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The Biochemistry of Transcription in Eukaryotes: A Paradigm for Multisubunit Regulatory Complexes

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The biochemistry of transcription in eukaryotes: a paradigm for multisubunit regulatory complexes

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SUMMARY

The control of gene expression during development, differentiation and maintenance of cellular function is governed by a complex array of transcription factors. We have undertaken a molecular dissection of the regulatory factors that direct transcription of protein coding genes by RNA polymerase II. Our early studies identified sequence-specific transcriptional activators that bind to enhancer and promoter sequences to modulate the transcriptional initiation event. However, the mechanism by which activators enhance transcription and mediate promoter selectivity remained unknown. Combining biochemical purification and *in vitro* assays, we have recently identified an essential class of transcription factors called TAFs that are tightly associated with the basal factor TBP (TATA-binding protein). We have found that TAFs are responsible for at least two regulatory functions. Some TAFs serve as coactivators capable of binding activators and mediating enhancing function. Other TAFs have been shown to confer template selectivity by binding directly to core DNA elements of the promoter. Thus different subunits of TBP/TAF complexes perform a variety of functions critical for transcriptional regulation in animal cells.

1. INTRODUCTION

In the last ten years, significant advances have been made towards deciphering the molecular events that govern eukaryotic gene regulation. In particular, there has been rapid progress in identifying the enzymatic machinery that has evolved to direct the surprisingly elaborate process of turning genes on and off. Although gene expression is subject to regulation at many different steps during the flow of genetic information from DNA to RNA to protein, this discussion will focus on one of the central control points: the copying of genes encoded in eukaryotic chromosomes into RNA by the process called transcription. More specifically, we will review events controlling the initiation of RNA synthesis which has emerged as a primary event that determines the patterns of gene expression in animal cells.

The transcriptional control regions of eukaryotic protein coding genes can be separated into at least two categories: a core promoter and upstream (or downstream) regulatory elements. Each gene carries a unique array of proximal and distal enhancer elements that are recognized by sequence specific DNA binding factors critical for activating or repressing transcription initiation (Tjian & Maniatis 1994) (figure 1). Eukaryotic promoters are regulated by a combination of sequence specific DNA binding proteins, general transcription initiation factors and associated accessory factors (figure 2). The sequence-specific transcription factors can be divided into several classes on the basis of the activation domains they possess, including those that are glutamine rich (Courey & Tjian 1988; Dreiver *et al.* 1989; Tanaka & Herr 1990) proline rich (Mermod *et al.* 1989) and acidic (Hope & Struhl 1986; Ptashne 1988). Furthermore, there are several activation domains that do not apparently belong to any of these three classes, suggesting that there are additional families of activating motifs. In addition to the site specific proteins, mammalian promoters directed by RNA polymerase II require at least five basal transcription factors (TFIIA, TFIIB, TFIID, TFIIE and TFIIF) to form an active initiation complex (Reinberg & Roeder 1987; Reinberg *et al.* 1987; Buratowski *et al.* 1989).

2. THE BASAL MACHINERY

Early studies had demonstrated that purified RNA pol II is incapable of promoter recognition. Importantly, sequence specific enhancer and/or promoter binding proteins such as Sp1 alone could not direct purified RNA pol II to discriminate between different promoters. Clearly there were additional components necessary to reconstitute a transcription initiation complex that could respond appropriately to enhancer binding factors. The next logical step was to identify the accessory factors required to assemble an active initiation complex. It seemed likely that one or more of the components associated with RNA pol II in the initiation complex might be the targets of activators. A battery of experiments carried out by several laboratories in the mid 1980s identified a cadre of accessory factors (TFIIA, B, D, E, F and H) that help assemble an initiation complex (figure 2) (Matsui *et al.* 1980; Samuels *et al.* 1982; for review see Zawel & Reinberg 1992). Of these basal transcription factors, TFIID stood out as a likely target of site specific DNA enhancer factors, because it is thought to be the first

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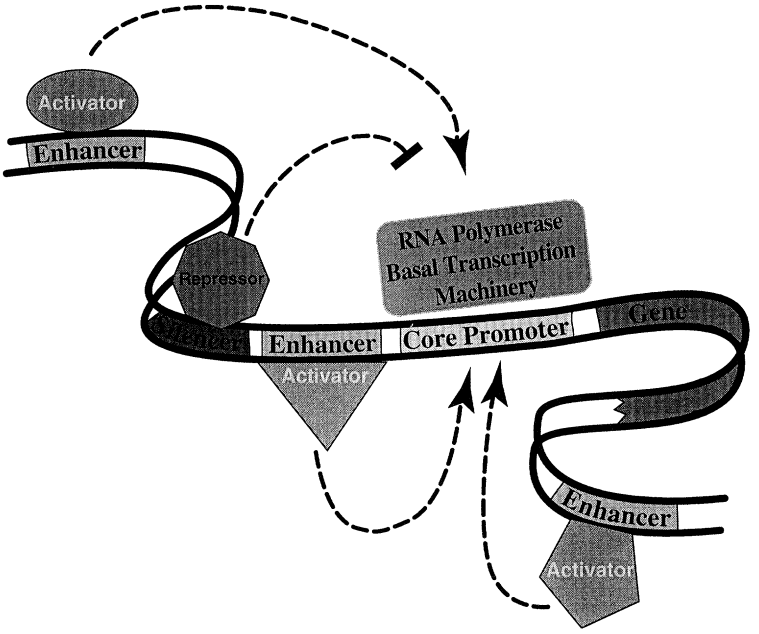

Figure 1. Transcriptional control region of a eukaryotic gene. This diagram illustrates a typical arrangement of regulatory sequences flanking a protein-coding gene. Multiple enhancers and silencers can be located both upstream and downstream from the proximal core promoter, which contains binding sites for the TATA binding protein and the assembled RNA pol II initiation complex. Various activators and repressors bind to the enhancers (or silencers), and in a combinatorial fashion, directs transcription by RNA pol II.

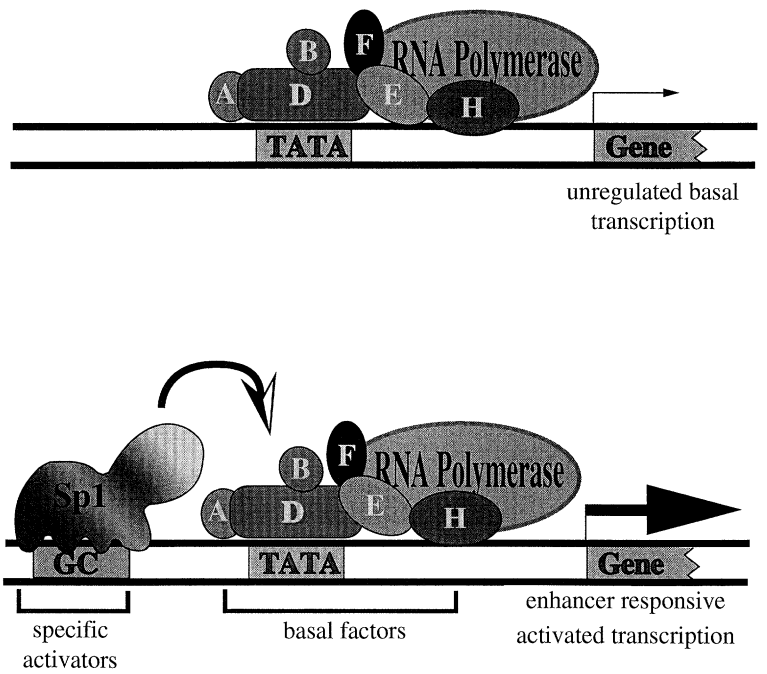

Figure 2. The RNA pol II basal transcriptional initiation complex. The top diagram depicts the collection of basal factors (TFIIA, B, D, E, F and H) that assemble along with RNA pol II to form the initiation complex. The lower diagram illustrates activation of transcription by Sp1, which is thought to occur via direct interactions between the glutamine rich activation domain of Sp1 and specific components of the initiation complex.

component to assemble on the DNA template (Nakajima *et al.* 1988; Buratowski *et al.* 1989). In addition, TFIID was the only basal factor known to display sequence specific binding properties and to recognize the TATA box element of core promoters (Sawadogo & Roeder 1985). Studies using relatively crude preparations of TFIID reported an apparent interaction between activators and this basal factor (Horikoshi *et al.* 1988). Unfortunately, despite the efforts of many laboratories, the purification, subunit composition and biochemical properties of TFIID remained out of reach.

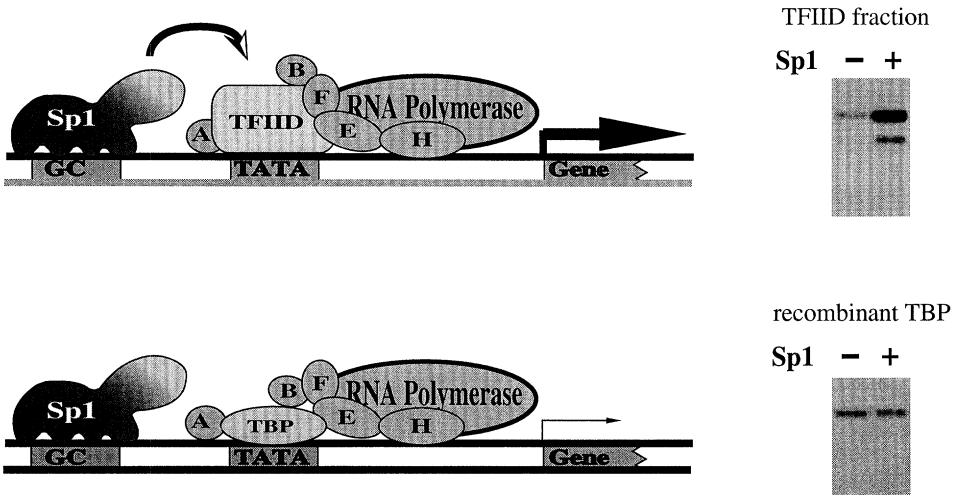

Figure 3. Coactivator requirements for transcriptional activation by Sp1. Top panel: *in vitro* reconstituted transcription reactions were performed with RNA pol II and chromatographic fractions containing the basal factors including TFIID which is required to mediate activation of transcription by Sp1. Bottom panel: if the TFIID fraction is replaced by purified recombinant TBP, Sp1 is no longer able to activate transcription, suggesting that one or more components, called co-activators, that are associated with TFIID mediates activation.

A breakthrough came in 1989 when several laboratories simultaneously reported the purification and molecular cloning of a TATA binding protein (TBP) from yeast (Hahn *et al.* 1989; Horikoshi *et al.* 1989). Yeast TBP was found to be a single polypeptide of 25 kDa, capable of binding selectively to the TATA box. Subsequently, Drosophila and human TBP were cloned and studied (Peterson *et al.* 1990). Most importantly, this single subunit was also able to restore basal levels of transcription *in vitro* using either yeast or mammalian transcription factors. It was therefore natural to assume that TBP and TFIID were identical and that a single polypeptide was sufficient to serve as the TATA binding factor to nucleate the assembly of RNA pol II initiation complexes.

Experiments were undertaken to determine whether purified recombinant TBP could meditate activation by Sp1 in reconstituted transcription reactions (Peterson *et al.* 1990). Surprisingly, neither yeast, Drosophila nor Hela TBP were able to substitute for TFIID in directing Sp1 activation (Pugh & Tjian 1990) (figure 3). Several other activators were also tested, including CTF-1, NTF-1 and GAL4-VP16, with no success (Dynlacht *et al.* 1991; Tanese *et al.* 1991). Many other failed attempts to recapitulate activator dependent transcription with the recombinant purified TBP, suggested that there must be some critical functional difference between TBP and TFIID. These experiments led to the idea that the partially purified TFIID must contain one or more activities, called coactivators, that are essential for transcriptional activation (Pugh & Tjian 1990). These coactivators would also represent a novel class of transcription factors because they are neither enhancer binding proteins nor are they required for basal transcription. It was further hypothesized that this new class of coactivators would serve as the direct targets of activators and mediate interactions between activators and the basal transcriptional machinery. Moreover, based on chromatographic properties of TFIID, it was likely that at least some of these coactivators are tightly associated with TBP to form a large multi-subunit complex with the functional properties of TFIID.

3. PURIFICATION OF TFIID AND IDENTIFICATION OF TAF SUBUNITS

Although recombinant versions of Drosophila and human TBP did not turn out to be the direct targets of Sp1 activation, they gave rise to valuable reagents, such as monoclonal and polyclonal antibodies, that could be used for biochemical purification and characterization of TFIID. Using antibodies against Drosophila and human TBP, it was established by coimmunoprecipitation that TFIID exists as a large stable complex with multiple subunits (figure 4) (Dynlacht *et al.* 1991; Tanese *et al.* 1991). Analysis of immunopurified Drosophila and human TFIID revealed the presence of some eight tightly associated polypeptides ranging in molecular mass from 20–250 kDa. These subunits of TFIID have been termed TAF's (TBP associated factors).

The discovery of TAFs as subunits of TFIID strongly suggested that one or more of these factors may also serve as coactivators. To test this idea, TAFs were chromatographically separated from TBP by differential elution of TFIID complexes tethered to antibody columns. After eluting the TAFs under denaturing conditions, they were renatured and reconstituted with either purified TBP or recombinant TBP, and then tested for their ability to restore activation by Sp1 (Dynlacht *et al.* 1991; Tanese *et al.* 1991). These experiments established that TAFs are essential for mediating activator dependent transcription. Thus it seemed likely that at least some of the TAFs in the TFIID complex were responsible for mediating activation.

These findings with Drosophila and human TFIID however led to a paradox. Why was yeast TFIID isolated chromatographically as a single polypeptide

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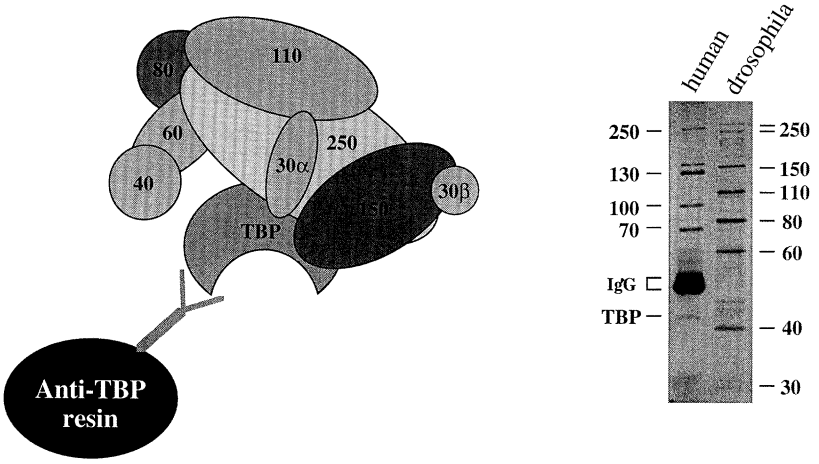

Figure 4. Purification and identification of TBP associated factors (TAFs). Using anti-TBP affinity chromatography, it was possible to immunopurify the TFIID complex from both human and Drosophila to homogeneity. The panel on the right shows a silver stained SDS polyacrylamide gel of immunopurified complexes, which contains TBP and at least eight TAFs ranging in molecular mass from 250–30 kDa that are subunits of Drosophila and human TFIID. The panel on the left shows a diagram depicting the various TBP-TAF and TAF-TAF interactions that have been identified from a series of protein:protein binding assays carried out *in vitro*.

(TBP), when TFIID in the human and Drosophila cells exist as a stable complex of some nine subunits? Are there in fact TAFs in yeast? The first clue to this question came with the purification, molecular cloning and characterization of Drosophila $TAF_{II}150$ (Verrijzer *et al.*, 1994). This large subunit of TFIID turned out to be the homologue of a previously identified yeast gene called TSM-1. Biochemical characterization of Drosophila $TAF_{II}150$ and yeast TSM-1 revealed that both proteins can bind directly to homologous or heterologous TBPs. Thus, yeast TFIID most likely also contained associated TAFs. Recent antibody purification of yeast TFIID confirmed that it is indeed a complex of TBP and TAFs. Purification of yeast TFIID identified several TAFs with amino acid sequences similar to TAFs 250, 80, and 60 in addition to 150 (A. Weil, personal communication; Reese *et al.* 1994). Thus it now seems clear that TFIID from yeast to man consists of TBP and multiple TAFs.

The purification and subsequent molecular cloning of all the TFIID subunits provided a valuable set of reagents. For example, in addition to revealing homologies between $TAF_{II}150$ and yeast TSM-1, genetic analysis of TSM-1 established this subunit as an essential gene product for viability in yeast. Another serendipitous connection that resulted from the cloning of TAFs, was the discovery that the largest subunit of the complex, $TAF_{II}250$, is identical to a previously isolated gene called CCG-1, which had been implicated in cell cycle control (Ruppert *et al.* 1993; Wang & Tjian 1994). Cells bearing temperature sensitive mutant CCG-1 arrest cell growth at the G1 to S boundary (Sekiguchi *et al.* 1988). These results not only confirm the essential activities carried out by TAFs *in vivo*, but also provide a powerful molecular genetic strategy for further analysis of TAF function.

4. MAKING CONTACTS BETWEEN ACTIVATORS AND TAFS

Three questions were addressed to test the co-activator hypothesis: (i) Do activators make direct and specific contacts with selected TAFs of the TFIID complex? (ii) Do the interactions between activators and TAFs represent an integral step in the signaling pathway that leads to transcriptional activation? (iii) Do different classes of activators (i.e. glutamine rich, isoleucine rich, acidic, proline rich, etc.) target distinct TAFs? The availability of recombinant purified subunits has enabled a detailed biochemical analysis of TAF function during transcription. For example, a variety of TAF/TBP and TAF/TAF interactions critical for the assembly of TFIID have been defined. More importantly, specific contacts between TAFs and activators have been documented (figure 5). The first of these studies established that Drosophila $TAF_{II}110$ and its human counterpart $TAF_{II}130$ interacts selectively with the glutamine-rich activation domains of Sp1 (N. Tanese, J.-L. Chen & R. Tjian unpublished result; Hoey *et al.* 1993). Analysis of mutant Sp1 activation domains also revealed a strong correlation between loss of transcriptional activity and failure to bind $TAF_{II}110$ (Gill *et al.* 1994). Indeed $TAF_{II}110$ not only binds Sp1, but also interacts with other glutamine rich activators including CREB and Button head (Ferreri *et al.* 1994; F. Sauer personal communication). Importantly, other classes of activators have been found to target different TAFs. For instance, the acidic activators, VP16 and p53 bind selectively to $dTAF_{II}$ 60 and 40 or their human counterparts $hTAF_{II}$ 70 and 32 (Goodrich *et al.* 1994; Thut *et al.* 1995). Similarly, the activator NTF-1 binds selectively to $dTAF_{II}150$ and 60 (Chen *et al.* 1994). Although these findings were consistent with the coactivator hypothesis, evidence for the role of individual TAFs in mediating transcriptional activation was lacking.

A powerful, but arduous, strategy was to reconstitute

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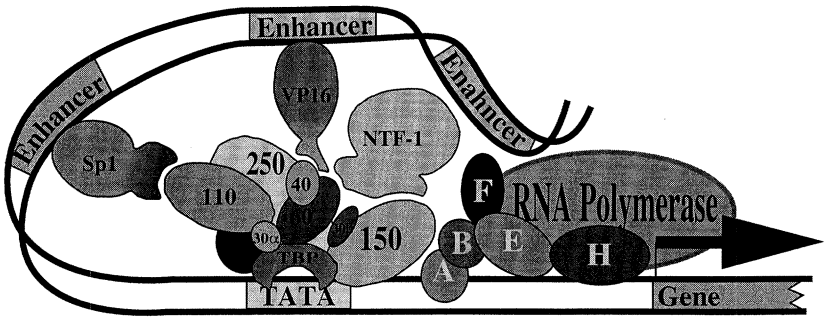

Figure 5. A hypothetical model for the assembly of an activated initiation complex. This diagram illustrates multiple distinct activators (Sp1, VP16, and NTF-1) bound to tandem enhancers on the template, each interacting with specific TAF targets in the TFIID complex to mediate activation of transcription.

partial complexes containing subsets of TAFs and TBP to demonstrate the function and specificity of individual TAF/activator interactions in mediating transcription. Using such an approach, it was found that a minimal complex containing TBP and $TAF_{II}250$ can direct normal levels of basal transcription but failed to respond to activators that were tested (Chen *et al.* 1994; Thut *et al.* 1995). In contrast, a triple complex containing TBP, $TAF_{II}250$ and 150 was able to support activation by the isoleucine rich activator, NTF-1, but not the glutamine rich activator, Sp1. However, a quadruple complex containing TBP, $TAF_{II}250$, 150 and 110 was highly active for both NTF-1 and Sp1. Curiously, a triple complex containing TBP, $TAF_{II}250$ and 110, but lacking 150, was not active for Sp1 transcription. This unexpected result suggested that some TAFs might serve a dual function. In the case of activation by NTF-1, $TAF_{II}150$ serves as the target for direct contact, but in the case of activation by Sp1, $TAF_{II}150$ might provide a function other than as a target, as Sp1 does not bind to $TAF_{II}150$. Nevertheless, transcriptional activation by Sp1 requires both $TAF_{II}150$ and 110. These results taken collectively provide the best available evidence that specific interactions between activators and TAFs represent a requisite step during the process of transcriptional activation by enhancer binding proteins. These results, using partial TFIID complexes and different classes of activators, also illustrate the selective nature of the TAF/activator interaction. Finally, it is evident that some TAFs, such as 150 and possibly 250, may perform multiple functions. They may serve as targets for activators, but also function in some other capacity, as yet unclear, that is required to relay activation signals directed by certain regulators.

Having succeeded in assembling transcriptionally active partial TFIID complexes containing subsets of TAFs, the next step was to attempt reconstitution of a complete TFIID complex using purified recombinant subunits. Applying the same strategy of tethering one subunit to a solid support and then loading each successive TAF one at a time, it was possible to reconstruct a complex containing TBP and all eight known TAFs (250, 150, 110, 80, 60, 40, 30α, 30β) (Chen *et al.* 1994). This so called holo-TFIID, when tested for transcriptional activity, was able to mediate activation by all of the activators that were tested (including Sp1, NTF-1, VP16, CTF-1 and p53). These findings lend support to the notion that promoter specificity and transcriptional control in eukaryotic cells depend on the ability of multiple upstream enhancer factors bound to the template to communicate with the basal machinery through contact with TAFs (figure 5).

The accumulated evidence to date points to the TBP/TAF subunits of the TFIID complex as central players in the control of transcription in eukaryotes. Early studies established that TFIID and, in particular, its TBP subunit is responsible for binding to the TATA box and directing basal levels of transcription (Zawal & Reinberg 1992; Conaway & Conaway 1993). Recent studies found that the TAF subunits of TFIID can act as coactivators that mediate transcriptional activation by upstream enhancer binding factors (Chen *et al.* 1994; Goodrich & Tjian 1994). Now we have provided direct evidence that at least some TAFs also play a critical role in core promoter selectivity by RNA pol II.

Using a combination of reconstituted transcription reactions as well as DNA binding studies, we determined that $TAF_{II}250$ and $TAF_{II}150$ can confer promoter selectivity by interacting specifically with proximal downstream elements that include the initiator. Our data suggest a close relation between the organization of core promoter elements and the subunit architecture of TFIID. Indeed, various core promoter elements can be viewed as an array of binding sites for distinct TFIID subunits. Depending on the precise arrangement and sequence in the core promoter, the TAFs can stabilize or destabilize the DNA binding of TFIID and modulate the preinitiation complex formation and intrinsic promoter strength (figure 6). The surprising discovery that TAFs can destabilize the binding of TFIID at certain promoters suggests that they might compensate for the rather low sequence specificity of TBP alone (Hahn *et al.* 1989) and prevent transcriptional initiation from occurring at weak or cryptic promoters in the genome. Thus the function of TAFs such as $TAF_{II}250$ and $TAF_{II}150$ in discriminating between different RNA pol II promoters is reminiscent of the prokaryotic σ-and Δ factors that direct promoter selectivity and prevent *Escherichia coli* RNA polymerase from initiating at incorrect sequences (Helman & Chamberlin 1988). Taken together, these findings reveal novel activities carried out by TAFs that modulate promoter utilization and selectivity.

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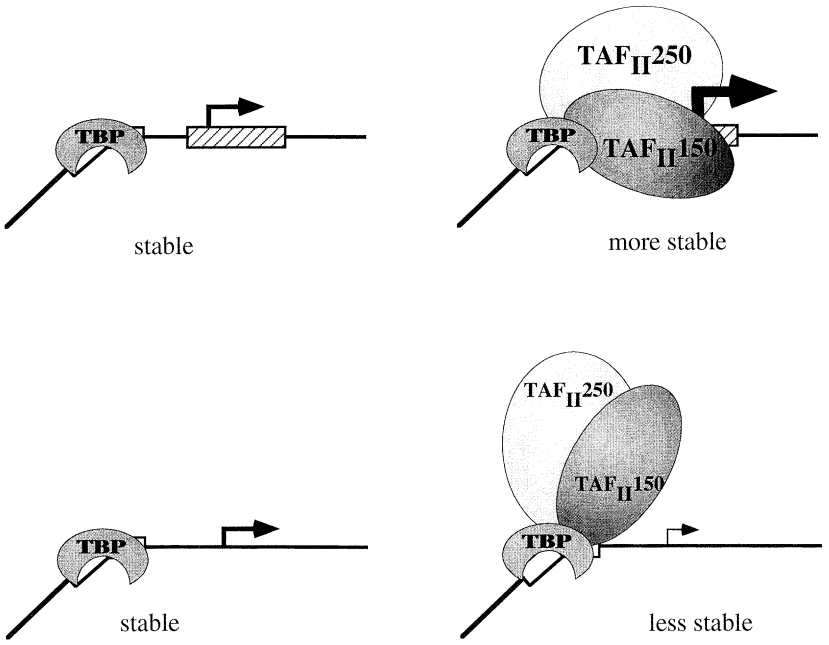

Figure 6. A schematic model for core promoter recognition by RNA polymerase II TAFs. TBP, TAF$_{II}$250 and TAF$_{II}$150 are indicated. The TATA box is represented by an open box, and the downstream regions of the core promoter, including the initiator, are represented by a hatched box. The arrow indicates the transcription start site. TBP can bind stably to the TATA box and support equal levels of transcription either in the absence or presence of downstream core promoter elements. In contrast, the TAFs in TFIID or a trimeric TBP, TAF$_{II}$250, TAF$_{II}$150 complex can recognize these core promoter sequence, increasing the stability of binding, and mediate a higher level of transcription. However, in the absence of the downstream elements, the TAFs can decrease the stability of TFIID promoter interactions, resulting in a reduced level of transcription.

Deciphering the role of TAFs suggests that the multiple subunits of TFIID, each with distinct structural and presumably functional characteristics, are necessary to provide the diversity and specificity of interfaces needed to receive regulatory inputs from a vast number of activators. Such an arrangement provides the opportunity for multiple activators, and perhaps also repressors, to interact coordinately with components of the transcriptional apparatus in directing tissue specific and developmental regulation. It is also possible to imagine that with additional studies, cell-type specific TAFs and coactivators may be discovered. It is, therefore, possible that TFIID functions as a biological central processing unit that helps integrate the gene regulatory information from a large number of specific enhancer and promoter bound transcription factors as a means of controlling the initiation of RNA synthesis in eukaryotic cells.

5. UNIVERSALITY OF TBP/TAF COMPLEXES

When the studies of TBP and TFIID first began there was a tacit assumption that TBP functions exclusively in the realm of RNA pol II transcription. Assuming the central role that has been attributed to TBP/TAF complexes is correct, one might expect that eukaryotes would not have evolved such an elaborate machinery only for transcription of mRNA. It would seem reasonable to expect that a similar arrangement of basal transcription factors might also serve as the relay station for activators that regulate rRNA and tRNA synthesis by RNA polymerases I and III, respectively. The first hint that TBP may also function as a transcription factor for other RNA polymerases came with the finding that transcription of U6 RNA by pol III required TBP (Lobo *et al.* 1991; White & Jackson 1992) . Initially, this finding was treated as an exception rather than the rule, because after all the U6 promoter was unusual and actually contained a TATA box. By contrast, most other pol III promoters (i.e. those directing tRNA transcription) and pol I promoters contained no obvious TATA element. However, a series of detailed biochemical analysis of rRNA transcription by RNA pol I led to the surprising conclusion that not only TBP but also a unique set a TAFs distinct from pol II TAFs play a critical role in transcription by RNA pol I (Comai *et al.* 1992).

Previous studies with human ribosomal transcription had revealed at least three chromatographically distinct fractions required to reconstitute accurate and selective transcription at the ribosomal promoter (Learned *et al.* 1985, 1986; Bell *et al.* 1988, 1990). These fractions were eventually purified and found to consist of RNA pol I, an upstream binding factor called UBF-1, and an essential promoter selectively factor called SL1. The subunit composition of human RNA pol I has been characterized and is similar to other classes of eukaryotic RNA polymerases. The enhancer binding factor, UBF-1, was purified to homogeneity and identified as a polypeptide of 96 kDa. cDNA clones encoding UBF-1 were obtained and shown to encode a sequence specific DNA binding factor with HMG-box DNA binding motifs (Jantzan *et al.* 1990). Recently, each of the SL1 subunits, consisting of TBP and three novel TAF$_I$s of 110, 63 and 48 kDa, were cloned and

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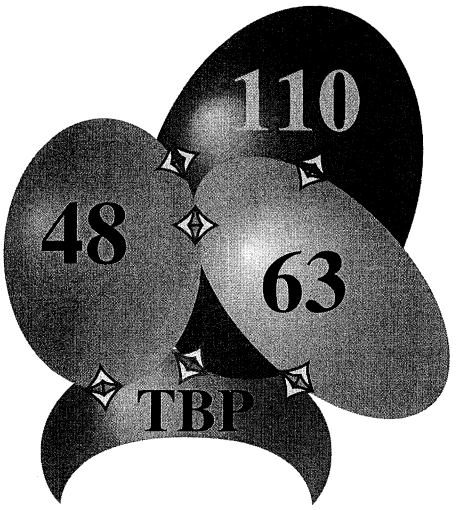

Figure 7. Subunit interactions between TAFs and TBP in the SL1 complex. *In vitro* protein : protein binding assays revealed that each of the TAF subunits of SL1 can make specific contacts with TBP as well as with each other. These multivalent interactions result in the formation of a stable TBP/TAF complex dedicated to the promoter selective transcription of ribosomal RNA by RNA polymerase I.

expressed as purified recombinant proteins (Comai *et al.* 1994). These three TAFs interact selectively with each other and can bind TBP to form a stable quadruple complex (figure 7). Reconstitution of SL1 from its subunits produced an active complex that directs transcription of the human ribosomal promoter *in vitro* with an activity indistinguishable from endogenous SL1 (Zomerdijk *et al.* 1994). Thus it is clear that this unique TBP/TAF complex serves essentially the same function for RNA pol I transcription as TFIID serves for RNA pol II.

These results with SL1 raise an interesting question: If both RNA pol I and RNA pol II TAFs can tightly associate with TBP to form SL1 and TFIID, respectively, is there the possibility for the formation of TBP/TAF complexes containing mixed subunits, some from SL1 and others from TFIID? Interestingly, immunopurification of TBP/TAF complexes using anti-TAF antibodies failed to reveal the presence of any chimeric complexes. Thus it seemed that *in vivo* some mechanism prevented the formation of mixed complexes. To probe those mechanisms that might prevent the formation of mixed complexes, a series of TBP/TAF binding experiments were carried out *in vitro* (Comai *et al.* 1994). In the first set of experiments, immobilized TBP was allowed to bind either $TAF_{II}250$ or 150 (two of the TFIID subunits known to bind TBP directly). These dimer complexes were than challenged with a molar excessive of either $TAF_{I}110$, 63 or 48. In each case, no binding of the SL1 subunits was observed, as if the presence of the pol II TAFs precluded an interaction of TBP with the pol I TAFs. In a second set of experiments the reciprocal reactions were carried out. TBP was first bound to either TAF_{I} 48, 63 or 110, and then challenged with TAF_{II} 250 or 150. Again no binding of the pol II TAFs was observed in complexes containing TBP and one or more of the pol I TAFs. These results suggest that the mutually exclusive binding of class specific TAFs to TBP provides a simple mechanism that might be operative in preventing the formation of mixed complexes. Apparently, the presence of pol I TAFs on TBP may be functionally incompatible for TFIID activity and vice versa. Assuming a similar arrangement of TBP/TAF interactions also plays a role in the formation of TFIIIB (the RNA pol III factor), then one can envision three alternative complexes formed by TBP and TAFs that

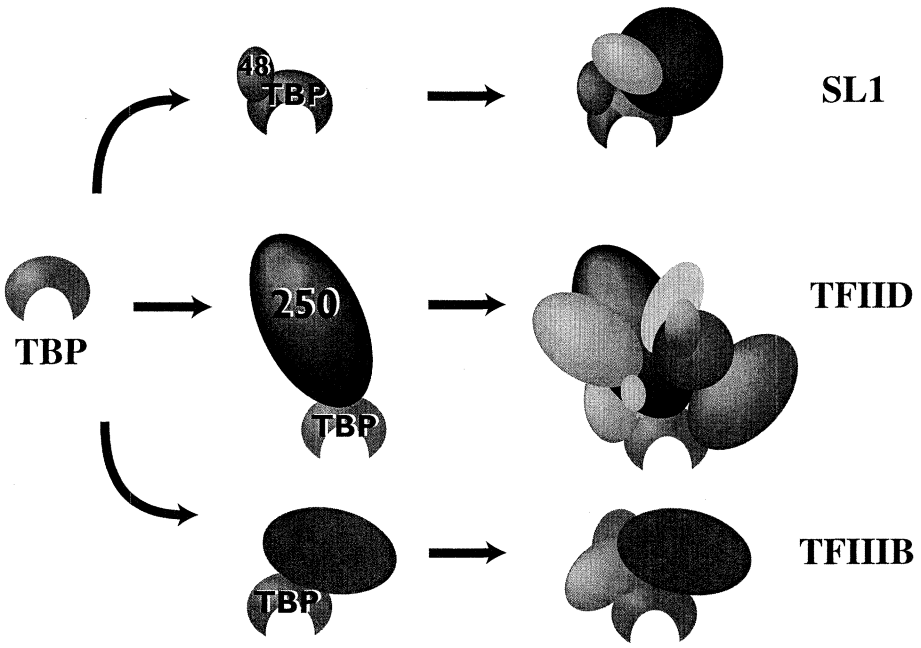

Figure 8. TBP is an integral component of transcriptional initiation complexes responsible for transcription by RNA polymerases I, II and III. This diagram depicts the assembly of three alternative TBP/TAF complexes: SL1, TFIID, and TFIIIB that direct transcription by RNA pol I, II, and III respectively. The initial binding of a subunit from one of the classes of TAFs apparently can preclude the interaction of TAFs from heterologous class of subunits thus preventing the formation of 'mixed' complexes.

specify differential promoter recognition by RNA polymerases I, II and III (figure 8) (For review see Gill & Tjian 1992; Goodrich & Tjian 1994).

6. FUTURE DIRECTIONS

The discovery of TAFs and their role as coactivators take us substantially closer to unraveling the mechanisms that regulate transcription and gene expression. However, TAFs as targets of activators and possibly repressors do not fully explain the molecular events that must occur during transcriptional regulation. Many questions remain and several new ones emerge as we assimilate the newly acquired insights concerning TFIID, TAFs and coactivators. For example, what happens after an activator makes contact with a TAF in the TFIID complex? Is binding of TAFs to activators sufficient to account for activation? Perhaps stabilization of the initiation complex or possibly recruitment of the TFIID complex to the activated promoter is sufficient to trigger the events leading to transcriptional initiation. Alternatively, activator/TAF interactions might induce specific conformational changes in TFIID, which in turn catalyze structural changes in other components of the basal apparatus. What is the role of TAFs that bind DNA, such as $TAF_{II}150$? Do repressors that bind to silencer elements also interact with TAFs to block the formation of productive transcription initiation complexes? What is the nature of the TAF/basal factor interactions? Which basal factors are in direct contact with TAFs? Do some of the TAFs bind directly to subunits of RNA polymerase in the manner reminiscent of σ-factors? Finally, what is the three-dimensional structure of activation domains? If most activation domains are unstructured, as is currently suspected, how do they direct TAF specific interactions? Do TAFs, which appear to contain unique secondary and presumably tertiary structures, help to induce activators into assuming distinct three dimensional surfaces? These are only a few of the many questions that can now be addressed. Obviously to answer some of these questions a combination of molecular genetics, biochemistry and structural techniques will be required. The challenge for the future then is to bring all of these tools of modern biology to bear on the critically important issue of how genes are regulated. Understanding transcriptional regulation will very likely have an impact on many other areas of biology (such as DNA replication, DNA repair, oncogenesis, signal transduction, etc.), and increasingly, problems in medicine are recognized to have a specific transcriptional regulatory component. We anticipate that within the next decade a great deal more will be learned about the process of transcriptional regulation and gene expression.

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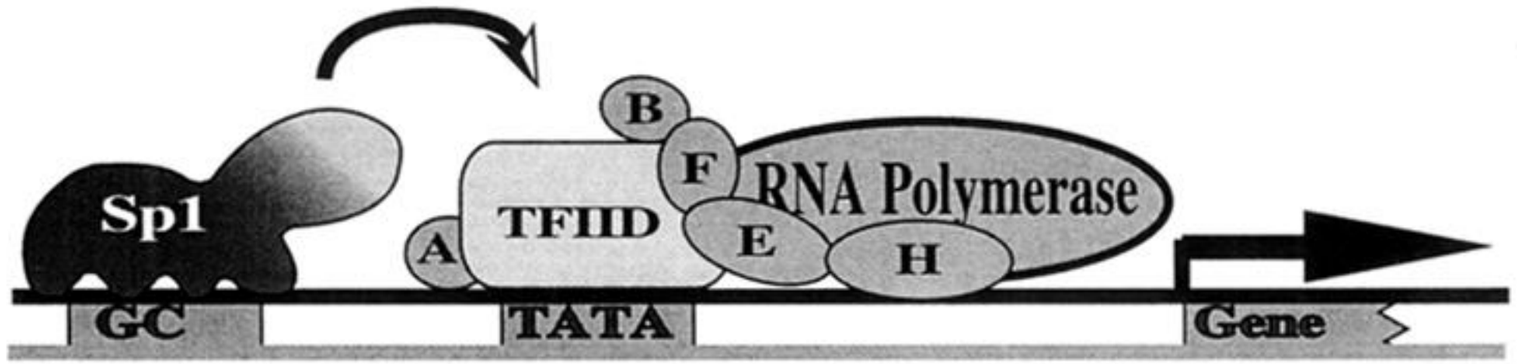
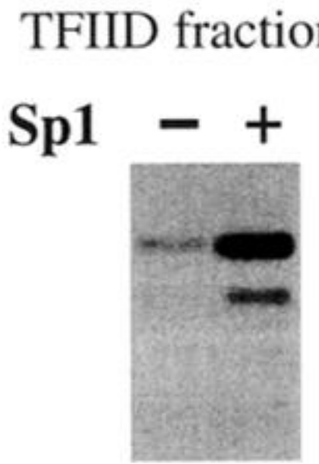
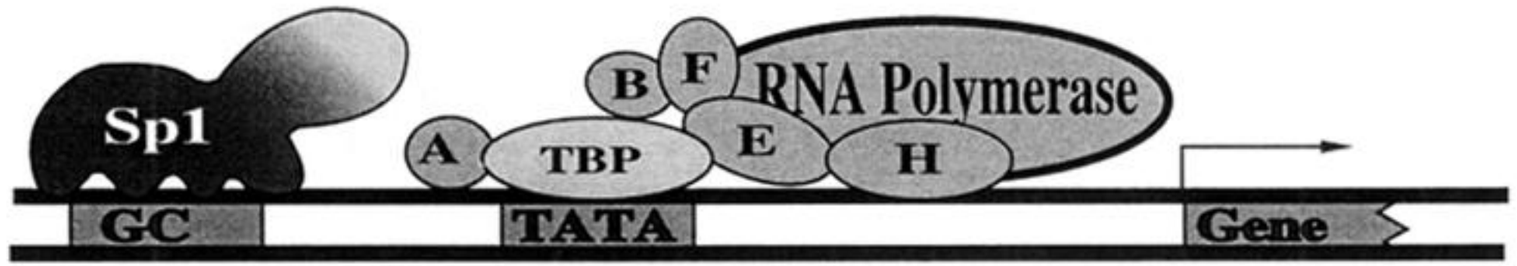
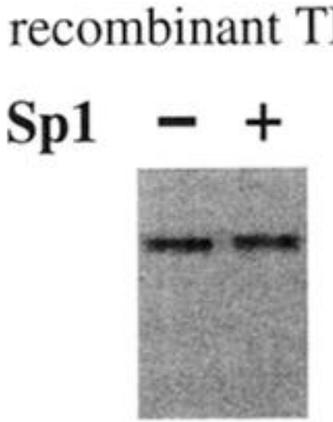
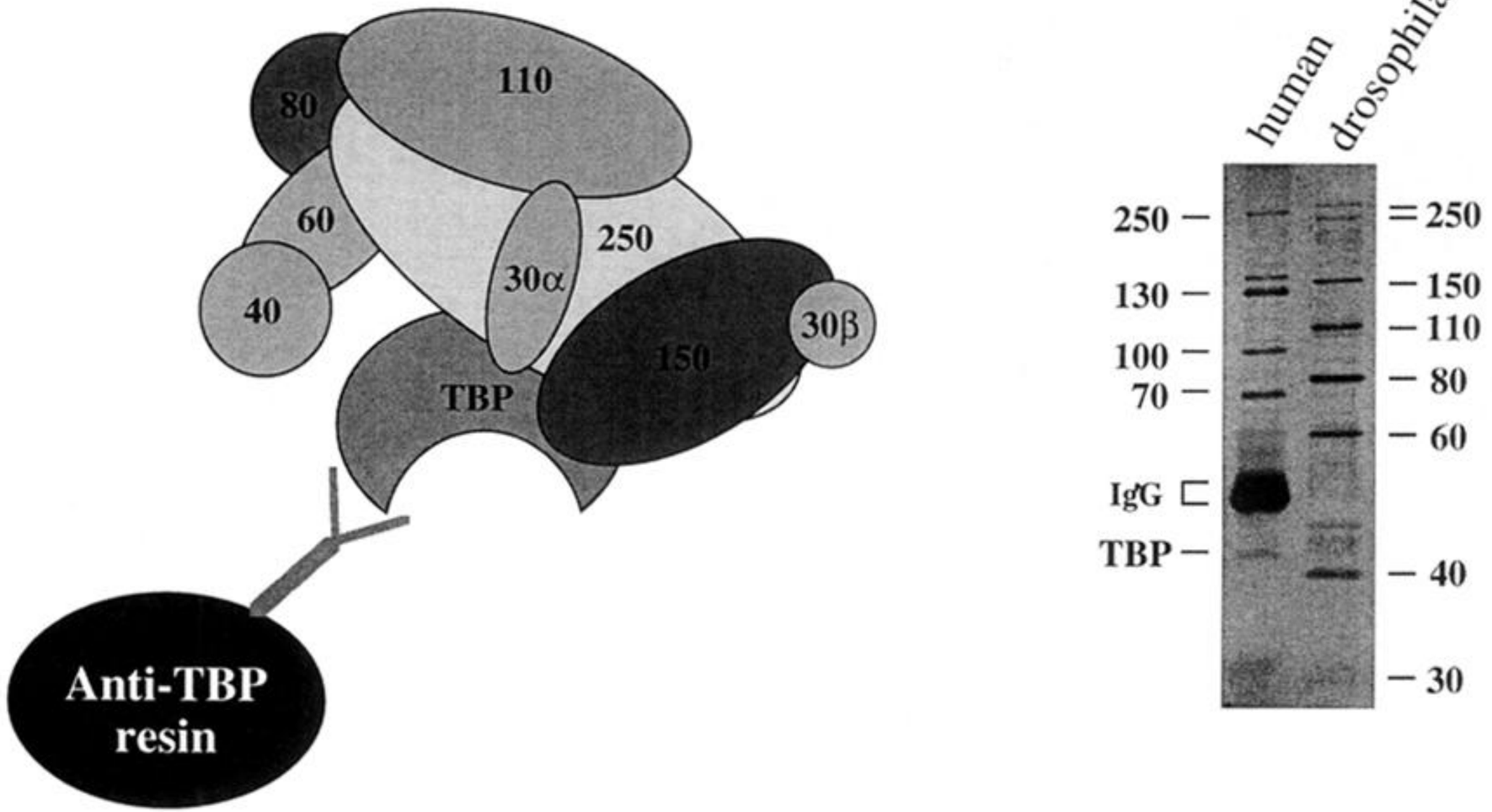








gure 3. Coactivator requirements for transcriptional activation by Sp1. Top panel: *in vitro* reconstituted anscription reactions were performed with RNA pol II and chromatographic fractions containing the basal factors cluding TFIID which is required to mediate activation of transcription by Sp1. Bottom panel: if the TFIID fraction replaced by purified recombinant TBP, Sp1 is no longer able to activate transcription, suggesting that one or more mponents, called co-activators, that are associated with TFIID mediates activation.



gure 4. Purification and identification of TBP associated factors (TAFs). Using anti-TBP affinity chromatography, was possible to immunopurify the TFIID complex from both human and Drosophila to homogeneity. The panel the right shows a silver stained SDS polyacrylamide gel of immunopurified complexes, which contains TBP and least eight TAFs ranging in molecular mass from 250–30 kDa that are subunits of Drosophila and human TFIID. he panel on the left shows a diagram depicting the various TBP-TAF and TAF-TAF interactions that have been entified from a series of protein:protein binding assays carried out *in vitro*.

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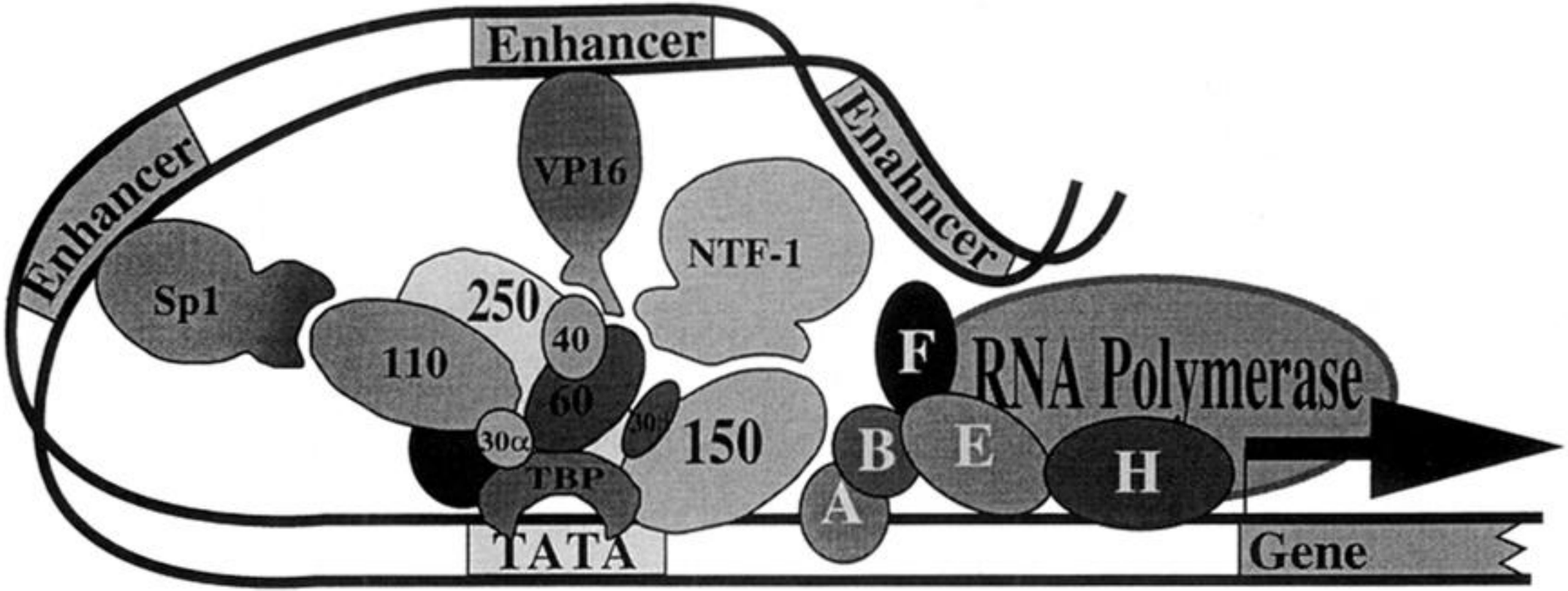
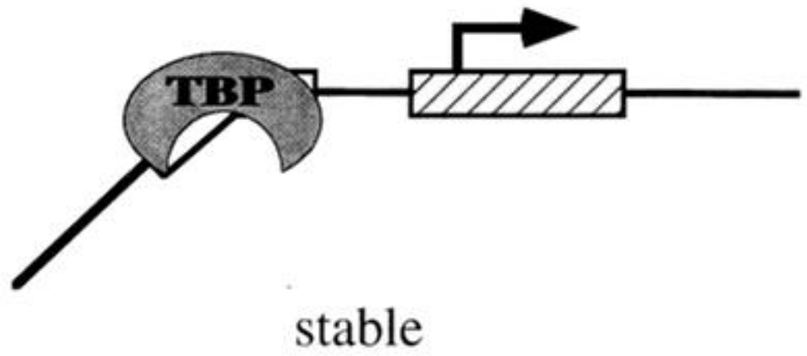


gure 5. A hypothetical model for the assembly of an activated initiation complex. This diagram illustrates multiple stinct activators (Sp1, VP16, and NTF-1) bound to tandem enhancers on the template, each interacting with ecific TAF targets in the TFIID complex to mediate activation of transcription.



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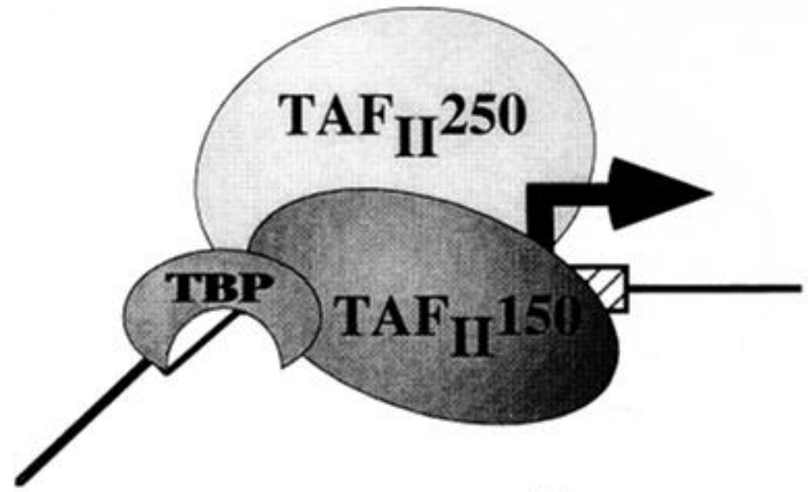

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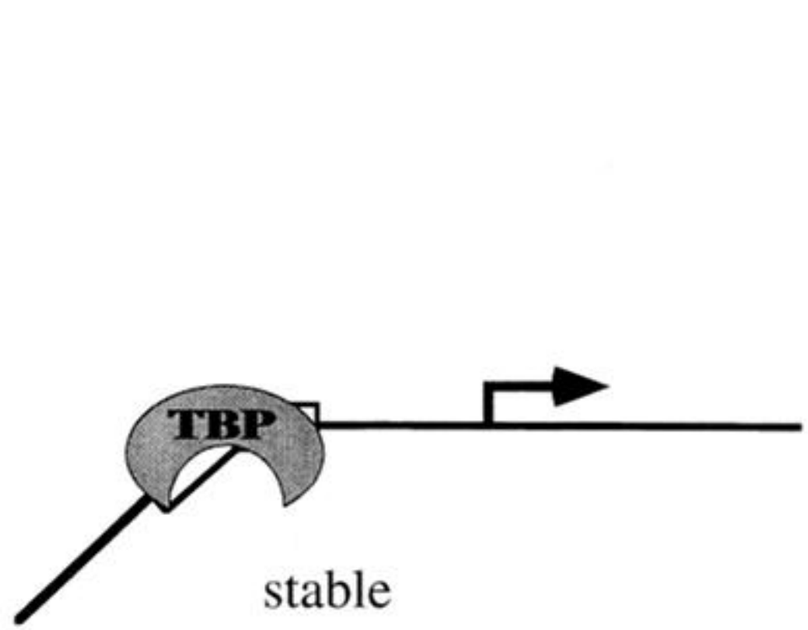

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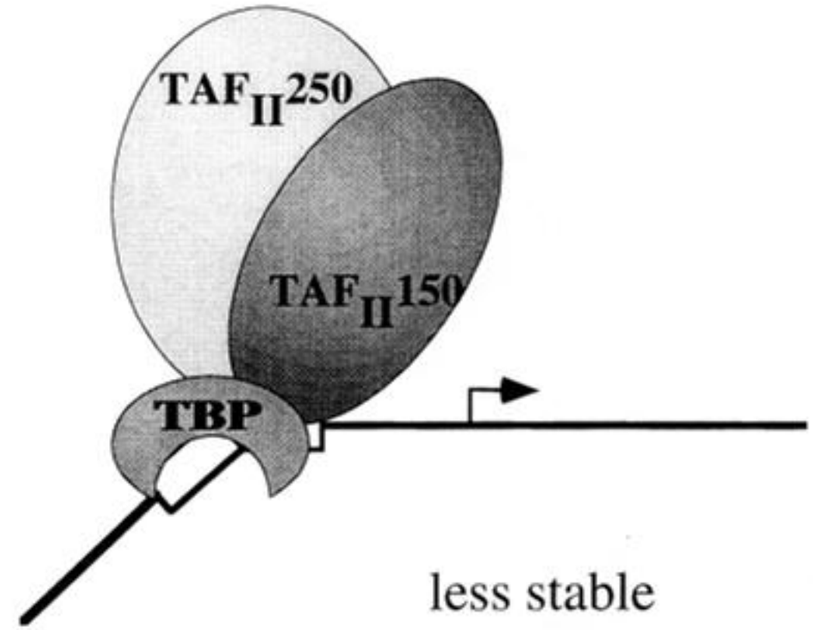
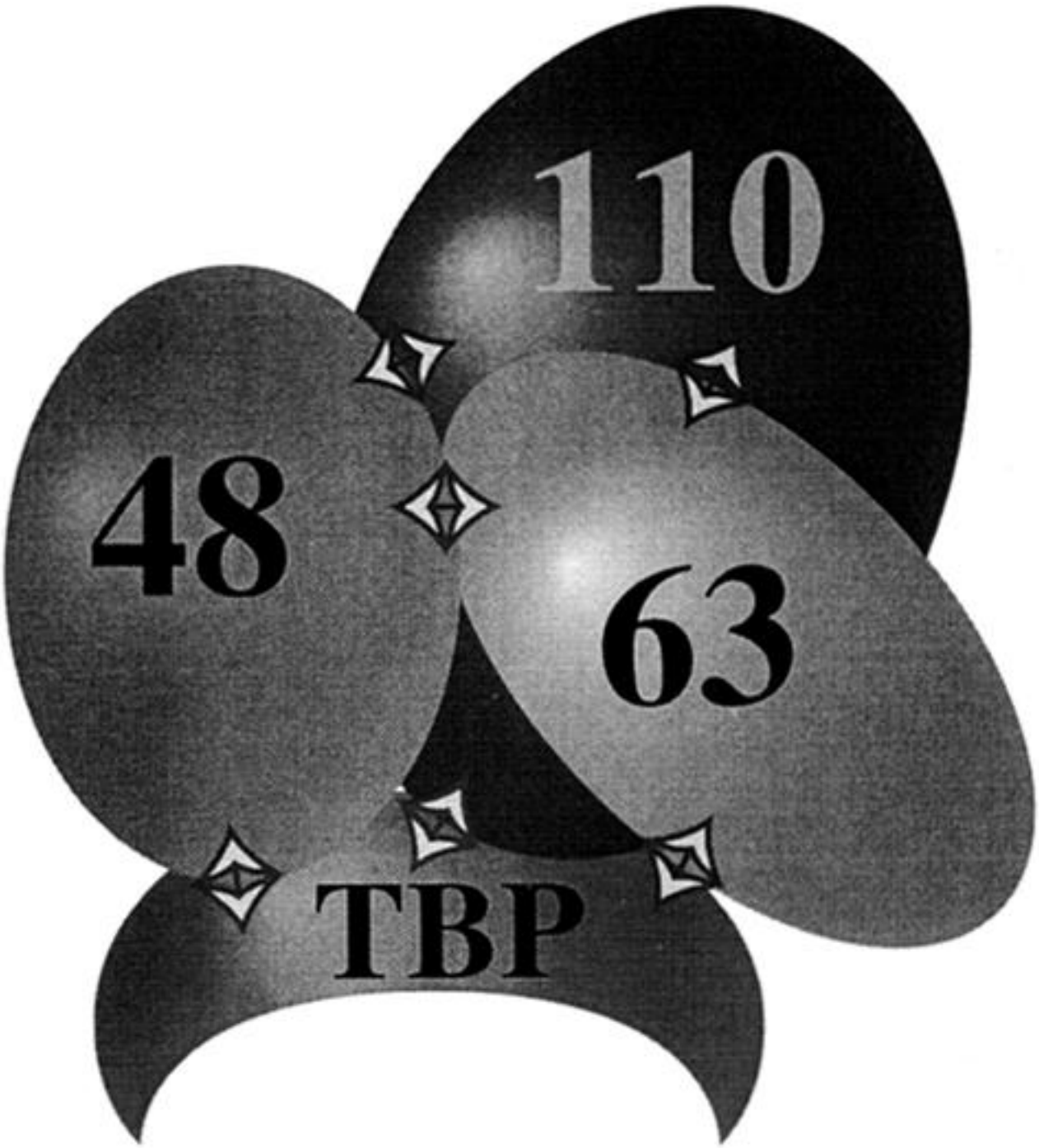


less stable

gure 6. A schematic model for core promoter recognition by RNA polymerase II TAFs. TBP, TAF$_{II}$250 and AF$_{II}$150 are indicated. The TATA box is represented by an open box, and the downstream regions of the core omoter, including the initiator, are represented by a hatched box. The arrow indicates the transcription start site. BP can bind stably to the TATA box and support equal levels of transcription either in the absence or presence of wnstream core promoter elements. In contrast, the TAFs in TFIID or a trimeric TBP, TAF$_{II}$250, TAF$_{II}$150 mplex can recognize these core promoter sequence, increasing the stability of binding, and mediate a higher level transcription. However, in the absence of the downstream elements, the TAFs can decrease the stability of TFIID omoter interactions, resulting in a reduced level of transcription.



gure 7. Subunit interactions between TAFs and TBP in the
1 complex. *In vitro* protein : protein binding assays revealed
at each of the TAF subunits of SL1 can make specific
ntacts with TBP as well as with each other. These
ultivalent interactions result in the formation of a stable
BP/TAF complex dedicated to the promoter selective
anscription of ribosomal RNA by RNA polymerase I.

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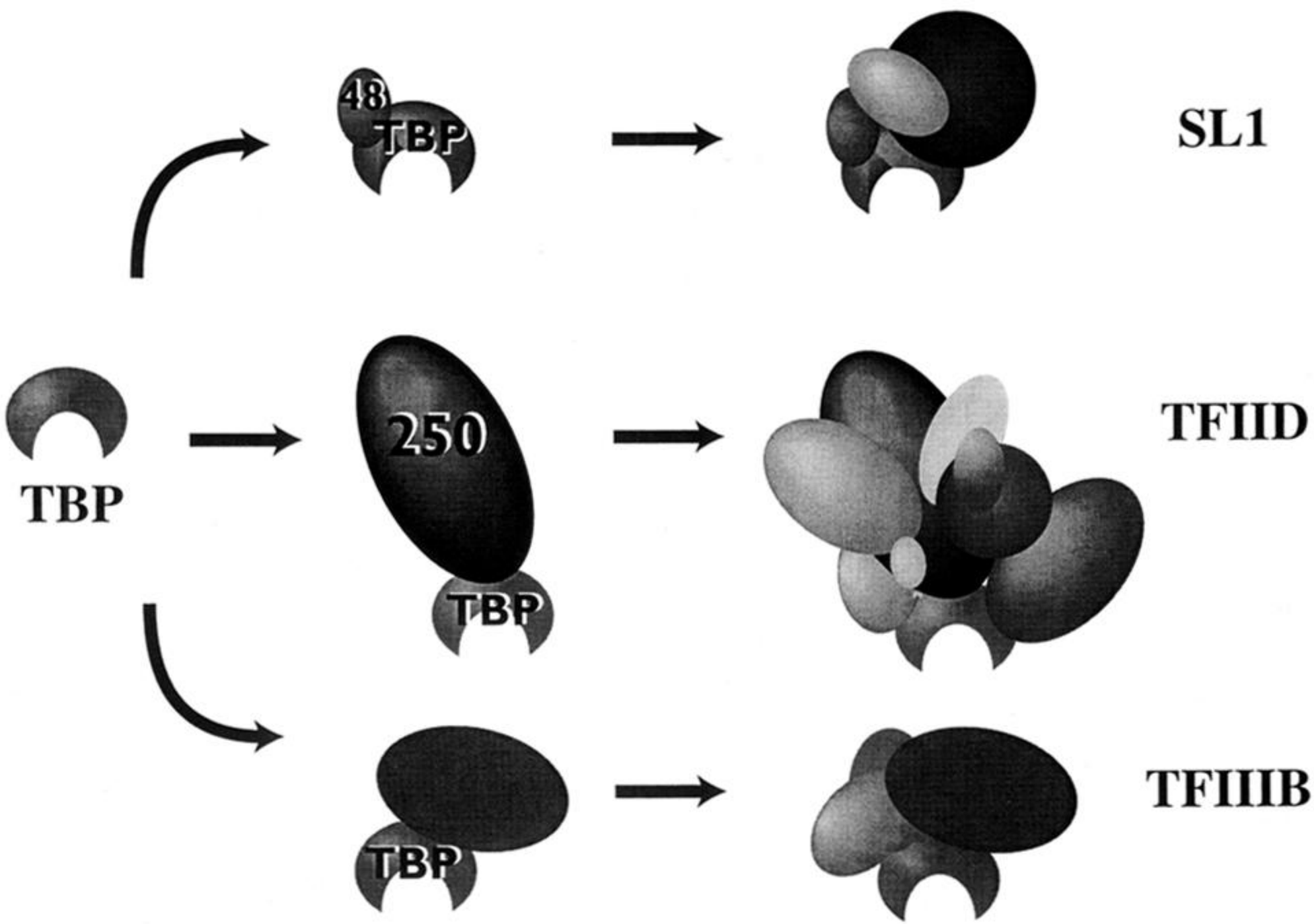

gure 8. TBP is an integral component of transcriptional initiation complexes responsible for transcription by RNA lymerases I, II and III. This diagram depicts the assembly of three alternative TBP/TAF complexes: SL1, TFIID, d TFIIIB that direct transcription by RNA pol I, II, and III respectively. The initial binding of a subunit from e of the classes of TAFs apparently can preclude the interaction of TAFs from heterologous class of subunits thus eventing the formation of 'mixed' complexes.