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Cisplatin- and UV-damaged DNA lure the basal transcription factor TFIID/TBP

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A connection between transcription and DNA repair was demonstrated previously through the characterization of TFIIH. Using filter binding as well as *in vitro* transcription challenge competition assays, we now show that the promoter recognition factor TATA box-binding protein (TBP)/TFIID binds selectively to and is sequestered by cisplatin- or UV-damaged DNA, either alone or in the context of a larger protein complex including TFIIH. Computer-assisted 3D structural analysis reveals a remarkable similarity between the structure of the TATA box as found in its TBP complex and that of either platinated or UV-damaged oligonucleotides. Thus, cisplatin-treated or UV-irradiated DNA could be used as a competing binding site which may lure TBP/TFIID away from its normal promoter sequence, partially explaining the phenomenon of DNA damage-induced inhibition of RNA synthesis. Consistent with an involvement of damaged DNA-specific binding of TBP in inhibiting transcription, we find that microinjection of additional TBP in living human fibroblasts alleviates the reduction in RNA synthesis after UV irradiation. Future anticancer drugs could be designed with the consideration of lesion recognition by TBP and their ability to reduce transcription.

Keywords: cisplatin/DNA repair/TBP/TFIID/UV irradiation

Introduction

Nucleotide excision repair (NER) is essential for the genomic repair of UV-induced pyrimidine dimers or bulky, helix-distorting chemical adducts caused by numerous compounds such as acetylaminofluorene (AAF) or the anticancer drug, cisplatin (Zamble and Lippard, 1995). Early investigations aimed at elucidating how cells respond to damage induced by such diverse agents demonstrated a transcriptionally linked subpathway of NER in which lesions in transcribed genes were repaired preferentially (Bohr *et al.*, 1985). A further bias was shown by the increased removal of damage in the coding versus the non-coding strand and was suggested to result from participation of the arrested RNA polymerase II (RNA pol II) elongation complex which might serve to recruit repair machinery (Mellon *et al.*, 1987; Leadon and Lawrence, 1991; Sweder and Hanawalt, 1992).

More recently, it was established that the basal transcription factor TFIIH, critical for transcription, was also an intricate component of NER (for reviews, see Hoeijmakers *et al.*, 1996; Svejstrup *et al.*, 1996). This dual function suggests that TFIIH, as part of the transcription initiation complex, is well positioned to assist in the rapid removal of lesions in transcribed genes. What remains unclear is how the TFIIH complex, an essential transcription factor as well as part of the core NER machinery required for both global and transcription-coupled repair, is shared between these two distinct processes. Difficulties in understanding the regulation/function of TFIIH in repair versus transcription reflect the involvement of numerous proteins and their relative interactions in each process. This is complicated further by differences in *in vitro* conditions required to support each process. As a result, studies concerned with the role of TFIIH during *in vitro* NER or transcription are usually performed in the absence of one process. However, the notion that tight connections exist between NER and transcription, and the dual involvement of components in both processes, prompted us to investigate whether damaged DNA produces a high affinity site which is able to sequester factors supporting NER and/or transcription (Iyer *et al.*, 1996). We test this hypothesis by developing a challenge *in vitro* transcription assay using an undamaged transcription unit as a template in the presence of either UV-irradiated or cisplatin-damaged DNA as a competitor. This assay still examines a selective function of TFIIH (transcription) but, through pre-incubation of damaged DNA with whole cell extracts or purified factors, allows processes of NER to be initiatied and allows the observation of the relative influence of interactions between such factors on the ability of these extracts to support transcription. Our results indicate that the presence of damaged DNA leads to an inhibition of transcription from an independent and transcriptionally viable template. The most likely interpretation of this *trans*-effect on transcription is the sequestration of TFIIH in repair events, rendering it unavailable for transcription initiation. However, administration of extra TFIIH had only a relatively minor effect on recovery of RNA synthesis. Unexpectedly, addition of the recombinant TATA box-binding protein TBP or the whole TFIID also appeared to largely abolish the inhibition of transcription. TBP was found subsequently to bind strongly to different types of damaged DNA. Computer-assisted 3D structural analysis reveals a

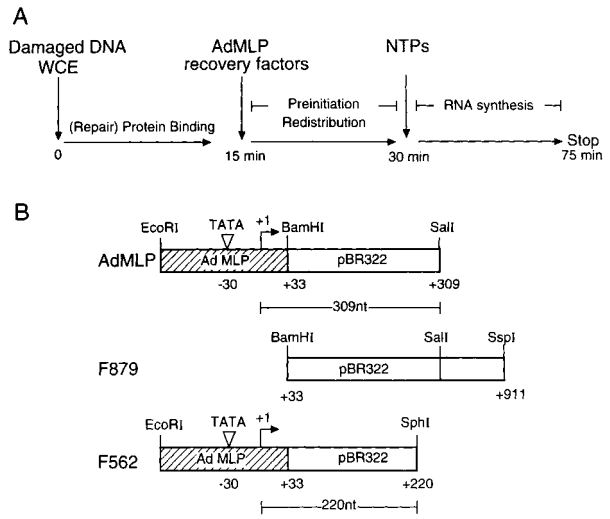

Fig. 1. (**A**) Design of transcription competition experiment. (**B**) Description of DNA fragments used for the *in vitro* transcription competition assay. AdMLP and F562 transcription templates are generated by *Eco*RI–*Sal*I and *Eco*RI–*Sph*I restriction digestion of plasmid pUC309 and give rise to 309 and 220 nt RNA transcripts respectively. Both contain the AdMLP promoter (hatched bars). Fragment F879 was created by restriction digestion of pUC309 with *Bam*HI and *Ssp*I. This fragment does not contain any promoter sequence.

remarkable similarity between the structure of the TATA box as found in its TBP complex and that of platinated oligonucleotides. Consistent with an involvement of damaged DNA-specific binding of TBP resulting in the inhibition of transcription, we also find that microinjection of additional TBP into living human fibroblasts alleviates the reduction in RNA synthesis after UV irradiation.

The sequestration of this crucial transcription factor by DNA lesions could partially explain the overall reduction in RNA synthesis observed *in vivo* after genotoxic treatment, and thus represents part of the cellular response to DNA damage.

Results

Addition of damaged DNA inhibits in vitro transcription

The presence of DNA lesions recognized by TFIIH, alone or in concert with other NER proteins, may reduce the availability of TFIIH or other transcription factors, thereby inhibiting the formation of a functional transcription initiation complex. To investigate this possibility, we developed a crude *in vitro* transcription competition assay (Figure 1A).

HeLa whole cell extract (WCE) was pre-incubated with various amounts of cisplatin- or UV-damaged DNA under conditions which only allow the formation of the pre-incision complex, one of the first steps of NER. The following incision/excision and resynthesis steps are inhibited by the low ATP concentration and the absence of dNTP respectively (Calsou and Salles, 1994; Moggs *et al.*, 1996). After the first 15 min, an AdMLP reporter template was introduced and the reactions were continued for an additional 15 min to allow the formation of pre-initiation transcription complexes and for any redistribution of factors including TFIIH between damaged DNA and transcription template. RNA synthesis was then initiated by addition of NTPs and quantified by the production of a 309 nt transcript. Pre-incubation of WCE with a UV-irradiated 879 bp fragment (F879 UV+) (containing ~3–4 lesions per DNA molecule, Jones and Wood, 1993) lacking promoter sequences (Figure 1B) inhibited transcription from the AdMLP template (Figure 2A, compare lanes 2–5 with lanes 6–9). Similarly, pre-incubation of the 3 kb pSK plasmid containing ~30 cisplatin-induced DNA crosslinks (Hansson and Wood, 1989) also inhibited transcription compared wirh undamaged DNA (Figure 2B, compare lanes 2–8 with lanes 9–15).

Quantification of the synthesis of RNA transcript (309 nt) from several experiments repeatedly shows that both UV- and cisplatin-damaged DNA inhibit transcription from an AdMLP template 3- to 4-fold more than undamaged DNA (see Figure 2A and B, lower panels). Note that 50% transcription inhibition is observed with a 2-fold excess of cisplatinated sites compared with TATA promoter sites, in the conditions described in Figure 2B.

To ensure that inhibition of the 309 nt transcript reflected a titration of factors on damaged DNA necessary to support transcription, we included a fixed concentration of a competitor DNA in the reaction but varied the ratio of damaged to undamaged fragment. Under these conditions, inhibition of transcription was observed as a function of the increase in damaged fragment present (Figure 2A, lanes 10 and 11). To demonstrate further that inhibition resulted from the loss of critical transcription factors on damaged DNA rather than non-specific protein–DNA or DNA–DNA interactions, we performed transcription competition experiments using two transcribable templates: the AdMLP reporter template (product = 309 nt) and a second AdMLP-containing template, F562, which produces a 220 nt transcript (Davison *et al.*, 1983). We reasoned that if a decrease in the production of the 309 nt transcript resulted solely from DNA–DNA or non-specific protein interactions, then transcription from a transcribable competitor may also be inhibited when presented in an undamaged form. However, we observed that pre-incubation of increasing amounts of UV-irradiated F562 resulted in a more effective inhibition of transcription from the AdMLP template than pre-incubation with undamaged F562 DNA (Figure 2C, compare the 309 nt transcript from lanes 8–13 with lanes 2–7). The presence of undamaged F562 decreased transcription somewhat from the AdMLP reporter template (309 nt), as might be expected since transcription factors would now be shared between two active promoters; however, the F562 template was still capable of being transcribed (220 nt), suggesting that the presence of two different DNAs in our reaction was not leading to interactions which rendered the templates unavailable for transcription. Only in the presence of UV-irradiated F562 did we also observe the absence of the 220 nt transcript (Figure 2C, lanes 8–13). The lack of the 220 nt product demonstrates that transcription factor(s) which are associated with damaged DNA (F562 UV+) are unable to support transcription from that template but, more importantly, are also unavailable to participate in transcription from an independent, undamaged template (AdMLP). Together with data presented in Figure 2A, these experiments demonstrate that the reaction conditions support transcription from two templates and that DNA

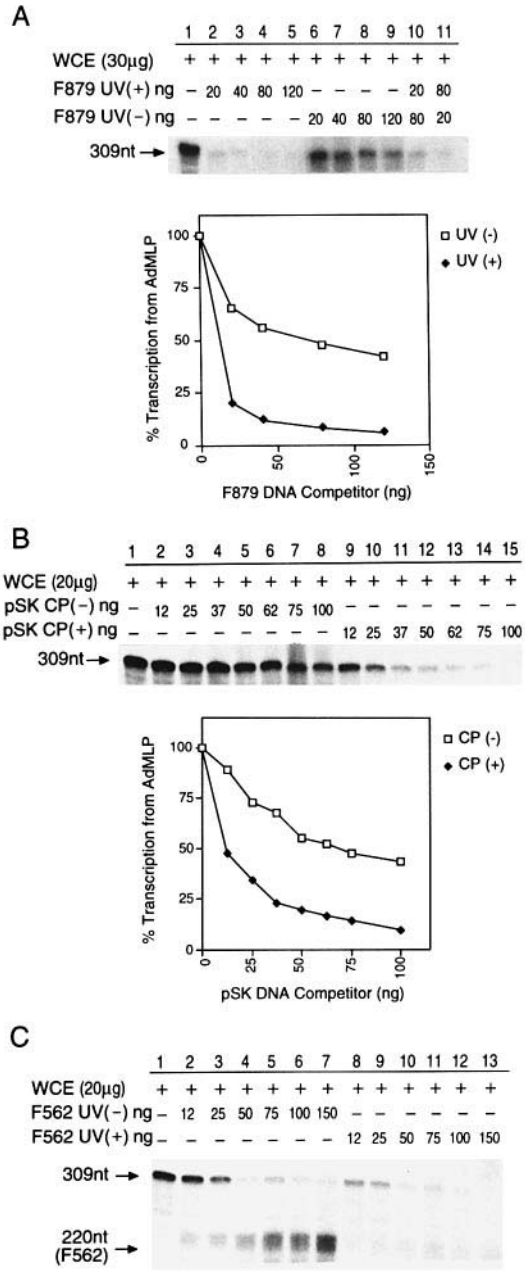

Fig. 2. Inhibition of *in vitro* transcription from the AdMLP by the presence of damaged DNA. (**A**) Transcription of AdMLP (50 ng) is performed with 30 µg of WCE in the presence of increasing amounts of UV-irradiated (3 kJ/m^2) (lanes 2–5) or non-irradiated (lanes 6–9) F879 DNA fragment. Upper panel: autoradiogram; lower panel: densitometric quantification of autoradiogram (309 nt band), presented as the percentage of transcription from the AdMLP as a function of the amount of F879 competitor DNA. Transcription in the absence of competitor equals 100%. (**B**) Transcription of AdMLP (50 ng) is performed with 20 µg of WCE in the presence of increasing amounts of plasmid pSK CP(–) (lanes 2–8) or CP(+) (lanes 9–15). Top panel: autoradiogram showing transcription from the AdMLP (309 nt); bottom panel: graph representing quantification by PhosphoImage analysis of the autoradiogram. (**C**) Inhibition of transcription by UV-irradiated F562 DNA. Transcription of AdMLP is performed with 20 µg of WCE in the presence of increasing amounts of non-irradiated (lanes 2–7) or UV-irradiated (1.5 kJ/m^2) (lanes 8–13) F562 DNA fragment. The size of each transcript is indicated.

containing UV- or cisplatin-induced lesions inhibits transcription because it interacts with components absolutely required for transcription from a TATA promoter.

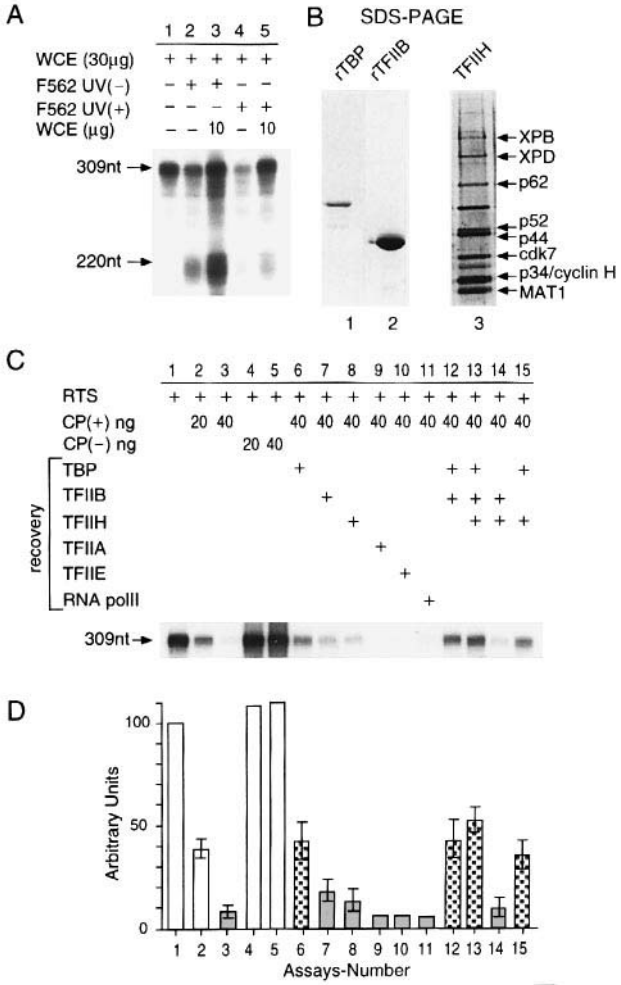

Fig. 3. Transcription factors are associated with damaged DNA. (**A**) UV-irradiated (1.5 kJ/m^2) (25 ng) or non-irradiated (25 ng) F562 DNA fragment was incubated as described in Figure 2C with 30µg of WCE. Recovery was obtained with the addition of 10 µg of WCE (lanes 3 and 5) to reactions inhibited by non-irradiated and UV-irradiated F562 DNA respectively. (**B**) Purified recombinant TBP and TFIIB as well as purified TFIIH from HeLa cells (hydroxyapatite fraction, Humbert *et al.*, 1994) were analysed by SDS–PAGE. (**C**) Transcription of AdMLP (50 ng) was carried out in a highly purified reconstituted *in vitro* transcription system (RTS). Cisplatin-damaged pSK DNA is pre-incubated with all components of the RTS before addition of AdMLP and either of the various transcription factors indicated at the top of the figure. The added amount of factors being tested for their ability to restore transcription was the same as that used to assemble the reconstituted assay, effectively doubling the concentration during the recovery. (**D**) The scanned transcription activities of (C) are reported together with values of two other experiments. In the shaded and chequered columns, 40 ng of damaged DNA is added; the chequered columns contain TBP. The means ± SE are indicated.

TBP is sequestered by DNA damage

In order to determine the specific transcription factors contributing to the loss of activity in the presence of damaged DNA, we carried out transcription competition experiments in which WCE or purified transcription factors were added to the transcription reaction together with the AdMLP fragment. Addition of WCE increases transcription from either AdMLP (309 nt) or F562 (220 nt) (Figure 3A, compare lanes 2 and 3 with lanes 4 and 5). When added to a reaction inhibited by UV-damaged DNA, WCE significantly restored the level of transcription (compare

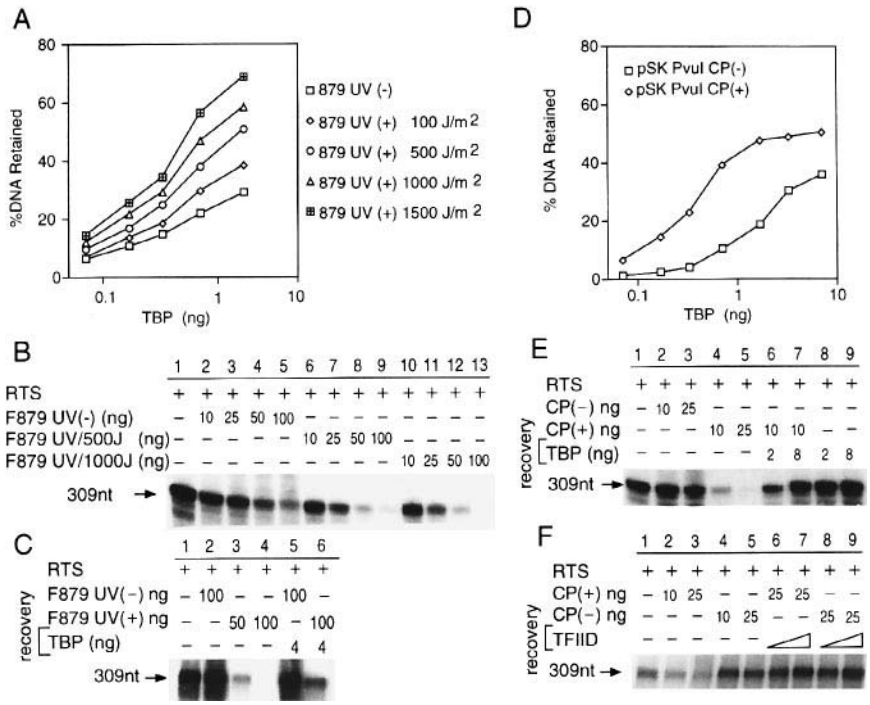

Fig. 4. Preferential binding of TBP to damaged DNA. (**A**) ^{32}P-Labelled F879 DNA was UV irradiated at different doses as indicated in the right part of the panel, incubated with various amounts of TBP and tested for its retention on nitrocellulose filters. Graphs represent the percentage of DNA retained on the filters as a function of the amount of TBP for four different doses of UV and for an undamaged F879 DNA fragment. Quantification was performed using a PhosphoImage analyser; 100% represents the total counts obtained when 1 µl of each DNA probe (input) was spotted onto Whatman paper and simultaneously exposed with the nitrocellulose filter. (**B**) Inhibition of AdMLP-dependent transcription in a highly purified reconstituted *in vitro* transcription system is dependent on the UV dose. The reconstituted transcription assay was carried out in the presence of increasing amounts of non-irradiated (lanes 2–5), 500 J/m^2 (lanes 6–9) and 1000 J/m^2 (lanes 10–13) UV-irradiated F879 DNA. (**C**) The transcription reaction was carried out in an RTS with F879 UV (+) (1000 J/m^2) or (–) DNA probe as competitor. Recovery of transcription after inhibition with competitor DNA was obtained by adding TBP. (**D**) The filter binding assay was performed as described in (A), except that a *Pvu*I restriction fragment (1084 bp) of pSK cisplatin-treated CP(+) or untreated CP(–) was used as the DNA probe. (**E**) The transcription reaction was carried out in an RTS with an increasing amount of cold CP(–) or CP(+) pSK *Pvu*I DNA probe (see D) as competitor. Recovery of transcription after inhibition with CP(+) DNA was achieved by adding various amounts of TBP. (**F**) The transcription reaction was carried out as in (E) except that purified TFIID was used in place of TBP. Recovery of transcription was obtained by adding various amounts of TFIID roughly corresponding to 6–15 ng of TBP according to Western blot analysis.

lanes 1, 4 and 5). The weak 220 nt product from the F562 UV+ template (lane 5) is either non-specific or results from residual undamaged template. Together, these results demonstrate that our assay is responsive to new factors and that we are below saturating conditions with respect to total protein (compare lanes 2 and 3 with lanes 4 and 5). Furthermore, these results also demonstrate that UV lesions in the damaged F562 template remain a significant block to transcription even in the presence of additional proteins (compare lanes 3 and 5).

Besides TFIIH, WCE contains many other factors which may contribute to the observed restoration of transcription. In addition, controlling the relative amounts of individual factors within a WCE is virtually impossible. We therefore used a reconstituted transcription system (RTS) in order to determine effectively the role of each transcription factor. Thus *in vitro* transcription competition experiments were performed with an RTS containing highly purified TBP, TFIIB, TFIIE, TFIIF, TFIIH, TFIIA and RNA pol II (Humbert *et al.*, 1994; see also Figure 3B). The RTS was pre-incubated with cisplatin-damaged DNA, and the ability of each purified transcription factor to restore activity was determined. Addition of TBP, and to a lesser extent addition of TFIIB and TFIIH, restored transcription (compare Figure 3C and D, lane 3, with lanes 6–8), whereas addition of TFIIA, TFIIE or RNA pol II had no effect (lanes 9–11).

However, the addition of TFIIH and TFIIB, either alone or in combination with TBP, did not lead to a significantly greater degree of restoration than that provided by TBP itself, suggesting that this was the limiting primary factor after incubation of the RTS with damaged DNA. In fact addition of increasing amounts of either TBP or TFIID, in an RTS already inhibited by cisplatin-treated DNA (Figure 4E and F), was able to restore activity to the initial level of transcription without inducing similar increases in the reaction performed in the absence of competitors (Figure 4E, compare lane 1 with lanes 8 and 9). Together these results suggest that TBP interacts directly with the DNA lesion, whereas TFIIH and TFIIB may either be associated with DNA lesions through the TBP, or drive transcription through stabilization of the TBP–promoter complex (see Discussion and Gérard *et al.*, 1991). Furthermore, TBP and TFIIB as well as purified TFIIH were shown to be free of repair proteins (Figure 3B; see also Aboussekra *et al.*, 1995), indicating that binding to damaged DNA as well as recovery of transcription is not the result of a contaminating repair factor. Although TFIIH has been reported to be recruited to the DNA lesion site in conjunction with other factors (Park

et al., 1995), the added presence in our reconstituted transcription system of recombinant xeroderma pigmentosum group A (XPA) protein did not significantly affect transcription (data not shown).

TBP interacts directly with damaged DNA

The ability of TBP, TFIIB and TFIIH to restore transcription inhibited by the presence of damaged DNA suggested that these factors may interact with DNA lesions. We investigate this possibility using a standard nitrocellulose filter binding assay. As illustrated in Figure 4A, treatment of F879 DNA, which does not contain a TATA element (Figure 1B), with increasing doses of UV irradiation (Figure 4B; 100–1500 J/m^2) resulted in a corresponding increase in the amount of DNA retained by a fixed concentration of TBP. The functional significance of this interaction is demonstrated further in the following experiment. The RTS, containing all of the basal transcription factors, was pre-incubated with F879 DNA fragment damaged by irradiation with either 500 or 1000 J/m^2, and assayed for its ability to support transcription from the AdMLP reporter template. In agreement with observations from the crude transcription assay and nitrocellulose filter binding assays, the presence of damaged DNA preferentially inhibited the production of the 309 nt transcript (Figure 4B, compare lanes 2–5 with 6–9 and 10–13). The inhibition can be reversed by back addition of TBP (Figure 4C).

TBP also recognizes cisplatin-damaged DNA, as judged by the nitrocellulose filter binding assay (Figure 4D). Pre-incubation of the RTS containing recombinant TBP with cisplatin-damaged DNA resulted in a specific inhibition of transcription from the AdMLP template (Figure 4E, compare lanes 2 and 3 with lanes 4 and 5). Transcription can be restored completely after readdition of TBP (lanes 6 and 7). It is worthwhile noting that our assay is saturated with respect to all individual components for a given concentration of AdMLP template. For example, we noticed that addition of TBP did not result in a general increase in the level of transcription (Figure 4E, compare lane 1 with lanes 8 and 9). When the same experiment was performed with an RTS containing TFIID (the native transcription factor that contains TBP) instead of TBP, cisplatinated DNA also inhibited the transcription reaction (Figure 4F, lanes 1–5); here too, transcription is restored upon addition of an excess of TFIID (lanes 6–7), thus demonstrating that in the context of TFIID, TBP can still bind to damaged DNA.

TFIIB, which was also able to restore transcription, was unable to retain a damaged or undamaged fragment (data not shown), suggesting that its contribution to the inhibition/restoration of transcription is indirect, most probably through stabilization of TBP on the promoter. Attempts to use filter binding assays to demonstrate a specific association of highly purified TFIIH alone with damaged DNA were also unsuccessful. Highly purified TFIIH retained, although weakly, similar levels of both damaged and undamaged DNA, suggesting that binding to damaged DNA probably occurs through interactions with other repair factors such as XPA (Park *et al.*, 1995) or the transcription factor TBP, as suggested by transcription recovery experiments.

The ability of purified TBP to bind to damaged DNA was also observed in standard electrophoresis mobility shift assays (EMSAs). A ^{32}P-labelled DNA probe, either undamaged or containing a single 1,3-GpTpG cisplatin crosslink, was incubated with rTBP and non-specific DNA (see Materials and methods), and complex formation was detected by a change in the migration pattern of DNA on acrylamide gels. EMSA revealed the formation of a TBP–DNA nucleoprotein complex that was specific for damaged DNA (Figure 5A, lanes 2 and 12). Moreover, the formation of a TBP–damaged DNA complex was reduced specifically with increasing concentrations of an unlabelled cisplatin-damaged *Pvu*I pSK DNA fragment as compared with a non-damaged fragment (compare lanes 7 and 8 with lanes 9 and 10). The addition of an unlabelled fragment containing a TATA box (TATA) also resulted in competition with TBP binding to the cisplatin-damaged DNA probe (compare lane 2 with lanes 5 and 6), supporting conclu-

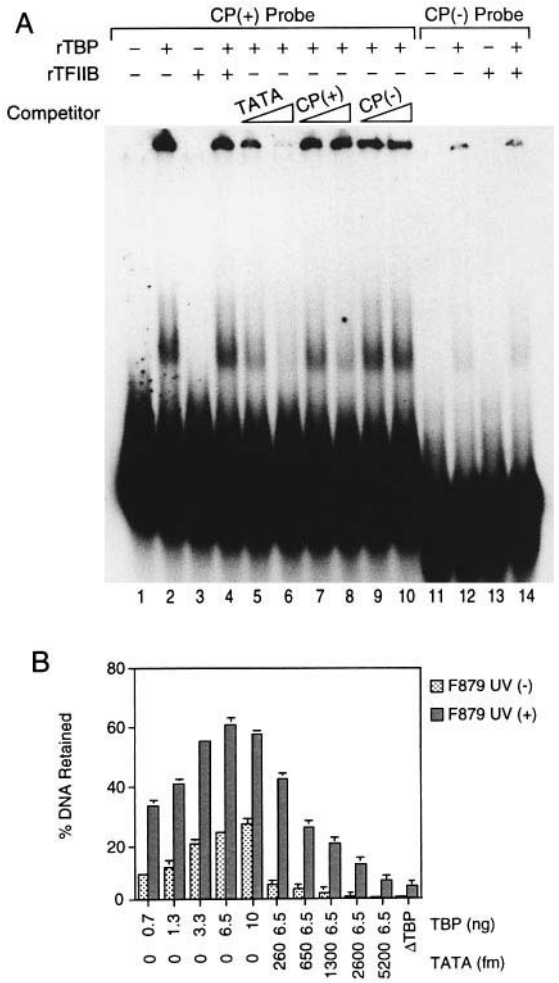

Fig. 5. The TATA box-containing fragment competes with damaged DNA. (**A**) Gel shift analysis of rTBP binding to cisplatin-damaged DNA. Binding was performed with the indicated cisplatin-damaged CP(+) or undamaged CP(–) 36mer DNA probe (0.5 ng; 10 000 c.p.m.), rTBP (20 ng) and/or rTFIIB (20 ng). Then 10 and 50 ng of a 64mer AdMLP fragment containing the TATA box (TATA) or 10 and 50 ng of *Pvu*I pSK CP(+) and CP(–) DNA fragment were added as competitors as indicated. Note that the radioactive material in the wells is due to recombinant TBP–DNA probe aggregates. (**B**) Inhibition of TBP binding to UV-irradiated (1.5 kJ/m^2) F879 DNA was performed by addition of increasing amounts of the TATA DNA fragment (64mer) also used in the EMSA. When heat inactivated (47°C), TBP interacts weakly with DNA.

Table I. Effect of TBPr injection on transcription in living human fibroblasts

Exp.	Microinjected sample[a]	UV irradiation (J/m^2)	Incubation time (h)[b]	RNA synthesis[c] (grains/nucleus)	DNA repair[d] (grains/nucleus)
1	400 ng/μl rTBP	16	1		20.0 ± 1.0
	Non-injected	16	1		21.0 ± 1.0
2	400 ng/μl rTBP	0	1	12.0 ± 1.02	
	40 ng/μl rTBP	0	1	14.0 ± 1.0	
	4 ng/μl rTBP	0	1	21.0 ± 1.0	
	Non-injected	0	1	22.0 ± 1.0	
3	16 ng/μl rTBP	0	1	74.0 ± 2.0	
	Non-injected	0	1	73.0 ± 2.0	
	16 ng/μl rTBP	16	1	54.0 ± 3.0	
	Non-injected	16	1	34.0 ± 1.0	
4	16 ng/μl rTBP	16	3	43.5 ± 3.5	
	50 ng/μl TFIIB	16	3	27.8 ± 1.0	
	Non-injected	16	3	27.0 ± 2.5	
5*	16 ng/μl rTBP	16	3	42.0 ± 3.0	
	Non-injected	16	3	25.0 ± 1.0	
6	16 ng/μl rTBP	30	3	34.0 ± 1.0	
	Non-injected	30	3	19.0 ± 1.0	

[a]Injections were performed in control fibroblasts (C5RO), except for Exp 5 where fibroblasts of a CS-B patient (CS1AN) are injected.
[b]Time of incubation after UV irradiation and before pulse labelling with radioactive nucleotides.
[c]RNA synthesis (with and without UV challenge), autoradiographically measured by [^{3}H]uridine incorporation (Vermeulen *et al*., 1994) expressed as grains/nucleus ± SEM (at least 50 nuclei for each sample are counted).
[d]UV-induced DNA repair synthesis (UDS), measured autoradiographically as the [^{3}H]thymidine incorporation, expressed as grains/nucleus ± SEM (>50 nuclei/sample).
*CS-B cells also presented in Figure 6B.

sions that the shifted probe corresponded to a TBP–DNA complex. TFIIB alone was unable to shift the damaged DNA probe, and no supershift was observed when TFIIB was added to the TBP–damaged DNA complex (see lanes 3 and 4 respectively). These conclusions were supported by additional filter binding assays assessing the relative affinity of TBP for a DNA lesion in the presence of the normal TATA sequence. UV-irradiated F879 DNA (Figure 1B) was incubated with TBP in the presence of the TATA element of the AdMLP (TATA). The results illustrated in Figure 5B confirm a preferential association of TBP with damaged DNA (column sets for TBP concentration 0.7–10 ng) and indicate that significantly greater amounts of the TATA fragment were required to compete the binding of TBP on UV-damaged DNA to similar levels to that observed with undamaged DNA (column sets for TATA competitor, concentration 260–5200 fmol). A rough estimation indicates that ~650 fmol of TATA box-containing fragment (~200-fold) is necessary to achieve a 50% competition inhibition of the association of highly purified TBP with the 3–5 fmol of damaged sites induced by UV irradiation (according to Jones and Wood, 1993) (see also Figure 3C). As a control, inactivated TBP, previously incubated for 15 min at 47°C (Nakajima *et al.*, 1988), shows no interaction with UV-damaged or undamaged DNA. Together, these results indicate for the first time that two types of DNA lesions, induced by either UV irradiation or cisplatin treatment, serve as binding targets for TBP.

TBP microinjection protects cells from UV-induced inhibition of RNA synthesis

UV-induced DNA damage in the genome causes a transient inhibition of overall transcription. One possibility is that this suppression is at least in part due to sequestration of TBP by DNA lesions. Therefore, we reasoned that microinjection of extra TBP might partly relieve this UV-induced inhibitory effect. To test this possibility, rTBP was microinjected into normal primary fibroblasts. The effect on NER, transcription and UV-induced inhibition of transcription was assessed by incubating the cells after rTBP injection, in the presence of ^{3}H-labelled thymine (for repair synthesis) or ^{3}H-labelled uridine (to measure transcription). NER and transcription were quantified by counting the number of autoradiographic grains above the nuclei of injected cells and compared with the non-injected cells on the same slide. Initial experiments (Table I, Exp 1 and 2; using rTBP at a concentration of 400 ng/μl) indicated that the injected rTBP by itself did not affect DNA repair (Exp 1) but caused a strong inhibition of transcription (Exp 2). Apparently, the excess TBP (we calculate that we injected ~40–60% of the total cellular TBP content) squelches factors interfering with normal transcription, a fact that was also observed after transient overexpression of TBP (S.Buratowsky and P.Chambon, personal communications). To avoid a dominant-negative effect on regular transcription, we titrated TBP to a concentration at which no inhibition was observed (4 and 16 ng/μl, see Exp 2 and Exp 3 respectively). As shown in Table I (Exp 3, 4 and 6), injection of 16 ng/μl rTBP induced a clear protective effect against the inhibition of transcription caused by different doses of UV irradiation (see also Figure 6A). The partial relief of transcription was observed 1 and 3 h after UV exposure and was independent of transcription-coupled repair, as injection in Cockayne syndrome (CS)-B fibroblasts (which exhibit a selective defect in transcription-coupled repair) also stimulated UV-suppressed RNA synthesis (Exp 5 and Figure 6B). Microinjection of TFIIB (50 ng/μl, Exp 4) and cdk7–cyclinH–MAT1 complex (50 ng/μl) failed to reverse the UV-induced transcription inhibition, indicating that the observed protection was TBP specific. These findings show that at least part of the suppression of transcription by UV can be overcome

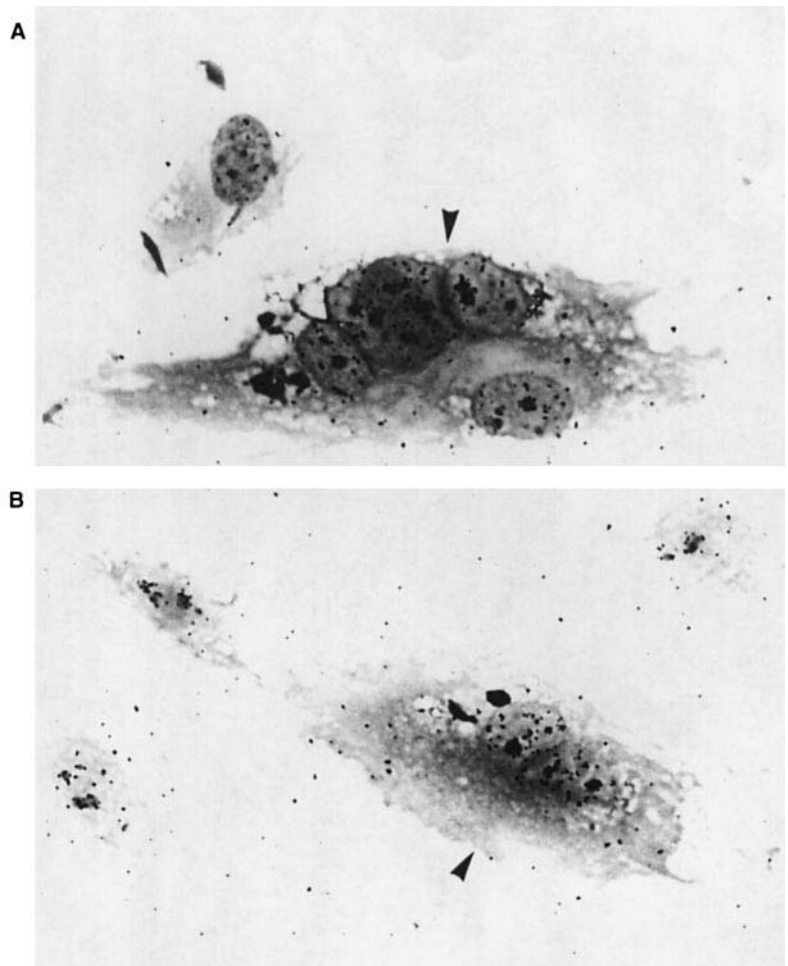

Fig. 6. Effect of TBP on transcription *in vivo*. Micrograph of C5RO normal (**A**) and CS-B patient (**B**) fibroblasts injected (indicated by an arrow) 1 h before UV irradiation (16 J/m^2) showing an increase in the overall RNA synthesis. In each panel, one has to compare the autoradiographic grains above the nuclei of injected cells (indicated by an arrow) with the surrounding nuclei.

by exogeneous TBP. This is consistent with the idea that after UV irradiation, sequestration of endogeneous TBP to DNA lesions takes place, contributing to the damage-induced inhibition of transcription.

Similarities between the TATA box and cisplatin-damaged DNA

We investigated the structural implications of these observations by comparing the structure of a platinated DNA with its TATA counterpart from the human TBP–TATA complex. The only available crystal structure of a 1,2-cisplatin adduct on an oligonucleotide is that of a double-stranded DNA dodecamer containing a central G∧G site (*cis*-[Pt(NH_3)$_2$-{d(GpG)-N7(G_6), N7(G_7)}] intrastrand crosslink) (Takahara *et al.*, 1995), hereafter referred to as GGPG. Superposition of GGPG onto the TATA box DNA (TATA) revealed a strikingly similar overall shape, especially in the central region, although the detailed conformations of the base pairs differ in the two structures (Figures 7A and 8A and B). The following analysis is based on the optimal superposition of the original structural data without any energy minimization. An r.m.s.d. of 2.1 Å was found between the backbone atoms of TATA and GGPG (compared with an r.m.s.d. of 8.8 Å between the backbone atoms of TATA and BDNA, the canonical B-DNA dodecamer, and an r.m.s.d. of 5.7 Å between the backbone atoms of GGPG and BDNA, compare Figure 7B and C). To calculate a realistic r.m.s.d. value, we have taken into account the different orientation of the base pairs in the two structures which leads to a shift of one nucleotide on one strand (Figure 7A). This explains how the two overall structures can fit so well in spite of important differences in base pair conformations. Both TATA and GGPG are bent towards the major groove and partially unwound without disrupting the Watson–Crick hydrogen bonding pattern. In the TBP–TATA complex, the saddle-shaped TBP core wraps around the DNA in the minor groove (Chasman *et al.*, 1993; J.L.Kim *et al.*, 1993; Y.Kim *et al.*, 1993; Juo *et al.*, 1996). The pronounced bend is induced by the insertion of phenylalanine side chains into the first and last steps of the TATA element, and is favoured by the intrinsic bendability of the TA steps. In the GGPG structure, a similar bend is produced by the coordination of the platinum ion to the N7 nitrogen atoms of two adjacent guanines on the same strand, which forces the destacking of the complementary bases. As a result, the cisplatin adduct mimicks the distorted conformation of TATA in its complex with TBP. In both molecules, the structure exhibits an abrupt B- to A-form transition near the bent portion, with a pronounced opening and flattening of the minor groove, as illustrated in the two by two superpositions of TATA, BDNA and GGPG (Figure 7).

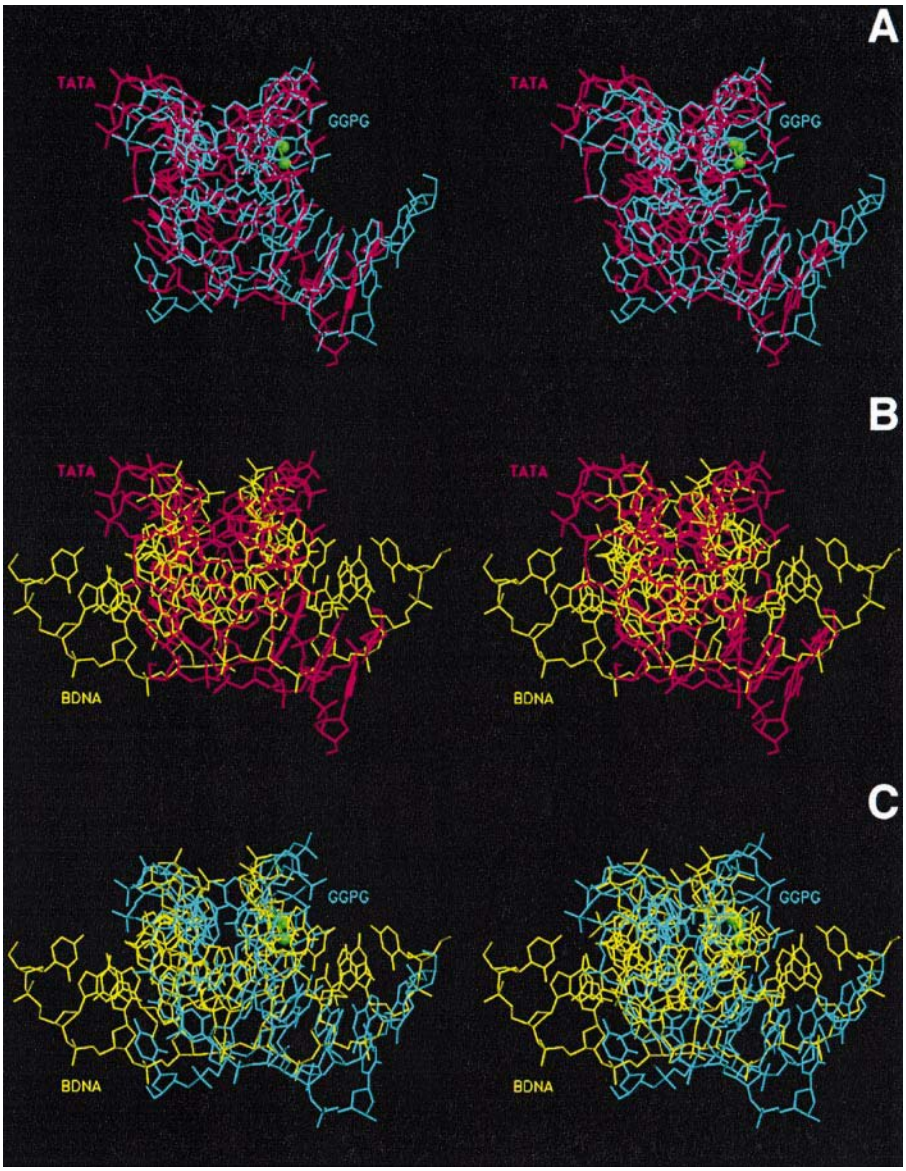

Fig. 7. Stereoviews of the two by two superpositions of the crystal structures of the TATA box from the human TBP–TATA complex (TATA, PDB code 1TGH; Juo *et al.*, 1996), of the DNA dodecamer containing a central *cis*-[Pt(NH_3)$_2$-{d(GpG)-N7(G_6), N7(G_7)} intrastrand crosslink (GGPG, PDB code 1GPG; Takahara *et al.*, 1995), and of a canonical B-DNA dodecamer (BDNA, PDB code 1BNA; Drew *et al.*, 1981). The superpositions were optimized using the LSQ options of O (Jones *et al.*, 1991) and displayed with SETOR (Evans, 1993). TATA is shown in red, GGPG in cyan, with the platinum ion and the nitrogen atoms of its amine ligands in green, and BDNA in yellow. (**A**) GGPG–TATA; (**B**) BDNA–TATA; (**C**) BDNA–GGPG.

Indeed, TBP appears to dock exceedingly well on GGPG (Figure 8A and B), the complex showing very few stereochemical clashes at the level of the inserted phenylalanines. An optimal fit would require a small adaptation of DNA with a minimal energy cost. The buried surface in the TBP–GGPG complex calculated from the docked structures is 2707 Å^2 compared with 3090 Å^2 in the TBP–TATA complex, the slightly lower value arising from the more pronounced bend in GGPG, as seen in Figure 8A. Note that the complex with GGPG has not been energy-minimized. In both cases, the contacts with TBP are essentially hydrophobic, with only a few exceptions such as the hydrogen bond bridges formed by Asn163 and Asn253 with the two central T·A base pairs in the TBP–TATA complex. When BDNA is docked with TBP, the interface drops down to 2241 Å^2, and the minor groove forms a cavity which suppresses the contacts between the protein side chains and the bases. Instead, phosphate groups point towards the protein surface, making the binding of TBP to DNA in a B conformation very unfavourable (Figure 8C). The importance of the interactions with the bases is supported by the observation that two TBP mutants at the 253 position are defective in DNA binding (Arndt *et al.*, 1995; Lee and Struhl, 1995). As a consequence, the helical twist is nearly zero at the central step, the two base pairs being stacked directly on top of one another with a 25° roll angle (Juo *et al.*, 1996). This value can be compared with the 26° roll angle observed between the two central G*C base pairs in GGPG (Takahara *et al.*, 1995). According to our docking experiment, the two asparagine residues are accommodated at the TBP–GGPG interface, providing different hydrogen bonding patterns,

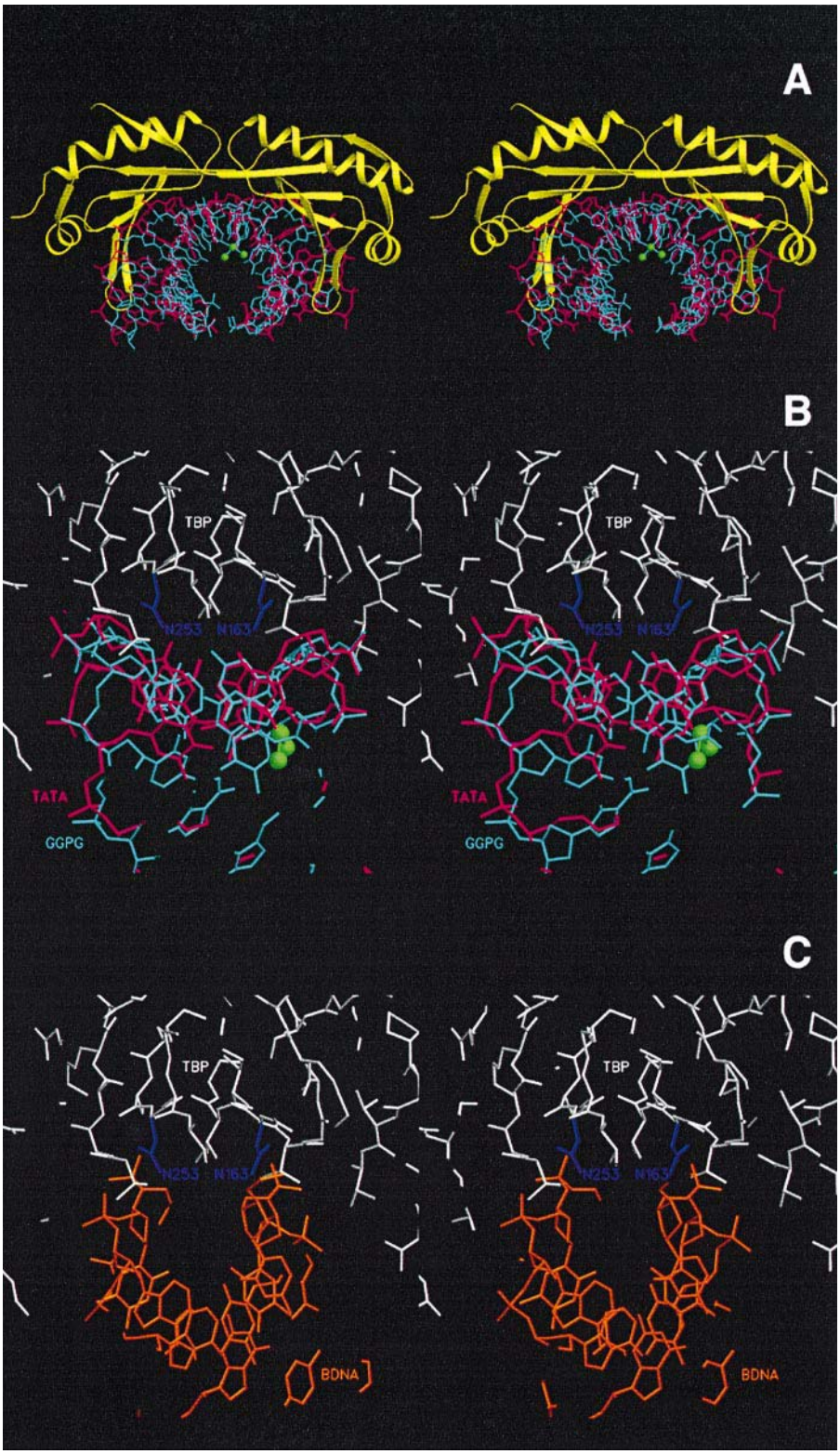

Fig. 8. Stereoviews of the TBP–DNA interfaces displayed with SETOR. (**A**) View along the long axis of TBP of the human TBP–TATA complex showing GGPG superposed to TATA as in Figure 6A. TATA is shown in red, GGPG in cyan, with the platinum ion and the nitrogen atoms of its ammine ligands shown in green, and TBP is displayed in yellow in a ribbon representation. (**B**) Close view in the same orientation as in Figure 6 showing TBP (in white) sitting on the minor groove. The Asn163 and Asn253 side chains are coloured in magenta. (**C**) View of BDNA docked with TBP in the same orientation as in Figure 7B, showing the cavity at the interface and the unfavourable orientation of the central phosphate groups.

Asn163 and Asn253 contacting N3 of G_6 and O2 of C_{19}, respectively. Thus these interactions do not discriminate between different sequences, but rather are involved in the recognition of a particular type of DNA structure.

Discussion

We present data which suggest that repair and transcription may be linked at a more fundamental level than previously

thought. Earlier work suggested a role for an arrested RNA pol II complex at a DNA lesion (Donahue *et al.*, 1994), followed by participation of TFIIH in the recognition and incision/excision of the DNA damage, leading to preferential repair (Moggs *et al.*, 1996). Our work presented here suggests that TBP/TFIID, an essential component which nucleates the formation of an active transcription complex, recognizes and binds directly to DNA lesions induced by UV irradiation or cisplatin treatment.

TFIID/TBP directly binds damaged DNA

Using *in vitro* transcription challenge competition assays, we show that TBP/TFIID, either in the context of a crude cellular extract (WCE) and therefore in the presence of all repair proteins, or in the presence of all the general transcription factors and RNA pol II, associates with damaged DNA. This association appears to be relatively rapid and persistent, since changing either the time of preincubation of WCE with damaged DNA or increasing the pre-initiation time in the presence of both the competitor and AdMLP DNA had little effect on the overall level of inhibition induced by damaged DNA (data not shown). The so-called TATA-binding protein, TBP, either free or associated with the TBP-associated factors, TAFs (named TFIID), directly interacts with damaged DNA as demonstrated by nitrocellulose filter binding, gel shift and transcription competition experiments. This was also observed in reactions containing a TATA box, indicating that a lesion can bind TBP efficiently even in the presence of its specific (and natural) binding site. Moreover, the strength of interaction of TBP with either UV-, cisplatin- (the present study) or AAF- (unpublished results) damaged DNA will be a function of the nature of the damage and the surrounding sequences, which will each contribute to the overall distortion in the DNA helix.

Transcription challenge competition assays show that not only TBP but also TFIIB and TFIIH, although to a lower extent, are required to restore AdMLP transcription activity previously inhibited by the presence of a damaged DNA fragment (Figure 3B). This is not particularly surprising in light of the fact that TFIIH contains subunits with zinc finger motifs (Humbert *et al.*, 1994; data not shown) and has been shown to interact with TBP (Gérard *et al.*, 1991). However, attempts to demonstrate a specific association, using gel shift experiments, between TFIIH or TFIIB alone or in combination with TBP and damaged DNA were unsuccessful (data not shown). TFIIH recruitment to the excision complex was shown to occur through other factors present in the crude cellular extract (Nocentini *et al.*, 1997). It is possible that the association of TFIIB and TFIIH with a DNA lesion or with a TBP-damaged DNA complex could be rather weak compared with the interactions which are required during the formation of an active transcription complex. Partial restoration by TFIIB and TFIIH may therefore reflect some interactions with DNA lesions or the ability of these factors to disrupt binding of TBP to damaged DNA. If TBP alone exhibits a stronger affinity for damaged DNA, rather than for its natural TATA target, addition of TFIIB or TFIIH may stabilize existing binary TBP–TATA box complexes, displacing the thermodynamic equilibrium away from the damaged DNA complex towards the formation of a functional transcription initiation complex. The inability of TFIIA, previously shown to stabilize TBP–TATA box interactions (Buratowski *et al.*, 1989), to recover transcription inhibited by damaged DNA competitor may reflect this being exclusively a promoter function. It also must be borne in mind that the damaged cisplatin structure is not completely identical to the TATA box structure and thus may not be targeted equally by all the basal transcription factors, e.g. TFIIA, TFIIB and TFIIH.

In vivo evidence for TBP/TFIID binding to damaged DNA

To investigate a possible *in vivo* role of TBP/TFIID in the cellular response to DNA damage, we performed microinjection into living cells. The absence of an effect on UV-induced DNA repair synthesis, under conditions in which transcription was strongly inhibited, indicates that both processes, although requiring the participation of common factors such as TFIIH, are largely independent. Furthermore, the lack of squelching by excess TBP on NER suggests that TBP does not interact with essential NER factor(s), and is therefore not implicated directly in the NER process. However, microinjection of a well-defined concentration of TBP was found to protect cells from the reduction in transcription (overall RNA synthesis) caused by UV exposure, whereas microinjection of either TFIIB or the three components of the CAK complex (Rossignol *et al.*, 1997) had no significant effect. One possible explanation for the relief provided by TBP is that when exogeneously added, this protein binds directly to lesions that would otherwise have trapped endogenous transcription-competent TFIID or other SL1 or TFIIIB complexes (two transcription factors which include TBP and are associated with RNA pol I and RNA pol III transcription respectively). The result of microinjection then is to increase the pool of TBP-containing complexes such as TFIID (Colgan and Manley, 1992) which would be available for damage and/or promoter recognition. This conclusion is supported by our filter binding studies. The fact that TFIIB microinjection did not relieve transcription inhibition may reflect the weak affinity of TFIIB for the damage and/or its preference for the transcription reaction in the context of an *in vivo* situation in which TFIIB plays a crucial role in the activation process. Although the microinjection experiments are not simple to interpret, they fit well with our model in which TBP/TFIID binding to lesions is at least partly responsible for the general drop in transcription exerted by UV irradiation and other DNA-damaging treatments.

TBP recognizes a typical 3D structure

The almost perfect match between the TBP core, as found in its complex with the TATA box, and GGPG strongly supports the present results of a specific binding of TBP to cisplatin 1,2-adducts. In the former case, the TBP–TATA interaction is an induced fit, while in the latter, TBP seems to bind to a pre-formed, bent DNA in a lock-and-key fashion. As noted by Juo *et al.* (1996), TBP recognizes the intrinsic bendability of the TATA box more than the base pair sequence *per se*. It is the opening of the minor groove induced by the protein which allows a snug fit of the concave surface of TBP against DNA. In the 1,2-cisplatin G∧G adduct, the minor groove is already

exposed, inviting TBP. The limited number of polar interactions with the bases, as discussed above, discards any strong sequence specificity and favours a structural recognition. As for the relationship with the UV-damaged DNA, it is of interest to note that the crystal structure of an oligonucleotide containing a cyclobutane-type thymine dimer in complex with T4 endonuclease V also exhibits a sharp kink around the thymine dimer portion, splitting the duplex into two halves of B-DNA with a 60° inclination between the two helical axes (Vassylyev *et al.*, 1995), i.e. two 30° bends. In this case too, the DNA shape is very reminiscent of that of TATA bound to TBP, although the thymine dimer undergoes different constraints from its interaction with the endonuclease, including the flipping-out of the adenine base complementary to the 5′-thymine of the dimer, which allows the excision process to take place. The structural similarity of the deformation indicates that this type of UV-damaged DNA could also bend towards the major groove through interaction with TBP, due to the instability of the TT step introduced by the crosslinking of the two adjacent thymines.

It thus appears that various genotoxic and antitumour agents could turn GC-rich sequences into potential sites for TBP. This structural correlation supports the experimental observation that several damaging factors have similar effects, suggesting that in all cases the DNA bendability, with a marked tendency to adopt locally an A-form conformation with a flat, widened minor groove, is the common property.

Implications of TFIID/TBP binding to damaged DNA

The structural similarities between the TATA box and DNA lesions implies an important basal role for TBP. Indeed, TBP, either directly or within the context of one of the multiprotein complexes SL1, TFIID and TFIIIB, allows the initiation of transcription from the three classes of promoters. The damage caused by agents such as UV irradiation and cisplatin treatment results in an altered 3D structure of the DNA similar to the one adopted by the TATA sequence. These different lesions, forming a kind of TATA-like 3D structure, may then be recognized by TBP, with functional implications; they may serve as a lure for TFIID/TBP, diverting it from its natural promoter target, explaining the loss of transcription observed in cells after DNA damage. In addition, binding of TBP to damaged DNA could also serve to alter the equilibrium of TFIIH associated with transcription or repair complexes. Less TBP bound to promoter sequences would result in a decrease in the number of pre-initiation complexes to which TFIIH may be recruited and would lead to an increase in the availability and association of TFIIH with repair proteins. In this manner, TBP may stimulate the repair function of TFIIH indirectly. However, such a hypothesis does not exclude a possible role, if any, for TBP in the first step of NER, simply through the recognition of the lesion in conjunction with XPA and RPA, and the recruitment of TFIIH. It remains to be determined how TBP damage recognition leads to a decrease in overall or selective transcription, resulting in apoptosis. Binding of TBP could shield the lesion from repair proteins unless it can be translocated efficiently. Furthermore, the persistent presence of bound TBP may be responsible for the increased cytotoxicity of DNA-damaging agents in CS cells (Mayne and Lehmann, 1982).

Although this kind of molecular decoy has been proposed previously [hUBF, a transcription factor involved in rRNA synthesis, was shown to be hijacked by cisplatin adducts (Treiber *et al.*, 1994)], this is the first time that a functional consequence of this type of interaction (hijacking) has been demonstrated. Depending on the outcome, future anticancer drugs could be designed with the consideration of lesion recognition by TBP, taking into account the specific type of lesion, its affinity for TBP and its tendency to compete transcription.

Materials and methods

Materials

HeLa WCEs, as well as all the components of the *in vitro* reconstituted transcription system, were as described in Humbert *et al.* (1994).

Substrates used for *in vitro* transcription or filter binding analysis were generated by restriction digestion of either pUC309 or pSK plasmid DNA. pUC309 was created by ligation of an *Eco*RI–*Bam*HI fragment, corresponding to sequences –677 to +33 of the AdMLP (Δ –372/–34), to the *Bam*HI–*Sal*I fragment from pBR322. The resultant fragment was cloned into the *Eco*RI–*Sal*I sites of pUC19, generating the pUC309 plasmid. Competitor DNA fragments of 879 (F879) and 562 bp (F562) were generated by restriction digestion of pUC309 with *Bam*HI–*Ssp*I and *Eco*RI–*Sph*I, respectively (Figure 1B). The final competitor used was the 3 kb Bluescript, pSK+ plasmid (Stratagene). F562 and F879 were damaged by UV irradiation, at 0.1 mW/cm^2 with a germicidal UV-C lamp. pSK was treated with cisplatin for 15 h in the dark at 37°C and at a drug:nucleotide ratio of 0.005 (Hansson and Wood,1989).

The CP(–) or CP(+) fragments used in the filter binding assays were generated by restriction digestion with *Pvu*I of pSK undamaged or damaged by cisplatin. The AdMLP DNA probe (64mer) was created by annealing synthesized, complementary oligonucleotides corresponding to regions –40 to +24 of the AdMLP.

The 32mer 5′-TCTTCTTCTTCTTCT**GTG**CACTCTTCTTCTCT-3′ containing a single GpTpG (highlighted sequence) was allowed to react with cisplatin (Moggs *et al.*, 1996). After ethanol precipitation, the presence of a 1,3-intrastrand cisplatin d(GpTpG) DNA crosslink was confirmed by analysis of the oligonucleotide on a 12% acrylamide gel. The 36 bp DNA probe used in EMSA was created by annealing the damaged CP(+) or undamaged CP(–) DNA with its complementary oligonucleotide, leaving a 5′ overhang at each extremity. The DNA was filled and radiolabelled with [^{32}P]dATP (3000 Ci/mmol) in the presence of Klenow and purified on G50 Sephadex columns.

Crude transcription assay

Approximately 15–30 µg of HeLa WCE were incubated with varying amounts of competitor DNA in a 50 mM Tris–HCl pH 7.9 buffer containing 10% glycerol, 1 mM EDTA, 0.5 mM dithiothreitol (DTT) and 5 mM $MgCl_2$. Reactions (final volume 20 µl) were incubated for 15 min at 28°C, at which point 50 ng of the AdMLP template (*Eco*RI–*Sal*I) were added and pre-initiation of transcription allowed to continue for 15 min. Transcription was then initiated by addition of NTPs including [α-^{32}P]CTP (400 Ci/mmol). The final volume of the reaction was 25 µl, and transcription was carried out for 45 min at 28°C. The RNA transcripts were then analysed by autoradiography and quantified directly by counting on a PhosphoImage analyser.

The reconstituted transcription assay containing purified transcription factors TBP, TFIIA, TFIIB, TFIIE, TFIIH, TFIIF and RNA pol II was modified to include an initial incubation step to allow the potential binding of transcription factors to a damaged or undamaged fragment and carried out as described above. TFIID was derived from a subfraction of the TFIIH purification procedure (Gérard *et al.*, 1991).

Filter binding assay

Purified recombinant human TBP was combined with various DNA probes: UV-damaged or undamaged 879 bp fragment was labelled with [^{32}P]dATP (3000 Ci/mmol) using the Klenow fragment of DNA polymerase; the damaged or undamaged *Pvu*I fragment from plasmid pSK was labelled by exploiting the exonuclease activity of Klenow in the presence of [^{32}P]dCTP (3000 Ci/mmol) and subsequent filling by

addition of cold nucleotides before purification. Approximately 1 ng of probe corresponding to 5000 c.p.m. was combined with varying amounts of TBP, in 20 µl of the transcription buffer containing 60 µg/ml bovine serum albumin (BSA), 500 ng of poly(dGdC) and 5 mM $MgCl_2$, for 30 min at 30°C. Reactions were applied to a 0.45 mm nitrocellulose membrane (Millipore) using the 96-well Hybri-dot Manifold (BRL), pre-soaked in 0.4 mM KOH, washed with distilled water and pre-equilibrated in the reaction buffer without BSA. Filters were air dried and directly exposed to a PhosphoImage screen, for quantification, or a Biomax film (Kodak). One µl of input DNA corresponding to the same volume used in each reaction was spotted on Whatman filter paper as a control for determination of the percentage of DNA retained on nitrocellulose filters. The amount of radioactivity retained in the presence of TBP was measured, background counts (radioactivity retained in the absence of protein) subtracted, and the amount was divided by the level of radioactivity present in the input.

Miroinjection of rTBP into human fibroblasts

Microneedle injection into homopolykaryons of fibroblasts derived from a repair-competent individual (C5RO) and of a CS-B patient (CS1AN) was performed as described earlier (Vermeulen *et al.*, 1994). RNA synthesis was determined by pulse labelling the cells for 1 h with [^{3}H]uridine (10 µCi/µl), whereas NER was determined by [^{3}H]thymidine (10 µCi/µl) incorporation after UV irradiation (16 J/m^2) and autoradiography. TPB and TFIIB were diluted into phosphate-buffered saline (PBS) containing BSA.

Electrophoretic mobility shift assays

EMSA reaction mixtures (20 µl) contained 0.2 ng of ^{32}P-labelled 36 bp DNA probe (10 000 c.p.m.), 500 ng of poly(dGdC) in a 50 mM Tris–HCl pH 7.9 buffer containing 80 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 500 ng of BSA, 10% glycerol, 0.5 mM DTT, 0.01% NP-40, and rTBP and/or rTFIIB, when indicated. After 30 min of incubation at 30°C, glycerol was added to a final concentration of 20% and applied to a 4% native polyacrylamide gel. Protein–DNA complexes were electrophoresed in 25 mM Tris–19 mM glycine buffer at room temperature. Gels were dried and exposed to Biomax film (Kodak).

Structural analysis

The crystallographic coordinates of the human TBP–TATA box complex (Juo *et al.*, 1996) and of a double-stranded DNA dodecamer containing a central G∧G site (*cis*-[Pt(NH$_3$)$_2$-{d(GpG)-N7(G$_6$), N7(G$_7$)}] intrastrand crosslink) (Takahara *et al.*, 1995) were extracted from the PDB (Bernstein *et al.*, 1977; access codes 1TGH and 1GPG respectively). In the platinated DNA crystal structure, two duplexes are found in the asymmetric unit but appear to be almost identical (r.m.s.d. value of 0.3 Å between the two molecules). The structure superpositions were done using the LSQ options of the program O (Jones *et al.*, 1991). The TBP–DNA interfaces were analysed and displayed with the program GRASP (Nicholls *et al.*, 1993). For comparison, the Dickerson's dodecamer (Drew *et al.*, 1981) was used as canonical B-DNA (PBD code 1BNA), and noted BDNA.

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References

Aboussekhra,A.M. *et al*. (1995) Mammalian DNA nucleotide excision repair reconstituted with purified protein components. *Cell*, **80**, 859–868.

Arndt,K.M., Ricupero-Hovasse,S. and Winston,F. (1995) TBP mutants defective in activated transcription *in vivo*. *EMBO J*., **14**, 1490–1497.

Bernstein,F.C., Koetzle,T.F., Williams,G.J.B., Meyer,E.F., Brice,M.D., Rodgers,J.R., Kennard,O., Shimanouchi,T. and Tasumi,M. (1977) The Protein Data Bank: a computer-based archival file for macromolecular structures. *J. Mol. Biol*., **112**, 535–542.

Bohr,V.A., Smith,C.A., Okumoto,D.S. and Hanawalt,P.C. (1985) DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. *Cell*, **40**, 359–369.

Buratowski,S., Hahn,S., Guarente,L. and Sharp,P.A. (1989) Five intermediate complexes in transcription initiation by RNA polymerase II. *Cell*, **56**, 549–561.

Calsou,P. and Salles,B. (1994) Properties of damage-dependent DNA incision by nucleotide excision repair in human cell-free extracts. *Nucleic Acids Res*., **22**, 4937–4942.

Chasman,D.I., Flaherty,K.M., Sharp,P.A. and Kornberg,R.D. (1993) Crystal structure of yeast TATA-binding protein and model for interaction with DNA. *Proc. Natl Acad. Sci. USA*, **90**, 8174–8178.

Colgan,J. and Manley,L. (1992) TFIID can be rate limiting *in vivo* for TATA-containing, but not TATA-lacking, RNA polymerase II promoters. *Genes Dev*., **6**(2), 304–315.

Davison,B.L., Egly,J.-M., Mulvihill,E.R. and Chambon,P. (1983) Formation of stable preinitiation complexes between eukaryotic class B transcription factors and promoter sequences. *Nature*, **301**, 680–686.

Donahue,B.A., Yin,S., Taylor,J.S., Reines,D. and Hanawalt,P.C. (1994) Transcript cleavage by RNA polymerase II arrested by a cyclobutane pyrimidine dimer in the DNA template. *Proc. Natl Acad. Sci. USA*, **91**, 8502–8506.

Drew,H.R., Wing,R.M., Takano,T., Broka,C., Tanaka,S., Ikatura,K. and Dickerson,R.E. (1981) Structure of a B-DNA dodecamer: conformation and dynamics. *Proc. Natl Acad. Sci. USA*, **78**, 2179–2183.

Evans,S.V. (1993) SETOR: hardware lighted three-dimensional solid model representations of macromolecules. *J. Mol. Graphics*, **11**, 134–138.

Gérard,M., Fischer,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. Chem*., **266**, 20940–20945.

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

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.

Iyer,N., Reagan,M.S., Wu.,K.-J., Canagarajah,B. and Friedberg,E.C. (1996) Interactions involving the human RNA polymerase II transcription:nucleotide excision repair complex TFIIH, the nucleotide excision repair protein XPG, and Cockayne syndrome group B (CSB) protein. *Biochemistry*, **35**, 2157–2167.

Jones,C.J. and Wood,R.D. (1993) Preferential binding of the xeroderma pigmentosum group A complementing protein to damaged DNA. *Biochemistry*, **32**, 12096–12104.

Jones,T.A., Zou,J.Y., Cowan,S.W. and Kjeldgaard,M. (1991) Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr*., **A47**, 110–119.

Juo,Z.S., Chiu,T.K., Leiberman,P.M., Baikalov,I., Berk,A.J. and Dickerson,R.E. (1996) How proteins recognize the TATA box. *J. Mol. Biol*., **261**, 239–254.

Kim,J.L., Nikolov,D.B. and Burley,S.K. (1993) Co-crystal structure of TBP recognizing the minor groove of a TATA element. *Nature*, **365**, 520–527.

Kim,Y., Geiger,J.H., Hahn,S. and Sigler,P.B. (1993) Crystal structure of a yeast TBP/TATA-box complex. *Nature*, **365**, 512–520.

Leadon,S.A. and Lawrence,D.A. (1991) Strand-selective repair of DNA damage in the yeast GAL7 gene requires RNA polymerase II. *J. Biol. Chem*., **267**, 23175–23182.

Lee,M. and Struhl,K. (1995) Mutations on the DNA-binding surface of TATA-binding protein can specifically impair the response to acidic activators *in vivo*. *Mol. Cell. Biol*., **15**, 5461–5469.

Mayne,L.V. and Lehmann,A.R. (1982) Failure of RNA synthesis to recover after UV irradiation: an early defect in cells from individuals with Cockayne's syndrome and xeroderma pigmentosum. *Cancer Res*., **42**, 1473–1478.

Mellon,I., Spivak,G. and Hanawalt,P.C. (1987) Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. *Cell*, **51**, 241–249.

Moggs,J.G., Yarema,K.J., Essigmann,J.M. and Wood,R.D. (1996) Analysis of incision sites produced by human cell extracts and purified proteins during nucleotide excision repair of a 1,3-intrastrand d(GpTpG)-cisplatin adduct. *J. Biol. Chem*., **271**, 7177–7186.

Nakajima,N., Horikoshi,M. and Roeder,R.G. (1988) Factors involved in specific transcription by mammalian RNA polymerase II: purification, genetic specificity, and TATA box-promoter interactions of TFIID. *Mol. Cell. Biol*., **8**, 4028–4040.

Nicholls,A. (1993) *GRASP: Graphical Representation and Analysis of Surface Properties*. Columbia University, New York.

Nocentini,S., Coin,F., Saijo,M., Tanaka,Y. and Egly,J.-M. (1997) DNA damage recognition by XPA protein promotes efficient recruitment of TFIIH. *J. Biol. Chem*., **272**, 22991–22992.

Park,C.H., Mu,D., Reardon,J.T. and Sancar,A. (1995) The general transcription-repair factor TFIIH is recruited to the excision repair complex by the XPA protein independent of the TFIIE transcription factor. *J. Biol. Chem*., **270**, 4896–4902.

Rossignol,M., Kolb-Cheynel,I. and Egly,J.-M. (1997) Substrate specificity of the cdk-activating kinase (CAK) is altered upon association with TFIIH. *EMBO J*., **16**, 1628–1637.

Svejstrup,J.C., Vichi,P. and Egly,J.-M. (1996) The multiple roles of transcription/repair factor TFIIH. *Trends Biochem. Sci*., **21**, 346–350.

Sweder,K.S. and Hanawalt,P.C. (1992) Preferential repair of cyclobutane pyrimidine dimer in the transcribed strand of a gene in yeast chromosomes and plasmids is dependent on transcription. *Proc. Natl Acad. Sci. USA*, **89**, 10696–10700.

Takahara,P.M., Rosenzweig,A.C., Frederick,C.A. and Lippard,S.J. (1995) Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin. *Nature*, **377**, 649–652.

Treiber,D.K., Zhai,X., Jantzen,H.-M. and Essigman,J.M. (1994) Cisplatin–DNA adducts are molecular decoys for the ribosomal RNA transcription factor hUBF (human upstream binding factor). *Proc. Natl Acad. Sci. USA*, **91**, 5672–5676.

Vassylyev,D.G., Kashiwagi,T., Mikami,Y., Ariyoshi,M., Iwai,S., Ohtsuka,E. and Morikawa,K. (1995) Atomic model of a pyrimidine dimer excision repair enzyme complexed with a DNA substrate: structural basis for damaged DNA recognition. *Cell*, **83**, 773–782.

Vermeulen,W. *et al*. (1994) 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.

Zamble,D.B. and Lippard,S.J. (1995) Cisplatin and DNA repair in cancer chemotherapy. *Trends Biochem. Sci*., **20**, 435–439.

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