested by the microinjection experiments.

The homology of XAB2 to SYF1 may provide a clue for understanding the role of XAB2. Dix et al. (47) described that the *SYF1* gene had been identified as a synthetic lethal mutant with the *CDC40*/*PRP17* gene, which is involved in S phase progression of the cell cycle and pre-mRNA splicing in *S. cerevisiae* and that ISY1, interactor of SYF1, was required for optimal pre-mRNA splicing in *S. cerevisiae*. In addition, McDonald *et al*. (48) reported that CWF3, the *S. pombe* ortholog of SYF1, is associated with CDC5, which is required for G_2/M progression of the cell cycle and essential for pre-mRNA splicing. We found that the reported partial amino acid sequences of CWF3 perfectly match to the *S. pombe* apparent homolog of

XAB2. Thus, it is possible that XAB2 is also involved in the processes associated with cell cycle control and pre-mRNA splicing in mammalian cells. The *SYF1* and *CWF3* genes have been found to be essential for viability in *S. cerevisiae* (52) and *S. pombe* (48), respectively. The requirement of XAB2 in transcription may account for the essential role of SYF1 and CWF3 for viability in yeast. The above findings in yeast fit nicely with our observation that a significant proportion of XAB2 is in a complex with the fraction of RNA polymerase II that is associated with CSB and is thought to be in an elongation mode (21). Since a tight coupling of transcription elongation and pre-mRNA splicing has been observed (49, 50), a potential involvement of XAB2 in pre-mRNA splicing may explain the inhibition of RNA synthesis observed after microinjection of anti-XAB2FL as a consequence of impaired splicing giving rise to arrested transcription. However, it has been reported that transcription may still occur at normal rates in the absence of efficient splicing of nascent pre-mRNA during transcription elongation in human cells (50, 51). Thus, it is likely that the inhibition of RNA synthesis by anti-XAB2FL resulted from impaired transcription rather than disturbed pre-mRNA splicing.

Based on our experimental data and the homology with CWF3 and SYF1, it is likely that XAB2 is a multifunctional protein involved in cellular processes such as cell cycle control and pre-mRNA splicing as well as TCR and transcription in mammalian cells. Das *et al*. reported that tandemly arranged TPR motifs are organized into a regular right-handed superhelix with a helical repeat of approximately seven TPR motifs (39). It is proposed that proteins with these structures could simultaneously interact with multiple target proteins, utilizing specific combinations of TPR motifs within the superhelix (39). Since XAB2 harbors 15 tandem arrays of TPR, a possible scaffolding function for XAB2 within cellular processes including NER and transcription is in line with its deduced amino acid sequence. XAB2 may function as a bridging protein, by simultaneously interacting with several other proteins or protein complexes. In addition, it would be of interest to find out whether defects in XAB2 also give rise to a human condition, since both CSA and CSB are associated with the severe neurodevelopmental, UV-sensitive TCR disorder Cockayne syndrome.

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