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Mechanisms of action of transcription activation and repression domains

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Abstract. Transcriptional regulators contain domains that either activate or repress transcription. Indeed, many cellular transcriptional regulators contain both activation and repression domains. Transcriptional regulators act at several stages in the transcription process, including assembly of the transcription complex, initiation and elongation. In order to influence these processes, the regulatory domains must interact with components of the transcription apparatus. This review will focus on our current understanding of the nature of transcriptional regulatory domains and their targets in the transcription machinery.

Key words. Transcription; preinitiation complex; promoter; RNA polymerase II.

The general transcription machinery

Transcription of a gene by RNA polymerase II (pol II) requires the assembly of the general transcription factors (GTFs) at the promoter to form a preinitiation complex (PIC; [1–3]; fig. 1). At most promoters, this begins with the GTF TFIID, which binds to the TATA box. TFIID is a multi-subunit factor composed of the TATA-binding protein (TBP) and TBP-associated factors (TAFs). TFIIA and TFIIB then join the complex, and their assembly provides a platform for the recruitment of pol II and TFIIF. The PIC is completed by the GTFs TFIIE and TFIIH. This ordered assembly process was elucidated using purified components. However, it is likely that several of the GTFs (and also many other factors) are normally associated with pol II into a holoenzyme [1, 2, 4, 5]. The content of the holoenzyme has proved to be highly variable depending upon the method of preparation. However, several of the GTFs are present in most of the holoenzyme preparations from nuclear extracts along with a host of other factors that are required for the regulation of transcription [5]. These include the SRB (suppressors of RNA polymerase B) and MED (MEDiator) proteins. Thus, PIC assembly may actually require only a limited number of recruitment events (fig. 1b). Such factors are important when considering how transcription is regulated and will be discussed further below.

The chromatin barrier

The PIC described above is sufficient to direct accurate transcription initiation of a naked DNA template in vitro. This is termed basal transcription. However, in a living cell, DNA is assembled into chromatin, which impedes the assembly of the PIC (fig. 2). Thus, chromatin needs to be remodelled before a PIC is able to nucleate at the promoter. Several chromatin remodelling activities have been described that fall into two classes: (i) enzymes that acetylate (e.g. CBP) or deacetylate histones and (ii) enzymes that remodel chromatin in an ATP-dependent manner (e.g. SWI/SNF) [1–3, 5–9]. Transcriptionally active regions of chromatin frequently exhibit high levels of acetylation at specific lysine residues located near the N-terminus of the histone proteins [9]. Several histone acetyl transferases (HATs) have been identified that are capable of acetylating these residues, including the coactivators CBP/p300, P/CAF and TAFII250 [9]. In addition, several histone deacetylases (HDACs) have been identified [8]. These enzymes have been found to be associated with known components of the transcription apparatus [7, 10]. Thus, the process of histone acetylation and deacetylation appears to be an integral step in gene regulation. A more thorough discussion of these enzymes is contained in one of the other reviews in this issue. Several ATP-dependent remodelling activities have also been de-

scribed, including SWI/SNF and NURF [6, 11–13]. These enzymes use ATP to catalyse a reaction that causes nucleosomes to slide along the DNA, thereby providing access to naked DNA [12]. The ATP-dependent and acetylase chromatin-remodelling activities can also be found as part of the same complexes, suggesting that they function together to modify chromatin [5, 14]. However, it is not yet clear whether they function simultaneously or at different stages during the transcription process.

Regulation of transcription

The transcription rate of a gene is subject to strict control. There are several levels at which this can occur. For example, during mitosis transcription is shut down, and this is caused by direct modification of the transcription machinery and thus affects all genes [1, 2]. There are other mechanisms of global transcriptional regulation. Dr1 is a general repressor of transcription and acts by blocking the assembly of the PIC by direct contacts with TFIID [1, 2, 15]. This review, however, will focus on transcriptional regulators that function in a gene-specific manner by interacting with DNA sequence elements associated with a particular promoter. There are several ways by which transcription can be regulated by gene-specific factors. One such mechanism is to regulate the regulator itself, for example by expression, posttranslational modification or interaction with inhibitory factors. These mechanisms are discussed elsewhere in this issue. This review will focus on how transcriptional regulators function once they have located at the promoter.

Transcription of a gene occurs in a chain of events that include several points that can be subject to regulation (fig. 3) [3]. First, as mentioned above, the transcription cycle begins with the modification of the nucleosome, which provides the foundation for PIC assembly. Once the PIC has formed, it must convert to an open complex, in which the DNA is melted to provide a single-stranded DNA template for the RNA polymerase. The PIC must then undergo a transition in which it converts to a processive enzyme. This event is marked by promoter clearance. Once pol II has cleared the promoter, various factors dissociate from the complex, and it converts to an elongating enzyme. Interestingly, TFIID appears to remain bound to the TATA element after

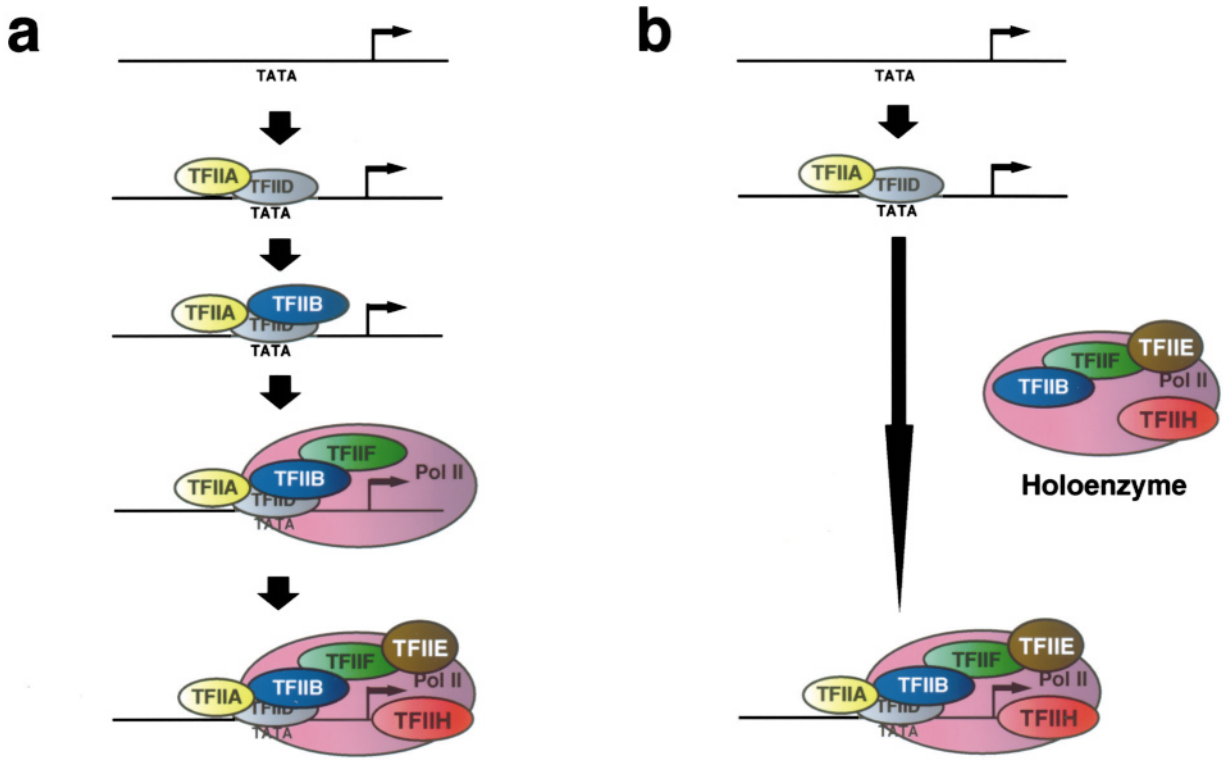

Figure 1. Preinitiation complex assembly. (*a*) The ordered assembly model of the preinitiation complex. (*b*) The holoenzyme model of preinitiation complex assembly.

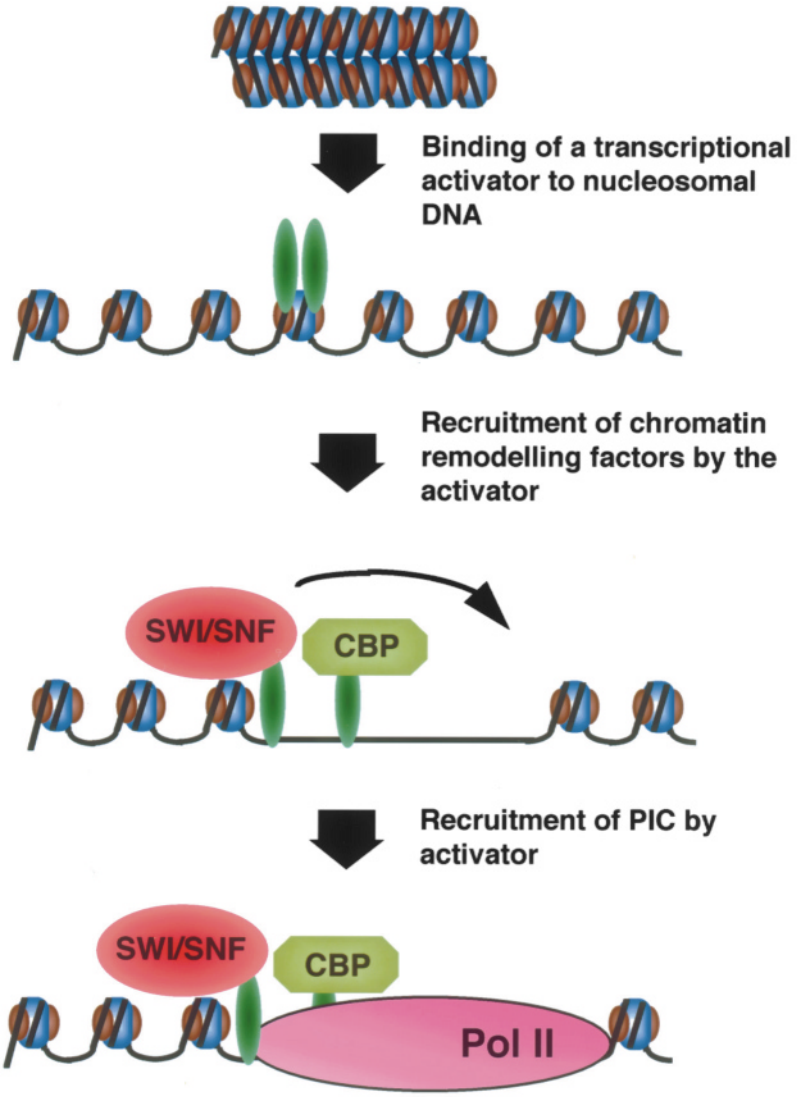

Figure 2. Steps in transcriptional regulation. Cartoon of the steps required for transcriptional activation of a gene. SWI/SNF and CBP are given as examples of the different chromatin-remodelling activities. The activator is shown in green.

transcription initiation [16, 17]. Thus, the assembly of a second PIC (reinitiation) does not undergo the same chain of events as the assembly of the first. Indeed, this probably represents most transcription events in vivo. The elongating complex is also a target for transcriptional regulation [18, 19]. Most of these events require the concomitant remodelling of nucleosomes.

Transcriptional regulators appear to affect all of the above processes. Transcriptional regulators have been classed as those that activate transcription (activators) and those that abate it (repressors). However, it has become increasingly clear that several transcriptional regulators are capable of performing both of these functions depending upon their cellular environment [20].

Transcriptional activation domains

Transcriptional activation domains have been loosely classed according to their amino acid composition [1–3]. Initial studies identified the acidic class of transcriptional activation domain typified by the potent activation domain of the herpesvirus VP16 protein and the yeast GAL4 protein [21]. Glutamine-rich (e.g. SP1) and proline-rich (e.g. CTF1) transcriptional activation domains are two other classes of transcriptional activa-

tion domains that were initially identified. Over a decade ago Ptashne and colleagues expressed random *Escherichia coli* DNA fragments fused to a DNA binding domain in yeast to identify and characterise those fragments that would encode activation domains [22]. The only common theme found was a bias toward the presence of acidic residues. It was predicted that such acidic domains may form an amphipathic α helix, with the acidic residues lying on one face. Adding evidence to this, an artificial acidic amphipathic helix (AH) formed a potent activation domain when fused to a heterologous DNA binding domain [22, 23]. However, although the artificial AH domain has the potential to form an α helix, the natural yeast GCN4 and GAL4 activation domains adopt β sheet-type structures [24].

The C-terminal 90 amino acids of VP16 contain two activation modules that are rich in both acidic and bulky hydrophobic residues [21, 25]. Extensive mutational analysis has been performed on these domains. In spite of the labelling of VP16 as an acidic activation domain, mutagenesis of acidic amino acids appears to have only marginal effects on the potency of transcriptional activation [26, 27]. However, several bulky hydrophobic residues are critical to the function of the activation domain, which has proved also to be the case for nonacidic activators such as the glutamine-rich activation domain of SP1 [28]. Hydrophobic interactions frequently play a greater role in the affinity of protein-protein interactions. Due to their energetic neutrality, electrostatic interactions are less critical in determining the affinity, but provide specificity to the interaction surface [29]. Analysis of the VP16 activation domain by nuclear magnetic resonance (NMR) showed the domain to be unstructured [30]. However, when the VP16 activation domain is complexed with a target factor (hTAFII32), an α-helical structure forms [31]. Biochemical evidence also suggests that the VP16 activation domain becomes structurally constrained upon interaction with GTFs [32]. This induced-fit mechanism has been identified in other activator-target interactions. Upon interaction with TBP, the otherwise unstructured c-Myc transactivation domain adopts an α-helical conformation [33]. Also, the kinase-inducible activation domain of cyclic AMP response element binding protein (CREB) undergoes a coil-to-helix transition upon binding to its docking site in the coactivator CREB binding protein (CBP) [34].

How transcriptional activation domains function is a central question in the field of gene expression. It is clear that activators can affect several steps in the process of transcription, including chromatin remodelling, PIC formation, promoter clearance and elongation. However, do all types of transcriptional activator function similarly, or are there a diverse array of mechanisms? There has been an explosion of information regarding the function of transcriptional activation domains, and our understanding of their function is becoming clearer but is by no means complete. Table 1 provides a summary of the various target factors of transcriptional activators that will be discussed here.

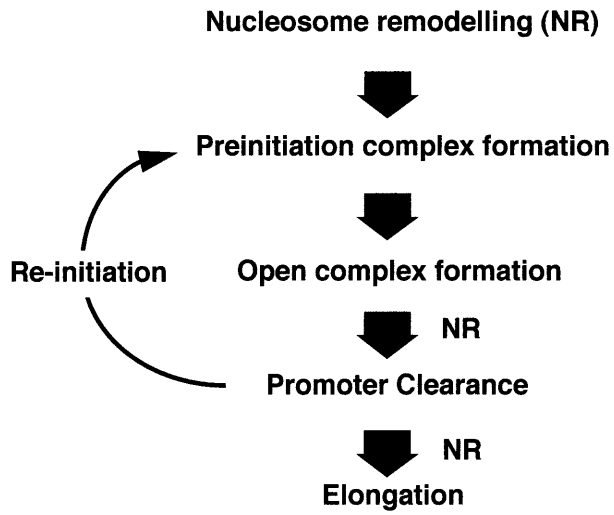

Figure 3. Points of regulation. The critical steps in the transcription cycle that can be regulated. NR, nucleosome remodelling.

Activators and chromatin remodelling

Many transcriptional activators are able to recognise and interact (albeit with reduced affinity) with DNA elements that are assembled into chromatin [11]. This is followed by the recruitment of chromatin remodelling activities to destabilise local nucleosomes and facilitate both the further assembly of transcriptional regulators and the recruitment of the basal transcription machinery [3, 9, 35–38]. Several chromatin-remodelling activities have been shown to interact with transcriptional activation domains [8, 9]. One well-studied example is the coactivator CBP, which was identified as a factor that interacts with the transcriptional activator protein CREB (see above) [39]. CBP and its homologue p300 have since been found to be coactivators for several transcriptional activator proteins. p300/CBPs, in addition interacting with GTFs, are HATs [38–40]. Thus, recruitment of p300/CBP to the promoter by transcriptional activators would result in the acetylation of histones and destabilisation of chromatin. Other HATs have been identified, including the p300/CBP-associated factor p/CAF and hTAFII250 [9, 41]. It has become apparent that some HATs are part of a large complex SAGA (Spt-Ada-Gcn5 acetyltransferase) that also con-

Table 1. The targets of transcriptional activation domains (see text for details).

Target factor		Example activators	Function
TFIID	TBP	VP16, Myc	
	hTAF32	VP16	recruitment to
	dTAF110	SP1, CREB	TATA
TFIIB		VP16, THRβ kruppel	recruitment to PIC/ conformational change
TFIIF		Fos, Myc androgen receptor	promote elongation?
TFIIH		VP16	recruitment/ initiation?
SWI/SNF		VP16	nucleosome remodelling
CBP/p300		CREB	HAT, contacts GTFs
NCOA		nuclear hormone receptors	HAT
SRB/MED complexes		E1a, SP1, VP16	recruitment/ mediates signalling pathways; nucleosome remodelling

tains other previously identified coactivators and TAFs [7, 10, 42]. Activators have also been shown to recruit ATP-dependent chromatin-remodelling activities to the promoter [6]. Thus, activators appear to able to recruit a diverse array of factors that can modify/displace nucleosomes from the region around which the PIC is to form.

Targets of transcriptional activation domains in the basal machinery

There is a great deal of evidence to support the idea that activators increase the rate and extent of PIC formation [3, 43, 44]. However, there has been much controversy over which GTFs are the targets of transcriptional activator proteins. The first step in PIC assembly, the binding of TFIID to the TATA element, has been reported by several groups to be a target of transcriptional activator proteins [2, 3, 45]. In purified in vitro systems, an activator can directly enhance the formation of a TFIID-TFIIA complex at the promoter. Moreover, activation domains can interact with several target factors within the TFIID complex, including TBP. Significantly, mutations in TBP that abolish the interaction with an activation domain disrupt transcriptional activation [46]. Two recent reports have shown that an activator recruits TFIID to the promoter in living yeast cells, providing significant evidence to suggest that activator-mediated recruitment of TFIID is a central process in transcriptional activation [47, 48]. Activation domains can also interact with the TAF subunits of TFIID [28, 49, 50]. Interestingly, different classes of activation domain appear to interact with different TAFs. This has led to the proposal that TFIID may be an integrator for the diverse transcriptional activation domains of higher eukaryotes. There is thus a large body of evidence that indicates TFIID (and TFIIA) assembly as the target of transcriptional activators. TFIIB, the next GTF to enter the complex, has also been proposed as a target of transcriptional activators [2, 3, 17, 43, 51, 52]. Activator proteins can interact directly with TFIIB, and mutagenesis suggests that this interaction is required for transcriptional activation both in vivo and in vitro [17, 51, 53]. Interestingly, recent studies have reported a role for TFIIB following PIC formation that appears to be required in yeast, but not human transcription systems [54–56]. TFIIH is another attractive target of transcriptional activators [2]. TFIIH contains enzymatic activities, including a protein kinase (CDK7) that is required for the phosphorylation of the C-terminal domain of pol II. This event occurs upon transcription initiation, suggesting that the interaction of activator proteins with TFIIH may facilitate the conversion of the PIC to an elongating complex [2]. The transcriptional activation domains of c-myc, androgen receptor and c-fos have been shown to interact with the RAP74 subunit of TFIIF [33, 57, 58]. RAP74 plays a major role in transcriptional elongation, suggesting that these interactions may be relevant to the processivity of the pol II machinery.

More coactivators

The term 'coactivator' refers to the very large group of factors that are required for the activation of transcription, but are not part of the basal transcription machinery [1–3]. We have already discussed the coactivators that are involved in chromatin remodelling. However, there are several coactivators that are required for transcriptional activation of a naked DNA template in vitro. The first such coactivators to be identified were the TAFs [49, 50]. This was based on the observation that in a reconstituted transcription system, recombinant TBP was unable to replace TFIID in transcriptional activation. It emerged that different TAFs engaged in contacts with different classes of transcriptional activation domains [49, 50, 59]. For example, the glutamine-rich activator SP1 interacts with dTAF110

(hTAF130), whereas VP16 interacts with hTAFII32 [28, 30]. Thus, the construction of partial TFIID complexes revealed that different activators required different complements of TAFs [49]. However, over the last few years there has been a great deal of controversy over the role of the TAFs in transcriptional activation in vivo [50, 59–61]. Some studies have found that the TAFs are only required for the activation of a small subset of genes. Moreover, this minimal requirement mapped to the core promoter rather than upstream activators. Indeed, TAFs do play a role in recognising core promoters [49]. However, recent studies suggest that at least some of the TAFs are globally necessary for transcriptional activation in yeast [5, 60]. Interestingly, dTAF230 interacts with the concave surface of TBP and can inhibit its ability to interact with the TATA box [62]. A potential role for this interaction in transcriptional activation has been proposed because VP16 competes with this interaction and can displace the inhibitory interaction of the N-terminus of dTAF230 with the DNA-binding surface of TBP. Adding further confusion, it has emerged that several of the TAFs are also present in complexes other than TFIID. The SAGA complex contains two of the histone-like TAFs complexed with HATs [42]. Significantly, these TAFs, the histone-like TAFs, are the ones that are globally required for transcriptional activation in vivo [63, 64]. It is still not clear whether the TAFs in TFIID are generally required for transcriptional activation. Most of our knowledge of TAF function in vivo has been derived from yeast, which do not contain the diversity of transcriptional activation domains seen in higher eukaryotes. As mentioned above, different mammalian TAFs are targeted by different classes of activator proteins in higher eukaryotes. Recent results from *Drosophila* support the idea of a more general requirement for TAFs in the response to upstream transcriptional activators [50, 65].

Another group of coactivators have been purified from a nuclear extract fraction termed USA (upstream stimulatory activity) that is required in addition to the GTFs to support high levels of transcription in vitro [66, 67]. Several of these factors have been cloned. The best-characterised of these is PC4, which interacts directly with TFIID [66]. Addition of PC4 alone to a purified transcription system containing TFIID is sufficient to support transcriptional activation by VP16. PC4 binds to both single- and double-stranded DNA, suggesting a potential topological role in transcriptional activation.

The mediator complex originally identified in yeast has also been purified from mammalian nuclear extracts as a complex required for transcriptional activation by E1a and also VP16 in vitro [68]. A very similar complex (CRSP) was also purified as an essential factor in SP1-mediated transcriptional activation [69]. These complexes also contain SRBs (suppressors of RNA polymerase B, originally identified as holoenzyme components). More complexes have also been identified that contain overlapping MED and SRB subunit compositions [5]. The precise relationships between these complexes and their role in mediating transcriptional activation is yet to be determined.

Activator-mediated recruitment of the holoenzyme

The holoenzyme was first purified from yeast as a complex containing pol II, the SRBs and a subset of the GTFs [4]. Upon the addition of recombinant TBP, this complex was able to support transcriptional activation in vitro. The holoenzyme has since been purified from mammalian cells by several laboratories, but the precise complement of factors appears to vary between preparations. This may not be so surprising given the size of the complex and the procedures required to extract it from nuclei. The existence of a holoenzyme provides the opportunity for activators to recruit a large proportion of the PIC in one fell swoop. A series of artificial recruitment studies have provided evidence that this may actually be the case [70–72]. In these experiments, a component of the holoenzyme is fused to a DNA binding domain that will tether it to a promoter and in doing so recruit the entire holoenzyme. Expression of such fusion proteins in both yeast and mammalian cells elicits transcriptional activation in a manner dependent upon the presence of the appropriate DNA binding sites in the promoter. In vitro experiments with the holoenzyme suggest that recruitment is a pivotal mechanism of action of activator proteins [71]. For example, increasing the concentration of the holoenzyme is able to substitute the function of the transcriptional activator. Thus, our current understanding suggests that the PIC is recruited in at least two steps (fig. 4). First, TFIID-TFIIA is recruited to the TATA element. This is followed by the activator-mediated recruitment of the holoenzyme. The precise target of an activator in the holoenzyme is not yet known. Indeed, it is possible that any of several of the holoenzyme components (including GTFs, MEDs, SRBs or pol II) may form the contact surface.

Activator synergy

An interesting feature of transcriptional activator proteins is that they function synergistically [23, 44]. Thus, multiple activators located at a promoter direct a much greater level of transcription than would be expected based on that observed with a single activator. The most likely explanation for synergy is that activators need to influence more than one step in PIC assem-

bly in order for efficient transcriptional activation to occur. Indeed, as described above, activation domains can contact several components of the PIC. One interesting proposal was that TAFs may be involved in activator synergy. The *Drosophila* activators Hunchback and Bicoid are each able to interact with different TAF subunits of TFIID [59]. Although each activator alone is able to elicit a moderate level of transcriptional activation, contact of each activator with their respective TAFs results in a synergistic activation of transcription.

Conformational considerations

One aspect of transcriptional activation that is becoming increasingly important is the prospect of a role for conformational alterations in the targets of transcriptional activation domains. In bacteria, an activator-mediated conformational change in the RNA polymerase holoenzyme is a pivotal step in transcriptional activation [73]. In a eukaryotic in vitro transcription system a TFIID-TFIIA-promoter complex that has been assembled in the presence of an activator protein is competent to complete an activated level of PIC assembly even if the activator is removed prior to the assembly of the other GTFs [45]. Thus, the activation domain was believed to have caused an isomerisation of the TFIID-TFIIA-promoter complex that committed this subcomplex to recruitment of the remaining PIC components to cause an activated level of transcription. Interestingly, this effect was activator-specific, indicating that activator-mediated recruitment of TFIID and the activator-induced isomerisation of the TFIID-TFIIA-TATA complex are separate events.

An activator can also induce a conformational change in the GTF TFIIB [53, 74–76]. TFIIB plays a pivotal role in PIC formation, providing a bridge between promoter-bound TFIID and pol II. TFIIB is composed of two domains that are engaged in an intramolecular interaction. These two domains contain the binding sites for TFIIF and pol II [74]. Thus, the TFIIB intramolecular interaction may limit the assembly of TFIIF/pol II into the PIC. Both biochemical and structural analyses have shown that upon binding to an activator protein, TFIIB undergoes a conformational change disturbing the intramolecular interaction. This conformational change exposes the binding sites in TFIIB for pol II/TFIIF and thus may drive PIC assembly forward.

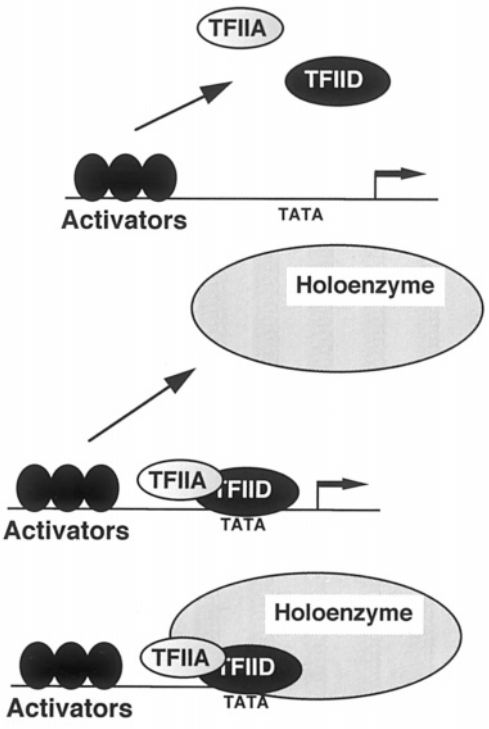

Figure 4. Two-step model for transcriptional activation. Activators first recruit TFIID and TFIIA to the promoter. The remaining GTFs are then recruited in the form of the holoenzyme.

The role of activators after PIC assembly

Our knowledge of the role of transcriptional activators after PIC assembly is very limited. Activators have been shown to stimulate open complex formation, and as mentioned above, it is possible that the interaction of transcriptional activators with TFIIH may promote the phosphorylation of pol II and conversion of the complex to an elongating enzyme [1, 2]. Activators can also stimulate transcription elongation by overcoming pauses of the elongating polymerase [18]. Significantly, as mentioned previously, RAP74 (which plays a major role in elongation) has been found to interact with several activation domains. A multitude of factors are required during transcription elongation that are not known to be necessary during the initial assembly of the transcription complex [19]. It is not yet clear whether these factors are also the targets of transcriptional activators. One factor, FACT (facilitates chromatin transcription), is required for continued transcription through chromatin-assembled DNA templates [77].

Repression of transcription

There are basically three general mechanisms by which transcription can be repressed in a gene-specific manner [78]. The first two of these involve the abatement of the function of transcriptional activators and will not be

Table 2. The targets of transcriptional repression domains (see text for details).

Target factor	Example repressors	Function
TFIID (TBP) interaction	Eve, Msx-1	block IID-TATA
TFIIB	THRb	block pol II recruitment
TFIIE	kruppel	block PIC assembly?
DR1	E4BP4 [104]	block TFIIB assembly
NcoR/SMRT	hormone receptors	recruit histone-deacetylases
HDAC	Rb	histone deacetylase

discussed in detail here. Briefly, a repressor can prevent the association of transcriptional activator with the promoter. Second, a repressor can suppress the function of a DNA-bound transcriptional activator protein. For example, the dorsal suppressor protein DSP1 binds to a DNA element adjacent to that occupied by the activator dorsal and inhibits the function of the dorsal transcriptional activation domain [79]. The third mechanism, so-called true repression, involves a distinct transcriptional repression domain that has negative effects on generation of the transcription complex. There is inevitably overlap in these mechanisms. For example, Rb both inhibits the activation domain of DNA-bound E2F and has an active repression domain that contacts HDACs [80, 81]. DSP1 also falls into this category. DSP1 sterically inhibits the dorsal transcriptional activation domain, but is also able to directly disrupt the formation of the PIC [82]. The remainder of this review will focus on 'true' transcriptional repression domains. Table 2 summarises the repressor-target interactions that will be discussed.

Transcriptional repression domains

The categorisation of transcriptional activation domains inevitably lead to efforts to similarly classify transcriptional repression domains. Indeed, a screen of random *E. coli* fragments that could function as repression domains in yeast leads to the finding that domains containing basic amino acids formed potent repressors when fused to heterologous DNA binding domains [83]. However, natural repressors have yet to fall into these neat categories, although the presence of hydrophobic amino acids is typical of most repression domains. For example, the potent transcriptional repression domain from *Drosophila* even skipped (Eve) protein contains an alanine-rich region [84–86]. Also, proline- and glutamine-rich stretches have been proposed to have negative effects on transcription in artificial fusion experiments [87]. Indeed, the transcriptional repression domains of Oct 2 and the Wilms' tumour suppressor protein are rich in proline residues that are required for efficient transcriptional repression [88, 89].

Repression domains and the basal transcription machinery

Our understanding of transcriptional repression is far less advanced than that of transcriptional activation. One of the first transcriptional repression domains to be studied in detail was Eve [84–86]. Eve is a potent transcriptional repressor in both *Drosophila* and mammalian cells. Interestingly, not only is an alanine-rich region of Eve required for transcriptional repression, but also the homeodomain itself, even when fused to a heterologous DNA binding domain. In vitro experiments have confirmed that Eve affects the assembly of PIC by preventing the interaction of TFIID with the TATA element. This may involve a direct interaction with the TBP subunit of TFIID. However, there are two distinct models by which this inhibition has been proposed to occur (fig. 5). In the first model, Eve interacts with DNA elements upstream of the promoter and the repression domain binds to TFIID, preventing its association with the TATA box [85]. In the second model, Eve binds similarly to elements upstream of the TATA box, but then recruits further Eve proteins that bind to low-affinity sites in the promoter [84]. Indeed, the Eve homeodomain does exhibit a low DNA binding specificity (to TA-rich regions). This binding to low-affinity sites around the TATA elements occludes the assembly of TFIID, thereby preventing PIC formation. It is interesting to note that the mammalian transcriptional repressor Msx-1 also requires an interaction between its homeodomain and TBP to repress transcription [90].

An interesting facet of Eve function is that it does not repress transcription synergistically [86]. The Wilms' tumour suppressor protein WT1 also does not repress transcription synergistically [89]. This is relevant mechanistically because it provides insights into how both repressors and activators function. The building of a transcription-competent complex is a multievent process that activators modulate at several steps. However, it is possible that repression requires the disruption of only one step in the process.

Is there any link between transcriptional repression and the holoenzyme? As mentioned above, the holoenzyme contains several SRB/MED components along with the GTFs [5, 91]. A complex NAT (negative regulator of activated transcription) has been identified that contains a subset of SRBs and MEDs [5]. The inhibitory function of this complex appears to reside in the SRB 10/11 heterodimer, which is able to phosphorylate the CTD of

pol II before assembly into the PIC [5, 70]. This generates a nonfunctional form of pol II, thus resulting in transcriptional repression. Whether this complex is a target of gene-specific transcriptional repressors is not known.

Repression domains and chromatin remodelling

A central function of transcriptional repressors is the restoration of a repressive chromatin configuration associated with a promoter. Several complexes have been identified that contain histone deacetylase activities and are the direct targets of several transcriptional repression domains [92]. Two complexes, NCoR (nuclear receptor corepressor) and SMRT (silencing mediator for retinoic acid receptor and thyroid hormone receptor) mediate transcriptional repression by diverse transcriptional regulators. These complexes contain HDAC1 and HDAC2 along with the Sin3 corepressors and several other polypeptides. SWI/SNF, the ATP-dependent nucleosome remodelling activity, can also manipulate nucleosomes in a manner that represses transcription [93]. Moreover, the NuRD (nucleosome remodelling and histone deacetylase) complex, contains a SWI/SNF family member along with HDACs 1 and 2 [94]. Interestingly, a DNA microarray screen found a large number of genes that are normally downregulated by SWI/SNF [93]. Not surprisingly, therefore, ATP-dependent chromatin remodelling activities have also been shown to be the target of transcriptional repressors [95].

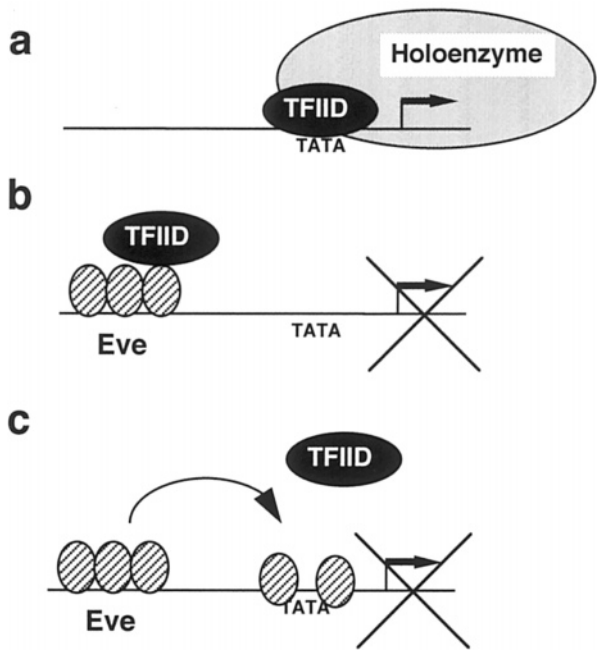

Figure 5. Repression by Eve. (*a*) Basal transcription of a gene in the absence of Eve. (*b*) Direct transcriptional repression by Eve by interacting the TFIID and preventing its association with the TATA element. (*c*) Indirect repression by Eve. The interaction of Eve with high-affinity sites upstream of the promoter leads to the recruitment of further Eve proteins that interact with low-affinity DNA elements around the TATA box. This prevents the association of TFIID with the TATA element.

Methylation and transcriptional repression

The methylation of DNA has long been known to have silencing effects on transcription and is usually associated with the underacetylation of histones. However, some recent studies have provided evidence that DNA methylation and histone acetylation are directly linked [96–98]. First, MeCP2, which specifically binds to methylated DNA, associates with histone deacetylase complexes [96, 97]. Moreover, MeCP2 can repress transcription in a manner that can be reversed by histone deacetylase inhibitors. The NuRD complex, which contains histone deacetylases and ATP-dependent chromatin remodelling activities, also contains MBD3, a protein that contains a methyl-CpG binding domain, providing a further connection between DNA methylation and chromatin remodelling [94]. It is therefore likely that DNA methylation and histone deacetylation synergise to silence gene transcription [98].

Dichotomous transcriptional regulators

Several cellular transcriptional regulators are able to either activate or repress transcription depending upon their cellular environment. One example is the *Drosophila* kruppel protein, which switches from an activator to a repressor of transcription as its concentration within a cell increases [20]. The molecular basis for this switch is due to the dimerisation of kruppel. As a monomer, kruppel activates transcription and as a dimer it represses transcription. The monomeric form of kruppel, but not the dimer, has been shown to engage in contact with TFIIB. Conversely, although the dimeric form of kruppel can interact with TFIIE, the monomeric form can not. Thus, upon dimerisation kruppel must undergo a conformational change that alters the nature of its contacts with the general transcription machinery.

Other forms of activator-repressor switching involve the interaction with additional factors. The Dorsal/DSP interaction mentioned above is an example of this, and also the E2F-Rb interaction [79, 80]. WT1 is also believed to be converted from an activator to a repressor by interaction with a soluble nuclear factor [99, 100]. Switching is not confined to the DNA-bound transcriptional regulators, but also to the coregulators. For example, MDM2 functions as a corepressor of p53 function, but it can also function as a coactivator for E2F [101].

Several nuclear hormone receptors are converted from transcriptional repressors to activators upon interaction with ligand [93]. The repressor forms of these hormone receptors interact with the corepressor complexes NCoR/SMRT/SIN3, which contain deacetylases. Other classes of transcriptional repressor also recognise these complexes. Upon interaction with hormone, the nuclear receptors switch to associate with coactivator complexes that contain the acetylases CBP/p300 and p/CAF [102]. In addition to this mechanism of switching, the thyroid hormone receptor β (THRβ) has been proposed to engage in a qualitatively different interaction with the GTF TFIIB depending upon the presence of ligand [20, 103]. In the unliganded form, THRβ engages in an interaction with TFIIB that would inhibit the interaction between TFIIB and the holoenzyme. However, upon addition of ligand, THRβ forms a productive interaction with TFIIB. Thus, activator-repressor switching can involve both alternative interaction partners and a shift in the nature of contacts with a single target factor.

Conclusions

Our understanding of the mechanisms of transcriptional regulation has made significant advances in recent years. Several additional proteins and complexes have been identified that have added to the diversity of mechanisms by which transcription can be regulated. Efforts are currently centred on providing a 'best-fit' model for how all these complexes are integrated. To this end, the use of DNA microarray chip technology will provide many answers along with detailed biochemical and genetic studies to dissect the function of these complexes.

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