REVIEW

The general transcription factors of RNA polymerase II

George Orphanides, Thierry Lagrange, and Danny Reinberg[1]

Howard Hughes Medical Institute, Department of Biochemistry, Division of Nucleic Acid Enzymology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, New Jersey 08854-5635 USA

Messenger RNA (mRNA) synthesis occurs in distinct mechanistic phases, beginning with the binding of a DNA-dependent RNA polymerase to the promoter region of a gene and culminating in the formation of an RNA transcript. The initiation of mRNA transcription is a key stage in the regulation of gene expression. In eukaryotes, genes encoding mRNAs and certain small nuclear RNAs are transcribed by RNA polymerase II (pol II). However, early attempts to reproduce mRNA transcription in vitro established that purified pol II alone was not capable of specific initiation (Roeder 1976; Weil et al. 1979). Selective initiation was achieved only when the polymerase was supplemented with a number of additional protein components that could be isolated from crude cell extracts by chromatography (Matsui et al. 1980).

In recent years, tremendous experimental effort has led to the identification of each of the factors required for transcription in vitro from a variety of organisms. Amazingly, organisms as diverse as human, rat, *Drosophila*, and yeast use the same set of conserved proteins to initiate mRNA synthesis. These protein factors—collectively known as the General Transcription Factors (GTFs)—have since been purified to homogeneity from HeLa cells, rat liver, *Drosophila*, and *Saccharomyces cerevisiae*, and have been named TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH (where TF stands for transcription factor) according to their chromatographic elution profiles and order of discovery (Matsui et al. 1980; for review, see Zawel and Reinberg 1993). The entire set of GTFs is composed of ~30 polypeptides, most of whose corresponding cDNAs have been isolated. The GTFs can assemble sequentially onto promoter DNA to form stable nucleoprotein complexes that recruit pol II (termed preinitiation complexes). Not surprisingly, the assembly of the GTFs is subject to regulation by activator and repressor proteins: Activators can recruit GTFs to a promoter, thereby accelerating the assembly process, whereas repressor proteins can inhibit transcription by blocking the assembly of GTFs.

The purification of the GTFs as separate entities with unique functions and the observation that they can assemble at a promoter in a specific order in vitro suggested that a preinitiation complex must be built in a stepwise fashion, with the binding of each factor promoting association of the next. The concept of ordered assembly recently has been challenged, however, with the discovery that a subset of the GTFs exists in a large complex with pol II and other novel transcription factors. The existence of this pol II holoenzyme suggests an alternative to the paradigm of sequential GTF assembly (for review, see Koleske and Young 1995). It is therefore possible that most or all of the polypeptides required for initiation associate with a promoter in a single step, analogous to the initiation of transcription by the bacterial RNA polymerase holoenzyme (Busby and Ebright 1994).

This review focuses on the properties of the GTFs and on the role of each GTF in transcription. While we acknowledge that the stepwise model may not be the path by which GTFs assemble at a promoter in vivo, we use this model to highlight the interplay between GTFs that contributes to the formation of the complete preinitiation complex. We also review the mounting evidence for the existence of pol II holoenzymes and their roles in regulating transcription. Finally, we describe how regulatory factors target the GTFs to directly modulate the rate of transcription initiation.

Initiation of pol II transcription can occur in a series of ordered steps

In its most general form, the conventional model for ordered transcription initiation by pol II is characterized by a distinct series of events: (1) recognition of core promoter elements by TFIID, (2) recognition of the TFIID-promoter complex by TFIIB, (3) recruitment of a TFIIF/pol II complex, (4) binding of TFIIE and TFIIH to complete the preinitiation complex, (5) promoter melting and formation of an "open" initiation complex, (6) synthesis of the first phosphodiester bond of the nascent mRNA transcript, (7) release of pol II contacts with the promoter ("promoter clearance"), and (8) elongation of the RNA transcript. The only GTF not included in this scheme is TFIIA, which can join the complex at any

[1]Corresponding author.

Table 1. *Subunit compositions and properties of the human GTFs*

General transcription factor	Subunits[a]	Properties
TFIIA	37 kD (α) 19 kD (β) 13 kD (γ)	Antirepression; Required for activation
TFIIB	35 kD	Recruits pol II/TFIIF, start-site selection, zinc-ribbon, activator-induced conformational change
TFIID	38 kD (TBP)	Binds TATA element
	250 kD (TAFII250)	HMG-box, bromodomains, serine kinase
	150 kD (TAFII150)[b]	Binds downstream promoter regions
	135 kD (TAFII135)	
	95 kD (TAFII95)	WD-40 repeats
	80 kD (TAFII80)	Histone H4 similarity
	55 kD (TAFII55)	
	31 kD (TAFII31)	Histone H3 similarity
	28 kD (TAFII28)	
	20 kD (TAFII20)	Histone H2B similarity
TFIIE	56 kD 34 kD	Zinc-binding domain; Promoter melting, recruits and modulates activity of TFIIH
TFIIF	58 kD (RAP74)	Stimulates elongation, phosphorylated in vivo
	26 kD (RAP30)	σ homology, cryptic DNA-binding, prevents spurious initiation
TFIIH	89 kD (ERCC3)	3′–5′ helicase (essential for transcription), excision repair
	80 kD (ERCC2)	5′–3′ helicase (dispensible for transcription), excision repair
	62 kD (p62)	Excision repair
	44 kD (hSSL1)	Zinc-binding domain, excision repair
	40 kD (cdk7)	CTD-kinase, component of cdk7-activating kinase (CAK)
	37 kD (cyclin H)	cdk7 partner
	34 kD (p34)	Zinc-binding domain, homologous to hSSL1
	32 kD (MAT-1)	Ring-finger zinc-binding domain, CAK assembly factor

[a]For references describing the isolation of cDNAs encoding subunits of TFIIA, TFIIB, TFIIE, TFIIF, and TFIIH, see text. References describing the isolation of cDNAs encoding TAFIIs are reviewed in Burley and Roeder (1996).
[b]A human homolog of TAFII150 has not been identified. However, evidence supporting its existence has recently been reported (Kaufmann et al. 1996).

stage after TFIID binding and stabilizes the initiation complex (see below). The subunit compositions and selected properties of the GTFs are shown in Table 1.

Distinct classes of DNA sequence elements direct transcription by pol II

Three distinct families of DNA sequence elements direct transcription by pol II. The first family includes the core, or basal, promoter elements found near the site where pol II initiates transcription. Two classes of core element have been identified: the TATA element, located ~25–30 base pairs upstream of the transcription start site (consensus TATAa/tAa/t), and the Initiator motif, a pyrimidine-rich sequence (consensus $PyPyA_{+1}NT/APyPy$) encompassing the transcription start site (for review, see Weis and Reinberg 1992; Smale 1994). The TATA and Initiator elements, which serve to nucleate the initiation complex, are recognized by components of the transcription machinery. They can function independently or synergistically and cellular promoters contain one element, both elements, or neither (for review, see Smale 1994). Two other families of *cis*-regulatory elements are the promoter-proximal elements, situated 50 to a few hundred base pairs upstream of the start site, and the promoter-distal elements (enhancers) found up to tens of thousands of base pairs away from the transcription start site (Ptashne 1988; Mitchell and Tjian 1989). Both of these elements contain binding sites for proteins that modulate transcription.

Recognition of a promoter by TFIID nucleates transcription initiation

Early studies established that the multisubunit TFIID recognizes TATA elements in a variety of promoters (Parker and Topol 1984; Nakajima et al. 1988). The subunit of TFIID responsible for TATA element recognition is the TATA-Binding-Protein (TBP; for review, see Hernandez 1993). TBPs have been identified in a wide vari-

ety of organisms, emphasizing the universal requirement for this protein. In fact, TBP is also essential for transcription by RNA polymerases I and III and, as such, can be considered a universal eukaryotic transcription factor (for review, see Sharp 1992; Hernandez 1993; Struhl 1994). TBPs range in molecular weight from 22 kD (*Arabidopsis*) to 38 kD (human and *Drosophila*) and can be divided into two structural domains. The conserved carboxy-terminal TATA-binding domain of TBP consists of two imperfect direct repeats, can direct efficient and specific transcription initiation in vitro when combined with the remaining GTFs and pol II (Hoey et al. 1990; Horikoshi et al. 1990; Peterson et al. 1990; Zhou et al. 1993), and is sufficient for formation of the complete TFIID complex (Zhou et al. 1993). The amino terminus of TBP is divergent among species and its function is unclear.

Recognition of the TATA element involves dramatic DNA distortion

The crystallographic structures of two complexes containing the carboxy-terminal core domain of TBP tightly bound to a TATA element gave the field its first insight into the molecular recognition events that drive formation of the pol II initiation complex (J.L. Kim et al. 1993; Y. Kim et al. 1993). The structure of TBP represents a novel protein fold consisting of a curved, 10-stranded, antiparallel β-sheet, forming a concave lower surface with four α-helices on its upper surface (Fig. 1). A loop, or "stirrup," is present at each end of the long axis of TBP and gives the molecule the appearance of a "protein saddle" (Nikolov et al. 1992, 1996; Nikolov and Burley 1994). As predicted from earlier biochemical experiments (Lee et al. 1991; Starr and Hawley 1991), TBP recognizes the minor groove of the 8-bp TATA element. However, these biochemical experiments did not predict the dramatic and unprecedented DNA distortion that accompanies the induced-fit mechanism of TATA element recognition. To facilitate DNA recognition, the minor groove of the TATA element is molded to follow the curved β-sheet on the underside of TBP's saddle. As a result, the TATA sequence is partially unwound and is bent in a smooth arc (Fig. 1).

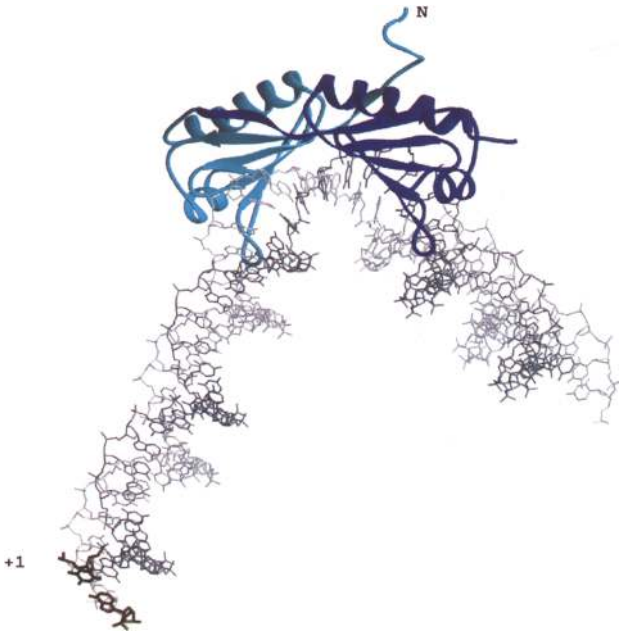

Figure 1. Structure of the TBP–TATA element complex. TBP is shown in the ribbon representation. The amino- and carboxy-terminal direct repeats of TBP are light blue and dark blue, respectively. DNA is drawn as an atomic stick model with the transcription start site indicated by +1. TBP is bound to the minor groove of the TATA sequence and induces a sharp bend in the DNA. Coordinates provided by D. Nikolov and S. Burley; figure by K. Das and E. Arnold.

What are the consequences of the DNA distortion brought on by TBP binding? The severe change in the path of the DNA may increase the proximity of proteins bound on either side of TBP. In addition, it has been suggested that this mode of TBP–TATA element binding is incompatible with the packaging of TATA elements into chromatin (Burley and Roeder 1996). When TATA-like sequences are positioned on the surface of nucleosomes, their minor grooves are compressed (for review, see van Holde 1989). Because this direction of DNA bending is opposite to that induced by TBP, nucleosomal packaging of a TATA element would preclude TBP binding. Conversely, preformed TBP-promoter (or TFIID-promoter) complexes would disfavor nucleosome packaging and would prevent chromatin repression of preformed initiation complexes.

The TBP-associated proteins ($TAF_{II}s$)

In addition to TBP, TFIID contains at least eight additional subunits—the TBP-associated factors ($TAF_{II}s$)—ranging in size from 20 to 250 kD (Table 1). The $TAF_{II}s$ are a phylogenetically conserved set of proteins identified in humans ($hTAF_{II}s$), *Drosophila* ($dTAF_{II}s$), and yeast ($yTAF_{II}s$) (for review, see Goodrich and Tjian 1994a; Sheldon and Reinberg 1995; Burley and Roeder 1996). The $TAF_{II}s$ are required for activator-dependent transcriptional stimulation in human and *Drosophila* systems in vitro. The role of $TAF_{II}s$ in transcriptional activation in vivo is currently unclear (discussed below).

A role for $TAF_{II}s$ in promoter recognition

Accumulating evidence is firmly establishing a role for $TAF_{II}s$ in promoter recognition. In early footprinting experiments, TFIID protected sequences extending from the TATA element to downstream of the transcription start site of certain promoters from nuclease digestion (Nakajima et al. 1988; Nakatani et al. 1990; van Dyke and Sawadogo 1990). Numerous studies since then have shown that efficient utilization of Initiator elements requires the TAF_{II} components of TFIID (Smale et al. 1990; Pugh and Tjian 1991; Zhou et al. 1992; Martinez et al. 1994). In addition, DNA footprinting and site-selection

experiments suggested that components of TFIID contact the Initiator region of a promoter (Kaufmann and Smale 1994; Purnell et al. 1994). However, these studies did not provide evidence for direct recognition of the Initiator by a TAF_{II}. The isolation of a cDNA encoding the 150-kD TAF_{II} of *Drosophila* TFIID permitted the first direct demonstration that a TAF_{II} can recognize promoter sequences (Verrijzer et al. 1994). In DNase I footprinting experiments, recombinant *Drosophila* $TAF_{II}150$ alone protects a region of the adenovirus major late promoter spanning from the transcription start site to 30–40 bp downstream. Furthermore, optimal utilization of sequences downstream of the transcription start site, which include the Initiator element, can minimally be accomplished by a *Drosophila* TFIID subassembly of TBP, $dTAF_{II}150$, and $dTAF_{II}250$ (Verrijzer et al. 1995). In addition, the ability of the *Drosophila* transcription apparatus to discriminate between the Initiator regions of two tandem *Adh* promoters depends on $dTAF_{II}150$ (Hansen and Tjian 1995). Despite these findings, it is still not clear whether $dTAF_{II}150$ directly recognizes the Initiator element, as no correlation between Initiator sequence requirement and $dTAF_{II}150$ binding has been demonstrated.

A human homolog of $dTAF_{II}150$ has not been cloned. However, a partially purified activity from HeLa cells that stimulates TFIID-dependent Initiator function contains a 150-kD polypeptide that is recognized by antibodies raised against *Drosophila* $TAF_{II}150$ (Kaufmann et al. 1996). Further evidence supporting a role for TAF_{II}s in core promoter recognition is the finding that affinity-purified *Drosophila* TFIID, but not recombinant TBP, recognizes a novel sequence motif [termed the downstream promoter element (DPE)] located ~30 base pairs downstream of the start site of a subset of TATA-less *Drosophila* promoters (Burke and Kadonaga 1996). Although the TAF_{II}(s) responsible for DPE recognition has not been identified, it is likely that $dTAF_{II}150$ is involved.

The connection between TAF_{II}s and the function of Initiator and downstream elements is gaining strength. The consensus from functional studies seems to be that TAF_{II}s recognize sequences downstream of the Initiator rather than the Initiator element itself. It will be interesting to determine whether a TAF_{II}-promoter interaction can nucleate the preinitiation complex in the absence of a TATA element. It is also pertinent to note that several other proteins have been implicated in Initiator recognition, including YY1 (Usheva and Shenk 1994), TFII-I (Roy et al. 1993), E2F (Means et al. 1992), USF (Du et al. 1993), and RNA polymerase II itself (Carcamo et al. 1991; Aso et al. 1994).

A histone-like octamer of proteins in TFIID?

Little is known about the organization of TBP and TAF_{II}s within the TFIID complex. Understanding the architecture of TFIID, together with the information regarding TFIID subunit–DNA interactions described above, would define the path of promoter DNA in the TFIID-promoter complex. The largest TAF_{II} ($hTAF_{II}250$, $dTAF_{II}250$, and $yTAF_{II}130$) is thought to interact with the upper surface of TBP and seems to be vital for tethering the remaining TAF_{II}s to TBP (Kokubo et al. 1993; Ruppert et al. 1993; Weinzierl et al. 1993; Reese et al. 1994; Poon et al. 1995). Surprisingly, Nakatani and coworkers have discovered that $hTAF_{II}250$ inhibits TATA element binding by TBP through a domain at its amino terminus (Kokubo et al. 1994b). Other TAF_{II}–TBP and numerous TAF_{II}–TAF_{II} interactions have also been reported (for review, see Burley and Roeder 1996). The vast range and redundant nature of TAF_{II}–TAF_{II} interactions may allow different TAF_{II} arrangements, thus accommodating conformational changes within TFIID.

The combination of biochemistry and crystallography has provided strong evidence that a histone octamer-like structure exists within TFIID. Sequence analysis of $dTAF_{II}40$ and $dTAF_{II}60$ (alternatively named $dTAF_{II}42$ and $dTAF_{II}62$ and homologous to $hTAF_{II}31$ and $hTAF_{II}80$, respectively) revealed that their amino termini bear homology to histones H3 and H4, which form a $(H3/H4)_2$ tetramer within a histone octamer (Kokubo et al. 1994a). These TAF_{II}s form a heteromeric structure in vitro (Hoffman et al. 1996; Nakatani et al. 1996) and in vivo (in the yeast two-hybrid assay; Nakatani et al. 1996). Direct evidence that these TAF_{II}s form histone-like structures recently has been provided by the Burley laboratory. The crystallographic structures of the amino-terminal regions of $dTAF_{II}40$ and $dTAF_{II}60$ (Xie et al. 1996) are remarkably similar to the corresponding regions of histones H3 and H4 and exhibit the classical "histone-fold" motif (Arents and Moundrianakis 1995). Moreover, these polypeptides form a $dTAF_{II}40/dTAF_{II}60$ heterotetramer in the crystal.

A TAF_{II} homolog with similarity to histone H2B has also been identified (Hoffmann et al. 1996): $hTAF_{II}20$ not only resembles histone H2B, but can interact with the human homologs of $dTAF_{II}40$ and $dTAF_{II}60$ and with histones H3 and H4. $hTAF_{II}20$ can also form homodimers, which may explain why no TAF_{II} homolog of histone H2A has been found (histone H2B forms a heterodimer with histone H2A in the histone octamer). Therefore, in the case of human TFIID, a putative $hTAF_{II}$ histone octamer-like structure would contain a heterotetramer of $hTAF_{II}31$ and $hTAF_{II}80$ sandwiched between two $hTAF_{II}20$ homodimers. The measured 4:1 stoichiometry of $hTAF_{II}20$:TBP is consistent with this model (Hoffmann et al. 1996).

What are the consequences of such a structure in TFIID? The histone-like TAF_{II}s retain the positively charged amino acids that interact with DNA in the nucleosome, suggesting that they may bind promoter DNA and, similar to histones, may even wrap DNA on their surface. In this way, these TAF_{II}s would contribute to the extended DNase I footprint of TFIID compared with that of TBP on certain promoters (for review, see Burley and Roeder 1996). Alternatively, the histone fold motifs of these TAF_{II}s may function solely to mediate protein–protein interactions within TFIID.

A kinase in TFIID?

In addition to participating in functionally relevant interactions with promoter DNA, the TAF_{II}s contact other GTFs in the preinitiation complex. TAF_{II}–GTF interactions include $dTAF_{II}40$–TFIIB (Goodrich et al. 1993), $dTAF_{II}110$–TFIIA (Yokomori et al. 1993), $dTAF_{II}250$–TFIIF (Ruppert and Tjian 1995), $hTAF_{II}80$–TFIIE (Hisatake et al. 1995), and $hTAF_{II}80$–TFIIF (Hisatake et al. 1995). Although these interactions have not been shown to be functionally significant, it is likely that some or all of them serve to stabilize the preinitiation complex. One particular TAF_{II}–GTF interaction has an additional twist. $hTAF_{II}250$ can interact specifically with the large subunit (RAP74) of TFIIF (Ruppert and Tjian 1995). Although they possess only weak homology to protein kinase sequence motifs, both $dTAF_{II}250$ and $hTAF_{II}250$ have been reported to contain intrinsic serine kinase activity with specificity for the RAP74 subunit of TFIIF (Dikstein et al. 1996). Although intriguing, the relevance of this observation to the process of transcription initiation remains to be established. Significantly, this kinase activity does not seem to be conserved in yeast: The yeast equivalent of $dTAF_{II}250$ ($yTAF_{II}130$) lacks the protein region responsible for this activity (Reese et al. 1994; Poon et al. 1995).

TFIIB recognizes the TFIID-promoter complex

The association of TFIID with promoter DNA nucleates the initiation process and is the only step in GTF assembly driven entirely by protein–DNA interactions. All subsequent steps entail recognition of preformed nucleoprotein complexes. The first of these steps is the binding of TFIIB to the TFIID-promoter complex, which results in a more stable ternary complex (the DB complex; see Fig. 2). Human TFIIB exists as a single polypeptide of ~35 kD (Ha et al. 1991). Homologs have been identified in *Drosophila* (Wampler and Kadonaga 1992; Yamashita et al. 1992), yeast (Pinto et al. 1992), and archaebacteria (Ouzounis and Sander 1992; Qureshi et al. 1995). The primary role of TFIIB is to physically link TFIID at the

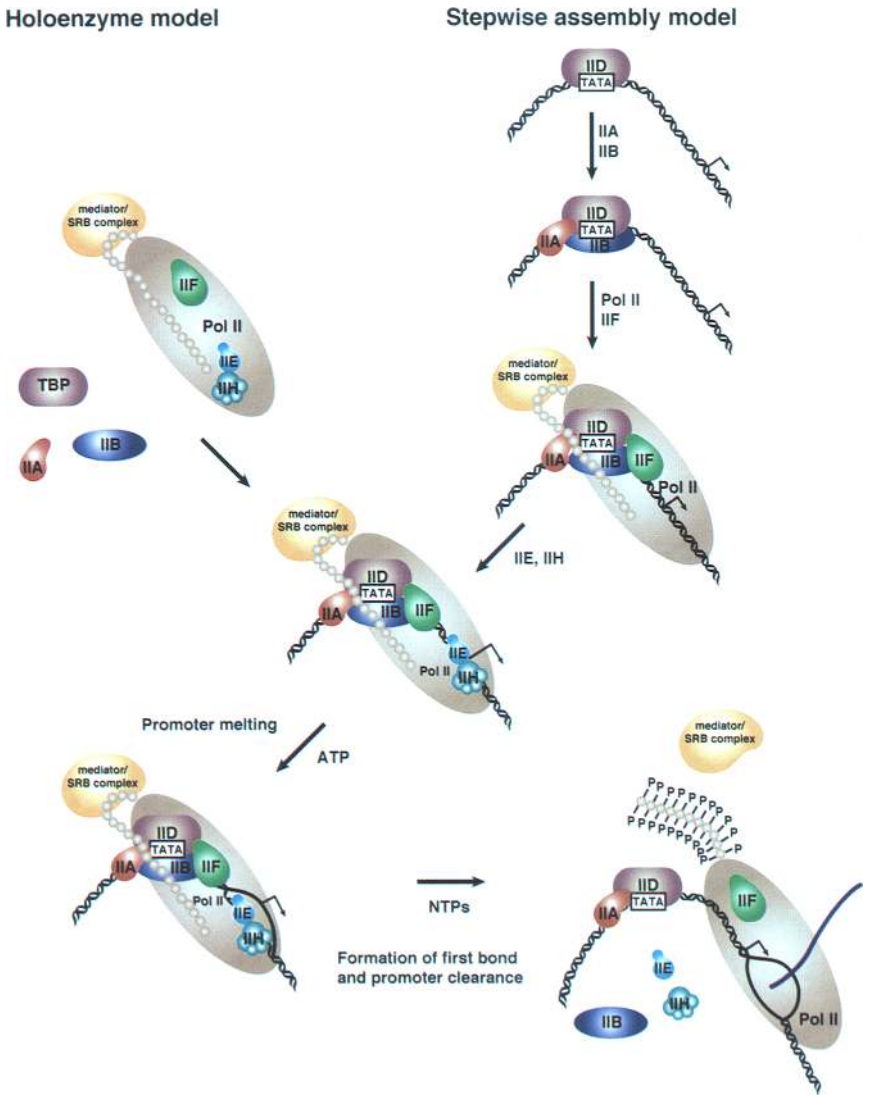

Figure 2. Model for the initiation of transcription by RNA pol II: holoenzyme model vs. the stepwise assembly model (see text for details). The TAF_{II}s of TFIID have been omitted for clarity. In the stepwise assembly model, TFIID first binds the TATA element of the promoter. TFIIA and TFIIB are next to bind. TFIIF then escorts pol II to the promoter. The preinitiation complex is completed by the binding of TFIIE and TFIIH. Upon the addition of ATP, the DNA strands at the start site of transcription separate (promoter melting), a process that involves TFIIE and TFIIH. Finally, in the presence of appropriate nucleotide triphosphates (NTPs), pol II begins transcription and subsequently breaks its contacts with the promoter (promoter clearance). The holoenzyme model represents an abbreviated form of the stepwise model in which a preassembled subset of GTFs (TFIIE, TFIIF, and TFIIH in the figure) and pol II bind the promoter in a single step.

promoter with the pol II/TFIIF complex. Consistent with this role, TFIIB contains binding sites for the TFIID–DNA complex (Buratowski et al. 1989; Maldonado et al. 1990), TFIIF (Ha et al. 1993), and pol II (Ha et al. 1993; Fang and Burton 1996).

Tethering pol II and TFIIF to the promoter is not the only role of TFIIB in initiation. Mutations in the *S. cerevisiae* TFIIB gene (*SUA7*) can dramatically alter the position at which pol II begins transcription (Pinto et al. 1992). A similar alteration in start sites accompanies mutations in genes encoding subunits of *S. cerevisiae* pol II (Hekmatpanah and Young 1991; Berroteran et al. 1994; Furter-Graves et al. 1994; Hull et al. 1995; Sun et al. 1996). This suggests that the interaction between TFIIB and pol II is crucial for specifying the start site of transcription. This assertion was substantiated by in vitro transcription experiments that suggested that TFIIB and pol II are solely responsible for specifying the start site of transcription (Li et al. 1994).

The primary structure of TFIIB reveals some interesting features. The amino terminus of the protein contains a cysteine-rich sequence that forms a zinc-ribbon domain (Zhu et al. 1996; see below). Most of the remainder of the protein consists of two imperfect direct repeats of ~75 amino acids. Protease digestion of TFIIB defined the amino- and carboxy-terminal regions to be separate domains (Barberis et al. 1993; Malik et al. 1993). The amino-terminal region is rapidly degraded by proteases, leaving a protease-resistant carboxy-terminal "core" region containing the two direct repeats of TFIIB. This carboxy-terminal region is able to bind the TBP–DNA complex, but is deficient in transcription, probably because it is unable to support efficient incorporation of the pol II/TFIIF complex (Barberis et al. 1993; Buratowski and Zhou 1993; Ha et al. 1993; Yamashita et al. 1993).

The TFIIB core region is an α-helical protein that resembles cyclin A

The structures of the carboxy-terminal core of TFIIB (TFIIBc) and the TFIIBc–TPB–DNA complex have been solved recently by NMR (Bagby et al. 1995) and X-ray crystallography (Nikolov et al. 1995), respectively (Fig. 3A). The solution and crystal structures of TFIIBc are similar. Each repeat of TFIIBc consists of five α-helices, which pack to form a compact globular domain. The only significant difference between the solution and crystallographic structures is a variation in the relative orientation of the two repeats (Fig. 3A). This suggests that TFIIB undergoes a change in conformation upon binding the TBP–DNA complex that may represent an important rate-limiting step in its binding.

As predicted from primary structure alignments (Gibson et al. 1994), the structure of TFIIBc resembles that of cyclin A (Jeffrey et al. 1995). In fact, each domain of TFIIB can be superimposed on the corresponding domain of cyclin A, although the two domains pack differently in the two proteins. This architectural resemblance suggests that these proteins evolved from a common ancestor or, alternatively, that the same protein scaffold is employed in proteins of different function.

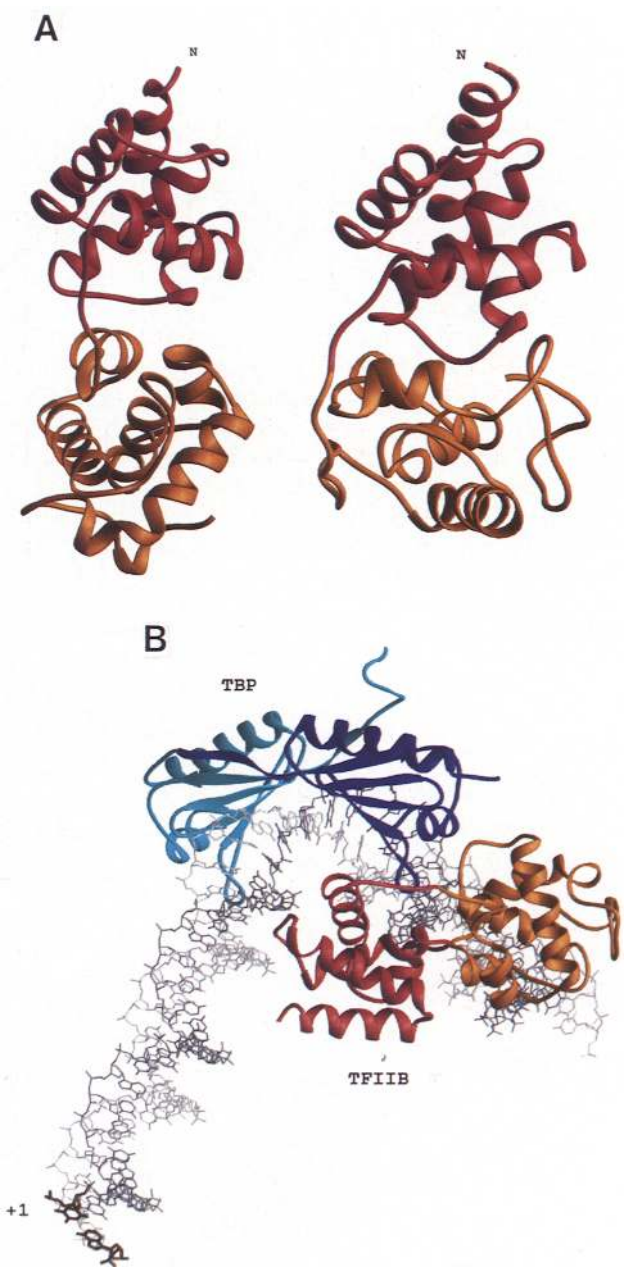

Figure 3. (*A*) Crystal structure (*left*) and solution structure (*right*) of TFIIBc. TFIIBc is shown in the ribbon representation. The amino- and carboxy-terminal domains of TFIIBc are red and orange, respectively. (*B*) Structure of the TBP–TFIIBc–TATA element complex. Proteins are shown in the ribbon representation; DNA is drawn as an atomic stick model with the transcription start site indicated by +1. The amino- and carboxy-terminal direct repeats of TBP are light blue and dark blue, respectively. TFIIBc binds to the carboxy-terminal repeat of TBP and contacts DNA upstream and downstream of the TATA box. Coordinates provided by D. Nikolov, S. Burley, S. Bagby, and M. Ikura; figures by K. Das and E. Arnold.

The TBP–TFIIB–TATA element complex: recognition of a preformed nucleoprotein complex

The crystal structure of the TBP–TFIIB–DNA ternary complex elegantly illustrates how TFIIB recognizes the preformed TBP–DNA complex (Nikolov et al. 1995). As suggested by footprinting (Lee and Hahn 1995) and cross-linking (Lagrange et al. 1996) experiments, TFIIB binds underneath and on one face of the TBP–DNA complex where it interacts with TBP and DNA (Fig. 3B). TBP–TFIIB contacts are mainly between the basic amino-terminal repeat of TFIIB and the acidic carboxy-terminal stirrup of TBP, in agreement with mutagenesis studies (T.K. Kim et al. 1994; Tang et al. 1996). The dramatic distortion of the TATA element by TBP allows TFIIB to interact with the phosphodiester backbone of DNA both upstream and downstream of the TATA sequence. Interestingly, recent studies demonstrate that TFIIB is a sequence-specific DNA-binding protein that recognizes a novel sequence element found in certain promoters (T. Lagrange, A. Kapanidis, H. Tang, D. Reinberg, and R.H. Ebright, in prep).

The two repeats of TFIIB make nonequivalent interactions with the TBP–DNA complex. This asymmetry in TFIIB binding ensures that transcription initiation can only occur downstream of the TATA element. Although the amino-terminal domain of TFIIB is missing from the structure, the position of the amino-terminal repeat of TFIIBc suggests that it lies close to the downstream DNA segment where it would be positioned to influence the position of the transcription start site. What do we know about the structure of this amino-terminal domain? Predictions that this region of TFIIB contains a zinc-binding domain, based on sequence analyses, were recently confirmed by NMR. A 49-amino-acid peptide derived from the amino terminus of an archaebacterial TFIIB assumes a zinc-ribbon structure (Zhu et al. 1996). A similar structure is present in the transcription elongation factor TFIIS (Qian et al. 1993). Interestingly, a portion of TFIIS that contains this zinc ribbon is able to bind both single-stranded and double-stranded DNA (Qian et al. 1993). Thus, it is tempting to speculate that the zinc ribbon of TFIIB binds DNA in the vicinity of the transcription start site and may stabilize the melting of the promoter that occurs prior to initiation of RNA synthesis (see below). Indeed, mutations in the region immediately adjacent to the zinc ribbon alter the start site (Pinto et al. 1994). Similarly, a truncation in the zinc-ribbon domain the Rpb9 subunit of pol II leads to a comparable change in the start site of transcription (Sun et al. 1996). Thus, it is likely that the zinc-ribbon domains of TFIIB and Rpb9 play a crucial role in start-site selection.

TFIIA: a close-up view of a once controversial factor

TFIIB is not the only GTF that can bind and stabilize the TBP-promoter complex. TFIIA also binds to the TBP–DNA complex (Buratowski et al. 1989; Cortes et al. 1992; Coulombe et al. 1992; Lee et al. 1992; see Fig. 1) and increases the affinity of TBP for the TATA element (Imbalzano et al. 1994b). cDNAs encoding TFIIA subunits have been isolated from yeast (Ranish et al. 1992), human (DeJong and Roeder 1993; Ma et al. 1993; Ozer et al. 1994; Sun et al. 1994; DeJong et al. 1995), and *Drosophila* (Yokomori et al. 1993, 1994). Human and *Drosophila* TFIIA consist of three subunits of 37 kD (α subunit), 19 kD (β subunit), and 13 kD (γ subunit). The α and β subunits are the products of a single gene, although is not clear whether they are produced by protein processing or are products of proteolysis during preparation of transcription extracts. Yeast TFIIA contains only two subunits, encoded by the TOA1 and TOA2 genes. The 32-kD TOA1 gene product is homologous to the human α subunit at its amino terminus and the human β subunit at its carboxyl terminus (Fig. 4A), whereas the 13-kD TOA2 product is homologous to the human γ subunit. Both the TOA1 and TOA2 genes are essential for yeast viability (Ranish et al. 1992).

The role of TFIIA in transcription is controversial

The precise role of TFIIA in transcription initiation has been the subject of much controversy, because the requirement for TFIIA in reconstituted transcription varies from system to system. Generally, reactions reconstituted with recombinant TBP do not require TFIIA (Cortes et al. 1992), while reactions with purified TFIID are stimulated by TFIIA (Ozer et al. 1994; Sun et al. 1994; Yokomori et al. 1994; Ma et al. 1996). The degree of requirement for TFIIA can often be correlated with the purity of the reconstituted transcription factors, with purer systems being less dependent on TFIIA. Much of this variability can be attributed to the ability of TFIIA to relieve the repressive effects of certain factors [including Dr1 (Inostroza et al. 1992), topoisomerase I (Merino et al. 1993; Ma et al. 1996), and MOT1 (Auble et al. 1994)] that may be present in cruder systems, a process known as antirepression (see below). However, the ability of TFIIA to stimulate transcription in reactions reconstituted with TFIID and essentially homogeneous preparations of the remaining GTFs implies a more fundamental role for TFIIA (Sun et al. 1994; Yokomori et al. 1994). The requirement for TFIIA in activated transcription is less controversial: In vitro transcription reactions generally require TFIIA to respond to activators.

TFIIA can neutralize repressors of transcription

An important function for TFIIA may be to relieve the effects of certain repressor molecules that inhibit transcription. Although the precise mechanism of antirepression by TFIIA is unknown, two distinct and possibly complementary mechanisms can be envisaged. First, by binding to TBP, TFIIA can displace repressors associated with TBP that prevent TBP from participating in other protein–protein interactions required to build a functional preinitiation complex. Consistent with this mechanism, TFIIA can bind to TPB in the absence of DNA (Ranish and Hahn 1991; Cortes et al. 1992; Coulombe et al. 1992) and can displace the repressor topoisomerase I from TBP (Merino et al. 1993; Ma et al. 1996). TFIIA also blocks the action of the MOT1 repressor, which targets

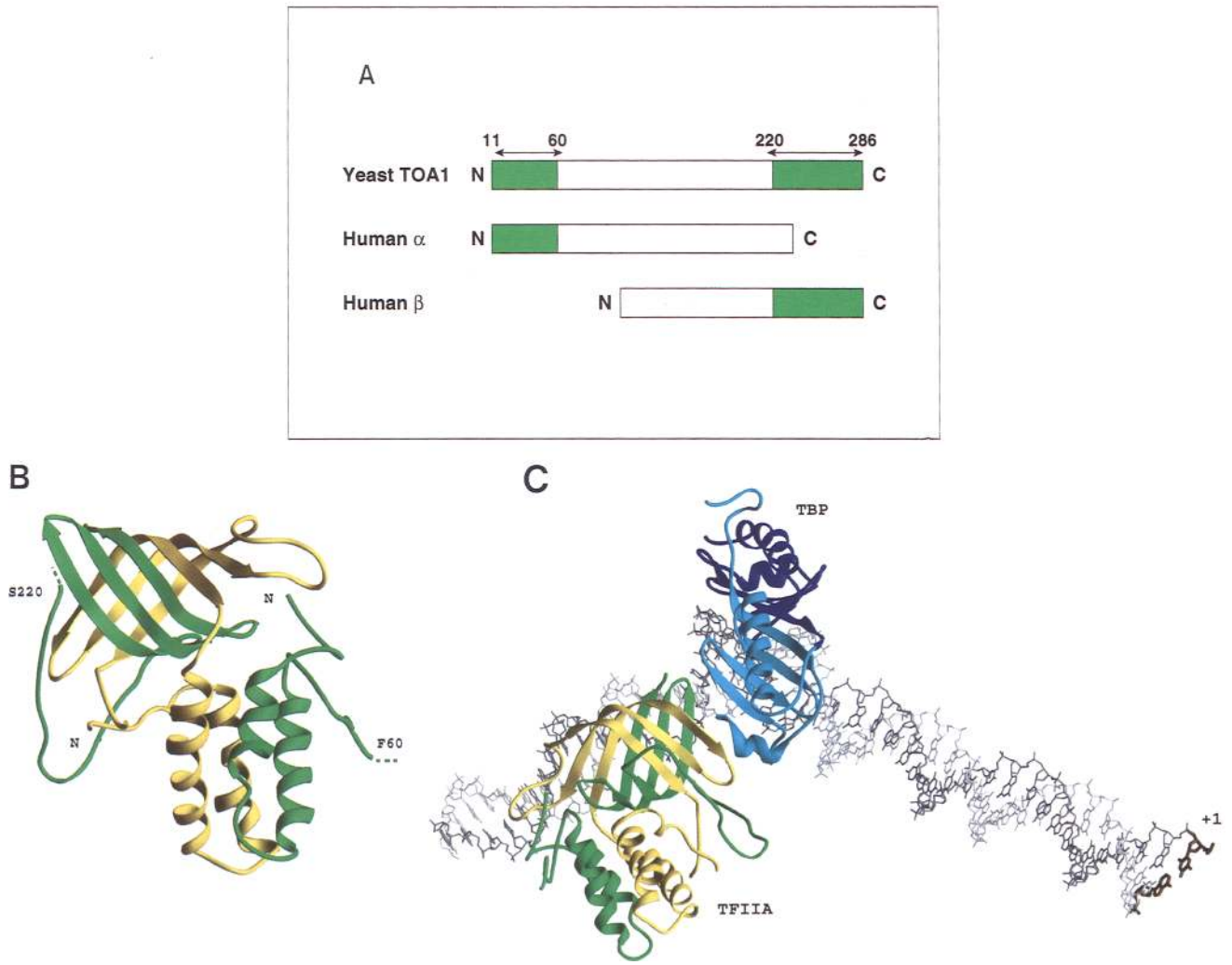

Figure 4. (*A*) Comparison of the TOA1 subunit of yeast TFIIA with the α and β subunits of human TFIIA. Conserved regions are green. Arrows indicate regions of TOA1 present in the models shown in *B* and *C*. (*B*) Ribbon drawing of the structure of yeast TFIIA. The TOA1 subunit is green and the TOA2 subunit is yellow. Regions of TOA1 included in the model span from residues 11–60 and 220–286 (see text). (*C*) Structure of the TBP–TFIIA–TATA element complex. Proteins are shown in the ribbon representation; DNA is drawn as an atomic stick model with the transcription start site indicated by +1. The amino- and carboxy-terminal direct repeats of TBP are light blue and dark blue, respectively. TFIIA binds to the amino-terminal repeat of TBP and contacts DNA upstream of the TATA element. Coordinates provided by D. Nikolov, S. Burley, J. Geiger, and P. Sigler; figures by K. Das and E. Arnold.

TBP (Auble et al. 1994). Second, by increasing the affinity of TBP for a promoter, TFIIA can enhance the ability of TBP to compete with nonspecific DNA-binding proteins bound at the promoter. In support of this assertion, TFIIA is required for TBP to bind a TATA element present in a nucleosome (Imbalzano et al. 1994a). The antirepression and activation functions of human TFIIA recently have been separated (Ma et al. 1996). A subcomplex of the β and γ subunits of human TFIIA is able to function in antirepression, while the full α–β–γ assembly is required for activation (Table 1).

The structure of the TBP–TFIIA–TATA element complex

Our molecular view of the preinitiation complex has been expanded recently by the laboratories of Sigler and Richmond, who have reported independent structures of the TBP–TFIIA–DNA complex (Geiger et al. 1996; Tan et al. 1996). Both groups used a yeast TFIIA variant containing the phylogenetically conserved amino and carboxyl termini of the large subunit TOA1 (homologous to the amino terminus of human α subunit and the carboxyl terminus of human β subunit respectively; see Fig. 4A) and the complete TOA2 small subunit (homologous to human γ subunit). The region of TOA1 used is the minimal sequence that can complement deletion of the TOA1 gene (Kang et al. 1995).

The structures from the two groups are very similar. TFIIA is two-domain protein that resembles an "L" in shape (Fig. 4B). The TOA1 and TOA2 polypeptides interact extensively along their lengths. The carboxyl terminus of TOA1 and the carboxyl terminus of TOA2 form a

compact β-sheet structure termed a β sandwich, or β barrel: Three antiparallel β strands from each polypeptide clasp and partially encircle each other to give the domain a hydrophobic core. The amino termini of TOA1 and TOA2 form a four-helix bundle that forms the other arm of the L.

TFIIA binds to the TBP–TATA element complex such that it interacts with the amino-terminal region of core TBP and with DNA upstream of the TATA element (Fig. 4C). The β-barrel domain of TFIIA is responsible for interactions with both TBP and DNA. TFIIA–TBP interactions are mainly between the edge of TOA2's β sheet and a β strand at the amino terminus of TBP's stirrup. This interaction essentially joins the β sheets of TBP and TFIIA, resulting in an extended β sheet reaching upstream of the TATA element.

TBP mutagenesis experiments suggest an additional TBP–TFIIA interaction surface not seen in the crystal structures. Mutations in the H2 helix on the upper surface of TBP abrogate the binding of human TFIIA (Lee et al. 1992; Buratowski and Zhou 1992; Tang et al. 1996). It is possible that a region of TFIIA not present in the crystallographic models contacts TBP. Indeed, such an unstructured region exists in the TOA1 subunit at the amino terminus of residues 220 and is a good candidate to interact with the TBP H2 helix.

As well as interacting with TBP, the β barrel of TFIIA also contacts the phosphate backbone of DNA within and upstream of the TATA element. Protein–DNA contacts extend to 3 bp upstream of the TATA element and are mediated by basic residues of TOA1. This is one aspect where the Sigler and Richmond structures differ. In the Richmond structure, three additional basic residues of TOA1 interact with the phosphate backbone of DNA. The same amino acids do not contact DNA in the Sigler structure, although they are perfectly positioned to do so.

What are the implications of these new structures for transcription initiation? The ability of TFIIA to interact simultaneously with TBP and DNA provides a simple explanation of how TFIIA stabilizes the TBP–DNA complex. TFIIA interacts with DNA phosphates that span the major groove. It has been suggested that these interactions are sensitive to the width of the major groove between the TFIIA contact points (Tan et al. 1996). Thus, the DNA sequence immediately upstream of the TATA element may influence the affinity with which TFIIA binds the TBP–DNA complex and may modulate the intrinsic strength of a promoter. The structures also help to explain why TFIIA binds upstream, and not downstream, of TBP. The interactions between TFIIA and TBP are stereospecific: TFIIA cannot make equivalent interactions with the symmetry-related carboxy-terminal region of the TBP saddle. Only a very small region of TFIIA interacts with TBP and DNA. This leaves a large surface area on TFIIA available to interact with other factors. The α-helical domain of TFIIA is particularly conspicuous in the ternary complex and is well placed to interact with other proteins, including transcription activators (see below).

The crystallographic model is compatible with the observation that the β and γ subunits of human TFIIA can function in antirepression, whereas the α subunit is additionally required for activation (Ma et al. 1996): The regions of yeast TFIIA homologous to the human β and γ subunits may be able to fold into the β-barrel structure that binds TBP and DNA. Activators, however, would interact with the four-helix bundle domain of TFIIA that includes the protein region homologous to the α subunit of TFIIA.

With the crystal structures of the TBP–TFIIB–DNA and TBP–TFIIA–DNA complexes in hand, one can model the TBP–TFIIA–TFIIB–DNA assembly (Fig. 5). The model shown here was constructed with the assumption that neither protein or DNA change in conformation between the ternary complexes and the quaternary complex. However, the results of recent site-specific protein–DNA photo-cross-linking experiments suggest a subtle change in conformation between the TBP–TFIIB–DNA and TBP–TFIIA–TFIIB–DNA complexes (Lagrange et al. 1996). In the model shown in Figure 5, TFIIB interacts with DNA both upstream and downstream of the TATA element, while TFIIA interacts with only the upstream DNA segment. Although TBP interacts with TFIIA and TFIIB, its upper surface is entirely free to interact with other polypeptides, such as the TAF_{II}s. As each polypeptide is added to the structural framework of the TPB–TATA element complex a general theme begins to emerge. Each additional factor uses up only a small portion of the available protein interaction surface while providing a much larger surface area for incoming polypeptides. It is likely that additional polypeptides will soon be added to this complex.

TFIIF escorts RNA polymerase II to the promoter and plays multiple roles in transcription

Once TFIID and TFIIB have assembled at the promoter, pol II can enter the preinitiation complex (for review on pol II, see Young 1991). However, pol II cannot stably associate with this GTF subassembly, and must be escorted to the promoter by TFIIF (forming the DBpolF complex; see Fig. 1). The subunits of TFIIF were first identified through their ability to interact with immobilized pol II (Sopta et al. 1985). Soon after, TFIIF was purified independently as an essential pol II initiation factor (Flores et al. 1988, 1989, 1990). In addition to its role in initiation, TFIIF performs additional duties that increase the specificity and efficiency of pol II transcription. By stably associating with pol II, TFIIF can increase the rate of transcription elongation (Flores et al. 1989; Price et al. 1989; Bengal et al. 1991; Izban and Luse 1992; Bradsher et al. 1993; Kephart et al. 1994) and, in this respect, resembles the general elongation factor SIII (see below; Aso et al. 1995b). In addition, TFIIF prevents spurious initiation by inhibiting, and reversing, the binding of polymerase to nonpromoter sites on DNA, drawing comparisons with the bacterial σ factors (Conaway and Conaway 1990; Killeen and Greenblatt 1992).

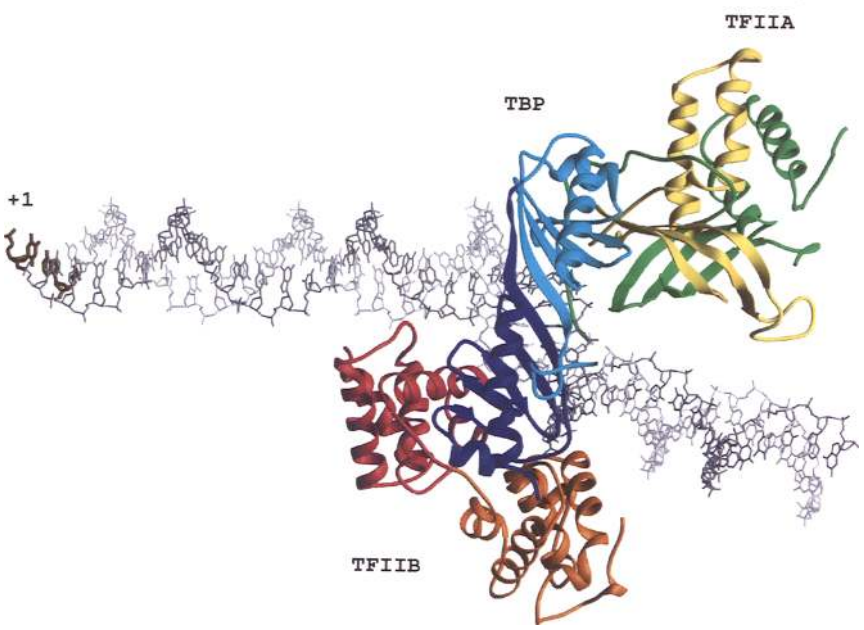

Figure 5. Model for the structure of the TBP–TFIIB–TFIIA–TATA element complex (top view). Proteins are shown in the ribbon representation; DNA is drawn as an atomic stick model with the transcription start site indicated by +1. The amino- and carboxy-terminal direct repeats of TBP are light blue and dark blue, respectively. The amino- and carboxy-terminal domains of TFIIBc are red and orange, respectively. The TOA1 and TOA2 subunits of (yeast) TFIIA are green and orange, respectively. Coordinates provided by D. Nikolov, S. Burley, J. Geiger, and P. Sigler; figure by K. Das and E. Arnold.

TFIIF is structurally and functionally related to bacterial σ factors

cDNAs encoding TFIIF subunits have been isolated from human (Sopta et al. 1989; 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). Human TFIIF is a heterotetrameric factor consisting of 26–kD (RAP30) and 58-kD (RAP74) subunits. Primary sequence analysis revealed that TFIIF is indeed structurally related to bacterial σ factors: The RAP30 subunit contains two distinct regions with sequence similarity to *Escherichia coli* σ factors (Sopta et al. 1989; Garrett et al. 1992). Moreover, one of the σ-homology regions of RAP30 is thought to interact with pol II, and TFIIF binds to the same surface on *E. coli* RNA polymerase as *E. coli* σ^{70} (McCracken and Greenblatt 1991). Some of the functions of TFIIF can be accomplished by RAP30 alone. RAP30 can deliver pol II to the promoter to support transcription initiation in the absence of RAP74. This is consistent with the ability of RAP30 to interact with TFIIB (Ha et al. 1993), pol II (McCracken and Greenblatt 1991), and DNA (Tan et al. 1994). It should be noted that on some promoters TFIIF is dispensable for transcription initiation, suggesting that pol II has weak affinity for the TFIID–TFIIB-promoter complex (Parvin and Sharp 1993; Parvin et al. 1994; Usheva and Shenk 1994). In agreement with this observation, under certain conditions pol II can bind the TBP–TFIIB complex in the absence of TFIIF (Buratowski et al. 1991; Tang et al. 1996).

As with the majority of the GTFs, the functional domains of RAP30 have been investigated extensively using mutagenesis strategies (Aso et al. 1992; Garrett et al. 1992; Yonaha et al. 1993; Kephart et al. 1994; Tan et al. 1994, 1995; Frank et al. 1995; Wang and Burton 1995). The most significant finding of these experiments was the discovery of a cryptic DNA-binding domain at the carboxyl terminus of the polypeptide (Tan et al. 1994). The fact that a cryptic DNA-binding domain also exists in the *E. coli* σ^{70} protein reinforces the functional link between bacterial σ factors and RAP30 (Dombroski et al. 1992). Strikingly, the DNA-binding domain of RAP30 is homologous to the domain of bacterial σ factors that recognizes the -35 region of bacterial promoters. It is likely that a RAP30–DNA interaction enhances the stability of the preinitiation complex. This idea is consistent with the photoaffinity cross-linking of RAP30 to sequences between the TATA element and the transcription start site (Coulombe et al. 1994).

The larger subunit of TFIIF, RAP74, is highly charged such that the 58-kD protein migrates as a 74-kD protein on SDS–PAGE gels. Although RAP30 can carry out many of the functions of TFIIF alone, the full complement of TFIIF activities requires the presence of RAP74. Most strikingly, RAP74 is required for stimulation of the rate of pol II elongation (Bengal et al. 1991; Chang et al. 1993; Kephart et al. 1994). In addition, the RAP30–RAP74 complex can remove pol II from nonspecific DNA sites, whereas RAP30 alone can only prevent it from binding to these sites (Conaway and Conaway 1990; Killeen and Greenblatt 1992). Intriguingly, RAP74 has been implicated recently in start site selection: A mutation in the yeast TFIIB gene (*SUA7*) that causes aberrant start site selection is suppressed by mutations in the gene encoding the yeast RAP74 homolog, restoring use of the normal start site (Sun and Hampsey 1995). A role for RAP74 in positioning the start site of transcription is consistent with its cross-linking to promoter sequences between the TATA element and the start site (Robert et al. 1996) and its ability to interact with TFIIB (Fang and Burton 1996).

Deletion mutagenesis experiments recently have re-

vealed a cryptic pol II-binding domain in RAP74 that may play a role during transcription elongation (Wang and Burton 1995). The discovery of a cryptic pol II-binding domain in RAP74 and a cryptic DNA-binding domain in RAP30 suggests that TFIIF undergoes a change in conformation, possibly during the transition between initiation and elongation.

RAP74 is heavily phosphorylated in vivo, in accordance with the presence of several potential phosphorylation sites in its primary sequence (Aso et al. 1992; Finkelstein et al. 1992). The kinase, or kinases, responsible for this phosphorylation in vivo have not been identified. However, recent studies suggest that the TFIID subunit $TAF_{II}250$ specifically phosphorylates RAP74 on serine residues (Dikstein et al. 1996). The precise role of RAP74 phosphorylation has not been established, although in vitro data suggest that both the initiation and elongation activities of TFIIF are stimulated by phosphorylation (Kitajima et al. 1994). It will be interesting to determine whether the phosphorylation state of RAP74 changes during the transcription cycle and whether it is subject to regulation.

TFIIE and TFIIH complete the preinitiation complex and kick-start transcription

As we have described, the delivery of pol II to the promoter by TFIIF creates the DBpolF intermediate. Even though pol II is stably incorporated, it is unable to initiate RNA synthesis and requires the presence of two additional GTFs, TFIIE and TFIIH. TFIIE exists as a heterotetramer of 34-kD and 56-kD subunits (Ohkuma et al. 1990; Inostroza et al. 1991). The 56-kD subunit contains a sequence with the potential to form a zinc-binding domain and a stretch of amino acids sharing homology with the catalytic loop of a kinase domain (M.G. Peterson et al. 1991). However, neither purified or recombinant TFIIE possesses kinase activity. TFIIE can enter the preinitiation complex after pol II and TFIIF. However, because TFIIE binds to pol II in solution with high affinity, it is likely that it joins the preinitiation complex concomitant with pol II and TFIIF. Once TFIIE is stably incorporated, it recruits TFIIH to the promoter (Flores et al. 1990). TFIIH is a complex, multisubunit assembly containing kinase and DNA helicase activity (see Table 1; for review, see Drapkin and Reinberg 1994). Cloning of cDNAs encoding TFIIH subunits has recently implicated TFIIH in DNA repair and cell cycle regulation (see below). The addition of TFIIE and TFIIH to the preinitiation complex completes the assembly process and renders the polymerase competent to initiate transcription (Fig. 1). As we discuss next, both factors play an active role in the initial stages of RNA synthesis.

Initiation of transcription by pol II requires energy

Transcription initiation requires separation of the DNA strands to grant the polymerase access to nucleotides of the template strand. This process is known as "promoter melting" and is followed by formation of the first phosphodiester bond of the RNA transcript (see Fig. 1). Extension of the RNA transcript results in disruption of polymerase contacts with the initiation complex—"promoter clearance"—and entry into the "elongation" phase of transcription in which the nascent RNA is extended as the polymerase embarks on its journey along the template. For pol II, the transition from initiation to elongation is accompanied by covalent modification of an unusual structure at the carboxyl terminus of its largest subunit. This evolutionarily conserved structure consists of multiple tandem repeats of the heptapeptide Tyr–Ser–Pro–Thr–Ser–Pro–Ser and has been named the RNA pol II CTD (carboxy-terminal domain) (for review, see Dahmus 1994). The number of times this sequence is repeated varies from 26 in yeast to 52 in human and seems to be directly related to genome complexity. Since five of the seven residues of the repeat can be phosphorylated, it is not surprising that phosphorylation of the CTD is central to the transcription mechanism of pol II. During initiation of RNA synthesis, the CTD becomes extensively phosphorylated on serine and threonine residues. The unphosphorylated form of pol II (pol IIA form) is the form recruited to the initiation complex (Laybourn and Dahmus 1989; Lu et al. 1991), whereas the elongating polymerase is found with a phosphorylated CTD (pol IIO form) (Bartholomew et al. 1986; Layburn and Dahmus 1989; Dahmus 1994; O'Brien et al. 1994; Zawel et al. 1995).

Unlike other eukaryotic RNA polymerases, initiation of transcription by pol II requires hydrolysis of the β–γ phosphoanhydride bond of ATP (Bunick et al. 1982; Sawadogo and Roeder 1984). The development of highly purified reconstituted transcription systems that stringently reproduce this requirement generated renewed interest in tracing the source of ATP utilization. Aside from the newly discovered kinase activity of $TAF_{II}250$ (Dikstein et al. 1996), TFIIH is the only GTF with ATP-requiring activities (see Table 1). TFIIH contains two ATP-dependent helicases (ERCC2 and ERCC3; Schaeffer et al. 1993, 1994; Drapkin et al. 1994a) and a protein kinase specific for the pol II CTD (Feaver et al. 1991; Lu et al. 1992; Serizawa et al. 1992). Hence, by a process of elimination, TFIIH is considered the most likely source of ATP dependence in transcription initiation. But where does the requirement lie, in its helicase activity or its CTD kinase? The CTD-kinase activity of TFIIH can be ruled out as a source of ATP dependence for two reasons. First, the kinase activity can accommodate GTP as a phosphate donor (Lu et al. 1992; Serizawa et al. 1992), but, even in the presence of GTP, initiation requires hydrolysis of the β–γ phosphoanhydride bond of ATP. Second, under conditions in which the TFIIH kinase is completely dispensable for transcription, ATP is still required (Serizawa et al. 1993; Mäkelä et al. 1995).

These arguments lead to the conclusion that one of the two helicases of TFIIH must play a crucial role in initiation and must, therefore, be the source of ATP dependence. Studies in yeast have shown that of the two helicases—ERCC2 and ERCC3—only ERCC3 helicase ac-

tivity is essential for transcription (Sung et al. 1988; Park et al. 1992; Feaver et al. 1993; Guzder et al. 1994; van Vuuren et al. 1994). Therefore, the ERCC3 helicase is widely believed to be the energy-requiring component of pol II transcription. But what could be the role of such a helicase in transcription initiation? Helicases unwind DNA to facilitate biological processes that require the DNA strands to be separated. Thus, the promoter melting stage is most likely to involve a helicase.

Are TFIIE and TFIIH required for promoter melting or promoter clearance?

The idea that a TFIIH helicase that functions during promoter melting is the source of ATP dependence was fueled by the studies of Parvin and Sharp, who found that TFIIE, TFIIH, and ATP were dispensable when transcription was performed using templates with high negative-superhelical density (Parvin and Sharp 1993). The free energy of negative supercoiling favors DNA unwinding and thus promoter melting and, therefore, was inferred to overcome the energetic requirement for strand separation at the promoter, obviating the requirement for TFIIH. These conclusions were supported by the work of Timmers, who, in addition, reported that AMP–PMP—which lacks a hydrolyzable β–γ phosphoanhydride bond—can support transcription on supercoiled but not topologically relaxed templates (Timmers 1994).

Further insight into the role of TFIIH during initiation was provided by Goodrich and Tjian (1994b), who resourcefully used a procedure previously used in studies of prokaryotic transcription mechanisms. The technique used was abortive initiation, which takes advantage of the observation that in the absence of a complete set of nucleotides initiation complexes can cyclically produce short RNA products specific to the transcription start site (McClure et al. 1978; McClure 1980). Thus, abortive initiation can be used to restrict the polymerase to formation of the first phosphodiester bond. Goodrich and Tjian found that TFIIE, TFIIH, and ATP were not required for formation of a short RNA product, but were required for a subsequent step in the transcription process—either promoter clearance or elongation (Goodrich and Tjian 1994b). Because it is well established that pol II elongation does not require TFIIE or TFIIH, the authors concluded that these factors are required for promoter clearance.

The proposal that TFIIE and TFIIH do not participate in promoter melting or during the initiation of RNA synthesis, but are required for promoter clearance, has since been challenged by studies from a number of laboratories, which contend that the energy requirement and, by extrapolation, TFIIH are required for promoter melting. Promoter melting can be detected by the ability of thymidines in single-stranded DNA regions to react chemically with permanganate. Gralla and colleagues used this technique to show that formation of the open complex requires hydrolysis of the β–γ bond of ATP (Wang et al. 1992). Moreover, in contradiction with the results of Goodrich and Tjian, the synthesis of short abortive transcripts has been reported to require hydrolyzable ATP (Dvir et al. 1996; Holstege et al. 1996). TFIIE and TFIIH were more directly linked to promoter melting by experiments in which the promoter was artificially premelted by creation of a short region of mismatched heteroduplex DNA at the start site. The use of these templates overcame the requirement for TFIIE, TFIIH, and hydrolyzable ATP (Pan and Greenblatt 1994; Tantin and Carey 1994; Holstege et al. 1995). A premelted region of 6 bp encompassing the transcription start site was minimally required to counteract the need for TFIIE and TFIIH (Holstege et al. 1996). On the basis of these and similar experiments, Timmers and coworkers have proposed an alternative to the Goodrich and Tjian model (Holstege et al. 1996). In the Timmers model, promoter opening occurs in two distinguishable steps: (1) restricted opening occurs in the presence of ATP and the complete set of GTFs (the DBpolFEH complex), and (2) formation of the first phosphodiester bond leads to extension of the melted region downstream of the start site.

What is the cause of this crucial discrepancy? Is TFIIH required for promoter melting or promoter clearance? It is possible that abortive initiation does not require full promoter melting and, therefore, does not faithfully recreate initial events in the synthesis of a long transcript. Consistent with the observation of Goodrich and Tjian (1994b) that supercoiling overcomes the requirement for TFIIH during promoter clearance, full promoter melting may be required at some stage after the synthesis of the initial phosphodiester bond. This would be consistent with the observation that promoter melting cannot be detected under conditions of dinucleotide priming (Holstege et al. 1996). It is possible that variability in specific assay conditions contributes to this discrepancy. In addition, nonspecific DNA-binding proteins might inhibit synthesis of short aborted products that are produced in the absence of TFIIH, and perhaps discrepancies regarding the requirement for TFIIH and ATP in abortive synthesis (see above) are a result of the presence of contaminating nonspecific DNA-binding proteins in some systems.

A direct role for TFIIE in promoter melting?

It has come to light recently that TFIIE may play a role in promoter melting in the absence of TFIIH. Although transcription from supercoiled templates does not require TFIIE or TFIIH, it is stimulated to various degrees by TFIIE alone (Goodrich and Tjian 1994b; Timmers 1994). In a systematic investigation of this phenomenon, a requirement for TFIIE could be created if reaction conditions were modified such that the DNA helix was stabilized and thus made more resistant to melting. Conversely, conditions that destabilized the DNA helix led to a decrease in TFIIE dependence (Holstege et al. 1995). Moreover, promoter-specific variations in the degree of TFIIE stimulation were found to depend on sequences spanning the transcription start site. A role for TFIIE in promoter melting is not surprising. The 56-kD subunit of TFIIE contains an essential zinc-ribbon motif similar

to those of TFIIB and the elongation factor TFIIS (M.G. Peterson et al. 1991; Maxon and Tjian 1994). The zinc ribbon of TFIIS is able to bind both single-stranded and double-stranded DNA (Qian et al. 1993). Moreover, the region of TFIIB containing its zinc ribbon has been implicated in positioning the start site of transcription and structural data predict that it lies in the vicinity of the melted region (Nikolov et al. 1995). Thus, it is tempting to speculate that in the preinitiation complex the zinc ribbons of TFIIB and TFIIE cooperate to stabilize the melted region of the promoter. The cross-linking of a TFIIE subunit between the TATA element and the transcription start site is consistent with this proposal (Robert et al. 1996).

At this stage, it is pertinent to ask why pol II initiation requires the input of energy in the form of ATP hydrolysis. Eukaryotic RNA polymerases I and III do not require ATP hydrolysis or helicase activity, so why should pol II? Furthermore, it is well documented that pol II can initiate transcription, and presumably melt DNA, when commencing transcription from DNA nicks or DNA ends. Therefore, the energy requirement for pol II transcription remains a mystery, leaving its significance open for speculation. Perhaps the energy requirement stems from an unusual conformation of promoter DNA that is unique to the pol II preinitiation complex. For instance, the conformation of DNA wrapped onto multiple protein surfaces—including those of TFIID (or TBP), TFIIB, TFIIA, and presumably other GTFs—may create an energetic barrier to promoter melting that requires the input of energy in the form of a helicase.

Phosphorylation of the pol II CTD

The CTD of pol II is heavily phosphorylated during the transition from initiation to elongation. Although it has been more than 10 years since the discovery of the CTD, we still do not completely understand the role of CTD phosphorylation in transcription. However, it is clear that the CTD is essential for cell viability (Nonet et al. 1987; Allison et al. 1988; Zehring et al. 1988) and that its partial deletion affects growth and the response of the transcription machinery to regulatory proteins (Nonet et al. 1987; Bartolomei et al. 1988; Allison and Ingles 1989; Scafe et al. 1990; C.L. Peterson et al. 1991). The fact that many reconstituted transcription systems do not require the CTD for transcription has contributed to our slow progress in unveiling the role of CTD phosphorylation. Indeed, the requirement for the CTD seems to vary between core promoters: The adenovirus major late promoter, for example, can be transcribed in the absence of a CTD, whereas the murine DHFR promoter is dependent on an intact CTD (Thompson et al. 1989; Buermeyer et al. 1992; Kang and Dahmus 1993; Akoulitchev et al. 1995). Transcription in the absence of a CTD can be correlated with the presence of a TATA element: Buermeyer et al. (1995) found that addition of a consensus TATA element to the CTD-dependent dihydrofolate reductase (DHFR) promoter abolished the requirement for an intact CTD. CTD dependence has also been shown to vary with the purity of the transcription system: Pol II lacking the CTD was active in a purified system, but inactive in a crude yeast extract (Li and Kornberg 1994).

Which kinases phosphorylate the CTD?

Many kinases are capable of phosphorylating the CTD in vitro (for review, see Dahmus 1994) without necessarily carrying out this role in vivo. It is not surprising that the CTD is a substrate for a multitude of kinases, because nearly half of its amino acids can be phosphorylated. The search for a physiological CTD kinase is complicated by the inevitable indirect consequences of disabling a particular kinase in vivo. Because CTD phosphorylation occurs early during the transcription cycle (initiation or promoter clearance), one would predict that the kinase responsible would be an integral part of the preinitiation complex. Thus, it was satisfying to discover that TFIIH purified from a variety of sources contained a kinase specific for the CTD (Feaver et al. 1991; Lu et al. 1992; Serizawa et al. 1992). Moreover, the observation that the TFIIH CTD kinase is stimulated by TFIIE provides a means by which its activity can be regulated in the preinitiation complex (Lu et al. 1992; Ohkuma and Roeder 1994).

The TFIIH kinase is cdk7

An intensive search led to the discovery that the TFIIH kinase activity resides within cdk7 (also known as MO15; Feaver et al. 1994; Roy et al. 1994; Serizawa et al. 1995; Shiekhattar et al. 1995), a kinase implicated in regulating progression through the cell cycle. In mammalian cells, cdk7, together with its cyclin partner cyclin H and an activating protein, MAT-1 (see below), comprise a factor known as cdk-activating kinase (CAK). This CAK complex is thought to play a pivotal role in cell-cycle regulation. Fundamental transitions during the cell cycle are coordinated by the actions of the cyclin-dependent kinases (cdks), whose activities are tightly regulated (for review, see Sherr 1993; Morgan 1995). Activation of at least three cdks (ckd2, cdk4, and cdc2) requires two events: binding of a cyclin partner and phosphorylation on a conserved threonine residue. The activating phosphorylation event is catalyzed by CAK, whose active component in mammalian cells is thought to be cdk7.

Is the cdk7 kinase activity required for transcription? Mäkelä et al. (1995) demonstrated that cdk7 was solely responsible for the CTD kinase activity of TFIIH, but found that its function was dispensable for transcription from the adenovirus major late promoter. However, the development of a highly pure in vitro transcription system using the TATA-less murine DHFR promoter allowed Akoulitchev et al. (1995) to demonstrate that the cdk7 kinase is required for transcription under conditions in which its substrate, the CTD, is required. Moreover, in this study both the CTD and cdk7 were required for productive transcription, whereas cdk7 was dispensable for synthesis of the first phosphodiester bond of the

RNA transcript. This suggests that cdk7 kinase is required at a later stage of transcription, perhaps promoter clearance.

The finding that CAK is an essential component of TFIIH links the initiation of pol II transcription with regulation of the cell cycle. This raises two important questions: (1) Is the phosphorylation of the CTD regulated during the cell cycle? and (2) Is TFIIH involved in cell-cycle progression? Presently, insufficient experimental evidence precludes a definitive answer to these questions. However, our current knowledge allows us to speculate on these points. TFIIH kinase activity does not vary during the cell cycle (Adamczewski et al. 1996). This lack of regulation may be due to MAT-1, a recently discovered component of mammalian CAK that is also present in TFIIH (Devault et al. 1995; Fisher et al. 1995; Drapkin et al. 1996). MAT-1 is a ring-finger protein that promotes the association of cdk7 with cyclin H. The presence of MAT-1 in CAK makes cdk7 unique in not requiring threonine phosphorylation for activation, making it constitutively active. The answer to the second question is more controversial. It is not clear whether TFIIH, through its CAK component, functions to regulate cell-cycle events. Part of this uncertainty stems from the finding that the TFIIH kinase and its regulatory subunits are not as tightly associated as other subunits of TFIIH and can be separated from other TFIIH subunits by chromatography (Shiekhattar et al. 1995; Svejstrup et al. 1995; Drapkin et al. 1996; Reardon et al. 1996). In fact, biochemical fractionation revealed that only 20% of the total cellular CAK is associated with TFIIH (Drapkin et al. 1996). Additional evidence against a role for TFIIH in cell cycle regulation comes from studies with the *S. cerevisiae* TFIIH kinase KIN28. Although KIN28 is the subunit of *S. cerevisiae* TFIIH that phosphorylates the CTD and is required for transcription in vivo, it does not possess CAK activity in vitro (Feaver et al. 1994; Cismowski et al. 1995; Valay et al. 1995). Moreover, biochemical and genetic evidence suggest that the *S. cerevisiae* CAK activity resides in a single polypeptide of ~42 kD termed Cak1p, or Civ1, which is not a component of TFIIH (Kaldis et al. 1996; Thuret et al. 1996). Therefore, whether TFIIH contains the physiological CAK activity remains an issue of controversy.

The subunit composition of TFIIH reveals a role in DNA repair

The complexity surrounding the biological function of TFIIH took another twist in 1993 when it was discovered that its 89-kD subunit was the DNA repair protein ERCC3 (Schaeffer et al. 1993). The ERCC3 protein (encoded by the XPB gene) is a DNA helicase that plays an essential role in the process of nucleotide excision repair (NER; Weeda et al. 1990). The link between TFIIH and NER does not stop at ERCC3; several other TFIIH subunits function in DNA repair. These include the other TFIIH helicase, ERCC2 (encoded by the XPD gene), the 62-kD subunit; the 44-kD subunit (SSL1); and, potentially, the 34-kD subunit (Drapkin et al. 1994a; Humbert et al. 1994; Schaeffer et al. 1994; Wang et al. 1995; see Table 1). Thus, five of the nine subunits of TFIIH for which cDNAs have been isolated have dual functions in transcription and DNA excision repair. It is well established that transcription and DNA repair are mechanistically coupled processes: Lesions in transcribed genes are repaired faster than those in nontranscribed genes (Mellon and Hanawalt 1989). It is likely that TFIIH plays a central role in the mechanism of coupling. It was initially imagined that TFIIH travels with pol II during elongation. However, recent studies demonstrate that this is not the case (Zawel et al. 1995). Because of space limitations, we shall not discuss further the issue of transcription-coupled DNA repair in this review. The reader is directed to reviews by Drapkin et al. (1994b), Hanawalt (1994), Friedberg (1996), and Sancar (1996).

RNA pol II holoenzymes: a challenge to the stepwise assembly model

In the model we have described above for the assembly of a preinitiation complex, the GTFs exist separately in solution and come together only upon association with promoter DNA. This model has been challenged recently by the discovery that a subset of the GTFs exist in a preassembled form in an RNA pol II "holoenzyme"—suggesting that the majority of the initiation machinery can bind to a promoter in a single step (Fig. 1). It should not come as a surprise that GTFs and pol II can associate away from the promoter: Numerous GTF–GTF and GTF–pol II interactions have been reported (see above). Nevertheless, a combination of genetic and biochemical experiments with the yeast *S. cerevisiae* not only uncovered the existence of RNA pol II holoenzymes, but also demonstrated their biological significance. The series of experiments leading to this discovery began when Richard Young and coworkers examined the effects of truncating the CTD of the largest pol II subunit on cell growth and viability. Removing all but 11 of the 26 heptapeptide repeats of the CTD produced colonies that were unable to grow at low temperatures (Nonet et al. 1987). The cold-sensitivity phenotype of these cells allowed Young to identify suppressing mutations in other genes that could overcome this defect (Thompson et al. 1993; Hengartner et al. 1995; Liao et al. 1995). The genetic tour-de-force that ensued led to the discovery of nine *srb* genes (for suppressor of RNA polymerase B) whose products were exclusively present in a large multiprotein complex that also contained the subunits of RNA pol II and the GTFs TFIIB, TFIIF, and TFIIH—the RNA pol II holoenzyme (Koleske and Young 1994; for review, see Koleske and Young 1995).

Concurrently, a yeast RNA pol II holoenzyme was identified in the laboratory of Roger Kornberg by use of a completely different approach. Kornberg used a biochemical approach to search for protein factors that would allow a purified yeast transcription system to respond to acidic activators. This led to the purification of a multisubunit complex—termed "mediator"—that was found to be associated with pol II and TFIIF (Y.-J. Kim et

al. 1994). It soon became apparent that the mediator complex purified by Kornberg and colleagues was similar to the SRB complex of Young. The mediator complex could be displaced from pol II using antibodies directed against the CTD. Thus, genetics and biochemistry had converged on the same complex. Furthermore, the link between the mediator/SRB complex and the CTD had been demonstrated both genetically, by virtue of suppressor mutations, and physically, by antibody displacement. Although the Young and Kornberg holoenzymes share functional similarities (the ability to respond to activators), and polypeptides (pol II, SRB/mediator subunits, and TFIIF), they differ significantly in the subset of GTFs that they contain: The Kornberg complex lacks TFIIB and TFIIH.

It is unfortunate that the primary structures of most of the SRB proteins reveal little about their function (exceptions are SRB10 and SRB11; see below). The sequences of the majority of SRB genes have no similarity to genes identified previously. However, it is clear that SRB proteins are essential for pol II transcription. In cells with temperature-sensitive *srb4* or *srb6* genes, transcription of many genes is shut down upon transfer to the nonpermissive temperature (Thompson and Young 1995). In this case, should the SRBs join the list of GTFs? Here lies a problem of terminology. The GTFs were purified and named as transcription factors that are essential for unregulated transcription in vitro. The SRBs, however, are not required for unregulated transcription in vitro, and, thus, we suggest that they should not be termed GTFs.

Pol II holoenzymes contain molecules that confer response to transcription regulators

A crucial property of the pol II holoenzymes is their ability to respond to transcriptional activators. This activity is thought to be conferred by the mediator/SRB complex, which contains molecules—known as coactivators—that physically link activators to the transcription machinery (for review, see Guarente 1995). In support of this model, the mediator/SRB complex interacts with the acidic activation domain of the viral activator VP16 (Hengartner et al. 1995). One coactivator present in the pol II holoenzyme is the product of the GAL11 gene (Y.-J. Kim et al. 1994) implicated in the activation of a variety of yeast genes (Suzuki et al. 1988; Fassler and Winston 1989). Another, albeit controversial, pol II holoenzyme coactivator is the SUG1 protein. Mutations in the SUG1 gene were isolated as suppressors of mutations in the activation domain of the yeast activator GAL4. Kim and colleagues have reported that the SUG1 gene product is a component of the yeast mediator/SRB complex (Y.-J. Kim et al. 1994). However, recently Rubin et al. (1996) reported that the SUG1 protein is a component of the 26S proteasome, suggesting that its association with the yeast pol II holoenzyme is an artifact.

A role for the mediator/SRB complex in regulating transcription is supported by the finding that genes involved in glucose repression in *S. cerevisiae* encode SRB proteins (Kuchin et al. 1995; Song et al. 1996). In addition, two proteins implicated in the repression of many genes—SIN4 and RGR1—were identified as components of a yeast pol II holoenzyme (Li et al. 1995). Thus, the mediator/SRB complex can be considered a transcriptional control panel, integrating and responding to signals from both activator and repressor proteins.

Mammalian pol II holoenzymes

The level of conservation between the transcription systems of yeast and higher eukaryotes and the fact that the sequence of the pol II CTD is highly invariant suggest that similar pol II complexes exist in mammals. The first tentative indication that such a complex exists in mammals came from the Schibler laboratory. Schibler and colleagues used antibodies directed against the cdk7/MO15 subunit of TFIIH to immunoprecipitate pol II and the entire set of GTFs, except TFIIA, from rat liver extracts (Ossipow et al. 1995). Not surprisingly, the immunoprecipitates could support pol II transcription in vitro. This was taken as evidence for a large pol II holoenzyme in mammals. However, a number of uncertainties cast doubt on this conclusion: (1) It was not determined whether the immunoprecipitated material represented a single holoenzyme complex or many TFIIH–GTF complexes [TFIIH can independently bind many of the GTFs (Gerard et al. 1991)]; (2) the complex could not respond to transcription activators; and (3) it was not demonstrated that the complex contained mammalian homologs of yeast SRB proteins.

Demonstrations of the existence of mammalian holoenzymes homologous to the yeast complex came from the laboratories of Young and Reinberg, who purified large pol II complexes from calf thymus and HeLa cells, respectively. The calf thymus complex contains the GTFs TFIIE and TFIIH and cannot respond to activators (Chao et al. 1996), whereas the HeLa cell complex contains TFIIF, TFIIE, and a substoichiometric amount of TFIIH, and its transcription is stimulated by activators (Maldonado et al. 1996). More significantly, both complexes contain homologs of yeast SRB proteins: hSRB7 (Chao et al. 1996; Maldonado et al. 1996) and cyclin C/cdk8 (hSRB10/hSRB11, Maldonado et al. 1996). The SRB proteins are the hallmarks of the yeast pol II holoenzyme and distinguish the mammalian complexes as true holoenzymes.

New implications for the role of CTD phosphorylation

The existence of the mediator/SRB complex puts a different slant on the role of CTD phosphorylation in transcription initiation. Before the discovery of the mediator/SRB complex the CTD was thought to be a naked structure, whereas we know now that it is adorned with 20 or so polypeptides. The CTD is extensively phosphorylated during elongation. The available evidence suggests that the holoenzyme contains the unphosphorylated (pol IIA) form of pol II (Ossipow et al. 1995; E. Maldonado, pers. comm.). Thus, the pol II holoenzyme is

probably the form of pol II competent for initiation, and not the elongating form. It is likely that the extensive CTD phosphorylation that occurs during initiation severs contacts between the CTD and the mediator/SRB complex, leaving elongating pol II with a naked CTD. In light of this model, it is relevant to note that TBP and TFIIE also interact with the unphosphorylated form of the CTD (Usheva et al. 1992; Maxon et al. 1994). It is possible that the phosphorylated CTD is a binding site for factors involved in postinitiation steps, such as elongation factors and RNA processing factors (Yuryev et al. 1996).

The identification of a kinase/cyclin pair in the SRB/mediator complex adds yet more complexity to this scenario. The *srb10* and *srb11* genes encode kinase and cyclin partners with homology to mammalian cdk8/cyclinC genes, respectively (Liao et al. 1995). *Srb10* mutations drastically reduce CTD phosphorylation in vivo and the yeast kinase can phosphorylate the CTD in vitro (Liao et al. 1995). Thus, the two identified CTD kinases present in the preinitiation complex are both cdks—cdk7/MO15 and cdk8. Significantly, this suggests that the events that regulate transcription initiation are similar to those that regulate the cell cycle. Alternatively, the assumption that these cdks play a role in cell-cycle regulation may be incorrect: These cdks may function solely in transcription.

Does pol II transcription occur in a "transcriptosome"?

To understand the sheer size of these pol II holoenzyme complexes requires only simple arithmetic addition. As if the 12 subunits of pol II, the 20 or so mediator/SRB polypeptides, and a number of GTF subunits (depending on the holoenzyme in question) were not enough, the holoenzymes harbor yet more proteins. A controversial component of the *S. cerevisiae* holoenzyme is the SWI/SNF complex, controversial because it is present in the Young holoenzyme (Wilson et al. 1996), but is not part of the complex described by Kornberg (Y.-J. Kim et al. 1994). The SWI/SNF complex is a multisubunit assembly whose function is thought to be to remodel chromatin to facilitate transcription activation (Peterson 1996). Adding further complexity, the holoenzyme purified from HeLa cells includes polypeptides implicated in DNA repair processes (Maldonado et al. 1996). Thus, the image that emerges is one of a ribosome-sized complex, with >70 polypeptides, containing the entire set of factors necessary for efficient transcription initiation in response to activators. Clearly, such a complex would be severely compromised in its ability to diffuse freely. It has been suggested that transcription initiation by this complex may occur in complex transcription factories, or "transcriptosomes", anchored to distinct nuclear locations (Halle and Meisterernst 1996). It is striking that a component of a mammalian pol II holoenzyme—YY1 (Maldonado et al. 1996)—is identical to the nuclear matrix-associated factor NMP-1 (Guo et al. 1995). Thus, YY1 may tether the mammalian RNA polymerase II holoenzyme to the nuclear membrane.

The GTFs and the regulation of transcription

We shall end this review with a brief description of the role of the pol II GTFs in the regulation of transcription. Before we begin, we must define some common terms. Under normal conditions, the minimal protein apparatus required for accurate transcription initiation consists of the GTFs and the subunits of pol II. This transcription is not dependent on the presence of regulatory molecules and is known as "basal" transcription. Transcription in a cell, however, is a tightly regulated process. DNA packaged into chromatin results in general gene repression. Transcription must be triggered by activator proteins that bind to specific DNA sequences and induce the expression of a gene or set of genes. This is known as "activated" transcription. Thus, basal transcription does not occur in vivo, and is purely an operational term defined by in vitro studies.

The fact that genes packaged into chromatin are in a repressed state provides a convenient means of regulation. Moreover, chromatin is not the only repressor of transcription; a host of other general and gene-specific repressor proteins operate in eukaryotic cells (for review, see Hanna-Rose and Hansen 1996). Thus, the level of transcription of a gene can be increased by large magnitudes simply by derepression. Once activators achieve this derepressed state, they can act on the basal transcription machinery to increase the efficiency of the transcription process. We do not mean to imply that these two processes are separate. As we discuss below, a single interaction between a DNA-bound activator and a component of the initiation machinery is sufficient both for derepression and the achievement of elevated transcription.

In theory, activator proteins can stimulate various steps along the pathway to the production of a molecule of mRNA (Fig. 6). These include (1) removal of repressor molecules from promoter DNA, (2) recruitment of GTFs and pol II to a promoter, (3) induction of conformation changes in the preinitiation complex, (4) induction of covalent modification of proteins in the preinitiation complex, and (5) stimulation of promoter clearance and elongation. As we describe next, many of these steps involve direct interactions between activators and GTFs.

(1) Removal of repressor molecules from promoter DNA Experiments performed primarily in vitro have demonstrated that the GTFs and pol II cannot efficiently bind promoters packaged into nucleosomes (for review, see Paranjape et al. 1994). In this case, promoter binding requires the assistance of factors that remodel nucleosomes and make promoter sequences more accessible. Nucleosome remodeling requires ATP and is thought to involve multisubunit complexes (for review, see Kingston et al. 1996). One candidate for this activity is the SWI/SNF complex, which has been implicated in chromatin remodeling by genetic studies (for review, see Peterson 1996). However, it is not clear how the SWI/SNF complex can be recruited to specific promoters. The recent report that a yeast pol II holoenzyme contains

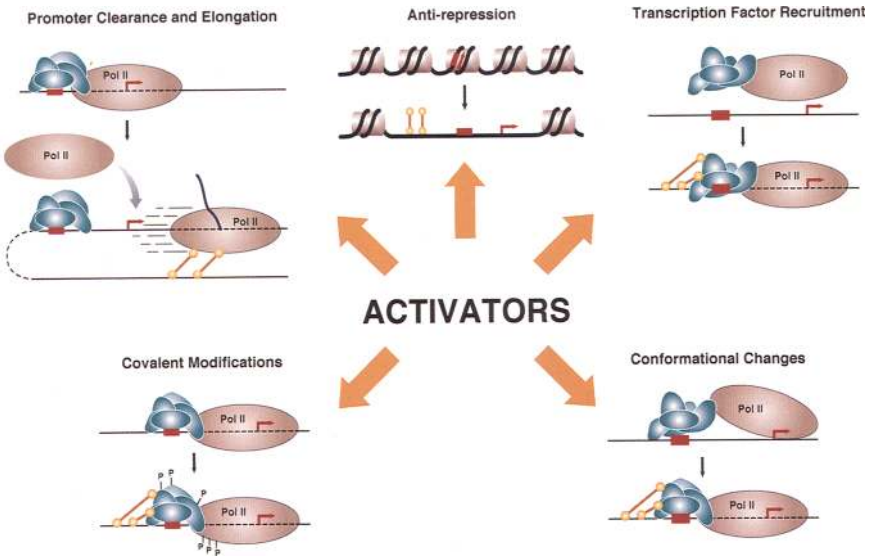

Figure 6. Activators can function during multiple stages of transcription (see text for details).

stoichiometric amounts of the SWI/SNF complex provides a convenient answer (Wilson et al. 1996). Also, the SWI/SNF complex apparently possesses weak, but significant, sequence/structure-specific DNA-binding activity, suggesting that it may assist in targeting the pol II holoenzyme to specific promoters (Quinn et al. 1996). By interacting with components of the SRB/mediator complex, activators could recruit pol II, the GTFs, and the SWI/SNF complex in a single step. It is unlikely, however, that the SWI/SNF complex functions universally to remodel nucleosomes because, in yeast, it is required for the induction of only a subset of genes. Another candidate remodeling activity is the complex known as NURF (Nucleosome remodeling factor). NURF is a four-subunit complex that can remodel nucleosomes in an ATP- and activator-dependent manner (Tsukiyama and Wu 1995; Tsukiyama et al. 1995).

(2) Recruitment of GTFs and pol II to a promoter The recruitment of GTFs by activator proteins is the best-documented phenomenon on this list of activator functions. A staggering number of GTF-activator interactions have been reported. Because of space limitations, we shall not discuss specific interactions here. The reader is directed to reviews by Triezenberg (1995), Zawel and Reinberg (1995), and Ranish and Hahn (1996). For most of these interactions, no correlation between interaction and transcription activation has been made. Nevertheless, it is clear that the binding of GTFs to a promoter in vivo can be rate-limiting, as artificially tethering TBP to a promoter overcomes the requirement for an activator to generate elevated levels of transcription (Chatterjee and Struhl 1995; Klages and Strubin 1995; Xiao et al. 1995). The importance of GTF recruitment is heightened by the discovery of a pol II holoenzyme. Theoretically, a single activator–holoenzyme interaction can recruit the entire initiation machinery to a promoter. Ptashne and colleagues have shown that this is indeed the case: Creating an artificial interaction between a DNA-binding protein and a holoenzyme component is sufficient for transcription activation (Barberis et al. 1995). Therefore, it seems that tethering any single component of the initiation machinery to a promoter can overcome chromatin-mediated repression and is sufficient for activation.

In addition to recruiting GTFs to a promoter, activators may function to keep them at the promoter after initiation. Studies performed in vitro have determined the fate of the GTFs subsequent to pol II initiation (Zawel et al. 1995). TFIID is the only factor that remains bound at the promoter after initiation. TFIIB dissociates during the early stages of pol II transcription. TFIIE is next to leave, followed by TFIIH when pol II has transcribed past the +30 mark of a gene. TFIIF also dissociates from the promoter but, consistent with its role in elongation, is able to reassociate with pol II. By interacting directly with promoter-bound GTFs, in particular TFIIA and TFIIB, activators may prevent their dissociation to facilitate subsequent rounds of initiation. A recent report, however, questions the relevance of activator–GTF interactions that have been defined in vitro (Tansey and Herr 1995). TBP interacts with the activation domains of Gal4-VP16 and p53 in vitro. However, TBP mutations that disrupt these interactions in vitro have no effect on activation by these proteins in vivo. In the future, perhaps we should await corroborating in vivo data before drawing firm conclusions.

Transcription activation in vitro using human or *Drosophila* systems requires the TAF_{II}s of TFIID, whereas basal transcription is supported by TBP. The role of TAF_{II}s in activation in these systems has been examined in a multitude of in vitro experiments using both partial and complete TFIID assemblies. Certain activators interact with specific TAF_{II}s, and, in general, the ability of an activator to stimulate transcription de-

pends on the presence of the TAF_{II}(s) with which it interacts (for review, see Goodrich and Tjian 1994a; Goodrich et al. 1996; Burley and Roeder 1996).

Although the requirement for human and *Drosophila* TAF_{II}s for transcriptional activation in vitro is firmly established, the role of yeast TAF_{II}s in vivo is controversial. Recent studies by Struhl, Green, and colleagues provocatively suggest that activation of many yeast genes does not require TAF_{II}s (Apone et al. 1996; Moqtaderi et al. 1996; Walker et al. 1996). Using a novel copper-inducible double-shutoff technique and strains with temperature-sensitive TAF_{II} alleles, respectively, the Struhl and Green labs show that depleting cells of TAF_{II}s generally has no effect on the ability of a number of yeast activators to stimulate transcription. In contrast, cells depleted in any of several GTFs show diminished transcription.

If yeast TAF_{II}s are not required for activation of all genes, for which genes are they required? Although TAF_{II} depletion generally has no effect on activation in vivo, transcription from promoters bearing a suboptimal TATA element is abated (Moqtaderi et al. 1996). This is consistent with in vitro studies demonstrating that TAF_{II}s recognize promoter elements distinct from the TATA motif (Verrijzer et al. 1994, 1995; Burke and Kadonaga 1996), and suggests that TAF_{II}s stabilize the preinitiation complex on promoters with weak TATA elements. In addition, an unexpected role for TAF_{II}s was revealed by Green and coworkers (Apone et al. 1996; Walker et al. 1996). Cells devoid of any one of a number of TAF_{II}s exhibit cell-cycle arrest phenotypes, indicating that TAF_{II}s are required for cell-cycle progression. This suggests that TAF_{II}s are required for the proper regulation of genes whose products are directly required for events that regulate the cell cycle.

Can these surprising findings be extended to the TAF_{II}s of higher eukaryotes? The high degree of functional conservation between the transcription systems of yeast and higher eukaryotes suggests that the answer is yes. However, there are several crucial discrepancies between the biochemical properties of yeast TAF_{II}s and those of higher eukaryotes that imply a functional difference. While *Drosophila* and human TFIIDs were purified as complexes containing TAF_{II}s and TBP, yeast TFIID was purified as a single polypeptide of yeast TBP (Cavallini et al. 1989; Hahn et al. 1989; Horikoshi et al. 1989; Schmidt et al. 1989). Moreover, in vitro transcription activation using yeast-derived factors can be accomplished by TBP in the absence of TAF_{II}s (Kelleher et al. 1992; Koleske and Young 1994; Y.-J. Kim et al. 1994), whereas human or *Drosophila* in vitro systems are absolutely dependent on TAF_{II}s. Thus, it is possible that the more complex, multicellular, higher eukaryotes have evolved additional functions for their TAF_{II}s. Nevertheless, the Struhl and Green laboratories have provided arguments against a general role for yeast TAF_{II}s in transcription activation and have suggested specific roles. Without a doubt, these findings will provoke intense experimentation that should soon identify the relevant functions of the TAF_{II}s.

(3) Induction of conformational changes within the preinitiation complex Transcription activation is thought to entail conformational changes in the preinitiation complex prior to initiation of RNA synthesis. This belief is based on a number of observations. First, the presence of an activator can dramatically alter the DNase I footprint of TFIID on a promoter (for review, see Burley and Roeder 1996). Second, the hybrid activator Gal4-VP16 induces a conformational change in TFIIB that is thought to disrupt an interaction between the amino and carboxyl termini of TFIIB and stimulates the recruitment of TFIIF and pol II to the preinitiation complex (Roberts and Green 1994). In addition, Carey and colleagues have reported recently that an activator-induced conformational change in the TFIIA–TFIID complex is a rate-limiting step in activation (Chi and Carey 1996). Although activation domain-induced conformational changes may enhance the activation process, they are not a prerequisite for activation, because recruiting TBP or TFIIB to a promoter in the absence of an activation domain can stimulate transcription (see above).

(4) Induction of covalent modifications to polypeptides of the preinitiation complex The complete preinitiation complex contains at least three protein kinases. Two of these—cdk7/MO15 of TFIIH and cdk8 of the mediator/SRB complex—phosphorylate the CTD of pol II (see above). Phosphorylation of the CTD is required for the polymerase to enter an elongation mode. Therefore, activators could increase the rate of initiation by promoting CTD phosphorylation. This is conceivable, as activators have been shown to interact with both TFIIH (Xiao et al. 1994; Tong et al. 1995) and the mediator/SRB complex (Hengartner et al. 1995). The third kinase reported to be present in the preinitiation complex is $TAF_{II}250$, which is reportedly specific for the RAP74 subunit of TFIIF (Dikstein et al. 1996). It remains to be seen whether activators can stimulate this activity.

(5) Stimulation of promoter clearance and elongation Initiation is not the only stage of pol II transcription that is stimulated by activators. Postinitiation steps, such as promoter clearance and elongation, are also subject to regulation. Stimulation of promoter clearance—the stage at which pol II breaks its contacts with the preinitiation complex—not only increases the rate of RNA synthesis but, significantly, facilitates the entry of a second polymerase to the promoter (see Fig. 6). A growing body of data suggests that many activation domains can stimulate both initiation and elongation stages of transcription (see Yankulov et al. 1994 and references therein). Indeed, the ability of activators to augment multiple events in RNA synthesis is likely to be crucial for their potency. A family of protein factors that stimulate pol II elongation has emerged recently (for review, see Aso et al. 1995a). These factors act by stimulating the catalytic rate of RNA synthesis [such as TFIIF (see above) and SIII (Aso et al. 1995b)] or by facilitating the passage of pol II through various arrest sites [such as TFIIS (Sekimizu et al. 1976; Reinberg and Roeder 1987)]. Conceivably, activators

could stimulate elongation by recruiting these factors to pol II.

Summary and perspectives

The past decade has witnessed the identification, purification, and cloning of the factors that direct pol II to its promoters and help it to initiate transcription (the GTFs). The ability to reconstitute pol II transcription with essentially homogeneous preparations of these factors has permitted a mechanistic dissection of the role of each GTF in the transcription of protein-encoding genes. Structural studies have presented us with a glimpse into the molecular recognition events that build a preinitiation complex, revealing novel and unprecedented nucleoprotein architectures. Also, we have gained insight into the mechanisms by which regulatory proteins exploit the assembly process to regulate gene expression. While in vitro transcription experiments led to the identification of the pol II transcription machinery, the awesome power of yeast genetics recently has revealed the existence of novel polypeptides that play essential roles in regulating gene expression, a good example of which are the SRB proteins associated with the pol II holoenzyme. We should not, however, underplay the value of in vitro experiments. They have revealed much about the mechanism of transcription regulation. As technology evolves, these in vitro experiments are gradually becoming more complex and sophisticated and we should welcome the move toward more physiological conditions, such as the use of chromatin templates and cellular activators and promoters that reproduce more realistically the regulatory processes that occur in the cell. We can be confident that, at the present rate of progress, the subtle mechanisms that underlie the regulation of pol II transcription will soon reveal themselves.

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