======================================= REVIEW =======================================

Structure and Functions of the Mediator Complex

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Abstract—Mediator is a key factor in the regulation of expression of RNA polymerase II-transcribed genes. Recent studies have shown that Mediator acts as a coordinator of transcription activation and participates in maintaining chromatin architecture in the cell nucleus. In this review, we present current concepts on the structure and functions of Mediator.

Mediator is an essential coactivator of transcription by RNA polymerase II (Pol II) in unicellular and multi-cellular eukaryotes. Mediator acts as a scaffold that binds and coordinates most transcription components [1]. Mediator interacts with general transcription factors, chromatin-modifying complexes, and numerous gene-specific activators [2]. It is involved in many signaling pathways [1] and controls various aspects of cell metabolism [3]. Mediator plays a key role in ontogenesis; mutations in the Mediator subunits are associated with the development of various pathologies [1, 4, 5]. It is not surprising that a complex with such diverse and broad functions has attracted considerable interest of researchers. In this review, we present recent data on the structure and functions of Mediator.

STRUCTURE OF MEDIATOR COMPLEX

Mediator is a large protein complex with a molecular mass of 1.4 MDa that consists of ~25 subunits in mammals (0.9 MDa and 20 subunits in yeast). The subunits form four major structural modules: head, middle, tail, and a mobile Cdk8 kinase module (CKM) (see table).

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Abbreviations: CKM, Cdk8 kinase module; CTD, *C*-terminal domain of RNA polymerase II Rpb1 subunit; IDR, intrinsically disordered protein regions; ncRNA, noncoding RNA; PIC, preinitiation complex; Pol I, II, III, DNA-dependent RNA polymerase I, II, and III, respectively; UAS, upstream activating sequence.

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The subunit composition of the modules and their relative position in the complex have been elucidated after solving the 3D structure of yeast Mediator with high resolution [6, 7] (Fig. 1a). The central role in the organization of Mediator complex belongs to the Med14 subunit, that connects the head, the middle, and the tail modules [8], and to the Med17 subunit, that forms multiple contacts with other subunits [9].

Recently, the structure of the Mediator complex has been resolved by X-ray analysis, cryoelectron microscopy (cryo-EM), and mass spectrometry of cross-linked subunits [9]. Most data have been obtained for yeast Mediator, including the results of cryo-EM that allowed reconstructing the 3D structure of the Mediator complex consisting of its three major structural modules (Fig. 1b). Although the structure of Mediator is conserved in all eukaryotes [10, 11], its details in higher eukaryotes have not been completely elucidated.

The structures of the Mediator core 15-subunit complex with general transcription factors [12] and of the complete Mediator—Pol II preinitiation complex (PIC) on the promoter [13] have been obtained recently. It was found that the Mediator head module forms multiple contacts with RNA polymerase; the most important contact is binding of the head module to the unphosphorylated *C*-terminal domain (CTD) of the RNA polymerase Rpb1 subunit [13]. The head and the middle modules are responsible for the interaction with general transcription factors on promoters. The tail module protrudes from the PIC toward the upstream DNA, thereby creating a platform for the interaction of PIC with transcriptional activators [14] (Fig. 1f). Taken together, these results corroborate the suggestion on

Mediator complex modules and subunits [5] with their known functions in transcription activation

Module	Subunits	Role in transcription activation
Head	Med6 Med8 Med11 Med17 Med18 Med20 Med22 Med30*	stimulates PIC assembly, interacts with Pol II and general transcription factors
Middle	Med1 Med4 Med7 Med9 Med10 Med14 Med19 Med21 Med31 Med26*	interacts with Pol II together with the head module; Med1 is target for many transcription factors; Med14 acts as a scaffold that bonds all three modules
Tail	yMed2/hMed29 yMed3/hMed27 yMed5/hMed24 Med15 Med16 Med23*	interacts with DNA-binding transcription factors; human Med24, Med27, and Med29 are supposed homologs of yeast Med5, Med3, and Med2
CKM	Med12 (hMed12L*) Med13 (hMed13L*) Cdk8 (hCdk19*) CycC	involved in transcription activation and repression; Med12, Med13, Cdk8 have paralogs in human (shown in parentheses)
?	Med25* Med28*	location in the Mediator complex is unknown

Notes: yMed, yeast Mediator subunits; hMed, human Mediator subunits.
    * *Metazoan*-specific subunits.

the major functional role of Mediator as a scaffold for PIC assembly and stabilization on a promoter [13].

An important feature of the Mediator complex is that its subunit composition can change depending on the biological context. The most extensively characterized alteration in the Mediator complex is reversible binding of CKM [1]. Mass-spectrometry analysis showed that relative contents of individual subunits in the Mediator complex preparations may differ. Also, the subunit composition of Mediator in differentiated cells becomes less diverse, as it was also described for TFIID complex [15]. The presence of particular subunits in the complex might be tissue-specific, as demonstrated for the Med26 subunit in *Drosophila* [16]. In yeast, the Mediator complex composition can vary as well. Thus, Med3 and Med15 subunits of the tail module can form amyloid-like aggregates under stress conditions, which changes the subunit composition of the whole complex [17]. These data suggest the existence of Mediator complexes of varying composition. Since different Mediator subunits bind different transcription factors, alternative forms of the complex

might be involved in the generation of alternative transcriptional responses in cells.

Mediator is a complex that is dynamic in its subunit composition and structure. Many subunits in this complex (both in humans and yeast) have intrinsically disordered regions (IDRs). It is possible that the presence of many IDRs provides Mediator with the ability to interact with structurally diverse transcriptional factors [18]. Structural rearrangements in the Mediator complex come along with its binding to the CKM and transcription factors or its interaction with PIC [8, 14]. It was suggested that structural rearrangements of Mediator are related to the sequence of processes during gene transcription activation and play an important role in the functioning of Mediator [8].

CYCLIN-DEPENDENT KINASE MODULE OF MEDIATOR

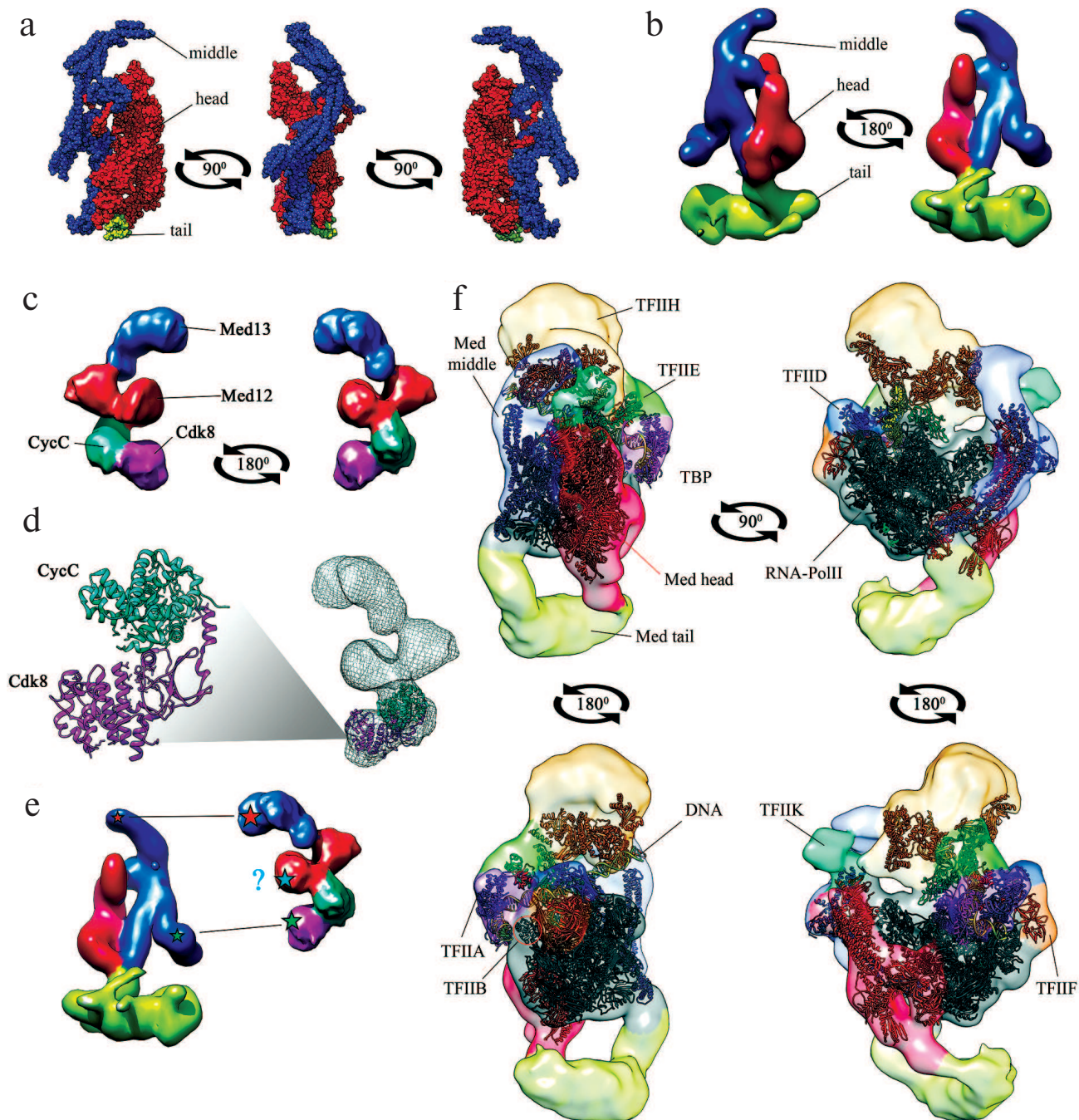The only enzymatic activity of Mediator is provided by the kinase module that is universally conserved in

Fig. 1. Three-dimensional structures. a) Mediator complex in free state based on data of X-ray analysis at 4.4 Å resolution [8] (RCSB PDB ID: 5u0p [150]). All subunits are shown except Med1. The structure of the tail module is incomplete because of limitations of the method. b) Yeast Mediator complex in free state based on data of cryo-EM at ~1.8 nm resolution [11] (ePDB EMDB ID: 2634 [151]). Structural modules (head, middle, tail) are shown with different colors. c) CKM in free state based on data of cryo-EM [20] (ePDB EMDB ID: 5588 [151]). d) Superposition of the structures of the complete CKM (cryo-EM) and Cdk8−CycC complex [152] (RCSB PDB ID: 3rgf [150]). e) Contacts between CKM and Mediator (asterisks, presumed areas of contacts; ?, interaction between Med12 and middle module was demonstrated for human Mediator but not for yeast Mediator [20]); f) Mediator-containing PIC, as obtained by superposition of cryo-EM data (resolution, 21.9 Å; ePDB EMDB ID: 8308 [151]) and X-ray analysis data (resolution, 15.3 Å; RCSB PDB ID: 5sva [150]). The figure shows DNA molecules, RNA polymerase II (Pol II), TATA-binding protein (TBP), and transcription initiation factors TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, and TFIIK. Structural modules (head, middle, tail) are shown with different colors.

eukaryotes [4]. Yeast CKM is a protein complex with a molecular mass of ~430 kDa that consists of four subunits: Cdk8, CycC, Med12, and Med13. In mammals, Cdk8, Med12, and Med13 subunits have also paralogs Cdk19, Med12L, and Med13L, respectively. The paralogs are required for the normal course of ontogenesis (in particular, neurogenesis); their presence in the CDK−Mediator complex is mutually exclusive [19].

CKM has an elongated structure; it binds to the Mediator middle module via the Med13 subunit in both mammals and yeast [20, 21] (Fig. 1, c-e).

Regulation of CKM binding to Mediator is not completely understood. CKM itself is involved in the interaction with transcription factors in plants, flies, and humans [22-26] and can be recruited to DNA independently, as demonstrated for several genes including yeast heat-shock genes [27, 28]. There are several mechanisms for the dissociation of CKM from the complex. In yeast, Cdk11 phosphorylates subunits Med4 and Med27, which results in CKM dissociation [29]. In mammals, CKM dissociation might be caused by specific degradation of Med13 [30]. PARP-1 regulates CKM dissociation from retinoic acid receptor target genes [31]. In plants, dissociation of CKM from the Mediator complex in the upstream regions of some genes depends on the degradation of the IAA14 transcription repressor [23]. All these data indicate that regulation of CKM binding to Mediator is species- and gene-specific.

Cdk8 is a serine/threonine kinase whose target proteins are SMAD1 and SMAD3 transcription factors [32], Notch ICD [33], SREBP [34], E2F1 [35], STAT1 [36], histone H3 Ser10 [37], and CTD of RNA Pol II [38]. Large-scale search for Cdk8/Cdk19 substrates using cortistatin A allowed to significantly expand this list [39]. Sixty-four most probable targets were identified, most of which were DNA-binding transcription factors, Pol II factors, mRNA-processing factors, and subunits of chromatin-modifying complexes. This kinase also phosphorylates several Mediator subunits including Med12 and Med13. Other targets of CKM are proteins involved in DNA replication and repair and other processes, which indicated that CKM functions in the nucleus are much broader than it was earlier suggested. It was found that in HCT116 cells, phosphorylation does not affect the stability of target proteins except Med13/Med13L. However, in other studies, Cdk8-dependent phosphorylation led to degradation of Notch ICD, SMAD1, SMAD3, and SREBP in higher eukaryotes [32-34] and Ste12p in yeast [40]. It is possible that phosphorylation still plays a role in the stability of the protein, but in a tissue-specific manner.

CKM can either activate or suppress gene expression. However, Cdk8 knockdown in human cells has a very slight effect on gene expression [39]. The functions of Cdk8 in gene expression control are context-dependent − in human cells Cdk8 regulates different genes in response to different factors [41, 42]. The role of cooperation between the module subunits in the regulation of gene expression remains obscure. In yeast, all four subunits control expression of a common set of genes [43]. In mammalian cells, Cdk8 and Med12 sometimes cooperate in the regulation of certain genes; in other cases, Cdk8 and CycC function independently of Med12 [44-46]. Knockdown of Med12/Med13 and CycC/Cdk8 in

Drosophila resulted mostly in the opposing effects on gene transcription; though rarely such knockdowns produced similar effects [47]. Two paralogs, Cdk8 and Cdk19, seem to have different gene targets, since knockdown of these subunits affected only slightly overlapping groups of genes in human cells [39].

Therefore, CKM often acts as an independent factor of gene expression regulation that functions in a context-dependent manner. The involvement of CKM in the expression regulation is determined not only by its kinase activity, but also through its interaction with other transcription factors (see below).

ROLE OF MEDIATOR IN ORGANIZATION OF TRANSCRIPTION

Localization of Mediator on chromatin in higher eukaryotes demonstrated that Mediator preferentially binds to enhancers throughout the whole genome [48]. Also, Mediator is typical for large enhancer clusters that initiate tissue-specific transcriptional programs (so-called super-enhancers) [49]. In yeast, Mediator was detected on the majority of the upstream activating sequences (UASs) that serve as the analogs of enhancers located upstream of the promoters [5, 50].

Mediator recruitment to enhancers occurs mostly via interaction of its tail and middle modules with transcriptional activators [2, 51, 52]. Deletion of individual subunits of the tail module results in the inability of Mediator to bind to UASs throughout the entire yeast genome [53, 54].

CKM can play different roles in the recruitment of Mediator to enhancers. In some loci, it acts as a binding antagonist. Thus, removal of the CKM Med13 subunit in yeast increases the amount of Mediator bound to certain UASs [54]. The kinase activity of CKM blocks Mediator-mediated activation of target genes in the EGFR/Ras/ERK signaling pathway in *Caenorhabditis elegans* [55]. Cdk8 and Cdk19 suppress the activity of super-enhancers in the AML cell line [51]. It is believed that in such cases CKM is required for the fine regulation of recruitment of Mediator to UASs and enhancers [5]. At the same time, CKM can play a role of coactivator for other genes. For example, Cdk8 is required for the activation of the estrogen receptor target genes in humans [56]. CKM is recruited to promoters of target genes during stimulation of the TRL9 receptor; it cooperates with the transcription factors C/EBPβ and NF-κB [57]. Cdk8 and CycC are coactivators of the *Drosophila* ecdysone receptor [58]. These facts may explain the gene-specific role of CKM in the regulation of gene expression.

Transcription activation in eukaryotes involves physical contacts between an enhancer and a promoter. Using sequential chromatin immunoprecipitation, Petrenko et al. [54] were first to demonstrate the existence of a single

Mediator complex that simultaneously associates with an enhancer and a promoter, thereby corroborating the concept that Mediator acts as a dynamic bridge for these two regulatory elements.

The presence of Mediator on yeast promoters is difficult to demonstrate by chromatin immunoprecipitation [59]. The amount of Mediator on a promoter significantly increases when TFIIH kinase is inhibited, what significantly stabilizes PIC [53, 54]. The reason for a weak signal from the promoter is that the interaction of Mediator with PIC is very short-termed (estimated time, 1/8 s) [60]. Despite its short duration, it is this involvement of Mediator in PIC formation that is believed to be its major genome-wide function [5, 61]. In yeast, promoter- and enhancer-bound Mediator complexes have different subunit composition (promoter-bound Mediator lacks the CKM) [53, 54]. The interaction between the enhancer and the promoter is provided by the Mediator in the absence of CKM.

The major target of Mediator complex on a promoter is Pol II CTD, which acts as a scaffold for many transcription factors and serves as a coordinator of the entire transcription process [62]. Besides, Mediator regulates recruitment and activity of other PIC components, such as general transcription factors TFIIA, B, D, E, and F [50, 63-65], and stimulates recruitment and enzymatic activity of TFIIH [66, 67]. Structural analysis revealed that Mediator forms direct contacts with these factors while being a part of the PIC [8, 12, 13] (Fig. 1f). In addition to a purely structural role in PIC assembly, Mediator is essential for coordination of the sequential processes involved in PIC assembly on the promoter [68, 69] — the role of Mediator in this process depends on the promoter architecture [63, 70].

At the initial stages of gene activation, Mediator forms transcriptionally inactive PIC that is then activated by CTD phosphorylation by TFIIH. This stage is stimulated by Mediator [69, 71] that dissociates from the polymerase (as phosphorylated CTD is incapable of Mediator binding [60, 72]) and leaves the promoter [60]. Hence, the interaction between Mediator and TFIIH is important for transcription initiation and Mediator dissociation from the promoter.

Mediator also provides rapid reinitiation of transcription. It was shown for the HIV-1 promoter that rapid Mediator-dependent reinitiation of transcription results in the appearance of polymerase convoys (groups of polymerase molecules that move along the gene one after another) and transcriptional bursting [73].

Beside transcription initiation, Mediator is involved in the regulation of elongation. For instance, mutations in the middle module of yeast Mediator do not affect recruitment of polymerase to the promoter but decrease its concentration in the gene transcribed region and prevent a decrease in the number of nucleosomes along the gene [74]. In vertebrates, inhibition of Cdk8/19 activity

results in the suppression of CTD phosphorylation and inhibition of transcription elongation on the NF-κB-controlled genes [38].

After dissociation from the promoter and synthesis of several tens of nucleotides of the transcript, RNA polymerase stops. This RNA polymerase pausing plays an important regulatory role [75]. Mediator is involved in this process via interacting with DSIF [76], cohesin [48], and TFIIS [77, 78]. Cooperation between Mediator and transcription elongation factor TFIIS helps to prevent the negative effect of a nucleosome at the start of the transcribed sequence [79]. Also, Mediator from multicellular organisms can initiate transcription *in vitro* if RNA polymerase contains the Gdown1/Pol2M subunit [80]. It was found recently that this subunit binds to polymerase after the start of the RNA synthesis and stabilizes the enzyme in the paused state [81]. Possibly Mediator overcomes the negative effect of Gdown1 and facilitates polymerase exit from pausing [5].

P-TEFb, one of the major factors stimulating transition to elongation, could be recruited to promoters through interaction with CKM [41] and Med26 [82]. Perhaps these two mechanisms of P-TEFb recruitment occur in different groups of genes [83]. Cooperative action of Mediator and P-TEFb also takes place on enhancers, as shown for the T cell-specific enhancer (both factors stimulated synthesis of enhancer ncRNA) [84], enhancers of Hippo/YAP signaling pathway target genes [85], and BRD4-controlled regulatory elements in AML cells [86].

Med26 interacts with the EAF subunit of the super elongation complex (SEC) to ensure initiation of elongation on some genes. Med26 knockdown does not impair PIC assembly but suppresses SEC recruitment [87]. Interestingly, Med26 also interacts with TFIID via the same domain that provides its binding to EAF [88]. Interaction of this subunit with alternating partners might be the mechanism for switching between transcription initiation and elongation. Med26 recruits the LEC initiation complex to genes encoding small nuclear RNAs [89]. SEC subunits also interact with the kinase module of Mediator [42]. Cdk8 knockdown inhibits transcription elongation and SEC recruitment to activated promoters [41, 42]. Cooperation between all three factors (Mediator, SEC, and P-TEFb) is essential for the regulation of transcription of genes controlling early embryonic development in *Drosophila* [90].

Therefore, Mediator is involved in all stages of transcription activation. Based on the existing data, the following mechanism of Mediator functioning was suggested [5]. Initially, the complete Mediator complex is recruited to enhancers via transcription factors; this stage is negatively regulated by the CKM. After CKM dissociation, Mediator is integrated into PIC, which results in PIC stabilization. As a component of PIC, Mediator stimulates kinase activity of TFIIH, thereby causing

phosphorylation of Ser5 residues in CTD and polymerase escape from the promoter. Mediator dissociates from the promoter and binds CKM, which stimulates recruitment of factors (e.g., P-TEFb) favoring the polymerase exit from pausing. P-TEFb phosphorylates Ser2 residues in CTD, and polymerase starts elongation.

CKM plays a dual role in this model. Most likely, its repressor function is not related to the enzymatic activity but results from the binding of CKM to Mediator, since Mediator can only bind either CKM or RNA polymerase [20]. The activator function of CKM is related to its ability to directly bind to some transcriptional activators and coactivators.

In the yeast nucleus, ~15% of all Mediator complexes interact with RNA Pol II, 15% are bound to CKM, and therefore excluded from this interaction, and the remaining 70% are in a free state [91].

It is important to emphasize that Mediator is not an obligatory component of PIC, however, it stimulates consequent stages of PIC maturation on the promoter. Also, some Mediator subunits can be functionally duplicated by other factors. Thus, deletion of individual Mediator subunits produces very moderate effect on yeast transcription, and only impairments in all three modules — head, middle, and tail — significantly disturb cell translation and viability [70].

Mediator is also involved in posttranscriptional events. Med23 interacts with mRNA-processing factors and regulates alternative splicing and alternative mRNA polyadenylation [92]. Mediator directly binds various factors regulating mRNA 3′-end processing and mRNA degradation [93]. The Med31/Med7N module interacts with the mRNA nuclear export factor TREX-2. Interestingly, the latter is required for the binding of CKM to Mediator and regulates Mediator binding to polymerase [94].

ROLE OF MEDIATOR IN REGULATION OF CHROMATIN STRUCTURE

In addition to direct interaction with transcription machinery components, Mediator controls gene activity by regulating chromatin structure. Mediator binds to chromatin through association with histone H3 and H4 tails [95, 96]. It also interacts with chromatin-remodeling and chromatin-modifying factors.

Mediator participates in keeping promoter regions of active genes in a nucleosome-free state. It was found to interact with the chromatin-remodeling complex SWI/SNF in yeast [97, 98]. In humans, CKM interacts with Brg1 [99]. The yeast Med15 subunit binds to the Hrp1 remodeling factor of the CHD1 family [100]; Med15 interacts with CHD1 in mouse cells, and the resulting complex is recruited by active genes [101]. Binding of Mediator to the yeast *HO* gene requires

recruitment of the SWI/SNF complex [102]. For some genes, on the contrary, recruitment of the SWI/SNF complex happens after binding of Mediator to the genes [97, 98, 103]. Therefore, cooperation between Mediator and remodeling factors is tissue-specific. Moreover, the presence of Mediator in the content of PIC on a promoter is important for DNA dissociation from nucleosomes irrespectively of the chromatin remodeling factors [104].

Mediator regulates epigenetic events. For instance, mediator and histone acetyltransferase p300 function synergistically during estrogen receptor transcription in human cells [105] and on active enhancers of mouse hemopoietic cells [106]. Mediator can be recruited to target genes of frog androgen and thyroid hormone receptors directly or via interaction with p300 [107]. In human cells, Mediator binds to the histone acetyltransferase Gcn5 [108]. The two proteins function cooperatively: Gcn5 together with Cdk8 perform tandem modification of histone H3 [109]. Yeast have promoters that recruit the histone acetyltransferase complex SAGA and Mediator in a coordinated manner [110, 111]. However, yeast cells also have promoters to which these complexes bind independently of each other [112, 113]. In higher eukaryotes, Mediator is recruited cooperatively with the histone acetyltransferase complexes STAGA [114, 115] and ATAC [116].

Mediator also plays a role in H2B ubiquitination. In human cells, Med23 recruits the corresponding RNF20/40 enzyme to chromatin [117].

Therefore, Mediator is involved in the recruitment of complexes that establish active chromatin markers. However, Mediator also participates in epigenetic repression, mostly via its kinase module. Gene-specific suppression of trimethylation of the H3K4 residue by CKM [118] was found in yeast. It was demonstrated for one of these genes that CKM blocks the binding of the chromatin-modifying Set1p/COMPASS activator complex [40]. In mammals, downregulation of neuronal genes outside of the nervous system is mediated by the Mediator-dependent recruitment of histone methyltransferase G9a, and this function of Mediator depends on CKM [119]. Repression of immune response genes in humans depends on the Mediator-mediated recruitment of arginine methyltransferase PRMT5; in this case, binding occurs also via the kinase module [120].

Mediator is involved in epigenetic transcriptional memory. In yeast, CKM binds to the *INO1* gene in the memory state (the gene is switched off but can be easily reactivated), which is important for polymerase recruitment and pausing for further transcription initiation. This mechanism is evolutionarily conserved — it was also found for the IFNγ-induced genes in human cells [121].

Mediator binds the Polycomb group complexes that repress certain genes in ontogenesis. Cdk8 knockdown in a mouse cancer model resulted in reduction in histone H3K27 trimethylation and derepression of the

Polycomb-regulated genes [122]. CKM binds the EZH2 and SUZ12 subunits of the PRC2 complex in humans; this interaction is essential for the timely activation of neuronal genes during development [123]. The CKM subunit Med12 operates together with PRC1 to silence key developmental genes in mouse pluripotent cells. During cell differentiation, Med12 dissociates from PRC1, and Mediator converts from a transcriptional repressor to a transcriptional enhancer [124]. There are also some evidences indicating that Mediator can counteract the Polycomb-dependent repression − its subunit Med25 blocks the binding of PRC2 to the gene targets of HFN4α [125].

Finally, Mediator is involved in the formation and maintenance of the structure of pericentric and telomeric heterochromatin [1] and in the establishment of borders between active and inactive chromatin in yeast [126]. In accordance with it, Med26 (but not other subunits) was found in the pericentric heterochromatin in *Drosophila* [16]. Perhaps this subunit has a specific function that differs from the functions of the complex. Mediator is also required for the synthesis and processing of centromeric ncRNAs involved in heterochromatin formation in yeast and plants [127-129]. In telomeric heterochromatin of yeast, Mediator operates together with histone deacetylase Sir2 [130].

Therefore, due to many interactions with enzymes that modify chromatin, Mediator acts as an important factor in epigenetic events in all genome regions in a broad range of living organisms.

ROLE OF MEDIATOR IN CHROMATIN ARCHITECTURE FORMATION

The presence of Mediator at a certain locus does not always correlate with the transcriptional activity of this locus. In both yeast and higher eukaryotes, Mediator has been found on many regulatory elements along the entire genome regardless of their activity [48, 53]. It was suggested that besides regulating gene activity, Mediator is important for maintaining general chromatin architecture [50]. Indeed, Mediator plays an essential role in the formation of contacts between gene regulatory elements in higher eukaryotes [131-134]. Such contacts are a structural feature of DNA packing in higher eukaryotes. Interactions between enhancer-anchored Mediator and promoter-bound PIC are short-termed, as mentioned above, and most probably cannot serve as a basis for the formation of stable contacts in chromatin.

One of the proteins involved in the formation of long-range contacts in chromatin is cohesin. In mouse cells, Mediator and cohesin interact, and the resulting complex binds to the factor Nipbl that provides cohesin docking on DNA. The loci that bind Mediator and cohesin form long-range contacts in a process that depends on Med12; the pattern of such contacts is tissue-specific [48, 108]. Synergistic action of Med12 and Nipbl was also shown in the *Danio rerio* zebrafish [135]. In human cells, Mediator and cohesin act synergistically to maintain cell type [136]. Analysis of chromatin packing hierarchy in mammalian cells showed that Mediator and cohesin are required for the formation of short-range (up to 100 kb) tissue-specific contacts between enhancers and promoters, while CTCF and cohesin are important for the establishment of stable long-range (over 1000 kb) contacts. Formation of short-range contacts depends on Med12. There are also small loops (600-1000 bp), the formation of which is regulated by Mediator, but not by cohesin [137]. At the same time, Mediator is not an obligatory component of the enhancer−promoter contacts in the genome − both Mediator-independent and cohesin-independent contacts were described for the *beta-globin* locus [138].

Although gene regulation in yeast does not proceed via formation of long-range contacts [139], in these organisms Mediator determines the distance over which UASs can activate transcription [140]. In yeast, Mediator is also involved in the formation of contacts between the 5′- and 3′-ends of genes [141], general packing of chromosomes, and formation of chromatin domains (lack of Mediator results in chromatin decompaction in the nucleus) [142]. Analysis of the distribution of Mediator in the yeast genome showed that it is more abundant at the boundaries of the so-called chromosomal interacting domain (CIDs), which are analogs of TADs (topologically associating domains) in higher organisms. Mediator is also associated with various factors involved in the organization of general chromatin structure in the yeast nucleus [93]. Therefore, Mediator has an evolutionarily conserved function involving regulation of distant genomic elements and control of chromatin packing in the nucleus of all eukaryotic organisms.

Other molecules required for the formation of long-range interactions are ncRNAs. It was found that ncRNA-a (one of the classes of mammalian ncRNAs involved in gene activation) interacts with Med12. Mediator is recruited to the sites of synthesis of this RNA; ncRNA-a and Mediator operate together to promote long-range interactions and to activate target genes [143]. In a similar manner, Mediator is required for the activity of enhancer RNAs synthesized on active enhancers, as it was shown for the target genes of androgen receptor [144] and PPARγ receptor [145].

Therefore, Mediator plays an important role not only in transcription, but also in the formation of contacts between distant genome loci. Perhaps such broad activity of Mediator is related to its ability to interact with different types of molecules, including ncRNAs.

Mediator is believed to be a structural integrating complex that participates in the coordinated recruitment
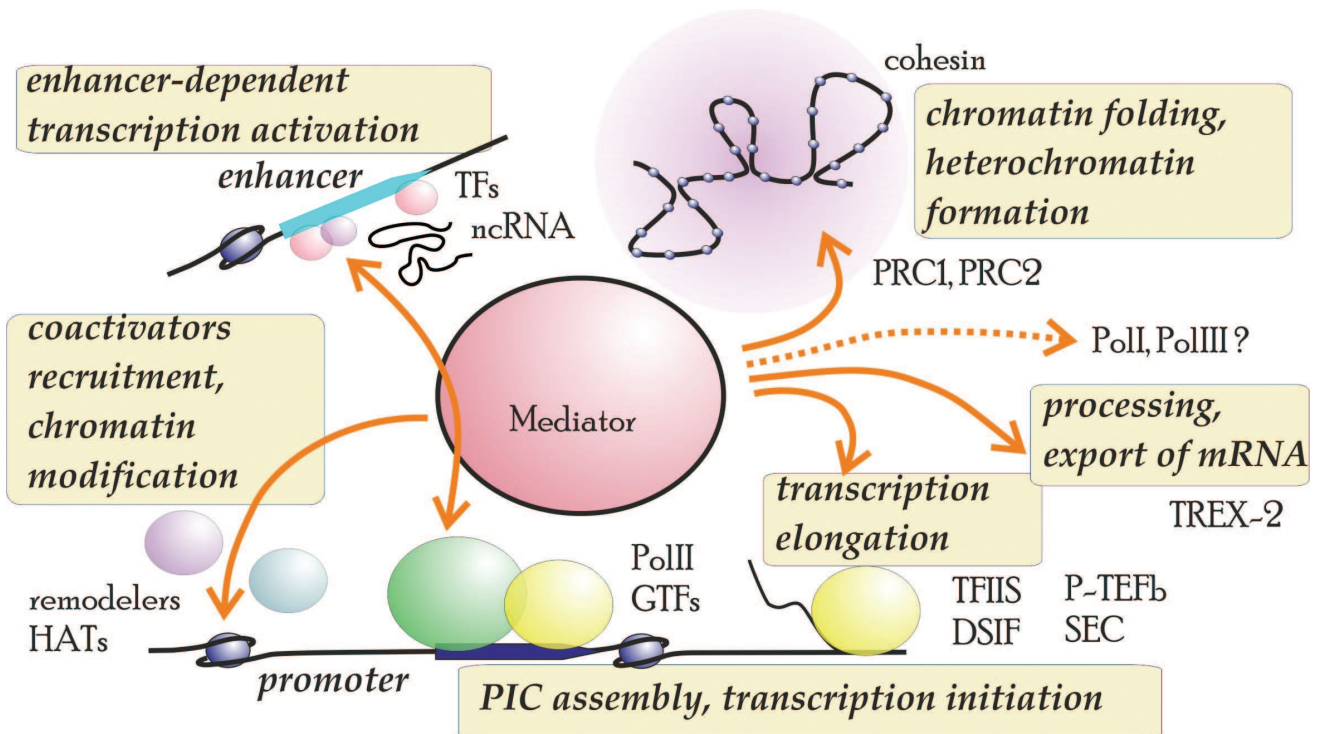
Fig. 2. Major functions and interaction partners of Mediator complex: GTFs, general transcriptions factors; HATs, histone acyltransferases; ncRNA, noncoding mRNA; remodelers, chromatin-remodeling factors; TFs, transcription factors. Putative involvement of Pol I and Pol III is indicated with a dashed line.

of various activities toward genomic loci. Recent description of the Mediator interactome in yeast corroborated and extended this model [91]. More than 400 Mediator-interacting proteins with different functions were identified. In addition to the earlier described partners, Mediator was found to interact with FACT chaperones Spt16 and Pob3, chromatin-remodeling factors ISWI and INO80, acetylated and trimethylated histones, and proteins involved in mRNA processing such as polyA-binding protein (PABP), proteins of RNA splicing apparatus, decapping proteins, and Xrn1, which is a protein participating in mRNA degradation. Interestingly, Mediator copurifies with Pol I and III; it interacts with all 14 subunits of Pol I, as well as with the initiator complex proteins and factors of RNA processing and ribosome biogenesis. In Pol III, Mediator interacted with its two large subunits and two components of the initiator complex — TFIIIB and TFIIIC. These data indicate that the role of Mediator in transcription is much broader than it had been believed before (Fig. 2).

Despite a considerable progress in understanding of the Mediator's functional role, many mechanisms of its action still require further investigation. Thus, the structure of Mediator complex in higher eukaryotes and contribution of subunits specific for the multicellular organisms remain poorly studied. It is still unclear if the complex has varying subunit composition. Among other unre-

solved problems are structural rearrangements that occur during binding of Mediator to activators and PIC and the role of these rearrangements in the activity of the complex. The mechanism of CKM binding to Mediator and the role of kinase in the regulation of gene activity are to be studied as well. The structural basis of interactions of Mediator with other factors often remains obscure. Thus, almost nothing is known about interactions of Mediator with ncRNAs. It remains unclear if Mediator's binding to RNA is sequence-specific, and how such binding affects the structure and functions of Mediator.

The involvement of Mediator in the formation of chromatin architecture is apparently not limited to cohesin binding. It was shown recently that formation of chromatin loops requires participation of many different factors [146]. It seems reasonable to suggest that Mediator acts as a recruiter for these factors. According to another hypothesis, interaction of Mediator with CTD stabilizes long-range contacts in the genome. Since CTDs of higher eukaryotes are long, perhaps they can stimulate formation of long-range enhancer–promoter contacts [1].

Another interesting hypothesis is that the Mediator could be involved in formation of transcription factories. The presence of IDR domains in subunits of the complex might indicate its ability to form a separate phase in the nucleus [1]. IDR domains are common in transcription factors [62, 147, 148], which might be the basis for the

concentration of RNA polymerase and general transcription factors into discrete sites inside the regions of transcription. The role of separate protein phase formation in the nucleus has been demonstrated recently for the heterochromatin protein HP1α [149].

Acknowledgments

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