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A 3′ → 5′ XPB Helicase Defect in Repair/Transcription Factor TFIIH of Xeroderma Pigmentosum Group B Affects Both DNA Repair and Transcription*

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XPB is a subunit of the basal transcription factor TFIIH, which is also involved in nucleotide excision repair (NER) and potentially in cell cycle regulation. A frameshift mutation in the 3′-end of the *XPB* gene is responsible for a concurrence of two disorders: xeroderma pigmentosum (XP) and Cockayne's syndrome (CS). We have isolated TFIIH from cells derived from a patient (XP11BE) who carries this frameshift mutation (TFIIHmut) and from the mother of this patient (TFIIHwt) to determine the biochemical consequences of the mutation. Although identical in composition and stoichiometry to TFIIHwt, TFIIHmut shows a reduced 3′ → 5′ XPB helicase activity. A decrease in helicase and DNA-dependent ATPase activities was also observed with the mutated recombinant XPB protein. The XPB mutation causes a severe NER defect. In addition, we provide evidence for a decrease in basal transcription activity *in vitro*. The latter defect may provide an explanation for many of the XP and CS symptoms that are difficult to rationalize based solely on an NER defect. Thus, this work presents the first detailed analysis of a naturally occurring mutation in a basal transcription factor and supports the concept that the combined XP/CS clinical entity is actually the result of a combined transcription/repair deficiency.

TFIIH, previously called BTF2, was originally defined as a mammalian transcription factor required for accurate transcription of protein-coding genes. In addition to TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and RNA polymerase II, TFIIH participates in the formation of the active transcription initiation complex on a TATA box-containing minimal promoter. Binding of TFIID to the TATA box sequence is thought to initiate the reaction, while TFIIH enters the complex late during its assembly (1–3). Recently, this factor was found to be engaged in one of the main DNA repair pathways, nucleotide excision repair (NER)[1] (4). It is also interesting to note that TFIIH contains two polypeptides, Cdk7 (MO15) and cyclin H, that were shown to play some role in cell cycle regulation (5). Purification of TFIIH to homogeneity demonstrated that it is a multisubunit protein complex that contains between five and nine polypeptides, including p89 (XPB-ERCC3) and p80 (XPD-ERCC2), two DNA helicases with opposite polarity (6–8) and with DNA-dependent ATPase activities (9); Cdk7 (MO15) (10) and cyclin H, components of a cyclin-dependent kinase-activating kinase (11); p44, the human homologue of yeast SSL1 (12) and p34, which possess zinc fingers motifs that may provide TFIIH with a DNA binding function (13). We (14) and others (15–18) recently demonstrated that at least four subunits, p89 (XPB), p80 (XPD), p62, and p44, are involved in NER. NER is the main repair system for the elimination of a wide range of structurally unrelated DNA lesions, including UV-induced damage and numerous bulky adducts. At least six steps are thought to take place in the NER reaction: lesion recognition, followed by template demarcation (including local melting of the DNA around the damage), dual incision of the injured strand (each site at some distance from the lesion), release of the 27–29-nucleotide damage-containing oligomer (19), gap-filling DNA repair synthesis, and finally sealing of the remaining nick (20). The NER reaction has two modalities: preferential repair of lesions that block ongoing transcription (transcription-coupled repair) and the slower and less efficient global genome NER subpathway. TFIIH appears to be a core component of the excision process implicated in both NER modes. One of the putative functions of the complex is the opening of the DNA required for the incision stage of the NER reaction.

Three hereditary, genetically heterogeneous human syndromes, xeroderma pigmentosum (XP), Cockayne's syndrome (CS), and trichothiodystrophy (TTD), have been recognized to be associated with mutations in TFIIH subunits and to carry defects in the NER pathway (21). Cell hybridization studies have identified many distinct complementation groups within and considerable overlap between these disorders: seven in XP (XP-A to XP-G, three of which also harbor patients with combined XP and CS (XP-B, XP-D, and XP-G)), two in the classical form of CS (CS-A and CS-B), and three in TTD (XP-B, XP-D, and TTD-A) (21, 22). The discovery of TFIIH as a focal point for the regulation and coordination of different cellular events has aided the interpretation of some unexplained clinical symptoms associated with defects in the genes for some of the TFIIH subunits. This is well illustrated by the case of the *XPB* gene encoding the p89 subunit of TFIIH. XP-B is a very rare form of XP of which only three cases have been described; two display a combination of XP and CS syndromes, and the third manifests TTD. In XP/CS patient XP11BE, the most severe XP-B

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[1] The abbreviations used are: NER, nucleotide excision repair; XP, xeroderma pigmentosum; CS, Cockayne's syndrome; TTD, trichothiodystrophy; Ab, antibody; CTD, C-terminal domain.

case, a C to A transversion in the last intron of the *XPB* gene generates a splice mutation at the RNA level (23) and a frameshift at the protein level changing the last 41 amino acids (see Fig. 1*B*). This patient had acute sun sensitivity with the pigmentation abnormalities characteristic of XP, including actinic keratoses and several cutaneous malignancies, in addition to all the clinical hallmarks of CS such as developmental and neurological abnormalities (dwarfism, microencephaly, and deafness) and impaired sexual development (24, 25). The cells of this patient have a severe defect in both subpathways of NER, with repair synthesis being only 5–10% of the activity of normal fibroblasts (26). In contrast, the patient's mother, heterozygous for the causative allele of the *XPB* gene, was clinically normal and had wild-type levels of DNA repair consistent with the recessive nature of the genetic defect.

It was proposed that many of the CS and TTD features (such as neurodysmyelination, brittle hair, and growth defect among others) unexplainable based only on an NER defect may be due to a deficiency in the transcription function of TFIIH. This dysfunction might cause a reduced expression of a specific set of genes particularly sensitive to a malfunctioning TFIIH (21). The aim of this study was to characterize at the biochemical level the defect in TFIIH that is responsible for the diverse set of severe clinical features. We have analyzed the enzymatic activities of TFIIH isolated from the lymphoblasts of XP11BE. Our results show that the mutated XPB polypeptide associated with TFIIH presents both helicase and ATPase deficiencies that directly or indirectly impair the NER reaction and, to a lesser extent, appear also to affect basal transcription. These results provide an explanation for the large range of clinical symptoms associated with CS and TTD, some of which are unrelated to an NER deficiency.

EXPERIMENTAL PROCEDURES

Purification of TFIIHmut and TFIIHwt—Extracts were prepared with 80 liters (0.7×10^6 cells/ml) of either GM2252A cells (lymphoblastoid cell line derived from an XP-B patient) or GM1855 cells (lymphoblastoid cell line derived from the patient's mother). Cells were grown in suspension in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 10% fetal calf serum and 40 μg/ml gentamicin and collected by centrifugation for a whole cell extract preparation. TFIIH purification was performed as described previously (30), except for the fourth purification step, which used a TSK-heparin-5PW column (0.75 × 7.5 cm, flow rate of 0.6 ml/min; Toso-Haas, Stuttgart, Germany). The transcriptionally active fractions of SP-5PW preparations (300 ml, eluted at 0.45 M KCl) were pooled and dialyzed against 50 mM Tris-HCl, pH 7.6, 50 mM KCl, 0.5 mM dithiothreitol, 0.1 mM EDTA, and 10% glycerol before being loaded onto the heparin-5PW column equilibrated in the same buffer. The TFIIH fraction was eluted with a 20-ml linear gradient from 0 to 0.9 M ammonium sulfate and tested repeatedly in *in vitro* transcription (28), helicase, CTD kinase, and DNA-dependent ATPase (29) assays. For each TFIIH, three different preparations were performed.

Bidirectional Helicase Assay—To prepare helicase substrate, 5 ng of an oligonucleotide corresponding to fragment 6219–6255 of single-stranded M13mp18(−) DNA was annealed to 1 μg of single-stranded M13mp18 DNA in 5 mM Tris-HCl, pH 7.9, 25 mM NaCl, and 2.5 mM $MgCl_2$ by incubation at 95 °C for 5 min followed by 30 min at 24 °C. The resulting heteroduplex was digested for 1 h at 37 °C with *Eco*RI (New England Biolabs Inc.), thus generating two duplex regions of 17 and 20 base pairs. The oligonucleotide was then elongated with DNA polymerase I Klenow fragment (5 units) in the presence of 50 μM TTP, 30 μM dGTP, 7 μCi of [α-^{32}P]dATP (3000 Ci/mmol) (Amersham International, Buckinghamshire, United Kingdom), and 4 mM $MgCl_2$. After 20 min at 23 °C, 50 μM dATP was added to the reaction mixture, which was then incubated for another 20 min at 23 °C. After phenol/chloroform extraction, the free nucleotides were removed by gel filtration on Sephadex G-50 (Pharmacia, Uppsala). The DNA helicase assay (25 μl) contained 20 mM Tris-HCl, pH 7.9, 4 mM $MgCl_2$, 1 mM dithiothreitol, 50 μg/ml bovine serum albumin, 2 mM ATP, and 1–3 ng of DNA substrate. Reactions were incubated at 37 °C for 45 min; stopped by the addition of 10 μl of 60 mM EDTA, 50% glycerol, 0.75% SDS, and 0.1% bromphenol blue; and loaded on a 10% nondenaturing acrylamide gel in 0.5 × Tris-borate/EDTA buffer, pH 8, at 100–200 V. The gel was then dried and analyzed by autoradiography. The substrate heated for 2 min at 100 °C was used as positive control.

Immunoprecipitation—The monoclonal antibodies to p62 and Cdk7 (MO15) were cross-linked to protein A-agarose. The TFIIHmut- and TFIIHwt-heparin-5PW fractions were incubated for 1 h at 4 °C with the antibodies in buffer A (50 mM Tris-HCl, pH 7.9, 10% glycerol, 0.1 mM EDTA, and 150 mM KCl) containing 1 mg/ml bovine serum albumin. The immunoadsorbed complexes were sequentially washed three times with buffer A containing 0.1% Nonidet P-40 and twice with buffer A containing 0.1% Nonidet P-40 and O.5 M KCl and equilibrated in 50 mM Tris-HCl, pH 7.9, containing 10% glycerol, 0.1 mM EDTA, and 50 mM KCl before being tested in the bidirectional helicase assay.

Preparation of Mutated Recombinant XPB—The XL-1 blue strain of *Escherichia coli* transformed with pSK(−) was used to generate a single-stranded DNA template. The XL-1 blue cells containing *XPBwt* in pSK(−) were grown with M13 phage at 37 °C in 2 × YT medium supplemented with 75 μg/ml ampicillin and 12 μg/ml tetracycline for 1–2 h. Kanamycin was added to a final concentration of 70 μg/ml, and the culture was shaken for 16–24 h or until growth had reached saturation. The cells were spun down for 5 min, and 0.1 volume of 20% polyethylene glycol, 2.5 M NaCl was added to the supernatant. After a 15-min incubation on ice, the supernatant was removed by centrifugation, and the pellet was resuspended in 0.3 M NaOAc, pH 6.0, 1 mM EDTA. The DNA was phenol/chloroform-extracted, ethanol-precipitated, and resuspended in 10 mM Tris-HCl, pH 7.6, 1 mM EDTA.

The mutated oligonucleotide (5′-AGCCAGGCCGGCATCTCGGC-3′) was phosphorylated at the 5′-end with 10 units of bacteriophage T4 polynucleotide kinase (30 min at 37 °C and then 5 min at 65 °C) and was annealed with the single-stranded *XPB* template by a 5-min incubation at 65 °C and then slow cooling to room temperature in 5 μl of 16 mM Tris-HCl, pH 7.5, 200 mM NaCl, and 80 mM $MgCl_2$. Second strand synthesis was continued in a 15-μl reaction mixture of 20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 5 mM dithiothreitol, 0.7 mM ATP, 0.7 mM dNTPs, 3 units of T4 DNA polymerase, and 6 Weiss units of T4 ligase (New England Biolabs Inc.) for 3 h. After ligation and transformation into competent Mut.L cells, plasmids isolated from randomly picked colonies were sequenced.

Overexpression of Wild-type and Mutated Recombinant XPB Proteins—E. coli strain BL21(DE3) containing the wild-type or mutated pGEX2TK-p89 plasmid was grown in LB medium supplemented with ampicillin (50 μg/ml) at 37 °C. Expression was induced with isopropyl-1-thio-β-D-galactopyranoside (0.4 mM) at an absorbance of 0.6 at 600 nm. After 3 h at 37 °C, the cells were collected by centrifugation, and the pellet was resuspended in 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.5% Triton X-100. The cells were frozen, thawed, and lysed by sonication on ice. After centrifugation for 10 min at 15,000 rpm in an R45Ti rotor, the soluble fractions were collected, and the pellets were resuspended in the same buffer as described above. Wild-type or mutated recombinant XPB was purified with a glutathione *S*-transferase affinity column (Pharmacia). All fractions were frozen in liquid nitrogen and stored at −80 °C.

Production of Antibodies—Ab-CTmut is a monoclonal antibody raised against the 43 C-terminal amino acids of XPBmut: QAGISALWHHEFYVWGRRHCVHGVPLIAEQGAQQTCTPALQAL. The anti-XPBwt and anti-p80 (7), anti-p62 (37), anti-p44 and anti-p34 (13), and anti-MO15 (38) antibodies were as described.

RESULTS

Purification and Characterization of TFIIH—To assess the effects of the frameshift mutation in XPB on the various enzymatic activities associated with TFIIH, extracts (27) prepared from lymphoblastoid cells derived from either the XP-B patient (XP11BE) or her mother (as a control) were subjected to several chromatographic steps of the TFIIH purification scheme (Fig. 1*A*; see "Experimental Procedures"). Using a monoclonal antibody directed against the recombinant XPB protein, we showed that this polypeptide is present in both cell lines and possesses the predicted molecular weight (Fig. 1*C*). An antibody raised against an oligopeptide corresponding to the frameshifted carboxyl terminus of the mutated XPB polypeptide of XP11BE (Ab-CTmut) (Fig. 1*B*) revealed the presence of the mutant *XPB* gene product in the TFIIH fraction derived from the patient's cell line (TFIIHmut) (Fig. 1*C*, *lane 9*). The mutated XPB

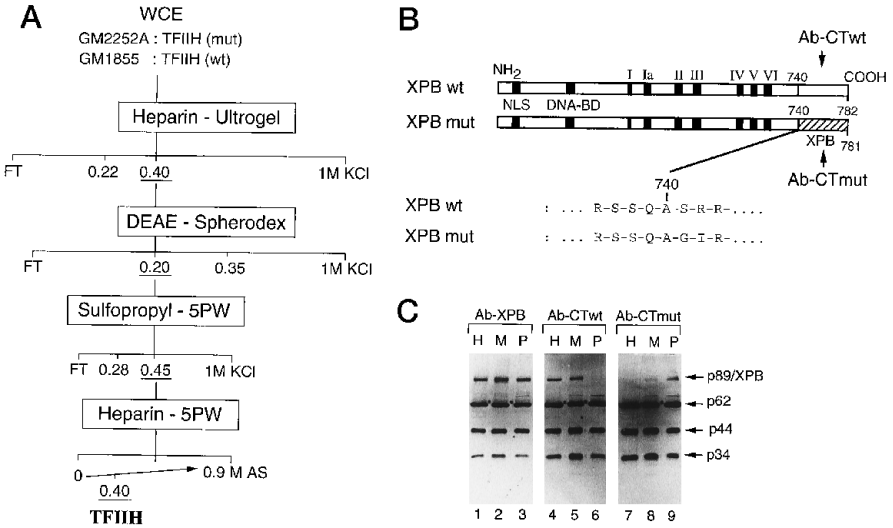

FIG. 1. *A*, purification scheme of TFIIHmut and TFIIHwt from GM2252A and GM1855 lymphoblast cells, respectively. *WCE*, whole cell extract. The salt concentration at which TFIIHmut and TFIIHwt were eluted from the various columns is *underlined*. *B*, schematic diagram of the wild-type and mutated XPB polypeptides. The putative character of the domains is indicated: the seven conserved helicase domains are marked by *solid boxes* numbered from *I* to *VI*; the nuclear localization signal (*NLS*) and the DNA-binding domain (*DNA-BD*) are designated (see Refs. 23 and 31). The peptides used to induce the antibodies raised against either the wild-type (Ab-CTwt) or the mutated (Ab-CTmut) C-terminal domain of XPB are indicated on the sequence (*arrows*). *C*, immunoblot analysis of TFIIH (heparin-5PW fraction) prepared from HeLa (*H*), GM1855 (mother (*M*)), and GM2252A (patient (*P*)) cells using antibodies against the recombinant XPB (Ab-XPB), wild-type (Ab-CTwt), or mutated (Ab-CTmut) XPB C-terminal oligopeptides. The *arrows* indicate the four subunits of TFIIH.

polypeptide was also present, although in lower amounts (10–15% of the amount found in the patient), in TFIIH prepared from the cell line of the mother (TFIIHwt) (*lane 8*). Furthermore, an antibody generated against the wild-type carboxyl terminus allowed us to detect wild-type XPB in the cells derived from the mother (*lane 5*), whereas this form was not detected in the patient's cells (*lane 6*). This is consistent with the observation that the paternal allele is not detectable at the RNA level in the patient and that the maternal allele is responsible for the clinical phenotype (23).

The purification of both TFIIHwt and TFIIHmut was done in parallel under the same conditions to obtain similar preparations and were monitored using the following enzymatic assays: a reconstituted *in vitro* transcription assay lacking TFIIH (28), a kinase assay (29), a bidirectional helicase assay (7), and a DNA-dependent ATPase assay (29). As expected from previous results using HeLa extracts (30), none of the enzymatic activities were detectable during the first two steps of the TFIIH purification. For adequate quantitation of the assays and further comparison between TFIIHwt and TFIIHmut, antibodies directed against either the p62 or XPB subunit were used; this allowed us to follow TFIIH during the purification and to measure quantitatively the relative amount of the XPB subunit present in each fraction. As previously shown, the transcription, CTD kinase, helicase, and DNA-dependent ATPase activities match the elution of the p62 and XPB TFIIH subunits during the last steps of the purification procedure (data not shown). Starting with 80 liters of lymphoblast cells (0.7×10^6 cells/ml) and after four purification steps, the estimated TFIIH yield was 10 times lower than that starting from HeLa cells (30).

A comparative analysis of TFIIHmut and TFIIHwt was then performed for each of the known enzymatic activities. According to the bidirectional helicase assay, we observed that the 3′ → 5′ XPB helicase activity of TFIIHmut was significantly lower (40%) than that of TFIIHwt (Fig. 2*A*, compare *lanes 5* and *6* with *lanes 3* and *4*) despite the fact that the mutation occurs outside of the seven helicase motifs and the putative DNA-binding domains (see Fig. 1*B* and Refs. 23, 29, and 31). It is interesting to note that the 5′ → 3′ XPD helicase activity, which may be used as an internal control, is almost identical in both TFIIHmut and TFIIHwt, a result that we repeatedly observed (Fig. 2*A*, *upper* and *lower panels*). This demonstrates that our TFIIH preparations do not contain contaminants that may inhibit the helicase activity.

To further confirm that the difference in XPB helicase activity between TFIIHmut and TFIIHwt was not the result of some contaminants that may have copurified with TFIIH in the heparin-5PW column, we immunoprecipitated in parallel TFIIHwt- and TFIIHmut-heparin fractions with antibodies (directed against either p62 or Cdk7 (MO15), two subunits of the TFIIH complex) previously coupled to protein A-agarose. The immunoprecipitates were sequentially washed with 0.15 and 0.50 M KCl adsorption buffer containing 0.1% Nonidet P-40. After being equilibrated in the helicase assay buffer, the immunoprecipitated TFIIH complexes were separately tested in the bidirectional helicase assay (7). As shown in Fig. 2*B*, the XPB/XPD helicase activity ratio measured in TFIIHmut is lower than in TFIIHwt, thus demonstrating once more that XPB helicase activity in TFIIHmut is weaker than in TFIIHwt. As also shown by the helicase assay (Fig. 2*A*), the XPD activities in TFIIHwt and TFIIHmut were identical. As an additional control, we analyzed the adsorbed fractions by SDS-polyacrylamide gel electrophoresis. Unfortunately, antibodies as well as part of protein A partially masked the subunits of TFIIH, thus rendering the silver-stained protein pattern unreadable. Nevertheless, we could perform Western blotting with the same fractions. Antibodies against XPD and XPB allowed us to demonstrate that both polypeptides were not degraded in either TFIIHwt or TFIIHmut and that the levels of XPB and XPD present in each immunoprecipitate were identical. All together, these results demonstrate the weaker XPB helicase activity in TFIIHmut compared with TFIIHwt.

In contrast, the DNA-dependent ATPase activity (0.18 pmol of P_i released per ng of protein/h) (Fig. 2*C*) as well as the CTD kinase activity (0.18 pmol of PO_4 incorporated per ng of protein/h) (Fig. 2*D*) of TFIIHmut appeared unaffected compared with TFIIHwt (0.18 pmol/ng/h for ATPase and 0.15 pmol/ng/h

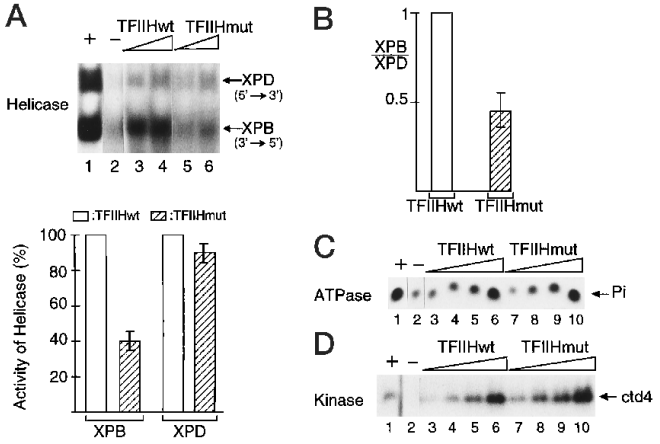

FIG. 2. **Comparison of TFIIH enzymatic activities.** The antibodies against XPB were also used to measure quantitatively the relative amount of XPB in each fraction; both fractions of TFIIHmut and TFIIHwt were then adjusted to contain the same amount of XPB/microliter. Partially purified TFIIHmut (7 ng/μl) and TFIIHwt (7 ng/μl) were tested for their enzymatic activities. *A*, in the bidirectional helicase assay (heparin fractions), 1 and 2 μl of TFIIHwt (*upper panel*, *lanes 3* and *4*, respectively) or TFIIHmut (*lanes 5* and *6*, respectively) were used. Controls were carried out with substrate heated for 2 min at 100 °C (+, *lane 1*) and with unheated substrate without proteins (−, *lane 2*). The XPB and XPD helicase activities of three independent purifications of TFIIHwt and TFIIHmut were measured and standardized to XPBwt and XPDwt (*lower panel*). The XPB and XPD values of TFIIHwt were each considered to be 100%. The scanned XPB and XPD values for TFIIHmut are reported in the *lower panel*. The means ± S.E. are indicated. The total level of available probe was determined by heat denaturation of substrate (*upper panel*, *lane 1*). XPB and XPD activities of TFIIHwt were measured as 4.3 and 1.9%, respectively, of bound oligonucleotide displaced per h/ng of TFIIH fraction standardized of XPB protein. *B*, the heparin fractions (100 μl) were immunoprecipitated, conjugated to protein A, extensively washed, and tested in the bidirectional helicase assay as indicated under "Experimental Procedures". The figure represents the XPB/XPD helicase activity ratio for TFIIHwt and TFIIHmut proteins. In this case, as previously stated, if the observed XPB and XPD helicase values are represented as 100%, the XPB/XPD ratio will be 1. The XPB and XPD activities of TFIIHmut were then determined by comparison with wild-type levels; the XPB/XPD ratio calculated is ~0.4. *C* and *D*, in DNA-dependent ATPase and kinase assays, 1, 2, 4, and 8 μl of TFIIHwt-heparin (*lanes 3–6*, respectively) or TFIIHmut-heparin (*lanes 7–10*, respectively) fractions were used. Controls were carried out without (−, *lane 2*) and with (+, *lane 1*) the HeLa TFIIH factor (6 and 2 μl, respectively, at a concentration of ~3–4 ng/μl). The *arrows* indicate the products from each assay, the inorganic phosphates (*Pi*), and the phosphorylated oligopeptide mimicking CTD (*ctd4*).

for CTD kinase). In this regard, one has to take into account that the DNA-dependent ATPase activity of TFIIH is the result of the combined action of the XPD and XPB helicases (7). Moreover, the C terminus frameshift in XPBmut does not alter the ATP- and DNA-binding domains of the protein. The kinase activity resides primarily in the Cdk7 (MO15) subunit of TFIIH (10). It is of interest to note that we observed reproducibly a slight difference between the kinase activities of TFIIHmut and TFIIHwt (Fig. 2*D*). This may reflect the change of mutated XPB, which modulates the affinity of TFIIHmut for the CTD substrate and may result in a slightly increased specific activity. This observation also strengthens the significance of the decrease in the helicase activity of TFIIHmut.

NER and Transcription Activities of TFIIHmut in Vitro and in Vivo—TFIIHmut and TFIIHwt were then tested for their ability to restore both the DNA repair and transcription reactions. When added to a reconstituted *in vitro* transcription assay lacking TFIIH, both TFIIHwt and TFIIHmut preparations restored transcription initiated from the adenovirus type 2 major late promoter as observed by the synthesis of a 309-nucleotide-long run-off transcript. However, TFIIHmut was 25–30% less active than TFIIHwt (Fig. 3*A*; see also the curves in Fig. 3*B*). This deficiency in transcription was observed reproducibly with several different preparations of TFIIHmut and thus seems to be a consistent intrinsic feature of the TFIIHmut complex.

When added to an XPB-deficient DNA repair extract, TFIIHmut was unable to rescue the repair reaction, whereas the addition of TFIIHwt restored the DNA repair activity (Fig. 3*C*, *lanes 3* and *4*, respectively). To assess the *in vivo* NER activity of TFIIHmut and TFIIHwt, TFIIH-heparin-5PW fractions were microinjected into living fibroblasts of different NER-deficient complementation groups (Table I). The effect of the injected TFIIH on the repair capacity of the cells was measured by UV-induced unscheduled DNA synthesis, determined immediately after microinjection and UV irradiation by incubation of the slides with the injected cells in the presence of [^{3}H]thymidine for a period of 2 h to label DNA repair patches. Incorporated [^{3}H]thymidine was visualized by *in situ* autoradiography and quantified by counting silver grains above nuclei (14). Microinjection of TFIIHwt into XPB-deficient cells (XPCS1BA) restored the UV-induced unscheduled DNA synthesis activity to the normal level (Table I), whereas microinjection of TFIIHmut had no effect (compared with noninjected cells). In contrast, microinjection of TFIIHmut in XPD- or TTD-A-deficient cells restored DNA repair to virtually normal levels. This supports the idea that some subunits of TFIIH can exchange during the repair reaction and are thus able to reconstitute an active TFIIH repair factor with wild-type *XPB*, *XPD*, and *TTD-A* gene products *in vivo* within a period of 2 h after microinjection. Microinjection of TFIIHmut or TFIIHwt fractions in XPD-deficient cells restored the NER activity; this demonstrates that TFIIHmut contains an active XPD subunit (Table I).

Recombinant XPBwt and XPBmut Activities—To exclude the possibility that other defects in the mutated cell line may result in a decrease in the 3′ → 5′ helicase of the TFIIHmut complex, we analyzed the intrinsic activity of the mutated XPB polypeptide. The mutation identified in patient XP11BE was reproduced by site-directed mutagenesis, and the modified (rXPBmut) and wild-type (rXPBwt) XPB recombinants were overexpressed as glutathione *S*-transferase fusion polypeptides in *E. coli*. Both polypeptides were purified by affinity chromatography through a glutathione-Sepharose column (29). The flow-through and eluate fractions obtained after extensive washing were assayed for 3′ → 5′ helicase and DNA-dependent ATPase activities. As shown in Fig. 4*A*, rXPBmut has a very weak helicase activity (*lanes 3* and *4*) compared with rXPBwt (*lanes 1* and *2*). Furthermore, while the ATPase activity of purified TFIIHmut appeared unaffected (Fig. 2*B*), rXPBmut was largely devoid of this activity (0.0045 pmol of P_i released per ng/h) (Fig. 4*B*, compare *lanes 8–11* with *lane 1*) compared with rXPBwt (0.018 pmol/ng/h). Note that the XPB recombinants present a much lower activity than TFIIH, likely because of incomplete unfolding or processing in *E. coli*; also, XPB may adopt a more favorable conformation when in the context of the whole TFIIH.

DISCUSSION

It was previously shown that a frameshift mutation at the 3′-terminus of the *XPB* gene leads to a virtually complete inactivation of the NER pathway (23). The function can be replaced by the introduction of the wild-type gene either by microinjection into XP-B primary fibroblasts or by gene transfection into cells from the UV-sensitive mutants of rodent complementation group 3, the Chinese hamster equivalent of XP-B (14). Patient XP11BE, the first documented case of XP-B,

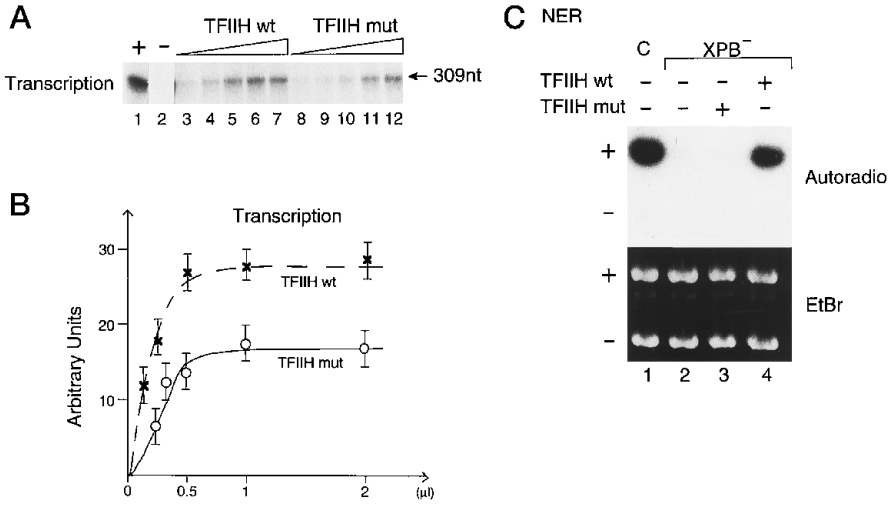

FIG. 3. **Transcription and NER activities of TFIIHmut and TFIIHwt.** *A* shows the transcription activity of increasing amounts (0.5, 1, 2, 4, and 8 μl; 7 ng/μl) of TFIIHwt (*lanes 3–7*, respectively) or TFIIHmut (*lanes 8–12*, respectively), and *B* shows the densitometer quantitation of the data. The values are expressed in arbitrary units. *Lane 1*, positive control (+) using highly purified HeLa TFIIH (~20 ng); *lane 2*, negative control (−). The *arrow* indicates the specific transcript (*nt*, nucleotides). In *C*, 100 ng of purified TFIIHwt (*lane 4*) or TFIIHmut (*lane 3*) was tested in an *in vitro* DNA repair assay for the ability to complement an XPB-deficient extract. Use of undamaged (−) and aminoacetylfluorene-damaged (+) DNA substrates is indicated. The *upper panel* shows the [α-^{32}P]dATP incorporation that indicates the repair activity (*Autoradio*), and the *lower panel* shows the ethidium bromide-stained DNA gel. *C*, positive control using HeLa whole cell extract.

TABLE I
Microinjection of TFIIHmut (XP11BE, GM2252A cell line) and TFIIHwt (GM1855 cell line) in various XP and TTD complementation groups

	UDS levels[a]		
	TFIIHmut	TFIIHwt	Noninjected
XPCS1BA (XP-B)	14	106	14
XP1BR (XP-D)	107	107	20
TTD1BR (TTD-A)	91	92	6

[a] Percent of unscheduled DNA synthesis (UDS) of normal fibroblasts assessed in parallel.

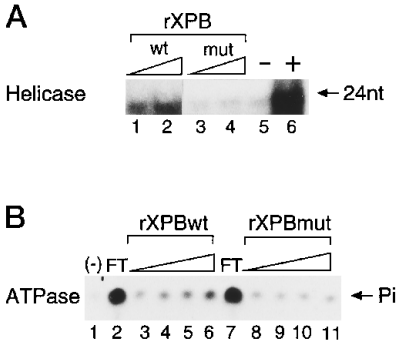

FIG. 4. **Helicase and ATPase activities of mutated (rXPBmut; 10 ng/μl) and wild-type (rXPBwt; 10 ng/μl) XPB recombinants.** *A*: *lanes 1* and *2*, 1 and 2 μl of rXPBwt, respectively; *lanes 3* and *4*, 1 and 2 μl of rXPBmut, respectively; *lane 5*, negative control (−); *lane 6*, positive control (+) (substrate heated for 2 min at 100 °C). *B*: *lane 1*, negative control (−); *lanes 3–6*, 1, 2, 4, and 8 μl of rXPBwt, respectively; *lane 7*, flow-through (*FT*) fraction from the glutathione *S*-transferase column; *lanes 8–11*, 1, 2, 4, and 8 μl of rXPBmut, respectively. The *arrows* indicate the displaced labeled oligonucleotide (*24nt*) and the P_i generated.

presented a perplexing combination of XP and CS features. The identification of two more cases, one with XP/CS and one with TTD (21), in this very rare group of XP has further broadened the range and heterogeneity of the clinical symptoms. The discovery that the *XPB* and *XPD* genes encode DNA helicases that are subunits of TFIIH, a multisubunit complex engaged not only in NER, but also in basal transcription and potentially linked with cell cycle regulation, has given a new perspective to these clinical conditions. This study provides evidence in accordance with the idea that the non-repair features associated with CS and TTD could be due to the deficiency of TFIIH at the level of transcription. TFIIH purified from XP11BE not only displays a profound NER defect, but also an impairment of the basal transcription function.

Biochemical Consequences of the XP11BE Mutation in XPB—The XP11BE frameshift, which converts the C-terminal 41 amino acids of the XPB polypeptide to a nonsense sequence, clearly decreases its 3′ → 5′ helicase activity even though this region of the C terminus does not harbor any of the sequence motifs characteristic of DNA or RNA helicases. It seems unlikely that a change in the carboxyl terminus of XPB such as in XP11BE modifies the composition of TFIIH since antibodies against both the wild-type and mutated carboxyl termini of XPB immunoprecipitate the entire TFIIH. Furthermore, immunoblot analysis demonstrates that the composition and stoichiometry of TFIIHmut and TFIIHwt are identical (Fig. 5). Finally, the diminished unwinding capacity is also observed using a free mutated recombinant XPB protein. Therefore, a conformational modification may be responsible for the decrease in XPB helicase activity. It is worthwhile to note the profound defect not only of the helicase activity, but also of the ATPase activity that reside in the XPB recombinant compared with purified TFIIHmut. Although the ATPase activity was affected by the mutation, it is also possible that the defective activity is due to an incomplete processing or to the fact that XPB is out of its TFIIH context. In purified TFIIHmut, a deficiency of the XPB DNA-dependent ATPase activity was not detected. Indeed, such a decrease may be masked by the XPD ATPase activity.

Different Levels of Inhibition between the Repair and Transcription Functions—The XPB function has to be considered in the context of the whole TFIIH. (i) Free XPB was never detected in the various protein subfractions generated during TFIIH purification (data not shown). TFIIH is absolutely required for both the *in vitro* transcription and the *in vitro* NER reaction (14, 15, 30, 32, 33), and the addition of the recombinant subunit to the TFIIH-lacking reaction mixture has no effect. (ii) Microinjection of antibodies against any of the TFIIH polypeptides (p89/XPB, p80/XPD, p44, and MO15) into normal

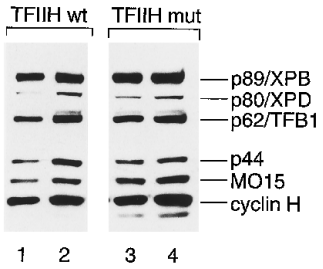

FIG. 5. **Western blot analysis of TFIIHwt (7 ng/μl) and TFIIHmut (7 ng/μl) eluted from the heparin column using the antibodies against p89/XPB, p80, p62, p44, MO15, and cyclin H.** *Lanes 1* and *2*, 1 and 2 μl of TFIIHwt, respectively; *lanes 3* and *4*, 1 and 2 μl of TFIIHmut, respectively. The *lines* indicate each subunit of TFIIH.

cell lines inhibits both *in vivo* NER and *in vivo* transcription. The same antibodies are able to deplete crude extracts used for *in vitro* assays (10, 13, 14). (iii) TFIIH contains at least four subunits (XPB, XPD, p62/TFB1-like, and p44/SSL1-like) that were shown to play a role in DNA repair (6, 7, 18).

The DNA repair synthesis in XP11BE-derived cells is only 5–10% of that in normal fibroblasts, and TFIIHmut is not able to restore the DNA repair reaction, neither when added in the reconstituted *in vitro* NER system nor when microinjected into cells derived from another XP-B patient (XPCS1BA). In basal transcription, the inhibition rate is ~30%. Although some variation is intrinsic to transcription activity measurements, our studies demonstrate a consistent decrease in TFIIH transcription stimulation with the mutant complex. Obviously, this can be due to a higher *in vitro* lability of the complex, *e.g.* during purification. However, our finding that the composition and stoichiometry of the complex were unaffected and that the other activities (kinase and DNA-dependent ATPase) were very similar compared with control TFIIH isolated in parallel argues against this explanation. Thus, we believe that the decreased *in vitro* transcription activity reflects a true consequence of the primary mutation in the XPB protein. In relation to this, it is worth mentioning that the doubling time of the mutated XPB cell culture is also significantly higher than that of the mother's control cells grown under identical conditions (data not shown). Although this could obviously be due to other trivial differences, the subtle transcription defect may also be the underlying cause. This observation is also in line with the severe growth defect displayed by patient XP11BE (24). The finding that the effect of the mutation in XPB is much more dramatic for the NER function than for transcription may be explained in different ways. The XPB helicase faces different DNA substrates in each process. In transcription, TFIIH will target a promoter that includes specific sequences such as a TATA box and an initiator site as well as a number of other basal transcription factors (3). In NER, TFIIH is thought to act at an early stage in the reaction where it is required for the elimination of, for example, UV-induced (6-4) photoproducts or cyclobutane pyrimidine dimers. In both reactions, the function of the complex may be the local opening of the DNA helix, but the requirements for this activity may be different for both processes. The change in the carboxyl terminus of XPB may prevent the binding of other repair factors, but may induce only a slight effect on the opening of the DNA template at the promoter level. This would result in a limited decrease in the rate of basal transcription. Another possibility is that XPBmut is compensated by XPD, another helicase associated with TFIIH.

Clinical Consequences and Putative Mechanisms—Some of the clinical symptoms of patient XP11BE cannot be simply explained on the basis of NER deficiency. The typical XP clinical features such as sun hypersensitivity and cancer predisposition are likely derived from a repair defect. The remaining features such as the neuromyelination defect, stunted growth, and impaired sexual development are likely due to a crippled transcription function. In the latter case, a subtle mutation in XPB as observed here may prevent the interaction between TFIIH and either the promoter or components of basal or activated transcription machinery (*e.g.* TFIIE (34), Gal-VP16 (35), and p53 (36)). As a consequence, transcription of a specific subset of genes may be critically hampered, impairing specialized functions and, for example, general development (see Refs. 20 and 21 for a more extensive discussion).

Thus, our work defines the enzymatic defects of a TFIIH mutant in 3′ → 5′ XPB helicase, NER, and transcription activities. In addition, it provides the first biochemical evidence for inherited deficiencies within the human population in one of the most fundamental cellular processes, basal transcription. Obviously, the translation of the molecular defect into any of the individual symptoms comprising the complex and pleiotropic CS and TTD phenotypes awaits the generation of experimental mouse models for these disorders and a more precise definition of the role of TFIIH in transcription. Work along both lines of research is ongoing.

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Nucleic Acids, Protein Synthesis, and Molecular Genetics:

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