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REVIEW

Toward understanding of the mechanisms of Mediator function *in vivo*: Focus on the preinitiation complex assembly

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ABSTRACT
Mediator is a multisubunit complex conserved in eukaryotes that plays an essential coregulator role in RNA polymerase (Pol) II transcription. Despite intensive studies of the Mediator complex, the molecular mechanisms of its function *in vivo* remain to be fully defined. In this review, we will discuss the different aspects of Mediator function starting with its interactions with specific transcription factors, its recruitment to chromatin and how, as a coregulator, it contributes to the assembly of transcription machinery components within the preinitiation complex (PIC) *in vivo* and beyond the PIC formation.

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Introduction

The discovery of Mediator originates from the observation of the activator interference phenomenon attributed to a competition between two activators for a common intermediate factor in yeast RNA polymerase II (Pol II) transcription *in vitro*.[1,2] These studies provided the first evidence for the existence of a Mediator of transcription regulation required for stimulation of transcription by activators. In parallel, genetic screens for suppressors of truncated C-terminal domain (CTD) of the largest Pol II subunit allowed to identify *SRB* (*Suppressors of RNA polymerase B*) genes. These genes corresponded to several Mediator subunits that interacted with Pol II CTD and were needed for activator-dependent transcription.[3-6] Multisubunit Mediator complex was then purified from yeast extracts. Mediator was shown to promote the assembly of general transcription factors (GTFs) TFIIA, B, D, E, F and H and Pol II into a preinitiation complex (PIC) on the promoter.[7,8] PIC formation represents a key intermediate in Pol II transcription and an important regulatory step. The Mediator complex was shown to enable activated transcription *in vitro*, but also to stimulate basal transcription in the absence of activator as well as Pol II CTD phosphorylation by Kin28/Cdk7 kinase of TFIIH.[9] Initial names for the yeast Mediator subunits derive from their identification in purified Mediator (Med) or genetic screens (Srb, Gal, Sin, Ssn, Nut, Rye). In human cells, complexes homologous to the yeast Mediator were purified via interactions with activation domains of different transcription factors, in particular nuclear receptors (NR).[10-17] Many names were initially used for human Mediator complexes depending on the purification procedures (TRAP, SMCC, CRSP, DRIP, ARC or PC2). Mediator has been then biochemically identified in many eukaryotes including *Drosophila melanogaster* and *Caenorhabditis elegans*, and more recently, in the plant *Arabidopsis thaliana*. A unified Mediator subunit nomenclature is now used in all eukaryotic organisms[18] (Table 1). Mediator is composed of 25 subunits in yeast and up to 30 subunits in human. Biochemical and structural studies revealed the modular organization of the complex. Four structural modules, head, middle, tail and Cdk8 kinase module, constitute the Mediator complex. The presence of a kinase module within the complex is transient. The

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Table 1. Mediator subunits in yeast *S. cerevisiae* and human.*

Subunit	Yeast *Sc*	Human	Module
Med1	Med1	Med1 (TRAP220, ARC/DRIP205, CRSP200, PBP)	Middle
Med2/29	Med2	Med29 (Hintersex)	Tail
Med3/27	Med3 (Pgd1, Hrs1)	Med27 (TRAP37, CRSP34)	Tail
Med4	**Med4**	Med4 (TRAP36, ARC/DRIP36, p34)	Middle
Med5/24	Med5 (Nut1)	Med24 (TRAP100, ARC/DRIP100)	Tail
Med6	**Med6**	Med6 (ARC/DRIP33, p32)	Head
Med7	**Med7**	Med7 (ARC/DRIP34, CRSP33, p36)	Middle
Med8	**Med8**	Med8 (ARC32)	Head
Med9	Med9 (Cse2)	Med9	Middle
Med10	**Med10** (Nut2)	Med10 (hNut2)	Middle
Med11	**Med11**	Med11 (HSPC296)	Head
Med12	Med12 (Srb8)	Med12 (TRAP230, ARC/DRIP240)	Kinase
Med12L	—	Med12L	Kinase
Med13	Med13 (Srb9, Ssn2)	Med13 (TRAP240, ARC/DRIP250)	Kinase
Med13L	—	Med13L	Kinase
Med14	**Med14** (Rgr1)	Med14 (TRAP170, ARC/DRIP150, CRSP150, p110)	Head/Middle/Tail
Med15	Med15 (Gal11)	Med15 (ARC105, PCQAP, TIG-1)	Tail
Med16	Med16 (Sin4)	Med16 (TRAP95, DRIP92, p96b)	Tail
Med17	**Med17** (Srb4)	Med17 (TRAP80, ARC/DRIP77, CRSP77, p78)	Head
Med18	Med18 (Srb5)	Med18 (p28b)	Head
Med19	Med19 (Rox3)	Med19 (LCMR1)	Middle
Med20	Med20 (Srb2)	Med20 (hTRFP, p28a)	Head
Med21	**Med21** (Srb7)	Med21 (hSrb7, p21)	Middle
Med22	**Med22** (Srb6)	Med22 (Surf5)	Head
Med23	—	Med23 (TRAP150β, ARC/DRP130, CRSP130, hSur2)	Tail
Med25	—	Med25 (ARC92, ACID1)	Unassigned (tail?)
Med26	—	Med26 (ARC70, CRSP70)	Unassigned (middle?)
Med28	—	Med28 (Fksg20)	Unassigned (head?)
Med30	—	Med30 (TRAP25)	Head
Med31	Med31 (Soh1)	Med31 (hSoh1)	Middle
Cdk8	Cdk8 (Srb10, Ssn3, Ume5)	Cdk8 (hSrb10)	Kinase
Cdk19	—	Cdk19	Kinase
CycC	CycC (Srb11, Ssn8, Ume3)	CycC (hSrb11)	Kinase

*Mediator subunits identified in yeast *Saccharomyces cerevisiae* and human complexes are indicated with the names according to the unified nomenclature. Alternative names previously used for Mediator subunits are present in brackets. Subunits of yeast Mediator that are essential for viability are shown in bold. The head, middle, tail and kinase module subunits are highlighted in orange, yellow, blue and green, respectively, with Med14 subunit that links all three main Mediator modules in red. Metazoan-specific subunits Med25, Med26 and Med28 are unassigned and a putative module is indicated in brackets. Kinase module subunits Med12L, Med13L and Cdk19, paralogous to Med12, Med13 and Cdk8, respectively, assemble in a mutually exclusive manner in vertebrates.

spatial organization of Mediator modules that was recently redefined will be discussed below.

Multiple sequence alignments and analysis of secondary structure features led to the conclusion that Mediator was conserved in nearly all eukaryotes.[19] Metazoan Mediator can have up to five additional subunits to the 25 found in yeast. Together with other coregulator complexes, Mediator is characteristic of eukaryotic organisms and is not found in bacteria or Archaea. In eukaryotes, multisubunit coregulators constitute, thus, an additional regulatory layer between specific transcription factors and basal Pol II transcription machinery (Fig. 1).

More than 20 years of intensive studies on the Mediator complex provided important information on its composition and function. General requirement of Mediator for Pol II recruitment and transcription has been demonstrated.[20-22] Mediator subunits were also implicated in human diseases including neurodevelopmental pathologies and different types of cancer.[23-26] In some cases, subunits of Mediator complex were proposed as potential therapeutic targets.[25,26] However, many open questions remain to be answered on the molecular mechanisms of Mediator function *in vivo*. In this review, we will discuss a current view of the molecular mechanisms in which Mediator is involved *in vivo* with a particular focus on its function in PIC assembly and raise some of the remaining open questions concerning its function in transcription regulation.

Mediator recruitment to chromatin and interactions with TFs

Mediator interactions with TFs

Mediator discovery has been conceptually associated with activated transcription *in vitro*. It is, thus, not surprising that several Mediator subunits interact with transcription activators. The Mediator tail module and, in particular, the Med2-Med3-Med15

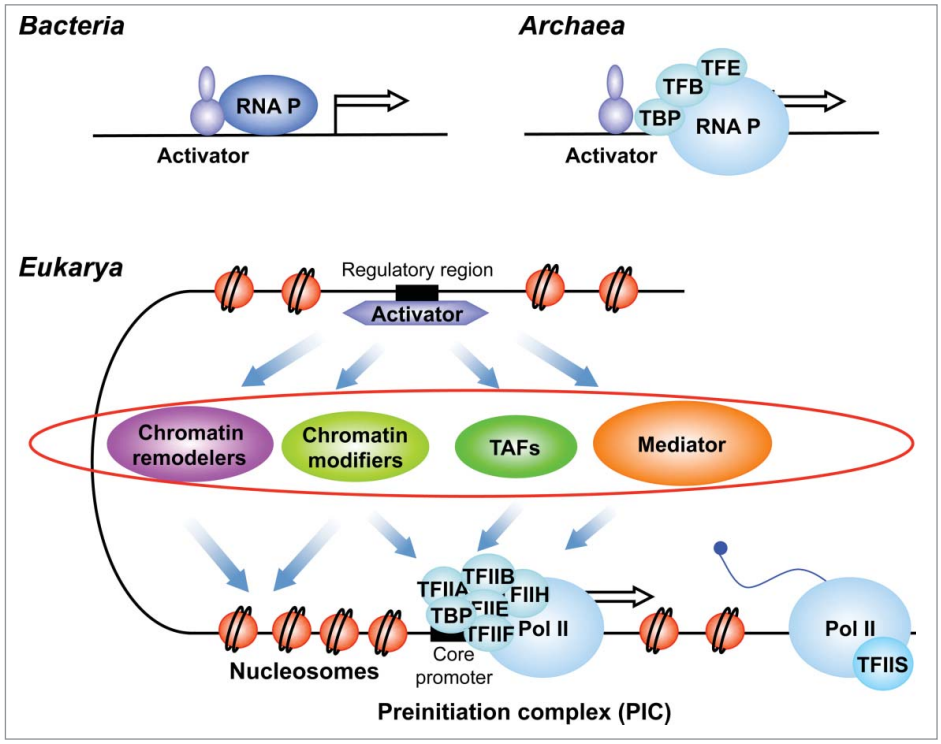

Figure 1. Regulation of Pol II transcription initiation. A simplified view of eukaryotic Pol II transcription regulation at the step of preinitiation complex (PIC) formation is shown. Unlike bacteria which have direct links between activators and RNA polymerase or Archaea that have an RNA polymerase closely related to the eukaryotic enzyme and three general transcription factors, eukaryotes have multisubunit coregulators. These complexes constitute an additional regulatory layer (indicated by a red mark), that emerged in the eukaryotic kingdom, operating between activators and basal Pol II transcription machinery in chromatin context. These multisubunit complexes include chromatin remodelers and chromatin modifiers that act on the promoter chromatin structure or TAFs-containing TFIID and Mediator that stimulate PIC assembly. Some complexes could have both activities.

(Gal11) submodule, was proposed to bind different activators since the deletion of some of Mediator subunits in yeast led to the loss of activator-dependent transcription response both *in vitro* and *in vivo*.[27-29] The fact that different Mediator subunits contact different TFs could, at least in part, reflect the differential involvement of Mediator subunits in regulation of a particular TF-dependent set of genes. The Med15 Mediator subunit in various species is involved in well-characterized interactions between Mediator and transcription activators. For example, NMR studies showed that a three-helix bundle "KIX" domain of the mammalian Med15 is engaged in Mediator interaction with SREBP-1a.[30] Med15 KIX domain is conserved in eukaryotes and, in fungi, is targeted by Oaf1 and Pdr1 activators.[31,32] Recently, the Med15 KIX domain — Pdr1 interaction was used to propose a target for antifungal strategies using molecules that disrupt the contact between the two proteins.[33] The KIX domain is not the only Med15 part that is implicated in contacts with activators. In yeast, Gcn4 binds Med15 through multiple low affinity interactions.[34,35] Structural analysis proposed a fuzzy protein interface between Gcn4 and Med15 in multiple conformations and orientations.[36,37] Med23, another tail module subunit specific to metazoan, has been initially identified in HeLa extracts as a target of adenovirus E1A protein.[10] This subunit was then shown to be required in mouse ES cells for proper PIC formation on gene promoters responsive to MAPK signaling TFs, E1A and Elk-1.[7,38] Interestingly, a recent work proposed a regulatory mechanism for Mediator-Elk-1 interaction through Med23 involving differential phosphorylation of Elk-1.[39]

Mediator interactions with TFs are not restricted to the tail module. For example, the yeast Med17 Mediator head subunit interacts with Gal4 *in vitro*[40] and Drosophila kinase module subunits interact with TFs involved in Wnt signaling pathway.[41] In mammals, Med1 Mediator middle subunit is involved in a large number of interactions with NR.[10-17] Med1 binds an

NR-activation domain through an LxxLL (Leucine XX Leucine Leucine) motif.[42] In addition, several studies have shown that Med14, linking tail, middle and head modules,[43-45] also interacts with different NRs.[46-48]

Molecular mechanisms of transcription activation through Mediator-TF interactions remain to be understood and could potentially involve conformational changes in Mediator complex induced by TF binding.[49] It should be also noted that in many cases several TFs bind to the same gene promoter *in vivo*.[50] It would be important to know how these different regulatory signals are integrated by the Mediator complex and other coregulators recruited by TFs. One of the open questions is how the regulatory information is transmitted between different Mediator subunits within the complex.[51]

Mediator chromatin binding

Direct binding of Mediator to DNA has not been documented. The Mediator complex is recruited to chromatin through its interactions with TFs, specific or general. Mediator enrichment can, thus, be expected both on regulatory regions, together with TFs, and on core promoter regions, where PIC components are located. Preferential association of yeast Mediator with upstream activating sequences of *GAL* genes[52] and Mediator recruitment to the promoter regions of constitutively expressed genes[53] were demonstrated by chromatin immunoprecipitation (ChIP). ChIP-chip genome-wide location analysis of different Mediator subunits in yeast revealed the presence of all four modules on the chromatin with a lower occupancy for the Cdk8 kinase module.[54] ChIP-seq approach allowed to gain in resolution for Mediator genome-wide distribution in yeast[55-58] and mammals.[59-61] Altogether, these studies demonstrated that Mediator associates essentially to the regulatory sequences with low signals on core promoters.[52-62] Mediator occupancy on transcribed regions was previously suggested, but several laboratories argued that Mediator ChIP signals on transcribed regions of highly expressed genes are non-specific.[55,56,63-66] Taking advantage of an independent technique, called chromatin endonuclease cleavage (ChEC)-seq, a recent study mapped the genome-wide yeast Mediator occupancy exclusively on the regulatory sequences.[67] Nevertheless, a transient Mediator association to core promoters was clearly evidenced by its stabilization within the PIC under conditions where Pol II CTD phosphorylation by TFIIH and therefore Pol II promoter escape were inhibited by depleting or inactivating Kin28 TFIIH kinase.[63,66] The model proposes that following Mediator recruitment to chromatin via Mediator-activator interactions on regulatory sequences, Mediator associates transiently with PIC components on core promoters to allow PIC formation. Mediator is then released upon CTD phosphorylation and Pol II promoter escape.

Important extension of these studies provided evidence and mechanistic clues to understand how Mediator composition evolves during transcription activation.[68,69] Mediator kinase module is known for its negative role in gene expression both *in vitro* and *in vivo*.[20,70-72] Its association with core Mediator is mutually exclusive with Pol II-Mediator interaction.[73-75] However, previous genome-wide location analysis of Mediator showed that the entire Mediator including kinase module is recruited to chromatin.[54] Based on the genome-wide occupancy of several Mediator subunits from all modules under Pol II CTD phosphorylation inhibition, recent studies proposed that the full Mediator complex is recruited to regulatory regions essentially through tail module interactions with TFs and then, kinase module is evicted to allow Mediator interactions with core promoter-bound PIC components. This mechanism involves changes in Mediator composition and emphasizes a regulatory function for the Mediator kinase module.[68,69]

Contribution of structural studies to understanding Mediator mechanisms in PIC formation

Here, we will discuss how structural models of Mediator alone or in complex with Pol II contribute to our understanding of its role in PIC formation. Since the Cdk8 module represses PIC assembly, we will not discuss data that pertain to this module.

Mediator is large and flexible and not purified easily in a homogenous form. Hence, it is not readily amenable to the crystallographic investigations. The first studies resorted to electron microscopy (EM) to image negatively stained samples of Mediator devoid of the Cdk8 kinase module.[76-78] The general shape of free Mediator was apparent, being around 350 Å high and 200 Å wide and roughly triangular with a distinct dense domain at the base. Upon association with Pol II, Mediator assumed an elongated shape, embracing

the enzyme. Tentative assignment of Mediator modules in the complex with Pol II was proposed. This interpretation formed the basis for nearly all the structural work that followed until it was revisited in depth 15 years later (see below[79,80]). Nevertheless, the early studies provided the important notion that Mediator could undergo large movements upon association with Pol II. They also established that the general structure of Mediator was similar in yeast, mouse and human. In addition, it was suggested that the interaction of Mediator with transcription activators could be the signal inducing the transition to an elongated shape allowing the interaction with Pol II.[81]

Diffracting crystals could not be obtained for the entire Mediator complex. Nevertheless, the structures of several individual subunits expressed *in vitro* and then assembled in sub-complexes were resolved. Crystals of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* head modules that diffracted at 4.3 Å and 3.4 Å, respectively, were obtained.[82,83] The general shape of the head module was compared with a crocodile head (with two jaws) and neck. Most of the carbon backbones of the individual subunits could be traced on the *S. pombe* head module. Mutations that affect the function of *S. cerevisiae* Mediator by impairing interactions with Pol II CTD,[6] its Rpb3 subunit,[21] or TFIIH[84] could be mapped on the head structure, suggesting the positions of interaction surfaces implicated in PIC formation.[83] These structures indicate that the head can adopt several alternative 3D organizations that may be implicated in the progressive assembly of the PIC. The atomic structure of complete Mediator middle module has not been determined. Nevertheless, the structures of two sub-complexes have been solved,[85,86] and completed by cross-linking data that were used to model the middle module.[87]

Improvement of the biochemical purification of Mediator allowed for the analysis of its structure using cryo-EM.[79,80] Deletion mutants and tagging of Mediator subunits were used to investigate the position of the various subunits and modules. It was observed that Med14 subunit forms the backbone of Mediator, on which the head and middle modules associate. Med14 also connects to the tail. These findings were supported and expanded by an independent modeling of the yeast Mediator, based on integrative computational analysis, cross-linking data and available structural models.[44] These studies revisited the Mediator architecture and allowed for the reassignment of the modules both in isolated Mediator and in complex with Pol II. They also suggested intermediates in the formation of the Mediator-Pol II complex that may be relevant for PIC formation.

Recently, structures of Mediator alone or in complex with Pol II from *S. pombe* were further improved to 4.4 Å and 7.8 Å, respectively.[88] These structures included 16 out of the 21 subunits of the coregulator. This study further supports the notion that Med14, which extensively interacts with Med17, functions as a backbone on which the three modules are tethered. The formation of the Mediator-Pol II complex does not alter the conformation of Pol II contrary to that of Mediator in which the orientation of specific domains is altered due to Med14 change in conformation. These conformational changes allow the CTD to be engaged between the head and the middle modules and facilitate additional Mediator-Pol II contacts.[88] In yeast, the CTD consists of 25–26 heptad repeats. It could thus well interact when extended with several surfaces of Mediator and two other recent studies found that the CTD interacts with the head module.[45,89] All these works are in line with initial identification of Mediator by genetic studies indicating that its function is closely related to that of Pol II CTD.

Two recent studies have investigated the structure of the PIC and how Mediator participates in its organization.[45,90] A 15-subunit core Mediator composed of head and middle modules including Med14 was assembled on a core initially transcribing complex (cITC) of *S. cerevisiae* constituted of Pol II bound to an artificial transcription bubble together with TFIIB, TBP and TFIIF.[45] The structure of the Pol II-Mediator cITC has been solved at 9.7 Å[45]. Mediator-Pol II orientation proposed in *S. cerevisaie* cITC is similar to recently reported *S. pombe* Mediator-Pol II model[88] leading to a consensus Mediator-Pol II conformation that is stable under cryo-EM conditions. However, other conformations of Mediator-Pol II that could be functional at different steps of PIC assembly may remain to be determined. In addition to gaining insights on the Mediator interaction with Pol II Rpb3–11 and Rpb4-Rpb7 subunits dimers, and Pol II dock, the study uncovered contacts with TFIIB. Mediator also participates in the stabilization of cITC. A lower resolution structure of a complete PIC assembled *in vitro* containing the 21 subunits Mediator, Pol II, TBP, TFIIA, TFIIB, TFIIE, TFIIF and TFIIH was

also obtained from cryo-EM and cross-linking analysis.[90] It allowed to position all the components of the PIC and further supported the notion that the CTD-Mediator interface is the major contributor for the initial interaction of the coregulator and the enzyme.

Thus, structural information has proposed how Mediator functions to stimulate Pol II recruitment, to interact with CTD, to stabilize TFIIH and to interact with TFIIB. However, we are still far from an atomic understanding of the structure of Mediator or of the complete PIC. The preparations of the starting material (purified or recombinant) used in structural studies are different and it is likely that the assembly of the PIC entails several steps that might be captured by the different structures. One should also note that the models built from cryo-EM data are based on the selection of a limited number of images in the samples and represent only one possible conformation. In the future, we will need many more models representative of the alternative/successive steps leading to PIC assembly that will have to be supported by *in vivo* experiments. With this goal in mind, one should be able to relate the molecular function of Mediator *in vitro* with its role in PIC assembly on promoters in the chromatin context.

Mediator links with transcription machinery components in PIC assembly in vivo

Pioneering *in vitro* studies with purified PIC components led to the idea that the PIC is assembled starting with TFIID recruitment to promoter, followed by TFIIA and TFIIB incorporation, arrival of Pol II in association with TFIIF and final recruitment of TFIIH and TFIIE.[91,92] Mediator was absent from these initial experiments even though it is important for efficient PIC formation *in vitro*. Indeed, no stable PIC assembly on immobilized templates with nuclear extracts from yeast Mediator mutants is observed in its absence.[8] In a murine nuclear extract system, PIC assembly on promoter DNA *in vitro* was stimulated by activator–Mediator interactions.[7]

To stabilize the PIC, Mediator cooperates with GTFs through physical protein–protein interactions or functional links. Specifically, cooperative action of Mediator and TFIID in PIC assembly was documented *in vitro*.[93-97] Human Mediator and TFIID are required for both basal and activated *in vitro* transcription in nuclear extracts.[93] In immobilized template assays reconstituted with purified proteins, human Mediator and TFIID assemble cooperatively on promoter DNA suggesting reciprocal and synergistic interactions between template DNA, activators, TFIID and Mediator required for activated transcription.[94] Using biochemical approaches, physical interactions between Mediator and TFIID were identified in yeast[95, 96] and human.[97] Based on *in vitro* experiments, a functional interplay between human Mediator and TFIIB was also suggested[98] and interactions between yeast Mediator sub-complexes and TFIIB were shown.[99] Early Mediator studies demonstrated that *in vitro* Mediator stimulates the phosphorylation by TFIIH kinase of the Pol II CTD in yeast and mammalian cells,[9,100] emphasizing a functional link between Mediator and TFIIH complexes. As mentioned above, the discovery of Mediator complex is closely associated with its functional and direct interaction with Pol II.[4-6,9] These contacts were initially focused on the Pol II CTD and then extended to multiple subunits.[21,45,75,77,89,90,101-104]

Many questions remain to be completely answered on the Mediator contribution to PIC assembly pathways in chromatin context and in gene-specific manner *in vivo*. Complementary to *in vitro* studies, Mediator function, in promoting PIC assembly in concert with the GTFs, was uncovered *in vivo*. In line with cooperative recruitment and action of Mediator and TFIID, TBP recruitment depends on Med17, the central head subunit. Indeed, *med17–138* mutation led to a great decrease in TBP occupancy at several promoters.[105,106] Functional interactions between Drosophila Mediator and TFIID complexes were observed on metal-activated promoters.[107] More recently, an *in vivo* genome-wide study in Mediator Med17 mutants showed that Mediator selectively contributes to TBP recruitment or stabilization in the PIC.[56] Functional inteplay between Mediator and TFIIB was also proposed. Artificial tethering of TFIIB to the promoter led to Mediator recruitment and PIC assembly *in vivo*.[108] A recent genome-wide analysis of all PIC components occupancy showed that mutations in Med10 Mediator middle module that affect Mediator–TFIIB contact led to a pronounced decrease in TFIIB and had a global impact on Pol II recruitment and transcription.[57] This work demonstrated a role of Mediator in PIC assembly on a genomic scale at the step of TFIIB binding. Interestingly, the Mediator mutation effect on PIC assembly was related to

the promoter architecture, suggesting a mechanism for functional interplay between Mediator and TFIIB in the context of promoter chromatin. A direct and functionally important link between Mediator and TFIIH core module was also identified.[56,84,109] An analysis of mutants in Med11 and Med17 Mediator head subunits showed that Mediator stabilizes TFIIK kinase and TFIIH core modules independently.[56,84] In line with close physical and functional associations between Mediator and Pol II, Mediator mutations or downregulation of Mediator subunits were shown to strongly compromise Pol II recruitment and transcription in yeast, mouse and human cells.[20,27,53,56,57,84,110-112] A direct Mediator-Pol II interaction was shown to be generally required for Pol II recruitment and transcription.[21] Using *in vivo* photo-cross-linking strategy, this work identified Rpb3 Pol II subunit that crosslinked to Med17 Mediator head module subunit among the 80 pairwise Pol II-Mediator contacts tested. The functional importance of this contact for the genome-wide recruitment of Pol II in PIC *in vivo* was demonstrated by complementary genetic and genomic approaches suggesting that most transcriptional regulatory information is transmitted through the Mediator-Pol II interface.

The PIC composition extends beyond the GTFs, Pol II and Mediator. For example, TFIIS, a thoroughly characterized transcription elongation factor, was identified as a PIC component by a proteomic analysis.[113] Mediator was found to act in conjunction with TFIIS in PIC assembly for optimal Pol II recruitment.[114] This conclusion is consistent with TFIIS contribution to *in vitro* PIC assembly independently from its role in stimulation of Pol II cleavage activity in transcription elongation.[113] Mediator cooperates also with other coactivators for optimal PIC assembly. Recent proteomic analysis identified Mediator and SAGA as major coactivator complexes within the PIC in HeLa and mouse ES cells.[115] Integration of the Mediator function with those of different chromatin regulators and other coactivator complexes would be important for our understanding of transcription regulation mechanisms.

The model for the PIC assembly in a linear sequence that does not include Mediator cannot explain *in vivo* observations suggesting an independent behavior for PIC components. Based on *in vivo* studies of Mediator mutants in yeast *S. cerevisiae*, a model for PIC assembly through multiple pathways was proposed.[84] Mediator was suggested to independently orchestrate multiple steps of PIC assembly *in vivo*.[56] Several studies support non-linear PIC assembly *in vivo*.[116] In addition, a partial PIC has been proposed as a regulated intermediate in response to heat shock in yeast.[117] Interestingly, a recent single-molecule analysis with human transcriptional platform reconstituted from purified TFIID, TFIIA and TFIIB revealed that promoter binding of TFIIB is highly transient and dynamic.[118] These works do not support the step-wise assembly model of GTFs in the PIC but rather suggest more complex and dynamic relationships between the PIC components.

Mediator function beyond the PIC assembly

In vivo Mediator function in transcription regulation is not limited to promoting the efficient PIC assembly. The Mediator complex could also influence so-called post-recruitment steps of Pol II transcription and modulate the function and activity of the PIC after its formation. The best examples of such a role come from the Mediator studies in metazoan systems.[48,112,119,120] Mediator has been proposed to regulate promoter proximal pausing of Pol II, even though the exact mechanisms remain to be understood. For example, a model for Mediator function in Pol II transition to productive elongation has been proposed.[97] It has been suggested that metazoan-specific Med26 Mediator subunit can have a function helping to switch between transcription initiation to the elongation step by first contacting TFIID and then binding to the super-elongation complexes containing P-TEFb and other proteins.

A growing number of evidences suggest that *in vivo* Mediator function is closely related to chromatin architecture and that this complex can play an active role in 3D chromatin organization. In mouse cells, Mediator interacts with the cohesin complex in enhancer-core promoter loop formation that links very distant genome regions and activates transcription.[59] Mediator is highly enriched in groups of enhancers named super-enhancers important for regulation of lineage-specific genes.[60,61] In yeast, Mediator has been recently proposed as one of the complexes important for 3D genome organization.[121] A recent study suggests that Mediator can be implicated in gene positioning at the nuclear periphery

through a direct contact with TREX-2 complex.[122] Other interesting works suggest that non-coding RNAs (aRNA and eRNA) could be involved in cooperative contacts with Mediator and other nuclear complexes in enhancer-core promoter looping that might represent an important mechanism for transcription regulation.[123,124]

Concluding remarks and perspectives

Combined efforts taking advantage of complementarity between functional genome-wide, genetic, biochemical, structural and imaging approaches will continue to contribute to our understanding of complex mechanisms of Mediator function in transcription regulation. Integrative analyses and modeling will potentially constitute one of the challenges for the near future. It would be necessary to take into account multiple Mediator roles *in vivo*, from its contribution to the PIC assembly, to its function at post-recruitment steps of transcription, but also in chromatin organization and probably more generally in transcription-coupled events (Fig. 2). Specific Mediator subunits within the complex could be involved in different functions and a competition between different proteins to the same interaction interface on the Mediator complex could not be excluded. Future studies are needed to precisely define interaction interfaces involved. *In vitro* studies[125] and a recent work based on single-cell imaging[126] suggested also an active role for Mediator in re-initiation of transcription, but this phenomenon has not been observed *in vivo*. Mediator functions beyond transcription are emerging suggesting that Mediator might act as an assembly platform or a regulatory element in chromatin-related processes including DNA repair[55] or telomere maintenance.[127,128] The modular Mediator organization is central for its functions and the precise mechanisms of Cdk8 kinase module remain to be fully understood. It would be particularly interesting to precisely define how individual Mediator subunits contribute to the overall function of the complex. For the moment, *in vivo* Mediator complex assembly and its regulation remain completely unknown. The yeast model will continue to offer a lot of possibilities to study the molecular mechanisms of Mediator complex that are likely conserved in other eukaryotes including human. Studies in mammalian systems are essential to understand Mediator implications in differentiation, cell type-specific transcription programs and human diseases. In this regard, the focus on metazoan-specific Mediator subunits would be of particular interest.

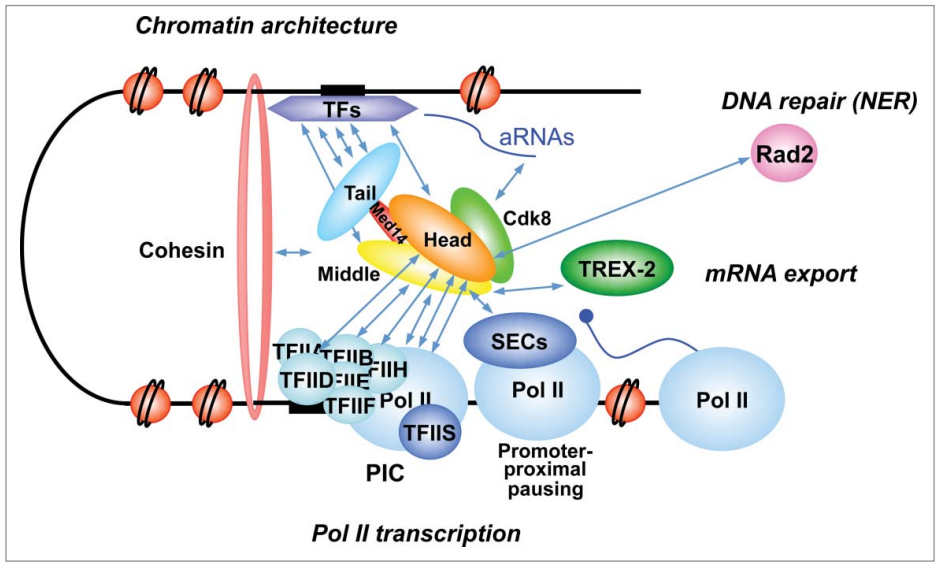

Figure 2. Mediator interactions within the nucleus. Mediator functions are closely related to its physical and functional interactions with nuclear proteins. Some of these contacts discussed in the review are summarized on the figure focusing on Pol II transcription (TF interactions, PIC assembly, promoter proximal pausing), but also extending to chromatin architecture, mRNA export or DNA repair. The cartoon represents a combined view from different studies in yeasts and metazoans. Identified interactions between Mediator and nuclear proteins and ncRNAs are shown. Mediator contacts with SECs, cohesin and ncRNAs were reported in metazoans. Mediator modules are shown in different colors with Med14 linking the three main modules (head, middle and tail).

Disclosure of potential conflicts of interest

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