

## p44 and p34 subunits of the BTF2/TFIIL transcription factor have homologies with SSL1, a yeast protein involved in DNA repair

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**The human BTF2 (TFIIL) transcription factor is a multisubunit protein involved in transcription initiation by RNA polymerase II (B) as well as in DNA repair. In addition to the previously characterized p62 and p89/ERCC3 subunits, we have cloned two other subunits of BTF2, p44 and p34. The gene encoding p44 appeared to be the human counterpart of *SSL1*, a gene involved in translation and UV resistance in yeast. Interestingly, the p34 subunit also has homology with a domain of *SSL1*, suggesting that it corresponds to an as yet unidentified protein involved in DNA repair. Both p44 and p34 possess zinc finger domains that may mediate BTF2 binding to nucleic acids.**

**Key words:** BTF2 (TFIIL)/DNA repair/SSL1/transcription factor

### Introduction

BTF2 (TFIIL) is an essential class II transcription factor that has recently been shown to play a role in nucleotide excision repair (NER) (Schaeffer *et al.*, 1993). This basal factor participates in the formation of the preinitiation transcription complex, which includes the TFIIA, TFIID, TFIIB, TFIIE, and TFIIF basal transcription factors and RNA polymerase II (B) onto a minimal promoter (containing the TATA box and cap site) (Conaway and Conaway, 1991; Zawel and Reinberg, 1992). BTF2, like its homologs the rat factor  $\delta$  and the yeast factor  $b$ , is absolutely required for the basal *in vitro* transcription of most protein coding genes. Little is known about the role of BTF2 in the transcription reaction, although a kinase activity which is capable of phosphorylating the carboxy-terminal domain (CTD) of the large subunit of RNA polymerase II, and a DNA-dependent ATPase activity have been found to be associated with BTF2 and its rat and yeast homologs (Bunick *et al.*, 1982; Sawadogo and Roeder, 1984; Conaway and Conaway, 1988; Feaver *et al.*, 1991; Dahmus and Dynan, 1992; Lu *et al.*, 1992; Serizawa *et al.*, 1992). These two activities may be responsible for the obligate ATP hydrolysis required for completing transcriptional initiation.

A role of BTF2 in NER was recently suggested when one of the subunits (the 89 kDa polypeptide; p89) was identified

as the *ERCC3* gene product, a presumed helicase associated with DNA repair (Weeda *et al.*, 1990; Schaeffer *et al.*, 1993; van Vuuren *et al.*, 1994). Mutations in this gene confer sensitivity to sunlight (UV) and a predisposition to skin cancer manifested by xeroderma pigmentosum group B (XP-B), a severe form of this repair syndrome, which also exhibits the clinical hallmarks of another DNA repair disorder, namely Cockayne's syndrome. Further experiments, using both an *in vivo* microinjection repair assay and an *in vitro* NER system based on cell-free extracts (Wood *et al.*, 1988), confirmed that the p89/ERCC3 subunit of BTF2 is directly involved in the excision repair reaction (van Vuuren *et al.*, 1994). More recent results have shown that the ERCC2 80 kDa polypeptide is also associated with the BTF2 complex, although not as tightly as p89 (Schaeffer *et al.*, 1994).

The dual function of BTF2 in these two important, but otherwise quite distinct, mechanisms of DNA metabolism has recently generated some controversy as to whether BTF2 is indeed a transcription repair coupling factor, or whether components of BTF2 function independently in each of the two processes (Sweder *et al.*, 1993). A means to resolve this disparity lies in the cloning of all subunits of BTF2, which will permit the elucidation of their individual roles and that of the entire complex in DNA repair and transcription. As a step toward this goal, we report here the cloning of two additional subunits of this multifunctional complex. Analysis of these components provides indications that BTF2 may be involved in yet another basic cellular process: translation.

### Results

#### **Cloning and expression of the p44 and p34 polypeptides of BTF2/TFIIL**

The BTF2 (TFIIL) transcription factor was purified as previously described (Gérard *et al.*, 1991). A concentrated hydroxyapatite fraction was subjected to SDS-PAGE and electrotransferred onto a PVDF membrane. The 44 kDa (p44) and the 34 kDa (p34) polypeptides were then individually digested with trypsin, before being resolved by reversed phase chromatography. Amino acid sequences obtained from tryptic digests of p44 or p34 were used to synthesize degenerate oligonucleotides for screening a HeLa  $\lambda$ ZAPII cDNA library. Multiple positive clones were obtained, and sequencing revealed that for each, one clone contained the entire open reading frame (ORF). The p44 and p34 cDNAs (Figure 1) possess ORFs of 1185 and 909 bp respectively that encode proteins of 395 and 303 amino acids, with calculated mol. wts of 44 451 and 33 920 Da respectively, which is in good agreement with the previously estimated mol. wt of the purified protein on an SDS gel (43 and 35 kDa respectively; Gérard *et al.*, 1991). Moreover, when overexpressed in *Escherichia coli*, both

A

p44 1 MDEEPERTKR..... WEGGYERTWEILKEDESGSLKATI  
 .  
 SSL1 50 RDKHVQRKKKKRLSNRNLQGSNGGYAWEDEIKRSWDLVKVDDEGDMASLV  
 35 EDILFKAKRKRVFEEHGQVRGLGMMRHLYVVVDSRTMEDQDLKPNRLTCT  
 .  
 100 ASIVEARKKRTAKKNITPYERGIIRSLILTLDCSEAMLEKDLRPNRHAMI  
 85 LKLLEYFVEEYFDQNPISQIGIIIVTKSKRAEKLTELSGNPRKHITSLKKA  
 .  
 150 IQYAIDFVHEFFDQNPISQMGIIIMRNGLAQLVSQVSGNPQDHIDALKSI  
 135 VDMTCHGEPSLYNSLSMAMQTLKHM**GHTS**REVLIIIFSSLTTCDPSNIYD  
 .  
 200 RKQEPKGNPSLQNALEMARGLLL**PVA**HCTREVLIVFGSLSTD**PGDIHQ**  
 185 LIKTLKAAKIRVSVIGLSA**EV**RVCTVLARET . . . . . . . . . .  
 .  
 250 TIDSLVSEKIRVKV**LG**LSAQVAICKELCKATNYGDESYKILLDETHLKE  
 231 LLTHHLSP.PPASSSECSLIRMGFPQHTIASLSDQDAKPSFSMAHLDGN  
 .  
 300 LFNEAVTPLPVNK**KG**FTLV**KG**FPTRIF . . . . . . . . . .  
 280 TEPGLTLGGYFC**Q**CRAKYCELPVECKICGLTLVSAPH**L**ARSYHHLFPLD  
 .  
 340 . . SKL**VYGGY**FC**PN**CHSKV**CSL**PTVC**PC**CD**ML**L**IL**STH**L**ARSYH**HL**PLK  
 330 AFQEIPPLEEYNGERF**CY**G**CQ** . . . . . . . . . . . . . . .  
 .  
 388 TFAEVPTTEKFRSED**C**FSC**Q**SRFPILKNH**K**NG**K**L**T**SS**R****Y****R****C****E****D****C****K****Q****E**  
 368 **CV**CD**D**V**F**V**H**DSL**H**CCPG**C**IHK**I**P**A**PSGV 395  
 .  
 437 **CV**CD**D**V**F**I**HE**IL**H**NC**G**ES**K**P**V**IT 461

B

p34 1 MVSDEDELNLLVIVVDANPIWWGKQALKESQFTLSKC**I**DAVMVLGNSHLF  
 51 MNRSNKLAVIASHI**Q**ESRFLY**P**GN**R**LG**D**FF**G**DP**G**NP**P**EF**N**PL**G**SK**D**KG  
 101 YELLTSANEVIVEEIKDLM**T**KS**D**IK**G**Q**H**T**E**T**L**LAGSLAKALCY**I**HR**M**N**K**  
 151 VKDNQEMKS**R**IL**V**IKAA**E**DS**A**Q**Y**MF**M**N**V**IFAA**Q**K**Q**N**I**L**D**AC**V**L**D**SD**S**  
 201 GLLQQACDITGGLYLKVP**Q**MP**S**SL**E**Y**Y**LLW**V**FL**P**D**Q**RS**Q**L**I**L**P**PPV**H**VD  
 251 YRAACF**CH**RL**I**E**I**GYV**C**V**C**LS**I**FC**N**SP**I**CT**T****C**ETAF**K**ISL**P**P**V**L**K**  
 301 KRN

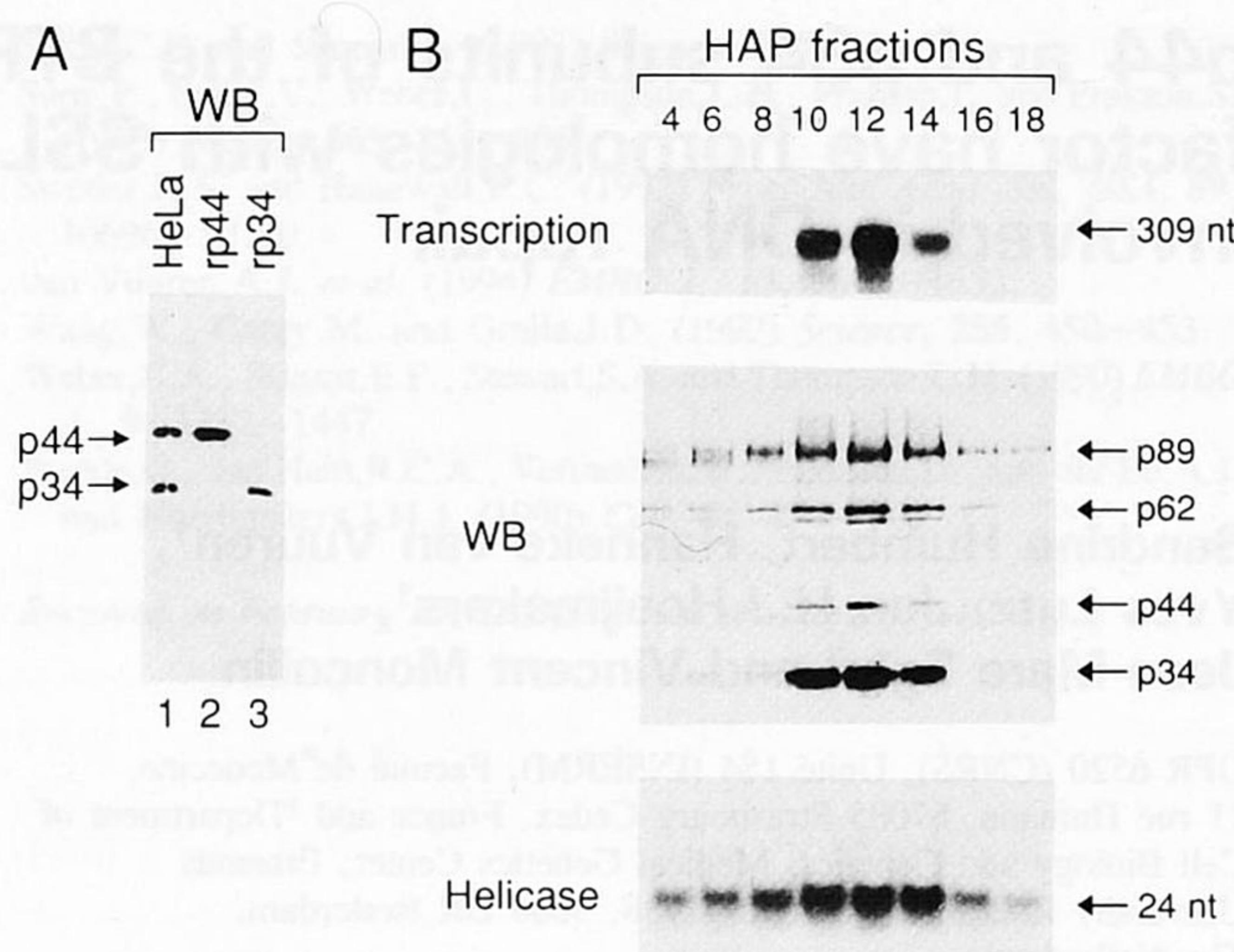
C

SSL1 332 PTFC**S**CH .  
 \*  
 p34 252 RAACF**C**H .  
 \*  
 p44 269 PSF**SM**A**H**LD**G**NTEPGL**T**LG**Y**FC**Q**CR**A**KY**C**EL**P**VE**C**KIC**G**LT**L**VS**A**

**Fig. 1.** Sequences of p44 (A) and p34 (B) BTF2 polypeptides, and alignments with SSL1 (A and C). Positions of the amino acids are indicated on the left. The peptides that were microsequenced from the purified 44 and 34 kDa polypeptides are underlined. The zinc finger motifs in p44 and p34 are boxed or in bold characters (see text). The SSL1 sequence has been aligned with the p44 sequence and homologies are indicated by | (threshold 1), : (threshold 0.5) or . (threshold 0.1). The nucleotide sequences of p44 and p34 subunits can be found in the GenBank database under the accession numbers Z30094 and Z30093, respectively.

recombinant polypeptides (rp44 and rp34) exhibit the same electrophoretic mobility on SDS-PAGE as the endogenous BTF2 p44 and p34 polypeptides according to immunoblotting experiments depicted in Figure 2A (compare lane 1 with lanes 2 and 3 respectively). In both cases the microsequenced peptides were observed in the deduced amino acid sequences confirming the identity of the clones (Figure 1A and B).

Searches in DNA and protein databases revealed a significant overall homology between p44 and the yeast protein SSL1 (Figure 1A). SSL1 is a yeast zinc finger protein previously identified as the product of a stem-loop suppressor gene associated with initiation of translation (Yoon *et al.*, 1992). At the amino acid level, p44 and SSL1 have a 58% similarity and a 40% identity. p44 as well as SSL1 contains the motif Tyr-X-Cys-X<sub>2</sub>-Cys-X<sub>3</sub>-Phe-



**Fig. 2.** Western blot analysis of endogenous and recombinant p44 and p34 (A) and copurification of BTF2 polypeptides with transcription and helicase activities over the hydroxyapatite gradient (B). (A) An SDS gel was loaded with either 2  $\mu$ l of a heparin HPLC fraction (lane 1), or 0.01  $\mu$ l of the pelleted fraction of either rp44 (lane 2) or rp34 (lane 3). After transfer to nitrocellulose, the polypeptides were detected using monoclonal antibodies raised against each subunit. (B) The BTF2 hydroxyapatite fractions were tested in a standard run-off transcription assay lacking BTF2 (2  $\mu$ l), in Western blot (10  $\mu$ l) for the presence of the four cloned subunits of BTF2 (p89, p62, p44 and p34) and in a helicase assay (2  $\mu$ l). The arrows on the right indicate respectively the 309 nucleotide transcript (Transcription), the BTF2 polypeptides (WB) or the oligonucleotide (24 nt) displaced from the single-stranded DNA (Helicase).

X<sub>8</sub>-His-X<sub>2</sub>-Leu-His (amino acids 358–380) characteristic of TFIIIA-like zinc finger proteins that have been demonstrated to interact with DNA (Jacobs, 1992; Berg, 1993). We also noticed in p44 as well as SSL1, a repeat of the motif Cys-X<sub>2</sub>-Cys-X<sub>4</sub>-Cys/His-X<sub>5</sub>-Cys-X<sub>2</sub>-Cys interrupted by a Cys-X<sub>2</sub>-Cys-X<sub>11</sub>/X<sub>22</sub>-Cys-X<sub>2</sub>-Cys domain. Surprisingly, the first type is also present in p34. Alignment of the three sequences in this region (Figure 1C) highlights the conservation of the cysteine residues among the three polypeptides, which also suggests that p44 and p34 must share some common function, e.g. in the interaction with the DNA template. Preliminary results suggest that p44 indeed interacts with the DNA promoter (unpublished results). No other known motifs were found, but we noticed that the p34 is rich in leucine and isoleucine residues (20% over the entire sequence) which confers a very hydrophobic character to the polypeptide.

#### **The p44 and p34 polypeptides are bona fide subunits of BTF2**

When overexpressed in *E. coli*, the p44 and p34 polypeptides remained in an insoluble form (up to 98%) and were therefore subjected to denaturation–renaturation with guanidium hydrochloride. In this condition a small part of the proteins was recovered in a soluble form that was used in the following experiments.

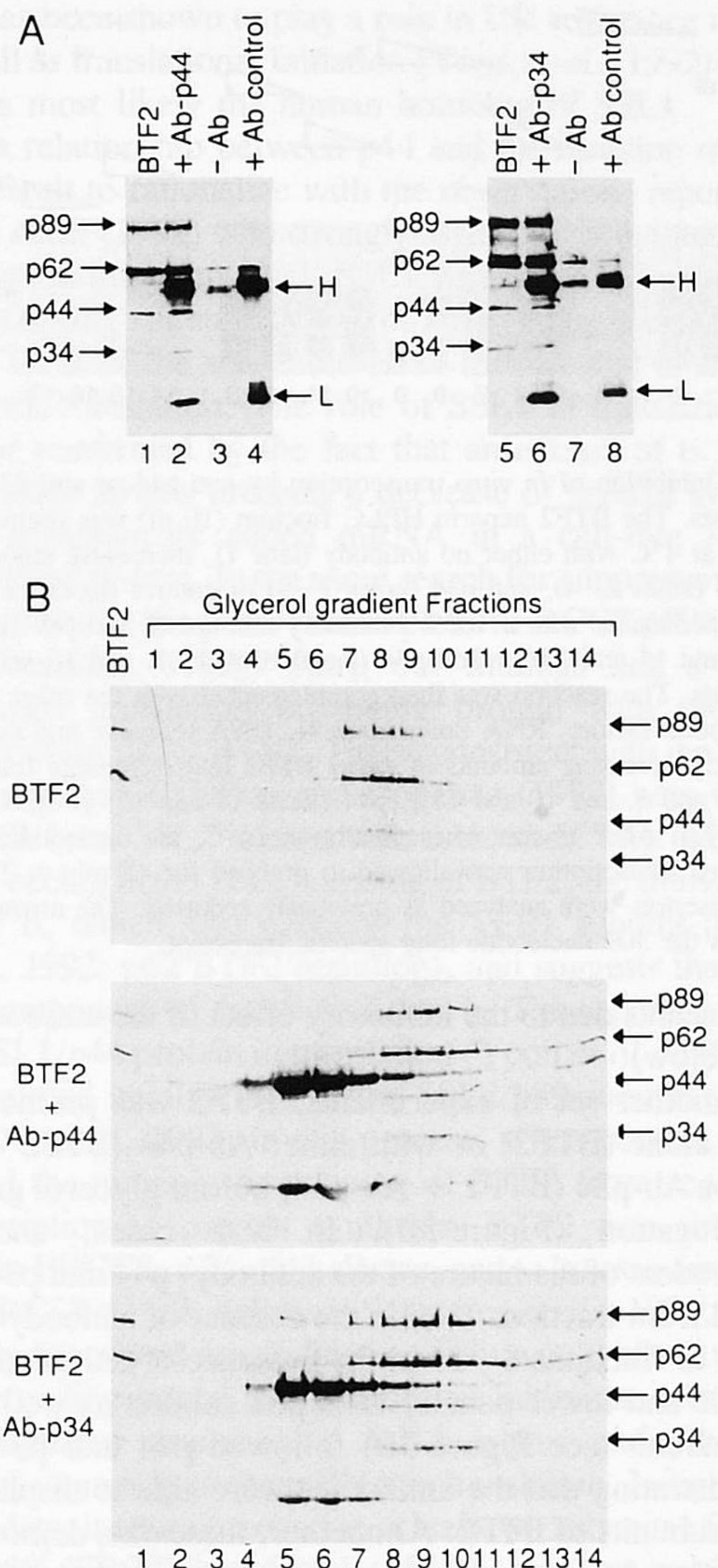
The recombinant polypeptides p44 and p34 did not substitute for the BTF2 activity when added either alone or in combination with the two other bacterially expressed BTF2 subunits (p62 and p89) in a BTF2-dependent *in vitro* transcription system (data not shown). Indeed, this was expected since our purification evidenced at least an

additional polypeptide in BTF2 (Gérard *et al.*, 1991).

Human BTF2 (TFIIFH), the rat factor  $\delta$  and the yeast b factor have been found to contain several activities including a DNA-dependent ATPase (Roy *et al.*, 1994), a CTD kinase capable of phosphorylating the CTD of the largest subunit of RNA polymerase II (Feaver *et al.*, 1991; Lu *et al.*, 1992; Serizawa *et al.*, 1992) and a helicase (Schaeffer *et al.*, 1993), thus suggesting the presence of putative helicase motifs, ATP-binding sites, or kinase motifs in the various BTF2 subunits. None of the above motifs was detected in either p44 or p34 and no kinase, ATPase or helicase activities could be found associated with p44 or p34 when tested in the three enzymatic assays under the conditions used for the native BTF2 complex (data not shown). Furthermore, no stimulation of any of these activities was observed upon addition of p44 or p34.

Despite our inability to detect any of the aforementioned biological activities associated with the two polypeptides, several lines of evidence indicate that both p44 and p34 are indeed subunits of BTF2 (TFIIFH). First, monoclonal antibodies raised against the p44 (Ab-p44) and p34 (Ab-p34) polypeptides recognized the 44 and 34 kDa polypeptides (Figure 2B, middle panel) that cofractionated with BTF2 transcription activity (upper panel) and the helicase activity (lower panel) through the HAP chromatography (sixth step of the purification; Gérard *et al.*, 1991) as well as on the glycerol gradient sedimentation step (data not shown; see also Figure 3B, upper panel). Furthermore, both p44 and p34 perfectly coelute with the previously described p62 and p89 (lanes 10–14) that have previously been shown to belong to BTF2 transcription factor (Fischer *et al.*, 1992; Schaeffer *et al.*, 1993); the CTD kinase and the DNA-dependent ATPase activities were also found to coelute with the four BTF2 subunits (unpublished results; Roy *et al.*, 1994). Identical results were also observed with the phenyl fractions (fifth step of our purification procedure; Gérard *et al.*, 1991). Thus, in the three last steps of the purification scheme (using three different separation techniques based either on the hydrophobic character, the charge or the sedimentation constant of the BTF2 complex), p44 and p34 are always found tightly associated with the various activities and polypeptides characteristic of BTF2. In addition, when the BTF2 multisubunit complex is treated with 1 M KCl before glycerol gradient sedimentation, the four polypeptides cosediment in a region corresponding to ~200–250 kDa (unpublished results).

Second, to confirm that p44 and p34 belong to the BTF2 multisubunit complex, two additional sets of experiments were performed: an immunoprecipitation (Figure 3A) and a glycerol gradient shift (Figure 3B) in which either of the two previously described monoclonal antibodies was used to precipitate or to shift all the BTF2 subunits. A partially purified BTF2 fraction eluted from the heparin column (Gérard *et al.*, 1991), was incubated either with protein A–agarose beads alone (– Ab, lanes 3 and 7) or with protein A–agarose beads to which anti-p44 (lane 2) or anti-p34 (lane 6) antibodies or an antibody against an unrelated polypeptide (Ab control, lanes 4 and 8) were bound. After extensive washing with a buffer containing 0.15 M KCl, the remaining proteins were analyzed by SDS–PAGE (Figure 3A). The anti-p44 or anti-p34 antibodies immunoprecipitate both p34 and p44 in addition to p62 and p89 (lanes 2 and 6, respectively), while the protein A–agarose, either alone



**Fig. 3.** The monoclonal antibodies raised against p44 or p34 immunoprecipitate BTF2 (A) and displace this complex in a glycerol gradient (B). (A) 50 µl of the BTF2 heparin HPLC fraction were incubated for 2 h at 4°C with either no antibody (lanes 3 and 7), anti-p44 antibody (lane 2), anti-p34 antibody (lane 6), or an unrelated control antibody (lanes 4 and 8). These mixtures were incubated with protein A–Sepharose. The beads were washed and loaded onto an SDS–polyacrylamide gel. After electrophoresis and transfer onto nitrocellulose, the various BTF2 subunits (as indicated by arrows on the left) were detected with the corresponding antibody. Lanes 1 and 5: 2 µl of BTF2 heparin HPLC fraction. (B) 100 µl of heparin HPLC BTF2 fraction were incubated for 2 h at 4°C with either no antibody (upper panel), anti-p44 antibody (middle panel) or anti-p34 antibody (lower panel). After centrifugation for 12 h at 300 000 g on a 10–30% glycerol gradient, 15 fractions were collected and analyzed by Western blotting using anti-p62, anti-p44 and anti-p34 antibodies. The arrows indicate the position of the four BTF2 subunits. The heavy (H) and light chain (L) subunits of the antibody are also illuminated by the second anti-mouse antibody.

or coupled to the control antibody, precipitated none or negligible amounts of p89, p62, p44 and p34 polypeptides (lanes 3, 4, 7 and 8). Thus, both of the antibodies raised against either p44 or p34 are able to coprecipitate p62 as well as p89 in addition to their corresponding antigenic p44 and p34 subunits. No transcription activity was recovered either from the protein A–agarose beads on which Ab-p44 or Ab-p34 were immobilized or from their corresponding

(van Vuuren *et al.*, 1994). Similar results were obtained using the anti-p44 antibody (data not shown).

## Discussion

Here, we report the cloning and characterization of two additional subunits of BTF2 that appear to be intimately associated with the p62 subunit which shares homology with the 74 kDa subunit (TFB1) of yeast transcription factor b (Gileadi *et al.*, 1992) and the p89 subunit that corresponds to the product of the *ERCC3* gene (Schaeffer *et al.*, 1993). All our data demonstrate unequivocally that both polypeptides are subunits of BTF2. First, p44 and p34 copurified not only with the other identified subunits of BTF2 (p89 and p62) but also with the previously characterized enzymatic activities of BTF2 (helicase, DNA-dependent ATPase and CTD kinase). Second, all subunits can be immunoprecipitated or shifted on a glycerol gradient with monoclonal antibodies raised against p44 or p34 (or p62; not shown). Third, the latter antibodies inhibit an *in vitro* transcription assay, and this inhibition can be relieved by addition of purified BTF2.

Preliminary experiments to reconstitute the transcription activity using the four recombinant subunits of BTF2 (p89, p62, p44 and p34) either alone or in combination were not successful. This may be due to the lack of correct folding of the protein in *E. coli*, but also, and most likely, to the absence of some other component(s) of the BTF2 factor. It also remains possible that additional polypeptides that are not strongly associated with BTF2 (three other polypeptides were found to coelute with the five polypeptides of BTF2; Fischer *et al.*, 1992) can modulate the function of the 'core' BTF2 to select its implication in either transcription or NER but it is still unclear which ones constitute the transcriptional 'core' or the repair function of BTF2. In a similar context, we also noticed that the most purified fraction of the  $\delta$  factor, the rat homolog of BTF2, contains at least seven polypeptides (Conaway and Conaway, 1989). Although we have demonstrated that the recombinant ERCC3, the p89 subunit of BTF2, contains a DNA helicase activity (Schaeffer *et al.*, 1993), we were unable to detect any enzymatic activity such an ATPase, or CTD kinase associated with p44 or p34. In fact, none of the characteristic motifs for such function were obvious when considering their amino acid sequence. Instead, both proteins contain various zinc finger motifs, such as Cys-X<sub>2</sub>-Cys-X<sub>12</sub>-His-X<sub>3</sub>-His, or Cys-X<sub>2</sub>-Cys-X<sub>10</sub>-Cys-X<sub>2</sub>-Cys, that may mediate the binding of BTF2 to the DNA template. More interesting is the observation that there is some common zinc finger to both p44 and p34 proteins that may suggest some similarity in their function. It will be interesting to determine which of the identified zinc fingers is responsible for the DNA binding and if so, if such a polypeptide displays sequence specificity.

The dual role of BTF2 in NER as well as in transcription initially suggested by the identification of ERCC3 as one subunit of BTF2, is further strengthened by the following observations. First, antibodies against p44 or p34 were able to deplete both the *in vitro* NER system and *in vitro* transcription. This strongly suggests that p44 and/or p34 are either directly involved or are associated with one or more polypeptides of the BTF2 protein complex that are absolutely required for either of the two reactions. Secondly, p44 has significant homology (58% similarity) with SSL1, a protein

that has been shown to play a role in UV resistance in yeast as well as translational initiation (Yoon *et al.*, 1992). Thus, p44 is most likely the human homolog of SSL1.

The relationship between p44 and the function of SSL1 is difficult to rationalize with the observations reported by Yoon *et al.* (1992) who strongly assert that SSL1 acts at the translation initiation level. SSL1 was identified in a search for screening for suppressors of an artificial stem-loop in the 5'UTR of the selectable HIS4 mRNA that is assumed to block translation. The role of SSL1 in translation was further confirmed by the fact that an extract of ts<sup>-</sup> SSL1 suppressor strains presents a decrease of protein synthesis from exogenously added mRNA in a cell-free *in vitro* translation system. In the same search for suppressors, they isolated SSL2, the yeast counterpart of ERCC3/p89 (Gulyas and Donahue, 1992). Thus, our findings link a second presumed translation regulating protein with the basal transcription factor BTF2. This is consistent with the genetic cross studies of Gulyas and Donahue (1992) who showed that SSL1 and SSL2 interact with each other. This interaction likely occurs in the yeast homolog of BTF2, the transcription factor b, which also contains the TFB1 subunit (Gileadi *et al.*, 1992; p62 BTF2 homolog), and suggests that SSL1 is a component of the yeast factor b. The reported role of the SSL1/p44 protein in translation (Yoon *et al.*, 1992) and the claimed involvement of the SSL2/p89 subunit (Gulyas and Donahue, 1992) in this process, when correct, would extend the multiple functioning of BTF2 from repair and transcription to protein synthesis. BTF2 was shown to contain ERCC3, a 3'-5' helicase, and to be associated with the ERCC2 5'-3' helicase (Schaeffer *et al.*, 1993, 1994). This bidirectional unwinding capacity would allow reading of the coding strand in transcription initiation, removal of the damaged sequence in DNA repair and ribosomal binding/scanning of the mRNA in translation. In light of all these data, it appears now that at least two subunits of BTF2, i.e. p89 (ERCC3) and p44 (SSL1), may be involved in several essential functions of the cell such as transcription, DNA repair and translation. The further characterization of BTF2 and the reconstitution of the whole complex will represent a major step towards the understanding of its role in eukaryotic gene expression.

## Materials and methods

### Isolation and sequence of cDNA clones

Microsequencing of the polypeptides, screening of the HeLa cDNA library constructed in the  $\lambda$ ZAPII vector (Stratagene) and sequencing of the positive clones were as described (Fischer *et al.*, 1992). The 1.2 kb p44 cDNA was generated by PCR using the following oligonucleotides: 5'-TGAAACATATGGATGAAGAACCTGTAAAGAACT-3' and 5'-GACCGGATCCTCAAACACCTGAAGGAGCTGGA-3'. The resultant PCR fragment was inserted into the *Nde*I-*Bam*HI site of pET11a (Novagene).

To subclone the p34 ORF, the pET3d plasmid (Novagene) cut by *Nco*I-*Bam*HI was ligated to an *Nco*I-*Bst*EII-*Bam*HI adaptor. The 1038 bp *Nco*I-*Bst*EII fragment containing the entire ORF of p34 was cloned in the modified pET3d digested by *Nco*I and *Bst*EII.

### Expression of recombinant p44 and p34

*E. coli* strain BL21(DE3) (Novagene) containing the pET11a-p44 or pET3d-p34 plasmids were grown in LB medium supplemented with ampicillin (100  $\mu$ g/ml) at 37°C. Cultures were induced with isopropyl- $\beta$ -D-thiogalactopyranoside (0.4 mM) at an optical density at 600 nm of 0.6. After 2 h at 37°C, the cells were collected by centrifugation and the pellet was resuspended in 50 mM Tris-HCl pH 8, 1 mM EDTA pH 8, 100 mM KCl. The cells were then frozen, thawed and lysed by sonication on ice.

Soluble and non-soluble fractions were collected after centrifugation. Renaturation of the recombinant polypeptides was performed as described (Hager and Burgess, 1980).

#### Production of antibodies

The antibodies against rp44 (1H5) and rp34 (2B1) were produced as described (Fischer *et al.*, 1992). The p44 antibody was raised to a polypeptide from amino acids 1 to 17. For p34, the entire protein that was overexpressed in *E.coli* was used. The anti-p62 and anti-p89 monoclonal antibodies were as described in Fischer *et al.* (1992) and Schaeffer *et al.* (1993) respectively. The monoclonal antibodies were purified from ascite fluids by caprylic acid and ammonium sulfate precipitations.

#### Immunoprecipitation

The BTF2 heparin fraction was incubated for 2 h at 4°C with the antibodies and then for 1 h with protein A-Sepharose FF (Pharmacia) in TG10EK150 (50 mM Tris-HCl pH 7.9, 10% glycerol, 0.1 mM EDTA, 150 mM KCl) containing 1 mg/ml BSA. Beads were washed twice with TG10EK150 containing 0.1% NP40 and 1 mg/ml BSA and once with TG10EK150 containing 0.15% NP40, and resuspended in SDS-PAGE loading buffer. After SDS-PAGE and transfer to nitrocellulose, the various polypeptides were detected with the corresponding antibodies.

#### Other techniques

The purification of BTF2 and all the basic transcription factors required for the transcription run-off assay were as previously described (Gérard *et al.*, 1991). The *in vitro* NER assay was as described (Weeda *et al.*, 1990). Mutants 27.1 (*ERCC3*<sup>-</sup>) and UV140 (*ERCC4*<sup>-</sup>) are as described by Wood and Burki (1982) and Busch *et al.* (1994) respectively.

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