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The Plant Mediator Complex in the Initiation of Transcription by RNA Polymerase II

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Abstract

Thirty years have passed since the discovery of the Mediator complex in yeast. We are witnessing breakthroughs and advances that have led to high-resolution structural models of yeast and mammalian Mediators in the preinitiation complex, showing how it is assembled and how it positions the RNA polymerase II and its C-terminal domain (CTD) to facilitate the CTD phosphorylation that initiates transcription. This information may be also used to guide future plant research on the mechanisms of Mediator transcriptional control. Here, we review what we know about the subunit composition and structure of plant Mediators, the roles of the individual subunits and the genetic analyses that pioneered Mediator research, and how transcription factors recruit Mediators to regulatory regions adjoining promoters. What emerges from the research is a Mediator that regulates transcription activity and recruits hormonal signaling modules and histone-modifying activities to set up an off or on transcriptional state that recruits general transcription factors for preinitiation complex assembly.

Contents

INTRODUCTION

In eukaryotes, RNA polymerase II (Pol II) synthesizes messenger RNA (mRNA), a universe of transcripts that includes protein-coding mRNAs and microRNAs (miRNAs). Other RNAs are synthesized by Pol I, which makes up the larger ribosomal RNAs, and Pol III, which synthesizes transfer RNAs, most of the small nuclear RNAs, and 5S ribosomal RNAs. In plants, two other RNA polymerases have evolved from Pol II, RNA polymerase IV and RNA polymerase V, which acquired novel and nonredundant functions in the synthesis of noncoding RNAs that enter in the RNA-directed DNA methylation (RdDM) pathway (reviewed in 102).

Pol II: RNA polymerase II

mRNA: messenger RNA

miRNA: microRNA

RdDM: RNA-directed DNA methylation

GTF: general transcription factor

PIC: preinitiation complex

TBP: TATA-box-binding protein

CKM: CDK kinase module

The process of transcription by Pol II is highly regulated. Once promoter accessibility is granted, Pol II assembles with general transcription factors (GTFs) to form the preinitiation complex (PIC) on the core promoter containing the TATA box consensus sequence (21). The transcription initiation site is set once the PIC is assembled. The PIC components are highly conserved in eukaryotes, Pol II, and GTFs, which include TFIIA, TFIIB, TFIID, TFIIF, TFIIE, and TFIIH. Recognition of the core promoter requires TFIID, which consists of the TATA-box-binding protein (TBP) plus 13 TBP-associated factors (TAF1 to TAF13). The PIC may be assembled in vitro and is sufficient for a basal level of transcription but does not lead to the precise level of transcriptional regulation required to build a eukaryote. The diverse signals that lead to eukaryotic PIC assembly need to be processed and transmitted to Pol II. This function is carried out by the Mediator complex.

Depending on the species, the Mediator complex comprises about 25 to 30 subunits, which form three modules—the Head, Middle, and Tail (126) (**Figure 1**)—and a separable regulatory module, the CDK kinase module (CKM), the only one with enzymatic activity. The Head and Middle modules interact with Pol II and GTFs, while the Tail module interacts with specific TFs, which may be either activators or repressors, and is able to transmit signals through the Head and Middle modules to the basal transcription machinery (104).

The purpose of this review is to integrate the recent advances in our understanding of the structure of the Mediator complex in yeast and mammals with the work done in plants, mostly

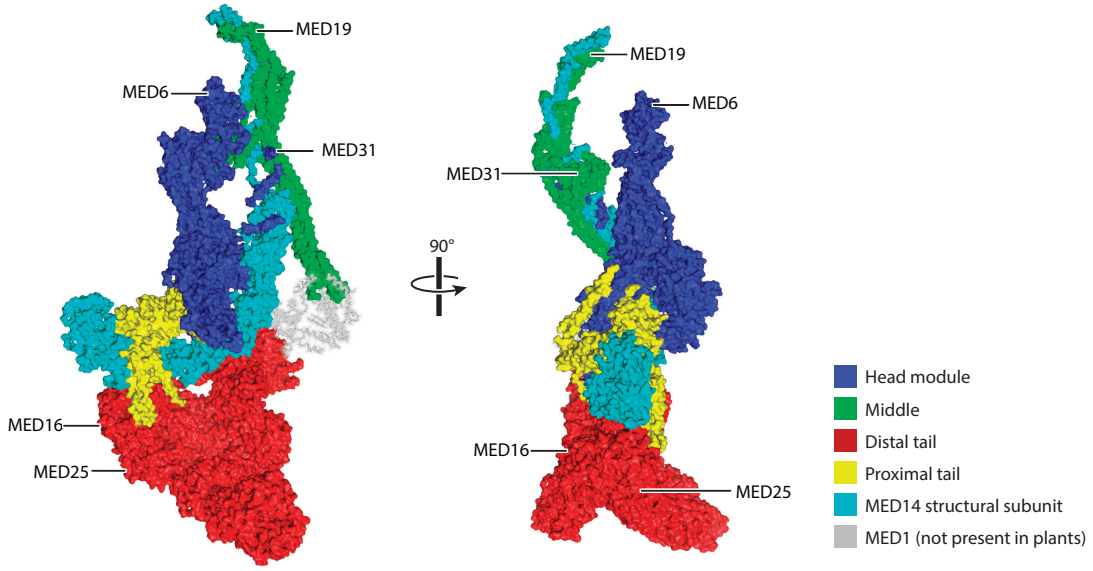


Figure 1

The structure of the mammalian Mediator complex. The mammalian structure was selected because it contains the same subunits as the *Arabidopsis* Mediator, except for MED1 (*light gray*), and because of its higher conservation of the primary structure of plant subunits in mammalian or human subunits than in yeast subunits. Data from Reference 33. Figure adapted from images created with the PyMOL Molecular Graphics System, Version 2.5.5 Schrödinger, LLC.

with genetic approaches, and offer a perspective of how this structural information may guide future research in the function of the plant Mediator complex in the initiation of transcription by Pol II.

BRIEF HISTORY

Mediator was discovered about 30 years ago as a fraction from a yeast extract that could be distinguished from general initiation factors and had a transcription-promoting activity, but only in the presence of transcriptional activators (36). Later, Mediator was purified and shown to promote the phosphorylation of the C-terminal domain (CTD) of Pol II by the TFIIH complex (70). In parallel experiments, suppressors of CTD truncations allowed the isolation of Mediator genes (*SRB* genes) in yeast (95). Afterward, their protein products were shown to copurify with a 1.2–Mega Dalton (Md) complex, which also included the TBP (121).

The importance of Mediator in vivo was determined by using thermosensitive *srb* mutants in yeast; at the restrictive temperature, mRNA transcription was essentially shut down, indicating that Mediator is essential for transcription by Pol II (54, 122).

The first electron microscopy images of Mediator revealed its shape (5) and started a fantastic journey toward the determination of the Mediator structure (reviewed in 126). Later, others revealed the modular nature of Mediator and proposed the Head, Middle, and Tail modules and that Mediator conformation could change upon activator binding (27, 32, 117). It took more than 10 years of structural research to correctly assign the modules in the Mediator images (124, 129). Eventually, purification from different cells using a battery of tagged Mediator subunits led to consistent results regarding the mammalian Mediator composition and suggested that differences in composition in earlier reports were due to methodological differences (110). Nevertheless,

CTD: C-terminal domain

some differences persisted and are likely to reflect the existence of functionally distinct forms of Mediator.

hMed: human Mediator complex

yMed: yeast Mediator complex

mMed: mammalian Mediator complex

cMed: core Mediator

Cryo-EM: cryogenic electron microscopy

Arabidopsis Mediator was initially purified from cell suspension cultures, and 21 subunits were identified (7, 91). Due to sequence divergence, several subunits were named as new subunits (**Table 1**), but refined bioinformatic searches revealed the similarities among subunits across eukaryotes, flagging those previously thought to be unrelated (14, 91). More recently, the use of transgenic lines carrying FLAG-tagged versions of different subunits and purification led to the consistent identification of 28 conserved subunits (46). The only subunits not found with respect to the human Mediator complex (hMed) were MED1 and MED26. MED1 was never found, while three paralogs of MED26, MED26A–MED26C, were identified in *Arabidopsis* (91). MED26 is present at substoichiometric levels in hMed (110, 119), and this might also be the case in *Arabidopsis*. In addition to the conserved subunits, the CREB-binding protein (CBP)/p300 histone acetyltransferases HAC1 and HAC5, a DNA helicase, ATPRP40A, ATFIB2, and HSP70-11/HSP70-12 were proposed to be part of the plant Mediator complex (7, 46). However, these proteins were not found across independent purifications of the plant Mediator complex, and we lack biochemical and structural studies to ascertain whether these proteins are genuine plant Mediator components.

The latest structural studies highlight the higher level of conservation of structural information regardless of the relatively low sequence identity of MED subunits across yeasts and mammals. Unfortunately, we lack structural information on the plant Mediator other than protein–protein interaction assays (46, 89).

THE STRUCTURE AND SUBUNIT COMPOSITION OF MEDIATOR

In the last 11 years, we have witnessed impressive advances toward the determination of the yeast Mediator complex (yMed) and mammalian Mediator complex (mMed) structures. The structure of the Head module was determined in both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* by X-ray crystallography at resolutions ranging from 3.4 Å to 4.3 Å (57, 79, 105) and the structure of the core Mediator (cMed; comprising 15 subunits including the Head and the Middle modules) was determined in *S. pombe* with a resolution of 3.4 Å (96). These X-ray structures fit into the cryogenic electron microscopy (cryo-EM) map of yMED, suggesting that individual modules are relatively stable within the Mediator complex and that their relative positions may vary as part of conformational changes in the whole complex (124).

Cryo-EM maps were obtained with increasing resolution for the core and complete Mediator complex structures of yeast, mouse, and human with a resolution of up to 4 Å (33, 124, 129, 143). The cryo-EM structure of the Mediator bound to Pol II was obtained for the thermophilic fungus *Chaetomium thermophilum* and *S. pombe* (123, 138). The comparison between free and Pol II–bound Mediators showed that Mediator changed its conformation, the hook in the Middle module moved toward the neck in the Head module (**Figure 1**), and the Tail rotated. These data suggest a coordination of movements across the entire Mediator through MED14, the structural subunit that connects the three modules (see the section titled The Structural Roles of MED14) (123, 138).

Models for the Mediator complex and the Mediator complex assembled with the PIC were also obtained for both the yMed and hMed, giving important insights on how Mediator can control transcriptional as well as cotranscriptional events, including the phosphorylation of the Pol II CTD (1, 22, 100, 103, 106).

The Head Module: Conservation, Roles, and Composition

Mediator and the Mediator Head module are essential for transcription. Disruption of the Mediator Head module by growing thermosensitive yeast *srb4* mutants, which encode a defective

Table 1 Mediator subunit orthologs in plants, yeast, and human

Module	Submodule(s)	Plant[a]	Identification	Yeast	Human
Head	Shoulder/arm (neck)/beam	MED6	AT3G21350	MED6	MED6
	Neck/movable jaw	MED8	AT2G03070	MED8	MED8/ARC32
	Neck	MED11	AT3G01435	MED11	MED11
	Neck/fixed jaw	MED17	AT5G20170	SRB4	MED17/TRAP80
	Movable jaw	MED18	AT2G22370	SRB5	MED18/SRB5/p28b
	Movable jaw	MED20	AT2G28230 MED20A, AT4G09070 MED20B, AT2G28020 MED20C	MED20/SRB2/HRS2	MED20/SRB2/TRFP
	Neck	MED22	AT1G07950	SRB6	MED22/SRB6/MED24/SURF5
Tail	Proximal Tail	MED28	AT3G52860	NA	MED28
	Proximal Tail	MED30	AT5G63480	NA	MED30/TRAP25
	Proximal Tail	MED 2/MED32	AT1G11760	MED2	MED29
	Proximal Tail	MED 3/MED27	AT3G09180	MED3/PGD1/HRS1	MED27/MED3/CRSP8/CRSP34
Backbone	Hook/beam/knob	MED14	AT3G04740	MED14/RGR1	MED14/CSRP/RGR1/CRSP2/ CRSP150/DRIP150/TRAP170
Tail	Distal Tail	MED15	AT1G15780	MED15/GAL11/ABE1/SPT13	MED15/TIG1/PCQAP/ARC105
	Distal Tail	MED16	AT4G04920	MED16/SIN4/GAL22	MED16/THRAP5/TRAP95
	Distal Tail	MED23	AT1G23230	NA	MED23/SUR2/CRSP3/MRT18/ SUR-2/CRSP130/DRIP130
	Distal Tail	MED33/MED5	AT3G23590 MED33A/ MED5A, AT2G48110 MED33B/MED5B	MED5/NUT1	MED24/MED5/ARC100/DRIP100
	Distal Tail	MED25	AT1G25540	NA	MED25/P78/ACID1/ARC92/PTOV2/ CMT2B2
Middle	Plank/knob	MED4	AT5G02850	MED4	MED4/TRAP36
	Knob/hook	MED7	AT5G03220 MED7A, AT5G03500 MED7B	MED7	MED7/ARC34/CRSP9/CRSP33
	Plank	MED9	AT1G55080	MED9/CSE2	MED9
	Hook	MED10	AT5G41910 MED10A, AT1G26665 MED10B	MED10/NUT2	MED10/L6/NUT2
	Hook	MED19	AT5G12230 MED19A, AT5G19480 MED19B	NA	MED19/LCMR1

(*Continued*)

Table 1 (*Continued*)

Module	Submodule(s)	Plant[a]	Identification	Yeast	Human
	Hook	MED21	AT4G04780	MED21/SRB7	MED21/SRB7
	Connector/hook	MED26	AT3G10820 MED26A, AT5G05140 MED26B, AT5G09850 MED26C	NA	MED26/CRSP7/CRSP70
	Knob	MED31	AT5G19910	MED31/SOH1	MED31/SOH1
CKM	NA	MED12	AT4G00450	MED12/SRB8/GIG1/NUT6/SSN5	MED12/FGS1/HOPA/ARC240/ TRAP230
	NA	MED13	AT1G55325	MED13/TRAP240/SRB9	MED13/ARC250/THRAP1/DRIP250/ TRAP240
	NA	CDK8	AT5G63610	CDK8/SSN3/GIG2/NUT7/RYE5/ SRB10	CDK8/K35
	NA	CYCC	AT5G48640 CYCC1;1, AT5G48630 CYCC1;2	SSN8/CNC1/GIG3/NUT9/RYE2/ SRB11	CycC/SRB11

[a] List of Mediator subunits found in *Arabidopsis*. All the subunits were identified after immunopurification of tagged versions (47), with the exception of MED26 paralogs, which were identified by bioinformatics (91). MED26 plays posttranscriptional roles and is found at substoichiometric levels (110, 119). MED1 was not included because it was not found in plants by either method. Some subunits include more than one Mediator subunit name because originally they were not assigned correctly to orthology groups due to low conservation of sequence. Those names and most commonly used names, but not all names, are also included.

Abbreviations: ABE, activator of basal expression; ARC, activator-recruited factor; CDK8, cyclin-dependent kinase 8; CKM, CDK kinase module; CRSP, cofactor required for Sp1; CSE, cold-sensitive chromosome segregation; CYCC, cyclinC; DRIP, vitamin D receptor-interacting proteins; FGS, FG syndrome; GAL, galactose negative phenotype; GIG, GlcNAc-induced gene; HOPA, human opposite paired; HRS, hyper-recombination suppression; K35, Protein kinase K35; LCMR, lung cancer metastasis-related protein; MED, Mediator; NA, not applicable; NUT, negative regulation of URS2; PGD1, poly-glutamine domain protein 1; RGR1, repressor of glucose-regulated genes 1; THRAP, thyroid hormone receptor (TR)-associated protein; SIN, switch independent; SOH, suppressor of hpr; SPT, suppressor of Ty; SRB, Suppressor of RNA polymerase B; SURF, surfeit locus protein; TRAP, thyroid hormone receptor-associated protein; TRFP, TRF-proximal protein homolog.

MED17, at the restrictive temperature brings transcription to a halt and impairs the association of all Mediator modules with promoters in vivo (118, 122).

The Head module is formed by seven subunits (MED6, MED8, MED11, MED17, MED18, MED20, and MED22) in all the systems studied through X-ray diffraction or cryo-EM: mouse, human, *S. cerevisiae*, *S. pombe*, and *C. thermophilum* (1, 19, 22, 33, 57, 79, 96, 100, 103, 105, 111, 124, 138) (**Figure 1**). Consistent with the essential role of the Head module, MED6, MED8, MED11, MED17, and MED22 are essential for mammalian and yeast cell viability. In *Arabidopsis*, loss-of-function mutants for *MED6*, *MED11*, and *MED22* were not reported. MED20 is encoded by three paralogs: *MED20a*, *MED20b*, and *MED20c*. MED20a and MED20b proteins are 76.26% identical. *MED20c* encodes a putative protein of 70 residues, which are 92.75% identical to the C-terminal portion of MED20a (71). A *med20a* mutant was isolated from an ethyl methanesulfonate (EMS) screening, but mutants for *MED20b* and *MED20c* were not described, and we do not know whether they are viable. The transcriptome of *med20a* was compared with that of the *nrpb2-3* mutant, which encodes a weak allele of the second largest subunit of Pol II. Downregulated genes in both genotypes were highly concordant, suggesting that the Head module is essential for transcription in plants also. Further, in *med20a* mutants, Pol II recruitment to promoters was affected, also suggesting that Mediator is important in the recruitment of Pol II to regulatory regions in plants (71).

When compared to other Head mutants, the phenotype of *med20a* mutants was similar to *med17-1* (SALK_102813) and *med18-1* (SALK_027178), which is consistent with all of those affecting the Head module. However, *med20a* displayed a stronger phenotype than *med17-1* and *med18-1*. Some level of *MED17* and *MED18* transcripts persisted, indicating that these two mutants are not null (71).

MED17 is a scaffold for Head module assembly in both mMed and yMed. MED17 is similar in size to yeast and human orthologs, and, despite low identity (17%), its AlphaFold (64) prediction displays a similar structure. In hMED, the N-terminal half of hMED17 contacts other Head module subunits, while the C-terminal half contacts the structural hMED14 subunit (see the section titled The Structural Roles of MED14) and the Tail module subunits hMED2/hMED29 and hMED15 (22). In plants, the N-terminal half of MED17 interacts directly with Head module subunits MED6, MED11, MED18, and MED22 (89) but also with MED14. Therefore, as expected, *med17* knockout mutants are inviable (28). However, in *med17-1* mutants, a truncated version of *MED17* mRNA is still expressed (41), which may produce a polypeptide of about 400 residues. This truncated MED17 could be sufficient to assemble the Head module, accounting for mutant viability, but could affect interactions with the Tail module subunits, also accounting for its phenotype. Unfortunately, we lack Mediator structural data to test these predictions.

The N-terminal portion of hMED8 forms part of the neck together with hMED6, while the C-terminal portion forms the movable jaw together with hMED18 and hMED20 (103) (see **Figure 1**). Human and yeast Head modules are highly conserved with respect to size [268 amino acids (aa) for hMED8 and 223 aa for yMED8], location, and interactions of MED8 (107, 109). *Arabidopsis med8-2* homozygous mutants are embryo lethal, confirming that MED8 is essential (51). *Arabidopsis* MED8 is larger (454 aa) and 19.83% and 19.42% identical to its human and yeast counterparts, respectively, but its identity is restricted to the first 200 residues. Truncation alleles *ceal5* (377 aa) and *med8-1* are viable in *Arabidopsis* (51), suggesting that the Q-rich C-terminal portion of MED8 is dispensable for viability and Mediator assembly and may have acquired novel functions in plants during evolution. *med8-1* mutants are more resistant to *Fusarium oxysporum* and more susceptible to *Alternaria brassicicola* and *Botrytis cinerea* (66). Interestingly, MED8 interacts with FAMA, a basic helix-loop-helix (bHLH) TF, and both seem to interact in an

interdependent manner in the *THIOGLUCOSIDE GLUCOHYDROLASE 1* (*TGG1*) regulatory region, to promote jasmonic acid (JA)-responsive expression of *TGG1* mRNA (35, 84).

The Proximal Tail: A Head–Tail Connection Conserved in Mammals and Plants

Four other subunits have been included in the hMed Head module: MED3/MED27, MED28, MED2/MED29, and MED30 (1, 22). Several pieces of evidence support this view. The C-terminal portion of MED14 (the scaffold subunit that connects all modules) wraps around the Mediator Head module, dividing the Head and the Tail modules and leaving the four subunits on the Head side (22) (**Figure 1**). It has been reported that these four subunits stabilize the cMed (103) and also that MED15 and MED27–MED30 remain associated with cMed upon loss of subunits of the large Tail (distal Tail) segment (33). Nevertheless, other evidence suggests that these four subunits are part of the Tail module and were proposed to form a proximal Tail or an upper Tail that connects the Head and lower Tail or distal Tail modules (33, 103, 143). They are organized in pairs (MED3/MED27-MED2/MED29 and MED28-MED30) and make extensive contact with both the Head and distal Tail modules. MED3/MED27 is positioned between the Head jaws and contacts subunits MED20 and MED17, while MED28 and MED30 C-terminal helices form a four-helix bundle with the C-terminal helices of MED11 and MED22 (103). On the other side, MED3/MED27 and MED2/MED29 contact MED16 in the distal Tail module, and two helices of the distal Tail subunit MED15, residues 617 to 649, are sandwiched between MED3/MED27 and MED2/MED29, forming an important interface between the proximal and distal Tail portions (103, 143). As we explain below, the MED15–MED3/MED27–MED2/MED29 interactions are conserved in yeast and *Arabidopsis*.

MED28 and MED30 were not found in yeast, but they are present in *Arabidopsis*, and they interact by two-hybrid assays (46). Therefore, as in mammals, there is a putative proximal Tail module in *Arabidopsis*. Mutants of these two subunits were characterized, and *med28* (Salk_037570) mutants are viable and show irregular downward-curled leaves and accelerated senescence (112). By contrast, *med30* knockouts are inviable, and MED30 is essential for the paternal control of early embryo development. Further, knockdown of *MED30* affected flowering but did not promote senescence (62). These contrasting phenotypes between *med30* and *med28* mutants suggest that, regardless of forming a pair in *Arabidopsis*, they may still assemble into the Mediator complex independently of each other.

The other two subunits that form the proximal Tail module, MED3/MED27 and MED2/MED32 (MED29 in mMed), connect the distal Tail module in *Arabidopsis*. In yeast, MED2, MED3, and MED15 form a submodule, suggested by their similar transcriptomes (125). The TFs HEAT SHOCK FACTOR 1 (HSF1) and GENERAL CONTROL FACTOR 4 (GCN4) recruit the MED2-MED3-MED15 submodule, independently of yMed, to their target promoters (4, 69, 137). The submodule has been observed by cryo-EM with a shape consistent with an elbow that connects the Tail with the Head modules, and the Tail is lost in Δ*med15* mutants (124). In plants, MED2 interacts directly with both MED3 and MED15a (89). Both *med2* and *MED15a* mutants display defective papillae on *Arabidopsis* leaf trichomes (37) and are affected in the defense response, and transcriptomic analysis suggests that they may also play a role in the salicylic acid (SA) response (17, 30). However, no other phenotypic similarities were found among *med2*, *med3*, and *med15a* mutants, leaving open the question of whether the MED2-MED3-MED15 submodule acts as a submodule in plants (17, 30, 31, 68, 127).

The Middle Module

yMed purified from a *med19* mutant produces a Middle module formed by subunits MED1, MED4, MED7, MED9, MED10, MED21, and MED31 in equimolar stoichiometry (9, 76).

Cryo-EM images also demonstrated that MED19 forms part of the Middle module's hook (124). The Middle module was crystallized as part of the 15-subunit cMed from *S. pombe* (96), also containing the Head module that displayed the same structure as the free form (79). The subunits MED4, MED9, MED7, MED21, MED31, MED10, the MED14 N-terminal, and MED19, listed here in the order they appear in the elongated Middle structure, form the plank, beam, connector, knob, and hook (**Figure 1**). The MED1 subunit probably dissociated during crystallization (96). Dissociation of the *S. pombe* Middle module revealed the MED4-MED9 and MED7-MED21 dimers and the trimer MED7-MED21-MED31 (76). These interactions were also shown by two-hybrid assays and are conserved in yeast and *Arabidopsis* (45, 46, 49, 76, 78, 89, 93) (**Supplemental Table 1**).

TF: transcription factor

MED14 in the Middle module contacts the Head module by interfacing with both MED20 in the Head movable jaw and MED17 in the fixed jaw, and MED6 in the Head module contacts the hook formed by MED10 and MED19. MED6 and MED17 also showed shared domains forming tethers: The C-terminal helix in MED6 and two N-terminal helices in MED17 associate with MED14 in the beam region of the Middle module. Interestingly, *Arabidopsis* MED14 interacts with both MED17 and MED6, suggesting that these Head–Middle tethers and contacts are conserved in *Arabidopsis* (89). This evidence suggests the conservation of the topology of both the Middle module and the Head–Middle interfaces in *Arabidopsis* and yeast.

The cryo-EM structure of mMed reveals that its Middle module is similar to the yMed counterpart. It is an extended structure, with most of its subunits being helical, and therefore predicted to be rigid with the potential to transmit structural rearrangements; MED1 is the exception (143). As MED1 is absent from plants, and several intersubunit interactions are conserved (**Supplemental Table 1**), we may infer that the Middle module will also be rigid and elongated in plants.

Supplemental Material >

Two paralogs encode MED7 in *Arabidopsis*: *MED7A* and *MED7B*, two proteins with 168 residues and 95.83% shared identity (77). They share about 35% identity with their human and yeast counterparts. Downregulation of both *MED7a* and *MED7b* leads to defects in hypocotyl elongation and, at the transcriptomic level, misexpression of brassinosteroid and auxin pathway genes, which explain their phenotype (77).

CENTRAL CELL GUIDANCE (CCG) and CCG-BINDING PROTEIN1 (CBP1) interact in the central cell to guide pollen tube growth during fertilization by inducing the expression of cysteine-rich peptide (CRP) transcripts. While CCG interacts with TBP1 and the CTD of Pol II, CBP1 interacts with both MED7 and MED9 and with MADS-box transcription factors (TFs) that are specifically expressed in the central cell and the endosperm. Li et al. (82) proposed that, in this way, CCG and CBP1 connect TFs with the Pol II machinery to regulate pollen tube attraction. They also proposed that CBP1 replaces the role of MED1 in plants, although MED1 is unrelated to CBP1 (82). Up to now, CBP1 has not been shown to copurify with the *Arabidopsis* Mediator complex in different preparations, suggesting that it is not a genuine MED1 subunit (7, 46).

Arabidopsis MED4 is encoded by a single gene; MED4 displays 20–22% identity with yMED4 and hMED4, although *Arabidopsis* MED4 is longer. Extra residue stretches appear along the MED4 protein and are not C-terminal extensions, as they are in MED8 and MED14.

Transfer DNA (T-DNA) insertion alleles of *Arabidopsis MED4*, *med4-1*, and *med4-2* (SALK_122082 and SALK_085899) did not produce homozygous mutants and segregated at a 1:1 ratio, suggesting that MED4 is essential for either pollen or ovule development (83). MED4 is 426 residues long; the sequence insertion data of SALK_122082 and SALK_085899 suggest that a truncated version of MED4 could possibly be produced but no longer than 235 aa. However, a viable mutant allele of *MED4* was recently obtained from EMS-treated plants. This novel *MED4* allele, *suppressors of cpr5* (*socp1/med4-3*), was isolated as a suppressor of the mutant *constitutive expression of PR genes 5* (*cpr5*). The *med4-3* lesion is a nonsense mutation that likely produces a

truncated form of MED4 (MED4*) with 277 residues (88). As in the cases of MED8*, MED14*, and MED17*, this MED4* protein might still assemble into the Mediator complex. The AlphaFold (64) prediction suggests that the N-terminal α-helices described in yMED4 (96) are conserved in *Arabidopsis* MED4 and present in MED4*. The C-terminal portion of MED4 interacts with MED9 in a two-hybrid system. Other contacts among subunits might act redundantly to maintain the overall structure of Mediator in *med4-3* mutants.

The reasons behind the suppression of *cpr5* by *med4-3* are presently unknown. CPR5 is an SR family protein with RNA-binding activity that negatively regulates plant immunity. *Cpr5* mutants show alternative splicing (AS) defects genome wide (98). It would be interesting to know if the AS defects observed in *cpr5* mutants are also suppressed by *med4-3*.

MED9 is encoded by a single gene, has 244 residues, and shares about 17% identity with yeast (149 aa) and human (146 aa) orthologs. A second partial copy is present in the *Arabidopsis* genome, but it is not expressed (26). MED9 interacts with MED4, and this interaction is conserved in yeast (45, 89) (**Supplemental Table 1**). Given the role of MED9 in the Middle module, and the fact that subunits MED4 and MED21 are also essential, it is unlikely that *MED9* knockouts would be viable. A single mutant has been characterized so far, an insertional mutant (SALK_029120) in which the T-DNA is inserted in the last intron of *MED9* (26) and could produce a polypeptide of at least 225 residues. This *med9* mutant shows a relatively weak phenotype, including a low number of differentially expressed genes (DEGs) when its transcriptome is compared with the wild type (WT) (26) (**Supplemental Table 2**).

Supplemental Material >

Two paralogs, *MED19a* and *MED19b*, encode MED19 in *Arabidopsis*. Both share 79% identity with each other and also 24–30% identity with yMED19 and hMED19 orthologs. Together with MED10 and the N-terminal portion of MED14, MED19 forms the hook. The MED19 C terminus points toward the tip of the hook, both in mMed and yMed (96, 143). Given that the primary sequence is relatively well conserved, their location in the hook might be conserved in *Arabidopsis*, but we lack the protein interaction data to confirm or dismiss this assertion.

Two alleles of *MED19a*, *med19a-1* and *med19a-2*, were characterized in *Arabidopsis*, and mutants resemble wild-type plants but with abnormally shaped siliques (16). *med19a-1* and *med19a-2* are more susceptible to the biotroph *Hyaloperonospora arabidopsidis* (*Hpa*), the causal agent of downy mildew. During infection, the HaRxL44 effector targets MED19a and promotes its degradation by the proteasome, reprogramming the defense transcriptome, reinforcing JA and ethylene (ET) signaling, decreasing SA-triggered immunity, and thus favoring infection by biotrophs.

Biotrophs inhibit senescence to increase host susceptibility (reviewed in 48). Interestingly, senescence is promoted by MED19a. The TF ORESARA1 (ORE1) physically interacts with MED19a and acts in the same pathway in an interdependent manner to induce senescence under nitrogen deficiency, integrating senescence signals through this interaction with MED19a (23). The C-terminal portion of MED19a is likely to be exposed at the tip of the Middle module hook, as we mentioned above. This C-terminal is therefore free to interact directly with ORE1. It seems that *Hpa* has evolved to manipulate this pathway to delay senescence and promote infection.

The MED19a–ORE1 interaction is also disrupted by *ELF18-INDUCED LONG NONCODING RNA 1* (*ELENA1*). ELENA1 is expressed in roots under nitrogen starvation and transported to the shoots to attenuate senescence promoted by MED19a and ORE1 (24).

Two paralogs, *MED10a* and *MED10b*, which share 77% aa identity, encode MED10. MED10a is the most conserved, with 21.33% and 36.57% similarity to yMED10 and hMED10, respectively, while MED10b shares 20% and 32.84% identity with them. Consistent with forming part of the hook, *Arabidopsis* MED10a interacts with the N-terminal domain of MED14 (89) but also with Middle subunits MED4 and MED7. The AlphaFold (64) prediction for MED10a and MED10b folding is very similar to the structure of yMED10 (96), suggesting that their location in the hook

as well as their exposure in the complex might also be conserved. This might be an important feature, as together with MED21 they form an exposed surface that may be bound by corepressors (44, 81).

Sw5b is a nucleotide-binding domain and leucine-rich repeat–containing (NLR) protein from wild tomato that confers resistance against the tomato spotted wilt virus. The N-terminal coiled-coil (CC) domain of Sw5b interacts with MED10b in both tomato and *Nicotiana benthamiana*. Using *N. benthamiana* as a model, researchers showed that Sw5b disrupts the interactions between NbMED10b and NbMED7 to relieve the repression of JA-responsive genes and induce immune responses (131).

A single gene encodes MED21; containing 139 residues, it is conserved in size with yeast and human orthologs, which comprise 140 and 144 residues and share 21.90% and 25.93% identity, respectively. The AlphaFold prediction for *Arabidopsis* MED21 folding is very similar to those for yMED21 and hMED21. The MED21-MED7-MED9 association is well conserved in yeast and mammalian Mediator complexes and seems to be conserved in *Arabidopsis* (89) (**Supplemental Table 1**), suggesting a structural role consistent with being an essential subunit (29). Interestingly, MED21 interacts with the RING E3 ligase HISTONE MONOUBIQUITINATION1 (HUB1). *hub1* mutants and RNA interference (RNAi) lines of MED21 are more susceptible to *B. cinerea* and *A. brassicicola*; therefore, it seems likely that MED21 contributes to defense against necrotrophic fungal pathogens, and this may occur through interaction with HUB1 (29). It would be interesting to know if the HUB1–MED21 interaction occurs in the context of the *Arabidopsis* Mediator complex.

Supplemental Material >

A single gene encodes MED31; it shares 41.22% and 36.2% identity with hMED31 and yMED31, respectively. Identity is limited to the central portion, where a series of helices predicted by AlphaFold are conserved. The location in the knob is consistent with two-hybrid assays in *Arabidopsis* (89) (**Supplemental Table 1**). MED31 is also larger (196 aa) compared with yMED31 (127 aa) and hMED31 (131 aa) and carries a C-terminal extension (aa 131–196) of 66 residues. MED31 is essential for both mammalian and plant development (141); RNAi was used to knockdown *Arabidopsis MED31*, and these RNAi plants showed stunted root growth and reduced root meristem size, phenotypes similar to those of *SHORTROOT* (*SHR*) and *SCARECROW* (*SCR*). SCR binds MED31, acts in the same pathway, and, depending on their relative abundances, forms a ternary complex with SHR to regulate *CYCLIND6;1* (*CYCD6;1*) expression and root patterning. More importantly, SCR was necessary to recruit MED31 to the *CYCD6;1* promoter (141). Determining if this interaction occurs in the context of the complete Mediator would be interesting.

The Tail Module: A Hub for Transcription Factor Binding

As mentioned above, the proximal Tail subunits of the hMed MED29/MED2, MED27/MED3, MED28, and MED30 connect the Head module to the distal Tail module (33, 103, 108, 143). In the mMed structure, these four subunits, which are relatively small, have adopted extended conformations and show large interfaces among themselves and with MED14 and the Head module. By contrast, the distal Tail large subunits MED5/MED24/MED33, MED15, MED23, MED16, and MED25 are mostly self-contained (143). Consistent with self-containment, phenotypes and transcriptomes of *Arabidopsis* distal Tail mutants are clearly different, although significant overlapping is also found (30) (**Supplemental Table 2**). We prefer to stick to the proximal/distal Tail classification, as it emphasizes the difference both with Head subunits and between Tail subunits, which is more representative of their roles.

The distal Tail subunits have been regarded as the sites for TF binding, although they are not exclusive in this role. In contrast to loss of Head subunits, the loss of Tail subunits did not affect the

viability of mammalian cells, although it affected doubling time. Tailless Mediator cells obtained by deletion of MED15, MED16, MED23, MED24/MED5, and MED25 led to only a 1.3-fold decrease in transcription. This indicates that they are essential for neither global Pol II activity nor cMed assembly (33). Similarly, in *Arabidopsis*, distal Tail mutants are all viable and seeds produce plants with the correct body plan, although in some cases fertility is affected.

Genetic Analysis of Distal Tail Subunits

MED16 or SENSITIVE TO FREEZING6 (SFR6) was originally characterized as a regulator of cold acclimation, drought, and osmotic stress (15, 73, 75); abscisic acid (ABA) transcriptional response (47); circadian clock–regulated and flowering time gene expression (74); and, most importantly, pathogen defense. MED16 controls SA- and JA-mediated defense gene expression (130). In *med16* mutants, the induction of JA- and ET-responsive genes is reduced, and plants are more susceptible to necrotrophic fungal pathogens *B. cinerea* and *A. brassicicola* (139). In summary, MED16 is a positive regulator of both SA and JA/ET signaling pathways.

More recently, MED16 was implicated in the development of papillae on trichomes (37) and metal homeostasis, including changes in root morphology, transporter expression, and production of exudates (53, 55, 101, 132, 133, 142). In short, MED16 seems to be involved in a myriad of responses and interactions with many TFs that may underlie the responses mentioned above. With 1,278 residues, it is about 30% larger than yMED16 and hMED16 but shares 18% and 21% identity, respectively.

MED25 was originally characterized for its role in flowering, light, and immune responses (18, 66, 72). It is the best-characterized subunit, and we refer the reader to recent reviews on its role (65, 135). As a paradigmatic subunit, it interacts with a large number of TFs. MED25 was not found in yeast, but its N-terminal von Willebrand factor type A (vWF-A) domain (aa 1–242) is conserved among plants and animals. In mMed, the MED25 vWF-A domain (aa 1–226) nestles between MED23, MED24, and MED16, but the remainder of the protein (aa 227–747) has several disordered regions and was not detected in the mMED cryo-EM map (143). *Arabidopsis* MED25 is 836 aa long, and if the vWF-A domain is also involved in anchoring MED25 to the Mediator complex, it leaves the remainder of the protein, composed of almost 600 aa, to interact with TFs, and this is precisely what has been found in *Arabidopsis*.

The situation of MED15A may be similar. Most of the first ~530 residues of mMED15 are expected to be disordered and were not detected in the mammalian MED cryo-EM map, but the C-terminal portion was well resolved and sat between the N-terminal portions of mMED23 and mMED24 (143). The C-terminal portion of *Arabidopsis* MED15A, which contains residues 1,224–1,313, aligns well with residues 702–788 of hMED15 and might also be involved in anchoring MED15A to the Mediator complex. MED15 is also larger than hMED15 (1,335 versus 788 residues). The first 800 residues form disordered regions, except for the KIX domain (aa 16–97) (120), which is bound by the plant-specific TF WRINKLED1 (WRI1) (68). Thus, as in MED25, a large portion of MED15A may remain free to interact with TFs.

MED15B is shorter than MED15A (1,335 versus 935 residues). They share 35% overall identity. The N-terminal portion is the most different, except for the conserved KIX domain (aa 20–103) (120). Mutants for the *MED15B* locus were not described, and if *MED15A* and *MED15B* act partially in a redundant form or if they have nonoverlapping roles is still unknown. The other three loci around *MED15A*, AT1G15770, AT1G15772, and AT1G15790, encode putative shorter versions of *MED15A*, but they were not characterized.

MED15A was originally identified from a genetic screening as *NON-RECOGNITION-OF-BTH4* (*NRB4*) (17). Three alleles were found, *nrb4-1*, *nrb4-2*, and *nrb4-3*, and all three are

missense mutations of the KIX domain but not predicted to be disruptive for the local secondary structure. They were defective in SA-dependent defense and pathogen-induced systemic acquired resistance (SAR) but displayed a normal response to methyl jasmonate (MeJA). Two other insertional alleles, *nrb4-4* and *nrb4-5*, are presumed null and displayed different phenotypes that are useful for the analysis of structure–function relations. *nrb4-4* and *nrb4-5* mutants were smaller and chlorotic, lacked papillae on trichomes, failed to properly control endoreduplication, and, despite a relatively normal initial growth, did not produce flowers after bolting (17). The trichome and endoreduplication phenotypes were observed in other Tail mutants, such as *med16* (37). Therefore, it is possible that the phenotypes of the KIX domain missense alleles are specific to MED15 because it may still be properly assembled into the Mediator Tail module, while the phenotypes of the T-DNA insertional mutants may arise from malfunctioning of other Tail subunits due to structural defects produced by the absence of MED15 in the structure.

CDK8: cyclin-dependent kinase 8

The *Arabidopsis* MED23 shares 22% identity with hMED23 and a similar predicted structure by AlphaFold (92). The mammalian MED23, MED5/MED24/MED33, and MED16 subunits seem to form a submodule since ablation of either MED5 or MED23 led to loss of all three subunits from the Mediator complex (61, 114). Further, the hMed cryo-EM structure revealed that hMED23 interacts closely with MED5/MED24 and MED16 within the human Tail module (1, 22). In *Arabidopsis*, *med23* and *med16* mutants suppress a dominant allele of *MED5* (*ref4-3*) (30, 31). As hMED23 and hMED5/hMED24 "form a rigid framework of the Tail" (22), the genetic data suggest a conserved location for MED23 within the *Arabidopsis* distal Tail module, closely associated with MED5. However, it is unlikely that loss of MED23 leads to MED16 dissociation from the Mediator Tail in vivo, as *med23* insertional mutants (SALK_119080) believed to be null alleles did not show the increased sensitivity to *Sclerotinia sclerotiorum* that *med16* mutants did (128).

MED5 is encoded by two paralogs in *Arabidopsis*, *MED5A* and *MED5B* (also *MED33A* and *MED33B*). MED5A and MED5B are of similar size and share 54% identity with each other and relatively low identity of 16–17% with their yeast and human counterparts. They were identified after positional cloning of *reduced epidermal fluorescence 4* (*ref4*) mutations that affect the phenylpropanoid pathway; the *ref4* alleles turned out to be semidominant alleles of *MED5B* (115). For this reason, *MED5A* was originally named *REF4 RESEMBLING 1* (*RFR1*). While the double *med5a med5b* mutants are relatively normal, the semidominant alleles of *MED5B* interfere with the activity of the TF PRODUCTION OF ANTHOCYANIN PIGMENT 1 (PAP1), which is a positive regulator of the phenylpropanoid pathway. When *ref4-3* is the only functional allele (*ref4-3*/*med5b*, *med5a*/*med5a*), it leads to a strong dwarf phenotype (13). A possible interpretation would be that MED5B G383S has to be assembled in the Mediator complex to exert its effects, and MED5A and MED5B wild-type proteins compete for assembly into the Mediator distal Tail module.

MED5A and MED5B repress the phenylpropanoid metabolism indirectly by upregulating a pair of F-box proteins that promote the degradation of phenylalanine ammonia lyase (PAL), the first enzyme in the phenylpropanoid pathway (12, 67). They also promote the expression of defense genes against necrotrophic fungi (127). Unfortunately, the direct transcriptional targets of MED5A and MED5B and how they achieve their roles are currently unknown.

Interestingly, cyclin-dependent kinase 8 (CDK8) loss-of-function alleles also suppressed some of the phenotypes of *ref4-3* mutants (90). The *cdk8* alleles suppressed the SA hyperaccumulation and the stunted growth and partially restored the transcriptome. More importantly, a kinase dead mutant of CDK8 carrying a D176A substitution did not complement *cdk8* loss of function, underscoring the importance of the CDK8 kinase.

The Structural Roles of MED14

ABRE: ABA-responsive element

The latest advances related to the structure of the Mediator complex show compelling evidence that MED14 plays a structural role, forming a backbone around which the Mediator complex is assembled (**Figure 1**). The N and C termini of MED14 are located at opposite ends within yMed, and MED14 forms a central interface connecting the fixed jaw in the Head module with the Middle and the Tail modules (107, 124). The beam in the Middle module is formed mainly of the central region of MED14, and it extends with its N-terminal domains and CTDs into the Middle's hook and Tail modules, respectively (96, 107). The cryo-EM structure of yMed showed that the Med14 C terminus connects the Tail to the Head module, likely involving some portion of the MED2-MED3-MED15 submodule (106, 124). This assembly is highly conserved from yeast to mammals but with some particularities. The mMED14 C terminus is much larger and involved in further interactions with the Tail module (143). In hMed, the MED14 N-terminal portion crosslinks with MED19 (the hook), then MED1 (the Middle module) and MED17 (the Head module). The central portion of MED14 (aa 541–766), which could be assimilated to the C-terminal portion of yMED14, crosslinks with MED3/MED27, MED30, and MED15, and the C-terminal region with MED2/MED29 (22).

Supplemental Material >

This structural information is not available for plants. Two-hybrid assays suggest that the MED14 N-terminal half interacts with MED10 (the Middle module's hook), MED6 (the Head module), and MED17 (the Head module), indicating that it could also play a role in connecting Middle and Head modules with the Tail module (**Supplemental Table 1**). We lack structural information about the MED14 C-terminal portion, which is larger than yMED14 and mMED14, and genetic evidence suggests a role in connecting with the Tail module, as we discuss below.

In *Arabidopsis*, only two viable mutant alleles of *MED14* were found. The *struwwelpeter* (*swp*) mutant carries a T-DNA insertion 250 base pairs (bp) upstream of the start codon and produces a larger transcript of *MED14* mRNA, starting within the T-DNA itself (6). It is recessive and therefore expected to be a loss-of-function or hypomorphic allele. Several other insertional mutants in the coding region of *MED14* were inviable (140); thus, it is likely that some MED14 protein is produced in *swp*, but reduced levels lead to a series of defects: reduced leaf size and number, increased cell size, dwarfism, stem fasciation, reduced cell number throughout development, a defect in meristem maintenance, and progressive loss of shoot apical meristem organization (6). The second viable allele *med14-1* carries a T-DNA insertion (line SAIL_373_C07) close to the end of the sixth exon, while the other three insertional mutants, carrying insertions in exons further upstream than *med14-1*, were not viable in homozygosity (140), consistent with MED14 being essential for Mediator assembly. The T-DNA insertion site in *med14-1* suggests that a truncated MED14 protein (MED14*) is produced. WT MED14 is 1,703 aa long while MED14* is expected to be at least 1,072 aa (10). Analysis of public RNA sequencing data reveals that the shorter transcript produced in *med14-1* is expressed at similar levels to the WT. Phenotypes reported for *swp* and *med14-1* are very different (6, 140). *med14-1* mutants were paler and their leaves flatter than the WT but otherwise looked relatively normal. However, they show decreased responsiveness to SA and are unable to establish SAR (140). Distal Tail mutants *med15a*, *med15b*, and *med16* are also compromised in plant immunity and SAR (17, 139, 140). The transcriptome changes in *med14-1* mutants show a high degree of overlap with the transcriptome of *med16* Tail mutants; 92% of DEGs in *med14-1* mutants also are DEGs in *med16* mutants (140); *med14-1*, *med16*, and *med2* are defective in the recruitment of Pol II to CBF-responsive cold-regulated genes (52) and these three Mediator subunits specifically promote transcriptional responses to ABA through the ABA-responsive element (ABRE) motif (80). These similarities between *med14-1* and Tail mutants suggest that MED14* might be sufficient to assemble Head and Middle modules but defective in the correct assembly

or the functioning of the Tail components because of the absence of its CTD. More biochemical and structural work is needed to support these propositions.

MECHANISMS OF TRANSCRIPTIONAL REGULATION BY MEDIATOR

The CDK Kinase Module: Structure and Regulatory Roles

The CDK8 module, also known as the CKM, is composed of four subunits: MED12, MED13, CDK8, and CYCLIN C (CycC). Two paralogs encode CycC in *Arabidopsis*: *CycC1;1* and *CycC1;2*. The roles of these genes and the phenotypes of the corresponding mutants were recently reviewed (2). In this section and henceforth, we focus on the molecular aspects of both the positive and negative roles of the CKM in transcription.

In *S. pombe*, the CKM binds to Mediator and blocks its interactions with Pol II (34). Also, in preparations of mMed, Mediator interacts with either Pol II or the CKM but not with both simultaneously (33). With purified recombinant preparations of the yCKM, yMed, and Pol II, it was recently shown that Pol II competes with the CKM for Mediator binding. Cross-linking experiments also show extensive surface interactions between MED12 and MED13 with the knob and hook domains of the Middle module and CDK8 and CycC with the Head-neck and moveable jaw domains, respectively, clearly showing why CKM-bound Mediator does not assemble into a PIC (97). Further, in vitro, CDK8 phosphorylates residues in the interface of the CKM and Mediator, weakening the interaction and releasing the CKM. In vivo, inhibition of CDK8 activity abolishes gene induction in response to heat shock but not the expression of nonresponsive genes, demonstrating a positive role for the CKM, and for CDK8 activity, in the induction of transcription after a stimulus (97).

A cryo-EM map of the yCKM at near-atomic resolution was recently reported (85). It revealed that MED12 functions as a scaffold that connects Cdk8 and CycC through its N-terminal portion, which is important to stimulate CDK8 kinase activity. The extended conformation of the CKM exposes a large surface that can be bound by TFs that offer an opportunity to regulate CDK8 kinase activity. On the opposing face, the complex contacts Middle and Head modules in surfaces that also bind Pol II and TFIIH (see the section titled Mediator-Dependent Recruitment of Enzymatic Activities to Promoters and Regulatory Regions) and thus block PIC assembly. At one end of the structure, the position of CDK8, close to the Head movable jaw and the proximal Tail module, may facilitate phosphorylation of Tail subunits or TFs bound to the Tail module (43, 125). The folding of MED13 is typical of ARGONAUTE, but the central channel that binds nucleic acids is occupied by a linker domain. Therefore, it is possible that the CKM interacts with nucleic acids in a different conformation (85).

In yeast, the CKM-Mediator complex is found at upstream activating sequences (UASs) through the Tail subunits that bind TFs (99), but not at the PIC. The repressive role of the CKM is exerted at the UAS and depends on CDK8 kinase activity; Mediator association to UASs is increased on both ΔMED13 and CDK8 kinase dead mutants but does not affect binding of Mediator to core promoters (63, 99).

The transcriptomes of individual CKM deletion mutants are virtually identical and also similar to the Δ*MED19* profile (125), supporting the notion that the CKM acts as a whole and MED19 connects the CKM to the Middle module. Furthermore, the anticorrelation between the ΔMED2 with ΔCKM transcriptomes and the epistasis of ΔMED2 over ΔCDK8 suggest that CDK8 and the CKM are negative regulators of MED2 and that Tail subunits work downstream of CKM and CDK8 activity (125). Indeed, MED2 and MED3 are substrates of CDK8 (43, 125).

Some lines of evidence support the conservation of the CKM architecture in *Arabidopsis*, binary protein–protein interactions (60, 145), and immunoaffinity purification of the complex

Supplemental Material >

SMG: seed maturation gene

followed by mass spectrometry (86, 144). The CKM complex from *Arabidopsis* also shows overlapping transcriptomes and phenotypes. MED12 and MED13 transcriptomes are very similar (39, 86) (**Supplemental Table 2**); loss-of-function alleles are late flowering and show overlapping developmental phenotypes in *Arabidopsis* and pea (39, 40, 50, 145).

In a study, *Arabidopsis* Pol II subunits did not copurify with MED12-YFP-FLAG, but most Mediator subunits did (86), while the CKM did not copurify with Mediator–Pol II fractions (7), suggesting that the CKM competes with Pol II for Mediator binding in *Arabidopsis* also. This competition may explain how the CKM works during early seedling development. After germination, two transcriptional repressors, HIGH-LEVEL EXPRESSION OF SUGAR INDUCIBLE GENE 2 (HSI2) and HSI2-like 1 (HSL1), inhibit the expression of seed maturation genes (SMGs). Researchers suggested that HSI2 and HSL1 recruit MED13 to repress transcription on SMG promoters and HISTONE DEACETYLASE 6 (HDA6) to start deacetylation as a first step toward silencing the seed maturation program (25). It would be interesting to test whether MED13 interaction with HSI2 and binding to SMG chromatin occurs in the context of the full CKM and Mediator. If this hypothesis holds true, one could imagine that HSI2/HSL1 could stabilize the CKM-Mediator complex to block both the accessibility of Pol II and assembly of the PIC while simultaneously recruiting histone-modifying enzymes to start long-term silencing.

Earlier reports also suggested that the corepressor LEUNIG (LUG) could repress gene expression by recruiting HDA19 and interacting with MED14 and CDK8, although at that time there was no information on the TFs involved and the association of these proteins with regulatory regions (42). Later, in the section titled Mediator-Dependent Recruitment of Enzymatic Activities to Promoters and Regulatory Regions, we discuss the repressive signals produced by the CKM that inhibit lateral root formation mediated by auxin (59).

In other developmental programs, the CKM plays a positive role in transcription. The lipid content of seeds is lower in *cdk8* mutants, which seems to result from low activity of WRI1 (136). Two SMG-encoded TFs, ABI3 and FUS3, promote the expression of WRI1 in a CDK8-dependent manner, but a direct interaction is unknown. However, Zhai et al. (136) reported very low WRI1 in *cdk8* mutants. They ruled out a direct physical interaction between CDK8 and WRI1 to explain its low levels and activity (136). However, WRI1 recruits MED15 to UASs to promote the transcription of genes involved in lipid synthesis (68). Therefore, in the context of the CKM and Mediator, CDK8 could phosphorylate WRI1 or MED15 or both when bound to UASs, promoting their stability, activity, or both, similar to the way it occurs in yeast (43, 63, 99, 125).

CDK8 amplifies the spectrum of potential target TFs by interacting with other MED subunits. CDK8 and MED25 interact in a CDK8 kinase activity–independent manner to regulate the expression of JA-responsive genes (145). The recruitment of CDK8 to regulatory regions suggests that it is indeed occurring in the context of a full Mediator complex. Interestingly, in the case of the *ACCT1* promoter, CDK8 was indeed associated specifically with the 500-bp upstream region but not the TATA box. This would support the notion that the CKM is evicted from Mediator upon PIC assembly in *Arabidopsis* also.

The N-terminal vWF-A domain of MED25 interacts with CDK8 and the MED25-BINDING RING-H2 PROTEIN1 (MBR1) and MBR2 proteins, which target MED25 for degradation (58, 145). mMED25 is assembled into mMED through its vWF-A domain. Therefore, the aforementioned interactions may antagonize each other, leading to CKM eviction after Mediator is recruited to the UAS. Interestingly, if MED25 is first recruited by TFs to the UAS and degradation of MED25 is then required for the eviction of the CKM from Mediator to allow PIC assembly, then the interaction of CDK8 with MED25 may explain the phenomenon of activation by destruction (58).

The CKM also promotes light-induced gene expression. MED12 enrichment increased over a subset of light-inducible genes, and this increase was necessary for the transient gene induction observed in dark-adapted plants in response to a light treatment. Researchers proposed that CKM might play a role in the induction of gene expression when repressed genes transit toward an active state, through recruitment of histone acetylases (86).

Finally, CDK8 interacts with coactivator NONEXPRESSOR OF PR1 (NPR1) and WRKY18 in the *PR1* regulatory region to promote the expression of *PR1*, as part of the SA and SAR responses (20).

Mediator–Transcription Factor Interaction as a Prelude for PIC Assembly or Repression

Although Pol II assembles with the PIC in vitro without Mediator, tracking single molecules both in vivo and in vitro suggests that it occurs otherwise. By using yeast nuclear extracts and fluorescently labeled components, research has shown that Pol II, TFIIF, and TFIIE associate with upstream regulatory regions in a way that is independent of the core promoter (8). Finally, TFIIH binds in a core promoter–dependent manner with a lag phase, and after Pol II, TFIIF and TFIIE are transferred from the UAS to the core promoter, suggesting that TFIIH joins the PIC assembled in the core promoter. Tracking of single molecules in vivo in yeast cells, coupled to specific depletion of GTFs, Pol II, or Mediator components, indicates that TFIID and TFIIA associate with chromatin independently of Pol II and Mediator, while Mediator associates with upstream regulatory elements and recruits Pol II and then TFIIB, TFIIF, and TFIIE, in a TFIID-independent manner (94) (**Figure 2**). This is consistent with the behavior of the CKM discussed above.

If this model is correct, then the loss of specific Tail subunits would affect the recruitment of the Mediator complex to specific regulatory regions. For example, MED16 is required for the recruitment of both Mediator and Pol II to C-REPEAT BINDING FACTOR (CBF)-responsive cold-regulated genes, and CBF1 is able to bind cold-responsive promoters independently of MED16 (52). It is necessary to test whether loss of CBFs affects the recruitment of the Mediator complex to these regulatory sites.

In the JA/ET-defense response, *PDF1.2* and *ORA59* marker genes are induced by MeJA plus 1-aminocyclopropane-1-carboxylic acid (ACC) treatment. WRKY33 physically associates with MED16, which it requires to induce the expression of *PDF1.2* and *ORA59*, suggesting that WRKY33 might recruit Mediator through MED16 (128).

The response to low phosphate requires a physical interaction between MED16 and the TF SENSITIVE TO PROTON RHIZOTOXICITY 1 (STOP1), which coregulate a group of low-phosphate-induced transcripts (101, 132). Whether STOP1 recruits the Mediator complex to the UAS in a MED16-dependent manner remains to be seen.

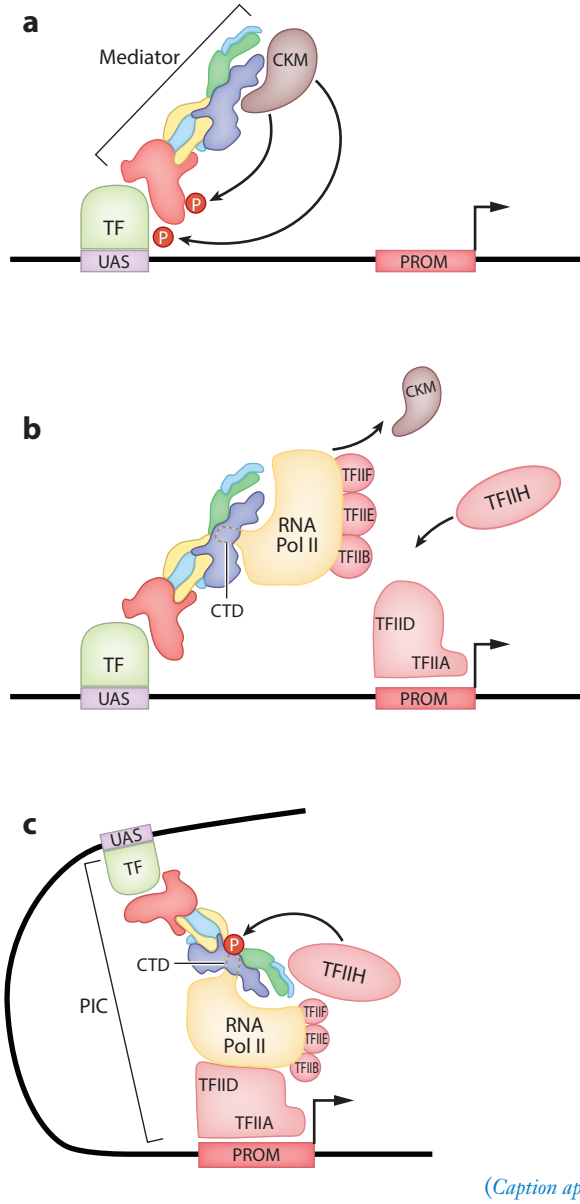
Under low-iron conditions, MED16 physically interacts with DEFICIENCY-INDUCED TRANSCRIPTION FACTOR (FIT) to induce the expression of the iron uptake genes *FERRIC REDUCTASE/OXIDASE 2* (*FRO2*) and *IRON-REGULATED TRANSPORTER 1* (*IRT1*). Both FIT and MED16 interact with *FRO2* and *IRT* chromatin, and in *med16* mutants FIT association with *FRO2* and *IRT* chromatin diminishes (142). It seems that stabilization of FIT on *FRO2* and *IRT* chromatin depends, to some extent, on MED16 (and possibly CKM). As in the previous cases, it would be interesting to know if recruitment of Mediator to *FRO2* and *IRT* promoters occurs in *fit* and *med16* mutant backgrounds.

In other cases, MED16 can aid in the repression of transcription. *Arabidopsis med16* mutants display larger organs due to increased endoreduplication. MED16 was shown to interact directly with the transcriptional repressor DP-E2F-LIKE1/E2Fe (DEL1) to repress the expression of the anaphase-promoting complex/cyclosome activator CELL CYCLE SWITCH52 A2 (CCS52A2).

The recruitment of MED16 to the *CCS52A2* promoter was dependent on DEL1, while recruitment of DEL1 was partially dependent on MED16, suggesting that Mediator is recruited by DEL1 to the *CCS52A2* promoter to repress its expression (87). It is possible that the CKM remains attached to Mediator in this case, and a second stimulus or TF is required for derepression.

Mediator-Dependent Recruitment of Enzymatic Activities to Promoters and Regulatory Regions

Besides CDK8 kinase activity, Mediator can nucleate events to bring other enzymatic activities and signaling modules to regulatory regions. For example, the histone acetyltransferases HAC1



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

A model of Mediator in action during the activation of gene expression. (*a*) TFs recognize and bind to UASs in the vicinity of promoters. Through interaction with Mediator subunits, they recruit the full Mediator complex. At this stage, the CKM may repress gene expression and recruit histone deacetylases or may activate gene expression by stabilizing the DNA-bound complex through interactions or phosphorylation of targets that may include other Mediator subunits or TFs. (*b*) Phosphorylation of interaction surfaces also weakens the interaction of the CKM with the core Mediator complex, releasing the CKM and exposing interaction domains for RNA polymerase II–TFIIF binding and recruitment of TFIIB and TFIIE. (*c*) PIC assembly continues via the assembly of Pol II–Mediator with TFIID and TFIIA bound at the core promoter, which may include promoter DNA looping. Mediator positions the CTD of Pol II to facilitate phosphorylation by the CAK subcomplex, which is part of TFIIH. TFIIH incorporation sets up PIC formation, bringing not only CTD phosphorylation activity but also the helicase and ATPase activities required to open a DNA bubble. After CTD phosphorylation, RNA Pol II escapes from the promoter, initiating transcript synthesis. Abbreviations: CAK, CDK-activating kinase; CKM, CDK kinase module; CTD, C-terminal domain; P, phosphate group; PIC, preinitiation complex; Pol II, RNA polymerase II; PROM, promoter; TF, transcription factor; UAS, upstream activating sequence.

and HAC5 copurify with Mediator through association with MED8 and MED25 (46). MED25 participates and recruits JA-signaling modules to the UAS, recruits histone acetylases, and regulates DNA looping at promoter targets. We refer the reader to a recent review (135), and we focus on other mechanisms.

CDK8 interacts with the ETHYLENE RESPONSIVE FACTOR/APETALA2 (ERF/AP2)-type TF (RAP2.6) to promote the transcription of ABA-responsive genes. RAP2.6 also binds SnRK2.6, a kinase involved in ABA signaling (144). The three proteins form a subcomplex. Although CDK8 does not seem to phosphorylate RAP2.6, SnRK2.6 does. The implications are that Mediator can nucleate ABA signaling events at promoters, a mechanism with the potential to rapidly change transcription in response to a stimulus.

In other reports, a mechanism for rapid response to a hormonal stimulus was clearly shown. The work of Ito et al. (59) shows that Mediator interacts with auxin regulatory modules directly on the UAS and proposes a model for rapid gene induction by the auxin hormone. The *solitary-root-1* (*slr-1*) allele of *INDOLE-3-ACETIC ACID INDUCIBLE 14* (*IAA14*) produces a stable IAA14 that converts the ARF7 and ARF19 TFs into auxin-insensitive repressors that suppress lateral root formation. Mutations in *CKM* subunits, *MED25*, or *MED17* suppress this effect and restore auxin sensitivity in lateral root formation. Mutations in *TOPLESS* (*TPL*) have a similar effect as those in *MED13*, suggesting that both work in the same pathway. But the interaction between MED13 and TPL only occurs when IAA14 is stabilized by using an auxin antagonist. Researchers studying the *LATERAL ORGAN BOUNDARIES-DOMAIN16* (*LBD16*) regulatory regions showed that MED13 associates to auxin-responsive elements (AuxRE) only when auxin signaling is inhibited or IAA14 is stabilized by using its mutated form, and, further, both mIAA14 and MED13 associate with the same regions (59). The authors also found physical interactions of ARF7 and ARF19 with MED25 as well as MED25 association with AuxRE. Auxin signaling led to the dissociation of MED13 but not MED25 from the AuxRE region (59). These results suggest that upon auxin-promoted IAA14 degradation, the CKM is evicted and allows engagement of Mediator with Pol II and PIC assembly at the core promoter. It remains to be tested if MED25 dissociates from Mediator in this process, remaining bound to AuxRE ready to recruit Mediator again, or if DNA forms a loop, connecting the ARF7/ARF19-bound AuxRE with Mediator and the PIC.

Gibberellins also act in a Mediator-dependent manner to repress flowering. RGA binds to the TF SQUAMOSA PROMOTER-BINDING PROTEIN-LIKE15 (SPL15) at the UAS and blocks its interaction with MED18. Under gibberellin presence, RGA is degraded and SPL15 recruits MED18 to promote the transcription at SPL15 targets (56). This SPL15-MED18 module brings

another possibility. SPL10 also promotes flowering by recruiting MED2 to targets in common with SPL15 (134). When multiple UASs are found at regulatory regions, is it possible that different TFs recruit the same Mediator complex by interacting with different subunits? May this work as a signal integration mechanism?

MED25 regulates shade avoidance signaling by binding PIF4 (38, 113, 116) and also recruits JA signaling modules (3). Maybe MED25 plays a role in the observed trade-off of shade and defense responses (11).

CONCLUDING REMARKS

The structural information obtained through cryo-EM is invaluable for future research. But we still lack specific information for plant systems. Mediator has been purified from plants, but this is only the first step.

Several transcription factors were shown to bind Mediator. Now it is important to understand if they interact in the context of the full Mediator, if they are required to recruit Mediator to UASs, and if this process is dependent on both the TF with DNA-binding activity and the cognate-interacting subunit in Mediator. Once the complex is assembled in chromatin, is this activity regulated by Mediator? Does the CKM have any role? What is the signal that triggers CKM eviction and Pol II recruitment? Once the PIC is assembled, does DNA always bend to join the UAS with the core promoter? Or might the Mediator dissociate from the Tail to leave part of the Tail module associated to the UAS to recruit Mediator again? Does Mediator integrate signals from different TFs bound to different subunits? When several UASs are set in tandem, does transcription increase because of the larger number of TF-Mediator complexes assembled close to the core promoter? Is this a limiting step for transcription initiation?

Some reports suggest that Mediator recruits hormone signaling modules to UASs and actively participates in signaling per se. Is this a general rule? In vitro systems following single molecules were set up in yeast. It would be fantastic if these systems could be assembled in plants to study the dynamics of hormone signaling in regulatory regions located upstream of promoters.

Finally, our focus here has been on the events that occur before transcription initiation and in close proximity of promoters. Other promising areas of research emerge well before and after transcription initiation. How is Mediator involved in establishing domains in the nuclear space and long-range interactions? What are the roles of Mediator after the polymerase leaves the promoter? There are studies working on these questions. Fortunately, there is still a lot of work to be done.

SUMMARY POINTS

1. The *Arabidopsis* Mediator is formed by 29 subunits.
2. The Tail module is assumed to bind transcription factors (TFs), but the Head and Middle modules also have significant roles.
3. Mediator assembles with TFs at upstream activating sequences and recruits RNA polymerase II (Pol II) before the preinitiation complex is assembled at promoters.
4. The CDK kinase module (CKM) may act to activate or repress transcription.
5. The CKM is evicted from Mediator before Pol II is recruited.
6. High-resolution models show that Mediator positions the C-terminal domain to facilitate phosphorylation.

7. MED14 functions as a structural component, providing the backbone for the assembly of the Mediator complex.
8. CKM kinase activity is necessary for transcription regulation.
9. Mediator nucleates hormone signaling at gene regulatory regions.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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