Interconversion between active and inactive TATA-binding protein transcription complexes in the mouse genome

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ABSTRACT

The TATA binding protein (TBP) plays a pivotal role in RNA polymerase II (Pol II) transcription through incorporation into the TFIID and B-TFIID complexes. The role of mammalian B-TFIID composed of TBP and B-TAF1 is poorly understood. Using a complementation system in genetically modified mouse cells where endogenous TBP can be conditionally inactivated and replaced by exogenous mutant TBP coupled to tandem affinity purification and mass spectrometry, we identify two TBP mutations, R188E and K243E, that disrupt the TBP–BTAF1 interaction and B-TFIID complex formation. Transcriptome and ChIP-seq analyses show that loss of B-TFIID does not generally alter gene expression or genomic distribution of TBP, but positively or negatively affects TBP and/or Pol II recruitment to a subset of promoters. We identify promoters where wild-type TBP assembles a partial inactive preinitiation complex comprising B-TFIID, TFIIB and Mediator complex, but lacking TFIID, TFIIE and Pol II. Exchange of B-TFIID in wild-type cells for TFIID in R188E and K243E mutant cells at these primed promoters completes preinitiation complex formation and recruits Pol II to activate their expression. We propose a novel regulatory mechanism involving formation of a partial preinitiation complex comprising B-TFIID that primes the promoter for productive preinitiation complex formation in mammalian cells.

INTRODUCTION

Accurate initiation of transcription by RNA polymerase II (Pol II) requires the assembly of the multiprotein preinitiation complex (PIC) on the core promoter around the mRNA start site (1–3). Amongst the basal transcription factors in this process is the TFIID complex comprising the TATA binding protein (TBP) and a set of 13–14 TBP-associated factors (TAFs) (4–7). Two additional TBP-containing complexes involved in Pol II transcription have been described, the B-TFIID complex, where TBP is associated with BTAF1 and TAC, a complex found in undifferentiated embryonic stem cells, where TBP is associated with an unprocessed form of basal transcription factor TFIIA (8). BTAF1 and its yeast orthologue Mot1p belong to the SNF2 superfamily of ATPases and they can dissociate TBP from DNA in an ATP-dependent manner (9–12). While it was first proposed that Mot1p may be a transcriptional repressor, genome-wide studies show that it is associated with active promoters where it may use ATP hydrolysis to promote a dynamic equilibrium of promoter occupancy between a transcriptionally inactive Mot1p–TBP–NC2 complex and an active TFIID complex (13–15).

The highly conserved TBP C-terminal core domain binds DNA and interacts with basal transcription

factors and a host of other regulatory proteins (16). The structure of TBP bound to TATA-containing DNA, as well as with the basal transcription factors TFIIA, TFIIB, NC2, BRF and the N-terminal domain of TAF1 have all been described (17–21). Surfaces involved in these interactions have been mutated as part of a systematic mutagenesis of all solvent-exposed residues (22–25). The properties of the mutant TBPs have been evaluated *in vitro* with respect to transcription using various Pol II and Pol III promoters, binding to DNA, interaction with co-factors and the ability to form higher-order DNA complexes *in vitro* with its partners, and their ability to support activated transcription in transfected mammalian cells (22,26).

While previous studies provided considerable insight into the structure–function relationships of TBP in mammalian cells, their scope has been limited to *in vitro* assays or transfections with artificial promoters that may not reproduce the complexity of the *in vivo* situation with the diversity of promoters in mammalian cells. We have developed a unique approach to study TBP function *in vivo* using mouse embryonic fibroblasts (MEFs) where one allele of the TBP gene has been inactivated and the other has been floxed such that endogenous TBP can be inactivated by Cre-recombinase. We have performed complementation assays showing that TBP mutations affecting critical interactions *in vitro* can complement loss of endogenous TBP, but lead to impaired cell proliferation. We identify TBP mutations which disrupt the TBP–BTAF1 interaction and show that loss of B-TFIID complex formation does not affect the global genomic distribution of TBP, but positively or negatively affects PIC formation and Pol II recruitment at a selected set of promoters.

MATERIALS AND METHODS

Isolation of the $Tbp^{lox/-}$ MEFs

Mouse ES cells with a null allele of TBP (27) were re-targeted with a second vector to introduce LoxP sites around exon III of the *Tbp* gene. These ES cells were used to generate mice with the corresponding genotype. MEFs were derived from E10.5 embryos by standard procedures and immortalized using large T antigen from SV40.

Generation and characterization of cells expressing mutant TBPs

$Tbp^{lox/-}$ MEFs were first infected with pBABE retro-viruses expressing WT or mutant N-terminal Flag-HA tagged TBP. After puromycin selection, cell extracts were prepared and expression of the exogenous TBP verified by immunoblot. Cells were reinfected with a second retrovirus vector expressing the tamoxifen inducible Cre-ER^{T2}. After blasticidine selection, cells were treated with hydroxy-tamoxifen (OHT) and clonal lines established from limiting dilutions of cells. The deletion of the *Tbp* alleles was verified first by triplex polymerase chain reaction (PCR) genotyping and then cell extracts were prepared from the selected cell clones

and TBP expression verified by immunoblot. Cell proliferation analysis, Affymetrix microarrays, qPCR and immunoblot were all performed by standard methods as previously described (28). TBP was detected using the 3G3 antibody that recognizes an epitope at the extreme N-terminus shared between mouse and human TBP (29).

Chromatin immunoprecipitation -sequencing

Chromatin immunoprecipitation (ChIP) and ChIP-seq experiments were performed according to standard protocols as previously described (30,31). Briefly, ChIP-seq was performed using an Illumina GAIIx sequencer and the raw data analysed by the Illumina Eland pipeline V1.6. Peak detection was performed using the MACS software (http://liulab.dfci.harvard.edu/MACS/) (32) under settings where the GFP ChIP was used as a negative control. Peaks were then annotated using GPAT [(33), http://bips.u-strasbg.fr/GPAT/Gpat_home.html] using a window of ±10 kb with respect to the coordinates of the beginning and end of RefSeq transcripts. Global clustering analysis and quantitative comparisons were performed using seqMINER [(34) (http://bips.u-strasbg.fr/seqminer/]. Further details are provided in the Supplementary Data. ChIP was performed with the following antibodies; TBP (Abcam, AB28175), Pol II, MED6, XPB, TFIIB (Santa Cruz SC-9001, SC-9434, C18-CS225, respectively), H3K4me3 (Upstate 07-473). The TAF1 antibody was a kind gift from Dr. L. Tora.

Proteomics analysis

MEF cell nuclear extracts were prepared as previously described and subjected to tandem Flag-HA affinity immunopurification using Flag M2 beads, epitope peptide elution followed by reprecipitation with HA-beads as previously described (35). The precipitated proteins were digested with trypsin, and the tryptic peptides separated by tandem strong cation exchange. Peptides were then analysed by nanoflow-LC coupled to an LTQ-FTICR mass spectrometer (Thermo Fisher Scientific). Spectra were processed with Bioworks (version 3.1, Thermo Fisher Scientific) and the subsequent data analysis was carried out using Mascot (version 2.2.1, Matrix Science). Mascot results files were filtered to contain only peptides above a Mascot ion score of 15 using in-house written software. Scaffold (version 2.01.02, Proteome Software) was used to validate protein identifications. Protein identifications were accepted if they could be established at >99.9% probability and contained at least two identified peptides. Human TBP and its variants were identified following essentially the same procedure, but using a manually generated database containing sequences of mouse TBP (Swiss-Prot accession number P29037), human TBP (Swiss-Prot accession number P20226), and those of the TBP mutants used in this study. Further details are described in the Supplementary Data.

RESULTS

Mutations in TBP complementing loss of wild-type TBP in mouse cells

Using homologous recombination, we generated ES cells and mice harbouring a null allele in the *Tbp* gene by insertion of a hygromycin resistance cassette in exon III and a floxed allele, in which exon III is surrounded by LoxP sites [(27) and see Supplementary Figure S1A]. From these mice, we generated an embryonic fibroblast *Tbp*$^{lox/-}$ cell line in which TBP expression can be inactivated by expression of the Cre recombinase leading to cell death (see Supplementary Figure S1C and S1D). We adopted a two-step strategy to generate *Tbp*$^{-/-}$ cell lines expressing human (h)TBP. Cells were first infected with pBABE retrovirus vectors expressing Wt hTBP or a series of mutants in the TBP core region (Figure 1A), all of which carry a Flag-HA tag on their N-terminus. Analysis of infected cell populations revealed the presence of endogenous mTBP and exogenous hTBP in most lines (Figure 1B, lanes 2, 3 and 5), but for several mutants we were reproducibly unable to observe stable expression of mutant hTBP (lanes 1 and 4, summarized in Figure 1D). These mutants comprise radical single amino acid substitutions on the DNA binding surface of the first repeat (R203E and T210K), the H2 helix (R239E) or the TFIIB interaction surface on the C-terminal stirrup (E286R, Figure 1A).

Cells expressing endogenous mTBP and exogenous hTBP were then infected with a second retrovirus expressing the four hydroxy-tamoxifen (OHT) inducible Cre-ER^{T2}. Subsequently, multiple clonal populations from the OHT treated cells were isolated and genotyped by PCR to identify the *Tbp*$^{-/-}$ clones (Supplementary Figure S1B). At least two independent clones where expression of the endogenous mTBP was lost and replaced by the exogenous hTBP were isolated (Figure 1C). Each mutant hTBP was expressed at levels comparable, but not identical to that of the endogenous mTBP in the *Tbp*$^{lox/-}$ cells, with V162A, Q242A and R318A showing higher expression. These results indicate that all of these mutants are capable of supporting cell viability, irrespective of their previously characterized properties *in vitro* or in transfection assays (see refs 22, 23 and 25) summarized in Figure 1D. Although many mutant hTBPs can complement loss of mTBP, the majority of cell lines display a moderately or strongly reduced proliferation (Supplementary Figure S2, summarized in Figure 1D). The R186E mutation, on the other hand, led to increased proliferation. At least two independent clones for each mutant were analysed to ensure the reproducibility of their phenotypes. Slow growth is particularly severe for the L212A, K243E and R294A mutants. Analysis of several mutant lines by fluorescence-activated cell sorting (FACS) analysis did not however reveal specific G1/S or G2/M checkpoint arrest, rather a general lengthening of all phases of the cycle. The slow growth seems to result from defects in Pol II transcription as none of the mutations had a significant effect on Pol I and/or Pol III transcription (Supplementary Figure S3).

TBP mutations that disrupt the B-TFIID complex

The properties of the hTBP mutants used here have been previously evaluated in various *in vitro* assays for their ability to interact with DNA, TFIIA, TFIIB, NC2 and BTAF1 and/or for their ability to form ternary complexes comprising TBP, DNA and one of the additional cofactors [(22–25), summarized in Figure 1D]. We took advantage of the N-terminal Flag-HA tag to perform tandem affinity purification of Wt and mutant hTBPs from nuclear extracts of the corresponding cell lines to address how these mutations affect TBP interaction with its partners. Mass spectrometry identified TBP and all TFIID TAFs in purifications from cells expressing Wt hTBP, but not in control purifications from untagged *Tbp*$^{lox/-}$ cells (Figure 2A and Supplementary Dataset S1 showing results of two independent experiments, Wt hTBP and R188E with a negative control, and Wt hTBP, a negative control and a series of mutants). BTAF1, BRF and all four SL1/TIF1B subunits were also present in Wt hTBP samples. Under the purification conditions used we did not detect any spectra for NC2 or TFIIB, and only low numbers of spectra for TFIIA (Supplementary Dataset S1). In these experiments, spectra for more than 600 proteins were identified (Supplementary Dataset S1). In addition to the known TAF partners, only a small number of other proteins showed a pattern consistent with a specific association with TBP, i.e. absent in the control samples, but present in the Wt and in several of the mutant samples (Supplementary Dataset S1).

Analysis of the mutant TBP samples showed that none of the tested mutations significantly affected interaction of TBP with TFIID-TAFs. Several mutations appeared to affect interaction with the SL1 subunits, in particular TAFIC, but the number of recorded spectra for these subunits is in general low and a significant loss of this interaction could not be confirmed by immunoblotting (Supplementary Figure S3B). In contrast, two mutations (R188E and K243E) clearly resulted in a complete loss of BTAF1 interaction. No spectra corresponding to this protein were found in these samples, while abundant spectra were observed with Wt hTBP (Figure 2A). Loss of interaction was confirmed by immunoblotting where BTAF1 coprecipitates with Wt hTBP, but not with R188E and K243E, whereas TAF4 and TAF6 coprecipitated with all three TBPs (Figure 2B). We also noted significantly reduced BTAF1 levels in nuclear extracts from the R188E and K243E cells not due to changes in *Btaf1* mRNA level, but more likely due to its instability when not associated with TBP. Nevertheless, we did not detect residual BTAF1 in the R188E and K243E TBP immunoprecipitations. Amongst the partners detected by mass spectrometry, these two mutations, therefore, lead to a specific loss of interaction with BTAF1 disrupting B-TFIID complex formation in mammalian cells. Their effect on other partners such as TFIIA could not be assessed in this assay.

Further evidence for a role of the R188 and K243 residues for interaction with BTAF1 comes from examination of the structure of the Encephalitozoon Cuniculi (Ec) TBP–BTAF1 complex that has been recently
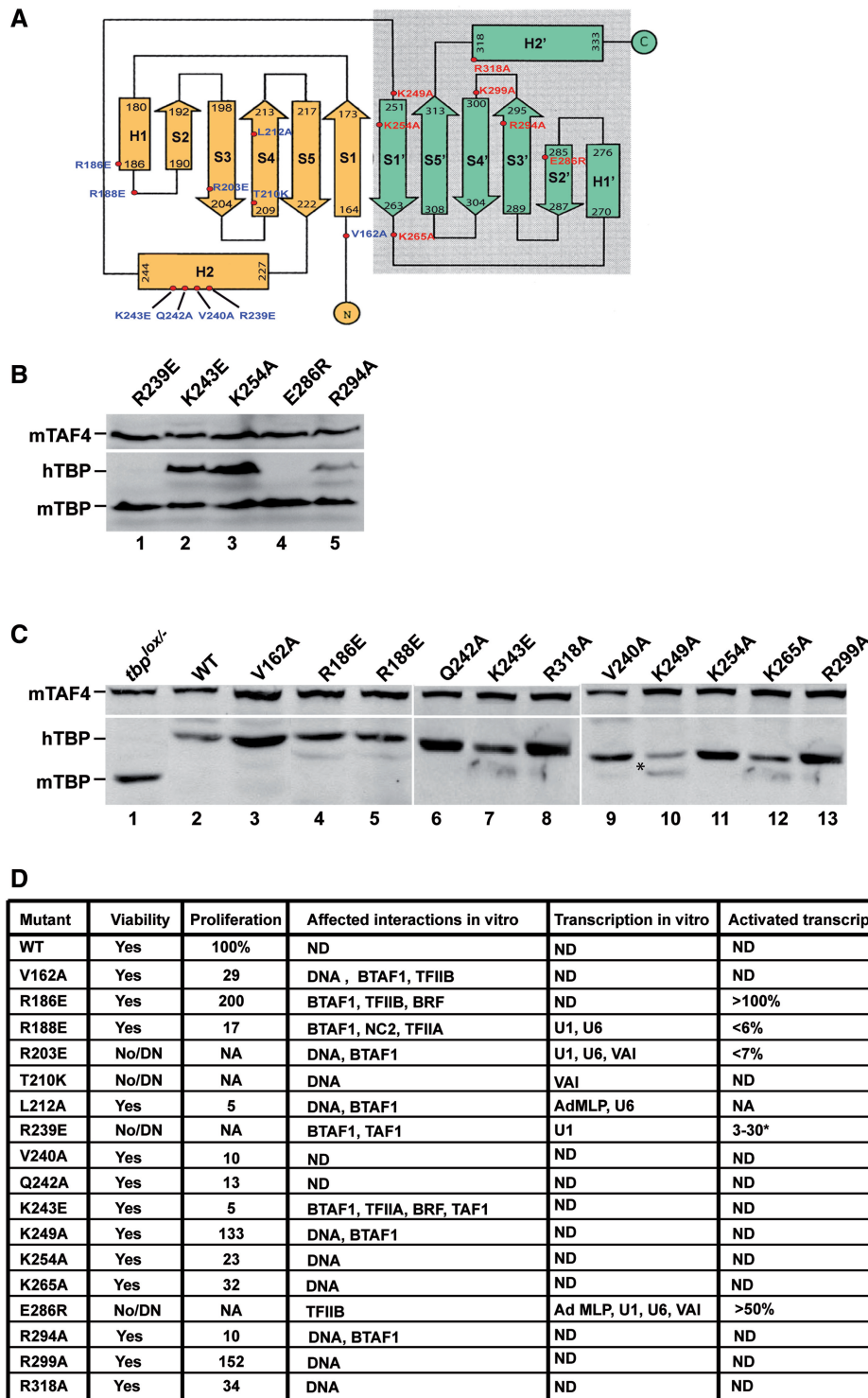
Figure 1. Viability and proliferation properties of MEFs expressing mutant TBP. (**A**) Locations of the tested mutations on a two-dimensional representation of the ternary structure of the TBP core domain. (**B**) Immunoblot analysis of endogenous mouse and exogenous human TBP in MEFs infected with retroviruses expressing the TBP mutants shown above each lane. Endogenous mouse TAF4 is shown as loading control. (**C**) Immunoblot analysis of cloned cell lines expressing the indicated exogenous hTBP mutants. The asterisk indicates that in some extracts the Flag-HA tag has been cleaved from TBP during extract preparation. (**D**) Summary of cell lines expressing mutant TBPs, showing the mutated amino acid, cell viability, cellular proliferation and interactions shown to be affected in *in vitro* assays (effects on TBP/DNA complex formation, Pol II/Pol III transcription from an assayed promoter and on activation in transient expression assays). ND is not determined and DN is dominant negative.
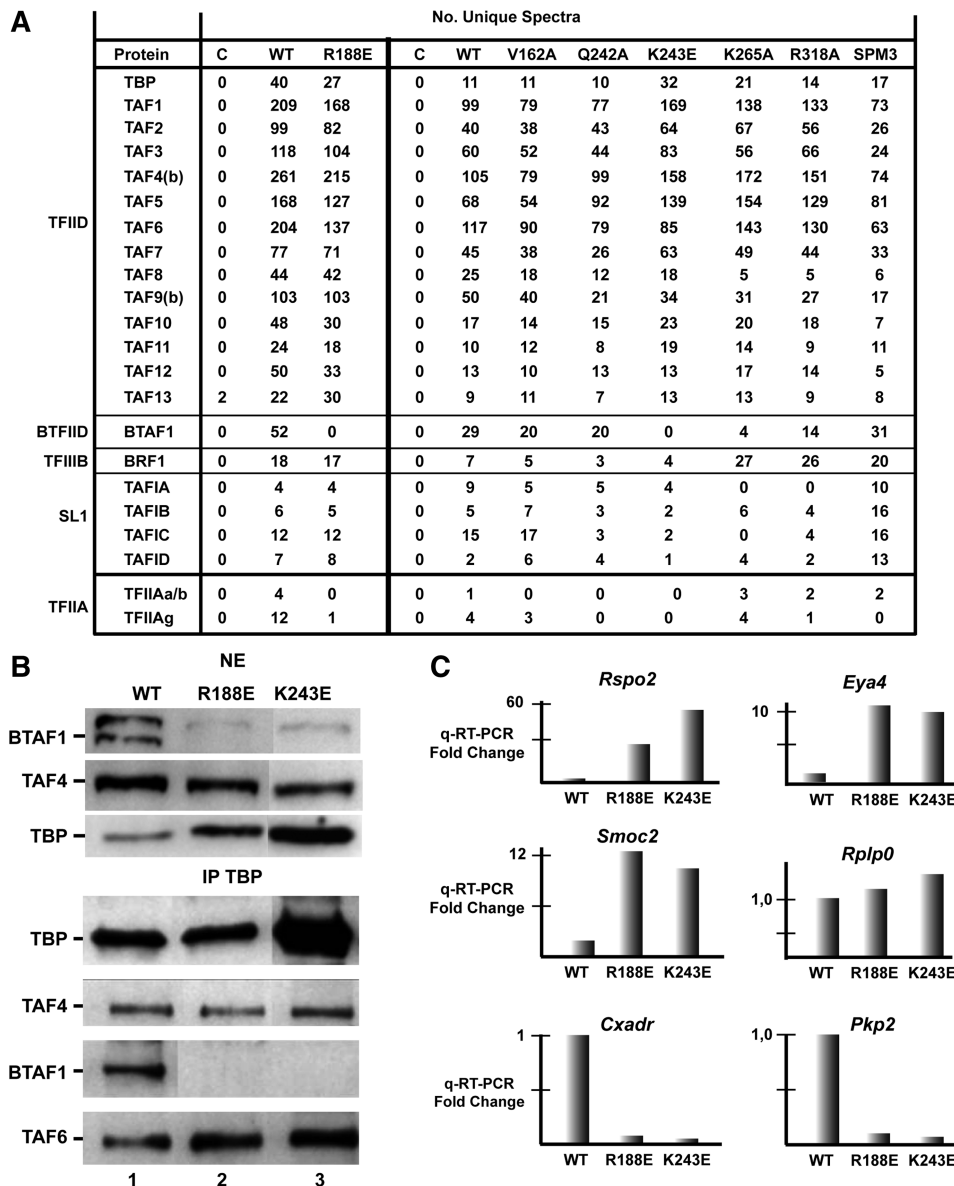
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	Protein		No. Unique Spectra									
		C	WT	R188E	C	WT	V162A	Q242A	K243E	K265A	R318A	SPM3
TFIID	TBP	0	40	27	0	11	11	10	32	21	14	17
	TAF1	0	209	168	0	99	79	77	169	138	133	73
	TAF2	0	99	82	0	40	38	43	64	67	56	26
	TAF3	0	118	104	0	60	52	44	83	56	66	24
	TAF4(b)	0	261	215	0	105	79	99	158	172	151	74
	TAF5	0	168	127	0	68	54	92	139	154	129	81
	TAF6	0	204	137	0	117	90	79	85	143	130	63
	TAF7	0	77	71	0	45	38	26	63	49	44	33
	TAF8	0	44	42	0	25	18	12	18	5	5	6
	TAF9(b)	0	103	103	0	50	40	21	34	31	27	17
	TAF10	0	48	30	0	17	14	15	23	20	18	7
	TAF11	0	24	18	0	10	12	8	19	14	9	11
	TAF12	0	50	33	0	13	10	13	13	17	14	5
	TAF13	2	22	30	0	9	11	7	13	13	9	8
BTFIID	BTAF1	0	52	0	0	29	20	20	0	4	14	31
TFIIIB	BRF1	0	18	17	0	7	5	3	4	27	26	20
SL1	TAFIA	0	4	4	0	9	5	5	4	0	0	10
	TAFIB	0	6	5	0	5	7	3	2	6	4	16
	TAFIC	0	12	12	0	15	17	3	2	0	4	16
	TAFID	0	7	8	0	2	6	4	1	4	2	13
TFIIA	TFIIAa/b	0	4	0	0	1	0	0	0	3	2	2
	TFIIAg	0	12	1	0	4	3	0	0	4	1	0

Figure 2. Effects of TBP mutations on the interactions with partner proteins. (**A**) Mass spectrometry analysis showing the number of unique spectra obtained for the each partner proteins after purification of the indicated TBP mutant. C is a parallel control immunoprecipitation from non-tagged cells. Two independent series of experiments are shown along with corresponding negative control. Spectra for TAF4 and TAF4b, TAF9 and TAF9b are grouped together. Partner proteins are grouped by complexes. (**B**) Immunoblot analysis of TBP, BTAF1, TAF4 and TAF6 in immunoprecipitates from cells expressing the indicated TBP mutants. (**C**) RT-qPCR analysis of mRNA expression. The relative expression levels of the indicated genes in the cells expressing TBP R188E and K243E are shown.

resolved (36). The R188 and K243 residues are conserved in EcTBP (R48 and K103, respectively, in Figure 3A) and are at the interface (Figure 3B) where they make contacts with residues in BTAF1 (Figure 3C and ref. 36). These structural observations confirm our present results and previous *in vitro* biochemical studies on the essential role of these TBP residues in B-TFIID complex formation.

Changes in gene expression in cells lacking B-TFIID

To determine the effect of loss of the TBP–BTAF1 interaction on gene expression, we performed a detailed transcriptome analysis on two independently isolated Wt hTBP and R188E mutant lines. Compared to MEFs expressing Wt hTBP, 274 and 200 genes were up- or down-regulated 2-fold or more, respectively, in R188E TBP MEFs (Supplementary Dataset S2) corresponding to ~4% of the total expressed genes. Amongst these, are genes that are either strongly induced or repressed. The mRNA profiling results were verified by RT-qPCR confirming that expression of R-spondin 2 (*Rspo2*), eyes absent 4 homolog (*Eya4*) and SPARC related modular calcium binding 2 (*Smoc2*) are up-regulated in the TBP R188E cells, while plakophilin 2 (*Pkp2*) and coxsackie
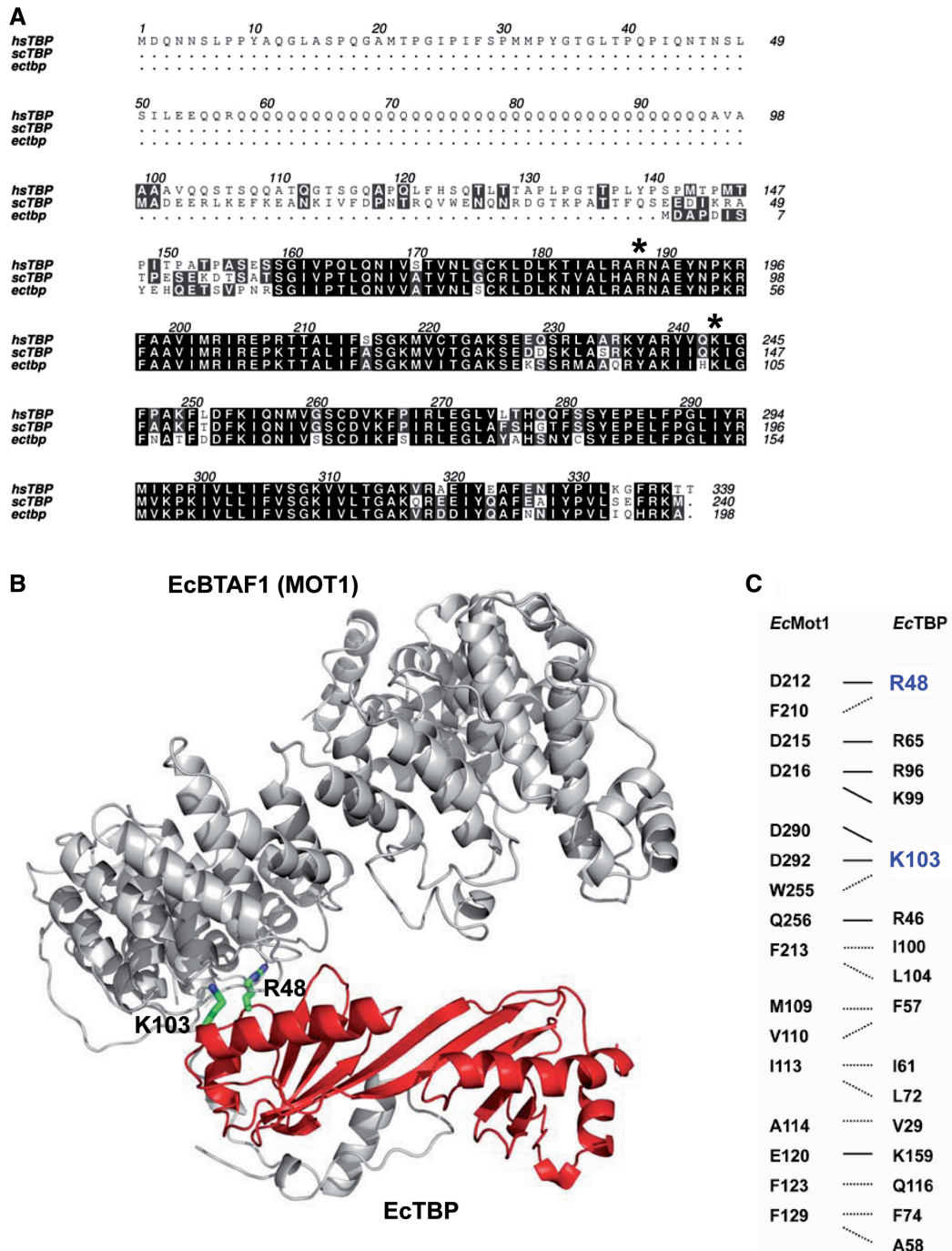
Figure 3. Role of the R188 and K243 residues in TBP–BTAF1 interactions. (**A**) Alignment of human, yeast (*S. cerevisiae*) and the parasite Encephalitozoon Cuniculi (Ec) TBPs showing the conservation of the R188 and K243 residues (asterisks). (**B**) Ribbon representation of the structure of EcTBP and BTAF1 (Mot1p). The side chains of EcTBP R48 and K103 are indicated. (**C**) The contacts made between EcTBP R48 and K103 and the EcBTAF1 residues are summarized. Figure adapted from ref. (36).

virus and adenovirus receptor (*Cxadr*) were down-regulated (Figure 2C). *Rplp0*, encoding an acidic ribosomal protein, was unchanged and is used as a control in subsequent experiments (Figure 2C and see below). Thus, the R188E mutation that destabilizes formation of the B-TFIID complex does not have a general effect on transcription, but rather positively or negatively affects expression of a selected set of genes.

Comparison of genome-wide occupancy of Wt and R188E TBP

To determine whether loss of B-TFIID integrity affects TBP promoter occupancy, we performed ChIP-seq experiments on cells expressing Wt or R188E mutant TBPs. ChIP was performed with antibodies against TBP, Pol II and trimethylated lysine 4 of histone H3 (H3K4me3) and

the ChIPed DNA analysed by Illumina sequencing. Peak detection ('Materials and Methods' section and Supplementary Data), identified ~13 000 binding sites for Wt TBP in the genome. The majority of these are located at the transcription start sites (TSS) of annotated genes, although TBP binding sites can also be found in intergenic regions with or without associated Pol II (Supplementary Figure S4A and S4B). The specificity of the ChIPs was verified by ChIP-seq with an anti-GFP antibody and by independent ChIP-qPCR experiments (Supplementary Figure S5A). Analysis of the sequences at the TBP occupied sites showed that around 5% contained the 5′-TATAW-3′ consensus for TBP binding. This is similar to the general frequency of TATA elements in Pol II promoters (37). Moreover, this sequence is not enriched in the 1000 most occupied sites indicating that TATA-containing promoters do not show higher than average TBP occupancy (Supplementary Figure S5B).

At the control TATA-containing *Rplp0* promoter comparable TBP occupancy can be seen in the Wt and R188E-expressing cells along with Pol II occupancy at the promoter and transcribed regions and H3K4me3 at the 5′ region (Figure 4A). Analogous profiles for Wt TBP, Pol II and H3K4me3 are seen at the majority of expressed genes (Supplementary Figure S5C). A global comparison of the ChIP-seq data for each cell type by read density clustering analysis shows that TBP and Pol II are present at approximately 10 000 RefSeq start sites corresponding to the different classes of expressed genes (Figure 4B). In addition, there is no gross quantitative re-distribution of TBP at the TSS in cells expressing the hTBP R188E mutation (Supplementary Figure S5C), consistent with the observation of only a small number of genes showing altered expression. Thus, loss of B-TFIID complex formation does not result in a global redistribution of TBP at TSS. Nevertheless, comparison of TBP Wt and R188E occupied loci revealed a group of more than 4000 sites that appeared enriched in the R188E expressing cells (Supplementary Figure S6A). Further re-clustering of this group identified 1334 loci that are enriched in the R188E cells and 397 loci that display a low occupancy of R188E TBP, but show no significant signal for Wt TBP. This is confirmed by a quantitative comparison showing that these later sites are weakly occupied by TBP R188E, but show no significant occupancy by Wt TBP (Supplementary Figure S6B). The vast majority of these sites are located in intergenic and intragenic regions, rather than at the TSS of annotated genes.

Differential Pol II recruitment at R188E TBP-regulated genes

To understand the molecular basis underlying the changes in gene expression in the R188E TBP cells, we more closely examined TBP and Pol II occupancy at the loci of 172 most differentially regulated genes. A quantitative comparison of the up-regulated genes indicated a correlation with an increased Pol II occupancy, while changes in TBP occupancy are in general less marked (Figure 4C). This suggests that Wt TBP is present at these promoters without a subsequent recruitment of Pol II. For example,

at the *Rspo2* promoter in Wt TBP cells, both H3K4 trimethylation and TBP occupancy can be clearly observed, but Pol II is absent (Figure 5A). In the R188E-expressing cells, increased signals for TBP and H3K4me3 are observed, but Pol II is now strongly recruited to the promoter. These observations were confirmed in independent ChIP-qPCR experiments showing the presence of a basal level of Wt TBP that is increased with R188E TBP, whereas Pol II is recruited only by TBP R188E (Figure 5B). A similar situation was seen at the *Eya4* promoter where Pol II is observed only in the R188E TBP cells (Figure 5C and D).

One interpretation of these observations is that, while TFIID containing Wt TBP can initiate PIC formation, only TFIID containing R188E TBP is capable of recruiting Pol II to the *Rspo2* and *Eya4* promoters. Alternatively, it is possible that Wt TBP occupies these promoters in the B-TFIID complex, while in the R188E-expressing cells they are occupied by TBP present in TFIID. ChIP-qPCR experiments detected TFIIB and MED6 occupancy at both promoters in the Wt and R188E TBP cells showing that PIC formation had been initiated in each case (Figure 5B and D), while TFIIE is seen only in the R188E TBP cells. In contrast, BTAF1 is detected at the *Rspo2* and *Eya4* promoters in the Wt, but not in the R188E mutant cells, whereas the TFIID-specific TAF1 is recruited only in the TBP R188E cells. Similar results were seen for each of these factors in cells expressing the K243E mutation (Figure 5B and D), which also affects B-TFIID formation and expression of *Rspo2* and *Eya4* is strongly activated in these cells (Figure 2C).

As the R188E and K243E mutations have also been reported to affect interaction with TFIIA (22,23,25), we asked if TFIIA could be differentially recruited to the *Rspo2* and *Eya4* promoters. Anti-TFIIA ChIP readily detected TFIIA at the *Rplp0* promoter in both Wt and mutant TBP expressing cells (Supplementary Figure S5A), but no significant signal for TFIIA could be seen at the *Rspo2* and *Eya4* promoters in any cell type (data not shown). Thus, TFIIA does not seem to be recruited to these promoters, whereas the transcriptional effects that are seen correlate with differential B-TFIID and TFIID recruitment.

To further show that TFIIB and BTAF1 or TAF1 co-occupy the above promoters, we performed a TFIIB ChIP and then reChIPed the eluate with either BTAF1 or TAF1 antibodies (Figure 6A). Co-occupancy of TFIIB and TAF1, but not BTAF1, was seen at the *Rplp0* promoter in TBP Wt and R188E and K243E mutant cells (Figure 6B). In contrast, TFIIB–BTAF1 co-occupancy was seen at the *Eya4* and *Rspo2* promoters in TBP Wt cells, whereas TFIIB–TAF1 co-occupancy was seen in the TBP R188E and K243E expressing cells. No significant signal was seen with any combination at the control *Tnp1* promoter.

The combination of ChIP-seq, ChIP-qPCR and ChIP-reChIP show that, in cells expressing Wt TBP, B-TFIID initiates formation of an incomplete PIC comprising TFIIB and the Med complex, but not TFIIE, whereas in cells expressing mutant TBPs not competent to interact with BTAF1, TFIID replaces B-TFIID allowing
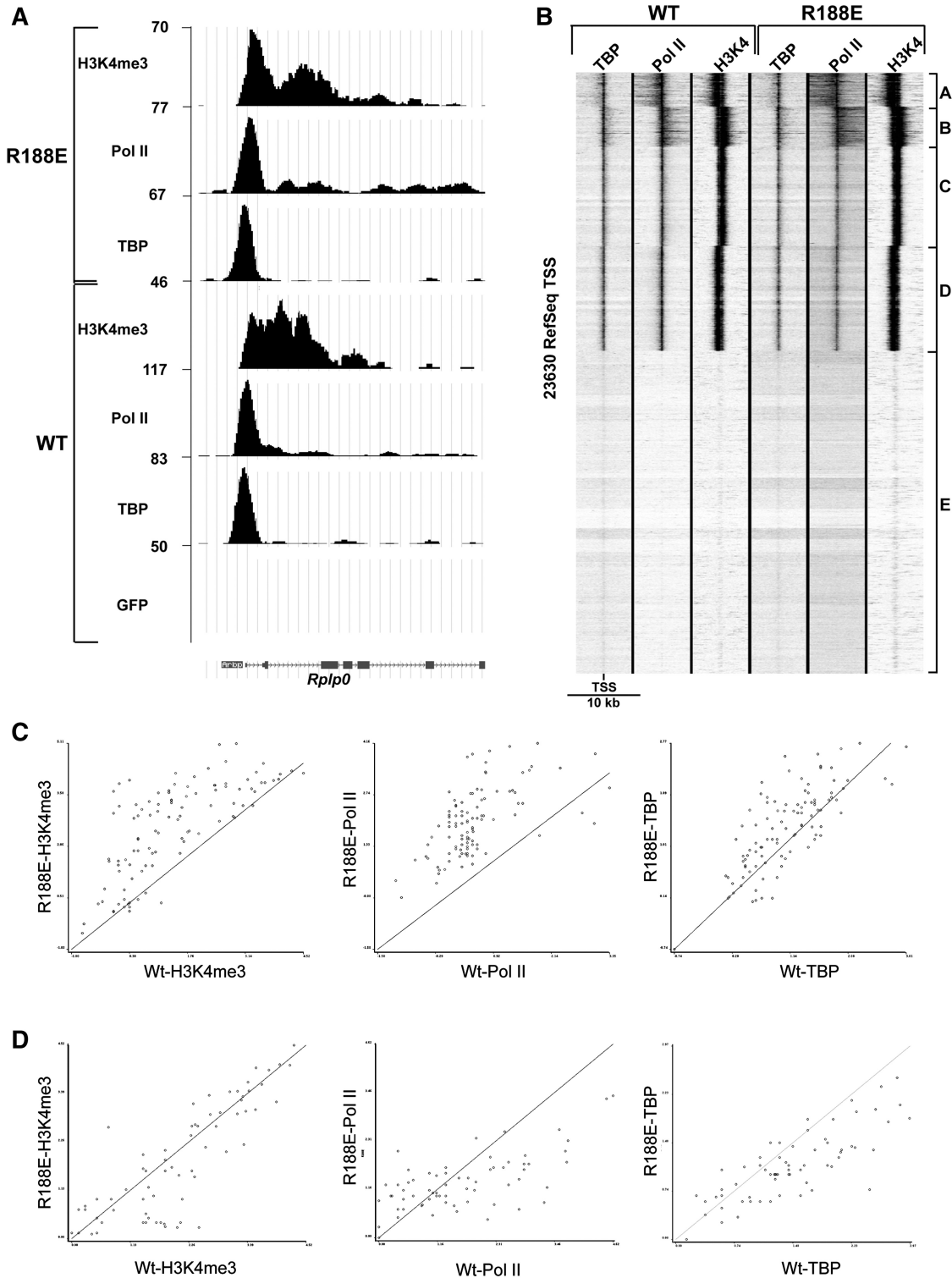
Figure 4. Genome-wide distribution of TBP in Wt and R188E expressing cells. (**A**) Screen shots of .Wig files of the TBP, Pol II and H3K4me3 ChIP-seq from cells expressing Wt and R188E TBPs at the *Rplp0* locus. (**B**) Comparative clustering of ChIP-seq data at the 23 630 RefSeq TSS in Wt and R188E TBP cells. Tag densities from each ChIP-seq dataset were collected in a window of 10 kb around the TSS. The collected values were subjected to *k*-means clustering coupled to ranked based-normalization. This clustering identifies distinct classes: (A and B) Highly transcribed anti-sense and sense, (**C** and **D**), moderately transcribed anti-sense and sense, (E) no occupancy and not transcribed. (C and D) Differential occupancy at the promoters of de-regulated genes. Quantitative analysis of TBP, Pol II and H3K4me3 at the promoters of (C) genes up-regulated and (D) down-regulated in R188E TBP cells.
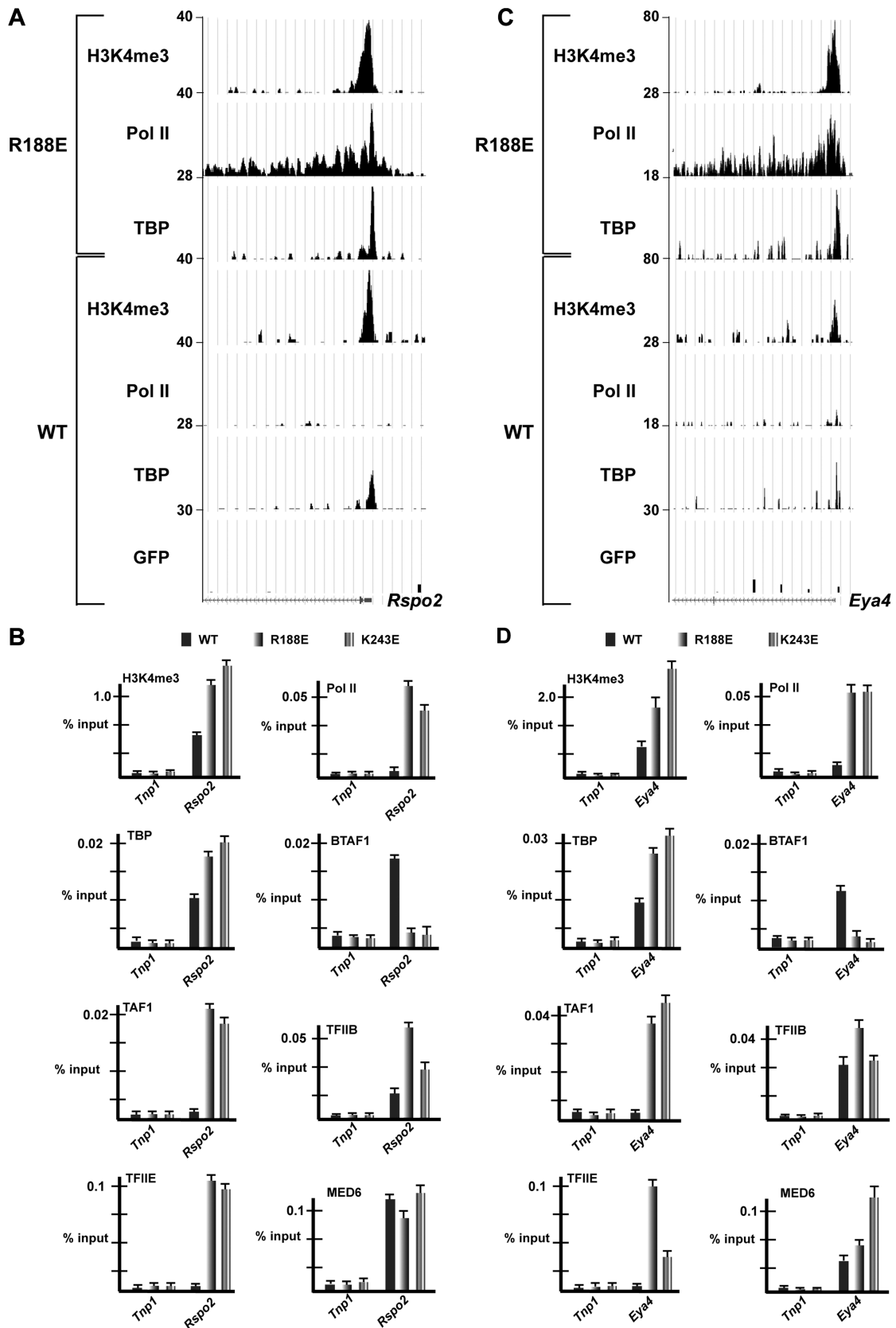
Figure 5. Binding of PIC components at the promoters of up-regulated genes. (**A** and **C**) UCSC screen shots of TBP, Pol II and H3K4me3 ChIP-seq results at the *Rspo2* and *Eya4* loci, respectively. (**B** and **D**) ChIP-qPCR with the indicated antibodies in the Wt, R188E and K243E cells at the *Rspo2* and *Eya4* promoters using the haploid cell-specific transition protein 1 (*Tpn1*) promoter as negative control.
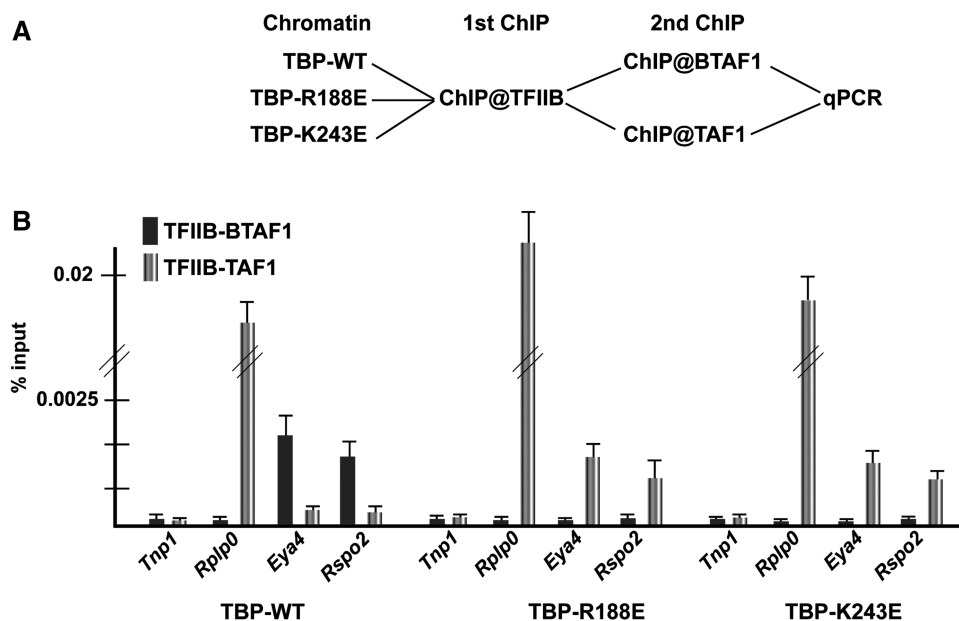
Figure 6. Exchange of B-TFIID for TFIID at the *Eya4* and *Rspo2* promoters. (**A**) Schematic description of the ChIP-reChip strategy. (**B**) Results of ChIP-reChip-qPCR experiments showing the presence of a TFIIB–BTAF1 complex at the *Eya4* and *Rspo2* in TBP Wt cells and a TFIIB–TAF1 complex at these promoters in the TBP R188E and K243E cells.

recruitment of TFIIE along with Pol II to activate *Rspo2* and *Eya4* expression.

At genes showing down-regulation, there is a strong reduction in both TBP and Pol II occupancy, while H3K4me3 is less affected (Figure 7A and B and a more global comparison in Figure 4D). Examples of this are seen at the plakophilin 2 (*Pkp2*) or coxsackie virus and adenovirus receptor (*Cxadr*). At both promoters, the TBP, Pol II, TFIIB and MED6 levels present in Wt cells are almost completely lost in the R188E and K243E TBP cells, but significant residual H3K4me3 levels persist (Figure 7A and B and data not shown). Interestingly, however, differential recruitment of Wt and mutant TBP does not always lead to changes in gene expression. For example, R188E TBP is strongly and selectively recruited to the promoter of the divergent potassium voltage-gated channel, shaker-related subfamily, beta member 2 (*Kcnab2*) and nephronophthisis 4 (*Nphp4*) genes, but there is no recruitment of Pol II and no expression of these genes (Supplementary Figure S7).

Taken together, our data show that TBP recruitment does not necessarily lead to gene activation and reveal the existence of a set of promoters where TBP in the form of B-TFIID assembles a partial PIC that maintains them in a non-productive state, but primes them for activation.

DISCUSSION

Genome-wide studies of TBP function and distribution have previously been mainly performed in yeast (14,38). In this study, we analysed mammalian TBP function in an experimental system allowing substitution of Wt TBP by mutated variants identifying two mutants exclusively affected in B-TFIID formation. Comparative genome-wide occupancy analysis of the wild-type and mutant cells identifies a novel primed promoter state at a subset of genes in mammalian cells.

TBP surfaces required for function in mammalian cells

We have investigated the ability of hTBPs bearing single amino acid substitutions to complement lethality upon loss of endogenous mTBP. Several mutant TBPs could not be expressed even in cells harbouring endogenous TBP probably reflecting a dominant negative activity of these mutants. Mutation R203E has been shown to affect interaction with DNA (22,23) and TFIIA (22), while T210K is a radical mutation in the first half of the DNA-binding surface. These mutant TBPs interact normally with the TAF partners, but they cannot bind TATA-containing promoter DNA explaining their dominant negative behaviour. E286R is a radical mutation in the TFIIB interaction surface (22). This mutant interacts with TAFs and binds promoter DNA, but fails to interact with TFIIB showing that TBP–TFIIB interaction is essential for viability of mammalian cells. The basis of the dominant negative effect of the R239A mutation on the H2 helix remains to be determined.

Mutations that strongly compromise specific TBP interactions or activated transcription in transfected cells are nevertheless capable of complementation. V162A, R188E, L212A and K249A. V162A have been shown to prevent formation of the TBP–TFIIB–DNA complex *in vitro* (23). R188E affects formation of the TBP–NC2–DNA complex, strongly reduces *in vitro* transcription from the U1 and U6 promoters by Pol II and Pol III, respectively (25), affects interaction with TFIIA and abolishes activated transcription (22,26). L212A strongly down-regulates U6 transcription by Pol III *in vitro* (25), while K249A
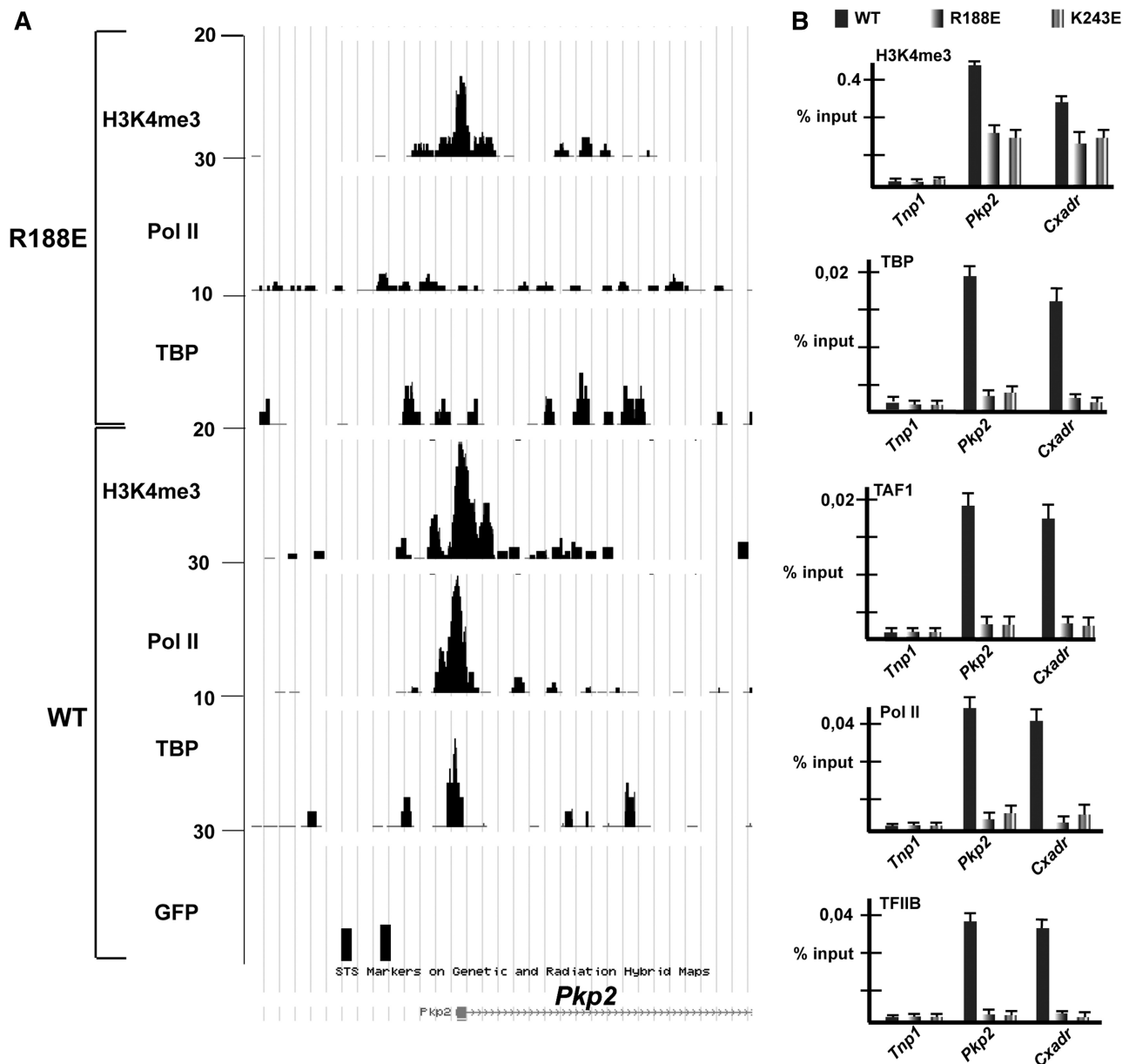
Figure 7. Loss of TBP and Pol II recruitment at down-regulated genes. (**A**) UCSC screen shot of TBP, Pol II and H3K4me3 ChIP-seq data at the *Pkp2* locus. (**B**) ChIP-qPCR with the indicated antibodies in the Wt, R188E and K243E cells and these promoters using the *Tpn1* promoter as negative control.

inhibits formation of the NC2/TFIIA/TFIIB–TBP–DNA complexes (23). Despite the effects of these mutants *in vitro*, they support MEF cell viability. Thus, while mutations may affect a specific interaction *in vitro*, in the context of the living cell additional interactions with modified histones (39,40) and/or the presence of TAFs may stabilize the PIC and overcome weakened interactions of TBP with DNA or its protein partners.

Interaction of TBP with BTAF1 plays a limited, but specific role in transcription regulation

Viable TBP mutants associate normally with the TFIID- and SL1-TAFs as well as with BRF1 of TFIIIB. Under the same conditions, however, mutations R188E and K243E selectively disrupt the TBP–BTAF1 interaction and hence formation of B-TFIID. These mutations were previously shown to affect TBP–BTAF1 interactions *in vitro* (23). The R188E mutation had a milder effect *in vitro* than other mutations, such as V162A, or R186E that do not affect B-TFIID integrity in cells. *In vitro* assembly of B-TFIID from recombinant TBP and BTAF1 has therefore different requirements from the regulated assembly in living cells. The role of the R188E and K243E residues in TBP–BTAF1 interactions has recently been confirmed by the resolution of the structure of the TBP–BTAF1 complex from Encephalitozoon Cuniculi (36). These two residues lie at the interface

between TBP and BTAF1 and contribute to the interaction.

Gene expression profiling shows no general effect on transcription by Pol II upon loss of B-TFIID, but the R188E and K243E mutations selectively affect expression of a subset of genes. In agreement with the specific effects on gene expression, loss of B-TFIID does not result in a global change in TBP occupancy. A subset of mostly inter- and intra-genic loci showed higher occupancy in the R188E expressing cells in agreement with previous proposals that Mot1p can remove TBP from non-promoter sites mobilizing it for use at promoters (41,42).

The R188E and K243E mutations may also affect interactions with other cofactors such as TFIIA. We were unable to determine the effects of these mutations on TBP–TFIIA and TBP–NC2 interactions by mass spectrometry as these proteins did not purify with TBP under the conditions used. Nevertheless, while these mutations have been reported to affect interaction with TBP *in vitro* (22,23,25), they did not alter TFIIA occupancy at the *Rplp0* promoter in cells and we did not see significant TFIIA occupancy at the *Rspo2* and *Eya4* promoters in TBP Wt or mutant cells. Hence, at promoters where we observe TFIIA, we do not see BTAF1 and *vice versa* in agreement with the fact that TBP–TFIIA and TBP–BTAF1 interactions are mutually exclusive (36). These observations suggest that the transcriptional effects seen at these promoters are principally due to differential recruitment of B-TFIID and TFIID and not of TFIIA.

The effects seen upon loss of TBP–BTAF1 interaction differ from those seen upon knockdown of BTAF1 expression which has been shown to lower TBP mobility in the nucleus (43). In human cells, BTAF1 knockdown was shown to increase TBP occupancy at several promoters (43) and maybe also more globally in the genome (44). However, although we observed reduced BTAF1 levels in the R188E and K243E mutant cells, we did not observe increased TBP promoter occupancy. As our experiments were performed in MEFs, while the knockdown experiments were performed in U2OS and HeLa cells, it possible that loss of BTAF1 does not have the same effects in all mammalian cell types.

Evidence for a novel primed promoter state controlling productive PIC formation *in vivo*

Analysis of genes up-regulated in cells expressing mutant TBPs showed that Wt TBP and BTAF1 are present at their promoters, but assemble an incomplete PIC lacking TFIIE and Pol II. In contrast, in the R188E and K243E cells, TFIIE and Pol II are recruited and they are transcribed into stable mRNA. Importantly, the TFIID subunit TAF1 is recruited to these promoters only in the R188E and K243E mutant cells, while BTAF1 is lost. Wt TBP is therefore recruited to these promoters in the form of B-TFIID that nucleates formation of a partial PIC, whereas R188E and K243E TBP are recruited as TFIID that is competent to form a full PIC, recruit Pol II and initiate transcription.

In contrast to the above, we also identify genes that are down-regulated by the R188E and K243E mutations. At many of these loci, we observe a total or partial loss of TBP and Pol II, but not a complete inactivation as H3K4me3 persists at these loci. These observations suggest that the TBP–BTAF1 interaction is required for normal TBP recruitment and PIC formation at these loci.

Only a small number of cellular promoters show differential positive and negative regulation in the R188E cells and we have not been able to determine common characteristics (for example, consistent presence or absence of a TATA or other promoter elements) that would confer this type of regulation. Similarly, gene ontology analysis did not reveal obvious related functions (like cell-cycle progression) for the deregulated genes.

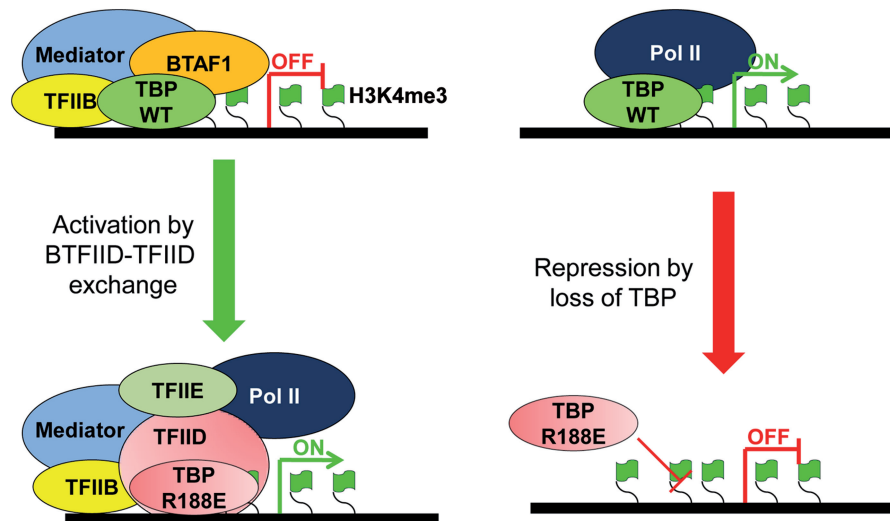Together, our data demonstrate that the formation of a stable B-TFIID complex is not essential for cell viability

Figure 8. Cartoons illustrating models for the differential effect of mutations in TBP on PIC recruitment and gene expression. Genes may be activated by promoting exchange of B-TFIID for TFIID. In contrast, at other promoters the same TBP mutations can lead to a loss in TBP occupancy and gene expression.

and at least in MEFs is not generally required for the transcriptional programme. Our genome-wide occupancy analysis of Wt and R188E mutant TBP and the activating H3K4me3 histone modification indicates that certain mammalian promoters are occupied by transcriptionally inactive forms of TBP. At a subset of promoters, B-TFIID assembles a partial and transcriptionally inactive PIC. The inactivity of these promoters is not due to high levels of NC2 binding as, while we detect NC2 occupancy at promoters such as the *Rplp0* control, it is below the level of detection at the repressed promoters (our unpublished data). This defines a new mechanism controlling mRNA synthesis involving 'stalled preinitiation complex assembly' (Figure 8). This is reminiscent of the mechanism whereby full PIC formation takes place, but Pol II remains in a paused state downstream of the TSS to repress productive mRNA synthesis (1). This, together with our study, shows that transcription can be regulated at several steps through formation of a partial PIC lacking Pol II or following complete PIC formation by pausing of Pol II at the promoter.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Methods, Supplementary Figures S1–S7, Supplementary Datasets S1 and S2, Supplementary References [30–34,45–47].

ACKNOWLEDGEMENTS

FUNDING

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