

Putting the brakes on pollen wall development: A conserved negative feedback loop regulates pollen exine formation in flowering plants

Pollen grains are covered by a complex multi-layered structure, the pollen wall. Exine, the outermost layer of this wall, is made from the biopolymer sporopollenin that exhibits remarkable physical and chemical stability. In different plants, exine, which shields pollen from harsh environmental stresses, has very diverse morphologies and forms different patterns (Wang and Dobritsa, 2018). It also contributes to pollination, pollen–stigma interaction, pollen hydration, and eventual release of the pollen tube. Formation of exine begins after male meiosis when four microspores are arranged in a tetrad configuration and enclosed by the callose wall (Ariizumi and Toriyama, 2011). At this stage, microspores secrete primexine, the precursor of exine that accumulates between the microspore plasma membrane and the callose wall, providing a scaffold for exine patterning (Wang et al., 2021). As the callose wall gradually gets degraded, sporopollenin is secreted by the nearby tapetal cells and deposited onto the primexine template to form mature exine. This process requires coordination of activities between microspores and the surrounding tapetum.

Exine formation is controlled by the regulatory cascade of tapetal transcriptional activators. In *Arabidopsis* and rice, where this cascade has been the most studied, it is respectively referred to as AtDYT1–AtTDF1–AtAMS–AtMS188/MYB80–AtMS1 (Wang et al., 2018) and OsUDT1–OsTDF1–OsTDR–OsMS188/MYB80–OsPTC1 (Han et al., 2021). In this cascade, each transcription factor (TF) induces the expression of the next TF, and, collectively, they activate genes required for exine formation and tapetal activity. Although these cascades have been well studied, some questions remain. One intriguing question concerns the discovery that loss-of-function mutations in some of these activators lead to increased levels of their own transcripts and those of the upstream-acting TFs (Ferguson et al., 2017; Yang et al., 2019). This paradoxical finding suggested the presence within this cascade of an unknown negative-feedback regulator. In a recent study, Hou and colleagues (Hou et al., 2023) have unveiled a previously missing piece of this puzzle. In a tour-de-force study, they identified a repressor of the tapetal cascade and showed that it creates a conserved negative feedback loop that regulates timely tapetal degeneration and precise exine formation in flowering plants.

Hou et al. (2023) started by identifying the gene disrupted in maize *ms1* mutants. Compared with the wild type, the *ms1* loss-of-function mutants have an unusually thickened exine (Figure 1A) and delayed tapetal programmed cell death (PCD), implicating the affected gene in preventing excessive exine deposition and controlling the timing of tapetal activity. The causal gene of the

ms1 mutant, dubbed *ZmMs1/ZmLBD30*, was found to encode a LOB-binding domain (LBD) TF specifically expressed in tapetum at stages S9–S10 of anther development. In contrast to its loss-of-function phenotypes, premature expression of *ZmMs1/ZmLBD30* led to the formation of a very thin exine (Figure 1A) and early tapetal degeneration, consistent with *ZmMs1/ZmLBD30* controlling the timing of exine deposition and tapetal PCD.

The authors' group had previously reported the involvement of several maize TFs in tapetal development and exine formation (Zhang et al., 2018; Jiang et al., 2021). Like *ZmMs1/ZmLBD30*, three of these TFs (*ZmHLH51*, *ZmMYB84*, and *ZmMS7*) have their expression peaks in stage S9 anthers. Notably, these three TFs were orthologs of the downstream members of the tapetal activation cascades in *Arabidopsis* (*AtAMS–AtMS188/MYB80–AtMS1*) and rice (*OsTDR–OsMS188/MYB80–OsPTC1*), prompting Hou et al. (2023) to ask if the cascade also functioned in maize and activated *ZmMs1/ZmLBD30*. Indeed, they found that these three TFs form an activation cascade: *ZmHLH51* activates *ZmMYB84*, which in turn activates *ZmMS7*; *ZmMYB84* and *ZmMS7* interact to induce expression of *ZmMs1/ZmLBD30* (Figure 1B). Furthermore, the three activators were upregulated in the *ms1* loss-of-function mutant but were downregulated when *ZmMs1/ZmLBD30* was expressed prematurely, suggesting that *ZmMs1/ZmLBD30* provides negative feedback to their expression. Subsequent experiments revealed that *ZmMs1/ZmLBD30* acts as a transcriptional repressor and recognizes a specific DNA motif within the promoter regions of all three activators to directly repress their transcription, thereby creating a negative feedback loop in the tapetal cascade (Figure 1B).

In both *Arabidopsis* and rice, the tapetal cascades orchestrate the expression of many genes involved in tapetal PCD and exine formation (Li et al., 2017; Han et al., 2021). To gain deeper insights into the mechanisms by which *ZmMs1/ZmLBD30* governs tapetal PCD and exine thickness, Hou et al. (2023) selected and analyzed six genes related to sporopollenin formation, as well as three genes involved in scavenging of reactive oxygen species (ROS). All these genes displayed contrasting expression patterns in the *ms1* loss-of-function mutants (upregulated) and the premature-expression mutants (downregulated), indicating that *ZmMs1/ZmLBD30* negatively regulates their transcription. Further studies uncovered that *ZmMs1/ZmLBD30* directly represses two sporopollenin-related genes

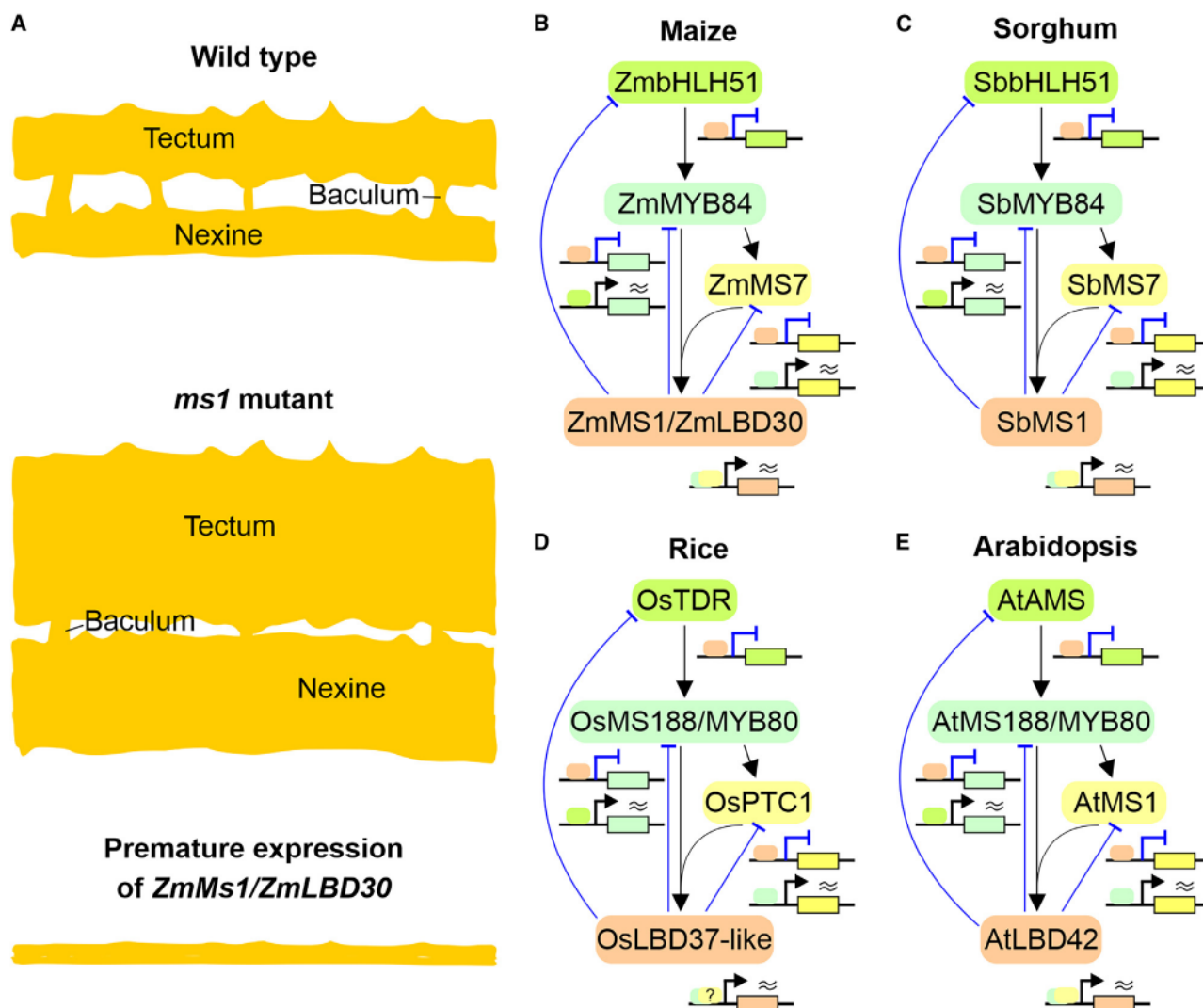


Figure 1. Exine thickness is controlled by a conserved transcriptional regulatory network.

(A) Compared with the wild type, loss-of-function *ms1* mutations lead to the formation of thicker pollen exine, whereas premature expression of *ZmMS1/ZmLBD30* leads to thinner exine. Exine parts are indicated.

(B–E) *ZmMS1/ZmLBD30*-orchestrated feedback repression loop is conserved in maize **(B)**, sorghum **(C)**, rice **(D)**, and *Arabidopsis* **(E)**. Using maize as an example, ZmbHLH51, ZmMYB84, and ZmMS7 act as transcriptional activators, while ZmMS1/ZmLBD30 functions as a transcriptional repressor. ZmbHLH51 binds to the *ZmMYB84* promoter to activate its transcription. ZmMYB84 binds to the promoters of *ZmMS7* and *ZmMS1/ZmLBD30* to activate their transcription. ZmMYB84 also interacts with ZmMS7 to form a complex that enhances the expression of *ZmMS1/ZmLBD30*, even though ZmMS7 does not activate *ZmMS1/ZmLBD30* expression on its own. In its turn, ZmMS1/ZmLBD30 binds to the promoters of these three transcriptional activators and directly represses their transcription. Orthologous transcription factors from different species are highlighted with identical colors.

and one ROS scavenging gene. Additionally, it indirectly represses other genes by repressing ZmMYB84 (which activates all nine genes) and ZmMS7 (which activates one of them) (An et al., 2020). These results suggested that the *ZmMS1/ZmLBD30*-mediated feedback loop inhibits sporopollenin formation and ROS scavenging to ultimately contribute to the precise timing of tapetal degeneration and accurate exine deposition.

The functional conservation of the tapetal activation cascade in maize, rice, and *Arabidopsis* brought up the intriguing question of whether the *ZmMS1/ZmLBD30*-orchestrated repression loop was similarly conserved in other species. To address this, Hou et al. (2023) first identified the closely related sorghum orthologs

of ZmbHLH51, ZmMYB84, ZmMS7, and *ZmMS1/ZmLBD30* and demonstrated the existence of the TF cascade SbbHLH51-SbMYB84-SbMS7-SbMS1 in sorghum (Figure 1C). Notably, they found that all three activators in this cascade can be directly repressed by SbMS1, confirming the existence of the SbMS1-orchestrated repression loop. Unlike sorghum, rice and *Arabidopsis* do not have easily identifiable clear orthologs of *ZmMS1/ZmLBD30*. Still, two anther-expressed LBD-type TFs in these species, rice OsLBD37-like and *Arabidopsis* AtLBD42, were found to act as transcriptional repressors and exert feedback repression activity on the corresponding tapetal activation cascades (Figures 1D and 1E). Premature expression of OsLBD37-like and AtLBD42 in rice and *Arabidopsis* disrupted

pollen development, causing dominant male sterility. Collectively, these findings suggest that the ZmMS1/ZmLBD30-orchestrated negative feedback loop is conserved in flowering plants.

This study identified the first regulator that puts the brakes on exine production in maize and uncovered a conserved feedback mechanism that ensures the timely tapetal PCD and proper exine formation in flowering plants. Since exine formation requires an unusual partnership between microspores and adjacent tapetal cells, it will be interesting to explore the signaling between these distinct cell types for coordinating this process. Hou et al. (2023) discovered that ZmMS1/ZmLBD30 potentially directly targets 569 genes and indirectly represses 694 genes. Studying these genes could provide valuable insights into the communication between tapetum and microspores during exine formation. In addition, other TFs, like AtDYT1-AtTDF1 in *Arabidopsis* and OsUDT1-OsTDF1 in rice, act in the upstream part of the tapetal cascade (Wang et al., 2018; Han et al., 2021). It would be important to find out if these TFs and their maize orthologs, ZmMS32 and ZmMS9 (Jiang et al., 2021), are also repressed by AtLBD42/OsLBD37-like/ZmMS1 and incorporated into the negative feedback loop. Exines of different plants assume fascinating and markedly distinct patterns on their pollen surfaces, contributing to the efficiency and species specificity of pollination (Wang and Dobritsa, 2018). For instance, *Arabidopsis* pollen exhibits a reticulate outer surface with an open tectum, whereas rice pollen features a closed tectum without reticulate morphology. Also, across species, exine thickness can vary significantly. Future studies should allow more insights into the mechanisms that guide formation of exines with diverse patterns and precise thickness in different plant taxa.

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Yuan Zhou¹ and Anna A. Dobritsa^{1,*}

¹Department of Molecular Genetics, Ohio State University, Columbus, OH 43210, USA

*Correspondence: Anna A. Dobritsa (dobritsa.1@osu.edu)
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