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Review

Interactions between subunits of the Mediator complex with gene-specific transcription factors

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ABSTRACT

The Mediator complex forms the bridge between gene-specific transcription factors and the RNA polymerase II (RNAP II) machinery. Mediator is a large polypetide complex consisting of about thirty polypeptides that are mostly conserved from yeast to human. Mediator coordinates RNAP II recruitment, phosphorylation of the C-terminal domain of RNAP II, enhancer-loop formation and post-initiation events. The focus of the review is to summarize the current knowledge of transcription factor/Mediator interactions in higher eukaryotes and illuminate the physiological and gene-selective roles of Mediator.

Contents

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1. Introduction

In eukaryotes, all protein-coding genes are transcribed by RNA polymerase II (RNAP II). The initiation of transcription and its regulation requires the five general initiation factors (GTFs) TFIIA, -B, -D, -E, -F, and -H, DNA-binding transcription factors as well as transcription coactivators. The Mediator complex is the central coactivator complex that forms the bridge between transcription factors bound at the upstream regulatory elements and the general transcription machinery at the core promoter region [1–4].

1.1. Discovery of the Mediator complex

The Mediator complex was originally discovered in *Saccharomyces cerevisiae* by Kornberg and coworkers as a requirement for transcriptional activation in a reconstituted system [5,6]. The initial evidence came from the observation of "activator interference", that one gene activator may interfere with the activity of another [7]. This interference could not be relieved by addition of purified RNA polymerase II and general initiation factors, but rather by a partially purified fraction from yeast proposed to contain a common intermediary factor which interacts with different activators, and was therefore termed "Mediator" [6]. In parallel, the Young group genetically identified the products of Srb genes as supressors of truncations of the C-terminal domain of RNAP II [8]. When Mediator was purified from yeast to near homogeneity as a complex of 20 polypeptides, or as holoenzyme together with RNAP II, it turned out that many Mediator subunits are indeed Srb proteins [9].

1.2. Isolation of the mammalian Mediator complex

The first mammalian Mediator complex was isolated by affinity purification of an epitope-tagged thyroid hormone receptor (TR) from HeLa cells grown in the presence of a cognate ligand and thus designated as thyroid hormone receptor-associated proteins (TRAPs) [10]. This was followed by the isolation of other Mediator-like complexes termed ARC (activator recruited factor) [11], DRIP (vitamin D receptor interacting protein) [12], CRSP (cofactor required for Sp1 activation) [13], PC2 (positive cofactor) [14], NAT (negative regulator of activated transcription) [15] and human Mediator complex [16] as well as mouse Mediator [17] and rat Mediator [18].

Despite the initial controversy about the composition of these independently isolated complexes, a subsequent proteomic analysis using multidimensional protein identification technology (MudPIT) suggested that nearly all of the previously described mammalian Mediator subunits were identified by this method and are thus bona fide Mediator subunits [19]. In addition, bioinformatic analyses of Mediator subunits across many species have identified short regions of similarity between subunits of mammalian Mediator complex and all but three yeast Mediator subunits (MED2, MED3 and MED5) [20]. The existence of conserved Mediator complexes from diverse eukaryotic species has led to a unified nomenclature for eukaryotic Mediator subunits [21,22].

1.3. Structure of the Mediator complex

Given that both the yeast and the mammalian Mediator complex are multi-component polypeptide complexes in the megadalton range, electron microscopy (EM) is the only method that allows structural studies of the entire complex.

Initial EM studies in yeast outlined the overall architecture of Mediator in complex with RNAP II [23–25]. Mediator is composed of four separate domains called head, middle tail and kinase domains [23,26,27]. Upon incubation with RNAP II, Mediator undergoes a conformational change from a compact- to a fully extended conformation, in which the three modules are easily visualized. The structures of both murine and human Mediator complexes were also obtained and displayed an overall similarity to the yeast complex [24]. Moreover, in human, structures of both a large (TRAP or ARC-L, including the dissociable Cdk8 module involved in transcription repression) and a small (without Cdk8 module) form of Mediator were revealed [28]. Interestingly, the association of Mediator with different activators like VP16 or SREBP showed that Mediator is not rigid but rather a high degree of conformational flexibility. The Taatjes lab recently extended their studies to look at human Mediator–RNAP II in presence or absence of general transcription factor TFIIF [29]. The Mediator–RNAP II–TFIIF ternary complex is more stable than the binary Mediator–RNAP II complex. The authors concluded that TFIIF is required to stably orient RNAP II within the RNAP II/Mediator/TFIIF ternary assembly.

In addition to EM, an impressive list of submodules of yeast Mediator have been crystallized by the Cramer group allowing the first glimpses at the atomic level: the MED8/MED18/MED20 submodule of the head-domain of Mediator including sites of known yeast mutants (srb-mutants), suggest that the Mediator head contains a multipartite TBP-binding site that can be modulated by transcriptional activators [30]. Futhermore loss-of-function studies followed by microarray analysis lead to similar changes in the yeast transcriptome [31].

When studying two other Mediator submodules, MED11/22 heterodimer [32] and MED7/21 subcomplex [33] both these complexes form a four-helix bundle domain. The authors predicted that the four-helix bundles is a building block within Mediator, which might provide important insights into the evolution of Mediator.

Futhermore, two independent studies using NMR gain the first insights into Mediator/activator structure. The structure of Mediator subunit MED25 bound to potent herpes simplex 1 viral transcriptional activator VP16 was revealed by Vojnic et al. [34]

and Milbradt et al. [35]. Unlike other known activator targets, MED25/ACID forms a seven-stranded β-barrel framed by three helices. These two studies provide the framework for subsequent functional approaches.

1.4. Mediator is conserved throughout evolution

Bacteria lack Mediator and comparable transcription initiation cofactors. In eubacteria a single sigma factor fulfills the tasks of eukaryotic general transcription factors. The Mediator complex was biochemically isolated from *S. cerevisiae* [9], yeast *S. pombe* [36], plants [37], *C. elegans* [38] and *D. melanogaster* [39].

Comparative genomics supports the deep evolutionary origin of Mediator [40,41]. The *S. cerevisiae* Mediator remains the best-studied complex (as reviewed in [42]) and the common nomenclature is based on the original yeast MED proteins [21]. The phylogenetic biochemical and structural studies point to a "universal" eukaryotic Mediator, that might have significantly contributed to the diversification of transcriptional programs as discussed in depth in [40].

1.5. The Mediator complex in transcriptional regulation

The mediator complex is generally considered as a coactivator in transcriptional regulation, which functions at multiple steps during transcription, such as RNAP II recruitment, preinitiation complex (PIC) formation, post-recruitment processes and DNA loop formation, reviewed in [3] and see Fig. 1. Initial ChIP studies at model promoters in yeast showed that Mediator is found at enhancers rather than at core promoters [43,44]. Subsequent genome-wide studies of yeast Mediator subunits using ChIP-on-chip technology have revealed that Mediator bound to upstream intergenic regions of both active and inactive genes, and also the coding regions of many genes [45,46], suggesting a role of Mediator complex as a general transcription factor.

Several lines of evidence support that Mediator functions by binding to RNAP II and thus facilitating its recruitment to target gene promoters. RNAP II holoenzyme containing Mediator subunits has been purified from yeast [9,47]. Similarly, human RNAP II–Mediator complexes have also been isolated [19,48]. Moreover, it has been reported that Mediator enhanced basal or activator-dependent transcription by facilitating Mediator-dependent recruitment of RNAP II and the GTFs to the promoter, and therefore the formation of PIC [49,50].

In addition to the role in RNAP II recruitment and PIC complex formation, it has also been suggested that Mediator is required for post-recruitment processes. Using immobilized template containing hepatocyte nuclear factor 4 (HNF4) cognate sites, it was demonstrated that the interaction between HNF4 and Mediator was essential for transcriptional activation by HNF4 but not for RNAP II recruitment to HNF4 binding sites, suggesting that HNF4 might coordinate with Mediator to promote post-recruitment activation of transcription [51]. By studying transcription factor Elk1-dependent activation of early growth factor 1 (Egr1) in wild type and MED23$^{-/-}$ ES cells, the Berk group demonstrated that MED23 increased RNAP II recruitment to Egr1 promoter by 3-fold, in contrast, Egr1 transcription level was 13-fold higher, indicating that Mediator may perform alternative roles in addition to RNAP II recruitment [52].

Activation of gene expression is accompanied by DNA loop formation which communicate the enhancer-bound transcription factors and the general transcription machinery at the core promoter region. The evidences in supporting this concept come from chromosome conformation capture (3C) experiments, which have confirmed that some enhancers are brought into promoter region upon gene activation [53–55]. ChIP-seq analysis of Mediator occupancy in murine embryonic stem cells (ES cells) revealed that Mediator was found at both the enhancers and core promoters of actively transcribed genes. These sites were also co-occupied by cohesin and cohesin loading factor Nipb1, which coordinated with Mediator for proper gene transcription in ES cells. Interaction between the enhancer and the core promoter was observed by 3C suggesting that Mediator–cohesin–Nipb1 complex promoted cell type-specific gene activation through enhancer-promoter DNA looping [56].

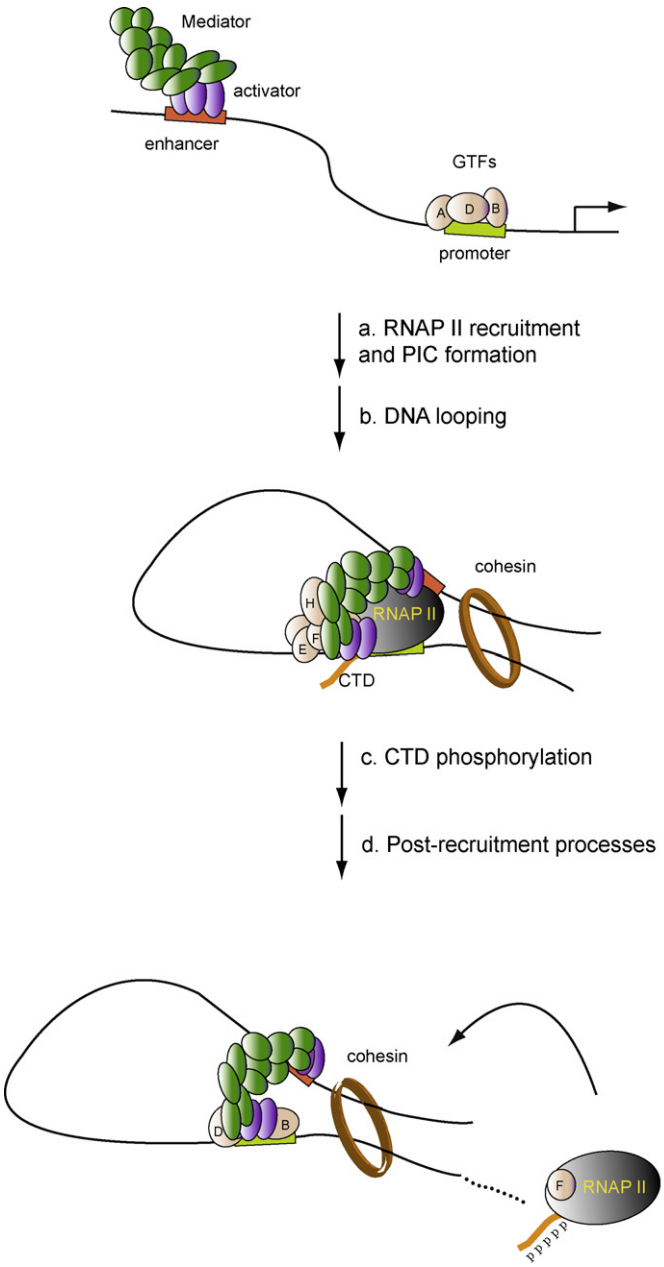

Fig. 1. Functional roles for Mediator in transcriptional activation: The Mediator complex transduces regulatory signals from enhancer bound activators to the general transcription machinery at core promoters. Mediator can facilitate RNAP II recruitment and enhance pre-initiation complex formation by (a) assembling an enhancer/core promoter loop complex (b) containing activators, general transcription factors (GTFs), RNAP II and cohesins. Subsequently, Mediator enhances the phosphorylation of the C-terminal domain (CTD) of RNAP II (c) mediated by general transcription factor TFIIH. Several post-recruitment events (d) such as the release of stalled RNAP II, enhancement of reinitiation and coordination of RNA capping, splicing and polyadenylation are also potentially regulated by the Mediator complex.

Table 1
Interaction between Mediator subunits and transcriptional activators.

Mediator subunits	Transcriptional activators
MED1	TRα/β, PPARα/γ, RARα, RXRα [57,58]; PPARγ [59]; VDR [12,57]; ERα/β [60–63]; RORα [64]; GR [65,66]; FXR [67]; AHR [68]; HNF4 [51]; GATA-1 [69,70]; PGC-1α [71]; BRCA1 [72]; GABPα [73]; Pit-1 and GATA2 [74]; C/EBPβ [75]; p53 [76–78]
MED12	SOX9 [79]; RTA [80]; Gli3 [81]; β-catenin [82]; G9a [83]; AICD [84]
MED14	GR [65]; HNF4 [51]; STAT2 [85]; SREBP-1α [86]; PPARγ [87]
MED15	Smad2/3/4 [88]; SREBP-1α [89]
MED16	DIF [90]
MED17	VP16 [91,92]; p53 [91]; HSF [90,92,93]; DIF [90,92]; RXR [92]; STAT2 [85]; p65 [94]
MED19	REST [95]
MED21	TRα/β [96]
MED23	E1A-CR3 [16]; Elk1 [49,97]; DIF and HSF [90]; ESX [98]; C/EBPβ [99]
MED25	VP16 [100,101]; DIF and HSF [90]; RARα [102]; HNF4 [103]; SOX9 [104]
MED26	REST [95]
MED28	Merlin and Grb2 [105]
MED29	DSX^F [106]
CDK8	c-Myc [107]

Mediator complex is also shown to be involved in transcriptional repression through the Cdk8 submodule that consists of MED12, MED13, cyclin C and Cdk8 which will be discussed in the section 4. All in all, these findings imply that Mediator complex is a versatile regulator of transcription, which is involved in transcriptional activation and repression, regulating multiple steps of both transcriptional initiation and elongation (Fig. 1).

2. Mediator interaction with transcription factors

Given its large size, Mediator provides multiple interfaces for protein–protein interactions. The studies over the last decade reveal that different transcription factors target distinct Mediator subunits, leading to gene-specific physiological effects. Here we review exemplary interactions between transcription factors and several well-defined Mediator subunits and the potential physiological consequences. Further transcription factor/Mediator interactions are comprehensively summarized in Table 1.

2.1. MED1 interaction with transcription factors

Mediator subunit MED1 is so far the best-studied Mediator subunit. Although MED1 is not required for cell viability, several studies show that MED1 is essential for transcriptional activation for several hormone receptors, GATA factors and C/EBPβ.

2.1.1. MED1 in the Mediator complex

Genetic ablation of MED1 in mice leads to embryonic lethality at day 11.5 dpc mainly due to placental insufficiency and impaired cardiovascular development [108–110]. Although MED1 is essential for animal development, it is dispensable for the integrity of the Mediator complex. A stable, endogenous Mediator complex naturally devoid of MED1 has been isolated [111] and MED1-deficient MEFs possessed a Mediator complex that was relatively intact [112]. This is further supported by the fact that Med1 existed only in a subpopulation of the Mediator complexes (less than 20% of the total) that was tightly associated with a near stoichiometeric level of RNAP II [113]. The phosphorylation of Med1 driven by MAPK signaling promoted its association with Mediator through Med7 subunit [114].

2.1.2. MED1 interaction with nuclear receptors

MED1 was first isolated in a yeast two-hybrid screen of mouse liver cDNA library using PPARγ ligand binding domain as bait and was therefore named as PBP (PPAR binding protein) [58]. Later on, cDNA encoding a human homolog TRAP220 was cloned on the basis of amino acid sequences derived from cognate polypeptides in the immunopurified TR–TRAP complex [57]. The physical and functional association of MED1 with various nuclear hormone receptors such as the vitamin D receptor, hepatocyte nuclear factor 4α, glucocorticoid receptor, estrogen receptor and aryl hydrocarbon receptor was further confirmed by numerous independent studies, as summarized in Table 1. The nuclear receptor recognition motifs (LxxLL motifs) of MED1 are essential for both the physical interaction and the *in vitro* transactivation. To investigate the *in vivo* role of the LxxLL motifs, the Roeder group generated Med1 LxxLL motif-mutant knockin mice recently and reported that these mice were grossly normal but exhibited profound defects in pubertal mammary gland development. In line with this phenotype, ER target genes were downregulated in Med1-mutant mammary epithelial cells and could no longer respond to estrogen stimulation [115].

The reader is also referred to the review Dr. W. Chen and Dr. R. Roeder in this issue covering in depth Mediator-dependent nuclear receptor functions with a particular focus on the Mediator subunit Med1.

2.1.3. MED1 interaction with GATA family of transcription factors

In addition to the well-defined physical interaction of MED1 with various nuclear receptors, it has been shown that MED1 also interacts with GATA family of transcription factors [70]. It has been shown by our group that MED1 physically interacts with the erythroid master regulator GATA-1 and functions as a coactivator for GATA-1 in erythropoiesis. MED1-deficient hematopoietic progenitor cells had a defect in forming erythroid burst-forming units (BFU-E) and colony-forming units (CFU-E), but not in forming myeloid colonies [69]. The essential role of MED1 in erythroid lineage is further emphasized by a subsequent study using Mx-Cre-mediated MED1 conditional knockout mice. A specific block in erythroid development was observed highlighted by the complete absence of β-globin gene expression, while the myeloid and lymphoid development was grossly normal [116]. In another study, it was shown that MED1, Pit-1 and GATA-2 interact reciprocally in thyrotropes and co-occupy the TSHβ proximal promoter to maximize TSHβ gene expression [74].

2.1.4. MED1 interaction with C/EBPβ

Like the ligand-dependent interaction with nuclear receptors, MED1 interacts directly with C/EBPβ and this interaction can be enhanced by IFNγ-stimulation. This IFNγ-induced dynamic association of MED1 with C/EBPβ serves as a regulatory aspect for C/EBPβ functions. The expression of C/EBPβ target genes irf9 and dapk1 upon IFNγ-stimulation was suppressed in the absence of MED1. MED1 recruitment to the dapk1 promoter after IFNγ induction was dependent on C/EBPβ and was subjected to the regulation by ERK signaling [75].

These studies about Mediator subunit MED1 clearly demonstrate that a ubiquitous factor like a Mediator subunit can have surprisingly selective physiological defects. This is in line with a lot of genetic data from yeast *S. cerevisiae*, *Drosophila* and *C. elegans* where Mediator subunits regulate only a specific set of genes. These studies also show that a single Mediator subunit can serve as the interface for several diverse transcriptional regulators suggesting a high degree of plasticity even for single Mediator subunit.

2.2. MED15 interaction with transcription factors

2.2.1. MED15 in TGFβ signaling

MED15 physically associates with Smad2/3/4 in response to TGFβ/Activin and specifically mediates TGFβ/Nodal/Activin/Smad2/3 signaling. Overexpression of MED15 enhanced TGFβ/Activin responses in a dose-dependent manner in a luciferase assay, whereas knockdown of MED15 decreased the amount of Mediator associated with Smad2/3-Smad4. Therefore, MED15 functions as a communicator between Smad2/3-Smad4 and the Mediator complex to activate gene expression mediated by TGFβ/Nodal/Activin signaling [88].

2.2.2. MED15 in lipid homeostasis

Sterol regulatory element binding protein (SREBP) family transcription factors are critical regulators of cholesterol and lipid homeostasis. Structural analysis revealed that the N-terminal region of MED15 contained a predicted KIX domain with marked sequence similarity to the CBP/p300 KIX domain. MED15 strongly bound to the activation domain of SREBP-1α through this domain. Knockdown of MED15 by RNA interference significantly decreased cholesterol-regulated SREBP target gene expression, as well as the promoter activity of the target genes [89]. Moreover, the *C. elegans* MED15 homologue MDT-15 was also required for fatty acid homeostasis, suggesting an evolutionary conservation of MED15 function in lipid homeostasis [117].

2.3. MED23 interaction with transcription factors

2.3.1. MED23 in MAPK signaling pathway

The mammalian MED23 was originally identified by the Berk and coworkers when searching for coregulators of adenovirus E1A protein [16]. Overexpression of MED23 inhibited activation by E1A and Elk via interfering the interaction between these activators and the Mediator complex in a luciferase assay [16]. Similarly, it was shown that in MED23$^{-/-}$ embryonic stem cells, E1A-CR3 activation and ELK-1 activation in response to MAPK signaling were defective [97]. To elucidate the molecular mechanism, an *in vitro* factor-binding assay was performed using an immobilized DNA template. E1A and phosphorylated ELK1 activation domain-stimulated binding of Mediator, RNAP II and the general transcription factors TFIIB, TFIIE, TFIIH to promoter DNA was abolished in nuclear extracts from MED23$^{-/-}$ ES cells indicating that the interactions of the E1A and ELK1 activation domains with MED23 promote the assembly of preinitiation complex on promoter DNA [49]. *In vivo* study of ELK1-dependent transcriptional activation of Egr1 gene in wild-type and MED23$^{-/-}$ ES cells demonstrated that MED23 increased RNAP II recruitment by 3-fold, in contrast, Egr1 transcription level was 13-fole higher, indicating that the interaction between phospho-ELK and MED23 was essential for both RNAP II recruitment and post-recruitment events, in this case transcription initiation rate [52].

2.3.2. MED23 in adipogenesis

It was shown by the Wang group that MED23$^{-/-}$ MEFs were resistant to hormone-induced adipogenesis. In the absence of either ELK-1 or MED23, adipogenesis was inhibited and the expression of Egr2, an immediate early gene induced by insulin during adipogenesis, was abolished. RNAP II at the Egr2 promoter could efficiently initiate transcription upon insulin stimulation only in the wild-type cells, not in the MED23-deficient cells, implying that MED23 is essential for both RNAP II recruitment and post-recruitment process also in the fibroblast cells [118].

2.4. MED25 interaction with transcription factors

2.4.1. MED25 interaction with VP16

MED25 physically interacts with the herpes simplex activator VP16. Depletion of MED25-containing Mediator complex using anti-MED25 antibody from HeLa cell nuclear extract abolished VP16-activated transcription *in vitro* [100]. VP16 binding domain of MED25 competed effectively for the binding of VP16 and was a dominant-negative inhibitor for VP16 transactivation. Additionally, RNA interference-mediated knockdown of MED25 resulted in the inhibition of Gal4-VP16 gene activation in human cells [101].

2.4.2. MED25 in lipid metabolism through the interaction with HNF4

MED25 physically interacts with HNF4, which controls multiple metabolic pathways. Comparison of immunoprecipitated HNF4 complexes from HepG2 cells or primary hepatocytes with either overexpression or knockdown of MED25 demonstrated that MED25 was essential for the association of HNF4 with Mediator and RNAP II. ChIP experiments showed that the recruitment of RNAP II to the promoter region of the drug-metabolizing enzymes required the association of MED25 with HNF4. Interestingly, the coactivator function of MED25 for HNF4 is highly selective with only a subset of HNF4 target genes related to drug and lipid metabolism was selectively affected [103].

2.4.3. MED25 in chondrogenesis

Sox9 is a transcriptional activator essential for chondrogenesis. Wwp2, an E3 ubiqutin-ligase, was identified as a direct target of Sox9. Sox9, Wwp2 and MED25 interact reciprocally and form a transcriptional complex to augment Sox9 transcriptional activity, whereas knockdown of either Wwp2 or MED25 resulted in down-regulation of chondrocyte-specific gene expression. In zebrafish, morpholino-mediated knockdown of either Wwp2 or MED25 led to palatal malformation, which resembled the palatal phenotype of Sox9 mutants [104]. It would be interesting to learn more about the physiological function of Med25 using knockout mice.

Collectively, the studies on individual Mediator subunit show a surprisingly specific contribution of the ubiquitously expressed Mediator complex and also provide mechanistic insights like the role for Mediator in post-recruitment events.

3. Mechanistic insights into the Mediator–activator interactions

3.1. Synergy of several different Mediator subunits in gene-specific regulation

A variation of the bipartite interaction of one transcription factor/one Mediator subunit is that particular transcription factors evolved to interact with several Mediator subunits. We provide here as examplatory studies on transcriptional regulators REST, the glucocorticoid receptor and PPARγ.

3.1.1. REST

REST (RE-1 silencing transcription factor) plays essential role in suppressing the nonspecific expression of neuronal genes in terminally differentiated nonneuronal cells. The internal repression domain in REST exerts the repressive function through recruitment of G9a, a histone methyltransferase responsible for generating the transcriptional repressive H3K9me2 mark around RE1 sites within repressed genes. The Boyer group showed that MED12 is required for REST-directed G9a recruitment to RE1 silencing elements. siRNA mediated MED12 knockdown abolished REST and G9a-dependent neuronal gene repression. Additionally, Mediator, G9a and REST interact reciprocally and exist in a trimeric complex [83]. Later

on, it was shown by the same group that MED19 and MED26 subunits serve as the interface of Mediator in physical association with REST. Combined depletion of both MED19 and MED26 disrupted the association of REST with Mediator and impaired REST-mediated recruitment of Mediator and G9a to RE1 silencing elements leading to de-repression of the target genes [95]. Therefore, MED12, MED19 and MED26 coordinate synergistically to stabilize the trimeric complex formed by Mediator, G9a and REST to repress neuronal gene expression in nonneuronal cells.

3.1.2. Glucocortecoid Receptor

MED1 and MED14 interact with the ligand binding domain (LBD) and activation function-1 (AF-1) domains of glucocortecoid receptor (GR), respectively. Luciferase assay showed that MED1 cooperated with MED14 in enhancing GR transactivation activity [65]. When looking at endogenous GR target genes in ligand induced fashion, a more articulated view of the role of MED1 and MED14 in respect to GR target gene expression emerged [119]. In this setting, ligand-induced GR target genes can be categorized into three classes: MED1/MED14-dependent, MED14-dependent and MED1/MED14-independent genes [119]. MED1 LxxLL motif mutations completely abolished its interaction with GR, whereas reduced rather than eliminated the expression of GR target genes. This was further confirmed by analysis of endogenous GR target genes in MED1$^{-/-}$ MEFs implying that other Mediator subunits like MED14 or other coactivators such as p160/SRCs are involved in GR transcriptional activities [66].

3.1.3. PPARγ

MED1 was first isolated in a yeast two-hybrid screen as a PPARγ-interacting protein [58]. The importance of MED1 in PPARγ-mediated transcriptional activation was corroborated by the finding that MED1$^{-/-}$ MEFs were defective in PPARγ2-stimulated adipogenesis, but not in MyoD-stimulated myogenesis. This defect could be restored by ectopic expression of MED1 [59]. However, further investigation about the molecular mechanism involved showed that a conserved N-terminal region of MED1 lacking the nuclear receptor recognition LxxLL motifs could rescue PPARγ2-stimulated adipogenetic defect in MED1$^{-/-}$ MEFs. This indicated that MED1 LxxLL motif-mediated interaction between PPARγ and Mediator was not essential for PPARγ2-stimulated adipogenesis. Moreover, MED1 was dispensable for PPARγ-mediated target gene activation in undifferentiated MEFs suggesting the existence of alternative pathway required for Mediator recruitment and PPARγ function [120]. Using an adenoviral PPAR expression system, it was shown that acute target gene activation by PPARγ/α was independent of MED1. Instead, MED14 was identified as a potential Mediator component for PPARγ-mediated transactivation by a small interfering RNA (siRNA)-based approach. As in the case for GR, MED14 interacts directly with PPARγ N-terminus in a ligand-independent manner. Knockdown of MED14 compromised the ability of PPARγ to activate a subset of target genes and impaired the adipogenesis in 3T3-L1 cells [87]. However, although MED14 could serve as the anchoring point between PPARγ and Mediator for a subset of PPARγ target genes, other Mediator subunits or other coactivators must be involved for the gene-specific regulation of other PPARγ target genes.

Thus, the distinct interaction domain, and variable ligand dependency of nuclear receptors with MED1 and MED14 implies a general complementary potential of these two Mediator subunits, although the requirement for MED1 and/or MED14 is gene-specific. The studies summarized above mark the end of monopoly era for MED1 interaction with nuclear hormone receptors, suggesting that the requirement of Mediator for nuclear hormone receptor functions is far more than the mere physical interaction mediated by MED1 nuclear receptor motifs.

3.2. Activator-induced conformational changes

Using EM (electron microscopy)-based analysis and single-particle reconstruction techniques, distinct structures were obtained for activator-free Mediator and different activator (VP16 or SREBP-1α)-bound Mediator [28]. Localization of the VP16 and SREBP-1α binding sites on Mediator indicated that the activation domains of VP16 and SREBP-1α bound distinct regions on Mediator and targeted distinct Mediator subunits (VP16 targeted MED17 and MED25; SREBP-1α targeted MED14 and MED15) [28]. Subsequent analysis of TR- and VDR-bound Mediator structures revealed that TR and VDR (targeted the same Mediator subunit MED1)-bound Mediator complexes shared highly related (but not identical) conformational features that were distinct form either VP16 or SREBP-1α-bound Mediator structures. Consistent with the same target subunit, both receptors bound to the same region on Mediator [121]. Recently, the Taatjes group has demonstrated two functional consequences of such structural alterations. On the one hand, analysis of subunit compositions of activator-free and VP16 or SREBP-1α-bond Mediator by MudPIT have provided clear evidence that activator-induced structural shifts trigger specific Mediator–cofactor interaction, which can modulate Mediator activity [122]. On the other hand, structural analysis of p53-Mediator has revealed different conformational changes when binding to either p53AD (activation domain, targeted MED17) or p53CTD (C-terminal domain, targeted MED1). p53AD-Mediator structural shifts enabled the formation of a large pocket domain in Mediator and allowed the transition of RNAP II complexes from a stalled state to a productive elongation state, whereas p53CTD-Mediator lacked this RNAP II pocket domain and represented an inactive structural state [76]. All these studies indicate that Mediator has high plasticity and undergoes substantial conformational alterations upon binding to different activators. This structural shift can lead to subsequent recruitment of additional activators or cofactors to the preinitiation complex or regulation at the post-recruitment processes, representing another regulatory aspect of the Mediator functions.

3.3. Variable association of Cdk8 submodule with the core Mediator

Two distinct Mediator complexes have been purified, the large complex containing Cdk8 submodule is transcriptionally inactive and fails to interact with the CTD of RNAP II, whereas the smaller complex is highly active and interacts directly with the CTD of RNAP II [28,123]. The association of these two distinct Mediator complexes and transcriptional activities has been exemplified by two independent studies. First, C/EBPβ is an intrinsic repressor that is converted into an activator by Ras-mediated MAPK signaling. It was shown by the Leutz group that activation of C/EBPβ by Ras induced an exchange of a transcriptional active Mediator (the core Mediator devoid of Cdk8) for a transcriptional inactive Mediator (Cdk8-containing Mediator) at the target gene promoters [99]. In another study from the Reinberg group, to clarify the mechanism of the dependency on the DNA-binding protein PARP-1 in RA (retinoid acid)-induced expression of RARβ, a dynamic association of the core Mediator with or without Cdk8 submodule at the RARβ promoter was observed. In the resting state, Mediator containing Cdk8 submodule was present, whereas upon RA induction, Cdk8 submodule dissociated with the core Mediator contaminant with the active gene expression. In PARP-1$^{-/-}$ MEFs, Cdk8 submodule was retained even upon induction consistent with the repression of gene expression, indicating that PARP-1 might function as a

RA-induced switch of Mediator from the inactive (associated with Cdk8) to the active state (devoid of Cdk8) in RAR-dependent transcription [124].

Although the role of Cdk8 submodule has become much more complicated with a flurry of recent reports deciphering distinct functional aspects of Cdk8 submodule (as discussed in the next section), the association of Cdk8 submodule with the core Mediator is still a possible mechanism for Mediator functions in a gene-specific context.

4. Cdk8 submodule: implication in transcription repression and activation

Cdk8 submodule consists of four Mediator subunits MED12, MED13, cyclin C and Cdk8 and is variably associated with the core Mediator complex. The four-subunit composition is conserved from human to yeast and is also in yeast a biochemical entity [125]. Cdk8 submodule can act as co-repressor or co-activator depending on the specific scenarios. The studies in yeast, *Drosophila* and human cells provide multiple mechanistic insights related to the Cdk8 submodule.

4.1. Cdk8 submodule in transcriptional repression

Historically, numerous evidences indicate that Cdk8 submodule is a negative regulator during multiple steps of gene transcription and are summarized as follows:

4.1.1. RNAP II binding

Electron microscopy structures of the *S. pombe* Mediator with and without Cdk8 module showed that the Cdk8 submodule bound to the RNAP II-binding cleft of Mediator, therefore sterically blocked RNAP II interaction [126]. In a reconstituted transcription system on chromatin template, the Cdk8 subcomplex could repress activated transcription independent of its kinase activity. This repression can be released by supplementation of additional core Mediator, but not extra TFIIH and RNAP II and highly dependent on the existence of MED12 and/or MED13. Chromatin sedimentation assay of assembled PIC in the presence or absence of Cdk8 submodule and MudPIT analysis of Mediator samples purified with anti-Cdk8 or anti-Med1 antibodies demonstrated that the existence of the Cdk8 submodule blocked RNAP II association with Mediator [127].

4.1.2. Phosphorylation of CTD, TFIIH and activators

Srb10 (the yeast homologue of mammalian Cdk8) can phosphorylate the CTD prior to the formation of the preinitiation complex, which disrupts Mediator–RNAP II interaction with consequent inhibition of transcription [128]. Cdk8/cyclin C negatively regulates transcription by phosphorylating cyclin H subunit of the general transcription factor TFIIH, therefore represses both the ability of TFIIH to active transcription and its CTD kinase activity [129]. Upon activation of the Notch signaling pathway, the Notch intracellular domain (NICD) translocates into the nucleus and complexes with DNA binding proteins and the coactivator Mastermind to activate target gene expression. Using this system, Fryer et al. showed that Cdk8:cyclin C was recruited with NICD and coactivators such as Mastermind to the promoter of the Notch target gene HES1, which in turn mediated the phosphorylation and ubiquitin-dependent degradation of the NICD to control the length of the activation time [130].

4.1.3. Exchange of Cdk8-Mediator for core Mediator

As discussed in Section 3.3, an exchange of Cdk8-Mediator for the core Mediator has been observed in both Ras/MAPK-induced activation of C/EBPβ [99] and RA-stimulated activation of RARβ2 [124].

4.2. Cdk8 submodule in transcriptional activation

Genome-wide analysis of Mediator binding sites by ChIP-on-chip in yeast reveals that Cdk8 submodule displays similar binding patterns as the core Mediator subunits, where they bind to the upstream regions of both active and inactive genes [45,46], arguing against the correlation between Cdk8 submodule association and gene repression. Using a chemical genetics strategy, Srb10 (the yeast homolog of Cdk8) and Kin28 (the yeast homolog of Cdk7) were engineered to respond to a specific inhibitor. It was shown that both kinases cooperatively promote the transcription *in vitro* and *in vivo* and are essential for the dissociation of the preinitiation complex into the scaffold complex, namely transcription re-initiation [131]. Direct evidence for the positive role of Cdk8 comes from the knockdown of Cdk8 using RNA interference, where the phosphorylation of RNAP II CTD at serine 2 and serine 5 was repressed and the transcriptional activation by Gal4-VP16 was counteracted in a luciferase-assay [132]. Recent studies on the role of Cdk8 submodule in the positive-regulation of specific gene expression shed light on the molecular mechanism and physiological consequences of this submodule and are summarized as follows:

4.2.1. RNAP II recruitment

Using *in vitro* transcription assay and cellular approaches, the Fondell group demonstrated that Cdk8 and its kinase activity are essential for the transcriptional activation of TR target gene type I deiodinase (DioI). Mediator containing the Cdk8 submodule is specifically recruited into the PICs at the DioI promoter in concomitant with RNAP II in a TR- and T3-dependent manner. Cdk8 knockdown decreases RANP II occupancy and DioI mRNA expression, which could be rescued by wild-type but not the kinase-inactive mutant of Cdk8 [133]. In another study, to clarify the mechanism of stimulus-specific transcriptional activation within the p53 network, the Espinosa group compared the landscape at the p21 locus in UVC- and Nutlin-treated cells. The recruitment of histone acetylation marks, TFIIA, p53 and core Mediator subunits is independent of the stimuli, whereas the enrichment of TFIIB, TFIIF, RNAP II, phosphorylated RNAP II and Cdk8 submodule (Cdk8, MED12, cyclin C) relies on Nutlin treatment and associates with the transcriptional activation status of p21 suggesting that Cdk8 submodule positively regulate p21 expression. This was further confirmed by the biochemical studies showing that Cdk8 knockdown impairs p21 expression and ectopic expression of Cdk8/cyclin C enhances the p21 reporter activity [134].

4.2.2. RNAP II elongation

Within the serum response network, Cdk8 is a positive regulator of immediate-early genes. ChIP experiments with Cdk8 knockdown cell line showed that Cdk8 was dispensable for RNAP II recruitment to immediate-early genes but caused a decrease in RNAP II CTD phosphorylation at Ser2 and Ser5 indicating a potential role of Cdk8 in transcriptional elongation. Nuclear run-on assay further confirmed that Cdk8 is required for assembly of elongation complexes, namely the recruitment of Cdk7, Cdk9 and subsequent RNAP II CTD phosphorylation [135].

4.2.3. Cdk8 as an oncogenic gene

Cdk8 was identified in two loss-of-function screens in human colon cancer cells displaying the requirement for both β-catenin activity and colon cancer cell proliferation. In colon cancer cells characterized by high levels of Cdk8 and β-catenin, Cdk8 kinase activity is required for both colon cancer oncogenesis and β-catenin

driven transcription by facilitating β-catenin binding at the proximal promoter region of the target genes [136].

4.2.4. Cdk8 histone H3 kinase activity

Using biochemical approaches, several novel substrates of recombinant Cdk8 subcomplex including histone H3, MED13 and Cdk8 itself were identified. Cdk8 could phosphorylate H3 within chromatin only when incorporated into Mediator and MED12 is essential to activate Cdk8 kinase activity. Since H3S10 phosphorylation is tightly associated with active gene expression, this serves as a possibility by which Cdk8 positively regulates gene expression. But the detailed mechanism still awaits further investigation [137].

5. Perspectives

The Mediator complex forms the bridge between many transcription factors and RNAP II machinery. Over the last few years, genetic, biochemical and structural studies on different transcription factor/Mediator interactions provided major insights into Mediator mechanism. Further studies are required to understand how Mediator facilitates enhancer/promoter loop formation. Dynamic systems will unravel the sequence of events required for the initiation of transcription (i.e. binding of activator, Mediator, cohesin, GTFs and RNAP II at enhancer and core promoter elements). So far, the information of post-initiation events that require Mediator is scarce. Possibly, not only further cell-free functional and structural studies but also localization of Mediator in the nucleus will help to substantiate the initial studies.

Analogous to the many genetic experiments performed in yeast, genetic studies in mice will also be key to understand Mediator function in higher eukaryotes (see also the review by Dr. K. Hentges in this issue). A combination of such genetic and structural studies will be powerful.

Many of the transcription factors described to interact with Mediator are involved in development and disease (see also Spaeth et al. in this issue). Therefore, it is tempting to foresee that disrupting activator/Mediator interactions by small molecules has therapeutic potential. The prediction is by disrupting a specific activator/Mediator interface, only a subset of target genes is affected.

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