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Review article

Mediator: A key regulator of plant development

Manuel Buendía-Monreal, C. Stewart Gillmor *

Laboratorio Nacional de Genómica para la Biodiversidad (Langebio), Unidad de Genómica Avanzada, CINVESTAV-IPN, Irapuato, Guanajuato, Mexico

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ABSTRACT

Mediator is a multiprotein complex that regulates transcription at the level of RNA pol II assembly, as well as through regulation of chromatin architecture, RNA processing and recruitment of epigenetic marks. Though its modular structure is conserved in eukaryotes, its subunit composition has diverged during evolution and varies in response to environmental and tissue-specific inputs, suggesting different functions for each subunit and/or Mediator conformation. In animals, Mediator has been implicated in the control of differentiation and morphogenesis through modulation of numerous signaling pathways. In plants, studies have revealed roles for Mediator in regulation of cell division, cell fate and organogenesis, as well as developmental timing and hormone responses. We begin this review with an overview of biochemical mechanisms of yeast and animal Mediator that are likely to be conserved in all eukaryotes, as well as a brief discussion of the role of Mediator in animal development. We then present a comprehensive review of studies of the role of Mediator in plant development. Finally, we point to important questions for future research on the role of Mediator as a master coordinator of development.

1. Introduction

Mediator is a large protein complex that serves as a molecular bridge between gene-specific transcription factors bound at enhancers, and RNA polymerase II (RNA pol II). In yeast, Mediator consists of 25 subunits; in mammals approximately 31 subunits; and in plants, approximately 34 subunits (reviewed in Allen and Taatjes (2015), Samanta and Thakur (2015)). Mediator was first discovered in yeast as a large protein complex that was required for transcription (Kelleher et al., 1990; Flanagan et al., 1991), and was subsequently purified from human cells (Fondell et al., 1996), and from plant cells (Bäckström et al., 2007). Because of the low sequence conservation between Mediator subunits from different species (typically as low as 20% amino acid identity), many initial studies of Mediator in yeast and animals did not recognize that proteins that had been isolated based on their differing effects on transcription, were indeed Mediator components, and in some cases, the same Mediator subunit from different organisms (Sato et al., 2004; reviewed in Kornberg (2005)). This discovery led to a unified nomenclature for Mediator subunits in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and the animals *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Homo sapiens* (Bourbon et al., 2004), which was also used for the *Arabidopsis thaliana* Mediator (Bäckström et al., 2007). Shortly thereafter, Mediator components were identified from genomic sequences of many eukaryotes, indicating that Mediator has been widely conserved in evolution (Bourbon, 2008).

Structural studies of Mediator complexes have classified Mediator as having four different modules, referred to as the Head, Middle, Tail, and Cyclin Dependent Kinase 8 (CDK8) modules (reviewed in Chadick and Asturias (2005), Conaway et al. (2005)) (Fig. 1). The Head module is thought to have the most important initial interactions with RNA pol II, while the Middle module serves a structural function as well as interacting with RNA pol II once Mediator's conformation changes after its initial interaction with RNA pol II. The Tail module is thought to play an especially important role in interacting with gene-specific transcription factors (Tsai et al., 2014; Robinson et al., 2015). In yeast, animals, and plants, Mediator has been purified in two forms: as a complex of the Head, Middle and Tail modules (commonly referred to as Core Mediator), and as a larger complex containing Core Mediator and the CDK8 module. Core Mediator preparations support transcription in vitro, while Core Mediator preparations containing the CDK8 module do not (reviewed in Björklund and Gustafsson (2005)). The CDK8 module consists of 4 proteins: MED12, MED13, Cyclin C (CycC), and Cyclin Dependent Kinase 8 (CDK8). The MED12 and MED13 subunits are both about 2000 AA, much larger than most other Mediator subunits (Table 1) (Samuelsen et al., 2003). The large size of MED12 and MED13 may be related to their role as signal integrators, allowing large surface areas for protein interactions, as well as protein modifications that can affect their stability.

* Corresponding author.
E-mail address: stewart.gillmor@cinvestav.mx (C.S. Gillmor).

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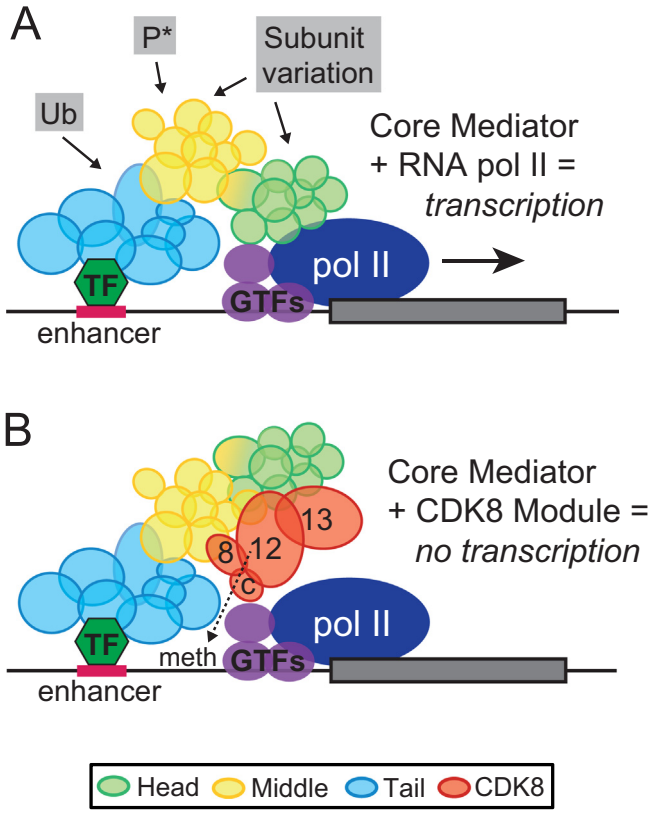

Fig. 1. Regulation of transcription by Core Mediator and the Cyclin Dependent Kinase 8 (CDK8) module of Mediator. A simplified representation of the role of Core Mediator and the CDK8 module of Mediator in regulation of transcription, based on literature cited in this review. (A) Core Mediator (composed of Head, Middle and Tail modules) serves as a molecular bridge between transcription factors (TF) bound at enhancers, and RNA polymerase II (pol II) and general transcription factors (GTFs) at the transcription start site. Individual subunits of each module are represented by colored circles. The composition of Core Mediator is dynamic, varying between different target genes (Subunit variation). Stability and activity of Mediator subunits can be regulated by ubiquitination (Ub), and by phosphorylation (P*). (B) The CDK8 module (composed of CDK8 (8), CyclinC (C), MED12 (12) and MED13 (13)) often acts to prevent transcription, either by steric inhibition of interactions between Core Mediator and RNA pol II, or through increasing epigenetic marks that inhibit transcription (such as H3K9me^2), or reducing epigenetic marks that promote transcription (such as H3K4me^3).

The size of MED12 and MED13 is also almost certainly related to their mechanism of action. Initial studies of the CDK8 module of Mediator reported that its effect was to prevent transcription by steric hindrance of interactions between Core Mediator and RNA pol II (Elmlund et al., 2006). A recent report expanded on earlier work by demonstrating that the yeast CDK8 module interacts with certain Head and Middle module Mediator subunits, in order to occupy the RNA pol II binding cleft of Core Mediator, preventing the initial association of RNA pol II and Core Mediator that leads to activation of transcription (Tsai et al., 2013) (Fig. 1). The MED13 protein plays the most important role in this interaction. The other CDK8 module components can repress gene expression through alternate methods, recruiting histone methylation marks that repress transcription, as well as decreasing histone marks that promote transcription (Gonzalez et al., 2007; Ding et al., 2008; Chaturvedi et al., 2012; Tsutsui et al., 2013; Law and Ciccaglione, 2015). In the absence of the CDK8 module, RNA pol II is able to interact with the Head and Middle domains in the RNA pol II binding pocket. Through mechanisms that are still poorly understood, the conformation of the Middle and Tail domains changes until RNA pol II occupies a site at the Middle domain, adjacent to the Tail domain (Tsai et al., 2013, 2014; Robinson et al., 2015). In addition to RNA pol II complex assembly, Core Mediator participates in multiple steps of transcription, such as RNA pol II initiation, pausing and elongation, and reinitiation. Core Mediator can also promote the formation of super enhancers, and alter genome architecture by looping DNA to put distant enhancers (with bound TFs) in close proximity to promoters, a mechanism that includes non-coding RNAs (Kagey et al., 2010; Whyte et al., 2013; Pelish et al., 2015; reviewed in Allen and Taatjes (2015)). Core Mediator has also been shown to be required for transcription of some siRNA precursors, as well as miRNA precursors (Kim et al., 2011).

Since the discovery of Mediator about 25 years ago, the vast majority of research has focused on biochemical and structural studies of Mediator preparations purified from yeast or human cells (comprehensively reviewed in Poss et al. (2013)). These studies have focused primarily on the activities of the whole Core Mediator complex as a transcriptional co-activator, or in the case of CDK8 module, as a repressor. Meanwhile, developmental biology studies, particularly genetic screens for mutants affecting a particular process of interest, have discovered discrete roles for animal Mediator subunits from all three modules of Core Mediator, and in particular for the Kinase (CDK8) module (reviewed in Yin and Wang (2014), Grants et al. (2015)). This research has demonstrated an essential role for Mediator as a signal integrator and specificity factor, with discrete Mediator subunits specific to certain developmental pathways. Mediator has been discovered to play an essential role in some of the most important signaling pathways in animals, including Wnt-β-catenin (Carrera et al., 2008; Rocha et al., 2010; Yoda et al., 2005), Hedgehog (Janody et al., 2003; Mao et al., 2014; Zhou et al., 2012), RAS-MAPK (Pandey et al., 2005; Balamotis et al., 2009; Grants et al., 2016), and TGFβ-SMAD signaling (Kato et al., 2002; Alarcón et al., 2009; Zhao et al., 2013; Huang et al., 2012). Mediator components have also been found to interact with several Sox transcription factors, which in turn bind to β-catenin and GLI, downstream components of the Wnt-β-Catenin and Hedgehog signaling pathways (reviewed in Kamachi and Kondoh (2013), Rau et al. (2006), Nakamura et al. (2011) and Hong et al. (2005)). Thus, Mediator serves as a transcriptional activator or repressor in a pathway-dependent manner, and can interact with components of signaling pathways like β-catenin (Kim et al., 2006), as well as cofactors of signaling pathway effectors such as Pygopus (Carrera et al., 2008), and Sox transcription factors (Zhou et al., 2002).

In plants, almost all research on Mediator has been performed with *Arabidopsis thaliana*; unless otherwise specified, all studies on plants mentioned in this review were conducted with that species. Mediator has been shown to regulate basic cellular processes such as cell proliferation, cell growth, and organ growth; as well as developmental timing, and hormone responses (Fig. 2 and Table 1). Similar to animals, transcription factors have been discovered which interact with specific plant Mediator components (Table 1), suggesting that many of the mechanisms of Mediator function are likely to be conserved between yeast, plants and animals, though the specific pathways in which they act differ between different kingdoms.

2. Mediator is involved in basic cellular processes in plants

2.1. Cell proliferation

In *hen3 [cdk8]* mutants, loss of CDK8 activity results in smaller leaves, which have approximately the same cell number per area as larger wild type leaves, indicating that CDK8 regulates cell proliferation at the level of the organ (Wang and Chen, 2004). *cct [med12]* and *gct [med13]* mutants also affect cell proliferation and organ growth, delaying the initiation of cotyledon primordia in embryos (Gillmor et al., 2010), and decreasing the rate of leaf

Table 1
Mediator functions in plant development and other processes.

Mediator submodule	Subunit	Arabidopsis gene names	Length (AA)		Functions in development	Other functions	Interacting Proteins
			At	Hs			
Head	MED6	At3g21350	298	246			
	MED8	At2g03070 SETH10 (Lalanne et al., 2004)	524	268	**Floral transition** (Kidd et al., 2009), **root development** (Sundaravelpandian et al., 2013), **cell expansion and organ size** (Xu and Li, 2012), **cell wall composition** (Seguela-Arnaud et al., 2015), **sugar signaling** (Seguela-Arnaud et al., 2015), **male gametophytic development** (Lalanne et al., 2004), **lateral root development** (Ito et al., 2016), **auxin responses** (Ito et al., 2016)		**ARF7**, **ARF19** (Ito et al., 2016)
	MED11	At3g01435	115	117			
	MED17	At5g20170	653	651	**Floral transition** (Kim et al., 2011), **lateral root development** (Ito et al., 2016)	**Non-coding RNA production** (Kim et al., 2011)	
	MED18	At2g22370	219	208	**Floral transition** (Lai et al., 2014; Zheng et al., 2013), **redox homeostasis** (Lai et al., 2014), **floral organ development** (Zheng et al., 2013), **ABA signaling in germination** (Lai et al., 2014)	**Non-coding RNA production** (Kim et al., 2011)	**SUF4, ABI4, YY1** (Lai et al., 2014); **SPL15** (Hyun et al., 2016)
	MED20a/ MED20b/ MED20c	At2g28230; At4g09070; At2g28020	219/ 219/ 70	212	**Floral transition (MED20a)** (Kim et al., 2011)	**Non-coding RNA production** (Kim et al., 2011)	
	MED22	At1g16430, MED22a; At1g07950, MED22b	154	200			
	MED28	At3g52860	156	178	**Redox homeostasis in root and senescence** (Shaikhali et al., 2015a, 2015b)		
	MED30	At5g63480	189	178			
Middle	CBP1 (MED1?)	At2g15890	203	1581	**Pollen tube guidance** (Li et al., 2015a)		**CCG, RNA Pol II, MED7a, MED7b, MED9, several AGL factors** (Li et al., 2015a)
	MED4	At5g02850	426	270	**Vegetative phase change (miR156 pathway)** (Li et al., 2015b)		**SAD1 (RPA34.5)** (Li et al., 2015b)
	MED7	At5g03220, MED7a; At5g03500, MED7b	168	233			
	MED9	At1g55080	244	146			
	MED10	At5g41910, MED10a; At1g26665, MED10b	189	135			
	MED19	At5g12230	221	244		**Pathogen resistance** (Caillaud et al., 2013)	**HaRxL44** (Caillaud et al., 2013)
	MED21	At4g04780	139	144		**Defense response to fungal pathogens** (Dhawan et al., 2009)	**HUB1** (Dhawan et al., 2009)
	MED26	At3g10820, MED26a; At5g05140, MED26b; At5g09850, MED26c	580 / 436 / 353	600			
	MED31	At5g19910	226	131			
Tail	MED14	At3g04740, SWP (Autran et al., 2002)	1703	1454	**Cell proliferation in leaf development** (Autran et al., 2002)	**Plant immunity** (Zhang et al., 2013), **cold response** (Hemsley et al., 2014)	**LUG** (Gonzalez et al., 2007)
	MED15	At1g15780, NRB4 (Canet et al., 2012)	1335	788	**Floral transition** (Canet et al., 2012)	**Salicylic acid response** (Canet et al., 2012), **lipid biosynthesis in seeds** (Kim et al., 2016)	**WRI1** (Kim et al., 2016)
	MED16	At4g04920, SFR6 (Knight et al., 2008), YID1(Yang et al., 2014), IEN1 (Zhang et al., 2012)	1278	877	**Floral transition** (Knight et al., 2008), **cell wall composition** (Sorek et al., 2015)	**Plant immunity** (Wang et al., 2015; Wathugala et al., 2012; Zhang et al., 2013, 2012), **iron homeostasis** (Yang et al., 2014), **cold response** (Hemsley et al., 2014; Knight et al., 2009)	

Table 1 (*continued*)

Mediator submodule	Subunit	Arabidopsis gene names	Length (AA)		Functions in development	Other functions	Interacting Proteins
			At	Hs			
	MED23	At1g23230	1615	1368			
	MED24/33/5	At3g23590, MED33a/MED5a/RFR1; At2g48110, MED33b/MED5b/REF4 (Bonawitz et al., 2012)	1309	989	**Cell wall composition** (Anderson et al., 2015; Bonawitz et al., 2014, 2012)		
	MED25	At1g25540; PFT1 (Cerdán and Chory, 2003)	836	747	**Floral transition** (Cerdán and Chory, 2003; Iñigo et al., 2012a, 2012b), **cell expansion and organ size** (Xu and Li, 2011), **auxin signaling** (Raya-González et al., 2014), **redox homeostasis in root** (Sundaravelpandian et al., 2013), **cell wall composition** (Seguela-Arnaud et al., 2015), **sugar signaling** (Seguela-Arnaud et al., 2015), **ABA signaling in germination** (Chen et al., 2012), **lateral root development** (Raya-González et al., 2014; Ito et al., 2016)	**Jasmonate-dependent defense** (Çevik et al., 2012; Chen et al., 2012; Kidd et al., 2009), **sulfate assimilation** (Koprivova et al., 2014), **iron homeostasis** (Yang et al., 2014)	**COP1&HY5** (Klose et al., 2012); **MBR1&MBR2** (Iñigo 2012b); **CDK8** (Zhu et al., 2014); **ERF1**, **MYC2**, **MYC3**, **MYC4**, **BZS1**, **POSF21**, **ORA59**, **WRKY10**, **MYB104**, **ERF15**, **DREB2A** (Çevik et al., 2012); **DREB2A**, **ZFHD1**, **PHL1** (Elfving et al., 2011); **MYC2 & ABI5** (Chen et al., 2012); **EIN3 & EIL1** (Yang et al., 2014); **ARF7**, **ARF19**, **IAA14** (Ito et al., 2016)
	MED27/3	At3g09180	402	311			
	MED29/32/2	At1g11760	151	200	**Redox homeostasis in root and senescence** (Shaikhali et al., 2015a, 2015b)	**Cold response** (Hemsley et al., 2014)	
CDK8	MED12	At4g00450, *CCT* (Gillmor et al., 2010), *CRP* (Imura et al., 2012)	2253	2177	**Embryo polarity and patterning** (Gillmor et al., 2010), **embryo to seedling transition** (Gillmor et al., 2014), **vegetative phase change (miR156 pathway)** (Gillmor et al., 2014), **floral transition** (Gillmor et al., 2014; Imura et al., 2012), **auxin signaling** (Gillmor et al., 2010; Imura et al., 2012; Ito et al., 2016), **lateral root development** (Ito et al., 2016)		
	MED13	At1g55325, *GCT* (Gillmor et al., 2010), *MAB2* (Ito et al., 2011)	2001	2174	**Embryo polarity and patterning** (Gillmor et al., 2010; Ito et al., 2011), **embryo to seedling transition** (Gillmor et al., 2014), **vegetative phase change (miR156 pathway)** (Gillmor et al., 2014), **floral transition** (Gillmor et al., 2014), **auxin signaling** (Gillmor et al., 2010; Ito et al., 2011; Imura et al., 2012; Ito et al., 2016), **lateral root development** (Ito et al., 2016)		**CYCC1;2** (Ito et al., 2011), **TPL** (Ito et al., 2016), **TPR2** (Ito et al., 2016), **IAA14** (via **TPL**) (Ito et al., 2016)
	CDK8 (CDKE)	At5g63610, *HEN3* (Wang and Chen, 2004)	470	464	**Floral organ development** (Wang and Chen, 2004), **auxin signaling** (Ito et al., 2016), **lateral root development** (Ito et al., 2016)	**Mitochondrial retrograde regulation** (Ng et al., 2013)	**RNA Pol II** (Wang and Chen, 2004); **CYC1;2** (Ito et al., 2011); **CYC1;1&CYCC1;2, WIN1** (Zhu et al., 2014); **LUG** (Gonzalez et al., 2007)
	CYCC1;1/CYCC1;2	At5g48640; At5g48630	253/256	283			**MED13+CYCC1;2**, **CDK8+CYCC1;2** (Ito et al., 2011); **CDK8+CYCC1;1&CYCC1;2** (Zhu et al., 2014)
Unassigned	MED34	At1g31360, RECQ2 (Kobbe et al., 2008)	705			**3′- > 5′ DNA helicase** (Kobbe et al., 2008)	
	MED35	At1g44910, PRP40a (Kang et al., 2009)	958			**RNA processing** (Kang et al., 2009)	**RNA Pol II** (Kang et al., 2009)
	MED36	At4g25630, FIB2 (Barneche et al., 2000)	320			**Processing of rRNA** (Barneche et al., 2000)	**PRMT1** (Barneche et al., 2000)
	MED37	At5g28540, BIP1 (Maruyama et al., 2010)	668		**Female gametophyte development** (Maruyama et al., 2010)		**BRI1–5** (Hong et al., 2008)

Length of Mediator subunits from *Homo sapiens* (Hs) was taken from Allen and Taatjes (2015). Length of Mediator subunits from *Arabidopsis thaliana* (At) was taken from TAIR database (www.arabidopsis.org).

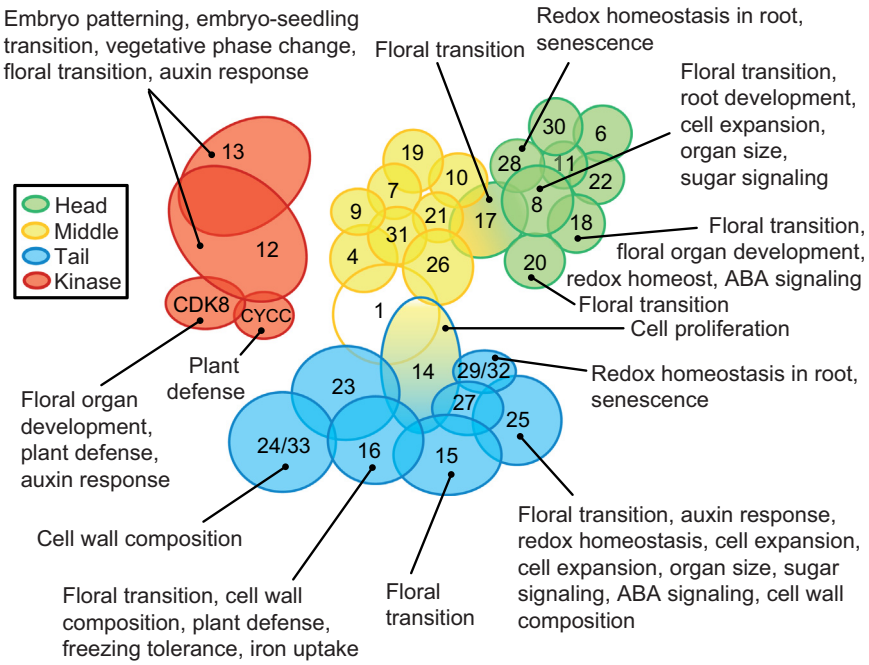

Fig. 2. Mediator functions in plant development. Submodular structure of the plant Mediator complex is depicted on the basis of tridimensional reported structures of yeast Mediator and human Mediator (Robinson et al., 2015; Tsai et al., 2014). Subunit sizes are according to predicted protein length (see Table 1). Note that Med14 and Med17 are represented in split color since the Med14 C terminal domain (CTD) belongs to the Tail module, and the Med14 N terminal domain (NTD) belongs to the Middle module. The Med17-NTD belongs to the Middle module and Med17-CTD to the Head. Med1 is absent in plants, although it has been suggested that CBP1 could act as a tetramer to play the role of Med1 in plants (Li et al., 2015a).

growth (Gillmor et al., 2014). *med8* mutants have smaller organs, due to reduced cell expansion (Xu and Li, 2012). By contrast, *med25* mutants have larger organs (with more and larger cells), as a result of an increased period of cell proliferation and cell expansion, but normal ploidy levels; plants overexpressing *MED25* show smaller organs (Xu and Li, 2011). *pft1 [med25]* mutants also have longer primary roots, and more and longer lateral roots as a result of increased cell division and cell elongation, indicating that MED25 restricts cell expansion and cell proliferation (Raya-González et al., 2014). *struwwelpeter (swp) [med14]* mutants reduce cell numbers in aerial organs by affecting the timing or window of cell proliferation, resulting in smaller aerial organs (Autran et al., 2002).

2.2. Cell wall and cell growth

MED25, along with *MED8*, *MED16*, *MED33A/MED5A*, and *MED33B/MED5B* regulate cell wall composition and growth in plants. Mutations in both *MED25* and *MED8* alter glucose-responsive gene expression, suppressing a cell elongation defect resulting from the arabinose deficiency of *mur4* seedlings (Seguela-Arnaud et al., 2015). Loss of *MED16* allows seedlings to be more resistant to perturbations in cellulose organization, partly through upregulation of pectin methyl esterification inhibitors (Sorek et al., 2015). *MED33A/MED5A* and *MED33B/MED5B* are important for synthesis of lignin, a class of phenylpropanoid polymer that plays an essential role in plant growth through its interaction with cellulose, but which also interferes with extraction of polysaccharides from plant biomass, which consists primarily of cellulose. *med33a* and *med33b* mutants result in increased expression of phenylpropanoid biosynthetic genes and hyperaccumulation of phenylpropanoids, the precursors of lignins (Bonawitz et al., 2012). The stunted growth and lignin biosynthesis mutant *reduced epidermal fluorescence 8 (ref8)*, is partially rescued by mutations in a *med5a;med5b* double mutant, because the loss of these Mediator subunits alters the lignin biosynthetic pathway, resulting in a novel lignin which interferes less with polysaccharide extraction from cellulose. Thus, *med5a;med5b;ref8* triple mutants allow production of biomass and facilitate polysaccharide extraction (Bonawitz et al., 2014).

MED25 also participates in regulation of reactive oxygen species (ROS). This was first discovered due to the role of *MED25* in root hair growth: *pft1* mutants have short root hairs, a defect that can be rescued by application of H_2O_2, which activates Ca^{2+} channels to focus tip growth (Foreman et al., 2003). MED25 was found to promote levels of H_2O_2 producing peroxidases, which in turn regulate cell wall modifying enzymes that promote cell elongation (Sundaravelpandian et al., 2013). A recent study provided a mechanistic link between ROS and Mediator. MED10A, MED28 and MED32, representatives of each core Mediator module (Fig. 2), form covalent oligomers linked by intermolecular disulfide bonds which can be reduced by thioredoxin (TRX)- and glutaredoxin (GRX)-dependent systems, implicating a redox regulation of Mediator function (Shaikhali et al., 2015b). *med28* and *med32* mutants show phenotypes in processes regulated by redox changes: senescence and root development, respectively. *med28* mutants showed an early senescent phenotype associated with earlier upregulation of the *SENESCENCE ASSOCIATED GENE 12 (SAG12)* and elevated levels of H_2O_2 in leaves. On the other hand, the reduction in root length in response to H_2O_2 treatment was significantly stronger in *med32* mutants compared to WT plants; this effect correlates with a defective redox behavior of MED32 protein, which is probably oligomerized in oxidizing conditions (Shaikhali et al., 2015a).

3. *MED12, MED13, MED18,* and *MED25* are major regulators of plant development

Since the biochemical identification of the Mediator complex in *Arabidopsis thaliana* (Bäckström et al., 2007), there have been an increasing number of reports on the role of Mediator in different

plant processes, mostly in Arabidopsis. Besides its function in plant immunity (reviewed in An and Mou (2013)) and sensing environmental nutrients like iron (Yang et al., 2014; Zhang et al., 2014), Mediator is important for regulation of developmental timing during the plant life cycle, as well as hormone responses.

3.1. Regulation of developmental phase transitions

Plants and animals go through multiple developmental phases during their life cycle, including embryogenesis, a post-embryonic (juvenile) phase, and an adult (or reproductive) phase. Seed plants have a more complicated transition from the embryo to juvenile phase, as seed desiccation and subsequent germination are superimposed upon the transition from embryogenesis to vegetative growth. After germination, the vegetative phase of plants consists of juvenile and adult stages, the length of which can vary greatly between species (reviewed in Poethig (2013)). In plants, much research on the role of Mediator has focused on regulating temporal aspects of development, with the majority of research on the timing of the vegetative to reproductive transition.

The Arabidopsis Mediator CDK8 module subunits *CENTER CITY (CCT) [MED12]* and *GRAND CENTRAL (GCT) [MED13]* were first identified based on their regulation of the timing of pattern formation in early embryogenesis: *cct* and *gct* mutants delay specification of the shoot apical meristem, vascular tissue, and ground tissue (Gillmor et al., 2010). Subsequently, *MED12* and *MED13* were shown to regulate the seed-to-seedling transition: *cct* and *gct* mutants show heterochronic misexpression of numerous late embryogenesis seed storage genes in seedlings, with *cct;gct* double mutants having a synergistic effect on seed gene misexpression (Gillmor et al., 2014). Surprisingly, *cct;gct* seedlings show a complete growth arrest. This result suggests that one of the principal roles of *MED12* and *MED13* is to promote the seedling (growth) program by repressing the seed (dormancy) program after germination (Gillmor et al., 2014). Phytohormones have long been known to play a key role in regulating this seed-to-seedling transition: abscisic acid (ABA) promotes late embryo identity and seed dormancy, while gibberellin (GA) promotes germination, and represses embryo identity (reviewed in Holdsworth et al. (2008)). *MED12* and *MED13* repress seed gene expression in seedlings in parallel with GA (Gillmor et al., 2014). *MED18* promotes ABA responses by increasing levels of *ABSCISIC ACID INSENSITIVE 4 (ABI4)* and *ABSCISIC ACID INSENSITIVE 5 (ABI5),* two transcription factors required for ABA responses; loss of *MED18* makes seed insensitive to germination inhibition by ABA (Lai et al., 2014). *MED25* has the opposite role of *MED18*, as *MED25* represses transcriptional responses to ABA by decreasing ABI5 protein abundance. ABA responsive genes are greatly increased in the *med25* mutant (Chen et al., 2012), and thus *MED25* may serve to repress seed specific genes during seedling development, similar to *MED12* and *MED13*.

The transition from the juvenile to adult vegetative phase is controlled by the microRNA miR156 and its targets, the *SQUAMOSA PROMOTER BINDING PROTEIN LIKE* (*SPL)* transcription factors. miR156 levels are high in the early vegetative stage, and decrease during shoot development, with a concomitant increase in *SPLs*. *SPLs* trigger adult leaf traits and flowering partly through increasing transcription of the microRNA miR172 (reviewed in Poethig (2013)). *MED12* and *MED13* regulate the juvenile to adult vegetative transition by fine tuning the levels of miR156 during vegetative development. *cct* and *gct* mutants have higher levels of miR156, decreased levels of *SPL* transcription factors, and decreased miR172, resulting in an extended juvenile vegetative phase (Gillmor et al., 2014) (Fig. 3). Similar to *cct* and *gct*, rice *super apical dormant (sad1)* mutants show higher levels of miR156 and a decrease of miR172, resulting in a delayed juvenile-to-adult transition. Interestingly, *SAD1* encodes an RNA Pol I subunit that interacts with Mediator through direct binding to the MED4 subunit, linking Mediator regulation of vegetative phase change to rRNA production (Li et al., 2015b).

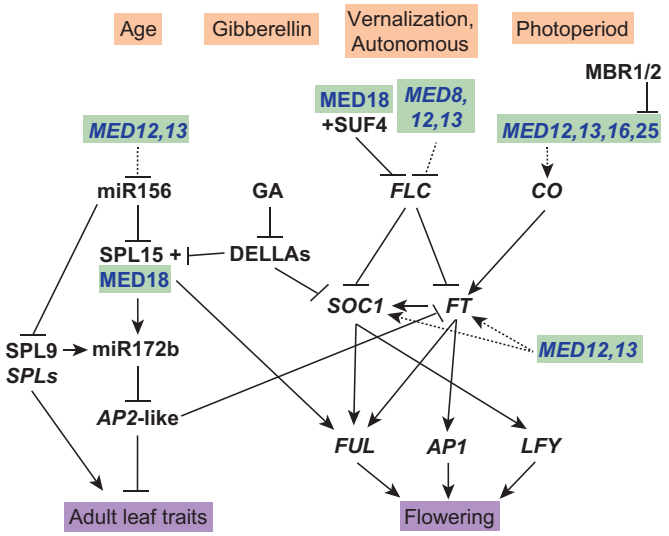

Fig. 3. Mediator regulation of vegetative and reproductive transitions. A simplified model of the genetic network regulating vegetative phase change and the transition to flowering, showing Mediator regulation of components of the network discussed in this review. Mediator regulation of transcription that has not been determined to be direct or indirect is shown with dotted lines. Direct regulation of transcription or protein stability is shown with solid lines. Protein-protein interactions are denoted with '+'. The different pathways controlling vegetative and reproductive transitions are shown with an orange background, Mediator components are shown with a green background, and phenotypic outputs are shown with a purple background. Figure modified from Kim et al., (2009).

The transition from the vegetative to the reproductive stage is controlled by at least five genetic pathways: photoperiod, vernalization, gibberellin, aging, and the autonomous pathway (Fig. 3). The photoperiod pathway responds to day length and light quality, through regulation of the flowering gene *CONSTANS* (*CO*) by photoreceptors and circadian clock-related genes. Vernalization refers to the induction of flowering by exposure to a long period of cold, which leads to epigenetic silencing of the flowering repressor *FLOWERING LOCUS C (FLC)*. Gibberellin signaling is essential for flowering, inducing the floral integrator genes *LEAFY (LFY)* and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)*. The aging pathway is mediated by miR156, via *SPLs*, which promote transcription of the floral integrators *LFY* and *FRUITFULL (FUL)* and the microRNA miR172. The autonomous pathway represses *FLC* expression by regulating chromatin modification and RNA processing (reviewed in Srikanth and Schmid (2011)).

Various Mediator mutants have late flowering phenotypes, specifically *med8* (Kidd et al., 2009), *cryptic precocious (crp)/cct [med12]* (Imura et al., 2012; Gillmor et al., 2014), *macchi-bou2 (mab2)/gct [med13]* (Ito et al., 2011; Imura et al., 2012; Gillmor et al., 2014), *med15* (Canet et al., 2012), *med16* (Knight et al., 2008), *med17* (Kim et al., 2011), *med18* (Kim et al., 2011; Zheng et al., 2013; Lai et al., 2014), *med20a* (Kim et al., 2011) and *phytochrome and flowering time 1 (pft1) [med25]* (Cerdán and Chory, 2003). Due to the complex and interconnected nature of flowering control, some of these Mediator mutants affect multiple flowering pathways (Fig. 3).

MED25 participates in the photoperiod pathway. *pft1* mutants suppress the early flowering phenotype of *phyB* mutants, indicating that *MED25* is essential for the phyB regulation of flowering. *pft1* mutants have reduced transcript levels of *CO* and *FT*, whereas the protein levels of phyA and phyB remain unaffected, suggesting that *MED25* regulates *FT* downstream of *PHYB* (Cerdán and Chory, 2003). Given that the relative contributions of the three

phytochromes phyB, phyD and phyE remained unchanged in *pft1* mutants compared to WT plants, and that the quadruple *phyB phyD phyE pft1* mutant flowered significantly later than the triple *phyB phyD phyE* mutant, *MED25* can still promote flowering in the absence of these three phytochromes (Iñigo et al., 2012a). MED25 promotes flowering by enhancing light sensitivity (through its interaction with COP1 and HY5), and by modulating phyB function (Klose et al., 2012). The induction of flowering by MED25 is coupled to a mechanism called "activation by destruction", where two RING-H2 proteins, called MED25-BINDING RING-H2 PROTEIN1 (MBR1) and MBR2, target MED25 for degradation (Iñigo et al., 2012b).

MED16 is thought to act upstream of the circadian clock. The *sensitive to freezing6 (sfr6) [med16]* mutant is late flowering, and shows reduced expression of the circadian clock genes *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*), *GIGANTEA (GI)*, *FLAVINBINDING, KELCH REPEAT, F-BOX1 (FKF1)*, *ZEITLUPE* (*ZTL)* and *TIMING OF CAB1 (TOC1)*, which in turn regulate the flowering genes *CO* and *FT* in the photoperiodic flowering pathway (Knight et al., 2008, 2009).

MED8, *MED18, MED12*, and *MED13* all act to promote flowering through downregulation of *FLC*, a floral repressor that participates in both the vernalization and autonomous pathways. *med8* and *med18* mutants are late flowering, and have increased levels of *FLC* and decreased levels of the *FLC* target *FT* (Kidd et al., 2009; Zheng et al., 2013; Lai et al., 2014). MED18 represses *FLC* expression by binding directly to the *FLC* promoter and interacting with SUPPRESSOR OF FRIGIDA 4, a transcription factor that promotes *FLC* expression (Lai et al., 2014). MED18 also acts downstream of miR156 in the age-dependent pathway, promoting flowering in short day (SD) conditions by interacting with SPL15 at the promoters of *FUL* and *MIR172B* (Hyun et al., 2016). MED18 and SPL15 directly increase expression of *FUL*, and indirectly increase expression of *FT* by promoting levels of mir172b, which represses APETALA 2-like (AP2-like) transcription factors which otherwise repress *FT* expression (Hyun et al., 2016). CDK8 module subunit mutants *crp/cct [med12]* and *mab2/gct [med13]* also result in delayed flowering. Analysis of dominant and loss of function mutants of *crp/cct* and *mab2/gct* show that *MED12* and *MED13* promote flowering in part by repressing the floral repressor *FLC*, allowing for transcription of the downstream floral activators *FT* and *SOC1* (Imura et al., 2012; Gillmor et al., 2014).

Indeed, *MED12* and *MED13* play an important role as integrators of multiple flowering pathways, and at multiple regulatory levels. In addition to allowing expression of *FT* and *SOC1* by repressing their repressor *FLC*, *MED12* and *MED13* also promote *FT* and *SOC1* expression independently of *FLC*, as *med12;flc* and *med13;flc* double mutants only partially restore *FT* and *SOC1* expression to WT levels (Imura et al., 2012; Gillmor et al., 2014). *MED12* and *MED13* also regulate the photoperiod pathway through increasing CO expression: flowering of *crp/cct* and *mab2/gct* mutants is greatly delayed under long day conditions, but is normal under SD conditions (Imura et al., 2012; Gillmor et al., 2014). *MED12* and *MED13* likely act in parallel with the gibberellin pathway, as GA treatment of *cct* and *gct* mutants can mitigate their late flowering phenotypes, while double mutants between *cct* or *gct,* and a deletion allele *(ga1–3)* of the enzyme encoding the first committed step to GA biosynthesis, have an additive effect on flowering (Gillmor et al., 2014). *MED12* is also required for normal transcript levels of *LFY*, one of the key targets of the gibberellin pathway (Imura et al., 2012). Finally, *MED12* and *MED13* regulate the aging pathway, through repression of miR156 levels, and promotion of *SPL* and miR172 expression. Part of the decrease in *LFY* and *AP1* levels in *crp/cct* and *gct* mutants is likely due to decreased levels of *SPLs* in these mutants (Imura et al., 2012; Gillmor et al., 2014).

3.2. Auxin responses

The phytohormone auxin controls many cellular and developmental processes in plants. Auxin is actively transported through plant tissues by PINFORMED (PIN) auxin efflux transporters and AUXIN RESISTANT1/LIKE AUX1 (AUX1/LAX) influx transporters. Transcriptional responses to auxin are mediated by AUXIN RESPONSE FACTOR (ARF) transcription factors, and AUX/IAA proteins, which impede ARF function. Auxin responsive gene transcription occurs when auxin binds to TRANSPORT INHIBITOR RESPONSE (TIR1) receptors, which mark AUX/IAA proteins for degradation by the Ubiquitin pathway, allowing ARFs to function (reviewed in Enders and Strader (2015)). *MED12*, *MED13* and *MED25* have all been implicated in auxin transcriptional responses. In early embryogenesis, *cct [med12]* and *gct [med13]* mutants have phenotypes characteristic of *ARF* and *IAA* mutants such as *monopteros* and *bodenlos* (Gillmor et al., 2010). Consistent with this, *mab2 [med13]* embryos have decreased transcriptional auxin responses, *mab2* and *crp [med12]* plants have auxin deficient phenotypes, and *mab2* and *crp* enhance the phenotype of *pinoid*, a mutant that affects the polarity of auxin transport (Ito et al., 2011; Imura et al., 2012). Thus, directly or indirectly, *MED12* and *MED13* promote auxin transcriptional responses. *MED25* was recently shown to have the opposite role, repressing auxin dependent transcription. *pft1* mutants increase primary and lateral root growth (processes that are promoted by auxin), while overexpression of *PFT1* has the opposite effect. As expected from their phenotypes, *pft1* seedlings have increased auxin responsive transcription in their roots (including increased *PIN1* expression), while *35S::PFT1* seedlings have decreased auxin responsive transcription (Raya-González et al., 2014). These results raised the possibility that *MED12* and *MED13* act through *MED25* to regulate auxin-responsive gene transcription.

A very recent study examined the molecular basis of Mediator regulation of auxin-responsive gene expression in plants (Ito et al., 2016). Double mutants between *solitary root-1 (slr-1)* (a dominant mutant allele of *IAA14* which is immune to auxin-induced degradation), and *mab2 [med13]*, *crp [med12]* and *cdk8,* showed that these mutants are epistatic to *slr-1*. Using an auxin resistant IAA14 transgene, the Tail module component MED25, as well as the Head module subunit MED17, were also shown to be required to transmit the repressive signal from IAA14. These genetic data are consistent with a model in which IAA14 transmits its repressive function through the CDK8 module, to the Head and Tail module, to inhibit auxin-responsive gene expression via ARF7 and ARF19. Ito et al. (2016) demonstrated the molecular output of this system using *LATERAL ORGAN BOUNDARIES-DOMAIN16 (LBD16),* a target of ARF7 and ARF19, whose expression is repressed by MED13, and promoted by the interaction of MED25 and ARF7. Both MED13, and MED25 (in cooperation with ARF7), were demonstrated to bind to the auxin-responsive element upstream of *LDB16,* in order to repress (MED13) or promote (MED25) auxin responsive gene expression. MED13 and IAA14 were shown to interact in vivo via the transcriptional co-repressor TOPLESS (TPL), which had previously been shown in regulate auxin responsive gene expression through interation with IAA proteins (Szemenyei et al., 2008). All together, these results suggest a model of auxin regulated gene expression via Mediator, where in low auxin conditions the repressive signal from an IAA protein is transduced through TPL to MED13 and the CDK8 module of Mediator, which sterically prevents interation of Core Mediator with RNA pol II, preventing transcription. Higher levels of auxin in the cell would induce degradation of the IAA protein, somehow causing disassociation of the CDK8 module and possibly conformational changes in Core Mediator, allowing ARF proteins, through their association with MED25, to promote transcription of auxin target genes such as LBD16 (Ito et al., 2016). These data are satisfying because they fit with the general idea of

the CDK8 module as a repressor of gene expression, and Core Mediator as a promoter of gene expression (Allen and Taatjes, 2015).

However, when we consider all functional data for the role of *MED12*, *MED13* and *MED25* in auxin-responsive gene expression, the picture becomes more complex. As mentioned above, *med12* and *med13* embryos have multiple phenotypes demonstrating that, during embryogenesis, *MED12* and *MED13* promote auxin responses (Gillmor et al., 2010; Ito et al., 2011; Imura et al., 2012). By contrast, *med25* mutants were shown to have an increase in the auxin responsive markers DR5::GFP and PIN1-GFP, while overexpression of *MED25* had the opposite effect, decreasing auxin responsive marker expression (Raya-González et al., 2014). Thus, the previously reported phenotypes of *med12*, *med13* and *med25* mutants are not what would be expected based on the molecular mechanism above. Taken together, the data suggest that Mediator subunits may promote or repress auxin-responsive gene expression in a tissue-specific manner, perhaps with outputs that depend on the specific IAA-ARF module active in a particular tissue.

4. Mechanistic studies of Mediator in plants

Several recent studies on the role of Mediator in regulating hormone and pathogen responses have contributed to a mechanistic understanding of how Mediator subunits function to regulate gene expression at the molecular level.

4.1. MED18, MED25 and CDK8

Lai et al. (2014) dissected the function of MED18 in repression of *FLC* and activation of *ABI5*. MED18 is found at the *SUPPRESSOR OF FRIGIDA 4 (SUF4)* binding site in the *FLC* promoter, the TATA box, the coding region, and the terminator region. *SUF4* promotes *FLC* expression, and MED18 can interact with SUF4, suggesting that MED18 interacts with SUF4 at the *FLC* promoter to prevent SUF4 activation of *FLC* transcription. Consistent with a repressive role of *MED18* on *FLC* transcription, Histone 3 Lysine 36 tri-methylation ($H3K36me^3$) in *FLC* was increased in *med18* mutants compared to WT, demonstrating that MED18 acts to decrease the presence of this positive mark of transcription. MED18 and SPL15 interact at the promoters of *FUL* and *MIR172B* to promote their expression; this interaction is inhibited in the presence of REPRESSOR OF GA (RGA) (Hyun et al., 2016). By contrast, MED18 promotes *ABI5* transcription, and is constitutively present at the ABI4 binding site in the *ABI5* promoter region. In the presence of ABA, MED18 was also recruited to the TATA box, coding region, and terminator of *ABI5*. MED18 interacts with ABI4, suggesting that perhaps ABA induces ABI4 to recruit MED18 to the *ABI5* gene. MED18 was also shown to be required for RNA Pol II occupancy at *ABI5*, and for recruitment of $H3K36me^3$, a positive mark for transcription. Thus, MED18 was shown to positively and negatively regulate transcription by interaction with transcription factors, occupancy at regulatory and coding regions of genes, RNA Pol II recruitment, and recruitment of epigenetic marks (Lai et al., 2014).

MED25 has also been shown to repress ABA-responsive transcription (Chen et al., 2012). *med25* seed are more sensitive than WT seeds to ABA inhibition of germination. This increase in ABA sensitivity is not due to an increase in *ABI5* mRNA transcripts in *med25* mutants, yet the ABA sensitivity of *med25* mutants is indeed due to *ABI5*, because analysis of *med25;abi5* double mutants showed that the ABA insensitive phenotype of *abi5* mutants is epistatic to the *med25* ABA sensitive phenotype. Further experiments showed that MED25 protein acts to decrease ABI5 protein levels in the absence of ABA, possibly through proteosome-mediated degradation. At low levels of ABA, MED25 and ABI5 interact strongly, and MED25 is present at higher levels at the promoter of the ABA responsive gene *EM6*. At high ABA concentration, there is less MED25-ABI5 interaction, and ABI5 predominates at the *EM6* promoter (Chen et al., 2012). These results suggest that, when ABA levels are low, MED25 exerts its effect on ABA responsive gene expression by targeting ABI5 for proteolysis, and also by preventing ABI5 access to the *EM6* promoter. High ABA levels would prevent this interaction. Whether ABA affects the stability of MED25 protein, or the ability of MED25 to cause degradation of ABI5, remains to be determined. MED25 has also been shown to interact with proteins involved in drought responses, through its ACID domain (Elfving et al., 2011). MED25 interacts with DROUGHT RESPONSIVE ELEMENT PROTEIN B 2A (DREB2A), ZINC FINGER HOMEODOMAIN 1 (ZFHD1), and PHOSPHATE STARVATION RESPONSE LIKE1 (PHL1); the genes encoding all of these proteins are induced in response to drought stress. *med25* mutants are more resistant to drought stress than WT plants, and show upregulation of drought responsive genes (Elfving et al., 2011). This is consistent with the role of *MED25* in repressing ABA-responsive transcription, as ABA is one of the principle hormones involved in promoting drought stress (Chen et al., 2012).

Another recent study of the role of Mediator in disease resistance has illuminated a number of ways in which *CDK8* regulates gene expression. *CDK8* (also known as *CDKE* in plants) was originally identified through mutant analysis as *HUA ENHANCER3 (HEN3). CDK8 [HEN3]* is required for AGAMOUS expression in flowers, and HEN3 was demonstrated to have CDK8 kinase activity (Wang and Chen, 2004). CDK8 was also demonstrated to repress gene expression by interaction with the co-repressor LEUNIG and its partner SEUSS (Gonzalez et al., 2007). Zhu et al. (2014) recently discovered that *CDK8*, as well as *CYCC*, *MED12*, and *MED13* regulate the response to both bacterial and fungal pathogens, though the four components did not always have the same function for all pathogens. CDK8 was shown to physically interact with MED25, and also with CYCCA (CYCC1;1) and CYCCB (CYCC1;2), the two CYCC proteins of Arabidopsis. Both CDK8 and MED25 were demonstrated to interact with WAX INDUCER1 (WIN1) in order to promote epidermal wax deposition; WIN1 is part of the ERF transcription factor family, many members of which have previously been shown to interact with MED25 (Çevik et al., 2012). In response to pathogen attack, CDK8 was shown to increase its occupancy at the upstream regulatory region, TATA box, and terminator sites of *PLANT DEFENSIN1.2 (PDF1.2)*. CDK8 was also shown to recruit RNA Pol II to these same sites, and this recruitment depended on the kinase activity of CDK8. However, kinase activity was not required for resistance to all pathogens, nor for all genes controlled by *CDK8*, demonstrating that CDK8 regulation of transcription can be both dependent or non-dependent on its kinase activity (Zhu et al., 2014).

4.2. Does Med1 exist in plants?

The middle module subunit Med1 plays multiple roles in animal development, due to its ability to interact with transcription factors such as PPAR-γ and GATA-1, through its LxxLL motif (Crawford et al., 2002; Zhu et al., 1997). Interestingly, this subunit is apparently absent in plants, with the exception of the red alga *Cyanidioschyzon merolae*, suggesting Med1 functions are lost in plants or they are carried out by other subunits (Mathur et al., 2011).

A recent study showed a role for Mediator in pollen tube guidance, and suggests that CCG BINDING PROTEIN 1 (CBP1) plays the role of Med1 in plants. CENTRAL CELL GUIDANCE (CCG) and CBP1 are essential proteins for pollen tube attraction; both genes positively co-regulate cysteine-rich peptides (CRPs) in the central cell and the synergid cells, contributing to pollen tube attraction. CBP1

interacts with CCG, Mediator subunits, RNA Pol II and central cell-specific AGAMOUS-like transcription factors. Thus, it has been proposed that the interaction of CBP1 with CCG recruits Mediator and the transcription initiation machinery to the promoters of *AGAMOUS*-like transcription factors to promote pollen tube guidance to the central cell. The interaction of CBP1 with AGL80 and AGL81, proteins involved in endosperm development, suggest that CBP1 also has a role in endosperm development (Li et al., 2015a). Since CBP1 interacts with MED7 and MED9, similar to Med1 in yeast and human, CBP1 may play the role of Med1 in plants. Although MED1 is five times larger than CBP1, the latter can form a tetramer in vitro. CBP1 shows almost no sequence similarity to MED1, but Mediator subunits between different species show low sequence identity due to the high proportion of disordered regions in Mediator proteins (Li et al., 2015a). The essential role of CBP1 in sexual reproduction resembles the essential role of Med1 in spermatogenesis, placenta and embryo development in animals (Huszar et al., 2015; Ito et al., 2000; Landles et al., 2003).

5. Mediator subunits with no known function in development

Plant-specific subunits such as MED34, MED35, MED36 and MED37 have functions distinct from transcription, such as DNA replication and RNA processing, and so far have no described function in plant development (Barneche et al., 2000; Kang et al., 2009; Kobbe et al., 2008). In addition, all subunits of the Middle module of Core Mediator, as well as several Head module subunits, have yet to be ascribed any function in development (Fig. 2 and Table 1). One possibility is that these subunits might serve a primarily structural (essential) function, where their loss would affect Core Mediator activity in many or all contexts. For example in animals, MED11 and MED22 serve a more ubiquitous function by stabilizing the transcription initiation complex (Seizl et al., 2011), MED26 has docking sites for both transcription elongation factors and for the general transcription initiation factor TFIID, and functions as a switch from initiation to elongation (Takahashi et al., 2011), and MED17 plays an essential role in switching between transcription and DNA repair by Nucleotide Excision Repair (NER) (Kikuchi et al., 2015). Mutations in subunits with a ubiquitous function might be expected to lead to gametophytic or embryo lethal phenotypes, and would not have been recovered in genetic screens targeting other phases of development. A simple way to test this hypothesis would be to systematically look for developmental phenotypes of mutations in all Mediator subunits in Arabidopsis.

6. Questions and challenges for identifying specific roles of Mediator in plant development

Research on Mediator in plants is currently confronting a universal issue in gene regulation and developmental biology: how are cellular and tissue-specific signals perceived and transduced into transcriptional outputs? In some cases, Core Mediator is recruited to target genes through interaction of one subunit of Mediator with a specific transcription factor already bound to a promoter or enhancer, for example in the case of MED18 recruitment by SPL15 (Hyun et al., 2016). Specific Mediator subunits may also continually present at their target genes, and act in cooperation with another transcription factor: MED18 is constitutively present at several sites in the *FLC* and *ABI5* genes, where it can interact with SUF4 or ABI4 to repress or promote transcription of *FLC* and *ABI5* (respectively) (Lai et al., 2014). In the future, it will be important to gain a mechanistic knowledge of interactions between the many TFs whose functions have been described in plants, and specific Mediator subunits.

Of all the Mediator subunits, the proteins of the CDK8 module may be the most mysterious, and also the most important for development. Biochemical and structural studies have demonstrated that the CDK8 module can act as a repressor by sterically blocking the initial association of Core Mediator with RNA pol II (Elmlund et al., 2006; Tsai et al., 2013). MED12 has also been demonstrated to recruit histone methylation in order to silence gene expression (Ding et al., 2008). Yet the CDK8 module, and in particular CDK8 itself, have also been shown to promote gene expression (reviewed in Nemet et al. (2014)). In yeast, the CDK8 module was shown to be present at most protein coding genes (Andrau et al., 2006). Thus, regulation by the CDK8 module may be more complex than just proximity to target genes. A couple of examples from yeast have shown that CDK8 module components regulate other Mediator components, or are themselves targeted by signaling pathways. CDK8 regulates the iron homeostasis pathway through phosphorylation of MED2 (van de Peppel et al., 2005), while MED13 itself is a target of the Ras pathway, which targets MED13 for degradation via Ubiquitination (Chang et al., 2004; Davis et al., 2013), demonstrating that targeted degradation is one mechanism for CDK8 module regulation.

Which components of Mediator play discrete roles in developmental biology, and which are factors required for general regulation of transcription? One difficulty in judging the extent of pleiotropy, at least based on morphological criteria, is masking of more subtle phenotypes by severe ones. In animals, especially Drosophila, it is common to study gene function using mosaics of wild type and mutant tissue (Xu and Rubin, 2012). In plants, there are fewer examples of sector analysis (e.g. Poethig and Sussex, 1985; Heidstra et al., 2004). Single tissue or inducible loss of function studies of Mediator subunits in plants would be one way to determine their role in discrete aspects of development, as have previously been useful in studying the role of Med1 in animal development (Landles et al., 2003; Chen et al., 2010; Huszar et al., 2015). Another important tool to determine interactions between individual Mediator subunits and their target DNA elements will be genome level molecular studies at single cell type resolution (Adrian et al., 2015; Efroni et al., 2015). Given the increasing interest in the role of Mediator in plants, the next few years should lead to important insights into the mechanism of Mediator function, as well as its role in development.

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