

Mediator special issue

Structure of eukaryotic Mediator complexes

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Mediator, a macromolecular complex comprising ~20 different protein components, is largely responsible for the tight control of transcription that underpins cell development, differentiation, and maintenance in eukaryotes from yeast to human. In the past five years, macromolecular electron microscopy has been used to characterize the structure of Mediator, and of the complexes it forms with other components of the transcription machinery. The results reveal how Mediator interacts with RNA polymerase II, and suggest that regulatory information could be conveyed through changes in Mediator conformation that would influence the transcription initiation process.

Introduction

As the simplest eukaryotic organism, the budding yeast *Saccharomyces cerevisiae* has long been used as a model for the study of transcription and its regulation in higher organisms. Despite reports of direct interaction between activators and components of the basal transcription machinery [1], transcription assays in a reconstituted yeast system that included RNA polymerase II (RNAPII) and five general transcription factors (TBP, TFIIB, TFIIE, TFIIIF and TFIIH) revealed that these components were not enough to recapitulate activation. An activity in a partially purified yeast fraction turned out to restore response to activators, and was therefore termed Mediator [2–4]. Further purification efforts led to isolation of a large multi-protein complex that stimulated basal transcription and enabled activation, and that also stimulated the kinase activity of TFIIH [4,5]. Although it was initially argued that Mediator might exist only in yeast and that other complexes would enable transcription regulation in higher cells [6–8], it is now established that Mediator complexes with limited composition and sequence homology are present in all eukaryotic organisms [9], and that they are the primary conduits for conveying regulatory information to the basal transcription machinery. Here we review current knowledge on the structure of Mediator complexes isolated from several different eukaryotes.

Structure of yeast Mediator

Purification of yeast Mediator revealed a large complex of 21 polypeptides, with a combined molecular weight of ~1 MDa [10]. Purification of the complex was quickly

followed by structural characterization using single-particle electron microscopy (EM). Images of yeast Mediator particles preserved in (uranyl acetate) stain showed a discrete complex with a well-defined structure. Most Mediator particles appeared in approximately the same orientation in the EM samples, enabling definition of a 2D map of Mediator by alignment and averaging of individual particle images. This projection map (2D), with a limited resolution of ~40 Å, revealed that Mediator had an elongated, roughly conical shape, ~400 Å in length, with a large, separate domain forming the bottom portion of the structure (subsequently termed the ‘head’ domain) [11]. Extension of the initial EM studies eventually resolved the 3D structure of the yeast Mediator complex from images of particles preserved in stain [12] (Figure 1a).

An alternative Mediator conformation is required for interaction with RNA polymerase II

In an attempt to understand from a structural point of view how Mediator conveys regulatory information to the basal transcription machinery, the complex was incubated with RNAPII, with the expectation of obtaining images of an anticipated Mediator–RNAPII complex. Upon incubation with RNAPII, Mediator particles went from the compact conformation observed for the free complex, to several conformations showing varying degrees of unfolding. Most of the Mediator particles (>70%) interacted closely with RNAPII to form a complex (the holoenzyme) in which Mediator adopted an extended conformation around the central RNAPII density [11]. Further extension of the EM work on the yeast holoenzyme complex determined the 3D structure of the holoenzyme (Figure 1b). No holoenzyme complexes were observed after incubation of Mediator with RNAPII lacking the carboxy-terminal domain (CTD) of Rpb1 (the largest RNAPII subunit). This domain was previously shown to be necessary for activation of transcription by Mediator [13]. However, incubation of Mediator with a recombinant CTD polypeptide prompted no structural change, suggesting that more than the CTD is required to facilitate the conformational change necessary for interaction of Mediator with RNAPII. The Mediator–RNAPII interaction was species specific because no holoenzyme was formed when yeast Mediator was incubated with mammalian RNAPII, despite significant similarity in CTD sequence (the yeast and mammalian CTDs differ only in the number of repeats of a conserved heptapeptide sequence) [11]. Examination of

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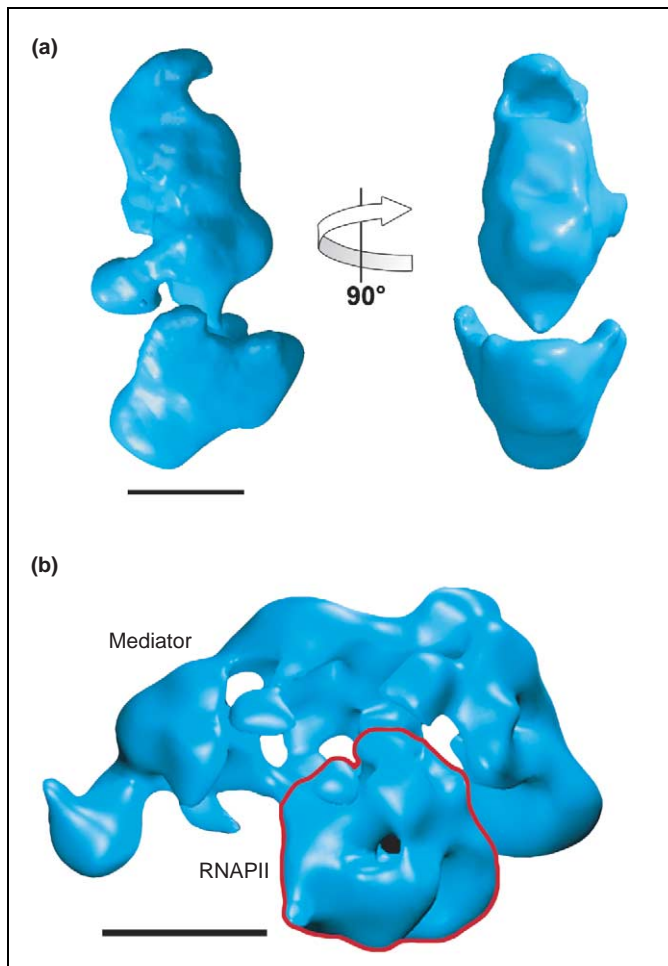


Figure 1. Structure of the yeast Mediator and holoenzyme complexes. (a) A 3D reconstruction of the yeast Mediator structure was calculated from images of individual particles imaged in an electron microscope after preservation in stain. Mediator has a compact, roughly triangular shape. A large domain at the bottom is linked by a thin connection to the top portion of the structure. The resolution of the reconstruction is ~ 35 Å, and the scale bar represents 100 Å. (b) Structure of the Mediator-RNA polymerase II holoenzyme complex calculated from electron microscope images of individual particles preserved in stain. Previous characterization of the polymerase and Mediator structures led to identification of the Mediator and RNA polymerase II (red outline) portions of the holoenzyme structure. In the holoenzyme, Mediator adopts an extended conformation, embracing the central polymerase density. The resolution of the reconstruction is ~ 35 Å, and the scale bar represents 100 Å. Part (b) reproduced, with permission, from Ref. [19].

the extended structure adopted by Mediator upon interaction with RNAPII revealed three distinct structural modules. The structure of the 'head' domain of Mediator remained constant, but the top portion of the original structure unfolded, revealing two separate domains later termed 'middle' and 'tail'. Detailed comparison of the compact and extended Mediator structures, along with examination of partially extended Mediator particles present in Mediator-RNAPII samples, led to a possible model for the conformational transition, which is illustrated in Figure 2a.

Biochemical identity and localization of modules in the yeast Mediator structure

Evidence from chromatographic [14], deletion mutation [15], reconstitution [16], and two-hybrid screen studies [17] of yeast Mediator indicates that the constituent polypeptide subunits are associated into four distinct

modules. One of them, the Cdk8 subcomplex that includes Mediator subunits Cdk8 (also known as Srb10), CycC (Srb11), Med12 (Srb8), and Med13 (Srb9), is comparatively labile, and was not present in the Mediator preparations used for the EM studies. A probable correspondence between the three remaining biochemically-defined modules and the three structural domains apparent in the extended Mediator structure was established by analyzing the structure in projection of a holoenzyme complex purified from a mutant yeast strain lacking *MED16* (*SIN4*, GenBank accession number: 855485). In the projection map for this mutant holoenzyme, the tail Mediator module was absent, indicating that this portion of the extended Mediator structure is formed by a set of physically interacting subunits that are lost upon deletion of *Med16* (*Sin4*), including Med16 (*Sin4*), Med15 (*Gal11*), Med2, and Med3 (*Pgd1*). The tail subunit Med16 (*Sin4*) is physically associated *in vivo* [18] with subunit Med14 (*Rgr1*), implying that Med14 (*Rgr1*) and related subunits (identified by several lines of evidence) must reside in the middle Mediator domain. Lastly, the remaining subunits would constitute the head Mediator domain. The conclusions derived from this analysis are illustrated in Figure 2b. It is important to emphasize that no individual yeast Mediator subunits have been directly localized, and that the mapping illustrated in Figure 2b is derived from localization of the tail Mediator subunit module and the extensive information about subunit interactions provided by a variety of studies [14–17].

Mediator-RNAPII interaction in the yeast holoenzyme

One of the most important questions about the structure of the yeast holoenzyme regards the interaction between Mediator and RNAPII. Unfortunately, the low resolution of the 3D holoenzyme structure (in addition to limitations related to staining and imaging artifacts) prevented a direct, definitive determination of the precise orientation of RNAPII in the complex. However, the problems could be circumvented by examining a projection map of the holoenzyme complex with higher resolution. Cross-correlation analysis between the RNAPII portion of the yeast holoenzyme structure in projection and projections of an independently determined EM reconstruction of RNAPII, revealed the specific orientation of polymerase in the holoenzyme complex [19]. The tail Mediator domain extends away from RNAPII and only its proximal portion contacts RNAPII directly, but there are multiple contacts between RNAPII and the middle and head Mediator domains (Figure 1b). Upon establishing the polymerase orientation it became apparent that the Mediator-RNAPII interactions involve the back face of the polymerase, are centered on the Rpb3-Rpb11 subunit complex [19], and involve portions of subunits Rpb1, Rpb2, Rpb6 and Rpb12 (Figure 3). This result is consistent with reports indicating that mutations in Rpb3 affect activated transcription [20]. Moreover, the bacterial counterpart of Rpb3-Rpb11, the α_2 complex, is involved in regulation in bacteria [21], pointing to an apparent conservation of the polymerase surface involved in regulation. Interestingly, current structural models for organization of the pre-initiation complex (PIC) [22,23] suggest that the back

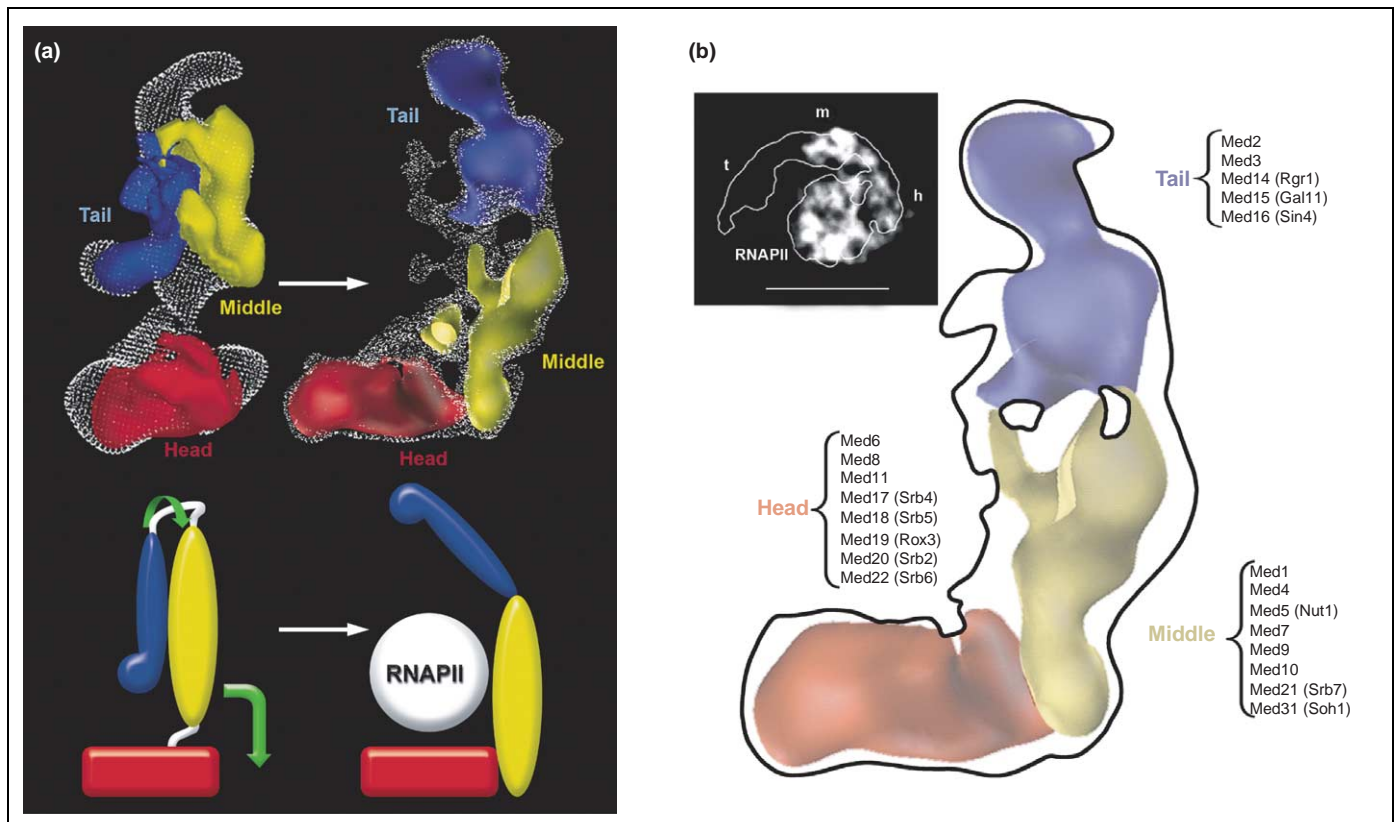


Figure 2. Correspondence between the compact and extended Mediator conformations, and distribution of Mediator subunit modules. **(a)** Upon incubation with RNA polymerase II, Mediator undergoes a large-scale conformational change and adopts an extended conformation in which three separate structural domains are apparent. Fitting portions of the extended Mediator structure (shown as solid colored shapes) into the compact structure of the complex (shown as a dotted white surface) suggests how the unfolding that leads to the extended conformation might take place. **(b)** Biochemical characterization of Mediator indicated that the component subunits are organized in modules. A projection map of Mediator isolated from a mutant *Med16* (*Sin4*) deletion yeast strain (see insert, scale bar 200 Å) lacked the tail portion of the extended Mediator structure apparent in the wild-type holoenzyme. Deletion of *Med16* (*Sin4*) results in loss of three additional Mediator subunits (Med15 (Gal11), Med2, and Med3 (Pgd1)), which, along with Med16 (*Sin4*) must constitute the tail domain. Considering this in combination with biochemical information about inter-subunit contacts and the composition of Mediator subunit modules suggests that subunits might be distributed as illustrated. Part (a) reproduced, with permission, from Ref. [19].

surface of RNAPII involved in interaction with Mediator is also involved in interaction with upstream promoter DNA, which would therefore be positioned along the Mediator–RNAPII interface in the holoenzyme complex.

Although the contacts between RNAPII and Mediator are extensive, a large fraction of the RNAPII surface (~75%) remains available for interaction with other components of the PIC. It is worth noting that the variety of Mediator–RNAPII contacts in the holoenzyme complex only include portions of the RNAPII structure that are not particularly mobile, and it seems unlikely that the role of Mediator in RNAPII transcription regulation would involve a direct effect on the conformation of the enzyme. Interestingly, whereas RNAPII and Mediator always interact in the same manner (with polymerase binding in a pocket generated when Mediator adopts an extended structure), statistical analysis of holoenzyme images suggests that the orientation of polymerase is poorly defined in a significant fraction of the holoenzyme complexes (~50%) [19]. This is probably related to the absence of additional components of the PIC that might be required to stabilize the Mediator–RNAPII complex fully, and suggests that a more complete understanding of the interaction between Mediator and RNAPII will require structural characterization of complexes that resemble more closely the targets of Mediator *in vivo*.

Structure of Mediator homologs in higher organisms

The initial EM study of the structure of yeast Mediator also included information about a functionally equivalent murine complex, identified on the basis of limited sequence homology [24]. Similarly to the yeast Mediator, the purified murine complex included a large number of component subunits, interacted with the CTD of RNAPII, and stimulated CTD phosphorylation by TFIID [24]. Structurally, the murine complex showed a rough resemblance in projection to the yeast Mediator [11]. Complexes that are functionally equivalent to the yeast Mediator have now been identified in several eukaryotic organisms, including humans [9,25]. That there has been a significant degree of evolutionary divergence is indicated by the rather limited sequence homology between component subunits of the different complexes. However, it has been proposed that sequence homology between Mediator subunits from different organisms might be most pronounced in regions involved in inter-subunit contacts, which would point to a conservation of the overall subunit organization across eukaryotes [17]. Three-dimensional low-resolution structures of murine [12] and human [26,27] Mediators have been reported (illustrated in Figure 4). It is important to note that the dissociable Cdk8 subunit complex involved in transcription repression in yeast has a human homolog, which includes the human Mediator

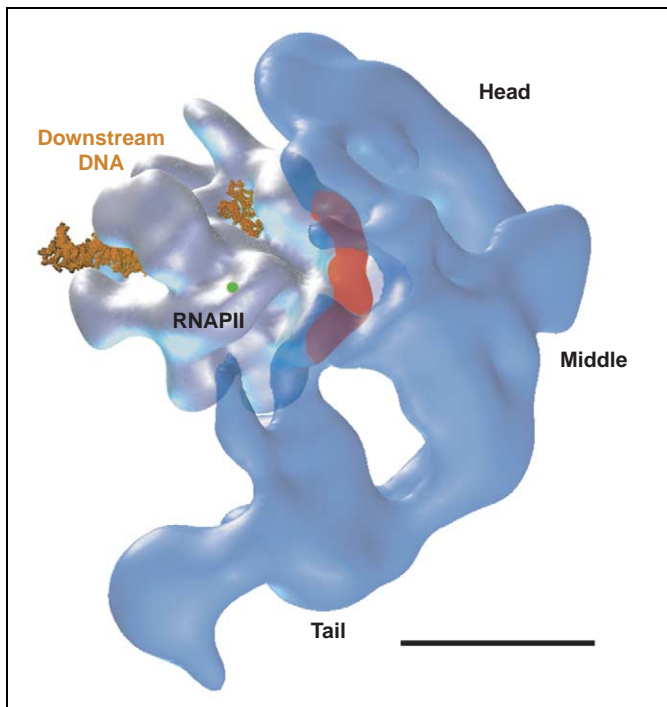


Figure 3. Interaction of Mediator and RNA polymerase II (RNAPII) in the holoenzyme complex. The precise orientation of RNAPII in the holoenzyme complex was established by 2D cross-correlation analysis between holoenzyme and RNAPII projections. The figure shows a cryoelectron microscopy reconstruction of polymerase fitted into the extended Mediator structure in the orientation determined by cross-correlation analysis. Multiple contacts between Mediator and RNAPII are established in the holoenzyme complex, involving mostly the head and middle domains, and distributed around the Rpb3–Rpb11 polymerase subunits (highlighted in red). The small green circle indicates the point where the carboxy-terminal domain of Rpb1 (the largest polymerase subunit), crucial for Mediator–polymerase interaction, emanates from the surface of the enzyme. The bacterial homolog of the Rpb3–Rpb11 complex, the α_2 homodimer, is involved in transcription regulation in bacteria, suggesting a conservation between prokaryotes and eukaryotes of the RNA polymerase surface involved in regulation. The scale bar represents 100 Å. Reproduced, with permission, from Ref. [19].

subunits Cdk8, cyclin C, Med12 (TRAP230) and Med13 (TRAP240) [26]. The Cdk8 complex was not present in the published structures of yeast or murine Mediators. However, structures of both a large [12] (TRAP or ARC-L, including the Cdk8 module) and a smaller [26] (CRSP, not including the Cdk8 module) form of human Mediator have been published. Interestingly, even the differences between the ARC-L and CRSP structures are multifarious, and seem to go beyond what would be anticipated solely on the basis of differences in subunit composition (Figure 4).

A simple examination and comparison of the different low-resolution Mediator structures is not particularly informative, with matters further complicated by the differences in composition and functionality among the complexes. Comparison of the human ARC-L Mediator structure with the yeast Mediator structure would suggest an apparent super-structural conservation of the head domain. However, whereas the yeast Mediator structure corresponds to a transcriptionally active form of the complex, only the smaller CRSP human complex is transcriptionally active [26]. There is a general resemblance between the structures of the yeast, murine, and small human Mediators, however the murine complex shown in Figure 4 only stimulated the kinase activity of TFIIF and had no effect on basal or activated

transcription levels. The different Mediator complexes are clear functional homologs but, as suggested by sequence analysis [17], structural similarities might be limited to a conservation of the overall organization of the complexes.

It is apparent that more than just the structures of isolated Mediator complexes must be examined to understand their role in transcription regulation. The conformational dynamics of the Mediator complexes, and their mode of interaction with components of the basal machinery must also be analyzed. Thus far there is little overlap between experiments carried out in the yeast and human systems, making it difficult to draw any general conclusions. For example, the only change in Mediator conformation characterized in the yeast system is the one prompted by interaction with RNAPII, there are no data on how the complex responds to other components of the transcription machinery. Although there is preliminary evidence from several reports regarding a putative human Mediator–RNAPII complex [12,28], no structural information about it is available. The effect of activators and repressors on the structure of yeast Mediator has not been investigated, but in human Mediator several structural changes have been reported [26,28]. Binding of different activators to human Mediator triggers specific conformational changes, and bound activators seem to target different patches on the surface of the complex, as would be expected by biochemical data indicating that they target different Mediator subunits (Figure 5) [26–28]. It has been suggested that the changes in human Mediator conformation might be specifically linked to the type of activator bound to the complex [28]. Further complexity in transcription in higher metazoans might come from endogenous forms of Mediator with varying subunit composition [29].

The changes in the conformation of human Mediator brought about by activator binding are relatively small when compared with the dramatic conformational change observed when yeast Mediator interacts with RNAPII. The functional significance of these activator-induced conformational changes in human Mediator has yet to be determined, but they could facilitate fine control of the expression of specific genes. If that were the case, the conformation of Mediator triggered by an activator could modulate interaction of the complex with additional components of the transcription machinery, including other activators, thereby providing a possible mechanism for the signal integration function carried out by Mediator in transcription regulation. Multiple forms of Mediator, with multiple activator targets, coupled with various possible conformational outcomes, and the interplay with several additional, equally complex coactivator complexes, presents itself as an elegant solution for maintaining tight, highly specific control of the expression of a large number of genes [30,31]. The significance of the structural changes reported in yeast and human Mediator complexes would be significantly bolstered if similar changes could be detected in both systems, which would then be identified as elements of a general mechanism for transcription regulation by Mediator.

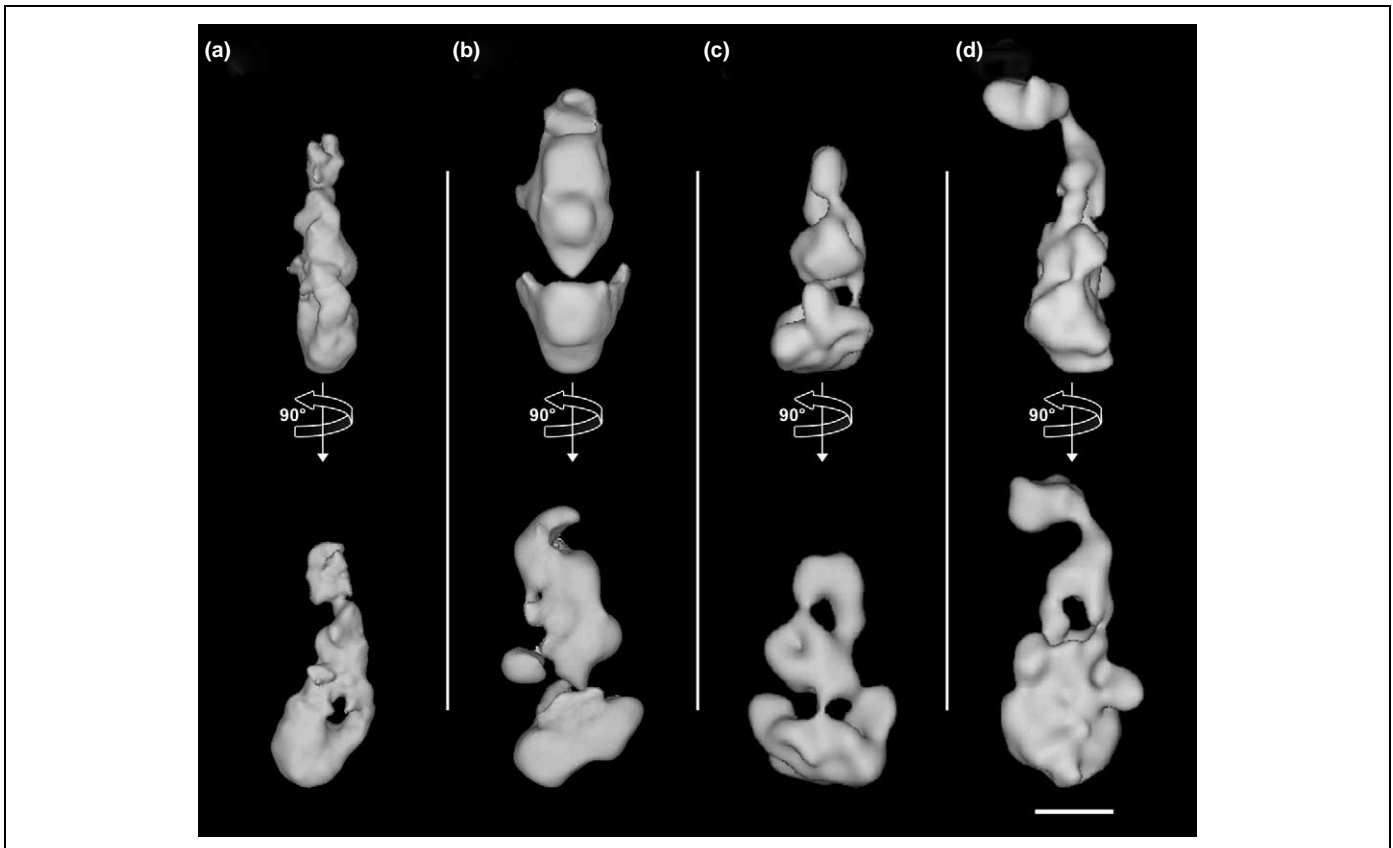


Figure 4. Comparison of eukaryotic Mediator structures. Mediator complexes have been identified in mouse and human cells on the basis of functional characterization, and limited sequence homology of the component subunits to yeast Mediator subunits. The structure of all complexes was determined using macromolecular electron microscopy and image analysis. The structures of the murine Mediator (a), the yeast Mediator (b) and two forms of human Mediator (c,d) are shown. The two forms of the human Mediator differ in their subunit composition. The small human complex [CRSP, (c)] lacks subunits in the Cdk8 module, and is the form of human Mediator that is transcriptionally active. The large human complex [TRAP or ARC-L, (d)] includes subunits in the Cdk8/cyclin C module, and is also bound to the VP16 activator. The subunit composition of the yeast Mediator (b) corresponds to that of the small form (CRSP) of the human Mediator (c). The two complexes are functionally homologous and, as illustrated, there is a similarity between their structures.

Mediator and the basal machinery: a possible mechanism for regulation

A particularly interesting revelation from the structural analysis of yeast Mediator and its interaction with

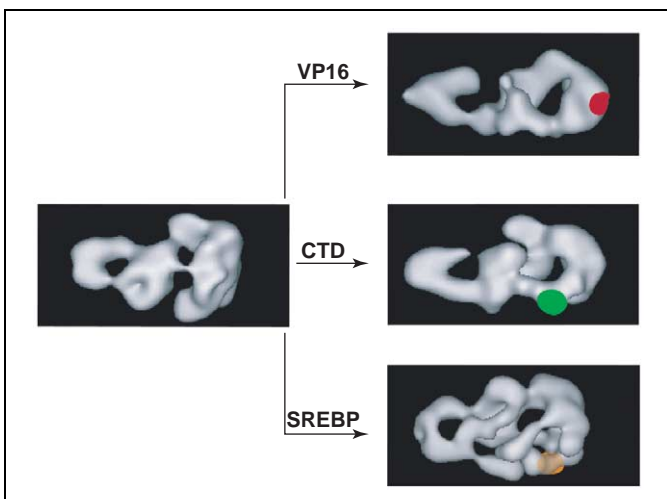


Figure 5. Conformational changes in human Mediator. Incubation of human Mediator with the carboxy-terminal domain of the largest RNA polymerase II subunit (required for Mediator–polymerase interaction), or with the VP16 or SREBP activators, induces significant and specific conformational changes. The location of the respective binding sites is indicated by the colored dots included in the panels. In the case of SREBP, the binding site is actually located on the back face of the Mediator view shown in the figure.

RNAPII is that Mediator unfolding seems to generate a surface for recruitment and assembly of components of the basal transcription machinery. Unfortunately, no structural information about the interaction of other Mediator complexes with the respective RNA polymerases is available at the moment. However, it is tempting to speculate that generation of a surface for PIC assembly through a large change in Mediator conformation could be an important general feature of the Mediator regulation mechanism. This speculation seems to be supported by some similarity between the structures of the active forms of yeast and human Mediator (Figure 4b,c). Evidently, because RNAPII alone is incapable of interacting with a promoter or initiating transcription, and because the actual target of Mediator must minimally include RNAPII and the basal transcription factors, obtaining structural information about larger complexes that include Mediator and additional components of the basal machinery will be essential for elucidating the mechanism by which Mediator affects transcription regulation [32]. Nonetheless, considering what is known, it seems possible that the regulation of transcription by Mediator is brought about through its effect on assembly of the PIC. A major change in Mediator conformation would generate a surface for assembly. Smaller activator-induced changes in Mediator structure (such as those observed for the human Mediator)

would influence the process by modulating the transition in Mediator conformation and/or the stability of the PIC.

Since the structure of the yeast Mediator–RNAPII complex was first examined, much has been learned about the organization of a functionally significant minimal PIC subcomplex including RNAPII, TFIIF, and TFIIB and TBP. The components in this minimal PIC can

be considered the catalytic core of the transcription machinery, as they are capable of promoter-directed initiation under conditions that favor transient promoter opening (e.g. use of a supercoiled plasmid promoter) [33,34]. Several models of this minimal PIC have been proposed on the basis of X-ray [35], EM [22], and site-specific cleavage and chemical crosslinking studies [23]. All of these models

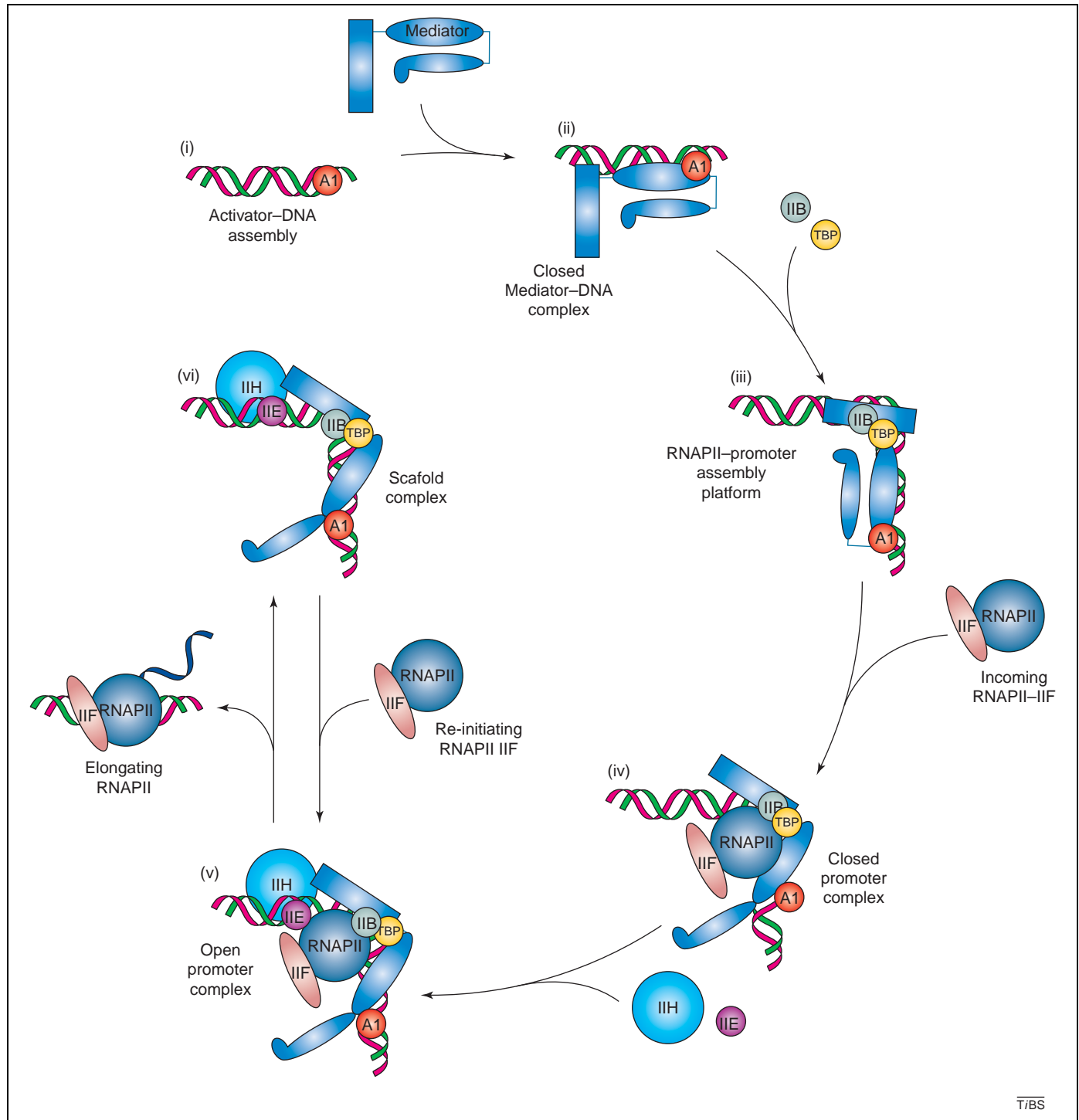


Figure 6. A possible mechanism for transcription regulation by Mediator. (i) A DNA-bound activator (A1) recruits Mediator to the transcription initiation site (ii). Transcription factors IIB and IID (including TBP) interact with DNA and Mediator and establish a platform for the recruitment of RNA polymerase II (RNAPII) (iii). Recruitment of RNAPII and IIF concomitantly (or the RNAPII–IIF complex) would prompt a large conformational change in Mediator that enables RNAPII–IIF to bind to the Mediator–DNA–TBP–IIB complex (iv). Factors IIE and IIH would join the complex and the helicase activity of IIH would result in promoter opening. The DNA template strand would then reach the RNAPII active site (v). After the abortive transcription phase, RNAPII (or the RNAPII–IIF complex) would escape the promoter, leaving behind a platform that would facilitate rapid assembly of a new preinitiation complex and re-initiation by a new incoming RNAPII–IIF complex [(vi), then back to (v)]. (IIB, IIE, IIF and IIH refer to TFIIB, TFIIE, TFIIF and TFIH, respectively.)

place TBP and TFIIB, the general transcription factors that mediate interaction of RNAPII with key promoter elements, on the back face of the enzyme, in the general vicinity of the Rpb3–Rpb11 polymerase subunit complex. Promoter DNA upstream of the transcription start site would run down the same back face of RNAPII and, along with TFIIB and TBP, would occupy the Mediator–RNAPII interface in the holoenzyme complex. Regulatory elements would recruit Mediator to the promoter (or to an upstream activating sequence [36]), and modulate Mediator conformation. Mediator opening would generate a binding surface that would facilitate assembly of the PIC. Components of the basal transcription machinery (including TBP and TFIIB) could then interact with the assembly surface provided by Mediator and facilitate recruitment of RNAPII and additional factors.

Consistent with this scenario for the role of Mediator in regulation, it seems that Mediator is recruited to a promoter independently of RNAPII [36–40]. Independent recruitment of Mediator and RNAPII to a promoter would be required given the structure of the holoenzyme, in which upstream promoter DNA is sandwiched between the two [22]. Moreover, a subset of PIC components, including Mediator, TBP, TFIIE, and TFIIH remain at the promoter after RNAPII clears the promoter and switches to elongation. The PIC components left behind, referred to as the ‘Scaffold’ complex, can facilitate assembly of a PIC and accelerate a new round of transcription (re-initiation) [41,42] (Figure 6). The reported effect of Mediator on basal transcription in both the yeast [42] and human [43] systems is consistent with the idea of Mediator having a key role in assembly of the basal transcription machinery.

Concluding remarks

Structural studies of yeast and human Mediator complexes suggest that conformational changes prompted by binding of activators and repressors could explain the capacity of Mediator to integrate regulatory information, and be essential for its mechanism. A large-scale rearrangement of the structure of Mediator could constitute a crucial control point for regulation, by generating a surface that would facilitate assembly of the PIC. Moreover, the role of a Mediator-based Scaffold complex in facilitating transcription re-initiation could prove as significant for regulation as any considerations regarding the effect of Mediator in factor recruitment and PIC assembly.

Understanding the mechanism of regulation will require structural characterization by macromolecular electron microscopy of assemblies that include not only Mediator and the components of the basal machinery, but also other complexes involved in the initiation process, such as those responsible for chromatin remodeling and modification. Combining information about the structure of these large complexes with high-resolution information about individual components obtained by X-ray crystallography and NMR will ultimately reveal the molecular details of the regulation process.

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