The role of general initiation factors in transcription by RNA polymerase II

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Transcription initiation on protein-encoding genes represents a major control point for gene expression in eukaryotes, and is mediated by RNA polymerase II and a surprisingly complex array of general initiation factors (TFIIB, TFIH, TFIIA, TFIIH) that are highly conserved from yeast to man. Elucidation of structural and functional features of these factors on model promoters has revealed insights into biochemical mechanisms and provides a basis for understanding their regulation on diverse promoters by gene- and cell-specific activators.

GENES TRANSCRIBED BY RNA polymerase II (Pol II) typically contain (1) common core-promoter elements that are recognized by general transcription initiation factors, and (2) gene-specific DNA elements that are recognized by regulatory factors, which in turn modulate the function of the general initiation factors. While natural constraints (such as limiting-factor concentrations, weak promoter-binding sites, chromatin structure, negative co-factors, etc.) restrict the function of general initiation factors in vitro, RNA Pol II and cognate general initiation factors alone have an intrinsic ability to effect low levels of accurate transcription from core promoters in vivo (defined as 'basal transcription'). This property has made it possible to study the fundamental aspects of transcription initiation mechanisms that are prerequisite for understanding the superimposed regulatory mechanisms.

Analogous to the situation in prokaryotes, eukaryotic transcription can be described in terms of pre-initiation complex (PIC) assembly, PIC activation (DNA melting), initiation, promoter clearance, elongation and termination steps. However, a major distinction is the remarkably greater complexity of the general transcription machinery in eukaryotes, an obvious consequence of which is a more diverse set of mechanistic steps and targets for regulatory factor interactions.

Core-promoter elements and general transcription initiation factors

Core-promoter elements are defined as 'minimal DNA elements that are necessary and sufficient for accurate transcription initiation by RNA Pol II in reconstituted cell-free systems'. The most common of these elements, which can function independently or synergistically, are the TATA box (consensus TATAA/TAT), located near position –30 to –25, and a pyrimidine-rich initiator (Inr, consensus YYAN/AYY) located near the transcription start site. Despite the complexity (12 evolutionarily conserved subunits) of RNA Pol II, it nonetheless requires additional factors for accurate transcription from even the strongest core promoters. This requirement was first demonstrated with purified RNA Pol II and a crude fraction from human cells in 1979 (Ref. 2), and subsequent fractionation studies led to the first demonstration of multiple RNA Pol II-specific factors in 1980 (Ref. 3). At present, there are six well-characterized general initiation factors that have now been isolated from human, rat, Drosophila and yeast: 

- TBP (TATA-binding polypeptide subunit of TFIIA, TFIB, TFIE, TFIIH and RNA Pol II. The ability of the small TBP subunit to substitute for the intact TFIIA complex in this reaction, with the concomitant elimination of any TFIAR requirement, has greatly simplified the analysis of core-promoter (basal) transcription mechanisms. This was also of historical importance, in that it allowed the initial identification and cloning of the gene encoding TBP from yeast (reviewed in Ref. 4), which in turn facilitated the direct cloning of TBP species from higher organisms and the examination of corresponding affinity reagents for the isolation and characterization of the other components from yeast to man. In relation to the fact that prokaryotic σ factors are directly responsible for promoter selection by prokaryotic RNA polymerases, it is worth noting that TFIIID is the only general initiation factor with components capable of sequence-specific binding to eukaryotic promoter DNA. These components include TBP, which directly recognizes the TATA element', and certain TBP-associated factors (TAFs), which have been implicated directly or indirectly in sequence-specific binding.

In the context of this review the general transcription initiation factors are defined as 'those factors that are necessary, in naturally occurring forms, for accurate transcription initiation from a broad group of core promoters - and not just the potent TATA- and INR-containing Adenovirus Major Late (AdML) promoter used for their initial characterization. Importantly, as described below, the specific factor or factor subunit requirements might vary somewhat, depending upon specific core-promoter sequences, template topology, the integrity of the general initiation factors (notably TFIIID) and the presence of TBP-binding negative co-factors. The broader group of general transcription factors (GTFs) includes those involved in initiation, elongation (reviewed in D. Reines et al., this issue) and termination.

TATA-directed initiation with minimal basal factors

The clearest picture of the fundamentals of PIC assembly, structure and function has come from studies of a minimal set of factors, or components thereof, that suffice for transcription from the consensus TATA element of the AdML promoter. These so-called 'basal' factors include, under normal conditions with relaxed DNA templates, the TBP (TATA-binding polypeptide) subunit of TFIIA, TFIIH and RNA Pol II. The ability of the small TBP subunit to substitute for the intact TFIIA complex in this reaction, with the concomitant elimination of any TFIAR requirement, has greatly simplified the analysis of core-promoter (basal) transcription mechanisms. This was also of historical importance, in that it allowed the initial identification and cloning of the gene encoding TBP from yeast (reviewed in Ref. 4), which in turn facilitated the direct cloning of TBP species from higher organisms and the examination of corresponding affinity reagents for the isolation and characterization of the other
Figure 1
Model for pre-initiation complex (PIC) assembly and function on a TATA-containing core promoter. In the simple situation (with TATA-binding protein [TBP]) a minimal set of basal factors (TFIIA, -E, -F, -H and RNA Pol II) suffice, in purified form, for the stepwise assembly in vitro of a functional PIC through a consensus TATA element, and stable intermediates corresponding to each step of assembly are demonstrable. Short solid bars indicate documented protein-protein interactions between the minimal basal factors. Although not essential for formation of a functional PIC with minimal factors, TFIIA can enter the PIC at any point after TBP to form corresponding intermediates; and in situations with weak TBP-TATA box interactions, TFIIA might be essential for stable complexes. In the more physiological situation, with TFIIID in place of the derived TBP, additional components [TFIIA and TBP-associated factors (TAFs)] modulate PIC assembly by stabilizing interactions (short open bars) with TBP and, most likely, with each other and with other initiation factors. Downstream TAF-DNA interactions (see Fig. 3) might also contribute to complex stability. In this situation, and on different core promoters, the assembly pathway might vary with respect to the number of stable intermediates and requirements for other essential factors. In the natural situation, a number of general factors (in addition to TFII) might enter the PIC in association with RNA Pol II in the form of a holoenzyme complex that also contains various coactivators. Following formation, the stable (closed) form of the PIC is activated in an ATP-dependent manner to form an unstable (open) complex. The presence of NTPs then results in initiation, promoter clearance, elongation and recycling of PIC components, as indicated and as detailed in the text. DNA melting and phosphorylation of the carboxy-terminal domain (CTD) of RNA Pol II are dependent upon TFIIH activities, although there is still some uncertainty about the timing and exact function (e.g., in promoter clearance) of the CTD phosphorylation events. Phosphorylation of the CTD is represented by the presence of green dots.

TFIID subunits (TAFs). As implied above, the TAFs, like TFIIA, are not essential for basal transcription with purified factors from conventional TATA-containing promoters, but do have either coactivator functions or basal factor functions on other promoters that are discussed later. TFIIA is also discussed here, in conjunction with minimal basal factors, because of its well-documented interactions with TBP and its effect on basal transcription under specific conditions.

Pre-initiation complex assembly and structure. Consistent with earlier studies of PIC assembly with TFIIID and other factors, later studies with purified minimal components (many recombinant) have established a stepwise in vitro assembly pathway on the AdML promoter, and identified corresponding intermediates that remain stable to various nuclease-protective, electrophoretic mobility shift and template-challenge assays. Further information regarding the underlying intermolecular interactions, and the protein domains involved (not detailed here), has been provided by binary interaction assays coupled with in vitro mutagenesis techniques. More recently, and beginning with the structure of TBP
in 1992, these studies have been complemented with high resolution X-ray structural studies of early intermediates in PIC assembly with minimal factors (reviewed in Refs 7, 11). The pathway (Figs 1, 2; Table I) includes the following steps:

(1) Binding of TBP to the TATA element, through minor-groove contacts, forms a stable complex with an unprecedented DNA distortion and a resulting bend that brings sequences upstream and downstream of the TATA element into closer apposition. While not important for PIC assembly in the minimal system, TFIIA nonetheless can bind stably to this complex through direct contacts both with TBP and with upstream DNA sequences. When weakened by altered ionic conditions or mutations in the DNA-binding surface of TBP (Ref. 15), TBP–DNA interactions can be greatly stabilized by TFIIA—of potential importance for basal transcription on other promoters with weak TATA elements.

(2) Binding of TFIIIB, through direct interactions with TBP and with DNA sequences both upstream and downstream of the TATA element, results in a complex in which the amino-terminal direct-repeat domain of core TFII B is oriented towards the downstream initiation site (Fig. 2). This is consistent with an essential role for more amino-terminal sequences (not present in the crystal structure) in TFII F–RNA Pol II recruitment, but not for TBP–TFII B complex formation (see below), and with the established function of TFII B in start-site selection by RNA Pol II (reviewed in Refs 6, 17). A possible physical basis for the latter is suggested by a two-dimensional crystallographic analysis of a TFII F–RNA Pol II complex, which indicates that the minimal distance between TFII B and the presumptive active site of the enzyme corresponds roughly to that between the TBP–TATA complex and the initiation site on the DNA (Ref. 17).

Like TFIIA, TFII B can stabilize TBP–TATA interactions that are weakened by suboptimal binding conditions. TFII B can bind simultaneously with TFII A to a TBP–TATA complex. As revealed by the combined X-ray structures of the DNA–TBP–TFII B and DNA–TBP–TFII A complexes (Fig. 2), TFII A and TFII B show no overlapping contacts with TBP or with DNA (the upstream DNA contacts residing on opposite faces of the DNA helix) and no direct contacts with each other. This is consistent with distinct, but interdependent, functions of TFII A and TFII B.

Thus, TFII B is involved directly in RNA Pol II–TFII F recruitment (below), whereas TFII A has both an anti-repression function, involving dissociation of TBP-bound negative co-factors that prevent TFII F binding to the PIC (reviewed by K. Kaiser and M. Meisterernst, this issue), and a separable activation function in TFII D-dependent transcription.

(3) Binding of a pre-formed TFII F–RNA Pol II complex, through direct interactions of TFII B with both TFII E and RNA Pol II, follows the binding of TFII B (Refs 6, 17). Although TFII F clearly plays a direct role in promoter targeting of RNA Pol II through these interactions, it also plays an indirect role by reducing RNA Pol II binding to non-specific sites in DNA (Refs 4, 5). RNA Pol II (form II A) that is not phosphorylated on the carboxy-terminal domain (CTD) of its largest subunit is recruited to the PIC in preference to the phosphorylated form (I0). Although the small subunit (RAP30) of TFII F appears sufficient for this recruitment, genetic and biochemical studies suggest functions for both the small (RAP30) and large (RAP74) subunits in stabilization of the PIC intermediate, through TFII F interactions, and in transcription initiation.

Interestingly, TFII F also serves as a transcription elongation factor, although the large and small subunit domains implicated in elongation are largely different from those implicated in initiation (reviewed by D. Reines et al. in this issue). Various nuclease protection studies have indicated TFII F and/or RNA Pol II contacts with DNA sequences (in the PIC intermediate) that lie between the downstream TFII B contact sites (positions −23 to −14) and position +17 (Refs 4, 5, 10). Consistent with these results, photo-crosslinking studies have localized RAP30 (and the two large RNA Pol II subunits) to a position near a downstream TFII B–DNA contact site (position −19) and RAP74 to an adjacent 3′ region (near positions −15 to −5) on the same face of the DNA helix.

(4) Binding of TFII E, through direct interactions with RNA Pol II (Refs 5, 17, 23), and potentially TFII F and TBP (Refs 5, 23), is the next step in the pathway. Within the PIC, the small subunit of TFII E has been localized, by photo-crosslinking, to a region just upstream of the start site, consistent with the proposed functions (below) of TFII E in promoter melting.

(5) Binding of TFII H completes assembly of the PIC (Refs 4–6) and is mediated by direct contacts with TFII E (Refs 23, 24). Consistent with this finding, TFII H has been reported to stabilize
Finally, it is important to mention reports of pre-assembled RNA Pol II-general initiation factor-coactivator complexes ('holoenzymes') that are presumed to be capable of promoter recognition and transcription without the necessity of a step-by-step assembly of all the above-described intermediates on the promoter (reviewed in Refs 27, 28). Although such complexes have been of interest principally because of the associated co-factors (including, in yeast, the SRBs and GAL I); reviewed in Ref. 27 and by S. Björklund and Y-J. Kim in this issue), variable levels of associated general initiation factors have been reported by different laboratories for both yeast and mammalian holoenzymes.27-28 Clearly a mechanism involving pre-assembly of RNA Pol II and all, or a subset of, general initiation factors could simplify the overall PIC-assembly process on the promoter, but this would still depend upon the various DNA-protein and protein-protein interactions deduced from studies of the stepwise assembly pathway. Depending upon the specific promoter and/or the limiting step(s) in transcription, as well as the exact composition of the RNA Pol II holoenzyme, this might or might not have consequences for the kinetics of promoter activation.

**PIC function.** In the presence of ATP and other ribonucleoside triphosphates, assembly of the complete PIC is followed rapidly by DNA melting (PIC activation), initiation and promoter clearance (Fig. 1; reviewed in Refs 4-6). A long-known requirement for hydrolysis of the β-γ bond of ATP (or dATP) before initiation15 is now ascribed to a TFIH helicase activity (reviewed by J. Q. Svejstrup et al. in this issue) that facilitates melting of a -10 bp region just upstream of the start site and forms an activated, but unstable PIC (Refs 29-31). This conclusion is supported by the demonstration that the requirements for TFIH and TFIIE (and in some cases TFIIF), as well as ATP hydrolysis, can be circumvented by the use either of supercoiled templates (ostensibly providing energy for promoter opening by other components, such as the RNA polymerase) or of pre-opened heteroduplex templates (reviewed in Ref. 29). However, using heteroduplex templates with melted regions of variable sizes, it has also been shown that TFIIE has TFIH-independent functions in promoter melting that are promoter specific (depending on sequences near the start site) and potentially involved in stabilization of single-stranded regions near the start site.
Concomitant with transcription initiation (formation of the first phosphodiester bond), the melted region in the open complex is extended downstream several basepairs. With limited transcription elongation, the melted region (transcription bubble) moves further downstream, with resulting renaturation of the open region at the initiation site. Continued elongation results in promoter clearance, as assayed by open-complex formation and RNA Pol II re-entry at the initiation site. The long-standing observation that phosphorylation of the RNA Pol II CTD is associated with the transition from initiation to elongation has suggested a role for CTD phosphorylation in promoter clearance, and the TFIH kinase activity is now known to mediate this modification (reviewed by J. Q. Svejstrup et al. in this issue). Although a role for CTD phosphorylation in promoter clearance has not yet been shown for the AdML promoter in the purified basal factor system, in which CTD phosphorylation is not required for transcription, a recent study of Adenovirus E4 (AdE4) promoter transcription in nuclear extracts supports this view. A related study has shown that transcription from the dihydrofolate reductase (DHFR) promoter requires the CTD kinase activity of TFIH at a post-initiation step.

From a mechanistic point of view, TFIH-mediated phosphorylation of the CTD within the PIC, which can be stimulated by TFIE (reviewed in Ref. 24), is proposed to result in conformational changes that release RNA Pol II from general initiation factor contacts thought to be important for either PIC assembly, activation or initiation steps. Earlier-described interactions that are reversed by phosphorylation, and potentially related to the proposed CTD function, include CTD-TBP (Ref. 5) and RNA Pol II-TFIE (Ref. 23) interactions. However, more recent studies have shown that novel co-factors in the RNA Pol II holoenzyme complex (above) interact with RNA Pol II through the CTD, such that CTD phosphorylation might also reverse these interactions once the accessory functions of the co-factors (e.g. in activator-stimulated PIC assembly or initiation) are complete (reviewed in Ref. 27 and by S. Björkland and Y-J. Kim in this issue). This would help explain the lack of a CTD requirement for transcription in purified systems lacking these specific co-factors.

**PIC recycling.** Another important question concerns the fate of transcription factors and promoter complexes during the transcription cycle (Fig. 1). In vitro studies in the purified human system have shown that TBP or TFIID remains stably associated with the promoter following initiation and promoter clearance, thus facilitating secondary initiation events, while other factors are either sequentially dissociated (TFIIA and TFIE by the time of RNA Pol II advancement to nucleotide position +10, and TFIIH during transit from positions -30 to +68) or remain associated with the elongating RNA Pol II (TFIIJ). Consistent with renaturation of the melted region at the initiation site and release of TFIIH in the first round of transcription, re-association on residual stable complexes again requires ATP βγ bond hydrolysis. The release of TFIIH after TFIE is surprising, but, in view of possible inhibitory effects of TFIIIE or the two TFIH helicases, it might allow enhanced TFIIH-helicase activity for post-initiation functions.

At or following termination, dephosphorylation of the CTD by a CTD-specific phosphatase returns the polymerase to a state (form IIA) competent for PIC assembly (Fig. 1). Finally, it should be emphasized that while partial pre-initiation complexes might, in some cases, be stable and used for multiple rounds of initiation in vitro, this remains to be demonstrated both for a wider range of natural promoters and in vivo where other activities might promote complete complex dissociation. Studies with Drosophila extracts that can undergo multiple rounds of transcription have failed to provide evidence for complexes stable throughout the

**Figure 3**
Promoter recognition through different core-promoter elements. Various types of functional core promoters consisting of individual core-promoter elements, or combinations thereof, are indicated, along with factors known or proposed to directly recognize the corresponding elements, and to facilitate TFIID recruitment. (a) Recognition of simple TATA-containing promoters by the TATA-binding protein (TBP) component of TFIID, with DNA interactions restricted to a ~20 bp region around the TATA element. (b) Hypothetical recognition of simple initiator (Inr)-containing promoters by a factor (X), which could be either a TBP-associated factor (TAF) or a separate Inr-binding protein (IBP) that recruits TFIID through TBP or TAF interactions. Although certain factors (e.g. TFIIA, RNA Pol II, plus other general transcription initiation factors) have been shown to facilitate TBP recruitment dependent only upon Inr elements, this has not yet been demonstrated for TFIID on bona fide natural TATA-less promoters. (c) Recognition of composite TATA- and Inr-containing core promoters by TBP and a distinct factor (X) as described in (b). Partially purified or near-homogenous TFIID can bind over extensive regions of such promoters, with footprints extending from about position -45 to +35 (Refs 39, 42, 46, 49, 58). However, the presence, in several cases, of strongly interacting sequences downstream of the Inr (Refs 42, 43, 46) has precluded any clear demonstration of primary Inr recognition through a TAF on a natural promoter. (Although Inr-dependent downstream interactions have been demonstrated with crude TFIID preparations, the possible contribution of an associated factor has not been excluded.) (d) Recognition of composite downstream-promoter element (DPE)- and Inr-containing core promoters by TAFs and a distinct factor (X) as described in (b). Although a conserved DPE element, which most likely interacts with a specific TAF(s), is best described in Drosophila, the TFIID-interacting downstream regions in certain TATA- and Inr-containing promoters, such as AdML (Ref. 58), human gfa (Ref. 42) and Drosophila hsp70 (Refs 43, 46) might be analogous. Arrows indicate regions of DNase protection that might not represent sequence-specific interactions.
transcription cycle, consistent with such effects also being promoter specific\textsuperscript{38}.

Initiation from diverse core promoters: broader general initiation factor requirements

As free TBP does not represent the natural (intact) form of the TATA-binding factor, and as the model AdML promoter is an intrinsically strong promoter with both consensus TATA and strong Inr elements, it is important to consider PIC assembly and function in the context of natural TFIIID and on a broader variety of natural core promoters. In fact, TFIIID was employed in the earliest studies of PIC assembly, which first documented discrete steps and stable intermediates and served as important precedents for the later studies with TBP (Refs 9, 35). However, the availability of better-defined factors, including near homogeneous preparations of TFIIID, has facilitated more detailed studies.

**TATA-less promoters.** An increasingly large and important group of genes lack TATA elements, but contain functional initiator elements. The TATA-less promoter of the murine terminal deoxynucleotidyl transferase (TdT) gene contains a pyrimidine-rich Inr element that is sufficient to promote low levels of specific transcription in vitro (reviewed in Refs 1, 5, 39) and has served as an important model. Recent studies of the natural TdT core promoter in reconstituted human systems have shown that basal transcription requires all of the natural general initiation factors, including TFIIA and TFIIID, as well as two additional factors that have been partially purified and show no activity with TBP in place of TFIIID (Ref. 40; E. Martinez and R. G. Roeder, unpublished). These results, including the inability of TBP to substitute for a near homogeneous TFIIID, have established a novel absolute requirement for both TAFs and TFIIA in core-promoter function. The TFIIA requirement is consistent with a common TFIIA requirement for optimal TFIIID (but not TBP) function on several other (TATA-containing) promoters (reviewed in Refs 5, 19).

Pyrimidine-rich Inr elements in *Drosophila* promoters have not yet been shown to function independently in *Drosophila* transcription systems. However, a recently described downstream promoter element (DPE) in *Drosophila* TATA-less promoters has been shown to function synergistically with Inr (but not TATA) elements\textsuperscript{5}. The DPE element mediates Inr-dependent site-specific binding of TFIIID (through undefined

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**Figure 4**

Models for activator effects on TFIIID (TBP–TAFs) binding and TFIIID–promoter topology and for activator synergy. (a) Activators, through TATA-binding protein (TBP) or TBP-associated factor (TAF) interactions that might involve TFIIA, enhance TFIIID recruitment. Increased promoter occupancy results in increased pre-initiation complex (PIC) assembly through TBP-mediated recruitment of other general initiation factors according to the minimal assembly pathway of Fig. 1. (b) Activators, through TBP or TAF interactions that might involve TFIIA, alter the conformation of TFIIID or the TFIIID–DNA complex, increased PIC assembly results either from the creation of a more efficient docking platform for RNA polymerase II (Pol II) and other initiation factors or from direct TAF–general factor/RNA Pol II interactions, or from both. The activator-induced downstream interactions of TFIIID might be analogous, at least in part, to the intrinsic downstream interactions observed on promoters with Inr and/or DPE elements (see Fig. 3). (c) Multiple activators, through concerted interactions with different general initiation factors and coactivators, exert a synergistic effort on transcription through enhanced recruitment and/or conformational changes in the PIC. The figure also emphasizes the fact that activator function requires both TAFs and additional cofactors (e.g., PC4) that serve as adaptors between activators and general initiation factors (reviewed by K. Kaiser and M. Meisterernst in this issue).
TAFs) to the corresponding region of the promoter. This provides the first evidence that TAFs can directly recruit TFIIID to a promoter in the absence of a consensus TATA element.

Consistent with the existence of alternative TFIIID recruitment mechanisms, it has also been shown that the TATA-binding ability of TBP is not required for the function of TFIIID on mammalian Inr-dependent TATA-less promoters. Moreover, as TBP binding to the TATA element induces a DNA bend that might be essential for TFIIIB binding (Fig. 2), these results raise the possibility either that such a bend is not essential on TATA-less promoters or that it is achieved by other means.

**TATA- and Inr-containing promoters.** Although TBP can elicit basal activity on composite core promoters (e.g. AdML) containing both TATA and Inr elements, transcriptional activity at comparable molar inputs is usually greater with TFIIID than with TBP (Refs 20, 39, 40, 42-44; T. Oelgeschlager and R. G. Roeder, unpublished). As the enhanced activity with TFIIID, but not the TBP activity, is dependent on the Inr19,40,44 and, in some cases, other downstream elements9,44, a TAF requirement for Inr function, in conjunction with TATA, is also apparent. However, Inr function in this context is also dependent upon the presence of TFIIA and at least one other factor in both human65 (E. Martinez and R. G. Roeder, unpublished) and Drosophila44 systems. The human factor might correspond to one of those (above) required for Inr function in the absence of a TATA element, and has been reported, by one group65, to contain a homologue of the Drosophila 150 kDa TAF (TAF150) that is not stably associated with TFIIID. In Drosophila, TAF150 has been shown to be important for the function of both Inr [distal alcohol dehydrogenase (Adh) promoter]44 and downstream sequence (hsp70 promoter) elements45. However, although Drosophila TAF150 interacts specifically with downstream sequence elements in some promoters45, it does not appear to specifically recognize Inr elements. This is further indicated by the fact that near-homologous human TFIIID preparations, apparently lacking a Drosophila TAF150 homologue, show normal downstream interactions with TATA- and Inr-containing promoters (reviewed in Ref. 45).

**Mechanisms for Inr function.** What are the proteins that specifically interact with the Inr element to effect TFIIID recruitment and/or function? Current models40 (Fig. 3) include specific TAFs (Refs 39, 46), Inr-binding factors such as TFIIJ (Ref. 47), and possibly RNA Pol II (Ref. 48). Inr-binding factors that have been identified include YY1, TFIIJ, USF and E2F, but their relevance to the TFIIID-dependent Inr function described here is unclear, as some (TFIIJ and YY1) have been shown to function in the absence of TAFs and might represent factors that act through distinct initiator sequences or in other contexts (reviewed in Refs 39, 45).

A second important question concerns the requirement for TFIIA in basal transcriptions from Inr-containing promoters. This might reflect an effect of TFIIA in countering the inhibitory effects of TBP-interacting negative co-factors present in nuclear extracts or in TFIIID preparations (K. Kaiser and M. Meistererent, this issue). Alternatively, it might reflect an independent effect of TFIIA in stabilizing otherwise weak interactions of TFIIID with TATA elements, with Inr elements or associated factors, or with other general initiation factors. In this regard, TFIIA has recently been shown to alter the relative disposition of TAFs and DNA in a TFIIID-promoter complex49. These varied effects of TFIIA might reflect interactions not only with TBP, but also with specific TAFs — in particular Drosophila TAF6110 (Ref. 7) or the human TAF135 homologue49.

Finally, it is important to note that TAFs appear to be capable of eliciting either positive or negative effects on basal transcription, depending upon the circumstances. Thus, they might enhance TFIIID binding and/or transcription in the presence of appropriate downstream promoter sequences (above); conversely, they might antagonize TFIIID binding and function on some TATA elements in the absence of such sequences9,43. Possibly related, an inhibitory effect of TAF250 on TBP binding has been reported50.

**PIC assembly and stable intermediates.** A recent study has confirmed earlier reports28,35 that TFIIID and TFIIA are sufficient to form a highly stable complex (resistant to template challenge) on the AdML promoter, but has also demonstrated that the formation of stable complexes on a variety of other promoters requires TFIIID, TFIIIA, TFIIIB and RNA Pol II (and to a lesser extent TFIII)51. This was shown for various TATA- and/or Inr-containing promoters, including some with consensus TATA elements identical to that in the AdML promoter. Hence, the context of a given promoter element can have important consequences for PIC assembly. Further, while TFIIIB readily binds to TBP-TFIIA promoter complexes, it appears to bind poorly to TFIIID-TFIIA complexes on both AdML (Ref. 9) and AdE4 (Refs 52, 53) promoters. Thus, in the more natural situation, TAFs might restrict TFIIID-TFIIIB interactions such that efficient PIC assembly might necessitate conformational changes in TFIIIB (Refs 26, 54) or in the TFIIID-TFIIA promoter complex (below) in response to activators or other general initiation factors. These findings also provide a possible rationale for pre-assembly of holoenzyme complexes containing multiple general initiation factors.

Overall, the results summarized above indicate that PIC assembly pathways might vary from promoter to promoter, depending on both the specific elements involved and the promoter context, at least with respect to the formation of stable intermediates and general factor requirements. Hence, the minimal pathway with several stable intermediates (Fig. 1) should be viewed as a flexible framework for PIC assembly with other promoters and natural factors. Moreover, these results emphasize that, in considering a broad spectrum of promoters, the basal factors must be redefined to include all the commonly recognized general initiation factors (including TFIIID and TFIIA), and possibly other factors (for Inr function) as well.

Lastly, variable core promoter structures and general factor requirements are consistent with the possibility of selective gene regulation through core promoter-specific functions of certain activators and repressors (reviewed in Refs 41, 45).

**General initiation factors as activator targets**

Apart from possessing intrinsic capabilities for basal transcription, it is obvious that the general initiation factors must also represent the ultimate targets of various gene specific activators (or repressors). Consistent with its key function in transcription initiation, TFIIID was implicated as a target for gene-specific activators in the earliest studies of activation mechanisms50-52. Thus, following the initial demonstration of distinct TFIIID interaction patterns on different core promoters58, these studies showed both quantitative effects56 and qualitative effects (induction of downstream DNA interactions)57 on TFIIID binding (in response to activators), which have been correlated with increased recruitment and function of other general initiation factors. These varied effects can now be understood in terms of specific activator
interactions either with TBP (reviewed in Ref. 59) or with TAFs (reviewed in Ref. 7 and by C. P. Verrijzer and R. Tjian in this issue). The TAFs are most commonly viewed as coactivators because of their dispensability for basal, but not activated, transcription on some promoters.

In relation to the role of activator–TFIID interactions, two basic mechanisms, which are not mutually exclusive, are evident: (1) increased recruitment of TFIID, which in turn enhances recruitment of the other basal factors mainly through subsequent TBP-directed interactions (Fig. 4a). There is clear evidence that TBP/TFIID binding can be limiting for transcription both in vivo and in vitro (reviewed in Ref. 7). (2) An altered TFIID or TFIID–promoter topology that facilitates recruitment of other general factors through secondary stabilizing interactions either with TAFs or with a TAF–DNA docking platform (Fig. 4b). In support of this mechanism, recent studies52,53 with homogenous TFIID have confirmed the earlier studies54 of activator-induced changes in TFIID interactions with downstream DNA sequences, but shown a concomitant TFIIF requirement. Recent studies have also shown both DNA wrapping in TFIID–AdML promoter complexes, as well as the above-mentioned TFIIF-induced changes in the relative disposition of TAFs and DNA in these complexes. In addition, interactions of specific TAFs with TFIIF, TFIIH, TFIII, TFIF and TFIIF have been reported (reviewed in Ref. 7), although their functional relevance remains to be determined. It is significant that, in one instance, it has been observed that an activator is required only transiently for induction of the downstream TFIID interactions, and potentially for activation—consistent with a primary function of this activator in TFIID interactions, but also suggesting a means to propagate the active state of a gene in the absence of the inducer.55

Not surprisingly, activators have also been shown to interact with a number of other general initiation factors that include TFIIF, TFIIH, TFIF and TFIH (reviewed in Ref. 7). The functional relevance of the various activator–general initiation factor interactions has not yet been proven in all cases (e.g. by reciprocal activation-specific mutations in both the activator and the interacting general factor component). However, multiple activator–general factor interactions do provide a basis for the observed synergism between multiply bound activators under saturating binding conditions. Thus, we are left with the view that activators might interact with general initiation factors either directly or, indirectly, using coactivators (Fig. 4c). It is important to note, however, that factors (e.g. TAFs or TFIIA) that technically might be viewed as coactivators on some promoters might be essential basal factors on others.

Another area of increasing importance concerns the role of chromatin remodeling (nucleosome rearrangement), by various factors, in the function of activators (reviewed in Ref. 61). In this regard, it is important to note the potentially important role played in this process by TFIID, by virtue of its ability to exclude nucleosomes when pre-bound to the promoter and by virtue of an internal complex of histone-like TAFs (Reifs 7, 62) that might help to destabilize nucleosome structures and/or to stabilize TFIID-promoter interactions through nucleosome-like DNA wrapping.

Conclusions and perspectives

Since the discovery of Class II general initiation factors over 15 years ago, we have come to appreciate their remarkable complexity, phylogenetic conservation and common functions on a variety of model and natural promoters. The increasing ability to study these factors in completely purified and/or in recombinant forms has allowed more definitive biochemical, biophysical (structural) and genetic studies, and provided increasingly deeper insights into their mechanisms of action. A continuation of these efforts must be directed towards more detailed studies of both the structure and the dynamics of the PIC during formation and function, modulation of these parameters in response to gene- and cell-specific activators and repressors, both on diverse natural promoters and natural (chromatin) templates, and attempts to relate the in vitro mechanistic studies to the natural situation in the living cell.

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Mediator of transcriptional regulation

Stefan Björklund and Young-Joon Kim

A multi-protein complex, termed mediator, has been isolated from yeast, based on its requirement for transcriptional activation in a system reconstituted from pure RNA polymerase II and general transcription factors. Mediator polypeptides include the products of many genes previously recovered from screens for mutations affecting transcription. This connection between biochemical and genetic studies reveals that mediator is important for both activation and repression of transcription, and that mediator plays a role in transcriptional regulation in vivo as well as in vitro. Mediator binds the carboxy-terminal domain of RNA polymerase II, forming a polymerase holoenzyme, whose possible association with additional proteins is a subject of some controversy.

MOST GENES in eukaryotes show a regulated pattern of expression during development, during the course of the cell cycle or in response to changes in the cellular environment. Expression is controlled by regulatory proteins that bind to specific sequences in the vicinity of a promoter and influence the initiation of transcription. While it was at first thought that positive regulatory proteins, referred to as activators, exerted their effects directly, through physical contact with components of the transcription apparatus, it is now believed that the effects are indirect, involving intermediary factors that convey signals from activators to transcription proteins. The failure of systems reconstituted with purified transcription proteins to respond to activators supports this idea, and the isolation of intermediary factors allowing a response in these systems provides proof. Three types of intermediary factors have been described:

1. TAFs, which associate with TATA-binding protein (TBP) to form TFID; and
2. mediator, which associates with RNA polymerase II (Pol II) to form a holoenzyme; and
3. a chromatin factor from human cells, termed Upstream Stimulatory Activity (USA), whose resolution is still in progress. Here, we focus on the mediator and its characterization with regard to subunit structure and functional activities.

Mediator of transcriptional regulation

The existence of mediator was originally inferred from experiments in Saccharomyces cerevisiae by Kornberg and co-workers. They employed a squelching assay in which the addition of one activator could interfere with the stimulation of transcription by another, suggesting competition between the two activators for a common target. Squelching was specific for the activation domains of the two activators and could be relieved by the addition of a crude yeast protein fraction, but not by the addition of an excess of any known components of the transcription apparatus. These findings identified an activity in the crude fraction that was termed mediator and evidently resided in a novel protein.

Subsequent studies revealed co-fractionation of mediator activity with RNA Pol II and with TFIIID (Refs 7, 8). Removal of the polymerase, with the use of antibodies against its carboxy-terminal domain (CTD) immobilized on a solid support, yielded an apparently homogeneous mediator preparation. This mediator contained about 20 polypeptides and displayed three functional activities: support of transcriptional activation, five- to tenfold stimulation of basal transcription, and 30-50fold stimulation of TFIIID-dependent phosphorylation of the polymerase CTD.

RNA Pol II holoenzyme

Mediator associates with the polymerase CTD to form a complex referred to as holoenzyme, and on purification of polymerase II from yeast, a substantial fraction is recovered as holoenzyme. The existence of a higher form of polymerase II was previously recognized by Young and co-workers in studies of CTD-interacting proteins termed Suppressors of RNA polymerase B