RNA polymerase II holoenzyme and transcriptional regulation
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RNA polymerase II holoenzymes isolated from yeast and mammalian cells are large, preassembled complexes that contain some or all of the general transcription initiation factors and many other polypeptides. Recent experiments suggest that these holoenzymes may mediate alterations in chromatin structure and play a key role in regulatory mechanisms that influence transcriptional initiation, RNA chain elongation, RNA processing and transcription termination.

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Abbreviations
CBP CREB-binding protein
CDK cyclin-dependent kinase
CPSS cleavage and polyadenylation specificity factor
CREB cAMP response element binding protein
CTD carboxy-terminal heptapeptide repeat domain
FICP TFIIF-associated CTD phosphatase 1
GTF general transcription factor
HAT histone acetyltransferase
polII RNA polymerase II
SNF sucrose nonfermenting
Srb suppressor of RNA polymerase II
SWI switch
TAF TBP-associated factor
TBP TATA-binding protein
TFII transcription factor II

Introduction
Although the general transcription factors (GTFs) and RNA polymerase II (polII) can be assembled in vitro onto a promoter in an ordered, stepwise pathway [1*], transcriptional initiation may normally involve promoter binding by a preassembled holoenzyme complex containing polII and many or all of the GTFs that are essential for initiation. A polII holoenzyme was first identified by Richard Young and his colleagues [2] in the yeast Saccharomyces cerevisiae as a consequence of a hunt for suppressors of a mutation that shortened the carboxy-terminal heptapeptide repeat domain (CTD) of the largest subunit of polII and conferred a conditional growth phenotype. These suppressor of rna polymerase II (srb) mutations occurred in genes SRB2 and SRB4–SRB11, whose protein products were found in a holoenzyme complex containing TFIIA, TFIIF, TFIIH, all the Srb proteins and polII [3,4].

Promoter-specific transcription in vitro with crude yeast nuclear extracts requires at least some of the Srb proteins [2]. Moreover, temperature-shift experiments with temperature-sensitive srb4 and srb6 mutants indicated that some Srb proteins are needed for most or all transcription by polII in vivo [5*]. As Srb proteins were found exclusively in the holoenzyme complex [3,4], it was concluded that most or all transcription in yeast necessarily involves this preassembled holoenzyme. In this review, I shall assume that any genetic or biochemical experiment dealing with one or more of the Srb proteins necessarily involves the polII holoenzyme.

An important role for the yeast polII holoenzyme in transcriptional regulation was first revealed when Roger Kornberg and his colleagues [6] purified a complex, termed the ‘mediator’, that stimulated basal transcription and was required for transcriptional activation in vitro. This mediator copurified with a subfraction of the polII, the holoenzyme, and could be separated from polII by passage of the holoenzyme through a column containing an immobilized antibody directed against the CTD. The purified mediator fraction contained TFIIF, several of the Srb proteins (Srbs 2, 4, 5, 6 and 7), and a number of other proteins. It did not include Srbs 8, 9, 10, and 11 [6]; R Kornberg, personal communication), which are therefore not required for activation in vitro and whose presence in the holoenzyme is still controversial. Also missing were TFIIA and the TBP (TATA-binding protein)-associated factors (TAFII15) of the TFIIID complex which are essential for activated transcription in vitro in fractionated human and Drosophila systems [7] and in reactions containing crude yeast nuclear extracts [8]. That at least some Srb proteins are generally required for transcription in vivo [5*], while the yeast TAFII15s are not [9*,10*] and perhaps play more specialized roles in transcription (e.g. in the transcription of specific genes involved in cell cycle control [11]), has emphasized the critical role of the holoenzyme in transcriptional regulation. Association of the mediator with the CTD in the polII holoenzyme may explain earlier observations linking the CTD to the efficiency of transcriptional activation [12].

Transcription by polII in vivo can be limited by chromatin structure [13], recruitment of the GTFs and polII to the promoter [11,14–16,17*,18], isomerizations that occur in the partly or fully assembled preinitiation complex [19,20*], or promoter clearance and chain elongation [21,22]. Activator proteins probably stimulate transcription by overcoming one or more of these barriers to transcription. Recent experiments have suggested that the holoenzyme mediates activating and repressing signals
that regulate several stages of the transcription process. For example, activators may recruit holoenzyme as a complex to the promoter [17**,18]. The holoenzyme may also mediate alterations in chromatin structure that enable the initiation complex to bind to a promoter [23**,24**]. It is also likely that the holoenzyme transduces activating and repressing signals that alter the state of phosphorylation of the CTD and consequently stimulate or inhibit promoter clearance and chain elongation downstream of the initiation site ([25*]; see below). Finally, the holoenzyme may contain a CTD phosphatase [26-28] that is important for transcription termination and other proteins involved in RNA processing that are carried along in association with the CTD in the elongation complex [29**]. In this article, I shall review the many ways in which the polII holoenzyme has been implicated in transcriptional regulation recently.

Which GTFs are present in the holoenzyme?
The GTFs present in various holoenzyme preparations have varied as a function of the source of the holoenzyme and the method used in its purification. In the case of S. cerevisiae, one preparation contained TFIIB, TFIIF, and TFIIH [3] while another contained only TFIIF [6]. Moreover, an earlier version of the former preparation also contained TBP, the TATA box binding subunit of TFIID [2]. The mammalian holoenzymes have only been purified during the past year [30*,31,32*]. One preparation, from human HeLa cells [32*], contained stoichiometric amounts of TFIIE and TFIIF and a small amount of TFIIH, as well as the human homologs of Srb7, Srb10 (the human homolog is cyclin-dependent kinase [CDK]8), and Srb11 (the human homolog is cyclin C). It also contained some of the proteins that are involved in nucleotide excision repair and DNA double strand break repair. Assuming that these proteins did not copurify fortuitously, this raises the interesting possibility that there are different holoenzymes with specialized functions (e.g., a special form of holoenzyme that is involved in initiation or chain elongation that contains DNA-repair proteins). Another preparation from calf thymus contained Srb7, but only substoichiometric amounts of TFIIE and TFIIF [31].

In each of these cases, an extensive series of columns was used for the purification. In contrast, purification by immunoprecipitation with an antibody against CDK7, one of the subunits of TFIIH, led to the isolation from in vivo labeled nuclei extracts of a holoenzyme containing all five of the essential GTFs, including the TBP and TAF[II] components of the TFIID complex [30*]. If indeed this enzyme was a single complex, which was not demonstrated, it was truly a holoenzyme in the sense that it was capable of promoter-specific initiation on its own. Curiously, only the mammalian, and not the yeast, holoenzyme preparations contained TFIIE. One possibility is that the most abundant form of eukaryotic holoenzyme contains all the essential GTFs, and that various GTFs other than TFIIF, which binds directly and tightly to polII [33], are weakly bound and can be partially or entirely lost upon exposure of the complex to elevated ionic strengths or various ion exchangers. Consistent with this idea, purification of the human holoenzyme by a different single-step procedure involving affinity chromatography on a column containing the immobilized elongation factor TFIIIS also led to the isolation of a holoenzyme complex containing all five essential GTFs in near-stoichiometric amounts (G Pan, J Greenblatt, unpublished data). This large 60S complex was similar in size to the major polIII-containing complex observed when HeLa nuclear extract was directly sedimented on a sucrose density gradient [32*] and was substantially larger than the 40S complexes that were purified by multicolumn procedures [31,32*]. The yield of holoenzyme suggests it includes about 10-20% of the soluble polII and GTFs in yeast nuclear extracts [3,4] and at least 5-10% of the soluble polII and GTFs found in HeLa extracts (G Pan, J Greenblatt, unpublished data). The existence of holoenzyme complexes containing all the essential GTFs raises the possibility that polII can be recruited to a promoter in conjunction with the promoter-recognizing multisubunit factor TFIID and all the other essential GTFs (Fig. 1a).

Binding of polymerase to the promoter: evidence for the involvement of the holoenzyme

There is strong evidence that recruitment of the GTFs and polII to some promoters in S. cerevisiae is rate-limiting for transcription in vivo [11,14-16,17**,18,34]. For example, tethering TBP or TAF[II]90 to a yeast promoter by artificially fusing it to a heterologous DNA-binding domain (such as that from the bacterial LexA protein) leads to a high level of transcription in the absence of an activator [14-16]. In most studies, neither TBP nor TFIID is thought to be a component of the holoenzyme [3,6,31,32*], but high levels of transcription approaching those stimulated by a strong activator have also been achieved by tethering to a promoter many proteins that are more commonly thought to be components of the yeast holoenzyme, including TFIIB [11], Gal11 [17**,18], components of the SWI/SNF (switch/sucrose nonfermenting) complex [35], Sin4 [36], and Srb9, Srb11, and Rnx3 [37*]. Moreover, recruiting holoenzyme to a promoter is sufficient for activation: strong activation was also achieved when the gal11/p mutation in the holoenzyme enabled it to artificially interact with a promoter-bound portion of the DNA-binding domain of yeast Gal4 [17**,18]. These observations are most simply explained by assuming that TBP or TFIID can be recruited to the promoter as a component of the holoenzyme (Fig. 1a). They also imply that interaction of an activator with many, if not most, of the components of the holoenzyme could cause the holoenzyme to bind to the promoter and lead to an elevated level of transcription (see Fig. 1). The mediator may be important for activation in vitro [6] because it aids recruitment of the holoenzyme to the

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promoter. The acidic activation domain of the herpes simplex virus activator protein VP16 binds the yeast holoenzyme [4], but the actual target(s) of VP16 in the holoenzyme complex remains to be determined.

The idea that transcriptional initiation involves recruitment of holoenzyme to the promoter seems inconsistent with observations made in vitro in the human and Drosophila systems. These observations show that an activator can cause recruitment to the promoter DNA of TFIIA and the promoter-recognizing factor TFIIID, a process in which the TAFII subunits of TFIIID are particularly important [38,39]. As the TAFII subunits are not needed for transcription activation in yeast [9*,10*], one
possibility is that transcriptional activation in yeast and the higher eukaryotes is fundamentally different. Another possibility (Fig. 1b,c) is that activation proceeds in two steps, namely, the recruitment of TFIID and TFIIA and then the recruitment of a holoenzyme complex lacking TFIID and TFIIA [40]. An equally plausible and simpler alternative is that TFIID associates with holoenzyme in vivo [2,30*] and that recruitment of TFIID in vitro reflects interactions with the TAF{115} in TFIID [7] that may sometimes be important for recruiting holoenzyme in vivo (Fig. 1a).

Another important observation is that an activator can cause recruitment to the promoter of TFIIB, the other GTFs, and polIII when the TFIID concentration is not limiting in the reaction [20*,41]. In that case, the activator may alter the conformation of promoter-bound TFIID in such a way as to facilitate the recruitment of TFIIB and the other factors [19,20*]. Apparently, proper interaction of TBP with TFIIB is important for activation [42,43]. This sort of phenomenon requires a different kind of explanation, and one possibility is also illustrated in Figure 1. Once holoenzyme has bound to the promoter and polIII has initiated transcription, TFIID and TFIIA would presumably remain bound at the promoter. Reassembly of the preinitiation complex could then proceed by one of two possible pathways. One pathway (Fig. 1c) would involve binding of a holoenzyme complex lacking TFIID; the other pathway (Fig. 1f) would involve stepwise recruitment of TFIIB, the other GTFs, and polIII from the large pools of unassembled factors. Both of these pathways might require continued interaction of the activator with TFIID and/or TFIIA [44*]. According to this model, the observed activator-induced isomerization of the TFIID complex followed by stepwise recruitment of GTFs and polIII in vitro [19,20*,41,45] could reflect one of two possible pathways for reinitiation in vivo.

The holoenzyme and remodeling of chromatin

Transcription in vivo is repressed by nucleosomes [13]. This effect varies in a manner that can depend on nucleosomal positioning and the ability of activator proteins and TFIID to bind nucleosomal DNA. Chromatin structure is altered when the core histones are acetylated by histone acetyltransferases (HATs) [46]. There are also several multisubunit ATP-utilizing enzymes (e.g. SWI/SNF, NURF [nucleosome-remodeling factor], and RSC [remodel the structure of chromatin]) that can remodel chromatin structure in vitro [13], and this remodeling can facilitate the binding of sequence-specific DNA-binding proteins to nucleosomal DNA [47-49]. The SWI/SNF complex is not essential for the growth of S. cerevisiae, although mutational loss of the complex causes reduced expression of a subset of yeast genes [50]. Conversely, the much more abundant RSC complex is essential in S. cerevisiae [51*].

How various chromatin-modifying enzymes are targeted to individual genes has been an interesting and largely unresolved question. There is evidence that certain HATs, like the yeast ADA/GCNS complex and the mammalian CBP (CREB-binding protein)/p300 family of coactivator proteins and their associated cofactor (P/CAF), can be targeted to particular promoters by interacting with DNA-binding activator proteins [52,53*]. The discovery that the TAF{115} subunit of human TFIID and the corresponding Drosophila and yeast TAF{115} contain a HAT activity [24**] suggests that recruitment of TFIID to the promoter on its own or as a component of holoenzyme could lead to histone acetylation in the vicinity of that promoter and thus facilitate the binding of holoenzyme (Fig. 2).

Figure 2

Chromatin-remodeling enzymes may be recruited, by the activator, to the promoter together with polIII holoenzyme. Human TAF{115} and its equivalents in other organisms have histone acetyltransferase activities [24**]. There is also evidence that the SWI/SNF chromatin-remodeling enzyme [50] may be an associated substoichiometric component of the holoenzyme [23**,51*]. Either or both of these proteins may be involved in the disassembly of the nucleosome array that occurs upon recruiting holoenzyme to the yeast PHO5 promoter [55*].
Targeting of holoenzyme to a promoter via an artificial interaction between the Gal4 DNA-binding domain and the mutant holoenzyme component Gal11P leads to elimination of positioned nucleosomes at the yeast PHO5 promoter even in the absence of a TATA box [55**]. Therefore, recruitment of holoenzyme to a promoter by an activator should lead automatically to alterations in chromatin structure that may be necessary for transcription (see Fig. 2). Curiously, however, perturbation of the PHO5 nucleosomes by recruiting holoenzyme does not depend on the SWI/SNF complex unless the enhancer (upstream activation sequence) is weakened [55**]. Therefore, critical chromatin-remodeling components of yeast holoenzyme may yet remain to be identified.

**Activating elongation by RNA polymerase II also involves the holoenzyme**

Although recruiting holoenzyme to certain promoters is apparently sufficient for high levels of transcription in *S. cerevisiae* [17**,18], there is important evidence that promoter clearance and chain elongation by polII can also be rate-limiting for transcription in *vivo* and that these processes are strongly stimulated by particular activators [21,22]. For example, there is a transcriptionally engaged polII molecule that is paused 25–35 nucleotides downstream of the initiation sites on *Drosophila* *hsp70* (*heat-shock protein 70*) genes even in the absence of induction by heat shock [56]. Loading of the initiation complex onto these promoters depends on binding sites for the GAGA factor, while further elongation by polII depends on heat shock and the heat-shock factor (HSF). In this case, HSF is regulating promoter clearance by polII. Paused polymerase molecules were also found downstream of the promoter on many other genes when nuclear run-on experiments were used to investigate this phenomenon [22]. Similarly, the transactivator protein Tat of HIV-1 almost exclusively stimulates chain elongation downstream of position +60 on HIV-1 DNA [57,58*]. Here, polymerase loading depends on binding sites for the human activator protein Sp1 in the HIV-1 promoter. Although Sp1, GAGA, and CTF (CCAAT box transcription factor) activate only initiation and Tat stimulates only elongation, strong acidic activators like herpes simplex virus VP16, human p53 and E2F1, and yeast Gal4 activate both initiation and elongation by polII [25**,58*,59].

There is increasingly strong evidence that activation of elongation by polII depends on phosphorylation of the CTD. Firstly, the paused polymerase molecules downstream of the *hsp70* promoters have unphosphorylated CTDs, while the elongating polymerase molecules examined after a heat shock have phosphorylated CTDs [60]. Secondly, DRB (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole), which inhibits the CTD kinase activity of the CDK7 subunit of TFIIH, inhibits chain elongation by polII, and so does a specific antibody against TFIIH in microinjected Xenopus oocytes [61]. Thirdly, a *Drosophila* factor, P-TEFb (positive transcription elongation factor b), which stimulates chain elongation by polII in *vivo*, is also a CTD kinase that is inhibited by DRB [62]. Finally, the acidic activation domains and Tat, all of which stimulate elongation, all bind TFIIH [58*,63], and Tat was recently shown to directly stimulate phosphorylation of the CTD by TFIIH [64**]. Perhaps phosphorylation of the CTD can prevent an RNA-release factor from acting on polII [65]. Alternatively, it may somehow enable polII to transcribe through nucleosomes that would otherwise arrest RNA-chain elongation by polII [66].

Involvement of the CTD in regulating chain elongation could implicate the holoenzyme, whose mediator subcomplex is associated with the CTD [2–4,6], in chain elongation. Recent nuclear run-on experiments with *S. cerevisiae* by David Bentley and his colleagues have suggested this may indeed be the case [25*]. At the *GAL1* promoter and at a *CYC1* promoter with upstream Gal4-binding sites, in the absence of induction by galactose, there were polII molecules engaged in transcription downstream of the promoters, but only near the 5′ ends of the genes and not near the 3′ ends. Induction by galactose substantially increased the number of polymerase molecules engaged in transcription at only the 3′ ends of the genes. Therefore, an effect of induction in these experiments was to increase the elongation competency of promoter-proximal polymerase molecules. As transcription in the absence of inducer was limited by paused polymerase molecules near the 5′ ends of the genes, these experiments could not have measured the effects of the Gal4 activator protein on the frequency of initiation by polII.

Interestingly, efficient elongation was prevented by CTD truncation, by a temperature-sensitive mutation in the *KIN28* gene, which encodes the cyclin-dependent CTD kinase subunit of yeast TFIIH, and by mutations in the *SRB2* and *SRB10* genes whose products are hallmarks of the holoenzyme [2–4,6]. These experiments therefore imply that efficient elongation in yeast depends on CTD phosphorylation and that holoenzyme-specific polypeptides transduce the elongation-enhancing signal from the acidic activator Gal4 (see Fig. 3). The effect of deleting the *SRB10* gene on elongation is particularly interesting as the *SRB10* gene product, like that of the *KIN28* gene, is a cyclin-dependent kinase that can phosphorylate the CTD [67*]. Mutations in the *KIN28* and *SRB10* genes each globally reduce phosphorylation of the CTD in *vivo* [67*,68]. Whether Gal4 directly stimulates Kin28 and/or Srb10 to phosphorylate the CTD is not yet clear. In any case, it would appear that Gal4 can activate transcription by two different mechanisms, both of which involve the holoenzyme. Firstly, Gal4 is likely to interact with a component(s) of the holoenzyme, possibly TBP
or TFIIIB [69], in order to recruit it to the promoter (Fig. 1a); secondly, it is likely to interact with a different holoenzyme component(s), probably TFIIH [58*,63,64**], in order to stimulate CTD phosphorylation and enhance promoter clearance or elongation by polymerase (Fig. 3). In this view, CTD phosphorylation by Srb10 might be necessary for efficient elongation even if Srb10 activity is not directly regulated by the activator.

Many other holoenzyme components, including Gal11, Sin4, Rgr1, Rox3, and Srb11, are important for efficient activation by Gal4 and other activators [3,4,6,17**,18,37*,70,71*]. Whether these polypeptides influence initiation and/or chain elongation is not yet known. Consistent with the idea that the mediator may control elongation, as well as initiation, in vivo, it greatly enhances phosphorylation of the CTD by TFIIH in vitro [6].

Transcriptional repression: another link to the holoenzyme

A system that represses transcription of a number of unrelated genes in S. cerevisiae involves the Tup1 and Ssn6 (Cyc8) proteins, which are targeted to particular genes (e.g., HO, CYC7 and SUC2) by interacting with particular site-specific DNA-binding proteins like Mig1 (which is involved in glucose repression) and α2 (which is involved in a-specific repression in α cells) (see [37*] and references therein). Mutations that partly prevent repression in these systems have been identified in genes that encode a large number of holoenzyme components, specifically SIN4, RGR1, GAL11, ROX3, and SRBs 8, 9, 10, and 11 [71*]. Remarkably, most of these holoenzyme components are also needed for efficient activation by Gal4. In view of the involvement in repression of the cyclin dependent kinase complex encoded by the SBR10 and SBR11 genes [72–74], and the observation that Srb10 is needed for Gal4 to stimulate efficient elongation in vivo [25*], it seems probable that the Tup1/Ssn6 system in S. cerevisiae acts, at least in part, to inhibit efficient elongation by polII. As suggested in Figure 3, interaction of Tup1 with a holoenzyme component may lead to inhibition of Srb10 activity, causing target genes to be transcribed by an underphosphorylated polymerase that elongates poorly [67*].

Figure 3

A model for the involvement of the polII holoenzyme (dark gray shapes plus associated white tombstone-shaped mediator-containing complex bound at the promoter) in the control of RNA-chain elongation, RNA processing, and transcription termination. Holoenzyme-specific polypeptides constitute a multiprotein 'signal transducer' that controls the efficiencies both of activation by various activators and of transcriptional repression by DNA-bound complexes containing yeast Tup1. Activation of initiation (not shown) by holoenzyme in vitro involves polypeptides in the mediator complex (e). Activation (a) and repression by Tup1 (b) of elongation in vivo involve at least some of the mediator proteins and the additional holoenzyme-associated proteins Srb8, 9, 10 and 11 [5*,37*,71*]. This signaling complex containing the Srb proteins and various other proteins in the tombstone-shaped diagram controls phosphorylation of the polII CTD by Srb10 (CDKB) and Kin28 (CDK7) [6,25*] (c) and, therefore, controls elongation by polII (d) [25*]. The holoenzyme initiation complex may also contain CPSF [29**], which recognizes the polyadenylation signal AAUAAA in the nascent RNA (e), and FCP1, a component of a CTD phosphatase (f) that is stimulated by TFIIF (arrow from F) and inhibited earlier in the RNA-elongation process by TTD (bar from D) (I27, J Archambault et al., unpublished data). This model supposes that CPSF and FCP1 are also present in the elongation complex, at least until the RNA sequence AAUAAA is synthesized. Dephosphorylation of the CTD should lead to termination of transcription further downstream (not shown). The presence in polII holoenzymc of CPSF and FCP1 provides a biochemical link between initiation events at the promoter and the control of RNA processing and transcription termination. Ladder represents DNA; gray line represents mRNA; circled Ps represent phosphorylation. The elongation complex (represented by the two right-hand polII-containing complexes) is thought not to contain most of the polypeptides that are in the initiator complex (left-hand complex to which the mediator complex is attached).
Does the holoenzyme also integrate initiation with RNA processing and transcription termination?

Recent experiments have functionally linked the CTD of polII to RNA splicing and 3'-end formation (i.e. cleavage and polyadenylation). Firstly, CTD peptides and an anti-CTD antibody inhibit splicing in vitro [75]. Secondly, hyperphosphorylated polII was found to be associated with splicing complexes assembled in vitro and was partly localized to nuclear 'speckle' domains enriched in splicing factors [76]. Finally, splicing and 3'-end formation were very inefficient in transfected cells when the RNA was synthesized by polymerase molecules with truncated CTDs [29**]. As the mediator complex is associated with the CTD [6], involvement of the CTD in RNA processing raises the possibility that particular splicing and/or cleavage/polyadenylation factors might interact either with a form of the holoenzyme that is involved in elongation or cleavage with the holoenzyme complex at the promoter and then be carried along in association with the phosphorylated CTD during chain elongation (Fig. 3). For the cleavage and polyadenylation specificity factors CPSF and CstF (cleavage stimulatory factor), two observations are consistent with either possibility. First, CPSF and CstF bind to columns containing immobilized unphosphorylated or phosphorylated CTDs [29**]. Second, human polII holoenzyme purified by TFIIIS-affinity chromatography also contained some associated CPSF and CstF [29**]. Although subsequent experiments have revealed that the CPSF and CstF are substoichiometric (E Wilson, J Greenblatt, unpublished data), the holoenzyme may well integrate transcription initiation with RNA processing, and future experiments should reveal whether mutations in genetically defined subunits of the holoenzyme can influence RNA processing in vivo.

If CTD phosphorylation is critical for efficient elongation [25*,58*,60-63,64**, then dephosphorylation of the CTD by a CTD phosphatase may be necessary for termination of transcription downstream of the polyadenylation signal. Transcription termination is linked to the initiation complex and, by inference, to the holoenzyme for two reasons: firstly, the nature of 3'-end formation depends on the type of promoter utilized by polII [77]; and secondly, downstream termination is functionally linked to cleavage and polyadenylation (which are linked in the holoenzyme via CPSF) because it fails if the polyadenylation signal is mutated [78] or if 3'-end formation is inhibited by CTD truncation [29**]. CTD phosphatases have been purified from HeLa and yeast cell extracts [26-28], and recent experiments have revealed that an essential component of the human CTD phosphatase, FCP1 (TFIIF-associated CTD phosphatase 1), interacts with TFIIF and is present in the human holoenzyme (J Archambault et al., unpublished data). This may explain why human RNA polymerase II initiates transcription with an unphosphorylated CTD. In the elongation complex, the phosphatase may be activated to dephosphorylate the CTD only when the polyadenylation signal in the nascent RNA is synthesized and associated with the CPSF in the transcription complex to activate cleavage and polyadenylation (see Fig. 3).

Conclusions

This has been an exciting year for research that relates the polII holoenzyme to transcriptional regulation. Mammalian holoenzymes were described for the first time [30*,31,32*], and one of them contained TFIID [30*]. New discoveries that TAF1250 has a HAT activity [24**] and that the chromatin-remodeling SWI/SNF complex may be a substoichiometric component of the yeast holoenzyme [23**] were followed by experiments showing that recruitment of holoenzyme to the promoter can alter chromatin structure [55**]. Initial observations that the mediator complex in the yeast holoenzyme was important for gene regulation [6] were extended by new experiments suggesting that the mediator integrates activating and repressing signals [37*,67*,71*] that stimulate or inhibit both promoter binding [17**,18] and chain elongation [25*]. Also, recent experiments have implicated the CTD, and possibly the holoenzyme, in the RNA processing events that accompany transcription [29**]. In the year to come, we may learn precisely which chromatin-remodeling enzymes are recruited with the holoenzyme to the promoter and which polypeptides in the holoenzyme are important for initiation and chain elongation. We should also begin to learn how the holoenzyme integrates the activating and repressing signals that it receives from various regulatory proteins, and better appreciate the relationships among the holoenzyme, RNA processing, and transcription termination. As so many yeast holoenzyme components have been identified and genetically characterized in a preliminary way, and because of the genetic power of that system, it is likely that most critical observations on the holoenzyme will continue to emerge from experiments with S. cerevisiae.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest  ** of outstanding interest


This definitive review on the GTFs summarizes the properties of many of the GTF polypeptides that are components of polII holoenzyme.
The holoenzyme component could be the target of a natural activator. Mutant protein to bind to amino acids 58-97 of yeast Gai4. In cells with the GAL7 7P mutation, this portion of Gal4, when tethered to DNA, can function for transcription from TATA-less promoters.

Yeast holoenzyme in vitro can be activated in the absence of TAF1p [6].

In yeast cells with temperature-sensitive TAF10s or expressing particular TAF10s, both poly (A)+ mRNA synthesis in S. cerevisiae. This implies that the mediator in the holoenzyme is generally required for transcription by pol II in yeast.


Yeast with temperature-sensitive TAF10s or expressing particular TAF10s under the control of the GAL10 promoter are depleted of those TAF10s when the cells are shifted to high temperature or transferred to growth medium containing glucose. In these circumstances, transcriptional activation is still normal for most genes. This observation rationalizes why transcription by the yeast holoenzyme in vitro can be activated in the absence of TAF10s [8].


Individual yeast TAF10s under the control of the copper-repressible ANB1 promoter and tagged with an amino-terminal recognition signal for ubiquitin-dependent degradation are destroyed when the amino-terminal recognition protein is induced with copper. Remarkably, many yeast activators still function normally in the absence of TAF10s. TAF10s seem to be more important for transcription from TATA-less promoters.


The GAL11p mutation in the holoenzyme component Gal11 enables the mutant protein to bind to amino acids 58-97 of yeast Gal4. In cells with the GAL11p mutation, this portion of Gal4, when tethered to DNA, can function as a strong 'activation domain'. This argues that recruitment of Gal11 to a promoter suffices for high levels of transcription in S. cerevisiae. Almost any holoenzyme component could be the target of a natural activator.


Transient interaction of the Epstein—Barr virus ZEBRA transactivator with promoter-bound human TFIID—TATA complex is shown to stably convert the ternary complex to an 'activated form' that can bind TIF10, the other GTFs, and TBP. Would recruitment of TFIID to promoter through yeast [17'-18], bypass this isomerization requirement for activation, or does activation in a higher eukaryote require interaction of an activator with TIF10 and/or TFIID?


Copurification and coimmunoprecipitation experiments are used to show that the SWI5/SNF chromatin-remodeling enzyme can associate with yeast pol II holoenzyme, but the claim that SWI5/SNF is a stoichiometric component of the holoenzyme or a stable bound component of the mediator has been contested [51]. Cooperative interaction of SWI5/SNF with holoenzyme and chromatin would lead to chromatin remodeling and DNA binding by TBP when holoenzyme is recruited to the promoter.


A portion of TAF10p250, expressed as a recombinant protein, has HAT activity. Histone acetylation in nucleosomes around the promoter may be induced when TFIID is recruited to the promoter on its own as a component of holoenzyme or when it interacts with holoenzyme in the vicinity of the promoter.


Nuclear run-on experiments with yeast are used to show that pol II bound and transcriptionally engaged at the CYC7 or GUS promoter can efficiently escape the promoter.


Transcription of a gene in transfected cells by a poll1 that lacks most of the polymerase II holoenzyme or when it interacts with holoenzyme in the vicinity of the promoter.


A purified human pol II complex or set of similar complexes contains TFIIE, TFIIH, SRB proteins, a small amount of TFIIH, and many DNA-repair proteins. This suggests that holoenzyme-specific polypeptides may link transcription to DNA repair.


37. Song W, Treich I, Gian N, Kuchin S, Carlson M: SSN genes that affect transcription repression in Saccharomyces cerevisiae encode Sin4, Rox3, and SRB proteins associated with RNA polymerase II. Mol Cell Biol 1996, 16:115–120. Four yeast genes that contribute to glucose repression are shown to encode the holoenzyme components SrBb, SrB9, Sin4, and Rox3. Previous studies showed that mutations in the SRB10 and SRB17 genes had similar effects on glucose repression. A strong implication is that holoenzyme-specific polypeptides transduce the repressing signal emanating from the Tup1 repressor of this control system.


44. Ho SN, Biggar SR, Spencer DM, Schreiber SL, Crabree GR: Dimeric ligands define a role for transcriptional activation domains. Mol Cell Biol 1995, 15:823–826. A lipid-soluble binary ligand molecule capable of mediating the association of a DNA-binding domain and an activation domain is used for activating transcription. Use of a monomeric ligand to disrupt the interaction shows that continued function of the activation domain is needed for continued activated transcription in yeast and mammalian cells.


HIV Tat stimulates purified TFIIH to phosphorylate the recombinant polII CTD. Citrate inhibits both CTD phosphorylation and efficient elongation by polII in reactions containing HeLa nuclear extract. Both effects of citrate are reversed by Tat, suggesting that Tat stimulates elongation by causing TFIIH to phosphorylate the CTD.


Two polypeptides that copurify with the yeast polII holoenzyme constitute a kinase-cyclin pair. These polypeptides are Srb10 (CDK9) and Srb11 (cyclin T1) and they can phosphorylate the polII CTD in vitro. Cells lacking Srb10 have defects in activation and repression and contain polII with an underphosphorylated CTD.


The yeast mediator complex is shown to contain Sin4 and Rgr1 proteins, which were implicated previously in activation and repression. Sin4 and Rgr1 are present in a subcomplex containing Gal11 and a 50 kDa polypeptide. Therefore, the mediator is a 'signal transducer' that mediates responses to both activators and repressors.


78. Whiteaw E, Proudfoot N: Alpha-thalassaemia caused by a poly (A) site mutation reveals that transcriptional termination is linked to 3' end processing in the human alpha 2 globin gene. EMBO J 1986, 5:2915-2922.