Cell, Vol. 84, 179–182, January 26, 1996, Copyright ©1996 by Cell Press

Chromatin Structure and RNA Polymerase II Connection: Implications for Transcription

Minireview

Kevin Struhl
Department of Biological Chemistry and Molecular Pharmacology
Harvard Medical School
Boston, Massachusetts 02115

Activator proteins bind to enhancer elements of eukaryotic promoters and stimulate transcription by RNA polymerase II (pol II). How does this highly conserved process of transcriptional activation occur in living organisms under physiological conditions? Because activated transcription can be reconstituted in vitro with highly purified protein factors and DNA templates, a large part of the answer lies in the interactions between activator proteins and components of the pol II machinery. However, the physiological significance and relative importance of these protein–protein interactions are poorly understood. In addition, chromatin is the physiological template, and biochemical and genetic evidence indicate that chromatin structure plays an active role in transcription.

Although there is a great deal of information on the relationship between chromatin structure and transcriptional activation, molecular mechanisms connecting them have been elusive. In this issue of *Cell*, Wilson et al. (1996) have uncovered a surprising and provocative connection. Specifically, the pol II holoenzyme contains stoichiometric amounts of Swi/Snf, a multiprotein complex with the capability to disrupt nucleosomes. This provides an obvious link between protein contact and chromatin models for activation that are typically viewed as distinct. In this minireview, I discuss three views of transcriptional activation from the perspective of what occurs under physiological conditions. Studies in yeast will be emphasized, as this organism is ideal for performing experiments under conditions in which all proteins are present at physiological concentrations and the DNA template is in the form of chromatin. The combination of genetic and biochemical analysis, performed primarily in flies and mammals, has lead to a coherent framework for understanding the mechanism of transcriptional activation.

A TFIID-Centered View of Transcriptional Activation

The minimal set of protein factors necessary for accurate transcription in vitro (TFIIB, TFIID, TFIIE, TFIIF, TFIIH, pol II, and, to a lesser extent, TFIIA) can assemble in a stepwise fashion on a promoter (Figure 1). The first step in this process is binding to the TATA element by TFIID, a complex containing the TATA-binding protein (TBP) and approximately ten TBP-associated factors (TAFs). In most organisms, TFIID is extremely stable and hence is the form of TBP that interacts with TATA elements in vivo. In yeast, it is unclear whether TFIID or TBP (or both) is the TATA-binding entity in vivo, because TBP can be easily separated from the TAFs.

In vitro transcription experiments strongly implicate TAFs as being specifically involved in the response to activators (Tjian and Maniatis, 1994). TFIID and TBP support comparable levels of "basal" transcription, but only TFIID can respond to activators in in vitro transcription assays. Moreover, different classes of activation domains interact with distinct TAFs, and there is an excellent correlation between the presence of the relevant TAF and the ability to stimulate transcription. Activator proteins can stimulate formation of a TFIID–TFIIA–TATA element complex (Chi et al., 1995), and multiple contacts between activation domains and TAFs can strongly increase TFIID binding to the TATA element and synergistically activate transcription (Sauer et al., 1995). The affects of TAFs on the assembly of an activator-dependent transcription complex can also occur after binding of TFIID to the TATA element (Choy and Green, 1993).

In yeast, recruitment of TBP (or TFIID) to the TATA element is an important step in transcriptional activation under physiological conditions (Struhl, 1995). Activation domains can stimulate recruitment of TBP to promoters, and artificial recruitment of TBP by physical connection to a promoter-bound protein bypasses the need for an activation domain. TBP mutants specifically defective in the response to activators are impaired in their interactions with TATA elements or TFIIA, a protein that stabilizes the TBP–TATA interaction. An efficient TBP–TATA interaction is essential for the response to strong activators, because weak TATA elements are functionally saturated at lower levels of activation. In accord with these results, TBP binds extremely poorly to TATA elements in chromatin templates.

Although the activation mechanism in yeast cells clearly involves TBP and TFIIA, the role of TAFs has yet to be demonstrated in vivo. TAFs are essential for cell growth, but it is unclear whether this reflects a general requirement for activated transcription or a more specialized function. In this regard, it is striking that TAF mutations have not been identified in the numerous genetic screens for mutations that affect transcription. In

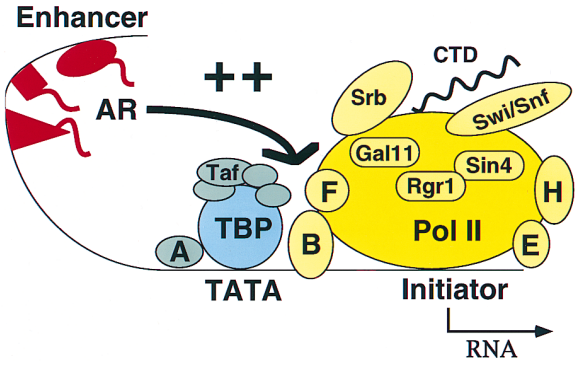

Figure 1. Proteins at Typical Eukaryotic Promoter

Activators (red) bound to enhancer elements stimulate transcription via activation domains (wavy lines) by protein–protein interactions (arrow) with components of TFIID (blue) and the pol II holoenzyme (yellow). Of the basic factors defined by in vitro transcription, TFIIA is considered here as part of the TFIID group, whereas TFIIB, TFIIE, TFIIF, TFIIH, and core pol II are considered part of the pol II holoenzyme.

contrast, mutations have been identified in other TBP-interacting proteins, basic transcription factors, components of the pol II holoenzyme, and histones.

A Pol II Holoenzyme–Centered View of Transcriptional Activation

In yeast cells, a considerable portion of pol II is found in a large multiprotein complex termed the pol II holoenzyme (Koleske and Young, 1995). Although its precise composition varies among individual preparations, it is clear that the pol II holoenzyme contains most of the basic transcription factors. This suggests that active transcription complexes may be formed on promoters by recruitment of a preassembled complex rather than by stepwise recruitment of individual components. However, it will be extremely difficult to prove whether such preassembled complexes truly associate with promoters in vivo or whether they represent stable complexes formed by sequential assembly of individual factors or subcomplexes. By either assembly pathway, the relevant protein–protein and protein–DNA interactions are likely to be similar, and synergistic activation due to activators contacting multiple components is expected.

Although the possibility of a preassembled transcription machinery has attracted most of the attention, perhaps a more significant aspect of the pol II holoenzyme is the presence of many other proteins that were unrecognized, and apparently absent, from the basic transcription machinery defined in vitro (Figure 1). Most of these holoenzyme components (e.g., Srb2 to Srb11, Gal11, Sin4, Rgr1) were originally identified by mutations that cause various transcriptional affects in yeast, and indeed these mutations were the key in discovering the pol II holoenzyme. These additional components, particularly the Srb proteins, are associated with and can phosphorylate the C-terminal domain (CTD) of the largest pol II subunit. Moreover, a subcomplex containing Srb and probably other proteins, termed mediator (Kim et al., 1994), can be separated from core pol II (the 12-subunit enzyme).

The pol II holoenzyme is almost certainly the molecular entity that initiates transcription in vivo (Koleske and Young, 1995). Srb proteins are found essentially only in the pol II holoenzyme, and Srb4 and Srb6 are essential for all pol II transcription in vivo. Thus, under physiological conditions, Srb4 and Srb6 behave indistinguishably from classically defined basic transcription factors, and they (along with the other Srb proteins) should be considered as part of the basic transcription machinery. Consequently, in vitro transcription experiments using core pol II and other basic factors are being performed in the absence of essential components of the basic transcription machinery that are required in vivo.

The pol II holoenzyme, in combination with TBP, can respond to activator proteins in vitro. In contrast with reactions containing core pol II, such activation-competent reactions do not appear to contain TAFs. Instead, activation requires the mediator component of the pol II holoenzyme, thereby implicating Srb (or other) proteins as potential targets (either direct or indirect) of activators. Consistent with this view, some *SRB* mutations confer transcriptional phenotypes consistent with a defect in the response to activators, and artificial recruitment of a holoenzyme component (Gal11) bypasses the need for an activation domain (Barberis et al., 1995). These observations strongly suggest that the mediator component of the pol II holoenzyme is important for the response to at least some activators in vivo. Nevertheless, it appears that the mediator is not essential for TAF-dependent activation in vitro.

The Swi/Snf View of Transcriptional Activation

The chromatin template in living cells severely restricts the access of transcriptional regulatory proteins to promoters. Nucleosomal repression affects all genes, although differences in intrinsic nucleosomal positioning and in the ability of activators and TBP to bind nucleosomal templates can affect the extent to which individual genes are affected (Felsenfeld, 1992). In vivo, activators can perturb chromatin structure in the absence of a functional TATA element and transcription (Struhl, 1995), and there are a number of biochemical activities that alter chromatin structure in vitro.

Perhaps the best example of such a chromatin disruption activity is Swi/Snf, a highly conserved complex that contains approximately ten proteins, including many identified by mutations that affect transcription in vivo (Peterson and Tamkun, 1995). The Swi/Snf complex is a DNA-stimulated ATPase, and it disrupts nucleosomal arrays in an ATP-dependent manner in vitro. This alteration in chromatin structure can facilitate binding of activator proteins or TBPs to their target sites on nucleosomal templates (Côté et al., 1994; Imbalzano et al., 1994). However, it is unclear how Swi/Snf perturbs nucleosomes and whether its affects on activator and TBP binding are relevant in vivo.

In vivo, the Swi/Snf complex is important for transcription of selected genes, including some dependent on activator proteins, but it is not required for cell growth. Swi/Snf affects chromatin structure in a manner that is independent of the transcriptional status of the promoter. Transcriptional defects caused by loss of Swi/Snf function can be alleviated by mutations in histones and other proteins that affect chromatin. These observations suggest that Swi/Snf stimulates transcription by virtue of its affects on chromatin structure. However, it is unclear how the Swi/Snf complex selectively affects the transcription of some genes and whether it is directly or indirectly related to the transcriptional activation process.

The Swi/Snf Complex Is an Integral Component of the Pol II Holoenzyme

The Swi/Snf complex is stoichiometrically present in the purified pol II holoenzyme (Figure 1), and, importantly, it endows the holoenzyme with the ability to disrupt nucleosomes (Wilson et al., 1996). Further, Swi/Snf is a component of the mediator subcomplex that contains Srb and other proteins and that is physically and functionally associated with the pol II CTD. These striking observations are consistent with, and indeed explain, several observations in vivo. First, strains containing CTD truncations, *SWI/SNF* mutations or some *SRB* mutations have phenotypic similarities, including a poor response to activator proteins. This weak activation response is particularly suggestive, given the requirement of the mediator subcomplex for transcriptional activation in vitro. Second, loss of Swi/Snf activity can be

suppressed by mutations in Sin4, another component of the holoenzyme. Third, as with Gal11, transcriptional activation can occur when Swi/Snf proteins (and, presumably, the entire holoenzyme) are artificially recruited to promoters. Of particular significance, activation by artificial recruitment depends on the ATPase activity, and presumably the chromatin disruption function, of Swi/Snf (Laurent et al., 1991).

The presence of Swi/Snf in the pol II holoenzyme solves the problem of how Swi/Snf is brought to promoters. Although other specific mechanisms may also occur, it is very likely that the major (and perhaps the sole) mechanism is recruitment of Swi/Snf with pol II. However, the apparent requirement for the pol II holoenzyme for transcription of all genes and the stoichiometric presence of Swi/Snf in the holoenzyme strongly suggests that Swi/Snf is brought to all functional promoters in vivo. Why then does Swi/Snf only affect a small subset of genes, and why is it important for the response to activators?

Although the answer to these questions is unknown, there are several possible explanations. One possibility is that other nucleosome-destabilizing activities, such as Nurf, which is highly conserved and is structurally related to Swi/Snf (Tsukiyama et al., 1995), may be functionally redundant except at a selected set of promoters. Alternatively, Swi/Snf function might be specifically involved in the response to activators. Nucleosome disruption by Swi/Snf might increase activator or TBP binding (or both) or stabilize the association of pol II holoenzyme at the initiation site, thereby amplifying the affects of protein–protein interactions mediated by activators. However, Swi/Snf-dependent effects on chromatin are not sufficient for the response to activators because they are observed in transcriptionally inactive promoters. Finally, Swi/Snf might not be specific for activation, but rather be important only at a subset of promoters where nucleosomes are unfavorably located for interactions of promoter DNA with activators, TBP, or holoenzyme.

The Triad Model of Transcriptional Activation

In considering the mechanism of activation in vivo, it is useful to think of three basic components: activator proteins bound to enhancer elements, complexes containing TBP and associated proteins bound to the TATA element, and the pol II holoenzyme bound to the initiation site (Figure 2). These three components correspond to the three distinct classes of eukaryotic promoter elements and macromolecular entities that exist in vivo. However, as noted above, this view does not preclude the sequential assembly of some components of the pol II holoenzyme.

Because pol II can not recognize promoters or initiate transcription on double-stranded DNA, the process of activation can be viewed as recruitment of pol II to the promoter in an active form. In principle, interactions between any two legs of the triad will lead to increased recruitment of pol II to the promoter. However, activator proteins play a particularly important role because they represent the component with the highest affinity and specificity for promoter DNA sequences. Indeed, when proteins bound to enhancer elements are artificially connected to TBP or components of the pol II holoenzyme,

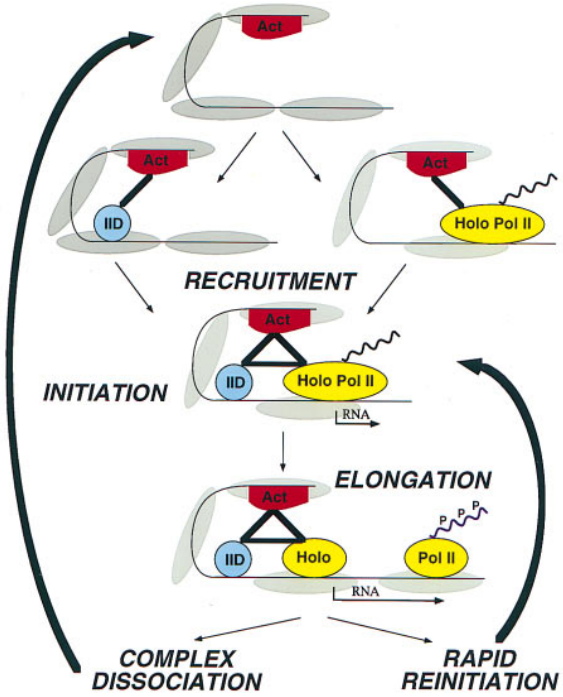

Figure 2. Triad Model for Transcriptional Activation

An activator (red) bound to its cognate site in chromatin (gray ovals, depicting nucleosomes) can recruit TFIID (blue) or pol II holoenzyme (yellow), yielding intermediate states. Recruitment of the other component leads to a stable triad capable of initiation (arrow with RNA). By virtue of Swi/Snf, recruitment of pol II holoenzyme alters chromatin structure (nucleosomes depicted by lighter gray, altered shape, and fewer numbers) in an unknown manner, perhaps leading to stabilization of activators, TBP, or holoenzyme at some promoters. Upon CTD phosphorylation and conversion to the elongating form, pol II disengages from the initiation complex. At this stage, a new pol II molecule (possibly with other components) could associate with the remaining metastable complex and rapidly reinitate transcription, or the complex could dissociate, thereby requiring reassembly of the triad. Related models involving interaction of TBP or holoenzyme with the promoter as the first step or sequential assembly of some components of the pol II holoenzyme on the promoter are not excluded. The relative importance of individual protein–protein interactions and nucleosome disruption will depend on the promoter.

transcriptional activation occurs in the absence of normal connections mediated by activation domains. TBP (and presumably TFIID) is inherently less sequence specific, and it interacts extremely poorly with TATA elements in the context of nucleosomal templates. Nevertheless, TFIID plays an important role, because strong activators cannot overcome transcriptional defects caused by weak TATA elements, and artificial recruitment of the pol II holoenzyme does not bypass the need for TFIID to efficiently bind the TATA element.

Because the legs of the triad are connected, protein–protein interactions or chromatin structural changes that strengthen any one connection will increase the overall stability of the complete transcription machinery at the promoter (Figure 2). Thus, enhanced recruitment of the pol II holoenzyme can be achieved by a variety of protein–protein interactions involving activators. In particular, TBP is associated with numerous proteins (e.g.,

TAFs in TFIID, TFIIA, Mot1, Spt3), and the pol II holoenzyme contains at least 25 polypeptides in addition to the core subunits. Activators could directly interact with any of these proteins or could cause a conformational change in one protein that increases interaction with another. Moreover, the presence of Swi/Snf in the pol II holoenzyme suggests the possibility that initial recruitment of the holoenzyme could result in changes in chromatin structure that increase the stability of one or more of the triad components with DNA. This initial recruitment of the holoenzyme might not require TFIID, thereby accounting for the activator- and Swi/Snf-dependent effects on chromatin structure that occur in the absence of transcription. Thus, while the relative importance of the various protein–protein interactions and disruptions of chromatin structure will vary according to the promoter, these distinctions might represent variations of a common mechanism of transcriptional activation.

Unresolved Questions

Although the triad model provides a useful framework, it does not address which proteins are direct and physiologically relevant targets of activators or how Swi/Snf affects chromatin structure and activation in vivo. The fact that the normal requirement for activation domains can be bypassed by artificial connections to TBP and holoenzyme components does not shed light on the proteins contacted by activation domains in native promoters. Similarly, the selective activation defects caused by mutations that disrupt TBP–TATA or TBP–TFIIA interactions or that inactivate Swi/Snf or other components of the mediator subcomplex merely indicate that these components are important for activation. However, because these mutations perturb the natural process, they do not provide information about the relative importance of these interactions or about rate-limiting steps in wild-type cells.

Another important, yet poorly understood, issue is whether each initiation event involves de novo assembly of the entire triad or whether pol II can initiate multiple times from a completely or partially assembled complex (Figure 2). Although the holoenzyme, whether sequentially or preassembled, is the form of pol II necessary for initiation, elongation is likely to involve CTD phosphorylation and disengagement of the core enzyme from the remainder of the machinery. Highly active promoters in yeast and flies initiate transcripts every 6 s, yielding a pol II density of one molecule per 100 bp. This argues against de novo assembly of the entire triad for each initiation event and suggests that there are two phases of activation: initial recruitment of TFIID and the pol II holoenzyme to the promoter, and metastable subcomplexes on the promoter that allow multiple initiation events. For strong promoters, it is likely that some or most of the assembled complex remains upon disengagement of the core enzyme, thereby permitting rapid reinitiation by a new pol II molecule. In promoters with weak activators or TATA elements or in situations with mutations in any relevant component, the lowered stability of the triad will result in fewer rounds of initiation per complex and increased reliance on the slower process of assembling the entire triad on an unoccupied promoter. A molecular understanding of these and other issues awaits future biochemical and genetic analyses.

Selected Reading

Barberis, A., Pearlberg, J., Simkovich, N., Farrell, S., Reinagel, P., Bamdad, C., Sigal, G., and Ptashne, M. (1995). Cell *81*, 359–368.

Chi, T., Lieberman, P., Ellwood, K., and Carey, M. (1995). Nature *377*, 254–257.

Choy, B., and Green, M.R. (1993). Nature *366*, 531–536.

Côté, J., Quinn, J., Workman, J.L., and Peterson, C.L. (1994). Science *265*, 53–60.

Felsenfeld, G. (1992). Nature *355*, 219–224.

Imbalzano, A.N., Kwon, H., Green, M.R., and Kingston, R.E. (1994). Nature *370*, 481–485.

Kim, Y.-J., Bjorklund, S., Li, Y., Sayre, M.H., and Kornberg, R.D. (1994). Cell *77*, 599–608.

Koleske, A.J., and Young, R.A. (1995). Trends Biochem. Sci. *20*, 113–116.

Laurent, B.C., Treitel, M.A., and Carlson, M. (1991). Proc. Natl. Acad. Sci. USA *88*, 2687–2691.

Peterson, C.L., and Tamkun, J.W. (1995). Trends Biochem. Sci. *20*, 143–146.

Sauer, F., Hansen, S.K., and Tjian, R. (1995). Science *270*, 1783–1788.

Struhl, K. (1995). Annu. Rev. Genet. *29*, 651–674.

Tjian, R., and Maniatis, T. (1994). Cell *77*, 5–8.

Tsukiyama, T., Daniel, C., Tamkun, J., and Wu, C. (1995). Cell *83*, 1021–1026.

Wilson, C.J., Chao, D.M., Imbalzano, A.N., Schnitzler, G.R., Kingston, R.E., and Young, R.A. (1996). Cell *84*, this issue.