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

Received for publication, June 7, 2000, and in revised form, August 2, 2000
Published, JBC Papers in Press, August 15, 2000, DOI 10.1074/jbc.M004936200

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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 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, cachetic 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, XPF, 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.
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 *in vivo* (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 CSA 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 recombinant XAB2, obtained using and pull-down assays using GST, GST-XAB2, or GST-CSA fusion protein, respectively.

**GATATGCTGGGGTTTTTGTC-3**

**cells with RT-PCR using an upper primer (5′-TCCATAGGCAAGGTCGGTCA-CAGC-3′) and P2 primer (5′-TGGTCGCCAGGGCCCTTACATC-3′) according to the protocol supplied by the manufacturer. The full-length cDNA of XAB2 was reconstructed in pBluescript SK− by insertion of the EcoRI-KpnI fragment from the rapid amplification of cDNA ends product into the EcoRI and KpnI 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′-GCAATCTGCGATGATCGGTTTGGTC-3′) with an EcoRI site and a lower primer (5′-GTTGCTAGACCTGTGATGTGATGTG-3′) insert into pBluescript SK− and pGEX-5X-2 for *in vitro* translation and GST, respectively.

**Anti-XAB2 Antisera—**Anti-XAB2FL was raised against the full-length recombinant XAB2, obtained using Bac-to-Bac Baculovirus Expression System (Life Technologies, Inc.). The EcoRI-Xhol 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. Six constructs (1 × 10^6 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 disrupted with PBS. The cell pellets were recovered 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. The pellets were subjected to electrophoresis on 7% polyacrylamide gel. To visualize the recombinant XAB2, the gel was stained with CBB-R250 and destained in water. The gel strip 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 protein in *E. coli*. The BglII–Xhol fragment of XAB2 cDNA was inserted into *BamHI* and *Xhol* 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-SV1CSA cells. CS3BE-SV expressed no endogenous CSA, while CS3BE-SV1CSA (expressing a wild-type CSA) has a functional double-tagged CSA at physiological levels showed a normal UV sensitivity. 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+-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 confirmation of XAB2 with RNA polymerase II, HeLa WCE (4 mg), prepared with NETN, was incubated with anti-RNA polymerase II mouse monoclonal antibody (SWG16; a kind gift from Dr. J. M. Egli, 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 10% SDS-polyacrylamide gel electrophoresis and subjected to immunoblot analyses. The WCEs (20 μg) were also subjected to immuno blot 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 (CSR0) and XP21RO (XP-C) cells obtained after cell fusion as described (21). NER (unscheduled DNA synthesis (UDS) was measured 24 h after injection. After microinjection, cells were UV-irradiated at 15 J/m2 and then subjected to a 2-h incubation in culture medium containing [3H]uridine (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/m2 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 [3H]uridine (10 μCi/ml; specific activity, 50 Ci/mmol), fixed, and processed for autoradiography. Overall normal RNA synthesis (transcription) per cell was determined as described (29), 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.
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), we isolated a cDNA encoding a novel protein, designated XAB2 (XPA-binding protein). The specific interaction of XPA and XAB2 in yeast (Fig. 1A) was confirmed by in vitro pull-down assays using GST-XPA fusion protein and in vitro translated XAB2 (Fig. 1B).

**RESULTS**

**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 3A). Sequence homology searches using NCBI PSI-BLAST to match the XAB2 sequence against sequences in nonredundant protein databases revealed three apparent homologs of *Drosophila melanogaster* (ORF of CG6197, accession number AAF53848; 60.6% identity), *Caenorhabditis elegans* (ORF of CSF02.3, accession number AAB37794; 47.5% identity), and *Schizosaccharomyces pombe* (ORF of SPBC121.02c, accession number CAB75410; 40.5% identity) (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 and SYF1 across species (23.8% identical) suggests 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. 4A, 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. 4B), 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/His₆-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. 5A, 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. 5A, 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. 5B), 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. 5B, 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. 5C). RNA polymerase II large subunit was detected in the immunoprecipitated fraction with anti-XAB2 antiserum but absent in the fraction with control serum (Fig. 5C, a).
Conversely, anti-RNA polymerase II (large subunit) antibodies but not anti-HA control antibodies precipitated part of XAB2 from HeLa WCE (Fig. 5C, 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. 2.

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

anti-CSB antisera 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 antisera, anti-XAB2FL antisera induced a significant inhibition of normal RNA synthesis (Fig. 6C 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 antisera 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 vitro. Anti-XAB2FL antiserum was injected into the cytoplasm of binucleate 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<sup>a</sup> | RRS<sup>b</sup> | Transcription<sup>c</sup> |
| Preimmune | 100 | 100 | 100 | 100 |
| Anti-XAB2FL<sup>a</sup> | 94–98 | 30–55 | 25–35 | 41–64 |
| Anti-XAB2C<sup>a</sup> | 96–98 | 74 | 40–55 | 95–106 |
| Anti-CSB | 100 | 20 | 37 | 100 |
| Anti-ERCC1 | 3–10 | ND<sup>f</sup> | 21 | 100 |

<sup>a</sup> Unscheduled DNA synthesis (DNA repair synthesis) levels expressed as a percentage of UDS compared with noninjected neighboring cells.

<sup>b</sup> Percentage of the residual UDS in XP-C cells was 25 ± 5% of normal level.

<sup>c</sup> Percentage of RNA synthesis recovery after UV exposure in injected cells compared with noninjected neighboring cells.

<sup>d</sup> Percentage of overall RNA synthesis in nonirradiated injected cells.

<sup>e</sup> 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.

<sup>f</sup> 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. 4B and 5C). This is consistent with the fact that CSA and CSB appear to reside in different protein complexes (21).
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 fracion 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 super-helix 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 super-helix (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 neurological deficit of NER and transcription is in line with its deduced amino acid sequence. 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 neurological deficit of NER and transcription is in line with its deduced amino acid sequence.