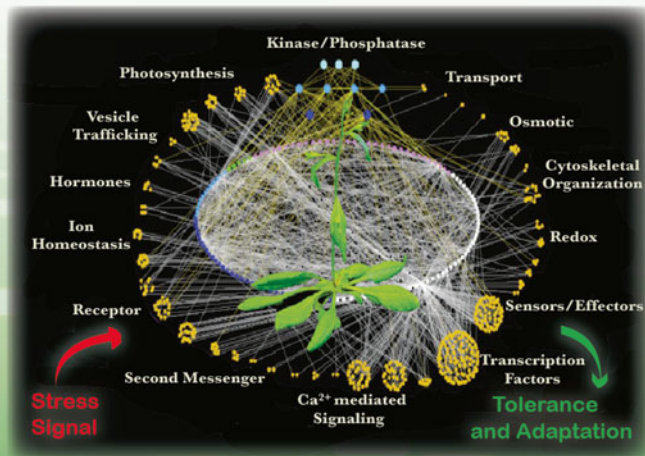Girdhar K. Pandey *Editor*

Elucidation of Abiotic Stress Signaling in Plants

Functional Genomics Perspectives, Vol. 2

Springer

Elucidation of Abiotic Stress Signaling in Plants

The above image represents a depiction of activation of different signaling pathways by diverse stimuli that converge to activate intricate signaling and interaction networks to counter stress (top panel). Since environmental stresses influence most significantly to the reduction in potential crop yield, progress is now largely anticipated through functional genomics studies in plants through the use of techniques such as large-scale analysis of gene expression pattern in response to stress and construction, analysis and use of plant protein interactome networks maps for effective engineering strategies to generate stress tolerant crops (top panel). The molecular aspects of these signaling pathways are extensively studied in model plant *Arabidopsis thaliana* and crop plant rice (*Oryza sativa*) (below).

Girdhar K. Pandey

Editor

Elucidation of Abiotic Stress Signaling in Plants

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Springer

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Preface

Plants are considered the backbone of life on earth. The colorful life on this planet has emerged as a consequence of over 3.5 billion years of unceasing evolution. Life on earth cannot sustain without plants, as they harness solar energy to produce sugars and oxygen, the primary constituents for supporting life. Humans are primarily dependent on plants and have developed a systematic discipline called "agriculture" to cultivate or domesticate plants over a period of time for food, biofuel, and fodder. At present time, crop productivity faces a major challenge from rapidly growing population and diminishing fertile land due to excessive anthropogenic activities. In addition, expanding human population and climate changes due to increased exploitation of natural resources imposes several major unfavorable conditions that reduce the crop productivity. These unfavorable conditions are primarily categorized as physical (or *abiotic*) and biological (or *biotic*) variables hindering normal growth and development in plants. Interestingly, stress perceived by one plant species may not be a stress factor for another plant species due to different growth habits and adaptation acquired during the course of evolution. Because of domestication and cultivation of crop plants by humans over a period of 10,000 years, many of these wild traits responsible for adaptive responses were lost, increasing the vulnerability of crop plants to biotic and abiotic stresses. Under abiotic stresses, limitation of water (drought), extremes of temperature (both high and low temperatures), nutrient deficiency, and soil contaminated with salt and heavy metals or pollutants are the major environmental factors contributing to crop losses worldwide.

In the past, agriculture has relied on breeding approaches to develop high yielding crop varieties which can grow optimally under stress conditions without affecting crop yield and productivity. In an effort to find an alternative tool faster than the traditional breeding approach, the last two decades has seen the advent and development of genetic engineering. This technique involves the identification, transfer, and stable integration of desired genes into genomes of crop plants to generate transgenic plants, exhibiting improved trait for tolerance against one or other stress factors in contained experimental conditions such as green houses.

However, plants are constantly exposed to a multitude of stresses at any given time in the natural environment, and not much has been achieved till now to generate crop varieties that can tolerate these multiple stresses without yield penalty. In order to develop stress-tolerant crop varieties with the ability to withstand multiple stresses in their environmental growth condition, an in-depth and systematic understanding of stress sensing, signal transduction, and generation of response is required.

Evolutionarily, the major distinction between plants and animals in sensing and responding to a plethora of stresses is due to their sessile versus mobile nature, respectively. In the case of animals, the primary response against a particular stress is avoidance of stress, whereas in plants, due to their immobilization, development of stress tolerance is the only escape response. Moreover, plants lack a well-defined brain and nervous system unlike their animal counterpart, leading to development of higher degree of plasticity in their communication skills by numerically expanding their signal transduction machinery. Despite the variances amid plants and animals, many of the signal transduction components can be found to be conserved. These include receptors, second messengers, signal-transducing molecules like kinases, phosphatases, small and large G-protein, and others, which finally affect the activity of either transcription factors to regulate the gene expression or transporters/channels, metabolic enzymes, and cytoskeletal proteins to directly change the physiology of the cell. Additionally, analogous to networking in the nervous systems, the signaling pathways in plants also exhibit scale-free web of networks instead of linear or definite pathways. These scale-free networks constitute extremely connected points called *nodes* and *hubs*, which are responsible for efficient processing, channeling, and integration of multiple signaling pathways at a given time to generate specificity as well as cross talk in the signaling networks.

Plants primarily rely on the complex, intertwined, and dynamic signal transduction pathways for developing a higher order of networks. This involves sophisticated control circuits like the nervous system of animals, where they learn, generate memory, alter behavior, and develop intelligence, which make them ready for future challenges. In nutshell, the complex interplay of signal transduction networks and machinery in plants leads them to sense, process, and integrate the signals they confront in their environment. Plants also develop behavioral changes accordingly or develop cognition and storage of processed information to adapt in rapidly changing or variable environment.

Identification of the role of a single or set of genes involved in signal transduction pathway has enabled researchers to understand and develop linear or complex signaling pathways, or maps in response to particular stimuli. However, because of the complete genome sequencing of many plant species including crop plants, a drift towards understanding the stress-signaling pathways involved in single or multiple stresses using high-throughput approaches has emerged. In the post-genomic era, the development of -*omic*-based approaches such as transcriptomic, proteomic, metabolomic, interactomic, and phenomic in several model organisms have laid the foundation of functional genomics. This area of plant science deals with the

understanding of large network of genes and proteins and integration of transcript data to proteins which then go to metabolite, and the complex and dynamic interaction develops a response or phenotype.

Elucidation of Abiotic stress signaling in Plants: *Functional Genomics Perspectives* comprises 30 chapters divided into two volumes (Volume I and II) in which some of the world's most well-known plant biologists have contributed in the field of stress signaling in plants with a special emphasis on functional genomics aspects. This book provides timely research in the field of stress-mediated signaling to develop a better and holistic understanding of stress perception and its transduction followed by the generation of response. In spite of the advent of different approaches to develop stress-tolerant crops towards multiple stress conditions in the field, the success in achieving this goal is still unsatisfactory. This is because stress tolerance is a very complex process involving plethora of components starting from stress sensing to generation of final adaptive response. As mentioned above, there are several factors, which act as nodes and hub in the signaling pathways, also serving as master-control switches in regulating a myriad of stress-signaling pathways by affecting diverse target genes or gene products to finally bring about a stress tolerance response. Therefore, in-depth understanding of these master-control switches and key components in signal transduction pathway will be highly beneficial for designing crop plants tolerant to multiple stresses in the field.

Towards achieving this goal, this book is divided into two volumes comprising five sections. Volume I consists of two sections with 14 chapters. The first section "Functional Genomics Approaches in Signal transduction" discusses three chapters on various approaches used to understand the signal transduction networks. These chapters will aware the readers on practical aspect of various "Omic"-based approaches such as transcriptomic, proteomic, phosphoproteomic, metabolomic, interactomic, and phenomic to understand the functions of genes and gene networks in signaling under stress.

The next section "Components of Signal Transduction" comprises 11 chapters discussing the different components of signal transduction pathways. The first three chapters focus on calcium signaling by describing the genes encoding for CAX (calcium-H$^+$-exchanger) involved in sequestration of calcium ions into vacuoles and maintenance of Ca^{2+} homeostasis. Chapters 5 and 6 discuss the role of Ca^{2+} signal decoding components like sensor and effector proteins. Here, CBLs, CIPKs, and CDPKs gene families have been extensively worked out in model plant Arabidopsis under abiotic stress condition and their role in other crop plant is being elucidated. Chapter 7 describes the role of ROS as redox signaling component in regulating multiple stress responses and in manipulation of ROS levels for imparting stress tolerance in crop plants. The role of MAP kinases as crucial signaling components in biotic as well as abiotic stresses has been discussed in Chapter 8. MAP kinases act as converging points for several signaling pathways, involving the phosphorylation-based relay of information to regulate a large number of targets such as transcription factors, other kinases, and cytoskeletal proteins in stress

signaling. The functional role of small and large G-protein acting as molecular switches to regulate both biotic and abiotic stresses has been discussed in Chapter 9. Chapter 10 deals with the molecular analysis of ABA receptor and ABA signaling in both biotic and abiotic stresses and genetic engineering of ABA receptor for developing stress-tolerant crop varieties. Auxin has been very well known as a plant growth regulator for several decades, and its emerging role in regulating stress signaling and responses is covered extensively in Chapter 11. SA (salicylic acid) is majorly involved in regulating biotic stress, but its role is also appreciated well in abiotic stresses as described in Chapter 12. In Chapter 13, the newly emerging role of methyl glyoxal (MG), which is a cytotoxin generated from both enzymatic and nonenzymatic pathways of metabolic reaction, has been discussed during several abiotic stresses. Chapter 14 discusses the role of immunophilins in diverse biological processes including development and stress management.

Volume II is divided into three sections encompassing 16 chapters. The first section of volume II emphasizes the gene expression regulation of stress signaling, with four chapters discussing the role of transcription factors (mediator complex in Chapter 1 and transcription factors of legumes in Chapter 2) and non-coding and small RNA (Chapters 3 and 4) in regulating abiotic stress responses.

Section two of volume II, comprises ten chapters, discusses the functional genomics aspect of heat/high temperature (Chapter 5), cold/freezing (Chapter 6), drought and dehydration (Chapter 7), flooding and submergence (Chapter 8), salinity (Chapter 9), UV-light (Chapter 10), heavy metal (Chapter 11), nitrogen (Chapter 12), and aging/senescence (Chapter 13) stress signaling responses. In this section, a detailed emphasis has been given in elaborating the respective stress-signaling pathway with a goal of potential candidate genes, which could be used for development of tolerant crop varieties by genetic manipulation and molecular breeding approaches. Moreover, cross talk or overlap in execution of several common signaling components open the scope for taming multiple stresses in future biotechnological intervention.

In the last section of volume II, Chapters 14–16 focus on the development of stress-tolerant crops and sustainable agriculture by utilizing the genes of signal transduction pathways. With the in-depth understanding of several signal transduction components and signaling pathways, the ultimate goal is to utilize the mechanistic knowledge and translate into useful tools to generate the crop varieties by either genetic manipulation of these signaling components or utilization of this knowledge for molecular marker-assisted breeding, ultimately augmenting stress tolerance in crop plants without compromising crop productivity.

Despite rigorous attempts, not every aspect of signaling pathways and components could be discussed here. Nevertheless, I strongly believe that two volumes covering signal transduction machinery and their components in stress condition, with a special emphasis to functional genomics, will be enormously useful to students, teachers, and research scientists.

I am indebted to all the contributors of this work, which could not be possibly compiled without their significant contributions. At last, I would like to express my sincere thanks to Dr. M. C. Tyagi and Dr. Amita Pandey for critical reading and help in copy-editing of this book. I also express my thanks to Ms. Manisha Sharma for designing the theme page.

New Delhi, India   Girdhar K. Pandey, Ph.D.

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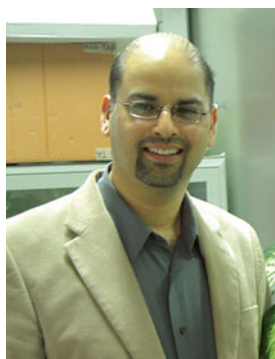
Girdhar K. Pandey, Ph.D.

Girdhar K. Pandey born in Almora, Uttrakhand, India. He received his B.Sc. (Hon.) in Biochemistry from Delhi University in 1992 and M.Sc. in Biotechnology in year 1994 from Banaras Hindu University (BHU). Subsequently, he joined Ph.D. in the School of Life Sciences, Jawaharlal Nehru University (JNU) and worked in the field of calcium signal transduction under abiotic stresses in plants. He was awarded the Ph.D. degree in year 1999 and then pursued postdoctoral career at Department of Plant and Microbial Biology, University of California Berkeley in year 2000. There, he extended his work in the field of calcium-mediated signaling in Arabidopsis by studying CBL-CIPKs, phosphatases, channels/transporters, and transcription factors involved in abiotic stresses. He has been working as Associate Professor in the Department of Plant Molecular Biology, Delhi University South Campus since October 2007.

Pandey's research interests involve detail mechanistic interplay of signal trans-duction networks in plant under mineral nutrient deficiency (mostly potassium, cal-cium, and nitrate) and abiotic stresses such as drought, salinity, and oxidative stresses induced by heavy metals. His laboratory is working on the coding and decoding of mineral nutrient deficiency and abiotic stress signals by studying several signaling

components such as phospholipases (PLA, PLC, and PLD), calcium sensors such as calcineurin B-like (CBL) and CBL-interacting protein kinases (CIPK), phosphatases (mainly PP2C and DSP), transcription factors (AP2-domain containing or ERF, WRKY), transporters and channels proteins (potassium and calcium channels/transporters), small GTPases, and Armadillo domain containing proteins in both Arabidopsis and rice. The long-term goal of his research group is to establish the mechanistic interplay and cross talk of mineral nutrient-deficient conditions and different abiotic stress signaling cascades in Arabidopsis and rice model system by using the advance tools of bioinformatics, genetics, cell biology, biochemistry, and physiology with greater emphasis on functional genomics approaches.

He has been awarded with Far Eastern Regional Research Organization (FERRO) fellowship to work at Beltsville Agricultural Research Center (BARC), United States Department of Agriculture, Beltsville, MD (1998). Later, he was awarded with Indian National Science Academy (INSA)-Deutsche Forschungsgemeinschaft (DFG) bilateral exchange visiting scientist fellowship in 2011. Also Department of Biotechnology (DBT), India, has awarded him with prestigious DBT-CREST Award (Cutting-edge Research Enhancement and Scientific Training) in 2011–2012. See Pandey's web page for further information about his lab and research work: https://sites.google.com/site/gkplab/home; http://www.dpmb.ac.in/index.php?page=girdhar-pandey.

Part I
Gene Expression Regulation of Stress Signaling

Chapter 1
Role of Plant Mediator Complex in Stress Response

Subhasis Samanta and Jitendra Kumar Thakur

Abstract Class II gene loci of eukaryotes are transcribed by RNA Polymerase II, which functions in coordination with several other proteins like transcription factors, general transcription factors, and cofactors. Recently, Mediator complex, a multi-subunit, megadalton size protein complex has gained lots of attention as an important component of RNA pol II transcriptional machinery because of its essentiality in the regulation of most of the class II genes. Like yeast and other metazoans, plants also possess the Mediator complex across the kingdom, and its isolation and subunit analyses have been reported from the model plant, *Arabidopsis*. Recent times have experienced a flurry of scientific papers containing the functional information of individual Mediator subunits in plants, although many were reported earlier without consideration of their association with the Mediator complex. Among its diverse functional aspects, several reports have established the Mediator complex as an important integrative hub of different biotic and abiotic stress signaling pathways, which have been discussed in this chapter from the functional genomics perspectives. Although reports are emerging in support of its inclusion as a component of the basic transcriptional machinery, the gene selective roles of the individual Mediator subunits are proven and indisputably accepted.

Keywords Transcription • RNA Polymerase II • Mediator complex • Mediator subunit • Biotic stress • Abiotic stress • Defense signaling • *Arabidopsis* • Rice

Abbreviations

BR	Brassinosteriod
ChIP	Chromatin immunoprecipitation
JA	Jasmonic acid
LC-MS/MS	Liquid chromatography-mass spectrometry
MED	Mediator
MudPIT	Multidimensional protein identification technology

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RNAP II   RNA Polymerase II
SA   Salicylic acid
TAP   Tandem affinity purification

1.1 Introduction

The process of transcription in eukaryotic organism is a highly orchestrated and immensely complex phenomenon and mediated by a plethora of proteins with the prime role played by RNA Polymerase II (RNAP II) (Lee and Young 2000). RNAP II with the basal transcription factors forms the heart of the transcription machinery. Over the time, several cofactors have been discovered, which offer the basic transcriptional machinery diverse regulatory avenues in terms of controlling gene expression (Woychik and Hampsey 2002). Among these cofactors, Mediator, a multi-subunit protein complex, has been proved to be quintessential in RNAP II-mediated gene expression (Myers and Kornberg 2000; Conaway et al. 2005; Kornberg 2005; Malik and Roeder 2005). Mediator complex, an ensemble of around 25–30 Mediator subunits, could be imagined as a bridge connecting the basic transcription machinery with the *cis*-element bound transcription factors (Fig. 1.1). However, Mediator does not act simply as a scaffold protein, rather as a subtle and complex modulator of gene expression during transcription. Although far from a clear and detailed understanding, the binding of transactivator or repressor with the Mediator complex might bring about certain conformational changes, which are transmitted to the RNAP II resulting into the desired changes in the level of gene expression. Apart from transcription factors (transactivator and repressor), Mediator complex also acts as docking site for several other proteins, which elicit their regulatory roles through Mediator-induced structural changes on RNAP II machinery (Meyer et al. 2010; Taatjes 2010). Since the discovery of plant Mediator in 2007, its subunits have been implicated in several biological processes. Recently, role of Mediator in growth and development was reviewed (Kidd et al. 2011). Here, in this chapter, we discuss the current status of Mediator research in plants from functional genomics perspectives with special emphasis on its role in biotic and abiotic stresses.

1.2 Discovery of Mediator Complex

Until now, Mediator complex has only been reported in eukaryotes. The complex was first isolated from the yeast as a factor required for enhanced transcription in a cell-free, in vitro transcriptional system, composed of RNAP II and general transcription factors in *Saccharomyces cerevisiae* (Kim et al. 1994; Myers et al. 1998) as well as in *Saccharomyces pombe* (Spahr et al. 2000). Later, the complex was isolated from almost all the eukaryotic organisms ranging from human (Fondell et al. 1996; Ito et al. 1999), *Drosophila* (Park et al. 2001), *Caenorhabditis* (Park et al. 2001) to even plant (Backstrom et al. 2007). The yeast Mediator complex was
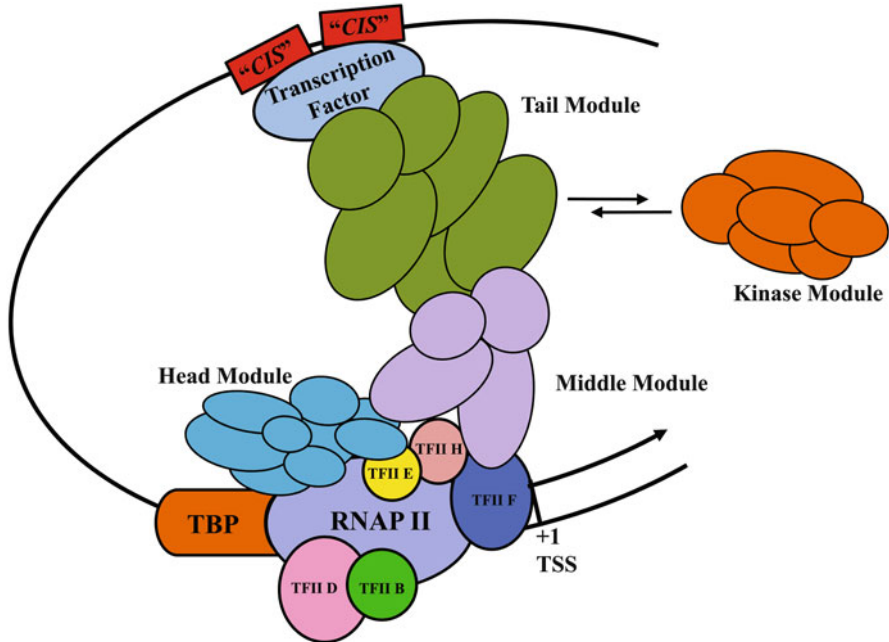
Fig. 1.1 Modular structure of Mediator complex and its interaction with transcriptional machinery. Head, Middle, and Tail modules form the Mediator complex along with a separable kinase module. Generally, the tail module interacts with the *cis*-element bound transcription factors, whereas the Head and Middle modules bind to the components of the basic transcriptional machinery of class II genes. In response to different signals, the Mediator complex helps the transcription factors transmit the messages encrypted in the regulatory DNA elements and engages the transcription apparatus to the promoter of the transcribing genes. **RNAP II**: RNA Polymerase II, **TBP**: TATA-box binding protein, **TFII**: transcription factor II, **TSS**: transcription start site

isolated using the principles of traditional biochemistry, i.e., fractionation of total protein through a series of chromatography based on different principles, and then immunoprecipitating the Mediator complex from the Mediator enriched chromatographic fractions. Using similar techniques, the first biochemical purification of Mediator complex among plants was reported from *Arabidopsis* (Backstrom et al. 2007). After protein fractionation by two different chromatographic techniques, the final step was performed by immunoprecipitation with antibody raised against a Mediator subunit, AtMED6. Apart from *Arabidopsis*, the bioinformatics analyses encompassing 16 plant species across the entire plant kingdom revealed the ubiquitous presence of this important regulatory complex in every plant groups included in the study (Bourbon 2008; Mathur et al. 2011). The presence of almost all the fungal/metazoan Mediator subunits in one or other plant species using HMM (Hidden Markov Model) profile of Mediator subunits was predicted (Bourbon 2008; Mathur et al. 2011). However, some plant-specific Mediator subunits are also reported. Thus, it seems that Mediator subunits have emerged at the very early stages of eukaryotic evolution and some extra subunit might have been added or lost in different lineages in course of evolution (Conaway and Conaway 2011).

1.3   Functions of Mediator Complex

RNAP II along with the components of preinitiation complex (PIC) is the minimum requirement to start any successful transcription event at the initiator region of a gene. In order to achieve increased or activated level of transcription, the requirement of Mediator complex has been proved quintessential almost for every gene of eukaryotes (Myers and Kornberg 2000). In fact, Mediator complex was first discovered as an entity required for enhanced transcription of an in vitro transcription system, which included RNAP II and other accessory factors (Kim et al. 1994). Very recently, critical role of Mediator was explained in the function of super-enhancers in increased level of gene expression to establish and maintain cell identity (Loven et al. 2013; Whyte et al. 2013). However, the inhibitory role of Mediator complex in the repression of gene functions has also been reported and is discussed in a later section. But, the controversial aspect of Mediator function as a cofactor or a basal transcription factor is still debatable (Taatjes 2010). There are evidences, which support the dual role of Mediator function, i.e., as a part of the basal transcriptional machinery as well as a selective regulator of gene function. The Mediator complex can support basal level of transcription as evidenced by its significant roles in the assembly of PIC and in the initiation of transcription (Mittler et al. 2001; Baek et al. 2002). On the other hand, Mediator complex enhances the RNAP II recruitment to the protein coding genes and provides stability to the transcription machinery assembled at the promoter region (Cantin et al. 2003; Baek et al. 2006). The repression of almost all the protein coding genes in yeast conditional mutant *MED17* corroborates the essentiality of Mediator complex in RNAP II-mediated transcription (Thompson and Young 1995; Ansari et al. 2009). In plants, the essentiality of Mediator complex in RNAP II-mediated gene expression became evident when 84 % of downregulated genes in *nrpb2–3* (second largest subunit of RNAP II) and *MED20A* mutant *Arabidopsis* plants were found to be common (Kim et al. 2011). Thus, literature evidences suggest that the Mediator complex is as important as the RNAP II and could be regarded as an integral component of the basal transcriptional machinery in eukaryotes. Nevertheless, reports of severe specific functional abnormalities, be it in growth and development or in the response to biotic and abiotic stresses, when a particular Mediator subunit gene is deleted, are proving that Mediator subunits do possess specific functions (Kidd et al. 2011). Although initial emphasis was laid in the crucial role of Mediator in the assembly of transcription initiation complex (Cantin et al. 2003; Johnson and Carey 2003; Wang et al. 2005), the more recent reports suggest its function in almost every steps of transcription such as promoter escape (Malik et al. 2007; Cheng et al. 2012; Jishage et al. 2012), elongation (Takahashi et al. 2011; Conaway and Conaway 2013; Galbraith et al. 2013), termination (Mukundan and Ansari 2011, 2013), and other related RNA-processing events (Kim et al. 2011; Huang et al. 2012; Oya et al. 2013). In last few years, Mediator has also been implicated in epigenetic modification of chromatin leading to changes in gene expression (Ding et al. 2008; Kagey et al. 2010; Zhu et al. 2011; Fukasawa et al. 2012; Liu and Myers 2012; Tsutsui et al. 2013; Zhang et al. 2013a; Lai et al. 2013).

1.4 Modular Organization and Composition of Mediator Complex in Plants

Mediator is a multi-protein complex, which is composed of several subunits. The number of subunits varies according to the species. The yeast Mediator complex is composed of 25 subunits whereas the metazoans possess 25–30 Mediator subunits (Boube et al. 2002; Bourbon 2008). On an average, the plants contain around 30–35 Mediator subunits (Backstrom et al. 2007; Mathur et al. 2011; Pasrija and Thakur 2012). However, expansion of some subunits has also been observed in plants. Apart from the orthologs of the yeast Mediator subunits, the plants also contain a unique set of Mediator subunits, which are not present either in yeast or in metazoans. Plants are sessile organisms and the Mediator complex assisted gene regulation seems to be more complicated in plants. This could be corroborated by the fact that plants possess increased number of transcription factors (Riechmann et al. 2000; Riechmann and Ratcliffe 2000). As the Mediator complex elicits its gene regulatory action by forging a bridge between the *cis*-element bound transcription factors and the RNAP II, the increased number of Mediator subunits might have evolved to interact with increased number of transcription factors in plants. Another important discovery is the presence of increased number of paralogs of some Mediator subunits in plants. Certain species of yeast like *Candida glabrata* and the metazoans do possess paralogs of MED15 and kinase module genes, respectively. But the possession of nine paralogs of MED15 in *Populus trichocarpa* and quite a few in other plant species is a distinguishing feature for the plant Mediator complex in general (Mathur et al. 2011; Pasrija and Thakur 2012). At present, the presence of all the paralogs of a particular Mediator subunit at the same time has not been validated. However, from the functional perspective, the spatial and temporal regulation of the expression level of different paralogs of a particular Mediator subunit has been reported (Mathur et al. 2011; Thakur et al. 2013). The rice *OsMED31_1* exhibits pronounced expression level in the leaves whereas *OsMED31_2* exhibits higher expression level only during early stages of panicle development. In *Arabidopsis*, there is only one *AtMED31* gene and it shows higher expression in reproductive organs including flower and seed. In rice, *OsMED15_1* showed seed preferential expression whereas *OsMED15_2* is expressed at similar level in vegetative and reproductive tissues (Thakur et al. 2013). Thus, the presence of multiple paralogs and the spatiotemporal regulation of Mediator subunits make the Mediator structure more dynamic depending upon the external milieu and the growth and developmental phases of the plants. Mediator subunits have been grouped into four modules according to the biochemical and structural evidences obtained from the 3D structure of the yeast Mediator complex (Asturias et al. 1999; Dotson et al. 2000; Chadick and Asturias 2005) assembled from the EM structure of the purified yeast Mediator complex. The following is a brief account of the Mediator complex subunits according to their arrangement in specific modules.

1.4.1 Head Module

The head module consists of MED6, MED8, MED11, MED17, MED18, MED19, MED20, MED22, MED28, and MED30. The head module subunits can establish direct contacts with RNAP II and with other components of the basic transcriptional machinery and, alone could stimulate transcription rate over the basal rate, but it does not support activator-dependent transcription (Takagi et al. 2006; Cai et al. 2010). Disruption of the head module leads to the dissociation of the Mediator complex from the promoter of the transcribing genes (Lariviere et al. 2006). Apart from the direct interaction of head module with the RNAP II (Soutourina et al. 2011), it also interacts with the components of the basic transcriptional machinery. The interaction between head module and TFIIH is probably mediated by an interface created by MED11/MED22 heterodimer of the head module, whereas the interaction with TBP is mainly through MED8 (Kim et al. 1994; Lariviere et al. 2006; Imasaki et al. 2011; Seizl et al. 2011). In yeast, MED17 performs the task of maintaining a link with the middle module through its interaction with the MED21 from the middle module. Similarly, MED17 also interacts with the reversible kinase module via its interaction with CDK8 (Guglielmi et al. 2004). MED17 is the most important Mediator subunit of the head module, as mutation in this gene in yeast affects the expression of most of the protein coding genes just like the deleterious effects caused by mutations in RPB1 subunit of RNAP II (Thompson and Young 1995). Given the fact that head module subunits establish direct contacts with the components of the RNAP II machinery and form the core of the Mediator complex, the head module subunits are thought to be the most conserved Mediator subunits of the complex. In general, structural analysis of the Mediator complex has been impeded by the low expressibility of the Mediator subunit proteins and by the inherent difficulties in the in vitro assembly of the Mediator complex. However, assembly of the head module has become feasible with the recent advances in heterologous protein expression technology, and a low resolution, EM structure of the head module has already been reported (Cai et al. 2010). More recently, a seven subunit partial backbone structure of the head module has been resolved with the help of X-ray crystallography (Imasaki et al. 2011). Only a limited number of head module Mediator subunits have been addressed functionally in plants. Among the significant ones, AtMED8 has been implicated in flowering and root hair biogenesis (Kidd et al. 2009; Sundaravelpandian et al. 2013) whereas AtMED17, 18, and 20 were found to be involved in siRNA and non-coding RNA production (Kim et al. 2011).

1.4.2 Middle Module

The Mediator subunits, MED4, MED7, MED9, MED10, MED21, and MED31 form the middle module. Although MED1 is an important middle module constituent in yeast and metazoans as it regulates many important genes by binding to their respective transcription factors, so far bioinformatics analyses from different

studies in different organisms have never been able to find its orthologs in plants except in a distantly related red algae (Ito and Roeder 2001; Bourbon 2008; Mathur et al. 2011). The apparent absence of plant MED1 suggests that either MED1 has been lost in course of evolution or its function might have been acquired by some other Mediator subunit. Middle module subunits, MED1 and MED10, interact with the tail module subunit, MED14, which happens to be at the interface of middle and the tail modules (Li et al. 1995; Lee et al. 1999; Guglielmi et al. 2004). The interaction between MED21 and MED3 also strengthens the connection between middle and tail module (Guglielmi et al. 2004). A combination of biomolecular techniques including small angle X-ray scattering revealed that a high degree of intrinsic flexibility and the elongated shape are the characteristic features of the middle module (Koschubs et al. 2010). MED4 and MED7 are probably the most important middle module subunits as they form three heterodimeric subcomplexes, Med7N/21, Med7C/31, and Med4/9 (Koschubs et al. 2010). Large-scale structural changes in the Mediator subunits are effected by a flexible hinge formed by MED7 and MED21 in the middle module (Baumli et al. 2005). The Med7C/31 is characterized by a novel conserved fold and is essential for activator-dependent transcription (Koschubs et al. 2009). Most of the middle module subunits are conserved in plants too, except a "poly Pro" region in AtMED31C, followed by a nuclear localization signal, which is absent in yeast and human (Mathur et al. 2011). Except AtMED21 whose involvement in pathogenesis is discussed in latter section, function of no other plant Mediator subunits from the middle module has been characterized.

1.4.3   Tail Module

The tail module is arguably the least conserved and functionally most significant Mediator module. The subunits from the tail module maintain direct contacts with the *cis*-element bound transcription factors and accordingly recruit the DNA bound Mediator complex to the RNAP II machinery. The module includes MED2/29/32, MED3/27, MED5/24/33, MED14, MED15, MED16, and MED23. Structural analysis revealed that MED14 occurs at the interface of middle and tail module. In yeast, heterodimer of MED2 and MED3 interacts with MED15 to form a triad (Zhang et al. 2004). Similarity analyses among the tail module subunits revealed that MED2 and MED3 of plants, are more similar to human as compared to yeasts (Mathur et al. 2011). Size of MED15 in plants is bigger than that of fungi and animals, though its amino terminal KIX domain is conserved in them (Thakur et al. 2013). Functionally, the KIX domain seems to be very important domain of MED15 proteins and so, structurally the most well-investigated one (Thakur et al. 2008, 2009, 2013, 2014, Lariviere et al. 2012). A myriad of transcription factors have been reported to interact with MED15 via KIX domain regulating diverse pathways in different organisms (Malik and Roeder 2005; Thakur et al. 2014). Despite poor structural similarity except in the N-terminal transcription factor interacting KIX domain among the MED15 proteins, the crucial amino acid residues of the KIX domain are surprisingly conserved among human, yeast, and *Arabidopsis*, three

important model organisms from three different kingdoms (Mathur et al. 2011). The importance of the tail module subunits in the transcriptional regulation could be well imagined by the fact that the maximum numbers of subunits, whose functions are elucidated, belong to this module. Although no interaction has been reported among them, an intriguing hypothesis regarding the formation of a triad consisting of MED15, MED16, and MED14 in plant defense signaling has been recently put forward (Zhang et al. 2013b). Apart from its gene-specific role, the tail module has recently been implicated in many different aspects of transcriptional regulations as a separate entity. The TATA-box containing and SAGA-regulated genes are much more dependent on the tail module for their transcription as compared to the TFIID-dependent gene expression (Ansari et al. 2012; Ansari and Morse 2012). Interestingly, a role of Mediator tail module in the maintenance of heterochromatin region of chromosome telomere has also been reported (Peng and Zhou 2012).

1.4.4   Kinase or CDK8 Module

The stimulatory role of Mediator complex in gene regulation has become complicated with the discovery of kinase module, which can reversibly associate with the core part of the Mediator complex. The kinase module is composed of MED12, MED13, Cyclin-Dependent Protein Kinase 8 (CDK8), and Cyclin C (CycC). All the kinase module subunits were discovered in yeasts in a screen for suppressor of RNAP II CTD mutation (Liao et al. 1995). Basically, the association of kinase module with the core complex inhibits its interaction with the RNAP II machinery (Akoulitchev et al. 2000; Knuesel et al. 2009a). Also, initial genetic studies revealed negative effect of this module on a subset of genes (Holstege et al. 1998; Samuelsen et al. 2003). However, recent reports contradicted these observations and showed the positive regulation of some genes by the Mediator complex, which had the kinase module associated with it (Donner et al. 2007, 2010; Belakavadi and Fondell 2010). Thus, the CDK8 kinase module can modulate the transcription factor activity in both positive and negative way (Taatjes 2010). Among the regulators of CDK8 kinase activity, MED12 has been established as the most significant one as CDK8 requires MED12 for its kinase activity (Knuesel et al. 2009a). Moreover, MED12 might directly interact with transcription factors for recruiting CDK8 to the chromosomal loci. MED13 helps in association and recruitment of kinase module to the Mediator complex via its interaction with the tail module (Knuesel et al. 2009a). Mediator also regulates kinase activity of CDK8 on chromatin by restricting its association with it (Knuesel et al. 2009b). The bioinformatics analyses have revealed the presence of kinase module in almost all the plant groups analyzed. Like in mammals and other metazoans, paralogs of the kinase module subunits have also been discovered in plants, which raise the possibility of combinatorial control of Mediator function in plants too. Since the kinase module bound Mediator complex accounts for only a small fraction of the total Mediator, the absence of kinase module

subunits in the first-ever Mediator complex purified from *Arabidopsis* is not so surprising (Backstrom et al. 2007). Among the four members of the kinase module, CDK8, Cyclin C, and MED12 have not been found to interact with any other subunit of the Mediator complex (Guglielmi et al. 2004). However, a comprehensive analysis in different organisms needs to be done before making any conclusion.

1.4.5   Plant-Specific and Module-Unassigned Mediator Subunits

Positions of MED25 and MED26 have not been understood yet. Similarly, the plant-specific Mediator subunits, MED34, MED35, MED36, and MED37, have not been assigned any module (Backstrom et al. 2007). Other two plant-specific Mediator subunits, MED32 and MED33, identified during biochemical purification of Mediator complex from *Arabidopsis*, have reported to be apparent homologs of MED2 and MED5, respectively (Mathur et al. 2011). MED26, which remained unreported for a long time from any plant species, has been reported from all the plant species using a rigorous HMM search except algal group (Mathur et al. 2011). Most of these MED26 proteins have been described as transcriptional elongation factors especially in rice and *Arabidopsis* databases, probably because of the presence of TFIIS helical bundle in them (Mathur et al. 2011). This helical bundle is a characteristic feature of RNAP II elongation factors, TFIIS and Elongin A. Thus, MED26 of the Mediator complex contributes to the elongation step of RNAP II-mediated transcriptional event, which is unusual as compared to the canonical role of the Mediator complex in the assembly of initiation complex (Takahashi et al. 2011; Conaway and Conaway 2013). As of now, in plants, MED25 is the most well-characterized Mediator subunit, which has been described to function in different biotic and abiotic stresses and in diverse developmental processes like root development, flowering, and fruit development. As the plants are sessile organisms, perhaps the gene regulatory mechanisms in plants are more diverse and complicated. Several transcription factors function as the master regulator of the cellular and physiological processes, and so their complex network can contribute immensely to the complexity of gene regulation. The genome analysis of plants revealed that plants contain more number of transcription factors as compared to animals (Riechmann et al. 2000; Riechmann and Ratcliffe 2000). As Mediator functions by interacting with the transcription factors, the increased number Mediator subunits in plants might have evolved to cover more number of transcription factors. Also, plant-specific Mediator subunits might be targeted by plant-specific transcription factors conferring the plants better transcript alteration ability in response to diverse internal and external cues. On the other hand; as the basic, overall structure of the Mediator complex is same in all the organisms, most of the plant-specific subunits, if not all, will predictably occupy the tail module of the Mediator complex, bestowing the plants with seemingly unlimited gene regulatory potential.

1.5   Transcriptomics of Mediator Genes

Most of the total protein-coding genes in eukaryotes require the contribution of Mediator complex even to sustain basal level of transcription proves unequivocally that Mediator constitutes the part of the basal transcriptional machinery (Ansari et al. 2009; Kim et al. 2011; Lacombe et al. 2013). At the same time, the increasing numbers of reports describing the effects of mutation in specific subunit on the transcription of specific set of genes strongly suggest that Mediator could also act as selective gene regulator (Taatjes 2010; Kidd et al. 2011). This raises the possibility of regulation of specific *MED* genes in response to specific signals. In order to address this, expression analyses of *MED* genes were performed by different research groups in animals and plants. In human endothelial progenitor cells, expression of *MED12* and *MED30* increased and decreased, respectively, after L-arginine treatment (Rienzo et al. 2010). Additionally, Mediator subunit genes were also found to undergo alternative splicing in tissue-specific manner (Rienzo et al. 2012). In plants too, alternatively spliced isoforms of *MED* transcripts are predicted. In rice, *MED* genes are more pronouncedly expressed in seeds of different stages as compared to shoot and root (Mathur et al. 2011). This is in accordance with the enrichment of seed storage-specific promoter elements on certain *MED* genes indicating the important regulatory role of MED subunits during seed development and maturation. A genome level transcriptome analysis of *MED* transcripts in response to different stresses like drought, cold, and salinity did not reveal much perturbations except that in *OsMed37_6*, which exhibited around twofold changes in transcript abundance in response to different stresses (Mathur et al. 2011). In *Arabidopsis*, some hormones such as brassinosteroid (BR) and ABA affect the stoichiometric concentrations of a set of MED subunits by regulating their transcript abundance (Pasrija and Thakur 2012). However, other hormones like auxin, jasmonic acid (JA) affect very few *MED* genes. *AtMED37*, which has been discovered as a plant-specific Mediator subunit, is highly up-regulated in response to BR treatment. A significant transcriptomic reprogramming of the Mediator subunit genes in *Arabidopsis* happens in response to stresses like salinity, cold, high light and continuous dark and is summarized in Table 1.1 (Pasrija and Thakur 2012). Additionally, in *Arabidopsis*, tissue- and organ-specific analyses revealed changes in transcriptome profile of several *MED* genes during development and maturation of tissues and organs (Pasrija and Thakur 2013). On the basis of their studies, apart from spatiotemporal regulations of individual Mediator subunits, enrichment of specific structural arrangement composed of specific Mediator subunits during certain developmental stages can be predicted. In the following section, we describe the change in the transcript abundance of individual Mediator subunits according to their module occupancy, at different developmental stages and in response to different environmental cues.