The EMBO Journal Vol.16 No.5 pp.1093–1102, 1997

Cloning and characterization of p52, the fifth subunit of the core of the transcription/DNA repair factor TFIIH

Jean-Christophe Marinoni, Richard Roy, Wim Vermeulen[1], Pierre Miniou[2], Yves Lutz, Geert Weeda[1], Thierry Seroz, Denise Molina Gomez[2], Jan H.J.Hoeijmakers[1] and Jean-Marc Egly[3]

Institut de Génétique et de Biologie Moléculaire et Cellulaire, BP 163, 67404 Illkirch Cedex, Université Louis Pasteur, Strasbourg, France, [1]Department of Cell Biology and Genetics, Medical Genetics Center, Erasmus University Rotterdam, PO Box 1738, 3000 DR Rotterdam, The Netherlands and [2]Unité 383 (INSERM) Hôpital Necker-Enfants Malades, 49 rue de Sèvres, 75015 Paris, France

[3]Corresponding author

J.C.Marinoni and R.Roy contributed equally to this work

TFIIH is a multiprotein factor involved in transcription and DNA repair and is implicated in DNA repair/transcription deficiency disorders such as xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy. Eight out of the nine genes encoding the subunits forming TFIIH have already been cloned. We report here the identification, cDNA cloning and gene structure of the 52 kDa polypeptide and its homology with the yeast counterpart TFB2. This protein, along with p89/XPB, p62, p44 and p34, forms the core of TFIIH. Moreover, using *in vitro* reconstituted transcription and nucleotide excision repair (NER) assays and microinjection experiments, we demonstrate that p52 is directly involved in both transcription and DNA repair mechanisms *in vitro* and *in vivo*.
Keywords: DNA repair/p52/TFIIH/TFIIH subunits localization/transcription

Introduction

TFIIH, a multisubunit transcription/DNA repair factor, is currently on the point of being fully characterized: eight subunits ranging from 89 to 32 kDa are now cloned and sequenced. TFIIH is the only basal transcription factor known to possess several enzymatic activities (for reviews, see Bhatia *et al.*, 1996; Svejstrup *et al.*, 1996a). The p89/XPB and p80/XPD subunits are a 3′–5′ and a 5′–3′ ATP-dependent helicase respectively; cyclin-dependent kinase 7 (cdk7), cyclin H and MAT1 form a ternary complex with a cdk-activating kinase (CAK) activity that was shown to use (at least *in vitro*) an array of substrates required in transcription such as the TATA binding protein (TBP), TFIIE and the carboxy-terminal domain (CTD) of the largest subunit of RNA polymerase II (RNA pol II) (for review, see Nigg, 1996). In addition to the XPB and XPD helicases, the p62 and p44 subunits of TFIIH were shown to be involved in the nucleotide excision repair (NER) mechanism by *in vivo* and *in vitro* complementation experiments using normal and NER-deficient mammalian cells (Vermeulen *et al.*, 1994a) or by induction of thermosensitive mutations in yeast (Wang *et al.*, 1995). p44 and p34 contain putative zinc finger motifs (Humbert *et al.*, 1994) that could help TFIIH target the promoter and/or the DNA lesion. TFIIH possesses cdk7 and cyclin H that can phosphorylate cdc2 and cdk2, two regulators of cell cycle progression (Morgan, 1995). While direct evidence that TFIIH participates in cell cycle regulation is lacking, an involvement in this or other cellular events is certainly not excluded (Hoeijmakers *et al.*, 1996). In contrast, the key roles of the complex in the initiation of transcription by RNA pol II and in the removal of DNA damage are well documented.

The multiple roles of TFIIH in the cell might explain why and how genetic defects in TFIIH subunits give rise to a wide clinical variety of human syndromes and symptoms. These include the rare autosomal recessive disease xeroderma pigmentosum (XP), known to be due to an NER deficiency, the main symptoms of which are cutaneous abnormalities such as UV photosensitivity and predisposition to UV-induced skin cancer. Other TFIIH-related disorders are Cockayne syndrome (CS), which is predominantly a neuro-developmental disease, and the CS-like, brittle hair disease trichothiodystrophy (TTD). These latter two conditions are thought to be due, at least in part, to a crippled transcription function of TFIIH affecting a specific set of genes (Vermeulen *et al.*, 1994a). The various degrees of photosensitivity exhibited by most CS and TTD patients are probably caused by an additional, but variable, TFIIH-related defect that impairs the ability to remove UV-induced DNA damage. The disorder-specific mutations in two of the TFIIH subunits, XPB and XPD, associated with either XP, XP/CS or TTD, reveal the clinical intricacies and pleiotropic effects of various TFIIH deficiencies (Vermeulen *et al.*, 1994a; Broughton *et al.*, 1995; Hoeijmaker *et al.*, 1996). The occurrence of mutations leading to disease in genes encoding the other subunits of TFIIH has not been documented. However, an NER defect in cells of a single photosensitive TTD patient (TTD-A) constitutes a separate NER-deficient complementation group, that is clearly corrected by microinjection of purified TFIIH. This patient does not carry mutations in either the *XPD* or *XPB* genes (our unpublished results); therefore, it is likely that a subunit of TFIIH is affected.

Mutations in TFIIH subunits may also give rise to syndromes resulting from a defect in transcription rather than DNA repair. Preliminary results have shown that the two p44 genes are located in the proximal vicinity of the human survival motor neuron (*SMN*) gene, a mutation of which results in spinal muscular atrophy (SMA) (Lefebvre *et al.*, 1995). Analysis of ~100 unrelated SMA patients revealed that one of the p44 genes is involved in large

© Oxford University Press

scale deletions associated with the severe forms of SMA, also called the Werdnig–Hoffman disease (L.Bürglen and J.Melki, personal communication).

In this study, we describe the cloning of a gene encoding the 52 kDa subunit that co-purifies with the eight other characterized subunits of TFIIH. We further demonstrate that this polypeptide is indeed a subunit of TFIIH by co-immunoprecipitation experiments and sedimentation shift assays in glycerol gradients using antibodies raised against p52. Microinjection of this antibody into normal fibroblasts or addition to *in vitro* reconstituted transcription and NER assays results in inhibition of both transcription and DNA repair. These results demonstrate that p52 is physically and functionally associated with TFIIH both *in vivo* and *in vitro*.

Results

Cloning of the cDNA encoding the p52 polypeptide of TFIIH

Eight out of the nine or more TFIIH subunits which are visible on SDS–PAGE of the hydroxyapatite column (the last step of our purification procedure; Gérard *et al.*, 1991; Figure 1, lanes 4 and 6 of the SDS–PAGE) have been cloned previously. We now focus our attention on the polypeptide (now called p52) migrating above the p44 subunit and co-purifying with the various enzymatic activities (ATPase, CTD kinase and helicase) associated with TFIIH transcription activity through each step of our purification procedure. After microsequencing of p52 tryptic digests, degenerate oligonucleotides were designed and used to screen several cDNA libraries. One apparently full-length clone was isolated from a human preB-cell λZAPII cDNA library.

Sequencing revealed the existence of an open reading frame (ORF) of 1386 bp that encodes a protein of 462 amino acids (Figure 2) with a calculated isoelectric point of 9.76 and a theoretical mol. wt of 52.186 kDa. When overexpressed in *Escherichia coli*, the recombinant polypeptide exhibits the same electrophoretic mobility on SDS–PAGE as the endogenous p52 and is recognized by Ab-p52 (data not shown). An in-frame stop TAA codon is found 48 nucleotides upstream of an ATG codon which is surrounded by a translational consensus sequence (Kozak, 1986); this indicates that this ATG is the translational start site of the p52 protein. All microsequenced oligopeptides were found in the deduced amino acid sequence of p52, thus confirming the identity of the clone. Moreover, since some of the TFIIH subunits have been shown to contain specific sequences (helicase or kinase motifs) typically associated with the various enzymatic activities (XPB, XPD and cdk7), or indicative of zinc finger (p44 and p34; Humbert *et al.*, 1994) or RING finger domains (MAT1; Devault *et al.*, 1995; Tassan *et al.*, 1995), we performed computer searches using the MOTIFS software of the GCG package. First, we found two highly basic domains (amino acids 274–280 and 451–459) rich in lysine and arginine residues and sharing homology with the consensus sequence for nuclear localization signals (Boulikas, 1994). Second, three putative conserved helix structures were detected from analysis of the human and the yeast proteins (amino acids 20–59, 210–240 and 369–412; Figures 2A and B; see also below). No other motifs

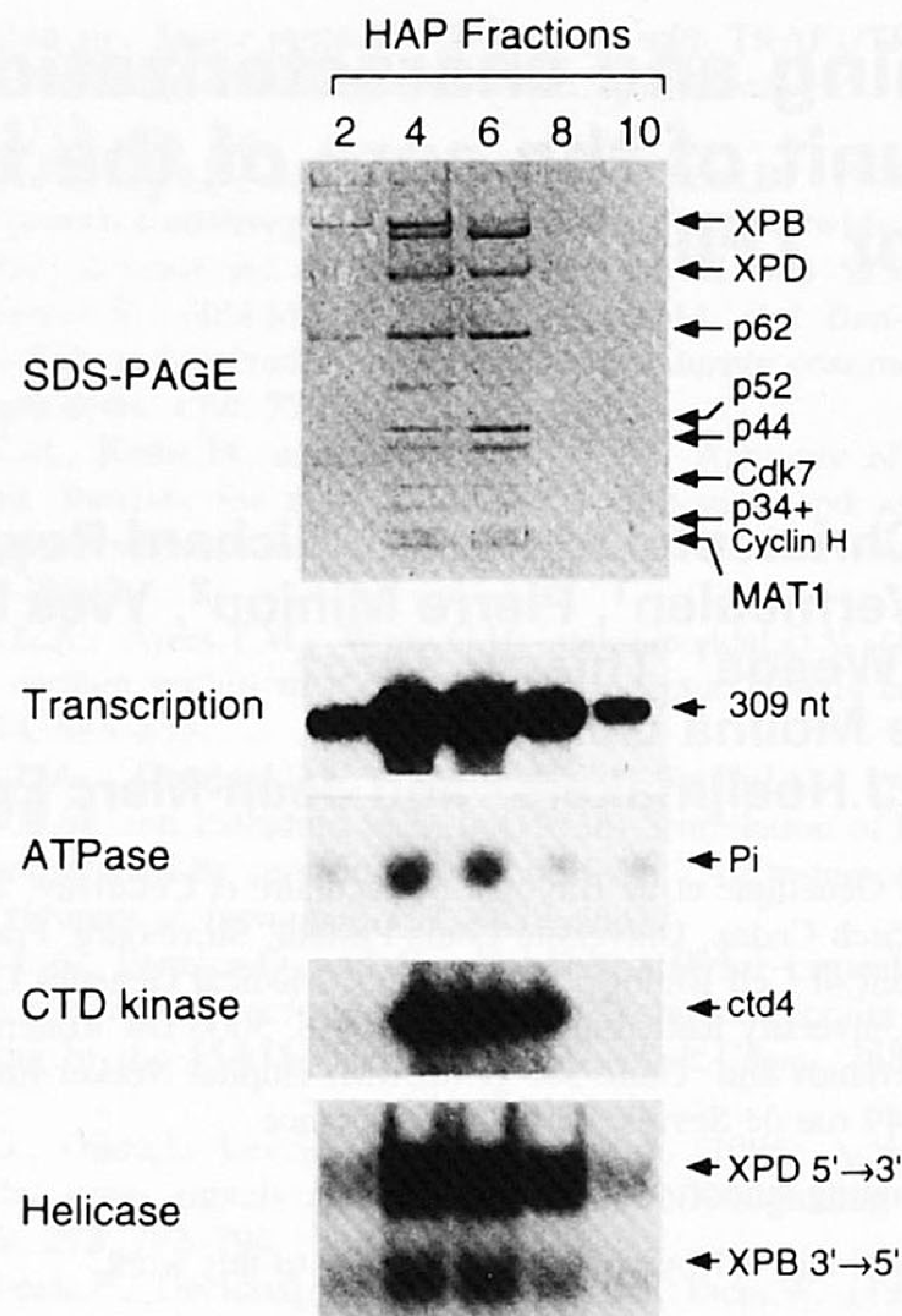

Fig. 1. The p52 polypeptide contained in the highly purified TFIIH eluted from an hydroxyapatite column co-purifies with the other eight identified subunits as shown on the right of the panel (silver staining of an 11% SDS–PAGE) and with transcription, ATPase, the CTD kinase and both 5′–3′ XPD and 3′–5′ XPB helicase activities of TFIIH (assays described in Fischer *et al.*, 1991; Adamzcewski *et al.*, 1996).

such as a leucine zipper, kinase signature or nucleotide binding domain were found. Furthermore, searching in the GenBank, EMBL as well as in a *Caenorhabditis elegans* expressed sequence tag (EST) databanks, we found high homologies to one *C.elegans*, one human and one *Brassica campestris* translated EST, with accession Nos T55635 (35% identity and 68% similarity), YK70F 5.3 (40% identity and 67% similarity) and L38200 (45% identity and 70% similarity) respectively (Figure 2B). In addition, a mouse EST (GenBank accession No. W61868) was found to share 98% identity with the human p52, and very likely corresponds to the mouse homolog of p52. Sequence searches in GenBank (accession No. U43503) and in the *Saccharomyces* Genome Project databank (genomic clones P310, P315 and P320 of yeast chromosome XVI), as well as information from the laboratory of R.Kornberg, detected matches with TFB2 (55 kDa, 513 amino acids), a newly cloned subunit of the yeast TFIIH. Comparison of human p52 with the yeast TFB2 revealed 37% identity and 61% similarity, indicating that p52 is indeed the human counterpart of the *S.cerevisiae* TFB2.

The p52 genomic structure and chromosomal assignment

To characterize further the gene encoding p52, in terms of its structural organization and regulatory aspects as well as to obtain probes for its chromosomal localization, isolation of genomic clones was undertaken. Using the cloned cDNA as a probe to screen a placental genomic λGEM12 library, 12 positive clones were isolated from which a 2.5 kb *Eco*RV and a 5.5 kb *Eco*RI fragment were subcloned and subjected to sequence analysis. The coding

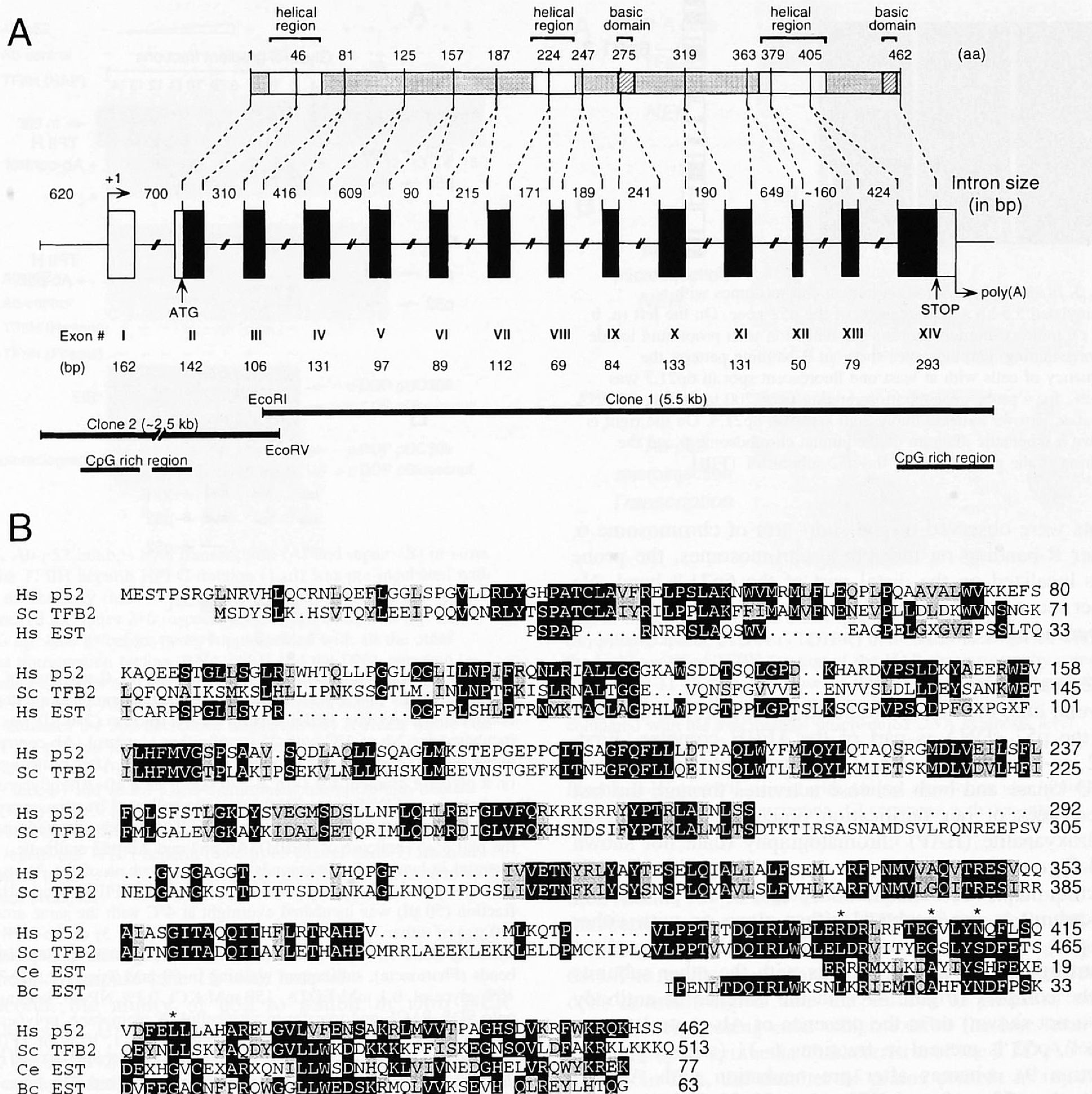

Fig. 2. The gene encoding p52 and its product. (**A**) Organization of the p52 gene deduced from the two clones. Complete exon–intron organization of the p52 untranslated region (in white) and coding region (in black) relative to the protein sequence are indicated. The helical regions of the p52 polypeptide are in white and the basic domains are hatched. (**B**) Protein sequence comparison between human (Hs p52), *S.cerevisae* (Sc TFB2; GenBank U43503), and alignment with related human (Hs EST; EMBL, T55635), *C.elegans* (Ce EST; Sanger Center, YK70F5.3) and *B.campestris* (Bc EST; EMBL, L38200) translated ESTs. Identical residues are boxed in black; similar residues are in grey.

sequence of p52 is contained within a 7 kb region and is split into 13 exons (numbered II–XIV); the size of each is indicated in Figure 2A. The sequence corresponding to the 5′-untranslated region was located within exons I and II. The translation start (ATG) is located in exon II. The sequences of the exon–intron junctions fit the splice donor and acceptor consensus sequences 5′ AGgt(a,g)agt 3′ and 5′ (c,t)(c,t)agG 3′ respectively. A potential polyadenylation signal was found 17 bp upstream from the actual polyadenylation site as deduced from the analysis of the cDNA. Three CpG-rich regions were found: the first is 408 bp long and located in the promoter, thus suggesting a putative housekeeping role for this gene. By its position as well as by its length and high CpG content, this region proves characteristic of class II gene promoters (Gardiner-Garden and Frommer, 1987). In addition, this region contains a CAAT box and a putative TATA box. The second CpG-rich region is 292 bp long and contained in the first exon and intron 1. The third CpG-rich region spans 692 bp over the end of intron 13, exon 14 and the 3′-untranslated region (Figure 2A).

To characterize p52 and its putative involvement in genetic disorders further, chromosomal localization was performed. The 5.5 kb *Eco*RI genomic fragment containing part of the p52 gene was biotinylated and used as a probe in fluorescence *in situ* hybridization (FISH) experiments. As shown in Figure 3, the p52 genomic probe shows one site of hybridization on the human karyotype. Fluorescent

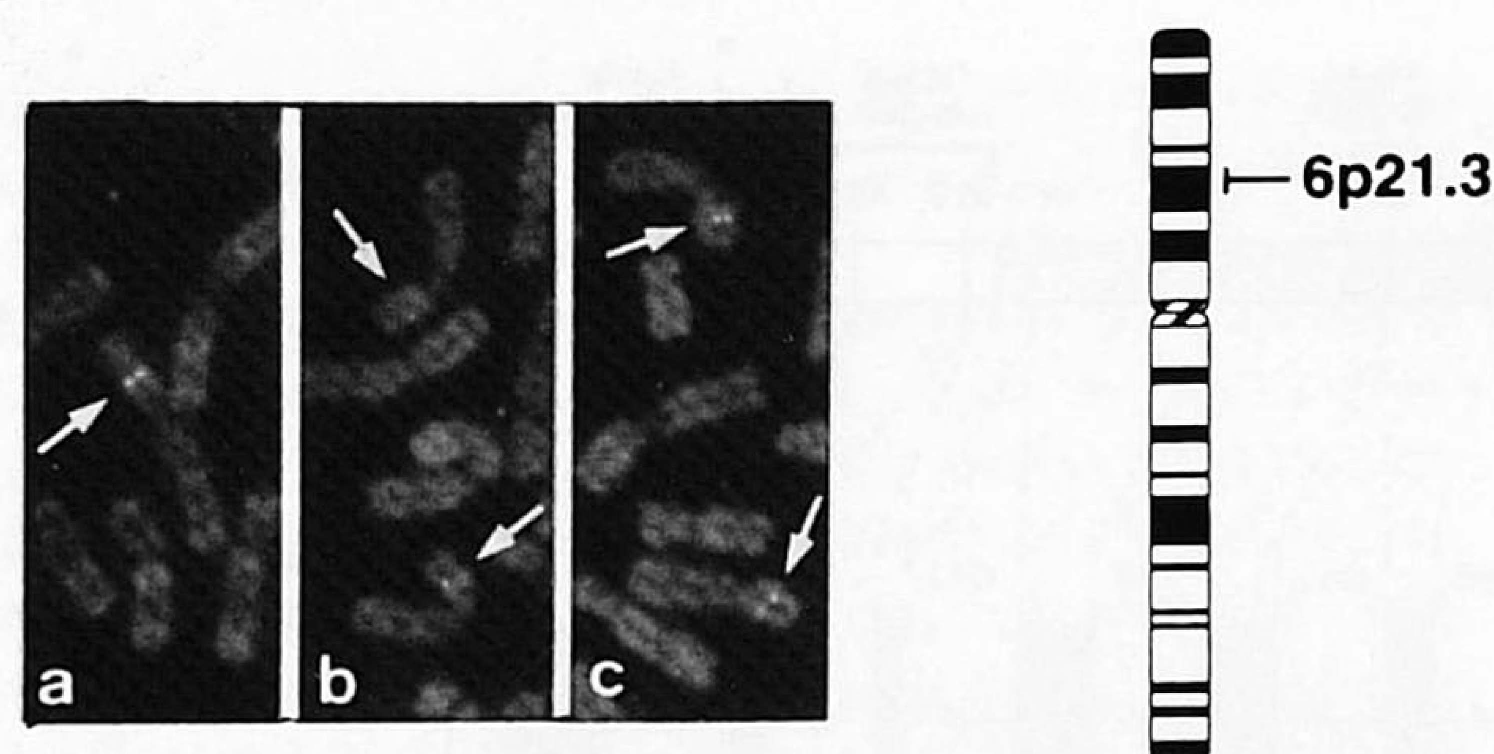

Fig. 3. *In situ* hybridization on human chromosomes with a biotinylated 5.5 kb genomic probe of the p52 gene. On the left (**a**, **b** and **c**), indirect immunofluorescence detection with propidium iodide counterstaining: chromosomes show an R-banding pattern; the frequency of cells with at least one fluorescent spot in 6p21.3 was >80%, for a probe concentration ranging from 200 to 300 ng of DNA per slide; arrows indicate fluorescent spots on 6p21.3. On the right is shown a schematic diagram of the human chromosome 6 and the position of the gene encoding the p52 subunit of TFIIH.

spots were observed on the short arm of chromosome 6. After R-banding on metaphase chromosomes, the probe was localized on the distal part of the 6p21.3 band. No other secondary hybridization site was detected under our experimental conditions.

p52 is a subunit of TFIIH

Several lines of evidence indicate that the protein encoded by the p52 cDNA is part of the TFIIH complex. First, p52 co-fractionates with TFIIH transcription, ATPase, CTD kinase and both helicase activities through the last three steps of our purification procedure including the hydroxyapatite (HAP) chromatography (data not shown and Figure 1). Second, when a partially purified TFIIH fraction (heparin HPLC fraction; step 4 of our purification procedure) is pre-incubated either alone or with either Ab-p52 or Ab-control antibody before glycerol gradient centrifugation, p52 co-sediments with the other subunits of the complex (Figure 4A). In the absence of antibody (data not shown) or in the presence of Ab-control (upper panel), p52 is present in fractions 6–11 (with a peak in fraction 9), whereas after pre-incubation with Ab-p52 antibody, p52, p62 and XPB (the p89 3′–5′ helicase of TFIIH) are co-shifted towards the bottom of the gradient (fractions 7–14, with a peak in fraction 10–11; lower panel). Third, immunoprecipitation of the same heparin HPLC fraction was carried out with either Ab-p52 (Figure 4B, lane 3), Ab-XPB (lane 4) or Ab-control (lane 2) previously incubated with protein G–Sepharose beads. After washing (0.15 M KCl), the remaining proteins were separated on SDS–PAGE and immunoblotted with Ab-p52, Ab-XPB and Ab-p62 antibodies (Figure 4B). Ab-p52 immunoprecipitated p52 as well as XPB and p62, two subunits of TFIIH, as did Ab-XPB (see lanes 3 and 4 respectively). Together, these experiments demonstrate that p52 is strongly associated with the previously characterized subunits of TFIIH as well as with all the enzymatic activities exhibited by this transcription factor.

p52 is involved in both transcription and NER reactions in vitro

Since the Ab-p52 was able to immunoprecipitate not only p52 but also the other subunits of TFIIH, we investigated

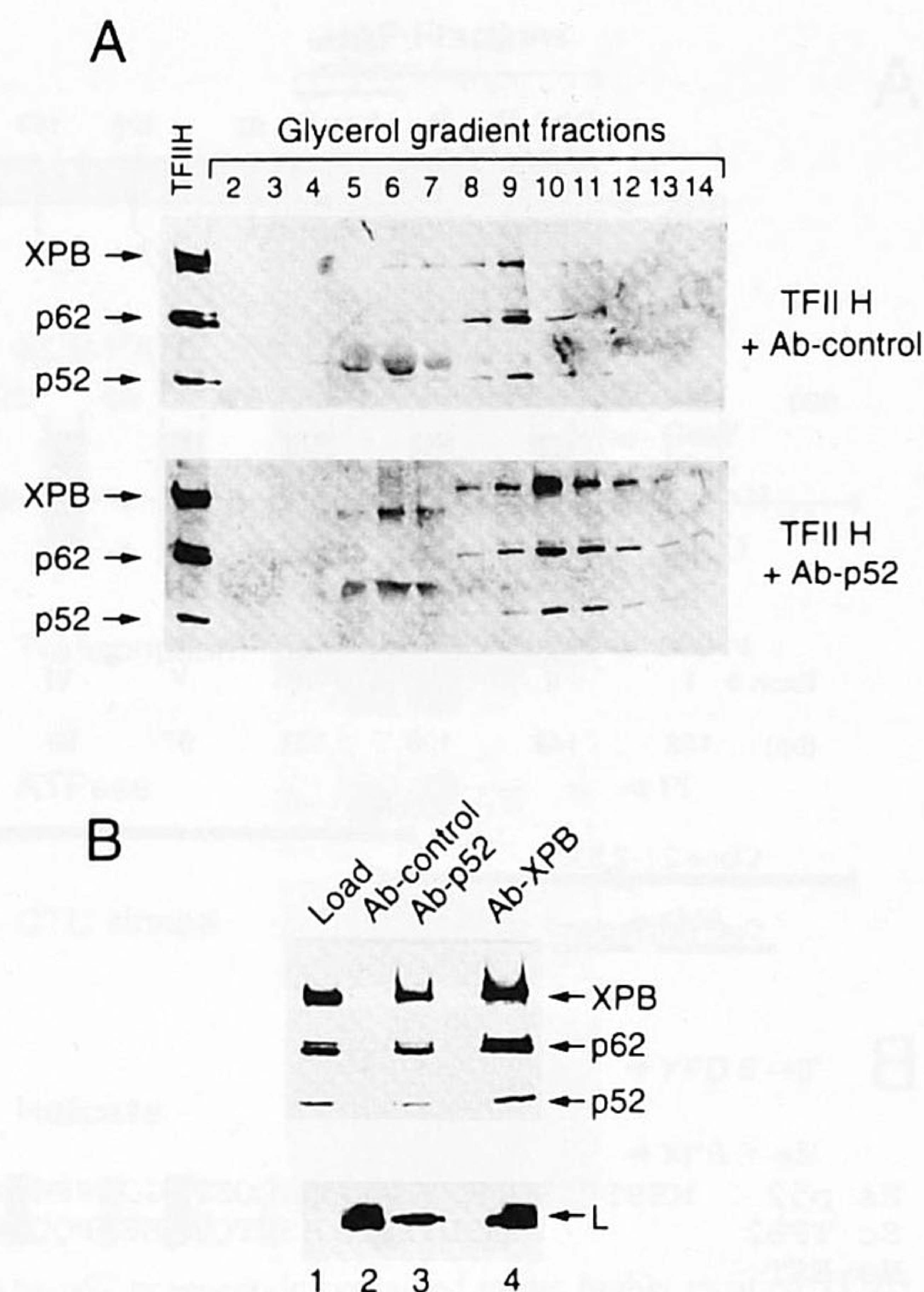

Fig. 4. The monoclonal antibody raised against the C-terminus of p52 displaces TFIIH in a glycerol gradient (A) and immunoprecipitates this complex (B). (**A**) The TFIIH heparin HPLC fraction (250 μl) was incubated for 2 h at 4°C with 25 μg of either a control (Ab-control; upper panel) or an Ab-p52 antibody (lower panel). After centrifugation on a glycerol gradient for 14 h at 270 000 *g* on a 10–30% glycerol gradient in an SW60 rotor (Beckman), the collected fractions were analyzed by immunoblotting using Ab-XPB (antibody directed against the p89 3′–5′ helicase of TFIIH), Ab-p62 and Ab-p52 antibodies. The material in lanes 5–7 corresponds to the heavy chain of the Ab used in each assay and/or protein contaminants. (**B**) The TFIIH heparin HPLC fraction (50 μl) was incubated overnight at 4°C with the same amount (20 μg) of either Ab-control (lane 2), Ab-p52 (lane 3) or Ab-XPB antibody (lane 4). After incubation for 2 h with protein G–Sepharose beads (Pharmacia), subsequent washing in 50 mM Tris–HCl, pH 7.9, 10% glycerol, 0.1 mM EDTA, 150 mM KCl, 0.1% NP-40, loading onto SDS–PAGE and transfer to nitrocellulose membrane, polypeptides were revealed with the described antibodies. Lane 1, 10 μl of TFIIH heparin HPLC fraction; L, light chain of antibody; XPB (the p89 3′–5′ helicase of TFIIH), p62 and p52 subunits are indicated. The lower yield observed with Ab-p52 may reflect the quality of the antibody (compared with Ab-XPB).

whether this polypeptide was associated with the transcription and repair functions of TFIIH. First, increasing amounts of Ab-p52 were pre-incubated with a fixed amount of partially purified TFIIH (heparin HPLC fraction), before being added to an *in vitro* transcription system containing RNA pol II, the adenovirus 2 major late promoter (Ad2 MLP) template and all the basal transcription factors except TFIIH (Figure 5A). Under these conditions, transcription was reduced as a function of the concentration of Ab-p52 (lanes 2–6, compared with lane 1), whereas transcription was not inhibited when increasing amounts of Ab-control were added (lanes 7 and 8). To establish further that this inhibition resulted from the specific interaction between Ab-p52 and TFIIH, increasing amounts of a highly purified fraction of TFIIH (the HAP fraction, see Figure 1) were added (lanes 11–15) to the *in vitro* transcription reaction that previously was 75% inhibited after addition of Ab-p52 (lane 10). Under such

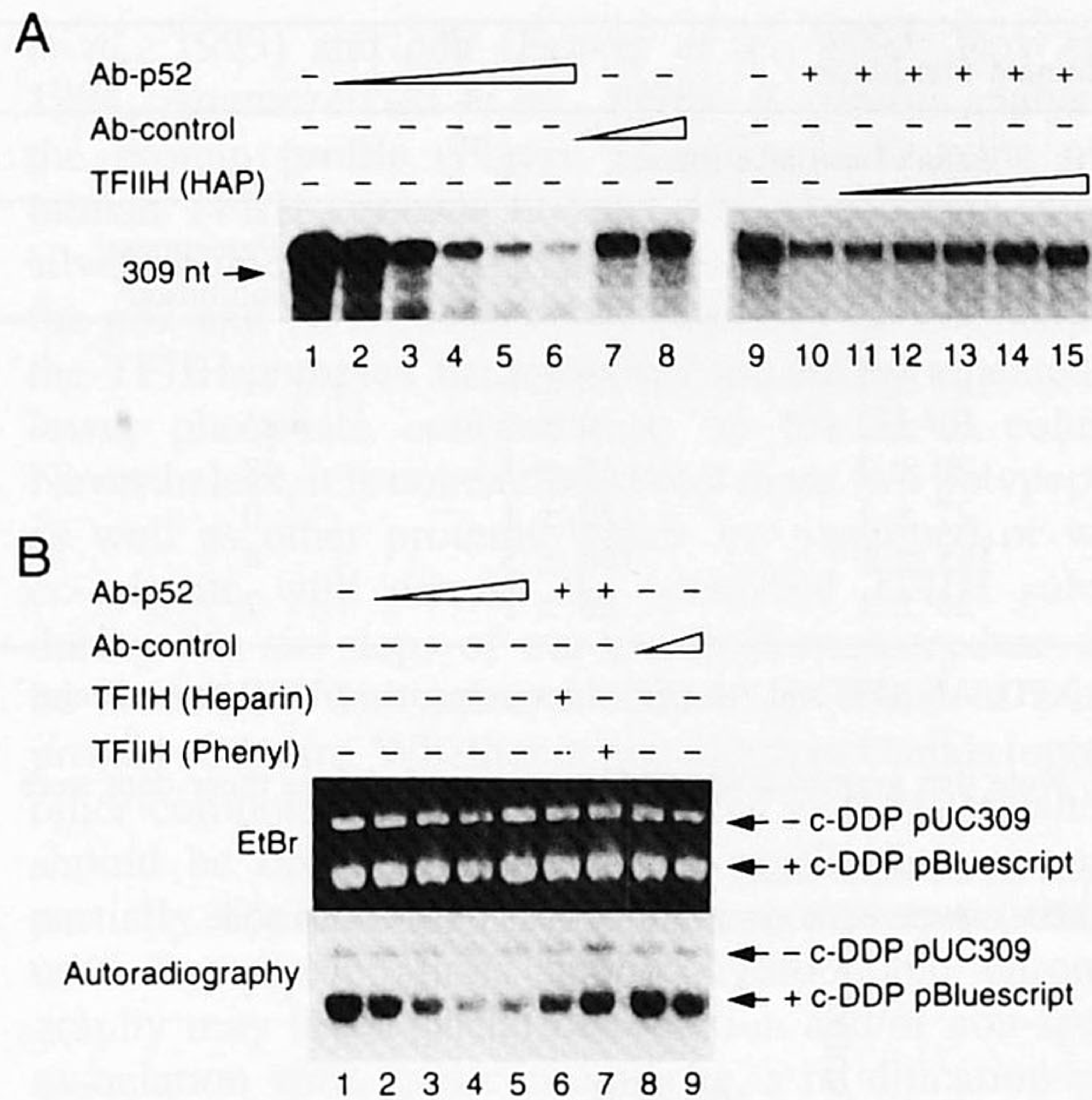

Fig. 5. Ab-p52 inhibits both transcription (A) and repair (B) *in vitro*. (**A**) The TFIIH heparin HPLC fraction (1 µl) was pre-incubated with either no antibody (lane 1), or increasing amounts of Ab-p52 (0.1, 0.5, 1, 5 and 10 µg; lanes 2–6 respectively), or Ab control (5 µg; lane 7 and 10 µg; lane 8) before being supplemented with all the other general transcription factors, RNA pol II and the DNA template. Increasing amounts of TFIIH HAP fraction were added to the 75% inhibited (5 µg of Ab-p52; lane 10) transcription (lanes 11–15). The arrow indicates the 309 nucleotide transcript. (**B**) A HeLa whole cell extract (10 µl) was incubated with increasing amounts of either Ab-p52 (0.5, 1, 5 and 10 µg; lanes 2–5) or Ab-control (5 µg; lane 8 and 10 µg; lane 9) and added to *cis*-diamminedichloroplatinum-treated (+ c-DDP) pBluescript and untreated (– c-DDP) pUC309 DNA and tested for repair activity (see Materials and methods). To restore the DNA repair, the TFIIH heparin (lane 6) or phenyl (lane 7) fraction was added to the 75% (5 µg of Ab-p52) inhibited NER reaction. EtBr, ethidium bromide.

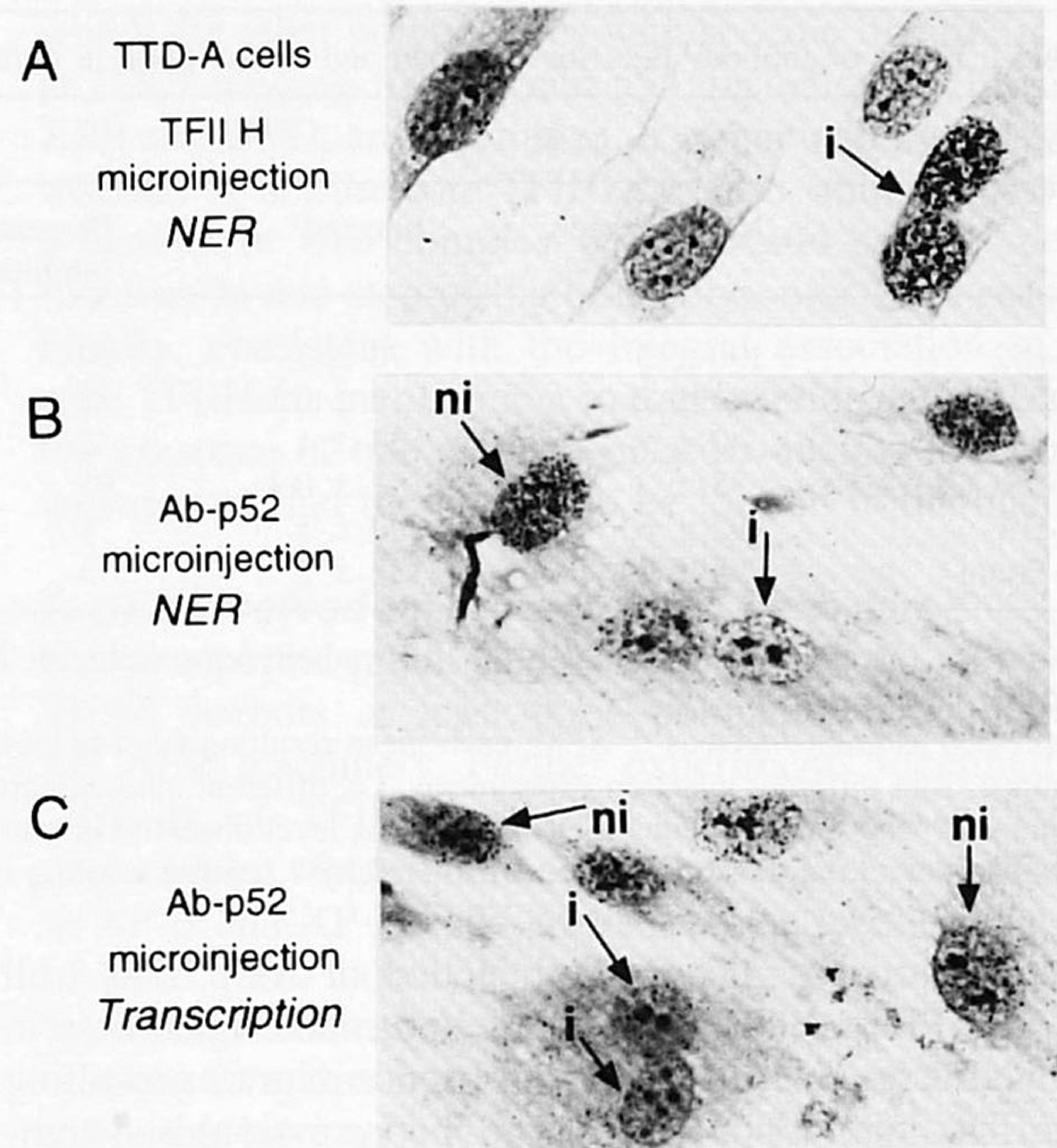

Fig. 6. Effect of Ab-p52 on transcription and NER *in vivo*. (**A**) Micrograph of TTD-A (TTD1BR) fibroblasts microinjected with the highly purified TFIIH fraction of the HAP chromatography column (see Figure 1), showing induction of the UV-dependent unscheduled DNA synthesis in the injected multinucleated cell ('i'; arrow) compared with the low residual unscheduled DNA synthesis level typical of TTD-A as shown by the surrounding uninjected mononuclear cells. (**B** and **C**) Micrographs of normal human fibroblasts injected ('i', arrow) with Ab-p52 showing *in vivo* inhibition of both NER (B) and transcription (C) compared with non-injected cells ('ni', arrow).

conditions, it was possible to increase the level of RNA synthesis (compare lane 15 with lane 9).

Second, the ability of p52 to inhibit *in vitro* NER was assessed. Undamaged (pUC309) and damaged DNA (c-DPP-treated pBluescript) were added to a HeLa whole cell extract previously pre-incubated with either Ab-p52 or Ab-control and tested for repair activity. The incorporation of labeled [α^{32}P]dATP to replace the damaged DNA was decreased as a function of the amount of Ab-p52 (Figure 5B, lanes 2–5 compared with lane 1) whereas Ab-control did not affect NER (lane 8–9). As shown for the transcription reaction, addition of TFIIH from either heparin (lane 6) or phenyl (lane 7) fractions to an already 75% inhibited NER reaction restores the repair activity (compare lanes 6 and 7 with lane 4). Taken together, these results demonstrate that p52 participates in the transcription and NER reactions as a component of TFIIH.

Ab-p52 inhibits both transcription and DNA repair in vivo

To measure the ability of TFIIH to participate in NER, a highly purified TFIIH preparation from the final stage of purification (HAP fraction, see Figure 1) was injected into the cytoplasm of repair-deficient TTD-A fibroblasts. These cells carry a mutation in a still undefined component of TFIIH causing a severe NER defect (Stefanini *et al.*, 1993). After injection, the cells were UV irradiated and their ability to restore DNA repair was monitored by incubation in the presence of [^{3}H]thymidine, followed by autoradiography to visualize the repair patches (Figure 6A). The results show that the number of grains in injected cells is much higher than the number of grains of non-injected neighboring cells which exhibit the low level of residual repair synthesis characteristic of the TTD-A NER defect. In fact, the number of grains present in injected cells is similar to that of repair-competent cells assayed in parallel, indicating that TFIIH restores DNA repair to the wild-type level (data not shown). Similar results were obtained when TFIIH was microinjected in XPB- and XPD-deficient cells (Vermeulen *et al.*, 1994a, and data not shown).

Since p52 is a component of TFIIH, we thus wondered if a mutation in p52 could be responsible for the form of TTD observed in the TTD-A patient. Unfortunately, the pSG513-p52 expression vector, which overexpresses p52, was not able to restore the NER activity when microinjected in TTD-A-deficient cells (data not shown), indicating that p52 is not deficient in TTD-A cells. Additionally, sequence analysis of the p52 cDNA in cells of patient TTD-A did not reveal any mutations.

To understand the role of p52 *in vivo*, the effect of microinjection of Ab-p52 into normal human fibroblasts on NER and transcription was studied. DNA repair and transcription were quantitated by counting the number of autoradiographic grains in the nuclei of the injected (as indicated by 'i' and arrow) compared with the non-injected

Table I. Effect of antibody injection on repair and transcription in living normal human fibroblasts

Microinjected antibody	Effect on NER			Effect on transcription		
	Non-injected[a]	Injected[a]	Percentage inhibition[b]	Non-injected[a]	Injected[a]	Percentage inhibition[c]
Ab-p52	190 ± 5	70 ± 7	64	197 ± 7	58 ± 6	71
Ab-XPD	46 ± 2	6 ± 0.5	87	61 ± 2	9 ± 1	85
Ab-XPB	40 ± 2	23 ± 1	42	29 ± 2	15 ± 1	48
Ab-ERCC1	38 ± 2	1 ± 0.1	97	27 ± 1	28 ± 1	0
Ab-HA	192 ± 6	190 ± 5	1	195 ± 6	188 ± 5	4
Ab-Preim	38 ± 2	37 ± 2	3	28 ± 1	26 ± 1	7

Ab-ERCC1 (an antibody raised against a NER protein not residing in TFIIH), Ab-XPD, Ab-XPB and Ab-p52 (three subunits of TFIIH), Ab-Preim or Ab-HA were used.
[a]Expressed as grains/nucleus ± SEM, each mean resulting from at least 25 nuclei. Note that grains/nucleus differ per sample, since these data were obtained from different injection experiments, i.e. different autoradiograms.
[b]Compared with the unscheduled DNA synthesis level observed in non-injected cells on the same slide.
[c]Compared with the transcription level (assayed by 1 h pulse labeling with [^{3}H]uridine) observed in non-injected cells on the same slide.

cells ('ni' and arrow). Microinjection of Ab-p52 as well as Ab-XPB and Ab-XPD results in a marked decrease in both NER (as determined by the absence of grains resulting from UV-induced [^{3}H]thymidine incorporation) and transcription (as determined by the absence of grains resulting from the incorporation of [^{3}H]uridine), whereas mock antibodies (Ab-Preim and Ab-HA) showed no effect (see Figure 6B and C respectively and Table I). The residual activity observed could be due to either incomplete interaction with the antigen or an insufficient titer of the antibody. Interestingly, microinjection of antibodies against ERCC1, a factor exclusively devoted to the DNA repair reaction, had no effect on RNA synthesis (Table I). This demonstrates unequivocally that p52, as part of TFIIH, participates in both transcription and DNA repair reactions *in vivo*.

p52 belongs to the core of TFIIH

The above results strongly suggest that p52 is a bona fide subunit of TFIIH and, as such, participates in the transcription and DNA repair activities of TFIIH. Previous results obtained with yeast TFIIH favor the hypothesis that TFIIH is divided into two subcomplexes. One is the kinase complex TFIIK containing Kin28 and two forms of Ccl1 (Feaver *et al.*, 1994; Svejstrup *et al.*, 1996b), the yeast counterparts of cdk7 and cyclin H, whereas the second subcomplex in yeast (called the core of TFIIH) contains the other six subunits (for review, see Bathia *et al.*, 1996). To localize p52 further among the polypeptides that form this multisubunit complex, TFIIH was immunoprecipitated with Ab-XPB and washed with either 0.1 or 1 M KCl. When pre-incubated at high salt concentration, TFIIH could be partially dissociated (Adamczewski *et al.*, 1996). At high salt concentration, immunoblotting experiments using the corresponding antibodies show that only XPB, p62, p44, p34 and p52 remain adsorbed on the Ab-XPB cross-linked onto protein A–agarose beads, whereas XPD and cdk7 as well as the two other polypeptides that form the kinase complex (cyclin H and MAT1) are resolved from the other subunits (Figure 7, compare lanes 2 and 3 respectively). Indeed, antibodies directed against any of the three components of the kinase complex detect the corresponding subunits in the low salt fraction but not in the high salt fraction (data not shown) as previously demonstrated (Gérard *et al.*, 1991; Adamczewski *et al.*, 1996). This demonstrates that p52 is part of the TFIIH complex and is tightly associated with the four other polypeptides that form the core factor.

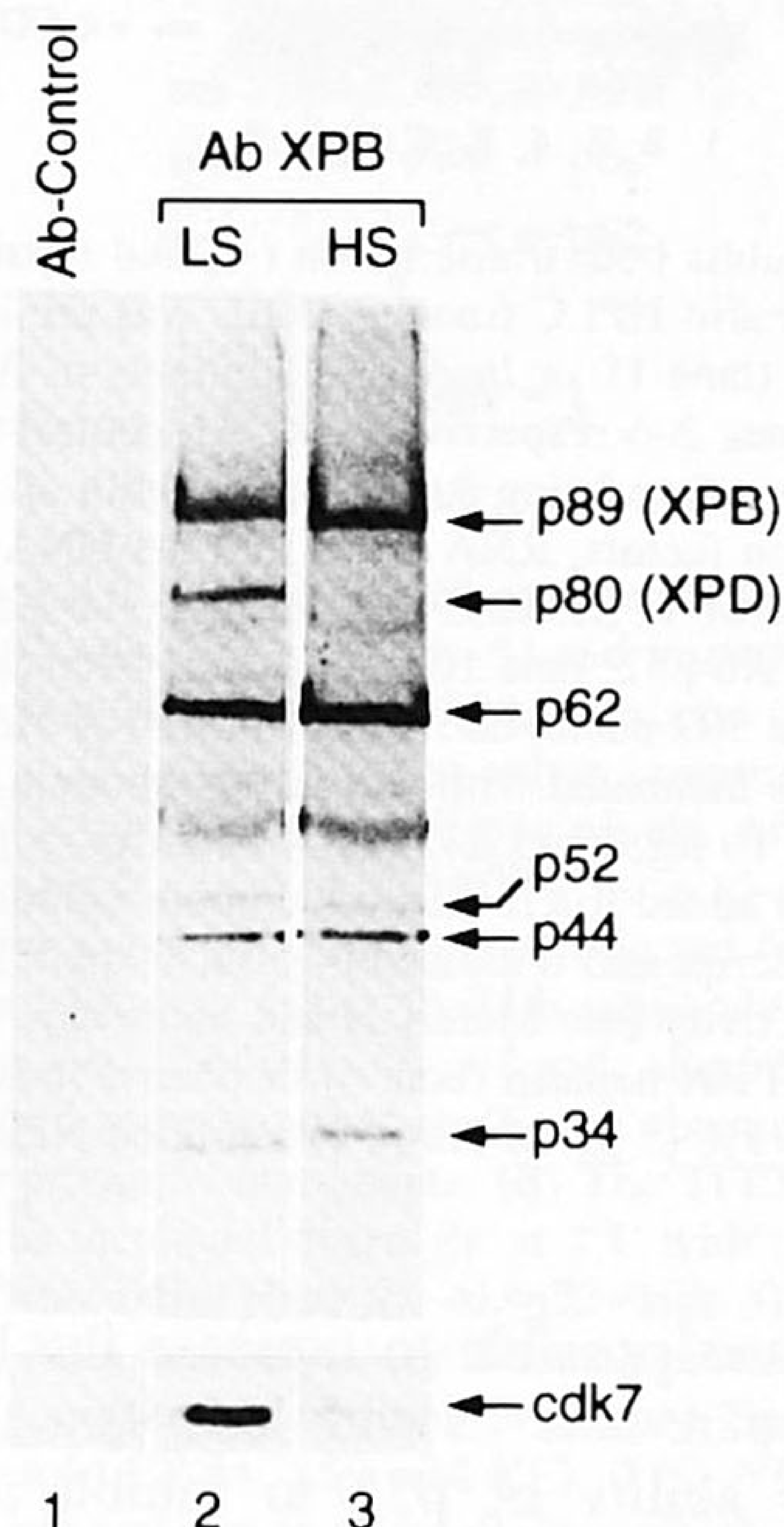

Fig. 7. p52 belongs to the core of TFIIH. The TFIIH heparin HPLC fraction (50 µl) was incubated with 20 µg of either Ab-control or Ab-XPB cross-linked to protein A–Sepharose (Roy *et al.*, 1994). After washing, either at low (0.1 M KCl, LS) or at high (1 M KCl, HS) salt concentrations (lanes 2 and 3 respectively), the immunoadsorbed polypeptides, as indicated at the right of the panel, were analyzed by immunoblotting using Ab-XPB, Ab-XPD, Ab-p62, Ab-p52, Ab-p44, Ab-p34 (upper panel) and Ab-cdk7 (lower panel).

Discussion

In an effort to understand the function of each of the TFIIH subunits in the diverse fundamental cellular processes in which TFIIH has been shown to participate (transcription, DNA repair and possibly cell cycle regulation), research in our laboratory has been aimed at systematically cloning and characterizing all TFIIH subunits. Several functions have been inferred for individual components. These activities include DNA helicases (Sung *et al.*, 1993; Drapkin *et al.*, 1994; Schaeffer *et al.*, 1994), ATPase (Roy

et al., 1993) and cdk (Feaver *et al.*, 1994; Roy *et al.*, 1994; Adamczewski *et al.*, 1996). A careful analysis of the protein profile (Figure 1) indicates that the purest human TFIIH contains at least nine Coomassie blue (or silver)-stained bands. The two polypeptides located above the p52 and XPB respectively probably do not belong to the TFIIH complex because they are eluted repeatedly at lower phosphate concentration on the HAP columns. Nevertheless, it is not excluded that these two polypeptides as well as other proteins which are unstained or which co-migrate with one of the identified TFIIH subunits during the six steps of our purification procedure could be associated with some functions of TFIIH in a larger protein structure. Whether or not additional bands represent other components of TFIIH remains to be established. It should be borne in mind that a purification process is partially denaturating: high salt concentrations that are used for precipitations and/or hydrophobic chromatography may favor partial dissociation and/or non-specific association with, as a consequence, a modification of the composition of the 'native protein complex'. For this reason, the definition of a multiprotein complex is often a matter of controversy. TFIID and the 'RNA polymerase holoenzyme' are excellent examples. For these complexes, the composition is not defined unequivocally and may vary as a function of the sample preparation. Indeed, it is not surprising to find associated with the TBP or with RNA pol II, polypeptides that are either directly (in the case of general transcription factors) or indirectly (in the case of activators) involved in transcriptional regulation of protein coding genes: these compounds often bind TBP or RNA pol II at one of the numerous steps of the transcription reaction. In all cases, as for TFIIH, proteins that belong to the core of a multiprotein complex have to be differentiated from the ones that are transiently associated. Concerning p52, we present strong arguments supporting the idea that it belongs to TFIIH.

p52 is de facto part of TFIIH

Our conclusion that p52 does belong to TFIIH is based on the following arguments. First, p52 co-purifies with the other subunits of TFIIH. Second, antibodies directed towards p52 or any subunit of TFIIH co-immunoprecipitate or co-shift on glycerol gradients a complex which contains p52 in addition to the eight other subunits of TFIIH: XPB, XPD, p62, p44, p34, cdk7, cyclin H and MAT1. Third, p52 antibody inhibits not only *in vitro* and *in vivo* protein coding gene transcription but also NER, a feature that was shown previously for the other identified subunits. Inhibition of *in vitro* transcription and NER can be relieved by supplementing the assay with purified TFIIH (HAP fraction for transcription and phenyl fraction for NER). Transcription and NER inhibition experiments demonstrate that p52 is in the complex(es) which participate(s) in both reactions either through a structural function or due to some enzymatic activity not yet identified.

In addition, we find that p52 together with the four other subunits, XPB, p62, p44 and p34, forms the core of TFIIH since the four others subunits (XPD and the ternary kinase complex) were dissociated at high salt concentration. p52, as is the case with p62, does not contain any known specific motif or enzymatic activities. Therefore, it may only have an architectural role, around which the other polypeptides with specific functions, such as DNA binding for p44 and p34, or helicase/ATPase for XPB and XPD, are assembled. It remains to be elucidated whether or not the core TFIIH complex (minimal) reflects a distinct *in vivo* complex which would have a specific function in one step of the transcription or NER reaction. Finally, consistent with the integral association of p52 with TFIIH, its involvement in transcription and repair and the presence of one or more putative nuclear localization signals, we find the protein to be located in the nucleus.

Is p52 involved in some genetic disorders?

It was reported previously (Vermeulen *et al.*, 1994a) that TFIIH harbors at least three polypeptides involved in the NER machinery. Further experiments identified two subunits of TFIIH (p89/XPB and p80/XPD) that are responsible, when mutated, for the NER defect observed in XP-B and XP-D cells. Moreover, the most severe form of SMA (also called Werdnig–Hoffmann disease) results from large scale deletions (Lefebvre *et al.*, 1994) that involve, in addition to the *SMN* gene, one of the two p44 genes that have arisen from a recent duplication (L.Bürglen and J.Melki, personal communication). Altogether these findings and the observations of the wide clinical heterogeneity and pleiotropy associated with XP, CS and TTD strongly suggest that mutations in some of the TFIIH subunits do not result exclusively in the appearance of NER syndromes, but also in a wider class of disorders that have been called transcription syndromes (Bootsma and Hoeijmakers, 1993).

To find possible clues as to whether mutations in other TFIIH subunits are associated with other mapped genetic disorders, we have determined the chromosomal localization of the genes for all previously unmapped TFIIH components (summarized in Table II). Genetic loci have already been published for XPD, XPB, p62 and cdk7 (Mohrenweiser *et al.*, 1989; Weeda *et al.*, 1991; Heng *et al.*, 1993). In fact for cdk7, two localizations have been proposed: 2q22–q24 and 5q12–q13 (Darbon *et al.*, 1994). In our hands, the localization has been refined to 5q13.1. Interestingly, cyclin H lies within the same 5q13–q14 region that harbors the X-ray-complementing Chinese hamster gene 4 (*XRCC4*) involved in double strand DNA break repair (Otevrel *et al.*, 1995). The gene encoding p44 is also located in 5q13.1. As stated above, p44 may be associated with the neurodegenerative disorder SMA. Genes associated with this condition lie in a 2 cM interval in the 5q12–q13 region (Lefebvre *et al.*, 1994). By searching the databanks, the p34 gene was found to be encoded by the complementary strand of the gene encoding eIF2b translation factor, located on chromosome 12. *In situ* hybridization experiments are under way to refine this localization.

The p52 gene has been assigned to 6p21.3. This particular location on chromosome 6 harbors numerous genes involved in immunity, such as the genes of the complement component 2, 4A and 4B and the major histocompatibility complex (HLA A–C) cluster, but also numerous genes encoding proteins involved in transcription, such as the octamer binding transcription factor 3 (OCT3), the retinoid X receptor β (RXRβ), the high mobility group protein 1 (HMG1) and the α-subunit of the transcription factor NF-γ (NF-YA). However, no obvious

Table II. Homologies between human and yeast (*S.cerevisiae*) TFIIH subunits

Human				Yeast			Percentage homology[a]
Name	MW (kDa)	aa	Chromosome localization	Name	MW (kDa)	aa	
XPB	89	782	2q21	SSL2/RAD25	95	843	55 (71)
XPD	80	760	19q13.2–13.3	RAD3	85	778	53 (73)
p62	62	548	11p15.1–p14	TFB1	75	642	22 (44)
p52	52	436	6p21.3[c]	TFB2	55	513	37 (61)
p44	44	395	5q13.1[c]	SSL1	50	461	41 (58)
p34	34	303	12[c]	TFB4[b]	37	338	31 (55)
cdk7	40	346	5q13.1	KIN28	35	306	47 (68)
cyclin H	34	323	5q13-q14[c]	CCL1[d]	47; 45	393	31 (52)
MAT1	32	309	n.d.	TFB3	33	321	31 (55)

The molecular weight (MW), the amino acid (aa) composition and the chromosomal localization are indicated.
[a]Identities are given and similarities are shown in parentheses.
[b]The TFB4 yeast homolog of human p34 (GenBank Z49219) is still being characterized (Bhatia *et al.*, 1996).
[c]Chromosomal localization detected or refined (cyclin H) in our hands.
[d]The *CCL1* gene produces two polypeptides of 45 and 47 kDa (Svejstrup *et al.*, 1996b).

candidate disorders for TFIIH defects have been mapped to this localization.

Since microinjection of a highly purified TFIIH was able to restore NER in TTD-A-deficient cells (from the only recorded patient), it was tempting to relate the gene encoding the p52 subunit of TFIIH to this still unexplained genetic disease. Unfortunately, microinjection of p52 cDNA in TTD-A cells does not restore NER, suggesting that this subunit is not responsible for the NER defect when mutated. However, it has to be noted that injection of the purest TFIIH preparation (HAP fraction, see Figure 1), induces only a weak or partial correction of the repair defect in TTD-A, XP-B and XP-D cells immediately after injection and a much stronger effect at later times. Similar findings were observed when individual recombinant XPB protein was injected into XP-B fibroblasts (our unpublished results). This suggests that either one subunit of TFIIH or a polypeptide associated with this factor is missing in the purest TFIIH, or that TFIIH (from the HAP fraction) needs to undergo some kinetically slow modification before being able to function fully in NER. This possibility could not be excluded since we know that p62 and cyclin H (our unpublished data) are phosphorylated proteins and that phosphorylation activates cdk7 (Fisher and Morgan, 1994). That the p52 gene product needs some type of post-modification to exert its role in NER or is responsible for still unidentified disorders remains an interesting possibility.

TFIIH in human and yeast

The recent completion of work on the TFIIH subunit composition reveals an extraordinary structural and functional conservation of this factor from yeast to humans. In both species, p52 is found associated with TFIIH (Table II). However, there are some discrepancies between the human and the yeast TFIIH complex. Nine subunits have been identified in humans whereas only eight were identified in yeast. The yeast TFB4, the counterpart of human p34, exists in the *Saccharomyces* Genome Project databank (genomic clones P665 and P670) and GenBank (accession No. Z49219) and possesses 31% identity and 55% similarity with its human homolog; whether or not this subunit is part of yeast TFIIH has yet to be demonstrated. Moreover, the human kinase complex contains three subunits (cdk7, cyclin H and MAT1; Tassan *et al.*, 1995; Adamczewski *et al.*, 1996) and exhibits a cdk-activating kinase activity (CAK), whereas the yeast TFIIH-associated kinase complex (also called TFIIK) contains only Kin28 and Ccl1 and does not possess CAK activity (Feaver *et al.*, 1994). These findings are in line with the high conservation of other basic transcription and repair factors between human and yeast.

With the study and cloning of p52, the characterization of the TFIIH complex is almost completed. It is now possible to focus on the reconstitution of a TFIIH complex and investigate its role in one of the various cellular events in which it is involved.

Materials and methods

Microsequencing of p52 and cloning of the cDNA and the gene encoding p52

TFIIH was purified as previously described (Gérard *et al.*, 1991). A concentrated HAP fraction was subjected to SDS–PAGE and electrotransferred onto a PVDF membrane. The p52 polypeptide was digested with trypsin, before being resolved by reverse phase chromatography. Amino acid sequences obtained from tryptic digests of p52 were used to design degenerate oligonucleotides. A preB-cell cDNA library constructed in the λZAPII vector was screened and the positive cDNA clones were sequenced (Fischer *et al.*, 1992). A human placental genomic library constructed in λGEM12 was screened using the random-primed full-length cDNA as a probe (Chalut *et al.*, 1995).

Fluorescence in situ hybridization (FISH)

In situ hybridization on human chromosomes was performed essentially as described (Viegas-Péquignot *et al.*, 1989). Briefly, the 5.5 kb p52 genomic probe was nick-translated in the presence of biotin-11-dUTP. The biotinylated probe (200–300 ng) resuspended in 15 μl of hybridization buffer was denatured for 10 min at 98°C with 10–20 μg of human placental DNA as competitor before addition to slides. After an overnight hybridization, slides were rinsed for 2 min in 50% formamide/2× SSC and for 1 min in 2× SSC at 37°C.

Antibodies used in the following assays

A monoclonal mouse antibody (hereafter Ab-p52) was raised against a polypeptide corresponding to the C-terminus of p52. Other antibodies used in these assays are: Ab-Preim (a pre-immune serum of the same mice), Ab-control (a mouse monoclonal antibody directed against GST), Ab-HA (an antibody against hemagglutinin), Ab-XPB, Ab-XPD, Ab-p62, Ab-p44, Ab-p34 and Ab-cdk7 (monoclonal mouse antibodies directed against the known subunits of TFIIH, previously described in Humbert

et al., 1994) and Ab-ERCC1 (a monoclonal mouse antibody against ERCC1, a protein involved in the NER process).

Inhibition of in vitro transcription

One µl of the TFIIH heparin HPLC fraction was pre-incubated for 1 h at 4°C with either no antibody, increasing amounts of Ab-p52 or Ab-control, or a 75% inhibitory amount of Ab-p52. The reaction was then complemented with the other basal transcription factors, RNA polymerase II, 50 ng of linearized Ad2-MLP DNA template and, when needed, increasing amounts of a TFIIH HAP fraction to restore the transcription activity. After 15 min of incubation at 25°C, the nucleotides were added and transcription was allowed to proceed for 45 min at 25°C. The 309 nucleotide transcripts were analyzed after electrophoresis of the samples through a sequencing gel (Humbert *et al.*, 1994).

Inhibition of in vitro repair

A pBluescript plasmid was treated with *cis*-diamminedichloroplatinum (c-DDP) to obtain ~20 platinum adducts per plasmid, as described in Hansson and Wood (1989). Standard 50 µl reaction mixtures contained 250 ng of non-treated (pUC309) and treated DNA in addition to 150 µg of protein from HeLa whole cell extract previously incubated for 1.5 h at 4°C either alone or with Ab-p52 or Ab-control. Reactions were incubated for 3 h at 30°C, with or without addition of TFIIH (heparin HPLC or phenyl fraction). Plasmid DNA was purified from the reaction mixtures, linearized with *Hin*dIII and loaded onto a 1% agarose gel. The DNA was visualized by ethidium bromide staining and the NER reaction was quantitated by autoradiography.

Microneedle injection and assays for UV-induced unscheduled DNA synthesis and RNA synthesis

Microneedle injections of repair-deficient (TTD1BR, XPCS1BA, XP6BE) and normal (C5RO) human diploid fibroblasts were performed as described (Vermeulen *et al.*, 1994b). Briefly, at least 3 days prior to injection, cells were fused with the aid of inactivated Sendai virus, seeded onto coverslips and cultured in Ham's F-10 medium, supplemented with 12% fetal calf serum and antibiotics. Protein preparations (purified TFIIH, final HAP chromatography) and antibodies were injected into the cytoplasm, whereas the cDNAs (pSG513-p52 construct) were delivered into one of the nuclei of polykaryons. After injection of at least 50 homopolykaryons, cells were incubated for the desired time (24–48 h for cDNA, 6–20 h for antibodies and 0–4 h for TFIIH injections) in normal medium before being assayed. NER activity was determined after UV-C light irradiation with 15 J/m^2, incubation for 2 h in a culture medium containing [^{3}H]thymidine (10 µCi/ml; sp. act. 50 Ci/mmol), fixation and exposure to autoradiography. RNA synthesis was determined after labeling with [^{3}H]uridine (10 µCi/ml; sp. act. 50 Ci/mmol) during a pulse labeling period of 1 h in normal culture medium. Grains within each nucleus (>100) were counted and represent a quantitative measurement for NER or overall transcription activity.

Nucleotide sequence accession number

The EMBL accession number for p52 reported here is Y07595.

Acknowledgements

We are grateful to D.Bootsma and P.Chambon for their unfailing support. We highly appreciated the very fruitful discussions we had with A.Lehmann, J.Feaver and R.Kornberg. We also are particularly indebted to A.Poterszman, P.van der Spek, D.Heard and P.Vichi for their computer expertise and criticisms and to S.Rademakers and G.S.Winkler for some of the cDNA microinjection experiments. We thank A.Fery for technical assistance and A.Staub for the protein microsequencing. J.C.M. was supported by a 'Ligue Nationale Contre le Cancer' fellowship. This work was supported by grants from the INSERM, the CNRS, the Ministère de la Recherche et de l'Enseignement Supérieur, DRET, the Association pour la Recherche sur le Cancer, the Dutch Cancer Society and a fellowship of the Royal Dutch Academy of Science to G.W.

References

Adamczewski,J., Rossignol,M., Tassan,J.P., Nigg,E., Moncollin,V. and Egly,J.M. (1996) MAT1, cdk7 and cyclin H form a kinase complex which is UV light sensitive upon association with TFIIH. *EMBO J.*, **15**, 1877–1884.

Bhatia,P., Wang,Z. and Friedberg,E.C. (1996) DNA repair and transcription. *Curr. Opin. Genet. Dev.*, **6**, 146–150.

Bootsma,D. and Hoeijmakers,J.H.J. (1993) DNA repair. Engagement with transcription. *Nature*, **363**, 114–115.

Boulikas,T. (1994) Putative nuclear localization signals (NLS) in protein transcription factors. *J. Cell Biochem.*, **55**, 32–58.

Broughton,B.C. *et al.* (1995) Molecular and cellular analysis of the DNA repair defect in a patient with xeroderma pigmentosum complementation group D with the clinical features of xeroderma pigmentosum and Cockayne syndrome. *Am. J. Hum. Genet.*, **56**, 167–174.

Chalut,C., Gallois,Y., Poterszman,A., Moncollin,V. and Egly,J.M. (1995) Genomic structure of the human TATA-box binding protein. *Gene*, **61**, 277–282.

Darbon,J.M., Devault,A., Taviaux,S., Fesquet,D., Martinez,A.M., Galas,S., Cavadore,J.C., Dorée,M. and Blanchard,J.M. (1994) Cloning, expression and subcellular localization of the human homolog of p40^{Mo15} catalytic subunit of cdk-activating kinase. *Oncogene*, **9**, 3127–3138.

Devault,A., Martinez,A., Fesquet,D., Labbé,J., Morin,N., Tassan,J.P., Nigg,E., Cavadore,J.C. and Dorée,M. (1995) MAT1 ('ménage à trois') a new RING finger protein subunit stabilizing cyclin H–cdk7 complexes in starfish and *Xenopus* CAK. *EMBO J.*, **14**, 5027–5036

Drapkin,R., Reardon,J.T., Ansari,A., Huang,J.C., Zawel,L., Ahn,K.J., Sancar,A. and Reinberg,D. (1994) Dual role of TFIIH in DNA excision repair and in transcription by RNA polymerase II. *Nature*, **368**, 769–772.

Feaver,W.J., Svejstrup,J.Q., Henry,N.L. and Kornberg,R.D. (1994) Relationship of CDK-activating kinase and RNA polymerase II CTD kinase TFIIH/TFIIK. *Cell*, **79**, 1103–1109.

Fischer,L., Gérard,M., Chalut,C., Lutz,Y., Humbert,S., Kanno,M., Chambon,P. and Egly,J.M. (1992) Cloning of the 62-kilodalton component of basic transcription factor BTF2. *Science*, **257**, 1392–1395.

Fisher,R. and Morgan,D. (1994) A novel cyclin associates with MO15/CDK7 to form the CDK-activating kinase. *Cell*, **78**, 713–724.

Gardiner-Garden,M. and Frommer,M. (1987) CpG islands in vertebrate genomes. *J. Mol. Biol.*, **196**, 261–282.

Gérard,M., Fisher,L., Moncollin,V., Chipoulet,J.M., Chambon,P. and Egly,J.M. (1991) Purification and interaction properties of the human RNA polymerase B (II) general transcription factor BTF2. *J. Biol. Cell.*, **266**, 20940–20945.

Hansson,J. and Wood,R.D. (1989) Repair synthesis by human cell extracts in DNA damaged by *cis*- and *trans*-diamminedichloroplatinum(II). *Nucleic Acids Res.*, **17**, 8073–8091.

Heng,H., Xiao,H., Shi,X.M., Greenblatt,J. and Tsui,L.C. (1993) Genes encoding general initiation factors for RNA polymerase II are dispersed in the human genome. *Hum. Mol. Genet.*, **3**, 61–64.

Hoeijmakers,J.H.J., Egly,J.M. and Vermeulen,W. (1996) TFIIH: a key component in multiple DNA transactions. *Curr. Opin. Genet. Dev.*, **6**, 26–33.

Humbert,S., van Vuuren,H., Lutz,Y., Hoeijmakers,J.H.J., Egly,J.M. and Moncollin,V. (1994) p44 and p34 subunits of the BTF2/TFIIH transcription factor have homologies with SSL1, a yeast protein involved in DNA repair. *EMBO J.*, **13**, 2393–2398.

Kozak,M. (1986) Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell*, **44**, 283–292.

Lefebvre,S. *et al.* (1995) Identification and characterization of spinal muscular atrophy-determining gene. *Cell*, **80**, 155–165.

Mohrenweiser,H.W., Carrano,A.V., Fertitta,A., Perry,B., Thompson,L.H., Tucker,J.D. and Weber,C.A. (1989) Refined mapping of the three DNA repair genes ERCC1, ERCC2, and XRCC1, on chromosome 19. *Cytogenet. Cell Genet.*, **52**, 11–14.

Morgan,D.O. (1995) Principles of CDK regulation. *Nature*, **374**, 131–134.

Nigg,E. (1996) Cyclin-dependent kinase 7: at the cross-roads of transcription, DNA repair and cell cycle control? *Curr. Opin. Cell Biol.*, **8**, 312–317

Otevrel,T. and Stamato,T.D. (1995) Regional localization of the *XRCC4* human radiation repair gene. *Genomics*, **27**, 211–214.

Roy,R., Schaeffer,L., Humbert,S., Vermeulen,W., Weeda,G. and Egly,J.M. (1993) The DNA-dependent ATPase activity associated with the class II transcription factor BTF2/TFIIH. *J. Biol. Chem.*, **269**, 9826–9832.

Roy,R., Adamczewski,J., Seroz,T., Vermeulen,W., Tassan,J.P., Schaeffer,L., Hoeijmakers,J.H.J. and Egly,J.M. (1994) The MO15 cell cycle kinase is associated with the TFIIH transcription–DNA repair factor. *Cell*, **79**, 1093–1101.

Schaeffer,L., Moncollin,V., Roy,R., Staub,A., Mezzina,M., Sarasin,A., Weeda,G., Hoeijmakers,J.H.J. and Egly,J.M. (1994) The ERCC2/DNA repair protein is associated with the class II BTF2/TFIIH transcription factor. *EMBO J.*, **13**, 2388–2392.

Stefanini,M. *et al.* (1993) A new nucleotide-excision repair gene associated with the disorder trichothiodystrophy. *Am. J. Hum. Genet.*, **53**, 817–821.

Sung,P., Bailly,V., Weber,C., Thompson,L., Prakash,L. and Prakash,S. (1993) Human xeroderma pigmentosum group D gene encodes a DNA helicase. *Nature*, **365**, 852–855.

Svejstrup,J.Q., Vichi,P. and Egly,J.M. (1996a) The multiple roles of transcription/repair factor TFIIH. *Trends Biol. Sci.*, **249**, 346–350.

Svejstrup,J.Q., Feaver,W.J. and Kornberg,R.D. (1996b) Subunits of yeast RNA polymerase II transcription factor TFIIH encoded by the *CCL1* gene. *J. Biol. Chem.*, **271**, 643–645.

Tassan,J.P., Jaquenoud,M., Fry,A., Frutiger,S., Hughes,G. and Nigg,E. (1995) *In vitro* assembly of a functional human CDK7–cyclin H complex requires MAT1, a novel 36 kDa RING finger protein. *EMBO J.*, **14**, 5608–5617.

Vermeulen,W. *et al.* (1994a) Three unusual repair deficiencies associated with transcription factor BTF2 (TFIIH). Evidence for the existence of a transcription syndrome. *Cold Spring Harbor Symp. Quant. Biol.*, **59**, 317–329.

Vermeulen,W. *et al.* (1994b) Clinical heterogeneity within xeroderma pigmentosum associated with mutations in the DNA repair and transcription gene *ERCC3*. *Am. J. Hum. Genet.*, **54**, 191–200.

Viegas-Péquignot,E., Dutrillaux,B., Magdelenat,H. and Coppey-Moisan,M. (1989) Mapping of single-copy DNA sequences on human chromosomes by *in situ* hybridization with biotinylated probes: enhancement of detection sensitivity by intensified-fluorescence digital imaging microscope. *Proc. Natl Acad. Sc. USA*, **86**, 582–586.

Wang,Z., Buratowski,S., Svejstrup,J.Q., Feaver,W.J., Wu,X., Kornberg,R.D., Donahue,T.F. and Friedberg,E.C. (1995) The yeast *TFB1* and *SSL1* genes, which encode subunits of transcription factor IIH, are required for nucleotide excision repair and polymerase II transcription. *Mol. Cell. Biol.*, **15**, 2288–2293.

Weeda,G., Wiegant,J., van der Ploeg,M., Geurts van Kessel,A., van der Eb,A. and Hoeijmakers,J.H.J. (1991) Localization of the xeroderma pigmentosum group B-correcting gene *ERCC3* to human chromosome 2q21. *Genomics*, **10**, 1035–1040.

Received on July 5, 1996; revised on August 27, 1996