MED5 and CDK8 play a role in lignin-modification-induced dwarfing in Arabidopsis

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Abstract

Changes in lignin content and composition hold great promise for the improvement of lignocellulosic biomass in the context of biofuel production. Unfortunately, such modifications frequently result in decreased growth and yield penalties that would be unacceptable for the widespread implantation of this technology. Although some examples of lignin-modification-induced dwarfing may be the result of mechanical issues affecting water transport, others instances are now thought to be caused by alterations in metabolite levels and gene expression resulting from those metabolic engineering strategies.

Plant metabolic networks are precisely regulated by the spatial and temporal expression of suites of genes. Among the various transcription (co)factors, a multi-protein complex, Mediator, has been identified as a hub for transcription regulation. The core Mediator complex, comprising the head, middle and tail domains, functions as a bridge between transcription factors and basal transcription machinery, whereas the CDK8 kinase module plays a repressive regulatory role. It is still unclear, however, how the kinase module represses target genes especially in plants. Using a forward genetic screen, our lab determined that MED5, an Arabidopsis Mediator tail subunit, is required for maintaining phenylpropanoid homeostasis. A semi-dominant mutant (ref4-3) characterized by a single amino acid substitution in MED5a (G383S) was isolated as a strong suppressor of phenylpropanoid pathway, indicated by decreased soluble phenylpropanoid metabolite accumulation, reduced lignin content and dwarfism. In contrast, knocking out MED5a and MED5b results in the accumulation of increased levels of phenylpropanoid pathway derivatives. Considering that the CDK8 kinase module is a repressive module in Mediator, we tested the hypothesis that Arabidopsis MED5 represses phenylpropanoid pathway by interacting with CDK8. To test this hypothesis, CDK8 knockout lines (cdk8-1) were crossed with ref4-3, and the phenylpropanoid content of the resulting double mutants was evaluated. In ref4-3 cdk8-1 plants, the concentration of sinapate esters and total lignin content are as low as in ref4-3 yet the growth defect in ref4-3 is largely rescued. To further determine the genes targeted by MED5 and CDK8 in maintaining proper plant growth, we performed an RNA-seq analysis which identified that a majority of the genes involved in salicylic acid (SA) biosynthesis and signaling are up-regulated in cdk8-3 compared to wild type and ref4-3 cdk8-1. Consistent with this observation, both free and total SA, both of which have been previously implicated in dwarfing in lignin-modified plants, are accumulated at elevated levels in ref4-3 but not in wild type and ref4-3 cdk8-1.

The tail module of the Mediator complex is evolutionarily divergent

The core Mediator complex functions as a bridge between transcription factors (TFs) and RNA polymerase II (Pol II), which thereby facilitates transcription regulation. Arabidopsis MED subunits have been assigned to Head (blue), Middle (green), Tail (yellow) and CDK8 kinase modules (pink) according to a connection map from Saccharomyces cerevisiae (S. cerevisiae). The size of each subunit depicted in the model corresponds to its molecular weight.

MED5a (REF4) and MED5b (RFR1) are required for phenylpropanoid homeostasis

(A) The phenylpropanoid pathway generates a series of compounds from the precursor phenylalanine, including soluble metabolites such as sinapylmalate and monomers for lignin biosynthesis. (B) Plants with ref4-1 or ref4-3 point mutations have growth defects and accumulate lower levels of phenylpropanoids. MED5a/ MED5b knockout plants are of normal stature and have enhanced accumulation of phenylpropanoids. (C) MED5a (G383S) is evolutionarily divergent compared to wild type and ref4-3 cdk8-1. Consistent with the low phenylpropanoid accumulation in ref4-3 and ref4-3 cdk8-1, the majority of phenylpropanoid pathway related genes are repressed in ref4-3 and ref4-3 cdk8-1 compared to wild type.

RNA-seq was used to identify differentially expressed genes

(A) Knocking out CDK8 partially rescues the growth phenotype of ref4-3. cdk8-1 is a T-DNA insertion line of CDK8, a subunit of the Mediator kinase module. (B) The low sinapylmalate level in ref4-3 is not rescued by knocking out CDK8. (C) Lignin content and composition are similar in ref4-3 cdk8-1 compared to ref4-3 according to thiglycolic acid (TGA) lignin analysis (C) and derivatization followed by reductive cleavage (DFRDC) measurement (D) respectively.

Salicylic acid (SA) biosynthesis and signaling are activated in ref4-3 but not in ref4-3 cdk8-1

(A) SA is synthesized from the precursor chorismate, and can either be stored as its glucoside form or serve as signal molecules for plant development and stress responses. (B-C) SA biosynthetic genes, catabolic genes and SA signaling marker genes are up-regulated in ref4-3 but not in ref4-3 cdk8-1. (D) Free SA and total SA are significantly increased in ref4-3 compared to wild type (P < 0.05), while they are not in ref4-3 cdk8-1.

Knocking out ICS1 is not sufficient to rescue the dwarfism of ref4-3

Disruption of SID2 does not rescue the stunted growth of ref4-3. The SID2 gene encodes the SA biosynthetic enzyme isochorismate synthase ICS1.

Conclusions

• Knocking out CDK8 rescues the growth defect in ref4-3, but not the soluble metabolites and lignin content.
• Most phenylpropanoid biosynthetic genes are repressed in ref4-3 and ref4-3 cdk8-1.
• SA signaling is activated in ref4-3, but not in ref4-3 cdk8-1.
• Knocking out ICS1 fails to rescue the stunted growth of ref4-3.

References

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