

- Introduction
- Basal and Activated Transcription
- Activators and Enhancers
- Interaction of Activators with the Pol II Transcription Complex
- Activation by Recruitment
- Postrecruitment Steps of Transcription Activation

Transcription Activation in Eukaryotic Cells

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Transcription activation is the first step in eukaryotic gene expression. Gene regulation, which is about turning on and off expression of specific protein-coding genes in response to environmental changes, during development and in differentiated cells, widely occurs at the level of transcription activation. This is mostly achieved by DNA-binding proteins that can control the specificity of the RNA polymerase II enzyme.

Introduction

Expression of protein-coding genes in eukaryotes is a multistep process that starts with initiation of transcription by RNA polymerase II at specific DNA sites called promoters. This step is followed by transcription elongation and termination, and by various RNA processing events that eventually produce a messenger RNA, which is then transported out of the nucleus for translation. While all these steps can be specifically regulated, much of gene expression is controlled at the level of transcription initiation. Thus, understanding gene regulation implies deciphering how RNA polymerase II is directed to the set of genes that must be transcribed at the right time and at the proper level in particular cell types, and how its competence for accomplishing this task is ensured.

Basal and Activated Transcription

Several genes in many organisms have been described as having so-called basal and activated levels of transcription. 'Basal transcription' of a given gene usually refers to its constitutive transcription, mostly low level, which can be enhanced such as to reach an 'activated transcription' level. Common to both levels of transcription is the requirement of a promoter sequence comprising the gene-specific transcription start point. The terms 'basal' and 'activated transcription' are broadly used to describe the transcriptional activity of a gene either in an *in vitro* system or *in vivo* (in isolated cells or in whole organisms). However, the type and number (complexity) of factors required, the state of the DNA template, and some aspects of the mechanisms of basal and activated transcription can vary considerably between *in vitro* and *in vivo* systems. We begin with the description of the mechanisms of basal and activated transcription in a typical *in vitro* reconstituted transcription experiment.

Contrary to the ability of bacterial RNA polymerase to specifically transcribe a bacterial gene *in vitro*, purified RNA polymerase II (Pol II) incubated in a test tube with purified DNA cannot initiate transcription specifically (**Figure 1a**). Additional factors have been found to be necessary for this process. These are the so-called general transcription factors (GTFs) that interact, directly or indirectly, with the RNA polymerase II. GTFs are required to position the RNA polymerase II at the gene promoter, help melting (opening) of the two DNA strands, and confer to the enzyme the full competence for initiating and elongating transcription. As their name suggests, these transcription factors are generally required at promoters used by RNA polymerase II. So far, this class of proteins comprises the TATA-binding protein (TBP), TFIIA, TFIIB, TFIIE, TFIIIF and TFIIH. These proteins contribute different activities necessary for the transcription process. For example, TBP recognizes the so-called TATA box, an AT-rich short sequence characteristic of many polymerase II promoters. TFIIH is a protein complex that contains a helicase that unwinds DNA, and a kinase that phosphorylates the C-terminal domain (CTD) of the RNA polymerase. This modification is necessary to release the polymerase from the promoter (i.e. from the promoter-bound transcription complex) to begin transcribing the gene. A detailed description of the GTFs and their functions can be found in the review article by Lee and Young (2000). In the presence of these general transcription factors, purified RNA polymerase II incubated with purified DNA can specifically bind a promoter sequence and initiate (and then elongate) transcription *in vitro* (**Figure 1b**). The level of transcription achieved by the combination of these reagents *in vitro* is referred to as basal transcription. It has been shown that this *in vitro* basal transcription can be enhanced (increased) by the addition of DNA-binding proteins that interact with specific sequences usually located upstream of the promoter region recognized by the RNA polymerase II in association with the GTFs. These sequence-specific proteins, which are

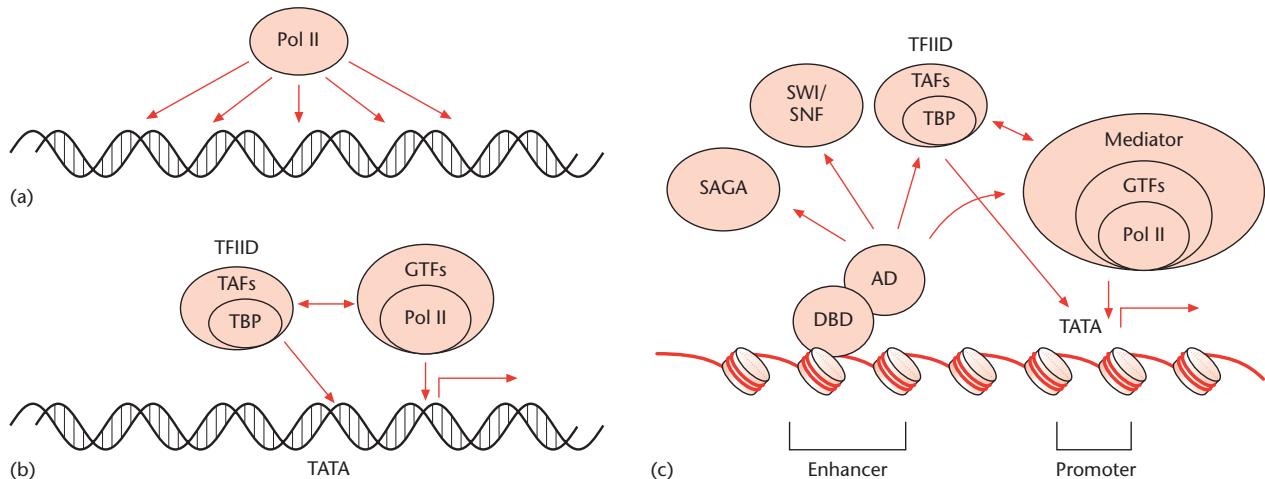


Figure 1 Transcription factor requirement for specific promoter binding and transcription initiation by RNA polymerase II. (a) Purified RNA polymerase II (Pol II) has affinity for DNA but is unable to recognize promoter sequences and specifically initiate transcription. (b) General transcription factors (GTFs), including TFIIID, which is composed of the TATA-binding protein (TBP) and the associated factors (TAFs) and can interact with some of the other GTFs, help the Pol II to specifically bind promoter sequences and initiate transcription on naked DNA template *in vitro*. (c) Additional factors are required for specific transcription initiation and activation *in vivo* on DNA template that is packaged into nucleosomes (cylinder-shaped histone octamer complexes with DNA wrapped around them). These additional factors include the Mediator complex, which interacts with Pol II, the nucleosome remodelling factors SWI/SNF and SAGA, and the sequence-specific transcriptional activators, which are typically composed of a DNA-binding domain (DBD) and an activation domain (AD) that can potentially interact with all the above factors to stimulate transcription. Arrows indicate potential molecular interactions, while the bent arrow on the DNA outline indicates specific initiation of transcription. The promoter region is where the transcription complex binds and transcription initiates. Enhancer is the binding site for transcriptional activators.

known as transcriptional activators, are therefore responsible for the activated level of transcription. Under the *in vitro* conditions just described, activated transcription is generally only a few times higher than the basal level and it requires additional factors such as, the TBP-associated factors TAFs or the so-called Mediator complex, which are believed to provide important interaction sites for activators (see below) (Myers and Kornberg, 2000; Verrijzer and Tjian, 1996). The role and the precise *in vitro* conditions for the requirement of these additional factors, which may change from laboratory to laboratory, are still a matter of debate.

In eukaryotic cells, DNA is not 'naked' as in a test tube but is packaged into so-called nucleosomes (histone octamer complexes with DNA wrapped around them). As a consequence, accessibility to DNA of the RNA polymerase and the GTFs is significantly reduced compared to the situation *in vitro*. Transcription initiation and elongation *in vivo* is therefore more complex and requires more factors than on naked DNA (Figure 1c). Most importantly for our understanding of gene regulation, transcriptional activators are generally required for transcription initiation to occur from promoters of protein-coding genes *in vivo*. Deletion of activator-binding sites located upstream of 'core' promoters (in a typical case, the TATA box and the initiation site) causes transcription from that promoter to drop below detectable levels in most of the cases, in particular in metazoan cells. This means that the transcription complex that contains the RNA

polymerase II is, on its own, usually unable to recognize a promoter sequence (or to be active on it) *in vivo*, in contrast to its ability to do so on naked DNA *in vitro*. Thus, basal transcription as described above for a naked gene rarely occurs *in vivo*, if at all. (It follows that when we describe the level of transcription of a given gene *in vivo* as basal, we mean that it is constitutive but, in most cases, still dependent on sequence-specific transcription factors that are constitutively bound in the vicinity of the promoter; one typical example of a constitutive and ubiquitous transcriptional activator is SP1.) The results of two complementary sets of experiments support the view outlined above and emphasize the importance of transcriptional activators and their interplay with nucleosomes to regulate the accessibility to DNA of the RNA polymerase II transcription complex. First, *in vitro* reconstitution of nucleosomes with a DNA template abolished basal transcription as described above, thus supporting the notion that nucleosomal DNA is not accessible to the transcription complex (Robinson and Kadonaga, 1998). Addition of sequence-specific transcriptional activators binding upstream of the promoter activated transcription of the nucleosomal DNA *in vitro*, thus indicating that this type of transcription factors can counteract the inhibitory effect of the nucleosomes (Robinson and Kadonaga, 1998). Second, disruption of nucleosomes *in vivo* by conditional ablation of histone proteins in yeast showed that transcription of several genes increased even in the absence of their specific transcrip-

tional activators (Durrin *et al.*, 1992). Thus, disruption of nucleosomes *in vivo* apparently created a situation that, in some cases, mimicked that of naked DNA *in vitro*, namely higher accessibility of promoter sequences to the transcription complex even in the absence of activators.

From these observations, taken together with results from a variety of experiments, we note that transcription from a promoter is not inherently an all-or-nothing process (or, inherently, one basal level and one activated level). Indeed, levels of transcription can be made to vary gradually, from very low to very high, by changing, for example, the state of the DNA template (from tight nucleosomal to naked DNA), the number and type of transcription factors helping the polymerase (including factors that can modify nucleosomes, the so-called chromatin remodelling factors), the number and strength of transcriptional activators, and the number of their binding sites on DNA. Thus, the level of transcription of any given gene in an organism will be determined by all these parameters applied to that gene at its chromosomal location. We note that in nature there are genes that have evolved to be gradually activated (e.g. activation of the copper-inducible *CUP1* gene in yeast by increasing concentrations of copper) as well as genes that are activated in a nearly all-or-nothing fashion (e.g. the *GAL1* gene in yeast, which reaches full activation over a narrow range of galactose concentrations).

Activators and Enhancers

Initiation of DNA transcription by RNA polymerase II is activated by *cis*- and *trans*-acting elements cooperating with each other. As the name suggests, *cis*-acting elements are DNA sequences that can influence transcription of a gene. They can be functionally subdivided into promoter and enhancer elements, both of which are necessary for the specific and activated expression of a gene (Figure 1c). *Trans*-acting elements are proteins (transcriptional activators) that bind specific *cis*-acting elements to activate transcription. It is important to note that, although not a topic of this article, there are also negative *cis*-acting elements that bind transcriptional repressors to turn down expression of genes.

Enhancers

DNA elements that activate the expression of genes upon specific stimuli are called enhancers. Enhancers are responsible for the tissue- and time-specific activation of transcription of various inducible genes in metazoans. Whereas promoters serve as binding surfaces for the general transcriptional apparatus to position the polymerase at the transcription start site, enhancers are specifically bound by transcriptional activators (Figure 1c). Typical

enhancers are composed of a number of specific sequences that can bind different transcriptional activators. One characteristic of these regulatory elements is that they can activate transcription from remote positions (several thousands of base pairs) upstream or downstream of a gene, and independently of their orientation (Muller *et al.*, 1988). Gene regulatory sequences in yeast that are functionally equivalent to the metazoan enhancers are called upstream activating sequences (UASs). Yeast UASs show a much lower degree of flexibility than their metazoan counterparts, but can nevertheless activate transcription from a distance of up to about 1200 base pairs from the promoter (Barberis *et al.*, 1995).

The mechanisms by which enhancers can activate transcription from distant promoters remain elusive. Several models have been proposed. The three most popular models have been dubbed 'scanning', 'linking' and 'looping'. The scanning model (also known as sliding or tracking) proposes that enhancer-bound transcriptional activators have a high affinity for the RNA polymerase II transcription complex, or parts of it, and tether it to DNA. Subsequently, the transcription complex scans the DNA until it reaches a promoter sequence competent for transcriptional initiation. In the linking model (also known as oozing), the binding of activators to enhancer sequences induces the formation of a DNA–protein structure that spreads from the enhancer until it reaches proteins bound to a transcriptionally competent promoter, which acts as a boundary to the further spread of this structure, and where transcription initiation by RNA polymerase II is stimulated. The looping model proposes that the contacts between enhancer-bound activators and transcription factors working at promoters bring the enhancer and promoter elements into close proximity (direct communication through physical apposition), with concomitant looping-out of the intervening DNA to accommodate the reaction. Such DNA looping might be facilitated by a variety of protein–protein and protein–DNA interactions, including those that affect the chromatin structure.

Activators

Eukaryotic transcriptional activators are modular proteins that are typically composed of a sequence-specific DNA-binding domain and an activating region (or domain). It is now generally accepted that activators interact through their activating regions with components of the RNA polymerase II transcriptional apparatus that bind promoters (see next section). It has been shown that some activators can also interact with factors that modify or remodel nucleosomes (chromatin). According to their amino acid constitution, activation domains have been classified as acidic (e.g. the viral VP16 and the yeast Gal4), glutamine-rich (e.g. SP1) or proline-rich (e.g. CTF). Binding of the activator to DNA serves merely to locate

it to a position from which the activation domain can activate transcription. DNA binding per se is clearly not sufficient for activating transcription. Many genes, especially in higher eukaryotes, are activated not by just one but by several activators that act in concert. The use of several, and often different, activators to activate one gene establishes a combinatorial control that allows differential regulation of many genes with a relatively small number of transcriptional activators (Ptashne and Gann, 2002).

So far we have dealt with transcriptional activators regulating other genes; how are these proteins themselves regulated? A wide variety of mechanisms has been devised by nature, some of which are described here. One mechanism to silence an activator is to keep it outside the nucleus away from the target genes. This mode of activator control is, for instance, realized in the case of NF κ B. This activator is kept outside the nucleus by its inhibitor I κ B. Through phosphorylation of I κ B upon specific signals, NF κ B dissociates and enters the nucleus, where it binds and activates its target genes. Another regulatory mechanism is to mask and thus to inactivate the activation domain by another protein. The activator Gal4, for example, can be inactivated by Gal80, a protein that binds to the activation domain of Gal4 and interferes with protein interactions necessary for its activation function (Ptashne and Gann, 2002). It has been shown that individual activators can also be shut down immediately after they have stimulated transcription from a promoter. For example, the yeast transcriptional activator Gcn4 is shut down by such a mechanism dubbed 'black widow'. Once bound to DNA, activators contact the transcriptional apparatus binding to the promoter (see below). Each time a Gcn4 protein contacts the transcriptional machinery to activate transcription, the kinase Srb10, which is a general component of the transcription complex, phosphorylates Gcn4 and signals it for destruction by ubiquitin-mediated proteolysis (Chi *et al.*, 2001). The ability of the transcriptional machinery to mark activators for destruction provides an efficient mechanism to limit the number of times that a DNA-bound activator can activate transcription from a gene promoter.

Interaction of Activators with the Pol II Transcription Complex

In vitro, several activation domains can interact with different components of the transcription complex, as well as with factors that can modify nucleosomes. Interactions of the Pol II transcription complex with activators have been shown for members of the Mediator complex, members of the TFIID complex (TBP and TAFs), and some of the other GTFs (Figure 1c). Interactions between activators and chromatin remodelling factors have been

shown for members of the SAGA complex as well as members of the SWI/SNF complex (Figure 1c) (Lee and Young, 2000; Myers and Kornberg, 2000; Verrijzer and Tjian, 1996). For example, the acidic activation domain of the yeast activator Gal4 can specifically interact *in vitro* with TBP, TFIIB, Srb4 (a component of the Mediator complex) and the SAGA complex (Ptashne and Gann, 2002). The physiological relevance of many of these interactions remains unclear. Also, the function of all these interactions in the process of transcription activation is a matter of debate. The following observation supports a potential relevance of multiple interactions established by activators. Different activators binding and activating a given gene generally show a synergistic rather than an additive effect on transcription. Synergistic transcription activation could be a consequence of cooperative DNA binding by different activators interacting with each other. However, cooperativity between interacting activators cannot be the only mechanism to explain synergism, since activators that evidently do not interact with each other are still capable of synergistic activation. Thus, a plausible and additional explanation for synergistic activation is provided by the multiple and perhaps simultaneous interactions that activators can establish with their targets (Ptashne and Gann, 2002).

Activation by Recruitment

Transcriptional activators need to bind DNA and to bear an activation domain in order to stimulate transcription. We have seen that activation domains can interact with a variety of components of the transcription complex, which binds to gene promoters, as well as with proteins that can remodel chromatin. What is the mechanism by which these interactions cause transcription activation? We imagine that DNA-bound transcriptional activators work by one or both of the following mechanisms.

1. **Recruitment.** The activator interaction with one or more transcription factors attracts the transcription complex in the vicinity of a gene promoter, thereby increasing its local concentration and facilitating binding to the promoter. Proper formation of this protein–DNA complex at the promoter provides the signal for the polymerase to initiate and elongate transcription of the cognate gene.
2. **Conformational change.** The transcription complex is bound to a promoter in an inactive conformation, and transcriptional activators, through interaction with 'specialized' targets, induce a conformational change that activates the RNA polymerase for transcription initiation and elongation.

These opposite hypothetical mechanisms for eukaryotic transcription activation have been inspired in part by advances in prokaryotic gene regulation, which provide

examples of pure recruitment and pure conformational changes, as observed with different classes of activators working on distinct promoters (Ptashne and Gann, 2002). However, so far, no evidence has been provided that can reasonably support the pure conformational change model to explain transcription activation in eukaryotes. Rather, a number of results from *in vivo* as well as *in vitro* experiments support the recruitment model. A selection of these is presented.

In yeast cells that carry a point mutation in Gal11, a component of the transcription complex, a derivative of the transcriptional activator Gal4 devoid of any classical activation domain is turned into a strong activator (Figure 2a,b). This activating function acquired by an otherwise silent DNA-binding protein is solely due to a novel and fortuitous interaction between the mutant Gal11, called Gal11P (potentiator), and a fragment of the Gal4 DNA-binding domain. This interaction has been generated by the P mutation, which otherwise has no effect on the normal function of Gal11. The simplest explanation for these results is that tethering Gal11 to DNA recruits the Pol II transcription complex to the promoter, which induces transcription by the polymerase (Figure 2b) (Barberis *et al.*, 1995). The following results provide strong support for this explanation. (i) The strength of the interaction between various Gal11P alleles (different hydrophobic amino acids at the same position) and the Gal4 DNA-binding domain, as determined *in vitro*, correlates with the level of gene activation measured *in vivo*. (ii) Fusion of a DNA-binding domain to Gal11, at a different position from the P mutation, creates a particularly powerful activator. A number of experiments have indicated that this fusion protein activates transcription of genes bearing its binding site upstream of a promoter by inserting the Gal11 component into the Pol II holoenzyme and recruiting the complex to DNA (Figure 2c) (Barberis *et al.*, 1995). Similar results of so-called ‘activator bypass’ experiments have been obtained with fusion proteins constructed with some other components of the transcription complex. (iii) The Gal11P and Gal4 interacting regions can be swapped, to have the Gal11P region on a DNA-binding domain and the interacting Gal4 region on Gal11 (i.e. on the side of the transcription complex), without loss of activity. (iv) Biochemical experiments have shown that Gal4 (1–100), which contains the Gal4 DNA-binding domain and the dimerization region contacted by Gal11P, but not the activating domain, can stimulate transcription in the presence of purified Pol II transcription complex bearing the mutant Gal11P component, but not when a similar complex containing wild-type Gal11 instead of Gal11P is used. Conversely, DNA-tethered classical activating domains such as those of Hap4 and VP16 can stimulate transcription *in vitro* when complexes containing either wild-type Gal11 or Gal11P are used. The stimulatory function of these activators is dependent on the concentration of the Pol II complex; at higher concentrations of this

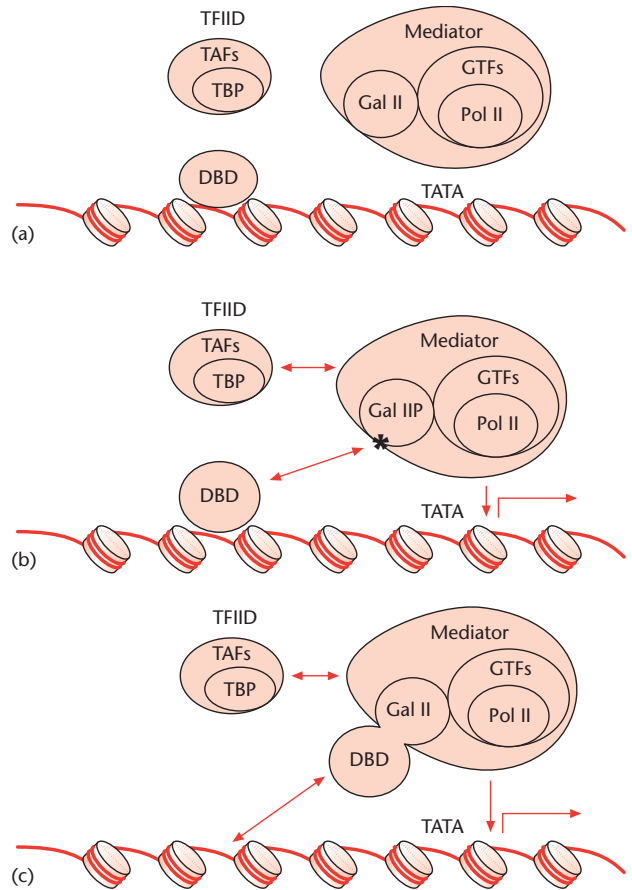


Figure 2 Transcription activation by recruitment of the RNA polymerase II transcription complex. (a) A sequence-specific DNA-binding protein (DBD) lacking an activation domain does not activate transcription in yeast cells containing wild-type Gal11 in the Mediator complex. (b) This DNA-binding protein becomes a strong transcriptional activator in the presence of the mutant Gal11P as part of the Mediator complex because it specifically interacts with this transcription factor and thereby recruits the entire transcription complex to the promoter. (c) The same DNA-binding domain (DBD) directly fused to Gal11 (or to Gal11P) specifically recruits the transcription complex to the promoter and activates transcription. The TATA-binding TFIIID, which is required for this instance of transcription activation, is believed to bind the promoter cooperatively with the rest of the transcription complex when this is recruited to DNA.

complex, the activators no longer have an effect on transcription (Gaudreau *et al.*, 1998). In other words, the increased concentration of the transcription complex mimics the activator function and overcomes its requirement to reach higher transcription rates.

Additional support for the recruitment model comes from the work of Keaveney and Struhl (1998), who showed that activation of transcription does not occur when an activation domain is physically disconnected from the enhancer-bound protein and transferred to components of the RNA polymerase II machinery. Thus, the transcriptional machinery seems inherently unable to activate

transcription *in vivo*, even when it bears a functional activating region.

All these results strongly argue in favour of the 'recruitment model' and thus against the 'conformational change model' for transcription activation in eukaryotes. What natural activators directly recruit is still a matter of debate and intense research. We have seen above that several activators are capable of contacting a variety of transcription factors and chromatin remodelling proteins (Figure 1c). These different interactions might recruit different activities, perhaps even in a sequential manner. For example, chromatin remodelling proteins could first be recruited to clear the promoter region, followed by the Pol II transcription complex that stably binds at the promoter, and finally by additional components that might regulate the activity of the polymerase for initiation, promoter clearance, elongation and termination. The results of the experiments with Gal11P described above also suggest that at least at some promoters, simple recruitment of the Pol II transcription complex can suffice to bring into place all necessary activities for strong transcription activation. For example, it has been shown that recruitment of the Pol II complex by the Gal4–Gal11P interaction suffices to remodel nucleosomes at the *PHO5* promoter in yeast.

Postrecruitment Steps of Transcription Activation

Recruitment is generally intended as the step that leads to the formation of a stable transcription initiation complex at the promoter. Clearly, formation of a stable initiation complex must be followed by promoter clearance and elongation to effectively produce an RNA transcript. It is now widely accepted that modification of the C-terminal domain (CTD) of the largest subunit of polymerase II by phosphorylation provides at least part of the signal for the switch from initiation to elongation. Concomitant with the CTD phosphorylation, probably as a consequence of this modification, there is an exchange of factors associated with the polymerase, which mark the elongating complex and distinguish it from the initiation complex (Lee and Young, 2000). While Pol II molecules of the promoter-bound initiation complex lacking phosphate on their CTDs are associated with the so-called Mediator complex (see above), which is believed to provide the major contact sites for transcriptional activators (Myers and Kornberg, 2000), Pol II molecules with phosphorylated CTDs are found associated with DNA sequences downstream of promoters and with different protein complexes, such as the elongator complex and various RNA processing factors. Indeed, as transcription elongation proceeds under the control of so-called elongating factors, RNA capping, splicing and ultimately polyadenylation are carried out by factors that have been found associated

with the phosphorylated Pol II (Lee and Young, 2000). Do transcriptional activators also control post recruitment steps? Several results indicate that at least some of these regulatory proteins can affect the phosphorylation state of the Pol II CTD by interacting with complexes carrying CTD kinases. For example, the general transcription factor TFIIF, which contains kinase and helicase activities, as mentioned above, is essential for the escape of Pol II from the promoter. It has been shown that members of one class of transcriptional activators, those with acidic activation domains, can interact with TFIIF, in addition to several other transcription factors (Ptashne and Gann, 2002). Thus, TFIIF may be actively brought into the vicinity of Pol II by transcriptional activators also. An additional example is provided by P-TEFb, a kinase/cyclin pair originally identified in *Drosophila*, which has been shown to phosphorylate the CTD and to stimulate transcription elongation *in vitro*. The *Drosophila* heat shock factor HSF, a sequence-specific DNA-binding activator, has been shown to stimulate elongation by interacting with P-TEFb (Ptashne and Gann, 2002). In human cells, P-TEFb interacts with the HIV Tat protein, which is known to stimulate elongation of transcription from the promoter contained within the virus LTR sequence. Thus, it is plausible that recruitment of this CTD kinase by Tat, which does not bind DNA but specifically interacts with the nascent RNA near its 5' end, stimulates transcription elongation by increasing the efficiency of Pol II phosphorylation (Ptashne and Gann, 2002).

In conclusion, transcription activation by sequence-specific activators in eukaryotes involves the recruitment of different factors with different activities that can affect the chromatin structure, lead directly to the formation of the Pol II initiation complex at promoters, and influence the efficiency of promoter clearance and elongation. Thus, all these multiple interactions of activators concur in conferring to the polymerase the specificity and the competence required for correct and productive transcription of specific genes.

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Further Reading