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The General Transcription Machinery and General Cofactors

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ABSTRACT In eukaryotes, the core promoter serves as a platform for the assembly of transcription preinitiation complex (PIC) that includes TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, and RNA polymerase II (pol II), which function collectively to specify the transcription start site. PIC formation usually begins with TFIID binding to the TATA box, initiator, and/or downstream promoter element (DPE) found in most core promoters, followed by the entry of other general transcription factors (GTFs) and pol II through either a sequential assembly or a preassembled pol II holoenzyme pathway. Formation of this promoter-bound complex is sufficient for a basal level of transcription. However, for activator-dependent (or regulated) transcription, general cofactors are often required to transmit regulatory signals between gene-specific activators and the general transcription machinery. Three classes of general cofactors, including TBP-associated factors (TAFs), Mediator, and upstream stimulatory activity (USA)-derived positive cofactors (PC1/PARP-1, PC2, PC3/DNA topoisomerase I, and PC4) and negative cofactor 1 (NC1/HMGB1), normally function independently or in combination to fine-tune the promoter activity in a gene-specific or cell-type-specific manner. In addition, other cofactors, such as TAF1, BTAF1, and negative cofactor 2 (NC2), can also modulate TBP or TFIID binding to the core promoter. In general, these cofactors are capable of repressing basal transcription when activators are absent and stimulating transcription in the presence of activators. Here we review the roles of these cofactors and GTFs, as well as TBP-related factors (TRFs), TAF-containing complexes (TFTC, SAGA, SLIK/SALSA, STAGA, and PRC1) and TAF variants, in pol II-mediated transcription, with emphasis on the events occurring after the chromatin has been remodeled but prior to the formation of the first phosphodiester bond.

KEYWORDS core promoter elements, general transcription machinery, transcription cofactors, preinitiation complex, CTD phosphorylation, pol II ubiquitination, E3 ubiquitin ligase, TAF variants

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INTRODUCTION

Discovery of Eukaryotic RNA Polymerase I,II,III, and IV

The view that genetic information flows from DNA to RNA to protein, known as the Central Dogma (Crick, 1958), has been expanded due to the

discovery of additional pathways to perpetuate genetic information, *i.e.*, from RNA to DNA (Baltimore, 1970; Temin and Mizutani, 1970) and to conformational changes induced by proteinaceous infectious agents (Prusiner *et al.*, 1982). The production of RNA, termed transcription, is a highly coordinated process mediated by RNA polymerase, whose enzymatic activity was first discovered by Weiss and Gladstone in 1959 from rat liver nuclei. This enzyme could synthesize RNA in a DNA-dependent manner, as evidenced by the observation that, upon addition of DNase, incorporation of [α-^{32}P]CTP into RNA was drastically reduced (Weiss and Gladstone, 1959). A similar RNA-synthesizing activity was later identified from *Escherichia coli* (Hurwitz *et al.*, 1960; Stevens, 1960; Chamberlin and Berg, 1962), thereby establishing a universal role of RNA polymerases in transcribing DNA in both eukaryotes and prokaryotes. To date, four different RNA polymerases have been identified in higher eukaryotes. In contrast, only one RNA polymerase is found in prokaryotes and archaea.

Three eukaryotic RNA polymerases (I, II, and III, or named A, B, and C, respectively) were first identified by Roeder and Rutter, based on the chromatographic fractionation of sea urchin embryo nuclei on a DEAE-Sephadex column. RNA polymerase I came off the column first at the lowest salt concentration, whereas RNA polymerase III eluted at the highest salt concentration (Roeder and Rutter, 1969). Although the existence of three eukaryotic RNA polymerases was established in 1969, their functions remained elusive until Chambon's and Roeder's groups found that the specific activity of each RNA polymerase could be resolved based upon their differential sensitivities to α-amanitin (Gniazdowski *et al.*, 1970; Kedinger *et al.*, 1970; Weinmann and Roeder, 1974; Weinmann *et al.*, 1974), a drug isolated from the death cap fungus, *Amanita phalloides*, that inhibits 50% activity of RNA polymerase II at low concentrations (0.02 μg/mL) and that of RNA polymerase III at high concentrations (20 μg/mL; Weinmann *et al.*, 1974). Using α-amanitin sensitivity assay with endogenous RNA polymerases present in isolated nuclei, Roeder and Rutter (1970) discovered that RNA polymerase I is primarily involved in transcribing 18S and 28S ribosomal RNAs, while RNA polymerase II transcribes mRNAs, and RNA polymerase III is responsible for synthesis of cellular 5S rRNA, tRNAs, and adenovirus VA RNAs. These results were consistent with the finding that RNA polymerase I is localized within nucleoli, the sites for rRNA synthesis, whereas RNA polymerase II and III are normally present in the nucleoplasm (Roeder and Rutter, 1970; Zylber and Penman, 1971; Weil and Blatti, 1976).

The fourth RNA polymerase, recently identified in plants to facilitate the production of small interfering RNA (siRNA) involved in RNA-directed DNA methylation and transcriptional silencing and formation of heterochromatin, is also present in the nucleus and is resistant to α-amanitin (Herr *et al.*, 2005; Kanno *et al.*, 2005; Onodera *et al.*, 2005). Although this plant-specific RNA polymerase IV appears to be nonessential for viability, it exhibits unique properties not shared by other nuclear RNA polymerases. Interestingly, an α-amanitin-resistant single-polypeptide nuclear RNA polymerase (spRNAP-IV), structurally unrelated to the multisubunit plant RNA polymerase IV, has been additionally found in human HeLa cells (Kravchenko *et al.*, 2005). This spRNAP-IV enzyme, encoded by an alternative transcript derived from the mitochondrial RNA polymerase gene (*POLRMT*) that also encodes the single-polypeptide mitochondrial RNA polymerase (mtRNAP), lacks the N-terminal 262-amino acids encompassing the mitochondrial targeting sequence but contains the same C-terminal 968 amino acid catalytic region of mtRNAP. While spRNAP-IV appears to be structurally distinct from α-amanitin-sensitive RNA polymerase II, it represents a second RNA polymerase able to transcribe a subset of mRNA-encoding genes that do not contain the core promoter elements and regulatory sequences commonly found in RNA polymerase II-transcribed genes.

Clearly, the findings that each of these eukaryotic RNA polymerases has unique biochemical properties, as exhibited by their distinct chromatographic elution profile, nuclear localization, and differential sensitivity to α-amanitin, and that RNA polymerase I has 14 subunits, while RNA polymerase II and III possess 12 and 17 subunits, respectively, suggest that these three classical eukaryotic RNA polymerases and the newly identified plant RNA polymerase IV and human spRNAP-IV are unlikely to play a functionally redundant role in the cell. However, these enzymes do share a common property in transcribing a diverse set of DNA sequences, although they lack sequence-specific recognition ability to correctly specify the transcription start site unique to each gene. For site-specific initiation, additional proteins are necessary to form an initiation-competent RNA polymerase complex. Here, we review

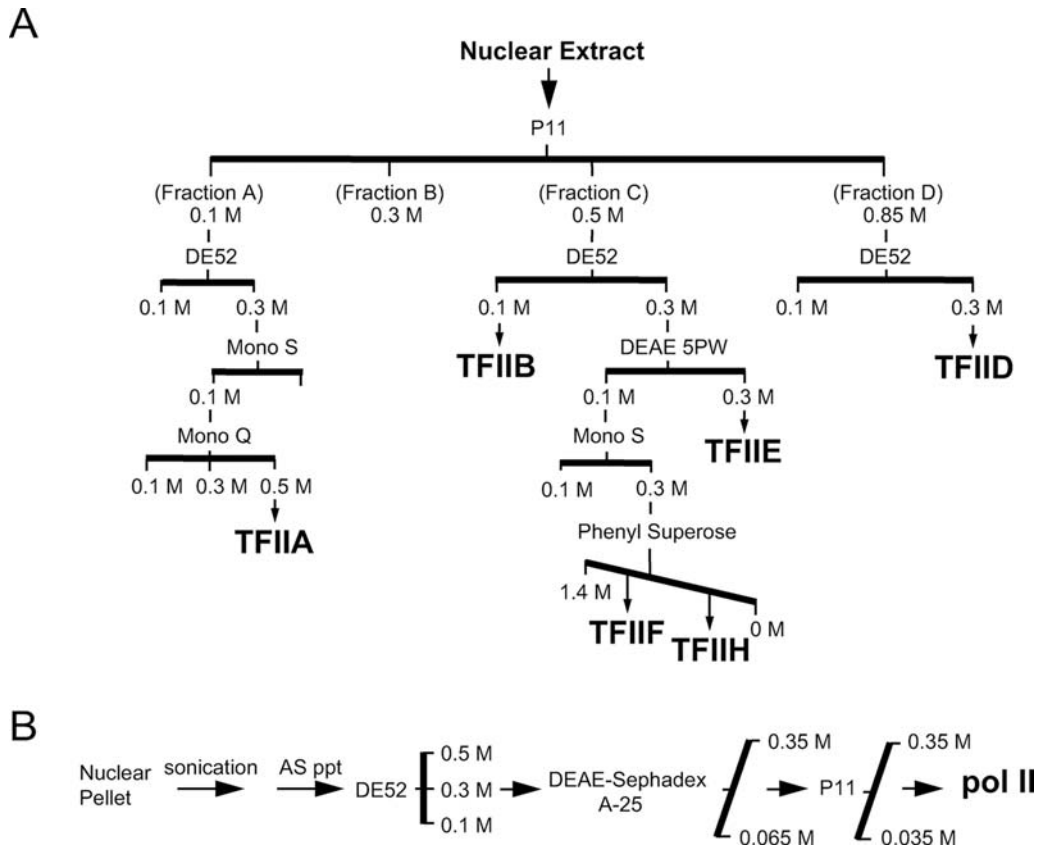


FIGURE 1 **Purification scheme for partially purified general transcription factors. Fractionation of HeLa nuclear extract (*Panel A*) and nuclear pellet (*Panel B*) by column chromatography and the molar concentrations of KCl used for elutions are indicated in the flow chart, except for the Phenyl Superose column where the molar concentrations of ammonium sulfate are shown. A thick horizontal (*Panel A*) or vertical (*Panel B*) line indicates that step elutions are used for protein fractionation, whereas a slant line represents a linear gradient used for fractionation. The purification scheme for pol II, starting from sonication of the nuclear pellet, followed by ammonium sulfate (AS) precipitation is shown in Panel B. (Figures are adapted from Flores *et al.,* 1992 and from Ge *et al.,* 1996)**

the functional properties of general transcription factors and general cofactors that help RNA polymerase II engage in the transcriptional process in a site-specific and gene-specific manner, with emphasis on the steps regulating preinitiation complex assembly that is often the focal point for gene regulation.

The General Transcription Machinery

Biochemical evidence of accessory factors necessary for site-specific initiation by RNA polymerase II (hereafter referred to as pol II) became evident when purified pol II, supplemented with crude subcellular fractions, was able to accurately transcribe the native adenovirus DNA template *in vitro* (Weil *et al.*, 1979). Further purification of these subcellular fractions over a Whatman P11 phosphocellulose ion exchange column yielded four enzymatically active fractions (A, B, C and D), which corresponded to the nuclear proteins sequentially eluted by 0.1, 0.3, 0.5 (or 0.6), and 0.85 (or 1.0) M KCl-containing buffer. Components within fractions A, C, and D were necessary for accurate initiation of transcription by pol II (Matsui *et al.*, 1980; Samuels *et al.*, 1982). The protein factors present in the A and D fractions required for pol II-mediated transcription were named TFIIA and TFIID, respectively (Figure 1A). The C fraction was subsequently fractionated into accessory factors TFIIB, TFIIE, TFIIF, and TFIIH (Sawadogo and Roeder, 1985a; Reinberg and Roeder, 1987; Flores *et al.*, 1989; Flores *et al.*, 1992; Ge *et al.*, 1996). These accessory factors (TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH) were collectively defined as general transcription factors (GTFs) using the following nomenclature: TF represents Transcription Factor, the Roman numeral II indicates pol II-driven transcription, and the "letter" generally corresponds to which chromatographic fraction the specific GTF was isolated from (Figure 1A). Nuclear pellets, the residual chromosomal fraction following extraction of soluble nuclear components (*i.e.*, nuclear extract) at 0.42 M KCl-containing buffer, were frequently used for pol II purification as outlined

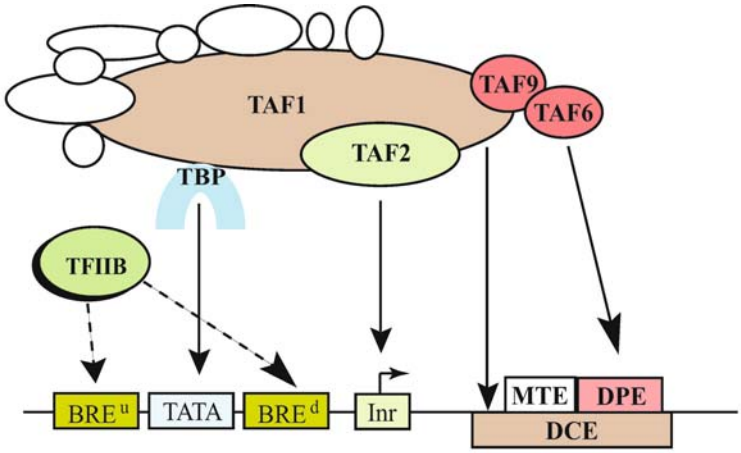
TABLE 1 **Components of the human general transcription machinery**

Factor	Protein composition	Function
TFIIA	p35 (α), p19 (β), and p12 (γ)	Antirepressor; stabilizes TBP-TATA complex; coactivator
TFIIB	p33	Start site selection; stabilize TBP-TATA complex; pol II/TFIIF recruitment
TFIID	TBP + TAFs (TAF1-TAF14)	Core promoter-binding factor Coactivator Protein kinase Ubiquitin-activating/conjugating activity Histone acetyltransferase
TFIIE	p56 (α) and p34 (β)	Recruits TFIIH Facilitates formation of an initiation-compentent pol II Involved in promoter clearance
TFIIF	RAP30 and RAP74	Binds pol II and facilitates pol II recruitment to the promoter Recruits TFIIE and TFIIH Functions with TFIIB and pol II in start site selection Facilitates pol II promoter escape Enhances the efficiency of pol II elongation
TFIIH	P89/XPB, p80/XPD, p62, p52, p44, p40/CDK7, p38/Cyclin H, p34, p32/MAT1, and p8/TFB5	ATPase activity for transcription initiation and promoter clearance Helicase activity for promoter opening Transcription-coupled nucleotide excision repair Kinase activity for phosphorylating pol II CTD E3 ubiquitin ligase activity
pol II	RPB1-RPB12	Transcription initiation, elongation, termination Recruitment of mRNA capping enzymes Transcription-coupled recruitment of splicing and 3′ end processing factors CTD phosphorylation, glycosylation, and ubiquitination

(Figure 1B). Accessory factors necessary for site-specific initiation by pol II were similarly purified by different groups from HeLa cells (Gerard *et al.,* 1991), rat liver (Conaway *et al.,* 1991), *S. cerevisiae* (Sayre *et al.,* 1992), and *Drosophila* (Parker and Topol, 1984; Heberlein *et al.,* 1985; Price *et al.,* 1987). The protein composition and functional properties of each GTF and pol II are briefly summarized in Table 1 and will be further elaborated in the later sections.

Core Promoter Elements

Studies on eukaryotic promoters have thus far identified seven core promoter elements (Figure 2), which are characteristic DNA sequences required for promoter function and for proper assembly and orientation of the transcription preinitiation complex (PIC). The TATA box is an A/T-rich sequence located approximately 25 to 30 nucleotides upstream of the transcription start site (+1) in humans. It contains a consensus sequence, TATA(A/T)A(A/T)(A/G), whose recognition by the TATA-binding protein (TBP) or the TBP subunit present in the human TFIID complex, nucleates PIC formation (reviewed by Smale and Kadonaga, 2003). A second core promoter element, the initiator (Inr), contains a pyrimidine-rich sequence, $PyPyA_{+1}N(T/A)PyPy$, surrounding the transcription start site. The Inr is capable of directing accurate transcription initiation either alone or in conjunction with a TATA box or other core promoter elements. The TAF1/TAF2 components of TFIID have been implicated in Inr recognition, as the Inr sequence was preferentially selected for binding by the immobilized human TAF1 and *Drosophila* TAF2 dimeric complex in random DNA-binding site selection assays (Chalkley and Verrijzer, 1999). The downstream promoter element (DPE) with a consensus sequence, (A/G)G(A/T)CGTG, represents the third core promoter element, which is located 28 to 34 nucleotides downstream of the transcription start site in many *Drosophila* TATA-less promoters (Burke and Kadonaga, 1996, 1997; Kutach and Kadonaga, 2000), but is situated between +29 and +35 in the human TATA-less TAF7 promoter (Zhou and Chiang, 2001, 2002). The finding that both *Drosophila* TAF6 and TAF9 can be cross-linked to the



Core Promoter Element	Position	Consensus Sequence (5' to 3')	Bound Protein
BRE^u	-38 to -32	(G/C)(G/C)(G/A)CGCC	TFIIB
TATA	-31 to -24	TATA(A/T)A(A/T)(A/G)	TBP
BRE^d	-23 to -17	(G/A)T(T/G/A)(T/G)(G/T)(T/G)(T/G)	TFIIB
Inr	-2 to +5	PyPyAN(T/A)PyPy	TAF1/TAF2
MTE	+18 to +29	C(G/C)A(A/G)C(G/C)(G/C)AACG(G/C)	n.a.
DPE	+28 to +34	(A/G)G(A/T)CGTG	TAF6/TAF9
DCE	3 subelements +6 to +11 +16 to +21 +30 to +34	core sequence: S_I CTTC S_{II} CTGT S_{III} AGC	TAF1

FIGURE 2 **Recognition of core promoter elements by TFIID and TFIIB. The upper figure depicts the interactions between TFIID and TFIIB with the seven core promoter elements discussed in this review. The table in the lower panel lists the consensus sequence and positions for each of these core promoter elements. n.a., not available.**

DPE (Burke and Kadonaga, 1997) and the human TAF6 and TAF9 dimeric complex exhibits DPE-binding specificity in electrophoretic mobility shift assays (Shao *et al.,* 2005) indicates that TFIID is the primary GTF recognizing the DPE. Interestingly, negative cofactor 2 (NC2 or Dr1-Drap1), initially characterized as a TBP-inhibitory factor when assayed with a TATA-containing promoter (Meisterernst and Roeder, 1991; Inostroza *et al.,* 1992; Goppelt *et al.,* 1996; Mermelstein *et al.,* 1996), has also been shown to facilitate transcription from DPE-driven *Drosophila* promoters (Willy *et al.,* 2000). It seems that TFIID and NC2, two of the DPE-targeting factors, may work synergistically through the DPE, although their functional relationship remains to be elucidated.

In additional to the DPE, two other core promoter elements, MTE (motif ten element; Ohler *et al.,* 2002; Lim *et al.,* 2004) and DCE (downstream core element; Lee *et al.,* 2005b), are also situated downstream of the transcription start site. The MTE with a consensus sequence C(G/C)A(A/G)C(G/C)(G/C)AACG(G/C) found between +18 to +29 normally functions in conjunction with the Inr to enhance pol II-mediated transcription. It can also functionally substitute for the loss of the TATA box and/or DPE as well as work synergistically with the TATA box and DPE, in an Inr-dependent manner, to strengthen the promoter activity (Lim *et al.,* 2004). However, the protein factors that act through the MTE have not yet been defined.

In contrast to the sequence continuity seen with other core promoter elements, the DCE contains three discontinuous subelements: S_I, S_{II}, and S_{III}, with a core sequence of CTTC, CTGT, and AGC, respectively, spanning from +6 to +34 (Lee *et al.,* 2005b; see Figure 2). The presence of DCE and DPE seems to be mutually exclusive, as indicated by the sequence analysis of human promoter databases (Lee *et al.,* 2005b). Interestingly, the TAF1 component of TFIID is able to contact each subelement as revealed by the crosslinking study, suggesting that TFIID indeed plays a

fundamental role in establishing downstream promoter contacts first observed by DNase I footprinting studies (Sawadogo and Roeder, 1985a; Nakajima *et al.,* 1988; Zhou *et al.,* 1992; Chiang *et al.,* 1993; Purnell *et al.,* 1994).

The sixth and seventh core promoter elements are recognized by a different GTF, TFIIB, through promoter contacts with DNA sequences flanking the TATA box, normally following TBP-induced DNA bending. The upstream TFIIB-recognition element (BRE^u) with a consensus sequence, (G/C)(G/C)(G/A)CGCC, is contacted by the helix-turn-helix (HTH) DNA-binding motif of TFIIB (Lagrange *et al.,* 1998). This TFIIB-BRE^u interaction can occur independently of TBP and helps orient the directionality of the PIC (Lagrange *et al.,* 1998; Tsai and Sigler, 2000). In contrast, TFIIB binding to the downstream TFIIB-recognition element (BRE^d) with a consensus (G/A)T(T/G/A)(T/G)(G/T)(T/G)(T/G) via its recognition loop that includes the evolutionarily conserved G153 and R154 residues appears to be TBP-dependent (Deng and Roberts, 2005). Although initially defined in TATA-containing promoters, both BRE^u and BRE^d can also be found in many TATA-less promoters based on bioinformatics analysis (Deng and Roberts, 2005; Gershenzon and Ioshikhes, 2005), suggesting that TFIIB is likely to enhance TFIID binding to the core promoter region by providing additional contact points.

The identification of these seven core promoter elements provides a molecular architecture underlying the diversity of eukaryotic promoters. With available computational tools, the abundance of each core promoter element can be calculated in a genome-wide basis. A previous analysis of the *Drosophila* promoter database reveals that 57% of the *Drosophila* promoters do not contain a TATA box, whereas the DPE is found in approximately 40% of the *Drosophila* promoters (Kutach and Kadonaga, 2000). A similar inspection of 1031 human genes with putative transcription start sites indicates that $TATA^+Inr^+$, $TATA^+Inr^-$, $TATA^-Inr^+$, and $TATA^-Inr^-$ genes constitute 28%, 4%, 56%, and 12%, respectively, of the screened human genes (Suzuki *et al.,* 2001). This analysis suggests that 1/3 of human genes contain a functional TATA box, whereas the majority (~68%) of human promoters are in fact TATA-less. Following a more comprehensive promoter analysis of EPD (eukaryotic promoter database) and DBTSS (database of human transcriptional start sites), it is found that less than 22% of the human genes contain a TATA box, and among these TATA-containing promoters, ~62% have an Inr, ~24% include a DPE, and ~12% hold a BRE^u (Gershenzon and Ioshikhes, 2005). The same study also indicates that more than 78% of the human genes are TATA-less, and among these TATA-deficient promoters, ~45% possess an Inr, ~25% have a DPE, and ~28% harbor a BRE^u. While these bioinformatics analyses provide an overview of the relative abundance of each core promoter element present in a given species, the functional role of these core promoter elements in dictating the promoter strength, the efficiency of PIC assembly, and as targeting regions for enhancer activity remain to be investigated. From the functional studies, it seems that the promoters of human housekeeping genes, oncogenes, growth factors, and transcription factors often lack a TATA box (Zhang, 1998; Zhou and Chiang, 2001). Moreover, differential utilization of alternative promoters that use a distinct combination of core promoter elements also plays a critical role in regulating gene expression in a spatial, temporal or lineage-specific manner (Hansen and Tjian, 1995; Ren and Maniatis, 1998). It is interesting to note that DPE and TATA motifs not only specify the initiation site for transcription, but also act as regulatory elements for enhancer function (Butler and Kadonaga, 2001; Smale and Kadonaga, 2003). Undoubtedly, the dissection of transcription complex assembly pathways occurring on the core promoter region that serves as a converging point for regulatory events is of vital importance for our understanding of the transcription mechanism unique to each gene.

The Sequential Assembly Pathway

The pathways leading to productive PIC assembly at the promoter region has been intensively studied for over a decade. In earlier studies, *in vitro* transcription experiments performed with chromatographic fractions derived from HeLa cells suggested that an initiation-competent PIC could be assembled in a stepwise manner and was stable, prior to the addition of ribonucleoside triphosphates (NTPs), and refractory to challenges by competitor DNA (Fire *et al.,* 1984), Sarkosyl (Hawley and Roeder, 1985), and poly(dI-dC:dI-dC) (Samuels and Sharp, 1986). There appeared to be multiple steps leading to formation of a stable promoter-bound complex, since transcription signals varied depending on the order that pol II, individual fractions, template and challenge DNA were added into the reactions. In later studies, it was found that

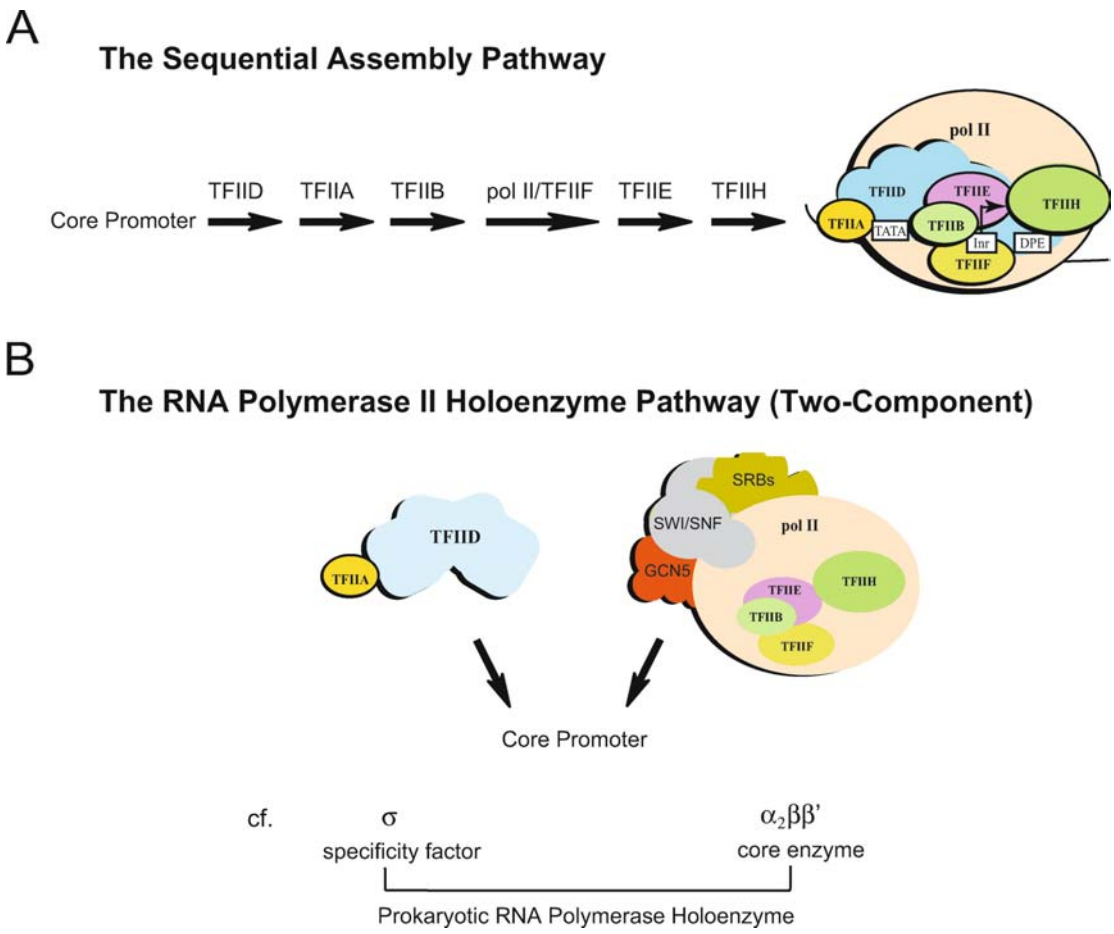


FIGURE 3 **Pathways for preinitiation complex assembly. Preinitiation complex *(PIC)* formation may occur by stepwise recruitment of the general transcription machinery (*Panel A*, the sequential assembly pathway) or by recruitment of preassembled pol II holoenzyme and TFIID complexes as depicted in the two-component pathway (*Panel B*). The eukaryotic two-component pathway resembles the prokaryotic RNA polymerase holoenzyme system where a dissociable σ factor directs the entry of the bacterial RNA polymerase core enzyme $\alpha_2\beta\beta'$.**

preincubation of partially purified TFIID with the adenovirus major late promoter template, before inclusion of the remaining transcription components, resulted in a PIC that was resistant to template challenge by a second adenovirus major late promoter-containing DNA (Van Dyke *et al.,* 1989). The hierarchical nature of GTF assembly at the promoter region was further defined by Buratowski and colleagues (1989) using native gel electrophoresis and DNase I footprinting to establish the order of entry and relative position of GTFs at the adenovirus major late promoter, thus suggesting an order-of-addition model for PIC formation. Specifically, TFIID first recognizes the TATA box, followed in a stepwise manner by the entry of TFIIA, TFIIB, pol II and TFIIE (note: TFIIF and TFIIH had yet to be identified). Once all of the GTFs, including TFIIF and TFIIH, were identified and purified to near homogeneity, this stepwise GTF assembly pathway was updated as: TFIID first binds to the promoter region, followed by the entry of TFIIA and TFIIB that help stabilize promoter-bound TFIID, and then the recruitment of pol II/TFIIF. After formation of a stable TFIID-TFIIA-TFIIB-pol II/TFIIF-promoter complex, TFIIE is then recruited, with the subsequent entry of TFIIH. This stepwise manner of PIC assembly became known as the sequential assembly pathway (Figure 3A).

The Pol II Holoenzyme Pathway

An alternative pathway for PIC formation was uncovered when several laboratories discovered that pol II could be purified as a preassembled holoenzyme complex containing pol II and SRBs (suppressors of RNA polymerase B mutations; Kim *et al.,* 1994; Koleske and Young, 1994), with or without a subset of GTFs; and other proteins involved in chromatin remodeling, DNA repair, and mRNA processing (Ossipow *et al.,* 1995; Cairns *et al.,* 1996b; Chao *et al.,* 1996;

Maldonado *et al.,* 1996; Wilson *et al.,* 1996; Yuryev *et al.,* 1996; McCracken *et al.,* 1997; Nakajima *et al.,* 1997; Cho *et al.,* 1998; Wu and Chiang, 1998; Wu *et al.,* 1999; Liu *et al.,* 2001b). Although the composition of pol II holoenzyme complexes isolated from different laboratories appeared to vary according to the methods of purification and the sources of materials, the human pol II holoenzyme complex isolated in our laboratory contains pol II, TFIIB, TFIIE, TFIIF, TFIIH, GCN5 histone acetyltransferase, SWI/SNF chromatin remodeling factor, and SRBs, but is devoid of TFIID and TFIIA (Wu and Chiang, 1998; Wu *et al.,* 1999; see Figure 3B). The identification of a TFIID-deficient pol II holoenzyme complex suggests that TFIID, as a core promoter-binding factor, may facilitate the entry of pol II holoenzyme to the promoter region, a scenario analogous to the prokaryotic RNA polymerase system where a dissociable σ factor recruits core RNA polymerase ($\alpha_2\beta\beta'$) to the promoter region for PIC assembly (Figure 3B). However, whether eukaryotic pol II indeed exists as a holoenzyme complex in the cell or is recruited in a stepwise manner as a separate entity, along with individual GTFs, to the promoter region remains to be elucidated. It is likely that both assembly pathways exist *in vivo*, and, depending on specific signaling molecules involved and the promoter context, either pathway may be employed in responding to environmental cues. Indeed, evidence supporting both models has been reported for different regulatory systems (Orphanides *et al.,* 1996; Hampsey, 1998; Parvin and Young, 1998; Lee and Young, 2000; Lemon and Tjian, 2000).

TFIID

TFIID is one of the first GTF that binds to the core promoter and nucleates PIC assembly through either the sequential assembly pathway or the two-component pol II holoenzyme pathway. It is a multiprotein complex comprising TBP and approximately 13 TBP-associated factors (TAFs), with molecular weights ranging from 250 kDa to 15 kDa (Table 2). That TBP and some TAF components of TFIID bind distinct core promoter elements (see Figure 2) classifies TFIID as a core promoter-binding factor. The TBP subunit of human TFIID contacts the TATA box allowing TFIID to recognize TATA-containing promoters, and the interaction between TAF-Inr, TAF-DPE, and TAF-DCE also confer TFIID the ability to recognize TATA-less promoters. It should be noted that although TFIID was initially defined as a TATA-binding factor by *in vitro* DNA-binding assays, yeast and human TFIID seem to act primarily through TATA-less promoters *in vivo* (Basehoar *et al.,* 2004; Kim *et al.,* 2005a).

Identification of TATA-Binding Activity

As described earlier, TFIID was originally identified as a chromatographic fraction necessary to support site-specific transcription by pol II *in vitro* (Matsui *et al.,* 1980; Samuels *et al.,* 1982). This TFIID chromatographic fraction was relatively crude, and many laboratories sought to isolate the key component(s) in TFIID. Interestingly, TFIID was shown to play a key role in binding the TATA box, initially from *Drosophila* (Parker and Topol, 1984), then mammals (Sawadogo and Roeder, 1985b) and yeast (Buratowski *et al.,* 1988; Cavallini *et al.,* 1988). Once TFIID bound to the TATA box, it was believed to serve as a scaffold upon which the PIC could assemble (Fire *et al.,* 1984; Workman and Roeder, 1987; Hai *et al.,* 1988; Horikoshi *et al.,* 1988; Moncollin *et al.,* 1992). Eventually a single polypeptide possessing TATA box-binding activity (later termed TBP) was purified (Buratowski *et al.,* 1988; Cavallini *et al.,* 1988; Horikoshi *et al.,* 1989a) and cloned from yeast (Cavallini *et al.,* 1989; Hahn *et al.,* 1989; Horikoshi *et al.,* 1989b; Schmidt *et al.,* 1989; Hoffmann *et al.,* 1990a), *Drosophila* (Hoey *et al.,* 1990; Muhich *et al.,* 1990), and humans (Kao *et al.,* 1990; Peterson *et al.,* 1990; Hoffmann *et al.,* 1990b). The immediate question that followed was whether this single polypeptide TBP is the functional equivalent of TFIID. In an elegant experiment assaying for transcriptional activation dependent on the transcriptional activator Sp1 (specificity protein 1), it was shown that Sp1 could stimulate transcription from TATA-containing promoters only in the presence of partially purified TFIID, but not with recombinant *Drosophila* or yeast TBP (Pugh and Tjian, 1990). Furthermore, glycerol gradient sedimentation and immunoprecipitation analyses of partially purified TFIID, which supported activator-dependent transcription, indicated that TFIID was a multiprotein complex with a native size of $\sim$ 750 kDa, rather than a single polypeptide of 38 kDa (Dynlacht *et al.,* 1991; Pugh and Tjian, 1991; Timmers and Sharp, 1991). Thus, it was proposed that additional proteins (*i.e.,* cofactors) were required to work in conjunction with TBP to potentiate transcriptional activation by Sp1. It was subsequently

TABLE 2 **Nomenclature of TAFs involved in RNA polymerase II-mediated transcription**

New name	*H. sapiens*	*D. melanogaster*	*S. cerevisiae*	*S. pombe*	*C. elegans*	
					New name	Previous name
TAF1	$TAF_{II}250$	$TAF_{II}230$	Taf145/130	$TAF_{II}111$	*taf-1*	*taf-1*
TAF2	$TAF_{II}150$	$TAF_{II}150$	Taf150	T38673*	*taf-2*	*taf-2*
TAF3†	$TAF_{II}140$	$TAF_{II}155$	Taf47		*taf-3*	C11G6.1*
TAF4†	$TAF_{II}130/135$	$TAF_{II}110$	Taf48	T50183*	*taf-4*	*taf-5*
TAF4b†	$TAF_{II}105$					
TAF5	$TAF_{II}100$	$TAF_{II}80$	Taf90	$TAF_{II}72$	*taf-5*	*taf-4*
TAF5b				$TAF_{II}73$		
TAF5L	PAF65α	Cannonball				
TAF6†	$TAF_{II}80$	$TAF_{II}60$	Taf60	CAA20756*	*taf-6.1*	*taf-3.1*
TAF6L	PAF65α	AAF52013*			*taf-6.2*	*taf-3.2*
TAF7	$TAF_{II}55$	AAF54162*	Taf67	$TAF_{II}62$/PTR6	*taf-7.1*	*taf-8.1*
TAF7L	TAF2Q				*taf-7.2*	*taf-8.2*
TAF8†	BAB71460*	Prodos	Taf65	T40895*	*taf-8*	ZK1320.12*
TAF9†	$TAF_{II}32/31$	$TAF_{II}40$	Taf17	S62536*	*taf-9*	*taf-10*
TAF9L	$TAF_{II}31L$					
TAF10†	$TAF_{II}30$	$TAF_{II}24$	Taf25	T39928*	*taf-10*	*taf-11*
TAF10b		$TAF_{II}16$				
TAF11†	$TAF_{II}28$	$TAF_{II}30\alpha$	Taf40	CAA93543*	*taf-11.1*	*taf-7.1*
TAF11L					*taf-11.2*	*taf-7.2*
TAF12†	$TAF_{II}20/15$	$TAF_{II}30\alpha$	Taf61/68	T37702*	*taf-12*	*taf-9*
TAF13†	$TAF_{II}18$	AAF53875*	Taf19	CAA19300*	*taf-13*	*taf-6*
TAF14			Taf30/ANC1#			
TAF15	$TAF_{II}68$#					
BTAF1	$TAF_{II}170$	Hel89B	Mot1	T40642*	*btaf-1*	F15D4.1*

The TAF nomenclature for *C. elegans* is indicated on the right two columns, shaded in grey. (Adapted from Tora, 2002).
†TAFs with histone fold domains.
*Accession numbers for TAFs not yet published or biochemically characterized.
#Non-conserved TAFs yet to be found in other species.

determined that TFIID is a multiprotein complex composed of TBP and TAFs (Dynlacht *et al.*, 1991; Tanese *et al.*, 1991). Isolation of TFIID was later facilitated by immunoaffinity purification with antibodies directed against TBP or an epitope tag linked to the TBP-coding sequence (Dynlacht *et al.*, 1991; Zhou *et al.*, 1992; Chiang *et al.*, 1993; Poon and Weil, 1993; Reese *et al.*, 1994; Sanders *et al.*, 2002a; Auty *et al.*, 2004). This approach has greatly simplified the purification scheme for TFIID and further facilitated the identification and cloning of TAFs.

In humans, at least 14 TAFs have been identified (Burley and Roeder, 1996; Hahn, 1998; Albright and Tjian, 2000; Green, 2000; Tora, 2002), most of which are highly conserved in *S. cerevisiae*, *S. pombe*, *C. elegans*, and *D. melanogaster* (Burley and Roeder, 1996; Albright and Tjian, 2000). Since individual TAFs were originally named by virtue of their apparent molecular weights, which often differ among species, a common name for most of the conserved TAFs was proposed (Tora, 2002). This unified nomenclature facilitates cross-species comparisons of TAFs, designated as TAF1 to TAF15 for TFIID and a unique TBP-associated factor found in B-TFIID as BTAF1 (see Table 2). The general view regarding the role of TFIID is that it functions as: (1) a core promoter-binding factor for both TATA-containing and TATA-less promoters, (2) a coactivator in mediating interactions between activators and the general transcription machinery to enhance PIC assembly, and (3) an enzyme to post-translationally modify chromatin and protein factors involved in transcriptional control.

TBP Recognition of the TATA Box

TATA recognition by the free form of TBP or the TBP subunit of TFIID initiates PIC assembly on TATA-containing promoters. The TATA box, with an 8-bp

consensus sequence TATA(A/T)A(A/T)(A/G), is recognized by the C-terminal region of TBP, which is phylogenetically conserved and is made up of ~180 amino acids (Hernandez, 1993; Nikolov and Burley, 1994; Burley and Roeder, 1996). The crystal structures of yeast TBP in complex with the TATA box of the yeast CYC1 promoter (Kim *et al.,* 1993b) as well as *Arabidopsis* TBP (Kim *et al.,* 1993a) and human TBP (Nikolov *et al.,* 1996) bound to the TATA sequence of the adenovirus major late promoter, all indicate that the DNA is severely bent upon TBP binding. In the bound and unbound state, TBP resembles a molecular "saddle" with a pair of "stirrups" flanking the DNA-binding surface that helps bend the DNA. This saddle-shaped TBP molecule is composed of four α-helices and ten β-strands (Nikolov *et al.,* 1992), which are organized into a bipartite DNA-binding surface. Each half consists of five antiparallel β-strands located on the concave underside of TBP that straddles the DNA and one α-helix forming the side stirrup. The other α-helix situated on the convex upperside interacts with transcription factors (Kim *et al.,* 1993a; Kim *et al.,* 1993b; Nikolov *et al.,* 1996). This concave surface of TBP, with two pairs of phenylalanine residues (Phe284/Phe301 and Phe193/Phe210 in humans) situated at its outermost edges, binds the TATA box in the minor groove of the DNA double helix and induces sharp kinks (more than 80°) into the DNA via intercalation of Phe284/Phe301 at the 5′ end and Phe193/Phe210 at the 3′ end of the TATA box (Juo *et al.,* 1996; Nikolov *et al.,* 1996). Moreover, amino acid residues 190 to 194 and 281 to 285 of human TBP, which make up the two side stirrups, accentuate the DNA bend (Juo *et al.,* 1996; Nikolov *et al.,* 1996). These structural studies, revealing a widened minor groove, and a compressed major groove, are consistent with TBP-TATA electrophoretic mobility shift assays using permuted DNA fragments (Horikoshi *et al.,* 1992).

The N-terminal region of human TBP is highly divergent among species and appears dispensable for TFIID complex assembly (Zhou *et al.,* 1993). Human TBP possesses a contiguous but variable stretch of glutamine residues (starting at residue 58), ranging in length from 29 to 42 between individual alleles, followed by three imperfect Pro-Met-Thr (PMT) repeats located around amino acids 142 to 150 preceding the C-terminal core region of TBP. Expansion of the polyglutamine tract beyond 42 residues is frequently linked to human neurodegenerative diseases, such as spinocerebellar ataxia SCA17 (Nakamura *et al.,* 2001). Although not directly involved in activator-dependent transcription by pol II (Zhou *et al.,* 1993), this N-terminal region of human TBP is necessary for the recruitment of small nuclear RNA-activating protein complex (SNAPc) to the U6 promoter transcribed by RNA polymerase III (pol III). The N-terminal region also inhibits TATA binding by the C-terminal region of TBP on both pol III- (Mittal and Hernandez, 1997) and pol II-dependent promoters (Zhao and Herr, 2002). Since TBP binding to the TATA box is suggested to be a two-step process involving an initial binding of TBP to the TATA box without bending the DNA and followed by a slow transition into a more stable bent TATA-TBP complex, it is interesting to find that deletion of the glutamine-rich domain and PMT repeats in the N-terminal inhibitory region promotes formation of the stable bent TBP-DNA complex. Moreover, it seems that TFIIB's ability to enhance TBP binding to the TATA box is likely due to induced stabilization of the bent TATA-TBP complex following TFIIB binding to the solvent-exposed surface on the convex side of the TBP core (Zhao and Herr, 2002).

Regulation of TBP Promoter Binding Activity by Homodimerization

Although TBP's specificity for DNA binding is $\sim 10^3$ weaker than most major groove-binding proteins (Coleman and Pugh, 1995), TBP does exhibit nonspecific DNA-binding activity, sometimes leading to formation of nonproductive transcription complexes on scattered AT-rich sequences. To prevent spurious transcription events initiating at nonpromoter sequence elements, TBP may form a homodimer that is inactive in DNA binding (Figure 4A). In addition, association with TAFs may reduce nonspecific DNA-binding activity of TBP and concurrently increase TFIID specificity at TATA-containing and particularly TATA-less promoters. Formation of TBP homodimers was initially observed in the crystal structures of the C-terminal core of *Arabidopsis* TBP (Nikolov *et al.,* 1992; Nikolov and Burley, 1994), yeast TBP (Chasman *et al.,* 1993), and human TBP (Nikolov *et al.,* 1996), in the absence of DNA, and further confirmed by biochemical analysis of full-length or the C-terminal core of TBP using gel filtration/glycerol gradient fractionation (Kato *et al.,* 1994), photon correlation spectroscopy (Nikolov *et al.,* 1996) and chemical cross-linking studies (Icard-Liepkalns, 1993; Coleman *et al.,* 1995; Jackson-Fisher *et al.,* 1999). Dimerization of TBP is mediated

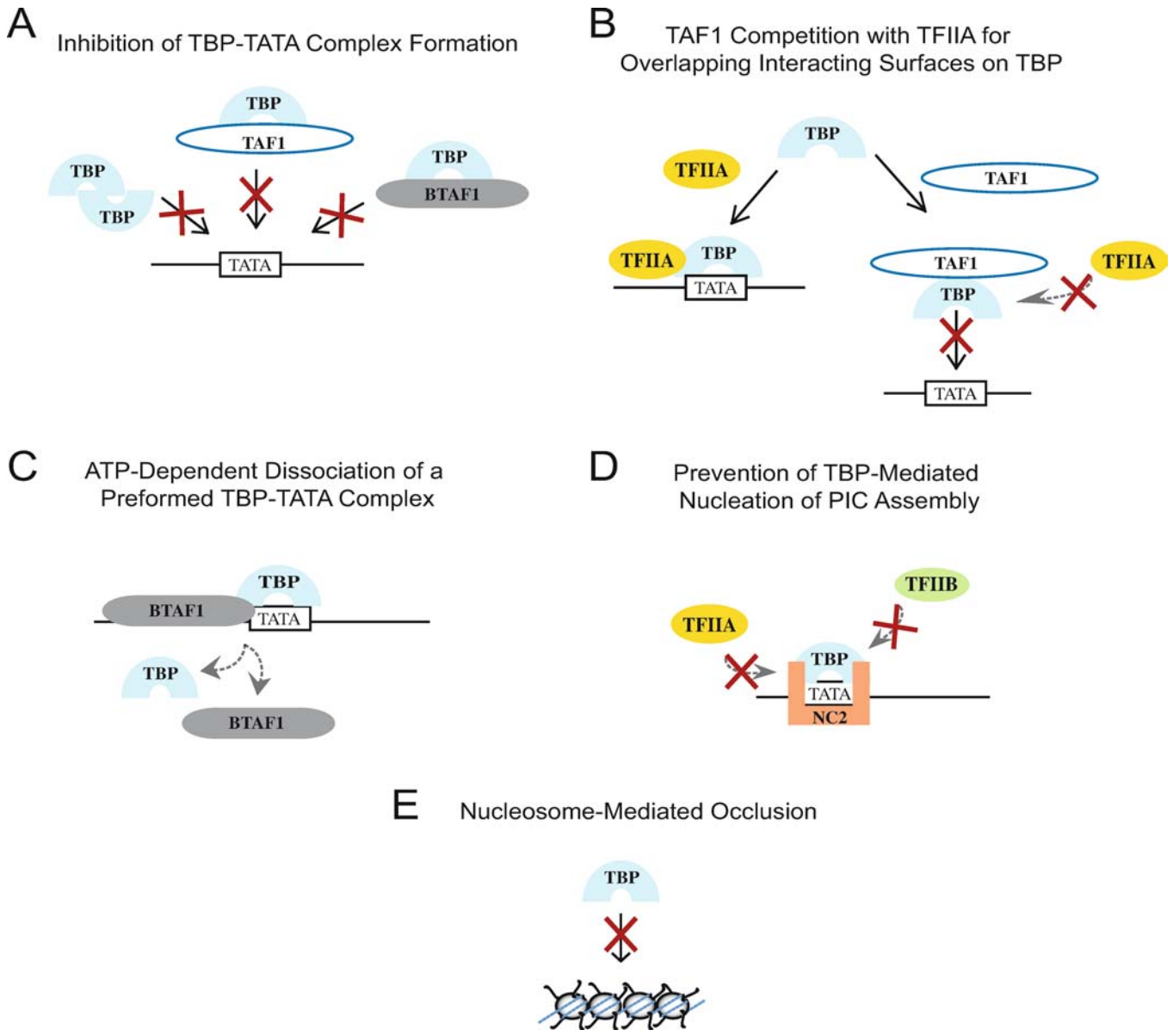


FIGURE 4 **Negative regulation of TBP-TATA complex formation. Multiple mechanisms are employed by transcription regulatory proteins to prevent TBP-TATA complex formation.**

through extensive contacts between the concave surfaces of each TBP monomer, thereby masking the DNA-binding domain also located in the concave region. Clearly only the monomeric form of TBP can bind to the DNA, as evidenced by the structural analysis of the TBP-TATA complex (Kim *et al.,* 1993a; Kim *et al.,* 1993b; Nikolov *et al.,* 1996). In yeast, these contacts are located in the deepest part of the concave surface, including amino acid residues N69, V71, V122, T124, N159, V161, V213, and T215, since substitutions of these amino acids individually with a bulky charged arginine amino acid result in destabilization of TBP dimers (Kou *et al.,* 2003) and, as shown with the V161R mutant, also significantly reduce the half-life of TBP *in vivo* (Jackson-Fisher *et al.,* 1999). The ability of TBP to form dimers also leads to the formation of TFIID homodimers, which can be detected by size exclusion column chromatography and by chemical cross-linking (Taggart and Pugh, 1996).

Although dimerization of the TBP core is clearly observed *in vitro*, dimerization of the full-length TBP is not always detectable under different conditions. By examining self-association of yeast TBP over a wide range of salt (60 mM to 1M KCl), protein concentrations (2.6 μM to 31 μM), and temperature (4°C to 37°C), Daugherty and colleagues (1999, 2000) found by

analytic ultracentrifugation that only monomeric rather than dimeric TBP is in equilibrium with tetrameric and octameric forms. Also by sedimentation equilibrium studies, Campbell and coworkers (2000) observed that at micromolar protein concentrations (6 μM to 60 μM) only monomeric yeast TBP was detected when 10% glycerol that prevents TBP aggregation was included in the buffer. Moreover, by using chemical crosslinking and gel filtration with 2.6 μM of TBP, Vanathi and colleagues (2003) noted that the monomeric form of TBP was favored over the dimeric form when 3 mM of Mg^{2+} was added in 100 mM to 500 mM KCl-containing buffer. Clearly, TBP self-association is a dynamic event influenced by ions, salt, buffer, and protein concentrations, wherein the N-terminal region of TBP somehow modulates these transitions.

Inhibition of TBP Promoter Binding Activity by TAF1

The DNA-binding activity of TBP is likewise subject to negative regulation by TAF1, the largest subunit of TFIID (Kokubo *et al.,* 1993). At the N-terminus of *Drosophila* TAF1 are two separate regions able to contact TBP: amino acid residues 11 to 77 (TAND1, for TAF N-terminal domain 1) interacting with the concave underside of TBP to block TATA recognition (Liu *et al.,* 1998; see Figure 4A) and amino acid residues 82 to 156 (TAND2) binding to the convex surface of TBP to compete with TFIIA that would otherwise facilitate TBP-TATA complex formation (Kokubo *et al.,* 1998; see Figure 4B). Interestingly, the solution structure of *Drosophila* TAND1 bound with yeast TBP shows a remarkable resemblance to the structure of the TBP-TATA complex (Liu *et al.,* 1998), as TAND1 exhibits an arch-shaped surface contacting the concave underside of TBP through both hydrophobic and electrostatic interactions. The negatively charged side chains of TAF1 (Asp29, Glu31, Glu51, Glu70, and Asp73) interact with the conserved lysine and arginine residues of TBP that also contact the phosphate backbone of the TATA sequence. Competition for the same binding surface underlies the mechanism for TAF1-mediated inhibition of TBP binding to the TATA box (Kokubo *et al.,* 1998), which may also account for reduced TATA-binding and transcription activity of TFIID, in comparison with TBP, *in vivo* and *in vitro* (Ozer *et al.,* 1998b; Wu and Chiang, 1998; Wu *et al.,* 1998). Likewise, TAND2-mediated inhibition of TFIIA binding to TBP is due to competition between TAF1 and TFIIA for the same conserved positive charge residues on the convex surface of TBP (Kokubo *et al.,* 1998; see Figure 4B). This TAF1-mediated effect on TBP-TFIIA and TBP-TATA interactions appear to be functionally conserved, as the same observation is also seen with experiments performed with homologous yeast and human proteins (Kokubo *et al.,* 1998; Ozer *et al.,* 1998b; Banik *et al.,* 2001).

Regulation of TBP-Promoter Complex Formation by BTAF1

Other than interacting with TAFs as part of TFIID, TBP also forms a distinct complex involved in pol II-mediated transcription. This "TFIID-like" complex, found in the P11 0.3 M KCl (or "B") fraction, is named B-TFIID, which is composed of TBP and a unique TBP-associated factor called BTAF1 (Timmers *et al.,* 1992). This BTAF1 protein was formerly named $TAF_{II}170$ and TAF-172 in humans (Timmers *et al.,* 1992; van der Knapp *et al.,* 1997; Chicca *et al.,* 1998), Mot1 in yeast (Poon *et al.,* 1994), and 89B helicase in *Drosophila* (Goldman-Levi *et al.,* 1996). As observed with TAF1, human BTAF1 binds the concave and convex surfaces of TBP likely in a reversible manner (Pereira *et al.,* 2001). The TBP concave-binding region, located at the N-terminal amino acid residues 290 to 381 of BTAF1, not only hinders TBP-DNA complex formation, but also blocks TAF1 interaction with the concave underside of TBP (Pereira *et al.,* 2001; see Figure 4A). While the N-terminus of BTAF1 interacts with TBP, the C-terminus possesses enzymatic activity allowing BTAF1 to induce the dissociation of TBP-TATA complexes in an ATP-dependent manner (Chicca *et al.,* 1998; see Figure 4C). This ATPase domain, with a conserved signature DEGH box within the Walker A motif that classifies BTAF1 as a member of the DNA-dependent SWI2/SNF2 ATPase family (Pereira *et al.,* 2003), however, is not necessary for BTAF1 binding to TBP (Auble *et al.,* 1997; Gumbs *et al.,* 2003). Thus, it is apparent that both the N-terminal TBP interaction domain and the C-terminal ATPase domain of BTAF1 are needed to remove TBP from the TATA box. This DNA-translocating activity also allows BTAF1 to clear TBP from nonpromoter AT-rich sequences, thereby enhancing the frequency of TBP binding to functional TATA elements. The redistribution of TBP to correct promoter regions may partially account for the coactivating

function of BTAF1 in stimulating both basal and activator-dependent transcription (Collart, 1996; Li *et al.,* 1999; Muldrow *et al.,* 1999; Andrau *et al.,* 2002; Dasgupta *et al.,* 2002; Geisberg *et al.,* 2002). Concurrently, BTAF1 may compete with other TBP-interacting factors, such as NC2 (see below) and TFIIA, along TBP's convex surface (Geisberg *et al.,* 2002; Klejman *et al.,* 2005) to further modulate promoter activity. An additional control mechanism lies in the observation that, in yeast, Mot1-TBP seems to exist as a transcriptionally inactive promoter-bound complex under normal growth conditions and is later activated by associating with other GTFs and pol II following environmental stress (Geisberg and Struhl, 2004). The dual role of BTAF1 in gene activation and repression is in agreement with the genome-wide transcriptional profiling data indicating that ~10% and ~5% of yeast genes are, respectively, more than two-fold upregulated and downregulated by BTAF1 (Dasgupta *et al.,* 2002; Geisberg *et al.,* 2002).

Although BTAF1 recruitment to TATA-containing promoters seems to be TBP-dependent (Geisberg and Struhl, 2004), BTAF1 can also stimulate transcription in a TBP-independent manner by facilitating chromatin remodeling via the use of its ATPase activity (Topalidou *et al.,* 2004). This coactivating activity of BTAF1 may account for the requirement of BTAF1 for transcription from some yeast TATA-less promoters (Collart, 1996). Nonetheless, whether TBP is somehow involved in BTAF1 recruitment to promoter regions lacking a canonical TATA box is yet to be resolved. A recent finding that BTAF1 is capable of recruiting TBP concave surface mutants defective in TATA binding to promoter fragments containing either wild-type or mutated TATA sequences (Klejman *et al.,* 2005) further suggests that BTAF1 may alter the DNA-binding specificity of TBP, allowing TBP to associate with TATA-less promoters. This intriguing possibility remains to be explored.

NC2 Regulation of TBP-TATA Complex Formation

A third TBP-interacting factor able to regulate TBP binding to the promoter region is negative cofactor 2 (NC2), which is conserved in eukaryotes and is essential for yeast viability (Inostroza *et al.,* 1992; Goppelt and Meisterernst, 1996; Gadbois *et al.,* 1997). Human NC2 consists of NC2α (Drap1; 22 kDa) and NC2β (Dr1; 20 kDa) that interact with each other through histone fold motifs, resembling the dimerization domain found in histones H2A and H2B (Goppelt *et al.,* 1996; Kamada *et al.,* 2001b). Both NC2α and NC2β subunits are required for stable NC2-TBP-TATA complex formation in electrophoretic mobility shift assays and also for NC2 to repress transcription from TATA-containing promoters in reconstituted cell-free transcription assays (Goppelt *et al.,* 1996). The structural analysis of NC2-TBP-TATA ternary complex, revealed by X-ray crystallography at 2.6 Å resolution, shows that the N-terminal regions of both NC2 subunits bind DNA on the underside of the preformed TBP-TATA complex and the C-terminus of NC2β additionally contacts the convex surface of TBP, hence giving NC2 the appearance of a molecular "clamp" that grips both upper and lower surfaces of the TBP-TATA complex (Kamada *et al.,* 2001b; see Figure 4D). As further demonstrated by electrophoretic mobility shift assays using NC2α mutants with an adenovirus major late TATA-containing promoter fragment, the C-terminus of NC2α indeed contributes to TBP-NC2-DNA complex formation (Gilfillan *et al.,* 2005). Obviously, this molecular clamp is able to block PIC assembly by inhibiting TFIIA and TFIIB binding to the upper side of TBP, an observation consistent with electrophoretic mobility shift assays and *in vitro* transcription experiments performed with recombinant proteins (Inostroza *et al.,* 1992; Kim *et al.,* 1995; Goppelt *et al.,* 1996). Indeed, some transcription factors, such as hypoxia-inducible factor 1α (HIF-1α), are able to inhibit transcription by inducing NC2 activity that, in turn, blocks PIC assembly (Denko *et al.,* 2003).

In addition to the repressive activity normally seen with TATA-containing promoters (Meisterernst and Roeder, 1991; Inostroza *et al.,* 1992), NC2 can also function as a coactivator in stimulating transcription from some TATA-less promoters (Lemaire *et al.,* 2000; Willy *et al.,* 2000). The dual role of NC2 in transcription allows it to regulate expression of ~17% of yeast genes, among which nearly half is either upregulated or downregulated (Geisberg *et al.,* 2001). Perhaps one of the underlying mechanisms for NC2 coactivator function is that NC2 works in conjunction with TFIID through the DPE to enhance TATA-less gene transcription (Burke and Kadonaga, 1997; Willy *et al.,* 2000). The observation that NC2 recruitment coresides with TBP on a number of TATA-containing and TATA-less human and yeast promoters, as revealed by *in vivo* chromatin immunoprecipitation (ChIP) assays, suggests that

NC2 may facilitate PIC formation by modulating TBP accessibility to the promoter region (Geisberg *et al.,* 2001; Cang and Prelich, 2002; Creton *et al.,* 2002; Gilfillan *et al.,* 2005). This finding is consistent with those of *in vitro* DNA-binding studies showing that the TATA-binding defects of TBP concave mutants can be restored by NC2, as also observed with BTAF1, in electrophoretic mobility shift assays (Klejman *et al.,* 2005). It is important to note that this NC2-induced TBP conformational change leading to productive PIC formation is likely distinct from the inhibitory clamp that prevents TBP association with TFIIA and TFIIB. As alluded to earlier in the discussion of BTAF1, NC2 likely competes with BTAF1 for the overlapping region on the convex surface of TBP, as illustrated by ChIP and *in vitro* DNA-binding assays showing that NC2-TBP and BTAF1-TBP complexes cannot be detected simultaneously on the same promoter (Geisberg *et al.,* 2002; Klejman *et al.,* 2005). Alternatively, NC2 may positively regulate transcription by targeting events downstream of the initiation step (*e.g.,* elongation), since NC2 association with the hyperphosphorylated form of pol II seems necessary for Gal4-VP16-mediated activation from the TATA-containing HIV-1 promoter (Castaño *et al.,* 2000). However, the exact mechanism underlying the coactivating function of NC2 during the transcription process remains to be elucidated.

In addition to forming a heterodimeric NC2 complex, NC2α and NC2β are also individually involved in the control of specific gene transcription. It seems that free forms of NC2α and NC2β are mainly found in exponentially growing yeast cells, whereas stable NC2α and NC2β complex is only detected after glucose depletion (Creton *et al.,* 2002). Interestingly, NC2α appears to co-reside with TBP at transcriptionally active promoters and NC2β is mainly associated with TBP-bound promoters at transcriptionally repressed genes (Creton *et al.,* 2002). This is not surprising considering that NC2, binding to the upper surface of TBP blocks TFIIB association and the available NC2α structure shows only binding to the underside of the TBP-TATA complex presumably without affecting PIC assembly (Kamada *et al.,* 2001b). Unexpectedly, the free entity of human NC2α, but not NC2β, or the NC2 complex, can interact with BTAF1 and in turn stimulates BTAF1 association with TBP on the DNA, as detected by *in vitro* DNA-binding assays (Klejman *et al.,* 2004). This finding suggests that NC2α and BTAF1 may bind at the same time to the underside and the upper region of the TBP-TATA complex, respectively. The functional role of this unusual quaternary complex, however, has not yet been addressed. Obviously, the presence of multiple forms of NC2-TBP complexes and the observations that NC2 can bind nonspecifically to DNA and is susceptible to post-translational modification by casein kinase II (CK2; Goppelt *et al.,* 1996; Creton *et al.,* 2002) provide an additional level of complexity for regulating promoter recognition by TBP and TFIID.

Positive Regulators that Promote TBP-TATA Complex Formation

For TBP to nucleate the assembly of a functional PIC, it must overcome molecular impediments that prevent productive TBP-TATA complex formation. Some of the impediments, as mentioned in the previous sections, include dimerization of TBP, inhibition of TBP binding to the TATA box exerted by TAF1 and BTAF1, and prevention of TFIIA and TFIIB association with the TBP-TATA complex by NC2 (see Figure 4A–D), as well as by nucleosomes assembled on the promoter region (Figure 4E). Alleviation of these inhibitory activities can be achieved by transcriptional regulators, which may function at the promoter recognition step by enhancing TBP binding to the TATA box, antagonizing repressor binding for the overlapping surfaces on TBP, stabilizing the bent TATA-TBP complex, or by modifying the chromatin structure surrounding the promoter region.

In general, TFIIA and TFIIB enhance TBP binding to the TATA box and further stabilize the TBP-promoter complex via distinct mechanisms (Figure 5A). TFIIA increases the likelihood of precise promoter recognition by TBP via promoting the dissociation of TBP dimers and thus facilitating the loading of monomeric TBP onto the TATA box (Coleman *et al.,* 1999; see Figure 5B). TFIIA also competes with the inhibitory domain of TAF1 for overlapping binding regions on TBP, hence alleviating TAF1-mediated inhibition of TATA recognition (Kokuba *et al.,* 1998). Furthermore, incorporation of TFIIA into the TBP-TATA complex renders the ternary complex resistant to BTAF1-mediated dissociation of the TBP-TATA complex (Auble and Hahn, 1993). Similarly, TFIIB can enhance TBP binding to the TATA box (Imbalzano *et al.,* 1994b) and also stabilize the bent TBP-TATA complex (Zhao and Herr, 2002), thereby reducing the dissociation rate of TBP from the promoter region (Wolner and Gralla, 2001).

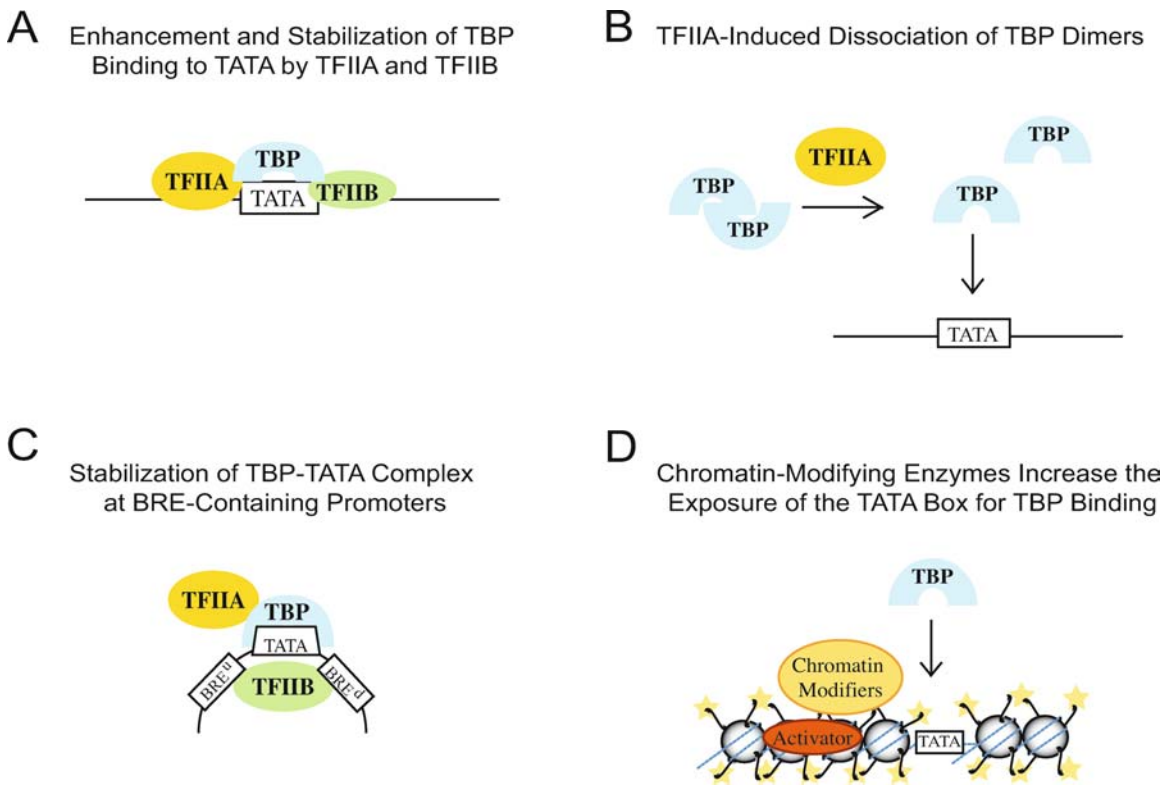


FIGURE 5 **Positive regulation of TBP DNA-binding activity. (*A*) TBP binding to the TATA box can be stabilized by TFIIA and TFIIB. (*B*) Inhibition of TBP binding to the TATA box can be alleviated by TFIIA-induced dissociation of TBP dimers. (*C*) TFIIB binding to BRE may provide additional contact points for further stabilization of TBP-TATA complex formation. (*D*) Chromatin-modifying enzymes that are recruited by activators may facilitate TBP loading onto the TATA box.**

Besides protein-protein interaction observed between TFIIB and TBP, additional DNA contacts provided by TFIIB-BRE^{u} and TFIIB-BRE^{d} likely contribute to this stabilizing effect (Figure 5C). Clearly, TFIIA and TFIIB help form a stable TBP-TATA complex, yet both protein factors may not efficiently enhance TBP binding to the TATA box when the promoter is in a nucleosome configuration.

Considering that approximately 146 base pairs of DNA are wrapped around each core histone octamer *in vivo*, the promoter region may be incorporated into a nucleosome, rendering the TATA box inaccessible for TBP binding thus suppressing basal transcription (Imbalzano *et al.,* 1994a; see Figure 4E). Undoubtedly, additional protein factors are needed to alleviate transcriptional repression from nucleosome-embedded promoters. In this regard, chromatin-modifying enzymes play a key role in enhancing TBP access to its recognition sequence by altering chromatin structure surrounding the TATA box (Martinez-Campa *et al.,* 2004; see Figure 5D). Chromatin modifiers may reconfigure nucleosome structure by covalently modifying histones to reduce protein-DNA interactions (Fischle *et al.,* 2003) or by using the energy of ATP hydrolysis to alter histone-DNA contacts in a process known as chromatin remodeling (Narlikar *et al.,* 2002). Since the interactions between the TATA box and nucleosomal core histones are regulated by acetylation of lysine residues at the N-terminal tails of core histones, acetylation of nucleosomal core histones at the promoter region creates a less compact chromatin structure, allowing TBP to bind the TATA box (Sewack *et al.,* 2001). SWI/SNF is an example of an ATP-dependent chromatin remodeler that enhances TBP access to the TATA box situated in a nucleosome (Imbalzano *et al.,* 1994a). The action of SWI/SNF following the acetylation of histones by GCN5 histone acetyltransferase is seen *in vivo* as well, as exemplified by SWI/SNF-facilitated TBP binding to the β-interferon promoter (Agalioti *et al.,* 2000). Interestingly, loading of TBP onto both TATA-containing and TATA-less promoters can be further enhanced by TBP association with other cellular proteins to form multiprotein complexes, such as TFIID and SAGA (see discussion of TAF-containing

complexes), both of which contain acetyltransferase activity.

TAF Recognition of Distinct Core Promoter Elements

The association of TBP with the TAF components of TFIID not only helps TBP to overcome chromatin barriers (Wu *et al.,* 1999), but also significantly broadens the scope of promoter recognition by this important core promoter-binding factor. Previous DNase I footprinting assays revealed that TFIID generates an extended protection over the TATA box on the adenovirus major late promoter when compared to that of TBP (Zhou *et al.,* 1992; Chiang *et al.,* 1993), indicating that TAFs may provide additional contacts with core promoter sequences (Oelgeschläger *et al.,* 1996; Verrijzer and Tjian, 1996). As described earlier in the discussion of core promoter elements, TFIID may contact the Inr via TAF1/TAF2, the DPE via TAF6/TAF9, and the DCE via TAF1, highlighting the importance of TFIID in core promoter recognition (see Figure 2). These additional TFIID-DNA contacts are particularly important at promoters lacking TATA sequences, as TAFs are required for transcription from TATA-less promoters (Pugh and Tjian, 1991; Martinez *et al.,* 1994; Orphanides *et al.,* 1996; Smale, 1997; Huisinga and Pugh, 2004). Recent findings that human TAF6 and TAF9 display sequence preference for the DPE present in the human interferon regulatory factor-1 promoter (Shao *et al.,* 2005) and that TAF1 can be crosslinked to individual DCE subelements (Lee *et al.,* 2005b), distinct from the previously identified upstream DNA contacts by TAF4, TAF5, TAF7 and an undefined 30 kDa TAF (Oelgeschläger *et al.,* 1996), in the adenovirus major late promoter further sustain the view that TFIID is a *bona fide* core promoter-binding factor. The extensive promoter contacts established by TFIID may provide additional stability for TFIID to act as a commitment factor. Indeed, as revealed by ChIP assays performed in HeLa cells, TFIID remains bound to active gene promoters during mitosis (Christova and Oelgeschläger, 2002), even though TFIID is likely inactivated due to mitosis-specific phosphorylation of some TFIID components (Segil *et al.,* 1996). Thus, TFIID may function as a promoter-marking factor for transcriptional memory in order to distinguish active from inactive genes as cells proceed through the cell cycle.

Histone-Like TAFs in TFIID

Many of the TAFs present within TFIID contain histone fold domains that contribute to the recognition of core promoter elements and to the integrity of TFIID complexes. Among many histone fold-containing TAFs identified thus far (*i.e.,* TAF3, TAF4, TAF4b, TAF6, and TAF8-13), four of them (TAF6, TAF9, TAF12, and TAF4/TAF4b) share sequence similarity to core histones H4, H3, H2B, and H2A, respectively (Gangloff *et al.,* 2001). A histone fold motif, consisting of a long α helix (α2) flanked on each side by a random coil loop (L1 or L2) and a short α helix (α1 or α3), is organized in the following order: α1-L1-α2-L2-α3 (Hoffmann *et al.,* 1996; Xie *et al.,* 1996; Birck *et al.,* 1998). This motif present in histone-like TAFs suggests that a TAF9-TAF6 heterotetramer and two of the TAF4b-TAF12 heterodimer may form a histone octamer-like structure (Selleck *et al.,* 2001), similar to H3-H4 tetramers and H2A-H2B dimers found in a nucleosome (Luger *et al.,* 1997). Indeed, initial crystal structures of *Drosophila* TAF9-TAF6 complexes revealed a heterotetramer, resembling the H3-H4 heterotetrameric core of the histone octamer. This finding indicates that TFIID may contain a histone octamer-like substructure (Xie *et al.,* 1996). Further supporting evidence for a histone-like octamer was demonstrated when it was found that yeast TAF9-TAF6-TAF12-TAF4 may reconstitute an octamer *in vitro* (Selleck *et al.,* 2001). The immediate question that follows is whether the histone-like octamer of TAFs is able to contact DPE in the context of a nucleosome-like structure. In a study investigating protein-protein interactions among human TAF9, TAF6, TAF4b, and TAF12, which contain sequences related to histones H3, H4, H2A, and H2B, respectively, it was found that these TAFs indeed form an octamer-like complex, which enhances both sequence-specific and nonspecific DNA-binding activities of TAF9-TAF6 and TAF4b-TAF12 pairs, respectively (Shao *et al.,* 2005).

In contrast to the interaction studies, which suggest that TFIID may contain a histone-like octamer, low-resolution electron microscopy (EM) studies of human and yeast TFIID complexes do not unveil an octamer-like structure within TFIID (Andel *et al.,* 1999; Brand *et al.,* 1999a; Leurent *et al.,* 2002, 2004). These EM studies revealed TFIID to be a trilobed (termed lobes A, B, and C), horseshoe-shaped structure with TBP sitting in the central cavity, while TFIIA and TFIIB bound to opposite lobes of the horseshoe-shaped structure

(Andel *et al.,* 1999; Brand *et al.,* 1999a; Leurent *et al.,* 2002). Each of the globular domains found in the TFIID EM structure is almost equivalent in size to the histone octamer, but none are large enough to accommodate all histone motif-containing TAFs (Brand *et al.,* 1999a). Therefore, a histone octamer-like structure containing all the TAFs with histone fold motifs may not exist in TFIID. However, the possibility that fewer TAFs may form a nucleosome-like structure remains to be explored. On the other hand, the histone folds in TAFs are likely to be important for protein-protein interactions for maintaining the structural integrity of TFIID, as pairwise interactions observed between histone-like TAFs can indeed be detected in yeast TFIID (Leurent *et al.,* 2002). From TAF localization studies of yeast TFIID revealed by immuno-EM, it appears that TAF5 plays a central role in connecting the three lobes of TFIID, with each lobe harboring a subset of histone-like TAFs (Leurent *et al.,* 2004). Consistent with the functional studies showing that TAND1 blocks TBP binding to the TATA box (Liu *et al.,* 1998) and TAF7 inhibits the histone acetyltransferase activity of TAF1 (Gegonne *et al.,* 2001), it was found that the N-terminal domain of TAF1 is spanned over TBP in the central lobe, whereas the histone acetyltransferase domain of TAF1 is colocalized with TAF7 in the same side lobe (Leurent *et al.,* 2004). The characterization of TFIID structures undoubtedly will further shed light on the molecular mechanism of TFIID function in PIC assembly.

Enzymatic Activities of TFIID

The presence of TAF1 in TFIID not only modulates DNA binding by TBP, but also confers on TFIID multiple enzymatic activities to post-translationally modify histones and transcription factors, thus allowing TFIID to serve as a core promoter-binding factor in the context of chromatin and as a coactivator mediating activator response. Human, *Drosophila* and yeast TAF1, a TFIID-specific subunit, is known to function as a histone acetyltransferase (HAT) in acetylating histones H3 and H4 *in vitro* (Mizzen *et al.,* 1996). *Drosophila* TAF1 has also been shown to act as a kinase in phosphorylating histone H2B (Maile *et al.,* 2004) and as a histone-specific ubiquitin-activating/conjugating enzyme in mediating monoubiquitination of linker histone H1 (Pham and Sauer, 2000) both *in vitro* and *in vivo*. These histone-modifying activities indicate that TFIID may be specifically needed for transcription from nucleosome-embedded promoters. Indeed, only TFIID, rather than TBP, can work in conjunction with a TFIID-deficient pol II holoenzyme complex to facilitate activator-dependent transcription from an *in vitro*-reconstituted chromatin template, suggesting a unique involvement of TAFs in chromatin transcription (Wu *et al.,* 1999). The importance of the acetyltransferase activity of TAF1 for gene transcription has also been demonstrated for MHC class I genes, where suppression of TAF1 enzymatic activity by TAF7, which is a TAF1-interacting protein able to contact multiple transcriptional activators (Chiang and Roeder, 1995; Lavigne *et al.,* 1999), leads to inhibition of transcription (Gegonne *et al.,* 2001). The involvement of TAF1 HAT activity in gene transcription, however, appears to be promoter-specific, as hypoacetylation of H3 due to TAF1 inactivation in the ts13 hamster cell line was only observed at the cyclin D1 promoter, but not the *c-fos* promoter (Hilton *et al.,* 2005). Similar to some HATs, higher eukaryotic TAF1 contains two tandem bromodomains recognizing acetylated lysine 14 (K14) of histone H3 as well as acetylated K5, K8, K12, and K16 of histone H4 (Jacobson *et al.,* 2000; Kanno *et al.,* 2004). This bromodomain-mediated interaction might enhance TFIID binding to acetylated nucleosomes at promoters previously modified by activator-recruited HATs. The structure of the human TAF1 double bromodomain spanning amino acid residues 1359 to 1638, resolved by X-ray crystallography at 2.1 Å resolution, reveals that two acetylated lysine residues of histone H4, separated by 7 or 8 amino acids (*i.e.,* K5/K12 or K8/K16), are preferentially recognized by the double bromodomain, where the N^{ε}-acetyllysine-binding pocket is situated within the amino acid residues of the two loops connecting the four antiparallel α-helices that constitute the core of each bromodomain (Jacobson *et al.,* 2000).

Other than acting on histone substrates, TAF1, either as a free entity or part of TFIID, can also covalently modify other general transcription factors and cofactors, as demonstrated by acetylation on TFIIEβ (Imhof *et al.,* 1997), phosphorylation on RAP74 (Dikstein *et al.,* 1996), serine 33 of histone H2B (Maile *et al.,* 2004), PC4 (Kershnar *et al.,* 1998; Malik *et al.,* 1998), and the β subunit of TFIIA (Solow *et al.,* 2001), and presumably ubiquitination on TAF5 and itself as well (Auty *et al.,* 2004). Interestingly, TAF1-mediated phosphorylation of histone H2B correlates with gene activation

(Maile *et al.,* 2004). Thus, the ability of TAF1 to modify distinct histones by acetylation, phosphorylation, or ubiquitination may account for TFIID involvement in chromatin transcription (Wu *et al.,* 1999).

TFIID as a Coactivator

Characterization of TAFs revealed that many transcriptional activators interact directly with specific TAFs (reviewed by Burley and Roeder, 1996; Verrijzer and Tjian, 1996). For example, the activation domain of Sp1 was found to interact with *Drosophila* TAF4 (Hoey *et al.,* 1993), whereas the DNA-binding domain of Sp1 was shown to contact human TAF7 (Chiang and Roeder, 1995). The finding that distinct domains in an activator are capable of contacting different TAFs suggests that TFIID may modulate activator function through multiple domain interactions (Chiang and Roeder, 1995), presumably by enhancing activator binding to the target site and further stabilizing TFIID-promoter contacts. That human TAF7 is able to interact with different transcriptional activators, including Sp1, YY1, USF, CTF, adenovirus E1A, and HIV-1 Tat proteins through its N-terminal domain, and with TAF1 via its central region indicates that TAF7 indeed acts as a coactivator bridging the activator and the general transcription machinery (Chiang and Roeder, 1995). Additional contacts between TAF7 and other TFIID components, such as TAF5, TAF11, TAF12, and TAF13, have also been demonstrated in human TFIID (Lavigne *et al.,* 1996) and have been verified by immuno-EM structural analysis performed with yeast TFIID (Leurent *et al.,* 2004). These activator-TAF and TAF-TAF interactions imply that activators may function by recruiting TFIID to the promoter in order to nucleate PIC formation (Burley and Roeder, 1996; Verrijzer and Tjian, 1996; Albright and Tjian 2000; Näär *et al.,* 2001). Consistent with its role as a coactivator, only purified TFIID, but not TBP, supports activator-mediated transcription in partially purified cell-free transcription systems (Dynlacht *et al.,* 1991; Chiang *et al.,* 1993). Thus the *in vitro* biochemical concept that TFIID was a universal coactivator required for all gene transcription was formed.

When the role of TAFs was later examined in yeast, it was found that transcription from selective genes *in vivo* could occur in the absence of TAFs (Moqtaderi *et al.,* 1996; Walker *et al.,* 1996). Additional evidence supporting the view that TAFs may not be universally required for gene function *in vivo* came from studies performed with hamster ts13 cells harboring an amino acid substitution G690D (Hayashida *et al.,* 1994; human equivalent G716D) in TAF1 (Wang and Tjian, 1994; Suzuki-Yagawa *et al.,* 1997) and with chicken DT40 TAF9-conditional knockout cells (Chen and Manley, 2000). Indeed, two distinct classes of yeast genes (TAF-dependent and TAF-independent) have been identified based on their differential requirement for TFIID TAFs (Kuras *et al.,* 2000; Li *et al.,* 2000). Although TAF1 was originally reported to play a limited role in transcription for only a subset (~16%) of yeast genes (Holstege *et al.,* 1998), more refined genome-wide gene profiling studies have indicated a broader involvement of TAF1 for transcription from the majority (~90%) of yeast and human genes whose promoters are predominantly TATA-less (Huisinga and Pugh, 2004; Kim *et al.,* 2005a) and primarily for housekeeping, such as the ribosomal protein-encoding gene promoters (Mencia *et al.,* 2002). This is in agreement with a similar requirement of TAF9 for the majority (~67%) of yeast gene transcription (Apone *et al.,* 1998; Holstege *et al.,* 1998; Michel *et al.,* 1998; Moqtaderi *et al.,* 1998). It should be noted that histone fold-containing TAF9 is present not only in TFIID, but also in several other TAF-containing complexes, including TFTC, SAGA, SLIK, and STAGA (see discussion of TAF-containing complexes). Therefore, inactivation of TAF9 may affect the function of multiple protein complexes.

The finding that TAFs may not be essential *in vivo* for every gene function has also been verified *in vitro* by cell-free transcription studies using highly purified and well-defined transcription systems. In these studies, TBP, in the absence of TAFs but in conjunction with other GTFs, pol II and general cofactor PC4, is able to direct activated transcription from TATA-containing promoters in an activator-specific manner with nucleosome-free DNA templates (Oelgeschläger *et al.,* 1998; Wu and Chiang, 1998; Wu *et al.,* 1998, 1999; Fondell *et al.,* 1999). TBP, free from TAFs of TFIID, is indeed found in yeast (Kuras *et al.,* 2000; Li *et al.,* 2000) and can be biochemically separated from TAFs during *in vitro* fractionation of yeast TFIID (Sanders *et al.,* 2002a). Although TFIID is the predominant form found in mammalian cells, the free form of TBP may transiently exist under some stressed conditions (Wu *et al.,* 1998) and may further associate with other cellular proteins to form distinct TBP complexes. Clearly, activator-regulated steps of transcription complex assembly are likely to be different,

depending on whether TBP or TFIID is used as the TATA-binding factor.

TAF-Dependent Activation

In TATA-less promoters where TAFs are needed for promoter recognition, it is plausible that the interactions between activators and TAFs are involved in the recruitment of TFIID to the Inr, DCE, and DPE promoter elements characterized in higher eukaryotes, hence modulating the level of transcription. Indeed, specific DPE has been shown to be essential for the function of some transcriptional activators in *Drosophila* embryos (Butler and Kadonaga, 2001), likely reflecting unique contacts between gene-specific activators and distinct components of TFIID that allosterically modulate TAF6-TAF9 recognition of the DPE. In addition, specific TFIID contacts with additional promoter sequences can be induced by the presence of activators, as illustrated by ATF-induced extension of TFIID footprinting to the downstream region of the adenovirus E4 promoter (Horikoshi *et al.,* 1988). Obviously, activators may facilitate TFIID binding to the core promoter region, which is often the rate-limiting step for the assembly of a functional transcription complex in both TATA-containing and TATA-less promoters, as documented by many DNA-binding and transcription studies (see Wu and Chiang, 2001a, and references therein). Normally, TFIID binds less efficiently to the TATA box compared to that of TBP, due to TAF1-mediated inhibition of TBP-promoter contacts. Interestingly, TAFs also contact several GTFs, including TFIIA (Yokomori *et al.,* 1993), TFIIB (Goodrich *et al.,* 1993), TFIIE (Hisatake *et al.,* 1995), the RAP74 subunit of TFIIF (Ruppert and Tjian, 1995), and both the RPB1 and RPB2 subunits of pol II (Wu and Chiang, 2001a). These TAF-mediated protein-protein interactions facilitate the entry of the remaining GTFs and pol II through either the sequential assembly pathway or the preassembled pol II holoenzyme pathway, thereby shifting the rate-limiting step for PIC assembly from the entry of downstream factors to promoter recognition by TFIID (Figure 6, *top*).

TAF-Independent Activation

The concept that TAFs are not absolutely required for pol II-dependent transcription has been supported by both yeast genetic analysis (Apone *et al.,* 1996; Moqtaderi *et al.,* 1996; Walker *et al.,* 1996) and biochemical reconstitution studies (Oelgeschläger *et al.,* 1998; Wu and Chiang, 1998; Wu *et al.,* 1998; Fondell *et al.,* 1999). A dispensable role of TAFs for selective gene transcription is also consistent with earlier microarray analyses indicating that only a subset of yeast genes, ranging from 8% to 20%, are affected by functional inactivation of TAF1, TAF5, TAF6, TAF10, and TAF12, individually (Green, 2000). For those genes that do not rely on TAFs, TBP appears to serve as the TATA-binding factor for TATA-containing gene transcription. Since TAF1-imposed inhibition of TATA binding is no longer observed with TBP, the rate-limiting step for PIC formation regulated by transcriptional activators may shift from promoter recognition to the entry of downstream factors, especially the pol II recruitment step (Figure 6, *bottom*). However, other regulatory steps occurring prior to TBP binding to the TATA box—such as histone modification, chromatin remodeling, TBP dimer dissociation, and activator modulation—are likely to take place on different promoter context. This mechanism underlying TAF-independent activation at the step of pol II recruitment is supported by order-of-addition transcription experiments and factor recruitment assays performed with individually purified general transcription factors, PC4 cofactor, pol II and activator, mimicking the assembly of transcription complexes via the sequential assembly pathway (Wu and Chiang, 2001a).

TBP, besides forming different classes of TAF complexes such as selectivity factor 1 (SL1) and TFIIIB used as core promoter-binding factors for RNA polymerase I and III, respectively, can also interact with BTAF1, NC2, or itself to downregulate the formation of TBP-TATA complexes. Moreover, TBP is able to associate with the γ and unprocessed $\alpha\beta$ subunits of TFIIA to form the TAC (TBP-TFIIA-containing) complex, which is detectable in P19 embryonal carcinoma cells but not in differentiated cells (Mitsiou and Stunnenberg, 2000). Intriguingly, exogenous expression of p300 in monkey kidney COS-7 cells induces TAC formation from endogenous TBP and TFIIA components, correlating with the observation that acetylated TFIIA$\alpha\beta$ is preferentially found in the TAC complex (Mitsiou and Stunnenberg, 2003). Obviously, the mechanism by which TAC regulates transcription and the identity of TAC-regulated promoters remain to be uncovered.

It is important to note that, in the absence of TAFs, TBP can also function *in vitro* with other general cofactors, such as PC4 (Wu and Chiang, 1998; Wu *et al.,* 1998), Mediator (Wu *et al.,* 2003), and the coactivating

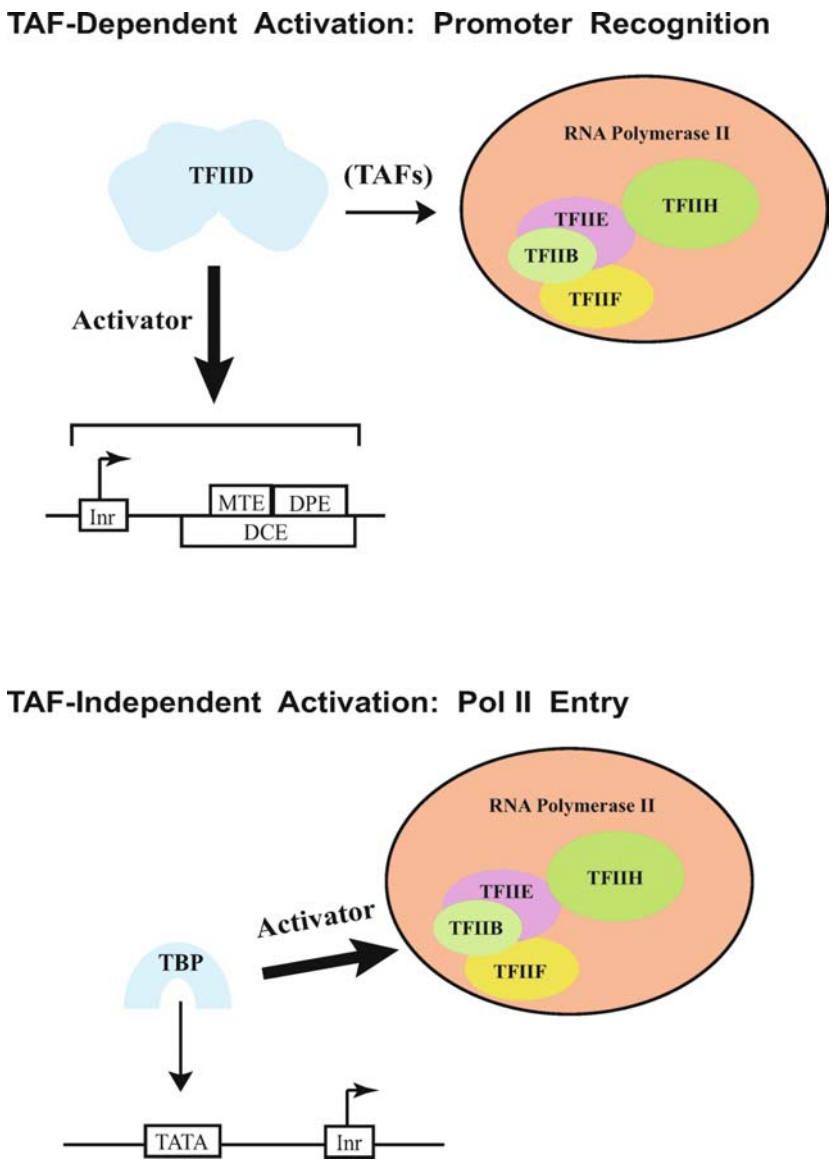


FIGURE 6 **TAF-dependent (*i.e.*, TFIID-mediated) *vs.* TAF-independent (*i.e.*, TBP-mediated) activation. Promoter recognition by TFIID is usually the rate-limiting step (*thick arrow; upper panel*) facilitated by transcriptional activators. In contrast, when TBP is used as the TATA-binding factor, pol II entry often becomes the rate-limiting step (*thick arrow; lower panel*) facilitated by activators. For simplicity, a preassembled pol II holoenzyme complex is depicted. (Adapted from Wu and Chiang, 2001a)**

activity of TFIIA and TFIIH (Wu *et al.*, 1998), during the activation process. These general cofactors are capable of functionally replacing TAFs in conveying regulatory signals between activators and the general transcription machinery, although the precise mechanisms have not yet been elucidated.

VARIANTS OF TBP AND TAFs

Some components of TFIID, including TBP and TAFs, have gene paralogs (*i.e.*, related proteins encoded by different genes belonging to the same family) often expressed in a tissue- and development-specific manner. These TBP-related factors (TRFs) and TAF variants likewise play an important role in pol II-dependent transcription. In addition, multiple forms of TAF-containing complexes exhibiting HAT activity involved in chromatin dynamics have been identified. These protein factors will be discussed briefly here.

TBP-Related Factors

All multicellular organisms contain at least two genes coding for TBP family members that share sequence homology at their C-terminal 180 amino acid core DNA-binding domains. While the first gene encodes

TBP itself, considered a universal TATA-binding transcription factor present in all eukaryotes and archaea, the second gene encodes TBP-related factor 2 (TRF2), also called TBP-related protein (TRP), TBP-like factor (TLF) or TBP-like protein (TLP). Although TRF2 shares ~60% sequence homology and 41% identity within the C-terminal core with that of TBP, it recognizes a sequence element distinct from the TATA box due to changes in some conserved amino acids, including substitutions of three out of four phenylalanine residues that kink the TATA box (Dantonel *et al.,* 1999; Berk, 2000; Hochheimer and Tjian, 2003). However, amino acid residues important for interactions with TFIIA and TFIIB are mostly unaltered, allowing TRF2 to associate with TFIIA and TFIIB (Maldonado, 1999; Moore *et al.,* 1999; Rabenstein *et al.,* 1999; Teichmann *et al.,* 1999) and thus assembling into a functional PIC able to transcribe some TATA-less promoters via an undefined TRF2-binding element (Ohbayashi *et al.,* 2003; Chong *et al.,* 2005). This property of TRF2 also enables it to function as a repressor, preventing PIC formation initiated by TBP or TFIID on TATA-containing promoters (Moore *et al.,* 1999; Teichmann *et al.,* 1999; Chong *et al.,* 2005), likely due to competition for limiting amounts of TFIIA and TFIIB. Not surprisingly, inactivation of TRF2 in *C. elegans* (Dantonel *et al.,* 2000; Kaltenbach *et al.,* 2000) and *Xenopus laevis* (Veenstra *et al.,* 2000) by RNA interference fails to support embryogenesis, indicating that TRF2 is essential for normal cell development as expected from its dual role in pol II-dependent transcription. In contrast to embryonic lethality seen with *C. elegans* and *X. laevis*, TRF2-null mice are perfectly viable and only exhibit defects in spermiogenesis, suggesting that the biological role of TRF2 is species-specific (Martianov *et al.,* 2001; Zhang *et al.,* 2001).

Other than TRF2, three species-specific TRFs (TRF1, TRF3, and TRF4) have also been identified. TRF1 (Crowley *et al.,* 1993), so far only identified in neuronal and germ cells of *Drosophila* (reviewed in Berk, 2000; Hochheimer and Tjian, 2003), exhibits 63% amino acid sequence identity to TBP at its C-terminal DNA-binding core which maintains most of the conserved residues important for interactions with the TATA box, TFIIA and TFIIB (Rabenstein *et al.,* 1999). It is thus expected that TRF1 binds TFIIA and TFIIB and can partially substitute for TBP in directing pol II-dependent transcription from some TATA-containing promoters *in vitro* (Hansen *et al.,* 1997). Interestingly, a TRF1 target gene, *Tudor*, whose expression is driven by two tandem promoters containing a TRF1-responsive upstream promoter and a TBP/TFIID-responsive downstream promoter, has been identified in *Drosophila* cells. A TC-rich sequence, located between −22 and −33 relative to the transcription start site of this upstream TRF1-responsive *Tudor* promoter preferentially nucleates TRF1-mediated PIC assembly and transcription. *In vivo*, promoter selectivity by TRF1 is likely enhanced by some transcriptional activators or by its association with neuron-specific TRF1-associated factors (nTAFs) to form a multiprotein complex, which is distinct from TFIID (Holmes and Tjian, 2000). That TRF1 does not interact with TFIID-specific TAFs provides a rationale why TRF1 and TBP are not interchangeable at respective TRF1-responsive upstream and TBP-responsive downstream *Tudor* promoters (Holmes and Tjian, 2000). While TRF1 clearly plays an important role in pol II-mediated transcription, the majority of cellular TRF1 is in fact associated with the TFIIB-related factor BRF, which is a component of TFIIIB involved in pol III-dependent transcription (Takada *et al.,* 2000). Similar to the pol II system, only addition of the TRF1-BRF complex, but not TBP-BRF, could restore pol III-dependent transcription of the tRNA, 5S RNA and U6 RNA genes in TRF1-depeleted extracts (Takada *et al.,* 2000).

TRF3, which shows 93% amino acid sequence identity to TBP at the C-terminal core region and has the same sequence as TBP at all of the conserved residues involved in TATA binding and interactions with TFIIA and TFIIB, is unique to vertebrates, ranging from fish to humans, but is not present in urochordate *Ciona intestinalis* and lower eukaryotes, such as *Drosophila* and *C. elegans* (Persengiev *et al.,* 2003). Unlike TRF1 and TRF2, which are expressed only in selective tissues, TRF3 is ubiquitously expressed in every cell type examined, similar to that of TBP, and is present as a protein complex with a molecular size of approximately 200 kDa. Since TAF1 does not cofractionate with the TRF3 complex, it is likely that polypeptides constituting the TRF3 complex are different from TAFs defined in TFIID and nTAFs associated with TRF1. The transcriptional properties of TRF3 and its regulated genes remain to be characterized.

TRF4, the only TBP-related protein found in the genomes of the unicellular human parasite *Trypanosoma brucei* (Tb) and several Tb-related family members where DNA sequences corresponding to *bona fide* TBP cannot be identified, displays only 31% amino acid sequence identity to *Drosophila* TBP at the C-terminal

core domain (Ruan *et al.,* 2004). Since amino acid residues critical for TFIIA and TFIIB interactions, but not the phenylalanine residues involved in TATA recognition, are still conserved in TRF4, it is likely that TRF4 may interact with TFIIA but fails to bind the TATA sequence. Indeed, a six-subunit complex containing the ~30 kDa TRF4 protein, three small nuclear RNA-activating complex (SNAPc) subunits (identified as TbSNAP50, TbSNAP2, TbSNAP3), the 25 kDa Tb ortholog (TbTFIIA-2) of the small TFIIA subunit γ, and a 75 kDa protein exhibiting weak sequence homology to the unprocessed $\alpha\beta$ subunits of TFIIA has been isolated. This TRF4 complex is able to recognize the upstream sequence element (USE) of the TATA-less spliced leader (SL) RNA gene and drives transcription of the SL RNA that is critical for mRNA maturation in Trypanosomatid parasites (Schimanski *et al.,* 2005). The finding that TRF4 is universally required for transcription by all three nuclear RNA polymerases suggests that TRF4 may functionally replace the classically defined TBP in these human parasites. However, it is yet to be resolved how transcription can proceed in these organisms lacking the genes coding for the other GTFs, such as TFIIB, TFIIF, and TFIIE.

In contrast to the functionally characterized TRFs present in eukaryotes and in Trypanosomatid parasites described above, very little is known about TBP-related family proteins in archaea despite the fact that multiple genes potentially encoding TBPs are found in several sequenced archaeal genomes. It was reported that the archaeon *Halobacterium* species NRC-1 has six predicted genes (Ng *et al.,* 2000b) that code for TBP family proteins (TBPa, TBPb, TPBc, TBPd, TBPe, and TBPf). Except TBPa, the other five members all have two direct repeats forming a saddle-like structure with two side stirrups, as found in the C-terminal region of their eukaryotic counterparts (Baliga *et al.,* 2000). TBPa has only one direct repeat and is thus unlikely to bind the TATA box as a monomer. Similar findings of multiple TBP family genes have also been described in other archaea, including *Methanosarcina acetivorans* (Galagan *et al.,* 2002) and *Haloferax volcanii* (Thompson *et al.,* 1999). The identification of a TBP gene family in archaea, similar to the discovery of multiple σ factors in bacteria (Borukhov and Nudler, 2003; Gruber and Gross, 2003), raises an interesting possibility that a combination of multiple TBPs and TFBs (the archaeal counterpart of TFIIB) is employed with its single RNA polymerase to generate diverse transcription complexes for recognizing different archaeal gene promoters. This is certainly an open area waiting to be explored.

TAF-Containing Complexes

Similar to TBP that forms SL1, TFIID, TFIIIB, and TAC complexes, some TAF components in TFIID are also present in distinct complexes such as TBP-free TAF_{II}-containing complex (TFTC), TFTC-related PCAF/GCN5 complexes, Spt-Ada-Gcn5 acetyltransferase (SAGA), SAGA-like complex (SLIK; also named "SALSA" for SAGA altered, Spt8 absent), Spt3-TAF9-GCN5L (*i.e.,* the long form of GCN5) acetylase (STAGA), and polycomb repressive complex 1 (PRC1). These TBP-lacking TAF-containing complexes are involved in diverse aspects of pol II-dependent transcription. Except PRC1 whose role is mainly involved in gene silencing, the other TAF-containing complexes are mostly implicated in activator-dependent transcription likely due to the HAT activity inherent to each complex.

TFTC, originally identified in HeLa cells using a monoclonal antibody against human TAF10 (Wieczorek *et al.,* 1998), contains TAF2, TAF5, TAF5L, TAF6L, TAF7, and some histone fold-containing TAFs, including TAF4, TAF6, TAF9, TAF10, and TAF12. In addition, TFTC has TRRAP (transformation-transactivation domain-associated protein), SAP130 (spliceosome-associated protein 130), GCN5L HAT enzyme, Ada3 adaptor protein, and histone fold-containing Spt3 (Cavusoglu *et al.,* 2003), TAF9L (also called TAF9b; Frontini *et al.,* 2005), and ataxin-7 (Helmlinger *et al.,* 2004). The three-dimensional structures of TFTC and TFIID, both resolved at 35 Å resolution by electron microscopy and single-particle image analysis, resemble a macromolecular clamp consisting of five globular domains (for TFTC) or three lobes (for TFIID) organized around a solvent-accessible groove that may accommodate a DNA duplex (Andel *et al.,* 1999; Brand *et al.,* 1999a). This configuration suggests that TFTC may adapt a DNA-binding conformation similar to that exhibited by TFIID. Indeed, TFTC is able to support basal and Gal4-VP16-mediated transcription *in vitro* from TATA-containing adenovirus major late and rabbit β-globin promoters and also basal transcription from the TATA-less transcription enhancer factor-1 (TEF-1) promoter, presumably via TAF recognition of the core promoter and interaction with other components of the general transcription machinery (Wieczorek *et al.,* 1998). The coactivator function of TFTC is in part mediated

by direct protein-protein contacts between activators and multiple subunits of TFTC, including TAF5, TAF6, TAF10, SAP130, Spt3, GCN5L, TRRAP, and ataxin-7 (Hardy *et al.,* 2002; Yanagisawa *et al.,* 2002; Helmlinger *et al.,* 2004; Palhan *et al.,* 2005). The TRRAP component of TFTC seems to act as a direct target for several liganded nuclear hormone receptors, including ERα, ERβ, VDR, and PPARγ (Yanagisawa *et al.,* 2002), as observed in the SAGA complex (see below). This activator-TFTC interaction may also facilitate p300-mediated transcription from a Gal4-VP16-dependent chromatin template (Hardy *et al.,* 2002), probably resulting from preferential acetylation of nucleosomal core histone H3 by the GCN5L component of the TFTC complex (Brand *et al.,* 1999b). Besides the HAT component, the presence of many histone fold-containing proteins, including Spt3, TAF4, TAF6, TAF9, TAF10, and TAF12, further contributes to the integrity of the TFTC complex. Recently, ataxin-7, the human homolog of yeast Sgf73 that is an integral component of yeast SAGA and SLIK complexes (see below), has also been shown to be essential for the structural integrity of TFTC and its related STAGA complex (Helmlinger *et al.,* 2004). Both wild-type and mutant ataxin-7, which contains polyglutamine expansion at its N-terminus and is linked to spinocerebellar ataxia type 7 (SCA7) neurodegenerative disorder, can be incorporated into TFTC and STAGA complexes whose structural components are largely unaltered (Helmlinger *et al.,* 2004; McMahon *et al.,* 2005; Palhan *et al.,* 2005). Presumably, incorporation of either form of ataxin-7 will alter the functional properties of these HAT complexes (see below).

Similar to TFTC, the yeast SAGA also lacks TBP but contains some common subunits, such as Tra1 (the yeast homolog of human TRRAP), adaptor protein Ada3, GCN5 (short form), TAF5, and histone fold-containing proteins Spt3, TAF6, TAF9, TAF10, and TAF12. The unique components present in SAGA, but not yet documented in TFTC, include Ada1, Ada2, Spt7, Spt8, Spt20 (also called Ada5), Chd1 (for chromo-ATPase/helicase-DNA binding domain 1), Ubp8 (for ubiquitin-specific processing protease 8), Sgf11 (for SAGA-associated factor 11 kDa), Sgf29, and Sgf73 (Grant *et al.,* 1998; Sanders *et al.,* 2002b; Henry *et al.,* 2003; Daniel *et al.,* 2004; Powell *et al.,* 2004; Ingvarsdottir *et al.,* 2005; Lee *et al.,* 2005c; McMahon *et al.,* 2005; Pray-Grant *et al.,* 2005). Five major functions attributed to SAGA include: activator interaction, contacting TBP, recognition of methylated histone H3, HAT activity, and deubiquitinating activity. These SAGA components are organized into five distinct domains with an overall shape analogous to the structure of human TFTC, as resolved by electron microscopy at 31 Å resolution (Wu *et al.,* 2004). The largest subunit of SAGA, Tra1, is located in the outermost globular domain able to contact transcriptional activators, as evidenced by the fluorescence resonance energy transfer (FRET) technique measuring direct *in vivo* association between yeast Gal4 acidic activator and Tra1 (Bhaumik *et al.,* 2004). This protein-protein interaction was also demonstrated by *in vitro* crosslinking experiments, in which the Tra1 subunit of SAGA was shown to serve as a direct target for both Gal4 and GCN4 transcriptional activator (Fishburn *et al.,* 2005; Reeves and Hahn, 2005). The illustration of Tra1 as a "true" target for transcriptional activators in *S. cerevisiae* is also consistent with the finding that human TRRAP, which shows 27.3% amino acid identity and 58.9% similarity with its yeast homolog, also interacts with c-Myc and E2F-1 oncoproteins implicated in mammalian transformation and transactivation (McMahon *et al.,* 1998). The c-Myc-recruited TRRAP apparently associates with GCN5 HAT activity that is important for c-Myc-induced cellular transformation (McMahon *et al.,* 2000). However, whether recruitment of TRRAP and GCN5 occurs in the context of human SAGA-like complexes, such as TFTC, PCAF and GCN5 complexes (Ogryzko *et al.,* 1998), remains to be investigated. Interestingly, approximately 10% of yeast genes, which are mostly driven by TATA-containing promoters and which are stress-induced, appear to be SAGA-dependent (Lee *et al.,* 2000; Huisinga and Pugh, 2004). These TFIID-independent yeast promoters are recognized by the free form of TBP that works in conjunction with SAGA at the targeted promoters via direct interaction between TBP and the Spt3 and Spt8 subunits of SAGA (Belotserkovskaya *et al.,* 2000; Larschan and Winston, 2001; Bhaumik and Green, 2002; Warfield *et al.,* 2004). The recruitment of SAGA by transcriptional activators likely enhances TBP binding to the promoter region, as found in the yeast PHO5 promoter (Barbaric *et al.,* 2003), or increases factor accessibility by GCN5-mediated acetylation of nucleosomal core histones at the promoter region (Grant *et al.,* 1997), thus leading to gene activation. Moreover, it was recently found that the 19S regulatory subcomplex of the 26S proteasome is able to enhance activator-dependent recruitment of the yeast SAGA

complex, apparently in an ATPase-dependent manner, by stimulating SAGA's nonspecific DNA-binding and HAT activities (Lee *et al.,* 2005a). These effects may further contribute to the coactivating function of the SAGA complex. Interestingly, SAGA can also negatively regulate basal transcription, in the absence of activator, by either inhibiting TBP binding to the *HIS3* and *TRP3* TATA boxes (Belotserkovskaya *et al.*, 2000) or by competing with TFIIA for TBP binding on the *HIS4* promoter (Warfield *et al.,* 2004). This inhibitory effect of SAGA on TBP binding to the *HIS3* and *TRP3* genes is also mediated by the Spt3 and Spt8 subunits of SAGA. Clearly, SAGA plays a dual role in transcription as typically exemplified by a general cofactor. Other than serving as a bridging factor for interactions between activators and TBP or HAT substrates, some components of the SAGA complex, such as Spt7, Spt20, Ada1, TAF5, TAF10, TAF12, and Sgf73 are necessary for the structural integrity of SAGA, as mutations in these subunits result in disruption of the holo complex (Grant *et al.,* 1998; Sterner *et al.,* 1999; Durso *et al.,* 2001; Kirschner *et al.,* 2002; McMahon *et al.,* 2005). In addition, Sgf73 is important for regulating GCN5 HAT activity in SAGA, as substitution of wild-type yeast protein with a polyglutamine-expanded pathogenic human Sgf73 mutant impairs GCN5-mediated acetylation of nucleosomal histones likely by reducing interactions between Ada2, Ada3, Spt3 and TAF12 with the core complex without altering the incorporation of GCN5 and other subunits (McMahon *et al.,* 2005). The Ubp8 and Sgf11 subunits of SAGA seem to function together as nonessential structural components to remove the monoubiquitin moiety from lysine 123 of histone H2B (Henry *et al.,* 2003; Daniel *et al.,* 2004; Ingvarsdottir *et al.,* 2005; Lee *et al.,* 2005c), since deletion of either the *UBP8* or *SGF11* gene in yeast results in similar effects on a common subset of SAGA-regulated genes (Powell *et al.,* 2004; Ingvarsdottir, 2005) and increased ubiquitination on histone H2B both *in vivo* and *in vitro* (Ingvarsdottir, 2005; Lee *et al.,* 2005c). At the chromatin level, the recent identification of Chd1 as a component of SAGA further provides a functional link between histone methylation and acetylation, given that Chd1 binding to methylated lysine 4 in histone H3 apparently enhances GCN5-mediated acetylation on chromatin (Pray-Grant *et al.,* 2005).

SLIK, with protein composition similar to that of SAGA, contains a SLIK-specific subunit Rtg2 (retrograde 2) and a C-terminal truncated Spt7 protein, but lacks Spt8 (Pray-Grant *et al.,* 2002; Sterner *et al.,* 2002). The remaining polypeptides common to both SAGA and SLIK complexes include GCN5, Ada1, Ada2, Ada3, TAF5, histone fold-containing TAF6, TAF9, TAF10 and TAF12, as well as Tra1, Spt3, Spt20, Chd1, Ubp8, Sgf11, Sgf29, and Sgf73 (Pray-Grant *et al.,* 2002; Daniel *et al.,* 2004; McMahon *et al.,* 2005; Pray-Grant *et al.,* 2005). It should be noted that TAF5 appears to be a major scaffold protein connecting different structural domains in both SAGA and TFIID complexes (Leurent *et al.,* 2004; Wu *et al.,* 2004) and likely SLIK as well. In addition, TAF5 can be post–translationally modified by ubiquitination (Auty *et al.,* 2004) and sumoylation (Boyer-Guittaut *et al.,* 2005), suggesting a critical role of this subunit in regulating the function of TFIID, SAGA, SLIK and other TAF5-containing complexes. With many common subunits as found in SAGA, it is not surprising that SLIK can interact with activators and TBP, and can regulate chromatin transcription by binding methylated histone H3, acetylating histones, and deubiquitinating histone H2B (Pray-Grant *et al.,* 2002; Henry *et al.,* 2003; Daniel *et al.,* 2004; Jazwinski, 2005). As seen with SAGA, the 19S regulatory subcomplex also enhances the affinity of SLIK for activator-bound DNA (Lee *et al.,* 2005c). However, there are some functional differences between SLIK and SAGA, due to a slight variation in their protein composition. In yeast, deletion of the *Rtg2* gene results in the disruption of SLIK but not SAGA, indicating that Rtg2 is indeed a SLIK-specific subunit (Pray-Grant *et al.,* 2002). Moreover, that Rtg2, rather than Spt8, is involved in *CIT2* gene activation that regulates biosynthetic and metabolic responses in mitochondria (Chelstowska and Butow, 1995) suggests that the presence of Rtg2 enables SLIK to regulate a subset of genes distinct from that of SAGA. This is consistent with the observation that SLIK does not repress *HIS3* basal transcription, presumably due to the absence of Spt8 caused by truncation of the Spt7 C-terminal region needed for Spt8 recruitment (Belotserkovskaya *et al.,* 2000; Sterner *et al.,* 2002; Wu and Winston, 2002).

STAGA, a human counterpart of yeast SAGA initially isolated from HeLa nuclear extracts using polyclonal antibodies against human TAF9 (Martinez *et al.,* 1998), contains many homolog found in yeast SAGA, including TRRAP, Ada1, Ada2, Ada3, Spt3, Spt7, and histone fold-containing TAF9, TAF10, and TAF12. While additional components of STAGA, TAF5L, TAF6L, SAP130, ataxin-7 and GCN5L are also present

in TFTC, the identities of the other subunits (such as STAF36, STAF42, STAF46, STAF55, STAF60, and STAF65γ) remain to be characterized (Martinez *et al.,* 2001; Helmlinger *et al.,* 2004; Palhan *et al.,* 2005). Pertinent to this, whether many of the STAGA components are commonly found in the related human PCAF/GCN5 complexes (Ogryzko *et al.,* 1998) remains to be examined. The conservation of histone fold-containing Spt3, TAF9, TAF10 and TAF12 in both SAGA and STAGA suggests that these subunits are likewise important for the structural integrity of these distinct HAT complexes. As seen with yeast SAGA, the existence of TRRAP, GCN5L, Ada1, Ada2, Ada3, and ataxin-7 in STAGA likely accounts for the coactivating activity of STAGA in supporting Gal4-VP16-mediated activation from a Gal4-driven chromatin template (Martinez *et al.,* 2001) and cone-rod homeobox (CRX)-stimulated transcription from the CRX-targeted rhodopsin promoter (Palhan *et al.,* 2005). Clearly, Ada proteins and ataxin-7 facilitate the access of GCN5L to the activator-occupied chromatin template, allowing acetylation of nucleosomal core histones (Balasubramanian *et al.,* 2002; Palhan *et al.,* 2005). That adaptor proteins are additionally required for activator-facilitated chromatin targeting of GCN5 is further supported by the observation that recombinant GCN5 protein is unable to potentiate activator-dependent transcription from preassembled chromatin templates (Thomas and Chiang, 2005). From our understanding of the yeast SAGA complex, it is likely that the Spt3 component of STAGA may contact TBP to facilitate transcription from STAGA-dependent promoters.

Unlike HAT-containing TFTC, SAGA, SLIK and STAGA complexes involved in gene activation, the TAF-containing *Drosophila* PRC1 complex is implicated in repression of homeotic genes that govern body segmentation and the developmental process. This complex, initially isolated from *Drosophila* embryos using a monoclonal antibody against epitope-tagged polyhomeotic (PH) or posterior sex comb (PSC) protein (Shao *et al.,* 1999), contains approximately 30 subunits, including TAF1, TAF4, TAF5, TAF6, TAF9, TAF11, PH, PSC, PC (polycomb), RING1, Zeste, HSC4, SMRTER, Mi-2, Sin3A, Rpd3, p55, Sbf1, DRE4/Spt16, p90, HSC3, Modulo, Reptin, DNA topoisomerase II, p110, tubulin, actin, Ribosome RS2, and Ribosome RL10 (Saurin *et al.,* 2001). Although this holo complex has histone deacetylase (HDAC) Rpd3, chromatin remodeling ATPase Mi-2, and other corepressor components (SMRTER, Sin3A, and p55) likely contributing to repression of transcription and inhibition of chromatin remodeling, it is surprising to see that a PRC1 core complex (PCC) containing only PH, PSC, PC, and RING1 is sufficient for transcriptional silencing (King *et al.,* 2002) and for blocking SWI/SNF-mediated mobilization of a nucleosomal array (Francis *et al.,* 2001). This finding, plus the fact that a sequence-specific transcription factor Zeste is present in PRC1, suggests that PRC1 may use different mechanisms to target PRC1-regulated gene transcription, irrespective of the presence or absence of a Zeste-binding element (Mulholland *et al.,* 2003). Given that the identified human PRC1 complex containing only homologs of the *Drosophila* core complex (PC, PH, PSC, and RING1) is able to inhibit SWI/SNF-mediated chromatin remodeling (Levine *et al.,* 2002) and that the other characterized *Drosophila* PRC2 (Ng *et al.,* 2000a; Tie *et al.,* 2001) and human PRC2 (Cao *et al.,* 2002; Kuzmichev *et al.,* 2002), PRC3 (Kuzmichev *et al.,* 2004), and PRC4 (Kuzmichev *et al.,* 2005) complexes do not seem to contain TAFs, the functional roles of TAFs and the other subunits constituting PRC1 await further investigation.

TAF Variants

Some components of TFIID are present in a substoichiometric ratio relative to the other TAFs. These TAFs, such as TAF4b, TAF5L, and TAF7L, are often found in a tissue-specific manner and likely confers TFIID unique properties functioning in a specialized environment. TAF4b, a paralog of TAF4 initially identified in TFIID purified from B cells and later found expressed at low levels in every cell types but specifically enriched in the testes and ovary, appears to function in a gonad-specific manner, as knockout of this TAF variant severely affects ovarian development in female mice (Freiman *et al.,* 2001) and also spermatogenesis in male mice (Falender *et al.,* 2005). Likewise, TAF5L and TAF7L, paralogs of TAF5 and TAF7, respectively, are implicated in male gametogenesis (Hiller *et al.,* 2001; Pointud *et al.,* 2003). The presence of these tissue-specific TAF variants likely enables TFIID to work in conjunction with germ cell-specific transcription factors, cofactors, or other components of the general transcription machinery, such as TFIIA$\alpha\beta$-like factor (Upadhyaya *et al.,* 1999; Ozer *et al.,* 2000). Related to this, a unique TFIID subunit, TAF8, is induced during adipocyte differentiation (Guermah *et al.,* 2003). Other than the tissue-specific expression

pattern observed with these TAF variants, functional inactivation of TAF1 (Hisatake *et al.,* 1993; Ruppert *et al.,* 1993), which is present in TFIID and PRC1, and of TAF10 (Metzger *et al.,* 1999), found in TFTC, STAGA, and SAGA-like complexes, causes cell cycle arrest, indicating a general role of selective mammalian TAFs in modulating cell growth. Undoubtedly, the presence of TAF variants further expands the general properties of TFIID to specialized needs in differentiated tissues.

TFIIA

Protein Composition

Human TFIIA is composed of α, β, and γ subunits with molecular weights of 35 kDa, 19 kDa, and 12 kDa, respectively. These three subunits are encoded by two genes: TFIIA$\alpha\beta$ and TFIIAγ in higher eukaryotes, and TOA1 and TOA2 in yeast. Human TFIIA$\alpha\beta$ encodes a 55-kDa precursor protein that is highly conserved with TOA1 in yeast within the N-terminal 54 amino acids and the C-terminal 76 amino acids, but is less conserved in the central part of TFIIA which has been shown to be dispensable for function (Ranish *et al.,* 1992; Kang *et al.,* 1995). The smallest subunit of TFIIA, TFIIAγ, is homologous to yeast TOA2 (Ozer *et al.,* 1994; Sun *et al.,* 1994). In higher eukaryotes, TFIIA$\alpha\beta$ is proteolytically cleaved into TFIIAα and TFIIAβ subunits (DeJong and Roeder, 1993; Ma *et al.,* 1993; Yokomori *et al.,* 1993). Originally it was thought that the three independent subunits (α, β, and γ) constitute TFIIA activity, since only the cleavage products were detected in cell extracts in association with one another. Recently, intact TFIIA$\alpha\beta$ protein was detected in association with TFIIAγ along with TBP in P19 embryonal carcinoma cells (Mitsiou and Stunnenberg, 2000, 2003), and likely in human parasites as a more divergent form such as the TRF4/SNAP$_c$/TFIIA complex identified in *Trypanosoma brucei* (Schimanski *et al.,* 2005). The site for proteolytic cleavage within human TFIIA$\alpha\beta$ was determined by Edman degradation; TFIIAβ was found to start at Asp278 (Høiby *et al.,* 2004). Furthermore, cleaved TFIIAα and TFIIAβ were more efficiently degraded than the unprocessed precursor via the ubiquitin-proteasome pathway, suggesting that cleavage and degradation of TFIIA control the level of TFIIA within the cell to perhaps adapt more rapidly to the transcriptional needs in responding to environmental changes (Høiby *et al.,* 2004). Similar to TAF4b, a cell type-specific TFIIA$\alpha\beta$-like factor (ALF) has been identified in human testis (Upadhyaya *et al.,* 1999; Ozer *et al.,* 2000) and is shown to work in conjunction with TFIIAγ to stabilize TBP binding to the promoter (Upadhyaya *et al.,* 2002). Further experiments indicate that ALF is also found in immature oocytes of the frog *Xenopus laevis*, in which ALF replaces TFIIA during oogenesis (Han *et al.,* 2003).

TFIIA as an Antirepressor, Not a GTF

The role of TFIIA as a GTF has been controversial. As with TFIID, TFIIA was initially identified as a phosphocellulose column fraction necessary for pol II-mediated transcription *in vitro* (Matsui *et al.,* 1980). Early *in vitro* experiments showed that TFIIA was essential for transcription (Reinberg *et al.,* 1987), while later *in vitro* studies demonstrated that TFIIA was largely dispensable for basal level transcription (Van Dyke *et al.,* 1988; Wu *et al.,* 1998). It has also been suggested that TFIIA stimulates both basal and activated transcription *in vitro* two-to tenfold, but generally only when TFIID, instead of TBP, is used as the promoter-binding factor (Orphanides *et al.,* 1996; Hampsey, 1998; Warfield *et al.,* 2004). In our laboratory, we found TFIIA was not required for either basal or activator-dependent transcription in a highly purified transcription system reconstituted with recombinant TFIIB, TFIIE, TFIIF, PC4 coactivator, and epitope-tagged multiprotein complexes (pol II, TFIID, and TFIIH), irrespective of whether TFIID or recombinant TBP was used in the assay (Wu *et al.,* 1998). Nevertheless, TFIIA indeed became essential for transcription in a reconstituted system containing partially purified fractions obtained according to the purification scheme outlined in Figure 1. Collectively, these studies suggest that TFIIA mainly functions as an antirepressor to overcome inhibitors present in crude fractions, likely by increasing the affinity of TBP or TFIID for DNA (Buratowski *et al.,* 1989; Lee *et al.,* 1992; Imbalzano *et al.,* 1994b; Kang *et al.,* 1995), thus enhancing PIC assembly. TFIIA stabilizes TBP-TATA box interactions through direct contacts with both TBP (on the surface of TBP opposite to the TFIIB-binding side) and the DNA sequence immediately upstream of the TATA box (Geiger *et al.,* 1996; Lagrange *et al.,* 1996; Oelgeschläger *et al.,* 1996; Tan *et al.,* 1996). Binding of TFIIA to TBP dimers has also been shown to induce TBP monomer formation and accelerate the kinetics of TBP binding to DNA (Coleman *et al.,* 1999). TFIIA can counteract the repressive effects of negative cofactors, such as NC2 (Xie *et al.,*

2000), HMGB1 (Ge and Roeder, 1994b), and DNA Topoisomerase I (Merino *et al.,* 1993), as well as the inhibitory activity of TAF1 and BTAF1 on TBP binding to DNA (Auble and Hahn, 1993; Kokubo *et al.,* 1998). Interestingly, TAF1 interaction with TFIIA may modulate TFIIA activity, since it has been shown that TAF1 phosphorylates human TFIIAβ on serine residues important for TBP binding and transcription activity (Solow *et al.,* 2001). Additionally, experiments have shown that the TFIIA-TBP-DNA complex may also be regulated by the transcriptional coactivator p300 through acetylation of TFIIA (Mitsiou and Stunnenberg, 2003).

TFIIA as a Coactivator

In addition to the antirepressing activity, TFIIA can also act as a coactivator to stimulate overall transcription by directly contacting activators, general cofactors, and components of the general transcription machinery. Many studies have demonstrated that TFIIA interacts with activators, such as Gal4-VP16 (Kobayashi *et al.,* 1995; Dion and Coulombe, 2003), Zta (Ozer *et al.,* 1994; Kobayashi *et al.,* 1995), and HTLV-1 Tax (Clemens *et al.,* 1996), or with transcriptional cofactors such as PC4 (Ge and Roeder, 1994a) and HMG2 (Shykind *et al.,* 1995). TFIIA has also been shown to interact with components of TFIID, including TBP (Maldonado *et al.,* 1990; Ranish and Hahn, 1991; Usuda *et al.,* 1991; Cortes *et al.,* 1992; Lee *et al.,* 1992; Yokomori *et al.,* 1993; Sun *et al.,* 1994), TAF1 (Solow *et al.,* 2001), TAF4 (Yokomori *et al.,* 1993), TAF11 (Kraemer *et al.,* 2001; Robinson *et al.,* 2005), and other GTFs, such as TFIIEα, TFIIEβ, and RAP74 (Langelier *et al.,* 2001). The observed interaction between TFIIA and TBP-related proteins, including TRF1 (Hansen *et al.,* 1997), TRF2 (Moore *et al.,* 1999; Rabenstein *et al.,* 1999; Teichmann *et al.,* 1999), and TRF4 (Schimanski *et al.,* 2005), further suggests that TFIIA plays a more general role in facilitating PIC assembly mediated by different core promoter-binding factors. In one proposed model of TFIIA function, interaction of TFIIA with both activators and TFIID may stimulate and stabilize TFIID binding to DNA as part of an activator-TFIID-TFIIA-DNA complex, thereby enhancing a rate-limiting step for promoter recognition (Wang *et al.,* 1992; Lieberman and Berk, 1994; Chi *et al.,* 1995; Kobayashi *et al.,* 1995; Ranish *et al.,* 1999; Dion and Coulombe, 2003). Moreover, TFIIA may upregulate PIC formation at a step post TFIID binding to the DNA by stimulating the functions of both TFIIE and TFIIF (Langelier *et al.,* 2001). In TAF-independent transcriptional activation experiments using a highly purified transcription system, TFIIA could potentiate TBP-mediated activation, suggesting that TFIIA may function as a coactivator especially in the absence of TAFs (Wu *et al.,* 1998). Several studies using immunodepletion of TFIIA subunits *in vitro* and mutational studies abolishing TFIIA-TBP interactions in yeast have indicated that, similar to TAFs, TFIIA is important for transcription only from a subset of genes and does not seem to be universally required for all gene transcription (Kang *et al.*, 1995; Ozer *et al.*, 1998a; Liu *et al.,* 1999; Stargell *et al.,* 2000).

TFIIB

TFIIB Stabilizes TFIID Promoter Binding

Once TFIID or TBP is bound to the promoter in the absence or presence of TFIIA, TFIIB is the next GTF to enter the PIC assembly pathway. Binding of TFIIB to promoter-bound TBP results in a more stable ternary complex composed of TBP-TFIIB-DNA (Orphanides *et al.,* 1996). Besides stabilizing TFIID/TBP binding to the promoter region, TFIIB plays an important role in recruiting pol II/TFIIF to the ternary complex and in specifying the transcription start site (Orphanides *et al.,* 1996; Hampsey, 1998; Hahn, 2004). In humans, TFIIB has 316 amino acid residues and exists as a single 33-kDa polypeptide (Ha *et al.,* 1991; Malik *et al.,* 1991), which shares sequence homology with a 38-kDa *Drosophila* TFIIB protein and also with a 38-kDa yeast protein encoded by the *SUA7* gene (Pinto *et al.,* 1992; Wampler and Kadonaga, 1992; Yamashita *et al.,* 1992). TFIIB is evolutionarily conserved among various species, and exhibits amino acid and structural conservation with archaeal TFB (transcription factor B) and eukaryotic pol III accessory factor BRF (TFIIB-related factor) at both the N-terminal pol II/TFIIF-interacting zinc-ribbon domain and the C-terminal DNA-binding domain containing two imperfect direct repeats (Orphanides *et al.,* 1996; Hampsey, 1998, Bell and Jackson, 2001; Hahn, 2004; see Figure 7A). These two functional domains in TFIIB were originally identified through protease digestion, as the N-terminus is rapidly degraded leaving behind a protease-resistant C-terminal "core" that retains the two imperfect direct repeats of TFIIB (Barberis *et al.,* 1993; Malik *et al.,* 1993).

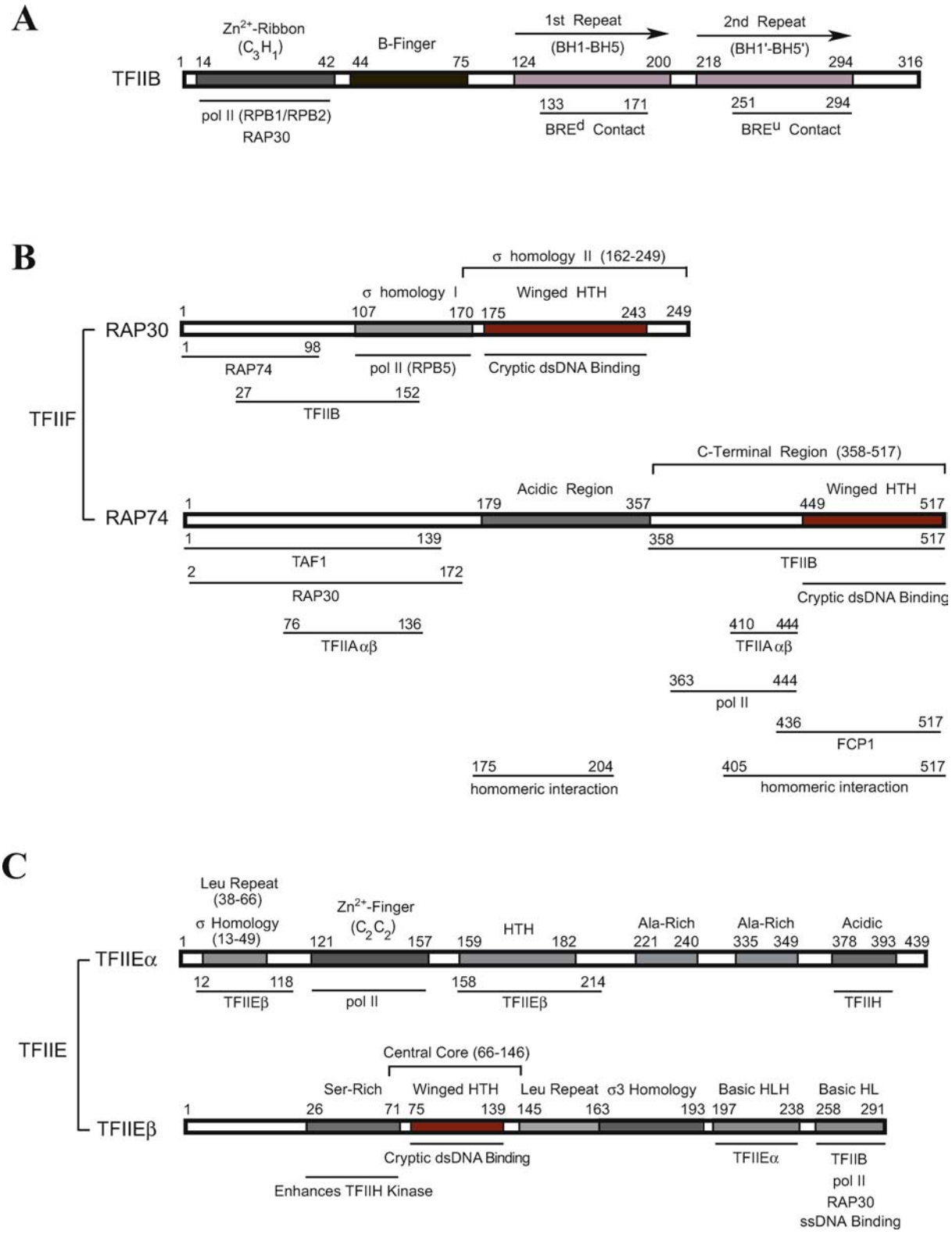


FIGURE 7 **Structural domains and interacting regions on TFIIB, TFIIF, and TFIIE. Schematic representation of the structural domains is depicted for TFIIB (*A*), TFIIF (*B*), and TFIIE (*C*). Solid lines below each protein correspond to the amino acid residues within TFIIB, TFIIF, and TFIIE regions that are shown to interact with other GTFs and pol II or contact DNA. Unless specified, the boundaries of amino acid residues for the solid lines are the same as indicated for the structural domains. It should be noted that, although not shown, RAP30 in panel B also interacts with TFIIEβ, whereas TFIIEα and TFIIEβ in panel C contact TFIIAγ and TFIIA$\alpha\beta$, respectively, through undefined regions.**

TFIIB-TBP-Promoter Structure

The C-terminal core of TFIIB comprises nearly two-thirds of the protein (amino acids 106 to 316) and contains two imperfect repeats each consisting of 5 α-helices, termed BH1, BH2, BH3, BH4, and BH5 in the first repeat (amino acids 124 to 200) and BH1′, BH2′, BH3′, BH4′, and BH5′ in the second repeat (amino acids 218 to 294; Bagby *et al.,* 1995; Tsai and Sigler, 2000). The C-terminal core of TFIIB, as revealed in the structure of a TATA-TBP-TFIIB ternary complex (Nikolov *et al.,* 1995; Tsai and Sigler, 2000), bound beneath and to one face of the TATA-TBP complex, consistent with hydroxyl-radical footprinting (Lee and Hahn, 1995) and photocrosslinking experiments (Coulombe *et al.,* 1994; Lagrange *et al.,* 1996). Structural evidence also exists for TFIIB competing with NC2 for overlapping binding surface on TBP (Kamada *et al.,* 2001b; see previous NC2 section). The conserved C-terminus of TFIIB interacts with both TBP and DNA, making DNA contacts with sequences flanking the TATA box, in particular the major groove upstream and the minor groove downstream of the TATA box (Nikolov *et al.,* 1995; Tsai and Sigler, 2000). Experiments also indicate that TFIIB alone without TBP can make sequence-specific DNA contacts with the BRE^u (Lagrange *et al.,* 1998; see also the Core Promoter Elements section) via a nonconserved helix-turn-helix (HTH) motif, which is a trihelical bundle composed of BH3′, BH4′ and BH5′ in the second direct repeat, and further stabilizes the ternary TFIIB-TBP-promoter complex (Evans *et al.,* 2001; Wolner and Gralla, 2001). Recent studies also show that TFIIB can contact the BRE^d sequence, situated downstream of the adenovirus E4 TATA box, via a separate DNA-binding domain located at a recognition loop linking BH2 and BH3 in the first direct repeat of TFIIB, apparently in a TBP-dependent manner (Fairley *et al.,* 2002; Deng and Roberts, 2005). These TFIIB-DNA interactions can be modulated by transcriptional activators, such as Gal4-VP16, which work in part by disrupting intramolecular interactions between N- and C-terminal domains of TFIIB (Hawkes *et al.,* 2000), thereby exposing the DNA recognition loop of TFIIB for BRE^d recognition. Concurrently, this activator-induced conformational change may weaken interactions between the HTH DNA-binding domain of TFIIB and the BRE^u (Evans *et al.,* 2001). Although the role of activator in disrupting TFIIB-BRE^u interactions seems contradictory with the previously proposed role of the BRE^u in stabilizing the TFIIB-TBP-DNA ternary complex, it appears that the BRE^u may play an inhibitory role in PIC formation or during the transition from the initiation to the elongation stage. Clearly, activators may stimulate transcription by regulating multiple steps. The precise mechanism by which activators, TFIIB, and BRE interactions affect PIC assembly, initiation, and promoter clearance remains to be investigated.

TFIIB N-Terminal Domain

The TFIIB amino terminus contains a zinc ribbon motif (amino acids 14 to 42 in humans) with a Cys-X_2-His-X_{15}-Cys-X_2-Cys sequence forming a protease-resistant ZnC_3H_1 structure comprising of three antiparallel β strands important for the structural integrity of TFIIB (Chen *et al.,* 2000; Ghosh *et al.,* 2004). This zinc ribbon motif interacts with the RPB1 and RPB2 subunits of pol II at the dock domain near the RNA exit channel (Chen and Hahn, 2003; Bushnell *et al.,* 2004) and also with the RAP30 subunit of TFIIF (Ha *et al.,* 1993; Fang and Burton, 1996; see Figure 7A). These interactions facilitate the recruitment of pol II/TFIIF to the TFIID-bound promoter region (Buratowski and Zhou, 1993; Ha *et al.,* 1993; Malik *et al.,* 1993; Yamashita *et al.,* 1993; Fang and Burton, 1996; Bangur *et al.,* 1997). Immediately adjacent to the N-terminal zinc ribbon motif is a highly conserved region called the charged cluster domain (CCD) or the B-finger, spanning amino acids 44 to 75 of human TFIIB (Bushnell *et al.,* 2004; see Figure 7A). It is believed that the B-finger domain acts as a molecular switch to regulate the conformational change of TFIIB, thereby modulating TFIIB function in promoter recognition, start site selection, and transcriptional activation (Pinto *et al.,* 1994; Pardee *et al.,* 1998; Hawkes and Roberts, 1999; Wu and Hampsey, 1999; Hawkes *et al.,* 2000; Faitar *et al.,* 2001; Elsby and Roberts, 2004).

B-Finger-Induced Conformational Changes

As mentioned previously, TFIIB undergoes a conformational change when it interacts with DNA or activators, and the B-finger plays a vital role in this conformational change (Hawkes *et al.,* 2000; Fairley *et al.,* 2002; Elsby and Roberts, 2004). The activator Gal4-VP16 has been shown to disrupt the intramolecular interaction between the B-finger and the second repeat of the

C-terminal domain (Elsby and Roberts, 2004). Evidence with corresponding mutations in the B-finger, which favors N- and C-terminal intramolecular interactions, also shows defects in activator-mediated recruitment and in transcriptional activation *in vivo* and *in vitro* (Hawkes *et al.,* 2000; Glossop *et al.,* 2004; Elsby and Roberts, 2004). These B-finger mutations, however, are competent in PIC assembly, indicating that mutations in the B-finger do not affect TFIIB interactions with TBP/DNA and pol II/TFIIF, mediated individually by the C-terminal core and the zinc ribbon motif. Related studies in yeast TFIIB also suggest that conformational changes in TFIIB regulate the stability of the TFIIB-TBP-promoter ternary complex (Bangur *et al.,* 1999).

Role of the B-Finger in Start Site Selection

The involvement of yeast TFIIB (Sua7) in start site selection has been observed on *CYC1*, *ADH1*, and many other genes (Pinto *et al.,* 1992; Berroteran *et al.,* 1994; Pardee *et al.,* 1998). Besides its role in mediating activator-dependent transcription, the B-finger also has a critical role in directing accurate initiation of transcription. The functions of the B-finger in transcriptional activation and start site selection are located on different amino acid residues (Hawkes and Roberts, 1999). Similar to the activation-defective B-finger mutants, distinct mutations in the B-finger with aberrant transcriptional start site selection also do not show defects in PIC assembly (Hawkes and Roberts, 1999; Fairley *et al.,* 2002). It has been proposed that these distinct B-finger mutations, which cannot undergo proper conformational changes, alter TFIIB's interaction with the pol II catalytic center, thus shifting the transcription start site. Recent photo crosslinking experiments have shown that TFIIB helps position the path of promoter DNA across the central cleft of pol II (Chen and Hahn, 2003, 2004). Supporting crystallographic data has also indicated that the B-finger of TFIIB forms a finger-like structure projecting into the active center of pol II (Bushnell *et al.,* 2004), at a location close to the RAP74 subunit of TFIIF (Chen and Hahn, 2004). This configuration enables TFIIB to work in conjunction with pol II/TFIIF for start site selection. Although mutations in the B-finger may not be directly involved in PIC formation, conformational changes in TFIIB does play an essential role in transcriptional activation, promoter recognition, and also start site selection.

Role of TFIIB Post PIC Assembly

In addition to its role in facilitating promoter recognition by TBP/TFIID and in recruiting pol II/TFIIF, TFIIB also modulates transcription at a step after PIC assembly. From the structural analysis of the pol II-TFIIB complex, it is obvious that the B-finger of TFIIB, situated in the RNA exit channel, is likely to block the extension of newly synthesized RNA transcripts (Bushnell *et al.,* 2004). Consistent with this prediction, transcript elongation by newly-initiated pol II complexes that retain TFIIB is strongly inhibited beginning at +7. This block is reduced when human TFIIB bearing a mutation in the B-finger (R66L) is substituted for wild-type TFIIB (Pal *et al.,* 2005). This finding is consistent with yeast studies showing that mutations in a B-finger residue (E62G and E62K) exhibit a post PIC assembly transcriptional defect (Cho and Buratowski, 1999; Ranish *et al.,* 1999).

TFIIF

Discovery of TFIIF

TFIIF was not initially identified when nuclear proteins were fractionated by P11 ion-exchange chromatography simply into four (A, B, C, and D) fractions (Matsui *et al.,* 1980). The discovery of TFIIF was made possible only after further purification of the C fraction and following the identification of TFIIE (Flores *et al.,* 1988). Human TFIIF was found to be composed of previously identified RNA polymerase II-associated proteins 30 (RAP30) and 74 (RAP74) isolated from calf thymus extracts as well as from human and mouse cell lines (Sopta *et al.,* 1985; Flores *et al.,* 1988). Further characterization of TFIIF by size exclusion column chromatography suggested that TFIIF is a heterotetramer comprising two subunits each of RAP30 and RAP74 (Flores *et al.,* 1990). The cDNAs for RAP30 and RAP74 had been cloned from humans (Aso *et al.,* 1992; Finkelstein *et al.,* 1992), *Drosophila* (Kephart *et al.,* 1993; Frank *et al.,* 1995; Gong *et al.,* 1995), and yeast (Henry *et al.,* 1994). The yeast TFIIF contains three subunits (Henry *et al.,* 1992; Henry *et al.,* 1994): Tfg1 (105 kDa) and Tfg2 (54 kDa) are essential for yeast viability and are counterparts of human RAP74 and RAP30, respectively; the third subunit, Tfg3 (30 kDa), is non-essential and is identical to yeast TAF14/ANC1, which is also present in yeast TFIID and SWI/SNF chromatin remodeling complexes (Cairns *et al.,* 1996a). Since Tfg3 exists in multiple complexes and is able to interact directly with

TFIIB and TBP (Kimura and Ishihama, 2004), it is probable that Tfg3 may serve as an intermediary factor to facilitate interactions between SWI/SNF and components of the general transcription machinery.

Structure and Functional Domains of TFIIF

Human RAP30 (249 amino acids, calculated 26 kDa, apparent mass by SDS-PAGE ~30 kDa) shares two regions of sequence homology with bacterial σ factors (Figure 7B): a central domain (σ homology I, residues 107 to 170) that interacts with pol II, and a C-terminal domain (σ homology II, residues 162 to 249) possessing cryptic DNA-binding activity (Sopta *et al.,* 1989; McCracken and Greenblatt, 1991; Garrett *et al.,* 1992; Tan *et al.,* 1994). RAP30 also contains an N-terminal RAP74-interacting domain spanning amino acids 1 to 98 and a TFIIB-interacting region located within amino acids 27 to 152 (Fang and Burton, 1996). Interestingly, human TFIIF prebound to *E. coli* RNA polymerase core enzyme ($\alpha_2\beta\beta'$) can be displaced by bacterial σ^{70} factor presumably via the same region in σ^{70} that shares homology with RAP30 (McCracken and Greenblatt, 1991). The RPB5 subunit of human pol II has been shown to interact with the central domain of RAP30 spanning amino acids 107 to 170 *in vivo* and *in vitro* (Wei *et al.,* 2001). The solution structure of the C-terminal 86 amino acid residues (164 to 249) of RAP30, revealed by multinuclear NMR spectroscopy, shows that the DNA-binding domain of RAP30 belongs to the eukaryotic "winged" HTH family of DNA-binding domains, similar to that found in histone H5 and the hepatocyte nuclear transcription factor HNF-3γ (Groft *et al.,* 1998). This "winged" HTH motif encompasses amino acid residues 175 to 243 of RAP30 (see Figure 7B). Within this region is an α-helix (H1, amino acids 179 to 193) followed by a short strand (S1, residues 196 to 197) of antiparallel sheet leading to the HTH motif, which is composed of an α-helix (H2, amino acids 199 to 205), turn (amino acids 206 to 209), and another α-helix (H3, amino acids 210 to 219). Following the HTH are two antiparallel β-sheets (S2, amino acids 222 to 225; and S3, 231 to 234) that are connected by a short loop of 5 amino acids (residues 226 to 230), forming the "wing" of RAP30 (Groft *et al.,* 1998). The DNA contacts in the winged HTH domain has been mapped to specific amino acid residues in helices H1 (Arg177, Ala178, Lys180-His182) and H2 (Asn198-Lys200), turn (Lys207, Gln208), helix H3 (Val210, Val211, Glu215, Lys218), and the wing (Lys226, His229, Asn231). It should be noted that RAP30 also interacts with TFIIEβ, although the interaction domain on RAP30 has not yet been described (Okamoto *et al.,* 1998).

The larger subunit of TFIIF, RAP74 (517 amino acids, calculated 58 kDa, apparent mass by SDS-PAGE ~74 kDa), has three functional domains able to interact with RAP30, the TAF1 subunit of TFIID, TFIIA$\alpha\beta$, TFIIB, pol II, and a protein phosphatase FCP1 (Ruppert and Tjian, 1995; Fang and Burton, 1996; Archambault *et al.,* 1998; Okamoto *et al.,* 1998; Langelier *et al.,* 2001; Abbott *et al.,* 2005a; see Figure 7B). The RAP30-interacting region is mapped to the N-terminal globular domain of RAP74 spanning amino acids 1 to 172 (Fang and Burton, 1996). The structure of the RAP30-RAP74 heterodimer from respective protein-protein interaction domains (RAP74, amino acids 2 to 172; and RAP30, residues 2 to 119), resolved at 1.7 Å by X-ray crystallography, reveals a triple-barrel β-structure comprising 16 parallel and antiparallel β-strands and some less localized loops and α-helices (Gaiser *et al.,* 2000). The N-terminal domain of RAP74 that encompasses amino acids 1 to 139 interacts with TAF1 (Ruppert and Tjian, 1995), which can phosphorylate RAP74 through either the N-terminal or the C-terminal kinase domain of TAF1 (Dikstein *et al.,* 1996). This TAF1-mediated interaction and phosphorylation of RAP74 can also be detected in the TFIID complex (Rossignol *et al.,* 1999; Wu and Chiang, 2001a). The TFIIA$\alpha\beta$-interacting regions on RAP74 have been mapped to two independent domains spanning amino acids 76 to 136 and 410 to 444 (Langelier *et al.,* 2001). Clearly, complex formation mediated by the N-terminal domain of RAP74 with RAP30, TFIIA$\alpha\beta$, and TAF1/TFIID is likely important for TFIIF involvement in PIC assembly, initiation and elongation (Lei *et al.,* 1998; Langelier *et al.,* 2001; Funk *et al.,* 2002).

The central region of RAP74 spanning amino acids 179 to 357 is enriched in Glu and Asp acidic amino acid residues (Aso *et al.,* 1992; Finkelstein *et al.,* 1992). This region, significantly lacking hydrophobic residues, is hypothesized to be externally exposed and unstructured within the PIC (Yong *et al.,* 1998) and can be phosphorylated by TAF1 and CK2 (Yonaha *et al.,* 1997; Rossignol *et al.,* 1999). In addition, it was reported that RAP74 has serine/threonine kinase activity capable of autophosphorylating itself at serine 385 and threonine 389, which may down-regulate pol II elongation in "run-off"

transcription assays (Rossignol *et al.,* 1999). The significance of these phosphorylation events in the context of transcriptional complexes has not been deciphered.

Similar to RAP30, RAP74 also contains a cryptic DNA-binding domain belonging to the winged HTH family, in which the structure spanning the C-terminal 69 amino acid residues of RAP74 (amino acids 449 to 517) has been resolved by X-ray crystallography at 1.02 Å resolution (Kamada *et al.,* 2001a). Within this C-terminal region of RAP74, there is an α-helix (H1, amino acids 456 to 465) and a short β-strand (S1, amino acids 467 to 469) leading to the HTH motif, which is composed of a second α-helix (H2, amino acids 470 to 475), a long loop (amino acids 476 to 485), and a third α-helix (H3, amino acids 486 to 500). This HTH motif is followed by two antiparallel β-sheets (S2, amino acids 503 to 507, and S3, residues 510 to 514) connected by a short turn (amino acids 508 to 509) that forms the "wing," similar to the winged HTH structure described earlier for RAP30. In analogy with the winged HTH domain of RAP30 that exhibits nonspecific DNA-binding activity with preference for double-stranded DNA sequences located between the TATA box and the Inr (Tan *et al.,* 1994; Forget *et al.,* 2004), the winged HTH domain of RAP74 likely contributes to the DNA-binding activity of RAP74 in the PIC (Robert *et al.,* 1998; Forget *et al.,* 2004). Indeed, the presence of the winged HTH domain significantly extends the promoter contacts by the N-terminal region of RAP74 further upstream of the TATA box and downstream of the Inr (Forget *et al.,* 1997; Robert *et al.,* 1998).

Besides binding DNA, the C-terminal region of RAP74 spanning amino acids 358 to 517 can also interact with TFIIB, TFIIA$\alpha\beta$, pol II, and FCP1 (Figure 7B). The interaction with TFIIB has been mapped to amino acids 358 to 517 of RAP74, which competes with TFIIB for overlapping binding surfaces at the N-terminal region of RAP30 (Fang and Burton, 1996). The pol II- and FCP1-interacting regions have been located at amino acids 363 to 444 and 436 to 517 of RAP74, respectively (Fang and Burton, 1996; Archambault *et al.,* 1998; Abbott *et al.,* 2005a), suggesting that pol II and FCP1 may bind simultaneously to non-overlapping surfaces at the C-terminal domain of RAP74 (see Figure 7B). The interaction between RAP74 and FCP1, which removes phosphorylation on serine 2 at the carboxy-terminal domain (CTD) of the pol II RPB1 subunit to regenerate a hypophosphorylated form of pol II competent for initiation or reinitiation (Cho *et al.,* 2001), is necessary and sufficient for FCP1 phosphatase activity *in vitro* (Archambault *et al.,* 1997, 1998) and may account for the requirement of the C-terminal region of RAP74 for multiple rounds of transcription (Lei *et al.,* 1998). Undoubtedly, the FCP1 phosphatase activity is likely modulated by some RAP74- and FCP1-interacting proteins. Indeed, TFIIB can inhibit FCP1 activity by displacing RAP74 from FCP1 via competition with RAP74 for the same binding surface on FCP1 (Chambers *et al.,* 1995; Kobor *et al.,* 2000; Nguyen *et al.,* 2003). In contrast, the FCP1 phosphatase activity is enhanced by CK2-mediated phosphorylation of FCP1 that in turn enhances the interaction between FCP1 and RAP74 (Palancade *et al.,* 2002; Abbott *et al.,* 2005b). Other than these heteromeric protein-protein interactions, RAP74 also shows homomeric interactions with itself, through either the C-terminal region (amino acids 407 to 517) or a linker region (amino acids 172 to 205) situated between the N-terminal RAP30-interacting domain and the central acidic domain (Robert *et al.,* 1998; see Figure 7B). As with other GTFs, TFIIF may be recruited to the promoter through interaction with some transcriptional activators, such as serum response factor (SRF; Joliot *et al.,* 1995), c-Jun/c-Fos (Martin *et al.,* 1996), and androgen receptor (AR; McEwan and Gustafsson, 1997; Reid *et al.,* 2002). The interaction with SRF has been mapped to the central acidic domain of RAP74 (Joliot *et al.,* 1995), whereas the interaction with AR has been mapped to the N-terminal 136 amino acids and C-terminal 155 residues of RAP74 (Reid *et al.,* 2002). These findings indicate that each domain of RAP74 can serve as a target for transcriptional activators, thereby facilitating TFIIF recruitment to the transcriptional complex.

Multiple Roles of TFIIF

TFIIF plays multiple roles during PIC assembly. First, TFIIF tightly associates with pol II (Sopta *et al.,* 1985; Price *et al.,* 1989). The RAP74 subunit of yeast TFIIF has been shown to contact the dissociable RPB4/RPB7 subunits of yeast pol II, based on structural comparison between pol II-TFIIF and pol II by cryo-electron microscopy (Chung *et al.,* 2003), and to interact with the RPB9 subunit of yeast pol II (Ziegler *et al.,* 2003; Ghazy *et al.,* 2004). This stable TFIIF-associated pol II complex accounts for ~50% of pol II isolated from *Saccharomyces cerevisiae* nuclear extracts and is active in supporting multiple rounds of

transcription (Rani *et al.,* 2004). Human RAP30 has also been shown to interact with the RPB5 subunit of human pol II (Wei *et al.,* 2001). The interaction between TFIIF and pol II facilitates the recruitment of pol II to the promoter-bound TFIID-TFIIB complex (Flores *et al.,* 1991). Second, TFIIF serves as a stability factor to enhance the affinity of pol II for the TBP-TFIIB-promoter complex by providing additional protein-DNA contact surfaces and likely by inducing changes in DNA topology which causes the promoter to wrap around pol II (Robert *et al.,* 1998). This TFIIF-induced conformational change creates a stable TBP-TFIIB-pol II-TFIIF-promoter DNA complex that may confer resistance to inhibition by transcriptional repressors that target PIC assembly to negatively regulate gene transcription (Hou *et al.,* 2000). In the study of human papillomavirus (HPV) E2-mediated transcriptional repression, we found that E2 could still inhibit HPV transcription when added to a preformed TFIID (or TBP)-TFIIB-pol II-promoter DNA complex; however, E2 failed to inhibit transcription once a TFIID (or TBP)-TFIIB-pol II-TFIIF-DNA complex was formed, presumably a surface targeted by E2 for repression was masked by TFIIF-induced DNA wrapping on this intermediate PIC (Hou *et al.,* 2000). Third, TFIIF is necessary for subsequent recruitment of TFIIE and TFIIH (Orphanides *et al.,* 1996) likely via direct interactions with TFIIE (Maxon *et al.,* 1994). Fourth, TFIIF, together with pol II and TFIIB, plays a role in transcription start site selection (Fairley *et al.,* 2002; Ghazy *et al.,* 2004), due to the close proximity of TFIIB and TFIIF near the active center of pol II (Bushnell *et al.,* 2004; Cramer, 2004; Hahn, 2004; Freire-Picos *et al.,* 2005). Fifth, TFIIF has been implicated in facilitating pol II promoter escape (Yan *et al.,* 1999). Following this transition, TFIIF further enhances the efficiency of pol II elongation (Price *et al.,* 1989; Shilatifard *et al.,* 2003), likely by promoting forward NTP-driven translocation at the active center of pol II (Zhang *et al.,* 2005). When pol II pauses during RNA synthesis, TFIIF is able to work in conjunction with TFIIS, a transcription elongation factor that stimulates pol II cleavage activity on nascent RNA, to overcome this elongation block (Zhang and Burton, 2004). Lastly, TFIIF increases the specificity and efficiency of pol II transcription, similar to bacterial σ factors, by preventing spurious initiation through inhibiting and/or reversing the binding of pol II to non-promoter DNA sequences (Orphanides *et al.,* 1996; Hampsey, 1998). It is clear that TFIIF has multiple roles in the pol II transcription process.

CORE POL II

Subunit Composition

Pol II is the key catalytic enzyme in the PIC responsible for transcription of protein-coding genes in eukaryotes. Yeast and human pol II both contain 12 subunits, designated RPB1 to RPB12 by decreasing order of their molecular mass (Young, 1991). In general, the 12 subunits of pol II are highly conserved in sequence, architecture, and function. Indeed, 7 subunits of human pol II can either partially (RPB4, RPB7, and RPB9) or completely (RPB6, RPB8, RPB10, and RPB12) substitute for the function of their yeast counterparts in complementation assays (McKune *et al.,* 1995; Khazak *et al.,* 1998). Of the 12 pol II subunits, 5 (RPB5, RPB6, RPB8, RPB10, and RPB12) are commonly shared among pol I, pol II and pol III (Woychik *et al.,* 1990; Carles *et al.,* 1991; Young, 1991; Hampsey, 1998), whereas 4 subunits (RPB1, RPB2, RPB3, and RPB11) have sequence-homologous counterparts in pol I and pol III. Only RPB4, RPB7, RPB9 and the CTD of RPB1 are unique to pol II. In addition, RPB1, RPB2, RPB3, and RPB6 share similar primary sequences with bacterial RNA polymerase subunits β', β,α and ω, respectively (Tan *et al.,* 2000; Minakhin *et al.,* 2001; Mitsuzawa and Ishihama, 2004). A prokaryotic α-like sequence also exists in RPB11 (Woychik *et al.,* 1993; Ulmasov *et al.,* 1996). The primary sequence similarity between RPB1 and β' as well as between RPB2 and β also corresponds to functional similarity: RPB1 and β' are involved in DNA binding, while RPB2 and β bind nucleotide substrates (Hampsey, 1998). Analogous to their bacterial counterparts, RPB1 and RPB2 are responsible for most of the catalytic activity of polymerase and are essential for phosphodiester bond formation (Hampsey, 1998; Lee and Young, 2000).

Structure of Pol II

Recently, there has been a wealth of structural information on prokaryotic and eukaryotic RNA polymerases provided by photocrosslinking, X-ray crystallography, NMR, and cryo-electron microscopy. The structures of a yeast pol II 10-subunit enzyme minus RPB4 and RPB7 in the absence of DNA (Cramer *et al.,* 2000, 2001) and of a complete 12-subunit pol II have recently been resolved by X-ray crystallography (Armache *et al.,* 2003, 2005; Bushnell and Kornberg, 2003). RBP4 and RBP7 were not included in the original crystals

since the heterodimeric RPB4/RPB7 module is found in substoichiometric amounts in pol II and may dissociate from the "core" 10 subunits. Furthermore, the RPB4/RPB7 heterodimer, although required for PIC formation and initiation of transcription, is dispensable for RNA chain elongation (reviewed by Hampsey, 1998; Lee and Young, 2000; Cramer, 2004; Hahn, 2004). The structure of the 12-subunit pol II complex pinpoints the location of RPB4/RPB7 close to the RNA exit channel and also suggests a role of this heterodimer in transcriptional initiation (Armache *et al.,* 2003, 2005; Bushnell and Kornberg, 2003). The CTD (amino acids 1535 to 1733 in yeast), representing an unstructured extension from the catalytic core of pol II, is flexibly linked to a region adjacent to the RNA exit channel via an 80-residue linker and provides a platform for interacting with many proteins involved in 5′ capping, mRNA splicing, termination and 3′-end processing (Meinhart *et al.,* 2005). Comparison of the structures between the 10-subunit free core enzyme and a transcribing elongation complex containing the same core enzyme in complex with 9 base pairs of an RNA-DNA hybrid within a partially unwound DNA duplex has revealed detailed information regarding subunit-subunit and protein-nucleic acid contacts both within and outside the catalytic center of the enzyme (Cramer *et al.,* 2001; Gnatt *et al.,* 2001). Furthermore, contact residues between pol II and distinct domains of TFIIB, based on the structural information, have been defined by photocrosslinking experiments (Chen and Hahn, 2003, 2004). The structures of pol II complexed with part of the zinc ribbon motif and the B-finger of TFIIB (Bushnell *et al.,* 2004), with elongation factor IIS (Kettenberger *et al.,* 2003), and with IIS in the presence of NTPs and a transcription bubble-mimicking DNA-RNA hybrid (Kettenberger *et al.,* 2004) have also been elucidated by X-ray crystallography. Low-resolution cryo-electron microscopy has resolved structures for a pol II-Mediator complex (Davis *et al.,* 2002) and for pol II interaction with TFIIF (Chung *et al.,* 2003). The implications of these structural studies have been the focus of many recent reviews (Woychik and Hampsey, 2002; Asturias, 2004; Cramer, 2004; Hahn, 2004; Boeger *et al.,* 2005).

CTD Phosphorylation

An essential feature of all pol II complexes involved in transcription and mRNA processing resides in the CTD of RPB1, the largest subunit of pol II. The CTD contains a tandem repeat of a heptapeptide: Tyr-Ser-Pro-Thr-Ser-Pro-Ser (YSPTSPS), which repeats 52 times in humans, 42 times in *Drosophila*, and 26 to 29 times in yeast, depending upon the species (Dahmus, 1995; Hampsey, 1998; Lee and Young, 2000). The CTD is disordered in pol II structures and tends to be degraded by proteases. Depending on the phosphorylation state and the presence or absence of the CTD, three forms of human pol II (IIO, IIA, and IIB; Figure 8) can be easily isolated and distinguished (Kershnar *et al.,* 1998). The IIA form of pol II contains a hypo- or unphosphorylated CTD normally implicated in PIC assembly and transcription initiation (Lu *et al.,* 1991; Serizawa *et al.,* 1993). The IIO form of pol II, involved in transcript elongation and termination, has a highly phosphorylated CTD with phosphorylation occurring primarily at serine residues 2 and 5 that are subject to a cycle of phosphorylation and dephosphorylation throughout the transcriptional process. The IIB form of pol II, representing a proteolytic derivative, does not have the CTD but remains transcriptionally active for at least the adenovirus major late promoter (Kim and Dahmus, 1989; Buratowski and Sharp, 1990; Kang and Dahmus, 1993; Kershnar *et al.,* 1998), the human rep-3b TATA-less promoter (Buermeyer *et al.,* 1992) and some TATA-containing cellular promoters derived from human histone H2B (Buermeyer *et al.,* 1995) and *Drosophila* HSP70 and actin 5C genes (Zehring and Greenleaf, 1990).

Several protein kinases implicated in CTD phosphorylation have been identified in humans, including cyclin-dependent kinase 7 (CDK7) associated with TFIIH (Feaver *et al.,* 1991b), CDK8 found in general cofactor Mediator, and CDK9 present in positive transcription elongation factor b (P-TEFb). The activities of these CTD kinases are regulated by their associated cyclins that form CDK7-cyclin H, CDK8-cyclin C, and CDK9-cyclin T pairs. Similar CTD kinases have been identified in yeast and include Cdk7/Kin28, Cdk8/Srb10, the CTD kinase 1 (CTDK-I), and Sgv1/Bur1 (Prelich, 2002). Both Bur1 and the catalytic subunit of CTDK-I, Ctk1, show sequence homology to mammalian CDK9. Phosphorylation of serine 5 by Cdk7/Kin28 following PIC assembly leads to initiation of transcription (Serizawa *et al.,* 1993) and later recruitment of mRNA-capping enzyme guanylyltransferase (Cho *et al.,* 1997; Komarnitsky *et al.,* 2000; Rodriguez *et al.,* 2000; Schroeder *et al.,* 2000; Pei *et al.,* 2001). Phosphorylation of serine 2 by other

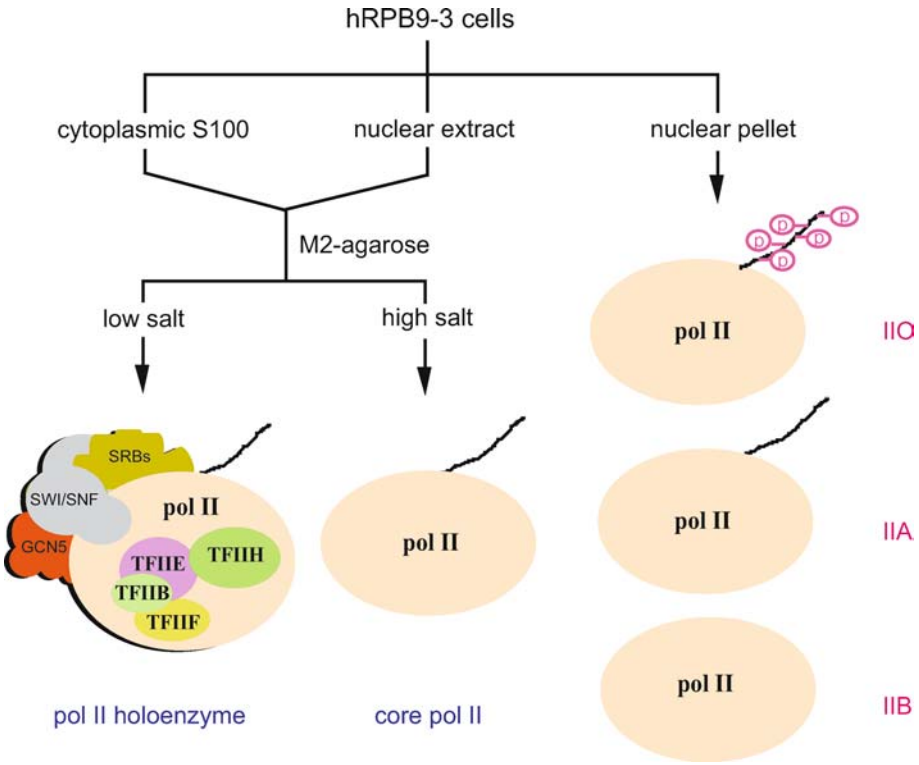


FIGURE 8 **Purification of human RNA polymerase II complexes. Four different forms of RNA polymerase II (pol II) are purified from a stable cell line (hRPB9-3) conditionally expressing FLAG epitope-tagged human RPB9 (Wu and Chiang, 2001b). Pol II holoenzyme is normally purified from the cytoplasmic S100 or nuclear extract fraction. Similarly, the IIA (*i.e.*, containing hypo- or unphosphorylated CTD) form of core pol II comprised of RPB1 to RPB12 subunits can also be isolated from the same S100 or nuclear extract fraction, but under high salt wash conditions (Kershnar *et al.,* 1998). The IIO (*i.e.*, containing hyper-phosphorylated CTD) and the IIB (*i.e.*, CTD-truncated) forms of pol II can be additionally purified from the nuclear pellet. The tail represents the CTD (carboxy-terminal domain) found in the RPB1 subunit of pol II.**

CTD kinases, such as CTDK-I or P-TEFb (Cho *et al.,* 2001; Zhou *et al.,* 2000; Shim *et al.,* 2002), results in transcription-coupled recruitment of 3′-end processing factors (Komarnitsky *et al.,* 2000; Ahn *et al.,* 2004). Two other CTD kinases, Cdk8/Srb10 (Hengartner *et al.,* 1998) and c-Abl (Baskaran *et al.,* 1993), have also been implicated in phosphorylation of serine 2 and tyrosine 1, respectively, although the functional effects remain to be defined.

Protein phosphatases that remove the phosphate group on serine 2 or serine 5 have likewise been identified. Yeast Ssu72 (Krishnamurthy *et al.,* 2004), plant *Arabidopsis thaliana* CTD phosphatase-like proteins AtCPL1 and AtCPL2 (Koiwa *et al.,* 2004), and human small CTD phosphatase 1 (SCP1) protein (Yeo *et al.,* 2003) are able to dephosphorylate serine 5 *in vitro*, whereas TFIIF-associated CTD phosphatase 1 (FCP1) isolated from yeast (Archambault *et al.,* 1997; Kimura *et al.,* 2002) and humans (Archambault *et al.,* 1998; Cho *et al.,* 1999) is mainly implicated in serine 2 dephosphorylation (Cho *et al.,* 2001; Meinhart *et al.,* 2005). FCP1 interacts with TFIIB (Chambers *et al.,* 1995; Kobor *et al.,* 2000), the RPB4 subunit of pol II (Kimura *et al.,* 2002), and the RAP74 component of TFIIF (Chambers *et al.,* 1995; Kobor *et al.,* 2000). It has been shown that TFIIF can stimulate FCP1 phosphatase activity and may thus accelerate reinitiation of transcription by enhancing the conversion of pol II from the elongating IIO form back to the initiating IIA form (Chambers *et al.,* 1995; see TFIIF section). Although TFIIB is able to inhibit TFIIF-stimulated FCP1 phosphatase activity, the functional role of this inhibition is still unclear (Chambers *et al.,* 1995). Undoubtedly, the counteracting activity between Ctk1-FCP1 and TFIIH-Ssu72 on CTD phosphorylation must play an important role in initiation, elongation, termination, and transcription-coupled mRNA processing.

CTD Glycosylation

In addition to phosphorylation, the CTD can also be modified by glycosylation via covalent linkage of a monosaccharide, N-acetylglucosamine (GlcNAc), onto the side chain hydroxyl group of serine or threonine

residues in the heptapeptide repeat (Kelly *et al.,* 1993). The β-O-linked N-acetylglucosamine (O-GlcNAc) is transferred predominantly to the fourth position (usually a threonine in the consensus YSPTSPS repeat, but occasionally a serine in nonconsensus sequences) of the heptapeptide repeat (Kelly *et al.,* 1993) by an O-GlcNAc transferase (OGT), with the reverse deglycosylation reaction carried out by an O-GlcNAc-specific β-N-acetylglucosaminidase (O-GlcNAcase; Iyer *et al.,* 2003 and references therein). The glycosylated Ser/Thr residues can be found in the heptapeptide sequence spread throughout the entire CTD domain (Kelly *et al.,* 1993). Glycosylation is only found in the IIA form of pol II and appears to be mutually exclusive with phosphorylation on the CTD (Comer and Hart, 2001; Iyer *et al.,* 2003). It is likely that glycosylation on Thr/Ser sterically blocks kinase accessibility to the CTD and thus regulates pol II activity during the early stage of the transcriptional process. Alternatively, the extent of glycosylation may alter CTD conformation, thereby modulating protein dynamic interaction on the CTD scaffold. The exact functional role of CTD glycosylation remains to be elucidated.

Pol II Ubiquitination

Ubiquitination is the third posttranslational modification that has been characterized on pol II. Yet, this important subject has not been extensively covered thus far in any reviews. In general, ubiquitination on pol II occurs at a low level under normal growth condition and can be readily detected when cells are under stress, particularly in response to specific DNA-damaging agents, or when pol II encounters elongation blocks during the transcriptional process independent of DNA damage. Treating cells with UV irradiation or the chemotherapeutic drug, cisplatin, induces DNA damage and causes pol II arrest at sites of DNA lesions (Bregman *et al.,* 1996). Under this circumstance, arrested pol II becomes polyubiquitinated, and the proteins involved in the nucleotide excision repair (NER) pathway are recruited to the damage sites. This transcription-coupled repair (TCR) process correlates with pol II ubiquitination and appears to require transcription-repair coupling factors Cockayne syndrome A and B (CSA and CSB), which are involved in the TCR pathway facilitating removal of DNA lesions preferentially from the transcribed strand of an active gene. Indeed, ubiquitination on the RPB1 subunit of pol II was detected within 15 minutes in UV-irradiated or cisplatin-treated human HeLa cells and persisted for ~8 to 12 hours; in contrast, RPB1 was not ubiquitinated in CSA- or CSB-deficient human fibroblasts derived from patients with Cockayne syndrome who exhibited defects in the TCR pathway (Bregman *et al.,* 1996). This finding suggests that CSA and CSB are essential for pol II ubiquitination in response to UV irradiation and cisplatin treatment. However, when TCR was induced by hydrogen peroxide (H_2O_2), which is a different DNA-damaging agent that triggers the base excision repair (BER) pathway rather than the NER, CSA and CSB were not required for pol II ubiquitination (Inukai *et al.,* 2004), indicating that ubiquitination of pol II can occur via the TCR pathway following treatment of cells with different types of DNA-damaging agents that activate either the NER or the BER pathway.

In yeast, Rad26, which is a homolog of human CSB and a member of the SWI2/SNF2 family of DNA-dependent ATPase, is implicated in TCR and appears to assist pol II in overcoming the elongation block, likely by enhancing an open (*i.e.,* remodeled) chromatin structure at sites of DNA lesions, thereby allowing TCR and resumption of pol II elongation (Lee *et al.,* 2002b; Woudstra *et al.,* 2002; Bucheli and Sweder, 2004). However, when damage is beyond repair, Rad26 then recruits Def1 (degradation factor 1), which in turn interacts with pol II and enhances ubiquitination and subsequent degradation of pol II (Woudstra *et al.,* 2002; Reid and Svejstrup, 2004). In general, DNA damage-induced RPB1 ubiquitination results in pol II degradation by the 26S proteasome (Beaudenon *et al.,* 1999; Inukai *et al.,* 2004), a view consistent with *in vivo* ChIP assays showing that the 26S proteasome can be recruited to stalled pol II at sites of UV-induced DNA damage (Gillette *et al.,* 2004). It should be noted that the association of pol II with only the 19S regulatory subcomplex, but not the 20S catalytic core, of the 26S proteasome appears to enhance pol II elongation during regular transcription, apparently in a nonproteolytic fashion (Ferdous *et al.,* 2001). Clearly, the association of the 19S subcomplex with pol II likely provides a platform for subsequent assembly with the 20S core to form the 26S proteasome leading to pol II degradation, when ubiquitination of pol II occurs at the unrepaired damage sites.

Three enzymes involved in the thioester cascade for pol II ubiquitination have been identified in yeast: E1 ubiquitin-activating enzyme Uba1, E2 ubiquitin-conjugating enzyme Ubc5 (or Ubc4), and E3 ubiquitin

ligase Rsp5 (Somesh *et al.,* 2005). Rsp5 (reversion of Spt phenotype; an extragenic suppressor of mutations in the *Spt3* gene) is the only E3 ubiquitin ligase in yeast known to ubiquitinate pol II through interaction with the CTD of RPB1 (Huibregtse *et al.,* 1997; Beaudenon *et al.,* 1999; Lommel *et al.,* 2000). This interaction is mediated through recognition of the PXY motif (X can be any amino acid) present in contiguous heptapeptide repeats (YSPTSPSYSPTSPS) of the CTD by the second or third WW domain of Rsp5 (Wang *et al.,* 1999; Chang *et al.,* 2000). The WW domain is a conserved 38 to 40 amino acid module containing two conserved tryptophan (W) residues folded into a three-stranded antiparallel β-sheet with a hydrophobic binding pocket for PXY recognition (Macias *et al.,* 2002). Although the CTD is essential for interaction with Rsp5, ubiquitination of yeast pol II occurs outside the CTD at lysine 695 of RPB1 (Somesh *et al.,* 2005) and also in the RPB7 subunit (Kus *et al.,* 2005). It appears that multiple components of pol II can serve as targets for ubiquitination, leading to pol II degradation induced by different DNA-damaging agents.

In mammalian cells, multiple E3 ubiquitin ligases, including human receptor potentiation factor 1 (hRPF1), von Hippel-Lindau tumor suppressor protein (pVHL) complex, breast cancer gene 1-encoded protein (BRCA1), and the CSA complex, have been implicated in pol II ubiquitination. The WW domain-containing hRPF1 protein, also named hNEDD4 (human neural precursor cell-expressed and developmentally down-regulated), is the human homolog of yeast Rsp5 (Imhof and McDonnell, 1996; Gajewska *et al.,* 2003) and is able to interact with PXY-containing proteins, such as the p45 subunit of NF-E2 transcription factor (Gavva *et al.,* 1997) and the CTD domain of RPB1 (Beaudenon *et al.,* 1999). While the catalytic HECT (homologs to the E6-AP carboxyl terminus) domain of Rsp5, and likely hRPF1, is essential for pol II ubiquitination, this ubiquitin ligase activity is dispensable for the coactivator function of hRPF1 in stimulating hormone-dependent activation by human glucocorticoid receptor (hGR) and by progesterone receptor isoform B (hPR-B; Imhof and McDonnell, 1996). It is interesting to note that co-transfection of Spt3, which is found in yeast SAGA and SLIK, as well as human TFTC and STAGA complexes (see discussion of TAF-containing complexes), with either Rsp5 or hRPF1, synergistically activate transcription (Imhof and McDonnell, 1996), suggesting that these two E3 ubiquitin ligases may function through TAF-containing complexes to regulate target gene transcription. The finding that Rsp5 is also present in some yeast TFIID preparations and that TAF1 and TAF5 can be ubiquitinated (Auty *et al.,* 2004) further indicates that ubiquitination on TAF-containing complexes may play an important role in fine-tuning the activity of these coactivator complexes. This intriguing possibility is yet to be explored.

The pVHL tumor suppressor protein associates with Elongin B, Elongin C, Cullin 2 (Cul2), and RING-box protein Rbx-1 (also called ROC1 for Regulator of Cullins, SAG1 for Sensitive to Apoptosis Gene, Hrt1 for High-level expression reduces transposition by the Retrotransposon Ty3; Kamura *et al.,* 1999; Ohta *et al.,* 1999; Skowyra *et al.,* 1999; Seol *et al.,* 1999; Tan *et al.,* 1999) to form an E3 ubiquitin ligase complex involved in the ubiquitination of RPB1 (Kuznetsova *et al.,* 2003) and RPB7 (Na *et al.,* 2003). In general, a cullin-based E3 ubiquitin ligase contains a substrate recognition protein, adaptors, and an E2-binding module formed by a cullin family member and a RING (Really Interesting New Gene; Saurin *et al.,* 1996) protein possessing two Zn^{2+}-binding fingers. This class of RING finger-containing E3 ligases, unlike the HECT E3 ligase family proteins such as Rsp5 and hRPF1, does not form a covalent thioester bond with ubiquitin and mainly serves as a scaffold bridging the E2 enzyme and the substrate (Pickart, 2004). Together, Cul2 and Rbx1 form a module that interacts with different E2 ubiquitin-conjugating enzymes, including UbcH5a, UbcH5b, and UbcH5c (Jensen *et al.,* 1995; Iwai *et al.,* 1999). UbcH5c and its yeast homologs (*i.e.,* Ubc5/Ubc4) have been shown to work in conjunction with the HECT family E3 ubiquitin ligase Rsp5 and the E1 enzyme Uba1 to ubiquitinate pol II RPB1 (Somesh *et al.,* 2005), suggesting that the UbcH5 family has the capacity to function with both HECT and RING finger E3 ubiquitin ligases. Elongin B and Elongin C, which are also present in the Elongin complex involved in pol II elongation control (Shilatifard *et al.,* 2003), are the adaptors forming a subcomplex that bridges the Cul2/Rbx1 module with the substrate recognition unit pVHL (Petroski and Deshaies, 2005). The pVHL protein with 213 amino acid residues has two distinct domains: an N-terminal domain rich in β sheet spanning amino acids 63 to 154 that mainly interact with substrates destined for ubiquitination, and an α-domain comprising of three α-helices spanning amino acids 155 to 192 that contact Elongin C (Stebbins *et al.,* 1999). The N-terminal

region (amino acids 54 to 113) of pVHL, including part of the β-domain, binds RPB7, correlating with the finding that naturally occurring pVHL mutations (P86H and Y98H) within the β-domain display both reduced interaction and ubiquitination of RPB7 (Na *et al.,* 2003). Since RPB7 in complex with RPB4 may form a dissociable module with the 10-subunit pol II core, it remains to be investigated whether ubiquitination and degradation of RPB7 occurs mainly as a free module or in the context of pol II. In contrast, the ability of pVHL to bind human RPB1 requires a hyperphosphorylated CTD and a hydroxylated proline (P1465) in the L(XX)LAP motif (where X represents any amino acid; Huang *et al.,* 2002) residing in the linker region (Kuznetsova *et al.,* 2003). The requirement of an oxidized P1465 for pVHL binding is reminiscent of that seen with P564 of HIF-1α in oxygenated cells (during normaxia), in which recognition of the hydroxylated proline (mediated by prolyl-4-hydroxylase) by the hydrophobic pocket located at the β-domain of pVHL (Hon *et al.,* 2002; Min *et al.,* 2002) eventually leads to the ubiquitination and degradation of HIF-1α (Jaakkola *et al.,* 2001; Ivan *et al.,* 2001). It is important to note that pVHL-mediated degradation of RPB1, which occurs in a proline hydroxylation-dependent manner in response to UV-induced DNA damage, is distinct from that used for degradation of RPB7, which lacks the L(XX)LAP motif and is thus likely to be degraded through a proline hydroxylation-independent and DNA damage-independent pathway.

BRCA1 is the third mammalian E3 ubiquitin ligase implicated in RPB1 ubiquitination and pol II degradation upon UV-induced DNA damage (Kleiman *et al.,* 2005; Starita *et al.,* 2005). Human BRCA1 has 1863 amino acid residues and belongs to the RING finger E3 ubiquitin ligase family. The E2-binding region of BRCA1 lies in the RING motif that encompasses N-terminal amino acid residues 23-76 of BRCA1 (Brzovic *et al.,* 2001; Ruffner *et al.,* 2001). This motif, flanked on each side by an α-helix, contains two Zn^{2+}-binding sites (Cys24, Cys27, Cys44 and Cys47 for the first finger, and Cys39, His41, Cys61 and Cys64 for the second finger) organized in the following manner (Brzovic *et al.,* 2001): the first Zn^{2+}-binding loop (residues 23 to 34, a C_2C_2 type finger) is followed by two short antiparallel β-strands (residues 35 to 37 and 42 to 44) and a central α-helix (residues 46 to 53), which leads to the second Zn^{2+}-binding loop (residues 54 to 73, a C_3H_1 type finger) and a third short β-strand (residues 74 to 76). Part of this RING domain, including two Zn^{2+}-binding loops and the central α-helix, forms a surface cleft binding UbcH5c (Brzovic *et al.,* 2001; Brzovic *et al.*, 2003), while the C-terminal region of BRCA1 (residues 1560 to 1863) interacts with the RPB2, RPB12, and hyperphosphorylated RPB1 subunits of pol II (Schlegel *et al.,* 2000; Krum *et al.,* 2003). The identification of cancer-predisposing mutations in human BRCA1 at C24R, C39Y, C61G, and C64Y within this RING finger domain further indicates the importance of these cysteine residues in maintaining an active conformation of BRCA1 (Ruffner *et al.,* 2001). Interestingly, human BRCA1-associated RING domain 1 (BARD1), with 777 amino acid residues, also contains a RING finger domain at its N-terminus (residues 49 to 100) exhibiting a similar feature as that of BRCA1 but lacking the central α-helix. Thus, the RING domain of BARD1 cannot form a surface cleft, as seen with BRCA1, for binding UbcH5c (Brzovic *et al.,* 2003). However, BARD1, in complex with BRCA1, can stimulate BRCA1 function as an E3 ubiquitin ligase (Xia *et al.,* 2003). Dimerization of BRCA1 and BARD1 is mediated by hydrophobic contacts made between the N-terminal α-helices preceding the RING domain of each protein (Meza *et al.,* 1999; Morris *et al.,* 2002; Brzovic *et al.,* 2003). Together, BRCA1 and BARD1 form an active E3 ubiquitin ligase able to work in conjunction with the E2 ubiquitin-conjugating enzyme UbcH5c to promote substrate ubiquitination (Hashizume *et al.,* 2001; Brzovic *et al.,* 2003) and likely pol II degradation. Indeed, it has been shown that hyperphosphorylated RPB1 is more susceptible to UV-induced ubiquitination and degradation by cotransfecting BRCA1 and BARD1 expression plasmids into HEK-293T cells (Starita *et al.,* 2005), in agreement with the observation that RPB1 stability was increased by knocking down both endogenous BRCA1 and BARD1 in UV-irradiated HeLa cells (Klieman *et al.,* 2005). These experiments further stress the physiological importance of BRCA1 and BARD1 in regulating pol II turnover in human cells responding to UV-induced DNA damage.

Another mammalian E3 ubiquitin ligase able to regulate pol II ubiquitination and degradation is the CSA complex. In addition to functioning as a transcription-repair coupling factor in the UV- or cisplatin-induced TCR pathway, CSA usually associates with other cellular proteins under normal growth condition. This CSA complex, first isolated from nuclear extracts of FLAG/HA-tagged CSA-expressing HeLa cells

(Groisman *et al.,* 2003), consists of Rbx1, Cullin 4A (Cul4A), damaged DNA-binding protein 1 (DDB1), and a dissociable module comprising all the 8 subunits of COP9 signalosome (CSN). DDB1 is known to bind DNA lesions and facilitates the recruitment of NER components to the damage sites (Wakasugi *et al.*, 2002), whereas Rbx1 and Cul4A likely form the E2 ubiquitin-conjugating enzyme-binding module. The CSN components negatively regulate the ubiquitin ligase activity of CSA-Rbx1-Cul4A, as the ubiquitin ligase activity of the CSA complex could only be detected after dissociation of the CSN module (Groisman *et al.,* 2003). During purification, it was also found that a substoichiometric amount of hyperphosphorylated RPB1 is present in the CSA complex following UV-induced DNA damage without altering the level of hypophosphorylated RPB1 already associated with the CSA complex prior to irradiation. Interestingly, while the CSN module is not detected in the chromatin-bound CSA complex under normal condition, it becomes associated with the CSA complex after UV irradiation, exhibiting the same kinetics as hyperphosphorylated RPB1 for association and dissociation with the CSA complex. It is likely that the association of CSN with CSA-Rbx1-Cul4A-DDB1 upon UV irradiation may temporarily inhibit pol II ubiquitination, giving TCR components time to repair DNA lesions. If damage cannot be efficiently repaired, hyperphosphorylated pol II is then ubiquitinated and degraded, following the dissociation of the CSN module. This strategy ensures pol II can be readily available to resume transcription after TCR, unless it is otherwise destined for degradation when damage is beyond repair. Whether CSN indeed acts as a molecular switch modulating ubiquitin ligase activity of the CSA complex on stalled pol II remains to be defined.

Ubiquitination of pol II is a prerequisite for proteasome-mediated degradation. In general, degradation by the 26S proteasome requires recognition of substrate-linked polyubiquitin chains formed between the extreme C-terminal Gly76 of a ubiquitin molecule with internal Lys48 (major site) or Lys29 (minor site) of a preceding ubiquitin, although nonproteolytic linkage through the other 5 lysine (K) residues, such as K6, K11, K27, K33, and K63, may also occur (Weissman, 2001). At present, little is known regarding the function of K11-, K27-, and K33-linked polyubiquitination, whereas polyubiquitin-linked K63 chains often leads to nonproteolytic pathways such as endocytosis (Galan and Haguenauer-Tsapis, 1997), error-free postreplicative DNA repair (Spence *et al.,* 1995; Hofmann and Pickart, 1999; Hoege *et al.,* 2002), activation of the IκB kinase in the NFκB pathway (Deng *et al.,* 2000; Wang *et al.,* 2001), and enhancement of ribosome translational efficiency (Spence *et al.,* 2000). Among the E3 ubiquitin ligases known to ubiquitinate pol II, each ligase is able to assemble a distinct pattern of polyubiquitin chains unique to individual substrates. For examples, Rsp5 shows preference for assembling K48-linked polyubiquitin chains on WW domain-binding protein 2 (WBP2; Kee *et al.,* 2005) and K63-linked polyubiquitin chains on the yeast plasma membrane protein uracil permease (Galan and Haguenauer-Tsapis, 1997); the pVHL complex promotes K48-linked polyubiquitination leading to HIF-1α degradation (Ohh *et al.,* 2000); BRCA1/BARD1 enhances K6-linked polyubiquitination on nucleophosmin (Sato *et al.,* 2004) as well as autoubiquitination on BRCA1 through K6, K29, K48, or K63 linkage (Chen *et al.*, 2002; Wu-Baer *et al.,* 2003; Xia *et al.,* 2003; Nishikawa *et al.,* 2004). Although the specific lysines in polyubiquitinated pol II by these E3 ligases have not yet been characterized, it is likely that some of these modifications may play a degradation-independent role to coordinate pol II activity in TCR or DNA damage-independent pathways. Indeed, it has been found that transcriptional arrest induced by α-amanitin, which blocks nucleoside triphosphates incorporation into the nascent transcript, promotes pol II polyubiquitination at K6, K48, and K63 of ubiquitin, in the absence of DNA damage (Lee and Sharp, 2004; Jung and Lippard, 2005). It will be of interest to investigate how these different E3 ubiquitin ligases regulate both degradation-dependent and degradation-independent activities of pol II via promoting different lysine-linked polyubiquitination.

Unlike glycosylation that is inhibited by CTD phosphorylation, ubiquitination of pol II seems to depend on prior phosphorylation of the CTD, as evidenced by the observation that polyubiquitination of pol II in mammalian cells correlates with enhanced CTD phosphorylation upon DNA damage induced by UV or cisplatin that triggers the NER pathway (Bregman *et al.,* 1996) or by H_2O_2 that induces the BER pathway (Inukai *et al.,* 2004). That hyperphosphorylated pol II provides a signal for polyubiquitination is further supported by the finding that pol II ubiquitination was suppressed when a CTD kinase inhibitor H8, which blocks phosphorylation on serine 5 by Cdk7 and

phosphorylation on serine 2 by Cdk9, was included in the ubiquitination reactions with phosphorylation on serine 2 by HeLa nuclear extracts (Mitsui and Sharp, 1999). Indeed, ubiquitination of pol II *in vitro* and in UV-irradiated mammalian cells by both pVHL and BRCA1/BARD1 RING finger-containing E3 ubiquitin ligase complexes was detected only when serine 5 of the CTD was phosphorylated (Kuznetsova *et al.,* 2003; Starita *et al.,* 2005), consistent with the result showing UV-induced association of hyperphosphorylated RPB1 with the CSA complex (Groisman *et al.,* 2003). Nevertheless, experiments performed *in vitro* with yeast Rsp5 and human RPF1, both belonging to the HECT E3 ubiquitin ligase family, indicates a phosphorylation-independent interaction and ubiquitination of RPB1 (Huibregtse *et al.,* 1997; Beaudenon *et al.,* 1999), correlating with *in vivo* studies demonstrating that under UV-induced DNA damage in yeast, serine 5 dephosphorylation by CTD phosphatase Ssu72 makes pol II prone to polyubiquitination and degradation, whereas phosphorylation on serine 5 by Kin28 confers protection to pol II from ubiquitination (Somesh *et al.,* 2005). While the requirement for CTD phosphorylation appears to vary depending on the species (yeast versus mammalian cells) and the type of E3 ubiquitin ligases involved, the CTD undoubtedly plays a central role in coordinating the signaling events induced by different DNA-damaging agents. It is important to note that the particular lysine residues linking polyubiquitin chain formation and the nature of DNA damage-independent transcriptional pausing induced by α-amanitin or during elongation also determine the functional outcome of a stalled pol II complex. To be or not to be degraded will surely be an important issue warranting further investigation for years to come.

TFIIE

Structures and Functional Domains

After formation of a TFIID-TFIIB-pol II/TFIIF-promoter complex, the next step in the sequential PIC assembly pathway is the recruitment of TFIIE and TFIIH. TFIIE consists of two subunits, α and β, which form an $\alpha_2\beta_2$ heterotetramer (Ohkuma *et al.,* 1991; Sumimoto *et al.,* 1991; Peterson *et al.,* 1991). In humans, TFIIEα is the larger subunit consisting of 439 amino acids with a molecular weight of 56 kDa, while TFIIEβ is 291 amino acids with an approximate molecular weight of 34 kDa. The N-terminal half of TFIIEα is necessary for interactions with TFIIEβ and pol II via nonoverlapping regions (Figure 7C), for basal transcription, for stimulating TFIIH-mediated CTD phosphorylation, and for the transition from initiation to elongation. The C-terminal region, which appears to be nonessential in yeast, is involved in TFIIH interaction likely to facilitate TFIIH entry into the PIC (Ohkuma *et al.,* 1995; Kuldell and Buratowski, 1997; Okuda *et al.,* 2004). From amino acid sequence analysis, TFIIEα contains multiple structural features (Ohkuma *et al.,* 1995; see Figure 7C), including a bacterial σ factor homology region (residues 13 to 49), a leucine repeat (residues 38 to 66), a zinc-finger motif (residues 121 to 157), an HTH motif (residues 159 to 182), two alanine-rich sequences (residues 221 to 240 and 335 to 349), and a C-terminal region rich in aspartic acids and glutamic acids (residues 378 to 393). The NMR structure of the zinc-finger motif present in a protease-resistant core between amino acids 113 and 174 of human TFIIEα is comprised of one α-helix and five β-strands, which is distinct from conventional zinc finger structures (Okuda *et al.,* 2004). This unusual zinc-finger motif, with a central α-helix (H1) preceded by an antiparallel β-sheet (S1 and S2) and followed by three β-strands (S3–S5), is organized in the following order: β-strand (S1, residues 126 to 129), turn (t1, residues 130 to 133), β-strand (S2, residues 134 to 136), α-helix (H1, residues 138 to 144), β-strand (S3, residues 145 to 146), loop (residues 147 to 150), β-strand (S4, residues 151 to 154), turn (t2, residues 155 to 158), and a fifth β-strand (S5, residues 159 to 164). Binding of the Zn^{2+} ion is coordinated by Cys129, Cys132, Cys154, and Cys157, located adjacent to and within the two turns of this structure. This C_2C_2 zinc-finger is not involved in binding the small subunit of TFIIE, given that mutations introduced into this zinc-finger motif does not impair TFIIEα binding to TFIIEβ based on *in vitro* GST pull-down assays (Okuda *et al.,* 2004). However, the zinc-finger motif of TFIIEα can enhance TBP binding to adenovirus major late and E4 TATA boxes, as evidenced by DNase I footprinting assays in which only wild-type TFIIEα, but not the C154A mutant, increases TATA recognition by TBP (Yokomori *et al.,* 1998). The structural information for the HTH motif of TFIIEα is derived from its archaeal homolog. The TFE protein from archaeal bacteria with 178 amino acids shows sequence homology to the N-terminal half of TFIIEα (Bell *et al.,* 2001), but lacks the C-terminal domain

present in TFIIEα found in *S. cerevisiae, C. elegans, D. melanogaster, X. laevis,* and *H. sapiens* (Ohkuma *et al.,* 1995; Kuldell and Buratowski, 1997). The winged HTH structure of TFE, resolved by x-ray crystallography at 2.8 Å (Meinhart *et al.,* 2003), is composed of three α-helices and three β-strands in the following order: α1-β1-α2-α3-β2-β3. Similar to the winged HTH motif seen with RAP74 and RAP30, the "wing" of TFE is formed by a loop connecting the antiparallel β2 and β3 strands. However, the TFE wing does not appear to exhibit any DNA-binding activity, as otherwise observed with most proteins possessing this structural motif.

Similar to TFIIEα, human TFIIEβ also contains several functional domains (Okamoto *et al.,* 1998; Okuda *et al.,* 2000): an N-terminal serine-rich region (amino acids 26 to 71) able to enhance TFIIH-mediated phosphorylation of the CTD, a central core domain (residues 66 to 146) exhibiting a winged HTH structure (residues 75 to 139) capable of binding double-stranded DNA, a leucine repeat (residues 145 to 163) with unknown function, a σ3 region (residues 163 to 193) having sequence homology with the bacterial σ factor subdomain 3, and a C-terminal domain containing two basic regions in which the first one (residues 197 to 238) is a basic helix-loop-helix motif contacting TFIIEα and the *Drosophila* Krüppel transcription factor and the second one (residues 258 to 291) has a basic helix-loop sequence interacting with single-stranded DNA, pol II, TFIIB, the RAP30 subunit of TFIIF and Krüppel (see Figure 7C). The multiple protein-protein and protein-DNA interactions observed with the C-terminal domain of TFIIEβ may account for its involvement in the transition from transcription initiation to elongation by pol II (Watanabe *et al.,* 2003). Since the N-terminal half of TFIIEα is also implicated in promoter clearance, it will be of interest to define whether the TFIIEα N-terminal region and the TFIIEβ C-terminal region function independently or cooperatively in aiding pol II transition from initiation to elongation. With respect to the structural analysis, the winged HTH motif in the central core domain (amino acids 66 to 146) of human TFIIEβ, revealed by NMR, is comprised of three α-helices and an antiparallel β-sheet (Okuda *et al.,* 2000): H1 (residues 75 to 90), S1 (residue 97), H2 (residues 99 to 105), H3 (residues 113 to 120), turn (residues 121 to 125), β-strand (S2, residues 130 to 133), loop (residues 134 to 135), and a third β-strand (S3, residues 136 to 139), similar to other winged HTH motifs with double-stranded DNA-binding activity. The region between residues 79 and 111 has sequence homology with the pol II-binding domain of RAP30, although interaction between this region of TFIIEβ and pol II has yet to be demonstrated. It should also be noted that TFIIEα and TFIIEβ could interact with TFIIAγ and TFIIA$\alpha\beta$, respectively (Yokomori *et al.,* 1998; Langelier *et al.,* 2001; Yamamoto *et al.,* 2001). Without doubt, the respective interaction domains need to be further defined.

TFIIE Function

As seen with other GTFs, TFIIE may be recruited to the promoter through direct interaction with gene-specific transcriptional activators, such as the Krüppel zinc finger protein (Sauer *et al.,* 1995) and the Antennapedia and Abdominal-B homeodomain proteins (Zhu and Kuziora, 1996). Once recruited, TFIIE interacts directly with both subunits of TFIIF, TFIIB, pol II, promoter DNA, and helps recruit TFIIH (Flores *et al.,* 1989; Maxon *et al.,* 1994; Okamoto *et al.,* 1998; Yokomori *et al.,* 1998; Watanabe *et al.,* 2003; Forget *et al.,* 2004). TFIIE binds to pol II near its active center and to the promoter DNA approximately 10 base pairs upstream from the transcription initiation site, where promoter melting begins (Douziech *et al.,* 2000; Kim *et al.,* 2000; Forget *et al.,* 2004). TFIIE can stimulate the ATPase, CTD kinase, and DNA helicase activities of TFIIH and thus facilitates the formation of an initiation-competent pol II complex (Ohkuma and Roeder, 1994; Serizawa *et al.,* 1994; Ohkuma *et al.,* 1995; Lee and Young, 2000). TFIIE and TFIIH are also essential for promoter melting and the transition from initiation to elongation (Holstege *et al.,* 1996). Consistent with TFIIE's role in promoter melting is its ability to bind single-stranded DNA (Kuldell and Buratowski, 1997). Interestingly, the requirement of TFIIE and TFIIH for gene transcription also depends on DNA topology and the promoter sequence (Parvin and Sharp, 1993; Goodrich and Tjian, 1994; Wu *et al.,* 1998), in agreement with the observation that TFIIE and TFIIH are not necessary for transcription from premelted promoter templates (Pan and Greenblatt, 1994, Holstege *et al.,* 1996).

TFIIH
Protein Composition

TFIIH is primarily recruited to the promoter region through association with TFIIE. Historically, TFIIH (also called BTF2; Gerard *et al.,* 1991) is a multiprotein

complex consisting of nine subunits: p89/XPB (gene defective in xeroderma pigmentosum patients complementation group B), p80/XPD (gene defective in xeroderma pigmentosum patients complementation group D), p62, p52, p44, p40/CDK7, p38/Cyclin H, p34, and p32/MAT1. TFIIH was initially purified from rat liver (Conaway and Conaway, 1989), HeLa cells (Gerard *et al.,* 1991; Flores *et al.,* 1992), and yeast (Feaver *et al.,* 1991a) by conventional column chromatography, and later by a single-step immunoaffinity purification method (Kershnar *et al.,* 1998; LeRoy *et al.,* 1998; Winkler *et al.,* 1998). It has three enzymatic activities required for transcription: DNA-dependent ATPase (Conaway and Conaway, 1989; Feaver *et al.,* 1991b; Roy *et al.,* 1994), ATP-dependent helicase (Schaeffer *et al.,* 1993; Serizawa *et al.,* 1993; Drapkin *et al.,* 1994), and CTD kinase (Feaver *et al.,* 1991b; Lu *et al.,* 1992; Serizawa *et al.,* 1992). In addition to the enzymatic activities essential for transcription, some components of TFIIH (*e.g.,* p89/XPB and p80/XPD) are involved in NER DNA damage response. Functionally, TFIIH can be separated into two subcomplexes: a cyclin-activating kinase complex (CAK) and a core complex. The CAK complex, responsible for phosphorylating pol II CTD, is consisted of CDK7, Cyclin H, and MAT1. The core complex contains XPB helicase, p62, p52, p44, and p34. CAK and core TFIIH are linked by the XPD helicase, which is essential for DNA repair activity of TFIIH but serves more or less a structural rather than an enzymatic role in transcription (Rossingnol *et al.,* 1997; Coin *et al.,* 1999). Mutations in either XPB or XPD lead to several human diseases, including xeroderma pigmentosum (XP), trichothiodystrophy (TTD), and Cockayne syndrome (CS) (Lee and Young, 2000; Lehmann, 2001; Zurita and Merino, 2003). Recent studies have identified a tenth subunit of TFIIH, TFB5, in both yeast (Ranish *et al.,* 2004) and humans (Giglia-Mari *et al.,* 2004). This small subunit (~8 kDa) has been implicated in the DNA repair function of TFIIH, as UV-irradiated yeast strains deficient in TFB5 exhibit a lower survival rate when compared with irradiated strains harboring wild-type TFB5 (Ranish *et al.,* 2004). Similarly, human TFB5 is involved in NER DNA damage response, given that cells derived from TTD patients with group A disorder are unable to repair UV-induced DNA lesions (Giglia-Mari *et al.,* 2004). This deficiency correlates with specific mutations in the gene coding for TFB5 and is likely caused by an increased degradation of TFIIH due to a lack of the TFB5 stabilizing subunit (Giglia-Mari *et al.,* 2004; Coin *et al.,* 2006). From *in vitro*-reconstituted experiments, human TFB5 has recently been shown to stimulate the XPB ATPase activity of TFIIH and further enhance the opening of damaged DNA facilitated by the XPB and XPD subunits of TFIIH (Coin *et al.,* 2006). It should be mentioned that many yeast core TFIIH components are also implicated in DNA damage response, as mutations in yeast genes encoding TFB1 (human p62; Matsui *et al.,* 1995; Wang *et al.,* 1995), TFB2 (human p52; Feaver *et al.,* 1997), Ssl1 (human p44; Wang *et al.,* 1995), and TFB4 (human p34; Feaver *et al.*, 1999) also exhibit defects in responding to UV irradiation.

DNA-Dependent ATPase Activity

The ATPase activity of TFIIH is required for transcription initiation and promoter clearance. Although an initial report indicated that TFIIH and ATP hydrolysis are only required for pol II promoter clearance, but not for transcription initiation and formation of the first phosphodiester bond in CpA dinucleotide-primed reactions (Goodrich and Tjian, 1994), subsequent studies found that TFIIH ATPase activity is indeed necessary for stable promoter opening and for first phosphodiester bond formation when natural nucleotide substrates were added with an excess amount of other GTFs, such as TFIIB, TFIIE, and RAP74 (that possess nonspecific DNA-binding activity), to initiate the transcription reactions (Holstege *et al.,* 1996, 1997; Kumar *et al.,* 1998). In general, without TFIIH, pol II tends to stall on the promoter-proximal region, leading to abortive transcription products; the addition of TFIIH in the presence of ATP significantly reduces the amount of the promoter-stalled pol II complex, indicating a direct involvement of TFIIH in promoter clearance (Dvir *et al.,* 1997; Kugel and Goodrich, 1998; Kumar *et al.,* 1998).

ATP-Dependent Helicase Activity

TFIIH contains two helicases, XPB and XPD which unwind the DNA in a $3' \rightarrow 5'$ and $5' \rightarrow 3'$ direction, respectively, making TFIIH a bidirectional DNA helicase (Schaeffer *et al.,* 1994). While XPB $3' \rightarrow 5'$ helicase activity is critical for both DNA repair and transcription, the XPD $5' \rightarrow 3'$ helicase activity is only required for DNA repair (Zurita and Merino, 2003). The

XPB helicase activity is essential for promoter clearance, which typically occurs within formation of the first 20 nucleotides and defines the transition from initiation and elongation. Once promoter clearance has occurred, TFIIH is no longer needed (Goodrich and Tjian, 1994). Consistent with this view, the requirement for TFIIH in transcription may be bypassed via the use of either negatively supercoiled or premelted templates (Parvin and Sharp, 1993; Pan and Greenblatt, 1994; Parvin *et al.,* 1994; Tantin and Carey, 1994), suggesting a role of TFIIH also in open complex formation. Nevertheless, it is unclear which activity (helicase or ATPase) of TFIIH is involved in open complex formation. Earlier reports suggested that the XPB helicase activity is needed for promoter opening, as a helicase-impaired human XPB protein (K346R) with a mutation in the ATP-binding domain and a temperature-sensitive yeast strain expressing a helicase-defective mutant of Rad25 (a yeast homolog of human XPB) both exhibited defects, respectively, in melting a number of promoters *in vitro* and *in vivo* (Guzmán and Lis, 1999; Tirode *et al.,* 1999). With better defined XPB mutants (Q647A and T478A in the helicase motif) that selectively inactivate helicase activity without impairing its ATPase activity, it was later found that while Q647A and T478A are defective in promoter clearance, they are fully functional in promoter opening (Lin *et al.,* 2005). This result is consistent with site-specific protein-DNA photocrosslinking experiments showing that XPB, in the context of a complete PIC, was not detected at the promoter region undergoing melting (*i.e.,* transcription bubble), which extends initially from −9 to +2 (Holstege *et al.,* 1997), in the adenovirus major late promoter with or without ATP, but rather was found at DNA sequences (+3 to + 25) downstream of the transcription bubble through contacts made exclusively with double-stranded DNA (Kim *et al.,* 2000). Thus, it is clear that the ATPase activity is involved in promoter opening and the helicase activity is crucial for promoter clearance. It is interesting to note that promoter clearance constitutes a regulatory step for some transcriptional regulators. The FUSE (Far Upstream Element)-binding protein (FBP) and FBP-interacting repressor (FIR), both playing an important role in modulating cell growth and differentiation in humans via regulating *c-Myc* gene transcription, can respectively stimulate or inhibit the helicase activity of XPB and thereby regulate transcription at the promoter clearance step (Liu *et al.,* 2000; Liu *et al.,* 2001a).

TFIIH and Nucleotide Excision Repair

NER is a process where damaged DNA is removed and replaced by newly synthesized DNA based on sequence information from the intact template strand. The finding that p89/XPB is identical to ERCC3, a DNA excision repair protein that is mutated in XP patients, led to the hypothesis that transcription might be coupled to DNA repair (Schaeffer *et al.,* 1993). Consistent with a dual role of TFIIH in transcription and DNA repair is the observation that transcriptionally active genes are preferentially repaired (Bohr *et al.,* 1985; Mellon and Hanawalt, 1989). For NER, the combined helicase activities of XPB/ERCC3 and XPD/ERCC2 seem to be required. Experiments have shown that microinjection of TFIIH into human XPD- or XPB-mutant cells led to complementation of the repair-deficient phenotype (van Vuuren *et al.,* 1994). Similarly, yeast cells with mutations in the XPD homolog (Rad3) were rescued by the addition of TFIIH but not by XPD alone, suggesting that the NER pathway requires a functional TFIIH (Wang *et al.,* 1994). Subsequent studies have shown that multiple components in TFIIH are required for DNA repair, including XPB, XPD, p62, p52, and p44 (Drapkin *et al.,* 1994; Humbert *et al.,* 1994; Schaeffer *et al.,* 1994; Wang *et al.,* 1995; Jawhari *et al.,* 2002). The XPB and XPD helicase functions are required for transcription-coupled NER, as defects in helicase activity are linked to human diseases including XP, TTD, and CS. Recent studies have unraveled the mechanism by which the XPB helicase subunit of TFIIH functions in NER and transcription. Experiments showing phosphorylation of the serine 751 residue of XPB leads to inhibition of NER activity, but does not prevent TFIIH from unwinding DNA (Coin *et al.,* 2004). Instead, phosphorylation of XPB serine 751 prevents the 5′ incision triggered by the ERCC1-XPF endonuclease (Coin *et al.,* 2004), providing convincing evidence that a separate but essential role of TFIIH is involved in both transcription and DNA repair.

Ubiquitin Ligase Activity

While XPB and XPD are directly involved in DNA repair, the yeast homolog of human p44 (Ssl1) in the TFIIH core complex has been implicated in DNA damage response likely by modulating the expression of proteins involved in the NER and BER pathways. A ubiquitin ligase activity of Ssl1 has recently been uncovered due to the presence of a RING finger domain

at its C-terminal region spanning residues 403 to 454, whose corresponding domain is present in human p44 (Takagi *et al.,* 2005). The NMR structure of the p44 RING finger domain (residues 321 to 395) shows two zinc-binding sites coordinated by eight cysteine residues (C345, C348, C360, C363, C368, C371, C382, and C385; Kellenberger *et al.,* 2005). These conserved cysteine residues are also found in the RING finger domain of Ssl1 and are critical for its E3 ubiquitin ligase activity, since mutations in the first two cysteine residues (C403A and C406A) abolished Ssl1 enzymatic activity in an *in vitro* polyubiquitination assays performed in the presence of E1 and E2 (Ubc4) enzymes and further reduced the yeast survival rate following UV irradiation or methyl methanesulfonate (MMS) treatment (Takagi *et al.,* 2005). The Ssl1 ubiquitin ligase activity can be enhanced by the inclusion of another RING finger-containing protein Tfb4 (the yeast counterpart of human MAT1), similar to BARD1-stimulated BRCA1 E3 ubiquitin ligase activity described earlier (see Pol II Ubiquitination section), or by the presence of other core TFIIH subunits, reminiscent of Rbx1 in the pVHL complex. However, whether TFIIH can polyubiquitinate pol II, in UV-irradiated cells at the sites of DNA lesions, or other protein components involved in transcription and DNA repair pathways remains to be explored.

TFIIH and CTD Phosphorylation

CDK7 is the kinase responsible for phosphorylating the serine 5 residue of the pol II CTD, whose activity is regulated by cyclin H, MAT1, TFIIE, Mediator (Svejstrup *et al.,* 1996), XPD (Keriel *et al.,* 2002), and U1 small nuclear RNA (snRNA; O'Gorman *et al.,* 2005). The CDK7-cyclin H-MAT1 CAK complex in the context of TFIIH has higher activity in phosphorylating the CTD compared with the free form of CAK (Yankulov and Bentley, 1997). Phosphorylation of serine 5 leads to the recruitment of 5′ capping enzyme (Cho *et al.,* 1997; Komarnitsky *et al.,* 2000; Rodriguez *et al.,* 2000; Schroeder *et al.,* 2000; Pei *et al.,* 2001) and is implicated in promoter clearance. That CTD phosphorylation regulates the transition from transcription initiation to elongation is supported by observations that pol II enters PIC assembly as the hypophosphorylated IIA form and escapes the promoter as the hyperphosphorylated IIO form (Hampsey, 1998). Besides phosphorylating CTD, TFIIH has been shown to phosphorylate transcriptional activators, such as p53 (Lu *et al.,* 1997), retinoic acid receptor α (Rochette-Egly *et al.,* 1997), retinoic acid receptor γ (Bastien *et al.,* 2000), Ets-1 (Drané *et al.,* 2004), estrogen receptor α (Chen *et al.,* 2000), and general cofactor PC4 (Kershnar *et al.,* 1998). The CTD kinase activity of TFIIH can also be stimulated via interaction with transcriptional activators (Jones, 1997).

TFIIH-Activator Interactions

Many activators have been shown to interact with TFIIH including Gal4-VP16, E2F1, Rb, p53, ERα, RARα, RARγ, and androgen receptor (reviewed by Zurita and Merino, 2003). Consistent with TFIIH's ability to interact with multiple activators is the finding that TFIIH can function as a coactivator in a reconstituted cell-free transcription system (Wu *et al.,* 1998). Perhaps activators work by enhancing the recruitment of TFIIH for PIC assembly or stimulating the enzymatic activities of TFIIH (Liu *et al.,* 2001a; Zurita and Merino, 2003). Conversely, TFIIH may covalently modify amino acid residues critical for activator function. For example, TFIIH has been shown to stimulate the transcriptional activity of the N-terminal activation domain (AF-1) of nuclear receptors RARα1 and RARγ via phosphorylation of specific serine residues in AF-1 (Rochette-Egly *et al.,* 1997; Bastien *et al.,* 2000). The kinase activity of TFIIH, which resides at its CDK7 subunit, appears to be regulated by XPD, as mutations in the XPD subunit impairs TFIIH's ability to phosphorylate RARα (Keriel *et al.,* 2002). Accordingly, XPD within the TFIIH complex can indirectly regulate nuclear receptor phosphorylation and transactivation activity and thus may account for its involvement in the transcriptional process besides its well-documented role in DNA repair. The cyclin H subunit of TFIIH is another target of the kinase module prone to regulation by other regulatory molecules. The U1 snRNA, involved in recognition of the 5′ splice donor site, is able to stimulate TFIIH CTD kinase activity via interaction with cyclin H (O'Gorman *et al.,* 2005), and also enhance first phosphodiester bond formation as well as transcription reinitiation (Kwek *et al.,* 2002). The finding that U1 snRNA can stimulate CDK7 kinase activity (O'Gorman *et al.,* 2005) and 7SK snRNA is inhibitory for CDK9 kinase activity (Nguyen *et al.,* 2001; Yang *et al.,* 2001) further implicates an important role of noncoding RNAs in modulating the transcriptional events through CTD kinases. Undoubtedly, this will be an interesting area for future studies.

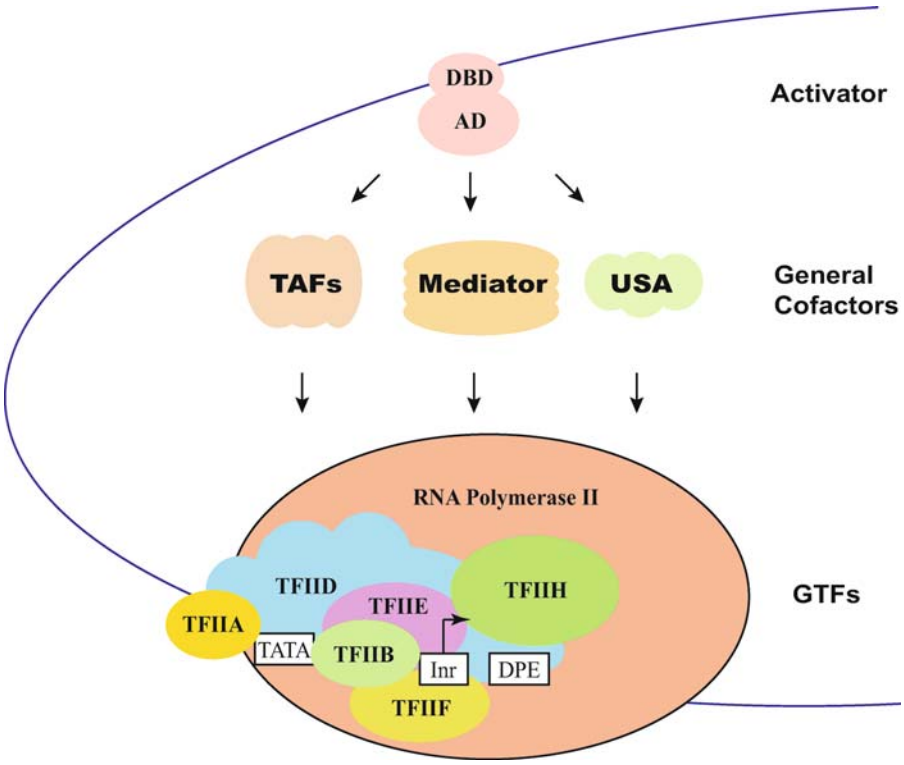


FIGURE 9 **General cofactors serve as molecular bridges in activator-dependent transcription. General cofactors (TAFs, Mediator, and USA) are required for transducing signals between gene-specific activators and components of the general transcription machinery. An activator normally contains a DNA-binding domain (DBD) contacting specific DNA sequences and an activation domain (AD) interacting with general cofactors or with components of the general transcription machinery. It should be noted that TAFs normally function as an integral part of TFIID, not as a free entity in mammalian cells as drawn here.**

GENERAL COFACTORS

Three classes of general cofactors are typically involved in gene activation to faciliate the communication between gene-specific transcription factors and components of the general transcription machinery (Figure 9). These general cofactors include TAFs found in TFIID, Mediator frequently associated with the CTD, and upstream stimulatory activity (USA)-derived positive cofactors (PC1, PC2, PC3 and PC4) and negative cofactor 1 (NC1). Similar to TAFs, some forms of Mediator and USA-derived components are capable of repressing basal transcription when activators are absent, and stimulating transcription in the presence of activators. Although other general cofactors implicated in transcription from higher-order chromatin structures, such as histone-modifying enzymes and chromatin-remodeling complexes, also exist, we will focus our discussion here on Mediator and USA-derived components originally defined in activator-regulated transcription using both genetic systems and nucleosome-free DNA templates.

MEDIATOR COMPLEXES

In addition to TFIID, Mediator represents the second class of general cofactors that transmit the regulatory signals from gene-specific transcription factors to the general transcription machinery (Björklund and Gustafsson, 2005; Kornberg, 2005). Mediator was first identified in yeast and found to consist of approximately 20 polypeptides (Kim *et al.*, 1994), of which 11 are essential for yeast viability (Rgr1, Rox3, Srb4, Srb6, Srb7, Med4, Med6, Med7, Med8, Med10/Nut2, and Med11) (Myers and Kornberg, 2000). While nine of the Mediator components were originally defined by genetic screens as proteins interacting with the CTD of pol II (*i.e.*, Srb2 and Srb4-11 for different dominant suppressors of RNA polymerase B; Nonet and Young, 1989; Thompson *et al.*, 1993), later biochemical purification of Mediator complexes from various species has identified additional conserved as well as species-specific subunits, for which a unified nomenclature has been proposed (Table 3; Bourbon *et al.*, 2004; Björklund and Gustafsson, 2005; Blazek *et al.*, 2005; Conaway *et al.*, 2005; Kim and Lis, 2005; Malik and Roeder, 2005).

TABLE 3 **Mammalian and yeast Mediator complexes**

New name	*S. cerevisiae*	TRAP/SMCC (Large)	ARC/DRIP (Large)	CRSP (Small)	PC2 (Small)	Mediator-P.5 (Large)	Mediator-P.85 (Small)
MED1	Med1	TRAP220	ARC/DRIP205	CRSP200	TRAP220	Med220	Med220
MED2	Med2						
MED3	Med3/Pgd1/Hrs1						
MED4	Med4	TRAP36	ARC/DRIP36		TRAP36	Med36	Med36
MED5	Nut1						
MED6	Med6	hMed6	ARC/DRIP33		hMed6	Med33	Med33
MED7	Med7	hMed7	ARC/DRIP34	CRSP33	hMed7	Med34	Med34
MED8	Med8		ARC32			Med31	Med31
MED9	Med9/Cse2						
MED10	Med10/Nut2	hNut2	hMed10		hNut2		
MED11	Med11						
MED12	Srb8	TRAP230	ARC/DRIP240			Med230	
MED13	Srb9/Ssn2	TRAP240	ARC/DRIP250			Med240	
MED14	Rgr1	TRAP170	ARC/DRIP150	CRSP150	TRAP170	Med150	Med150
MED15	Gal11		ARC105		PCQAP	Med105	Med105
MED16	Sin4	TRAP95	DRIP92		TRAP95	Med95	Med95
MED17	Srb4	TRAP80	ARC/DRIP77	CRSP77	TRAP80	Med78	Med78
MED18	Srb5						
MED19	Rox3						
MED20	Srb2	hTRFP			hTRFP		
MED21	Srb7	hSrb7	hSrb7		hSrb7		
MED22	Srb6						
MED23		TRAP150β	ARC/DRIP130	CRSP130	TRAP150β		
MED24		TRAP100	ARC/DRIP100	CRSP100	TRAP100	Med100	Med100
MED25			ARC92				
MED26				CRSP70			Med70
MED27		TRAP37		CRSP34	TRAP37		
MED30		TRAP25					
MED31		hSoh1			hSoh1		
CDK8	Srb10/Ume5/Ssn3	hSrb10	CDK8			CDK8	
CycC	Srb11/Ume3/Ssn8	hSrb11	CycC			CycC	

The identified protein composition of human Mediator-P.5 and Mediator-P.85 complexes (*grey shaded areas*) is described in Wu *et al*. (2003). Table adapted from Bourbon *et al*. (2004).

Isolation of Mediator Complexes

Human Mediator, first purified from HeLa cells as a protein complex that associates with the thyroid hormone receptor α (TRα) in a ligand-dependent manner, was able to potentiate TRα-mediated transcription *in vitro* (Fondell *et al.,* 1996). This TRα-associated protein complex (TRAP) contains many protein subunits subsequently found also present in other coactivator complexes, such as SRB/MED-containing cofactor complex (SMCC; Ito *et al.,* 1999), vitamin D receptor-interacting protein complex (DRIP; Rachez *et al.,* 1998), activator-recruited complex (ARC; Näär *et al.,* 1999), positive factor 2 (PC2; Malik *et al.,* 2000), cofactor required for Sp1 activation (CRSP; Ryu *et al.,* 1999), and negative regulator of activated transcription (NAT; Sun *et al.,* 1998). In humans, at least two forms of Mediator complexes, Mediator-P.5 and Mediator-P.85 isolated individually at 0.5 M and 0.85 M KCl fractions of the P11 phosphocellulose ion-exchange column, have been identified and demonstrated to enhance activator-dependent and basal transcription, respectively, in a highly purified transcription system reconstituted with recombinant GTFs and FLAG-tagged multiprotein complexes (Wu *et al.,* 2003). Mediator-P.5 represents a class of larger Mediator complexes, including TRAP/SMCC and ARC/DRIP, which contain a dissociable MED12-MED13-CDK8-CycC

module not found in the smaller Mediator complexes, such as Mediator-P.85, CRSP and PC2 (see Table 3). In contrast, the smaller Mediator complex has a unique polypeptide, MED26/CRSP70, normally absent in the larger complex. The rest of Mediator components seem to be commonly shared between large and small Mediator complexes, although the identities of some subunits remain to be characterized. The structures of yeast and murine Mediator as well as human TRAP, ARC and CRSP complexes have been resolved by electron microscopy at 30 to 40 Å (Asturias *et al.,* 1999; Dotson *et al.,* 2000; Taatjes *et al.,* 2002). Comparison of these structures reveals a similarity in the overall organization of Mediator complexes. In general, three visible domains (named "head," "middle," and "tail" in yeast Mediator) that may adapt to distinct conformations when in complex with activators or the CTD are distinguishable (Blazek *et al.,* 2005; Chadick and Asturias, 2005; Conaway *et al.,* 2005).

Head Module

The head module of Mediator, consisting of MED6, MED8, MED11, MED17, MED18, MED19, MED20, and MED22, forms the base of a roughly triangle-shaped Mediator complex. This triangular complex undergoes a drastic conformational change upon association with pol II, resulting in an arc-shaped structure in which the head module at the leading edge serves as a major docking site for pol II. This structural information is consistent with yeast genetic screens showing direct interaction between pol II and MED17/Srb4, MED18/Srb5, MED20/Srb2 and MED22/Srb6, respectively (Thompson *et al.,* 1993). Moreover, MED17 and MED18 also interact with transcriptional activators p53 and Gal4-VP16, respectively (Ito *et al.,* 1999; Lee *et al.,* 1999), suggesting that activator-induced conformational changes (Taatjes *et al.,* 2002) may further enhance head module-mediated recruitment of pol II.

Middle and Tail Modules

The middle module of Mediator contains MED1, MED4, MED5, MED7, MED9, MED10, MED21, and MED31, whereas the tail module includes MED2, MED3, MED14, MED15, and MED16 (Boube *et al.,* 2002; Guglielmi *et al.,* 2004). From the structures of yeast Mediator, it is obvious that, besides head module-pol II interaction, additional contacts are made between the Rpb1, Rpb3, Rpb6, and Rpb11 subunits of pol II with regions of Mediator extending from the head module to the intersection between middle and tail modules (Davis *et al.,* 2002). These extensive contacts may help Mediator unfold from its compact triangular shape, in which the middle and tail modules are not clearly visible in the absence of pol II, to a more extended conformation when bound to pol II. Despite the extended contacts, the DNA-binding cleft and interaction surfaces for other components of the general transcription machinery are still accessible on pol II (Davis *et al.,* 2002). Since MED1 in the middle module can interact with multiple nuclear receptors (Yuan *et al.,* 1998) and MED14 and MED15 in the tail module can associate with Gal4-VP16 (Lee *et al.,* 1999; Park *et al.,* 2000), it is likely that conformational changes induced upon activator binding to the middle and, especially the tail, modules further contribute to activator-facilitated recruitment of pol II by Mediator (Taatjest *et al.,* 2002).

MED12-MED13-CDK8-CycC Module

A dissociable module, which contains MED12/Srb8, MED13/Srb9, CDK8/Srb10 and CycC/Srb11 normally found in the large, but not small, Mediator complex (Borggrefe *et al.,* 2002; Samuelsen *et al.,* 2003), seems to contact mainly the middle module, via CDK8 interaction with MED1 and MED4 (Kang *et al.,* 2001), and also the head module, through MED13 interaction with MED17 (Guglielmi *et al.,* 2004). This CDK8 module, which could be isolated as a free entity, is able to phosphorylate serines 2 and 5 of the pol II CTD (Borggrefe *et al.,* 2002). That transcription could be inhibited by CDK8-mediated phosphorylation of the CTD occurring prior to PIC assembly (Hengartner *et al.,* 1998) or through phosphorylation on serines 5 and 304 of the cyclin H subunit of TFIIH by recombinant CDK8-CycC pair or by the NAT complex (Akoulitchev *et al.,* 2000) suggests that the CDK8 module may function as a repression module in the context of Mediator. This view is supported by biochemical evidence showing that the large form of ARC (ARC-L) is transcriptionally inactive (Taatjes *et al.,* 2002) and the coactivating activity of Mediator-P.5 was slightly enhanced when CDK8-CycC was immunodepleted from the large Mediator complex (Wu *et al.,* 2003). Clearly, removal of CDK8 in yeast by nutrient depriviation (Holstege *et al.,* 1998) or in mouse P19 embryonic carcinoma cells following all-*trans* retinoic acid (tRA) treatment (Pavri *et al.,*

2005) enhances transcription from a subset of cellular genes normally suppressed by CDK8. In the latter case, it was further demonstrated that dissociation of CDK8 following tRA treatment converts Mediator from a transcriptionally suppressed state to an activated complex at the tRA-targeted RARβ2 gene promoter, indicating that CDK8 indeed functions in the context of a repression module *in vivo*.

Functional Properties of Mediator

In addition to the inhibitory activity conferred by the CDK8 repression module, Mediator is an authentic coactivator able to stimulate both basal (Mittler *et al.,* 2001; Wu *et al.,* 2003) and activator-dependent transcription (Kim *et al.,* 1994). This stimulating activity of Mediator clearly relies on its ability to serve as a bridging molecule in transducing activation signals typically from activator-tail module to head module-pol II. Although Mediator can be isolated via its direct interactions with activators or with the CTD, direct biochemical evidence demonstrating that Mediator indeed functions by enhancing activator-facilitated entry of pol II to the PIC has only become possible after all the general transcription factors, cofactors and pol II are available in purified forms devoid of any contaminating activities (Wu *et al.,* 2003). From this *in vitro*-reconstituted transcription system, we learn that the large form of Mediator complexes, such as Mediator-P.5, has intrinsic coactivating activity able to stimulate activator-dependent transcription, whereas the small form of Mediator complexes, such as Mediator-P.85, only enhances basal transcription. Interestingly, the coactivator function of Mediator can occur in the absence of TFIID TAFs, suggesting that Mediator and TAFs may play some redundant roles in the transcriptional process. Indeed, it has been shown that TAFs can also enhance pol II entry to the PIC in the presence of transcriptional activators (Wu and Chiang, 2001a). Besides targeting pol II, Mediator has the ability to enhance TBP binding to the TATA box. This TATA-enhancing activity may help stabilize the promoter-bound scaffold complex, which contains TFIIA, TFIID, TFIIE, TFIIH and Mediator (Yudkovsky *et al.*, 2000), to facilitate reinitiation of transcription from the same promoter.

As found in TFIID, Mediator also exhibits multiple enzymatic activities. The kinase activity of Mediator, inherent to the CDK8 subunit, can phosphorylate the CTD of pol II (Hengartner *et al.,* 1998; Sun *et al.,* 1998; Borggrefe *et al.,* 2002), the cyclin H subunit of TFIIH (Akoulitchev *et al.,* 2000), and general cofactor PC4 (Gu *et al.,* 1999). In addition, yeast Mediator has been reported to exhibit HAT activity, residing in the MED5/Nut1 subunit, that preferentially acetylates histones H3 and H4 in the context of both free core histones and chromatin (Lorch *et al.,* 2000). However, no HAT activity has been reported in Mediator complexes purified from other species to date. Interestingly, human MED8 can associate with Elongin B, Elongin C, Cullin 2, and Rbx1 to form an E3 ubiquitin ligase complex able to assemble polyubiquitin chains *in vitro* in conjunction with Uba1 and UbcH5a (Brower *et al.,* 2002), similar to the pVHL complex described earlier that is capable of polyubiquitinating pol II (see Pol II Ubiquitination section). This finding suggests that MED8 may serve as a substrate recognition component in this MED8-containing ubiquitin ligase complex and functionally links the ubiquitin machinery to Mediator. It would be interesting to identify the natural substrates ubiquitinated by this MED8-containing ubiquitin ligase complex. Collectively, these enzymatic activities may contribute to Mediator functions in the transcriptional process, including a possible stimulation of TFIIH kinase activity on the CTD (Kim *et al.,* 1994). Many of these intriguing questions remain to be addressed.

USA-DERIVED COFACTORS

The third class of general cofactors, USA, was initially defined as a crude fraction derived from the P11 0.85 M KCl fraction of HeLa nuclear extracts able to stimulate activator-dependent transcription (Meisterernst *et al.,* 1991). Upon further fractionation of the USA fraction (Figure 10), several positive cofactors (PC1, PC2, PC3 and PC4) and a negative cofactor (NC1) were identified (Kaiser and Meisterernst, 1996). With ongoing research in the study of USA-derived cofactors, it is remarkable to see that each cofactor has a dual role to function as a coactivator in potentiating activator-dependent transcription and also as a repressor in inhibiting basal transcription when the activator is absent.

PC1

PC1 is a nuclear protein that is the functional equivalent of poly(ADP-ribose) polymerase-1 (PARP-1; Meisterernst *et al.,* 1997), an enzyme which is well studied for its role in DNA repair by binding to damaged

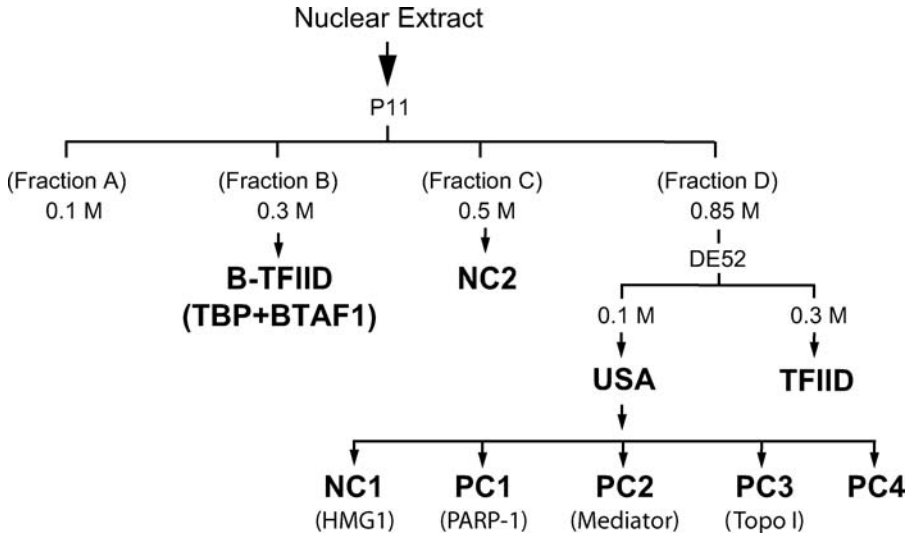


FIGURE 10 **Purification scheme for USA, NC2, TFIID, and B-TFIID. Fractionation of HeLa nuclear extract and the molar concentrations of KCl used for column chromatography are indicated.**

DNA and in nucleic acid metabolism by covalently modifying proteins involved in these pathways and also for its involvement in the maintenance of chromatin structure (Lindahl *et al.,* 1995; D'Amours *et al.,* 1999). These properties of PARP-1 explain why TFIIC (*i.e.,* PARP-1) only becomes necessary for site-specific initiation of transcription by pol II when nicked DNA templates were used for *in vitro* transcription assays, presumably due to suppression of nonspecific initiation from damaged DNA by TFIIC binding to the nicks (Slattery *et al.,* 1983).

Mammalian PARP-1, with a molecular size of approximately 114 kDa, catalyzes the transfer of ADP-ribose units from the donor nicotinamide adenine dinucleotide (NAD^+) to acceptor proteins, in a process known as poly(ADP-ribosyl)ation (Lindahl *et al.,* 1995; D'Amours *et al.,* 1999; Kraus and Lis, 2003; Kim *et al.,* 2005b). Not only does PARP-1 modify target proteins mediating DNA damage response, nucleic acid metabolism and chromatin dynamics, it also modifies transcription components, such as PC3 (*i.e.,* DNA topoisomerase I), high mobility group protein 1 (HMG1), histones (H1, H2A, H2B, H3, H4 and H5), TBP, pol II, TFIIF (RAP30 and RAP74), p53, YY1, Sp1, and several protein components (TLE1, TopoIIβ, Rad50, nucleolin, and nucleophosmin) in the TLE1 corepressor complex (Rawling and Alvarez-Gonzalez, 1997; Oei *et al.,* 1998; D'Amours *et al.,* 1999; Ju *et al.,* 2004). Moreover, ADP-ribose units can be transferred to PARP-1 itself. Auto(ADP-ribosyl)ation of PARP-1 is mediated through its central domain containing multiple glutamic acid residues, which serve as acceptor sites for poly(ADP-ribosyl)ation. This central domain links the N-terminal zinc-finger DNA-binding domain to the C-terminal NAD^+-binding catalytic domain (D'Amours *et al.,* 1999; Kraus and Lis, 2003). When disrupted, as observed during apoptosis where PARP-1 is cleaved by caspase death enzymes to release a 24-kDa N-terminal DNA-binding fragment and an 89-kDa C-terminal catalytic fragment, PARP-1 typically loses its enzymatic activity. For years, PARP-1 cleavage has been used as a diagnostic marker for programmed cell death.

Unlike the well documented roles of PARP-1 in DNA repair and apoptosis, the transcriptional role of PARP-1 has not been extensively studied (Kim *et al.,* 2005b). Obviously, the coactivator function of PARP-1 is mediated by its direct contact with distinct transcriptional activators, including the human T-cell leukemia virus type 1 Tax protein (Anderson *et al.,* 2000), human papillomavirus type 18 E2 (Lee *et al.,* 2002a), B-Myb (Cervellera and Sala, 2000), E2F-1 (Simbulan-Rosenthal *et al.,* 2003), AP2 (Kannan *et al.,* 1999), TEF-1 (Butler and Ordahl, 1999), RARα (Pavri *et al.,* 2005), TRα (Pavri *et al.,* 2005), and NFκB (Hassa *et al.,* 2003), and also with other transcriptional cofactors, such as p300, HDAC1-3, and components (Cdk8 and MED14) of Mediator (Hassa *et al.,* 2005; Pavri *et al.,* 2005). Since the C-terminal domain of PARP-1 is necessary for TEF-1- and NFκB-dependent transcription (Butler and Ordahl, 1999; Hassa *et al.,* 2003), but appears dispensable for Gal4-AH-mediated activation (Meisterernst *et al.,* 1997), it seems that the catalytic activity of PARP-1 is

differentially required for gene activation, depending on the specific activators and promoters examined. Not surprisingly, the coactivator function of PARP-1 is further modulated by posttranslational modifications, as phosphorylation of PARP-1 by CaMKII and acetylation of PARP-1 by p300 lead to HES1- and NFκB-mediated gene activation, respectively (Ju *et al.,* 2004; Hassa *et al.,* 2005). Using a cell-free transcription system performed with a Gal4-driven DNA template, it was shown that PARP-1 could stimulate PIC formation at a step post TFIID binding to the promoter region (Meisterernst *et al.,* 1997). However, the exact step regulated by PARP-1 during PIC assembly has not been elucidated.

Other than the coactivator function, PARP-1 also possesses repressing activity able to inhibit transcription through different mechanisms. First, PARP-1 can be incorporated into chromatin via its N-terminal DNA-binding domain to promote formation of a highly condensed chromatin structure, thereby inhibiting activator-dependent transcription (Kim *et al.,* 2004). Second, PARP-1 can be incorporated into a corepressor complex containing many protein components susceptible to modification by PARP-1, thus regulating the integrity of the corepressor complex on the target gene (Ju *et al.,* 2004). Third, PARP-1 is capable of inhibiting ligand-dependent transcription by TRα in transient reporter gene assays in a catalytic domain-dependent manner (Miyamoto *et al.,* 1999), suggesting that poly(ADP-ribosyl)ation of critical transcription components is likely involved in PARP-1-mediated transcriptional repression. This is consistent with the observation that poly(ADP-ribosyl)ation of sequence-specific DNA-binding proteins, such as p53, YY1, Sp1 and TBP, prevents binding to their cognate sequences (Malanga *et al.,* 1998; Oei *et al.,* 1998; Mendoza-Alvarez and Alvarez-Gonzalez, 2001). In the case of p53, the sites for poly(ADP-ribosyl)ation have been mapped to the DNA-binding and oligomerization domains of p53 (Malanga *et al.,* 1998). Interestingly, the enzymatic activity of PARP-1 is also critical for derepression to occur on some silenced loci, as seen by the association of PARP-1 and poly(ADP-ribosyl)ated proteins with decondensed chromatin structure at transcriptionally induced *Drosophila* polytene chromosome puffs (Tulin and Spradling, 2003). Clearly, PARP-1 can function as a molecular switch to convert a silenced gene into a transcriptionally active state, by first dissociating a corepressor complex via poly(ADP-ribosyl)ation (Ju *et al.,* 2004) or by removing a CDK8 repression module from the larger Mediator complex (Pavri *et al.,* 2005), and then enhancing activator-facilitated recruitment of chromatin-modifying enzymes, such as CBP or p300 HAT or ATP-dependent chromatin remodelers, to the targeted promoters.

PC2

PC2 (also termed USA100; Meisterernst *et al.,* 1991), originally isolated from HeLa nuclear extracts as a protein complex with a molecular size around 500 kDa (Kretzschmar *et al.,* 1994), corresponds to the smaller form of human Mediator complexes described earlier in the Mediator section. This protein complex can enhance transcription mediated by Gal4-AH and HNF4 in an *in vitro*-reconstituted transcription system with DNA templates (Malik *et al.,* 2000). The coactivator function of PC2 seems to require the presence of other general cofactors, since PC2 alone only weakly stimulates activator-dependent transcription and in conjunction with TAFs, PC3 and PC4, a synergistic activation of Gal4-VP16-mediated transcription recapitulating the stimulatory activity of the USA fraction is detected (Malik *et al.,* 2000). Likewise, PC2 is able to function together with PC3 and PC4 to regulate transcription mediated by TRα (Fondell *et al.,* 1999), NFκB, Sp1 (Guermah *et al.,* 1998), and the B-cell-specific Oct-1-OCA-B transcription complex (Luo *et al.,* 1998).

PC3

PC3, functionally equivalent to DNA topoisomerase I (Topo I; Kretzschmar *et al.,* 1993; Merino *et al.,* 1993), consists of 765 amino acids with a molecular size around 91 kDa (Leppard and Champoux, 2005). This protein is well known for its role in relaxing DNA by transiently introducing nicks, allowing strand passage and religation (Wang, 2002). Topo I has an unstructured N-terminal region containing nuclear targeting signals and surfaces for interaction with various transcription factors, such as p53 (Gobert *et al.,* 1999). This N-terminal region, although important for nuclear localization and protein-protein interaction, is dispensable for relaxing DNA *in vitro* (Stewart *et al.,* 1996). The enzymatic activity of Topo I is regulated posttranslationally by CK2- and protein kinase C-mediated phosphorylation, leading to increased relaxation activity and in responding to mitogenic stimuli (Wang, 1996). When Topo I encounters damaged DNA, it is stalled and must be removed

from the lesions in order to prevent formation of irreversible single- or double-strand breaks that impair genomic integrity (Leppard and Champoux, 2005). In this regard, PARP-1 can target Topo I for poly(ADP-ribosyl)ation and facilitate Topo I to remove itself from cleaved DNA and close the resulting gap (Malanga and Althaus, 2004).

Aside from its role in modulating DNA topology, Topo I can function as a transcriptional coactivator by contacting directly with activators, such as Gal4-AH (Kretzschmar *et al.,* 1993), AP2 (Kannan *et al.,* 1999), p53 (Gobert *et al.,* 1999), and c-Jun (Mialon *et al.,* 2005), and also with components of the general transcription machinery, such as TBP (Merino *et al.,* 1993), in order to stimulate transcription. The coactivator function of Topo I is in part due to its ability to enhance TFIID-TFIIA-promoter complex formation (Shykind *et al.,* 1997). In the absence of activator, Topo I functions as a repressor in inhibiting basal transcription. Although the transcription activity of Topo I is separated from its DNA relaxation activity (Merino *et al.,* 1993; Kretzschmar *et al.,* 1993), the precise mechanism by which Topo I regulates transcription remains to be elucidated. This is an area currently underexplored.

PC4

PC4 is a protein consisting of 127 amino acids with an N-terminal regulatory domain spanning amino acids 1 to 62 and a C-terminal single-stranded DNA-binding and dimerization domain located between amino acids 63 and 127 (Ge and Roeder, 1994a; Kretzschmar *et al.,* 1994; Brandsen *et al.,* 1997). The N-terminal region, important for PC4 interaction with distinct activation domains (Ge and Roeder, 1994a) and binding to double-stranded DNA in a non-sequence-specific manner (Kaiser *et al.,* 1995), contains two serine-enriched acidic (SEAC) domains located respectively at amino acids 9 to 22 and 50 to 61, separated by a lysine-rich region lying between amino acids 23 to 41 (Kaiser *et al.,* 1995). Both SEAC domains are susceptible to phosphorylation by several protein kinases, in particular CK2, which lead to inactivation of the coactivator function of PC4 likely by preventing PC4 interaction with TBP-bound TFIIA on the promoter region (Ge *et al.,* 1994; Kretzschmar *et al.,* 1994) and with transcriptional activators, such as the HIV Tat protein (Holloway *et al.,* 2000). The lysine-rich region, proposed to bind nonspecific double-stranded DNA (Kaiser *et al.,* 1995), may serve as acetylation sites for p300-enhanced PC4 binding to double-stranded DNA, consistent with the observation that CK2-mediated phosphorylation on the SEAC domains inhibits p300-dependent acetylation on PC4 (Kumar *et al.,* 2001). The C-terminal region, in which the structure has been resolved by X-ray crystallography at 1.74 Å resolution (Brandsen *et al.,* 1997), forms a dimer with each monomer composed of four antiparallel β-strands followed by a kinked α-helix and is able to bind with high affinity (Kd ~0.07 nM) two single strands of DNA running in opposite directions, as found in internally melted DNA duplexes. In the full-length protein, this single-stranded DNA-binding region is normally masked by intramolecular interaction with the N-terminal region and only becomes exposed after conformational changes induced, for instance, by CK2-mediated phosphorylation of the SEAC domains (Kaiser *et al.,* 1995). Phosphorylation-induced conformational changes lead to inactivation of PC4 coactivator activity and further inhibition of transcription mediated by the single-stranded DNA-binding activity of PC4 (Werten *et al.,* 1998).

The inhibitory activity of PC4 allows it to function as a repressor in suppressing basal transcription when activators are absent (Malik *et al.,* 1998; Werten *et al.,* 1998; Wu and Chiang, 1998). This inhibition usually occurs prior to PIC assembly in the absence of TAFs (Wu and Chiang, 1998) and can be alleviated by adding increasing amounts of TFIID, TFIIH and pol II holoenzyme in the transcription reaction, correlating with the ability of these multiprotein complexes to phosphorylate PC4 (Kershnar *et al.,* 1998; Malik *et al.,* 1998). However, since inactivation of the ATP-binding site of XPB/ERCC3 helicase, but not XPD/ERCC2 helicase or CDK7 kinase, impairs the ability of recombinant TFIIH to overcome PC4-mediated repression (Fukuda *et al.,* 2003), it remains unclear the precise mechanism used by components of the general transcription machinery to antagonize PC4 repressing activity.

The coactivator function of PC4 was evidenced by its ability to substitute for a crude USA fraction in mediating activator-dependent transcription in a cell-free transcription system reconstituted with recombinant GTFs (TFIIB, TBP, TFIIE, and TFIIF) and epitope-tagged multiprotein complexes (TFIID, TFIIH, and pol II; Wu *et al.,* 1998). In this system, where TAFs and Mediator are not essential for activator-dependent transcription, PC4 is the only general cofactor indispensable

for transcriptional activation mediated by Gal4-VP16 (Wu *et al.,* 1998), and human papillomavirus E2 (Wu and Chiang, 2001a; Hou *et al.,* 2002; Wu *et al.,* 2003). Not surprisingly, PC4 can interact with both transcriptional activators, such as Gal4-VP16 (Ge and Roeder, 1994a), BRCA1 (Haile and Parvin, 1999), AP2 (Kannan and Tainsky, 1999), HIV Tat (Holloway *et al.,* 2000), human papillomavirus E2 (Wu and Chiang, 2001a) and p53 (Banerjee *et al.,* 2004), and components of the general transcription machinery, such as TFIIA (Ge and Roeder, 1994a), TFIIH (Fukuda *et al.,* 2004), and pol II (Malik *et al.,* 1998), thereby serving as a bridging molecule to facilitate activator-dependent transcription likely through enhancement of PIC assembly on the promoter region. In addition, PC4 may promote sequence-specific DNA-binding activity of some activators, such as p53 (Banjeree *et al.,* 2004), stimulate promoter escape in a TFIIA- and TAF-dependent manner (Fukuda *et al.,* 2004), or enhance pol II elongation by modulating TFIIH kinase and FCP1 phosphatase activity on CTD phosphorylation (Calvo and Manley, 2005). Clearly, PC4 can work in conjunction with other general cofactors, such as TAFs (Wu and Chiang, 1998; Wu *et al.,* 1998) and Mediator (Fondell *et al.,* 1999; Malik *et al.,* 2000; Wu *et al.,* 2003), to synergistically mediate activator-dependent transcription. Whether phosphorylation of PC4, which accounts for 95% of total PC4 in the cell (Ge *et al.,* 1994), by TFIID, TFIIH, pol II holoenzyme (Kershnar *et al.,* 1998) and Mediator (Gu *et al.,* 1999), plays a role in different steps of the transcriptional process remains to be further defined.

Besides being a transcriptional coactivator, PC4 has also been implicated in other cellular processes, such as DNA repair and DNA replication. In the aspect of DNA repair, PC4 can prevent mutagenesis arising from oxidative DNA damage caused by the interaction of reactive oxygen species (ROS) with DNA, depending upon its single-stranded DNA-binding activity (Wang *et al.,* 2004). The involvement of PC4 in DNA replication appears to be more complicated, as PC4 can interact with replication protein A (RPA) on single-stranded DNA and facilitate T-antigen-mediated unwinding of DNA containing SV40 origin of replication, while it also inhibits RNA primer synthesis and DNA polymerase δ-catalyzed DNA chain elongation (Pan *et al.,* 1996). The biological significance of these *in vitro* reactions performed in the presence of PC4 requires further investigations.

NC1

NC1, also known as HMG1 or HMGB1 with a molecular size around 25 kDa, is a member of the highly conserved chromatin-associated proteins that bend DNA and bind preferentially to distorted DNA structures (Bustin, 2001; Thomas and Travers, 2001). HMGB1 is structurally divided into three domains: two homologous DNA-binding HMG-box domains A and B each containing approximately 80 amino acids, and a C-terminal tail containing a stretch of 30 acidic residues. Boxes A and B each forms an "L"-shaped structure with three α-helices constituting a minor-groove DNA-binding domain that preferentially binds distorted DNA, such as four-way junctions, cisplatin-modified DNA and bulged DNA, and induces DNA bending without sequence specificity (Thomas, 2001; Thomas and Travers, 2001). Both domains A and B contain an additional basic extension of amino acid residues that enhance the DNA-binding affinity of the HMG box. The C-terminal acidic tail modulates the DNA-binding activity of HMGB1 and seems to be inhibitory toward HMG box binding to DNA.

The transcriptional role of HMGB1 is similar to other USA-derived cofactors in that it normally functions as a repressor in the absence of an activator, but acts as a coactivator in activator-dependent transcription. The repressing activity of HMGB1 appears to work by promoting the formation of a stable HMGB1-TBP-promoter complex that prevents TFIIB entry (Ge and Roeder, 1994b), as the presence of HMGB1 increases the affinity of TBP for the TATA box by 20-fold (Das and Scovell, 2001). The interaction domains were mapped to the HMG box A of HMGB1 (Sutrias-Grau *et al.,* 1999) and the glutamine-rich region of TBP (Das and Scovell, 2001). TFIIA, as an antirepressor (see TFIIA section), can displace HMGB1 from the ternary complex and overcome HMGB1-mediated inhibition of PIC formation, thereby restoring transcription activity (Ge and Roeder, 1994b). The coactivator function of HMGB1 is attributed to its direct interaction with transcriptional activators, such as p53, steroid hormone receptors, Oct-1, HOX and Rel proteins (Thomas and Travers, 2001; Agresti and Bianchi, 2003), and with components of the general transcription machinery, including TBP (Ge and Roeder, 1994b) and TAF10 (Verrier *et al.,* 1997). Undoubtedly, the architectural role of HMGB1 in bending DNA will further contribute to its coactivator function typically by enhancing

sequence-specific recognition by these DNA-binding proteins.

CONCLUDING REMARKS

The finding of multiple core promoter elements (BRE^u, TATA, BRE^d, Inr, MTE, DPE and DCE) and core promoter-binding activity exhibited by TFIIB, TBP family members (TBP and TRF1-4) and distinct TAF complexes (TFIID, TFTC and SAGA/STAGA/SLIK) further exemplifies the diversity and specificity of core promoter recognition inherent in the eukaryotic genome. The recent discovery of RNA polymerase IV and spRNAP-IV implicated in transcriptional silencing and mRNA transcription, respectively, also broadens our view of pol II-mediated transcription processes beyond the traditional sets of RNA polymerases and their accessory factors. From the structural and functional analyses of GTFs, pol II, general cofactors, and the built-up transcription complexes, we have come across many unexpected properties of these transcription components. One of the most intriguing features is the uncovering of cryptic DNA-binding activity found in many of these proteins. In the case of TFIIB, activator-induced conformational changes unmask the recognition loop in the first direct repeat of TFIIB for BRE^d binding and concurrently reduce the affinity of its HTH DNA-binding domain, located in the second direct repeat, for contacting BRE^u. From the studies of TATA-containing and TATA-less promoters, TBP and some TAFs (TAF1, TAF2, and histone fold-containing TAF6 and TAF9) make sequence-specific contacts with the Inr, DPE, and DCE elements. However, the specific TAF(s) acting through the MTE has yet to be identified. Additional contacts made between GTFs and the promoter have also been observed presumably following TFIIF-induced DNA wrapping around a partially assembled TBP-TFIIB-pol II/TFIIF complex, due to cryptic DNA-binding activity exhibited by the winged HTH motifs present in RAP30 and RAP74. It is likely that the TAF components of TFIID will play a significant role in stabilizing this complex, if TFIID is substituted for TBP, and that the winged HTH motif of TFIIEβ will further stabilize the final PIC by providing extra DNA contacts. Undoubtedly, the efficiency and the functional property of the assembled PIC will be modulated by the presence of general cofactors, such as PC1, Topo I, PC4, and HMGB1, whose intrinsic DNA-binding activity may help reconfigure the topology and architecture of the promoter and, moreover, the recognition of damaged DNA templates.

The recent elucidation of pol II structures further provides mechanistic insight into the functional role of GTFs during PIC assembly. Besides the protein-DNA contacts contributed by sequence-specific recognition and cryptic DNA-binding activity as outlined above, the interactions amongst GTFs and with pol II also play an important role in stabilizing TBP/TFIID-promoter complex, alleviating repressing activity exerted by TAF1, BTAF1, and NC2, facilitating pol II entry, and forming a proper conformation for start site selection and later transition for pol II from initiation to elongation. The protein-protein contacts between the N-terminal zinc ribbon domain of TFIIB with TFIIF and pol II help recruit pol II/TFIIF to the promoter. Once assembled, TFIIF and the B-finger of TFIIB, situated by the catalytic center of pol II, facilitate start site selection. TFIIF and pol II then aid in the subsequent entry of TFIIE and TFIIH into the partially assembled PIC complex. The positioning of TFIIE at the active center of pol II allows TFIIE to stimulate TFIIH ATPase and DNA helicase activities, which facilitate promoter melting and promoter clearance. TFIIE also enhances the kinase activity of TFIIH, allowing TFIIH to phosphorylate serine 5 of the unstructured CTD, thereby marking the transition of pol II from initiation to elongation. Further elucidation of the structural mechanism for PIC assembly can be found in several recent reviews. Clearly, future structural studies with the inclusion of additional transcriptional components, such as TAFs, TFIIE, and TFIIH, into the TBP-TFIIA-TFIIB-pol II-TFIIF structure, and also with the permeation of small molecule inhibitors, such as DRB and α-amanitin, will provide a more complete understanding of PIC assembly and function.

Another intriguing feature that regulates promoter activity and the functional property of the PIC lies in the ability of these transcriptional components to be posttranslationally modified by, *e.g.,* phosphorylation, acetylation, glycosylation, ubiquitination, and poly(ADP-ribosyl)ation. In fact, many of these modifying activities are inherent to the transcriptional components themselves. TFIID, for example, possesses multiple enzymatic activities capable of phosphorylating H2B, TFIIAβ, RAP74, and PC4, acetylating H3, H4, and TFIIEβ, and ubiquitinating H1 and presumably TAF5 and TAF1 as well. TFIIH, besides its DNA helicase activity, can phosphorylate CTD, PC4 and a

number of transcriptional activators, and may also exhibit E3 ubiquitin ligase activity. Likewise, Mediator displays kinase, HAT, and likely E3 ubiquitin ligase activity, whereas PARP-1 can introduce poly(ADP-ribosyl)ation onto TBP, TFIIF, pol II, and other general cofactors and transcriptional activators. In many cases, however, the functional consequences of these modifications and critical substrates remain to be investigated. Related to this issue, many components of the general transcription machinery and general cofactors can be modified by exogenous enzymatic activities. An important but underexplored example is pol II ubiquitination, where pol II becomes polyubiquitinated in response to DNA-damaging agents or upon transcriptional arrest by different E3 ubiquitin ligases (Rsp5, hRPF1, pVHL-Elongin B/C-Cullin 2-Rbx1, BRCA1/BARD1, and the CSA complex) through polyubiquitin chain linkage via different lysine residues in the ubiquitin molecule. It would thus be interesting to see whether a transcriptionally stalled pol II complex can be polyubiquitinated by TFIIH and Mediator. This is certainly an exciting area for future exploration.

In conclusion, the assembly of an initiation-competent pol II complex is subject to multiple levels of regulation by a diverse set of protein factors and cofactors. There is no doubt that transcriptional activators have a significant impact in dictating the functional property of the PIC, regardless of which pathway (sequential assembly *vs*. pol II holoenzyme) is used for PIC assembly. The fact that the core promoter itself can be recognized by TBP, TRFs, TFIID and other TAF-containing complexes already lends flexibility for interaction with distinct transcriptional regulators as well as general cofactors which typically possess dual activities in repressing basal transcription and enhancing activator-dependent transcription in response to environmental cues. While tissue-specific TAFs and TRFs play an important role in regulating transcription during development, it remains a mystery what roles positive cofactors and Mediator play during embryonic development. Without doubt, the presence of TAF variants and multiple pathways for regulating PIC assembly provide an additional way to fine-tune the transcriptional events. Moreover, the alternative usage of general cofactors, such as TAFs, Mediator, and various USA-derived cofactors, and their functional redundancy and transcriptional synergy on both TATA-containing and TATA-less promoters provide an additional level of complexity in modulating eukaryotic gene transcription. Clearly, posttranslational modifications of activators through phosphorylation, acetylation, ubiquitination, and poly(ADP-ribosyl)ation by components of the general transcription machinery and general cofactors, such as TFIID, TFIIH, and Mediator, as well as by other cellular proteins functioning in response to extracellular signaling events will further regulate the functional property of the transcription complexes assembled on target genes. For sure, exciting stories will continuously be unraveled for years to come.

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