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The Mediator complex: a central integrator of transcription

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Abstract

The RNA polymerase II (pol II) enzyme transcribes all protein-coding and most non-coding RNA genes and is globally regulated by Mediator, a large, conformationally flexible protein complex with variable subunit composition (for example, a four-subunit CDK8 module can reversibly associate). These biochemical characteristics are fundamentally important for Mediator's ability to control various processes important for transcription, including organization of chromatin architecture and regulation of pol II pre-initiation, initiation, re-initiation, pausing, and elongation. Although Mediator exists in all eukaryotes, a variety of Mediator functions appear to be specific to metazoans, indicative of more diverse regulatory requirements.

Introduction

Eukaryotic transcription is governed, in part, by DNA-binding transcription factors (TFs), which assemble at promoter and enhancer sequences throughout the genome. A characteristic feature of promoters and enhancers is the presence of sequence motifs that allow multiple TFs to bind across the same region. In addition to sequence-specific DNA-binding domains, TFs typically possess separate activation domains that interact with transcription regulators. The **Mediator complex**, which is comprised of 26 subunits in mammals (up to 21 subunits in yeast; see Box 1), is a general target of TF activation domains; moreover, because different TFs bind to different Mediator subunits, multiple TFs might bind Mediator at the same time.

A basic function of Mediator is to communicate regulatory signals from DNA-bound TFs directly to the RNA polymerase II (pol II) enzyme. The precise mechanisms by which Mediator regulates pol II activity remain poorly understood, but clearly involve extensive protein-protein interactions between Mediator, pol II, and other general and gene-specific transcription regulatory factors. Mediator is also critical for the organization of genomic DNA into topological domains, including gene loops, which appear to be fundamental structures that enable the coordinated regulation of cellular transcription[1]. The large size of Mediator likely facilitates these diverse functional interactions.

Indicative of its functional versatility, Mediator is implicated in regulating at least some aspect of many fundamental processes involved in transcription, including transcription initiation, transcription elongation, chromatin architecture and **enhancer-promoter gene looping**. Throughout this Review we will emphasize the role of Mediator in some of these

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processes, and we direct readers to other recent reviews that address additional aspects of Mediator structure and function[2,3], or Mediator roles in selected human diseases[4-6].

Mediator composition and structure

A fundamentally important characteristic of Mediator is that its subunit composition can change; subunits can be lost or added to affect the biological function of the complex. Little is known about how subunit exchange is regulated and this remains an area of great interest and importance. Mass spectrometry (MS) analyses have indicated that although most Mediator subunits are present in similar numbers in human and yeast cells, select subunits are markedly over- or under-represented[7]. Furthermore, several labs have isolated mammalian Mediator complexes lacking one or more of the 26 core Mediator subunits[8,9]. These results suggest that a subset of endogenous Mediator complexes may lack specific subunits. Mediator complexes that lack specific subunits are incapable of transducing activation signals from TFs that would usually bind the missing subunit. For example, the thyroid hormone receptor (TR) binds the MED1 subunit and the Elk-1 TF binds the MED23 subunit[10,11]. In MED1 knockout murine embryonic fibroblasts (MEFs), activation of TR target genes was inhibited[12]; similarly, Elk-1 was unable to activate its target genes in MED23 knockout murine embryonic stem cells[11]. Importantly, however, the Mediator complexes lacking MED1 or MED23 could activate transcription in response to TFs that bind to other Mediator subunits[11,12].

These examples illustrate that different transcription factors — and thus the signaling pathways that activate them — rely on different Mediator subunits to orchestrate their transcriptional responses. In support of this, it appears that the subunit composition of Mediator is simplified in differentiated cells[13,14]. Whereas the phenomenon of simplified subunit composition in differentiated cells is best defined for the multi-subunit transcription factor IID (TFIID) complex[15], smaller Mediator complexes with fewer than the typical 26 subunits would also likely adopt more specialized functions. This could help enforce the expression of a restricted set of genes in differentiated cells; by contrast, proliferating cells, including cancer cells or stem cells, generally express all 26 core Mediator subunits in addition to the CDK8 module subunits (see below).

CDK8-Mediator

The most well-characterized example of Mediator subunit exchange is the reversible association, with Mediator, of the four-subunit **CDK8 module**. The CDK8 module can stably associate with Mediator, which results in a biochemically distinct pool of **CDK8-Mediator** complexes in cells[16,17]. Notably, the MED26 subunit is not present in CDK8-Mediator, resulting in a 29-subunit CDK8-Mediator complex. Upon association with Mediator, the CDK8 module dramatically alters Mediator structure and function[18,19]. The molecular mechanisms underlying the reversible CDK8 module-Mediator association are incompletely understood, but recent studies indicate that MED13 phosphorylation, ubiquitylation, and protein turnover are involved[20]. Targeted degradation of MED13 is expected to affect CDK8 module association with Mediator, as MED13 was shown to directly link the CDK8 module to Mediator in both yeast and humans[18,19].

CDK8 module subunits have been implicated in a growing number of cancers and developmental diseases (Box 2)[21-24]. Whereas some mechanistic details have been delineated[25,26], much remains to be discovered regarding the altered function of pathogenic mutations in CDK8 module subunits. The general lack of understanding stems from insufficient structural data, and the fact that connections between CDK8 module subunits and human diseases have been made relatively recently. In addition, CDK8 appears to function in context-specific ways, such that its biological function may vary in different cell types or in response to distinct stimuli. For example, in HCT116 cells, CDK8 regulates different genes during hypoxia or in response to serum[27,28].

As a kinase that reversibly associates with Mediator, CDK8 seems poised to broadly regulate gene expression. Although few CDK8 kinase targets have been identified, DNA-binding TFs represent common targets in yeast and human cells, suggesting that CDK8 may serve as a general regulator of TF function. Phosphorylation by yeast or human CDK8 appears to regulate TF activity and/or target TFs for proteasomal degradation[29,30]. As examples, Cdk8 phosphorylation of Gal4 or Sip4 activates these yeast TFs[31,32], whereas CDK8 phosphorylation of the human TFs SREBP-1c or E2F1 appears to repress their activity[33,34].

Another important consideration is the presence of CDK19, MED12L, and MED13L, paralogs of CDK8, MED12, and MED13, that have emerged in vertebrate organisms. Little is known about the biological roles of these paralogs, but they appear to assemble in a mutually exclusive fashion[35]. Specifically, mass spectrometry (MS) analyses have revealed that MED12 and MED12L will not associate in the same kinase module, nor will MED13 and MED13L or CDK8 and CDK19. Whereas CDK19, MED12L, and MED13L have been linked to neuronal and developmental disorders[36,37], their basic biological importance relative to CDK8, MED12, or MED13 remains unclear.

Mediator structural dynamics

Mediator is not only dynamic in its subunit composition, it is also structurally dynamic. An abundance of computationally predicted **intrinsically disordered regions** (IDRs) are observed in both yeast and human Mediator subunit sequences[38], and a high degree of structural flexibility is evident based upon electron microscopy (EM) analyses of Mediator[17,39,40]. In addition, the structural state of Mediator can shift dramatically upon binding other proteins or protein complexes. Examples of this are shown in Figure 1. In each case, Mediator structural shifts correlate with changes in Mediator function. Structural shifts that occur upon TF-Mediator binding (FIG. 1a), which appear to propagate throughout the entire Mediator complex[17], correlate with activation of pol II transcription[41]. Large-scale structural shifts also occur upon Mediator binding to pol II (FIG. 1b) or the CDK8 module (FIG. 1c); these structural changes may ensure that pol II and CDK8 module binding to Mediator is mutually exclusive. Numerous structural, functional, and biochemical assays have demonstrated that pol II-Mediator binding precludes association of the CDK8 module with Mediator and, conversely, that the CDK8-Mediator complex does not associate with pol II. For instance, Mediator complexes bound to the pol II CTD lack any detectable CDK8 module subunits[42], and proteomic data for CDK8-Mediator yield no detectable co-purifying pol II subunits[43]. Because existing structural data for the large assemblies depicted in Figure

1 are of low resolution, the precise nature of the structural shifts remain unknown. It is not clear how many subunits are involved in a given TF-Mediator structural shift, for example, or which subunit-subunit interfaces are changed. TF-induced structural changes appear to be a conserved feature of Mediator[44] and the general conservation of IDRs[38] and conformational flexibility from yeast to humans suggests that structural plasticity is a fundamental aspect of Mediator's cellular functions.

Indicative of its structural complexity, recent EM studies have completely redefined previous architectural models of yeast Mediator, which assigned subunits to co-called **head**, **middle**, and **tail** sections of the complex. A key feature of the more recent studies was the implementation of EM labeling strategies to identify the location of select subunits for the first time. The resulting models, aided by **crystal structure docking** of individual Mediator subunits, allowed more accurate delineation of the head, middle and tail modules[44,45]. Whereas previous structural models placed tail domain subunits away from a pol II–Mediator binding site[46], the new models position the tail subunits adjacent to this site. These results clarify some confounding issues with the previous models, including how tail module subunits could influence pol II–Mediator interactions[47] and assembly of the pre-initiation complex (PIC; Box 3)[48-51], and how CDK8 module interaction with head module subunits were observed in yeast two-hybrid screens[52].

Whether the subunit organization of mammalian Mediator complexes is similar to yeast is complicated by the fact that humans possess additional Mediator subunits; however, recent success with reconstitution of a partial human Mediator complex (15 subunits) has provided the most information to date about this basic question[53]. Crosslinking-mass spectrometry studies of this reconstituted 15-subunit Mediator complex showed similarities and differences between yeast and human Mediator in terms of overall subunit organization[53]. Because existing subunit-subunit interaction data for yeast and human Mediator (e.g. via crystal structures or crosslinking-mass spectrometry) have, understandably, involved partial assemblies[53-57], it remains to be determined whether potential differences in subunit organization will persist upon comparison of fully assembled complexes. These questions should be answered in the next several years.

Mediator roles in pol II transcription

Mediator is generally required for PIC assembly (Box 3), as revealed by a host of biochemical, cellular, and genetic studies in human cells and model organisms. In agreement, structural studies have revealed that Mediator makes extensive contacts with the pol II enzyme[39,58,59], which serves as a central scaffold around which the rest of the PIC assembles. Mediator is also a general target for DNA-binding TFs, invoking a “molecular bridge” model in which Mediator integrates and communicates regulatory signals from DNA-binding TFs directly to the pol II enzyme and the rest of the PIC. This central and fundamental role for Mediator is consistent with its global requirement for pol II transcription[60,61].

PIC assembly

For gene-specific transcription, that is, regulated transcription, pol II must be recruited to specific sites on the genome. This is generally controlled by sequence-specific, DNA-binding TFs. Although TFs do not directly bind pol II, one mechanism by which they can promote pol II recruitment is by binding to the Mediator complex. Mediator enables pol II recruitment via interaction with the C-terminal domain (CTD) of the pol II RPB1 subunit[42,62-64]. The large size of Mediator likely promotes stable PIC formation by directly interacting with multiple PIC factors (FIG. 2a). Numerous labs have shown cooperative and even synergistic functions between Mediator and TFIID during PIC assembly and transcription activation[65-69]; moreover, Mediator helps regulate the recruitment and/or the activity of the PIC components TFIIA, TFIIB, TFIIE, and TFIIF, within promoter-bound PICs[70-72]. Mediator also helps recruit the multi-subunit TFIIH complex to the PIC, likely via an interaction with the MED11 subunit[73,74].

An additional role for Mediator in PIC assembly appears to involve pol II complexes that contain Gdown1, which is now considered to be a 13th subunit of pol II, called POLR2M. Gdown1/POLR2M was identified as a factor that repressed transcription in the absence of Mediator *in vitro*[75]. Later work has shown that POLR2M affects PIC assembly by inhibiting TFIIF binding to pol II[72,76]. In fact, structural and biochemical data indicate that binding of TFIIF and POLR2M to the free pol II enzyme is mutually exclusive[72,77]. In the presence of Mediator, however, it appears that TFIIF can associate with pol II complexes containing POLR2M and this, in turn, may promote formation of active PICs[72]. The central role of Mediator in regulating POLR2M and TFIIF interactions with pol II may also have important implications in pol II pausing and elongation[76], as described below.

Pol II initiation

Following PIC assembly and the formation of the **open complex**, pol II must break contacts with Mediator and the PIC to leave the promoter and transition to productive elongation. Although many mechanistic details remain to be discovered, phosphorylation of the CTD of pol II plays a key role in disrupting the Mediator–pol II interaction (FIG. 2a)[78]. In particular, the pol II CTD is phosphorylated by TFIIH (via its CDK7 kinase subunit), and this is accompanied by pol II (and thus transcription) initiation[79,80]. The ability of TFIIH to phosphorylate the pol II CTD is dependent on Mediator[41,62,81,82], but it is unknown whether Mediator regulates other enzymatic functions within TFIIH. It also remains unknown how many **CTD repeats** must be phosphorylated to sufficiently disrupt Mediator–pol II binding to enable pol II to escape from the promoter. Recent results in yeast have shown that TFIIH-dependent pol II CTD phosphorylation contributes to, but is not required for, the disruption of Mediator–pol II contacts at the promoter[83,84]. Whereas promoter-proximal paused pol II does not appear to be a common regulatory intermediate in yeast[85], these and other[86] findings suggest a conserved role for Mediator in regulation of pol II promoter escape.

Pol II pausing and pol II elongation

In metazoans, the pol II enzyme can pause after transcribing 30 – 60 nucleotides[87,88]; these “paused” pol II complexes are widely observed at promoter-proximal sites throughout the genome, which establishes paused pol II complexes as common regulatory

intermediates[89-91]. Although Mediator appears to regulate pol II pausing and/or pause release, the molecular mechanisms remain unclear[41,92-94]. For example, it is not known whether Mediator remains bound to paused pol II or whether paused pol II complexes dissociate from Mediator (FIG. 2b); if Mediator-pol II contacts are broken during early initiation and pausing stages, Mediator-dependent regulation of pol II pausing or pause release would require association with intermediary factors. A growing set of proteins and protein complexes are known to regulate pol II pausing or elongation in metazoans[95]. Among these, many are known to physically or functionally interact with Mediator, including Gdown1/POLR2M and the super elongation complex (SEC).

Whereas Mediator has been shown to regulate PIC assembly in the presence of POLR2M (see above), biochemical, gene expression, and **ChIP-Seq** data have implicated Mediator in regulating potential POLR2M functions in pol II pausing and/or elongation as well[72,76]. *In vitro* transcription assays indicate that POLR2M stabilizes pol II pausing, at least in part, through blocking pause termination by TTF2[76]. Furthermore, POLR2M co-localized with paused pol II across the genome, and POLR2M knockdown caused an increase in pol II density downstream of pause sites[76]. Because Mediator appears to be essential for activating PICs that contain POLR2M[72,75], these data suggest additional roles for Mediator in the regulation of pol II pausing and elongation.

The Super Elongation Complex (SEC)[96], in various forms[97], appears to be a general regulator of pol II elongation and contains factors such as P-TEFb, AFF4, and ELL. The metazoan-specific MED26 subunit of Mediator has been shown to interact with the SEC to facilitate pol II elongation at a subset of genes[68]. Interestingly, a region on MED26 to which the SEC binds was also shown to bind TFIID, suggesting that MED26 acts as a switch, perhaps initially binding TFIID to promote PIC assembly and then, in response to an activating signal, binding the SEC to control elongation[68]. Another mechanistic link has been observed between the SEC and CDK8-Mediator complexes (FIG. 2b). SEC subunits, including CDK9, CCNT1, and AFF4, have been shown to interact with CDK8-Mediator complexes[43], and knockdown of CDK8 negatively affects transcription elongation and the recruitment of SEC factors at serum response promoters[27]. Similarly, pol II elongation and SEC recruitment at hypoxia-inducible genes is highly sensitive to CDK8 protein levels[28]. These findings suggest that CDK8-Mediator stimulates pol II elongation. Because the association of the CDK8 module with Mediator is mutually exclusive with pol II, CDK8-Mediator regulation of pol II elongation likely occurs after pol II–Mediator interactions are disrupted. Under these circumstances, the SEC may function as an intermediary factor (FIG. 2b). Interestingly, MED26 and the CDK8 module are rarely associated in the same Mediator complex[43,98] and it will be interesting to determine if MED26 and CDK8-Mediator regulate the occupancy or activity of the SEC across the genome. Their varied association with Mediator suggests that MED26 and CDK8-Mediator regulate SEC function in mechanistically distinct ways.

Interestingly, the functional cooperativity between Mediator and POLR2M or the SEC have been proposed to involve a structural remodeling or switching mechanism[68,76]. Consistent with these proposals are mechanisms that involve structural shifts in Mediator that are induced by TF-Mediator binding. TF-Mediator binding triggers structural changes at the pol

II–Mediator interaction site that correlate with activation of pol II at the promoter[2]. In agreement, numerous laboratories have documented that the Mediator-dependent activation of pol II elongation coincides with the binding of TFs, such as HSF1, Elk-1, and p53, to promoters[41,99,100].

Much remains to be discovered regarding the mechanisms by which Mediator helps regulate pol II pausing and elongation. In addition to POLR2M and the SEC, other factors involved in regulating pol II pausing and elongation have been shown to have mechanistic links to Mediator, including cohesin[101,102], ELL3[103], TFIIS[104-106], and DSIF[107]. This elaborate and expanding network of factors, whose regulatory roles are still emerging, coupled with the dynamic and apparently combinatorial nature of the pol II initiation and elongation machinery, suggests that delineation of specific mechanistic details will require structural, cellular, and biochemical assays, including studies with reconstituted *in vitro* transcription systems.

Pol II re-initiation

After pol II transcription initiation and promoter escape, another pol II enzyme could bind the promoter and initiate another transcript (FIG. 2c). This process, called re-initiation, can generate multiple transcripts via the recruitment of additional pol II enzymes. Because a sustained transcriptional response likely requires efficient re-initiation, this likely represents a key regulatory stage at active genes[108]. *In vitro* experiments have demonstrated that transcription re-initiation occurs more rapidly than the initial round[109] and is facilitated by scaffold PIC assemblies that remain following pol II promoter escape[110]. These scaffold assemblies appear to be stabilized by DNA-binding TFs and retain most PIC factors, including Mediator, but lack pol II and TFIIF (FIG. 2c).

Although TF turnover via proteasome-mediated degradation represents one simple means to regulate transcription re-initiation (that is, additional TFs must then be physically present to replace degraded TFs)[111], the CDK8 module alone and as part of the CDK8-Mediator complex may also play an important role[18]. ChIP-Seq data suggest the CDK8 module co-localizes with Mediator across the genome[28,102]; however, **ChIP-reChIP** and biochemical assays indicate that the association of the CDK8 module with Mediator is transient and reversible[18,112]. In stark contrast to the core Mediator complex, CDK8-Mediator does not interact with pol II or the pol II CTD[42,43]; thus, CDK8 module–Mediator binding will prevent pol II from being incorporated into the PIC[18,113]. In addition, the occupancy of CDK8 and TFIIH appears to be mutually exclusive at the HIV-1 promoter, suggesting that CDK8-Mediator disrupts Mediator-TFIIH interactions as well[114]. Following transcription initiation and pol II promoter escape, binding of the CDK8 module to Mediator would prevent a second pol II enzyme from immediately re-engaging the promoter (FIG. 2b). Disruption of CDK8 module–Mediator interactions would then be required to enable the PIC to fully re-assemble for the re-initiation of transcription (FIG. 2c).

Because the CDK8 module can negatively regulate Mediator–pol II interactions, it is fundamentally important to understand how CDK8 module–Mediator binding is regulated. PARP-1 and the FBW7 ubiquitin ligase have been shown to regulate the CDK8 module–Mediator interaction in human cells[20,115]. Whereas the PARP-1 protein was shown to

promote CDK8 module release from Mediator, this did not depend upon its catalytic activity[115]. By contrast, ubiquitylation of MED13 by FBW7 (which required binding a metazoan-specific phospho-degron motif in MED13) promoted MED13 degradation by the proteasome[20]. Because MED13 directly links the CDK8 module to Mediator[18,19], inhibition of MED13 degradation resulted in an increased level of CDK8-Mediator in cells[20].

Mediator and chromatin architecture

In eukaryotes, genomic DNA is wrapped around histone proteins and this “packaged” genomic DNA is called chromatin. Chromatin provides a general barrier to gene expression; compared with unobstructed “naked” DNA, histone-bound DNA (that is, nucleosomal DNA), can block PIC assembly to suppress transcription. A basic role for Mediator may be as simple as maintaining nucleosome free regions at key regulatory loci, as demonstrated recently in yeast. Promoter occupancy of Mediator correlated with PIC assembly, which appeared to block nucleosome assembly[116]. This model is analogous to results in *D. melanogaster*, in which paused promoter-proximal pol II complexes helped maintain nucleosome-free regions to promote transcription activation[117]. Mediator also interacts with the SWI/SNF and CHD1 chromatin remodeling complexes[118-120] and appears to contribute to nucleosome displacement during transcription activation in both yeast and humans[92,106].

DNA looping

In all domains of life, the 3D organization of the genome plays basic roles in coordinating gene expression programs[121]. A common theme is the formation of loops that enable interaction between linearly separated DNA sequences. The architectural constraints and topological complexity appears to have increased substantially from bacteria to yeast and from yeast to mammals[122]. A general trend toward increasingly larger loops in more complex organisms (that is, loops with a greater number of DNA bases spanning the interacting sequences) has been observed, including interactions among sequence elements on different chromosomes in mammalian cells. Gene loops may represent a predominant regulatory mechanism in metazoans[1,123], and 3D chromatin organization may functionally replace **operons** to facilitate the coordinated regulation of sets of genes that are not adjacent along a chromosome[124].

Although Mediator is not required for the formation of gene loops *per se* — looping can be mediated by lacR in bacteria, which lack a recognizable Mediator complex — it plays a central role in formation and/or stabilization of looped DNA structures in eukaryotes (FIG. 3). In mammalian cells, long-range looping interactions between enhancer and promoter sequences appear to be important for driving high-level and cell type-specific gene expression[125-127]. Precisely how these loops are formed and stabilized remains uncertain, but Mediator, in conjunction with other factors, appears to play important roles[102,128-130]. Whereas yeast genes are not regulated via long-distance enhancer-promoter interactions[122], it is notable that Sin4 mutants (the yeast ortholog of Med16) were identified in *S. cerevisiae* that enabled upstream activating sequences to activate transcription at longer distances from the promoter than normally observed in yeast (normally only 100 – 200 base pairs from the promoter TATA sequence)[131]. Furthermore, Mediator has been implicated in formation of

looped DNA structures between the 5′ and 3′-ends of yeast genes[132]. These findings suggest an ancient origin for Mediator in long-range interactions with pol II.

Non-coding RNAs (ncRNAs) are key regulators of gene expression patterns and play important roles throughout human development. Mediator's large size and extensive surface area may allow it to interact with a diverse set of ncRNAs. In fact, some of the biological functions of ncRNAs overlap with known functions of the Mediator complex, such as their association with enhancer regions and their ability to regulate chromatin architecture. Recent studies suggest that these shared functions may result, in part, from functional cooperation between Mediator and ncRNAs. Indeed, a type of long intergenic ncRNA, called activator RNAs (aRNAs), increases the transcription of neighboring genes[133]. These aRNAs were shown to interact with Mediator, potentially via the MED12 subunit, and this interaction correlated with transcription activation and gene looping between the aRNA locus and target promoters (FIG. 3)[134]. Significantly, MED12 mutants that are linked to developmental disorders showed a reduced ability to form gene loops and associate with aRNAs[134]. Because MED12 is part of the CDK8 module, potential ncRNA–MED12 interactions may help tether the CDK8 module near the promoter. This could facilitate the reversible (on/off) CDK8 module–Mediator interaction at the promoter, thereby regulating pol II recruitment[18] and/or pol II elongation[27,28].

The transcription of enhancer sequences, which appears to be common in mammals, correlates with enhancer-promoter looping and gene activation[1,127,135]. In fact, enhancer transcription is considered to be an indicator of enhancer activity; that is, whether the enhancer will activate transcription at a distal promoter[136-138]. Although the study of enhancer RNAs (a.k.a. eRNAs) is in its infancy, several groups have shown that Mediator is required for eRNA-dependent enhancer-promoter looping and gene activation, suggesting a functional synergy analogous to aRNAs[139,140]. Whereas the biogenesis of aRNAs and eRNAs may be distinct[137], these findings broadly implicate Mediator-ncRNA interactions in the 3D organization of mammalian genomes. Many important questions remain, such as whether Mediator-ncRNA interactions are specific (that is, if select ncRNA sequences or structures target a specific Mediator subunit) or general (that is, if interactions are RNA sequence-independent), and whether or how ncRNA-Mediator binding might affect Mediator structure or function.

Mediator interactions with the pol II CTD may also help stabilize enhancer-promoter gene loops in mammals. Whereas the pol II CTD binds both yeast and mammalian Mediator complexes[42,62,64], the length of the CTD has increased from yeast (approximately 26 repeats, depending on species) to humans (52 repeats). This increase in CTD length correlates with an increase in the length of DNA looping interactions. Furthermore, it appears that a longer pol II CTD is required for cell viability in mammals compared with yeast[64,141]. Because the pol II CTD serves as a binding platform for RNA processing factors[79,80], this could reflect the greater extent and diversity of RNA processing events in mammalian cells (for example, increased reliance upon splicing, alternate splicing, alternate 3′-end processing, and so on). Not mutually exclusive is the possibility that an extended CTD helps establish long-distance enhancer-promoter gene loops that are prevalent in mammalian cells. In fact, it has been demonstrated that pol II CTD truncations

disproportionately affect enhancer-driven transcription in mammalian cells, whereas transcription directed from promoter-proximal elements was not significantly affected[142].

Heterochromatin

In addition to promoting the formation of looped chromatin architectures, studies in model organisms have provided fascinating insight into alternative ways in which Mediator regulates chromatin structure. In the yeast *S. pombe*, Mediator has been linked to **pericentromeric heterochromatin** formation[143]. These studies suggest that Mediator functions by coordinating pol II-dependent ncRNA transcription with the RNAi machinery to help form and maintain heterochromatin domains[143-145]. Similarly, Mediator has been implicated in **telomeric heterochromatin** maintenance in the budding yeast *S. cerevisiae*. Using genetic screens and cell biology techniques, several groups showed that *S. cerevisiae* Mediator associates with telomeric heterochromatin and may play basic roles in regulating heterochromatin structure at telomeres, as well as maintaining telomere length[146-149]. Whereas data linking Mediator to similar functions in mammalian cells has been lacking, it is notable that the *D. melanogaster* Med26 protein—but not other Mediator subunits—localizes to pericentric heterochromatin and appears to interact with the heterochromatin-associated Hp1 protein[150]. Furthermore, Med26 co-localized with pericentric heterochromatin on *D. melanogaster* polytene chromosomes, but other Mediator subunits did not[150]; this may reflect functional antagonism between the Hp1 protein and Mediator, as seen in human cells[151].

Mediator and Signaling Cascades

Different TFs are activated in response to distinct signaling pathways. For example, the STAT family of TFs is activated during infection and p53 is activated after DNA damage. Because different TFs activate transcription, in part, by interacting with different Mediator subunits, specific subunits must be able to convert the biological input (for example, developmental cues) to specific transcriptional outcomes (FIG. 4). In agreement with this, Mediator has been described as the endpoint of cell signaling pathways because it represents an ultimate, functional target for transcription factors[152]. Below, we highlight a few pathways that help illustrate this concept.

TGFβ–SMAD signaling

Signaling through the TGFβ receptors ultimately results in the formation of different SMAD TF complexes (for example, SMAD1/5 with SMAD4 or SMAD2/3 with SMAD4); these form in response to different ligands and control processes such as pluripotency or germ layer specificity during development. For instance, TGFβ signaling promotes the formation of the SMAD2/3 and SMAD4 complex, whereas BMP signaling promotes formation of the SMAD1/5 and SMAD4 complex[153]. The transcriptional outcomes of TGFβ signaling in zebrafish are shut down in the absence of MED15, whereas BMP signaling is unaffected[154]. Subsequent experiments confirmed that SMAD2/3 must interact with MED15 to activate its target genes, whereas SMAD1/5 targets a different, unknown Mediator subunit[155]. The MED12 subunit, perhaps as part of the CDK8 module, modulates TGFβ signaling by directly binding to the TGFβ Receptor 2 and inhibiting its activity[156]. Notably, MED12

regulates CDK8 kinase activity[26,157], and CDK8-dependent phosphorylation of SMAD1 or SMAD2/3 fully activates these TFs while also marking them for proteasomal degradation[29,158]. These observations suggest an intricate regulatory feedback involving Mediator and TGFβ signaling.

Wnt–β-Catenin signaling

The Wnt–β-catenin pathway is crucial for the development of a vast array of tissues, and misregulation of the pathway has been implicated in a variety of cancers[159]. From genetic studies, CDK8 module subunits MED12 and MED13 have been shown to be essential for activation of Wnt signaling in metazoans[160-163], and binding assays suggest a direct interaction between β-catenin and MED12[162]. In addition, the CDK8 module increases Wnt–β-catenin signaling by phosphorylating E2F1. The phosphorylation of E2F1 antagonizes its ability to repress the Wnt–β-catenin pathway via β-catenin degradation[33,164]. As one consequence, high levels of CDK8 expression can drive colon cancer progression[165]. The CDK8 module therefore facilitates Wnt–β-catenin signaling by two mechanisms: mediating the transcription of β-catenin target genes and repressing an opposing pathway through its kinase activity.

Ras–ERK–MAPK signaling

The Ras–ERK–MAPK pathway helps regulate cell growth, survival, and differentiation via a set of TFs (termed the ternary complex factor subfamily) that function in part through their interaction with Mediator[166]. Specifically, MED23 is required for Ras–Erk signaling through the TCF factor Elk-1 in mammals[11]. Certain cell types, however, are able to signal through the MAPK pathway without MED23. For instance, while MAPK signaling is almost completely dependent on MED23 in murine embryonic stem cells, knocking down MED23 in MEFs has only a moderate effect. This is explained by the higher expression of an alternate ternary complex factor, NET, in MEFs; because NET does not stimulate transcription through MED23, MED23 knockout MEFs are still able to respond to MAPK signaling[99]. The Elk-1 and MED23 interaction is necessary for angiogenesis in both MEFs and multipotent mesenchymal stem cells[167,168]. However, in mesenchymal stem cells, reducing the levels of MED23 leads to adipogenesis rather than angiogenesis[168]. This difference between Ras–Erk signaling in MEFs and mesenchymal stem cells is also attributed to a different ternary complex factor interacting with a Mediator subunit other than MED23[168]. Activation of the Ras–ERK–MAPK pathway additionally leads to MED1 phosphorylation, which increases its association with core Mediator and enhances transcription in response to specific TFs[169,170].

Concluding remarks

Because of its myriad roles in gene expression, Mediator represents fertile ground upon which to cultivate a deeper understanding of transcription regulatory mechanisms. Future work will no doubt expand upon the themes discussed here and are likely to reveal completely new structural or functional roles. Further studies of Mediator-dependent regulation of chromatin architecture and enhancer-promoter gene loops should yield fundamental insights about Mediator subunit functions and define additional roles in

transcription regulation. New insights may include roles for Mediator in establishing nuclear domains, such as transcription factories[171], whose functional relevance is congruent with increasingly sophisticated chromosome conformation capture experiments that suggest that sets of genes can be coordinately regulated via their nuclear co-localization. Interestingly, proteins that bind RNA or contain intrinsically disordered regions are capable of forming "phase domains" in vitro[172,173], which provides a means of spatial segregation in the absence of lipid membranes[174]. Formation of such structures requires RNA, and clusters of highly active genes would provide high local concentration of RNA that could help promote formation and stabilization of these domains. Mediator binds RNA[134] and its large size and abundance of intrinsically disordered domains[38] suggests that Mediator could help demarcate transcription factories by facilitating formation of structural barriers in the nucleus. Although this concept remains to be tested, it is consistent with Mediator's known roles in 3D chromatin organization at sites of active transcription[102,128].

Recent and future insights regarding Mediator structure and function should provide a framework for development of methods to manipulate Mediator function for therapeutic purposes. Improved structural data, which will increasingly rely upon advances in EM data collection and image processing, will enhance our mechanistic understanding of the complex and may identify strategies to target Mediator with small molecules. Although development of small molecules that bind specific motifs or domains within Mediator would represent only a first step toward molecular therapeutics, such reagents would likely serve as versatile mechanistic probes to interrogate Mediator function *in vitro* and *in vivo*. Finally, advances in genome editing tools, combined with increasingly reliable genomics and informatics approaches, should continue to transform our ability to study Mediator function in cellular and *in vivo* contexts.

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Box 1

The evolution of Mediator

Consistent with its role as a general transcription factor[175], Mediator's emergence in eukaryotes coincided with the emergence of other general transcription factors such as TFIIH and the TAF subunits of TFIID. Throughout eukaryotic evolution, however, the subunit composition, sequences[176], and function of Mediator has diversified[2]. The poor sequence conservation between yeast and human Mediator (see Table) may in part reflect the high proportion of predicted intrinsically disordered regions (IDRs) within its subunit sequences[38], which generally tend to diverge more rapidly over time[177]. As a general target of DNA-binding TFs, the divergence of Mediator sequences throughout evolution may have been partially driven by the increasing diversity of DNA-binding TFs. An intriguing hypothesis is that Mediator may have evolved as a type of cellular defense mechanism against “selfish” DNA elements from viruses and transposons[178]. The transcription of these DNA elements is deleterious to the host, and Mediator may help ensure that transcription activation utilizes host-specific factors, such as endogenous TFs and co-activators. This could be one basis for Mediator's ancient origins in eukaryotes[176].

Whereas many basic functions of Mediator are conserved from yeast to humans (for example, the ability of Mediator to bind the pol II CTD and TFs, and its requirement for pol II transcription genome-wide), Mediator also regulates and interacts with various chromatin- or pol II-associated factors that are not present in yeast genomes, such as Gdown1/POLR2M[72,75], NELF[179], and aRNAs[134]. Mediator also appears to be essential for long range enhancer-promoter interactions in mammalian cells[102,128], whereas similar enhancer-dependent regulatory mechanisms are not observed in yeast[122].

Box 2

Mediator and human disease

The number of diseases linked to Mediator continues to increase, with a growing number of mutations linked to developmental diseases and cancer[4,5,180]. Whereas this information is medically important, it can also help delineate cellular functions for specific subunits. For example, MED12 mutations found in **uterine leiomyomas** were shown to affect its interaction with the CDK8 module subunit CCNC, which negatively affected CDK8 kinase function[26].

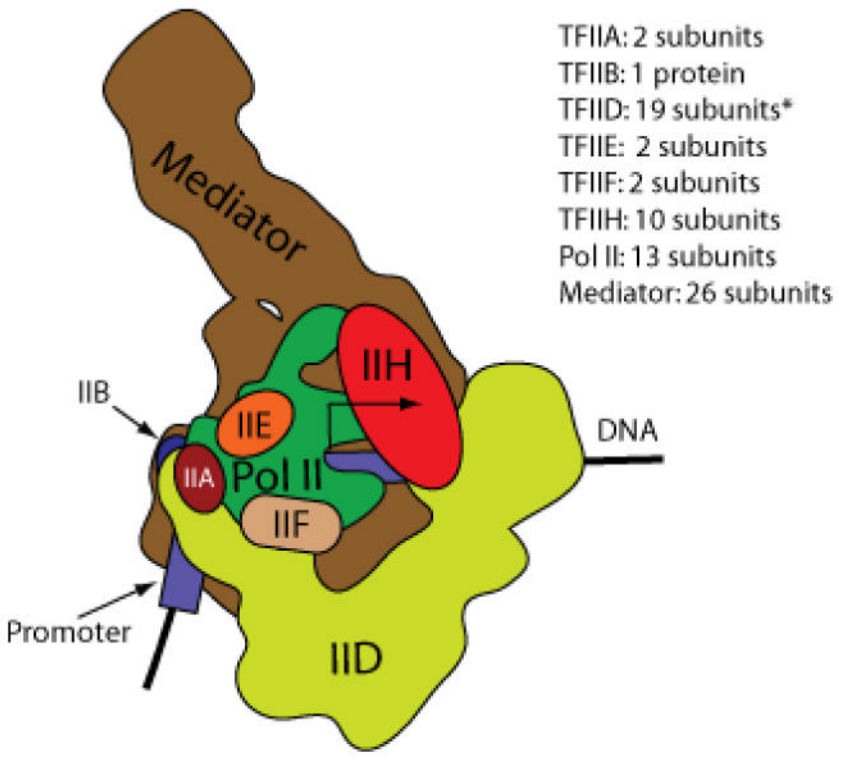
The biological and structural characteristics of Mediator suggest that it could represent an effective therapeutic target[181]. Because different TFs bind different Mediator subunits, blocking one TF-Mediator interaction site (for example, the site at which VP16 interacts with MED25) may not broadly inhibit cellular transcription. This is supported by the fact that knockout of single Mediator subunits can shut down transcriptional responses to specific signaling pathways, while leaving other pathways intact[11,12,154]. Moreover, in the few cases in which structural data are available, the TF binding site within a Mediator subunit is structured, with hydrophobic clefts or patches that could be selectively targeted with small molecules[182-184].

Genome-wide ChIP-Seq analyses in mammalian cells have uncovered enhancer elements that are unusually large and possess high-level occupancy of DNA-binding TFs and other transcriptional regulators such as cohesin and Mediator. These domains have been called super-enhancers[185,186], which appear similar to High Occupancy Target (HOT) domains[187,188] or Locus Control Regions (LCRs)[189] that have been identified in other studies. Super-enhancers are essentially clusters of enhancers and typically regulate the expression of genes that control cell identity; super-enhancers can also drive high-level expression of oncogenes in cancer cells. Mediator appears to play a key role in forming or maintaining super-enhancer structure and function[185]; in fact, perturbation of Mediator structure by knocking down key subunits, such as Med12, disproportionately affects genes regulated by super-enhancers[102,186], suggesting that targeting Mediator may selectively disrupt oncogene expression in cancer.

Box 3

The Pre-initiation Complex (PIC)

The eukaryotic RNA polymerase II (pol II) enzyme transcribes most non-coding RNA genes and all protein-coding genes. Unlike bacterial polymerases, however, pol II relies upon an array of auxiliary factors that ensure transcription initiation at the correct site in the genome, as well as subsequent RNA processing events leading to the production of a mature transcript[190]. At the transcription start site, pol II initiation is regulated by a protein assembly known as the Pre-Initiation Complex (PIC, see the Figure)[191]. Whereas the PIC can function in various forms (for example, simpler TFIID assemblies may operate in differentiated cells) it represents a fundamental intermediate that is targeted by an array of general and gene-specific regulatory factors. Each of the factors that comprise the PIC (TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, pol II, and Mediator) have varied functional and structural roles[192], but much remains unknown about how the PIC functions as a unit. Biophysical studies have begun to determine the overall structure and architecture of the PIC. Although current results suggest different architectures for human and yeast PICs[193,194], this remains an area of active investigation[195]. Asterisk: TFIID contains TBP and 13 different TAFs, five of which dimerize (TAF4, TAF5, TAF6, TAF9, TAF12)[196].



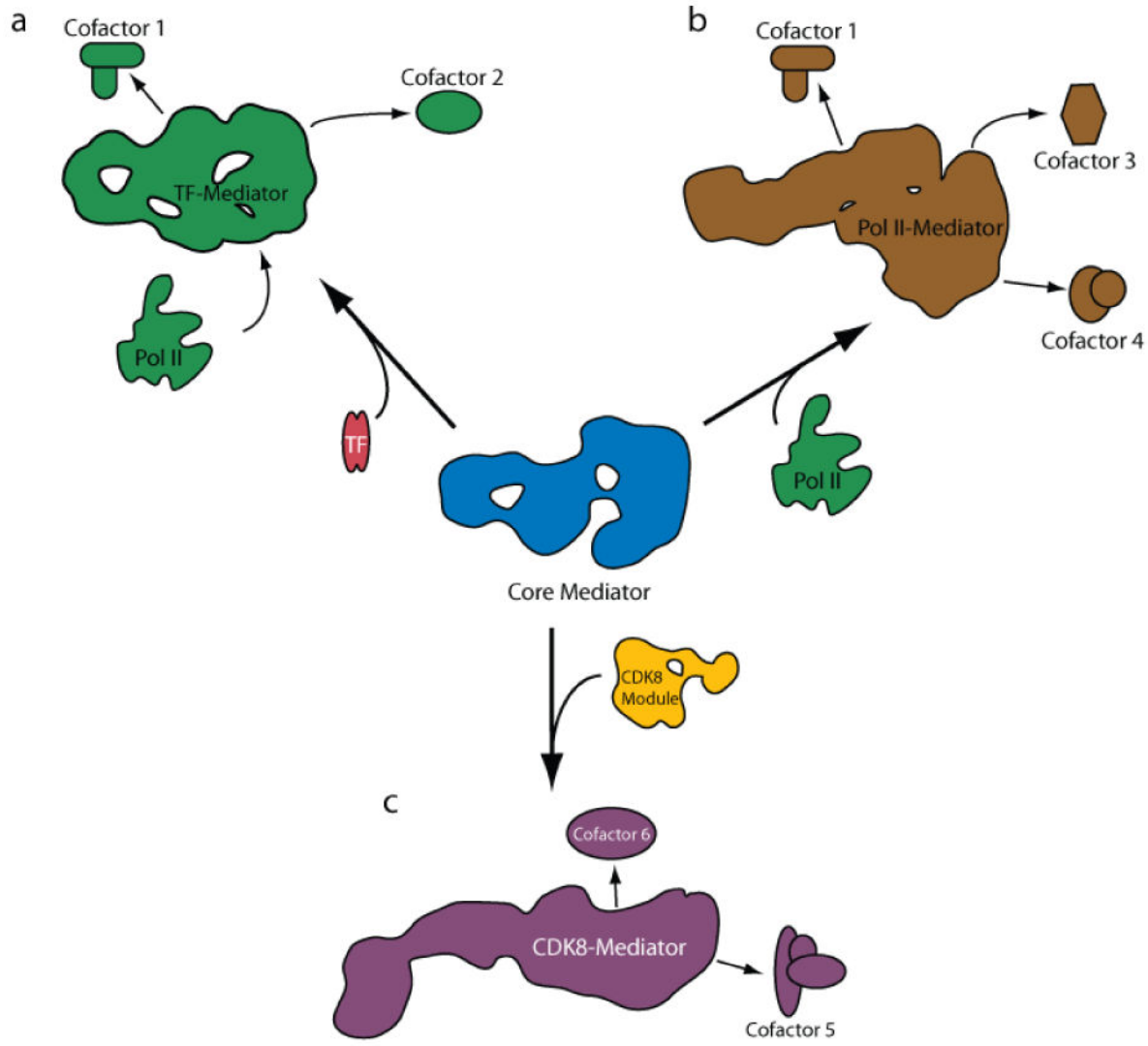


Figure 1. Structural changes in Mediator control its interactions with other transcription regulators

This schematic illustrates that as the structure of Mediator changes, its ability to interact with other regulatory proteins may also change. Such structural transitions could enable the same Mediator complex to adopt distinct functions at the appropriate stages of transcription (for example, initiation vs. elongation). Also, the model allows for some cofactors to bind Mediator in several different structural states. **a)** Upon binding a transcription factor (TF), Mediator undergoes structural changes that facilitate interactions with additional transcription regulators[43]. Structural changes associated with TF-Mediator binding also appear to promote stable association with pol II[197], and correlate with activation of transcription[41]. **b)** Binding of pol II triggers distinct structural changes throughout Mediator[58,59]; these might be important for functional interactions between Mediator and factors that promote pol II initiation and/or elongation, such as TFIIS, TFIIF, or POLR2M[72,76,104,105]. **c)** CDK8 module–Mediator binding also induces structural changes in Mediator that prevent pol II binding[18,198]; further, CDK8-Mediator associates with

elongation factors such as the SEC[27,43], which may regulate the timing of SEC recruitment at some genes.

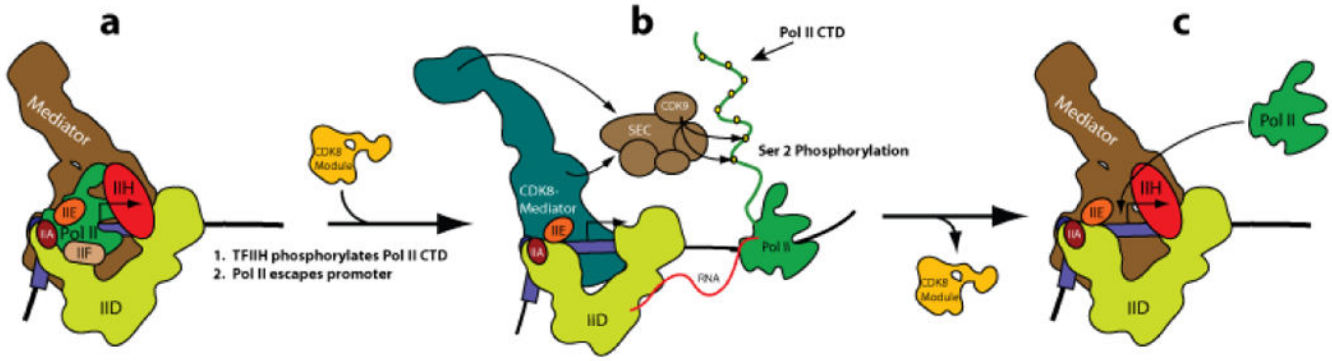


Figure 2. A simplified model for Mediator-dependent regulation of multiple stages of pol II transcription

a) Mediator plays key roles in the assembly of the Pre-initiation Complex (PIC). Mediator helps recruit pol II and other PIC factors to the promoter[191]. At this stage, the pol II CTD is not highly phosphorylated. During transcription initiation, the pol II CTD becomes highly phosphorylated by TFIIH[80], and the pol II enzyme begins to transcribe the gene. At some genes, pol II may pause after transcribing about 60 nucleotides[95]; whether this "paused pol II" remains associated with Mediator is not known. **b)** The CDK8 module may associate with Mediator after pol II-Mediator contacts are broken, and this may regulate pol II elongation or release from a paused state at some genes. CDK8-Mediator may regulate the recruitment or activity of the super elongation complex (SEC) to promote pol II pause release and elongation[27,28]. **c)** Because CDK8-Mediator cannot interact with pol II, re-initiation of transcription requires release of the CDK8 module. A scaffold PIC complex, which may be stabilized by a transcription factor, could facilitate rapid re-initiation[110].

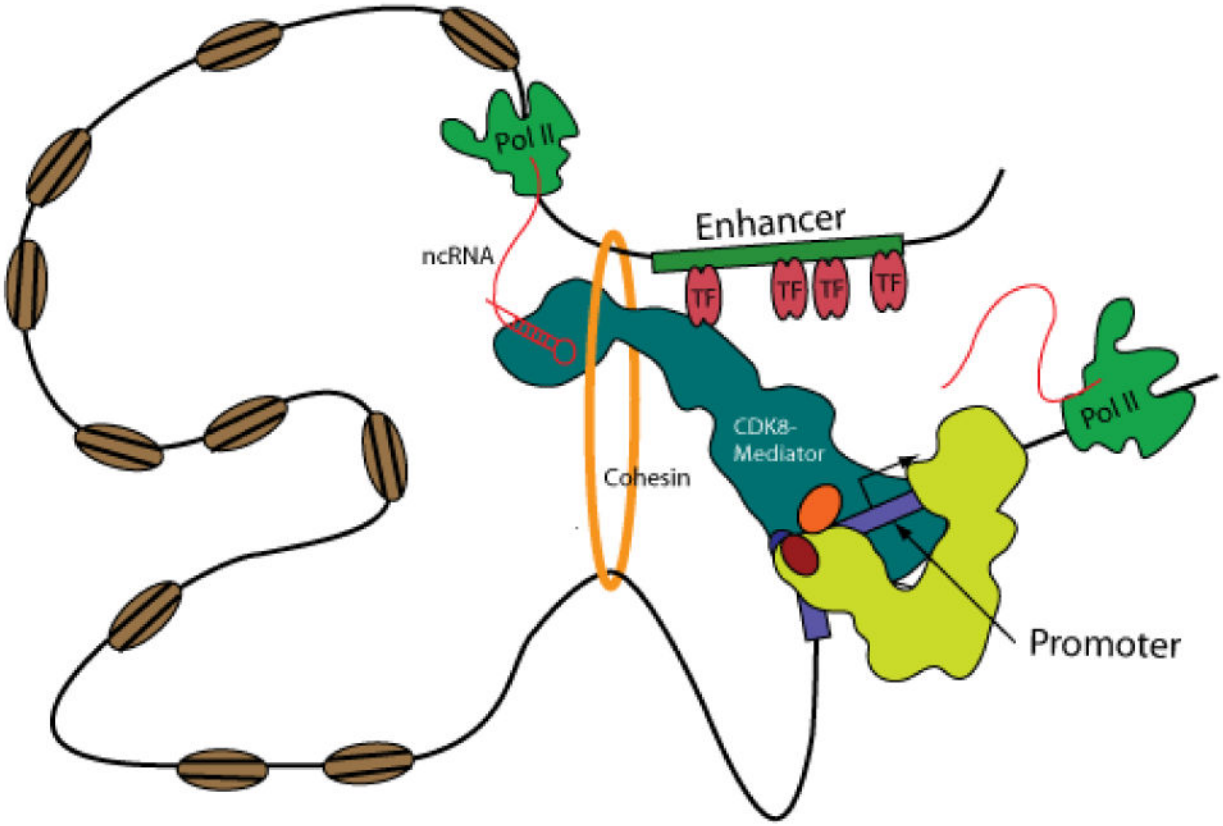


Figure 3. Mediator regulates chromatin architecture

The cooperative interactions between Mediator and transcription factors (TF), cohesin and non-coding RNAs (ncRNA) appear to stabilize enhancer-promoter loops[102,134,139,140]. An expanding set of factors influence the formation and stability of enhancer-promoter loops[1] and the formation of such structures correlates with high levels of transcription[125-127]. Because the CDK8 module must dissociate from Mediator to allow pol II–Mediator binding[18,19], a potential ncRNA–CDK8 module interaction, perhaps via the CDK8 module subunit MED12[134], may tether the CDK8 module near the PIC. This could facilitate re-formation of CDK8-Mediator complexes following pol II promoter escape.

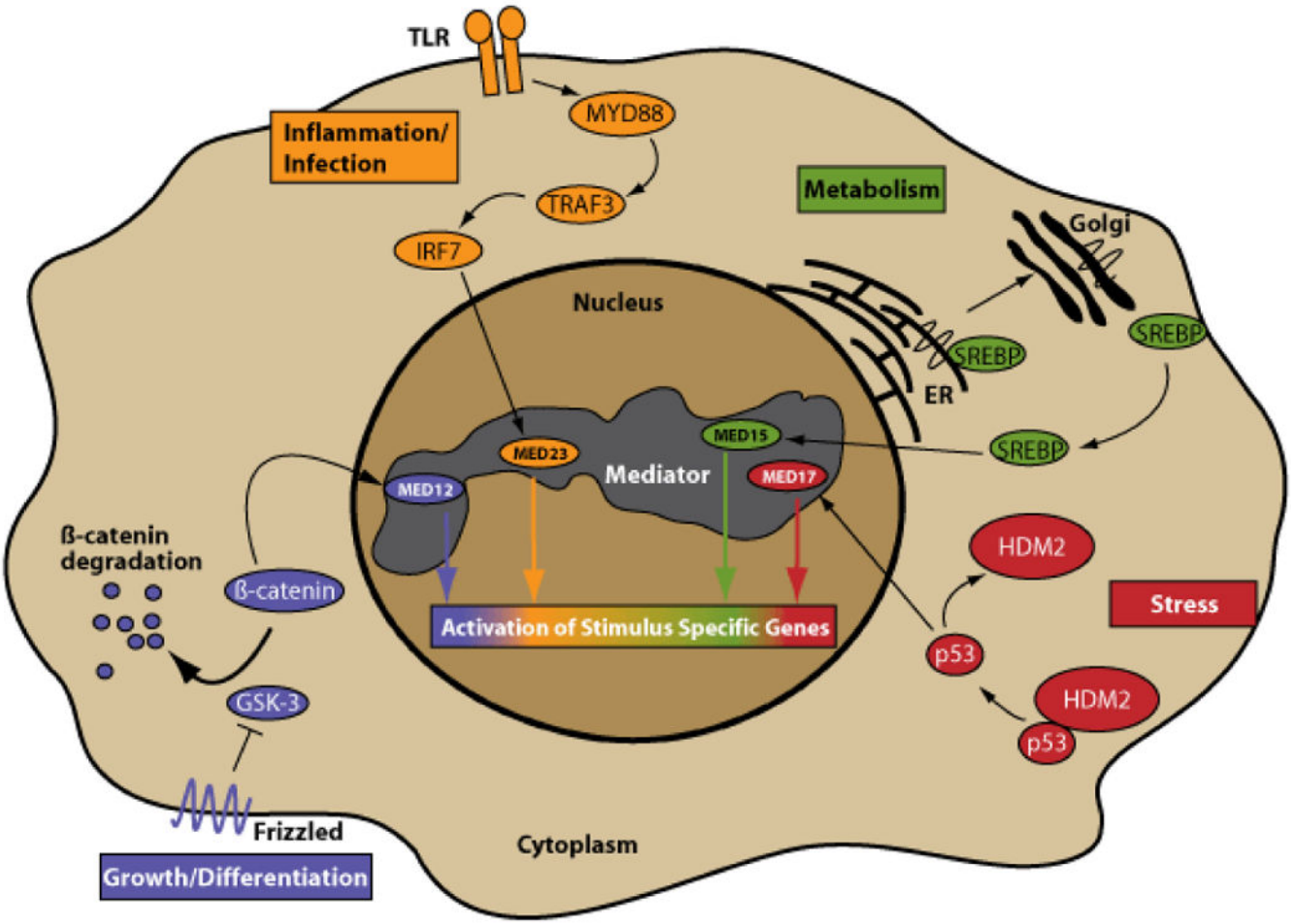


Figure 4. Mediator as an endpoint of signaling cascades

Four representative, simplified signaling pathways are shown. Each pathway is activated by different signals; for instance, p53 signaling is activated during cell stress, toll like receptors (TLR) are activated in response to infection, and the sterol regulatory element binding protein (SREBP) is activated in response to metabolic cues. Each pathway converges on a distinct transcription factor that targets a different Mediator subunit, helping to recruit and regulate Mediator activity at select genomic loci. The end result is transcription factor-dependent activation (or repression) of a specific set of genes that are important for the response to the stimulus. Due to Mediator's role in relaying and integrating these signals directly to the transcription machinery (including the pol II enzyme itself), Mediator can be considered an endpoint of signaling cascades[152].