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A structural perspective on Mediator function

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Gene transcription by RNA polymerase II requires the multiprotein coactivator complex Mediator. Mediator was identified two decades ago, but its molecular mechanisms remain poorly understood, because structural studies are hampered by its large size, modularity, and flexibility. Here we collect all available structural data on Mediator and discuss their functional implications. Progress was made in understanding the interactions of Mediator with gene-specific transcriptional regulators and the general transcription machinery. However, around 80% of the Mediator structure remains unknown and details on the Mediator–Pol II interface are lacking. In the future, an integrated structural biology approach may unravel the functional architecture of Mediator-regulated promoter assemblies and holds the promise of understanding a key mechanism of gene regulation.

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Introduction

In eukaryotes, the regulation of gene expression underlies fundamental biological processes such as cell differentiation, organism development, and biodiversity [1]. A major regulatory target during gene expression is the synthesis of messenger RNA (mRNA) by RNA polymerase II (Pol II). Large coactivator complexes integrate regulatory signals from positive and negative transcription factors and activate or repress transcription [2]. The central coactivator complex Mediator is required for transcription of most, if not all, protein-coding genes. It bridges between gene-specific transcription factors bound to regulatory DNA elements and the general Pol II machinery at the core promoter [3–5] (Figure 1). Mediator is required for the function of many medically important human transcription regulators, including hormone receptors, vitamin D receptor, and p53 [6,7]. Mutations in Mediator have been linked to human diseases, including cancer, cardiovascular diseases, metabolic and neurological disorders [8].

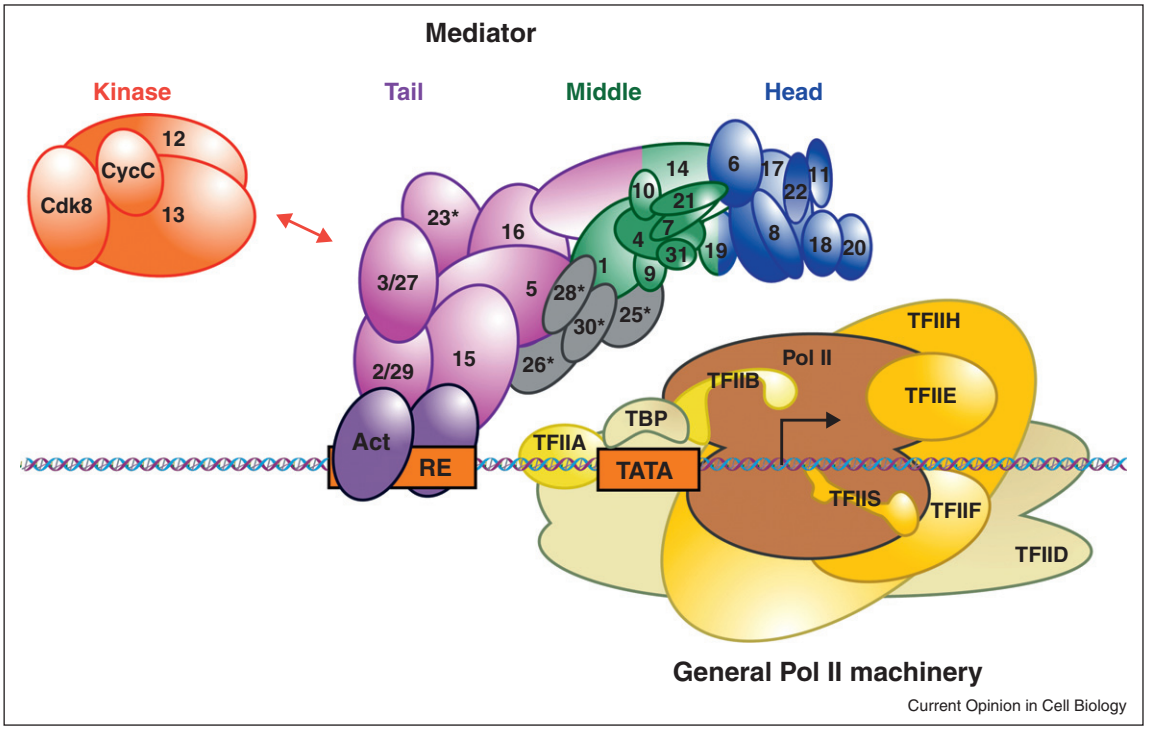
In the yeast *Saccharomyces cerevisiae*, Mediator has a total molecular weight of over 1 MDa and comprises 25 subunits. Five additional subunits were identified in metazoans. Based on electron microscopy (EM) [9–11], biochemical studies [12] and gene expression profiling [13], Mediator subunits were suggested to reside in four modules, the head, middle, tail, and kinase modules. The modular architecture and subunit composition is conserved from yeast to humans (Table 1) [14]. Over the past decade, structural and functional studies in several laboratories have contributed to the understanding of Mediator. Here we provide a complete collection of structural data on Mediator, but focus on recent structural advances and their implications on Mediator architecture and function.

Structural studies of Mediator

The size, intrinsic flexibility, low abundance, and heterogeneity of Mediator have thus far prevented high-resolution structure determination of the complete complex. However, the entire Mediator could be studied by EM at low resolution (Table 2). In addition, several Mediator subunits and subcomplexes were resolved by X-ray crystallography at high resolution. At present, 13 Mediator subunits have been structurally characterized at an atomic level at least partially (Table 3, Figure 2). These studies often relied on extensive engineering and trimming of subunits or subcomplexes, to overcome experimental difficulties owing to intrinsic flexibility or necessary co-folding with interacting subunits. The highly conserved head module, which interacts with the general Pol II machinery, is best characterized, whereas the tail module, which interacts with gene specific transcription factors, is least characterized.

Known crystal structures cover at least part of Mediator subunits Med1, Med6, Med7, Med8, Med11, Med15, Med18, Med21, Med22, Med25, Med31, CDK8, and CycC (Table 3). These studies revealed two heterodimeric four-helix bundles (Med11/Med22, Med7/Med21), a SPOC domain (Med25), a KIX domain (Med15), CYTH-like domains (Med18, Med20), a cyclin-dependent kinase-cyclin pair fold (CDK8/CycC), and an entirely novel fold (Med7/Med31). Most of these folds have not been observed in other transcription-related proteins. Some folds appear duplicated within Mediator, for example, Med18 and Med20 [15] or the four-helix bundle folds in Med11/Med22 and Med7/Med21 [16•]. The

Figure 1



RNA Polymerase II pre-initiation complex comprising Mediator and the general Pol II machinery at the promoter. Mediator bridges between gene specific activators (Act) bound to regulatory DNA elements (RE) and the general transcription machinery comprising Pol II and the general transcription factors TFIIA, TFIIB, TFIID/TBP, TFIIE, TFIIF, and TFIIH, and the factor TFIIS. The transcription start site is indicated with a black arrow. The Mediator modules head, middle, tail, and kinase are colored blue, green, purple, and orange, respectively. Mediator subunits Med14 and Med19 are probably bridging between modules and are therefore shown in two colors. Subunits that are not assigned to any module are colored gray. Yeast Mediator subunits Med2 and Med3 are identical to human Mediator subunits Med29 and Med27, respectively. Subunits present only in higher eukaryotes are marked with an asterisk.

latter are predicted to occur in additional subunits and thus represent a common building block that has been multiplied and functionally diversified during Mediator evolution [16•]. Several of the structurally characterized subcomplexes represent functional submodules that are connected to the rest of the Mediator through flexible linkers [17•,18]. Despite these new studies, still less than 20% of the total Mediator structure is presently known at atomic resolution.

Advances in recombinant co-expression of larger Mediator subassemblies in bacteria or insect cells have enabled preparation and structural studies of entire Mediator modules. Recombinant head module was obtained after co-expression of the subunits in insect cells, and was first studied by EM at low resolution [19]. Recently, a 7-subunit partial backbone model of the head module was derived by X-ray crystallography at 4.3 Å resolution [20••]. This model comprises 60% of the total head module, revealing the topology and interaction network between its subunits. The atomic model of Med18/Med20 [15] was placed into the medium-resolution electron density [20••]. The recent atomic structure of the Med11/Med22 heterodimer [16•] can now also be docked into the head module density. This docking indicates that the two N-terminal helices in Med11 have to be swapped and that the register in the Med22 N-terminal helix has to be adapted in the published backbone model (L Larivière *et al.*, unpublished). The middle module was obtained after co-expression of the subunits in *E. coli*, and its elongated shape and subunit interactions were revealed by small-angle X-ray scattering and ion-mobility and native mass spectrometry [21]. A low-resolution model of the free kinase module was obtained by EM [22]. Despite these advances, atomic structures of Mediator modules and information on the intermodular interactions are still lacking.

Interactions with gene-specific transcriptional regulators

Several studies identified Mediator subunits that are contacted by gene-specific transcriptional regulators [7]. Interactions usually occur between transactivation domains (TADs) in the transcriptional activator and activator-binding domains (ABDs) in Mediator. The interactions are generally weak, in the micromolar range

Table 1

Mediator subunit composition and modular architecture in the yeast *Saccharomyces cerevisiae* (*Sc*) and human (*Hs*). Subunits comprising the evolutionarily highly conserved core Mediator are in bold, subunits required for yeast viability are underlined (adapted from [14])

Module	Subunit	*Sc*	*Hs*
Head	**Med6**	Med6	hMed6/DRIP33/p32
	Med8	Med8	ARC32/mMed8
	Med11	Med11	HSPC296
	Med17	Srb4	TRAP80/DRIP77/CRSP77/p78
	Med18	Srb5	p28b
	Med20	Srb2	hTRFP/p28a
	Med22	Srb6	Surf5
Head/Middle	Med19	Rox3	LCMR1
Middle	Med1	Med1	TRAP220/DRIP205/CRSP200/PBP
	Med4	Med4	TRAP36/DRIP36/p34
	Med7	Med7	hMed7/DRIP34/CRSP33/p36
	Med9	Med9/Cse2	Med25
	Med10	Med10/Nut2	hMed10/hNut2
	Med21	Srb7	hSrb7/p21
	Med31	Soh1	hSoh1
Middle/Tail	**Med14**	Rgr1	TRAP170/DRIP150/CRSP150/p110
Tail	**Med2/29**	Med2	Hintersex
	Med3/27	Med3/Pgd1/Hrs1	TRAP37/CRSP34
	Med5/24	Nut1	TRAP100/DRIP100/CRSP100
	Med15	Gal11	ARC105/PCQAP/TIG-1
	Med16	Sin4	TRAP95/DRIP92/p96b
	Med23	–	TRAP150β/DRIP130/CRSP130/hSur2
Kinase	Med12	Srb8	TRAP230/DRIP240
	Med13	Srb9	TRAP240/DRIP250
	CDK8	Srb10/Ssn3/Ume5	hSrb10/CDK8
	CycC	Srb11/Ssn8/Ume3	hSrb11/CycC
Unassigned	Med25	–	ARC92/ACID1
	Med26	–	ARC70/CRSP70
	Med28	–	Fksg20
	Med30	–	TRAP25

[23•,24•,25•]. However, many weak, cooperative TADs-ABDs contacts may lead to a strong interaction. Several activator-binding DNA motifs are usually present upstream of the promoter, resulting in the simultaneous recruitment of multiple activators. Additionally, most of the TADs can target several ABDs in Mediator, for example, Gcn4 targets the tail subunits Med2, Med3, Med15 and Med16 [26•,27]. Finally, some Mediator subunits contain several ABDs, connected through flexible linkers [26•,28•].

These findings lead to two fundamental questions. First, how can very diverse TADs interact with the same ABDs? Second, how can diverse ABDs interact with the same TADs? Recent structural studies on ABD-TAD interactions addressed these questions [23•,24•,29,30••,31]. Although TADs are very diverse in sequence and ABDs exhibit unrelated folds (Figure 2), their interactions reveal common features. Most TADs are unfolded in their free state and form similar helical segments upon binding to their target ABDs [23•,25•]. These structural transitions are dynamic, such that TADs can adopt multiple conformations on the same target surface, forming a 'fuzzy' complex. This intrinsic conformational flexibility allows TADs to use distinct interaction modes to adapt to unrelated target surfaces.

In addition to recruiting Mediator to target genes, activator binding triggers large-scale structural rearrangements in metazoan Mediator. Consistent with this, a flexible hinge was revealed in the middle module subcomplex Med7/Med21 [32]. Conformational changes in Mediator are thought to promote interaction with Pol II and with other factors to activate transcription. The effects of various activators on Mediator structure have been studied by EM (Table 2). Binding to different activators seems to generate different rearrangements, potentially allowing for the recruitment of a different subset of factors [4,33•,34]. A common feature is the formation of a binding pocket for Pol II [33•,35,36].

Interactions with the general Pol II machinery

Saccharomyces cerevisiae and *Schizosaccharomyces pombe* Mediator complexes were first isolated together with Pol II in so-called holoenzymes, suggesting a direct Mediator–Pol II interaction [37–39]. A recent crosslinking

Table 2

EM reconstructions of Mediator, its modules and complexes with interacting factors

Module(s)	Subunit(s)	Org.[a]	Interacting factor(s)	Method[b]	Resolution (Å)	Ref.[c]
Head	6/8/11/17/18/20/22	*Sc*		NS	30–35	[19]
	6/8/11/17/18/20/22	*Sc*	Rpb4/7	NS	n.a.	[19]
	6/8/11/17/22	*Sc*		NS	n.a.	[19]
	6/8/11/17/22	*Sc*	TBP	NS	n.a.	[19]
	11/17/22	*Sc*		NS	n.a.	[19]
Kinase	12/13/CDK8/CycC	*Hs*		NS	38	[22]
	12/CDK8/CycC	*Hs*		NS	41	[22]
Head + Middle		*Sc*	Pol II	NS	n.a.	[11]
Head + Middle + Tail		*Sc*		cryo	28	[43]
		Sc		NS	30–35	[11]
		Sc		NS	~40	[9]
		Sc	Pol II	NS	~35	[10]
		Sc	Pol II	NS	~40	[9]
		Sp		NS	~25	[42]
		Sp	Pol II	cryo	~25	[42]
		Mm		NS	30–35	[11]
		Mm		NS	~40	[9]
		Hs		NS	32	[35]
		Hs		NS	31	[47]
		Hs		NS	30–35	[11]
		Hs	p53	NS	34	[33]
		Hs	p53 AD	cryo-NS	n.a.	[33]
		Hs	p53 AD	NS	34	[33]
		Hs	p53 CTD[e]	NS	38	[33]
		Hs	p53 ΔCTD[e]	NS	36	[33]
		Hs	Vitamin D receptor	NS	29	[36]
		Hs	Thyroid hormone receptor	NS	29	[36]
		Hs	SREBP-1a AD[d]	NS	32	[35]
		Hs	VP16 AD[d]	NS	32	[35]
		Hs	Pol II CTD[e]	NS	32	[48]
		Hs	Pol II + VP16 AD	cryo-NS	34 and 36	[41••]
		Hs	Pol II + TFIIF + VP16 AD	cryo-NS	36	[41••]
Head + Middle + Tail + Kinase		*Sp*		NS	~25	[42]
		Hs		NS	32	[35]
		Hs	TRAPP/GCN5L	NS	n.a.	[49]

[a] Organism: Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*; Mm, *Mus musculus*; Hs, *Homo sapiens*.
[b] NS, negative staining.
[c] None of the reported EM reconstructions and corresponding fits were deposited in public databases, except for [41••] where the reconstruction of the Pol II/TFIIF/Mediator complex was deposited in the EMDB under the accession number 5343 and the corresponding fits were deposited in the PDB under the accession code 3J0K.
[d] AD, activation domain.
[e] CTD, carboxy-terminal domain.

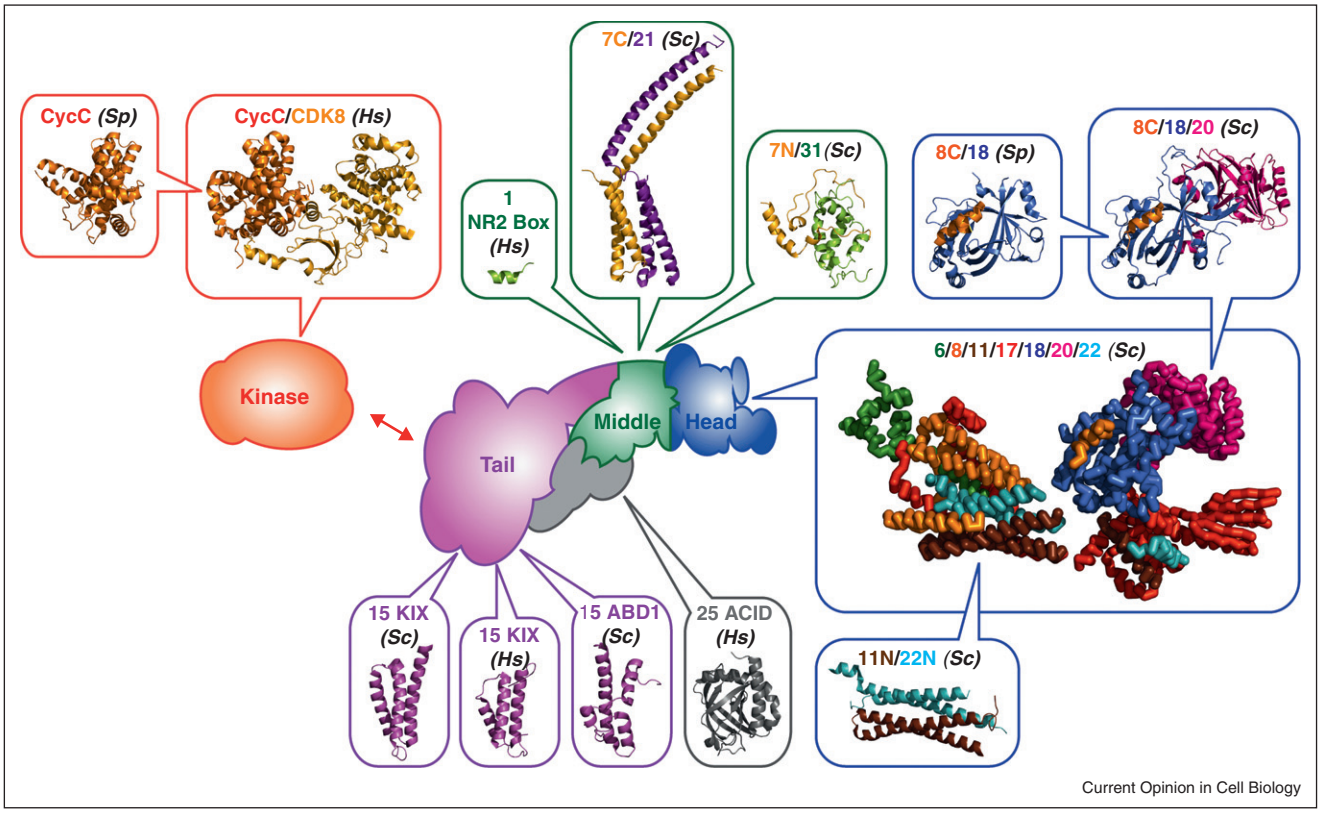
Table 3

Available crystal and NMR structures for Mediator subunit domains, subunits, and subcomplexes

Module	Subunit(s)	Org. [a]	Construct	Interacting factor(s)	Method	PDB code(s)	Resolution (Å)	Ref.
Head	6/8/11/17/18/20/22	*Sc*	6/8/11/17ΔN108/18Δ (109–140)/20/22		X-ray	3RJ1	4.3	[20••]
	8/18/20	*Sc*	8(190–210)/8Δ(109–140)/20		X-ray	2HZS	2.7	[15]
	18/20	*Sc*	18Δ(109–140)/20		X-ray	2HZM	2.4	[15]
	8/18	*Sp*	8(180–200)/18		X-ray	3C0 T	2.4	[18]
	11/22	*Sc*	11(5–89)/22(1–89)		X-ray	3R84	2.05	[16•]
Middle	7/21	*Sc*	7(102–205)/21		X-ray	1YKE, 1YKH	3.3, 3.0	[32]
	7/31	*Sc*	7(1–83)/31		X-ray	3FBI	2.8	[17•]
	1	*Hs*	1(640–652)	Vitamin D receptor LBD[b]	X-ray	2ZMI[c]	1.7–3.0	[50]
Tail	15	*Sc*	15(158–238)	Gcn4 central AD[d]	NMR	2KO4	–	[25•]
	15	*Sc*	15(6–90)		NMR	2K0 N	–	[30••]
	15	*Sc*	15(6–90)	SREBP-1a AD[d]	NMR-CSP[e]	–	–	[30••]
	15	*Sc*	15(6–90)	Pdr1p AD[d]	NMR-CSP[e]	–	–	[30••]
	15	*Sc*	15(2–100)	Gcn4 AD[d]	NMR-CSP[e]	–	–	[28•]
	15	*Hs*	15(5–78)		NMR	2GUT	–	[31]
	15	*Hs*	15(5–78)	SREBP-1a AD[d]	NMR-CSP[e]	–	–	[31]
Kinase	CycC	*Sp*	CycC		X-ray	1ZP2	3	[51]
	CDK8/CycC	*Hs*	CDK8(1–403)/CycC		X-ray	3RGF	2.2	[52]
Unassigned	25	*Hs*	25(394–543)[f]		NMR	2KY6, 2L23, 2L6U, 2XNF	–	[23•,24•,29,53]
	25	*Hs*	25(394–543)[f]	VP16 AD[d]	NMR-CSP[e]	–	–	[23•,24•,29]

[a] Organism: Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*; Hs, *Homo sapiens*.
[b] LBD, Ligand-binding domain.
[c] 17 structures were reported in total, 2ZMI corresponds to the highest resolution structure.
[d] AD, activation domain.
[e] NMR-CSP, NMR chemical shift perturbation analysis, not deposited in the PDB.
[f] Constructs vary slightly between the different structures.

Figure 2



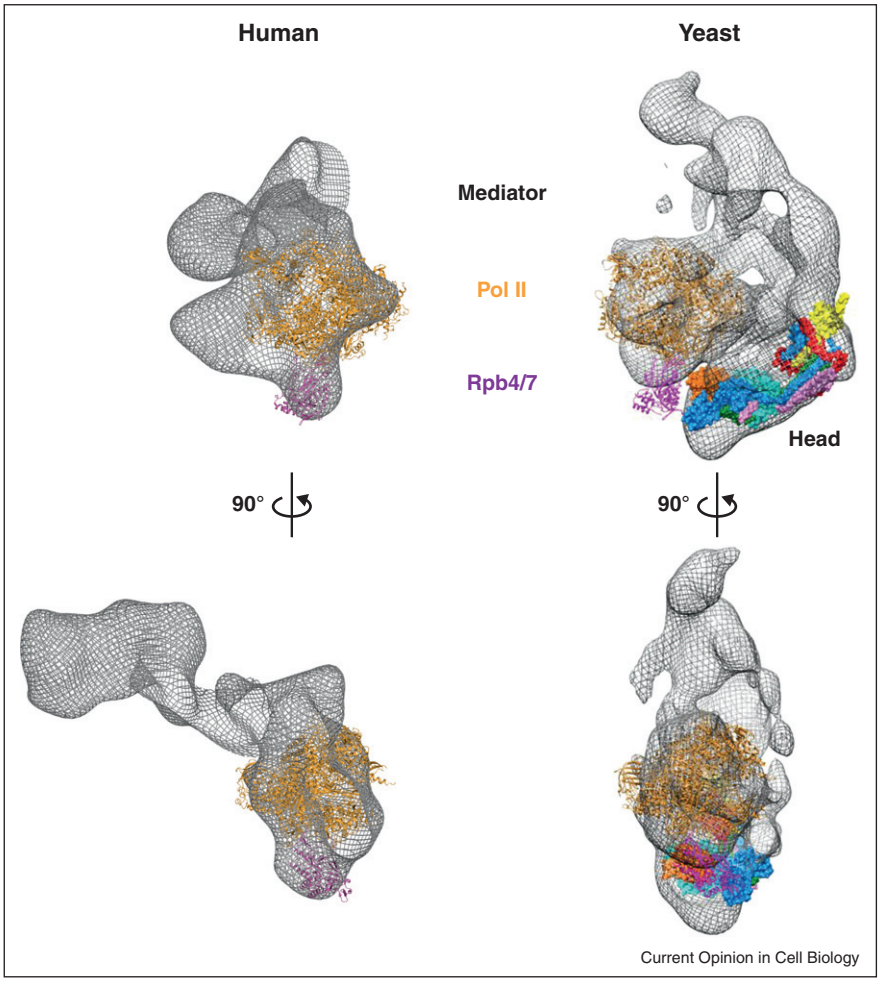
Atomic structures of Mediator subcomplexes. All currently available crystal and NMR structures (see Table 3) are shown indicating the respective locations within Mediator. In addition, the crystallographic backbone model of the complete *S. cerevisiae* head module is depicted [20••].

study supported the direct interaction and demonstrated its requirement for Pol II transcription [40••]. Although human Mediator and Pol II can be co-immunoprecipitated, the human Mediator–Pol II complex cannot be purified in large quantities directly from cells and thus has to be assembled *in vitro* (personal communication C Bernecky, [41••]). Different Mediator–Pol II complexes were investigated by EM (Table 2) [10,19,41••,42]. A common feature of yeast and human Mediator–Pol II complexes is a large structural change in Mediator upon Pol II binding. While EM reconstructions of free yeast and activator-bound human Mediator are similar [43], the various holoenzyme reconstructions differ (Figure 3).

Fitting of the Pol II X-ray structure into the cryo-EM reconstructions led to different locations and orientations of Pol II within the holoenzyme (Figure 3). In the *Saccharomyces cerevisiae* complex, Pol II was placed such that its Rpb3/Rpb11 subcomplex faces Mediator, but in two different orientations that differ by an approximately 90-degree rotation [10,43]. By contrast, Pol II was placed into an EM reconstruction of the human holoenzyme such that Mediator binds the Rpb2 side of Pol II at the lobe and protrusion domains, and the Rpb3/Rpb11 side is free [41••]. Similarly, the Mediator kinase module was reported to adopt very different positions on human and fission yeast Mediator [22,42]. This led to different conclusion, namely that the kinase module either prevents Pol II binding through steric hindrance or through an allosteric mechanism [41••,42]. The differences in Pol II and kinase module position could be due to the low resolution, but also due to limited sequence conservation in Mediator or to the presence of additional factors in holoenzyme preparations, like TFIIF in the case of the human complex.

Mediator not only interacts with Pol II, but also with general transcription factors, expanding its role in stabilizing the pre-initiation complex on the promoter. First hints on an interaction with TFIIH came from early experiments that showed that Mediator can stimulate the Pol II C-terminal domain kinase activity of TFIIH [37]. Later, it was reported that Mediator directly binds TFIIH through the head subunit Med11 and can recruit it to the promoter, even in the absence of Pol II [44]. It was recently shown that the Med11/Med22 heterodimer exhibits a conserved surface that may mediate this interaction [16•]. Interaction of TBP with the Mediator head module

Figure 3



EM reconstructions (black mesh) of Mediator from human (left, [41••]) and yeast (right, [20••]) with Pol II in a similar orientation (orange, Rpb4/7 in pink). The head module backbone model is placed in the yeast reconstruction (space-filling model).
Partially adapted from [20••].

was reported [12,45], and was shown later to occur at least partially through Med8 [15]. This is consistent with a recent EM study of a Mediator head–TBP complex [19]. In higher eukaryotes, an interaction between Mediator subunit Med26 and TFIID was recently identified [46]. These data suggest that Mediator stabilizes the preinitiation complex at the core promoter by tethering Pol II to TBP, TFIIH and TFIID.

Conclusions

Over the past decade, efforts in several laboratories and the application of different structural biology techniques have advanced our understanding of Mediator structure and function. However, most of the Mediator structure is still unknown and it is still unclear how Mediator interacts with the Pol II initiation complex and which structural transitions it may influence during transcription initiation. These points can probably only be addressed with an integrated structural biology approach that combines classical techniques such as cryo-EM, X-ray crystallography, and nuclear magnetic resonance with new technologies such as Förster resonance energy transfer and mass spectrometry-based mapping of chemical crosslinks. Such an approach poses a formidable challenge for the next decade. But it holds the promise of understanding key aspects of the mechanism of gene regulation.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Levine M, Tjian R: **Transcription regulation and animal diversity**. *Nature* 2003, **424**:147-151.

2. Sikorski TW, Buratowski S: **The basal initiation machinery: beyond the general transcription factors**. *Curr Opin Cell Biol* 2009, **21**:344-351.

3. Kornberg R: **Mediator and the mechanism of transcriptional activation**. *Trends Biochem Sci* 2005, **30**:235-239.

4. Taatjes D: **The human Mediator complex: a versatile, genome-wide regulator of transcription**. *Trends Biochem Sci* 2010, **35**:315-322.

5. Conaway RC, Conaway JW: **Origins and activity of the Mediator complex**. *Semin Cell Dev Biol* 2011, **22**:729-734.

6. Malik S, Roeder RG: **The metazoan Mediator co-activator complex as an integrative hub for transcriptional regulation**. *Nat Rev Genet* 2010, **11**:761-772.

7. Borggrefe T, Yue X: **Interactions between subunits of the Mediator complex with gene-specific transcription factors**. *Semin Cell Dev Biol* 2011, **22**:759-768.

8. Napoli C, Sessa M, Infante T, Casamassimi A: **Unraveling framework of the ancestral Mediator complex in human diseases**. *Biochimie* 2011 doi: 10.1016/j.biochi.2011.09.016.

9. Asturias FJ, Jiang YW, Myers LC, Gustafsson CM, Kornberg RD: **Conserved structures of mediator and RNA polymerase II holoenzyme**. *Science* 1999, **283**:985-987.

10. Davis J, Takagi Y, Kornberg R, Asturias F: **Structure of the yeast RNA polymerase II holoenzyme: Mediator conformation and polymerase interaction**. *Mol Cell* 2002, **10**:409-415.

11. Dotson MR, Yuan CX, Roeder RG, Myers LC, Gustafsson CM, Jiang YW, Li Y, Kornberg RD, Asturias FJ: **Structural organization of yeast and mammalian mediator complexes**. *Proc Natl Acad Sci USA* 2000, **97**:14307-14310.

12. Kang J, Kim S, Hwang M, Han S, Lee Y, Kim Y: **The structural and functional organization of the yeast mediator complex**. *J Biol Chem* 2001, **276**:42003-42010.

13. van de Peppel J, Kettelarij N, van Bakel H, Kockelkorn T, van Leenen D, Holstege F: **Mediator expression profiling epistasis reveals a signal transduction pathway with antagonistic submodules and highly specific downstream targets**. *Mol Cell* 2005, **19**:511-522.

14. Bourbon H-M: **Comparative genomics supports a deep evolutionary origin for the large, four-module transcriptional mediator complex**. *Nucleic Acids Res* 2008, **36**:3993-4008.

15. Lariviere L, Geiger S, Hoeppner S, Rother S, Strasser K, Cramer P: **Structure and TBP binding of the Mediator head subcomplex Med8-Med18-Med20**. *Nat Struct Mol Biol* 2006, **13**:895-901.

16. • Seizl M, Lariviere L, Pfaffeneder T, Wenzeck L, Cramer P: **Mediator head subcomplex Med11/22 contains a common helix bundle building block with a specific function in transcription initiation complex stabilization**. *Nucleic Acids Res* 2011, **39**:6291-6304.
Structural and bioinformatic analysis identifies a common helix bundle building block that has been multiplied and functionally diversified during Mediator evolution.

17. • Koschubs T, Seizl M, Lariviere L, Kurth F, Baumli S, Martin DE, Cramer P: **Identification, structure, and functional requirement of the Mediator submodule Med7N/31**. *EMBO J* 2009, **28**:69-80.
This study shows that Med7N/31 constitutes a structural and functional Mediator middle submodule required for the expression of a specific subset of genes.

18. Lariviere L, Seizl M, van Wageningen S, Rother S, van de Pasch L, Feldmann H, Strasser K, Hahn S, Holstege FC, Cramer P: **Structure-system correlation identifies a gene regulatory Mediator submodule**. *Genes Dev* 2008, **22**:872-877.

19. Cai G, Imasaki T, Yamada K, Cardelli F, Takagi Y, Asturias FJ: **Mediator head module structure and functional interactions**. *Nat Struct Mol Biol* 2010, **17**:273-279.

20. •• Imasaki T, Calero G, Cai G, Tsai KL, Yamada K, Cardelli F, Erdjument-Bromage H, Tempst P, Berger I, Kornberg GL *et al.*: **Architecture of the Mediator head module**. *Nature* 2011, **475**:240-243.
The partial backbone model of the essential Mediator head module reveals the intricate interaction network of its seven subunits.

21. Koschubs T, Lorenzen K, Baumli S, Sandstrom S, Heck AJ, Cramer P: **Preparation and topology of the Mediator middle module**. *Nucleic Acids Res* 2010, **38**:3186-3195.

22. Knuesel MT, Meyer KD, Bernecky C, Taatjes DJ: **The human CDK8 subcomplex is a molecular switch that controls Mediator coactivator function**. *Genes Dev* 2009, **23**:439-451.

23. • Vojnic E, Mourao A, Seizl M, Simon B, Wenzeck L, Lariviere L, Baumli S, Baumgart K, Meisterernst M, Sattler M *et al.*: **Structure and VP16 binding of the Mediator Med25 activator interaction domain**. *Nat Struct Mol Biol* 2011, **18**:404-409.
The studies of Vojnic *et al.* and Milbradt *et al.* show how Med25 is targeted by the viral activator VP16. Comparison with other VP16 targets reveal how a single transactivation domain can adapt to unrelated target surfaces.

24. • Milbradt AG, Kulkarni M, Yi T, Takeuchi K, Sun ZY, Luna RE, Selenko P, Naar AM, Wagner G: **Structure of the VP16 transactivator target in the Mediator**. *Nat Struct Mol Biol* 2011, **18**:410-415.
The studies of Vojnic *et al.* and Milbradt *et al.* show how Med25 is targeted by the viral activator VP16. Comparison with other VP16 targets reveal how a single transactivation domain can adapt to unrelated target surfaces.

25. • Brzovic PS, Heikaus CC, Kisselev L, Vernon R, Herbig E, Pacheco D, Warfield L, Littlefield P, Baker D, Klevit RE *et al.*: **The acidic transcription activator gcn4 binds the mediator subunit gal11/med15 using a simple protein interface forming a fuzzy complex**. *Mol Cell* 2011, **44**:942-953.
This structural study shows how an acidic activator binds to its target in multiple conformations and orientations using simple hydrophobic interactions.

26. • Herbig E, Warfield L, Fish L, Fishburn J, Knutson BA, Moorefield B, Pacheco D, Hahn S: **Mechanism of Mediator recruitment by tandem Gcn4 activation domains and three Gal11 activator-binding domains**. *Mol Cell Biol* 2010, **30**:2376-2390.
The studies of Herbig *et al.* and Jedidi *et al.* show how the archetypical activator Gcn4 recruits Mediator through multiple low-affinity protein–protein interactions with the tail subunit Med15.

27. Zhang F, Sumibcay L, Hinnebusch AG, Swanson MJ: **A triad of subunits from the Gal11/tail domain of Srb mediator is an *in vivo* target of transcriptional activator Gcn4p**. *Mol Cell Biol* 2004, **24**:6871-6886.

28. • Jedidi I, Zhang F, Qiu H, Stahl SJ, Palmer I, Kaufman JD, Nadaud PS, Mukherjee S, Wingfield PT, Jaroniec CP *et al.*: **Activator Gcn4 employs multiple segments of Med15/Gal11, including the KIX domain, to recruit mediator to target genes *in vivo***. *J Biol Chem* 2010, **285**:2438-2455.
The studies of Herbig *et al.* and Jedidi *et al.* show how the archetypical activator Gcn4 recruits Mediator through multiple low-affinity protein–protein interactions with the tail subunit Med15.

29. Bontems F, Verger A, Dewitte F, Lens Z, Baert JL, Ferreira E, de Launoit Y, Sizun C, Guittet E, Villeret V *et al.*: **NMR structure of the human Mediator MED25 ACID domain**. *J Struct Biol* 2011, **174**:245-251.

30. •• Thakur JK, Arthanari H, Yang F, Pan SJ, Fan X, Breger J, Frueh DP, Gulshan K, Li DK, Mylonakis E *et al.*: **A nuclear receptor-like pathway regulating multidrug resistance in fungi**. *Nature* 2008, **452**:604-609.
This study reveals a nuclear receptor-like gene regulatory pathway in fungi. This pathway relies on Mediator recruitment through its Med15 subunit for gene activation.

31. Yang F, Vought BW, Satterlee JS, Walker AK, Jim Sun ZY, Watts JL, DeBeaumont R, Saito RM, Hyberts SG, Yang S *et al.*: **An**

ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. *Nature* 2006, **442**:700-704.

32. Baumli S, Hoeppner S, Cramer P: **A conserved mediator hinge revealed in the structure of the MED7.MED21 (Med7.Srb7) heterodimer**. *J Biol Chem* 2005, **280**:18171-18178.

33. • Meyer KD, Lin SC, Bernecky C, Gao Y, Taatjes DJ: **p53 activates transcription by directing structural shifts in Mediator**. *Nat Struct Mol Biol* 2010, **17**:753-760.
EM analysis shows how the activator p53 can trigger different structural rearrangements within Mediator that modulate Pol II activity.

34. Ebmeier CC, Taatjes DJ: **Activator-Mediator binding regulates Mediator-cofactor interactions**. *Proc Natl Acad Sci USA* 2010, **107**:11283-11288.

35. Taatjes DJ, Naar AM, Andel F 3rd, Nogales E, Tjian R: **Structure, function, and activator-induced conformations of the CRSP coactivator**. *Science* 2002, **295**:1058-1062.

36. Taatjes DJ, Schneider-Poetsch T, Tjian R: **Distinct conformational states of nuclear receptor-bound CRSP-Med complexes**. *Nat Struct Mol Biol* 2004, **11**:664-671.

37. Kim YJ, Bjorklund S, Li Y, Sayre MH, Kornberg RD: **A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II**. *Cell* 1994, **77**:599-608.

38. Koleske AJ, Young RA: **An RNA polymerase II holoenzyme responsive to activators**. *Nature* 1994, **368**:466-469.

39. Spahr H, Beve J, Larsson T, Bergstrom J, Karlsson KA, Gustafsson CM: **Purification and characterization of RNA polymerase II holoenzyme from *Schizosaccharomyces pombe***. *J Biol Chem* 2000, **275**:1351-1356.

40. •• Soutourina J, Wydau S, Ambroise Y, Boschiero C, Werner M: **Direct interaction of RNA polymerase II and mediator required for transcription *in vivo***. *Science* 2011, **331**:1451-1454.
This study reveals a direct interaction *in vivo* between the Pol II subunit Rpb3 and the Mediator head subunit Med17.

41. •• Bernecky C, Grob P, Ebmeier CC, Nogales E, Taatjes DJ: **Molecular architecture of the human Mediator-RNA polymerase II-TFIIF assembly**. *PLoS Biol* 2011, **9**:e1000603.
The EM reconstruction of human Mediator–RNA polymerase–TFIIF complex provides a model for the entire human pre-initiation complex.

42. Elmlund H, Baraznenok V, Lindahl M, Samuelsen CO, Koeck PJ, Holmberg S, Hebert H, Gustafsson CM: **The cyclin-dependent kinase 8 module sterically blocks Mediator interactions with RNA polymerase II**. *Proc Natl Acad Sci USA* 2006, **103**:15788-15793.

43. Cai G, Imasaki T, Takagi Y, Asturias FJ: **Mediator structural conservation and implications for the regulation mechanism**. *Structure* 2009, **17**:559-567.

44. Esnault C, Ghavi-Helm Y, Brun S, Soutourina J, Van Berkum N, Boschiero C, Holstege F, Werner M: **Mediator-dependent recruitment of TFIIH modules in preinitiation complex**. *Mol Cell* 2008, **31**:337-346.

45. Koleske AJ, Buratowski S, Nonet M, Young RA: **A novel transcription factor reveals a functional link between the RNA polymerase II CTD and TFIID**. *Cell* 1992, **69**:883-894.

46. Takahashi H, Parmely TJ, Sato S, Tomomori-Sato C, Banks CAS, Kong SE, Szutorisz H, Swanson SK, Martin-Brown S, Washburn MP *et al.*: **Human Mediator subunit MED26 functions as a docking site for transcription elongation factors**. *Cell* 2011, **146**:92-104.

47. Taatjes DJ, Tjian R: **Structure and function of CRSP/Med2; a promoter-selective transcriptional coactivator complex**. *Mol Cell* 2004, **14**:675-683.

48. Naar AM, Taatjes DJ, Zhai W, Nogales E, Tjian R: **Human CRSP interacts with RNA polymerase II CTD and adopts a specific CTD-bound conformation**. *Genes Dev* 2002, **16**:1339-1344.

49. Meyer KD, Donner AJ, Knuesel MT, York AG, Espinosa JM, Taatjes DJ: **Cooperative activity of cdk8 and GCN5L within Mediator directs tandem phosphoacetylation of histone H3**. *EMBO J* 2008, **27**:1447-1457.

50. Nakabayashi M, Yamada S, Yoshimoto N, Tanaka T, Igarashi M, Ikura T, Ito N, Makishima M, Tokiwa H, DeLuca HF *et al.*: **Crystal structures of rat vitamin D receptor bound to adamantyl vitamin D analogs: structural basis for vitamin D receptor antagonism and partial agonism**. *J Med Chem* 2008, **51**:5320-5329.

51. Hoeppner S, Baumli S, Cramer P: **Structure of the mediator subunit cyclin C and its implications for CDK8 function**. *J Mol Biol* 2005, **350**:833-842.

52. Schneider EV, Bottcher J, Blaesse M, Neumann L, Huber R, Maskos K: **The structure of CDK8/CycC implicates specificity in the CDK/cyclin family and reveals interaction with a deep pocket binder**. *J Mol Biol* 2011, **412**:251-266.

53. Eletsky A, Ruyechan WT, Xiao R, Acton TB, Montelione GT, Szyperski T: **Solution NMR structure of MED25(391-543) comprising the activator-interacting domain (ACID) of human mediator subunit 25**. *J Struct Funct Genomics* 2011, **12**:159-166.