Activation and Repression Mechanisms in Yeast

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In eukaryotes, gene expression depends on activator proteins that bind enhancer elements and stimulate transcription by RNA polymerase II (pol II) (Struhl 1995; Zawel and Reinberg 1995). This general requirement for activators is inferred from numerous observations in vivo that intact promoters are much more efficiently transcribed than core promoter derivatives containing only the TATA and initiator elements. The pol II transcription machinery is complex and has a molecular weight comparable to that of a ribosome. The pol II machinery is composed of two basic components, TFIID and the pol II holoenzyme. The TFIID complex, which contains the TATA-binding protein (TBP) and TBP-associated factors (TAFs), specifically binds the core promoter region; TBP interacts with high affinity and specificity for TATA elements, whereas certain TAFs can interact with some specificity for initiator and downstream elements (Burley and Roeder 1996; Verrijzer and Tjian 1996; Burke and Kadonaga 1997). The pol II holoenzyme contains the core subunits of the enzyme, basic transcription factors (e.g., TFIIB), as well as Srb, Med, and a variety of other proteins (Koleske and Young 1995; Myers et al. 1998).

Activator proteins generally bind their cognate promoter elements with high specificity and affinity, and they can often bind their target sites in the context of nucleosomal templates, the physiologically relevant substrate (Kingston et al. 1996; Polach and Widom 1996). In contrast, the TBP moiety of the TFIID complex is virtually unable to bind TATA elements in nucleosomal templates, although weak binding is observed when chromatin is disrupted by histone acetylation or by nucleosome remodeling (Imbalzano et al. 1994). The pol II holoenzyme does not appear to recognize specific DNA sequences, and its association with promoters reflects protein-protein interactions with TFIID and/or activators.

Activators contain a DNA-binding domain that specifically recognizes enhancer elements and a physically separate activation domain that stimulates transcription (Struhl 1996; Ptashne and Gann 1997). Activation domains are functionally autonomous; they retain their functional activity when fused at different positions to a wide variety of heterologous DNA-binding domains and when tethered at different positions in the promoter region. Activation domains can interact directly with many components of the pol II machinery, and they can affect multiple steps in the assembly of an active transcription complex. However, the molecular mechanisms of transcriptional activation in vivo, particularly the physiological significance and relative importance of specific protein-protein interactions and mechanistic steps, remain to be clarified. For example, activators can interact with TBP or isolated TAFs, but there is no evidence for activator-TAF interactions in the context of TFIID or for activator-TBP interactions when TBP is bound to TATA elements.

This paper reviews our efforts to understand the molecular mechanism of transcriptional activation in yeast. These studies take advantage of the power of yeast genetics and molecular biology, and the experiments are typically performed under conditions where all proteins are present at physiological concentrations, and the DNA template is in the form of chromatin. In addition, we discuss activation and repression mechanisms in which changes in chromatin structure have a direct and active role in transcriptional regulation.

QUALITY OF ACTIVATION DOMAINS AND TATA ELEMENTS ARE LIMITING FOR TRANSCRIPTION IN VIVO

In considering the physiological mechanism of transcriptional activation, a critical issue is the nature of the limiting component or step in the process. By definition, a component of a chemical or biological process is limiting if small decreases in functional concentration or activity decrease the output of the process. The question of whether individual components of the pol II machinery are limiting for transcriptional activation in vivo is completely separate from the issue of whether such components are absolutely required for pol II transcription. Even if a component is essential (i.e., removal or complete inactivation eliminates transcription), it is not limiting if large decreases in its activity do not significantly affect the overall output.

Progressive deletion of the Gcn4 activation domain causes a series of small step-wise reductions of activity, rather than defining a position where there is a precipitous loss of activity (Hope et al. 1988). The strong correlation between the length of the Gcn4 activation region and the level of transcriptional activity is strongly suggestive of a repeating structure consisting of units that act additively. More specifically, the boundaries defining the step-wise reductions in transcription occur every seven residues, suggesting that α-helical character is important for activation domain. Along with many other results, these observations indicate that transcriptional activation regions

do not have a defined tertiary structure such as found in active sites or domains in a protein. Indeed, X-ray structural analysis indicates that activation domains become structured only upon specific interaction with another protein (Uesugi et al. 1997). Most importantly, the observation that progressive and subtle changes in the activation domain result in a gradual decrease in transcriptional output strongly argues that the quality of the activation domain is limiting for transcription in vivo.

A similar line of evidence suggests that the quality of the TATA element is also limiting for transcription in vivo. Detailed mutational analyses of the canonical TATA element in the *his3* promoter indicates that single-base-pair substitutions in this element results in a wide range of transcriptional outputs in vivo (Chen and Struhl 1988; Harbury and Struhl 1989) and in vitro (Wobbe and Struhl 1990). TATA elements in natural yeast promoters vary considerably in sequence, indicating that TATA element quality is physiologically important in determining relative levels of gene expression. It should be noted, however, that TATA element quality is not necessarily equivalent to TBP-TATA-binding affinity and that the level of TBP (or TFIID) may not be limiting. In particular, TFIIA and TFIIB recognize the TBP-TATA complex (Nikolov et al. 1995; Geiger et al. 1996; Tan et al. 1996), suggesting that the quality of the TATA element (particularly its ability to be structurally deformed) may influence the formation of transcriptionally relevant protein-DNA complexes that involve proteins in addition to TBP.

ACTIVATOR-DEPENDENT RECRUITMENT OF THE POL II MACHINERY TO PROMOTERS

Kinetic Evidence

The rate at which TBP interacts with the TATA element and promotes transcription in vivo was determined by rapidly inducing an altered-specificity TBP derivative (Strubin and Struhl 1992) and measuring transcription from promoters with appropriately mutated TATA elements (Klein and Struhl 1994). In the absence of an activator, transcription dependent on the altered-specificity TBP occurs only after a lag of several hours. In contrast, Gcn4-activated transcription occurs rapidly upon induction of the TBP derivative. This strongly suggests that accessibility of TBP to the chromatin template in vivo is limiting and that the Gcn4 activation domain can increase recruitment of TBP to the promoter.

Note that this experiment only measures the initial access of TBP to promoters, i.e., the difference in lag times in the nonactivated versus the activated situation. At steady state, the relative increase in transcription dependent on the altered-specificity TBP is equivalent in both situations (although, of course, the actual transcription level is higher in the activated case). This suggests that there is a difference in the ability of TBP to access a previously inactive template as opposed to a template that has been recently utilized.

Artificial Recruitment of the pol II Machinery Bypasses the Need for an Activation Domain

We (Chatterjee and Struhl 1995) and others (Klages and Strubin 1995; Xiao et al. 1995) showed that efficient activation can occur simply by physically connecting TBP to a heterologous DNA-binding domain; i.e., artificial recruitment of TBP to the promoter can bypass the normal requirement for an activation domain (Fig. 1). This suggests that interaction of TBP with the TATA element can be a limiting step for transcription in vivo, that natural activation domains can increase recruitment of TBP to the promoter, and that interactions between activation domains and general factors that function after TBP recruitment (e.g., TFIIB, TFIIF, and pol II) are not absolutely required for transcriptional activation.

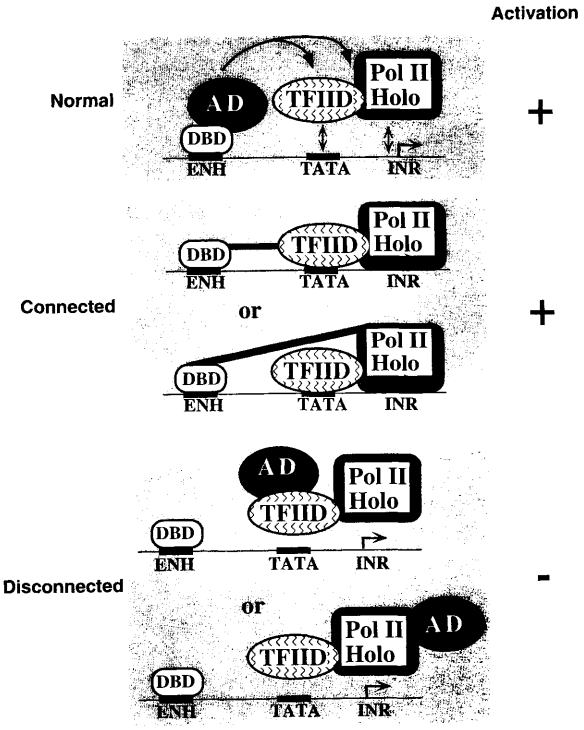

Figure 1. Transcriptional activation in yeast occurs predominantly by recruitment of the pol II machinery. (*Top*) In the physiologically relevant situation, activators bind enhancer elements via DNA-binding domains (DBD) and stimulate transcription via activation domains (AD). Arrows indicate the interactions between activation domains and the TFIID and/or the pol II holoenzyme complexes, although the direct targets within these complexes are not specified. Activator-dependent recruitment of the pol II machinery is depicted by arrows between TFIID and the TATA element and the pol II holoenzyme and the mRNA initiation site. (*Middle*) In the connected situation, activation is achieved in the absence of an activation domain by physically connecting (*thick bold line*) a component of either TFIID or the pol II holoenzyme to an enhancer-bound protein, thereby artificially recruiting the pol II machinery to promoters. (*Bottom*) In the disconnected situation, activation does not occur when the activation domain is transferred from its normal location on the enhancer-bound protein to a component of either TFIID or the pol II holoenzyme. (Reprinted, with permission, from Keaveney and Struhl 1998 [copyright Cell Press].)

Although initially demonstrated for TBP, activation by artificial recruitment occurs when an enhancer-bound protein is connected to virtually any individual component of the pol II machinery. Examples of such components include TFIIB (Gonzalez-Couto et al. 1997; Lee and Struhl 1997), TAFs (Apone et al. 1996; Gonzalez-Couto et al. 1997; Keaveney and Struhl 1998), and pol II holoenzyme subunits (Barberis et al. 1995; Farrell et al. 1996). However, the relationship of these artificial recruitment experiments to the physiological mechanism by which activation domains enhance transcription by the pol II machinery is unclear. Because the direct connection between the enhancer-bound protein and the pol II machinery is equivalent to exceptionally strong protein-protein interactions, artificial recruitment experiments might represent a bypass mechanism that is distinct from the physiological process that occurs with natural activators; i.e., the interaction of an activation domain with a single target in the pol II machinery might not be sufficient to mediate a significant degree of activation in vivo.

Activator-dependent Recruitment of the pol II Machinery Is the Predominant Mechanism for Activation in Yeast

If the physiological role of an activation domain is simply to recruit the pol II machinery, an activation domain within the machinery itself should not overcome the inherent inability of the pol II machinery to associate with promoters. To examine whether an activation domain in the preinitiation complex is sufficient for activation, we transferred activation domains from their normal location on the enhancer-bound protein to a variety of components of the pol II machinery (Keaveney and Struhl 1998). In this situation, the activation domain is physically disconnected from the enhancer-bound protein, and transcriptional stimulation does not occur (Fig. 1). However, complementation experiments indicate that the pol II machinery harboring a strong activation domain is transcriptionally competent and supports normal cell growth. This strongly suggests that the presence of an activation domain within the pol II machinery does not affect the transcriptional status of the majority of yeast genes.

In comparing the normal and "disconnected" situations, the components of the pol II machinery, the domains of the activator, the promoter, and cell physiology are identical, yet transcriptional output is dramatically different. As all of the ingredients for activation are available in the disconnected situation, the failure to activate almost certainly reflects an inability of the pol II machinery to interact with the promoter in vivo, not an inherent inactivity of the pol II machinery itself. Furthermore, unlike the activation domain, the requirement for the DNA-binding domain of the enhancer-bound protein cannot be bypassed, even though such DNA-binding domains are not usually involved in the transcriptional initiation process per se other than bringing activation domains to promoters.

These considerations indicate that (1) efficient activation requires firmly anchoring of the pol II machinery at the promoter, (2) the pol II machinery is inherently unable to associate stably with the promoter even if it carries an activation domain, (3) the DNA-binding domain provides the anchor for the pol II machinery to associate stably with the promoter, and (4) the predominant role of the activation domain is to provide the connection between the anchor and the enzymatically active entity. Thus, the location of the activation domain is important because most enhancer-binding proteins can directly associate with nucleosomal templates, whereas TFIID and the pol II holoenzyme cannot.

Activators Increase TBP Occupancy at Promoters

More recently, we have directly analyzed TBP occupancy at promoters in vivo by chromatin immunoprecipitation (L. Kuras and K. Struhl, in prep.). Specifically, cells containing an epitope-tagged TBP grown under appropriate conditions were treated with formaldehyde to cross-link proteins to DNA in situ. Following fragmentation of the DNA to an average length of 350 bp, protein-DNA complexes were immunoprecipitated with antibodies to the epitope, and the resulting DNA was quantitated by polymerase chain reaction (PCR). In all cases tested (>10 promoters, including those responsive to the Gal4, Ace1, Gcn4, and Hsf1 activators), TBP is not present at promoters in the absence of a functional activator. Moreover, the level of transcription correlates well with the degree of TBP occupancy. These results provide direct evidence that TBP association with promoters is a major limiting step in vivo, and they suggest that activators permit TBP to access chromatin templates. However, these experiments do not address the issue of whether TBP is a direct target of activators.

ROLE OF TBP, TFIIA, AND TFIIB IN RESPONSE TO ACTIVATORS

Mutations That Weaken the TBP-TATA Interaction Specifically Affect the Response to Strong Activators

Using a genetic strategy based on an altered-specificity TBP, we identified TBP derivatives that are impaired in the response to three acidic activators (Gcn4, Gal4, Ace1) but otherwise appear normal for pol II transcription (Lee and Struhl 1995). These activation-defective mutants affect residues that directly contact DNA and are defective for binding TATA elements. Similar activation-defective derivatives with mutations on the DNA-binding surface and a defect in TATA-element binding were identified in an independent genetic screen (Arndt et al. 1995). Thus, interactions at the TBP-TATA element interface can specifically affect the response to acidic activator proteins. However, activation deficiency does not simply reflect reduced affinity for the TATA element but rather involves more specific perturbations of the TBP-TATA interface (Lee and Struhl 1995).

The importance of the TBP-TATA interaction in the response to activators is also seen in a complementary set

of experiments involving the two *his3* TATA elements (Struhl 1986; Iyer and Struhl 1995a). The downstream TATA element contains the canonical TATAAA sequence (Chen and Struhl 1988; Wobbe and Struhl 1990), whereas the upstream TATA element is an extended region that lacks a conventional TATA sequence (Mahadevan and Struhl 1990) and is functionally equivalent to a weak TATA element (Iyer and Struhl 1995a). Differential *his3* TATA element utilization does not depend on specific properties of activator proteins, but rather is determined by the overall level of *his3* transcription (Iyer and Struhl 1995a). At low levels of transcription, the upstream TATA element is preferentially utilized even though it is inherently weaker than the downstream TATA element; this reflects an intrinsic preference for using upstream TATA elements. The TATA elements are utilized equally at intermediate levels, whereas the canonical TATA sequence is strongly preferred at high levels of transcription. These and other observations indicate that differential TATA utilization results from the functional saturation of weak TATA elements at low levels of transcriptional stimulation.

The importance of the TBP-TATA interaction for responding to strong activators might be related to transcriptional reinitiation (Struhl 1996). For promoters that depend on strong activators, it is likely that some of the assembled pol II machinery remains upon disengagement of the core enzyme and subsequent transcriptional elongation. In other words, efficient transcriptional activation in vivo might require the ability of an assembled preinitiation complex to initiate multiple rounds of transcription. We suggest that promoters with a compromised TBP-TATA interaction will result in fewer rounds of initiation per complex and increased reliance on assembling the entire triad on an unoccupied promoter.

Role of TFIIA in the Response to Acidic Activators

Using a different genetic strategy, we identified a TBP mutant that is specifically defective in the interaction with TFIIA (Stargell and Struhl 1995). This mutant supports transcription of most genes, but it is significantly impaired for the response to three different acidic activators, Gal4, Gcn4, and Ace1. Fusion of a TFIIA subunit to this TBP derivative corrects the phenotypic defects, indicating that the transcriptional activation defect is caused by the inability of this TBP derivative to interact efficiently with TFIIA. Interestingly, this TFIIA interaction mutant of TBP supports normal cell growth, suggesting that strong acidic activators may not be required for transcription of many yeast genes and for viability of the organism.

The properties of this TBP derivative suggests that the TBP-TFIIA interaction, and presumably TFIIA itself, is important for the response to acidic activators in vivo. In vitro, TFIIA stabilizes the TBP-TATA interaction, alters the conformation of TBP, and extends the DNase I footprint upstream of the TATA element and increases activator-dependent assembly of a TFIID-TFIIA-TATA complex (Lee et al. 1992; Lieberman and Berk 1994; Chi et al. 1995). Taken together, these observations suggest that the role of the TBP-TFIIA interaction in transcriptional activation reflects the ability of TFIIA to stabilize the interaction of TBP to the TATA element and to increase recruitment of TFIID to the promoter. However, TFIIA may also counteract repressor proteins (e.g., Mot1) that interact with TBP and block its interaction with the TATA element (Auble et al. 1994).

TFIIB Does Not Appear to be Generally Limiting for Transcriptional Activation

We analyzed the transcriptional properties of TBP derivatives in which residues that directly interact with TFIIB are replaced by alanines (Lee and Struhl 1997). A derivative with a 50-fold defect in forming TBP-TFIIB-TATA complexes in vitro supports viability and efficiently responds to activators in vivo. Another derivative, which is even more defective in the TBP-TFIIB interaction, retains the ability to respond to activators even though it does not support cell viability. Thus, a severely defective TBP-TFIIB interaction does not preclude transcriptional activation of most yeast genes in vivo.

In a complementary set of experiments, we analyzed the transcriptional effects caused by mutations on the DNA-binding surface of TFIIB that severely affect both TBP-TFIIB-TATA complex formation and interaction with the VP16 activation domain (Chou and Struhl 1997). In accord with the properties of the TFIIB-defective mutants of TBP, these TFIIB derivatives support viability, and they efficiently respond to Gal4-VP16 and natural acidic activators in different promoter contexts. One TFIIB derivative shows reduced transcription of *GAL4*, indicative of a selective transcriptional effect.

Taken together, these results argue that TFIIB recruitment is not generally a limiting step for transcriptional activation in wild-type cells. The growth phenotypes of the TFIIB mutants and the TFIIB-defective TBP mutants indicate that recruitment of TFIIB is limiting at some promoters in the mutant strains. Thus, even under conditions where TFIIB is artificially made to be limiting at a subset of promoters by virtue of mutations, there is little effect on a range of activated promoters. Nevertheless, the mutant TFIIB derivatives must be sufficiently stabilized at promoters in vivo, because TFIIB is generally required for pol II transcription (Moqtaderi et al. 1996b). Such stabilization might reflect TFIIB interactions with TAFs, TFIIF, and pol II (Zawel and Reinberg 1995) and/or recruitment as part of the pol II holoenzyme (Koleske and Young 1995). Finally, the mutant TFIIB derivatives might be stabilized at promoters simply because the concentration of TFIIB is sufficiently high to saturate ternary complex formation.

TBP Mutants Define Two Distinct Steps in the Transcriptional Activation Process

Steps in a complex biological process are often defined by mutations or inhibitors that block the process at dis-

tinct stages. In this vein, we used activation-defective TBP mutants to define two steps in the process of activation in vivo (Stargell and Struhl 1996). Specifically, we asked whether artificial recruitment of these TBP mutants could correct their transcriptional activation defects. Consistent with the ability of acidic activators to increase recruitment of TBP to the promoter, the activation defect of some TBP derivatives can be corrected by artificial recruitment. In contrast, the activation defect of the other TBP derivatives is not bypassed by artificial recruitment, suggesting that they are blocked in a postrecruitment step. Thus, these TBP mutants define two steps in the process of transcriptional stimulation by acidic activators: efficient recruitment to the TATA element and a postrecruitment interaction with a component(s) of the initiation complex.

The existence of mutations that block at distinct stages of the process indicates that the steps occur under physiological conditions in wild-type yeast cells. However, because mutations perturb the natural process, they do not provide information about which steps are limiting in wild-type cells. The two steps we have defined in vivo might correspond to the ability of acidic activators in vitro to stimulate the formation of a TFIID-TFIIA-TATA complex (Lieberman and Berk 1994; Chi et al. 1995) and to increase recruitment of TFIIB or subsequent factors to TBP(or TFIID)-TATA complexes (Lin and Green 1991; Choy and Green 1993). Another possibility, which is not mutually exclusive, is that the two steps of activation defined in vivo might correspond to in vitro activation reactions that depend either on TAFs or on the pol II holoenzyme. In this view, both the TAF- and the holoenzyme-dependent activation mechanisms observed in vitro would be required for the full response to acidic activators observed under physiological conditions.

PHYSIOLOGICAL ROLE OF TAFs

TFIID Is Not Generally Required for Transcriptional Activation

With the exception of TAF110, yeast cells contain homologs of all TAFs found in TFIID complexes in flies and humans (Moqtaderi et al. 1996a). Although all of these TAFs are essential for yeast cell growth, individual depletion of a variety of TAFs does not significantly affect transcriptional activation of the vast majority of genes, including those responsive to activators such as Gcn4, Gal4, Ace1, and Hsf1 (Apone et al. 1996; Moqtaderi et al. 1996b; Walker et al. 1996). Furthermore, depletion of TAF130 and TAF60 results in the dissolution of the TFIID complex in vivo (Moqtaderi et al. 1998). This suggests that the transcription observed in such TAF-depleted cells is mediated by the isolated TBP subunit, presumably in a manner related to TAF-independent activation in vitro. As transcription of essentially all yeast genes requires activator proteins, this result indicates that TAFs are not generally required for activation. This conclusion does not exclude the possibility that TAFs are targets for a limited subset of activators or that activator-TAF contacts are redundant with other protein-protein interactions mediated by activators (Struhl 1996).

TFIID Is Required for Core Promoter Function, Particularly at Promoters with Weak TATA Elements

Depletion of four TAFs (TAF130, TAF19, TAF40, and TAF67) causes a distinct profile of promoter-selective effects (Moqtaderi et al. 1996b, 1998). In particular, depletion of any of these TAFs differentially affects *his3* TATA element utilization; transcription from the nonconsensus TATA element is significantly reduced, whereas transcription from the consensus TATA sequence is unaffected. In addition, transcription of *trp3*, which contains a nonconsensus TATA element, is strongly decreased in these TAF-depletion strains. This subset of four TAFs is important for core promoter function, particularly at certain promoters containing weak TATA elements. In general accord with the role of TAFs in core promoter function, depletion of TAF130 also affects transcription of certain cell cycle and ribosomal protein genes, and analysis of hybrid promoters indicates that TAF function is associated with the core promoter, not the enhancer (Shen and Green 1997).

Very recently, it has been discovered that a subset of TAFs is present in the yeast SAGA and human PCAF histone acetylase complexes (Grant et al. 1998; Ogryzko et al. 1998; Struhl and Moqtaderi 1998). As a consequence, for these TAFs, physiological functions inferred from mutations or depletions could result from their presence in TFIID, SAGA, or both. Strikingly, the four TAFs with a common function at core promoters are exclusively found in TFIID. Moreover, TFIID is virtually devoid of TAFs upon TAF130 depletion, suggesting that the core promoter defects reflect the properties of the isolated TBP subunit. In accord with this suggestion, the transcriptional profile in these TAF-depleted strains is remarkably similar to that observed in yeast cells containing human TBP, which presumably interacts poorly with yeast TAFs (Cormack et al. 1994). Taken together, these observations suggest that the primary essential function of TAFs in TFIID is to facilitate transcription from certain kinds of core promoters. In weak promoters lacking consensus TATA elements, TAF interactions with initiator and/or downstream promoter elements (Burley and Roeder 1996; Burke and Kadonaga 1997) are likely to compensate for the weakened TBP-TATA interaction.

The Histone H3-like TAF Is Broadly, but not Universally, Required for Transcription

Unlike the case for all other TAFs tested, depletion of TAF17, which structurally resembles histone H3, causes a decrease in transcription of most genes (Moqtaderi et al. 1998). Although depletion of TAF17 causes the disintegration of TFIID in vivo, the results discussed above for TAF130-depleted or human TBP-dependent cells suggest that such disintegration is insufficient to account for the broad transcriptional affects. Instead, we suggest that the

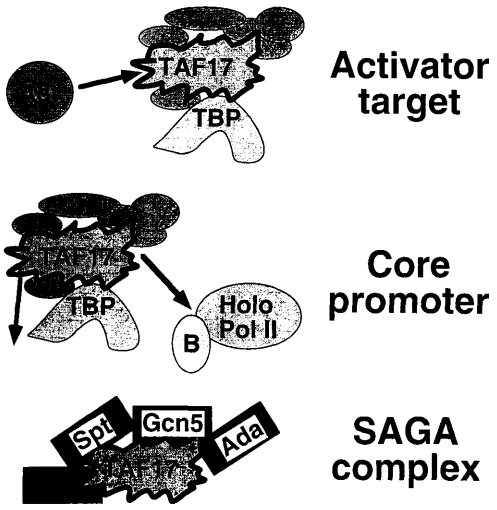

Figure 2. Models for TAF17 function. (*Top*) TAF17 in the context of TFIID interacting with the activation domain. (*Middle*) TAF17 in the context of TFIID interacting with promoter DNA and/or components of the pol II machinery such as TFIIB. (*Bottom*) TAF17 in the SAGA complex which could affect interactions with activators, TBP, or could affect histone acetylase activity. These models are not mutually exclusive.

TAF17-dependent effects on transcription are at least partly due to the presence of TAF17 in the SAGA histone acetylase complex (Fig. 2). Although mutational analyses suggest that the SAGA complex is nonessential for yeast cell growth (Roberts and Winston 1997), it is possible that SAGA has an essential role mediated by the TAFs. Alternatively, the broad transcriptional defects upon TAF17 depletion might reflect the simultaneous inactivation of the SAGA and TFIID histone acetylase complexes.

Although depletion of TAF17 broadly decreases transcription, copper-inducible (i.e., Ace1-dependent) transcription of *CUP1* is unaffected. More convincingly, TAF17-depleted cells efficiently activate heat shock genes after a brief temperature shift. Furthermore, a modified *his3* gene dependent on heat shock factor (Hsf) is inducible in TAF17-depleted cells, indicating that the immunity of the heat shock response to TAF17 depletion is due to Hsf itself, not some special property of the heat shock transcripts. Thus, TAF17-depleted cells are not fundamentally crippled for pol II transcription, and they can mediate de novo transcriptional activation by heat shock factor (Maqtaderi et al. 1998).

Our results with TAF17 are strikingly similar to those obtained previously for Kin28 (the CTD kinase subunit of TFIIH) and Srb4 (a pol II holoenzyme component) in that these proteins have broad transcriptional consequences but minimal affect on activation by Ace1 or Hsf (Lee and Lis 1998). We speculate that certain strong activators might efficiently use any of several targets and thus be less strictly dependent on any one. In contrast, a typical activator might entirely rely on a particular target or it might require multiple targets to generate a significant transcriptional response. In this view, TAF17 (or a closely associated protein such as the other histone TAFs) might be a general target of activators; loss of TAF17 would therefore affect most genes. In this regard, recent evidence has suggested that Srb4 might be an activator target (Koh et al. 1998).

ACTIVATION AND REPRESSION MECHANISMS THAT DIRECTLY INVOLVE CHROMATIN

Poly(dA:dT), a Ubiquitous Promoter Element That Stimulates Transcription via Its Intrinsic Structure

Many yeast promoters contain homopolymeric dA:dT sequences that affect nucleosome formation in vitro and are required for wild-type levels of transcription in vivo (Struhl 1985). Although typical promoter elements function as recognition sites for activator proteins, several lines of evidence indicate that poly(dA:dT) is a novel promoter element whose function depends on its intrinsic structure, not its interaction with activators (Iyer and Struhl 1995b). First, poly(dA:dT) stimulates Gcn4-activated transcription in a manner that is length-dependent and inversely related to intracellular Gcn4 levels. Second, Datin, the only known poly(dA:dT)-binding protein, behaves as a repressor through poly(dA:dT) sequences. Third, poly(dG:dC), a structurally dissimilar homopolymer that also affects nucleosomes, has transcriptional properties virtually identical to those of poly(dA:dT). Fourth, poly(dA:dT) function improves continuously when its length is increased by small increments. Fifth, *Hin*fI endonuclease cleavage in vivo indicates that poly(dA:dT) increases accessibility of the Gcn4-binding site and adjacent sequences in physiological chromatin. Thus, the intrinsic structure of poly(dA:dT) locally affects nucleosomes and increases the accessibility of transcription factors bound to nearby sequences.

The observed effects on chromatin structure in vivo are directly due to the effects of poly(dA:dT) on nucleosomes in vitro. The similar micrococcal nuclease cleavage patterns in the presence or absence of poly(dA:dT) suggest that altered nucleosome phasing or nucleosome-free DNA is not involved. The local perturbation of chromatin structure extends over a region of approximately 200 bp, which is somewhat larger than a single nucleosome. From these observations, we have suggested that a nucleosome covering poly(dA:dT) will be destabilized relative to adjacent and otherwise normal nucleosomes such that it will be less effective in competing with transcription factors for DNA (Fig. 3). In this view, longer dA:dT tracts would be more destabilizing to the relevant nucleosome, and the repressive effects of Datin might be rationalized by its occupancy of nucleosome-perturbing sequences. Aside from TATA elements, poly(dA:dT) is the most common sequence in yeast promoter regions; thus, it is very likely that poly(dA:dT) sequences are relevant for the expression of a significant fraction of yeast genes and hence have a major role in cell physiology.

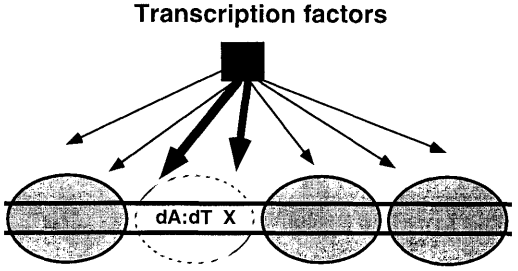

Figure 3. Model for poly(dA:dT) function. A stretch of DNA containing a poly(dA:dT) sequence and a binding site for a transcription factor (X) is coated by nucleosomes (*shaded ovals*); the position of the nucleosomes with respect to the dA:dT tract is arbitrarily drawn to reflect the apparent lack of nucleosome phasing. The nucleosome covering the dA:dT tract is shown as being perturbed (*lighter shading* and *dashed line*) in comparison to adjacent nucleosomes; this perturbation could reflect decreased stability and/or altered conformation of the nucleosome. DNA sequences covered by this nucleosome (e.g., X) on either side of poly(dA:dT) are preferentially accessible (*thicker arrows*) to transcription factors (*black box*). (Reprinted, with permission, from Iyer and Struhl 1995b.)

Transcriptional Repression by Targeted Recruitment of the Sin3-Rpd3 Histone Deacetylase Complex and Generation of a Locally Repressed Domain of Chromatin

The yeast Sin3-Rpd3 histone deacetylase complex is required for transcriptional repression by Ume6, a zinc finger protein that binds URS1 elements and regulates genes involved in meiosis and arginine catabolism (Kadosh and Struhl 1997). A short region of Ume6 interacts directly with Sin3 corepressor, and this region is necessary and sufficient for recruitment of the complex to promoters and for transcriptional repression. The Sin3-Rpd3 complex is not required for the function of the Tup1 and Acr1 transcriptional repressors under equivalent experimental conditions, indicating that repression by Sin3-Rpd3 requires recruitment to target promoters (Kadosh and Struhl 1997). Histone deacetylase activity is important for repression; Rpd3 mutants that are catalytically impaired in vitro, but competent for Sin3-Rpd3 complex formation, are severely or completely defective for transcriptional repression in vivo (Kadosh and Struhl 1998a). These observations strongly suggest that transcriptional repression occurs by targeted histone deacetylation. This mechanism is highly conserved, and it accounts for repression in mammalian cells by Mad, Rb, YY1, and steroid hormone corepressors (Pazin and Kadonaga 1997; Struhl 1998).

Direct analysis of the chromatin structure of repressed promoters in yeast cells indicates that recruitment of the Sin3-Rpd3 histone deacetylase complex and transcriptional repression are associated with localized histone deacetylation (Kadosh and Struhl 1998b; Rundlett et al. 1998). Decreased acetylation of histones H3 and H4 (preferentially lysines 5 and 12) is observed in wild-type strains but not in strains lacking the DNA-binding repressor (Ume6), Sin3 corepressor, and Rpd3 histone deacetylase. Mapping experiments indicate that the domain of histone deacetylation is highly localized, occurring over a range of one to two nucleosomes. The limited spread of histone deacetylation from the site of recruitment suggests that localized chromatin modification is an inherent property of the Sin3-Rpd3 complex that is relatively insensitive to the presence or absence of other promoter elements. Furthermore, the tethered Sin3-Rpd3 complex has a limited degree of flexibility that permits it to modify the nucleosome at the recruitment site and perhaps the neighboring nucleosome. Thus, the Sin3-Rpd3 complex defines a novel mechanism of transcriptional repression that involves targeted recruitment of a histone-modifying activity and localized perturbation of chromatin structure (Fig. 4).

Although the magnitude of histone deacetylation of individual lysines is modest (two- to threefold), the overall effect on chromatin structure is likely to be more substantial because at least two histones (H3 and H4) and multiple lysine residues are affected. The simplest model for transcriptional repression is that localized histone deacetylation generates a repressive chromatin structure that inhibits the binding of activator proteins or TFIID to their cognate promoter elements. However, in the promoter we have examined (Kadosh and Struhl 1998b), we

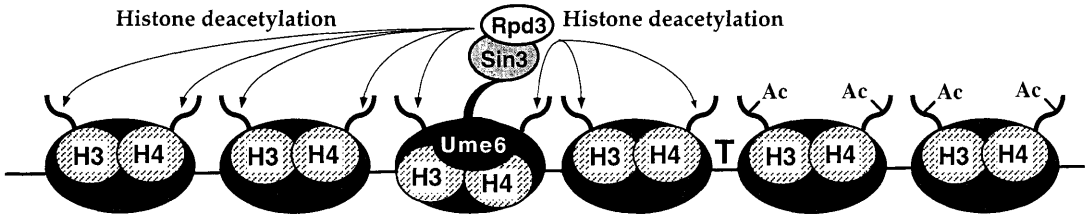

Figure 4. Transcriptional repression by targeted recruitment of the Sin3-Rpd3 histone deacetylase complex. The Ume6 repressor binds URS1 (shown as occurring in the context of a nucleosomal template) and recruits the Sin3-Rpd3 corepressor complex to the promoter. As a consequence, histones H3 and H4 (lysines 5, 12, and to a lesser extent 16) are deacetylated (lack of Ac) over a range of one to two nucleosomes from the site of recruitment. (*Arrows*) For the promoter tested, the region of local histone deacetylation includes the UAS element, but probably ends upstream of the TATA elements (T). Analogous regions of other Sin3-Rpd3-repressed promoters might vary in length and position. The figure is not intended to suggest any particular mechanism of repression (e.g., inhibiting access of activators, TFIID, or the pol II holoenzyme or inhibiting the communication between these components). (Reprinted, with permission, from Kadosh and Struhl 1998b.)

disfavor a direct effect on TBP binding because the domain of localized histone deacetylation is unlikely to extend as far as the TATA elements. Alternatively, locally deacetylated chromatin might not reduce the accessibility of activators or TBP per se, but rather interfere with the communication of these components with each other or with the pol II holoenzyme. More detailed information on the mechanism of transcriptional repression will require measurements of promoter occupancy of activators, TFIID, and pol II holoenzyme in vivo.

CONCLUDING COMMENTS

During the past few years, it has become increasingly clear that transcriptional activation and repression mechanisms are intimately connected with chromatin structure. For example, some histone acetylases are components of the pol II transcription machinery itself, whereas other histone acetylases are present in large multiprotein complexes that interact with activation domains, TBP, or the pol II holoenzyme (Struhl 1998). In addition, there is some evidence that the Swi/Snf nucleosome remodeling complex might interact with the carboxy-terminal tail of pol II (Wilson et al. 1996). Finally, as discussed here and elsewhere (Pazin and Kadonaga 1997; Struhl 1998), the Sin3-Rpd3 histone deacetylase complex mediates transcriptional repression in yeast and mammals by modifying chromatin upon being directly recruited to promoters by DNA-binding repressor proteins. With respect to the mechanism of transcriptional activation, it is clear that nucleosomal templates significantly block access of the pol II machinery (particularly TBP) to promoters in vivo. As such, a major function of activation domains is to increase recruitment of the pol II machinery to promoters in the context of chromatin. Recruitment is likely to involve both direct interactions to the pol II machinery itself and interactions with chromatin-modifying activities (which may or may not be directly associated with the pol II machinery) that alter the properties of the promoter template. However, it is still unclear which proteins are direct and physiological targets of natural activation domains.

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