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The Mediator complex subunit 8 regulates organ size in *Arabidopsis thaliana*

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Control of final organ size is a fundamental and core process of development of all multicellular organisms, but the mechanisms that set the final size of determinate organs are largely unknown. In a recent study, we demonstrated that the Mediator complex subunit 25 (MED25/PFT1), which is involved in the transcriptional regulation of gene expression, controls the final size of determinate organs by restricting both cell proliferation and cell expansion. *med25* mutants formed large organs with larger and slightly more cells, whereas plants overexpressing *MED25* produced small organs due to a reduction in both cell number and cell size. Here, we show that a loss-of-function mutation in the Mediator complex subunit 8 (MED8) causes small flowers as a result of reduced cell expansion. Analysis of the *med8 med25–2* double mutant reveals that *MED8* acts independently of *MED25* to regulate cell expansion and organ growth. Taken together, our findings show that MED8 and MED25 play an important role in regulating organ size. Further identification of upstream and downstream components of MED8 and MED25 will help understand how the Mediator complex is involved in organ size control in plants.

The Mediator complex was first identified in yeast as an activity required for the transcriptional activation and later as a multiprotein complex that functions to transmit various signals from activators, repressors, as well as general transcription factors, to the RNA polymerase II complex to initiate transcription.[1-3] The Mediator complex subunits are conserved in all eukaryotes from yeast to humans. In Arabidopsis, 21 conserved and six putative plant-specific Mediator subunits have recently been identified.[4] Several Mediator subunits were described to regulate flowering time, embryo development, stress responses and microRNA biogenesis in Arabidopsis.[5-9] The Mediator complex subunit 25 (MED25/PFT1) was first described as a positive regulator of shade avoidance and flowering time and later as a regulator of jasmonate-dependent defense and abiotic stress responses.[4,6,7,10] In a recent study, we discovered a new role of MED25 in plant organ size control through a genetic screen for mutations that enhance the floral organ size of the *da1-1* mutant; DA1 is a negative regulator of cell proliferation in Arabidopsis.[11,12] Loss-of-function mutants in *MED25* formed large organs, whereas plants overexpressing *MED25* produced small organs.[12] *med25* mutants predominantly increased cell expansion but also increased cell proliferation slightly. MED25 functions to restrict cell expansion and organ size independently of MED25-mediated phytochrome signaling and the jasmonate pathway.[12] We showed that cell enlargement in *med25* petals might, in part, result from increased expression of particular expansin genes.[12] In addition, our genetic analyses revealed that the *med25-2* mutation synergistically enhanced the cell number phenotype of *da1-1*,[12] indicating that *da1-1* is required for the dramatic effects of the *med25* mutations on cell proliferation and also suggesting that *MED25* acts redundantly with *DA1* to limit cell proliferation. Thus, MED25 may function as a hub that provides a link between cell proliferation and cell expansion pathways within the transcriptional machinery.

Previous studies showed that Arabidopsis *med25/pft1* and *med8* mutants appear to similarly affect both flowering time and pathogen resistance.[7] We therefore asked whether MED8 is involved in organ size regulation in Arabidopsis. To address this question, we obtained the *med8* mutant.[7] Surprisingly, in contrast to *med25* mutants, *med8* exhibited smaller flowers than wild type (**Fig. 1A and B**). Transformation of the *med8* mutant with a wild-type *MED8* cDNA driven by its own promoter restored a wild-type phenotype (**Fig. 1F**). To investigate the cellular basis of the decrease in flower size, we measured the number and size of adaxial epidermal cells in petals. The size of epidermal cells in the maximal width region of *med8* petals was significantly decreased compared with wild type (**Figs. 1C, D and 2B**), while the number of epidermal cells in *med8* petals was similar to that in wild-type petals (**Fig. 1E**), indicating that the *med8* mutation restricts cell expansion. To determine whether *MED8* and *MED25* function antagonistically in a common pathway to regulate cell expansion, we generated a *med8 med25-2* double mutant and analyzed its cell and organ size phenotypes together with those of the *med8* and *med25-2* single mutants. Genetic

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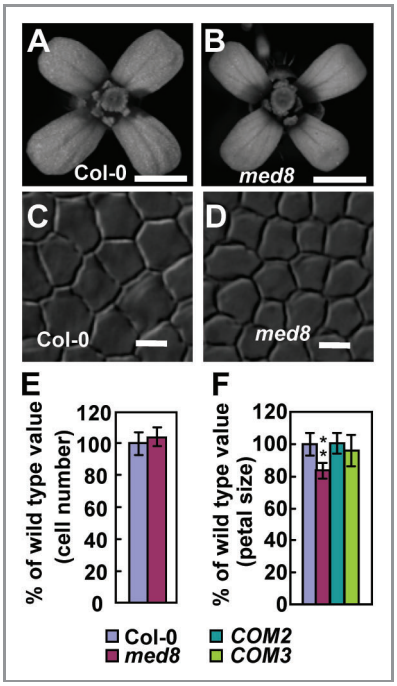

Figure 1. *med8* mutant forms small flowers. (A and B) Flowers of Col-0 and *med8*. (C and D) Adaxial epidermal cells in Col-0 and *med8* petals. (E) The number of adaxial epidermal cells in Col-0 and *med8* petals. Each value represents measurements from more than 10 petals. (F) Petal area of Col-0, *med8*, *COM2* and *COM3*. *COM* is *med8* transformed with *MED8* cDNA sequence driven by the 2362 bp *MED8* promoter. Petals from opened flowers (stage 14) were used to measure petal area. Each value for petal area represents measurements from more than 30 petals. Values (E and F) are given as mean ± standard deviation (s.d.) relative to the respective wild-type values. **, $p < 0.01$ compared with the wild type (Student's t-test). Scale bar, (A and B), 1mm; (C and D), 10μm.

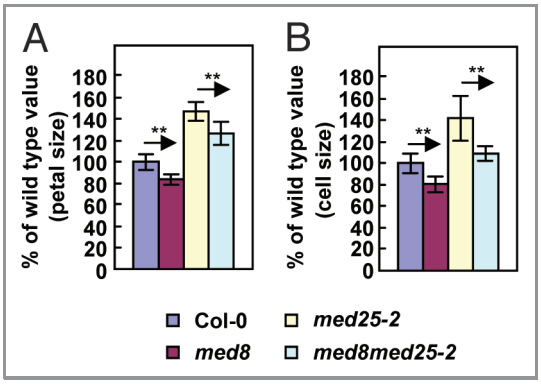

Figure 2. *MED8* acts independently of *MED25* to regulate cell and organ size. (A) Petal area of Col-0, *med8*, *med25–2* and *med8 med25–2* double mutant. Petals from opened flowers (stage 14) were used to measure petal area. Each value for petal area represents measurements from more than 30 petals. (B) The size of adaxial epidermal cells in the maximal width region of Col-0, *med8*, *med25–2* and *med8 med25–2* petals. More than 50 cells in the maximal width region of petals were measured. Each value represents measurements from more than 10 petals. Values (A and B) are given as mean ± s.d. relative to the respective wild-type values. **Difference indicated by the arrow is statistically significant at $p < 0.01$ (Student's t-test).

interactions between *med8* and *med25-2* were essentially additive for petal size and epidermal cell area compared with their parental lines (**Fig. 2**), suggesting that *MED8* acts to regulate cell expansion and organ growth separately from *MED25*. It is possible that MED8 and MED25 may transmit distinct signals from different classes of activators to the RNA polymerase II complex to initiate transcripts of their respective downstream target genes involved in organ size control. Therefore, further identification of their activators and downstream targets in organ-size control pathways will help understand how the Mediator complex controls organ size in plants.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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