# Regulation of RNA polymerase II transcription

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Transcription initiation plays a central role in the regulation of gene expression. Exciting developments in the last year have furthered our understanding of the interactions between general transcription factors and how these factors respond to modulators of transcription.

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## Introduction

Cellular growth and differentiation employ precise mechanisms to regulate the expression of various genes. One of the most rudimentary mechanisms for a cell to control the functional levels of a protein is to modulate the levels of mRNA encoding that polypeptide. It is therefore not surprising that most of the genetic programs that maintain the cell in a constant state of flux mediate their effects by impinging on mechanisms that control transcription initiation.

In contrast to prokaryotic RNA polymerase, eukaryotic enzymes require multiple accessory proteins to acquire promoter specificity. The synthesis of mRNA in eukaryotes is carried out by RNA polymerase II (RNAPII), a multisubunit enzyme that, to date, requires the presence of seven auxiliary factors, commonly known as basal or general factors, for the accurate initiation of transcription from class II promoters. These seven general transcription factors (GTFs) are TFIIA, IIB, IID, IIE, IIF, IIH and IIJ. Early studies [1] demonstrated that formation of a stable initiation complex occurs through the highly ordered assembly of the GTFs and RNAPII on class II promoter sequences. TFIID is the only GTF shown to have sequence-specific DNA-binding activity. The binding of TFIID to the TATA box, typically located 30 nucleotides upstream of the transcription start site, constitutes the initial step in the formation of a transcription-competent complex [2]. Mammalian TFIID is a multisubunit complex composed of the TATA-binding protein (TBP) and additional, tightly complexed, TBP-associated factors (TAFs) [3,4]. TFIIA associates with TBP and stabilizes the DNA–TFIID complex. TFIIB then enters the complex, resulting in the formation of a TFIID, IIA, IIB (DAB) complex. This pre-initiation complex intermediate serves as the nucleation site for the entry of the remaining GTFs and RNAPII. TFIIF mediates the entry of RNAPII into the complex, followed by the ordered association of TFIIE, TFIIH and TFIIJ. Formation of the DAB—polFEHJ complex, in the presence of each of four ribonucleoside triphosphates, enables RNAPII to clear the promoter region and initiate RNA synthesis from a specific start site [5].

The past year has seen intense activity aimed at elucidating the molecular mechanisms underlying transcription initiation. In particular, the interactions that GTFs can mediate, the GTF requirements for initiation, the role of RNAPII phosphorylation, and the phenomenon of antirepression in the process of activation have been the subject of many studies. These most recent developments are the focus of this review.

## Minicomplexes

Contrary to the dogma that all promoters require the full set of GTFs for basal transcription, the immunoglobulin heavy chain (*IgH*) gene promoter can be transcribed with a subset of GTFs. Initially, Parvin *et al.* [6] reported that transcription from the IgH promoter was independent of TFIIE. Recently, however, Parvin and Sharp [7••] found that the *IgH* promoter could be transcribed to high levels by RNAPII in the presence of only TBP and TFIIB. The ability of this subset of GTFs to transcribe the *IgH* promoter appears to be dependent on the DNA being negatively supercoiled. When the DNA was relaxed or linearized, transcription from the *IgH* promoter required the entire array of GTFs [7••]. This observation appears to be specific to the *IgH* promoter, as transcription from the adenovirus major late promoter (Ad-MLP) required all the previously described factors, independent of the state of the DNA. Kadonaga and colleagues [8] have also analyzed factor requirements for transcription of several *Drosophila* promoters as well as the Ad-MLP. Using TBP they found that a subset of promoters could be transcribed in the presence of TBP, TFIIB, RNAPII and

**Abbreviations**

**Ad-MLP**—adenovirus major late promoter; **CTD**—carboxyl-terminal domain; **DAB**—TFIID, IIA, IIB complex;
**DNA-PK**—DNA-dependent protein kinase; **GTF**—general transcription factor; **IgH**—immunoglobulin heavy chain;
**RNAP**—RNA polymerase; **SRB**—suppressor of RNA polymerase B; **TAF**—TBP-associated factor;
**TBP**—TATA-binding protein; **topo**—topoisomerase.

the small subunit of TFIIF, RAP30. Recent studies in our laboratory resulted in the following observations: first, the requirement for some basal factors may be a function of the length of the transcript analyzed; second, whereas minimal subsets of factors may be capable of mediating transcription, this process is very inefficient, especially for transcripts exceeding 70 nucleotides in length. Production of transcripts up to 70 nucleotides in length is extremely efficient; the complete set of factors was capable of producing RNA molecules approaching the number of template molecules. Interestingly, a subset of factors, namely TBP, TFIIB, RNAPII and TFIIH, was found to be sufficient for transcription and production of an RNA molecule 70 nucleotides in length. However, the extent of the reaction with this subset of factors was less than 5% of that observed with all the factors. The activity of this minicomplex, DB–polH, could not be demonstrated when analyzing for production of RNA molecules 400 nucleotides in length. The existence of a minicomplex capable of transcribing the Ad-MLP allowed us to analyze the effect of the other factors independently. It was found that TFIIE and the small subunit of TFIIF, RAP30, could independently stimulate transcription of the DB–polH minicomplex, while the large subunit, RAP74, had no effect. Nonetheless, transcription of a 400 nucleotide RNA was dependent on RAP74. These preliminary findings agree with previous observations suggesting a dual role for TFIIF in initiation and elongation [9,10]. Moreover, it was also observed that TFIIJ was not required for short transcript synthesis but drastically stimulates transcription of longer RNAs, suggesting a role in elongation. This finding is consistent with our failure to demonstrate TFIIJ commitment in template competition assays (L Zawel, P Kumar, D Reinberg, unpublished data).

Although these findings are exciting, the minicomplexes may only be competent to transcribe when TBP is used as a source of TFIID. The presence of TAFs may expand the requirement for seven GTFs. As TBP appears to always be associated with TAFs *in vivo*, the significance of these observations is questionable.

## Advances in general transcription factor interactions

Chromatographic studies indicate that endogenous TFIID consists of a multiprotein complex containing TBP and associated factors [3,4,11,12]. Although some of these factors can be dissociated from the TFIID complex under conditions of high ionic strength, a number of polypeptides, originally referred to as TAFs, remain tightly bound and require denaturing conditions for removal [3,13]. This convention accommodates the notion that under physiological conditions, some TAF proteins are always associated with TBP while others transiently interact with the TFIID complex to regulate its function in response to physiological cues. Progress in this area has resulted in the isolation of two *Drosophila* cDNA clones encoding dTAF110 [14••] and dTAF250 [15•]. These studies support the initial concept of TAFs, as it was found that dTAF250 interacts directly with dTBP. Although *Drosophila* TAF110 cannot bind directly to dTBP, it is tightly associated with dTAF250 and is also capable of interacting with the Sp1 activator. The human TAF250 appears to be similar to the *Drosophila* TAF250 as it can directly interact with TBP *in vitro* as well as *in vivo* [16•]. Interestingly, the cDNA encoding hTAF250 was found to be identical to the cell cycle regulator CCG1. This intriguing observation suggests that hTAF250/CCG1 may be involved in regulation of cell cycle progression. Based on this result, one can speculate that TFIID is a dynamic complex whose TAF composition varies during different physiological stages. Zhou *et al.* [17•] contend that, unlike dTAF110, hTAF125 also interacts directly with hTBP. These results may not necessarily be controversial as they may be a consequence of differences between species. Tanese *et al.* [4] demonstrated that there are three human TAFs in the 100 kDa range. It is possible that hTAF125 is not the homologue of dTAF110 but that one of the two remaining hTAFs in this range is. Interestingly, it was demonstrated that the divergent amino terminus of TBP is not required for the association of the TAFs. This implies that the evolutionarily conserved carboxyl terminus is sufficient not only for DNA binding, but also for the interaction with TAFs [18•].

One of the most exciting developments of the last year has been the determination of the X-ray crystal structure of TBP. The crystal structure of TBP-2 from *Arabidopsis thaliana* revealed a new, highly symmetrical DNA-binding fold resembling a saddle. The concave DNA-binding surface of the saddle is a curved antiparallel β-sheet, which may mediate specific and non-specific contacts with the DNA. Upon binding to DNA, the convex surface of the saddle is present for interaction with TAFs, GTFs and other regulatory proteins. We look forward to future crystallographic studies that will further our understanding of the DNA–TBP interaction, and explain how TBP can accommodate the vast number of interactions that biochemical studies have demonstrated [19••].

As previously mentioned, the association of RNAPII and the other GTFs on TATA-containing promoters requires the presence of a DAB complex. These observations have been extended to demonstrate that TFIIB can interact with three components of the basal transcription machinery, namely TBP, RNAPII and the small subunit of TFIIF. Discrete domains in TFIIB mediate these protein–protein interactions and may help to anchor RNAPII to the promoter. The amino terminus of TFIIB contains a putative zinc finger motif and the carboxyl terminus contains two imperfect direct repeats and a putative amphipathic α-helix, which is an important domain for contacting TBP. Specific residues mapping to the carboxyl terminus of the second direct repeat were found to be crucial for the interaction of TFIIB and RNAPII. Additionally, the interaction with the small subunit of TFIIF, was mapped to the amino terminus of TFIIB, which includes the zinc finger [20].

McCracken and Greenblatt [21] have demonstrated that RNAPII can interact with the small subunit of TFIIF. In addition, RNAPII has also been shown to interact with TFIIE

[9] and TFIIH [22] (Fig. 1). Recent studies suggest that the carboxyl-terminal domain (CTD) of yeast RNAPII interacts with a large multisubunit complex containing TBP and a number of tightly associated polypeptides termed suppressors of RNA polymerase B (SRBs; R Young, personal communication). SRB2 can interact directly with TBP, yet the significance of this interaction and the function of SRBs remains unknown. Although yeast TBP was originally thought not to contain TAFs, future studies may perhaps reveal that SRBs are the functional equivalents of TAFs in the yeast system.

## Phosphorylation of the RNAPII CTD

The CTD of the largest subunit of RNAPII is highly conserved and contains multiple tandem repeats of the consensus sequence, Tyr-Ser-Pro-Thr-Ser-Pro-Ser. The number of these repeats is important for viability [23]. Due to phosphorylation of the heptapeptide repeats *in vivo*, RNAPII can be resolved into two forms by gel electrophoresis, RNAPIIA and RNAPIIO: RNAPIIO is the phosphorylated form and RNAPIIA is unphosphorylated [24]. The functional significance of CTD phosphorylation was first analyzed by Laybourn and Dahmus [25] who reported that changes in CTD phosphorylation occur during the transcription cycle. Subsequent studies showed that RNAPIIA preferentially associates with the preinitiation complex [26], while RNAPIIO could be isolated from actively elongating complexes [27]. The conversion of RNAPIIA to RNAPIIO was found to occur before the formation of the first phosphodiester bond. Collectively, these experiments are consistent with a model in which RNAPIIA enters the preinitiation complex and subsequent phosphorylation of the CTD displaces the polymerase from the promoter and triggers elongation. This model is also favored by the finding that TBP cannot interact with RNAPIIO, but can associate with RNAPIIA [28•], presumably an interaction that occurs within the preinitiation complex.

Recent experiments have indicated that TFIIH can phosphorylate the CTD. TFIIH (BTF2) activity co-purifies with five polypeptides of 35, 41, 43, 62 and 89 kDa and with a unique kinase activity that mediates the phosphorylation of RNAPII at the CTD [29•]. The TFIIH kinase activity is greatly stimulated in the context of a complete preinitiation complex. In particular, TFIIE, whose association with the DAB–PolF complex precedes that of TFIIH, was found to stimulate the activity of TFIIH kinase both qualitatively and quantitatively. The p62 subunit of TFIIH was recently cloned [30]. No putative kinase domains are present in p62 but monoclonal antibodies directed against p62 were found to inhibit CTD phosphorylation and transcription *in vitro*.

A very recent and exciting development has been the cloning of the p89 subunit of TFIIH. Amino acid se-

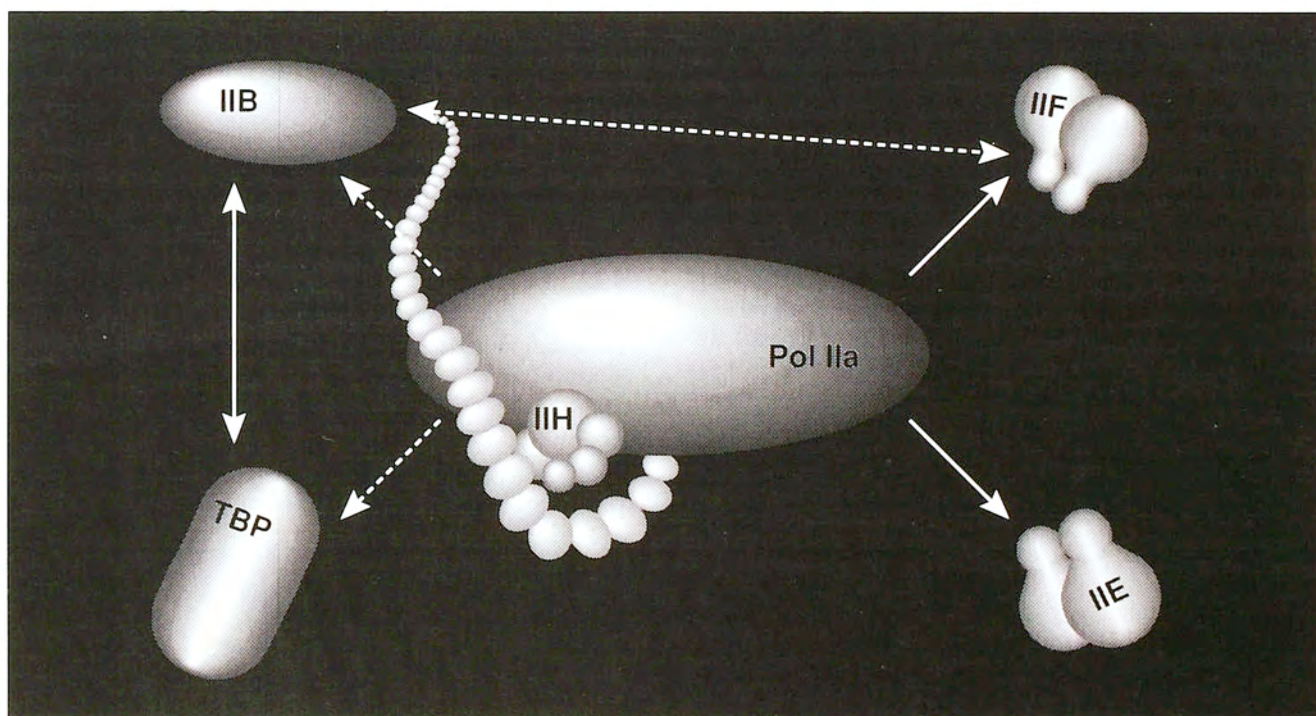


**Fig. 1.** Schematic model representing the currently demonstrated interactions between GTFs and RNAPII. These numerous interactions help explain both the cooperativity in preinitiation complex formation and the formation of a transcription complex on TATA-less promoters. The ability of RNAPII to recognize the initiator and accurately initiate transcription is thought to be enhanced by interactions with the GTFs. Solid arrows represent stronger interactions than the dotted arrows. The tail of RNA polymerase represents the unphosphorylated form of the CTD. In the coming year, we look forward to replacing the arrows denoting interactions with numbers representing the strength of these interactions.

quence analysis revealed that this polypeptide corresponds to the *ERCC-3* gene product, a presumed helicase implicated in the human DNA excision repair disorders xeroderma pigmentosum and Cockayne's syndrome. In fact, Schaeffer *et al.* [31••] demonstrated that a highly purified preparation of TFIIH contains an ATP-dependent DNA helicase activity, in addition to the CTD kinase activity. The relevance of this finding is made compelling by the studies of Selby and Sancar [32••], who demonstrated that the *Escherichia coli mfd* (mutation frequency decline) gene product functions as a transcription–repair coupling factor (TRCF). TRCF functions by recognizing and displacing RNAPs stalled at the DNA lesions in an ATP-dependent fashion. Furthermore, TRCF binds to the damage recognition subunit (UvrA) of the excision nuclease and stimulates repair of the transcribed strand. Both TRCF and ERCC-3 are DNA-dependent ATPases, contain helicase motifs, and confer ultraviolet sensitivity when mutated. We anxiously await studies defining the role of the TFIIH helicase in transcription.

Studies in the yeast *Saccharomyces cerevisiae* have identified factor b as the homologue of TFIIH [33]. This factor purifies as a heterotrimer with polypeptides of 85, 73, and 50 kDa and also contains a CTD-specific kinase activity. The 73 kDa protein, TFB1, shows sequence similarity with human p62 and, on this basis, is considered to be the homologue of p62. Interestingly, an ATP-binding site has been identified in the 85 kDa protein, suggesting that either this subunit contains a catalytic kinase domain or a helicase activity homologous to human p89.

Numerous other kinases have been shown to phosphorylate the CTD of RNAPII, suggesting that phosphorylation of RNAPII at the CTD may indeed be a key regulatory step in the initiation of transcription. A number of these putative CTD kinases, however, remain ill-defined, and some of these studies have not ruled out the possibility of TFIIH contamination [34,35]. Other investigators have purified a kinase using a DNA-targeted CTD substrate and found that the DNA-dependent protein kinase (DNA-PK) can phosphorylate the CTD [36,37]. It is not surprising that such an abundant nuclear kinase can phosphorylate a DNA-bound substrate. Though the role of DNA-PK may be important in regulating certain transcription factors, like Sp1 [38,39], its relevance in basal transcription remains speculative. Of novel interest, however, is the finding that DNA-PK co-purifies with a 350 kDa catalytic subunit and the previously characterized Ku autoimmune antigen [37,38].

The fact that the CTD can accommodate such a vast number of interactions suggests that it may have additional roles in gene expression [23]. Studies by Allison and Ingles [40] have alluded to a role for the CTD in response to certain activators. The rapid cycling that RNAPII undergoes during multiple rounds of transcription suggests the existence of a phosphatase that can modulate CTD phosphorylation. To date, none of the seven GTFs is thought to contain a phosphatase activity. However, Dahmus and co-workers [41] have identified such an activity in HeLa cell extracts. The functional significance of this phosphatase in the recycling of RNAPII remains to be delineated.

## GTF's response to activation: the phenomenon of antirepressin and true activation

Transcription of class II genes is controlled by myriad protein factors that include specific DNA-binding proteins analogous to those that bind within the operon region in bacteria [42,43]. Some of these proteins possess the ability to activate transcription.

Functionally, transcriptional activators bear two domains: a DNA-binding domain that confers specificity and an activator domain that directly or indirectly promotes transcription initiation [44]. Activation domains fall into several broad classes, including proline-rich, glutamine-rich and acidic. Of these, the acidic activators appear to be universal in that they can function in all eukaryotes tested. While the mechanism of activation remains unclear it is presumed that activators work by communicating with and influencing the basal transcription machinery [2]. Direct and specific interactions between regulatory factors and some of the GTFs have been demonstrated. A direct interaction between an acidic activator and TFIIB [45], TFIIH (J Greenblatt, D Reinberg, unpublished data) and TBP [46] has been demonstrated (Fig. 2). These results are made more compelling by the finding that mutations in the acidic domain of an activator, which weaken or eliminate activation of transcription, also reduce the extent of interaction with the particular GTF [45–47]. Conversely, mutations on critical residues of TFIIB that crucially affect the interaction with acidic activators (GAL4-AH, GAL4-VP16) abolish activated transcription, but only slightly decrease basal levels [48]. Despite these findings, physiological levels of activation cannot be demonstrated in a reconstituted transcription system composed of an activator and the basal factors.

It is becoming clear that in addition to activators, another class of factors exists that regulates gene expression by repression. Evidence for this class came from initial studies with histone H1 and chromatin. Kadonaga and coworkers [49] have shown that histone H1 interacts with naked DNA and renders it transcriptionally silent. Activators Sp1 and GAL4-VP16, among others, were shown to counteract H1 repression, a phenomenon now called antirepression [49].

*In vitro* studies by Workman and colleagues [50] showed that the presence of nucleosomes at the promoter precludes the binding of TBP to the TATA motif and inhibits other DNA-binding proteins from recognizing their respective sites. Some activators, however, like the yeast GAL4, can bind to nucleosomal templates [50]. In fact, the activation domain of GAL4 can alleviate nucleosomal repression of promoters presumably by altering some aspects of the chromatin architecture [50,51]. These data indicate that the acidic activation domains of activators like GAL4 stimulate transcription, in part, by enhancing the ability of basal transcription factors to compete with nucleosomes for occupancy of the promoter.

A growing family of negative regulators is also thought to mediate effects by interaction with the basal transcription machinery. Some of these transcriptional repressors include the recently characterized TBP-binding factors

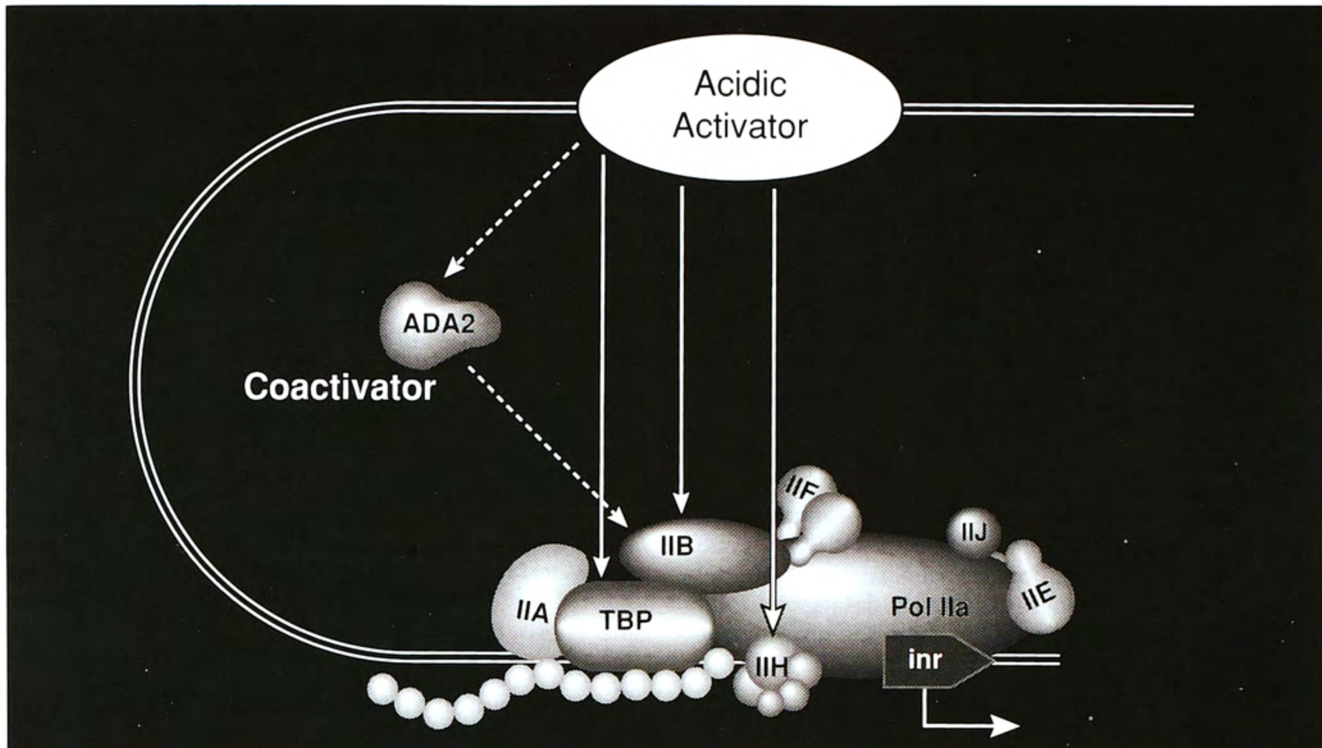


**Fig. 2.** Model for a competent transcription initiation complex at the promoter region. Solid arrows represent direct specific interaction between acidic activators and GTFs as determined by affinity columns. Dotted arrows indicate genetic evidence for adaptor molecules that bridge the interaction between TFIIB and acidic activators. inr, initiator.

Drl and Dr2 (A Merino, D Reinberg, unpublished data) [52•] and the previously described NC1 and NC2 fractions [53,54]. Drl is a 19 kDa nuclear phosphoprotein that can specifically associate with TBP as a monomer, dimer, trimer or tetramer. When Drl interacts with TBP as a monomer it precludes the association of TFIIB with TBP, but not that of TFIIA. This is presumably a result of Drl binding to the same site in TBP as TFIIB. When Drl associates with TBP as a tetramer, both TFIIB and TFIIA are prevented from interacting with TBP. In each scenario, basal and activated transcription are repressed. A search for activities capable of regulating Drl has resulted in the observation that the adenovirus E1A protein and the SV40 large T antigen are capable of preventing and/or displacing TBP-Drl interactions (J Nevins, D Reinberg, personal communication). Similar observations have been published by Meisterernst *et al.* [53,54] describing two negative regulators, NC1 and NC2 that also seem to interact directly with TBP and form a stable complex. The effect of NC1 and NC2 can be overcome by activators such as USF and Sp1. Recently, Merino *et al.* purified Dr2 to homogeneity and found that it is identical to human DNA topoisomerase (topo) I. Dr2/topo I specifically associates with TBP and prevents DA complex formation (A Merino, D Reinberg, unpublished data). Importantly, it was shown that the effect of Dr2 on basal transcription is independent of the DNA relaxation activity of topo I. The functional duality of Dr2/topo I fits well with a model in which Dr2/topo I, in the absence of activators, represses transcription by association with TBP. However, in the presence of activators, Dr2/topo I is translocated to the elongation apparatus and functions to remove superhelical torsion imposed by the elongating ternary complex. The association of NC1, NC2 and Dr2 with TBP represses transcription and, in the absence of an activator, TFIIA is capable of overcoming this repression (A Merino, D Reinberg, unpublished data) [53,54]. This finding agrees with results from Cortes *et al.* [55], who postulated that the function of TFIIA is to remove negative components in the TFIID complex. From these and other studies, it is clear that the process of transcriptional activation involves two independent, yet inter-related processes. The first step involves removal of factors that maintain genes transcriptionally silent, a process known as antirepression. The second step represents true activation where the levels of expression of a particular gene are increased well above basal levels.

In addition to antirepressing the effects of NC1, NC2, Drl, Dr2 and chromatin, some transcription activators must overcome more specific forms of repression. There are several examples of specific regulatory pathways: the nuclear exclusion of NF-κB by IκB (see Liou and Baltimore, this issue pp 477–487); competition for DNA binding between the WT1 tumor suppressor and the EGR family of activators [56]; attenuation of the DNA-binding activity of MyoD by the inhibitorof DNA binding, Id, and by protein kinase C phosphorylation [57,58]; or modulation of the transactivation domain of GAL4 by GAL80 [59].

True activation is the ability of an activator to stimulate transcription above levels conferred by antirepression. Transcriptional activators, known to occupy spe-

cific upstream DNA elements, stimulate transcription *in vivo* as well as *in vitro*. Biochemical as well as genetic evidence has postulated that such factors may mediate transcriptional activation by bridging the activator domains with the general transcription machinery. Further studies have shown that excessive amounts of these specific transcription factors decreased the activated levels of transcription, a phenomenon known as squelching [44]. It has been postulated that the non-DNA bound molecules of transcriptional activators may sequester factors required for optimal levels of activation from the promoter vicinity. Taking advantage of the squelching phenomenon, Guarente and colleagues [60] identified a gene in yeast, *ADA2*, that can suppress the negative effect of VP16 overexpression. *ADA2* is speculated to participate as a transcriptional adaptor for some acidic activators, such as GCN4 and GAL4-VP16, but not the acidic activator HAP4 [61••]. Interestingly, suppressors of *ADA2* mutants have been isolated in the *SUA-7* gene [62], the yeast TFIIB homologue (R Knaus, L Guarente, personal communication). This exciting finding supports the existence of a functional link between an activator and members of the basal transcription machinery (Fig. 2).

It has been observed that eukaryotic promoters contain a mosaic of regulatory DNA elements for several different activators/repressors. The effect of multiple regulatory proteins acting on the same promoter may lead to a synergistic effect on transcription initiation. Such an effect could result if transcriptional activators act in the same pathway, or in pathways that merge on a single transcriptional event. These pathways include alteration of chromatin structure that permits transcription factors to engage the promoter region, displacement of general and specific negative regulators, and favorable interactions with co-activators and members of the basal transcription machinery to enhance transcription [63].

## Conclusion

Numerous advances during the last year have shed new insight into the mechanisms of transcription initiation. The developments summarized make it increasingly clear that the regulatory factors modulating this process entail a complex network of interactions between DNA, chromatin, GTFs, repressors, activators and coactivators. Although a number of factors have been identified that repress basal transcription *in vitro*, the physiological relevance of these repressors remains to be determined. It is well established that the GTFs have the ability to direct basal transcription *in vitro*. However, their interactions *in vivo* are most likely limited by general and specific negative regulators. The resulting transcriptionally silent phenotype of many genes *in vivo* can be overcome in two steps. Certain activators can antirepress the effects of the negative regulators, permitting basal levels of transcription. Other activators not only antirepress but also activate transcription above basal levels. The reason why *in vitro* systems fail to simulate physiological levels of activation is most likely because they only score for activation above basal levels and not above silent levels. The exact role that repressors play during the three phases of transcription (silent, basal and activated) awaits future investigation *in vivo*. The coming year promises to yield a rich harvest of information that will enhance our understanding of the complex process of transcriptional regulation.

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