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Interaction map of *Arabidopsis* Mediator complex expounding its topology

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ABSTRACT

Understanding of mechanistic details of Mediator functioning in plants is impeded as the knowledge of subunit organization and structure is lacking. In this study, an interaction map of *Arabidopsis* Mediator complex was analyzed to understand the arrangement of the subunits in the core part of the complex. Combining this interaction map with homology-based modeling, probable structural topology of core part of the *Arabidopsis* Mediator complex was deduced. Though the overall topology of the complex was similar to that of yeast, several differences were observed. Many interactions discovered in this study are not yet reported in other systems. AtMed14 and AtMed17 emerged as the key component providing important scaffold for the whole complex. AtMed6 and AtMed10 were found to be important for linking head with middle and middle with tail, respectively. Some Mediator subunits were found to form homodimers and some were found to possess transactivation property. Subcellular localization suggested that many of the Mediator subunits might have functions beyond the process of transcription. Overall, this study reveals role of individual subunits in the organization of the core complex, which can be an important resource for understanding the molecular mechanism of functioning of Mediator complex and its subunits in plants.

INTRODUCTION

Mediator is a multi-subunit protein complex originally discovered in yeast as a molecular bridge between enhancer-bound transcription factors (TFs) and RNA pol II (1,2). Later, the complex was discovered in different animals and the model plant *Arabidopsis* (3,4). Genomics and proteomics studies revealed that the orthologs of almost all the yeast Mediator subunits are present in all other eukaryotes (5–7). Biochemical and electron microscopy-based structural analyses revealed that the Mediator subunits in yeast and human are organized in four different modules; head, middle, tail and kinase. However, there are few subunits which could not be assigned to any of these four modules and so are still called as unassigned module subunits. The modular arrangement of subunits was also supported by the interaction map of Mediator subunits (8–11). This modular organization seems to be very important as it helps the complex execute its function as a linker between TF and RNA pol II (12). Head and middle module subunits interact extensively with the components of RNA pol II transcriptional machinery including the CTD of RNA pol II (2,12). On the other hand, tail module subunits interact with different TFs (13–16). Thus, the Mediator complex plays critical role of relaying the transcriptional signals from TFs to the transcriptional machinery. Head, middle and tail modules constitute the core part of the complex whereas the kinase module can reversibly associate with the core part in response to different stimuli (12).

In yeast and human Mediator complexes, arrangement of subunits has been studied in great detail by using different biochemical techniques, yeast two-hybrid analysis, split-ubiquitin two hybrid assay, co-immunoprecipitation and pull-down assay (8,10,11,14,17–21). Different biophysical structural analyses including EM of Mediator complex have corroborated the arrangement of subunits (10,11,22–26). Apart from the overall 3D structure and models, high resolution crystal structures of head and middle modules and complete Mediator complex have been elucidated in yeast (27–29). The head module of yeast (Sc; *Saccharomyces cerevisiae*) Mediator complex was reconstituted *in-vitro* and the crystal structure was resolved up to 4.3 Å (28). Head module was found to form a crocodile head-like structure, which had a 'fixed jaw', a 'movable jaw' and a 'neck'. Med17 of head module is the most important subunit that makes extensive contacts with other head module subunits (27,28). C-terminal part of Med17 interacts with Med11 and Med22 to form the 'fixed jaw'. C-terminal region of Med17 also establishes contact with Med18. Supporting the interaction data, deletion of C-terminal region of Med17 dismantled

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the head module and resulted into loss of global transcription causing lethality (28). The flexible movable jaw, formed by the functionally distinct sub-module consisting of Med8, Med18 and Med20 subunits, interacts with TBP (29). Interaction of the Med18 with C-terminal part of Med17 and N-terminal region of Med11 is critical for the flexibility and important for attaining functional confirmations (23). The neck domain is mainly formed by N-terminal part of Med6 and different portions of Med8, Med11, Med17 and Med22. Structure of head module of another yeast (Sp; *Saccharomyces pombe*) was solved at even higher resolution going up to 3.4 Å (27). In the EM structures of yeast and human Mediator complexes, Med19 was found to form a hook-like structure at one end of the middle module (21). However, Med19 showed no association with the recombinantly expressed head and middle module subunits (10). Overall, the head module subunits of human interact extensively with each other. These studies unravel the conservation of head module structure in yeast and human. Apart from the central role of Med17 in maintaining the head module structure, Med17 was also found to link head and middle modules (8). Another head module subunit Med6 interacts with middle module subunit Med21 to provide flexibility at the head-middle junction (30). In yeast and human, it is proposed that the core middle module is formed by different interactions among Med7, Med21, Med4 and Med9 subunits (9,11). In yeast, Med4, Med7 and Med21 have been reported to interact with all other middle module subunits suggesting their importance in maintaining the middle module structure. Med21 was also found to provide the flexibility for head-middle junction (30). On the other hand, in human, Med31 was found to be present at the head-middle junction. In human, Med31 interacts with Med19 of head module (21). Crystal structure of head and middle modules together has been resolved at 3.4 Å (27). Head module formed same crocodile head like structure as was deduced by free head module in *S. pombe* (29), while middle module was found to form five sub-modular structures named as beam, plank, hook, knob, and connector (27). Plank was found to be formed by evolutionary conserved interaction of Med4–Med9 and the hook consisted of N-terminal part of Med14 and middle module subunit Med10. The hook is flexibly linked to the connector created by conserved Med21-Med7 interaction (27,30).

Different means of structural analyses have revealed unique folds in Mediator subunits. Med8, Med18 and Med20 of head module form a sub-complex in which the C-terminus of Med8 forms an α-helix that tethers β-barrel folds formed by Med18 and Med20 (29,31,32). A heterodimer formed by Med11 and Med22 consists of four helix bundle with C-terminal extensions that bind to Med17 (33). In the middle module, N-terminus of Med7 forms a sub-module with Med31 in which two proline rich regions of Med7N wraps around the four-helix bundle of Med31 (34). Though the structure of mammalian Mediator complex is not yet solved, few studies suggest that structure of human Mediator complex is similar to that of yeast (10,11,21). Yeast Med14 interacts with subunits of all the three modules and functions as a backbone to hold the entire complex (11). This role of Med14 is conserved in human Mediator complex (10). The whole Mediator structure seems to be supported over a beam formed by mainly Med14 (27). Various biochemical and structural studies suggest that Med14 interacts with many subunits across three modules to provide structural support for the whole complex both in yeast and human (10,11). N-terminal region of Med14 was found to interact with Med6 and Med17 of head module and Med10 of middle module. On the other hand, the C-terminal part of Med14 establishes contacts with the tail module subunits (10,11). In human, Med14, Med15 and Med16 was found to precipitate together in immunoprecipitation analysis suggesting their physical association (21). On the other hand, in yeast, a triad is formed by different tail module subunits; Med2, Med3 and Med15 (35).

Almost a decade ago, first plant Mediator complex was purified and characterized from *Arabidopsis* cell suspension culture (4). Since then, individual plant Mediator subunits have been studied in terms of their physiological relevance and function by different forward and reverse genetics approaches (36–39). They have been implicated in embryo development, organ development, flowering, response to different stresses and non-coding RNA production (37). However, there is no study providing the holistic picture of plant Mediator complex. Also, there is no information on the structure of plant Mediator complex or the relative positioning of Mediator subunits in the complex. Plant Mediator subunits are highly disordered and notoriously difficult to express at high levels, hampering their structural analysis. In this study, interaction map of core Mediator subunits of *Arabidopsis* has been analyzed to understand their arrangement in the complex. In several cases, interacting domains were delineated. On the basis of this interaction map, homology modeling and alignment with the yeast Mediator structure, a probable structural topology of the core part of *Arabidopsis* Mediator complex has been proposed. This study lays down a foundation for delineating structure-function of plant Mediator complex and understanding the molecular mechanism of its functioning.

MATERIALS AND METHODS

Strain, media and culture conditions

Seedlings of *Arabidopsis thaliana* ecotype Columbia (Col-0) were grown in culture room at 22°C under long day light photoperiod (16 h light and 8 h dark). The *Escherichia coli* strain DH5α was used as a host for all plasmid construction and maintenance. The bacterial strain was routinely grown in Luria-Bertani (LB) medium (Himedia) at 37°C. AH109 (MATa trp1-901 leu2-3, 112 ura3-52 his3-200, gal4Δ gal80Δ LYS2::GAL1UAS-GAL1TATA-HIS3, MEL1GAL2UAS-GAL2TATA-ADE2, URA3::MEL1UAS-MEL1TATA-lacZ) yeast strain was used to study one to one interaction, while Y187 (MATα gal4 gal80 his3 trp1-901 ade2-101 ura3-52 leu2-3, 112 URA3::GAL1::lacZ LYS2::GAL4(UAS)::HIS3 cyhR), was used to check the auto-activation of the subunits. The untransformed yeast strains were routinely cultured in YPD medium, at 30°C and 150 rpm. Transformants were cultured in selective dropout medium depending on the vector system used.

Plasmids and cloning

All one to one interactions of the Mediator subunits were studied using yeast two-hybrid vector system containing pGBKT7 and pGADT7 vectors (Clontech). For Gateway cloning, the full-length coding sequence and fragments of the subunits were first amplified and then cloned into pENTR™/D-TOPO® entry vector (Invitrogen) followed by mobilization into destination vector using LR reaction (Invitrogen). For subcellular localization studies, pSITE-3CA vector containing N-terminal yellow florescence protein (YFP) tag was employed (40) while for BiFC studies, vectors pSAT4-DEST-N(1–174) EYFP-C1 and pSAT4-DEST-C(175-end) EYFP-C1 were used (41). All the primers used in this study to amplify different Mediator subunits and their fragments are listed in Supplementary Table S1.

Characterization of activation property

The auto-activation strength of *Arabidopsis* Mediator subunits cloned into pGBKT7 was measured by two different assays, which measure the expression of *His*, *Ade* and *β-galactosidase* reporter genes in the yeast strain. The expression of *His* and *Ade* was measured by growing yeast cells on selective media lacking histidine and adenine. Growth of yeast colonies on selective media was recorded as positive for auto-activation while no growth was scored as negative.

The ONPG (*ortho*-nitrophenyl-β-galactosidase) assay was performed to check the trans-activation property of the *Arabidopsis* Mediator subunits cloned into pGBKT7 by checking the expression level of the *β-Galactosidase* gene. Transformed yeast cells were grown till the OD_{600} reached 0.5–0.8 in SD-Trp media. After that, medium was replaced with Z-buffer (1.6% (w/v) Na_2HPO_5, 0.55% (w/v) NaH_2PO_4, 0.075% (w/v) KCl and 0.025% (w/v) $MgSO_4$, pH 7) and cells were lysed by freeze-thaw cycles. 50 μl of 1.5 mg/ml of ONPG was added in the Z-buffer and initial OD was measured at 420 nm (42). The reaction mix was incubated at 37°C and OD_{420} was measured at regular interval of time using spectrophotometer. The time (t) taken for change in colour was noticed for every *Arabidopsis* Mediator subunit till 30 min and the *β-galactosidase* units were calculated using the following formula:

$$\beta - \text{galactosidase units} = 1000 \times OD_{420}/t \times OD_{600}$$

Yeast two hybrid assay

Interaction between two Mediator subunits was determined by using Matchmaker® Gold Yeast

Two-Hybrid System (Clontech) as per the instruction given in the manual. Coding sequences of *Arabidopsis* Mediator subunits were cloned in pGBKT7 and pGADT7 vectors and pair of recombinant plasmids were introduced into AH109 yeast cells. The double transformants were selected on SD-Trp/-Leu selection medium. Interactions were scored onto SD-Trp/-Leu/-His/-Ade, SD-Trp/-Leu/-His and SD-Trp/-Leu/-Ade media depending on the strength of the interaction. The transformants were grown at 30°C till the OD_{600} reaches 0.5–0.8 and from that 2μl of inoculum was spotted onto the selection plate. The plate was kept at 30°C for 4 days. Vector alone (pGBKT7 or pGADT7) was co-transformed with each of the Mediator subunits to eliminate the false positives.

Subcellular localization of *Arabidopsis* Mediator subunits

For subcellular localization studies, coding sequence of *Arabidopsis* Mediator subunits were cloned into pSITE3CA vector and transiently expressed as YFP tagged protein in the onion epidermal cells using biolistic method (Bio-Rad, USA). The onion epidermal cells were analyzed under TCS-SP2 Confocal Laser Scanning Microscope (Leica, Germany) for YFP fluorescence after 16 h of incubation in dark at 22°C.

Protein–protein interaction using bimolecular fluorescence complementation

Coding sequences of *Arabidopsis* Mediator subunits were cloned in Bimolecular Fluorescence Complementation (BiFC) vectors pSAT4-DEST-N (1–174) EYFP-C1 and pSAT5A-DEST-C (175-end) EYFP-C. These recombinant plasmids were bombarded onto onion epidermal cells using biolistic method (Bio-Rad, USA) in pairwise combination. The onion cells were analyzed under TCS-SP2 Confocal Laser Scanning Microscope (Leica, Germany) for YFP fluorescence after 16 h of incubation in dark at 22°C.

Structure Prediction, homology modeling and molecular dynamic simulations

Protein sequences of *Arabidopsis*, yeast and human Mediator subunits were obtained from UniProt and NCBI protein databases (https://www.ncbi.nlm.nih.gov/protein). Med domains were predicted in *Arabidopsis* and yeast Mediator subunits by MOTIF Search (https://www.genome.jp/tools/motif/) and CD-Search (43). ClustalW was used to align protein sequences and then percentage similarity was calculated (44). Homology models of *Arabidopsis* Mediator subunits were generated by SWISS-MODEL and Phyre2 server using crystal structure of yeast Mediator subunits as templates (Supplementary Table S2) (45,46). Structure quality of homology models was checked using PROCHECK and ProSA-web (Supplementary File 1) (47,48). Model structures with the best Z-score and maximum sequence coverage were selected (Supplementary Table S2). All *Arabidopsis* Mediator subunits were arranged by aligning with the PDB structure of yeast Mediator complex except AtMed9 and C-terminal of AtMed10. PyMol was used to visualize, align and arrange modelled complex (https://www.pymol.org). Molecular dynamic simulation was performed on atomic coordinates of modelled structure of *Arabidopsis* Mediator complex as well as crystal structure of yeast Mediator complex (PDB ID: 5N9J) using AMBER16 package with ff14SB force field in vacuo (49,50). Energy minimization of Mediator complexes were performed for 2000 steps using steepest decent algorithm for first 500 steps followed by conjugate gradient for remaining steps with distance-dependent dielectric constant of 4r and a non-bonded cut-off 12 Å. Molecular dynamic simulations were performed for 100 ns at 300 K temperature. Langevin thermostat was

used to maintain temperature with collision frequency of 5 ps^{-1} (51). Hydrogen bonds were constraint using SHAKE algorithm (52). Trajectories were written at 2 ps time interval. Backbone RMSD of Mediator complexes were calculated using cpptraj module. sander module of AMBER16 package was used to performed energy minimization and molecular dynamic simulation.

RESULTS

Trans-activation property of *Arabidopsis* Mediator subunits

Earlier studies by us and others have characterized Mediator subunits in *Arabidopsis thaliana* on the basis of sequence homology (3,6,7,53). Several of these subunits were earlier identified in the Mediator complex purified from *Arabidopsis* (4) and are mentioned in Supplementary Table S3. In order to identify interactions between *Arabidopsis* Mediator subunits (AtMed), each subunit was cloned in yeast two-hybrid (Y2H) vectors pGBKT7 and pGADT7 to express it as fusion protein with Gal4DBD (DNA binding domain of Gal4) and Gal4AD (activation domain of Gal4), respectively. Ability of *Arabidopsis* Mediator subunits to activate the AH109 reporter genes (*HIS3* and *ADE2*) was assessed by the growth of yeast cells harbouring the pGBKT7 construct on SD-Trp/-His/-Ade dropout medium (Supplementary Figure S1). None of the head module subunits showed transactivation property (Supplementary Figure S1). Among other module subunits, AtMed9, AtMed21 and AtMed25 full-length proteins were found to possess transactivation property (Supplementary Figure S1). Strength of transactivation ability was measured by scoring β-galactosidase activity using ONPG as a substrate (Figure 1A). Some of the subunits did not show transactivation ability when used as full-length protein, but their fragments were able to activate *LacZ* reporter gene in our assay. In AtMed4, region spanning 79–226 amino acid (AA) residues displayed transactivation property (Figure 1B and Supplementary Figure S2). Amino terminus region of 1–130 AA of AtMed9 was found to possess transactivation ability (Figure 1B and Supplementary Figure S2). In the case of AtMed10, activation domain was found to be present towards amino terminus within 1–102 AA (Figure 1B and Supplementary Figure S2). In AtMed21, the activation domain was found to be present within the stretch of 28–63 AA (Figure 1B and Supplementary Figure S2). Many of the tail module Mediator subunits of fungi and metazoans have been found to possess transactivation property (54,55). In yeast, ScMed2, ScMed3 and ScMed15 have been found to activate the reporter genes (55). However, in this study, we did not find any transactivation ability in AtMed2 and AtMed3. Even the fragments of AtMed2 and AtMed3 were not able to activate the reporter genes. In all, five Mediator subunits (Med4, Med9, Med10, Med21 and Med25) of *Arabidopsis* seem to possess transactivation ability (Figure 1A). There is a possibility that these subunits are targeted by different transcription factors for initiating the transcription of their target genes. AtMed4 and AtMed25 have been shown to interact with several transcription factors (7,56–58).

Sub-cellular localization of *Arabidopsis* Mediator subunits

In addition to transcription regulation, Mediator complex and its subunits have also been implicated in many other cellular processes. Usually function of a protein is decided by its subcellular localization, which determines its microenvironment and influences the function by controlling its access and availability to its interacting partners (59,60). In order to know the localization sites, each *Arabidopsis* Mediator subunit was transiently expressed as YFP-fusion protein in the onion epidermal cells. Surprisingly, most of the *Arabidopsis* Mediator subunits were found to be localized both inside and outside the nucleus, though the signal inside the nucleus was found to be more (Figure 2). All the head module subunits were localized both inside and outside the nucleus (Figure 2). Subcellular localization of AtMed6 and AtMed22 could not be studied as their fluorescence could not be observed. AtMed4 and AtMed10 subunits of middle module were localized specifically to the nucleus (Figure 2). Some of the *Arabidopsis* Mediator subunits are very disordered and unstructured (7). So, there is a possibility that these subunits are not expressed properly during transient expression in the onion epidermal cells. Nevertheless, overall pattern of subcellular localization suggested that some of the *Arabidopsis* Mediator subunits might also be involved in non-nuclear functions or they might be playing role in establishing link between cytoplasm and nucleus in some processes. Indeed, in fission yeast and human cell lines, role of some of the Mediator subunits outside the nucleus and other than the part of Mediator complex has been discovered (61,62). In *Arabidopsis*, Med4 and Med15 have been found to interact with several non-nuclear proteins (7,16).

Intra-modular interactions of *Arabidopsis* Mediator subunits

In order to understand the arrangement of Mediator subunits in the head, middle and tail modules of *Arabidopsis* Mediator complex, pair-wise interaction of subunits was studied by yeast two-hybrid (Y2H) analysis. *Arabidopsis* Mediator subunits cloned in pGBKT7 and pGADT7 were introduced into AH109 yeast cells in pairwise combinations. Strong interactions were scored by the growth of yeast cells on QDO (SD-Trp/-Leu/-His/-Ade) medium whereas weak interactions were scored on less stringent TDO (SD-Trp/-Leu/-Ade) medium. Using this approach, in the head module, we identified strong interactions of AtMed6-AtMed11, AtMed11-AtMed22 and AtMed18-AtMed20 (Figure 3A). BiFC analysis validated the AtMed11-AtMed22 and AtMed18-AtMed20 interactions (Figure 3B). In the head module of yeast and human Mediator complex, Med17 functions as an important subunit by interacting with several other Mediator subunits (8,10,11,21). Surprisingly, we did not get interaction of full-length AtMed17 with any head module subunit of *Arabidopsis* Mediator complex (Figure 3A). In the structure of yeast head module, four different helices of Med17 were shown to interact with other subunits (28). We aligned the modelled structure of AtMed17 with ScMed17 and highlighted the regions corresponding to these helices (Supplementary Figure S3). These four regions of AtMed17 (75–105 AA, 118–178 AA, 273–308 AA and 336–370 AA)

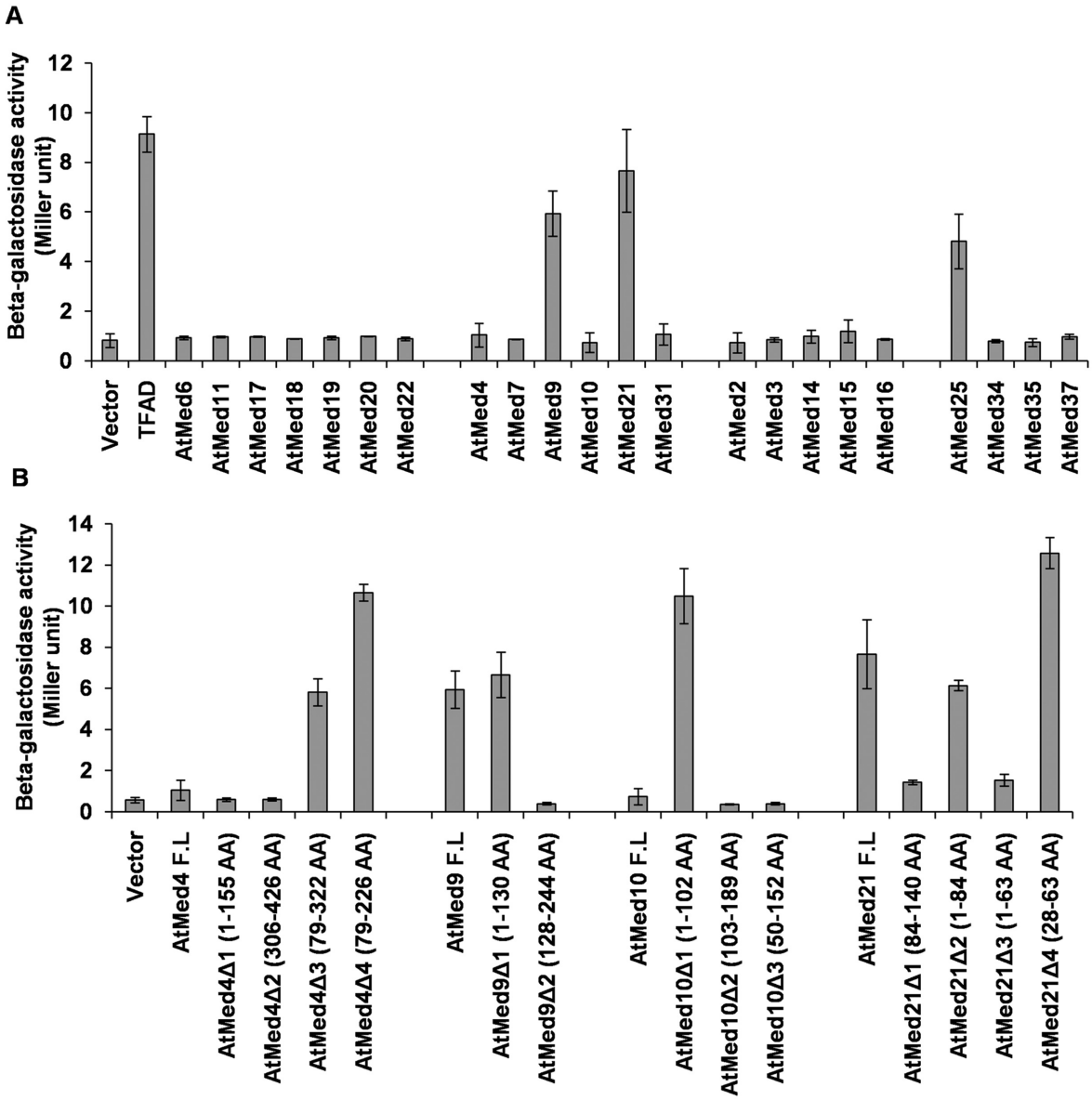

Figure 1. Trans-activation ability of *Arabidopsis* Mediator subunit proteins in yeast. Yeast cells expressing Mediator subunit fused in-frame to Gal4 DNA binding domain were checked for their ability to activate the LacZ reporter gene. The β-galactosidase activity was quantified using ONPG assay. (**A**) β-galactosidase activity in miller unit was quantified for full-length *Arabidopsis* Mediator subunits. TFAD (Transcription Factor Activation Domain) was taken as the positive control and pGBKT7 vector alone was used as the negative control. (**B**) Transcriptional ability of fragments of selected *Arabidopsis* Mediator subunits in terms of β-galactosidase activity. F.L. is full-length protein.

were checked for their interaction with other head module subunits of *Arabidopsis* Mediator complex. AtMed17 (75–105 AA) interacted with AtMed6, AtMed11, AtMed18 and AtMed22 (Figure 3C). AtMed17 (118–178 AA) interacted with AtMed18 whereas AtMed17 (273–308 AA) region interacted with AtMed6 (Figure 3C). AtMed17 (337–370 AA) was found to be engaged in interactions with AtMed6, AtMed11, AtMed18 and AtMed22 (Figure 3C). It should be noted that Mediator complex is a very dynamic and flexible structure (7,63). Indeed, Mediator complex has been reported to undergo conformational changes to regulate transcriptional process (15). So, all the interactions observed in this study for *Arabidopsis* Mediator subunits may not always occur but depend on their confirmation in a particular state. This could be the reason that interactions of Med17-Med6, Med17-Med11, Med17-Med18 and Med17-Med22 could not be detected with full-length AtMed17, indicating a possibility of regulatory mechanism involving masking of certain regions in AtMed17. This is similar to what was observed for certain Mediator subunits of yeast (8). In one other study, one full length large subunit of Pol III yielded no two-hybrid interactions with any other proteins (64). There is also a possibility that full length AtMed17 has these four interacting regions buried and not available to interact, especially in yeast, which might not have signals to open it. Half checker board in Figure 4A

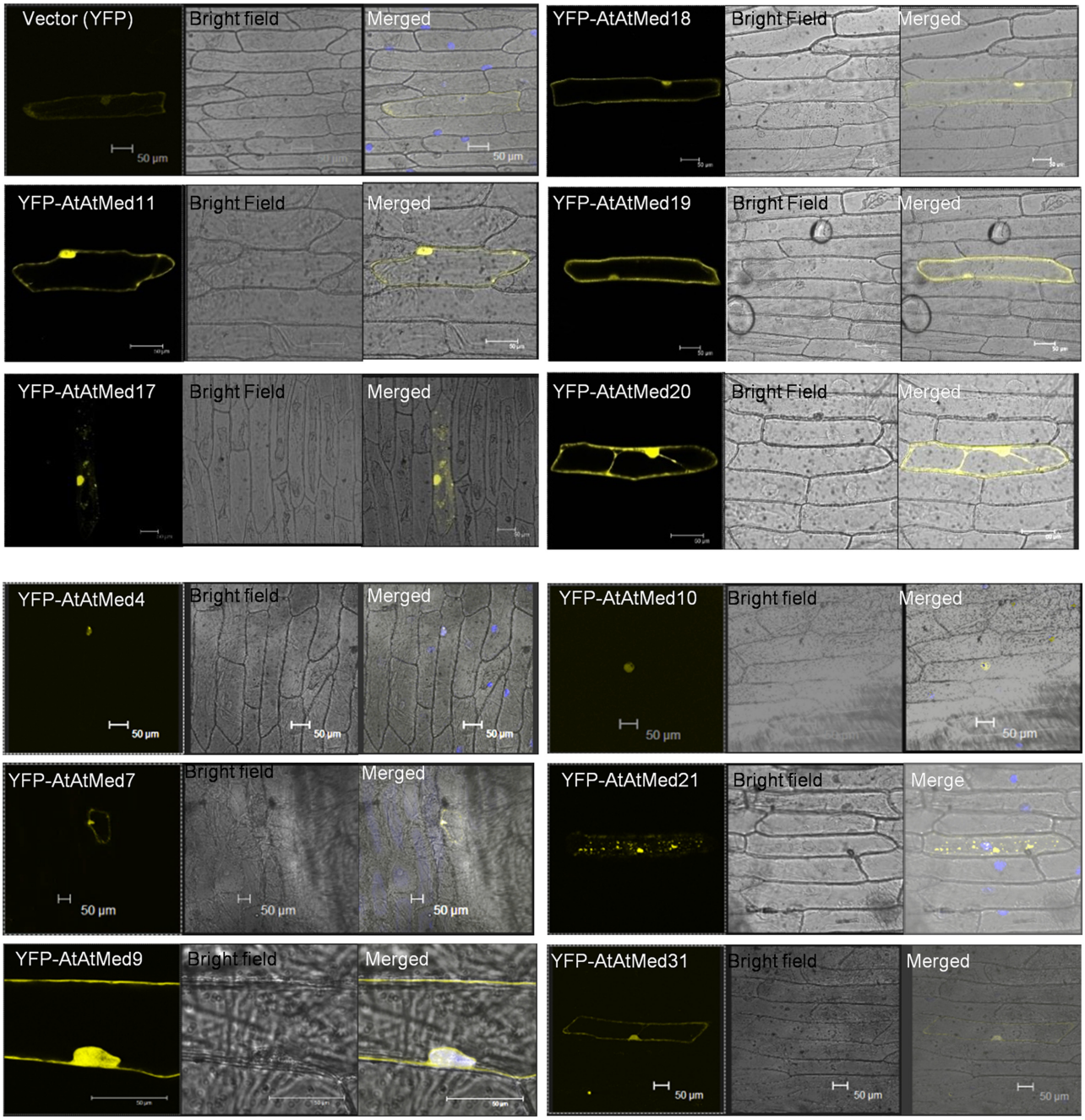

Figure 2. Localization of *Arabidopsis* Mediator subunits in onion epidermal cells. CDS of *Arabidopsis* Mediator subunits were cloned in-frame with YFP in pSITE-3CA vector plasmid. Recombinant plasmid was coated on gold particle and then bombarded onto onion epidermal cells. Expression of YFP of vector plasmid serves as a control. First panel shows the expression of individual *Arabidopsis* Mediator subunit fused in-frame to YFP. YFP signals are merged with bright field in the last panel. YFP was excited at 514 nm and emission was recorded at 530 nm.

mentions all the interactions and also highlights the regions of the subunits involved in those interactions. Mapping analysis revealed importance of long N-terminal region of AtMed11 in its interaction with AtMed22 (Figure 4A and Supplementary Figure S4). Similarly, N-terminal region of AtMed18 was found to be involved in its interaction with AtMed20 (Figure 4A and Supplementary Figure S4). Deletion of any small part of AtMed20 or AtMed22 protein abrogated its interaction with AtMed18 or AtMed11, respectively (Supplementary Figure S4). Interactions of Med11-Med22 and Med18-Med20 are also known in yeast (8,33). In this study, we found a novel interaction of AtMed6-AtMed11, which is not yet known in fungi and animals (Figure 3A). The ScMed17 has been reported to interact with ScMed6, ScMed11 and ScMed22. We found all these interactions in *Arabidopsis*. In addition, AtMed17-AtMed18 interaction was observed which is not yet found in yeast.

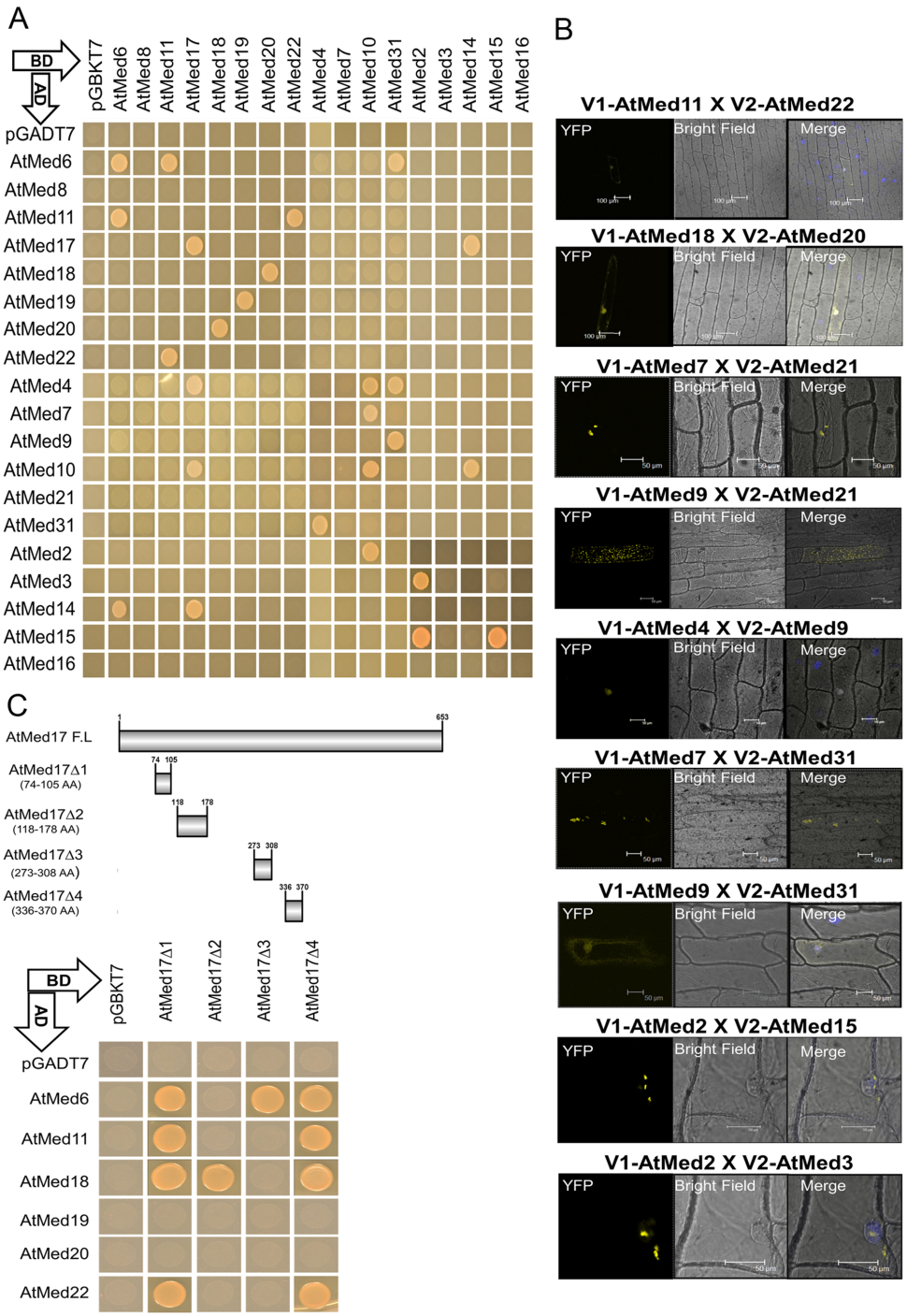

Figure 3. Pair-wise interaction between *Arabidopsis* Mediator subunits. (**A**) Interaction between subunits was studied by yeast two-hybrid. CDS of *Arabidopsis* Mediator subunits were cloned in-frame with Gal4BD (pGBKT7) and Gal4AD (pGADT7). Pair-wise combination of Gal4BD and Gal4AD plasmids were introduced into AH109 yeast cells. Strong interactions between the subunits were scored by growth of yeast colonies on SD-Trp/-Leu/-His/-Ade (QDO) stringent media. Weak interactions observed on less stringent SD-Trp/-Leu/-Ade (TDO) plates are shown in Supplementary Figure S5. (**B**) BiFC analysis to detect pair-wise interactions among *Arabidopsis* Mediator subunits. *Arabidopsis* Mediator subunits were cloned into BiFC vectors (V1- pSAT4-DEST-n(1–174)EYFP-C1 and V2- pSAT4-DEST-c(175-end)EYFP-C1) and bombarded onto onion epidermal cells in pair-wise combination as mentioned at the top of each panel. YFP signal shows the interaction of the subunits and the site of interaction within the epidermal cell of onion. YFP signals are merged with bright field in the last panel. YFP was excited at 514 nm and emission was recorded at 530 nm. (**C**) Yeast two-hybrid analysis of head module subunits with the fragments of AtMed17. Interaction were scored by growth of yeast colonies on QDO. For all yeast two-hybrid assays, photographs were taken after 4 days of growth on the selection media.

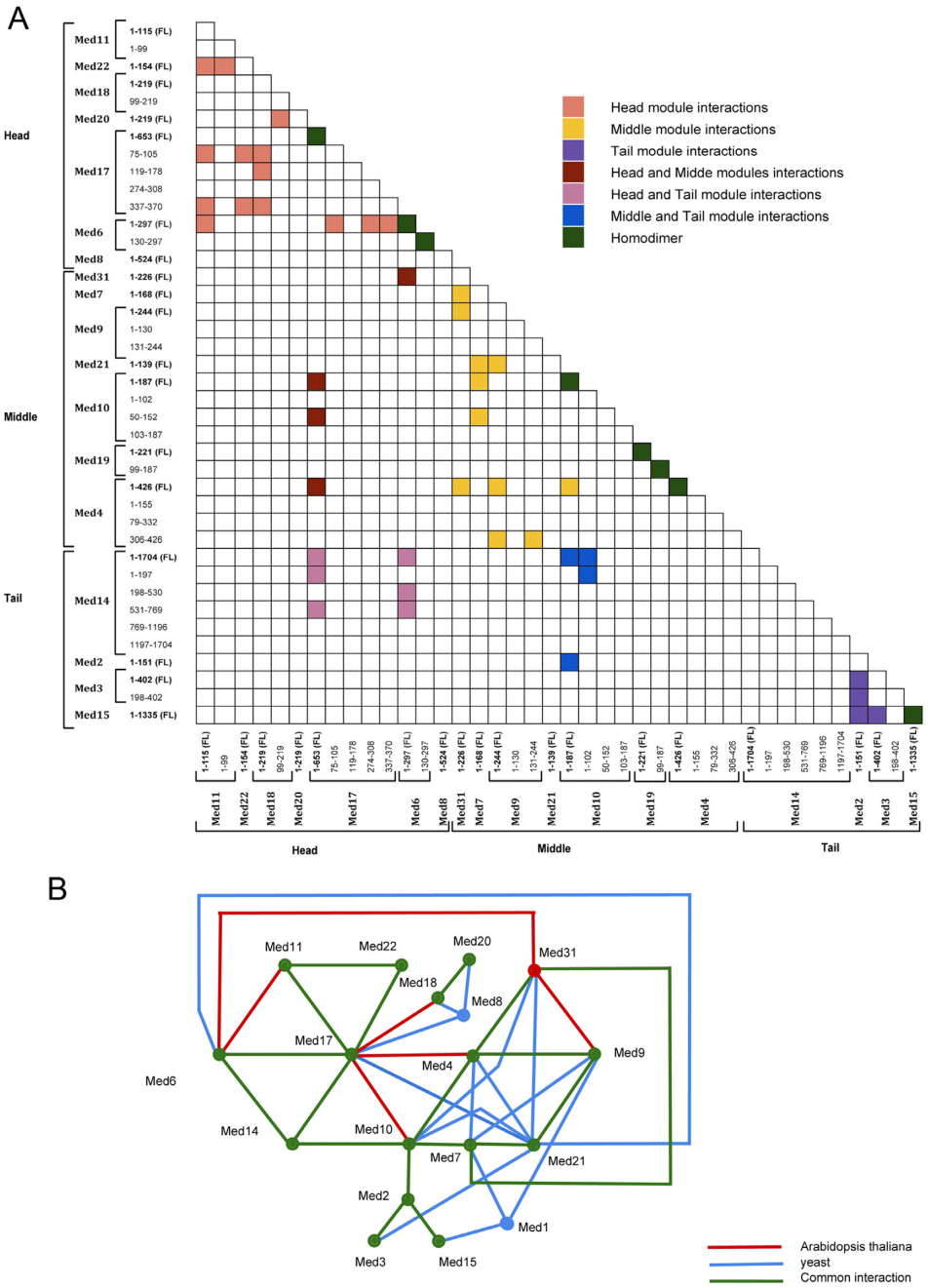

Figure 4. Interaction of subunits of *Arabidopsis* Mediator complex. (**A**) Half checker board summarizing the interactions of *Arabidopsis* Mediator subunits and their fragments. Intra- and inter-module interactions are indicated in different color as mentioned on the right side. (**B**) Connection map showing interactions of yeast and *Arabidopsis* Mediator subunits.

In middle module subunits, AtMed9 and AtMed21 fused to Gal4 DBD were omitted from the analysis because of their ability to activate the reporter genes on their own (Figure 1). The subunits of middle module of *Arabidopsis* Mediator complex were found to make extensive interactions among themselves. Combining the results of pair-wise Y2H and BiFC analyses, total of eight interactions were identified in between different subunits of middle module of *Arabidopsis* Mediator complex (Figure 3A and B). Strong interactions of AtMed4-AtMed10, AtMed4-AtMed31, AtMed7-AtMed10 and AtMed9-AtMed31 were identified in pair-wise Y2H analysis (Figure 3A). Weak interaction between AtMed4 and AtMed9 was observed on SD-Trp/-Leu/-Ade (TDO) (Supplementary Figure S5). Interactions of AtMed7-AtMed21, AtMed7-AtMed31 and AtMed9-AtMed21 were identified in pair-wise BiFC analysis (Figure 3B). Interactions of AtMed4-AtMed9 and AtMed9-AtMed31 were observed in both Y2H and BiFC analyses (Figure 3A and B). Interactions of AtMed7-AtMed21 and AtMed7-AtMed31 have been already re-

ported (7). Amino terminal region (1–155 AA) of AtMed4 was found to be involved in its interaction with AtMed31, whereas the C-terminal (306–426 AA) region interacted with AtMed9 and AtMed10 (Figure 4A and Supplementary Figure S6A). Middle region (50–152 AA) of AtMed10 interacted with AtMed4 (Figure 4A and Supplementary Figure S6B). In the interaction of AtMed7-AtMed10, both N and C termini were found to be involved (Supplementary Figure S6B). Most of the interactions identified within middle module of *Arabidopsis* Mediator subunits have been already discovered in fungi and animal systems, suggesting the conserved nature of arrangement of middle module Mediator subunits in different eukaryotic organisms (8,9,65). However, a novel interaction of AtMed9-AtMed31 which has not yet been reported in fungi or animals was observed both in Y2H and BiFC analyses in *Arabidopsis* (Figure 3A and B). AtMed9-AtMed31 required full-length proteins as deletions in any of them abolished their respective interactions.

In the case of tail module, interactions of AtMed2-AtMed3 and AtMed2-AtMed15 were identified both in Y2H and BiFC analyses, suggesting the existence of AtMed3-AtMed2- AtMed15 triad in *Arabidopsis* (Figure 3A and B). Triad of Med3-Med2-Med15 was earlier reported in yeast, suggesting that the triad is conserved across the plant and fungi kingdoms (35). Mapping analysis revealed involvement of C-terminal half (198–402 AA) of AtMed3 in its interaction with AtMed2 (Figure 4A and Supplementary Figure S6C). There are few subunits (AtMed25, AtMed35, AtMed36 and AtMed37) which are not yet assigned to any of the four modules of the Mediator complex. We did not observe any interaction within these unassigned module subunits (Supplementary Figure S7).

Homodimerization of *Arabidopsis* Mediator subunits

Homodimerization of Mediator subunits in yeast and human has not been reported. Recently, homodimerization and oligomerization of Mediator subunits Med10, Med28 and Med2 were reported in *Arabidopsis*, which was found to be dependent on the redox state of the plant cell (66). In our study we found that the AtMed6 and AtMed17 of head module, AtMed10 and AtMed19 of middle module and AtMed15 of tail module formed homodimers (Figure 3). AtMed4 was also found to form homodimer, which could be detected only on the less stringent medium of SD-Trp/-Leu/-Ade (TDO) (Supplementary Figure S5). In one of the earlier studies, it was found that the steady state transcript level of *Arabidopsis* Mediator genes did not change much in response to different stresses (53). So, we think that homodimerization could be a step to check and regulate the Mediator function in plant cell as the redox state of cell changes during stress conditions.

Inter-modular interactions in *Arabidopsis* Mediator complex

In order to characterize the subunits engaged in establishing links between different core modules of *Arabidopsis* Mediator complex, pair-wise interaction between subunits of one module with subunits of other module was checked. AtMed6 of head was found to interact with AtMed31 of middle module (Figure 3A). Interestingly, AtMed6 was also found to interact with AtMed14 of tail module (Figure 3A). AtMed17 of head module was found to interact with AtMed4 and AtMed10 of middle module and AtMed14 of tail module (Figure 3A). The C- terminal region of AtMed10 (103–187 AA) and N-terminal of AtMed14 were found to be involved in their interactions with AtMed17 (Figure 4A and Supplementary Figure S8A, B). Med17-Med10 interaction is not yet reported in yeast. However, in human, after crosslinking, association of Med17-Med10 was observed (10). Interaction of AtMed4-AtMed17 is novel and not been shown in any other organism. In the interaction between middle and tail modules, AtMed10 of middle module was found to interact with AtMed2 and AtMed14 subunits of tail module (Figure 3A). Mapping analysis revealed that N-terminal region (1–197 AA) of AtMed14 interacted with N-terminal region (1–102 AA) of AtMed10 (Figure 4A and Supplementary Figure S8B). Interaction of Med10-Med14 is already known in yeast (8). However, interaction of Med10 and Med2 has not been found in yeast and human, though cross-linking data suggested their proximity (65). Thus, AtMed6 and AtMed17 of head module, AtMed4, AtMed10 and AtMed31 of middle module and AtMed14 of tail module are important in joining the three core modules of *Arabidopsis* Mediator complex together.

Arrangement of subunits in *Arabidopsis* Mediator complex

Combining the data of one-to-one interactions of *Arabidopsis* Mediator subunits, an interaction map was drawn (Figure 4B). A half checkerboard detailing the regions of *Arabidopsis* Mediator subunits involved in the interactions is shown in Figure 4A. Like in yeast and human, AtMed17 seems to be the key component of head module of *Arabidopsis* Mediator complex interacting with seven other subunits spanning all the three core modules (Figure 4B). Amino side half region of AtMed17 (up to 370 AA) was involved in its interactions with head module subunits, leaving the C-terminal half to make contacts with other modules (Figures 3C and 4A). AtMed10 of middle module also seems to be very critical as it interacted with AtMed4 and AtMed7 which formed two different sub-modules, AtMed4-AtMed9-AtMed31 and AtMed7-AtMed21 (Figure 4B). It also established contacts with AtMed17 of head module and AtMed2 of tail module. So, unlike in yeast, Med10 may be one of the core subunits required to hold *Arabidopsis* Mediator complex. AtMed6 and AtMed14 are other subunits which displayed interactions with all the three modules. AtMed6 interacted with AtMed11 and AtMed17 of head, AtMed31 of middle and AtMed14 of tail (Figure 4B). AtMed14 of tail established direct contacts with AtMed6 and AtMed17 of head and AtMed10 of middle (Figure 4B). Here also, first half of AtMed14 (1–769 AA) was enough in accommodating all these interactions (Figure 4A and Supplementary Figure S8A). There are few other subunits, which seems to contribute in holding different modules together. For example, AtMed4 of middle interacts with three middle module subunits (AtMed9, AtMed10 and AtMed31) and AtMed17 of head module. Similarly, AtMed31 of middle also interacted with three other middle module subunits (AtMed4,

AtMed7 and AtMed9) and AtMed6 of head module (Figure 4B). Because of the large size and by virtue of its interaction with subunits from other two modules, AtMed14 covers the core complex from one side to other. Though we considered AtMed14 as a part of the tail module on the basis of earlier studies, our results suggest that it forms the scaffold for the core complex and so belongs to all the three modules. Subunits like AtMed6, AtMed10 and AtMed17 link all other head and middle module subunits with AtMed14. Tail module triad of AtMed3-AtMed2-AtMed15 can be connected to core head-middle part by AtMed2-AtMed10 interaction (Figure 4B).

In-silico structure modeling of *Arabidopsis* Mediator complex

Structure of head and middle modules of yeast Mediator is already known (27–29). In order to deduce the structural topology of core part of the *Arabidopsis* Mediator complex, first we looked for the similarity between the subunits of yeast and *Arabidopsis* Mediator subunits (Supplementary Table S4). In case of head module subunits, except AtMed8, all the *Arabidopsis* subunits showed presence of Med domains as characterized in yeast counterparts (Supplementary Figure S9). Individual structures of all these *Arabidopsis* Mediator subunits except AtMed8 and AtMed19 were modelled using the yeast counterparts as respective templates (Supplementary Figures S10, S11 and Supplementary Table S2). Interacting region of *Arabidopsis* Mediator subunits involved in pairwise interaction identified by mapping are highlighted in modelled structure of pairwise interaction (Supplementary Figures S12–S14). Next, according to the subunit-subunit interaction data and mapping of interacting regions, the modelled structures of *Arabidopsis* Mediator subunits were arranged (Figure 5). In order to validate the modelled structure of *Arabidopsis* Mediator complex, we generated Ramachandran plot of complete structure, which showed 86.2% residues present in the most favorable regions, 10.8% residues present in the additional allowed regions and 1.9% residues present in the generously allowed regions (Supplementary File 1). Further, to examine the stability of the *in-silico* modelled structure of *Arabidopsis* Mediator complex, MD (molecular dynamic) simulation was performed. For comparison, the solved crystal structure of yeast Mediator complex (PDB ID: 5N9J) was also subjected to similar MD simulation. Structures of both the Mediator complexes were refined by energy minimizing and simulated for 100 ns at the temperature of 300 K. To examine the conformational change in the structures of Mediator complexes, RMSD of backbone atoms from their first structure conformation was analyzed during 100 ns simulation. Less deviation in the conformation through simulation revealed stable structure of both the complexes (Supplementary Figure S15). We observed that the modelled structure of *Arabidopsis* Mediator complex reached equilibrium after ∼20 ns and remained stable for next 80 ns (Supplementary Figures S15). The yeast Mediator complex also reached equilibrium in ∼20 ns. Thus, the predicted structure of *Arabidopsis* Mediator complex was found to be as stable as yeast Mediator complex during MD simulation.

In the predicted structure, AtMed11-AtMed22 seems to form a bundle of four helices that interact with AtMed17 helix (75–105 AA) and AtMed6 forms the arm of *Arabidopsis* Mediator complex (Figures 5 and 6). AtMed18 was found to interact with three helices of AtMed17; 75–105 AA, 119–178 AA and 337–370 AA. AtMed17 helix (75–105 AA) was also found to interact with AtMed11, AtMed22 and AtMed6. In the modelled structure, AtMed18 was positioned next to the helix (337–370 AA) of AtMed17 closer to AtMed20 as AtMed18 was found to interact with both AtMed20 and 337–370 AA helix of AtMed17 (Figures 5 and 6). AtMed18-AtMed20 seems to form the movable part of Mediator complex similar to what was characterized in yeast Mediator complex (Figure 6). Other subunits make the shoulder, arm, spine and tooth of the complex as defined in the yeast structure (27).

Like yeast Mediator structure, *Arabidopsis* middle module can be divided into five sub-modules; the beam, plank, hook, knob and connector as defined in the recently solved crystal structure of yeast mediator complex (27). AtMed14 was found to interact with AtMed6, AtMed17 and AtMed10 which are important for inter- and intramodular interactions. So, AtMed14 forms the backbone of the whole Mediator complex. It extends from one side of the complex to the hook region of the other side (Figure 6). Amino side (1–197 AA) of AtMed14 was found to be interacting with the amino-terminal region of AtMed10 (1–102 AA) (Figure 6). Thus, similar to yeast structure, N-terminal four helical bundle structure of AtMed10 and AtMed14 forms part of the hook region (Figure 6). Like yeast, AtMed7-AtMed21 dimer structure forms the other part of the hook (Figure 6). Though AtMed4-AtMed31 interaction is similar to yeast Mediator complex, a new interaction of AtMed9-AtMed31 was identified in this study. Moreover, AtMed9 was also found to interact with carboxyl side of AtMed4 which was close to AtMed31. So, C-terminal helix of AtMed9 seems to be a part of another hook region and second helix extends to become the part of the connector in the *Arabidopsis* Mediator complex rather than plank as in the case of yeast Mediator complex (Figure 6 and Supplementary Figure S16). However, N-terminal bundle structure of AtMed4 seems to form the plank part of the *Arabidopsis* Mediator complex. AtMed7-AtMed21 (just like SpMed7-SpMed21) hinge structure forms the connector and is linked with the hook region (Figure 6 and Supplementary Figure S16).

DISCUSSION

Structure of Mediator complex is considered to be conserved across the eukaryotes. However, there are considerable variations among the subunit composition and their sequences (67,68). The primary amino acid sequences of Mediator subunits in different organisms show limited similarity and identity. This divergence is partially due to the presence of IDRs in many of the Mediator subunits (7). Study of subunit composition revealed presence of 19 subunits constituting Mediator of *S. pombe,* whereas *S. cerevisiae* complex consists of 21 subunits. In human, there are 26 Mediator subunits and in *Arabidopsis*, the number is even more (6,68). Interestingly, plants do not contain Med1 and yeasts

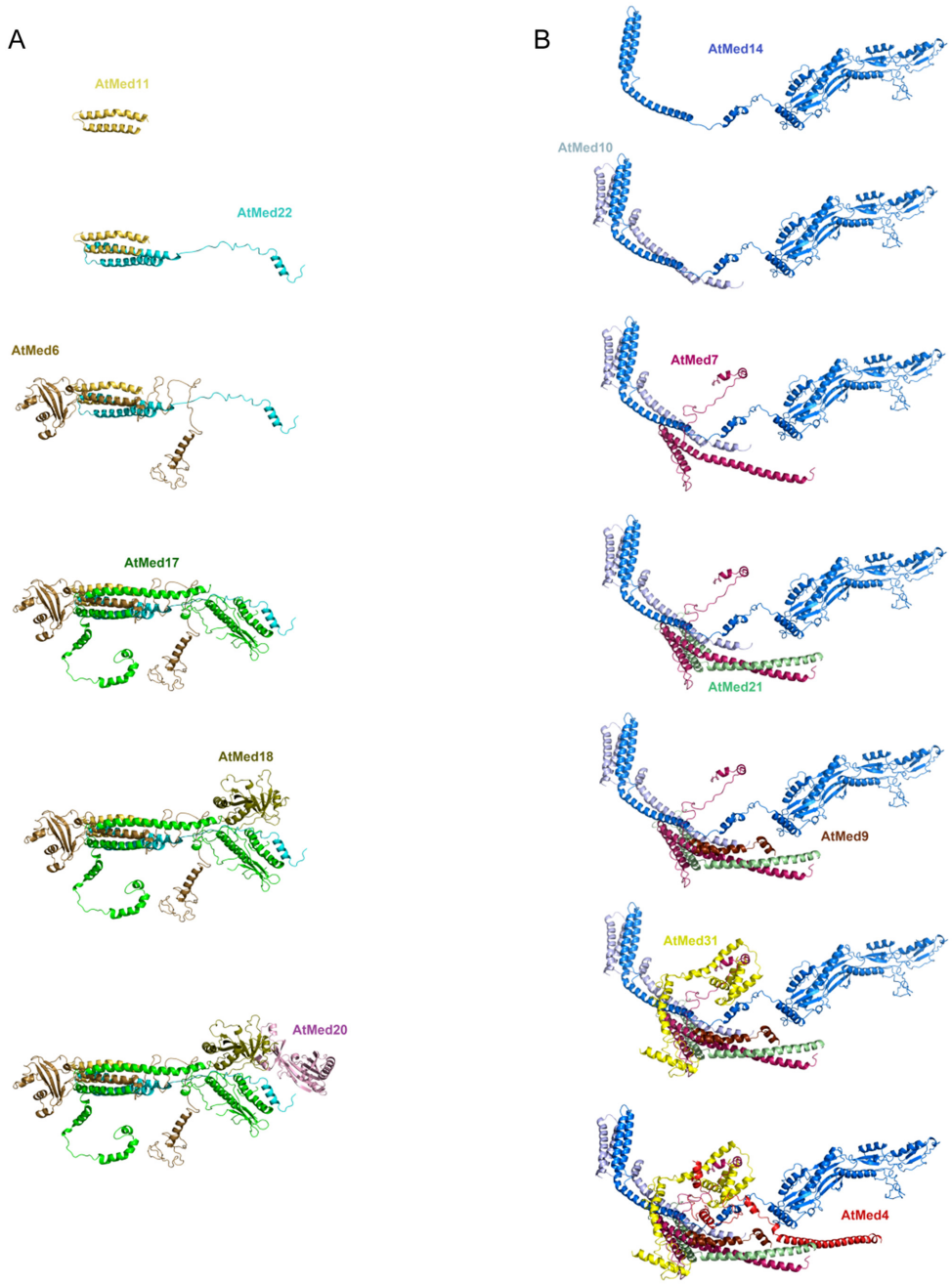

Figure 5. Placement of modelled structure of (**A**) Head and (**B**) Middle module subunits of *Arabidopsis* Mediator complex.

do not have Med23, Med25, Med26, Med28 and Med30 (6). Although it has not been studied yet, these differences might have important impact on overall structure and function of Mediator complex. We think that specialized and customized complexes might have important effect on gene expression that are not fully understood.

Pair-wise interactions between different subunits are very well explored in yeast and human (Figure 7A) (8,11,21,69). Till date, 28 and 35 Mediator subunit interactions are reported in yeast and human, respectively (Figure 7A). In this study, we have found 24 interactions among the subunits constituting Mediator complex of *Arabidopsis* (Figure 7A). There are also other reports explaining interactions of Mediator subunits in *Drosophila* and *C. elegans* (8). In all, twelve interactions observed in this study for *Arabidopsis* were found to be already characterized in yeast and human suggesting very conserved nature of Mediator complex in three eukaryotic kingdoms (Figure 7B). However, though there was significant overlap in subunit interactions, five interactions observed in *Arabidopsis* have not been reported yet in any other organism (Figure 7B). On the other hand, eight interactions reported in yeast could not be detected in *Arabidopsis* (Figure 7B). On the basis of immunoprecipitation experiments, eighteen unique interactions postulated in

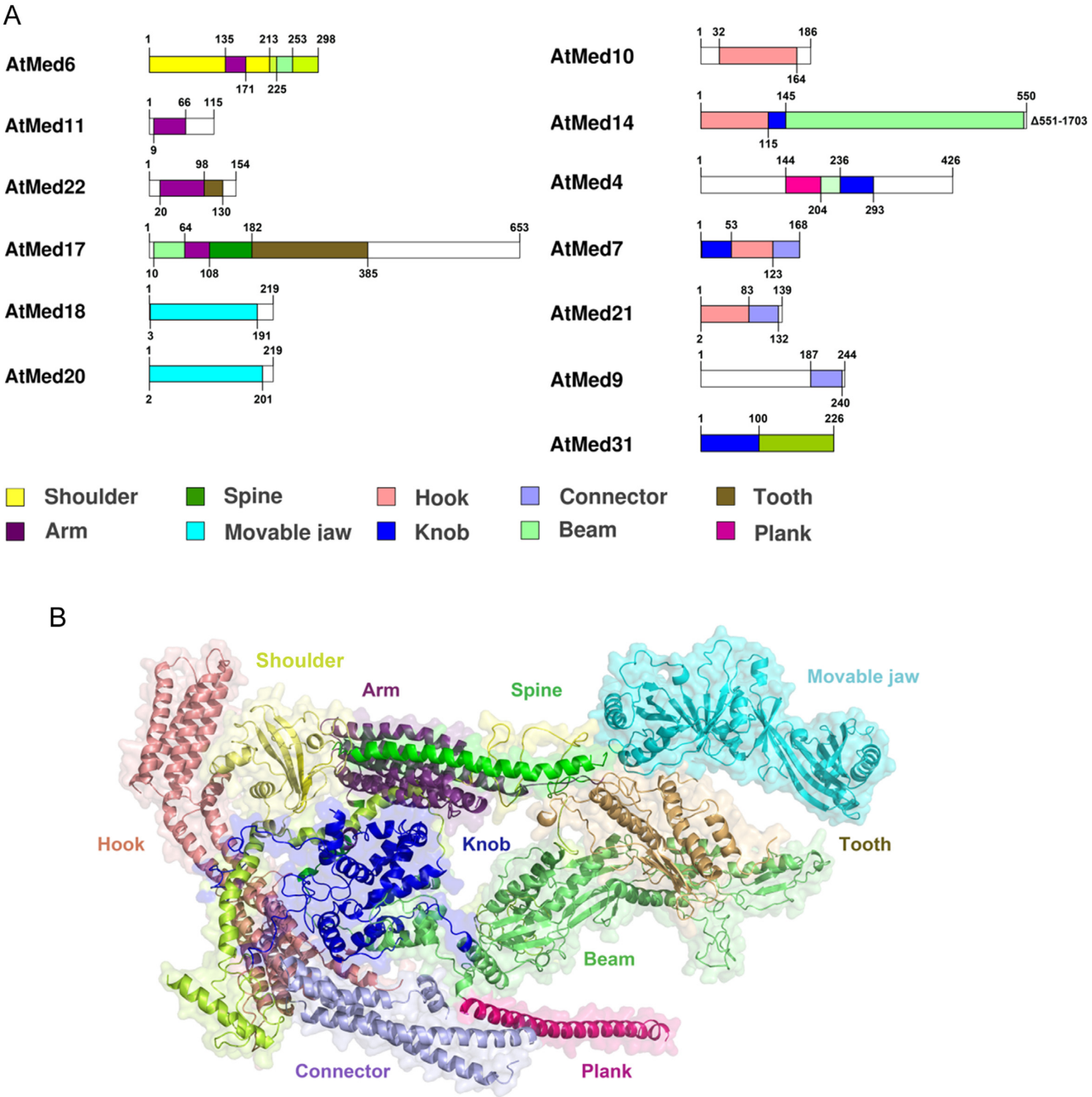

Figure 6. Modeled architecture of core *Arabidopsis* Mediator complex. (**A**) Arrangement of domains in different subunits making different parts of the Mediator complex (as mentioned in the color legend at the bottom). (**B**) Deduced structure of the core part of the *Arabidopsis* Mediator complex. All the movable parts corresponding to yeast structure are highlighted (27).

human could not be observed in this study (Figure 7B) (21). Four interactions of Med4-Med31, Med7-Med10, Med2-Med3 and Med2-Med15 which we found in *Arabidopsis* have been previously reported in yeast (Figure 7C). These interactions, however have not been detected in animals including human, *Drosophila* and *C. elegans* (Figure 7C). Similarly, two interactions (Med6-Med11 and Med17-Med18) were found to be common between *Arabidopsis* and human but not in yeast (Figure 7C). In head module, interactions of Med6-Med17, Med11-Med17, Med11-Med22, Med17-Med22 and Med18-Med20 are conserved in yeast, human and *Arabidopsis* (Figure 7C).

Engagement of Med17 with Med6, Med11 and Med22 suggests that importance of Med17 in head module is conserved in all the eukaryotic kingdoms. In yeast, head module consists of two main sub-modules; Med11-Med22-Med17 and Med8-Med18-Med20 (11). Interaction between Med8 and Med18 is exclusive to yeast and is reported in both *S. cervisiae* and *S. pombe* (8,70). Med17 connects the two sub-modules by interacting with Med8 of another sub-module. Interaction of Med8 and Med17 is also known

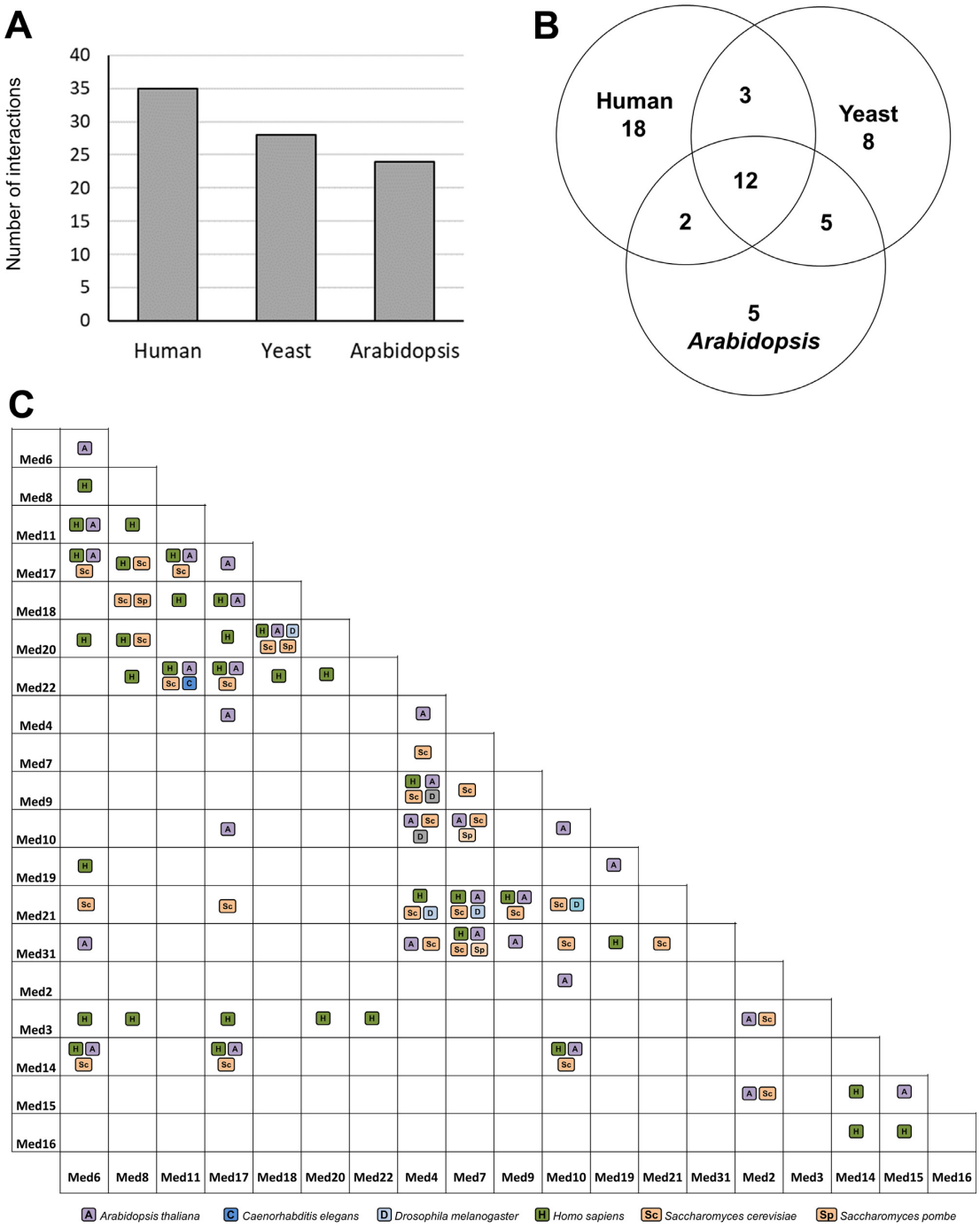

Figure 7. Comparison of Mediator subunit interactions in human, yeast and *Arabidopsis*. (**A**) Number of Mediator subunit interactions known in human and yeast. *Arabidopsis* bar shows number of Mediator subunit interactions discovered in this study. (**B**) Venn diagram showing the number of unique and conserved interactions among human, yeast and *Arabidopsis*. (**C**) Half checker board showing all the interactions discovered in this study and published earlier and discussed in the text.

in human (21). However, in *Arabidopsis*, we did not find any interaction engaging AtMed8 with any other subunits (Figures 3A and 4A). Instead, submodule of AtMed17-AtMed18 was found to connect with AtMed11-AtMed22-AtMed17 sub-module with AtMed18-AtMed20 interaction (Figure 7C). Interaction of Med6-Med11 found in *Arabidopsis* is already known in human, but not in yeast (Figure 7C). In yeast, the core middle module is formed by the interaction between four subunits Med7-Med21-Med4-Med9 and other middle module subunits are arranged over this tetramer structure (9,21). Most of these interactions such as Med4-Med9, Med7-Med21, Med7-Med31 and Med9-Med21 were found to be conserved in yeast, human and *Arabidopsis* (Figure 7C). Interactions of Med4-Med9 and Med7-Med21 are also reported in *Drosophila* (Figure 7C). Med7 and Med9 are two subunits that make contacts with

most of the other middle module subunits (9,21). In yeast, Med7 makes extensive contacts with all other middle module subunits (8,9). This makes Med7 an architecturally important subunit in yeast. Interactions of Med7 with Med4 and Med9 seem to be specific to yeast as they are not yet discovered in human and *Arabidopsis*. However, in *Arabidopsis*, Med7 was found to interact with only three other middle module subunits; AtMed10, AtMed21 and AtMed31 (Figure 7C). In *Arabidopsis*, we discovered a novel interaction of Med9-Med31that has not been discovered yet in any organism (Figure 7C). In *Arabidopsis*, AtMed10 turned out to be important as a connecting link between AtMed4-AtMed9 and AtMed21-AtMed7-AtMed31 submodules (Figure 7C). Interaction of Med10 with Med4 seems to be conserved as it is detected in yeast, *Drosophila* and *Arabidopsis*. On the other hand, interaction of Med10 with Med7 is conserved only in *Arabidopsis* and yeast. This interaction is not yet found in animal. Med21 is another subunit that interacts with all the other middle module subunits in yeast. Interaction of Med21 with Med4 could be detected in *Arabidopsis*, human and *Drosophila* (Figure 7C). Interaction of Med21 with Med4 and Med10, which seems to be conserved in yeast and *Drosophila* could not be detected in *Arabidopsis.* In middle module, it is Med31 that seems to establish specific interaction in different organisms. For instance, interactions of Med31-Med10 and Med31-Med21 have been reported only in *S. cerevisiae*. On the other hand, interaction of AtMed31-AtMed9 discovered in this study has not been found till date in yeast and animals. Similarly, interaction of Med31 with Med6 of head module has been detected only in *Arabidopsis* and Med31-Med19 has been reported only in human. Thus, the specific role of Med31 should be explored in detail to understand if this particular subunit has acquired specialized functions in different organisms. In yeast and human, Med6, a head module subunit, interacts with Med21 and Med19 subunits of the middle module (21,71). Med6 together with Med21-Med7 complex provides important structural flexibility to Mediator complex (71). This structural flexibility was found to be critical for Mediator and RNA pol II interaction. In our interaction studies, we did not find any interaction between AtMed6 and AtMed21. We hypothesize that the novel interaction of AtMed31-AtMed6 in *Arabidopsis* is important in maintaining the flexibility of head and middle interface.

The triad of Med3-Med2-Med15 was found to be conserved in yeast and *Arabidopsis* (Figure 7C). This triad structure has not been detected in human. Instead a separate triad of Med14-Med15-Med16 seems to exist in human (Figure 7C). In yeast, Med3-Med2-Med15 triad structure interacts with head module through Med14 (11). Going by the analogy and conservation pattern, we speculate that the triad of AtMed3-AtMed2-AtMed15 might be playing important role in *Arabidopsis* or other plant species as well. The tail module subunits are known to interact with enhancer-bound TFs. Since a set of TFs are unique to different kingdoms (for example Nuclear Receptors in animals, Zinc-cluster TFs in fungi and NAC and WRKY TFs in plants), the tail module might be conferring more specialized activities to cater organism-specific transcriptional responses (72–74). Interestingly, in human, Med27/Med3 has been shown to interact extensively with different head module subunits (21). We found AtMed2 interacting with AtMed10, and this interaction between tail and middle module subunits in *Arabidopsis* is novel (Figure 7C). Further, AtMed10 of middle was found to interact with AtMed14 of tail module, suggesting that these two subunits act as the connecting link between the two modules. Earlier, in yeast and human, evidences from yeast two-hybrid and crosslinking of Mediator subunits had revealed interaction of Med14 with both Med17 and Med10 (8,10,11). In this study, mapping of the interacting regions revealed involvement of N-terminal part of AtMed14 in its interaction with AtMed10, AtMed6 and AtMed17 (Figure 4A and Supplementary Figure S8). Thus, the N-terminus AtMed14 seems to hold the *Arabidopsis* Mediator complex together by interacting with middle and head modules. This leaves C-terminus of AtMed14 available to interact with tail module subunits. This suggests that like in yeast and human, N-terminus region of AtMed14 provides the backbone to hold the overall structure of *Arabidopsis* Mediator complex and C-terminus establishes link with the tail module. Indeed, knock-out of *Med14* results into lethality and reduction in its expression causes several growth and developmental defects in *Arabidopsis* (75).

In *Arabidopsis*, we have discovered inter-modular interactions of AtMed6-AtMed14, AtMed6-AtMed31, AtMed17-AtMed4, and AtMed17-AtMed14 (Figure 7C). Though these interactions are not yet reported in yeast, the subunits were found to be in close proximity in the EM and crystal structures (21,27). Also, after cross-linking of yeast Mediator complex, these interactions could be established (10,11). So, we suggest that these interactions of Med6-Med14, Med6-Med31, Med17-Med4 and Med17-Med14 that we observed in *Arabidopsis* are conserved in yeast as well. Interaction of AtMed10-AtMed17 seems to be important for linking two modules in *Arabidopsis* Mediator complex. This interaction seems to be absent in yeast as in the crystal structure, SpMed10 is distantly away from SpMed17 (27). Even in cross-linking experiment, this interaction was not reported in yeast (11). However, in human, interaction of HsMed10-HsMed17 was reported by cross linking analysis (10). In the predicted structure of *Arabidopsis* Mediator complex, amino terminal region of AtMed10 is involved in its interaction with AtMed14 (similar to SpMed10-SpMed14 interaction) whereas its carboxyl side is extended inside the complex along with AtMed7 for further interaction with AtMed17 (Supplementary Figure S17). AtMed17 seems to act as a hub for all the head module subunit interactions (Figure 4B). In addition, AtMed17 was also found to interact with AtMed10 and AtMed4 of middle module, which are novel interactions in plant and thus are important for inter module interactions. In the mapping analysis, the N-terminus of AtMed17 was found to interact with head, middle and tail modules (Figure 4A). In accordance to our finding of important position of AtMed17 in *Arabidopsis* Mediator complex, suppression of *AtMed17* affected expression of almost all the genes that are transcribed by RNA pol II transcriptional machinery (76). As mentioned earlier, in yeast, middle module subunits have been found to interact extensively with each other and the core of middle module seems to consist of Med7-

Med21-Med4-Med9 tetramer (9,77). It should be noted that AtMed9 consists of 244 AA as compared to 121 AA long yeast SpMed9 (7). In yeast, crystal structure revealed that SpMed9-SpMed4 forms a bundle of four helices to form plank structure of middle module (27). From this bundle of helices, SpMed4 was found to extend towards SpMed31-SpMed7 complex to form knob of middle module (27). As AtMed9 of *Arabidopsis* is much longer, there is a possibility that it is further extended from AtMed9-AtMed4 to reach and interact with AtMed31 of knob structure of *Arabidopsis* middle module (Figure 6). Thus, the plant specific interactions discovered in this study seem to have relevance in the structure of the plant Mediator complex.

CONCLUSION

In this study, we have characterized a detailed protein-protein interaction network of subunits of *Arabidopsis* Mediator complex. Though this interaction map is comparable to that of yeasts and metazoans, several novel interactions linking the three modules of the core *Arabidopsis* Mediator complex could be discovered. Expounded topological structure of *Arabidopsis* Mediator complex proposed in this study revealed that these novel interactions might have effect on the overall structure, making it little different from yeast and mammalian structures. As compared to animals, plants have much higher expansion rate of transcription factors (78). In *Arabidopsis,* around 10% of the genes code for the transcription factors, which is comparatively higher than that in yeast (3.5%) and human (6%) (72–74,78,79). Moreover, 45% of the transcription factors encoded by plant genomes are specific to plants (79). These transcription factors rely on Mediator complex for their transcriptional activities. In addition to the conserved interactions found in other eukaryote kingdoms, we also found many novel interactions which are not yet reported in fungi and metazoans. There is a possibility that these interactions could not be detected in other systems. On the other hand, it is equally possible that some of these interactions are plant specific. So, it will be interesting to explore the possibility of plant specific characteristic features of Mediator structure. We think that the comprehensive analysis of interaction map of subunits of *Arabidopsis* Mediator complex presented in this study is a valuable resource for understanding Mediator functioning in plants. At the same time, this study also raises the possibility that differences in the interaction map of Mediator subunits may lead to specialized function or adaptation of Mediator complexes in different eukaryotic organisms.

DATA AVAILABILITY

The protein interactions from this publication have been submitted to the IMEx (http://www.imexconsortium.org) consortium through IntAct and assigned the identifier IM-26688.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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