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Multiple Functions of General Transcription Factors TFIIE and TFIIH in Transcription: Possible Points of Regulation by *Trans*-Acting Factors

Yoshiaki Ohkuma[1]

Division of Cellular Biology, Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565

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General transcription factors together with RNA polymerase II assemble on the promoter DNA and initiate transcription accurately in response to a variety of signals. Such signals enhance preinitiation complex formation by targeting components thereof via several alternative pathways. Two components of the initiation complexes, TFIIE and TFIIH, are known to function at both a late stage of transcription initiation and the following promoter clearance. TFIIH has been studied extensively because of its multiple enzymatic activities, functioning not only in transcription but also in nucleotide excision repair and cell cycle control. Fewer data have been reported for TFIIE, but its potential regulatory function as to TFIIH warrants further attention. In this review, an overall perspective of the functional roles of TFIIE and TFIIH during transcription initiation and the following promoter clearance will be presented as it has emerged from recent studies.

Key words: general transcription factors, promoter clearance, RNA polymerase II, TFIIE, TFIIH.

Productive transcription initiation by RNA polymerase II (Pol II) plays a key role in the regulation of gene expression in response to various developmental and environmental signals. Numerous extra- and intracellular signals are transduced through various pathways into the nucleus, targeting transcriptional regulators as well as chromatin modulators. Four potentially distinct steps can be affected by these signals through direct and/or indirect pathways; they are: (i) formation of a preinitiation complex on the core promoter; (ii) transition from an inactive preinitiation complex to an active (competent) initiation complex, accompanied by promoter melting; (iii) transcription initiation (defined as the first phosphodiester bond formation); and (iv) transition to a productive elongation complex (promoter clearance) (reviewed in Refs. *1-3*). These steps and the relevant factors can be targets for transcriptional regulators.

In eukaryotes, six general transcription factors (TFIIA, IIB, IID, IIE, IIF, and IIH) as well as Pol II carry out transcription initiation on the promoter DNA, which typically contains core promoter elements, including the transcription initiation site, that are essential for formation of the preinitiation complex, and gene-specific DNA elements that are recognized by the transcriptional regulatory factors and modulate transcription (reviewed in Refs. *3-5*). These regulatory factors may have distinct roles at each of the steps mentioned above. Until recently, almost all efforts have been directed toward the identification of both general transcription factors and various sequence-specific transcriptional regulators, and cloning of the corresponding cDNAs to further our understanding of the mechanism of preinitiation complex formation. In particular, general transcription factors which are involved in the initial events of complex formation, such as TFIID, its DNA-binding subunit, the TATA box-binding protein (TBP), TFIIA, and TFIIB, have been studied extensively, including crystallographic elucidation of their molecular structures (*6*). Thus, the research focus has recently slightly expanded to include the factors that are involved in the later steps of initiation: TFIIF, Pol II, TFIIE, and TFIIH. With improved assay techniques, the structures and functions of these factors have been studied in detail. Here, this review covers recent progress in and perspectives of the multiple mechanisms of transcriptional regulation by focusing primarily on the later steps of transcription initiation, especially on the roles of TFIIE and TFIIH in a possible checkpoint for entry to the elongation phase and for completion of transcription. For this purpose, some background to all other general transcription factors and the mechanism of preinitiation complex assembly is presented first.

Concerted assembly of general transcription factors on the core promoter

Recent experimental work pertaining to the preinitiation complex has indicated that there may be alternative pathways: one may involve the stepwise assembly of multiple general transcription factors and Pol II, and the other may utilize a preformed Pol II holoenzyme containing several general transcription factors. Biochemical studies initially involved the isolation of pure general transcription factors and thereby the reconstitution of the transcription machinery *in vitro*. A consequence of this approach was the description of multiple steps in preinitiation complex

[1] Phone: +81-6-879-7978, Fax: +81-6-877-9382, E-mail: ohkumay@imcb.osaka-u.ac.jp

assembly. The model promoter in such studies contains a TATA element (box) located at −30 to −25 positions upstream from the transcription initiation site and a pyrimidine-rich initiator (Inr) element located near the transcription start site. As shown in Fig. 1, the core promoters of all class II genes can be categorized into five different classes based on the core promoter elements present: (i) TATA$^+$ Inr$^+$, (ii) TATA$^+$ Inr$^-$, (iii) TATA$^-$ Inr$^+$, (iv) TATA$^-$ Inr$^+$ DPE (downstream promoter element located about 30 base pairs downstream from the Inr, first identified in a subset of *Drosophila* promoters)$^+$ (*7*), and (v) TATA$^-$ Inr$^-$. However, at present, the *in vitro* reconstitution of a transcription system with clearly defined, purified factors has been successful only with TATA box-containing promoters of the first and second classes described above. As studies concerned with the other promoter classes are still far from complete (*8, 9*), I will focus here on the TATA box-directed transcription mechanism.

The stepwise assembly pathway starts with the association of TBP or TFIID (a multisubunit general transcription factor consisting of TBP and TAF_{II}s, TBP Associated Factors for Pol II) with the TATA box, stabilized by TFIIA through direct contact with TBP and upstream bound factors. The assembly pathway continues with the inclusion of the basal transcription factor TFIIB, a complex of TFIIF and Pol II, TFIIE, and finally TFIIH (Fig. 2).

According to the alternative model, a preassembled Pol II holoenzyme binds to the core promoter DNA and initiates transcription following open complex formation. Although it is based on the results of molecular genetic studies and biochemistry in the budding yeast (reviewed in Refs. *10* and *11*), similar but distinct holoenzyme complexes have also been isolated from human cells (*12, 13*). Further biochemical and functional characterization is required, however, to resolve inconsistencies in their molecular composition and physiological relevance.

TATA box-binding protein

Preinitiation complex assembly starts with TBP binding to the TATA element through minor-groove contacts, assisted by TFIIA followed by TFIIB. High resolution X-ray structural studies of TBP (*6*), and recent X-ray and NMR studies of early intermediates in preinitiation complex assembly have complemented biochemical studies involving deletion and/or point mutants to characterize intermolecular interactions and relevant interaction surfaces (reviewed in Refs. *4*, *5*, and *14*). More recently, structural information-based fine analyses of individual amino acid residues and nucleotides, that are essential for protein-protein and/or protein-DNA contacts, have become available (*15, 16*). Taking these results into consideration, we can now envision the structure and functioning of early steps in the preinitiation assembly of TBP, TFIIA, and TFIIB with the core promoter element.

Human TBP is a 38 kDa single polypeptide that has a

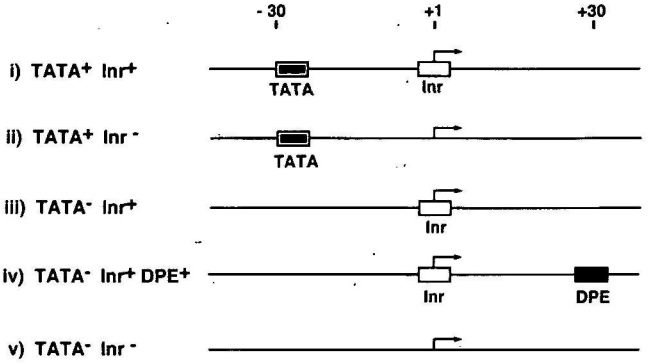

Fig. 1. **Representative core promoters for transcriptional initiation by RNA polymerase II.** There are five classes of functional core promoters known so far. (i) The core promoter with both TATA and initiator (Inr) elements. This is the strongest promoter for Pol II transcription, which efficiently binds TBP at the TATA element, and possibly TAF_{II}s at Inr. (ii) The core promoter with the TATA element alone. This is the second strongest promoter, which efficiently binds TFIID via TBP at TATA. (iii) The core promoter with the Inr element alone, which is known as the TATA-less promoter. TAF_{II}s or some other Inr-binding transcription factors regulate its transcriptional efficiency. (iv) The core promoter from *Drosophila* which was recently identified and possesses Inr and a downstream promoter element (DPE). DPE is located about 30 base pairs downstream from the transcription initiation site (*7*), and may bind to TAF_{II}s (not demonstrated yet). (v) The core promoter which does not possess either of these elements. This core promoter is usually regulated by sites located upstream and, sometimes, downstream of the transcription initiaiton site, and binds transcriptional activators.

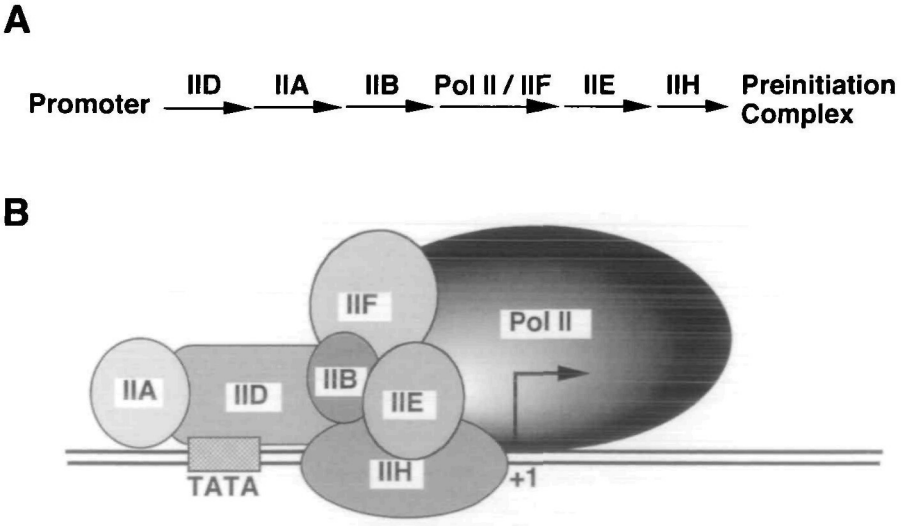

Fig. 2. **Stepwise assembly of a functional preinitiation complex on a TATA box-containing (TATA$^+$) promoter.** (A) The order of preinitiation complex assembly. At first, TFIID binds to the TATA box of the promoter. Then, the remaining general transcription factors assemble in an ordered fashion. (B) A schematic model of the functional preinitiation complex.

C-terminal core of 180 amino acids that is well conserved throughout eukaryotes, and an N-terminal, divergent sequence of 155 amino acids (Table I) (*17, 18*). The N-terminal region has several characteristic sequences, for example, a glutamine-run of more than 30 residues and an SPT domain (rich in serine, proline, and threonine). The C-terminal region is called the TBP core, that binds to the TATA element and consists of a symmetric structure of an approximately 90 amino acid repeat. As a "molecular saddle" structure, it recognizes the minor groove 6 bp of the TATA element and bends DNA with the underside of the saddle (*6, 19, 20*). Binding of TBP and subsequent DNA deformation may facilitate preinitiation complex assembly and activator-preinitiation complex interactions.

Transcription factor IID

As shown in Table I, human TFIID is a multisubunit complex consisting of TBP and 12 TAF_{II}s reported so far (reviewed in Refs. *14* and *21*). For transcriptional activation, a whole TFIID complex as well as the other general transcription factors are required. In other words, although TBP is sufficient for basal transcription from TATA-containing promoters, increased levels of transcription in response to activators require TAF_{II}s as well (reviewed in Refs. *4* and *22*). The difficulties in conducting structural and functional studies of TFIID as a whole have made it necessary to focus on binary interaction studies with individual TAF_{II} subunits, activators and DNA (*23*). Recently, however, three human TAF_{II}s, $hTAF_{II}20/15$, $hTAF_{II}31$, and $hTAF_{II}80$, were found to contain histone-fold motifs, homologous to those in H2B, H3, and H4 respectively (*24*, reviewed in Refs. *4* and *14*). *Drosophila* homologs of the latter two, $dTAF_{II}42$ and $dTAF_{II}62$, form a heterotetramer, as shown by crystallography (*14*). The former, present in two forms as $hTAF_{II}20$ and $hTAF_{II}15$, can form dimers (*25*) and interact with $hTAF_{II}80$ in a histone-like manner (*24*). Therefore, TFIID may contain a histone octamer-like structure consisting of a heterotetramer of $hTAF_{II}31$ and $hTAF_{II}80$ sandwiched between two $hTAF_{II}20/15$ dimers. In light of the results of an *in vitro* promoter DNA binding study with TFIID (*23*), these TAF_{II}s may play an important role in determining the DNA structure at the core promoter region and remodelling the chromatin structure in conjunction with various ATP-dependent macromolecular machineries or simply in response to the action of transcriptional activators.

The other intriguing finding pertains to the intrinsic enzymatic activities of $hTAF_{II}250$ and its *Drosophila* homolog, $dTAF_{II}250$ (*26, 27*). One is a histone acetyltransferase activity and the other a serine kinase activity. The former might be related to the role of TAF_{II}s with histone-fold motifs and/or to regulating the chromatin structure around the initiation region. The kinase, however, was found to target TFIIFα (also called RAP74, the large subunit of TFIIF) and, therefore, may function to affect preinitiation complex conformation during transcription initiation in concert with TFIIH, which is capable of phosphorylating TBP, TFIIEα, and TFIIFα (*28*). Possibly, acetylation may also be involved in this conformational change of the preinitiation complex. In addition, $hTAF_{II}$ 250, also known as CCG1, was found to play a role in cell cycle regulation especially at the G1 phase (*29-31*). In yeast, $TAF_{II}90$ was found to be required for cell cycle regulation through the G2/M phase (*32*). Although these observations provide evidence of a link between the cell cycle and transcription, its functional implications are currently unclear.

Transcription factor IIA as a coactivator

TFIIA stabilizes the TBP-DNA interaction through direct contact with both TBP and DNA bases upstream of the TATA element (reviewed in Refs. *3-5*). Human TFIIA consists of three subunits, of 35, 19, and 12 kDa, corresponding to the α, β, and γ subunits, respectively (Table I) (*33-35*). Recently, X-ray crystallography led to elucidation of the ternary structure of TFIIA with TBP bound to the TATA element. In the complex, TFIIA recognizes the N-terminal portion of the TBP core domain and interacts with DNA on the opposite surface to where TFIIB is in contact with DNA (*15, 16*). While TFIIA is not essential for basal transcription with TBP alone, stabilization of the TFIID-DNA complex by TFIIA might be important, especially for promoters with weak activity. One important function of TFIIA may be to remove certain transcriptional repressors from TBP or TFIID, and to mediate the effect of transcriptional activators (*36*). As such, TFIIA is not a general transcription factor but rather a transcriptional coactivator capable of binding to the preinitiation complex.

Transcription factor IIB

TFIIB recognizes the TBP-DNA and/or TBP-TFIIA-DNA complex and binds without effecting a conformational change as shown by an X-ray crystallographic study of the C-terminal TFIIB (cTFIIB)-TBP-DNA ternary complex

TABLE I. **General transcription initiation factors from human HeLa cells.**

Factor	Subunits (kDa)	Function
RNA Polymerase II	220, 150, 44, 32, 27, 23, 16, 14.5, 12.6, 12.5, 10, 10	Template-directed RNA synthesis
TFIIA	35, 19, 12	Stabilization of TFIID binding
TFIIB	35	Recruitment of Pol II-TFIIF; transcription initiation site selection
TFIID TBP	38	TATA binding; TFIIB recruitment
TAFs	250, 135, 95, 80, 55, 43, 31, 30, 28, 20, 18, 15	Transcriptional regulation; core promoter recognition
TFIIE	57, 34	TFIIH recruitment; regulation of TFIIH activities; open complex formation; promoter clearance; Pol II processivity
TFIIF (RAP 74, 30)	74, 30	Modulation of Pol II binding; suppression of Pol II pausing at transcription elongation
TFIIH	89, 80, 62, 52, 44, 40, 37, 34, 32	Open complex formation; promoter clearance; Pol II processivity; DNA repair; cell cycle regulation?

(reviewed in Refs. *4* and *5*). Human TFIIB is a 35 kDa single polypeptide (Table I). TFIIB interacts with TBP and DNA bases both upstream and downstream of the TATA element (*15, 16*). cTFIIB consists of two repeats that undergo nonequivalent interactions with the TBP-DNA complex (*37, 38*). This interaction mode might be important in determining the directionality of transcription. The N-terminal TFIIB contains a zinc-ribbon structure similar to the structure present at the C-terminus of general transcription elongation factor SII (also called TFIIS), that binds to both single-stranded and double-stranded DNA (*39*). Functionally, TFIIB has been shown to define the transcription initiation site, and to promote recruitment of Pol II and TFIIF to the TBP-DNA containing complex (*40, 41*).

Transcription factor IIF preassembled with RNA polymerase II

The general transcription factor, TFIIF, has unique roles in both transcription initiation and elongation (reviewed in Ref. *42*). Human TFIIF consists of two subunits, α (RAP74, 74 kDa) and β (also called RAP30, 30 kDa), and forms an $\alpha_2\beta_2$ heterotetramer (Table I). At initiation, TFIIF preassembles with Pol II and is important for two roles; the first role is to escort Pol II to the TBP-TFIIB-DNA ternary complex (promoter targeting of Pol II), and the second is to prevent nonspecific binding of Pol II to DNA (*43, 44*). Photocrosslinking studies involving the adenovirus 2 major late (AdML) promoter have localized TFIIFα at positions -15 and -5, and TFIIFβ at position -19 (*45*). These are just downstream of the TFIIB-DNA contact sites, and are located between the TATA element and the transcription initiation site. Interestingly, genetic analysis in budding yeast demonstrated a functional interaction between TFIIB and TFIIFα that affects initiation-site selection because one TFIIFα mutant was isolated as a suppressor of a TFIIB mutant defective in initiation-site selection (*46*). In addition, TFIIFβ directly binds to Pol II and TFIIB and, thus, might support the TFIIB function in determining the transcription start site. It has been demonstrated that the hypophosphorylated form of Pol II (form IIA), that is not phosphorylated at the carboxy-terminal domain (CTD) of its largest subunit, is preferentially recruited to the preinitiation complex (*47*).

At elongation, TFIIF remains on Pol II even after all other general transcription (initiation) factors have left the preinitiation complex, and functions as a general elongation factor (*48*). There are two classes of general elongation factors known so far, one that suppresses transient Pol II pausing and increases the elongation rate, and another that prevents Pol II arrest at intrinsic arrest sites (reviewed in Ref. *49*). It has been demonstrated biochemically that these two classes are functionally complementary. TFIIF belongs to the former class. Although the mechanism of TFIIF in transcription elongation has not been clearly elucidated, structure-function analyses of TFIIFα and β subunits revealed that the functional domains required for elongation and for initiation are separable.

Transcription factor IIE

TFIIE recruits TFIIH to the intermediate preinitiation complex comprising TFIIF and Pol II via interactions with all general transcription factors as well as Pol II, that stabilize and, ultimately, activate it (reviewed in Refs. *3* and *4*). Like TFIIF, human TFIIE also consists of two subunits, α (57 kDa) and β (34 kDa), that form an $\alpha_2\beta_2$ heterotetramer (Table I) (*50*).

Human TFIIEα contains 439 amino acids, is highly acidic (pI 4.5), and possesses several putative structural motifs and characteristic sequences, as shown in Fig. 3 (*51, 52*). TFIIEα binds to TBP, TFIIEβ, and TFIIH tightly, and Pol II, TFIIFα and β weakly. Amino acid sequence comparison with the homologues from other species (*Xenopus, Drosophila, C. elegans*, and *Saccharomyces cerevisiae*) indicated that all putative motifs and most characteristic sequences are well conserved and, therefore, might be functionally important (Y. Ohkuma, unpublished data). Indeed, the N-terminal half of TFIIEα, which contains all structural motifs, is essential for basal transcription (*53*). A putative leucine zipper motif and a similar hydrophobic repeat domain, which are separated by a putative zinc finger motif, are important for binding to the TFIIEβ subunit. Deletions of the zinc finger motif render the molecule dominant negative, that abolishes transcription activity of wild type TFIIEα when added in excess (*53*). However, the N-terminal half is not sufficient for transcription, and an acidic region located near the C-terminus binds to TFIIH and is required for basal transcription. Related to this, TFIIEα is important for stimulation of the CTD-kinase activity of TFIIH by TFIIE in both the absence and presence of all other general transcription factors together with promoter DNA.

Human TFIIEβ contains 291 amino acids, is highly basic (pI 9.5), and possesses several putative motifs and characteristic sequences different from those in TFIIEα (Fig. 3) (*52, 54*). Amino acid sequence comparison with the homo-

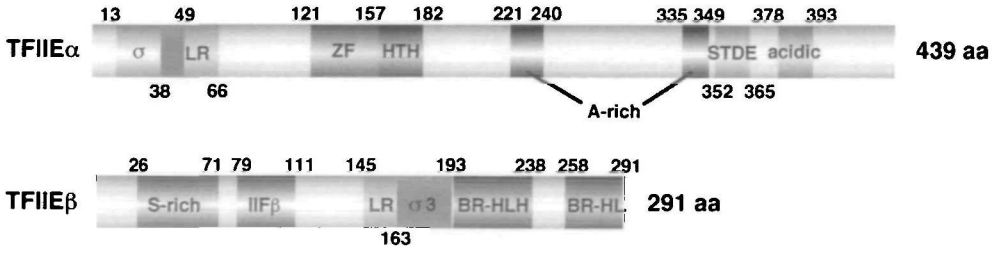

Fig. 3. **Structural features of TFIIEα and TFIIEβ.** The human TFIIEα subunit possesses motifs and sequences represented as follows: σ, similarity to bacterial sigma factors (subregions 2.1-2.2 and 4.1-4.2); LR, leucine repeat; ZF, zinc finger; HTH, similarity to the helix-turn-helix putative DNA-binding motif of the largest subunit of Pol II; A-rich, alanine-rich region; STDE, a region consisting of serine, threonine, aspartic acid and glutamic acid residues; acidic, acidic region (*51*). Human TFIIEβ possesses different kinds of motifs and sequences, except for a leucine repeat, as follows: S-rich, serine-rich region; IIFβ, similarity to the core Pol II-binding region of TFIIFβ (RAP30); LR, leucine repeat; σ3, similarity to subdomain 3 of bacterial sigma factors; BR-HLH, basic region-helix-loop-helix; BR-HL, basic region-helix-loop (*54*).

logues from other species indicated similar importance of the proposed domains to as in the case of TFIIEα (Y. Ohkuma, unpublished data). TFIIEβ binds strongly to Pol II, TFIIB, TFIIEα, TFIIFβ, and some of the TFIIH subunits. Recent structural characterization indicated that TFIIEβ binds to both TFIIB and TFIIFβ via a basic region near the C-terminus, which is located within the region essential for basal transcription. In summary, the two TFIIE subunits may have complementary roles in the recruitment of TFIIH and its functional regulation, as well as in the stabilization and activation of the preinitiation complex.

Transcription factor IIH

The last general transcription factor to join the preinitiation complex is TFIIH (reviewed in Ref. *55*). Human TFIIH consists of nine subunits with masses ranging from 32 to 89 kDa (Table I). Except for the hTAF$_{II}$250 subunit of TFIID, TFIIH is the only general transcription factor having various ATP-dependent catalytic activities that include CTD-kinase, DNA-dependent ATPase, and DNA-helicase activities. Two of these activities, the CTD-kinase and ATPase ones, were found to be positively regulated by TFIIE, while the DNA-helicase activity is negatively regulated by TFIIE (*28, 56*). Importantly, TFIIH contains subunits essential not only for transcription but also for nucleotide excision repair (NER) and cell cycle regulation (reviewed in Ref. *55*). Two of the large subunits, XPB (ERCC3) and XPD (ERCC2), have been demonstrated to possess ATPase and DNA-helicase activities, while CDK7 (MO15) possesses CTD-kinase activity as well as CAK (Cyclin-dependent kinase Activating Kinase) activity, that is important for cell cycle regulation. TFIIH itself has almost no Pol II binding activity and, therefore, TFIIE is important in recruiting and positioning TFIIH in the preinitiation complex in addition to regulating its activities (*28, 53*). The kinase activity of CDK7 is regulated by Cyclin H and MAT1, both of which are TFIIH subunits and components of CAK (reviewed in Ref. *55*). Interestingly, the specificity of the kinase changes depending on the

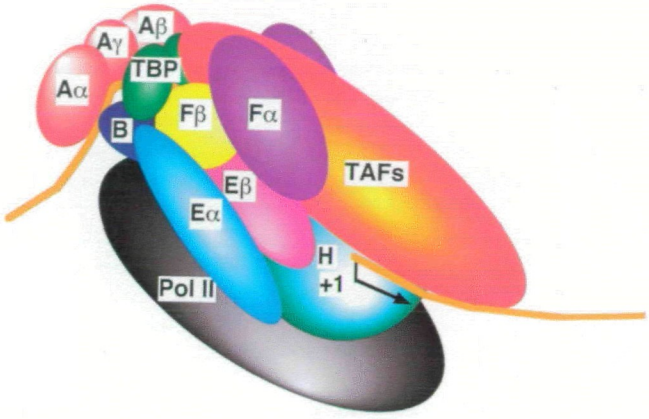

Fig. 4. **Schematic representation of a functional preinitiation complex on a core promoter.** The structure of the complex was derived from X-ray crystallographic data on TBP-TFIIA-DNA and TBP-TFIIB-DNA complexes, and from the predicted positions of TAF$_{II}$s, TFIIA, TFIIB, TFIIF, and TFIIE on both photocrosslinking and two-dimensional crystallography. TFIIE is located near the transcription start site and TFIIEα does not bind to DNA (*41, 45*). TFIIB and TFIIFβ (RAP30) bind to the C-terminus of TFIIEβ (Y. Ohkuma, unpublished observation).

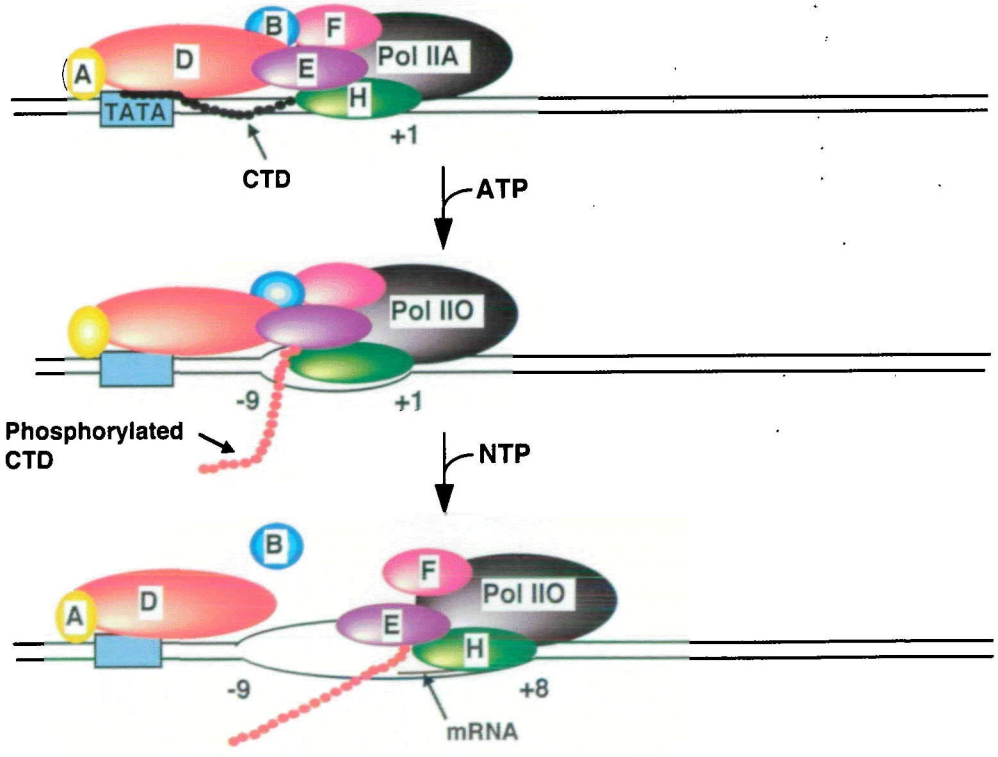

Fig. 5. **Two step model for transcription initiation.** DNA opening analysis involving a single strand-cutting reagent, $KMnO_4$, clearly demonstrated that there are two distinct opening steps (*59*). At the first step, before transcription initiation, the promoter DNA is opened in the preinitiation complex from -9 to $+1$ upon the addition of ATP. At the second step, DNA opening expands to $+8$ (from -9 to $+8$) depending on NTP addition. First phosphodiester bond formation (so called transcription initiation) starts at this second step. TFIIE and TFIIH might act at both steps. They stabilize and activate the preinitiation complex at the first step. CTD-phosphorylation of Pol II may occur at this step. Furthermore, they are assumed to function in open complex formation. At the second step, TFIIE and TFIIH function in promoter clearance to remove general transcription initation factors TFIID, TFIIA, and TFIIB from the Pol II-containing complex, and to assist the formation of an elongation complex with phosphorylated Pol II. The abbreviations are as follows: A, TFIIA; B, TFIIB; D, TFIID; E, TFIIE; F, TFIIF; H, TFIIH; Pol IIA, hypophosphorylated Pol II; and Pol IIO, phosphorylated Pol II.

composition of the complex; CTD-kinase activity is detected only for the whole TFIIH complex, and this activity phosphorylates TFIIEα, TBP, and TFIIFα as well (*28, 57, 58*). On the other hand, CAK activity is preferentially detected for a separate free form of the CAK complex.

Essential roles of TFIIE and TFIIH in transcription initiation

As shown in Fig. 2, TFIIE and TFIIH join the preinitiation complex last, completing preinitiation complex assembly and allowing activation of the complex through energy supplied by the hydrolysis of the β-γ phosphoanhydride bond of ATP (reviewed in Refs. *3* and *4*). RNA polymerases other than Pol II do not require ATP at transcription initiation. Preinitiation complex activation is called "open complex formation" and is accompanied by "promoter melting," in which the DNA double strands around the transcription initiation site are separated.

Photocrosslinking studies revealed that TFIIEβ binds between -14 and -2 of the core promoter, with TFIIF at almost the same position but on the opposite surface (between -19 and -5) (*45*). TFIIB and TFIIFβ bind upstream of -14, interacting with the C-terminal basic region of TFIIEβ. Photocrosslinking studies also indicated that TFIIEα does not bind to DNA directly (*45*). However, the direct interaction of TFIIEα with TFIIH allows TFIIE to stimulate the ATPase and CTD kinase activities of TFIIH within the preinitiation complex (*28*). Taken together, TFIIEα may act as a molecular chaperon for recruitment of TFIIH and two TFIIEβ subunits, which dock to the DNA in parallel, their C-termini facing upstream, properly positioning the TFIIE heterotetramer within the preinitiation complex.

Two-dimensional crystallography of yeast TFIIE with Pol II revealed that TFIIE actually interacts with the catalytic center of Pol II, that is located near the transcription initiation site (*41*). This conclusion agrees well with the results of photocrosslinking studies (*45*). Figure 4 shows a putative preinitiation complex drawn from the results of those studies. In addition, recent elegant studies demonstrated that short mismatched heteroduplex DNA around the initiation site in topologically relaxed templates abolishes the requirement for TFIIE, TFIIH, and ATP (*59*). In these studies, Timmers and colleagues found that premelting up to 6 bp (positions -4 to $+2$) of the AdML promoter was minimally sufficient to obviate the energy requirement, and that these premelted templates mimic the character of negatively supercoiled templates. Their results indicated a two step model for transcription initiation (Fig. 5). First, ATP-dependent promoter opening occurs upstream of the initiation site from -9 to $+1$. Second, depending on the presence of ribonucleoside triphosphates, formation of the first phosphodiester bond allows expansion of this region to $+8$ (-9 to $+8$). The late assembly of TFIIE and TFIIH into the preinitiation complex, and their role in the regulation of CTD-kinase activity are consistent with this model. Interestingly, the AdML promoter with the $-4/-1$ heteroduplex region does not show a TFIIH requirement but does partially require TFIIE. Taken together with results for other promoters that also show differential requirement for TFIIE and TFIIH (*60*), these data indicate the possibility of a TFIIH-independent role of TFIIE in transcription initiation. Precise studies defining such a role are still in progress.

Functional roles of TFIIE and TFIIH at transition from initiation to elongation

As described above, there is much evidence that strongly emphasizes the important roles of TFIIE and TFIIH in transcription initiation (*28, 56, 59*), although their requirement is alleviated by negative supercoiling of the template (*60-62*). On the other hand, TFIIE, TFIIH, and ATP are absolutely required for promoter clearance and efficient transition from transcription initiation to elongation, even on negatively supercoiled templates (*59*). At this step, many protein-protein and protein-DNA interactions that occur in the preinitiation complex are disrupted to disassemble the complex; instead, the elongation complex of Pol II with transcription elongation factors is newly formed. A promoter opening study demonstrated that the "open" region expands to $+8$ after the first phosphodiester bond formation (Fig. 5) (*59*). It is also noteworthy that TFIIE is released before position $+10$ from the transcription initiation site, and TFIIH, on the other hand, is released later between $+30$ and $+68$ (*48*). The transition from initiation to elongation occurs before the transcript reaches 10 nucleotides (*63*). Therefore, it is noteworthy that TFIIE is released at the transition step and TFIIH remains a little longer. These observations confirm the functional relevance of TFIIE and TFIIH at this step.

TFIIE and TFIIH function at transcription elongation?

The term "Pol II processivity" describes the potential of an RNA-polymerizing enzyme to complete transcription. It is currently not clear whether the general transcription factors, TFIIE and TFIIH, play direct roles in transcription elongation, but one idea is that these factors, in cooperation, enhance the processivity of Pol II by means of subunit modifications either before transcription initiation or on the transition from initiation to elongation.

Indeed, the phosphorylation of the C-terminal domain (CTD) of the largest subunit of Pol II distinguishes elongating Pol II from the hypophosphorylated form of the enzyme present at initiation (*64, 65*). Although the function of the phosphorylation has not been clarified mechanistically, there is evidence that the CTD is essential for both cell viability and transcription *in vivo*. TFIIH possesses CTD kinase activity and is the only such kinase present in the preinitiation complex, allowing it to phosphorylate the CTD at the time of transition from initiation to elongation. In addition, it has been demonstrated that the CTD kinase activity of TFIIH correlates with the basal transcription activity of this general transcription factor, and that TFIIE is essential for its regulation (*53*). Furthermore, TFIIH's involvement in transcription-coupled NER (described above) suggests that this general transcription factor may play an important role during transcription elongation, for example, to rescue stalled Pol II at damaged DNA sites. Multiple TFIIH interactions with many DNA repair factors have been reported (reviewed in Ref. *66*), that lend experimental support for such a model.

The function of TFIIE in NER, on the other hand, remains controversial (*67, 68*). However, TFIIE was found to interact with TFIIH, the NER factor, XPA, and the putative transcription-repair coupling factor, CSB (*53, 67,*

68). In addition, a specific antibody directed against TFIIEβ inhibits NER in a *Xenopus* oocyte nuclear extract (E. Ackermann, Y. Ohkuma *et al.*, unpublished data). These results may be interpreted as suggesting TFIIE involvement in the recruitment of NER factors to damaged DNA sites. In the case of transcription-coupled NER, TFIIE may in addition play a role in restarting Pol II transcriptional elongation. Indeed, it is currently unclear which factors are required to restart stalled Pol II. If the TFIIH kinase plays an important role in Pol II reactivation by phosphorylating the CTD, TFIIE may well be required as a regulator of that activity (*64, 65*). Although TFIIE and TFIIH have been shown to leave from Pol II within 10 bases after the transcription start site or between +30 to +68, respectively (*48*), these data do not rule out subsequent transient interactions with the stalled Pol II complex, during which TFIIE-mediated recruitment of TFIIH and regulation of its kinase activity leads to the rapid phosphorylation of the CTD and reactivation of a processive Pol II.

Transcriptional regulation through TFIIE and TFIIH?

TFIIE and TFIIH act both during transcription initiation and on the transition from initiation to elongation. These processes may involve the following steps that transcriptional regulators may be able to affect: before initiation, TFIIE binds to the general transcription factors, recruits TFIIH to the preinitiation complex and, ultimately, stabilizes and activates this complex. *In vitro* studies have demonstrated that TFIIE can stimulate TFIIH-derived ATPase and CTD-kinase activities even before the first phosphodiester bond formation (*28*). In addition, promoter opening is also induced by TFIIE and TFIIH when the energy state of the DNA topology is low (*e.g.* linear DNA) (*59*). Following open complex formation, transcription is initiated by activated, transcription competent Pol II with energy supplied through ATP-hydrolysis, presumably by the TFIIH-derived ATPase. At the next transition stage, both factors induce a conformational change in the transcription initiation complex and clear most of the general transcription factors from the Pol II-transcribing promoter, utilizing the energy from ATP hydrolysis. Then, as discussed above, TFIIE and TFIIH may regulate Pol II processivity (the ability to complete transcription) both directly and indirectly (*53, 58, 65, 70*).

There is an increasing number of regulatory factors that may target TFIIE and/or TFIIH to regulate one or several such steps. Activation may affect promoter opening either by stabilizing single stranded regions or by regulating the functions of TFIIE and/or TFIIH, which induce promoter opening. Alternatively, it may assist the functions of TFIIE and TFIIH later during promoter clearance. It has been demonstrated that transcriptional regulators, such as viral transcriptional activators VP16, Tat, EBNA2, and HBx, cellular tumor suppressor p53, putative transcription-DNA repair coupling factors CSA and CSB, and NER factor XPA, bind to TFIIH (*67-73*). Some of the same factors, for example EBNA2, CSB, and XPA, also bind to TFIIE (*67, 68, 74*). In addition, the *Drosophila* developmental gene product, Krüppel, the yeast transcriptional regulator, GAL11, the human GC-box binding transcription factor, BTEB2, and the proto-oncogene products, Fos and Jun, have also been reported to bind to TFIIE (*75-78*). Interestingly, not only transcriptional regulators but also putative transcription activation co-factors, such as the TFIID subunit $TAF_{II}80$, have been found to bind to TFIIEα (*79*). Thus, we may consider the possibility that TAFs transduce signals from activators not only to the factors involved in early preinitiation complex formation, as commonly assumed, but also to the factors affecting open complex formation and promoter clearance. Given the diversity of factors found to interact with TFIIH and TFIIE, the task ahead is elucidation of common mechanisms that involve these general transcription factors. Systematic deletion and point mutants may be important tools for an understanding of the functional relevance of these interactions.

Perspectives

In the last decade, a combination of biochemistry, molecular biology, genetics, and structural biology has made it possible to visualize —to a great extent— the mechanism of transcription from preinitiation complex assembly to initiation, and then to elongation. Remaining questions often concern the mechanism of transcriptional activation within the chromatin context. However, TFIIE and TFIIH are important in a number of transcriptional processes that remain poorly understood, for example, open complex formation, promoter clearance of general transcription factors, and Pol II processivity. As it has also become apparent that these factors may be involved in both NER and cell cycle regulation, we should develop appropriate experimental systems to answer the resulting question regarding functional coordination. Given the recent rapid progress, however, we might discover cues for these questions in the near future.

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