TFIID and human mediator coactivator complexes assemble cooperatively on promoter DNA

Kristina M. Johnson, Jin Wang, Andrea Smallwood, Charina Arayata, and Michael Carey

Department of Biological Chemistry, University of California, Los Angeles School of Medicine, Los Angeles, California 90095-1737, USA

Activator-mediated transcription complex assembly on templates lacking chromatin requires the interaction of activators with two major coactivator complexes: TFIID and mediator. Here we employed immobilized template assays to correlate transcriptional activation with mediator and TFIID recruitment. In reactions reconstituted with purified proteins, we found that activator, TFIID, and mediator engage in reciprocal cooperative interactions to form a complex on promoter DNA. Preassembly of the coactivator complex accelerates the rate of transcription in a cell-free system depleted of TFIID and mediator. Our data argue that this coactivator complex is an intermediate in the assembly of an active transcription complex. Furthermore, the reciprocity of the interactions demonstrates that the complex could in principle be nucleated with either TFIID or mediator, implying that alternative pathways could be utilized to generate diversity in the way activators function in vivo.

Key Words: TFIID; mediator; GAL4-VP16; activation; cooperativity

Received April 1, 2002; revised version accepted May 22, 2002.

Highly regulated signaling pathways and chromatin environments have evolved to modulate the activity of gene activators and to control their access to promoter DNA. A fundamental question is: How do activators stimulate high levels of transcription by RNA polymerase II after chromatin remodeling is complete? The current model is that activators function through recruitment of the general transcription machinery [Ptashne and Gann 1997]. The general machinery [Woychik and Hampsey 2002] comprises the general transcription factors (GTFs) required for basal transcription [TFIIB, TBP, TFIIIE, TFIIH] and two coactivator complexes: TFIID, with its TBP-associated factors (TAFs) [Hahn 1998], and the mediator complex [Myers et al. 1999, Malik and Roeder 2000].

Initial studies of transcription complex assembly in fractionated human systems indicated that binding of TFIID to the TATA box nucleates assembly of the preinitiation complex [PIC] [Buratowski et al. 1989, Van Dyke et al. 1989]. Incubation of template and crude TFIID and TFIIA fractions [the DA complex] with activators overcomes rate-limiting steps in transcription complex assembly [Wang et al. 1992b]. Indeed, numerous activators from USF to GAL4-VP16 recruit DA or stabilize its binding in vitro [Sawadogo and Roeder 1985, Horikoshi et al. 1988a, b; Lieberman and Berk 1994; Chi et al. 1995]. Interaction of the DA complex with activators generates a conformational change, which is both necessary and sufficient for gene activation [Horikoshi et al. 1988b, Chi and Carey 1996; Shykind et al. 1997].

Purified TFIID, however, fails to support robust activated transcription when incubated with the remaining GTFs (discussed in Baek et al. 2002). A fraction termed USA was found to contain coactivators that permit robust, activated transcription [Meisterernst et al. 1991]. Subsequent studies showed that the USA fraction contains a large, multisubunit assembly termed PC2 and several other positive and negative cofactors [Kretzschmar et al. 1994]. PC2 is a subcomplex of the human mediator [Malik et al. 2000].

The mediator coactivator complex was first purified from yeast as an activity that relieved squelching by the strong, model activator, GAL4-VP16 [Kelleher et al. 1990, Flanagan et al. 1991, Myers and Kornberg 2000]. Subunits of the same complex were also identified by a genetic screen for suppressors of mutations in the carboxyl terminal domain (CTD) of the largest subunit of RNA polymerase II [Thompson et al. 1993]. The yeast mediator is capable of supporting activated transcription when supplemented with purified general transcription factors and PolII [Kim et al. 1994; Myers and Kornberg 1998].
This discovery led to the model that activators interact with the mediator complex, which then mediates communication with the GTFs and PolII (Myers and Kornberg 2000).

The existence of a human equivalent of the yeast mediator [hMed] was indicated by the USA fraction [Meisterernst et al. 1991] and by later studies of thyroid receptor-associated proteins or TRAPs [Fondell et al. 1999]. Human mediator was eventually purified by several groups, who utilized different strategies for its isolation [for review, see Malik and Roeder 2000]. The complex has been variously termed TRAP [Fondell et al. 1999], SMCC [Gu et al. 1999], NAT [Sun et al. 1998], ARC [Naar et al. 1999], DRIP [Rachez et al. 1999], CRSP [Ryu et al. 1999], human mediator [Boyer et al. 1999], and PC2 of USA [Malik et al. 2000]. In this article we will use the Med subunit nomenclature proposed by Rachez and Freedman (2001). Cloning and analysis of hMed subunits revealed some minor but noteworthy homologies to yeast mediator counterparts [for review, see Malik and Roeder 2000].

Human mediator interacts directly with activators and undergoes specific conformational changes [Taatjes et al. 2002]. Recent reports indicate that two distinct forms of the mediator complex exist in mammalian cells, of which only one is highly active in vitro [Taatjes et al. 2002]. The current view, based on work in yeast, where deletion of a core subunit of yeast mediator, SRB4, causes global defects in transcription [Holstege et al. 1998], is that hMed is universally required for transcriptional activation. It is believed that individual activators can independently contact various mediator subunits [Ito et al. 1999].

Over a decade of work has provided strong evidence of the critical nature of both TFIID and mediator in activated transcription [Walker et al. 1996; Holstege et al. 1998]. Activators have been shown to interact directly with subunits of either or, in some cases, both the TFIID and mediator complexes in yeast and human (Hahn 1998; Malik and Roeder 2000). The mediator was shown to influence basal transcription [Mittler et al. 2001; Back et al. 2002), suggesting that it may contact GTFs directly in a physiological context. Further, mediator and TFIID act synergistically to support activated transcription in a reaction requiring TAFs [Guermah et al. 2001; Back et al. 2002]. In this paper we propose that activators recruit both of these coactivator complexes simultaneously via formation of a higher-order assembly. Cooperative interactions between activators, TFIID, and hMed are critical for formation of this higher-order complex, which stimulates PIC assembly and thus plays an essential role in regulated transcription.

The existence of an important, functional interaction between TFIID and mediator is supported by a study in yeast. This study indicated that the mediator and TFIID complexes, along with substoichiometric amounts of two GTFs, are bound together at a promoter in a reinitiation scaffold, whose stability is dependent on the strength of an activator [Yudkovsky et al. 2000]. Although its relevance to initiation was not demonstrated, this was the first indication that a stable interaction might exist between these two coactivator complexes—an observation critical to the argument for cooperative coactivator assembly.

The present study was designed to address whether purified TFIID and hMed coactivator complexes can cooperatively assemble on promoter DNA in a functionally relevant manner. We used the model activator GAL4-VP16 [Carey et al. 1990a] and an immobilized template system to study coactivator recruitment. Purified hMed is recruited to immobilized templates by GAL4-VP16 and, in turn, enhances recruitment of purified TFIID. Conversely, TFIID stimulates recruitment of hMed to promoter DNA. We present evidence that under conditions of cooperative recruitment of hMed and TFIID, preassembly of this higher-order coactivator complex overcomes a rate-determining step in activated transcription.

Results

GAL4-VP16 stimulates recruitment of both the human mediator complex and TFIID from nuclear extract to promoter DNA

Our study was designed to test the hypothesis that activated transcription involves cooperative recruitment of TFIID and hMed. To correlate transcriptional activity with recruitment, we employed an immobilized template system [Kim et al. 1998; Ranish et al. 1999; Yie et al. 1999]. The promoter we used contains five GAL4 DNA binding sites upstream of the adenovirus E4 TATA box: G5E4T [Fig. 1A; Carey et al. 1990a]. Linearized, biotinylated templates were immobilized via fusion to streptavidin-coated magnetic beads [Dynabeads]. These immobilized DNA fragments were used as templates for transcription and, in parallel, were used to isolate, visualize, and quantitate by immunoblotting components of the PIC bound to the template.

GAL4-VP16 binds to the immobilized template in amounts proportional to the number of GAL4 binding sites on the DNA [Fig. 1B]. GAL4-VP16 that is bound to an immobilized template bearing five GAL4 DNA binding sites can stimulate transcription in HeLa nuclear extract [NE] [Fig. 1C].

Parallel binding and transcription experiments show that recruitment of the transcription machinery [PIC assembly] by GAL4-VP16 correlates with transcriptional activity. Figure 1D illustrates that binding of GAL4-VP16 correlates with enhanced binding of all components of the transcription machinery tested [Fig. D, cf. lanes 1 and 2]. Note the increased presence of hMed, as represented by the subunits Med220/TRAP220/DRIP205/ARC205 [Yu et al. 1998], Med130/hSUR2/TRAP150/DRIP130/ARC130 [Boyer et al. 1999], and Med33/hMed6/DRIP33/ARC33 [Gu et al. 1999], and the stimulation of TFIID recruitment, assayed by the presence of TBP and TAF$_{250}$ [Hisatake et al. 1993; Ruppert et al. 1993]. To study the potential cooperative recruitment of the TFIID and human mediator coactivators, we
purified active complexes and subjected these to immobilized template recruitment analysis.

Composition and activity of purified human mediator

The human mediator coactivator complex (hMed) was isolated using two published affinity purification approaches (Rachez et al. 1998; Naar et al. 1999). First, we employed an affinity method initially described by Freedman and colleagues, which utilizes a GST-TRLBD fusion matrix (Rachez et al. 1998). Figure 2A, lanes 3–5 illustrate, in part, the composition of the purified fraction. As has been reported (Gu et al. 1999), we observe copurification of RNA polymerase II with hMed. We surmise based on work from the Roeder group, and our analysis of silver-stained gels of our hMed preparation, that hMed is depleted greater than 30-fold. Also, general transcription factors, including TBP and TAFs of TFIID, are present in the purified hMed fraction but are not present in the ΔNE fraction but are not present in the purified hMed fraction.

Figure 2. Composition and activity of purified human mediator [hMed] and mediator-depleted nuclear extract [ΔNE]. (A) HeLa nuclear extracts were fractionated on a GST-TRLBD affinity matrix. The starting [lane 1] and depleted extract [lane 2] along with increasing amounts of mediator fraction [lanes 3–5] were fractionated on 4%–15% SDS polyacrylamide gels and analyzed by immunoblot with antibodies against representative subunits of hMed and GTFs. Autoradiographs of the blots are shown. Note that hMed is depleted from the ΔNE. Med220 is depleted greater than 30-fold. Also, general transcription factors, including TBP and TAFs of TFIID, are present in the ΔNE fraction but are not present in the purified hMed fraction. (B) Purified hMed complements ΔNE for activated transcription. Twenty nanograms of pG5E4T template [lanes 1–6] was incubated with 35 µg ΔNE [lanes 1–4] and [lanes 3,4] or 1 unit purified hMed (see Materials and Methods) [lanes 5,6] in the absence [lanes 1,3,5] or presence [lanes 2,4,6] of 5 ng GAL4-VP16. Transcription was measured by primer extension [arrow]. Note that hMed alone cannot support transcription. GAL4-VP16-induced transcription is detected only when purified hMed is supplemented with ΔNE [lane 4]. (C) The VP eluate complements ΔNE. pG5E4T [lanes 1–8] was incubated with 35 µg HEla NE [lanes 1,2], 35 µg ΔNE [lanes 3–6] and/or 2 µg VP eluate [lanes 4–8] in the presence (+) or absence (−) of GAL4-VP16. Note that neither VP eluate nor ΔNE alone support transcription but the combination is active.
that the association of RNA PolII with the purified hMed is substoichiometric (Malik and Roeder 2000). Purified hMed alone is unable to support either basal or activated transcription [Fig. 2B, lanes 5 and 6] on a plasmid template. We also used an alternative method to isolate the mediator coactivator complex: a GST-VP16 affinity purification approach described by Tjian and colleagues (Naar et al. 1999). This is a somewhat more complicated mediator fraction that, in our hands, contains p300, SWI/SNF, and some TFIIIB [data not shown]. Mutations in the VP16 activation domain nearly eliminate coactivator association, demonstrating that purification of the complex is dependent on an intact activation domain [data not shown; Naar et al. 1999]. This fraction will herein be designated ”VP eluate.” The VP eluate alone, like the purified hMed described above, cannot support basal or activated transcription on plasmid templates [Fig. 2C, lanes 7,8].

To assess the activity of our purified hMed, we generated a mediator-depleted HeLa nuclear extract (ΔNE). ΔNE is specifically depleted of mediator complex subunits; GTFs, including TFIIID, are not depleted [Fig. 2A, cf. lane 1 [starting NE] and lane 2 [ΔNE]]. ΔNE alone is unable to support activated transcription [Fig. 2B, lanes 1,2, Fig. 2C, lanes 3,4]. Importantly, ΔNE is unable to support activated transcription at all concentrations tested—up to 80µg, the limit of our assay [data not shown].

Figure 2B demonstrates that our purified hMed is active. hMed can complement ΔNE, thereby enabling GAL4-VP16-activated transcription on G5E4T templates [Fig. 2B, lanes 3,4]. Figure 2C illustrates that the VP eluate contains an activity, which, in our assay, is functionally indistinguishable from hMed. Neither the VP eluate nor the ΔNE fractions alone can support activation by GAL4-VP16 [Fig. 2C, lanes 4,8] but, when combined, they enable activated transcription [Fig. 2C, lanes 5,6]. Having established that our purified hMed can support activation, we assessed whether activators directly recruit hMed to promoter templates.

Purified human mediator is directly recruited by GAL4-VP16 to promoter DNA

Figure 3A demonstrates that purified hMed is directly recruited to immobilized G5E4T promoter DNA by GAL4-VP16. This recruitment assay was performed in parallel to an in vitro transcription assay using the same immobilized template. Purified hMed can support GAL4-VP16-induced transcription on G5E4T immobilized templates when incubated with mediator-depleted nuclear extract [Fig. 3B, lanes 3,4]. Therefore, recruitment of hMed by GAL4-VP16 correlates with hMed-dependent, GAL4-VP16-induced transcription. As GAL4-VP16 has been observed to have only a modest effect on the recruitment of TFIIID to promoter DNA, we sought to determine whether recruitment of hMed by GAL4-VP16 to a DNA template might affect TFIIID recruitment. Alternately stated, is recruitment of TFIIID and hMed cooperative?

Recruitment of the human mediator from nuclear extract is affected by TFIIID

To begin our study of cooperative recruitment of hMed and TFIIID in activated transcription, we used an immobilized template without a functional TATA box to examine the effect of reduced TFIIID binding on hMed recruitment from HeLa nuclear extract. Our initial experiments revealed that under conditions where GAL4-VP16 is prebound and, thus, fully saturates the immobilized templates, hMed was equivalently recruited to both wild-type and TATAmut templates [data not shown]. This result is consistent with that of others (Ranish et al. 1999) and is not surprising given the substantial interaction between VP16 and hMed (Fig. 3B; Naar et al. 1999).

To observe an effect of TATAmut on hMed recruitment, we modified our assay conditions. Rather than prebinding GAL4-VP16, we incubated the activator in batch with the nuclear extract at a level optimal for in vitro transcription assays, which is, however, suboptimal
with respect to GAL4-VP16 binding site saturation on these templates [data not shown]. We assayed, by in vitro transcription, the activity of HeLa nuclear extract on these immobilized templates under these modified conditions. Figure 4A demonstrates a reduction in both basal and activated transcription on the TATA<sub>mut</sub> template relative to the wild-type template. An assay for recruitment of the transcription machinery was performed in parallel with this in vitro transcription assay. Predictably, we observed a significant reduction in TBP binding on the TATA<sub>mut</sub> template (Fig. 4B, cf. lanes 1 and 2). With the reduction in TBP binding on the TATA<sub>mut</sub> template, we observe a concomitant reduction in TBP recruitment to the TATA<sub>mut</sub> template (Fig. 4B, cf. lanes 1 and 2). Not unexpectedly, a decrease in TBP/TFIID recruitment to the TATA<sub>mut</sub> template also results in a decrease in GAL4-VP16 binding. Cooperative recruitment of TFIID and activators has been extensively studied (Sawadogo and Roeder 1985; Horikoshi et al. 1988a,b; Lieberman and Berk 1994; Chi et al. 1995; Ellwood et al. 1999). Consistent with these reports, lowering the concentration of TFIID decreases the affinity of GAL4-VP16 for GAL4-DNA binding sites in footprinting assays using G<sub>e</sub>E4T templates [data not shown]. The confounding effect of cooperativity between GAL4-VP16 and TFIID prevents us from determining whether the effect of reduced TFIID binding on the recruitment of hMed is direct, due to communication between TFIID and hMed, or if it is an indirect effect of the reduced recruitment of GAL4-VP16. However, the results presented in Figure 4B are suggestive of a higher-order connection between the TFIID and hMed coactivator complexes.

**Cooperative recruitment of TFIID and human mediator to promoter DNA**

A higher-order assembly of coactivators and activators might form as a result of a series of cooperative protein-protein and protein-DNA interactions (Johnson et al. 2001). A key aspect of this model is the potential for direct communication between hMed and TFIID coactivators. To determine if TFIID and hMed can stimulate each other’s recruitment to promoter DNA, we modified our immobilized template assay protocol to remove any impact of cooperative effects on GAL4-VP16 binding. By prebinding high, saturating levels of GAL4-VP16 to the immobilized template, we were able to study, in isolation, the potential for cooperative recruitment of purified TFIID and human mediator.

The activity of the immunopurified TFIID used in our cooperative recruitment assays was assessed in an hMed-dependent system. The in vitro transcription assay depicted in Figure 5A demonstrates that both immunopurified TFIID and purified hMed are required for restoration of GAL4-VP16-induced transcription in nuclear extract that is depleted of both TFIID and hMed [Fig. 5A, cf. lane 18 to lanes 12, 14, 16].

Figure 5B demonstrates that purified hMed can stimulate recruitment of purified TFIID to immobilized promoter DNA. TFIID was incubated with immobilized templates in the absence [Fig. 5B, lane 1] or presence [Fig. 5B, lane 2] of saturating levels of GAL4-VP16. Levels of TFIID recruitment in these first two lanes are below the detectable limit of the immunoblot. However, when the same amount of TFIID is incubated in the presence of saturating levels of purified hMed (Fig. 5B, lanes 5, 6), TFIID recruitment in the presence of GAL4-VP16 is substantially increased [Fig. 5B, cf. lanes 2 and 6]. The amount of TFIID used in this experiment is suboptimal with respect to TFIID template occupancy in the absence of hMed [data not shown]. However, in the presence of hMed, TFIID and hMed are recruited to the immobilized template in roughly equimolar amounts [Fig. 5B]. We also performed the experiment with an hMed fraction lacking PolII [a gift from T. Boyer and A. Berk] and observed a similar cooperative effect on TFIID binding [data not shown].

A model for cooperative interactions between TFIID and hMed implies the potential for mutual stabilization of these coactivator complexes on the DNA. Alternatively stated, if hMed can stimulate recruitment of TFIID to promoter DNA then the converse must also be
true: TFIID must be able to stimulate hMed recruitment. Demonstration of this proof of principle in experiments with our highly purified hMed required a modification of the recruitment assay conditions. Specifically, we used a higher concentration of TFIID and a lower concentration of hMed. Figure 5C depicts an experiment where, in the presence of GAL4-VP16, TFIID is saturating with respect to both template occupancy and transcriptional activity. Comparison of lanes 4 and 6 of Figure 5C demonstrates that saturating levels of TFIID stimulate the recruitment of subsaturating levels of hMed. The concentration of hMed used in this experiment is substantially below the optimal concentration used in the experiments shown in Figures 5B and 2B. It is our observation that GAL4-VP16 has a strong stimulatory effect on the recruitment of hMed and a relatively weak impact on the recruitment of TFIID. As such, we needed to manipulate the principle of mass-action and use substantially low concentrations of hMed in the experiment shown in Figure 5C to demonstrate that TFIID is capable of stimulating recruitment of hMed to an activator-bound template.

Our alternatively purified hMed fraction, the VP eluate, was also assayed for its ability to stimulate recruitment of TFIID to DNA. We demonstrate in Figure 5D...
that an increase in the concentration of VP eluate added to the immobilized template reaction results in a concomitant increase in TFIID recruitment (Fig. 5D, cf. lane 2 and lanes 10, 14). Conversely, incubation of TFIID with the VP eluate stimulates hMed recruitment to the immobilized template (Fig. 5D, cf. lanes 4 and 6, lanes 8 and 10, and lanes 12 and 14).

Figure 5D also demonstrates that at high levels of VP eluate, hMed binds to DNA independently of activator. This “basal” hMed binding is stimulated by TFIID (Fig. 5D, cf. lanes 7 and 9, and lanes 11 and 13) and, conversely, it stimulates TFIID binding (Fig. 5D, cf. lanes 1 and 13). These effects in the absence of activator are further demonstration of cooperative interactions between the TFIID and hMed coactivators.

**Preassembly of a complex containing both TFIID and hMed stimulates transcription**

There are several recent reports about the effects of the mediator complex on basal transcription (Mittler et al. 2001; Baek et al. 2002). An examination of the functional relevance of our TFIID/hMed basal complex is depicted in Figure 6A. TFIID alone, hMed alone, or both were preincubated for 30 min followed by a 4-min transcription reaction in the mediator-depleted extract. Preincubation of TFIID and hMed together results in a stimulation of transcription (Fig. 6A, cf. lane 3 to lanes 1, 2, 4). This indicates that formation of a TFIID, hMed complex is a rate-limiting step in basal transcription and establishes functional relevance of a TFIID/hMed coactivator complex.

Figure 6B demonstrates that preassembly of the TFIID/hMed complex also stimulates activated transcription. A TFIID-depleted, mediator-depleted nuclear extract (ΔNE) was generated by immunodepletion of TBP, TAF\textsubscript{i}250, and TAF\textsubscript{i}32 from mediator-depleted (ΔNE) HeLa nuclear extract. ΔNE is dependent on both TFIID and hMed for activated transcription (Fig. 6B, lanes 1, 2). After a 15-min preincubation of GAL4-VP16 and ΔNE with TFIID alone (Fig. 6B, lane 3), hMed alone (Fig. 6B, lane 4), or both together (Fig. 6B, lane 5), first-round transcription was assessed. Preassembly of both TFIID and hMed in the presence of GAL4-VP16 stimulates transcription [Fig. 6B, cf. lane 5 to lanes 3, 4, 6]. This provides evidence that formation of an activator/TFIID/hMed higher-order complex enhances the efficiency of PIC assembly, and thus plays an important role in activated transcription.

**Discussion**

Studies using GAL4-VP16 have provided significant insight into biochemical mechanisms of gene activation (Carey et al. 1990b). Like many activators, GAL4-VP16 accurately stimulates high levels of transcription from model templates, both in vitro (Carey et al. 1990a) and in vivo (Sadowski et al. 1988), on both naked (Carey et al. 1990b) and chloroplast templates (Pazin et al. 1994). Early studies demonstrated that GAL4-VP16 stimulated transcription via assembly of active transcription complexes.
Cooperative binding of TFII D and mediator

[Wang et al. 1992a]. Recruitment of TFII D and activator-stimulated conformational changes were suggested to be rate-limiting in activated transcription in vitro (Horikoshi et al. 1988b; Wang et al. 1992b; Chi et al. 1995; Chi and Carey 1996). GAL4-VP16 has a direct effect on TFII D recruitment, but this effect has been shown to be modest relative to other activators (Chi et al. 1995). If stable recruitment of TFII D to the promoter is a critical aspect of activator mechanism, how then does GAL4-VP16 fulfill this role? We propose that GAL4-VP16 directly recruits the human mediator complex, and that cooperative interactions between TFII D and hMed indirectly result in efficient GAL4-VP16 recruitment of TFII D.

GAL4-VP16 interacts with both TFII D and hMed directly. In the case of TFII D, the proposed subunit targets include both TBP and TAFI A32 (Klemm et al. 1995). The proposed hMed target of GAL4-VP16 is Med78/TRAP80 [Ito et al. 1999]. Our data support the functionality of this proposed GAL4-VP16-hMed interaction, as we have demonstrated that GAL4-VP16 directly recruits the human mediator coactivator complex to promoter DNA under conditions where purified mediator can support transcription. The novel contribution of this study is the demonstration of direct communication between the hMed and TFII D coactivators, which can lead to their cooperative recruitment to promoter DNA. We provide evidence that the direct recruitment of hMed by GAL4-VP16 can enhance recruitment of TFII D. As would be expected of truly cooperative interactions, we were able to demonstrate that the opposite effect is also possible; that is, that TFII D can stimulate hMed recruitment.

An interesting aspect of our study was the discovery of basal recruitment of the TFII D and hMed coactivator complexes. We believe this result supports our assertion that TFII D and hMed directly communicate. If, as we argue, the combination of TFII D and hMed recruitment to DNA serves as a scaffold for assembly of the PIC, we would expect to see that preassembly of TFII D and hMed in the absence or presence of activator would stimulate the rate of transcription [Johnson et al. 2001]. Indeed, assembly of a higher-order complex, which includes TFII D and hMed in the presence or absence of GAL4-VP16, stimulates transcription. This lends credence to the idea that cooperative assembly is of functional relevance.

Several previous studies have indicated the possibility of an interaction between TFII D and hMed. Two recent studies from the Roeder group report the existence of transcriptional synergy between mediator and TAFs [Guermah et al. 2001; Baek et al. 2002]. They propose a "close mechanistic linkage between [TAFs and mediator] that most likely operates at the level of combined effects on the general transcription machinery" [Baek et al. 2002]. Several older studies on the effects of activators on TFII D, when viewed in light of current knowledge of the critical nature of the mediator, also imply the potential for direct TFII D- mediator interactions.

The mammalian activator, SP1, contains a glutamine-rich activation domain that interacts directly with TFII D in a manner critical for in vitro activation [Hoey et al. 1993; Chen et al. 1994]. CRSP, a highly active mediator fraction, was isolated as an activity required for SP1 in vitro activation [Ryu et al. 1999]. In another study demonstrating an alternative method of mediator purification, it was shown that hMed does not directly interact with SP1 [Naar et al. 1999]. SP1 directly recruits only TFII D, but both TFII D and the hMed are required for SP1 activation [Ryu et al. 1999]. How, then, is the mediator complex brought to the promoter? Viewed in light of our model, we propose that TFII D can interact with the mediator complex, and thus recruit it to the promoter.

Our own studies with the Epstein-Barr virus activator, ZEBRA, show that it cooperatively recruits purified TFII D to promoter DNA [Chi et al. 1995] but does not strongly interact with purified hMed [data not shown], which is required for activation in in vitro transcription assays. As ZEBRA does not directly recruit hMed, we propose that the cooperative recruitment of TFII D by ZEBRA, through interactions with multiple TAFs, TBP, or TFII A, is responsible for recruitment of hMed. But, tressing this idea, older studies on the mechanism of ZEBRA and the IFN-β enhancersome indicate that the USA fraction, which contains the mediator, is required along with DAB complex for stable PIC assembly [Lieberman 1994; Kim and Maniatis 1997]. Formation of this stable, higher-order coactivator complex, similar to what was seen as a reinitiation scaffold by Hahn and colleagues [Yudkovsky et al. 2000], may be the true rate-limiting step in activation.

A further indication of cooperative assembly of the yeast mediator and TFII D comes from CHIP assays by the Struhl [Kuras and Struhl 1999] and Green groups [Li et al. 1999, 2000] and immobilized template recruitment experiments by Hahn and colleagues [Ranish et al. 1999]. Studies designed to evaluate the requirements of TAFs at individual promoters in vivo demonstrate that TBP binding at a promoter can be dependent on functional yeast mediator subunits [Kuras and Struhl 1999; Li et al. 1999, 2000]. Additionally, Ranish et al. [1999] indirectly demonstrated that the model activator GAL4-AH does not strongly interact with the yeast mediator but nevertheless recruits it to an immobilized template in a TFII D (and TFII A)- dependent manner. Further, Ranish et al. [1999] indicate that some GTFs, including TFII B, are required to facilitate this recruitment, suggesting that these GTFs may help stabilize the assembly when the mediator is recruited through TFII D. This may be reflective of the requirement for TFII B in the ZEBRA and IFN-β induced formation of the stable USA/TFII D/TFII A coactivator complex [Lieberman 1994; Kim and Maniatis 1997]. We demonstrate in our present study, however, that TFII B is not absolutely required for the direct interaction between TFII D and hMed, as our purified hMed does not contain TFII B.

We propose a model for transcriptional activation in which a series of cooperative, compensatory interactions between activators, coactivators, and promoter DNA occur [Lehman et al. 1998], leading to formation of a higher-order assembly, which is composed minimally of activator[s], TFII D, and the human mediator complex.
Figure 7. Model of cooperative recruitment of coactivators to DNA templates. Our hypothesis is that reciprocal, cooperative interactions between activator (or series of activators), TFIID, hMed, and template DNA determine the stability of a higher-order nucleoprotein complex. The presence of some form of this higher-order assembly is required for all activated transcription. A key feature of this model is direct communication between the hMed and TFIID.

[Fig. 7]. This cooperative assembly model allows for flexibility in how regulatory sequences surrounding the promoter may enable assembly of this higher-order coactivator complex, both DNA-protein interactions and protein-protein interactions are variables that may impact cooperative assembly [Johnson et al. 2001]. The subunit compositions of TFIID and hMed and the sequences within the core promoter could modulate the order in which the complex assembles. The chromatin status of a gene and its regulated remodeling program [Cosma et al. 1999; Krebs et al. 1999; for review, see Fry and Peterson 2001] may also influence accessibility of TFIID [Lomvardas and Thanos 2001] or activators to DNA, thus further influencing the way in which the PIC assembles. Indeed, a combination of all these influences enables tremendous diversity in the way activators could function in vivo [Johnson et al. 2001].

Synergistic activation of a gene in vivo may involve concerted chromatin remodeling and PIC assembly. However, synergy is observed in vitro on naked DNA templates [Carey et al. 1990b]. One can imagine various scenarios for how such synergistic gene activation could be achieved within the framework of this model. Multiple, simultaneous contacts of activators with the different subunits of mediator [Ito et al. 1999] may cooperatively recruit hMed, and, in so doing, facilitate TFIID binding. Conversely, multiple activator contacts with different TAFs, TBP, or TFIIA could recruit TFIID [Chi et al. 1995; Sauer et al. 1995], which then recruits hMed. Finally, one activator could interact with TFIID and another with mediator, as has been proposed for the synergy of activation by SREBP and SP1 on the LDLR promoter [Naar et al. 1998]. The collection of studies reviewed herein, when viewed in light of the data presented in the present study, indicate that cooperative recruitment of TFIID and hMed may be a critical aspect of the mechanism of action of many activators.

Materials and methods

Factor purification

Purification of recombinant GAL4-VP16 [Tantin et al. 1996], recombinant TFIIA [Ozer et al. 1994], and hemagglutinin (HA) epitope-tagged TFIIA from the HeLa cell line LTRko3 [Zhou et al. 1992] was as described previously. HeLa nuclear extracts were prepared as described [Dignam et al. 1983]. Human mediator was prepared essentially as described [Rachez et al. 1998] except that it was purified from HeLa cell nuclear extract and was eluted with a Med220 (DRIP205) NR2C peptide (Rachez et al. 2000). One unit of hMed is defined as the amount required to complement 35 µg of ΔNE to produce the same fmol amount of transcript as that produced by 35 µg HeLa NE. VP16 eluate was purified from HeLa nuclear extract via a GST-VP16 affinity resin as described except that bound proteins were eluted without Sarkosyl [Naar et al. 1999].

Mediator-depleted HeLa nuclear extract (ΔNE) was prepared by affinity depletion chromatography. Three milliliters of Glutathione-Sepharose Fast-Flow 4B (Amersham) beads, bound by GST-TRρ16D at 0.3 mg protein/mL of beads, were packed onto a 5-mL column and charged for 1 h with 10 mM T3 ligand in 0.1 M KCl binding buffer [Rachez et al. 1998]. Twenty-four milligrams of HeLa nuclear extract (~10 µM T3) in 3 mL was loaded onto the column at a flow rate of 3 mL/h. Peak fractions were identified by Bradford assays, pooled, and analyzed by both immunoblot and in vitro transcription. The extent of hMed depletion was determined by serial dilution and immunoblot.

Mediator-depleted, TFIID-depleted HeLa nuclear extract (ΔANΕ) was prepared by immunoaffinity depletion of the mediator-depleted HeLa nuclear extract (ΔNE). Forty µL protein G Sepharose beads [Amersham] were incubated with a cocktail of 20 µg TAF1250 (Santa Cruz), 20 µg TAF132 (Santa Cruz), and 20 µg TBP (Santa Cruz) antibodies for 1 h at room temperature. The beads were washed 3 times in buffer D [Dignam et al. 1983] and incubated with 800 µg ANE for 2 h at 4°C. The supernatant was collected and assayed by immunoblot and in vitro transcription. The extent of depletion of TBP, TAF1250, and TAF132 was determined by serial dilution and immunoblot. This approach is similar to that used by Oelgeschlager et al. [1998].

Immobilized template recruitment and in vitro transcription assays

Immobilized templates were generated essentially as described [Kim et al. 1998; Ranish et al. 1999; Yie et al. 1999]. Biotinylated fragments were amplified from pG,E4T [Carey et al. 1990a] with a 27-nucleotide, biotinylated primer positioned 205 bp upstream of the GAL4 sites and with a downstream T7 sequencing primer. The 650-bp biotinylated PCR products were fractionated on agarose gels and purified using a QIAquick gel extraction kit [QIAGEN]. The purified DNAs were bound to pG,E4T. Conversion of the six central alternating AT base pairs...
within the E4 TATA to a run of six CG base pairs was confirmed by DNA sequencing. Biotinylated TATA<sub>run</sub> fragments were generated from pG5E4T-TATA<sub>run</sub> and bound to Dynabeads™ as described above.

Immovilized template recruitment assays were performed as described [Ranish et al. 1999; Yie et al. 1999]. Typically, the immobilized templates were incubated with factors indicated in the figure legends at room temperature in standard transcription buffer (Tantin et al. 1996) lacking nucleoside triphosphates (NTPs). The beads were immobilized on a magnetic particle concentrator (MPC), washed, and resuspended in SDS-PAGE loading dye. Factors bound to the template were detected by immunoblot. Details of template and protein concentrations are described in the figure legends. Antibodies to the following polypeptides were obtained from the following sources: PolII CTD SWG16 (QED Bioscience), Med130/SUR2 (Pharmigen), TFII B [Chi et al. 1995]. All other antibodies were purchased from Santa Cruz Biotechnology.

The approximate stoichiometry of GAL4-VP16, hMed, and TFII D binding to immobilized templates was determined by the following: [1] Measurement of Med130, Med220, and TBP concentrations within their respective complexes by silver stain SDS-PAGE gel and comparison to BSA standards; [2] Determination of moles of protein bound to immobilized templates by immunoblot comparison to standard, known amounts of protein.

In vitro transcription and primer extension reactions with pG5E4T templates were performed as described [Tantin et al. 1996]. Details of protein concentration and preincubation of factors are described in the figure legends. Heat-treated nuclear extracts were prepared by incubating 15 µL aliquots of nuclear extracts from isolated mammalian nuclei. Biotinylated TATA<sub>mut</sub> fragments were incubated with factors indicated in the figure legends at room temperature in standard transcription buffer (Tantin et al. 1996) lacking nucleoside triphosphates (NTPs). The beads were immobilized on a magnetic particle concentrator (MPC), washed, and resuspended in SDS-PAGE loading dye. Factors bound to the template were detected by immunoblot. Details of template and protein concentrations are described in the figure legends. Antibodies to the following polypeptides were obtained from the following sources: PolII CTD SWG16 (QED Bioscience), Med130/SUR2 (Pharmigen), TFII B [Chi et al. 1995]. All other antibodies were purchased from Santa Cruz Biotechnology.

The approximate stoichiometry of GAL4-VP16, hMed, and TFII D binding to immobilized templates was determined by the following: [1] Measurement of Med130, Med220, and TBP concentrations within their respective complexes by silver stain SDS-PAGE gel and comparison to BSA standards; [2] Determination of moles of protein bound to immobilized templates by immunoblot comparison to standard, known amounts of protein.

In vitro transcription and primer extension reactions with pG5E4T templates were performed as described [Tantin et al. 1996]. Details of protein concentration and preincubation of factors are described in the figure legends. Heat-treated nuclear extracts were prepared by incubating 15 µL aliquots of nuclear extracts from isolated mammalian nuclei. Biotinylated TATA<sub>mut</sub> fragments were incubated with factors indicated in the figure legends at room temperature in standard transcription buffer (Tantin et al. 1996) lacking nucleoside triphosphates (NTPs). The beads were immobilized on a magnetic particle concentrator (MPC), washed, and resuspended in SDS-PAGE loading dye. Factors bound to the template were detected by immunoblot. Details of template and protein concentrations are described in the figure legends. Antibodies to the following polypeptides were obtained from the following sources: PolII CTD SWG16 (QED Bioscience), Med130/SUR2 (Pharmigen), TFII B [Chi et al. 1995]. All other antibodies were purchased from Santa Cruz Biotechnology.
fect of GAL4 derivatives on mammalian TFIIID-promoter interactions. *Cell* **54**: 665–669.

Horikoshi, M., Hai, T., Lin, Y.S., Green, M.R., and Roeder, R.G. 1988b. Transcription factor ATF interacts with the TATA factor to facilitate establishment of a preinitiation complex. *Cell* **54**: 1033–1042.

Ito, M., Yuan, C.X., Malik, S., Gu, W., Fendell, J.D., Yamamura, S., Fu, Z.Y., Zhang, X., Qin, J., and Roeder, R.G. 1999. Identity between TRAP and SMCC complexes indicates novel pathways for the function of nuclear receptors and diverse mammalian activators. *Mol. Cell* **3**: 361–370.

Johnson, K.M., Mitsuhashi, A., and Carey, M. 2001. Eukaryotic transcription: The core of eukaryotic gene activation. *Curr. Biol.* **11**: R510–R513.

Kelleher, R.J., 3rd, Flanagan, P.M., and Kornberg, R.D. 1990. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. *Cell* **61**: 1209–1215.

Kim, T.K. and Maniatis, T. 1997. The mechanism of transcriptional synergy of an in vitro assembled interferon-beta enhancerosome. *Mol. Cell* **1**: 119–129.

Kim, T.K., Kim, T.H., and Maniatis, T. 1998. Efficient recruitment of TFIIIB and CBP-RNA polymerase II holoenzyme by an interferon-beta enhancerosome in vitro. *Proc. Natl. Acad. Sci.* **95**: 12191–12196.

Kim, Y.J., Bjorklund, S., Li, Y., Sayre, M.H., and Kornberg, R.D. 1994. A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Cell* **77**: 599–608.

Klemm, R.D., Goodrich, J.A., Zhou, S., and Tjian, R. 1995. Molecular cloning and expression of the 32-kDa subunit of human TFIIID reveals interactions with VP16 and TFIIB that mediate transcriptional activation. *Proc. Natl. Acad. Sci.* **92**: 5788–5792.

Krebs, J.E., Rao, M.H., Allis, C.D., and Peterson, C.L. 1999. Cell cycle-regulated histone acetylation required for expression of the yeast HO gene. *Genes & Dev.* **13**: 1412–1421.

Kretzschmar, M., Stelzer, G., Roeder, R.G., and Meisterernst, M. 1994. RNA polymerase II cofactor PC2 facilitates activation of transcription by GAL4-AH in vitro. *Mol. Cell. Biol.* **14**: 3927–3937.

Kuras, L. and Struhl, K. 1999. Binding of TBP to promoters in vivo is stimulated by activators and requires Pol II holoenzyme. *Nature* **399**: 609–613.

Lehman, A.M., Ellwood, K.B., Middleton, B.E., and Carey, M. 1998. Compensatory energetic relationships between upstream activators and the RNA polymerase II general transcription machinery. *J. Biol. Chem.* **273**: 932–939.

Li, X.Y., Virbasius, A., Zhu, X., and Green, M.R. 1999. Enhancement of TBP binding by activators and general transcription factors. *Nature* **399**: 605–609.

Li, X.Y., Bhaumik, S.R., and Green, M.R. 2000. Distinct classes of yeast promoters revealed by differential TAF recruitment. *Science* **288**: 1242–1244.

Lieberman, P. 1994. Identification of functional targets of the Zta transcriptional activator by formation of stable preinitiation complex intermediates. *Mol. Cell. Biol.* **14**: 8365–8375.

Lieberman, P.M. and Berk, A.J. 1994. A mechanism for TAFs in transcriptional activation: Activation domain enhancement of TFIID-TFIIA–promoter DNA complex formation. *Genes & Dev.* **8**: 995–1006.

Lomvardas, S. and Thanos, D. 2001. Nucleosome sliding via TBP DNA binding in vivo. *Cell* **106**: 685–696.

Malik, S. and Roeder, R.G. 2000. Transcriptional regulation through Mediator-like coactivators in yeast and metazoan cells. *Trends Biochem. Sci.* **25**: 277–283.

Malik, S., Gu, W., Wu, W., Qin, J., and Roeder, R.G. 2000. USA-derived transcriptional coactivator PC2 is a submodule of TRAP/SMCC and acts synergistically with other PCs. *Mol. Cell* **5**: 753–760.

Meisterernst, M., Roy, A.L., Lieu, H.M., and Roeder, R.G. 1991. Activation of class II gene transcription by regulatory factors is potentiated by a novel activity. *Cell* **66**: 981–993.

Muller, G., Kremmer, E., Timmers, H.T., and Meisterernst, M. 2001. Novel critical role of a human Mediator complex for basal RNA polymerase II transcription. *EMBO Rep.* **2**: 808–813.

Myers, L.C. and Kornberg, R.D. 2000. Mediator of transcriptional regulation. *Annu. Rev. Biochem.* **69**: 729–749.

Myers, L.C., Gustafsson, C.M., Hayashibara, K.C., Brown, P.O., and Kornberg, R.D. 1999. Mediator protein mutations that selectively abolish activated transcription. *Proc. Natl. Acad. Sci.* **96**: 67–72.

Naar, A.M., Beaurang, P.A., Robinson, K.M., Oliner, J.D., Avizonis, D., Scheek, S., Zwickler, J., Kadonaga, J.T., and Tjian, R. 1998. Chromatin, TAFs, and a novel multiprotein coactivator are required for synergistic activation by Sp1 and SREBP-1a in vitro. *Genes & Dev.* **12**: 3020–3031.

Naar, A.M., Beaurang, P.A., Zhou, S., Abraham, S., Solomon, W., and Tjian, R. 1999. Composite co-activator ARC mediates chromatin-directed transcriptional activation. *Nature* **398**: 828–832.

Nakajima, N., Horikoshi, M., and Roeder, R.G. 1988. Factors involved in specific transcription by mammalian RNA polymerase II: Purification, genetic specificity, and TATA box-promoter interactions of TFIIH. *Mol. Cell. Biol.* **8**: 4028–4040.

Oecklschlagler, T., Tao, Y., Kang, Y.K., and Roeder, R.G. 1998. Transcription activation via enhanced preinitiation complex assembly in a cell-free system lacking TAFIs. *Mol. Cell* **1**: 925–931.

Ozer, J., Moore, P.A., Bolden, A.H., Lee, A., Rosen, C.A., and Lieberman, P.M. 1994. Molecular cloning of the small (gamma) subunit of human TFIIA reveals functions critical for activated transcription. *Genes & Dev.* **8**: 2334–2335.

Pazin, M.J., Kamakaka, R.T., and Kadonaga, J.T. 1994. ATP-dependent nucleosome reconfiguration and transcriptional activation from preassembled chromatin templates. *Science* **266**: 2007–2011.

Ptashne, M. and Gann, A. 1997. Transcriptional activation by recruitment. *Nature* **386**: 569–577.

Rachek, C. and Freedman, L.P. 2001. Mediator complexes and transcription. *Curr. Opin. Cell Biol.* **13**: 274–280.

Rachek, C., Suldan, Z., Ward, J., Chang, C.P., Burakov, D., Erdjument-Bromage, H., Tempst, P., and Freedman, L.P. 1998. A novel protein complex that interacts with the vitamin D3 receptor in a ligand-dependent manner and enhances VDR transcription activation in a cell-free system. *Genes & Dev.* **12**: 1787–1800.

Rachek, C., Lemon, B.D., Suldan, Z., Bromleigh, V., Gamble, M., Naar, A.M., Erdjument-Bromage, H., Tempst, P., and Freedman, L.P. 1999. Ligand-dependent transcription activation by nuclear receptors requires the DRIP complex. *Nature* **398**: 824–828.

Rachek, C., Gamble, M., Chang, C.P., Atkins, G.B., Lazar, M.A., and Freedman, L.P. 2000. The DRIP complex and SRC-1/p160 coactivators share similar nuclear receptor binding determinants but constitute functionally distinct complexes. *Mol. Cell. Biol.* **20**: 2718–2726.

Ranish, J.A., Yudkovsky, N., and Hahn, S. 1999. Intermediates in formation and activity of the RNA polymerase II preinitiation complex: Holoenzyme recruitment and a postrecruitment role for the TATA box and TFIIIB. *Genes & Dev.* **13**: 49–63.
Ruppert, S., Wang, E.H., and Tjian, R. 1993. Cloning and expression of human TAFII250: A TBP-associated factor implicated in cell-cycle regulation. Nature 362: 175–179.

Ryu, S., Zhou, S., Ladurner, A.G., and Tjian, R. 1999. The transcriptional cofactor complex CRSP is required for activity of the enhancer-binding protein Sp1. Nature 397: 446–450.

Sadkowski, I., Ma, J., Triezenberg, S., and Ptashne, M. 1988. GAL4-VP16 is an unusually potent transcriptional activator. Nature 335: 563–564.

Sauer, F., Hansen, S.K., and Tjian, R. 1995. Multiple TAFIIs directing synergistic activation of transcription. Science 270: 1783–1788.

Sawadogo, M. and Roeder, R.G. 1985. Interaction of a gene-specific transcription factor with the adenovirus major late promoter upstream of the TATA box region. Cell 43: 165–175.

Shykind, B.M., Kim, J., Stewart, L., Champoux, J.J., and Sharp, P.A. 1997. Topoisomerase I enhances TFIID-TFIIA complex assembly during activation of transcription. Genes & Dev. 11: 397–407.

Sun, X., Zhang, Y., Cho, H., Rickert, P., Lees, E., Lane, W., and Reinberg, D. 1998. NAT, a human complex containing Srb polypeptides that functions as a negative regulator of activated transcription. Mol. Cell 2: 213–222.

Taatjes, D.J., Naar, A.M., Andel III, F., Nogales, E., and Tjian, R. 2002. Structure, function, and activator-induced conformations of the CRSP coactivator. Science 295: 1058–1062.

Tantin, D., Chi, T., Hori, R., Pyo, S., and Carey, M. 1996. Biochemical mechanism of transcriptional activation by GAL4-VP16. Methods Enzymol. 274: 133–149.

Thompson, C.M., Koleske, A.J., Chao, D.M., and Young, R.A. 1993. A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell 73: 1361–1375.

Van Dyke, M.W., Sawadogo, M., and Roeder, R.G. 1989. Stability of transcription complexes on class II genes. Mol. Cell. Biol. 9: 342–344.

Walk, S.S., Reese, J.C., Apone, L.M., and Green, M.R. 1996. Transcription activation in cells lacking TAFII. Nature 383: 185–188.

Wang, W., Carey, M., and Gralla, J.D. 1992a. Polymerase II promoter activation: Closed complex formation and ATP-driven start site opening. Science 255: 450–453.

Wang, W., Gralla, J.D., and Carey, M. 1992b. The acidic activator GAL4-AH can stimulate polymerase II transcription by promoting assembly of a closed complex requiring TFIID and TFIIA. Genes & Dev. 6: 1716–1727.

Woychik, N.A. and Hampsey, M. 2002. The RNA Polymerase II machinery. Structure illuminates function. Cell 108: 453–463.

Yie, J., Senger, K., and Thanos, D. 1999. Mechanism by which the IFN-beta enhanceosome activates transcription. Proc. Natl. Acad. Sci. 96: 13108–13113.

Yudkovsky, N., Ranish, J.A., and Hahn, S. 2000. A transcription reinitiation intermediate that is stabilized by activator. Nature 408: 225–229.
TFIID and human mediator coactivator complexes assemble cooperatively on promoter DNA

Kristina M. Johnson, Jin Wang, Andrea Smallwood, et al.

*Genes Dev.* 2002, 16:1852-1864. Access the most recent version at doi:10.1101/gad.995702

References

This article cites 75 articles, 31 of which can be accessed free at: http://genesdev.cshlp.org/content/16/14/1852.full.html#ref-list-1

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.