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Pollen protection: TEX2 plays an important role in the formation of pollen grain exine

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Pollen houses the sperm cells of plants and is the fertilizing element of angiosperms, important for ensuring the continuation of the next generation of individuals. Pollen is surrounded by a complex multi-layered cell wall called exine, which serves to protect the internal sperm cells and enable pollination. Exine consists of a non-carbohydrate-based cell wall made up of the rigid polymer sporopollenin, and the exine architecture is generally created through the precise sporopollenin deposition of its elements: baculae rising like columns and tectum forming roof-like structures (Figure 1, A–D and I–L). The enzymatic steps in sporopollenin biosynthesis and the timing of events leading to exine development have recently been elucidated (reviewed in Blackmore et al., 2007), but the precise mechanisms of exine patterning and how sporopollenin precursors assemble into the pollen exine remains a topic of considerable interest.

Exine formation occurs through specific stages with the involvement of multiple genes. It starts with the four products of male meiosis, the microspores, kept in a tetrad formation under the common callose wall (Figure 1, A–D). The probaculae and protectum, precursors of the exine structures baculae and tectum, respectively, form on the surface of each microspore. When the microspores are released from the tetrad configuration, probaculae and protectum are converted to their mature structures through the deposition of large amounts of sporopollenin (Figure 1, I–K). Prior to the formation of mature exine, primexine, a transient layer between the microspore plasma membrane and the callose wall, forms and is involved in providing a scaffold for the developing exine (Ariizumi and Toriyama, 2011). Mutations leading to changes in primexine composition interfere with proper exine development. The defects in many Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*) mutants that lack exine and display pollen lethality seem to originate from primexine malformation (Paxson-Sowders et al., 2001). While the exact chemical structure of primexine is unknown, it is hypothesized to be carbohydrate based and to contain cell wall polysaccharides such as cellulose, xylan, pectin, and glycoproteins, with the involvement of several exine-patterning genes encoding polysaccharide metabolism-related enzymes.

In a recent study, Wang and Dobritsa (2021) elucidated the role of *THIN EXINE2* (*TEX2*; also known as *REPRESSOR OF CYTOKININ DEFICIENCY1* [*ROCK1*]) in exine formation. *TEX2* is one of the genes important for normal exine formation and belongs to the nucleotide sugar transporters protein family, the members of which transport monosaccharides from the cytosol into the endoplasmic reticulum and Golgi apparatus (Dobritsa et al., 2011). Inside these organelles, the transported sugars are incorporated into polysaccharides, glycoproteins, and glycolipids. TEX2 is thought to have two roles in this process: as a nucleotide–sugar transporter directly involved in the formation of primexine polysaccharide components, and to ensure proper protein folding in the secretory pathway of the endoplasmic reticulum (Niemann et al., 2015).

Wang and Dobritsa (2021) investigated the role of TEX2 through genetic, microscopic, and immunohistochemical analyses and showed that TEX2 functions at the intersection of several pathways involved in exine formation in pollen grains. In their study, the authors highlighted key differences between *tex2* and other primexine mutants. Primexine mutants are typically pollen-lethal, with their microspores degrading soon after being released from the tetrad stage. These mutants also exhibit defects with plasma membrane undulation and show large aggregates of sporopollenin near the surface of free microspores (Paxson-Sowders et al., 2001; Ariizumi et al., 2004; Guan et al., 2008; Chang et al., 2012; Xu et al., 2020). In contrast, *tex2* plants, despite their thin exine, still produce viable and fertile pollen, form a plasma membrane that undulates normally, and show small globules of

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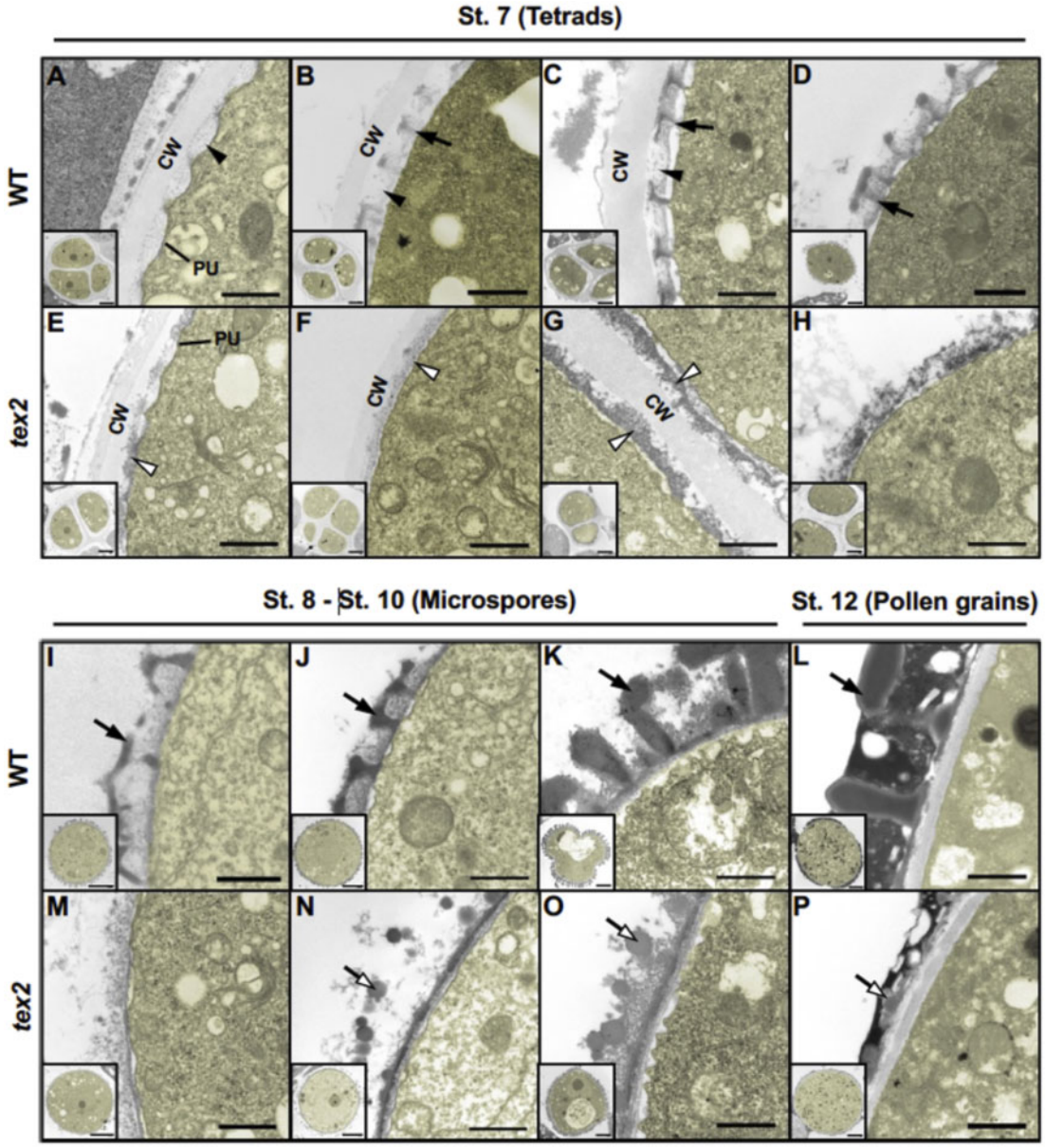

Figure 1 TEM micrographs of developing pollen cross-sections showing the difference between exine and primexine formation in *tex2* and wild-type Arabidopsis. Large images show Stages 7–12 (tetrads, microspores, and pollen grains), inserts show the cells from which the enlarged images were obtained. In (A–D), primexine (black arrowheads) and probaculae (black arrows) are visible between callose wall (CW) and undulating microspore plasma membrane (PU), while in (E–H) coarse primexine (white arrowhead) is present, while probaculae structures are absent. In (I–K), the developing exine on the surface of microspores is visible (black arrows), as is the mature exine on pollen grains (black arrow). Conversely, (M–O) shows the accumulation of granules (white arrows) representing unattached sporopollenin, and (P) clearly displays a very thin exine layer (white arrow). Cytoplasm of tetrads, microspores and pollen grains is false colored in yellow. Scale bars = 500 nm in (A–P) and 2.5 μm in inserts. Image taken from Wang and Dobritsa (2021), figure 1.

sporopollenin present in the free microspores. These differences between *tex2* and other primexine mutants suggest TEX2 may be required both for the formation of primexine and for sporopollenin transport out of the tapetum.

Primexine is difficult to study because it is produced in small amounts and is present only briefly before being converted into mature exine. Wang and Dobritsa (2021) used immunohistolabeling with glycan-directed antibodies to test the distribution of cell wall glycans in primexine. They found more arabinogalactan proteins in *tex2* mutants compared to wild-type plants, which likely leads to alterations in the primexine surrounding tetrads, inhibiting the formation of probaculae and protectum, and ultimately leading to defective exine formation. The authors imaged and compared pollen wall structures in *tex2* and wild-type plants at specific time points and clearly showed sporopollenin deposited on the surface of microspores in wild-type plants but not in *tex2* mutants (Figure 1).

This study elegantly displays the stepwise development of a crucial component of pollen grains and shows that loss of *TEX2* results in abnormal primexine formation, leading to failure of exine elements to properly assemble on the microspore surface and resulting in a thin exine layer. In addition, the authors have expanded our understanding of the composition of the short-lived primexine and revealed how phenotypic characteristics of *tex2* mutants can be used to piece together the genetic pathways leading to the formation of this vital pollen structure.

Conflict of interest statement. None declared.

References

Ariizumi T, Hatakeyama K, Hinata K, Inatsugi R, Nishida I, Sato S, Kato T, Tabata S, Toriyama K (2004) Disruption of the novel plant protein NEF1 affects lipid accumulation in the plastids of the tapetum and exine formation of pollen, resulting in male sterility in Arabidopsis thaliana. Plant J **39**: 170–181

Ariizumi T, Toriyama K (2011) Genetic regulation of sporopollenin synthesis and pollen exine development. Annu Rev Plant Biol **62**: 437–460

Blackmore S, Wortley AH, Skvarla JJ, Rowley JR (2007) Pollen wall development in flowering plants. New Phytol **174**: 483–498

Chang H-S, Zhang C, Chang Y-H, Zhu J, Xu X-F, Shi Z-H, Zhang X-L, Xu L, Huang H, Zhang S, et al. (2012) No primexine and plasma membrane undulation is essential for primexine deposition and plasma membrane undulation during microsporogenesis in Arabidopsis. Plant Physiol **158**: 264–272

Dobritsa AA, Geanconteri A, Shrestha J, Carlson A, Kooyers N, Coerper D, Urbanczyk-Wochniak E, Bench BJ, Sumner LW, Swanson R, et al. (2011) A large-scale genetic screen in Arabidopsis to identify genes involved in pollen exine production. Plant Physiol **157**: 947–970

Guan Y-F, Huang X-Y, Zhu J, Gao J-F, Zhang H-X, Yang Z-N (2008) RUPTURED POLLEN GRAIN1, a member of the MtN3/saliva gene family, is crucial for exine pattern formation and cell integrity of microspores in Arabidopsis. Plant Physiol **147**: 852–863

Niemann MCE, Bartrina I, Ashikov A, Weber H, Novák O, Spíchal L, Strnad M, Strasser R, Bakker H, Schmülling T, et al. (2015) Arabidopsis ROCK1 transports UDP-GlcNAc/UDP-GalNAc and regulates ER protein quality control and cytokinin activity. Proc Nat Aca Sci USA **112**: 291–296

Paxson-Sowders DM, Dodrill CH, Owen H A, Makaroff CA (2001) DEX1, a Novel Plant Protein, Is Required for Exine Pattern Formation during Pollen Development in Arabidopsis. Plant Physiol **127**: 1739–1749

Wang R, Dobritsa AA (2021) Loss of *THIN EXINE2* disrupts multiple processes in the mechanism of pollen exine formation. Plant Physiol **187**: 133–157

Xu D, Mondol PC, Ishiguro S, Shi J, Zhang D, Liang W (2020) NERD1 is required for primexine formation and plasma membrane undulation during microsporogenesis in Arabidopsis thaliana. BIOTECH **1**: 205–218