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PRIMER

The Mediator complex: a master coordinator of transcription and cell lineage development

Jing-wen Yin and Gang Wang*

ABSTRACT

Mediator is a multiprotein complex that is required for gene transcription by RNA polymerase II. Multiple subunits of the complex show specificity in relaying information from signals and transcription factors to the RNA polymerase II machinery, thus enabling control of the expression of specific genes. Recent studies have also provided novel mechanistic insights into the roles of Mediator in epigenetic regulation, transcriptional elongation, termination, mRNA processing, noncoding RNA activation and super enhancer formation. Based on these specific roles in gene regulation, Mediator has emerged as a master coordinator of development and cell lineage determination. Here, we describe the most recent advances in understanding the mechanisms of Mediator function, with an emphasis on its role during development and disease.

KEY WORDS: Mediator complex, Transcription, Cell fate, Lineage development, Disease

Introduction

Mediator is a large, multisubunit complex that was discovered following efforts to understand how RNA polymerase II (Pol II)-mediated transcription is regulated by transcription factors in yeast (Nonet and Young, 1989; Kelleher et al., 1990; Thompson et al., 1993; Kim et al., 1994). The complex, which consists of ~30 polypeptides (Fig. 1), shows conservation from yeast to humans and plays an indispensable role in regulating transcription. Multiple laboratories then used a variety of procedures to isolate mammalian Mediator complexes, which were named TRAP/SMCC, NAT, ARC, DRIP, Srb/MED, PC2, CRSP and mouse Mediator (Jiang et al., 1998; Sun et al., 1998; Boyer et al., 1999; Ito et al., 1999; Kingston, 1999; Näär et al., 1999; Rachez et al., 1999; Ryu et al., 1999; Malik et al., 2000). In 2004, a unified nomenclature for Mediator was established, consisting of MED1 to MED31, together with the cyclin-dependent kinase (CDK) 8-cyclin C pair and several paralogs such as MED1-like (MED1L), MED12L, MED13L and CDK19 (Bourbon et al., 2004). The Mediator complex can be divided into four distinct modules termed the head, middle, tail and CDK8 kinase module, which contains CDK8 (or its paralog CDK19), cyclin C, MED12 (or MED12L) and MED13 (or MED13L) subunits (Malik and Roeder, 2010; Taatjes, 2010). Importantly, the subunit composition of Mediator can vary and is not restricted to a single isoform. For example, immunodepletion of the Mediator complex from HeLa nuclear extracts with an anti-CDK8 antibody revealed that Mediator exists as at least two main isoforms, distinguished by the presence or absence of the CDK8 submodule (Wang et al., 2001). In addition, MED1 and MED26 are not present in all isolated isoforms (Malik and Roeder, 2010).

After the initial discovery of Mediator, research mainly focused on how the Mediator complex conveys signals from transcription factors to the Pol II machinery and general transcription factors (GTFs). These studies led to the formulation of the 'bridge' model, in which Mediator connects the transcription factor and Pol II machineries and promotes formation of the pre-initiation complex (Biddick and Young, 2005; Björklund and Gustafsson, 2005; Malik and Roeder, 2005). However, it soon emerged that multiple pathways responsible for cell growth, differentiation or tissue development were able to converge on one or more of the almost 30 subunits of Mediator through transcriptional regulators, suggesting that Mediator acts as a centralized 'hub' or 'integrator' for transcriptional regulation (Malik and Roeder, 2010; Carlsten et al., 2013). Recently, an increasing number of studies have revealed new functions for Mediator, highlighting its involvement in almost all stages of Pol II transcription, including epigenetic regulation, transcriptional elongation, termination, mRNA processing, noncoding RNA activation and super enhancer formation (Fig. 2). Recent evidence has also highlighted a role for the Mediator complex in developmental abnormalities, cancer and metabolic disorders. It thus seems that Mediator acts as a master coordinator that regulates multiple aspects of transcription to ensure the accurate intensity, pattern and timing of global gene expression both during development and in adults. Here, we describe the most recent studies of the Mediator complex, with an emphasis on its functions in development and disease.

Molecular mechanisms of Mediator function in transcriptional control

In addition to interacting with many transcription factors, an increasing number of studies have indicated that the Mediator complex can serve as the interface for multiple transcriptional co-factors, noncoding RNAs and other factors (Table 1). Below, we review selected interactions between well-defined co-factors/complexes and Mediator and discuss their potential effects on gene expression and their developmental consequences.

Interactions with master regulators of cell fate

Some DNA-binding transcription factors, also called 'master regulators', have the ability to determine lineage-specific transcriptional programs. For example, MyoD, PPARγ and Runx2 control the gene programs leading to differentiation into skeletal muscle, adipocytes and osteocytes, respectively (Davis et al., 1987; Tontonoz et al., 1995; Komori, 2002). More recently, four 'Yamanaka' factors [Oct4 (also known as Pou5f1), Sox2, c-Myc and Klf4] were shown to directly reprogram many types of somatic cells into pluripotent stem cells (Takahashi and Yamanaka, 2006; Wernig et al., 2008). In addition, many lineage-specific or non-specific transcription factors have been used to convert fibroblasts to particular cell fates,

State Key Laboratory of Cell Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China.

*Author for correspondence (gwang@sibcb.ac.cn)

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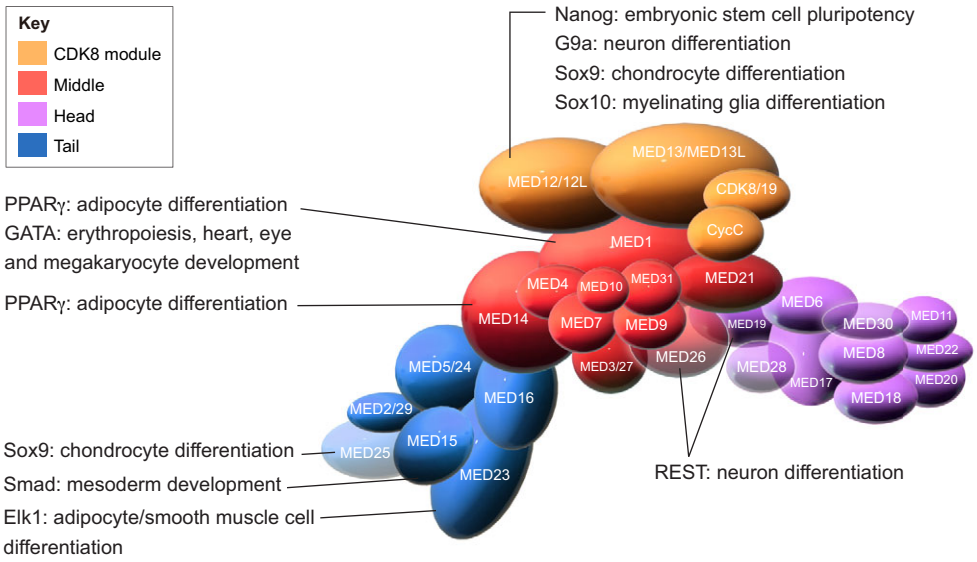

Fig. 1. Composition of the mammalian Mediator complex. The predicted composition of the complex and the relative sizes of individual subunits are based on the published literature. The positions of several subunits (e.g. MED25, MED26, MED28 and MED30) are postulated, as denoted by their transparency (see Larivière et al., 2012). Several representative transcription factor-Mediator subunit interactions and their involvement in developmental processes are indicated.

such as cardiomyocytes, blood cells, neurons and hepatocytes (Ieda et al., 2010; Szabo et al., 2010; Vierbuchen et al., 2010; Huang et al., 2011; Sekiya and Suzuki, 2011). A common theme seems to be that, once the key transcription regulator(s) had been identified and were overexpressed, the cell fate could be reprogrammed (i.e. changed). Since the discovery of the Mediator complex, many such master regulators have been found to target one or more Mediator subunits for their transcriptional activities, which led to the notion that the Mediator complex acts as a master coordinator of cell fate determination. For example, MED1 is targeted by the adipocyte master regulator PPARγ (Fig. 1) (Ge et al., 2002). Later research revealed that the association between MED1 and the erythroid regulator GATA1 determined blood cell lineage development (Stumpf et al., 2006). Importantly, the actions of PPARγ and of GATA1 are likely to be separated by developmental timing and space and it is thus unlikely that these two factors could simultaneously act upon a cell lineage and result in confused cell identity.

Mediator subunits can also play antagonistic roles in lineage specification. We recently demonstrated that the presence or absence of MED23 in mesenchymal stem cells can tune up or down two gene programs resulting in two distinct cell fates, namely adipocyte or smooth muscle cells (Fig. 1) (Wang et al., 2009; Yin et al., 2012). Additional examples of lineage regulation by Mediator include the interaction between the chondrogenesis master regulator Sox9 and MED12 and MED25 (Zhou et al., 2002; Nakamura et al., 2011), and the interaction between the pluripotency factor Nanog and MED12 (Fig. 1) (Tutter et al., 2009). Thus, in its role as an interface between transcription factors and the Pol II transcription machinery, the Mediator complex is able to orchestrate multiple master regulators for specifying distinct cell lineages, supporting the idea that Mediator may qualify as a master coordinator for cell lineage specification. It should be noted, however, that as a master coordinator, a Mediator subunit might be required for a particular master regulator to direct the cell lineage, but overexpression of the subunit should not be sufficient to change the cell fate.

Mediator and the cohesin complex for cell type-specific gene activity

DNA loop formation, which promotes communication between enhancer-bound transcription factors and the general transcription machinery at the core promoter region, plays an important role during gene activation. This looping can be mediated in part by the cohesin complex (Dorsett, 2011; Remeseiro et al., 2013). Chromatin immunoprecipitation followed by sequencing (ChIP-seq) and biochemical analyses revealed that Mediator collaborates with the cohesin complex to link enhancers to core promoters to activate transcription of different sets of genes in mouse embryonic stem cells (ESCs) and mouse embryonic fibroblasts (MEFs) (Kagey et al., 2010). Short hairpin RNA (shRNA)-mediated reduction of the components of either the Mediator complex or the cohesin complex yielded similar phenotypes: loss of the ESC state, as indicated by reduced Oct4 expression and disrupted ESC colonies (Kagey et al., 2010; Apostolou et al., 2013; Phillips-Cremins et al., 2013). The direct interaction between Mediator and the cohesin complex, together with the cell type-specific co-occupation of the Mediator-cohesin complex at distinct genomic regions, suggests that the Mediator-cohesin complex promotes cell type-specific gene activation through enhancer-promoter DNA looping.

Mediator mediates ‘super enhancer’ formation for cell identity

How does Mediator function with the master regulators of different lineage programs? Recent work by the Young group suggests a mechanistic relationship between the master regulators and the Mediator complex (Whyte et al., 2013). This study demonstrated that the Mediator complex can promote the formation of super enhancers, which are clusters of enhancers occupied by both Mediator and master regulator(s). Such super enhancers are usually established with different master transcription factors and act to control the key cell identity genes in different cell types such as ESCs, pro-B cells, myotubes, T helper cells and macrophages (Whyte et al., 2013). Accordingly, reduced levels of Mediator or master transcription factors result in preferentially reduced expression of lineage-specific genes. This role of the Mediator complex in establishing super enhancers provides novel mechanistic insights into how it might function in developmental gene regulation.

Mediator and epigenetic regulators

Cell-specific transcription patterns can be altered by modulating the activity or expression of a few master regulators of cell fate. However, epigenetics also plays an important role in cell type specification, and it is now clear that the differentiation process is accompanied by major changes at the chromatin level (Dambacher et al., 2013). Recent studies have begun to examine the relationship

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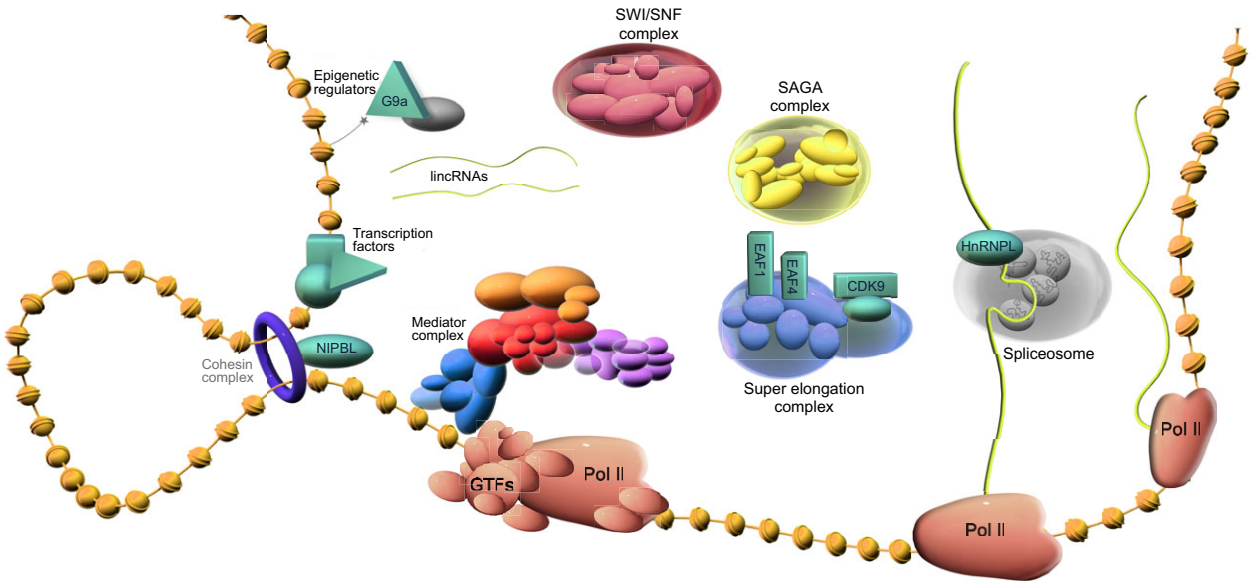

Fig. 2. Model summarizing interactions between the Mediator complex and other well-defined complexes/factors during transcription. Subunits of the Mediator complex interact with various transcription factors, long intergenic noncoding RNAs (lincRNAs), epigenetic regulators and other factors. As such, the Mediator complex plays a role in epigenetic modification, super enhancer formation, DNA loop formation, transcription initiation, elongation, termination, RNA splicing and noncoding RNA interaction. Other large complexes, such as the SAGA complex, the SWI/SNF complex, the spliceosome and the super elongation complex, also interact with Mediator. GTFs, general transcription factors; HnRNPL, heterogeneous nuclear ribonucleoprotein L.

between the Mediator complex and epigenetic regulators. For example, a neuronal-specific repressor, REST, binds to Mediator subunits MED19 and MED26, which then further recruit G9a (also known as Ehmt2), a histone H3K9 methyltransferase, through a MED12-G9a interaction (Fig. 1) (Ding et al., 2008). This complex can repress neuronal genes in non-neuronal cells. Furthermore, *MED12* mutations found in patients with X-linked intellectual disability attenuated the ability of Mediator to recruit G9a and induced abnormal neuronal gene expression (Ding et al., 2008). In another report, the two kinase subunits of Mediator, CDK8 and CDK19, were shown to interact with the histone arginine methyltransferase PRMT5 and WD-repeat protein 77 (WDR77; also known as MEP50), respectively, both of which are important for further recruitment of the DNA methyltransferase DNMT3A and subsequent repression of C/EBPβ-regulated genes (Tsutsui et al., 2013). Thus, individual Mediator components appear to exhibit specificity for distinct epigenetic events, such as the recruitment of specific histone or DNA modifiers which, in turn, influence specific epigenetic modifications.

Mediator and transcriptional elongation and termination

Promoter-proximal pausing of Pol II and the release of paused Pol II into productive elongation has emerged as an important mechanism of transcriptional control (Adelman and Lis, 2012). Accumulating evidence suggests that Mediator is involved in releasing the paused Pol II and prompting productive elongation. For example, it was recently demonstrated that Pol II elongation correlates with Mediator-dependent recruitment of super elongation complexes (SECs) that contain members of the eleven-nineteen lysine-rich in leukemia (ELL) family and their binding partners ELL-associated factors (EAFs), positive transcription elongation factor b (P-TEFb) and other proteins (Takahashi et al., 2011). In this study, it was observed that the N-terminus of MED26 contains docking sites for SEC and another ELL/EAF-containing complex, as well as the general transcription initiation factor TFIID. MED26 might therefore function as a molecular switch that first binds to TFIID in the pre-initiation complex and then exchanges to the SECs for Pol II elongation (Takahashi et al., 2011).

Recent studies suggest that the CDK8 module is also involved in transcriptional elongation. Originally, the CDK8 module was described as a co-repressor for transcription because the phosphorylation of Pol II C-terminal domain and TFIIH by CDK8 could block the assembly and function of the transcription initiation complex (Hengartner et al., 1998; Sun et al., 1998; Akoulitchev et al., 2000). However, it was previously found that CDK8 is also present at a serum-activated gene promoter (Wang et al., 2005), suggesting that CDK8 has a positive function during transcription. Moreover, the Espinosa group revealed a role for CDK8 in transcriptional elongation (Donner et al., 2010). CDK8 depletion did not affect recruitment of Pol II to gene promoters or overall Pol II intragenic occupancy but instead caused impaired recruitment of CDK7 and CDK9, which are needed for recruitment of P-TEFb and BRD4 for Pol II elongation. Most recently, it was demonstrated that hypoxia-inducible factor 1A (HIF1A) recruits CDK8-Mediator and a SEC containing the SEC scaffold protein AFF4 and CDK9 to alleviate Pol II pausing (Galbraith et al., 2013). This study showed that CDK8 is dispensable for HIF1A chromatin binding but is essential for the binding of SEC and Pol II elongation in response to hypoxia.

The MED23 Mediator subunit also regulates transcription and was previously shown to control the MAPK-Elk1-activated *Egr1* gene after pre-initiation complex formation, i.e. at a post-recruitment step (Wang et al., 2005). Under serum stimulation, MED23 depletion disrupts the Elk1-Mediator interaction, preventing the release of pre-bound Pol II into elongation (Wang et al., 2005). We recently demonstrated that under unstimulated conditions, Egr1 expression is largely reduced in MED23-depleted cells, while the occupancies of Pol II, GTFs, the Mediator complex, or the activator Elk1 at the *Egr1* promoter remain unchanged (Wang et al., 2013). However, MED23 depletion

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Table 1. Interactions between Mediator subunits and well-defined factors and their role in physiological/developmental processes

Mediator subunit	Interacting factor/complex	Developmental processes affected	References
Head (MED6/8/11/17/18/19/20/22)			
MED17	VP16, P53, HSF, DIF, RXR		(Ito et al., 1999; Park et al., 2001; Park et al., 2003)
	STAT2, P65		(Lau et al., 2003; van Essen et al., 2009)
MED19	REST	Neuron differentiation	(Ding et al., 2009)
Middle (MED1/1L/4/7/9/10/14/21/32)			
MED1	lincRNAs (ncRNA-a1/3/7)		(Lai et al., 2013)
	Rα/β, PPARα, RARα, RXRα, VDR		(Zhu et al., 1997; Yuan et al., 1998; Rachez et al., 1999)
	RORα, GR, FXR, AHR, HNF4		(Atkins et al., 1999; Hittelman et al., 1999; Malik et al., 2002; Pineda Torra et al., 2004; Wang et al., 2004b)
	ERα/β, PGC-1α, BRCA1, NR4A		(Zhu et al., 1999; Warnmark et al., 2001; Kang et al., 2002; Wallberg et al., 2003; Wada et al., 2004; Wansa and Muscat, 2005)
	GABPα, Pit-1, C/EBPβ, P53		(Drane et al., 1997; Gordon et al., 2006; Udayakumar et al., 2006; Li et al., 2008; Meyer et al., 2010)
	PPARγ	Adipogenesis	(Ge et al., 2002)
	GATA1	Erythropoiesis, heart, eye and megakaryocyte development	(Crawford et al., 2002; Stumpf et al., 2006)
MED14	GR, HNF4, SATAT2, SREBP-1α, ERα		(Hittelman et al., 1999; Malik et al., 2002; Lau et al., 2003; Toth et al., 2004; Lee et al., 2005)
	PPARγ	Adipogenesis	(Grontved et al., 2010)
MED21	TRα/β		(Nevado et al., 2004)
MED31	Elmo1		(Mauldin et al., 2013)
Tail [MED2(29)/3(27)/5(24)/15/16/23]			
MED2(29)	DSF		(Garrett-Engele et al., 2002)
MED15	SREBP-1α, NHR-49, OAF1, Prd1, Prd3		(Taubert et al., 2006; Yang et al., 2006; Thakur et al., 2008; Thakur et al., 2009)
	Smad2/3/4	Mesendoderm development	(Kato et al., 2002)
MED16	DIF		(Kim et al., 2004)
MED23	Splicing factor (HnRNPL)		(Huang et al., 2012)
	Elongation factor (CDK9)		(Wang et al., 2013)
	E1A-CR3, DIF, HSF, ESX, C/EBPβ		(Boyer et al., 1999; Asada et al., 2002; Kim et al., 2004; Mo et al., 2004)
	Elk1	Adipogenesis/SMC differentiation	(Wang et al., 2009; Yin et al., 2012)
Kinase (CDK8/19; MED12/12L/13/13L; cyclin C)			
MED12	lincRNAs (ncRNA-a1/3/7)		(Lai et al., 2013)
	RTA, Gli3, β-catenin, AICD, Pygopus		(Gwack et al., 2003; Kim et al., 2006; Zhou et al., 2006; Carrera et al., 2008; Xu et al., 2011)
	SOX9	Chondrogenesis	(Zhou et al., 2002)
	SOX10	Myelinating glia	(Vogl et al., 2013)
	Nanog	Pluripotency	(Tutter et al., 2009)
	Epigenetic regulator (G9a)	Neuron differentiation	(Ding et al., 2008)
MED13	Pygopus		(Carrera et al., 2008)
CDK8	c-Myc, PRMT5, WDR77/MEP50		(Eberhardy and Farnham, 2002; Tsutsui et al., 2013)
CDK19	PRMT5, WDR77/MEP50		(Tsutsui et al., 2013)
Unassigned (MED25/26/28/30)			
MED25	VP16, DIF, HSF, RARα, HNF4		(Mittler et al., 2003; Kim et al., 2004; Yang et al., 2004; Lee et al., 2007; Rana et al., 2011)
	PEA3, ERM, ER81		(Verger et al., 2013)
	SOX9	Chondrogenesis	(Nakamura et al., 2011)
MED26	Super elongation complex (EAF1/EAF4)		(Takahashi et al., 2011)
	REST	Neuron differentiation	(Ding et al., 2009)
MED28	Merlin, Grb2		(Wiederhold et al., 2004)

results in a significant decrease in P-TEFb and elongating Pol II (marked by serine-2 phosphorylation) at the coding region. Further experiments suggested that MED23 controls a basal level of transcription by recruiting elongation factor P-TEFb via a direct interaction with its CDK9 subunit (Wang et al., 2013). Taken together, these findings demonstrate that Mediator regulates transcriptional elongation, possibly by multiple subunits, through multiple mechanisms and in a gene-specific manner.

Recently, the relationship between Mediator and transcriptional termination has also been revealed. MED18 was proven to be important for termination in yeast (Mukundan and Ansari, 2011). In the absence of MED18, the recruitment of termination factors and Pol II to the 3′ end of genes was compromised, and a readthrough phenotype was found *in vitro*. Therefore, Mediator regulates not only transcriptional elongation but also termination.

Mediator and RNA processing

In higher species, alternative splicing affects the majority of protein-coding genes and creates a functional diversity of gene products to meet the needs of distinct cell types. Pre-mRNA splicing is largely

Table 2. Phenotype or developmental processes affected in Mediator subunit-deficient mice or cells

Mediator subunit	Disease/phenotype	Genetic state	References
MED1	Embryonic lethal at ~E11.5	KO	(Ito et al., 2000; Zhu et al., 2000)
	Erythroid development	KO	(Stumpf et al., 2006)
	Adipogenesis	KO	(Ge et al., 2002)
	Mammary gland development and luminal cell differentiation	LxxLL motif mutant knock-in	(Jiang et al., 2010)
	Embryonic lethal at ~E13.5, placental, hepatic and cardiovascular development	Conditional KO	(Landles et al., 2003)
MED12	Embryonic lethal at ~E7.5	KO	(Rocha et al., 2010)
	Embryonic lethal at ~E9.5, neural tube closure, axis elongation, somitogenesis and heart formation	Hypomorphic mutants	(Rocha et al., 2010)
	Embryonic stem cell function	KD	(Tutter et al., 2009)
	Neuron differentiation	KD	(Ding et al., 2008)
MED14	Adipogenesis	KD	(Grontved et al., 2010)
MED19	Neuron differentiation	KD	(Ding et al., 2009)
MED21	Embryonic lethal at blastocyst stage	KO	(Tudor et al., 1999)
	Keratinocyte differentiation	KD	(Oda et al., 2010)
MED23	Embryonic lethal at E9-10.5	KO	(Balamotis et al., 2009)
	Adipogenesis	KO/KD	(Wang et al., 2009)
	Smooth muscle differentiation	KO/KD	(Yin et al., 2012)
MED24	Embryonic lethal at E8.5-10.5	KO	(Ito et al., 2002)
MED25	Chondrogenesis	KD	(Nakamura et al., 2011)
MED26	Neuron differentiation	KD	(Ding et al., 2009)
MED28	Smooth muscle differentiation	KD	(Beyer et al., 2007)
MED31	Embryonic lethal at ~E16.5-18.5, chondrogenesis	Mutation causing degradation	(Risley et al., 2010)
CDK8	Embryonic lethal at ~E2.5-3.0	Gene trap insertion	(Westerling et al., 2007)

KO, knockout; KD, knockdown.

coupled with transcription, which permits immediate recognition of emerging splicing signals by the splicing machinery. However, despite extensive research this coupling mechanism is not fully understood. Using tandem affinity purification combined with mass spectrometry, we recently identified several pre-mRNA processing factors that specifically bind to MED23 (Huang et al., 2012). Among these was heterogeneous nuclear ribonucleoprotein L (HnRNP L), the interaction of which with MED23 was verified *in vitro* and *in vivo*. Functionally, MED23 and HnRNP L co-regulate a significant subset of alternative splicing and alternative cleavage and polyadenylation events (Huang et al., 2012). These findings demonstrate an important function of Mediator in the regulation of mRNA processing and reveal cross-talk between the Mediator complex and the splicing machinery, thus providing mechanistic insight into the coupling of transcription and splicing.

Interactions between Mediator and long intergenic noncoding RNAs

Recent advances have revealed a large number of transcripts, termed long intergenic noncoding RNAs (lincRNAs), that display no protein-coding potential but play widespread roles in multiple biological processes (Ørom et al., 2010; Ulitsky and Bartel, 2013). A recent study demonstrated that a particular class of lincRNAs, termed ncRNA-activating (ncRNA-a), which activate neighboring genes through a cis-mediated mechanism, interact with Mediator by tethering it to chromatin for gene activation (Lai et al., 2013). Furthermore, depletion of the MED12 subunit specifically and potently diminished ncRNA-a-induced activation of transcription, and disease-related MED12 mutations diminished the ability of Mediator to associate with ncRNA-a. These results demonstrated that the Mediator complex is able to mediate transcriptional activation through noncoding RNAs, providing additional insight into Mediator function during transcription.

Roles of the Mediator complex in development

Given the role of different Mediator subunits in cell fate-related gene expression programs, the involvement of Mediator in many developmental processes and human diseases is being increasingly recognized. In particular, studies of knockout mice harboring mutations in individual Mediator subunits (Table 2), together with studies in other model organisms (Table 3), have provided key insights into the developmental roles of various Mediator subunits. Below, we review these findings and discuss the key developmental processes that are influenced by individual Mediator subunits.

Insights from Mediator subunit knockout mice

Gene knockout (KO) technology is a powerful method for evaluating the importance of particular genes during development. Following the discovery of the Mediator complex, KO mice for several individual Mediator subunits have been generated (Table 2). Strikingly, all of these KO mice are embryonic lethal, either early or late with different defects, suggesting a general requirement for Mediator in many aspects of embryonic development.

Med1 null mice, for example, die at embryonic day (E) 11.5 due to placental insufficiency (Ito et al., 2000; Zhu et al., 2000). These mice show impaired heart formation, abnormal neuronal development, hepatic necrosis and hematopoiesis defects. *Med1* hypomorphic mutants with reduced MED1 levels survive until E13.5 and show developmental abnormalities similar to those seen in null mice at an early stage (Landles et al., 2003). This study demonstrated that MED1 is necessary for early extra-embryonic placental development, which is probably the reason for the embryonic lethality of null mice at E11.5. This embryonic lethality could be partially rescued by tetraploid aggregation, and the embryos remained alive until E13.5 but eventually died for similar reasons as the *Med1* hypomorphic mutants, which demonstrates that MED1 is also required for later multi-organ development.

Med12 hypomorphic mutants, by contrast, fail to develop beyond E10 and exhibit severe defects in neural tube closure, axis elongation, somitogenesis and heart formation (Rocha et al., 2010). Embryos that are incapable of expressing MED12 die at ~E7.5 and fail to establish the anterior visceral endoderm or activate brachyury

Table 3. Phenotype or developmental processes affected following Mediator subunit perturbation in animal models

Mediator subunit	Disease/phenotype	Genetic state	References
Zebrafish			
MED12	Brain, cartilage, ear, kidney, endoderm development; chondrogenesis	Mutation	(Rau et al., 2006; Hong et al., 2005; Wang et al., 2006; Shin et al., 2008)
MED23	Smooth muscle differentiation	MO	(Yin et al., 2012)
MED25	Chondrogenesis	MO	(Nakamura et al., 2011)
Drosophila			
MED6	Died in the third larval instar	Mutation	(Gim et al., 2001)
MED12/13	Retinal, wing and crystal cell development	Mutation	(Treisman, 2001; Lim et al., 2007; Janody et al., 2003; Carrera et al., 2008)
MED15	Wing development	Mutation	(Terriente-Felix et al., 2010)
MED31	Anteroposterior axis formation	Mutation	(Bosveld et al., 2008)
CDK8	Eye development	Mutation	(Loncle et al., 2007)
C. elegans			
MED12/13	Embryonic lethal, vuval development and T-cell division	Mutation	(Moghal and Sternberg, 2003; Wang et al., 2004a; Yoda et al., 2005)
MED23	Larval lethal, vuval development	Mutation	(Singh and Han, 1995)
Xenopus			
MED15	Mesendoderm development	MO	(Kato et al., 2002)

MO, morphant.

expression, and they do not complete gastrulation (Rocha et al., 2010).

Med21 KO mice, on the other hand, die as early as the blastocyst stage (Tudor et al., 1999). In addition, mouse ESCs harboring a *Med21* deletion do not survive, consistent with the requirement of Srb7, which is the yeast homolog of MED21, for Pol II binding and genome-wide gene expression (Chao et al., 1996), suggesting an important role for MED21 in the overall structure and function of Mediator. Therefore, MED21 is essential for cell viability and early embryonic development.

The development of *Med23* null embryos is delayed, and mutant embryos die between E9 and E10.5. All three germ layers develop in these mutant embryos and early organogenesis is initiated before death, which is likely to result from systemic circulatory failure (Balamotis et al., 2009). In contrast to *Med21*, *Med23* null ESCs survive well, and only the expression of a small subset of genes is changed (Stevens et al., 2002; Wang et al., 2005). The genetic ablation of mouse *Med24* revealed that it is not essential for cell viability; however, *Med24* null mice die at an early developmental stage, between E8.5 and E10.5, with severe hypoplasia (Ito et al., 2002). Specifically, yolk sac hematopoiesis is partially blocked, cardiac hypoplasia causes severe heart failure, vessels are ill developed, and the development of the central nervous system is abnormal. These results indicate that the phenotypic severity of *Med24* null embryos, which lack the submodule consisting of MED24, MED23 and MED16 (Ito et al., 2002; Stevens et al., 2002), is intermediate between that of the *Med1* and *Med21* mutations.

A mutation in the mouse *Med31* gene was identified from a screen assay. These mice were rarely recovered after E16.5, indicating late-gestation lethality (Risley et al., 2010). These *Med31* mutant embryos have fewer proliferating cells in the forelimb buds and display delayed chondrogenesis due to a lack of Sox9 and Col2a1 expression. In addition, embryonic fibroblast cells derived from the mutant embryos show a severe proliferation defect.

The kinase subunits of Mediator have also been targeted. Heterozygous mice harboring an inactivating gene trap insertion at the *Cdk8* locus have no phenotype (Westerling et al., 2007), but intercrossing these mice failed to produce homozygous *Cdk8* null offspring. Developmental analysis demonstrated embryonic lethality of the homozygous mice at E2.5 to E3.0, prior to implantation; the *Cdk8* null embryos have fragmented blastomeres and do not proceed to compaction (Westerling et al., 2007), suggesting an essential role of CDK8 in cell viability and early development.

In summary, although all MED KO mice are embryonic lethal, they die at different developmental stages with distinctive phenotypes, suggesting important and specific roles for individual Mediator subunits during development.

Mediating adipocyte differentiation: MED1, MED14 and MED23

The gene KO studies discussed above suggest that all Mediator subunits play essential roles in many aspects of embryonic development. Adipocyte differentiation is a good example of a developmental pathway that is regulated by Mediator. Three subunits of the Mediator complex, namely MED1, MED14 and MED23, are involved in regulating the differentiation of pre-adipocytes, acting via different mechanisms and at different stages. The analysis of MEFs derived from *Med1* KO mice revealed that MED1 is essential for PPARγ-driven adipogenesis but not MyoD-driven myogenesis (Ge et al., 2002). MED1 can interact with the C-terminal AF2 domain of many nuclear receptors, including PPARγ, through its LXXLL motif in a ligand-dependent manner (Zhu et al., 1997; Yuan et al., 1998). Unexpectedly, the expression of a mutant form of MED1 that lacks the LXXLL motif (and hence does not bind to PPARγ *in vitro*) in *Med1* null MEFs is sufficient to rescue PPARγ-driven adipogenesis, suggesting an LXXLL motif-independent mechanism of PPARγ recruitment to adipogenic genes (Ge et al., 2008). Further experiments revealed that MED14 can interact with the N-terminal AF1 domain of PPARγ independently of its ligand, suggesting that MED14 acts as an anchor for recruiting Mediator to PPARγ (Grøntved et al., 2010), which might also explain why the LXXLL MED1 mutant can rescue adipogenesis.

Insulin signaling also plays a crucial role in promoting adipogenesis, but the mechanism by which insulin signaling is transmitted to the adipogenic transcription cascade has remained unclear. Studies in our laboratory have revealed that MED23 and its binding to the transcription factor Elk1 are the missing links at this early differentiation stage. *Med23* KO or *Med23* knockdown in MEFs, adipocyte-derived stem cells, 3T3L1 cells and 10T1/2 cells inhibits hormone-induced adipogenesis (Wang et al., 2009; Yin et

al., 2012). In the absence of Elk1 or MED23, *Krox20* (also known as *Egr2*), a rapid-response gene that is stimulated by insulin during adipogenesis, becomes uninducible, and overexpressing Krox20 in MED23-deficient cells can rescue the adipogenetic defect. These observations suggest that MED23 plays a role at an earlier stage of adipogenesis by linking insulin signaling to the adipogenetic transcription cascade. These studies also support the notion that key lineage regulators control lineage-specific gene programs by targeting different subunits of Mediator, and that different Mediator subunits can also play distinct roles at different stages to assure proper lineage development.

Mediating neuronal differentiation: MED12, MED13, MED19 and MED26

Many human diseases are associated with neural degeneration, and this has prompted mechanistic studies of neural fate determination and maintenance. Recently, several subunits of the Mediator complex have been associated with neuronal gene expression and disease. As mentioned above, the neuronal-specific transcriptional repressor REST recruits the Mediator complex for neuronal gene suppression through its interaction with MED19 and MED26 (Fig. 1) (Ding et al., 2009). This suppression occurs via recruitment of the histone H3K9 methyltransferase G9a by the REST-MED19/26-MED12-G9a complex to neuronal gene promoters. Furthermore, *Med12* mutant mice show severe defects in neural tube closure (Rocha et al., 2010). Med12 was also identified in a large-scale genetic screen of mutant zebrafish exhibiting deficient neuronal development (Wang et al., 2006). Another screen for mutant zebrafish resembling the double-mutant phenotype of Sox9a/Sox9b (two key factors for neural crest development) also identified Med12 (Rau et al., 2006). Studies on MED12 and MED13 (also known as Kohtalo and Skuld) have also been carried out in *Drosophila* (see Table 3), where they play a role in retinal, wing and crystal cell development.

Mediating smooth muscle differentiation: MED23 and MED28

A previous study revealed the function of the MED28 subunit in smooth muscle development (Fig. 1). Knockdown of MED28 in NIH3T3 and myoblast C2C12 cells leads to upregulation of smooth muscle genes, whereas overexpression of MED28 represses the expression of these genes (Beyer et al., 2007). A head- or CDK8 module-related repression function was postulated, but the detailed mechanism underlying this regulation is not clear. *MED28* was previously identified as an endothelial cell gene and was named endothelial-derived gene EG-1 (Liu et al., 2002). Later, it was identified as a protein that interacts with the cytoskeletal protein merlin, localizes beneath the plasma membrane, and interacts with the actin cytoskeleton (Wiederhold et al., 2004). These observations suggest that MED28 might regulate smooth muscle gene expression through a cytoplasmic monomer isoform, not as a Mediator component.

Another subunit involved in smooth muscle differentiation is MED23. Our previous finding that MED23 depletion prevents adipogenesis (Wang et al., 2009) led us to investigate the effects of MED23 depletion from precursor cells on subsequent cell fate. The results of this study demonstrated that MED23-depleted mesenchymal stem cells are prone to differentiation into smooth muscle cells (Yin et al., 2012) . This type of 'Yin-Yang' regulation of adipogenesis and smooth muscle differentiation by MED23 has been examined in multiple cell types, such as MEFs, 10T1/2 cells and adipocyte stem cells (Fig. 1). Our recent study has demonstrated that MED23 controls the balance between Ras/ELK1 and RhoA/MAL signaling, which control adipogenetic and smooth muscle gene expression, respectively, thus oppositely directing the two distinct cell lineages (Yin et al., 2012). The possible relationship between MED23 and MED28 in smooth muscle differentiation remains to be investigated further.

Mediating chondrogenesis: MED12, MED25 and MED31

Sox9 is a transcriptional activator of cartilage-specific extracellular matrix genes (Lefebvre and de Crombrugghe, 1998). As such, it plays essential roles in chondrogenesis. It has been shown that MED12 functions as a co-factor of Sox9 and plays an important role in craniofacial chondrogenesis/endochondral bone formation during zebrafish development (Zhou et al., 2002). Recently, MED25 was found to be another direct target of Sox9, and morpholino-mediated knockdown of Med25 in zebrafish resulted in palatal malformation similar to that observed in *sox9* mutants (Nakamura et al., 2011). *Med31* mutant mice exhibit normal limb bud patterning but experience delayed chondrogenesis due to a lack of Col2a1 and Sox9 expression (Risley et al., 2010). Taken together, it appears that different Mediator subunits are able to function either upstream or downstream of the key transcription factor Sox9 to regulate chondrogenesis.

Human diseases related to Mediator function

Many mutations in Mediator subunits have been associated with human diseases (Table 4). For example, the missense mutations R961W and N1007S in *MED12*, which disrupt the interaction between MED12 and the transcription repressor REST, are responsible for both FG syndrome and Lujan syndrome, two X-linked genetic disorders characterized by intellectual disability (Ding et al., 2008). Another mutation related to neurological disorders is an R617Q mutation in *MED23*, which co-segregates with nonsyndromic autosomal recessive intellectual disability in families. This mutation specifically impairs the response of JUN and FOS immediate early genes to serum stimulation in patient-derived skin fibroblasts (Hashimoto et al., 2011). A summary of other studies of

Table 4. Mutations in Mediator subunits and their related human diseases

Mediator subunit	Disease/phenotype	Genetic state/mutation	References
MED12	FG intellectual disability syndrome	R961W mutation	(Risheg et al., 2007)
	Lujan intellectual disability syndromes	N1007S mutation	(Schwartz et al., 2007)
	Uterine leiomyomas	Distinct mutations in exon 2	(Makinen et al., 2011)
	Ohdo syndrome	Distinct missense mutations	(Vulto-van Silfhout et al., 2013)
MED13L	Transposition of the great arteries	Distinct missense mutations	(Muncke et al., 2003)
MED15	DiGeorge syndrome (DGS)/velocardiofacial syndrome (VCFS)	Chromosomal deletion including *MED15*	(Berti et al., 2001)
MED17	Infantile cerebral and cerebellar atrophy	L371P mutation	(Kaufmann et al., 2010)
MED23	Nonsyndromic autosomal recessive intellectual disability	R617Q mutation	(Hashimoto et al., 2011)
MED25	Charcot-Marie-Tooth disease	A335V mutation	(Leal et al., 2009)
CDK19	Congenital retinal folds, microcephaly and intellectual disability	Pericentric inversion	(Mukhopadhyay et al., 2010)

neurological disease-related mutations in Mediator subunits can be found in Table 4.

Mediator mutations have also been associated with congenital heart disease. For example, *MED15* is deleted in patients with DiGeorge syndrome (Berti et al., 2001), which is associated with various cardiovascular abnormalities. *MED13L* is interrupted in patients with chromosomal translocations, who display transposition of the great arteries (TGA) and intellectual disability, suggesting that MED13L is involved both in early heart and brain development (Muncke et al., 2003).

Changes in the level of Mediator expression are also frequently reported in diseases such as cancer. For example, MED23 is significantly overexpressed in lung cancer cell lines and clinical lung cancer samples with hyperactive RAS activities, whereas a lower MED23 expression level predicts better survival in RAS-active lung cancer patients (Yang et al., 2012), suggesting that the MED23 subunit might serve as a therapeutic target as well as a diagnostic marker for RAS-active cancers. Interestingly, MED15 is highly expressed in clinical breast cancer tissues, correlated with hyperactive transforming growth factor β (TGFβ) signaling, as indicated by SMAD3 phosphorylation (Zhao et al., 2013). Moreover, MED15 deficiency decreased the metastatic potential of a highly aggressive breast cancer cell line by attenuating TGFβ/Smad signaling. Interestingly, heterozygous mutations in exon 2 of the *MED12* gene have been described in 50-70% of uterine leiomyomas (Mäkinen et al., 2011). Overall, different Mediator components appear to control distinct types and stages of cancer development through distinct signaling pathways.

Conclusions

Mediator is an evolutionarily conserved protein complex with a large surface mediating diverse and dynamic protein-protein interactions. In addition to binding to an array of transcription factors, Mediator interacts with diverse co-factors and complexes as well as lincRNAs. Consequently, in addition to its classic role in establishing the pre-initiation complex, Mediator plays diverse roles at multiple stages of transcription, including elongation, termination, mRNA processing and epigenetic regulation. Mediator functions together with cohesin to establish the super enhancer loop for gene activation, which is important for cell identity. Therefore, Mediator can be considered as a master coordinator, orchestrating diverse developmental signaling and master regulators to specify distinct cell fates. It should be noted that the term ‘master coordinator’ is different to ‘master regulator’ in the sense that a master regulator is a driving force for cell lineage development, whereas a master coordinator just assists the master regulator in doing its job. In contrast to master regulators, the overexpression of a master coordinator (e.g. a Mediator subunit) cannot drive cell differentiation. Overall, as a master coordinator, Mediator coordinates transcription and cell lineage specification/development to ensure that the correct genes are expressed at the right time and place and with the necessary intensity and duration.

Our understanding of the molecular and developmental regulation of the Mediator complex has been greatly expanded in recent years. However, many questions remain. For example, what is the exact mechanism by which Mediator coordinates multiple transcription factors and co-factors? How is the dynamic composition and configuration of Mediator regulated in different conditions, cells and tissues? What are the relationships between Mediator and diverse chromosomal modifiers/remodelers? Among the many uncertainties, it is apparent that the identification of new capabilities of Mediator is inevitable.

Although KO mice for several Mediator subunits have been generated, their early or late embryonic lethality has prevented further investigation of the function of these subunits later in development. The detailed analysis of different subunits in development might thus require tissue-, cell type- or stage-specific KO mouse models. Furthermore, combining knock-in mouse models with known disease-related mutations might provide an in-depth understanding of how mutations within particular Mediator components are linked to various diseases.

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Competing interests

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