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DNA Repair Helicase: A Component of BTF2 (TFIIH) Basic Transcription Factor

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The human BTF2 basic transcription factor (also called TFIIH), which is similar to the δ factor in rat and factor b in yeast, is required for class II gene transcription. A strand displacement assay was used to show that highly purified preparation of BTF2 had an adenosine triphosphate–dependent DNA helicase activity, in addition to the previously characterized carboxyl-terminal domain kinase activity. Amino acid sequence analysis of the tryptic digest generated from the 89-kilodalton subunit of BTF2 indicated that this polypeptide corresponded to the *ERCC-3* gene product, a presumed helicase implicated in the human DNA excision repair disorders xeroderma pigmentosum and Cockayne's syndrome. These findings suggest that transcription and nucleotide excision repair may share common factors and hence may be considered to be functionally related.

Transcription initiation of protein coding genes requires, in addition to RNA polymerase II (B), two sets of transcription factors that control basal and activated transcription, respectively. The basal transcription factors along with RNA polymerase II are required for most if not all promoters (*1*). They function with the minimal promoter, which may or may not contain an initiator element (*2*) in addition to a TATA box, to form a multiprotein complex that, when nucleoside triphosphates (NTPs) are added, initiate transcription. Activated transcription is dependent on regulatory proteins that, by binding to specific cis-acting DNA elements, communicate directly or indirectly with the basal transcription machinery by way of the TATA binding protein (TBP) or through TBP-associated factors (TAFs) to activate or repress RNA synthesis (*3*).

Binding of the factor (TFIID/TBP), which recognizes the TATA box to the minimal promoter is thought to be the first step in the formation of a multiprotein complex containing at least six other general transcription factors TFIIA, TFIIB, TFIIE, TFIIF, TFIIJ/TFIIG, and BTF2 (TFIIH) (*4*). Once formed on the promoter, the conversion of this preinitiation complex to an active initiation complex capable of forming the first phosphodiester bond of the RNA transcript, requires the hydrolysis of the β-γ bond of adenosine triphosphate (ATP) (*5*). In eukaryotes, RNA polymerase II transcription factors or their associated proteins consume ATP. For example, the transcription factor b from yeast (*6*), factor δ in rat (*7*), and the human counterpart BTF2/TFIIH (*8, 9*) have a DNA-dependent ATPase activity (*7*), whereas the same factors were also found to consume ATP to phosphorylate the carboxyl-terminal domain (CTD) of the largest subunit of RNA polymerase II (*10–13*). It was thus postulated that such a phosphorylation of the CTD induces the transition from initiation to elongation (*14*). However, the absolute necessity of ATP hydrolysis for initiation of transcription in RNA polymerase II–dependent systems cannot be entirely accounted for by its consumption in the phosphorylation of the CTD, which can use both ATP and guanosine triphosphate (GTP) as substrates (*12, 13*), but rather by another ATP-dependent step that must occur during or after the kinase reaction and before the formation of the first phosphodiester bond (*14*). Such an activity could reside in a factor analogous to that responsible for the melting (opening) of the duplex DNA during the formation of the open complex in prokaryotes, a step that requires ATP (*15*). In eukaryotes, an affinity column strategy based on protein-protein interactions was used to show that an ATP-dependent DNA helicase activity was associated either with the transcription factor TFIIF (also called RAP30-RAP74) or with the RNA polymerase II, or both (*16*).

We present evidence that a helicase activity is closely associated with the BTF2/TFIIH transcription factor (*8, 9*), a multisubunit protein complex, which has a CTD protein kinase activity and includes a 62-kD polypeptide (*17*). Microsequencing of tryptic digests of the largest subunit (the 89-kD polypeptide) of BTF2 resulted in a series of oligopeptides also present in the human ERCC-3 gene product, which has been shown to participate in the nucleotide excision repair process in man, drosophila, and yeast (*18–20*) and whose sequence contains motifs similar to those of RNA and DNA helicases (*21*).

Coelution of helicase, protein kinase, and BTF2 transcription activities. The purification of the basal transcription factor BTF2 was performed as described (*8*), with the last three steps of the purification scheme, including chromatography with phenyl-5PW and hydroxyapatite (HAP) and a subsequent glycer-

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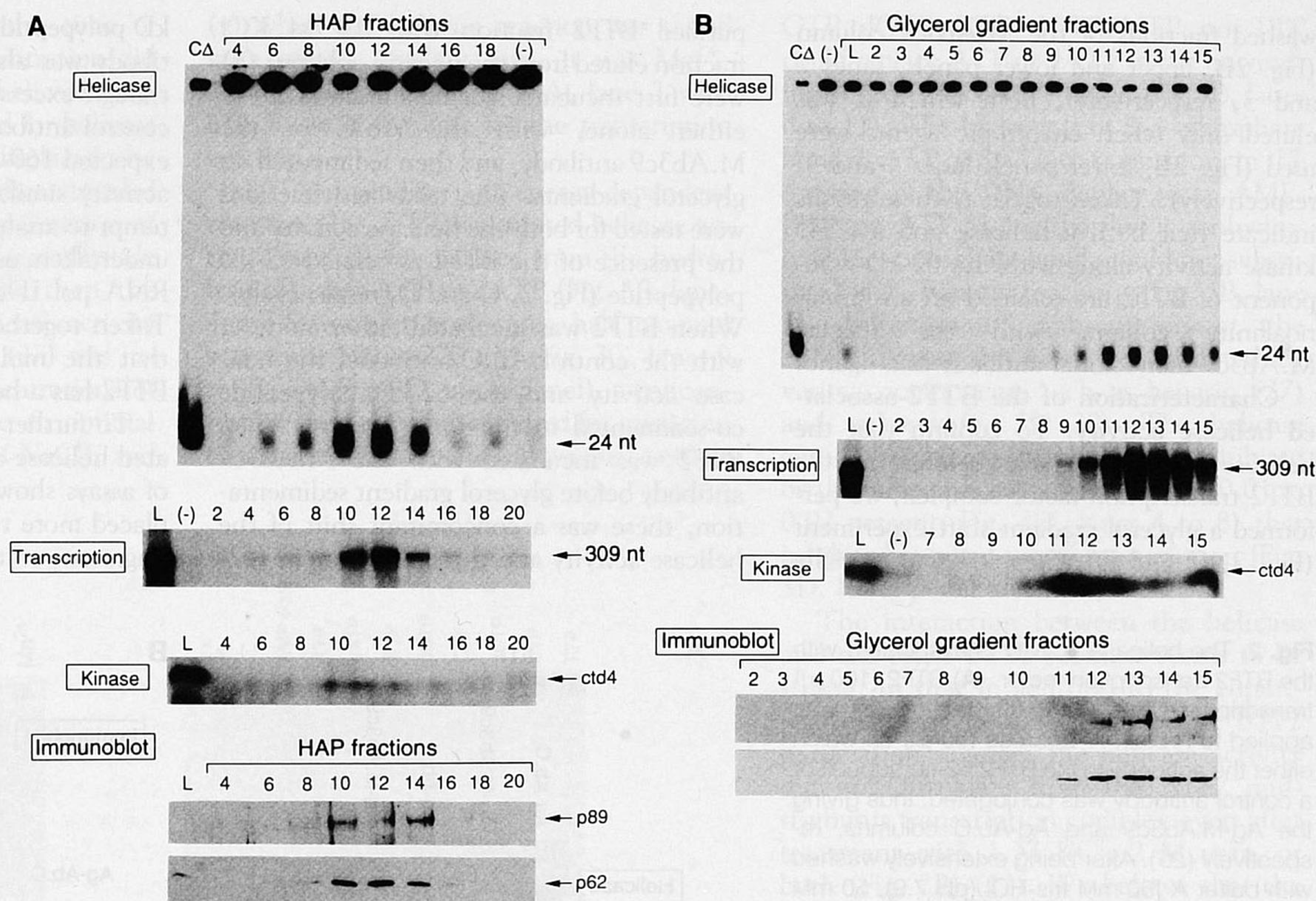

Fig. 1. The helicase activity (Helicase) coelutes with a CTD kinase activity (Kinase), the BTF2 transcription activity (Transcription), the 62-kD BTF2 subunit (p62), and the 89-kD polypeptide (p89) encoded by the *ERCC-3* gene (*21*). The BTF2 transcriptionally active fractions that were eluted from a phenyl-5PW column were successively applied on a hydroxyapatite (HAP) column (A) and a 15 to 35 percent glycerol gradient (B) as previously described (*8*). The protein fractions were tested in an in vitro helicase assay (*23*) (1 μl), in a protein kinase assay (*22*) (2 to 10 μl), in a standard runoff transcription assay lacking BTF2 (*17*) (2 to 10 μl), and by immunoblotting, with either a monoclonal antibody to the BTF2 62-kD polypeptide (*17*) (M.Ab3c9) or polyclonal antibody (329 B4) to a fragment of the 89-kD polypeptide (*25*). The fractions are indicated at the top of each panel. (**A**) (Lanes 2 to 20) Fractions eluted between 0.2 and 0.6 M phosphate from the HAP; only some of the gradient fractions are presented; (**B**) (lanes 2 to 15) fractions collected from the glycerol gradient. L, loaded material; (−), negative control; CΔ, heated DNA substrate for helicase assay. The arrows on the right indicate the displaced labeled oligonucleotide (24 nt) from the single-stranded DNA, the phosphate labeled heptarepeat (ctd4), and the RNA transcript (309 nt) in addition to the 89-kD (p89) and 62-kD (p62) polypeptides. The difference in intensities for each activity may reflect the linearity of the response, some partial degradation (artifact), the sensitivity to glycerol (samples contain 15 to 35% glycerol, final concentration), and presence of inhibitors, whereas immunoblot analysis determines the amount of polypeptide independent of the activity.

ol gradient sedimentation. The various fractions of the last two purification steps were tested (Fig. 1): (i) in an in vitro transcription runoff assay with the adenovirus major late promoter (Ad2MLP), RNA polymerase II, and all the general transcription factors with the exception of BTF2 (*17*); (ii) in a protein kinase assay in which a synthetic heptapeptide (ctd4) designed to mimic the CTD of the largest subunit of RNA polymerase II was used as a substrate (*22*); and (iii) in an in vitro helicase assay that detected the displacement of a labeled DNA fragment (24 nucleotides) from a single-stranded circular DNA molecule to which a labeled fragment had been annealed (*23, 24*). Immunoblotting procedures were used to probe the various protein fractions either with the monoclonal antibody M.Ab3c9, which recognizes the 62-kD polypeptide component of the BTF2 factor (*17*), or with polyclonal antibodies directed toward the ERCC-3 89-kD polypeptide [(*21, 25*) and as shown below]. A helicase activity that displaces the 3′ end-labeled 24-nucleotide fragment from the single-stranded M13mp18 DNA template, cofractionates with the BTF2 transcription activity, the corresponding CTD kinase activity, and the BTF2 protein (as judged from the 62-kD subunit) throughout the phenyl-5PW chromatography, HAP chromatography (Fig. 1A, fractions 10 to 14), and glycerol gradient centrifugation (Fig. 1B, fractions 11 to 15). The helicase activity cosedimented with BTF2-associated CTD kinase activity, BTF2 transcription activity, and the BTF2 protein (Fig. 1B) even after treatment with 1 M KCl (*11*).

An immunoaffinity chromatography was used to demonstrate that the helicase activity belongs to the BTF2 transcription factor complex (*26*). A partially purified BTF2 fraction eluted from a hydroxyapatite column (*8*) was loaded on either an agarose-M.Ab3c9 affinity column [which retains the native BTF2 factor (*17*)] or an agarose-Ab.C column, which served as a control (Fig. 2, A and B). After being washed with ten (column) volumes of adsorbtion buffer, the proteins were sequentially eluted with the same buffer supplemented with either 1 M KCl, 2 M urea or 1 M KCl, 4 M urea, and 0.1 percent NP-40. Almost all of the helicase activity (Fig. 2A, lane 1) and the transcription activity was recovered in the flowthrough of the control column, whereas essentially all of the helicase activity was retained on the specific agarose-M.Ab3c9 column (Fig. 2A, lane 2). Severe elution conditions are required to elute the BTF2 factor from the high affinity agarose-M.Ab3c9 column [either acidic pH (*17*) or high urea and salt concentration (*11*)]. Not surprisingly, these conditions were detrimental for the biological activity of BTF2. Since M.Ab3c9 did not inhibit the helicase activity or the phosphorylation of the ctd4 substrate in solution, we tested both the helicase and the CTD kinase activities of the BTF2 protein complex after immunoadsorption on the agarose-antibody columns prior to elution with urea (Fig. 2A). After the columns were washed with 20 (column) volumes of 1 M KCl buffer, the agarose beads recovered from the specific M.Ab3c9 column were not only capable of phosphorylating the ctd4 oligopeptide in the kinase assay, but were also able to displace the labeled oligonucleotide from the single-stranded circular DNA template (Fig. 2A, lane 4). Neither activity could be detected with the agarose beads taken from the control column (Fig. 2A, lane 3). The eluted affinity column fractions were also analyzed by protein immunoblotting (Fig. 2B). The 62-kD BTF2 subunit was present in the flowthrough fraction of the control (Ag-Ab.C) column, but was not detectable in the flowthrough or the

washed fractions of the M.Ab3c9 column (Fig. 2B, upper and lower panels, lanes 2 and 3, respectively), from which it was eluted only when chaotropic agents were used (Fig. 2B, lower panel, lanes 4 and 5, respectively). Taken together, these results indicate that both a helicase and a CTD kinase activity along with the 62-kD component of BTF2 are retained on an immunoaffinity column with the specific M.Ab3c9 monoclonal antibody as a ligand.

Characterization of the BTF2-associated helicase activity. To confirm that the helicase activity actually belongs to the BTF2 transcription factor complex, we performed a glycerol gradient shift experiment (Fig. 2, C and D). Samples of a partially purified BTF2 fraction (the 0.4 M KCl fraction eluted from the heparin column) (*8*) were first incubated for 30 minutes at 4°C either alone, with the Ab.C or the M.Ab3c9 antibody, and then sedimented on glycerol gradients. The recovered fractions were tested for both the helicase activity and the presence of the BTF2-associated 62-kD polypeptide (Fig. 2, C and D, respectively). When BTF2 was incubated either alone or with the control Ab.C antibody, the helicase activity and the 62-kD polypeptide co-sedimented in fractions 7 to 9. When BTF2 was incubated with the M.Ab3c9 antibody before glycerol gradient sedimentation, there was a concomitant shift of the helicase activity and the BTF2 protein (62-kD polypeptide). The specific M.Ab3c9 antibody was also shifted to some degree, although excess free M.Ab3c9 as well as the control antibody (Ab.C) sedimented in the expected 160-kD fraction. The CTD kinase activity similarly was shifted (*11*). No attempt to analyze transcriptional activity was undertaken, as M.Ab3c9 is known to inhibit RNA pol II–dependent transcription (*17*). Taken together, these results demonstrated that the multisubunit transcription factor BTF2 has a helicase activity.

To further characterize the BTF2-associated helicase activity, we performed a series of assays showing that: (i) the helicase displaced more than 80 percent of the labeled fragment at the highest concentration of

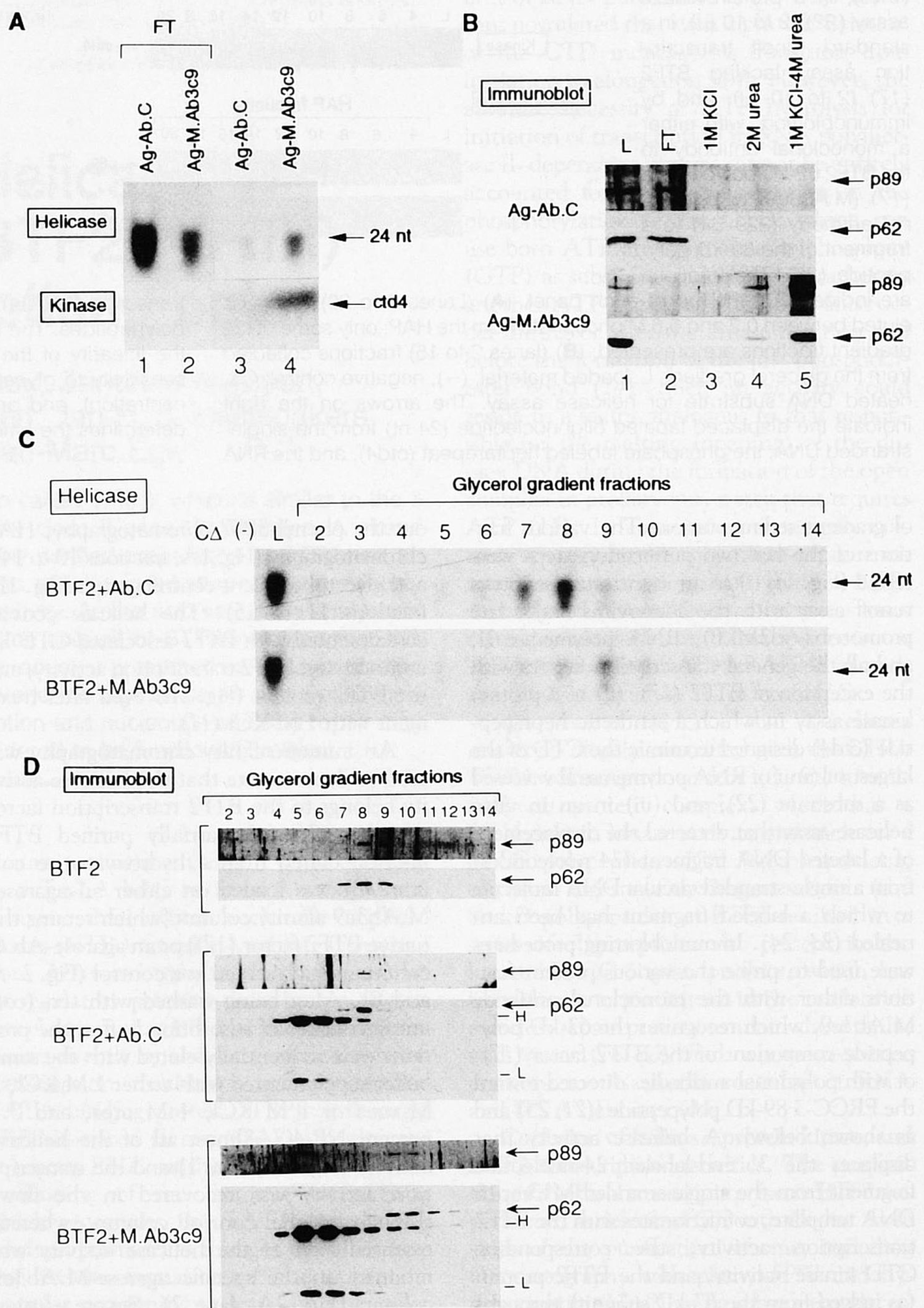

Fig. 2. The helicase activity is associated with the BTF2 transcription factor. (**A**) BTF2 (100 μl) transcriptionally active HAP fraction (*8*) was applied to Protein A–agarose (50 μl) on which either the antibody to the BTF2 62-kD subunit or a control antibody was conjugated, thus giving the Ag-M.Ab3c9 and Ag-Ab.C columns, respectively (*25*). After being extensively washed with buffer A [50 mM tris-HCl (pH 7.9), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 10 percent glycerol), part of the washed beads (10 μl) were tested either in an in vitro helicase assay (Helicase) or in a protein kinase assay (Kinase) (A, lanes 3 and 4), whereas the flowthrough of both columns were tested only for their helicase content (A, lanes 1 and 2). (**B**) Samples (3 ml) of BTF2 transcriptionally active heparin 5PW fractions (*8*) were applied to the same columns and then sequentially washed with ten volumes of buffer A, either alone or supplemented with 1 M KCl, 0.01 percent NP-40 (lane 3), 2 M urea, 0.01 percent NP-40 (lane 4), and 4 M urea, 1 M KCl, 0.1 percent NP-40 (lane 5). The load (L, lane 1), the flowthrough (FT, lane 2), as well as the eluted fractions from both columns were tested by immunoblotting for the presence of the 89-kD and 62-kD polypeptides (*17, 25*). (**C** and **D**) BTF2 transcriptionally active heparin 5PW fractions (*8*) were incubated either alone (BTF2), with Ab.C as a control (BTF2 + Ab.C), or with the M.Ab3c9 antibody (BTF2 + M.Ab3c9), before sedimentation through a glycerol gradient (10 to 30 percent glycerol, SW 60, 60,000 rpm, during 6 hours at 4°C). The collected fractions were tested (C) in an in vitro helicase assay (1 μl, Helicase) and (D) by protein immunoblotting (20 μl, Immunoblot) with M.Ab3c9 and 329 B4 antibodies for the presence of p62 and p89. Glycerol gradients collected fractions (tubes 2 to 14); L, load; CΔ, heated substrate; (−), negative control. The 89-kD and the 62-kD polypeptides and the heavy (H) and light (L) chains of the immunoglobulins are indicated by arrows.

enzyme used; (ii) increasing the time of incubation or the BTF2 concentration did not increase the extent of the helicase reaction; (iii) as previously observed for helicase II (*24*), the BTF2 helicase displaced the labeled oligonucleotide from the annealed single-strand circular DNA as a function of its size, with the shorter oligonucleotide being displaced more efficiently than the longer one (*11*); (iv) the helicase activity like the transcription reaction (*27*) was inhibited by NaCl or KCl concentrations of 200 mM (Fig. 3A, lanes 4 to 6; optimal activity occurred at 0 to 50 mM NaCl); and (v) when the helicase reaction was tested with several divalent ions, it was Mg^{2+}-dependent (compare Fig. 3A, lane 3 with lane 8) as is the case for the transcription reaction (*27*).

DNA unwinding is an energy-dependent reaction (*28*). BTF2-associated helicase activity used energy derived from the hydrolysis of either ATP or dATP (Fig. 3B, lanes 4 and 5) with, in both cases, half maximal activity at 150 to 200 μM (Fig. 3C, lanes 9 to 13, and Fig. 3D, upper panel), a concentration similar to that required for initiation of transcription (*5, 29*). However, neither GTP, dGTP, CTP, dCTP, UTP, nor TTP could replace ATP or dATP in the unwinding of the DNA double helix (Fig. 3B, lanes 6 to 11). The hydrolysis of β-γ-phosphate bond of ATP or dATP was obligatory in the opening of the DNA duplex since AMP-PNP, an ATP analog that has a nonhydrolyzable γ-phosphate bond, could not substitute for ATP in our reactions (Fig. 3B, lane 3). Initiation of transcription at the Ad2MLP was shown to be inhibited by various components such as heparin (*27*) and sarkosyl (*5, 29, 30*). The helicase activity associated with BTF2 was inhibited by the same sarkosyl concentration (0.01 to 0.02 percent; Fig. 3C, lanes 4 to 8) that inhibited in vitro runoff transcription [Fig. 3D, lower panel and (*30*)].

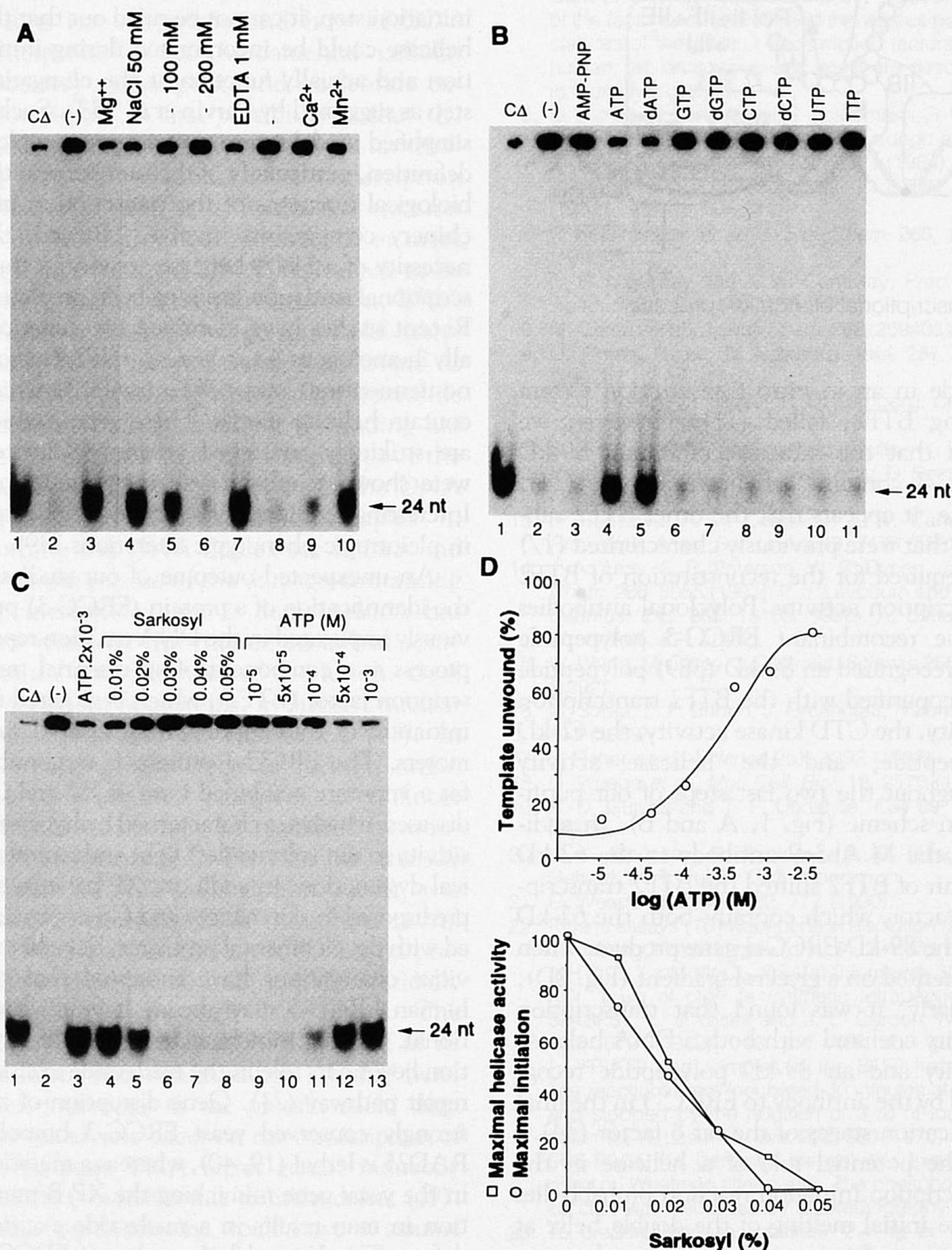

Fig. 3. Characterization of the BTF2-associated helicase. The effect of (**A**) increasing salt concentration, divalent ion (5 mM), (**B** and **C**) nucleoside triphosphate, and (C) sarkosyl of BTF2-associated helicase was tested (as indicated at the top of each panel) with BTF2 transcriptionally active hydroxyapatite fractions (*8*) in a strand displacement assay (*23*) as indicated at the top of (A), (B), and (C). Incubations were performed as described (*24*). Densitometer quantitation of the ATP requirement (**D**, upper part) and sarkosyl sensitivity (D, lower part) of the DNA helicase as shown in (B) and (C) are plotted in (D). The data presented in the lower part of (D), on the effect of sarkosyl on initiation at the Ad2MLP, are taken from Hawley and Roeder (*30*). This curve is very similar to that one presented by Sopta *et al.* (*16*). CΔ, heated substrate; (–), negative control; 24 nt, labeled strand.

The interaction between the helicase and the other BTF2 subunits seems to be strong, in that in addition to the copurification throughout six steps of the BTF2 purification scheme, the helicase activity did not dissociate from the BTF2 multisubunit transcription complex even after treatment with 1 M KCl, 2 M urea, or both (Fig. 2B) (*11*). We believe that the helicase activity is an intrinsic part of the BTF2 transcription factor, and not a component of the TFIIF transcription factor (*16*) for the following reasons: (i) the 89-kD polypeptide does not correspond to either one of the two subunits of the TFIIF (RAP30 and RAP74) and (ii) BTF2 and TFIIF are resolved on phenyl-5PW column (*17*). Neither RAP30 nor RAP74, which comprise the transcription factor TFIIF, could be detected in our purest preparation of BTF2 as evidenced by immunoblotting procedures with the two corresponding antibodies. Finally, neither of the two recombinant TFIIF polypeptides contain characteristic DNA or RNA helicase motifs nor have they been shown to exhibit, either alone or in combination, a DNA unwinding activity (*11, 16, 31*). However, an RNA polymerase-associated protein fraction, eluted from the RNA polymerase affinity column, has been shown to have a helicase activity and was found to contain, in addition to TFIIF, another polypeptide with a molecular mass of approximately 90 kD (*16*). The presence of such a helicase activity in the protein fraction eluted from the RNA polymerase column is not surprising because the BTF2 transcription factor interacts with both RNA polymerase II and TFIIF in solution (*8*) and utilizes RNA polymerase II as a substrate for phosphorylation (*11, 12*).

Presence of the repair protein ERCC-3, a presumed helicase, in the BTF2 complex. The purified BTF2 fraction obtained from either the HAP or the specific immunoaffinity column (*8, 26*) was resolved by SDS-PAGE, transferred onto a mem-

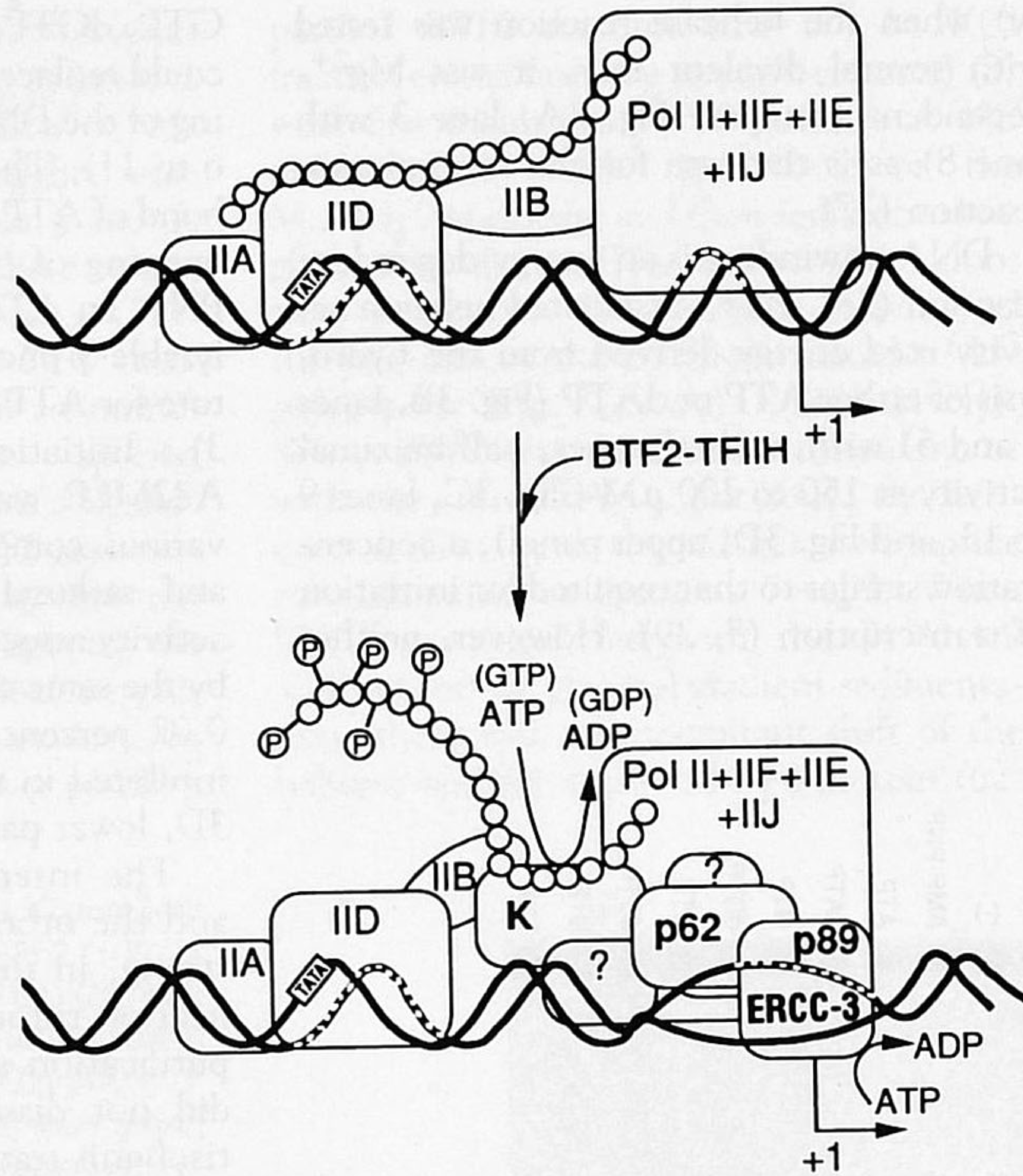

Fig. 4. A model illustrating the transition from a closed preinitiation complex to an open elongation-competent complex. RNA polymerase II (Pol II), containing a nonphosphorylated CTD, and all the basal transcription factors with the exception of the BTF2, including IIA, IIB, IID, IIE, IIF, and IIJ, are assembled into a closed complex on the promoter (upper part of the figure). Incorporation of the multisubunit transcription factor BTF2 (containing the p62 polypeptide) into the complex results in the melting of the DNA duplex at the start site due to the intrinsic DNA helicase activity, which appears to reside with its 89-kD component (p89), while CTD phosphorylation is probably carried out by one of the other BTF2 subunits (K, kinase). The resulting open, elongation-competent complex (lower part of the figure) can then dissociate from the promoter and enter the transcriptional elongation process.

brane, and stained with Coomassie blue; the region containing the 89-kD polypeptide was used to generate tryptic peptides, which were microsequenced (*17*). Our homology searches with the amino acid sequences of six peptides (Swiss-Prot data bank) identified the 89-kD polypeptide as the *ERCC-3* gene product (*21*). The deduced amino acid sequence of the *ERCC-3* cDNA (accession number M 31899) predicts a polypeptide of 782 amino acids (89,274 daltons). The *ERCC-3* gene encodes a presumed DNA helicase implicated in an early step of DNA excision repair process. ERCC-3 deficiencies in rodents result in phenotypes that closely resemble those expressed in patients with the human syndrome xeroderma pigmentosum (XP) and sometimes in Cockayne syndrome (CS). Functional domains within the nucleotide sequence included the following regions of interest (*21*, including references): (i) a potential nuclear localization signal (NLS); (ii) stretches of acidic domains typical to proteins that associate with chromatin (such as nucleolin) and high mobility group or transcription factors; (iii) a potential helix-turn-helix motif which may be involved in DNA binding; (iv) potential nucleoside triphosphate and a Mg^{2+} binding sites suggesting the possibility of ATPase activity; and finally (v) several amino acid stretches that correspond to consensus regions which are highly conserved between families of DNA and RNA helicases (*32*).

Attempts to reconstitute the BTF2 transcription activity with the recombinant 89-kD ERCC-3 (*33*), either alone or in combination with the 62-kD recombinant polypeptide in an in vitro transcription system lacking BTF2, failed (*11*). However, we found that the intact recombinant 89-kD ERCC-3 contains a helicase activity (*11*). Hence, it appears that the other BTF2 subunits that were previously characterized (*17*) are required for the reconstitution of BTF2 transcription activity. Polyclonal antibodies to the recombinant ERCC-3 polypeptide (*25*) recognized an 89-kD (p89) polypeptide that copurified with the BTF2 transcription activity, the CTD kinase activity, the 62-kD polypeptide, and the helicase activity throughout the two last steps of our purification scheme (Fig. 1, A and B). In addition, the M.Ab3c9 antibody to the 62-kD subunit of BTF2 shifted the BTF2 transcription factor, which contains both the 62-kD and the 89-kD *ERCC-3* gene product, when sedimented on a glycerol gradient (Fig. 2D). Similarly, it was found that transcription activity coeluted with both a DNA helicase activity and an 89-kD polypeptide recognized by the antibody to ERCC-3 in the final purification stages of the rat δ factor (*34*).

The potential role of a helicase in the transcription initiation reaction probably lies in the initial melting of the double helix at the onset of transcription. Although many details remain to be established, the finding of a BTF2-associated helicase activity sheds some light on the events that occur during or just before the transcription initiation step and can be briefly summarized as follows (Fig. 4). The TATA-box region is first targeted by TBP-TFIID which causes some conformational stress on the TATA region resulting in the bending of the DNA (*35*). Whether or not TFIIA is required for the stabilization or the activation of TFIID (or both) is still unclear (*1*). TFIIB then binds to TFIID (*4*, *36*). Assuming that RNA polymerase II is first recruited by TFIIF (*37*) to the DAB complex (previously defined as the result of the interaction between TFIID, TFIIA, and TFIIB) and is later joined by BTF2-TFIIH (*12*), it is possible that once incorporated in the complex, the BTF2-associated helicase can melt the DNA template, possibly in conjunction with the previously bent promoter, to facilitate the onset of the transcription reaction. Although the nucleotide specificity and the inhibition curves makes likely the potential involvement of the ERCC-3 or BTF2 in the initiation step, it cannot be ruled out that the helicase could be incorporated during initiation and actually function at the elongation step as suggested by Parvin *et al.* (*38*). Such a simplified model must await a more complete definition, particularly of the number and the biological functions of the transcription machinery components involved. Indeed, the necessity of a DNA helicase activity in transcriptional initiation has long been predicted. Recent studies have identified two functionally homologous yeast genes, SNF2 (sucrose nonfermenting) and SWI2 (switch), which contain helicase motifs. These genes, which are strikingly conserved among eukaryotes, were shown to play a role in transcription. Interestingly, mutations of these genes result in pleiotropic phenotypic alterations (*39*).

An unexpected outcome of our studies is the identification of a protein (ERCC-3) previously implicated in the DNA excision repair process as a component of an essential transcription factor (BTF2), which is required for initiation of transcription from class II promoters. The ERCC-3 protein is responsible for a very rare combined form of XP and CS diseases, which are characterized by hypersensitivity to sun (ultraviolet) light and neurological dysfunction. In addition, XP patients are predisposed to skin cancer and CS is associated with developmental problems. Several previous observations have suggested that the human ERCC-3 may already have an additional, although uncharacterized, vital function beyond its role in the nucleotide excision repair pathway (*21*). Gene disruption of the strongly conserved yeast ERCC-3 homolog RAD25 is lethal (*19*, *40*), whereas a mutation in the yeast gene mimicking the XP-B mutation in man results in a nucleotide excision defect. The *Drosophila* homolog of ERCC-3 appears also to play a vital function in addition to a role in DNA repair (*20*). This, in addition to our present results, might also pertain to two surprising recent findings related to ERCC-3 function. First, Gulyas and Donahue (*19*) identified *ERCC-3* in yeast as a potential suppressor that alleviates a block of expression of the His gene caused by an artificial strong stem-loop structure in its

5′ UTR, which hampers translation. In the case of this gene, the rate of transcription is also affected. Second, Mounkes *et al.* (*20*) uncovered *ERCC-3* as the gene responsible for "haywire" mutations in the *Drosophila* resulting in a reduction of the B2t tubulin gene expression that is required for normal spermatogenesis. In view of our findings, it is likely that alterations in the *ERCC-3* gene in both systems affect the transcription of the respective genes. Finally, a role of ERCC-3 in transcription, in addition to its involvement in the nucleotide excision repair process, may account for some of the clinical symptoms of XP-B, such as the severe growth defect (dwarfism and microcephaly).

It is not clear why and how ERCC-3 participates in two fundamental cellular processes, transcription and nucleotide excision repair. A first possibility is that the ERCC-3 protein catalyzes a DNA unwinding step in the context of transcription initiation as well as in nucleotide excision repair. Both processes require one or more stages in which the DNA duplex has to be melted. For transcription initiation, DNA melting is essential to permit RNA polymerase II to use the transcribed strand as template for RNA synthesis. For nucleotide excision repair, local DNA unwinding has been implicated in at least two steps of the reaction in *E. coli* (i) as part of the DNA translocation of the uvrAB complex in scanning the DNA duplex for lesions and (ii) in the release of the damage-containing oligonucleotide after a dual incision has been made in the injured strand at some distance on both sides of the lesion. Dual involvement of proteins, in distinct nuclear processes, is not unprecedented (*41*). In eukaryotic genes, DNA replication origin frequently contains promoter or enhancer elements which may be regulated by transcription factors (*42*). For example, the site specific CAAT transcription factor was shown to stimulate both transcription and DNA replication initiation. A second possibility raised by our finding is theoretically also tenable although less likely. The ERCC-3 function could be restricted to its role in transcription initiation revealed here. Some mutations in the protein could then have an adverse effect on the transcription of one or more DNA repair genes, indirectly resulting in a nucleotide excision defect (*19*). In this hypothesis one would have to assume that the strict dependence of such a repair gene on optimal functioning of ERCC-3 has been preserved in eukaryotic evolution from yeast to *Drosophila* and to man.

Future studies should discriminate between these and other possible alternatives and should further define the role (or roles) of BTF2 and ERCC-3 proteins in DNA transactions, in particular whether the whole BTF2 protein complex is involved in both initiation of transcription and DNA repair.

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22. Kinase assays (*13*) were done in a solution (20 μl) containing 20 mM Hepes (pH 7.9), 20 mM tris-HCl (pH 7.9), 7 mM $MgCl_2$, BSA at 0.5 mg/ml, 30 mM KCl, 1 μg of ctd4 [a synthetic tetrapeptide of SPTSPSY; L. J. Cisek and J. L. Corden, *Nature* **339**, 679 (1989)], 10 μM ATP, 2.5 μCi of [γ-^{32}P]ATP and samples of the BTF2 fractions. The reactions were incubated 30 minutes at 28°C and stopped by the addition of 3 μl of loading buffer (U. K. Laemmli, *ibid.* **227**, 680 (1970). After SDS-PAGE (15 percent), the gel was fixed and dried on Whatman filter paper. The phosphorylated ctd4 was visualized by autoradiography.
23. To prepare helicase substrate, 2.4 ng of 17-bp universal primer was annealed to 1 μg of single-stranded M13mp18 DNA in 5 mM tris-HCl (pH 7.9), 25 mM NaCl, 2.5 mM $MgCl_2$, by incubation at 95°C for 5 minutes and then for 30 minutes at 24°C. The primer was then elongated with DNA polymerase I–Klenow fragment (5 units) in the presence of 50 μM dGTP and dATP, 5 μCi [α-^{32}P]dCTP (3000 Ci/mmol) (Amersham), 4 mM $MgCl_2$. After 20 minutes at 23°C, 50 μM dCTP was added to the reaction mixture, which was then incubated for another 20 minutes at 23°C. After extraction with phenol and chloroform, the free nucleotides were removed by gel filtration on Sephadex G50 (Pharmacia, Sweden). The DNA helicase assay (25 μl) contained 20 mM tris-HCl (pH 7.9), 4 mM $MgCl_2$, 1 mM dithiothreitol (DTT), BSA at 50 μg/ml, 2 mM ATP, and 1 to 3 ng of DNA substrate. Reactions were incubated at 37°C for 45 minutes and stopped by the addition of 10 μl of 60 mM EDTA, 50 percent glycerol, 0.75 percent SDS, and 0.1 percent bromophenol blue; the reaction was then subjected to 10 percent polyacrylamide gel in 0.5× TBE (tris, borate, EDTA) buffer (pH 8) at 100 to 200 V. The gel was dried and analyzed by autoradiography.
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25. The rabbit polyclonal antibody 329 B4 was raised to a part of the ERCC-3 polypeptide from amino acids 126 to 314 that was overexpressed in *E. coli* as a fusion protein with Protein A in pRIT2T vector (Pharmacia).
26. Protein A–Sepharose F.F. (Pharmacia) was equilibrated in 50 mM sodium borate buffer (pH 9) containing 3 M NaCl. Monoclonal antibodies to the p62 subunit of BTF2 (M.Ab3c9) (*17*) or the F region of the retinoic acid receptor gamma (Ab.C) [C. Rochette-Egly *et al.*, *J. Cell Biol.* **115**, 535 (1991)] were covalently cross-linked to the Protein A with dimethyl pimelimidate [E. Harlow and D. Lane, *Antibodies, a Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988)].
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