p52 Mediates XPB Function within the Transcription/Repair Factor TFIIH*§

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Anass Jawhari‡§, Jean-Philippe Lainés, Sandy Dubaele, Valérie Lamour, Arnaud Poterszman, Frédéric Coin, Dino Moras, and Jean-Marc Egly¶

From the Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/Université Louis Pasteur, B. P. 10142, 67404 Illkirch Cedex, France

To further our understanding of the transcription/DNA repair factor TFIIH, we investigated the role of its p52 subunit in TFIIH function. Using a completely reconstituted in vitro transcription or nucleotide excision repair (NER) system, we show that deletion of the C-terminal region of p52 results in a dramatic reduction of TFIIH NER and transcription activities. This mutation prevents promoter opening and has no effect on the other enzymatic activities of TFIIH. Moreover, we demonstrate that intact p52 is needed to anchor the XPB helicase within TFIIH, providing an explanation for the transcription and NER defects observed with the mutant p52. We show that these two subunits physically interact and map domains involved in the interface. Taken together, our results show that the p52/Tfb2 subunit of TFIIH regulates the function of XPB through pair-wise interactions as described previously for p44 and XPD.

Human TFIIH is a multiprotein complex composed of nine subunits ranging from 89 to 32 kDa. These subunits are assembled into two subcomplexes: the core TFIIH, which is composed of six subunits (XPB, XPD, p62, p52, p44, and p34) and the cdk-activating kinase (CAK),1 which is composed of cdk7, cyclin H, and MAT1. Recently, the molecular structures of both human and yeast TFIIHs have been determined by electron microscopy and show similarities in size, shape, and architecture (1, 2). Originally identified as a basal transcription factor, TFIIH was subsequently found to contain XPB (Rad 25) and XPD (Rad 3), two helicases involved in nucleotide excision repair (NER) (3–8). Mutations in one of these subunits induce UV sensitivity in both human and yeast and are responsible for three rare human genetic disorders, xeroderma pigmentosum (XP), Cockayne syndrome, and trichothiodystrophy (9, 10). Many XP patients suffer from a high incidence of skin cancer due to their inability to remove lesions from their DNA. Further studies in yeast have shown that additional subunits of TFIIH, including p62 (Tfb1), p52 (Tfb2), and p44 (Ssl1) also have an essential role in DNA repair (11).

In an effort to understand the function of TFIIH in the diverse fundamental cellular processes in which it participates (transcription and DNA repair but also cell cycle regulation through the CAK complex), efforts have been aimed at systematically characterizing all TFIIH subunits. Several functions have been determined for individual components: XPB and XPD are ATP-dependent helicases indispensable for opening the DNA around a promoter and/or a lesion (12–16). Cdk7 is a serine/threonine kinase, which is regulated by cyclin H and MAT1 and phosphorylates several substrates including the C-terminal domain (CTD) of RNA polymerase II (see Ref. 17 and references therein). Recently, the N-terminal part of p44, a subunit of core TFIIH, has been shown to positively regulate XPD helicase activity, whereas the C-terminal part is involved in promoter escape (18). The XPD helicase regulation is lacking in a majority of XP-D patients because mutations in the C-terminal end of XPD abolish the XPD/p44 interaction (19). This can explain the UV sensitivity and NER defect harbored by yeast deleted for Ssl1, the p44 yeast homolog (20–23). The three remaining subunits, p62, p52, and p34, do not contain any known specific motif or enzymatic activities, and their function, as components of TFIIH structure, remains obscure.

Concerning p52, in vitro transcription and DNA repair assays, as well as microinjection experiments using p52 antibody, have demonstrated that p52, the last subunit of TFIIH cloned (24), is involved in both transcription and NER. Moreover, deletion of the C-terminal region of Tfb2, the yeast counterpart of p52 (64% similar and 40% identical), is detrimental for NER activity of yeast TFIIH in vivo and in vitro (25). In the present paper we have introduced a similar mutation in the human p52 subunit. We demonstrate that the C-terminal region of p52 is required to anchor XPB (the helicase involved in the opening of the promoter) within the core TFIIH. This study defines p52 as a preferential and indispensable partner of XPB in the TFIIH complex and helps to define the function of the XPB helicase in class II genes transcription and NER.

MATERIALS AND METHODS

Construction of Recombinant Baculoviruses—Baculoviruses expressing the TFIIH subunits XBP, His-XBP, XPD, p62, p52, His-p44, p44, p34, cdk7, His-cyclinH, and MAT1 were constructed as previously described (Ref. 13 and references therein). To obtain baculoviruses expressing p52 (13–135), p52 (137–296), p52 (1–358), p52 (381–462),
XPB (44–782), and XPB (208–782), the corresponding cDNAs were amplified by PCR and inserted into a modified pVL1392 baculovirus transfer vector that adds a 6-histidine tag at the N terminus of the open reading frame. To obtain baculoviruses expressing p52, p52 (1–358), and p52 (305–462) with a FLAG peptide (MTKDDDDKH) fused to its N terminus, the corresponding cDNAs were cloned in the PSK277 vector using a NdeI and BamHI restricted site as described (28). The vectors were then recombined with baculovirus DNA (Baculogold, Pharmingen), and the resulting viruses were plaque-purified and -amplified according to the manufacturer.

**Purification of Recombinant TFIIH Complexes**—Typically, 10^8 cells were infected with combinations of recombinant baculoviruses expressing combinations of XPD, XPD, p62, p52 or p52 (1–358), His-p44, p34, cdk7, His-cyclinH, and MAT1 as indicated and were collected 48 h after infection. Cells were washed in 1× phosphate-buffered saline, 30% glycerol and dounced in buffer B (20 mM Tris-HCl, pH 7.8, 5% glycerol, 150 mM NaCl, 0.1% Nonidet P40, 5 mM β-mercaptoethanol). After centrifugation at 14,000 °C for 30 min at 4 °C, clarified lysates were loaded on a heparin-Ultrogel column (Sepracor) pre-equilibrated in buffer A. After extensive washing with buffer A containing 300 mM NaCl, the proteins were eluted with the same buffer containing 500 mM NaCl. The eluted fractions were dialyzed for 2 × 2 h against 50 mM Tris-HCl, pH 7.9, 20% glycerol, 50 mM KCl, 0.1 mM EDTA, and 0.5 mM dithiothreitol and immunopurified using the 1H5 anti-p44 antibody at different ionic strengths.

Alternatively, to purify recombinant TFIIH by FLAG strategy, a virus derived from the pSK277 transfer vector expressing a p34 subunit with a FLAG peptide (MTKDDDDKH) fused at its N terminus was used. This virus was used together with viruses expressing p44, p52, p62, His-XPB, XPD, cdk7, His-cyclinH, and MAT1 as indicated and were collected 48 h after infection. Infection procedures were the same as above, but cells were collected, washed in 1× phosphate-buffered saline, 30% glycerol and dounced in buffer B (20 mM Tris-HCl, pH 7.8, 10% glycerol, NaCl 250 mM, 2 mM β-mercaptoethanol). DNA and cell membranes were pulled down by centrifugation at 14,000 × g during 30 min. The supernatants were incubated for 1 h at 4 °C with 1/40 fraction volume of cobalt chelate affinity resin (Talon, CLONTECH). After a 10-resin volume wash with buffer B containing 5 mM imidazole, TFIIH complexes were eluted using the same buffer containing 250 mM imidazole. The fractions from the Talon column, which contained TFIIH complexes, were incubated for 4 h at 4 °C with protein A-Sepharose beads cross-linked with anti-FLAG antibodies (FLAG-M2, Sigma). After three washes with buffer B containing 0.1% Nonidet P40, proteins were eluted in one bead volume of buffer B containing 1 mg/ml epitope peptide for 12 h.

**Protein-Protein Interaction Assays**—Pair-wise protein interactions were characterized by co-infection in S9B cells (2.5 × 10^7) with the corresponding recombinant baculoviruses at a multiplicity of infection of 5, collected 48 h after infection, washed in 1× phosphate-buffered saline, 30% glycerol and dounced in 2.5 ml of buffer A. Clarified lysates were obtained by centrifugation at 14,000 × g for 30 min at 4 °C. 50 μl of clarified lysate was adsorbed on 20 μl of protein G-Sepharose beads cross-linked with the appropriate monoclonal antibody (1B3, which recognizes the ATP binding site of XPB, and 1D11, which recognizes the N terminus of p52) in buffer C (20 mM Tris-HCl, pH 7.5, 10% glycerol, 50 mM KCl, 0.1 mM EDTA). After 1 h of incubation at 4 °C, the beads were washed extensively in buffer C containing 150 mM KCl and resuspended in Laemmli buffer. The proteins were resolved by SDS-PAGE (12.5% acrylamide) and revealed by Western blotting using the appropriate monoclonal antibodies.

**Nucleotide Excision Repair-Dual Incision Assay**—Circular DNA containing a single 1,3-intrastrand d(GpTpG) cisplatin-DNA cross-link (Pt-GTG) was prepared as described (27). Repair reactions were carried out in buffer containing 45 mM HEPES at 7.8, 70 mM KCl, 5 mM MgCl₂, 1 mM dithiothreitol, 0.3 mM EDTA, 10% glycerol, 2.5 μg of bovine serum albumin, and 2 mM ATP. Each reaction contained 50 ng of RPA, 22.5 ng of XPA, 10 ng of XPC-hHR23B, 50 ng of XPG, 20 ng of ERCC1-XPF NER factors, or 1.5 μl of purified TFIIH fractions. Following pre-incubation for 10 min at 30 °C, 50 ng of Pt-GTG was added and reactions were continued for 90 min at 30 °C. Reactions were stopped by rapid freezing. 6 ng of an oligonucleotide complementary to the excised DNA fragment (and containing four extra G residues at the 5′ end) was annealed to the excision products. Sequenase v.2.0 polymerase (0.1 unit) and 1 μCi of [³²P]dCTP were used to add four radiolabeled C residues to each excision product. The products were separated by electrophoresis on 14% polyacrylamide gel and visualized by autoradiography.

**TFIIH Enzymatic Assays**—The helicase substrate was obtained by annealing 5 ng of an oligonucleotide encompassing a sequence complementary to nucleotides 6219–6255 of single-stranded M13mp18 DNA to 1 μg of single-stranded M13mp18. The resulting heteroduplex was digested for 1 h at 37 °C with EcoRI (New England Biolabs) and then
extended to 21 and 20 bp, respectively, with the Klenow fragment (5 units) in the presence of 50 mM dTTP and 7 μCi of [α-32P]dATP (3000 Ci/mmol, Amersham Biosciences). The helicase assay was then performed as described (24).

ATP hydrolysis was monitored as previously described. Briefly, protein fractions were incubated for 2 h at 30 °C in the presence of 1 μCi of [γ-32P]ATP (7000 Ci/mmol, ICN Pharmaceuticals) in a 20-μl reaction volume containing 20 mM Tris/HCl, pH 7.9, 10% glycerol, 1 mM EDTA, 0.5 mM dithiothreitol, and 50 μg/ml bovine serum albumin. Reactions were stopped by adding EDTA to 50 mM. The reactions were then diluted 5-fold, spotted onto polyethylenimine TLC plates (Merck), run in 0.5 M LiCl/1 M formic acid, and autoradiographed.

Kinase assays (25) were carried out in a 20-μl reaction volume containing 20 mM HEPES, pH 7.9, 20 mM Tris/HCl, pH 7.9, 7 mM MgCl₂, 0.5 mg/ml bovine serum albumin, and 30 mM KCl, 1 μg of ctd4 (a synthetic tetrapeptide of SPTSPSY), and 2.5 μCi of [γ-32P]dATP. Samples were incubated 30 min at 25 °C, and reactions were stopped by the addition of 5 μl of loading buffer. After SDS-PAGE (15%), the gel was fixed and dried on Whatman filter paper. The phosphorylated ctd4 was visualized by autoradiography.

**KCNFO** Footprinting Assay—AdMLP template (20 ng) was incubated at 25 °C for 30 min with recombinant TBP, TFIIIB, TFIIIF, TFIIIE, highly pure pol II, and TFIIH as indicated in a 20-μl reaction that contained 50 mM Tris/HCl, pH 7.9, 10% glycerol, 1 mM EDTA, 0.5 mM dithiothreitol, and 5 mM MgCl₂. ATP and CTP (200 μM) were added for the last 5 min. Two μl of 160 mM KCNFO was added for 2 min, after which the reaction was stopped by the addition of 2 μl of 14.4 M β-mercaptoethanol. After phenol-chloroform extraction, DNA was recovered by ethanol precipitation, and wash, the sample was loaded onto a 6% sequencing gel. The gel was dried and autoradiographed.

**Proteolysis Assay**—For the proteolysis experiments, 30 μl of recombinant TFIIH purified by the FLAG strategy were incubated with 10 μl of chymotrypsin at various concentrations (0–0.25 mg/ml) and incubated 1 h at room temperature. The reaction was stopped by the addition of 5 μl of 100 mM Pefabloc (Roche Diagnostics), and the hydrolyzed peptides were analyzed by SDS-PAGE followed by Western blotting using monoclonal antibodies directed against either the N-terminal (1D11) or the C-terminal (5D6) part of p52.

**RESULTS**

**The C-terminal Deletion of p52 Is Detrimental for TFIIH Activity in Both DNA Repair and Transcription**—By taking advantage of recombinant technology, we have generated TFIIH complexes in insect cells. One of them (rIIH9/p52 (1-358)) contains a C terminus-truncated p52. Recombinant TFIIHs, (rIIH9wt and rIIH9/p52(1-358)) were produced in High-Five insect cells co-infected with baculoviruses expressing either the wild type or truncated forms of p52 as well as the eight other TFIIH subunits (13). The insect cell extracts were then loaded on a heparin-Ultrogel column, and the protein was eluted with 0.5 M KCl including rIIH9wt and rIIH9/p52(1-358), respectively, which were applied onto a cobalt chelate affinity resin. After extensive washing of the column under mild salt conditions (50 mM KCl), both rIIH9s were eluted with the same buffer containing 50 mM EDTA. As judged by immunoblotting, the stoichiometry of both rIIH9 complexes are almost identical (Fig. 1A). The mutated TFIIH differs only from the wild type by the presence of the truncated p52(1-358) protein (lane 2); p52 co-migrates with histidine-tagged p44 (lane 1).

Both recombinant TFIIHs were then tested for their DNA repair and transcription activities. As expected (25), the recombinant human TFIIH (rIIH9/p52(1-358)) was inactive in incision/excision (one of the first steps of NER) of cisplatin-damaged DNA when added to an *in vitro* assay (28) containing highly purified recombinant XPC/HR23h, XPA, RPA, ERCC1/XPF, and XPG (Fig. 1B). In addition, the transcription activity of rIIH9/p52(1-358) was strongly reduced (80%) relative to wild type (Fig. 1C, left panel) in an *in vitro* transcription assay.
containing the adenovirus major late MLP template, RNA pol II, and purified basal transcription factors except TFIIH.

Because CAK is anchored to the core TFIIH, we wondered if the p52 C-terminal deletion would have affected the stimulatory effect of CAK. Previous work has shown that for certain promoters such as MLP, dependence on the CAK subunits is not a function of the kinase activity of cdk7 but is rather due to the contribution of CAK to an optimal positioning of TFIIH within the transcription preinitiation complex (29, 30). Recombinant rIIH6/p52(1–358) and rIIH6wt were produced in Sf9 cells and purified as described above. Wild type CAK was then added to the reconstituted transcription assay containing either the rIIH6 or the rIIH6/p52(1–358) subcomplex (Fig. 1C, right panel). In both cases CAK stimulates rIIH6/p52(1–358) and rIIH6wt transcription activity by about 4–5-fold (Fig. 1C, right panel, compare lanes 2 and 4 with lanes 6 and 8). These results clearly indicate that the transcriptional defect associated with rIIH6/p52(1–358) is independent of CAK transcription stimulation and is instead intrinsic to the core structure of TFIIH.

p52 Mutations Affect Promoter Opening by XPB—We then wondered whether p52 mutations would affect the enzymatic activities of TFIIH that participate in transcription and DNA repair events. We then investigated the phosphorylation by the cdk7 subunit of TFIIH of peptide substrate (ctd4) that contains four copies of the hepta repeat found in the C-terminal domain of the largest RNA pol II subunit. Both TFIIHs phosphorylated ctd4 (Fig. 2A), showing that the cdk7 activity of CAK is not affected by the p52 mutation. In another set of experiments, we measured the overall ATPase activity of both rIIH9wt and rIIH9/p52(1–358). Neither the XPB nor the XPD ATPase activities were affected by the p52 mutation (Fig. 2B). Then, we asked whether mutation of p52 would impair the XPD or XPB unwinding activities. We found that the ATP-dependent XPD helicase activity of rIIH9/p52(1–358) is similar to rIIH9 wild type (Fig. 2C). As the XPB helicase activity cannot be measured using a standard strand displacement assay (31) (Fig. 2C) and XPB has been shown to be the helicase involved in promoter opening, we investigated whether rIIH9 retains its ability to direct promoter opening using a KMnO4 footprinting assay (27). In the presence of saturating amounts of basal transcription factors including HeLa TFIIH and RNA pol II, addition of both ATP and CTP (allowing the formation of the first phosphodiester bond) leads to an enhancement of the sensitivity of the thymidines around the AdMLP promoter (Fig. 2D, lanes 1 and 2, positions +3, +5, +7, and +8), indicating promoter opening around the transcription initiation site. When rIIH9/p52(1–358) is added instead of rIIH9wt, promoter opening is significantly decreased (Fig. 2D, lanes 2 and 3). This inhibition strongly correlates with the decrease in run-off transcription, indicating that this impairment of transcription can be directly linked to an impairment of promoter opening.

FIG. 3. p52 is required for the presence of XPB in the core TFIIH. Co-infected baculoviruses extract containing combinations of the nine TFIIH subunits (rIIH9 or rIIH9/p52(1–358) were immunoprecipitated under different salt conditions as indicated at the top of each panel (A). Subunits of the core TFIIH with (rIIH5) or without p52 (rIIH4) were immunoprecipitated and analyzed by Western blotting (C) or silver staining (B).
The Subunit p52 Is Required for the Integration of XPB in TFIH—Since promoter opening is impaired and XPB has been shown to be the helicase involved in the transcription initiation step (31), we then asked whether p52 mutation affects association of XPB with the TFIH multiprotein complex. Antibodies raised against p44 were used to immunoprecipitate TFIHs produced in insect cells co-infected with baculoviruses overexpressing wild type p52 or truncated forms and XPB were analyzed by Western blotting (A) and tested for ability to form a complex by immunopurification using an anti-XPB antibody; L, heavy chain (B). Recombinant TFIH was digested with increasing amounts of chymotrypsin (0.02, 0.1, 0.5, and 2.5 μg), subjected to SDS-PAGE (12.5% acrylamide) and analyzed by Western blotting using monoclonal antibodies directed against the N-terminal or C-terminal end of p52. A 43-kD protease-resistant fragment that contains the p52 N-terminal epitope accumulates, whereas the p52 C-terminal epitope is rapidly degraded (C). Schematic drawings of p52. The stretches of highly conserved residues in eukaryotes are indicated in black, and the regions involved in XPB binding are delimited (D).

p52 Mediates XPB Function in TFIH—The above data suggest a role for p52 in promoter opening and for the integration of XPB into TFIH. We therefore wondered if some connection between these two subunits could be identified. Sf9 insect cells were co-infected with two baculoviruses expressing wild type p52 or truncated forms and XPB, p62, p44, and p34. When performed at higher salt concentration (250 mM KCl), XPB did not co-precipitate with other TFIH subunits when p52(1–358) is present (Fig. 3A, lanes 2–4). Indeed, although treatment with 250 mM KCl does not dissociate TFIH subcomplexes (compare lanes 1–3), we notice that p52(1–358) prevents anchoring of XPB in the core TFIH (Fig. 3B, lane 2).

In another set of experiments, we demonstrate that p52 is required for the presence of XPB in the core TFIH. Indeed XPB could be co-immunoprecipitated from infected insect cells with p44, p62, and p34 only in the presence of p52 (Fig. 3C, compare lanes 2 and 4). Together these results show that the C-terminal domain of p52 determines the strength of the binding of XPB within TFIH whether or not CAK/XPD is present.

The p52 Subunit Directly Interacts with XPB—The above data suggest a role for p52 in promoter opening and for the integration of XPB into TFIH. We therefore wondered if some connection between these two subunits could be identified. Sf9 insect cells were co-infected with two baculoviruses expressing p52 and one of the five subunits of the core TFIH (XPB, XPD, p62, p44, and p34). Recombinant proteins of the core TFIH contained in cell lysates were then immunoprecipitated with the corresponding antibody. After extensive washing at 250 mM KCl, the immunoprecipitates were analyzed by Western blotting. Under these conditions, only XPB (Fig. 4B, compare lanes 1 and 2) and to a lesser extent p62 (data not shown) were able to significantly interact with p52. These results argue that XPB binds preferential to p52 within TFIH.
To map the region of p52 that is involved in the interaction with XPB, we generated a set of baculoviruses expressing truncated forms of p52 corresponding to residues 1–135, 137–296, 1–358, 1–304, 305–462, and 381–462 based on sequence alignments (see supplemental material at http://www.jbc.org). We then coexpressed each of the truncated p52s with wild type XPB (Fig. 4A). As a control, we confirmed that all proteins were successfully expressed (Fig. 4A). Ab-XPB is able to coimmunoprecipitate with XPB, p52(1–135), p52(1–304), and p52(1–358) as well as p52(305–462) but not p52(137–296) and p52(381–462) (Fig. 4B, lanes 3–14).

The above results show that p52 contains two distinct XPB binding regions that correspond to residues 1–135 and 304–381 (Fig. 4D) and raise questions concerning the role, if any, of the extreme C-terminal end of p52 in XPB interaction. To address this point, recombinant TFIIH containing the nine subunits produced in baculovirus-infected SF9 cells was subjected to limited proteolysis. At low protease concentration, a substrate protein would be expected to be cleaved preferentially at sites that are exposed to the solvent. It is generally accepted that protease-resistant polypeptides are involved in the formation of structural entities. Recombinant TFIIH was incubated with increasing amounts of chymotrypsin and analyzed by SDS-PAGE followed by immunoblotting. Using an antibody directed against the N terminus of p52, we noticed that p52 was proteolysed to result in a fragment of 43 kDa that corresponds approximately to 390–400 amino acids (Fig. 4C, left panel). Under these conditions, the C-terminal part of p52 is presumably degraded to very small fragments as the antibody directed against the C terminus of p52 does not detect any low molecular weight peptides (right panel). This experiment shows that the extreme C terminus of the protein is accessible to the protease and is in agreement with the observation that p52(381–462) does not interact with XPB.

The N Terminus of XPB Anchors the Helicase to Core TFIIH—To map in more detail the region of XPB involved in the interaction with p52, we generated several truncated XPB polypeptides including XPB(44–782) and XPB(208–782) and analyzed them as described above. Ab-p52 coimmunoprecipitates only XPBwt and XPB(44–782) but not XPB(208–782) (Fig. 5A). Ab-XPB does not precipitate p52 when coexpressed with XPB(208–782). It is also worth mentioning that when overexpressed together with p34, p44, p52, and p62 subunits, XPB(208–782) is unable to assemble into core TFIIH (Fig. 5B). Taken together, the above data demonstrate that the 1–381 portion of p52 interacts with XPB residues 44–208, which include the DNA binding motif (Fig. 5C).

DISCUSSION

Gene expression is regulated by the various transcription factors that are involved in RNA synthesis. Some of these transcription factors possess enzymatic activities that orchestrate the various events of the transcription reaction, such as chromatin remodeling, promoter opening, and RNA pol II phospho-/dephosphorylation.

It has been known for some years that the enzymatic activities of the cdk7 and XPD subunits of TFIIH are regulated. The activity of cdk7 kinase depends on the presence of cyclinH and MAT1, two other TFIIH subunits, as well as on the phosphorylation state of its T-loop (17). Similarly, upon interaction with p44, XPD helicase activity increases (19). Mutations in the C-terminal domain of XPD modify the contact with p44, explaining the NER defect associated with most of the XP-D patients (32). In addition, mutations in XPD or in p44 that modify the XPD-p44 interaction affect the composition of TFIIH by decreasing the amount of XPD and CAF1 subunits associated with the core and/or weakening the anchoring of
CAK to the core TFIIH (23, 31) with a consequent change in the rate of some hormone-responsive genes (33).

In the present study, we show that the p52 subunit is the privileged partner of the XPB helicase. Each of the enzymatic activities found in TFIIH now possesses its own regulatory partner within the protein complex. Using a recombinant TFIIH, with which the C-terminal domain of p52 was deleted, we have demonstrated that the p52 mutation is detrimental for both TFIIH-DNA repair and transcription activities. Indeed, several experiments, including permanganate footprinting, allow us to assign this transcriptional defect to an impairment in the opening of the DNA around the promoter. Having previously demonstrated that the XBP helicase was crucial in the promoter opening (12, 31), we further investigated the role of p52 in anchoring of XBP to core TFIIH. We first show that the integrity of p52 is a prerequisite for the presence of XBP in core TFIIH. Indeed, the ternary p34/p44/p62 complex does not interact with XBP in the absence of p52. In fact XBP anchoring is mediated by p52 and, as also observed in yeast (34), the two proteins interact directly. XBP binding involves two independent regions of p52: the first is located within residues 1–135 and contains a few residues strictly conserved among p52 orthologs. The second binding region includes residues 303–381, as p52(303–462) interacts with XBP, whereas p52(381–462) does not. This second binding region is highly conserved from human to yeast (Fig. 4 and supplemental material at http://www.jbc.org).

The fact that p52(381–462) does not interact with XBP correlates with the observation that the C terminus of p52 is rapidly digested in a mild proteolysis assay (Fig. 4D). It seems, however, that this domain is crucial for the architecture and/or the stability of TFIIH, as a deletion of residues 358–462 weakens the binding of XBP within the core TFIIH. This instability can explain the NER defect and transcriptional impairment as well as the phenotype observed in yeast cells containing a similar deletion. We propose that the C-terminal domain of p52 might possess a three-dimensional structure that locks up XBP inside TFIIH. The consequence of the p52 deletion would be a weaker and/or inappropriate positioning of the XBP helicase first within TFIIH itself and second within the transcription and/or DNA repair machinery. This defective association of XBP with TFIIH would result in a defect in one of the essential function of TFIIH, i.e. DNA opening.

Unlike the XBP and XPD genes, no human genetic disorders have been associated with the gene encoding p52. However, a change in the last 40 amino acids of XBP from XP-B/CS patients results in a similar defect in promoter opening and consequently RNA synthesis (31). Moreover, using photo-cross-linking experiments, we showed that the positioning of the mutated XBP helicase of TFIIH onto promoter DNA was impaired (35). Sequence analysis as well as deletion experiments suggest that XBP is composed of three modules: a N-terminal domain (also referred to as DNA binding domain), the catalytic core domain, which contains the helicase motifs, and the C-terminal domain (36). We have shown that the region of XBP that contacts p52 corresponds to the N-terminal putative DNA binding domain of this helicase (37, 38). It is likely that the C-terminal moiety of p52 allows trapping and stabilization of XBP within TFIIH. Whether deletion of the C-terminal region of p52 affects the accuracy of positioning and/or the association of TFIIH within the transcription preinitiation and NER complexes remains to be further investigated.

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