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YEAST TRANSCRIPTIONAL REGULATORY MECHANISMS

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

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ABSTRACT

Transcriptional regulation directly influences many biological phenomena such as cell growth, response to environmental change, development of multicellular organisms, and disease. Transcriptional regulatory mechanisms are fundamentally similar in eukaryotic organisms (93). Components of the basic RNA polymerase II (Pol II) machinery are highly conserved and, in some cases, functionally interchangeable. Transcription factors with similar structures and DNA-binding specificities are found throughout the eukaryotic kingdom, and acidic activation domains stimulate transcription across a wide range of species. Complex promoters with multiple protein binding sites are typical in all eukaryotic organisms, and efficient transcription generally requires the combinatorial and synergistic action of activator proteins that function at long and variable distances from the mRNA initiation site.

Molecular mechanisms of eukaryotic transcriptional regulation have been elucidated from the studies that involve a wide variety of genes, promoters, proteins, organisms, and experimental approaches. This review focuses on transcriptional regulatory mechanisms in the baker's yeast *Saccharomyces cerevisiae*. Studies in yeast have emphasized powerful genetic approaches that are not available in other eukaryotic organisms. As a consequence, yeast is particularly amenable for analyzing transcriptional regulatory mechanisms in vivo under true physiological conditions. Furthermore, classical and molecular yeast genetics has permitted the discovery and functional characterization of transcriptional regulatory proteins that were not identified in biochemical studies. Thus, genetic analysis in yeast has often generated information complementary to that obtained from biochemical studies of transcription in vitro, and it has provided unique insights into mechanisms of eukaryotic transcriptional regulation.

CONTENTS

PROMOTER ELEMENTS

Yeast Pol II promoters contain upstream (UAS), TATA, and initiator (Inr) elements whose properties have been reviewed previously (127). Upstream elements are short (10–30 bp), promoter-specific DNA sequences that are typically located 50 to 500 bp upstream of the initiation site. Yeast upstream elements are analogous, and in many cases homologous, to mammalian enhancer sequences. However, while yeast upstream elements function bidirectionally at variable distances upstream of the initiation site, they generally do not activate transcription when located downstream. In general, upstream elements are recognized by DNA-binding proteins that determine the particular regulatory properties of a given promoter. However, approximately 20% of yeast promoters contain homopolymeric dA:dT tracts that function as upstream elements by virtue of their intrinsic DNA structure, not by interacting with a specific DNA-binding protein (60).

Most yeast promoters contain TATA elements (consensus TATAAA) that are important for transcriptional initiation. Yeast TATA elements are located 40–120 bp upstream of the mRNA initiation sites; the precise distance is functionally unimportant. In contrast, TATA sequences in most other eukaryotes are invariably located 25–30 bp away from the initiation site. TATA elements are recognized by the TATA-binding protein (TBP), a component of the basic machinery that is required for Pol II transcription. For promoters that lack recognizable TATA elements, it is presumed that TBP binds in a nonsequence-specific manner that is stabilized by protein-protein interactions with other transcription factors.

In yeast, the initiator element is the primary determinant of where transcription begins. Initiator elements are located near the mRNA start sites, but unlike upstream and TATA elements, they do not have a strong sequence consensus. In comparison to upstream and TATA elements, initiator elements have a relatively modest effect on the level of transcription.

Some yeast promoters contain negative regulatory elements, termed operators, that repress transcription. Operators resemble upstream elements in that they are protein-binding sites that function bidirectionally at variable distances upstream of TATA elements. Some operators can repress transcription when located upstream of upstream elements, but repression is generally much more efficient when the operator lies between the upstream and TATA element. However, the mating-type silencer efficiently represses transcription when located 2 kb upstream or downstream from the initiation sites.

GENERAL TRANSCRIPTION FACTORS

Biochemical analysis of yeast and mammalian proteins has led to a reasonably good description of the remarkably complex Pol II machinery and the mechanism by which it initates transcription (15, 23). In yeast, the essential components for accurate initiation in vitro are Pol II, a 12-subunit enzyme (149), as well as general transcription factors TBP (53, 128), TFIIB (105), TFIIE (35), TFIIF (52), and TFIIH (36). TBP binds to the TATA element, TFIIB spans the region between the TATA element and mRNA initiation site, TFIIF interacts with Pol II and is important in recruiting Pol II to the promoter, TFIIH phosphorylates the C-terminal tail (CTD) of the largest subunit of Pol II, and TFIIE is important in promoter clearance. TFIIA, which influences binding of TBP to the TATA element, is important but not absolutely required for accurate initiation in vitro (65, 72). In addition, TBP-associated factors (TAFs) that are part of the TFIID complex (111) and components of the Pol II holoenzyme (Srb and other proteins)(72, 76) can be considered as general transcription factors even though they are not required for accurate initiation in vitro.

In vitro, accurate initiation and the response to activators can occur with recombinant or highly purified general transcription factors (36). Assembly of an active transcription complex can proceed by stepwise addition of individual factors. However, the existence of a very large complex containing most of the general transcription factors suggests the possibility that much of transcription apparatus (other than TBP and perhaps TFIIB) is preassembled before being recruited to the promoter (72, 76).

Although this biochemical description of the general Pol II machinery is essential for understanding transcriptional regulatory mechanisms, many issues are best addressed by genetic approaches. In particular, many of the general factors can interact with transcriptional activation domains in vitro, but the

physiological significance of these interactions remains to be established in most cases. This section emphasizes yeast genetic experiments that have made unique contributions to our understanding of the basic Pol II machinery.

TATA-Binding Protein, The Universal Transcription Factor

TATA-Binding Protein (TBP) is the most highly conserved eukaryotic transcription factor, with its C-terminal core domain (180 residues) showing greater than 80% sequence identity in a wide variety of species (53, 128). The yeast core domain is necessary and sufficient for normal cell growth, whereas the evolutionary divergent N-terminal region is functionally dispensible (24, 108). For reasons that are not apparent, overexpression of the core domain, but not full-length TBP, significantly inhibits cell growth (41, 151). The core domain is an intramolecular dimer of related, but not identical, subdomains that can be visualized as a saddle consisting of a curved, antiparallel β-sheet with four α helices lying on the upper surface (69, 71). The concave underside of the saddle binds to the TATA element in a manner that causes a dramatic distortion of the DNA helix in the immediate vicinity of the recognition sequence.

Although the overall structure of the TBP-TATA element complex is approximately twofold symmetric, TBP binds in a preferred orientation. Mutations in one TBP subdomain alter TATA-element specificity in a specific "half-site" of the TATA element (125). Moreover, equivalent mutations in the two TBP subdomains have distinct DNA-binding specificites (3). As a consequence of the oriented binding of TBP to the TATA element, the convex surface of the saddle and the α helices are also oriented with respect to the initiation site. Amino acid changes at specific positions on the convex surface or α helices can specifically affect interactions with TFIIA (16, 123), TFIIB (70), and Spt3 (32). Thus, the orientation of TBP on the TATA element defines the locations of interacting components of the basic machinery and hence imposes unidirectional transcription.

Although initially defined as a basic Pol II factor, TBP is required for transcription by all three nuclear RNA polymerases (53, 128). TBP carries out its multiple functions by associating with other proteins (TAFs) into distinct complexes (SL1, TFIID, TFIIIB) that are specific for the three RNA polymerase machineries. In vivo, TBP appears to be required for transcription of all genes, including those lacking TATA elements (26). Several TBP functions have been defined by TBP mutants with Pol-specific effects in vivo. Mutations affecting the DNA-binding surface indicate that the sequence-specific, TBP-TATA interaction is important for transcription from conventional Pol II promoters, but not from Pol I and III promoters (118). Conversely, human TBP supports transcription from TATA-containing Pol II promoters, but not from

Pol I, Pol III, and TATA-less Pol II promoters; presumably, this reflects evolutionarily diverged interactions with TAFs that are necessary for recruitment to promoters lacking TATA elements (25). TBP mutants specifically defective in Pol III transcription define an extensive surface that is likely to interact with a Pol III-specific TAF (27). Analysis of Pol III- and Pol II-specific mutations suggests that competition among TAFs for limiting quantitites of TBP determines the relative amounts of Pol II and Pol III transcription in vivo (27).

TBP-Associated Factors

Yeast TBP can associate with Pol II-specific TAFs into a complex that resembles TFIID from other eukaryotic species (111). As expected, yeast TFIID can support basal and activated transcription in vitro, whereas TBP is unable to respond to activator proteins. At least three proteins in yeast TFIID are homologous to Pol II-specific TAFs in other species (111, 144), and other components in the yeast TFIID complex will likely have counterparts in other organisms. All known Pol II-specific TAFs are essential for cell viability.

The biological roles of individual TAFs and the TFIID complex remain to be determined. It is unclear if the requirement of TAFs for cell viability is due to a general role in the transcription of most or all yeast genes, a more specific effect on transcriptional activation or repression, or a very specific effect on the transcription of one (or a few) essential genes. A related question is whether transcription from Pol II promoters is mediated by TBP or TFIID. These issues are of interest because, unlike the case in other organisms, yeast cells appear to contain a considerable amount of free TBP. Moreover, in experiments involving the Pol II holoenzyme (see below), it appears that activated transcription can be reconstituted in vitro under conditions where yeast TAFs and TFIID do not seem to be present (72).

Yeast contains other TBP-associated factors in addition to the Pol II-specific TAFs that are present in TFIID. Spt3 interacts directly with TBP, and this interaction is required for normal transcription patterns in vivo (32). Genetic analysis indicates that Spt3 is functionally related to Spt7 and Spt8, and these proteins may be part of a TBP complex that is distinct from TFIID (33). Mot1 is another TBP-interacting factor that inhibits binding to the TATA element in an ATP-dependent manner (4).

TFIIA

TFIIA associates with and alters the conformation of TBP, and it stabilizes the TBP-TATA element interaction, presumably by interacting with DNA just upstream of the TATA element. However, studies in vitro have generated considerable controversy about the role of TFIIA in basal and activated tran-

scription. Yeast TFIIA contains two subunits, both essential for cell viability (110). Depletion of TFIIA activity in vivo reduces transcription of several Pol II genes, but does not affect Pol III transcription (65). This suggests that TFIIA plays a general and important role in Pol II transcription, but is not absolutely required.

An efficient interaction between TFIIA and TBP appears to be essential for transcriptional activation in vivo. A TBP mutant that is specifically defective in interacting with TFIIA in vitro is unable to respond to acidic activators in vivo (123). This TBP derivative is fully competent to support transcription from many Pol II promoters, suggesting that the TBP-TFIIA interaction plays a less important (and perhaps no) role in constitutive Pol II transcription.

Selection of mRNA Initiation Site By TFIIB and Pol II

TFIIB and Pol II are the basal transcription factors that are primarily responsible for selecting the initiation site. As mentioned previously, *S. cerevisiae* differs from most other eukaryotic organisms in that the distance between the TATA element and initiation site is longer and more variable. In reconstituted transcription reactions using basic factors from *S. cerevisiae*, replacement of both TFIIB and Pol II by their *S. pombe* counterparts is necessary and sufficient to shift initiation to a site characteristic of that in *S. pombe* (87). In vivo, mutations affecting either TFIIB or the largest subunit of Pol II (Rpb1) can reduce initiation from the normal site while increasing initiation from more downstream sites (10, 106). Conversely, mutations affecting another Pol II subunit, Rpb9, shift initiation to more upstream sites (39, 58).

TFIIH Connects Pol II Transcription, DNA Repair, and Cell-Cycle Control

TFIIH is a complex transcription factor that phosphorylates the C-terminal tail (CTD) of the largest Pol II subunit. Remarkably, many TFIIH subunits had been previously identified by genetic screens, and this discovery revealed unexpected connections between Pol II transcription, DNA repair, and cell-cycle control. Initially, TFIIH was isolated as a five-subunit core that contains the DNA repair proteins Rad3, Tfb1, and Ssl1 (37). Although the TFIIH core can function in crude extracts, it does not phosphorylate the CTD or function in more purified systems. The fully functional transcription factor (termed TFIIH holoenzyme) contains Ssl2 and a complex of three proteins (termed TFIIK) that possess CTD kinase activity (36). CTD phosphorylation is mediated by Kin28 (36), which associates with Ccl1 to form a CDK/cyclin pair (143) that is homologous to a CDK activating kinase (CAK) involved in cell-cycle control. Although mammalian CAK was initially identified as a

standard CDK/cyclin pair, the CAK subunits are present in TFIIH and they mediate the associated CTD kinase activity (114).

In addition to its role in Pol II transcription, the TFIIH core plays a critical role in nucleotide excision repair. In this case, however, the TFIIH core does not interact with TFIIK, but rather with a large complex containing Rad1, Rad2, Rad4, Rad10, and Rad14, proteins required for nucleotide excision repair (130). Thus, by associating with distinct complexes, the TFIIH core exists in two forms that are specific for Pol II transcription and DNA repair. Interchangeability between these two forms may underlie repair-transcription coupling, the process in which DNA damage on the transcribed strand of active genes is preferentially repaired.

Pol II Holoenzyme

A considerable portion of yeast Pol II is found in a large multiprotein complex termed the Pol II holoenzyme (72, 76). Although its precise composition remains to be clarified, the Pol II holoenzyme contains TFIIF, and perhaps TFIIB and TFIIH. TBP may weakly associate with the holoenzyme but is probably not a true component, and TFIIE and TFIIA are not present. In addition, the Pol II holoenzyme contains Srb proteins (Srb2-Srb11), Gal11, and Sug1, all of which were initially identified by mutations that cause various transcriptional effects in vivo. Srb10 and Srb11 are of particular interest because they form a CDK-cyclin pair that phosphorylates the CTD (88). Although a Pol II holoenzyme and many of the components such as Srb proteins have yet to be described in other eukaryotic species, it seems almost certain that they will exist.

In vitro, the Pol II holoenzyme (but not core Pol II) responds to transcriptional activators (72, 76). A subcomplex of the holoenzyme that lacks the Pol II subunits, termed mediator, is required for activated transcription, and it also increases the level of basal transcription in reactions using highly purified components (72). Interestingly, in these most purified systems, transcriptional activation occurs in the apparent absence of the Pol II-specific TAFs that are components of TFIID.

The mutations that identified the various components of the Pol II holoenzyme have provided some information about their physiological functions. Some components (Srb4, Srb6, Srb7, and Sug1) are essential for cell growth, and loss of Srb4 or Srb6 function results in a general loss of Pol II transcription (136). These results indicate that the Pol II holoenzyme plays a critical role for transcription in vivo. Other holoenzyme components are not essential for cell growth, but they play distinct functional roles. The Srb proteins are functionally related to the CTD because the initial mutations were isolated as suppressors of transcriptional defects caused by CTD

truncations (88, 135). Moreover, mutations in the Srb10/Srb11 kinase cause reduced CTD phosphorylation, and are phenotypically similar to CTD truncations (88). Gal11 contributes to transcription of many genes including those activated by Gal4 and Rap1, but it does not seem to be required when the Gal4 and Rap1 binding sites are moved closer to the TATA element (102). Sug1, which was identified by mutations that relieve the requirement for the Gal4 activation domain, directly interacts with activation domains and TBP in vitro (131), suggesting that it may be part of the mediator component of the Pol II holoenzyme.

TRANSCRIPTIONAL ACTIVATION

Structure and Function of Yeast Activator Proteins

Yeast cells contain a wide variety of DNA-binding transcriptional activator proteins that specifically recognize upstream promoter elements via typical eukaryotic DNA-binding motifs (e.g. homeodomain, zinc finger, bZIP, bHLH). Although most upstream promoter elements are bound by a single transcriptional activator, some target sites (e.g. AP-1, ATF/CREB, Swi5/Ace2) are recognized by multiple proteins, while others are recognized by heteromeric complexes such as Hap2-Hap3-Hap5 (94) and Swi4-Swi6 (109). Many yeast activators are present at low intracellular concentrations, are not essential for viability, and interact with relatively few promoters. Some activators play discrete physiological roles, whereas others interact with genes of apparently unrelated functions. In addition, there is a class of activators (e.g. Rap1, Abf1, and Reb1) that are abundant, essential for cell growth, and interact with many unrelated promoters.

Upon binding to their cognate sites, activator proteins stimulate gene expression via a transcriptional activation domain that is functionally distinct, and usually physically separate, from the DNA-binding domain. Yeast activation domains are often defined by short acidic regions that function autonomously when fused to heterologous DNA-binding domains. Although negative charge is clearly important, the level of transcriptional stimulation is also influenced by hydrophobic residues and other features that are poorly understood at the structural level (47, 127). Some activation regions become acidic upon phosphorylation (121, 122), but it is unclear whether increased transcription is due simply to increased negative charge. Yeast activators are unlikely to contain glutamine-rich or proline-rich activation domains, because mammalian domains of this type do not function in yeast cells (81, 107). However, not all yeast activators contain regions that are acidic or are likely to be phosphorylated, suggesting that there are other types of yeast activation domains.

Mechanism of Transcriptional Enhancement by Acidic Activators

In yeast cells, activators can stimulate transcription by increasing recruitment of TBP to the promoter. Measurement of the rate at which TBP can productively access the chromatin template in vivo indicates that accessibility of TBP to the *HIS3* TATA element is a rate-limiting step that can be accelerated by the Gcn4 activation domain (74). Moreover, direct recruitment of TBP to the *HIS3* promoter by physically connecting TBP to a heterologous DNA-binding domain activates transcription (18, 73). This latter observation also suggests that interactions between activation domains and general factors that function after TBP recruitment (e.g. TFIIB, TFIIF, Pol II) can be bypassed for transcriptional activation. The hypothesis that activators stimulate TBP recruitment in vivo is attractive in light of observations in vitro that TBP binds very poorly to TATA elements in the context of chromatin (59).

Independent support for the importance of TBP recruitment to transcriptional activation comes from the analysis of TBP mutants that are specifically defective in the response to acidic activators in vivo. Several activation-defective derivatives are altered on the DNA-binding surface of TBP and are unable to bind TATA elements (2, 85). Although the mechanism is unknown, activation-deficiency does not simply reflect reduced affinity for the TATA element but rather involves more specific perturbations of the TBP-TATA interface (85). Another activation-defective TBP mutant is specifically defective in the interaction with TFIIA (123). Fusion of a TFIIA subunit to this TBP derivatives restores activation function, indicating that an efficient TBP-TFIIA interaction is required for transcriptional activation in vivo. The role of the TBP-TFIIA interaction in transcriptional activation likely reflects the ability of TFIIA to stabilize the interaction of TBP to the TATA element.

The mechanism(s) by which acidic activation domains increase recruitment of TBP to the TATA element is unknown. One possibility is that acidic activation domains directly interact with TFIIA and/or TBP, thereby stabilizing the TBP-TFIIA-TATA complex in the context of chromatin. In a related model, activation domains might interact with proteins that associate with TFIIA or TBP (e.g. TAFs or TFIIB). Alternatively, acidic activation domains might directly cause, or indirectly lead to, alterations in chromatin structure that increase accessibility of TBP to the promoter. Activation domains can perturb chromatin structure in the absence of a functional TATA element and transcription (5, 98). Moreover, mutations in histone H4 (31) or in the Swi/Snf complex that affects nucleosomes (see below) are impaired in transcriptional activation, suggesting that chromatin structure plays an active role in the response to acidic activators. These protein-protein interaction and chromatin

models are not incompatible and indeed may be synergistic in explaining increased TBP recruitment by acidic activators.

Although acidic activators can stimulate transcription by increasing recruitment of TBP, other mechanisms will likely be involved. For example, TBP is bound to the *CYC1* TATA elements in vivo in the absence of upstream activator proteins (19), and artificial recruitment of TBP to the *CYC1* promoter does not result in transcriptional activation (18). These observations suggest that, unlike the situation at the *HIS3* promoter, recruitment of TBP to the *CYC1* promoter is not a rate-limiting step for transcription. In support of this idea, transcriptional activation can be observed when a DNA-binding domain is artifically connected to Gal11, a component of the Pol II holoenzyme (8). Finally, alternative mechanisms are suggested by observations that acidic activation domains appear to be functionally distinct. Acidic activators can differ in their ability to activate transcription from certain TATA sequences (49), and they are selectively affected by *ada2* and *gcn5* mutations (9, 40) and by specific TBP derivatives (2).

Other Mechanisms of Transcriptional Activation

Although our knowledge about transcriptional activation in yeast is largely confined to acidic activation domains, there is increasing evidence for other types of activation domains that function by different molecular mechanisms. First, many yeast activators do not appear to contain highly acidic regions. Second, mutations altering TBP that severely impair the response to acidic activators do not affect the transcription of most Pol II genes and do not abolish cell growth (2, 85, 123). As normal transcription of most (and perhaps all) yeast genes requires activator proteins bound to upstream promoter elements, this observation is strongly suggestive of alternative modes of transcriptional activation. Third, abundant transcriptional activators such as Rap1, Abf1, and Reb1 also play important roles at DNA replication origins, telomeres, and the mating-type silencer (63, 92, 129). These proteins might not specifically interact with the Pol II machinery, but instead might carry out their diverse roles by affecting chromatin.

Some DNA-binding domains play additional roles in transcription beyond bringing the protein to the promoter. Positive control mutations in the Hap1 (140) and Adr1 (139) DNA-binding domains interfere with transcriptional activation while not affecting DNA binding. Some Hap1 derivatives are transcriptionally active only when bound at particular DNA sequences. DNA-binding domains also may be involved in the transcriptional synergy of multiple activator proteins bound to the promoter (103). DNA-binding domains might contribute to transcriptional activation by recruiting protein cofactors and/or altering chromatin structure. Multifunctional DNA-binding domains are likely

to be more prevalent than is currently appreciated because functional analysis of activator proteins by crude deletions or domain swapping is biased against identifying activation regions that are interdigitated with their DNA-binding domains.

Intermediary Transcription Factors

Intermediary factors do not bind promoter elements and are not components of the basic machinery; they have been identified by mutations that have selective effects on transcription. In principle, an intermediary protein with an activation domain could act as a co-activator that stimulates transcription upon association with a DNA-bound protein. Alternatively, an intermediary protein might be an adaptor/mediator that transduces the signal from the activation domain to a component of the basic machinery. Finally, intermediary proteins might increase transcription by altering chromatin structure. By the definition used here, intermediary proteins do not include those that are tightly associated with basic transcription factors (e.g. TAFs in the TFIID complex or components of the Pol II holoenzyme), or that modify or alter the level of transcription factors.

A complex containing Ada2, Ada3, and Gcn5 appears to function as a transcriptional adaptor for a subset of acidic activation domains (57). Mutations in any of these genes relieve the toxicity associated with the VP16 activation domain, and they reduce transcriptional activation by some acidic activators. However, the Ada2-Ada3-Gcn5 complex is not required for transcription of many genes and is not essential for cell growth. Ada2 interacts with a region of the VP16 activation domain, and it contains an Ada3-dependent transcriptional activation domain (119). These observations suggest that the Ada2-Ada3-Gcn5 complex might functionally connect some acidic activation domains to the basic machinery.

The Swi/Snf complex, which contains at least ten proteins including the genetically identified Snf and Swi proteins (17, 28), is important for transcription of a large set of genes including those stimulated by acidic activators (148). In vitro, Snf2/Swi2 (84) and the Swi/Snf complex (17, 28) have DNA-stimulated ATPase activity that is required for transcriptional activity in vivo (84). More importantly, the Swi/Snf complex alters chromatin structure in an ATP-dependent manner, and it facilitates the binding of activator proteins to their target sites in chromatin (28). A related complex from human cells has similar biochemical properties, and it also increases binding of TBP to nucleosomal templates (59). In vivo, the Swi/Snf complex affects chromatin structure in a manner that is independent of the transcriptional status of the promoter (56). These observations indicate that the Swi/Snf complex stimulates transcription by virtue of its effects on chromatin structure. However, given

its general ability to increase protein access in the context of chromatin, it is unclear how the Swi/Snf complex selectively affects the transcription of some genes, and whether it is directly or indirectly related to the transcriptional activation process.

TRANSCRIPTIONAL REPRESSION

Repression by Inhibiting the Function of Activators

Given the complexities involved in the basic initiation and transcriptional activation processes, it follows that transcription can be repressed by a variety of distinct mechanisms (55). The simplest forms of gene-specific repression involve specific binding of repressor proteins to operator DNA sites in the relevant promoters. Operator sites that inhibit transcription can be located at various positions in promoters. Repressor proteins can compete with activators by binding to coincident or overlapping sites; e.g. Acr1 and ATF/CREB activators compete for binding to ATF/CREB sites (145). Proteins bound between upstream and TATA elements also repress transcription, presumably by sterically inhibiting the communication between upstream activators and the basic machinery (13). In some cases, yeast repressors can function even when bound upstream of an intact promoter (62, 126), presumably by a mechanism distinct from simple steric hindrance (see below).

For non-DNA-binding proteins, the simplest form of repression involves protein-protein interactions that block the function of activators. Interaction of the repressor might simply mask either the DNA-binding and/or transcriptional activation function of the activator. For example, Gal80 represses transcriptional activation by Gal4 by functionally blocking the Gal4 activation domain (89). However, as Gal80 is tightly associated with Gal4 under all physiological conditions, repression is not simply due to masking but requires a conformational change in Gal4 and/or Gal80 (86).

While these repression mechanisms differ in molecular detail, they share important properties. First, they generally operate at the level of an individual gene or set of genes. Second, these repression mechanisms generally occur by inhibiting the function of activators, and they are fairly straightforward at the molecular level. Third, these repression mechanisms are indirect; i.e. they block the stimulatory function of specific activators rather than directly interfering with the basic transcription machinery or the chromatin template.

Global Repression by Chromatin

Eukaryotic organisms also have a variety of global repression mechanisms that negatively regulate the transcription of many apparently unrelated genes. The most general repression mechanism involves nucleosome coating of the DNA

template, which severely restricts access of transcriptional regulatory proteins to promoters (46). Disruption of the normal nucleosome structure by histone loss (48), change in histone dosage (21), histone mutations (91), or poly-(dA:dT) sequences (60) results in increased transcription. Nucleosomal repression is mechanistically simple and affects the transcription of all genes. However, differences in intrinsic nucleosome positioning and stability as well as differences in the ability of specific activators and TBP to interact with nucleosomal templates can affect the extent to which individual genes are affected in vivo.

Yeast contains several nonhistone proteins that may be involved in global repression by chromatin. These include Sin1(Spt2), which bears some similarity to the mammalian chromatin-associated HMG1 protein (79), Sin4 (61), and Spt4-Spt5-Spt6, which likely exists as a multiprotein complex (132). Mutations in these genes cause diverse transcriptional phenotypes that are very similar to those caused by certain histone mutations. In particular, increased transcription is observed from promoters lacking functional upstream elements. In addition, plasmids in *sin4* mutant strains have decreased superhelicity, possibly reflective of a change in nucleosomal density (61). The mechanism(s) by which these proteins affect chromatin structure remain to be established.

Global Repression by Direct Inhibition of the Basic Transcription Machinery

Another global repression mechanism involves proteins that interfere directly with a component(s) of the basic transcription machinery. As is the case with nucleosomal repression, inhibitors of basic transcription factors should repress all genes, although individual genes will be affected to various extents. Mot1, an ATP-dependent inhibitor of TBP binding to the TATA element, negatively affects the transcription of many, but not all, genes (4). Although the biochemical mechanism of repression seems clear, no obvious pattern distinguishes genes that are repressed from those that are not affected. Although repression is quantitatively modest (typically two- to fivefold) on individual genes, Mot1 is essential for cell growth, probably because it affects a wide spectrum of genes and alters the stoichiometry of essential macromolecular complexes.

The NOT complex, which contains Not1(Cdc39), Not2(Cdc36), Not3, and Not4(Mot2), acts as a global repressor (22). *not* mutations differentially affect TATA-element utilization in the *HIS3* promoter in a manner that does not depend on upstream promoter elements or transcriptional activators and that is distinct from chromatin-based repression. These observations strongly suggest that the Not complex inhibits the basic transcription machinery, with likely targets being TBP, TAFs, and perhaps TFIIA.

Transcriptional Corepressors That Affect Multiple Biological Pathways

The Cyc8(Ssn6)-Tup1 complex does not bind to specific promoter sequences, but it is required for transcriptional repression of genes regulated by glucose, oxygen, cell-type, and DNA-damage. Although repression of these distinct classes of genes requires pathway-specific DNA-binding proteins such as Mig1 (101), Rox1 (7), and α2 (44, 67), binding to their cognate operators is not sufficient (68). Conversely, both Cyc8 and Tup1 strongly repress transcription when artificially recruited to a functional promoter (68, 141). These observations indicate that Cyc8-Tup1 acts as corepressor that is recruited to promoters via pathway-specific DNA-binding proteins.

Different regions of the Cyc8-Tup1 complex are involved in recruitment of the corepressor to the various classes of promoters. For cell-type genes, recruitment occurs by the direct interaction of α2 with the WD motifs of Tup1 (77). However, distinct TPR motifs of Cyc8 are required for recruitment to oxygen-regulated (TPR4–7) and glucose-regulated (TPR8–10) genes; the WD motifs of Tup1 are not important (142). Although it is unknown whether these distinct TPR motifs directly interact with Rox1 and Mig1, TPR1–3 directly interacts with Tup1, indicating that the TPR motifs mediate specific protein-protein interactions (142). Thus, differential recruitment involves specific protein-protein interactions between distinct surfaces of Cyc8-Tup1 and structurally dissimilar DNA-binding proteins.

Cyc8-Tup1 inhibits transcription when recruited upstream of a heterologous promoter, suggesting that repression occurs by an active mechanism rather than by steric hindrance (68, 141). The transcriptional repression function of the Cyc8-Tup1 complex is mediated by a specific domain of Tup1 (141). Within this domain, short nonoverlapping regions with minimal sequence similarity can independently mediate repression, suggesting that the Tup1 repression domain interacts with a component(s) of the basic machinery. Furthermore, Cyc8-Tup1 can repress basal transcription in vivo (141) and in vitro (54). Although the mechanism of repression has yet to be elucidated, mutations affecting certain components of the Pol II holoenzyme partially alleviate repression by Cyc8-Tup1; perhaps these components functionally interact with the Tup1 repression domain (80, 146).

Transcriptional corepressors such as Cyc8-Tup1 have the potential to coordinate the regulation of distinct biological pathways. Sin3, a negative regulator of many genes, may represent another such corepressor. Like Cyc8-Tup1, Sin3 does not appear to bind DNA, and it represses transcription when artifically recruited upstream of a functional promoter (147). A mammlian homologue of Sin3 interacts directly with Mad and Mxi1, two DNA-binding proteins, and it is likely to be involved in Mad- and Mxi1-dependent repression in vivo (6,

117). Although presumed to exist, DNA-binding proteins that depend on Sin3 for transcriptional repression in yeast have yet to be identified.

Mating-Type and Telomeric Silencing By Heterochromatin

The mating-type silencer represses transcription when located > 2 kb upstream or downstream of promoters. Silencer function requires binding of Rap1 and Abf1, proteins that are transcriptional activators in other promoter contexts, and the DNA origin recognition complex (83, 113). Rap1 associates with Rif1 (50) and a complex of Sir proteins (97), and these interactions are critical for silencing, but not for activation. Artificial recruitment of Sir1 to a promoter can establish silencing in a manner that requires the other Sir proteins (20).

Silencing probably involves the establishment of a repressed chromatin state. Silencing is abolished by mutations in N-termini of histones H3 or H4 (66, 95, 138), and the repressed state is inherited epigenetically in strains that lack Sir1 or that have a partially disabled silencer (90, 104). The N-termini of histones H3 and H4 interact directly with Sir3 and Sir4, and mutations that disrupt these interactions prevent silencing (51). These observations suggest Rap1 recruits the Sir complex to the silencer, and that interactions between the Sir complex and the N-termini of histones H3 and H4 lead to a specialized chromatin structure that represses transcription over a relatively long distance (51). This specialized chromatin structure could repress the basic transcription machinery by blocking its accessibility to the promoter and/or by disrupting its communication with upstream activators.

Telomeric silencing, the repression of genes located near telomeres, occurs by a similar mechanism. As is the case for the mating-type silencer, telomeric silencing is epigenetically inherited (42), and it requires Rap1, Sir2–4, and histones H3 and H4 (1, 82, 138). Furthermore, Rap1 interacts with the $C_{1-3}A$ telomeric repeats, the DNA sequence necessary for repression that spreads continuously over a 3–5 kb range away from the telomere (112). Although Sir1, Abf1, Rif1, and the DNA origin recognition complex do not seem to be involved, artificial recruitment of Sir1 greatly improves telomeric silencing (20). The chromosome regions around the mating-type silencer and the telomere resemble heterochromatin in that they have a condensed chromatin structure, replicate in late S phase, are localized near the nuclear envelope, and repress transcription in an epigenetic manner (137). Hence, it appears that mating-type and telomeric silencing involve a heterochromatic state at particular chromosomal locations that is mediated by binding of Rap1 followed by recruitment of the Sir complex. A difference between mating-type and telomeric silencing involves Sir1, which converts this heterochromatic state into a form that is stably inherited.

TRANSCRIPTIONAL REGULATION

A variety of mechanisms are utilized to regulate the amounts or activities of many yeast transcription factors in response to environmental or developmental stimuli.

Regulation by Controlling Protein Level

Regulation of mating type genes is achieved by the appropriate transcription factors being present only in the correct cell type. Heteromeric complexes composed of cell type–specific (α1, α2, **a**1), and ubiqitously expressed proteins (Mcm1) play a critical role in this regulation. Diploid cells contain **a**1 and α2, homeodomain proteins that heterodimerize and mediate repression of haploid-specific genes. A surprising aspect of this interaction is that a short region of α2 causes a conformational change in **a**1 that greatly increases its inherent DNA-binding activity (124). In α cells, α2 interacts with Mcm1 to bind the complex operators that mediate repression of **a**1-specific genes. α2 and Mcm1 can independently bind to the operator, but a cooperative interaction between them is necessary for high affinity binding and transcriptional repression (120). In α cells, Mcm1 also cooperates with α1 to form a complex necessary for DNA-binding and transcriptional activation of α-specific genes (14). In this case, Mcm1 undergoes a conformational change upon binding (134).

The levels of several yeast activators are regulated in a manner that is consistent with and responsible for their biological functions. Gcn4, which activates many genes involved in protein synthesis, is subject to a novel translational control mechanism that ensures that its synthesis occurs only under conditions of amino acid starvation, UV irradiation, or rapid nutritional downshift (29, 34). By being regulated at the translational level, Gcn4 is sensitive to the process it controls. Gcn4 levels are also regulated in response to starvation by ubiquitin-dependent proteolysis (78). Glucose-regulated expression of Gal4 provides a sensitive switch for expression of the *GAL* genes, particularly in promoters where Gal4 binds cooperatively to multiple sites (45). Cell-cycle regulated expression of Swi4 (12), Swi5 (100), and Ace2 (30) contributes to restricting the transcription of target genes to specific times in the cell cycle.

Regulation by Small Molecules

Small molecules can directly regulate either the DNA-binding or transcriptional activities of a number of yeast activators. Binding of Hap1, which activates many genes in response to oxygen, requires heme. Heme unmasks the Hap1 DNA-binding domain by directly binding to short repeated sequences in an adjacent region of the protein (150). Ace1, an activator of a protein

critical for copper detoxification, requires copper for folding of the DNA-binding domain (38). Transcriptional activation, but not DNA binding, by Leu3 requires α-isopropylmalate, a metabolic intermediate of the leucine pathway (133).

Regulation by Protein Modification

Phosphorylation is the predominant mechanism by which yeast transcription is modified in response to environmental stimuli. The heat shock factor (HSF) activation domain is rapidly phosphorylated in response to high temperature, with the degree of phosphorylation being strongly correlated with transcriptional activity (122). Similarly, Ste12 becomes transcriptionally active upon the rapid phosphorylation of its activation domain in response to mating pheromones (121). Phosphorylation of Pho4 by the CDK-cyclin complex Pho80/Pho85 inhibits transcriptional activation (64). The CDK inhibitor Pho81 blocks this phosphorylation under conditions of low phosphate, thereby conferring phosphate-dependent regulation of the target genes (116). Protein kinase A, the ultimate effector of the Ras pathway, mediates growth-regulated expression of ribosomal protein genes by increasing the transcriptional activity of Rap1 (75); it also inhibits Yap1 DNA-binding activity under nonstressed conditions (43). In both cases, it is unknown if there is direct phosphorylation of the activator proteins. Phosphorylation of the Gal4 activation region in response to galactose is also observed, but this appears to be a consequence rather than a cause of increased transcriptional activity (115). As mentioned previously, galactose induction of Gal4 activity is mediated by a regulated interaction of its activation domain with Gal80.

Regulation by Nuclear Localization

Swi5, a transcription factor that controls mating-type switching through the *HO* endonuclease, is regulated at the level of nuclear entry. Swi5 is cytoplasmically localized throughout most of the cell cycle, but is translocated into the nucleus as cells enter G_1 (99). Cdc28 phosphorylates three serine residues in the Swi5 nuclear localization signal, but only when the protein is in the cytoplasm (96). Thus, dephosphorylation of the nuclear localization signal due to the cell cycle–dependent destruction of Cdc28 kinase is important for regulating Swi5-dependent transcriptional activation. A related transcription factor, Ace2, is likely to be regulated by a similar mechanism. Ace2 is required for cell-cycle specific expression of chitinase, but when overexpressed, it can partially subsitute for the function of Swi5 (30). Like Swi5, Ace2 is synthesized only during the G_2/M phases and is transported to nuclei only during G_1. Despite their similar structures and patterns of regulation, Swi5 and Ace2

differentially affect target genes (30), in part because of differences in binding cooperatively with proteins such as Grf10 (11).

CONCLUDING COMMENTS

Transcriptional regulation in yeast follows the same principles and uses the same basic mechanisms as in other eukaryotes. Indeed, yeast has contributed much of our current knowledge about eukaryotic transcriptional regulatory mechanisms. Future work will be directed at three major questions. First, how do activators and repressors affect the activity of the basic transcription machinery and/or the chromatin template under physiological conditions? A variety of mechanisms are likely to be utilized, but they remain to be elucidated. Second, how are the activities of transcriptional regulatory proteins altered to stimulate or repress gene expression? At present, there is limited information about the physiologically relevant signals, the pathways by which these signals are transmitted to the transcription factors, and the structural bases and biochemical consequences that occur when transcription factors are modified and/or associate with small molecules or other proteins. Third, how are the various regulatory pathways integrated to provide both the evolutionary stability of the organism and the ability to rapidly adapt to environmental change? Continued genetic and biochemical experiments combined with information from the genome sequencing project should increasingly converge and contribute to understanding the overall logic by which yeast cells regulate their genes.

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