The mediator coactivator complex: functional and physical roles in transcriptional regulation

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Summary
In vivo, the DNA is packed into chromatin and transcription is dependent upon activators that recruit other factors to reverse the repressive effects of chromatin. The response to activators requires additional factors referred to as coactivators. One such coactivator, mediator, is a multi-subunit complex capable of responding to different activators. It plays an key role in activation, bridging DNA-bound activators, the general transcriptional machinery, especially RNA polymerase II, and the core promoter. Its subunits are necessary for a variety of positive and negative regulatory processes and serve as the direct targets of activators themselves. In vivo and in vitro studies support various roles for mediator in transcription initiation, while structural studies demonstrate that it engages in multiple interactions with RNA polymerase II, and adopts conformations that are activator specific.

Key words: Transcription regulation, Mediator, Coactivator

Introduction
A significant goal in the field of transcriptional regulation is the elucidation of the mechanism of activator-dependent transcription. Over the past decade, the concept of the coactivator has emerged to explain how activators stimulate transcription. The TFIID complex was the first nominee for such a role, its recruitment to a promoter overcoming a rate-limiting step in the initiation of transcription. More recent work suggests that TFIID plays an important role in core promoter recognition as well as in transcription activation (Albright and Tjian, 2000).

Other coactivators are broadly defined by their histone acetyltransferase (HAT) activities. These include the GCN5-containing HATs and the nuclear hormone receptor (or related) HATs (Belotserkovskaya and Berger, 1999). As their name suggests, these coactivators acetylate nucleosomes and other transcription factors, thereby permitting additional access of the transcriptional machinery to the promoter as well as facilitating protein-protein interactions. A third class of coactivator includes the various chromatin-remodeling complexes (Vignali et al., 2000). These factors alter the structure of chromatin and, in common with the HATs, permit access of the transcriptional machinery to the DNA. The fourth complex, mediator, links the activator directly to the core promoter and the general transcriptional machinery (GTFs).

This large multiprotein complex is brought to the promoter by DNA-bound activators, is necessary for transcription in vivo, and stimulates high levels of activator-dependent transcription in vitro. There are several previous detailed reviews of mediator function and subunit composition (Boube et al., 2002; Carlson, 1997; Hampsey and Reinberg, 1999; Lee and Young, 2000; Lemon and Tjian, 2000; Malik and Roeder, 2000; Naar et al., 2001; Rachez and Freedman, 2001). Here, we describe the major findings that support a role for mediator as a coactivator and discuss more recent work in the field, proposing a functional role for the mediator complex.

Isolation of yeast and human mediator complexes
The mediator complex was initially identified in yeast by two approaches. The first approach used genetic screens for suppressors of truncations in the CTD (C-terminal domain) of the large subunit of yeast RNA polymerase II. This domain is a heptapeptide repeat, and the number of repeats correlates with the genome complexity: yeast CTD has 26 repeats, Drosophila CTD has 43 repeats, and human CTD has 52 repeats. These screens yielded the SRB (suppressors of RNA polymerase B) components, which reside in a 1-2 MDa complex containing substoichiometric amounts of RNA polymerase II (Hengartner et al., 1995; Koleske et al., 1992; Koleske and Young, 1994; Liao et al., 1995; Thompson et al., 1993). The second approach isolated mediator biochemically through a physical interaction with various activators and the CTD of RNA polymerase II (Hengartner et al., 1995; Liao et al., 1995; Myers et al., 1998). Other experiments showed that mediator can be purified through a physical interaction with various activators and the CTD of RNA polymerase II (Hengartner et al., 1995; Liao et al., 1995; Myers et al., 1998). Hence, these studies suggested that the mediator
Fig. 1. The yeast mediator model of activator-dependent transcription. Shown is a hypothetical promoter containing a canonical TATA box and interacting with the TFIIID complex. Additional TFIIID contacts are made with the initiator element (at the start site) and downstream of the start site. The arrow within the TFIIID complex represents the start site of transcription. The three activators (GCN4, VP16 and GAL4) are shown binding to their DNA sites and recruiting yeast mediator to the promoter via a physical interaction with a mediator module (indicated by the overlap between the activator and the respective mediator module) (Koh et al., 1998; Lee et al., 1999b). Additional overlap among the three yeast mediator modules represents physical interactions between the three modules. Lastly, overlap/physical interactions between the MED9/MED10 and SRB4 modules and RNA polymerase II are illustrated (Kang et al., 2001). The HEAD, MIDDLE and TAIL domains are indicated and are based on recent structural data (Asturias et al., 1999; Davis et al., 2002; Dotson et al., 2000b). Note that the negative regulation of TFIIH CAK (cyclin H/cdk7) by the SRB10/SRB11 (cyclin-C–CDK8) is specific to human and has not been observed in yeast (Akoulitchev et al., 2000).

is recruited to promoters through binding of an activator to its DNA-binding site and also interacts with RNA polymerase II (Fig. 1).

Human mediator complexes were purified using in vitro activator-dependent assays, immunopurification assays based on the various human Srb/Med homologues or an activator affinity purification step. These disparate purification procedures identified two complexes: a larger 2 MDa complex variously designated TRAP, DRIP, ARC, SMCC or NAT (Boyer et al., 1999; Fondell et al., 1996; Fondell et al., 1999; Ito et al., 1999; Naar et al., 1999; Rachav et al., 1999; Rachav et al., 1999; Sun et al., 1998) and a smaller 500-700 kDa complex termed PC2/CRSP (Malik et al., 2000; Ryu et al., 1999) (Table 1). Both complexes were shown to mediate activator-dependent transcription in vitro. Additionally, the NAT and SMCC complexes were shown to repress activator-dependent transcription, as well as basal transcription, but apparently through different pathways (Akoulitchev et al., 2000; Gu et al., 1999; Sun et al., 1998).

The differences between the various purification schemes are potentially significant. Tjian’s group demonstrated that both the GST-VP16 and SREBP-1α (sterol-responsive enhancer binding protein) activator affinity purifications produce not one, as was originally thought, but two different mediator complexes (Taatjes et al., 2002). The larger, 2 MDa complex, denoted ARC-L, appears to be identical to the previously identified TRAP/DRIP/ARC/SMCC/NAT complexes. The smaller complex is the 500 kDa CRSP complex. Affinity purification of CRSP from the mixture, using a CRSP-specific antibody, separates CRSP from the ARC-L complex. Interestingly, the ARC-L complex does not mediate activation on the SREBP-responsive promoter in vitro, whereas CRSP does.

Results obtained with ARC-L are consistent with those obtained from the isolation of the NAT complex, which was purified by affinity purification using antibodies to CDK8 (the human homolog of the yeast Srb10) and shown to inhibit transcription (Sun et al., 1998). Indeed, the larger 2 MDa mediator complexes contain the cyclin-C–CDK8 pair, MED230 and MED240, as does the ARC-L complex. These proteins are the homologues of four yeast proteins: Srb8 and Srb9 are MED230 and MED240 (Borggrefe et al., 2002; Boube et al., 2002), whereas Srb10 and Srb11 are CDK8 and cyclin C (Liao et al., 1995). Genetic studies in yeast suggest that these proteins are involved in the repression of certain genes (reviewed by Carlson, 1997). Moreover, they exist as a distinct complex that can be isolated from yeast (Borggrefe et al., 2002). One additional point is that CRSP70 is present only in the CRSP complex, suggesting that it may have a positive role in CRSP function (Taatjes et al., 2002).

Tjian and co-workers suggest that the larger complex is transcriptionally inert, and the smaller CRSP complex is the active species on the promoter (Taatjes et al., 2002), but these interpretations are based on in vitro assays and may not reflect mediator-promoter mechanisms in vivo. For example, the 2 MDa complex may still be recruited to promoters by activators and subsequent unknown events may alleviate its repressive effects (e.g. dissociation of the MED230, MED240, CDK8 or cyclin C subunits). Nevertheless, these data suggest that, contrary to expectations, the smaller CRSP complex is actually the ‘active’ complex, whereas, at least under the conditions assayed, the larger complex does not respond to an activator. It is, however, not clear whether ARC-L or CRSP mediates nuclear-receptor-dependent transcription.

Recruitment of mediator to the promoter

Is mediator recruited concomitantly with RNA polymerase II to the promoter or does it arrive at a different time? The answer to this question provides valuable insight not only into the role of mediator but also into the temporal recruitment of factors to specific promoters. In short, the answer to the question of temporal recruitment of mediator is that the timing of its recruitment depends on other factors, especially the regulatory context of a promoter.

Several recent studies show that mediator can be recruited...
Table 1. Components of the human mediator complex

| Human mediator subunit (yeast) | Activator | Mutant subunit defects |
|-------------------------------|-----------|------------------------|
| MED240 (Srb9)                | Eye-antennal disc development and sex-comb identity function (Drosophila) |
| MED230 (Srb8)                | See MED240 |
| MED220 (Med1)                | TR, VDR, GR, RARα, RXRα, PPARγ, ER, AR | Embryonic lethal (mouse); adipocyte-specific differentiation defects (mouse) |
| MED150 (Rgr1)                | E1A       | C. elegans Sur2: vulval developmental defects via RAS/MAP kinase pathway |
| MED130 (Gal11)               | p53, VP16 | Essential; sex-comb identity defect in combination with dMED240 (Drosophila) |
| MED70 (Med2)                 | Cyclin C (Srb11) | Necessary for expression of developmentally regulated genes (C. elegans) |
| MED36 (Med4)                 | MED34 (Med7) | Necessary for expression of developmentally regulated genes (C. elegans) |
| MED33 (Med6)                 | MED78 (Srb4) | Embryonic lethal (mouse) |
| MED10 (Med10)                | Cyclin C (Srb11) | Necessary for expression of developmentally regulated genes (C. elegans) |
| (Surf5) (Srb6)               | MED28b (Srb5) | Essential; sex-comb identity defect in combination with dMED240 (Drosophila) |
|                               | MED17 (Srb7) | Necessary for expression of developmentally regulated genes (C. elegans) |

The left column lists the components of the human mediator complexes. The mediator subunit nomenclature of Rachez and Freedman (Rachez and Freedman, 2001) has been adopted here. These subunits are those found in the smaller CRSP complex. Alongside each human subunit is listed its yeast counterpart; these assignments are largely based on two review articles (Boube et al., 2002; Rachez and Freedman, 2001). Listed in the middle column are the activators known to interact with the various mediator subunits. The activator/mediator interactions are described in the following reports: p53 and VP16 (Ito et al., 1999), E1A (Boyer et al., 1999), TR (Ren et al., 2000; Treuter et al., 1999), VDR (Rachez et al., 1999), RARα (Yuan et al., 1998), RXRα (Yuan et al., 1998), PPARγ (Yuan et al., 1998), ER (Yuan et al., 1998), AR (Wang et al., 2002) and GR (Hittelman et al., 1999). Listed in the right column are the defects known for several mediator subunit mutations (references can be found in the text).

An additional permutation is seen in the regulation of the yeast HO promoter. The binding of the Swi5 activator to the URS1 binding site is the first step in the events leading to the activation of HO transcription (Cosma et al., 1999). Mediator and Swi5 are concomitantly brought to the URS1 site early. Fifteen minutes later, mediator binds both URS1- and URS2-binding sites. This does not recruit RNA polymerase II, however, which arrives at the TATA region of the promoter only immediately before transcription starts. However, in the case of other promoters, such as PIR1, mediator and RNA polymerase II are recruited concomitantly with the activator Swi5 (Bhoite et al., 2001).

Clearly one may suggest from these studies that activators have temporal functions in transcription defined by promoter context. Each would have a temporally defined role in recruitment of particular coactivators to the promoter at specific times. These coactivators do not necessarily play a role in controlling the rate of transcription initiation itself. For example, the HAT coactivator SRC-1, possibly playing a role in chromatin remodeling, is recruited to promoters by thyroid receptor but much earlier than MED220/VDR. Its transient association with the promoter peaks at 15 minutes after ligand addition and then disappears. Meanwhile, the presence of MED220 correlates with transcription initiation at 60 minutes after ligand addition (Sharma and Fondell, 2002). The Swi5 data (discussed above, in the context of the HO and PIR1 promoters) suggest a second alternative: it is not the activator but the promoter context that dictates the timing of mediator recruitment. These distinctions can be resolved only once experiments that compare the same activator in different promoter contexts are performed. Note that promoter specificity could be due to the core promoter, various combinations of activators, or both of these parameters.

These recruitment data can be summarized and interpreted in another way. They suggest that three (and possibly more)
activator-dependent pathways exist and are reflected by the temporal order of recruitment of mediator and RNA polymerase II. The first is the initial recruitment of mediator followed by the arrival of RNA polymerase II and the concomitant initiation of transcription. The second is the co-recruitment of mediator and RNA polymerase II; transcription is initiated later. The third is the recruitment of RNA polymerase II and the later arrival of mediator, concomitantly with transcription initiation. These different temporal patterns of recruitment of mediator and RNA polymerase II may be necessary for the appropriate regulation of transcription of a particular gene, and reflect either the existence of multiple types of preinitiation complexes, or the recruitment of alternative mediator complexes (ARC-L or CRSP) and their subsequent conversion into transcriptionally active complexes.

Recruitment of mediator has also been analyzed in vitro to determine the role that mediator plays in preinitiation complex (PIC) formation in yeast. Using immobilized DNA templates bound to a support, the sequential assembly of the GTFs into the PIC can be monitored. Mutations in yeast Srb2, Srb4, and Srb5, and a CTD truncation all severely compromise transcription at the level of PIC formation: of all the GTFs, only TFIIA and TFIID were recruited to the promoter (Ranish et al., 1999). Importantly, the mediator mutants cannot support multiple rounds of transcription, which suggests that mediator functions in the reinitiation step of the transcription cycle. In fact, a reinitiation intermediate/scaffold that contains TFIIA, TFIID, TFIIH, TFIIE, and mediator can be isolated. This intermediate supports transcription after a brief incubation to supply the missing factors released through the process of transcription initiation (Yudkovsky et al., 2000). Lastly, mutation of members of the Sin4 module (Sin4 and Pgd1; see next section) also produces defects in transcription and PIC formation. Despite both components residing in the Sin4 module, only Pgd1 plays a role in the formation of a functional scaffold/reinitiation intermediate: in contrast to a Sin4 deletion, a Pgd1 deletion is unable to reinitiate transcription in vitro (Reeves and Hahn, 2003). Importantly, this scaffold was not observed in initial studies using immobilized template to analyze recycling of GTFs in the absence of mediator and activator (Zawel et al., 1993). Those studies demonstrated that the only factors that remain bound at the promoter after the first round of transcription are TFIIA (TBP) and TFIIA.

**Mediator modules**

It has been suggested that yeast mediator is functionally and physically divided into three modules: the Srb4 module (Srb2, Srb4, Srb5, Srb6, Rox3, Med8, Med11 and Med6) (Koh et al., 1998; Lee et al., 1999), the Gal11/Sin4 module (Gal11, Rgr1, Sin4, Pgd1 and Med2) (Kang et al., 2001), and the Med9/Med10 module (Med1, Med4, Med7, Srb7, Med9 and Med10) (Kang et al., 2001) (Fig. 1). This interpretation is based on biochemical, genetic and structural data.

Analysis of several mediator proteins has revealed physical and genetic interactions between certain mediator subunits. Specifically, a mutation in a particular mediator protein causes the loss of groups of other subunits. For example, mutations in Gal11 module components (Gal11, Rgr1, Sin4, Med2, and Pgd1) produce similar phenotypes (Jiang et al., 1995; Jiang and Stillman, 1995) and Rgr1 truncations result in loss of Gal11, Sin4 and Pgd1 (Myers et al., 1998). Additionally, a gal11 mutant lacks other members of the module and is activation defective (Lee et al., 1999b; Park et al., 2000). Structural studies of a Sin4-deletion mutant (see below) are also consistent with this notion: the entire tail domain is missing, leading the authors to conclude that the Sin4 module comprises the tail domain (Dotson et al., 2000) (see next section below and Fig. 1). Gal11 and Pgd1 bind to several activators in vitro, which suggests that the Gal11 module is a major recognition unit for activators (Bhoite et al., 2001; Han et al., 1999; Lee et al., 1999b; Myers et al., 1999).

The existence of an Srb4 module is supported by genetic and biochemical data. The Srb4 module can be reconstituted de novo (Koh et al., 1998); its assembly is consistent with genetic data showing that Srb5-null extracts require Srb2 and Srb5 to rescue in vitro activity (Thompson et al., 1993) and that suppressors of an Srb4 mutant are Med6 and Srb6 (Lee et al., 1998). Biochemically, the Srb4 module remains together during urea washes of immobilized mediator complexes (Lee and Kim, 1998). It is suggested to play a more general role in regulating transcription by modulating RNA polymerase II activity through the CTD (Lee and Kim, 1998), or perhaps by serving as a conduit for transferring activator ‘information’ from the Gal11/Sin4 module to RNA polymerase II.

The existence of the Med9/Med10 module is suggested by its de novo reconstitution from recombinant proteins. Assays of the reconstituted modules for interactions with the general transcriptional machinery revealed that the Med9/Med10 and Srb4 modules interact with the CTD of RNA polymerase II. TBP and TFIIH interact with both modules, whereas TFIIE interacts with Med9/Med10 (Kang et al., 2001). The interaction between the Srb4 module and TBP is possibly through Srb2 (Koleske et al., 1992). Finally, in vitro data indicate that the Srb10-Srb11 pair interacts with the Med9/Med10 module (Kang et al., 2001).

Srb8-Srb11 may also constitute a distinct module. Borggreve et al. isolated Srb8-Srb11 as a separate entity from yeast extracts (Borggreve et al., 2001), and its components have repressive functions in yeast (Carlson, 1997; Hengartner et al., 1998). Likewise, the human Srb10-Srb11 pair (CDK8–cyclin C) represses activator-dependent transcription in vitro (Akoulitch et al., 2000; Sun et al., 1998), and the large, transcriptionally inactive human ARC-L complex contains MED230, MED240, CDK8, and cyclin C (Taatjes et al., 2002) (Table 1). Thus, there is evidence in yeast and humans that these subunits are physically and functionally coupled (Kang et al., 2002).

**In vivo analysis of human mediator**

Considerable in vivo and genetic data supporting the existence of the yeast mediator complex (Carlson, 1997), as well as activator-mediator-subunit interactions, had been acknowledged in the field for some time. However, the studies of the mammalian mediator were biochemical in nature, and in vivo data were necessary to substantiate the in vitro interpretations. An important test of the mediator hypothesis is to assay the in vivo consequences of loss-of-function mutations in members of the mammalian mediator complex. Do these results confirm the biochemical analyses? Table 1 describes the many interactions between different activators and mediator
subunits defined biochemically; are these interactions also important in vivo?

MED220-null mice die by embryonic day 8.5, have heart and neural defects, and MED220-null cell lines grow slower than their wild-type counterparts (Ito et al., 2000; Zhu et al., 2000). Interestingly, these cells exhibit defective transcription stimulated by the thyroid receptor but not RAR, VP16 or p53 activators (Ito et al., 2000). MED220-null cells also exhibit defective adipogenesis and PPARγ-dependent activation, which can be rescued by ectopic MED220 expression (Ge et al., 2002). These results are consistent with others that showed that the adipocyte-specific nuclear receptor PPARγ physically interacts with MED220 (Yuan et al., 1998; Zhu et al., 1997). Furthermore, mediator isolated from MED220-null cells does not interact with PPARγ in vitro (Ge et al., 2002). Interestingly, the null cells can still differentiate into myofibers, which suggests that the MED220 defects are not global (Ge et al., 2002). There are several additional examples of mediator components that are necessary for developmentally specific events in Drosophila, C. elegans, and Xenopus (Boube et al., 2000; Kato et al., 2002; Kwon et al., 2001; Kwon and Lee, 2001; Kwon et al., 1999; Shim et al., 2002; Treisman, 2001).

Targeted ablation of MED100 produces results that contrast with MED220 ablation. Although the MED100-null mutation is embryonic lethal as well, studies of MED100+/− MEFs revealed that multiple activator-dependent and metal-induction pathways are defective in these mice (Ito et al., 2002). This suggests that the loss of MED100 has pleiotropic effects, seriously debilitating the response to activators. This is easily explained if the MED220+/− defects are restricted to a subset of activators, whereas the MED100 pleiotropic defects evident in the MED100+/− animals are the manifestation of the loss of multiple mediator functions and possibly its physical integrity (Ito et al., 2002).

Boyer et al. isolated human MED130 on the basis of its ability to interact with the viral E1A activator and stimulate E1A-dependent transcription in vitro and showed that it was part of the human mediator complex (Boyer et al., 1999). Immunoprecipitation of CDK8 from human MED130+/− cells revealed that, in addition to the lack of MED130, the CDK8 complex in these cells contains reduced amounts of MED100 and MED95, suggesting that MED130 is necessary for the incorporation of MED100 and MED95 into the complex. Transient expression assays indicated that E1A activation is defective in the absence of MED130, which is consistent with earlier work. In contrast, VP16 activation is similar in both wild-type and mutant cells (Stevens et al., 2002). This result is expected since VP16 does not interact with MED130 but, instead, the MED78 subunit; thus, mediator can still be recruited by VP16 to the promoter (Ito et al., 1999).

These in vivo experiments indicate that different mediator proteins seem to have activator-specific roles. This activator specificity is similar to that described for yeast mediator. For example, the yeast CUP1 activator is Srb4 independent, but Rgr1 dependent (Lee et al., 1999a). Mutations in Med2, Pgd1 and Sin4 all generate cells in which VP16 activation is defective but only a Sin4 mutant is GCN4 defective (Han et al., 1999). Additionally, some mediator ablations in mice result in loss of other subunits of mediator and the crippling of multiple activator-dependent pathways. Again, this is similar to the yeast complex, in which Rgr1 and Gal11 mutations cause loss of other members of the module (Lee et al., 1999b; Myers et al., 1998; Park et al., 2000). Although the data do not necessarily prove the existence of modules in the murine mediator complex, they do point to certain subunits maintaining the structural integrity of the complex through interactions with other subunits.

The structure of mediator

Several groups have determined the structures of the yeast, murine, and human mediator complexes, using EM data to generate 3D composites of the complexes. Yeast mediator by itself has a relatively compact triangular structure with a dense domain at its ‘base’ (Asturias et al., 1999). Importantly, in the presence of RNA polymerase II, an alteration of the mediator structure is observed, revealing an extended structure consisting of three domains: a tail followed by middle and head domains, the head and middle regions making contacts with RNA polymerase II (Asturias et al., 1999). Interestingly, a form of RNA polymerase II lacking the CTD repeats failed to induce this extended structure. Instead, mediator appeared partially extended, which suggests that a second interaction between mediator and RNA polymerase II occurs and is separate from that with the CTD (Asturias et al., 1999). This finding is consistent with initial biochemical studies demonstrating that mediator functions (activation and repression) and interaction with RNA polymerase II are CTD independent (Davis et al., 2002; Sun et al., 1998). These studies suggest that mediator interacts with RNA polymerase II through different domains. Interestingly, in the presence of the activator GAL4-VP16, the extended structure is not observed, which indicates that the binding of activator and RNA polymerase II to mediator might have different functional consequences and occurs through different domains (Asturias et al., 1999).

Additional studies showed that the yeast mediator tail domain corresponds to the Gal11/Sin4 module (Sin4, Gal11, Med2 and Pgd1) and suggested that this domain is linked to the middle domain through Rgr1. The head domain is postulated to contain the Srb4 module (Dotson et al., 2000) (Fig. 1). Asturias and colleagues suggest that one mediator–RNA-polmerase-II interaction (in addition to the CTD interaction) is centered on the Rpb3-Rpb11 heterodimer (Davis et al., 2002). This finding fits nicely with genetic studies demonstrating that mutations that result in defects in activator-dependent, but not basal, transcription map to Rpb3 (Tan et al., 2000).

The studies of the mammalian mediator complexes revealed a similar overall structure to that of yeast mediator. The smaller CRSP complex was purified on either CTD-containing or VP16 activator affinity resins. EM structures of the two preparations obtained are virtually identical but, importantly, conformational differences specific for a particular activator are evident (Fig. 2). The ARC structure of Taatjes et al. (Taatjes et al., 2002) clearly resembles that of the yeast mediator and the human TRAP complex (Dotson et al., 2000), with similar head, middle, and tail domains, whereas the smaller CRSP complex lacks the head domain as well as some protein density in the tail (Fig. 2). Lastly, the structure of the murine mediator closely resembles the yeast mediator structure (Asturias et al., 1999). Clearly, despite the evolutionary distances, the mediator structure is conserved between yeast and humans at least at the 30-40 Å resolution of these studies.
Conclusions and perspectives

The recent finding that the transcriptionally active human mediator complex is the smaller CRSP complex eliminates the contradictions posed by the identification of two types of mediator complex, both of which apparently stimulated transcription. One might conclude that the larger ARC-L type of complex is simply inactive, owing to the presence of the CDK8/cyclin C pair. This is perhaps an oversimplification, and does not adequately explain the presence of MED230 and MED240 in the large complex, or the presence of CRSP70 in the smaller CRSP complex. Experiments in Drosophila suggest that the MED230 and MED240 homologues are necessary for proper photoreceptor differentiation (Treisman, 2001) and that the Drosophila MED240 protein has a role in segment identity (Boube et al., 2000). Since cell growth was not affected in these experiments, these results suggest that MED230 and MED240 may play very specific roles in differentiation and/or activation.

The mouse in vivo ablation experiments support conclusions from yeast that some mediator subunits are activator specific and that mutations in one subunit do not necessarily affect other activator pathways. However, it is premature to conclude that the majority of activators operate through a mediator-dependent mechanism. Only a small number of activators have been assayed. In addition, there are several examples of activators that do not, at least physically, interact with mediator (Naar et al., 1999; Park et al., 2001a). This may not be a concern if one considers that the composite nature of the promoter, both in terms of its core promoter elements and activator sites, may allow the binding of mediator that is undetectable under more artificial experimental conditions. Nevertheless, since high levels of transcription appear to require mediator, one must ask whether there is a mediator interaction with a particular activator or whether it is possible to resurrect TFIIID as a coactivator in some cases. Several reports suggest that TFIIID (as opposed to the TATA-binding protein, TBP, which is a component of the TFIIID complex) is required, in addition to mediator, for maximal levels of transcription (Baek et al., 2002; Johnson et al., 2002). Perhaps then, in the end, a unification of mediator and TFIIID as coactivators is necessary. It is also entirely possible that other activator-dependent mechanisms of transcription initiation await discovery.

The in vivo and in vitro recruitment experiments suggest varying roles of mediator in transcription initiation and preinitiation complex (PIC) formation. This should be a particularly interesting and illuminating area of research. Does mediator play a different role depending on when it is recruited? Is there a difference between PICs on different promoters, as suggested by the in vivo recruitment experiments? Does promoter structure influence the role mediator plays in transcription? Can we define genes in terms of the timing with which they recruit mediator (or another GTF for that matter)?

The following model of mediator function takes some of these considerations into account. In part, mediator is recruited to the promoter in a manner defined by the promoter architecture (the composition and arrangement of its core promoter elements and activator binding sites). One function of mediator is to provide a scaffold for multiple rounds of transcription (Yudkovsky et al., 2000). Thus, the timing of its recruitment may be irrelevant for post-initiation events, although the in vivo recruitment studies suggest an important role of the timing of mediator recruitment in preinitiation events. After all, what is important in vivo is the number of RNA polymerase II molecules that can enter the transcription cycle in a defined period of time. The time required for the formation of the transcription initiation complex might change on different promoters, but after formation of the complex, the
then affect the magnitude of transcription at the level of PIC formation itself, the initiation of transcription, or subsequent re-entry of RNA polymerase II onto the preformed scaffold. Consequently, we suggest that the regulation and magnitude of transcription is a function of the promoter architecture and the activator’s influence on mediator conformation. All of these are intimately related to each other, each influencing the timing and magnitude of transcription.

The idea that mediator is organized into distinct physical and functional modules concisely combines a variety of genetic and biochemical observations. Yet, the assignment of specific functions is arguably incomplete and a more definitive assessment awaits mechanistic studies of mediator function. However, the EM structural data thus far has elegantly provided confirmation of some mediator module identities and the existence of significant structural alterations of mediator upon binding of RNA polymerase II, the CTD itself, or activators. These structural differences suggest that, mechanistically, activators force mediator into a unique structure that has implications for transcription. Further structural analysis using a variety of yeast mediator mutants, human and yeast activators, the addition of basal factors, and an increased resolution should provide a more detailed mechanistic model of mediator-dependent transcription.

The discovery and characterization of mediator has been a significant contribution to our understanding of transcriptional activation. Mediator has survived numerous tests of its function as a coactivator, and the identification of mediator has solved, at least partially, the problem of activator-dependent transcription. The continued study of this fascinating complex should further illuminate our understanding of the mechanism of transcriptional activation.

Fig. 3. A model of mediator function. This schematic model suggests that particular combinations of activators play a role in influencing the conformation of mediator. These different conformations would influence the re-entry of RNA polymerase II to the promoter to initiate subsequent rounds of transcription. The combination of activators in panel A forces a mediator conformation that only promotes the slow re-entry of RNA polymerase II to the promoter, while the combination in panel B promotes a faster RNA polymerase II re-entry via a more productive interaction with mediator. It is important to note that activator A, in the context of a different set of activators (X and Y in panel B), is capable of inducing a different conformation of mediator (Lefstin and Yamamoto, 1998). This is not meant to suggest that the number of activators is the key point in inducing a particular mediator conformation.

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