Cell, Vol. 84, 825–830, March 22, 1996, Copyright ©1996 by Cell Press

Contacts in Context: Promoter Specificity and Macromolecular Interactions in Transcription

Review

James A. Goodrich, Gene Cutler, and Robert Tjian
Howard Hughes Medical Institute
Department of Molecular and Cell Biology
University of California, Berkeley
Berkeley, California 94720

The coordinate and timely regulation of gene expression is essential to the proper growth and development of all organisms. Therefore, it is not surprising that transcription of protein-encoding genes is a finely tuned and highly controlled process. Work performed over the past decade has revealed much about the molecular machinery responsible for mRNA synthesis in eukaryotic cells. More than 40 different protein subunits function in a concerted manner to regulate transcription by RNA polymerase II at specific promoters in eukaryotic chromosomes. Recent experiments indicate that many of these transcription factors can interact with one another in highly specific ways to influence the activity of individual genes. Here we discuss recent insights in the field of RNA polymerase II transcription with an emphasis on aspects of promoter-specific recognition by the transcription machinery and mechanisms that govern transcription initiation.

The Eukaryotic and Prokaryotic Transcriptional Machinery: Similarities and Differences

In studying eukaryotic transcription, it is instructive to consider the analogous but less complex prokaryotic transcription systems. It is now well established that the level of transcription of any prokaryotic operon is intimately tied to the DNA sequence at the core promoter. Unregulated transcription initiation at many prokaryotic promoters requires only an RNA polymerase holoenzyme, consisting of four core subunits and a dissociable σ factor (Figure 1). It is the σ factor that interacts directly with the promoter DNA. The core enzyme cannot initiate transcription from promoters in the absence of a σ factor, but can elongate mRNA transcripts after initiation and dissociation of σ. Multiple σ factors have been identified, and each programs the core enzyme to transcribe from different classes of promoters and to respond to different types of transcriptional regulatory signals. Thus, σ factors are specificity factors for prokaryotic transcription, and a holoenzyme containing a specific σ factor can only function from a subset of promoters.

It seems likely that eukaryotic RNA polymerases will share some aspects of the core/σ factor architecture. Indeed, the core subunits of eukaryotic RNA polymerase II are able to catalyze RNA synthesis, but are not capable of gene-specific transcription. Instead, a host of accessory or general transcription factors (GTFs, including TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH) work in concert with RNA polymerase II to bring about promoter recognition and accurate transcription initiation (for review see Zawel and Reinberg, 1995). The complete set of GTFs (RNA polymerase II and TFIIA–TFIIH) is usually sufficient to direct basal levels of transcription in vitro from strong promoters (i.e., those containing TATA boxes). Thus, the eukaryotic transcriptional machinery is characterized by a core RNA polymerase and numerous accessory factors, which may as a group function in a capacity similar to prokaryotic σ factors.

Hidden Complexities of the Core Promoter

One important point that warrants special consideration is the diversity and uniqueness of core promoters utilized by RNA polymerase II. For example, not all mRNA promoters contain both a TATA box and an initiator element, the two core promoter elements identified so far. In addition, the sequences of these elements and the spacing between them vary significantly among RNA polymerase II promoters. The fact that core promoters are extremely diverse indicates that the interactions between the general transcription machinery and the promoter DNA will also vary from promoter to promoter. Observations made from a few isolated cases only serve to demonstrate possible mechanisms by which the RNA polymerase II machinery can recognize core promoter elements. General rules for predicting the relative activities of RNA polymerase II core promoters may eventually emerge once the functions of protein–DNA contacts at many different promoters have been analyzed.

Though no two core promoters are identical, it seemed reasonable to postulate that one or more subunits of the basal transcription machinery would contact one or more core promoter elements. The most prominent core promoter element thus far studied in detail is the TATA box, which is typically located upstream (−25 to −30) of the transcription initiation sites of many, but not all, eukaryotic genes. Indeed, one of the earliest examples of site-specific template recognition by a GTF came from the discovery that TFIID can bind to TATA boxes (Figure 2; for review see Hernandez, 1993). Subsequent studies identified a single subunit of TFIID as the TATA box–binding protein (TBP). This finding was consistent with TFIID being a eukaryotic RNA polymerase II promoter recognition factor. However, many eukaryotic promoters are TATA-less, and simple sequence comparisons did not reveal any highly conserved elements in TATA-less promoters that could serve in the absence of the TATA element as a tethering point for the transcriptional machinery.

A series of experiments performed by Smale and Baltimore (1989) identified a second core promoter element, the initiator. This element was initially identified in the TATA-less terminal deoxynucleotidyl transferase promoter, but has subsequently been found in many promoters of higher eukaryotes, both TATA-less and TATA containing. The initiator is a short, weakly conserved element that encompasses the transcription start site (for review see Weis and Reinberg, 1992). Mutational analysis demonstrated that this initiator element was important for directing the synthesis of properly initiated transcripts. But what GTF(s) recognizes the initiator? Once again, TFIID emerged as a likely candidate. It was

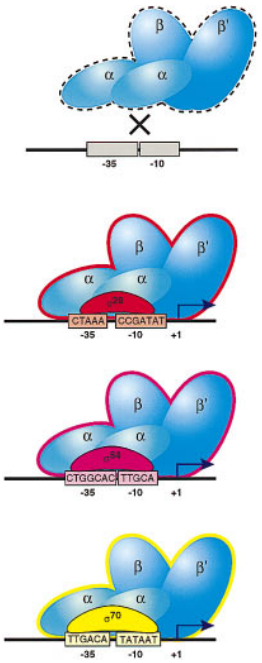

Figure 1. Different Prokaryotic RNA Polymerase Holoenzymes Recognize Distinct Promoter Sequences

Prokaryotic core RNA polymerases, consisting of four subunits, are not capable of promoter-specific transcription initiation. The association of one of the many prokaryotic σ factors with the core RNA polymerase results in an RNA polymerase holoenzyme that can recognize specific promoter sequences. For example, the Bacillus subtilis σ^{28}, the E. coli σ^{54}, and the E. coli σ^{70} subunits program RNA polymerase holoenzymes to initiate transcription from promoters with different DNA sequences.

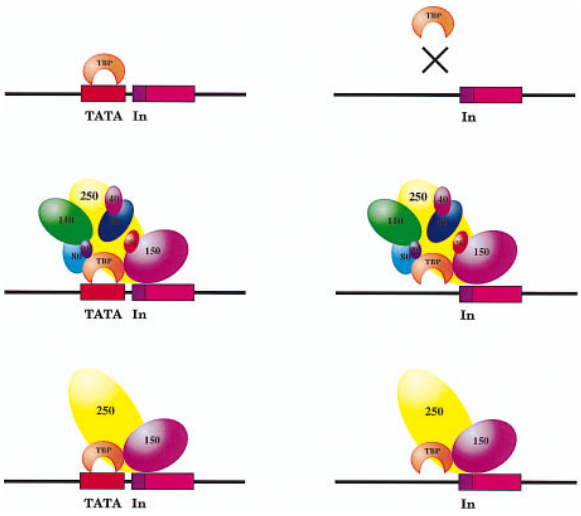

Figure 2. TFIID Is a Multifunctional Promoter Recognition Factor

The TBP subunit of TFIID binds specifically to TATA boxes found upstream of many, but not all, RNA polymerase II promoters. Whereas recombinant TBP cannot recognize TATA-less promoters, the complete TFIID complex can recognize some TATA-less promoters. Recent experiments demonstrate that the $TAF_{II}250$ and $TAF_{II}150$ subunits of TFIID can bind the initiator and downstream elements found in some RNA polymerase II promoters.

found that TFIID is required for transcription from TATA-less promoters and that recombinant TBP could not replace a TFIID fraction in directing transcription from TATA-less promoters containing initiator elements (Pugh and Tjian, 1990; Smale et al., 1990). Moreover, a series of footprinting experiments established that TFIID, but not TBP, could contact the initiator elements of both TATA-containing and TATA-less promoters (Kaufmann and Smale, 1994; Purnell and Gilmour, 1993). These results indicated that a single GTF, TFIID, could actually recognize two very distinct core promoter elements, the TATA box and the initiator.

Core Promoter Recognition Factors

Recent experiments have provided a possible explanation for the apparent multifunctional DNA binding properties of TFIID. It is now recognized that TFIID is actually a large and stable complex consisting of at least nine protein subunits, TBP and eight TBP-associated factors, or TAFs (for review see Goodrich and Tjian, 1994). Cross-linking studies with Drosophila TFIID (dTFIID) had suggested that several subunits of TFIID contact the initiator element DNA and that two of these subunits had apparent molecular masses of 60 and 150 kDa (Gilmour et al., 1990). The isolation of a cDNA encoding a TAF of molecular mass 150 kDa allowed experiments to be performed that tested the ability of recombinant $dTAF_{II}150$ to interact with the DNA around the start site of the adenovirus type 2 (Ad2) major late promoter (Verrijzer et al., 1994). Gratifyingly, this 150 kDa subunit of dTFIID alone was sufficient to recognize and bind to sequences overlapping the initiator of the Ad2 major late promoter. Indeed, a partial TFIID complex assembled from recombinant TBP, $dTAF_{II}150$, and $dTAF_{II}250$ selectively directed high levels of transcription only from templates containing wild-type initiator and downstream elements (Verrijzer et al., 1995). By contrast, recombinant TBP alone was not responsive to the presence of these elements in control experiments. Further support for the role of $dTAF_{II}150$ in promoter selection was obtained in a series of experiments aimed at understanding the molecular determinants responsible for the developmentally regulated selection of the distal over the proximal core promoter of the Drosophila alcohol dehydrogenase (*Adh*) gene in early embryos (Hansen and Tjian, 1995). Biochemical studies revealed that $dTAF_{II}150$ is required for differential *Adh* initiator recognition and thereby allows selective transcription from the distal promoter in response to developmental cues. Thus, dTFIID contains at least two DNA-binding proteins, TBP and $TAF_{II}150$, both of which can interact with core promoter elements and profoundly affect promoter utilization.

Although TFIID can be considered σ-like in that it is the GTF that interacts with the template at the core promoter during formation of RNA polymerase II transcription initiation complexes (for review see Zawel and Reinberg, 1995), TFIID is not the only GTF that contacts the promoter DNA. We presume that, at a minimum, subunits of RNA polymerase II itself must interact with the DNA in the region around and downstream of the transcriptional start site. In addition, TFIIH contains two subunits that are known to have helicase activity and,

as such, are likely to interact with the transcription template at some point during transcription initiation. Recent protein–DNA cross-linking experiments using the Ad2 major late promoter and purified basal transcription factors suggest that subunits of TFIIA, TFIIB, RNA polymerase II, and the small subunit of TFIIF (RNA polymerase II–associated protein 30 [RAP30]) are in close proximity to the promoter DNA, within 10 Å of the pyrimidine ring in the major groove (Coulombe et al., 1994). The C-terminus of RAP30 has also been found to contain a cryptic nonspecific DNA-binding domain that is essential for transcription (Tan et al., 1994).

Thus, it seems likely that many, if not most, of the GTFs have the potential to contact DNA during some steps of the transcription reaction. The roles of these potential multiple protein–DNA contacts in directing transcription from specific RNA polymerase II promoters remain to be determined. In addition, the functional importance of any potential protein–DNA contact on transcription will have to be examined in both the absence and presence of enhancer-binding proteins, because gene-specific transcriptional regulation is likely to result from the integration of multiple protein–DNA and protein–protein contacts within the extended regulatory DNA sequences of individual genes (i.e., core promoters and enhancer elements).

Signaling between Core Promoter Factors and Activators

Transcription from natural RNA polymerase II promoters is tightly controlled by the combined actions of positive and negative regulatory factors, including site-specific activators, repressors, and chromatin-associated proteins. For transcription of any gene, a rather complex array of signals must be integrated at the promoter to set the level of RNA production. One paradigm that has been established as a result of in vitro biochemical studies with RNA polymerase II transcriptional activators is the importance of protein–protein contacts between activators (more specifically, activation domains) and components of the general transcription machinery. Here too, parallels can be seen between eukaryotic and prokaryotic transcription systems. In Escherichia coli RNA polymerase, both the α and σ subunits have been identified as targets of transcriptional activators (for review see Busby and Ebright, 1994). To date, eukaryotic activators have been found to contact several GTFs, including subunits of TFIID, TFIIB, TFIIF, and TFIIH (for reviews see Triezenberg, 1995; Zawel and Reinberg, 1995). In addition, other cofactors for transcriptional activation, such as positive cofactor 4 (PC4), have been identified and found to interact with some transcriptional activators. However, demonstrating that an interaction can occur in isolation does not necessarily mean that the interaction will play a functional role in transcriptional activation. Indeed, it has been difficult to assess critically which of these putative interactions are essential for transcriptional activation.

Some coactivators and cofactors, including the TAFs, PC4, Dr2, and others, have been shown to be required for activated but not basal transcription (reviewed by Triezenberg, 1995; Zawel and Reinberg, 1995). This distinctive characteristic allows biochemical experiments to be performed that directly address the function of activator–coactivator interactions in mediating transcriptional activation. For example, the availability of recombinant TAFs and TBP allowed partial TFIID complexes containing subsets of the TAFs to be assembled and tested for activity in transcription experiments performed in vitro (Chen et al., 1994). The ability to assemble partial TFIID complexes containing select TAFs that were active in mediating transcriptional enhancement revealed an interesting correlation between the composition of a functional partial TFIID complex and the class of activators being used. For instance, complexes are only functional with transactivators such as Sp1 when they contain the subunit $dTAF_{II}110$, which is known to interact with glutamine-rich activation domains. Similarly, when present in partial TFIID assemblages, $TAF_{II}60$ and $TAF_{II}150$ render the complexes responsive to the activation domain of neurogenic element–binding transcription factor 1, or NTF1. Apparently, a complete set of eight TAFs is not required for activation by individual activators, at least in vitro. These observations provide strong evidence that activator–TAF contacts contribute an essential step during the process of transcriptional activation. Thus, the TFIID complex can be considered a multifaceted transmitter that receives signals from different classes of transcriptional activators and somehow relays signals to the basal transcription machinery that triggers activation from target promoters.

The observation that transcriptional activators target distinct components of the transcriptional machinery has interesting implications. One prediction is that mutations can be isolated in components of the general machinery that will affect activation by specific transcriptional activators. Indeed, such mutations have already been identified. For example, a single amino acid substitution in $TAF_{II}250$ (the ts13 mutation) reduces transcription of a subset of genes in vivo and severely decreases transcriptional activation by some, but not all, activators in vitro (Wang and Tjian, 1994). Interestingly, this mutation in $TAF_{II}250$ also causes hamster cells to be defective in progressing from G1 to S during the cell cycle (Hisatake et al., 1993). Another example is a double amino acid substitution in TFIIB that disrupts interaction between TFIIB and the transcriptional activation domain of the herpesvirus transactivator, virion protein 16 (VP16) (Roberts et al., 1993). The mutant TFIIB can replace wild-type TFIIB for basal transcription in vitro, but the transcription system is no longer responsive to the chimeric transcription factor GAL4–VP16. Curiously, the activation domain of VP16 has also been reported to interact with TBP. Mutations in yeast TBP that reduce levels of transcriptional activation by GAL4–VP16 in vitro have been identified, and one of these mutations inhibits the interaction between yeast TBP and the VP16 activation domain (Kim et al., 1994a). By contrast, a mutation in human TBP that disrupts interaction with GAL4–VP16 and GAL4–p53 did not affect transcriptional activation by these transactivators in mammalian cells (Tansey and Herr, 1995). These results suggest that the TBP–VP16 and TBP–p53 interactions identified in vitro may not be important for transcriptional activation in mammalian cells. Perhaps the mutation in yeast TBP (Kim et al., 1994a) disrupts not only interaction with the VP16 activation domain, but, in addition, another function(s) of TBP

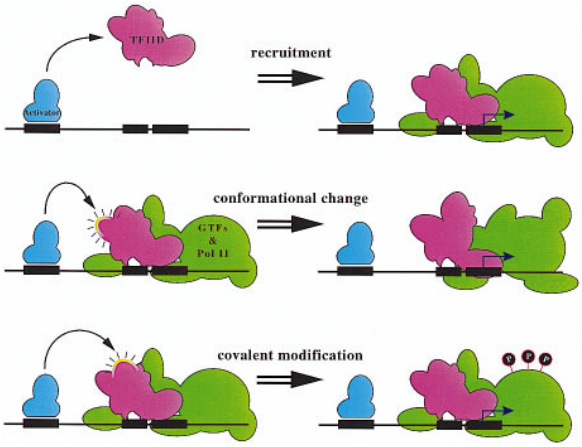

Figure 3. Proposed Mechanisms for RNA Polymerase II Transcriptional Activation

Shown are three of the possible mechanisms by which activators may increase levels of transcription from target promoters: recruiting TFIID or other GTFs to the promoter; causing a conformational change in the nucleoprotein initiation complex; and stimulating covalent modifications (such as phosphorylation) on components of the preinitiation complex.

required for activated but not basal transcription, for example association with TAFs.

Despite potential differences between yeast and mammalian transcription systems, yeast genetics should provide a useful tool for identifying functional contacts between activators and the general transcription machinery. For example, in yeast it should be possible to screen for allele-specific suppressors of down mutations in transcriptional activation domains. In addition, recent experiments with the *GAL11P* mutant yeast strain have demonstrated that a mutation in a component of the transcription machinery can create a fortuitous contact between the transcription machinery and normally inert regions of a sequence-specific transcription factor (Barberis et al., 1995). In this case, the yeast carries a single point mutation in GAL11P, a protein that has been found associated with a complex containing RNA polymerase II. Interestingly, this point mutation allows a contact to form between GAL11P and a region of the yeast GAL4 protein that is normally not involved in transcriptional activation. As a result, the normally defunct form of GAL4 becomes resurrected as a transcriptional activator, once again confirming the importance of protein–protein contacts in mediating transcriptional activation.

What Is Transcriptional Activation?

It is highly likely that a direct binding or contact between an activator and GTFs constitutes an early step in the process of transcriptional activation. But how do protein–protein contacts produce enhanced levels of transcription from target promoters? Here we will consider general mechanisms by which activator interactions with the general transcription machinery could result in increased levels of transcription (Figure 3). First, it is possible that in some cases activators function to recruit a GTF to the promoter directly. Indeed, evidence for this recruitment model is amassing. For example, activators have been shown to recruit TFIID, TFIID/TFIIA, and TFIIB to promoters (Klein and Struhl, 1994; Lieberman and Berk, 1994; Lin and Green, 1991; Sauer et al., 1995; Wang et al., 1992). The recruitment of these factors correlates with activator–GTF interactions and transcriptional activation and is thought to direct more efficient assembly of preinitiation complexes. Such a mechanism would most likely affect promoters with weak core DNA elements.

An activator–GTF contact may also alter the conformation of the existing component(s) of partially assembled preinitiation complexes (Figure 3B). The conformational change could be imparted to the promoter DNA, to a protein in the preinitiation complex, or to multiple components of the nucleoprotein complex assembled at the promoter. A conformational change could in turn cause subsequent events, such as the binding of other GTFs, to occur more efficiently. Consistent with this allosteric model is the finding that the sensitivity of TFIIB to proteolysis is altered in the presence of the VP16 activation domain (Roberts and Green, 1994). Another potential target that falls into this category of activation is TFIIH, a GTF known to have helicase activity that can affect transcription from some promoters. Interestingly, TFIIH has also been found to be a target of certain transcriptional activators. Perhaps the interaction between an activator or repressor and TFIIH can result in the TFIIH helicase changing the conformation of the DNA in the open complex, thereby increasing or decreasing transcription from the target promoter.

Finally, it is possible that transcriptional regulators can stimulate covalent modifications of the GTFs that have a positive or negative effect on transcription levels at target promoters. The activators themselves do not have to have enzymatic activity, but simply amplify the activity of enzymes, such as protein kinases that are associated with initiation complexes. Three kinases whose activities could potentially be affected by transcriptional activators are the Cdk7/MO15 subunits of TFIIH (Serizawa et al., 1995; Shiekhattar et al., 1995), the kinase/cyclin pair found associated with RNA polymerase II in yeast (Liao et al., 1995), and most recently the $TAF_{II}250$ subunit of TFIID, which was found to contain serine kinase activity (Dikstein et al., 1996). The first two of these kinases can phosphorylate the C-terminal domain of the large subunit of RNA polymerase II. By contrast, the bipartite kinase domain of $TAF_{II}250$ selectively phosphorylates the large subunit of TFIIF, RAP74, which binds to RNA polymerase II and is thought to remain associated with the enzyme during elongation. Perhaps at some promoters the activity of one or more of these kinases can be enhanced in response to signals from activators. The phosphorylation of the C-terminal domain, RAP74, and possibly other GTFs may in turn trigger subsequent events, such as the release of factors from preinitiation complexes or the clearance of RNA polymerase II from the promoter. The discovery that multiple subunits of the general transcription machinery contain kinase activities indicates that the regulated synthesis of mRNA in eukaryotic cells results from a complex process involving not only protein–protein contacts, but also covalent modifications.

Eukaryotic RNA Polymerase II Holoenzymes

The term "holoenzyme" has recently been used to describe RNA polymerase II–containing complexes isolated from yeast (Kim et al., 1994b; Koleske and Young, 1994) and mammals (Ossipow et al., 1995). The implication derived from the use of this terminology is that eukaryotes have a stable entity containing RNA polymerase II and all factors required to initiate transcription from RNA polymerase II promoters. However, the isolated RNA polymerase II holoenzymes do not appear to be complete in that the yeast complexes do not contain all of the factors required for promoter-specific transcription, and the complex isolated from mammalian nuclei has not yet been found to be responsive to transcriptional activators. We will therefore refer to these stable, but possibly incomplete, assemblages as RNA polymerase II complexes (RPCs). At present, a unified view of the protein components of the isolated yeast and mammalian RPCs is not available, nor has it been established how many different kinds of RPCs are present in eukaryotic nuclei. Even in prokaryotic cells, there are distinct RNA polymerase holoenzymes, only one of which is required to drive transcription from any one promoter. Since the eukaryotic transcriptional machinery is much more complex than its prokaryotic counterpart, there are likely to be many distinct assemblages consisting of core RNA polymerase II, GTFs, and associated factors. It will be of interest in the future to determine what functions these RNA polymerase II–associated proteins perform and how they influence transcriptional regulation. In the meantime, the discovery of RPCs has led us to reconsider potential mechanisms of transcriptional initiation and activation at eukaryotic promoters.

It should be noted that all of the protein–DNA contacts in the core promoter were identified using recombinant and highly purified GTFs. Under these conditions, preinitiation complexes can assemble in an ordered fashion, with GTFs binding the promoter in the following order: TFIID, TFIIA, TFIIB, TFIIF/polymerase II, TFIIE, and TFIIH. The isolation of stable RPCs containing GTFs has called into question the paradigm of ordered assembly of preinitiation complexes and fuels speculation that some of the identified core promoter–GTF interactions may not be important to transcription in the context of holoenzyme complexes. For example, the isolated yeast RPCs do not contain TAFs. Therefore, it will be interesting to see if the *TSM1* protein, the yeast homolog of $dTAF_{II}150$, plays a role in core promoter recognition.

How does the discovery of eukaryotic RPCs influence our understanding of transcriptional regulation? Earlier results demonstrated that preinitiation complexes could be assembled in vitro on test promoters by the sequential addition of GTFs. It was thought that activators might function to increase the assembly of active transcription complexes at promoters. With the isolation of RPCs in eukaryotes came the proposal that contact between a DNA-bound activator and any surface of the RPC may result in recruitment of the transcriptional machinery to a promoter. That this mode of activation is possible is supported by the GAL11P mutation, in which increased levels of transcription result merely from a fortuitous contact between GAL4 and the mutant form of GAL11, a protein associated with a yeast RPC (Barberis et al., 1995). Whether the complete assembly of RNA polymerase II and GTFs occurs at a promoter or in the nucleoplasm unattached to DNA, it seems reasonable to speculate that transcription initiation at eukaryotic promoters will result from an ordered series of steps. In prokaryotic transcription systems, in which holoenzyme compositions are well understood, the range of mechanisms for regulating transcription is enormous and includes alterations in the following steps: assembly of holoenzymes, association of polymerase with the promoter during closed complex formation, isomerization of closed to open complexes, clearance of the polymerase from the promoter, and regulation of the frequency of pausing and termination during mRNA synthesis. Although generalizations can be made, it is prudent to emphasize that each prokaryotic promoter is unique and that the limiting step in transcription from each promoter is dependent upon the DNA sequence and the availability of protein factors. Given the increased complexity of the eukaryotic transcriptional machinery and the diversity of eukaryotic promoters, not to mention the involvement of chromatin, we expect that even more elaborate mechanisms of transcriptional regulation will be identified in eukaryotes.

Concluding Remarks

Here we have provided a brief account of some recent advances in the study of eukaryotic transcriptional regulation. It now appears that at least a portion of the eukaryotic RNA polymerase II resides in preassembled complexes containing various GTFs and multiple other protein subunits. One of the GTFs, TFIID, is a multifunctional complex that participates in core promoter DNA recognition of both TATA boxes and initiator elements, while also acting as a mediator of transcriptional activation by contacting activation domains. The importance of protein–protein contacts in transcriptional activation is now well documented, but how these interactions ultimately result in enhanced levels of transcription will need further study. Finally, we must remember that most natural promoters contain complex regulatory regions with unique core promoter sequences and binding sites for multiple distinct activators and repressors. It is therefore increasingly evident that future studies of the intricacies of transcriptional regulation should include more detailed examinations of the role of gene-specific promoter elements and macromolecular contacts in directing transcription from native RNA polymerase II promoters.

References

Barberis, A., Pearlberg, J., Simkovich, N., Farrell, S., Reinagel, P., Bamdad, C., Sigal, G., and Ptashne, M. (1995). Contact with a component of the polymerase II holoenzyme suffices for gene activation. Cell *81*, 359–368.

Busby, S., and Ebright, R.H. (1994). Promoter structure, promoter recognition, and transcriptional activation in prokaryotes. Cell *79*, 743–746.

Chen, J.-L., Attardi, L.D., Verrijzer, C.P., Yokomori, K., and Tjian, R. (1994). Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. Cell *79*, 93–105.

Coulombe, B., Li, J., and Greenblatt, J. (1994). Topological localization of the human transcription factors IIA, IIB, TATA box-binding protein, and RNA polymerase II-associated protein 30 on a class II promoter. J. Biol. Chem. *269*, 19962–19967.

Dikstein, R., Ruppert, S., and Tjian, R. (1996). $TAF_{II}250$ is a bipartite protein kinase that phosphorylates the basal transcription factor RAP74. Cell *84*, in press.

Gilmour, D.S., Dietz, T.J., and Elgin, S.C.R. (1990). UV crosslinking identifies four polypeptides that require the TATA box to bind to the *Drosophila* hsp70 promoter. Mol. Cell. Biol. *10*, 4233–4238.

Goodrich, J.A., and Tjian, R. (1994). TBP-TAF complexes: selectivity factors for eukaryotic transcription. Curr. Opin. Cell Biol. *6*, 403–409.

Hansen, S.K., and Tjian, R. (1995). TAFs and TFIIA mediate differential utilization of the tandem *Adh* promoters. Cell *82*, 565–575.

Hernandez, N. (1993). TBP, a universal eukaryotic transcription factor? Genes Dev. *7*, 1291–1308.

Hisatake, K., Hasegawa, S., Takada, R., Nakatani, Y., Horikoshi, M., and Roeder, R.G. (1993). The p250 subunit of native TATA-binding factor TFIID is the cell-cycle regulatory protein CCG1. Nature *362*, 179–181.

Kaufmann, J., and Smale, S.T. (1994). Direct recognition of initiator elements by a component of the transcription factor IID complex. Genes Dev. *8*, 821–829.

Kim, T.K., Hashimoto, S., Kelleher, R.J., III, Flanagan, P.M., Kornberg, R.D., Horikoshi, M., and Roeder, R.G. (1994a). Effects of activation-defective TBP mutations on transcription initiation in yeast. Nature *369*, 252–255.

Kim, Y.-J., Björklund, S., Li, Y., Sayre, M.H., and Kornberg, R.D. (1994b). A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell *77*, 599–608.

Klein, C., and Struhl, K. (1994). Increased recruitment of TATA-binding protein to the promoter by transcriptional activation domains *in vivo*. Science *266*, 280–282.

Koleske, A.J., and Young, R.A. (1994). An RNA polymerase II holoenzyme responsive to activators. Nature *368*, 466–469.

Liao, S.M., Zhang, J., Jeffery, D.A., Koleske, A.J., Thompson, C.M., Chao, D.M., Viljoen, M., van Vuuren, H.J., and Young, R.A. (1995). A kinase-cyclin pair in the RNA polymerase II holoenzyme. Nature *374*, 193–196.

Lieberman, P.M., and Berk, A.J. (1994). A mechanism for TAFs in transcriptional activation: activation domain enhancement of TFIID-TFIIA-promoter DNA complex formation. Genes Dev. *8*, 995–1006.

Lin, Y.-S., and Green, M.R. (1991). Mechanism of action of an acidic transcriptional activator in vitro. Cell *64*, 971–981.

Ossipow, V., Tassan, J.-P., Nigg, E.A., and Schibler, U. (1995). A mammalian RNA polymerase II holoenzyme containing all components required for promoter-specific transcription initiation. Cell *83*, 137–146.

Pugh, B.F., and Tjian, R. (1990). Mechanism of transcriptional activation by Sp1: evidence for coactivators. Cell *61*, 1187–1197.

Purnell, B.A., and Gilmour, D.S. (1993). Contribution of sequences downstream of the TATA element to a protein/DNA complex containing the TATA-binding protein. Mol. Cell. Biol. *13*, 2593–2603.

Roberts, S.G., and Green, M.R. (1994). Activator-induced conformational change in general transcription factor TFIIB. Nature *371*, 717–720.

Roberts, S.G.E., Ha, I., Maldonada, E., Reinberg, D., and Green, M.R. (1993). Interaction between an acidic activator and transcription factor IIB is required for transcriptional activation. Nature *363*, 741–744.

Sauer, F., Hansen, S.K., and Tjian, R. (1995). Multiple TAFIIs directing synergistic activation of transcription. Science *270*, 1783–1788.

Serizawa, H., Makela, T.P., Conaway, J.W., Conaway, R.C., Weinberg, R.A., and Young, R.A. (1995). Association of Cdk-activating kinase subunits with transcription factor TFIIH. Nature *374*, 280–282.

Shiekhattar, R., Mermelstein, F., Fisher, R.P., Drapkin, R., Dynlacht, B., Wessling, H.C., Morgan, D.O., and Reinberg, D. (1995). Cdk-activating kinase complex is a component of human transcription factor TFIIH. Nature *374*, 283–287.

Smale, S.T., and Baltimore, D. (1989). The "initiator" as transcription control element. Cell *57*, 103–113.

Smale, S.T., Schmidt, M.C., Berk, A.J., and Baltimore, D. (1990). Transcriptional activation by Sp1 as directed through TATA or initiator: specific requirement for mammalian transcription factor IID. Proc. Natl. Acad. Sci. USA *87*, 4509–4513.

Tan, S., Pfeil, K., Conaway, R., and Conaway, J.W. (1994). Cryptic DNA-binding domain in the C terminus of RNA polymerase II general transcription factor RAP30. Proc. Natl. Acad. Sci. USA *91*, 9808–9812.

Tansey, W.P., and Herr, W. (1995). The ability to associate with activation domains *in vitro* is not required for the TATA box-binding protein to support activated transcription *in vivo*. Proc. Natl. Acad. Sci. USA *92*, 10550–10554.

Triezenberg, S.J. (1995). Structure and function of transcriptional activation domains. Curr. Opin. Genet. Dev. *5*, 190–196.

Verrijzer, C.P., Yokomori, K., Chen, J.-L., and Tjian, R. (1994). *Drosophila* $TAF_{II}150$: similarity to yeast gene TSM-1 and specific binding to core promoter DNA. Science *264*, 933–941.

Verrijzer, C.P., Chen, J.-L., Yokomori, K., and Tjian, R. (1995). Binding of TAFs to core elements directs promoter selectivity by RNA polymerase II. Cell *81*, 1115–1125.

Wang, E.H., and Tjian, R. (1994). Promoter selective transcriptional defect in cell cycle mutant ts13 rescued by $hTAF_{II}250$ *in vitro*. Science *263*, 811–814.

Wang, W., Gralla, J.D., and Carey, M. (1992). The acidic activator GAL4-AH can stimulate polymerase II transcription by promoting assembly of a closed complex requiring TFIID and TFIIA. Genes Dev. *6*, 1716–1727.

Weis, L., and Reinberg, D. (1992). Transcription by RNA polymerase II: initiator-directed formation of transcription competent complexes. FASEB J. *6*, 3300–3309.

Zawel, L., and Reinberg, D. (1995). Common themes in assembly and function of eukaryotic transcription complexes. Annu. Rev. Biochem. *64*, 533–561.