Mechanisms of transcriptional activation *in vivo*: two steps forward

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Transcriptional activation involves the regulated assembly of multiprotein complexes on promoter DNA in the context of the repressive effects of chromatin. How do activators orchestrate this complicated phenomenon in vivo? Recent genetic and biochemical advancements suggest that activator-dependent formation of the transcription machinery on the promoter involves at least two steps. First, the activator facilitates the recruitment of TFIID to the TATA element of the promoter. TFIID binding is then followed by the recruitment of the remainder of the transcriptional apparatus in the form of the RNA polymerase II holoenzyme.

A typical yeast RNA polymerase II (Pol II) promoter contains enhancer, TATA and initiator (Inr) elements. The Inr is important for start-site selection but plays a small role in the absolute level of transcription. Enhancer and TATA elements are both required for high rates of transcription. The enhancer is a collection of binding sites for promoter-specific factors, termed activators, with each gene having a unique arrangement and assortment of binding sites. In contrast, the TATA element is recognized by the TATA-binding protein (TBP), a component of the basic machinery that is required by all Pol II promoters. Assembly of the general transcription machinery is initiated by TBP binding, with an assortment of TBP associated factors (TAFs), in the form of TFIID. This is followed by the association of the remainder of the general factors and Pol II. Activator-dependent increases in gene expression are thought to be the result of multiple direct and indirect effects of the activator bound at the enhancer and the general transcriptional machinery complexed at the TATA and Inr elements. However, the physiological significance and relative importance of protein–protein interactions between activators and the general Pol II machinery are poorly understood. This review will discuss recent advances in our understanding of how transcriptional activation occurs *in vivo*. Many of these advances have come from genetic studies in the yeast *Saccharomyces cerevisiae*, an organism in which genes can be analyzed in their natural chromosomal location under conditions where all proteins are present at physiological concentrations.

Effects of activators on chromatin

Activators can stimulate transcription indirectly by preventing the repressive effects of chromatin. Occluding promoter sequences with nucleosomes results in decreased accessibility of transcriptional regulatory proteins to the promoter. This simple mechanism of inhibition affects the transcription of all genes. Disruption of the normal nucleosomal structure by histone loss, mutation, or change in dosage, or poly(dA–dT) sequences, results in increased transcription[1]. In addition to histones, several non-histone proteins, such as Sin1 and Spt4–6, might be involved in global repression by chromatin.

How does an activator deal with chromatin? First, some activators, such as Sp1 and Gal4, can bind to their site *in vitro* even when it is complexed into nucleosomes[2,3]. In contrast, TBP (and presumably TFIID) is essentially unable to bind the TATA element when template DNA is complexed into nucleosomes[4,5]. Second, activators can perturb chromatin structure. The acidic activator protein Gal4 can displace a nucleosome from the *GAL1* promoter *in vivo*[6,7]. This displacement is not dependent on a functional TATA element and occurs in the absence of transcription, therefore, the process of transcription is not the cause of these changes. Third, activators might recruit or utilize Gcn5, a histone acetylase[8], or the Swi/Snf complex, an ATP-dependent nucleosome remodeling activity[9,10] that is associated with the Pol II holoenzyme[11]. Mutations that eliminate Gcn5 or Swi/Snf activity can decrease the transcription level of certain genes[1]. Thus, one component of activator-dependent increases in transcriptional activity might be the elimination of the effects of negative factors that repress transcription; this would serve to increase the accessibility of the TATA and Inr elements to the rest of the transcription machinery.

Direct effects of activators on the transcription machinery

Activators can stimulate transcription on naked DNA templates in the absence of histones, presumably through direct protein–protein interactions with components of the initiation complex. *In vitro*, activation domains can interact directly with a number of components of the general transcription machinery. These include TBP (Ref. 12), TBP-associated factors (TAFs; Ref. 13), TFIIA (Ref. 14), TFIIB (Ref. 15), TFIIF (Ref. 16), and TFIIH (Ref. 17).

In line with this large number of potential interactions, activated transcription *in vitro* can be achieved using either TFIID (TBP and the TAFs) and purified components[13], or with TBP and the holoenzyme (see below). In stepwise assembly reactions, activators can stimulate formation of a TFIID–TFIIA–TATA-element complex[18,19], recruitment of TFIIB (Ref. 20) and recruitment of later-acting components[21]. The relative importance of the protein interactions and mechanistic steps involving activators is difficult to determine, particularly in light of evidence suggesting that many of the basic transcription factors can be preassembled into a Pol II holoenzyme (see below). Thus, genetic approaches are critical for establishing the biological importance of these interactions and determining the regulated steps of initiation.

Activation domains come in a variety of types, including those rich in acidic, glutamine or proline residues. Acidic activators function across eukaryotic species, indicating that their mechanism of action must involve highly conserved protein–protein interactions. In contrast, glutamine-rich and proline-rich activation domains do not function in yeast cells[22,23], indicating that the protein(s) responsible for interpreting these

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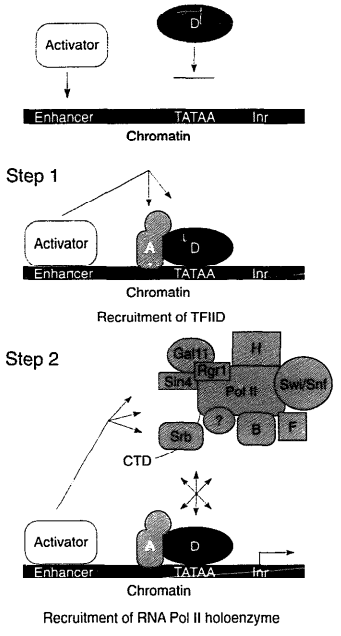

FIGURE 1. The two-step model of activated transcription *in vitro*. The activator can access the enhancer when complexed into chromatin (dark gray box), while TFIID (D) cannot bind the TATA element. Step 1: After binding, the activator alters chromatin (light gray box) and recruits TFIID, which is stabilized by TFIIA (A). Step 2: Activator–holoenzyme and TFIID–holoenzyme interactions recruit the holoenzyme. This results in high levels of transcription starting at the initiator sequence (Inr), indicated by the thick arrow. Holoenzyme components include RNA polymerase II (Pol II), TFIIB (B), TFIIF (F), TFIIH (H), Srb proteins interacting with the C-terminal domain (CTD) of Pol II, Gal11, Sin4, Rgr1, Swi/Snf and other unidentified proteins (?).

domains as activators are not conserved between yeast and higher eukaryotes. Interestingly, the glutamine-rich activator Sp1 specifically interacts with *Drosophila* TAF110 (Ref. 24), and a yeast homolog of this TAF has not been observed.

In considering the conserved mechanism utilized by acidic activators, it is noteworthy that TBP is the component of the basic transcription machinery that is most highly conserved throughout eukaryotic evolution. This striking sequence similarity (80% identity between yeast and human TBP) extends to the functional level. Yeast and human TBPs are interchangeable for basal Pol II transcription *in vitro*, and they have nearly identical sequence preferences for TATA elements[25]. Moreover, human TBP supports the response to acidic activators in yeast cells[26], and yeast TBP responds to activators in mammalian cells[27]. Taken together with the fact that TBP binding to the TATA element is the first step in assembly of preinitiation complexes *in vitro*, these observations implicate TBP as having an important role in transcriptional activation.

Step 1: recruitment of TFIID

Recruitment of TBP to the promoter can be a rate-limiting step of transcription that is enhanced by acidic activator proteins *in vitro*[28]. The kinetics of TBP recruitment *in vitro* were examined by rapidly inducing a TBP mutant with altered TATA-element specificity and measuring transcription from promoters with appropriately mutated TATA elements. In the absence of an activator, accessibility of the altered-specificity TBP to the *HIS3* promoter region was slow, in that transcription did not occur until 2–4 h after its expression was induced. In contrast, activator-stimulated transcription could be detected within 30 min after expression of the altered-specificity TBP. Although the mechanism by which activators increase recruitment of TBP is unknown, one possibility is that, as described above, the activator alters the chromatin structure of the promoter, thereby increasing the accessibility of TBP to the promoter. Increased recruitment might also reflect direct interactions between activation domains and TBP, its associated factors (TAFs) or TFIIA (see below). Most likely, the *in vitro* mechanism is a combination of all of these events (Fig. 1).

A simple prediction of such a critical role of TBP in the response to activators is the existence of mutants of TBP that, while functional for uninduced levels of transcription, are specifically defective for activated transcription. One class of such mutants might be defective for interacting with activation domains. However, the communication between the activator and TBP might not be direct, because a number of TBP mutants compromised for interactions with activation domains *in vitro* exhibit robust activation *in vitro*[29].

A mutant of yeast TBP that is specifically defective for the response to acidic activators *in vitro* has been described[30]. This mutant is specifically defective for interacting with TFIIA, and this defective TBP–TFIIA interaction is responsible for the activation defect *in vitro*. *In vitro*, TFIIA stabilizes the TBP–TATA interaction, alters the conformation of TBP and extends the DNaseI footprint upstream of the TATA element[31]. As mentioned above, acidic activation domains can interact directly with TFIIA *in vitro* and can stimulate the kinetics of the formation of the TFIID–TFIIA–TATA element interaction. The activator-dependent TFIID–TFIIA (DA) complex differs from an activator-independent DA complex in that it binds TFIIB stably[32]. Finally, *in vitro* crosslinking of an activator to TBP bound at a promoter can be strongly inhibited by TFIIA (Ref. 33), suggesting that TFIIA is positioned between the activator and TBP.

It should be noted that the functional interaction between an activator and TFIIA need not be direct. Indeed, biochemical studies have identified coactivator proteins that enhance transcriptional activation and interact with the activator and TFIIA (Ref. 34). Although

the requirement for the TBP–TFIIA interaction has not been tested for all activators, the above results suggest that increased recruitment of TBP by the activator to some promoters might be communicated via TFIIA. A simple model is that the activator-dependent DA complex is formed faster on the TATA element and is more stable, thereby serving as a better target for the remainder of the initiation complex.

If formation and stability of the TBP–TATA complex is important for the mechanism of activated transcription, one would predict that TBP derivatives defective for TATA-element interaction might also be activation defective. Using genetic screens, such TBP mutants have been isolated[35,36]. It could be that activators require the TBP molecule to be present at the promoter for a certain length of time before they can mediate their stimulatory affects. Alternatively, these TBP mutants might interact with the proteins responsible for recruitment, but once bound at the TATA element, dissociation is so rapid that productive interaction with later-entering members of the initiation complex cannot be achieved.

Combined, the results obtained with the TFIIA- and TATA-interaction-defective TBP mutants described above strongly suggest that an efficient TBP–TATA interaction is particularly important when transcription is initiated at a high level. While these TBP derivatives are artificial, these results are likely to be physiologically relevant because the affinity of wild-type TBP for natural TATA elements varies over a wide range. Further, canonical TATA elements achieve very high levels of activator-dependent transcription, whereas weak TATA elements become functionally saturated at lower levels of activation[37]. These observations are consistent with the described TBP mutants and weak TATA elements each being defective for an increase in recruitment of TBP by the activator.

If one function of the activator is to increase the rate-limiting step of TBP recruitment to the TATA element, then it is predicted that one could bypass the requirement for an activator by directly connecting TBP to a promoter-bound protein. In accord with this, artificial recruitment of TBP activates transcription to levels comparable to those observed with a bona fide activation domain[38,39]. This observation suggests that interactions between activation domains and general factors that function after TBP recruitment (for example, interactions between the activator and TFIIB, TFIIF or Pol II) are not required for transcriptional activation. This does not imply that these other factors are not involved in the process.

Step 2: recruitment of RNA Pol II holoenzyme

Although activators can stimulate transcription by increasing recruitment of TBP, there is considerable evidence for a post-TBP recruitment step in the mechanism of activated transcription *in vivo*. First, in the case of the *CYC1* promoter, TBP is bound at the TATA element in the absence of the activator protein[40]. This suggests that a second step in activator-dependent complex assembly can be slow and/or rate-limiting *in vivo*. Second, some activation-defective TBP mutants do not activate even when artificially recruited to the promoter via a heterologous DNA-binding domain, presumably owing to a defect after TBP is recruited to the TATA element[41]. Third, the C-terminal tail (CTD) of the largest Pol II subunit has been implicated in the process of transcriptional activation. Partial truncation of the CTD results in weakened responses to some activators[42], and extended CTDs can increase the function of weak activators[43]. The existence of a second step is also supported by biochemical studies indicating that activators function during multiple steps after TBP binds to the TATA element[21].

In yeast, approximately 10% of Pol II is found in a large multiprotein complex[11,44–46]. In addition to the ten subunits of the core enzyme, this Pol II holoenzyme includes most of the general transcription factors (but apparently not TBP), ten Srb proteins, Gal11, Rgr1, Sin4 and the Swi/Snf proteins (Table 1). Virtually all of the Srb proteins are required for normal cell growth, with Srb4 or Srb6 being essential for transcription from all promoters; this suggests that the Pol II holoenzyme is the entity that initiates transcription *in vivo*[47]. A subcomplex containing Srb, Swi/Snf and, perhaps, other proteins, termed mediator, is associated with the CTD (Refs 11, 45). *In vitro*, this mediator subcomplex can play a role in activation[45], suggesting that Srb or other proteins might be targets of activators. Taken together, these observations suggest that the holoenzyme is involved in the second step of transcriptional activation.

Independent verification of holoenzyme involvement comes from the observation that transcriptional activation occurs when Gal11, a holoenzyme component, is artificially recruited to a promoter by a DNA-binding domain[48]. Similarly, artificial recruitment of other holoenzyme components, Snf2, Snf5, Snf6 and Sin4, also results in activation[49,50]. As with artificial recruitment of TBP (TFIID), recruitment of holoenzyme bypasses the requirement for an activator. However, the TATA element (and, therefore, TBP or TFIID) is still essential for activated transcription. Thus, there are two steps required for activated transcription, recruitment of TFIID and recruitment of holoenzyme (Fig. 1).

The *in vivo* roles of some components of the Pol II holoenzyme are understood to a limited degree (Table 1). Genetic evidence indicates that TFIIB and certain subunits of Pol II are primarily responsible for selection of the initiation site[1]. Gal11, Sin4 and Rgr1 appear to exist as a subcomplex in holoenzyme, consistent with similarities between the phenotypes of mutations in the genes[46]. A subset of Srb proteins (Srb8–11) play a role in global repression by the Cyc8–Tup1 complex, because mutations in any of these genes leads to a partial relief of repression[51,52]. The Swi/Snf subcomplex is thought to facilitate activator function by antagonizing chromatin-mediated transcriptional repression[53]. Swi/Snf can enhance binding of activators or TBP (in concert with TFIIA) to nucleosomal templates *in vitro*[5,9,10], and it affects chromatin structure *in vivo*[54]. The molecular basis of the distinct, but selective, effects of individual holoenzyme components on transcription remains to be elucidated.

Concluding remarks

Taken together, the above evidence suggests that activators function at two steps. An apparent inconsistency between the *in vitro* transcription activation reactions could also be viewed as further support for this

TABLE 1. Factors involved in activated transcription

Factor	Subunits	Genetic function	Biochemical function
Activator	None; typically separable DNA-binding domains and ADs	Binds enhancer; alters chromatin structure; enhances level of transcription	ADs contact TBP, TAFs, TFIIA, TFIIB and TFIIH
TFIID	TBP and a large number of TAFs	Binds TATA element; nucleates initiation complex assembly; interaction with TATA element and TFIIA critical for activated transcription	TBP contacts TAFs, AD, TFIIA, TFIIB and TFIIF. TAFs contact AD, TFIIA, TFIIB and TFIIF[a]
TFIIA	Dimer of one α and one β subunit	Interaction with TBP necessary for activated transcription	Contacts TBP; stabilizes TBP–TATA interaction
Holoenzyme components[a]			
Pol II	10 subunits	Start-site selection; general transcription; truncation of the CTD of the largest subunit causes activation defect	RNA chain initiation and elongation
Srb	10 members	Interact with Pol II CTD; mutants suppress CTD cold-sensitive phenotype; required for transcription	Some Srb proteins phosphorylate CTD; others interact with TBP
TFIIB	Single polypeptide	Start-site selection	Binds TBP–TATA element co-structure; can interact with ADs and a TAF
TFIIH	Multisubunit complex	Involved in nucleotide-excision repair and cell-cycle control	Required for *in vitro* transcription
TFIIF	Three subunits, Rap74 (Tfg1), Rap30 (Tfg2) and Tfg3 (not essential)	Unknown	Recruits Pol II into preinitiation complex; Rap70 interacts with a TAF. Tfg3 has been detected in TFIID preparations
Swi/Snf	Approximately 10 members	Loss of function correlates with changes in chromatin structure and decreased potency of activators	DNA-stimulated ATPase activity; disrupts nucleosomal arrays in an ATP-dependent manner; facilitates TBP/TFIIA and activator binding to chromatin templates
Rgr1 sub-Rgr1, Gal11, Sin4, p50 complex		Loss of function diminishes full activation and glucose-repression of *GAL* genes, thus, positive and negative regulatory effects; alters chromatin	Unknown

[a]Response to activators has been achieved *in vitro* using either TFIID and highly purified general factors and Pol II, or with TBP and holoenzyme (see text).
Abbreviations: ADs, activation domains; CTD, C-terminal domain; Pol II, RNA polymerase II; TAFs, TBP-associated factors; TBP, TATA-binding protein.

view. In one type of reaction, the Pol II holoenzyme (but not core Pol II) responds to transcriptional activators[4,5]. Interestingly, TBP is sufficient in such reactions, suggesting that TAFs are not required for activation by the holoenzyme. In contrast, core Pol II can respond to activators, but only if TFIID is present[3]. Thus, activation can be achieved *in vitro* with either TFIID or the holoenzyme. This can be interpreted by proposing that the two *in vitro* transcription systems are assaying a different step of the process. A more accurate representation of the *in vivo* dynamics of activated transcription will be achieved when TFIID and holoenzyme preparations are both included in the biochemical assay system.

Although we present a scheme in which the activator recruits TFIID first and then recruits Pol II holoenzyme, different promoters could vary as to the order of these two events. One interpretation of the remodeling of chromatin observed after binding of the activator Gal4 (Refs 6, 7), is that it might reflect the action of Swi/Snf on activator recruitment of the holoenzyme. This remodeling occurs in the absence of a functional TATA element and is not correlated with transcription, suggesting that holoenzyme can be recruited in a TFIID-independent manner. Further, because artificial recruitment of holoenzyme components can bypass the requirement for the activation domain, the holoenzyme is clearly able to recruit TBP (TFIID). Likewise, TBP (TFIID) can recruit the holoenzyme in the absence of an activator. Thus, each of these multiprotein complexes can recruit the other, independent of the interaction with an activator. The physiologically relevant interactions between TFIID and the Pol II holoenzyme remain to be elucidated.

A major question not yet addressed is whether the initiation complex assembled at highly activated genes

in vivo is intrinsically different from that found at a promoter with a lower level of expression. For example, are certain TAFs associated with TBP only on highly active promoters? This would suggest steps in the biochemical pathway specific to activated transcription. Biochemical studies of activator–TAF interactions show that some activators have the potential to make TAF-specific contacts[13]. Alternatively, activated transcription could simply reflect faster recruitment and/or greater stability of an identical complex to that formed on an uninduced promoter. Thus, the same two-step process could be a universal feature of transcription initiation. A combination of genetic and biochemical approaches will be the means for understanding the choreography of these steps and the tempo at which they need to be performed in the complicated dance of activated transcription *in vivo*.

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