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The ERCC2/DNA repair protein is associated with the class II BTF2/TFIIH transcription factor

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ERCC2 is involved in the DNA repair syndrome xeroderma pigmentosum (XP) group D and was found to copurify with the RNA polymerase II (B) transcription factor BTF2/TFIIH that possesses a bidirectional helicase activity. Antibodies directed towards the 89 kDa (ERCC3) or the p62 subunit of BTF2 are able to either immunoprecipitate ERCC2 or shift the polypeptide in a glycerol gradient. Conversely, an antibody directed towards ERCC2 also retains or shifts BTF2. ERCC2 could be resolved from the other characterized components of BTF2 upon salt treatment, while its readdition enhanced BTF2 transcription activity. ERCC2, ERCC3 and p44 are three repair proteins found in association with BTF2. Two of them, ERCC2 and ERCC3, are responsible for atypical forms of XP disorders which confer a high predisposition to skin cancer. This includes clinical features that lack an adequate rationalization on the basis of nucleotide excision repair (NER) deficiency but which may now be explained better in terms of a partial transcription deficiency.

Key words: DNA repair protein/ERCC2/transcription factor/ xeroderma pigmentosum group D

Introduction

The nucleotide excision repair (NER) pathway is one of the principal pathways in the cell for eliminating a wide variety of unrelated lesions in a complex multi-step reaction. Because of the lesion-producing effects of genotoxin or UV light-induced DNA damage on the vital transcription process, their removal by NER must occur in a fashion closely coordinated with transcription. It has been shown that during transcription an RNA polymerase complex stalled in front of a lesion is displaced to give repair enzymes access to the injury (Hanawalt and Mellon, 1993). Two direct but distinct connections between NER and transcription have emerged from recent studies. In *Escherichia coli* it was shown that the protein encoded by *mfd* (mutation frequency decline) functions in DNA repair and in transcription by recognizing and displacing RNA polymerase that is stalled at DNA lesions (Selby and Sancar, 1993). Recently, we have shown that the largest subunit (89 kDa) of the human transcription factor BTF2/TFIIH is identical to the ERCC3 helicase known to be involved in the DNA repair mechanism (Schaeffer *et al.*, 1993; van Vuuren *et al.*, 1994). Mutations in this gene (Weeda *et al.*, 1990) confer UV light sensitivity and predisposition to skin cancers associated with a rare form of the DNA repair syndrome xeroderma pigmentosum group B, which exhibits all the clinical hallmarks of another DNA repair disorder, Cockayne's syndrome (Robbins *et al.*, 1974). Altogether, these data imply that a subset of proteins may play a pivotal role in two different processes: transcription and repair.

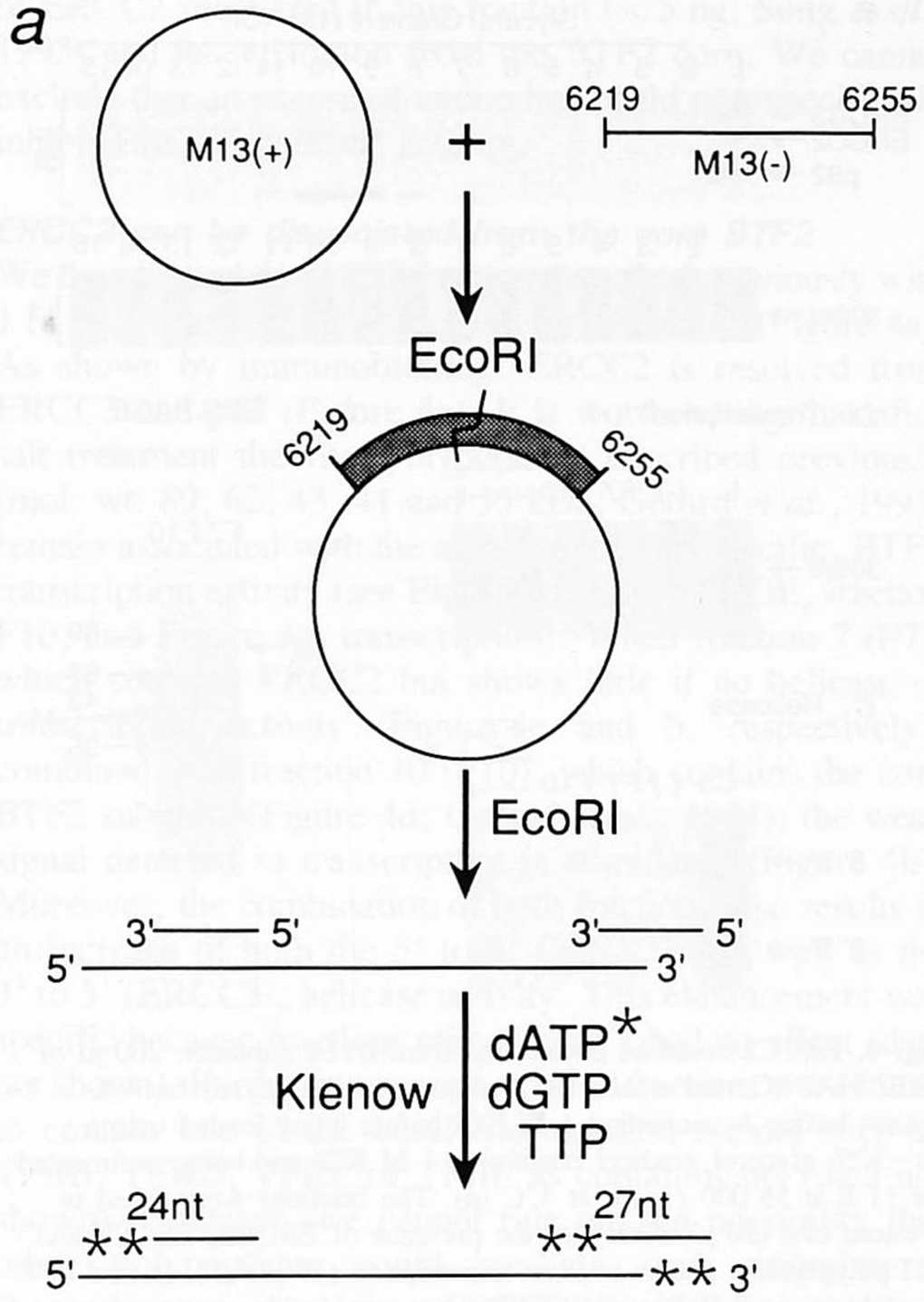
Four subunits, p89/ERCC3, p62, p44 and p34, were already shown to belong to BTF2 (Fisher *et al.*, 1992; Schaeffer *et al.*, 1993; Humbert *et al.*, 1994). In this report we identify an 80 kDa polypeptide, ERCC2, which is strongly associated with BTF2 and participates in both transcription and DNA repair.

Results

Cofractionation of two helicase activities with BTF2

To investigate further the putative pivotal role of BTF2 in transcription and repair, we characterized the BTF2 factor components. ENDFLTFDAMR and DQFQIR oligopeptides, resulting from trypsin hydrolysis of the 80 kDa polypeptide present in a highly purified BTF2 fraction, perfectly matched with the ERCC2 gene product (Weber *et al.*, 1990) also involved in the DNA repair process. The presence of ERCC2 was examined throughout the last three steps of purification of the BTF2 transcription factor [phenyl-5PW, hydroxy-apatite chromatography (HAP) and subsequent glycerol gradient sedimentation; Gerard *et al.*, 1991]. The various fractions were tested by immunoblotting using antibodies towards ERCC3 (AbERCC3), ERCC2 (AbERCC2; see legend to Figure 2) and p62 (Ab3c9; Fisher *et al.*, 1992) in an *in vitro* transcription run-off assay lacking BTF2 (Fisher *et al.*, 1992), and in a bidirectional helicase assay (Figure 1). Recombinant ERCC3 (Roy *et al.*, 1994) catalysed the displacement of the 24 nucleotide (nt) fragment from the linearized substrate (Figure 1b, compare lanes 2–4). However, when the BTF2 HAP fraction (Gerard *et al.*, 1991) is incubated with the double-labelled probe, both the 24 and 27 nt fragments are displaced (lanes 5 and 6), thus suggesting the presence of at least two helicase activities that translocate in opposite directions: a 3′ to 5′ helicase attributed to ERCC3 and a 5′ to 3′ helicase corresponding to ERCC2 (Sung *et al.*, 1993).

The p62 and ERCC3 (89 kDa) subunits of BTF2 (Schaeffer *et al.*, 1993), as well as ERCC2 as detected by immunoblotting, cofractionated with two helicase activities throughout the phenyl-5PW, HAP and subsequent glycerol gradient (Figure 2a, fractions 16–22; b, fractions 9–16;



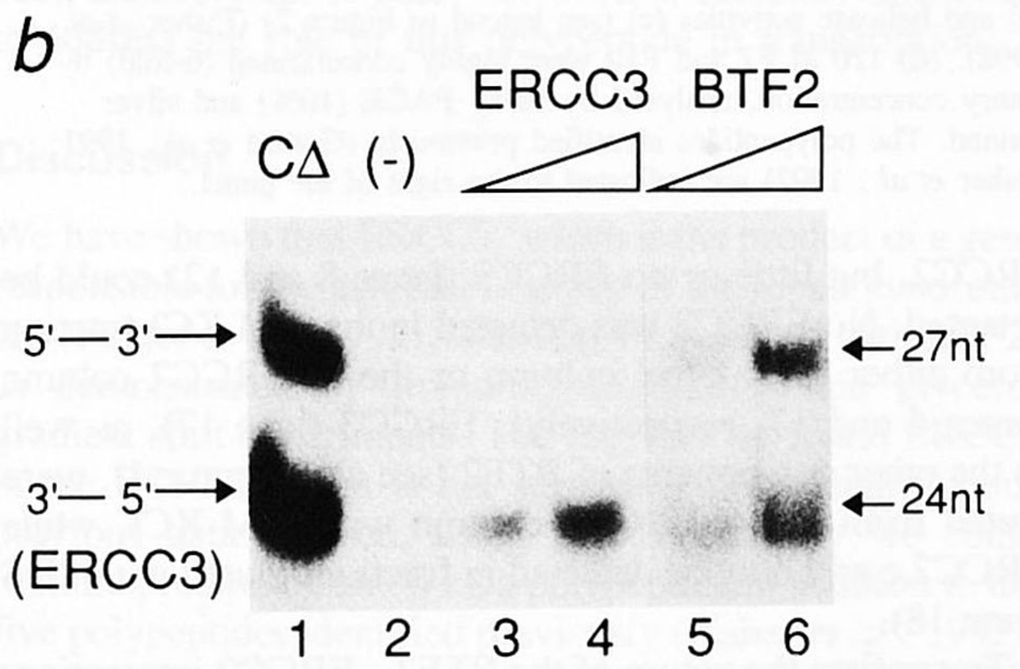
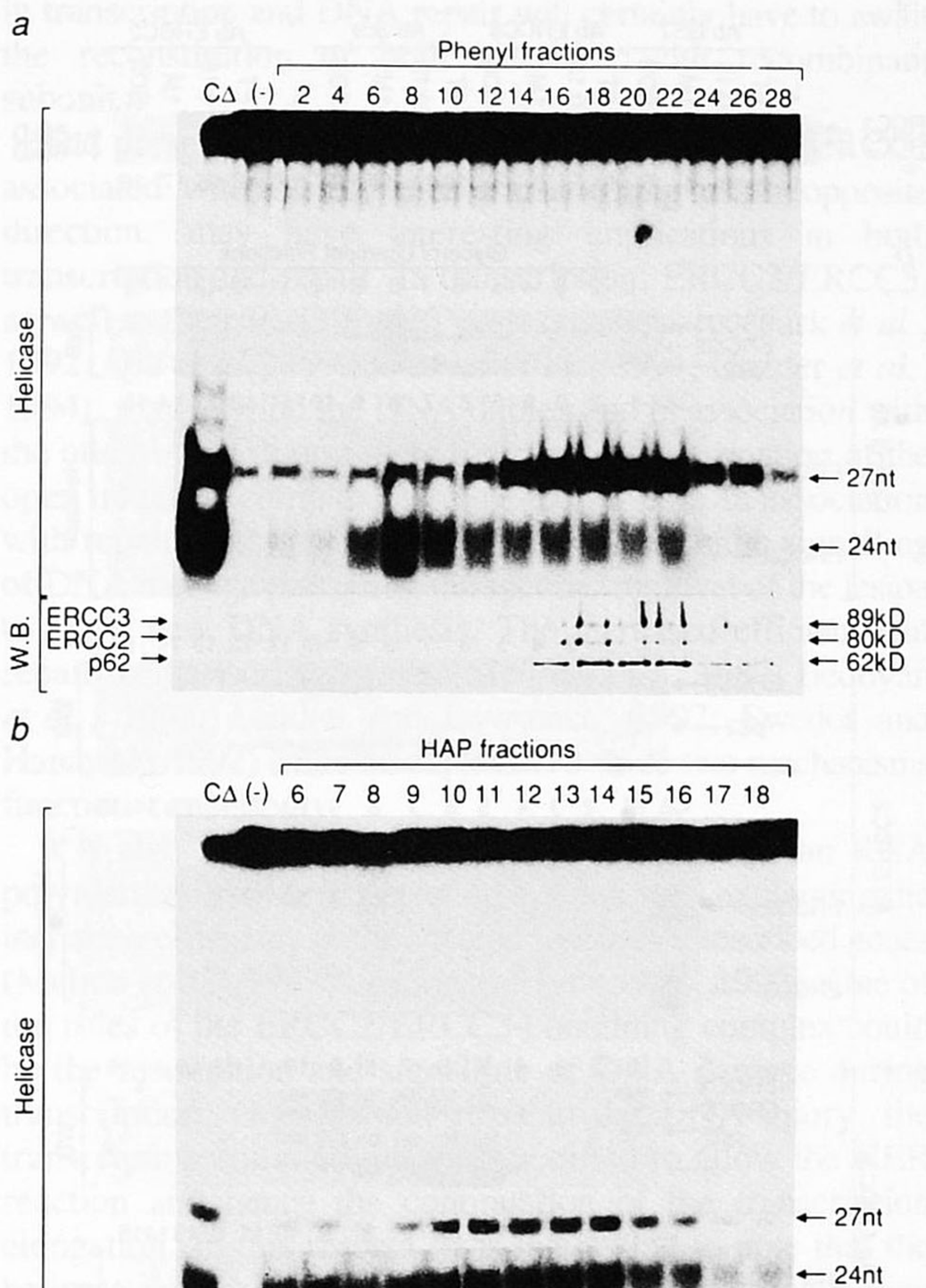


Fig. 1. BTF2 contains helicase activities that translocate in opposite directions. (**a**) The helicase substrate contains a duplex region at each extremity, either 24 or 27 nt in length. The 24 nt strand can only be displaced by a helicase translocating from 3′ to 5′, whereas to displace the 27 nt strand, a helicase which translocates in the opposite direction is required. (**b**) Helicase activity of ERCC3 and BTF2; lane 1, substrate heated for 2 min at 100°C (CΔ); lane 2, negative control (−); lanes 3 and 4, 10 and 30 ng of a GST fusion of ERCC3 purified on a glutathione−Sepharose column (Pharmacia, Sweden); lanes 5 and 6, 2 and 5 ng of a HAP fraction of BTF2 (Gerard *et al.*, 1991). The direction of translocation is indicated on the left of the panel.

and c, fractions 9−15, respectively). Contaminating helicase activities were resolved on the phenyl and HAP columns, and were distinct from ERCC3 and ERCC2 as determined by immunoblotting (Figure 2a and b). Note that BTF2 transcription activity also cosediments with the 3′ to 5′ (ERCC3) and the 5′ to 3′ helicase activities (Figure 2c, lower panel).



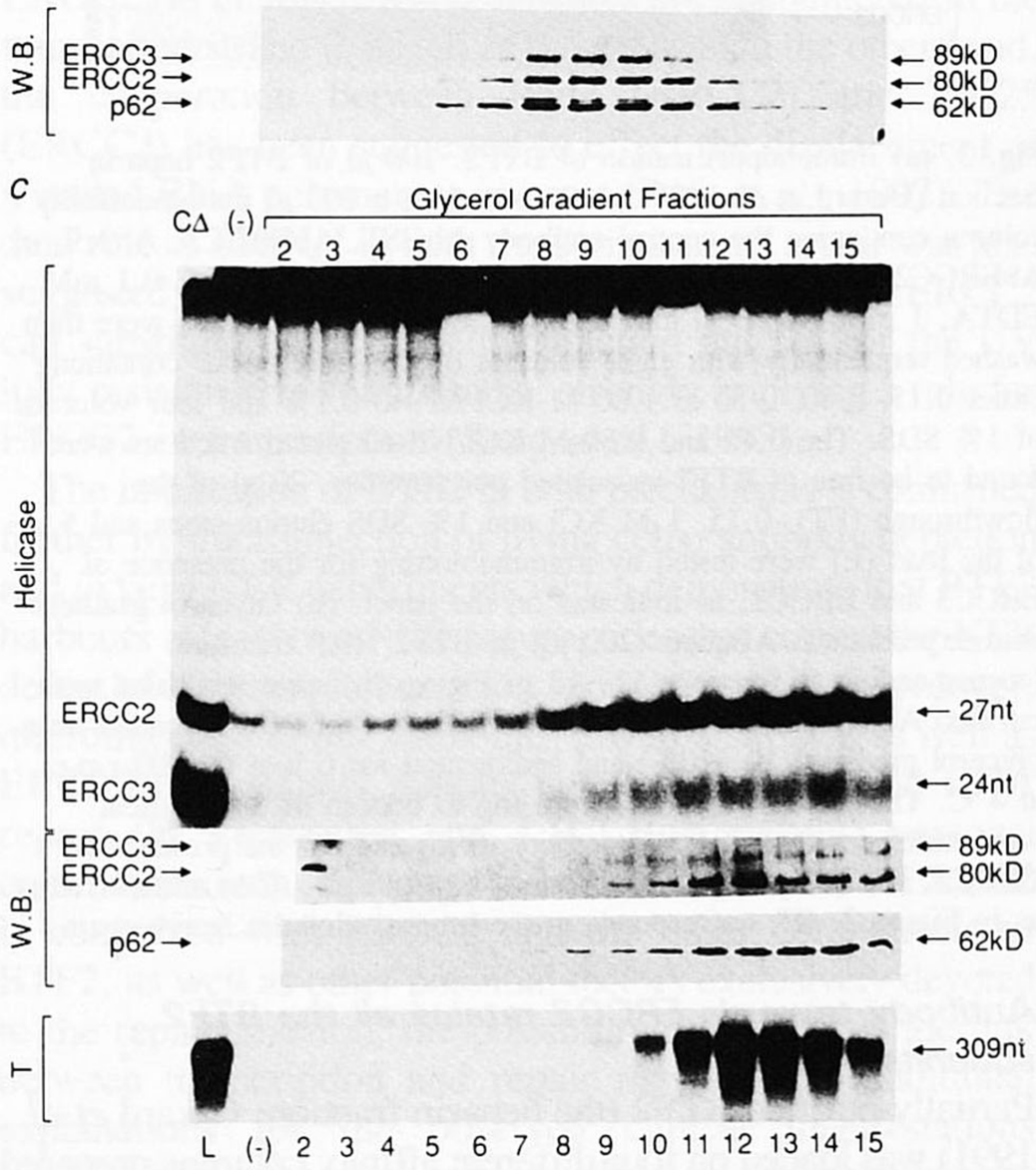


Fig. 2. The ERCC2, ERCC3 and p62 polypeptides detected by immunoblotting [Western blot (WB), 30 μl] were found to copurify with both helicase activities (Helicase, 8 μl) and BTF2 transcription activity (T, 15 μl) throughout the phenyl (**a**), the HAP (**b**) chromatography and the glycerol gradient (**c**). CΔ corresponds to the heated substrate and (−) to the negative control in the helicase test; the size of the transcript is indicated on the right (309 nt) of the panel; (L) represents the transcription activity of the loaded fraction and (−) the negative control.

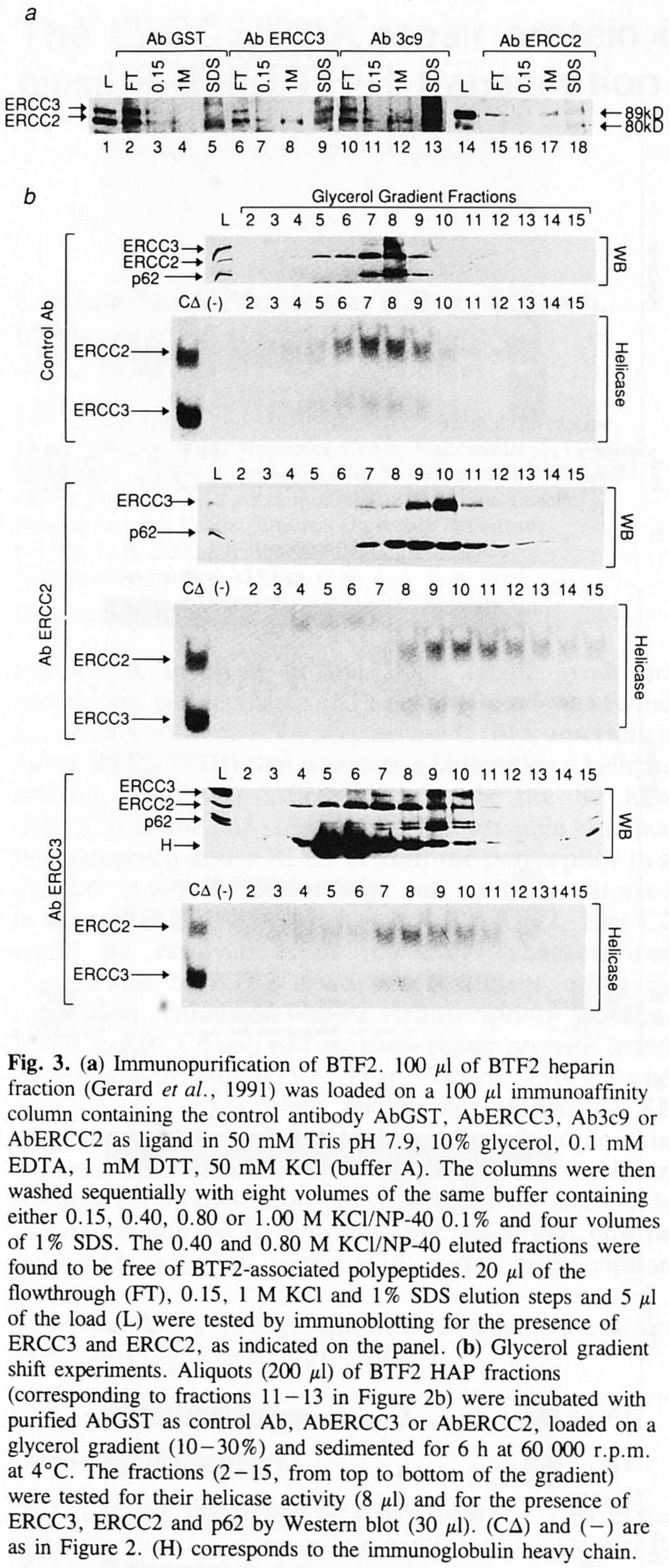


Fig. 3. (**a**) Immunopurification of BTF2. 100 μl of BTF2 heparin fraction (Gerard *et al.*, 1991) was loaded on a 100 μl immunoaffinity column containing the control antibody AbGST, AbERCC3, Ab3c9 or AbERCC2 as ligand in 50 mM Tris pH 7.9, 10% glycerol, 0.1 mM EDTA, 1 mM DTT, 50 mM KCl (buffer A). The columns were then washed sequentially with eight volumes of the same buffer containing either 0.15, 0.40, 0.80 or 1.00 M KCl/NP-40 0.1% and four volumes of 1% SDS. The 0.40 and 0.80 M KCl/NP-40 eluted fractions were found to be free of BTF2-associated polypeptides. 20 μl of the flowthrough (FT), 0.15, 1 M KCl and 1% SDS elution steps and 5 μl of the load (L) were tested by immunoblotting for the presence of ERCC3 and ERCC2, as indicated on the panel. (**b**) Glycerol gradient shift experiments. Aliquots (200 μl) of BTF2 HAP fractions (corresponding to fractions 11–13 in Figure 2b) were incubated with purified AbGST as control Ab, AbERCC3 or AbERCC2, loaded on a glycerol gradient (10–30%) and sedimented for 6 h at 60 000 r.p.m. at 4°C. The fractions (2–15, from top to bottom of the gradient) were tested for their helicase activity (8 μl) and for the presence of ERCC3, ERCC2 and p62 by Western blot (30 μl). (CΔ) and (–) are as in Figure 2. (H) corresponds to the immunoglobulin heavy chain.

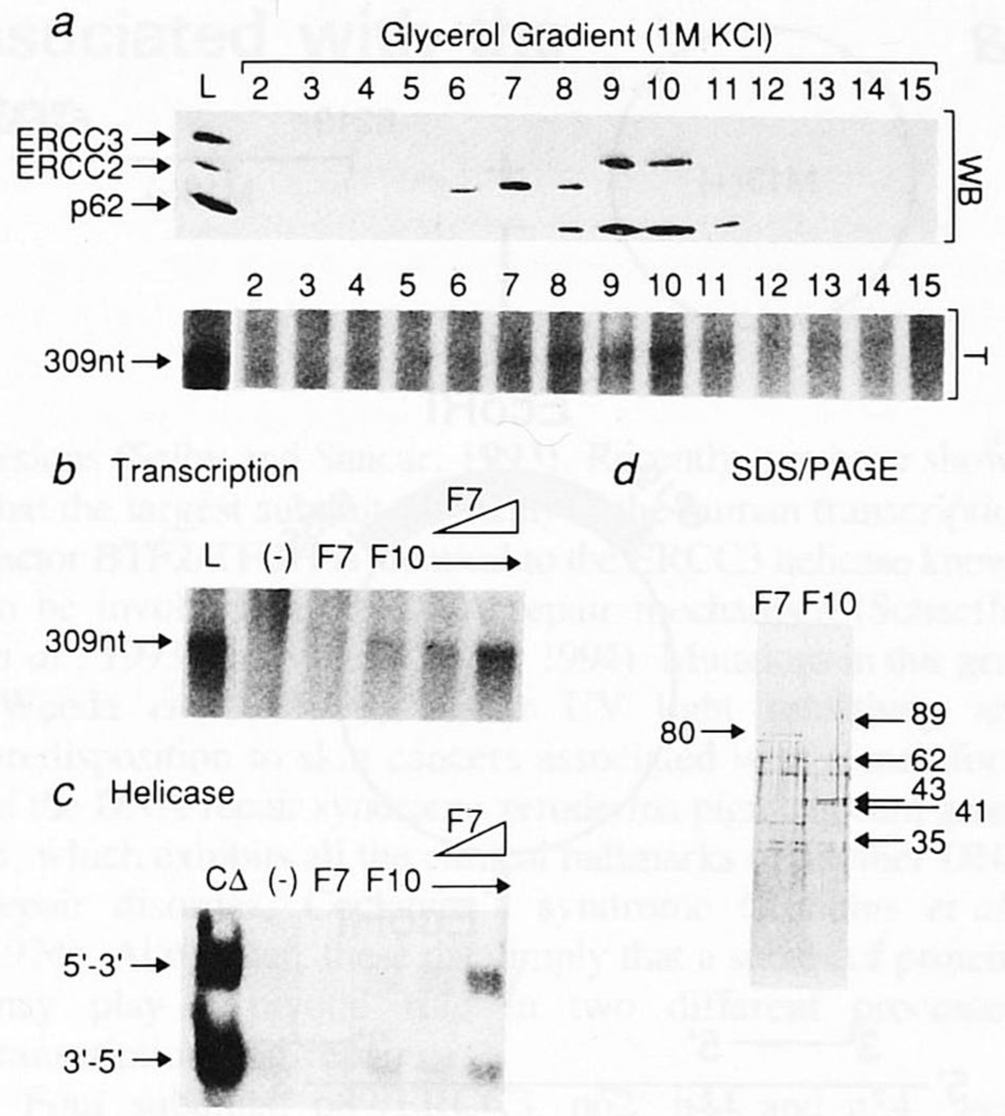


Fig. 4. ERCC2 could be dissociated from BTF2 subunits. 200 μl of BTF2 HAP (Gerard *et al.*, 1991) aliquots were dialysed for 4 h against buffer A containing 1 M KCl before being loaded onto a 10–30% glycerol gradient containing 1 M KCl and being sedimented for 11 h at 55 000 r.p.m. at 4°C (**a**). The fractions were tested in Western blot (20 μl) (WB) for the presence of ERCC3, ERCC2 and p62 polypeptides and in *in vitro* transcription (15 μl) (T). (**b** and **c**) 8 μl of fractions 7 (F7) and 10 (F10) were tested either separately or in combination (3 and 8 μl F7 was added to 8 μl F10) for transcription (b) and helicase activities (c) (see legend to Figure 2) (Fisher *et al.*, 1992). (**d**) 120 μl F7 and F10 were highly concentrated (6-fold) by rotary concentration, analysed by SDS–PAGE (10%) and silver stained. The polypeptides identified previously (Gerard *et al.*, 1991; Fisher *et al.*, 1992) are indicated to the right of the panel.

Antibody towards ERCC2 retains all the BTF2 subunits

Partially purified BTF2 (the heparin fraction; Gerard *et al.*, 1991) was loaded on four different affinity columns prepared with AbGST (used as a control), AbERCC3, Ab3c9 (Fisher *et al.*, 1992) and AbERCC2 as ligands (Figure 3). ERCC3/89 kDa and ERCC2/80 kDa were retained on the AbERCC3, Ab3c9 and AbERCC2 columns (Figure 3a, lanes 8 and 9, 12 and 13, and 17 and 18, respectively), whereas neither were retained on the control column (lanes 4 and 5). In addition, we found that fractions eluted at 1 M KCl from the AbERCC3 or the Ab3c9 column contained ERCC2, but little or no ERCC3 (lanes 8 and 12) could be detected. No ERCC2 was detected in the 1 M KCl fraction from either the control column or the AbERCC2 column (lanes 4 and 17, respectively). ERCC3 (lane 17), as well as the other components of BTF2 (see also Figure 4), were eluted from the AbERCC2 column with 1 M KCl, while ERCC2 could only be detected in fractions eluted with SDS (lane 18).

To confirm the nature of the BTF2–ERCC2 interaction, samples of a more purified BTF2 (11–13 HAP fractions; Figure 2b) were incubated with either the control Ab, AbERCC2 or AbERCC3 before glycerol gradient sedimentation (Figure 3b). When BTF2 was incubated with control Ab, the helicase activities, as well as ERCC3, ERCC2 and p62 polypeptides, were located in fractions 6–9. When BTF2 was incubated with either AbERCC2 or AbERCC3 it resulted in a shift of the two helicase activities and both ERCC3 and p62 to the bottom of the gradient. The fact that AbERCC3 did not shift ERCC2 in entirety could be explained by the presence of other complex(es) that contains ERCC2 but lacks ERCC3, or by the ability of AbERCC3 to partially disrupt the interaction of ERCC2 from the other BTF2 subunits. The 65–70 kDa band in the immunoblot is probably due to either non-specific interaction and/or degradation products (Figure 3b, upper and lower panels). The weak correlation between ERCC2 and helicase activity (Figure 3b, lower panel) could be due to low levels

of ERCC2 recovered in this fraction (<5 ng; Sung *et al.*, 1993) and its separation from the BTF2 core. We cannot exclude that an excess of antibodies could non-specifically inhibit ERCC2 helicase activity.

ERCC2 can be dissociated from the core BTF2

We then treated the BTF2 fraction described previously with 1 M KCl before glycerol gradient centrifugation (Figure 4a). As shown by immunoblotting, ERCC2 is resolved from ERCC3 and p62 (Figure 4a). It is worth noting that after salt treatment the five polypeptides described previously (mol. wt: 89, 62, 43, 41 and 35 kDa; Gerard *et al.*, 1991) remain associated with the albeit weak, but specific, BTF2 transcription activity (see Figure 4d, SDS–PAGE, fraction F10, and Figure 4a, transcription). When fraction 7 (F7), which contains ERCC2 but shows little if no helicase or transcription activity (Figure 4c and b, respectively), combined with fraction 10 (F10), which contains the core BTF2 subunits (Figure 4d; Gerard *et al.*, 1991), the weak signal detected in transcription is stimulated (Figure 4b). Moreover, the combination of both fractions also results in an increase of both the 5′ to 3′ (ERCC2), as well as the 3′ to 5′ (ERCC3), helicase activity. This enhancement was specific because fractions other than F7 had no effect (data not shown). Furthermore, none of these fractions were found to contain one of the basal transcription factors such as TFIIB, TFIID, TFIIE or TFIIF as contaminants (data not shown). However, we cannot rule out the possibility that other polypeptides could mediate such stimulation. Preparations of recombinant ERCC2 of sufficient purity have been unsuccessful to date, but work is currently underway to address the role of this polypeptide in transcription.

Discussion

We have shown that ERCC2, which is the product of a gene responsible for the atypical D group of the repair syndrome XP (Fletjer *et al.*, 1992), is strongly associated with BTF2, as demonstrated by immunoprecipitations and glycerol gradient shift experiments. The fact that we found ERCC2 in the BTF2 fraction is not surprising in the light of our previous data. Indeed, using Ab3c9 antibody we could immunoprecipitate an 80 kDa polypeptide in addition to the five polypeptides identified previously (Fisher *et al.*, 1992). This polypeptide was probably dissociated from the BTF2 core during the 60% ammonium sulfate (AS) precipitation preceding the original glycerol gradient sedimentation in the presence of 0.15 M AS, as described earlier (Gerard *et al.*, 1991). In the light of our present data, which demonstrate that ERCC2 could be dissociated from the other BTF2 polypeptides upon 1 M KCl treatment, and those from Gerard *et al.* (1991) it seems that BTF2 could be composed of (i) a core complex containing the subunits absolutely required for the BTF2 basal transcription activity (p62, Fisher *et al.*, 1992; p89/ERCC3, Schaeffer *et al.*, 1993; p44 and p34, Humbert *et al.*, 1994; and p41) and (ii) associated proteins like ERCC2 (in addition to p38 and p50, our unpublished results) that could modulate the transcriptional activity of BTF2. The multisubunit composition of BTF2 is not surprising *per se*, because two independent groups (Conaway and Conaway, 1989; Flores *et al.*, 1992) reported the presence of an 80 kDa polypeptide in the rat δ factor and TFIIH. The precise definition of the role of each subunit in transcription and DNA repair will certainly have to await the reconstitution of both activities with recombinant subunits.

The presence of the two helicases ERCC3 and ERCC2 associated with BTF2, each translocating in an opposite direction, may have interesting implications in both transcription and repair. In transcription, ERCC2/ERCC3, as well as their Rad3/Rad25 yeast counterparts (Park *et al.*, 1992; Qiu *et al.*, 1993; Feaver *et al.*, 1994; Guzder *et al.*, 1994), may unwind the DNA duplex and in association with the other transcription factors promote the formation of the open initiation complex (Wang *et al.*, 1992). In association with repair factors, they could be involved in the signalling of DNA damage preceding the specific removal of the lesion to allow new DNA synthesis. The increased efficiency of repair for transcribed genes (Mellon *et al.*, 1987; Bedoyan *et al.*, 1992; Leadon and Lawrence, 1992; Sweder and Hanawalt, 1992) could be explained if these two mechanisms function concertedly.

It is also possible that BTF2 may function as an RNA polymerase-associated detection system, thus explaining the increased efficiency of the specific repair of transcribed genes (Mellon *et al.*, 1987; Leadon and Lawrence, 1992). One of the roles of the ERCC2/ERCC3-containing complex could be the recognition and signalling of DNA damage during transcription. Once stalled close to the DNA injury, the transcription complex must be modified to allow the NER reaction and hence the continuation of the transcription elongation. In this respect, it is interesting to note that the helicase activity of yeast Rad3, the homologue of human ERCC2, is inhibited when damages are encountered in the transcribed strand (Naegeli *et al.*, 1992). On the other hand, the cooperation between Rad3 (ERCC2) and Rad25 (ERCC3) has been suggested to effect the displacement of a stalled RNA polymerase in yeast (Park *et al.*, 1992). The dual role of ERCC2 in both transcription and repair was also suggested by recent findings which demonstrate that ERCC2 can substitute in the viability function but not for the UV light resistance in yeast strains, strongly implying a role for ERCC2 in transcription (Sung *et al.*, 1993).

The implication of BTF2 in both mechanisms is confirmed further by microinjection of living cells, antibody depletion and *in vitro* NER experiments which demonstrate that BTF2 harbours at least two NER polypeptides that correct the NER defect of XP complementation groups B and D. Moreover, microinjection of our antibodies towards ERCC2 as well as ERCC3 dramatically inhibits both transcription and DNA repair (W.Vermeulen and J.H.J.Hoeijmakers, personal communication). Although it is still unclear how ERCC2 is connected with ERCC3 and the other components of BTF2, as well as other potential factors exclusively devoted to the repair reaction, the possibility that BTF2 is the link between transcription and repair may provide additional explanations for the observed clinical manifestations characteristic of atypical repair syndromes, which we suggest should be referred to as transcription/repair disorders.

Materials and methods

Bidirectional helicase assay

The helicase substrate was obtained by annealing 5 ng of an oligonucleotide corresponding to the fragment 6219–6255 of single-stranded M13 mp18 (–) DNA to 1 μg of single-stranded M13 mp18. The resulting heteroduplex was digested for 1 h at 37°C with *Eco*RI (New England Biolabs), thus

generating two duplex regions of 17 and 20 bp. The duplex regions were then extended to 24 and 27 bp, respectively, with the DNA polymerase I−Klenow fragment (5 U) in the presence of 50 μM TTP, 30 μM dGTP and 7 μCi [α-^{32}P]dATP (3000 Ci/mmol, Amersham). The helicase assay was performed as described previously (Schaeffer *et al.*, 1993). Briefly, reactions were incubated for 45 min at 37°C in the presence of 4 mM $MgCl_2$, 4 mM ATP, 50 μg/ml BSA and 1−3 ng DNA substrate. Reactions were stopped by the addition of EDTA and SDS, and loaded onto a 10% non-denaturing acrylamide gel. The gel was then dried and analysed by autoradiography.

Antibodies

AbERCC3 is a mAb raised towards a deletion mutant of ERCC3 (aa 82−480) (Roy *et al.*, 1994); AbGST is a mAb directed towards glutathione-*S*-transferase and was used as control antibody; AbERCC2 is a polyclonal rabbit antibody raised towards the 33 C-terminal amino acids of ERCC2; Ab3c9 is as described previously (Fisher *et al.*, 1992). The antibodies used for the glycerol gradient shift experiments were purified by caprylic acid and ammonium sulfate precipitation (Fisher *et al.*, 1992).

The immunoaffinity columns were prepared by covalently crosslinking the various antibodies to protein A−Sepharose beads (Pharmacia, Sweden) (Fisher *et al.*, 1992).

Other techniques

Purification of all the basic transcription factors required for the transcription run-off assay was as described previously (Gerard *et al.*, 1991). *In vitro* transcription, Western blots and microsequencing of the polypeptides were also as described previously (Fisher *et al.*, 1992).

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