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The plant Mediator and its role in noncoding RNA production

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Abstract

Mediator, a conserved multiprotein complex in animals, plants, and fungi, is a cofactor of RNA Polymerase II (Pol II). It is known to promote basal Pol II-mediated transcription as well as bridge sequence-specific transcriptional regulators and Pol II to integrate regulatory information. Pol II transcribes not only protein-coding genes but also intergenic regions to generate noncoding RNAs such as small RNAs (microRNAs and small interfering RNAs) and long noncoding RNAs. Intriguingly, two plant-specific polymerases, Pol IV and Pol V, have evolved from Pol II and play a role in the production of small interfering RNAs and long noncoding RNAs at heterochromatic regions to maintain genome stability through transcriptional gene silencing (TGS). Recent studies have defined the composition of the plant Mediator and evaluated its role in noncoding RNA production in relationship to Pol II, Pol IV and Pol V. Here, we review the functions of Mediator and that of noncoding RNAs generated by Pol II, Pol IV and Pol V in plants, and discuss a role of Mediator in epigenetic regulation via noncoding RNA production.

Keywords

small RNA; noncoding RNA; Mediator; Pol II; Pol IV; Pol V

Introduction

Dynamic changes in gene expression upon internal and external demands are first modulated at the transcription initiation step. Pol II, which is responsible for the transcription of protein-coding genes in eukaryotes, is recruited to promoters of genes (Sikorski and Buratowski, 2009). Biochemical studies show that Pol II alone is not sufficient to initiate transcription *in vitro* and imply the existence of co-factors for Pol II transcription initiation. One type of well-studied cofactors includes the basal initiation factors such as TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH (Reese, 2003; Thomas and Chiang, 2006). In the presence of these factors, which are so-called general transcription factors (GTFs), Pol II supports basal transcription *in vitro*. The assembly of the pre-initiation complex (PIC) including Pol II and GTFs on the core promoter is a critical step in transcription initiation. Genetic and biochemical studies have revealed another cofactor that plays an important role in transcription initiation: a multiple protein complex termed Mediator. It has been thought that Mediator bridges sequence-specific transcriptional regulators to the Pol II-containing PIC for transcription initiation in a passive mode (Kuras and Struhl, 1999; Struhl, 1996; Yudkovsky et al., 2000). Recently, Mediator is proposed to play a more active role as a signal integrator to transmit information from the input transcriptional regulators to the transcription machinery (Malik and Roeder, 2010).

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Pol II transcribes not only protein-coding genes but also genomic regions that give rise to noncoding RNAs. In addition, two plant-specific polymerases, Pol IV and Pol V, produce noncoding RNAs from repeats and transposable elements. The Pol IV- and Pol V-dependant noncoding RNAs are involved in the maintenance of genome stability through small interfering RNA (siRNA)-mediated transcriptional gene silencing (TGS) at repeats and transposable elements (Chen, 2009). Recently, it has been shown that Pol II is also required for siRNA-mediated TGS at a subset of heterochromatic loci (Zheng et al., 2009). A recent study in our laboratory has evaluated the role of Mediator in Pol II-, Pol IV-, and Pol V-dependent noncoding RNA production and TGS (Kim et al., 2011). In this article, we first review the general molecular functions of Mediator and the role of Pol II, Pol IV- or Pol V-dependant noncoding RNAs in TGS. Then we discuss roles of Mediator in noncoding RNA production and TGS and propose a working model.

Structure of the Mediator complex

Mediator is a large protein complex composed of 20-30 subunits (Table 1). It is functionally and structurally conserved in all eukaryotes although species-specific subunits exist ((Casamassimi and Napoli, 2007); Table 1). Mediator was first identified in yeast, *Saccharomyces cerevisiae*, an organism in which it remains to be the best studied (Carlson et al., 1981;Neigeborn and Carlson, 1984;Simchen et al., 1984;Stern et al., 1984;Suzuki et al., 1988). Structural studies of the yeast Mediator complex revealed that it is composed of three subdomains (Head, Middle and Tail) and a separable kinase module (Dotson et al., 2000;Guglielmi et al., 2004;Sato et al., 2003).

The head domain, composed of MED6, MED8, MED11, MED17, MED18, MED19, MED20 and MED22, interacts with a Pol II-TFIIF complex *in vitro* (Takagi et al., 2006) and constitutes the most extensive Pol II-interacting interface in Mediator. Disruption of the head domain results in dissociation of Mediator from transcriptionally active promoters (Lariviere et al., 2006). The middle domain, which includes MED1, MED4, MED7, MED9, MED10, MED21 and MED31, directly interacts with the C-terminal domain (CTD) of the largest subunit in Pol II (Rbp1) (Kang et al., 2001). It is believed that the middle domain transmits input signals from transcriptional regulators to the head domain. The tail domain consists of MED2, MED3, MED5, MED14, MED15 and MED16 and interacts with the DNA-bound transcriptional regulators (Han et al., 2001; Lee et al., 1999; Park et al., 2000). The detachable kinase module is composed of four proteins: MED12, MED13, cyclin-dependant kinase 8 (CDK8) and Cyclin C (CycC). Mediators containing the kinase module are referred as large Mediators, whereas variants without this module are called small Mediators (Malik and Roeder, 2000; Mittler et al., 2001; Naar et al., 2002; Sun et al., 1998; Taatjes et al., 2004). The CDK8 module is mainly involved in transcriptional repression probably through its kinase activity, which phosphorylates the Rbp1 CTD heptads, some Mediator subunits, GTFs, and transcriptional regulators (Chi et al., 2001; Hallberg et al., 2004; Hengartner et al., 1998; Hirst et al., 1999; Liu et al., 2004; Nelson et al., 2003; van de Peppel et al., 2005; Vincent et al., 2001).

Mediator is not a fixed complex - several isoforms or alternative forms exist in cells (Casamassimi and Napoli, 2007). The identification of large and small Mediators based on the presence of the CDK8 module has uncovered the functional flexibility of Mediator as either an activator or a repressor (Malik and Roeder, 2000; Mittler et al., 2001; Naar et al., 2002; Sun et al., 1998; Taatjes et al., 2004). In addition, new isoforms in several subunits have been identified and differences in the composition of complexes in the mammalian Mediator have been found (Mittler et al., 2001). It is not clear how many alternative Mediator forms exist in organisms. However, it is thought that the structural arrangement and complexity allow it to integrate a multitude of regulatory inputs.

Molecular functions of Mediator

Studies with the yeast *srb4* mutant suggest that Mediator is a GTF. *srb4* was isolated as a temperature sensitive mutant through a genetic screen aimed at the identification of regulators of transcription. Later, *SRB4* was found to be *MED17*, one of the head subunits of Mediator. In the *srb4/med17* mutant, the levels of more than 90% of Pol II-dependent transcripts are decreased under the restrictive temperature (Holstege et al., 1998; Thompson and Young, 1995). This suggests that Mediator acts as a general factor in Pol II transcription. Consistently, it has been shown that the head domain of Mediator stimulates basal transcription in the absence of activators *in vitro* (Baek et al., 2006; Mittler et al., 2001).

However, controversy exists over the role of Mediator in transcription initiation. A genome-wide analysis shows that Mediator occupancy is not tightly correlated with that of Pol II at many highly active Pol II promoters in yeast (Fan et al., 2006), thereby arguing against a role of Mediator as a GTF. Moreover, another study suggests that Mediator is associated with promoters in an activator- rather than Pol II-dependent manner (Fan and Struhl, 2009). In contrast to these results, another study argues that Mediator is associated with constitutively active genes and is required for the recruitment of Pol II as a GTF (Ansari et al., 2009). Therefore, it appears that further investigations are needed to better understand the functions of Mediator.

Besides the general role of Mediator in PIC formation, several pieces of evidence indicate that Mediator also acts at the chromatin level through interactions with chromatin modification factors such as histone acetyltransferases and methyltransferases. Mediator recruits the histone acetyltransferase p300 to a promoter bound by a transcription factor through direct interaction with p300 to allow acetylation of the local chromatin. Subsequent dissociation of p300 from the DNA promotes TFIID binding followed by PIC formation (Black et al., 2006). There is also evidence that MED12 mediates ternary complex formation with two other proteins, the silencing transcription factor REST and the methyltransferase G9a. The deposition of the H3K9 dimethylation repressive mark at target genes by G9a is thought to play a role in REST-mediated neuronal gene silencing in non-neuronal cells (Ding et al., 2008; Ooi and Wood, 2007).

Mediator in plants

Recently, the *Arabidopsis* Mediator was biochemically characterized and found to contain 21 subunits conserved in eukaryotes and six plant-specific subunits (Backstrom et al., 2007). Although the CDK8 module was not co-purified with Mediator in the experiment, *Arabidopsis* has homologs to *MED12, MED13, CDK8* and *CycC* genes encoding subunits of the CDK8 module. Prior to this purification of the *Arabidopsis* Mediator complex, several subunits had been studied genetically.

PHYTOCHROME and FLOWERING TIME1 (PFT1), now known as *MED25*, was identified as a factor of a *Phytochrome B* (*phyB*) signaling pathway that promotes flowering in response to shade (Cerdan and Chory, 2003). It was thought to be a transcriptional coactivator on the basis of its nuclear localization, the presence of a glutamine-rich domain and its transcription activation activity in yeast when fused to the LexA DNA-binding domain. A recent study suggested that *PFT1* negatively regulates the phytochrome signaling pathway rather than acting as a component in the pathway (Wollenberg et al., 2008).

STRUWWELPETER (SWP)/MED14 was reported as a nuclear protein playing a role in defining the duration of cell proliferation (Autran et al., 2002). An *swp* mutant exhibits dwarfism with an abnormal architecture such as a fascinated stem and abnormal floral

structures; some of the phenotypes being attributable to reduced cell numbers. Consistently, ectopic expression of *SWP* caused increased cell numbers. It was reported that the repressive activity of LEUNIG (LUG), a transcriptional corepressor, involves its interaction with SWP/MED14 and HUA ENHANCER3 (HEN3)/CDK8 (Gonzalez et al., 2007). *HEN3* was identified as a weak regulator of *AG*, which is a target of *LUG* (Liu and Meyerowitz, 1995; Wang and Chen, 2004). It is possible that *LUG* negatively regulates *AG* expression through the larger Mediator complex containing the HEN3/CDK8 module that has repressor activity.

Recent studies have suggested that Mediator acts as an integrator in response to environmental cues in *Arabidopsis* (Kidd et al., 2009). Another function of *PFT1/MED25* is that it is required for Jasmonic Acid (JA)-dependant defense gene expression and resistance to leaf-infecting necrotrophic fungal pathogens (Kidd et al., 2009). In addition to being late flowering, an *atmed8* mutant showed delayed symptom development upon infection by a root-infecting hemibiotrophic fungal pathogen (Kidd et al., 2009), indicating that *MED8* is a regulator of disease resistance and flowering time. In another study, it was shown that *MED21* is required for resistance to necrotrophic fungal pathogens (Dhawan et al., 2009). Interestingly, MED21 interacts with HISTONE MONOUBIQUITINATION1 (HUB1) that is also involved in defense against necrotrophic fungal pathogens, implying that *MED21* integrates pathogen-infection signaling through chromatin modifications.

Noncoding RNAs and Pol II, IV and V in plants

In recent years, small noncoding RNA-mediated gene silencing has been increasingly recognized to play crucial roles in a multitude of biological processes in plants and animals. Small RNAs of 20 - 30 nt in size serve as sequence-specific repressors of target gene expression. In plants, there are two major types of small RNAs: microRNAs (miRNAs; Fig. 1A) and small interfering RNAs (siRNAs; Fig. 1B) (Chen, 2009). Most plant miRNA genes (*MIR*) are located in intergenic regions and have their own promoters. It is thought that Pol II is responsible for *MIR* gene transcription in plants on the basis of common features between pri-miRNAs and mRNAs as well as the presence of TATA boxes in the promoters of *MIR* genes (Xie et al., 2005). Using a partial loss of function allele in the second largest subunit of Pol II (Zheng et al., 2009), we showed that miRNA accumulation indeed requires Pol II (Kim et al., 2011). We also showed that Pol II is present at the promoters of *MIR* genes (Kim et al., 2011), thus solidifying a role of Pol II in the transcription of *MIR* genes. In addition, several pieces of evidence support the transcriptional regulation of miRNA gene expression via transcription factor activity in plants (Bari et al., 2006;Kawashima et al., 2009;Megraw et al., 2006;Yamasaki et al., 2009).

Heterochromatic-siRNAs (hc-siRNA) are derived from repeats and transposable elements and represent the great majority of endogenous siRNAs in plants. Plants have two specialized polymerases, Pol IV and Pol V, which are required for the biogenesis and function of hc-siRNAs. Pol IV and Pol V are probably derived from Pol II because they are composed of 12 subunits that are paralogous or identical to those of Pol II (Huang et al., 2009; Lahmy et al., 2009; Ream et al., 2009). More than 90% of hc-siRNAs are Pol IV-dependent (Mosher et al., 2008; Zhang et al., 2007). Pol IV is presumed to transcribe transposable elements and repeated sequences into RNAs that serve as precursors to siRNAs (Herr et al., 2005; Kanno et al., 2005; Mosher et al., 2008; Onodera et al., 2005; Zhang et al., 2007). An *in vivo* transcriptional activity of Pol V is supported by the identification of Pol V-dependent long noncoding RNAs from some heterochromatic loci and by the presence of Pol V at these loci (Wierzbicki et al., 2008).

Biological functions of Pol II-, Pol IV- and Pol V-dependent noncoding RNAs in TGS

hc-siRNAs maintain genome stability by causing TGS of homologous sequences. The production of hc-siRNAs requires Pol IV, which is thought to transcribe heterochromatic loci into single-stranded noncoding transcripts that are subsequently converted into double-stranded RNAs (dsRNAs) by RDR2 (Xie et al., 2004). The dsRNAs are diced into 24 nt siRNAs by DCL3 and the small RNAs are methylated by HEN1 (Li et al., 2005; Xie et al., 2004). One strand of the hc-siRNA duplex is incorporated into an effector complex containing one of the AGO4-clade of argonaute proteins (Havecker et al., 2010; Zheng et al., 2007; Zilberman et al., 2003). Pol V also transcribes heterochromatic loci into long noncoding transcripts, also known as scaffold transcripts, which are thought to recruit the AGO4/siRNAs to homologous chromatin through base-pairing with siRNAs (El-Shami et al., 2007; Li et al., 2006; Wierzbicki et al., 2008). The AGO4/siRNAs in turn recruit chromatin-modifying factors such as the DNA methyltransferase DRM2 and histone modification enzymes to deposit repressive chromatin marks to result in TGS. Loss-of-function mutations in Pol IV, Pol V, or other genes in the pathway cause the transcriptional de-repression of repeats and transposable elements. Pol II is also required for endogenous siRNA-mediated TGS at some intergenic, low-copy-number loci. Like Pol V, Pol II generates noncoding scaffold transcripts, which recruit AGO4/siRNAs to homologous loci (Zheng et al., 2009). In addition, Pol II transcription recruits Pol IV and Pol V to different locations at heterochromatic loci to promote siRNA biogenesis and scaffold RNA production, respectively (Zheng et al., 2009).

Roles of Mediator in noncoding RNA production

Mediator is required for the transcription of protein-coding genes by Pol II. However, the function of Mediator in noncoding RNA production is largely unknown. A recent study shows that Mediator regulates the transcription of a subset of Pol II-dependant small nuclear RNA (snRNA) genes in mouse (Krebs et al., 2010), therefore revealing a role of Mediator in noncoding RNA production by Pol II. In plants, Pol II, Pol IV and Pol V are required for endogenous siRNA-mediated TGS to maintain genome stability through generating noncoding RNAs, raising the question of whether Mediator is required for Pol II, Pol IV or Pol V activities in noncoding RNA production. A recent study from our laboratory has addressed this question by analyzing *Arabidopsis* mutants in three Mediator genes, *MED17*, *MED18*, and *MED20a* (Kim et al., 2011). This study reveals that Mediator plays a role in *MIR* gene expression as well as TGS of repeats and transposons to maintain genome stability by promoting Pol II activity in *Arabidopsis*. Mediator promotes the transcription of *MIR* genes by recruiting Pol II to their promoters. In addition, Mediator is required for Pol II-mediated intergenic transcription to produce long noncoding scaffold RNAs that recruit siRNAs to chromatin in TGS.

Perspective

Several studies have begun to reveal functions of Mediator in noncoding RNA production in animals and plants. In *Arabidopsis*, promoters of *MIR* genes contain cis-regulatory elements (Megraw et al., 2006) that may be bound by transcription factors. Given the established role of Mediator in recruiting Pol II to the promoters of *MIR* genes (Kim et al., 2011), it is possible that Mediator bridges the interaction between Pol II and transcription factors that bind the cis-elements (Fig. 1A). Mediator also plays a role in silencing repeats and transposons since several loci known to be silenced through siRNA-mediated DNA methylation are de-repressed in *med17*, *med18*, and *med20a* mutants (Kim et al., 2011). Mediator does so by recruiting Pol II to some of these loci to produce long noncoding RNAs

that serve to recruit siRNAs to chromatin. However, it is not clear how broadly Mediator acts in the production of noncoding RNAs in TGS. The similarities in subunit composition among Pol II, Pol IV, and Pol V raise the possibility that Mediator also acts with Pol IV or Pol V to produce noncoding RNAs (Fig. 1B). But Pol IV- or Pol V-dependent noncoding RNAs were not affected in *med17, med18,* or *med20a* mutants (Kim et al., 2011). Given that these are not null mutants, a potential role of Mediator in Pol IV or Pol V transcription cannot be ruled out. Further studies are necessary to address the generality and specificity of the functions of Mediator in noncoding RNA production in TGS.

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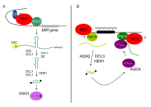

Figure 1.

Roles of Mediator in noncoding RNA production in plants. (A) A diagram of the miRNA biogenesis pathway in *Arabidopsis*. Mediator recruits Pol II to promoters of microRNA genes to promote the transcription of *MIR* genes possibly by bridging the interaction between transcription factors and Pol II. A *MIR* gene is transcribed by Pol II into a capped and polyadenylated primary precursor, which undergoes processing to give rise to a small RNA duplex containing the miRNA and the antisense miRNA*. The duplex is methylated on the 2′ OH of the 3′ terminal nucleotides, and the miRNA strand is bound by AGO1, the miRNA effector protein. Known factors in miRNA biogenesis are indicated. TF, transcription factor; CBC, cap-binding complex; DDL, DAWDLE; DCL1, DICER-LIKE1; HYL1, HYPONASTIC LEAVES1; SE, SERRATE; HEN1, HUA ENHANCER1; AGO1, ARGONAUTE1. (B) A diagram of siRNA biogenesis and siRNA-mediated transcriptional gene silencing at repeats and transposable elements. The black rectangle represents a repeats- or transposable element-containing locus that harbors DNA methylation (CH_3). Pol IV presumably transcribes the locus into a single-stranded noncoding RNA, which eventually gives rise to 24 nt siRNAs through the activities of RNA-DEPENDENT RNA POLYMERASE2 (RDR2), DICER-LIKE3 (DCL3), and HEN1. The siRNAs are bound by AGO4, a major siRNA effector protein. The AGO4/siRNA complexes are thought to be recruited back to homologous chromatin by noncoding scaffold transcripts produced by Pol V, and at some loci, Pol II. It is not known whether Mediator assists Pol V in the production of noncoding RNAs, but it is required for noncoding RNA production by Pol II. The small black dots in (A) and (B) indicate the methyl group on the 3′ terminal ribose of small RNAs.

Table 1

Mediator composition from diverse eukaryotes

Mammalian	S. cerevisiae	A. thaliana	Module
MED1	MED1	-	Middle
MED2	MED2	-	Tail
MED3	POLYGLUTAMINE DOMAIN 1 (PGD1)	-	Tail
MED4	MED4	MED4	Middle
MED5	NEGATIVE REGULATION OF URS TWO 1 (NUT1)	-	Tail
MED6	MED6	MED6	Head
MED7	MED7	MED7	Middle
MED8	MED8	MED8	Head
MED9	CHROMOSOME SEGREGATION 2 (CSE2)	MED9	Middle
MED10	NEGATIVE REGULATION OF URS TWO 2 (NUT2)	MED10	Middle
MED11	MED11	MED11	Head
MED12	SUPPRESSOR OF RNA POLYMERASE B MUTATIONS 8 (SRB8)	CRYPTIC PRECOCIOUS (CRP)	CDK
MED13	SUPPRESSOR OF RNA POLYMERASE B MUTATIONS 9 (SRB9)	MED13	CDK
MED14	RESISTANT TO GLUCOSE REPRESSION 1 (RGR1)	STRUWWELPETER (SWP)	Tail
MED15	GALACTOSE METABOLISM 11 (GAL11)	MED15	Tail
MED16	SWITCH INDEPENDENT 4 (SIN4)	MED16	Tail
MED17	SUPPRESSOR OF RNA POLYMERASE B MUTATIONS 4 (SRB4)	MED17	Head
MED18	SUPPRESSOR OF RNA POLYMERASE B MUTATIONS 5 (SRB5)	MED18	Head
MED19	REPRESSOR OF HYPOXIC GENES 3 (ROX3)	MED19	Head
MED20	SUPPRESSOR OF RNA POLYMERASE B MUTATIONS 2 (SRB2)	MED20	Head
MED21	SUPPRESSOR OF RNA POLYMERASE B MUTATIONS 7 (SRB7)	MED21	Middle
MED22	SUPPRESSOR OF RNA POLYMERASE B MUTATIONS 6 (SRB6)	MED22	Head
MED23	-	MED23	not identified
MED24	-	-	not identified

Mammalian	S. cerevisiae	A. thaliana	Module
MED25	-	PHYTOCHROME AND FLOWERING TIME 1 (PFT1)	not identified
MED26	-	-	not identified
MED27	-	MED27	not identified
MED28	-	MED28	Head
MED29	-	-	Head
MED30	-	-	Head
MED31	SUPPRESSOR OF HPR 1 (SOH1)	MED31	Middle
CDK8	SUPPRESSOR OF RNA POLYMERASE B MUTATIONS 10 (SRB10)	HUA ENHANCER 3 (HEN3)	CDK
Cyclin C	SUPPRESSOR OF RNA POLYMERASE B MUTATIONS 11 (SRB11)	-	CDK