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Research Highlight

# The secret of fertilization in flowering plants unveiled

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As recapitulated in the famous Chinese fairy tale *Journey to the West*, the Buddhist monk Xuanzang of the Tang Dynasty (Tangseng) took a heroic journey in a quest for India Buddhist sacred scriptures. The long and dangerous journey was made possible only with the help of the powerful Monkey King, who had been pinned down under a mountain by the Buddha as a punishment for his prior misdemeanor. The Buddha had to secure the mountain with a magic paper seal so that the Monkey King would not break loose. The Monkey King burst out from the mountain as soon as Tangseng lifted the seal, so that he could escort Tangseng through the journey (Fig. 1).

To some extent, the secret process of fertilization in flowering plants has much in common with this legendary story. Unlike animals, the sperm cells of angiosperm are unable to swim and must be transported to female gametes to accomplish the sexual reproduction. These sperm cells are enclosed as a cargo in a pollen grain [1]. Upon landing on the stigma, the tip of female reproductive organ, the pollen grain germinates and forms a growing pollen tube that travels a long distance through the female tissue to reach ovule, where it ruptures to release sperms and fertilizes the egg cell inside ovule (Fig. 2). The process takes place with remarkable precision and includes guided growth of pollen tube, prevention of pre-mature rupture of pollen tube before reaching the ovule, accurate localization of ovule opening, repression of pollen tube growth upon arrival to the ovule, and the rapture of pollen tube at the final stage [2]. Sophisticated communications between male and female reproductive tissues are crucial for the execution of each step, and any miscommunication results in failure of fertilization and aborted seed-set. A number of small peptides produced by pollen tube and female tissues serve as signals coordinating this complex process. For instance, small CYSTEINE-RICH PEPTIDES (CRPs), such as tomato LAT52 and STIG are known to regulate pollen tube growth [3,4]. The Arabidopsis LUREs [5] and maize ES1-4 [6] which are also CRPs, guide pollen tube growth toward the ovule. Members of another class of CRPs called RAPID-ALKALIZATION FACTORS (RALFs) have been reported to arrest pollen tube growth once inside the ovule [7–9]. Identification of peptides and their receptors controlling pollen tube journey is a major task in the field of plant reproduction research.

Synergid cells, a pair of cells neighboring the egg cell, are known to be a key source of female signals. LUREs [5] and ES1-4 [6] produced by synergid cells are key attractants for pollen tube guidance in *Arabidopsis* and maize respectively. Several Leucine-Rich Receptor-Like Kinases (LRR-RLKs) including PRKs and MDIS1, and MIK1/2 likely form a receptor complex for the perception of LUREs [10]. Two closely related receptor-like cytoplasmic kinase (RLCK) named LOST IN POLLEN TUBE GUIDANCE 1 (LIP1) and LIP2 are required for LURE-regulated pollen tube guidance [11], likely by acting downstream of the LURE receptor complex.

The *Arabidopsis* RLK protein FERONIA (FER), which belongs to the *Catharanthus roseus* RLK-like (CrRLK1L) family, also plays key role in pollen tube reception [12]. FER is highly expressed in synergid cell plasma membrane and is required for the repression of pollen tube elongation in the ovule [7]. Later research discovered FER as a major receptor for RALFs to suppress cell elongation [8]. Whether specific members of RALFs regulate the FER-mediated pollen tube growth-arrest remains unknown.

Controlled rupture of pollen tube tip is not only required for the release of sperm cells in the ovule, but also ensure pollen tube reaching the ovule without premature rupture. ANXUR1 (ANX1) and ANX2, two closely-related CrRLK1L family members localized at the tip of pollen tube, are required for the integrity of pollen tube [13,14]. The pollen tube of *anx1 anx2* double mutant bursts spontaneously after pollen germination and is male sterile. These findings suggest an involvement of transmembrane signaling in the control of pollen tube rupture. Consistent with this notion, the RLCK protein MARIS (MRI) and a pair of NADPH oxidases have been shown to act downstream for the ANXs-mediated suppression of pollen tube rapture [15,16]. It is not known, however, whether ANX1/2 are receptors or part of the receptor complex perceiving an unknown ligand to prevent premature rupture. Moreover, it is unknown how the ANX-mediated suppression is removed to allow pollen tube rupture in the ovule.

A newly published study sheds light on these important questions. Qu and colleagues show that, a pair of CrRLK1L members are essential to prevent premature rupture of pollen tube [17]. Inspired by the Monkey King story, the authors name these proteins Buddha's Paper Seal 1 (BUPS1) and BUPS2. Several lines of evidence indicate that BUPS1/2 and ANX1/2 act together to control pollen tube integrity. The *bups1/2* double mutants are completely sterile, and their pollen grains phenocopy that of *anx1/2* double mutants and burst spontaneously after germination. Like ANX1/2, BUPS1/2 are expressed in pollen grains and pollen tubes.

Because BUPS1/2 and ANX1/2 belong to the CrRLK1L family and are homologous to FER, the authors hypothesized that BUPS1/2

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**Fig. 1.** (Color online) The cartoon depicts a legendary story told in *Journey to the West.* The Buddha traps the Monkey King under the mountain and secures it with a paper seal. Tangseng lifts the seal, so that the Monkey King can break loose and escort his master to the west in a quest for Buddha's script. The drawing is credited to Xuefei Wang of the College of Life Sciences, Peking University.

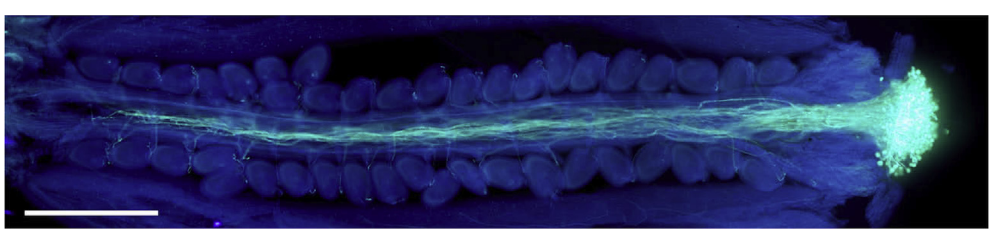

**Fig. 2.** (Color online) Pollen tube behavior in the pistil of wild-type by aniline blue staining after twenty hours pollination. Scale bars, 500 μm. Image courtesy of L.-J. Qu.

and ANX1/2 are also involved in the perception of RALF peptides. Because *bups1/2* and *anx1/2* pollen grains germinated *in vitro* burst in the absence of female signals, it is logical to postulate that these peptides are produced by pollen grains. Indeed, transcriptome analysis showed that several RALFs, including the closely related RALF4 and RALF19, are expressed in pollen grains and tubes, RALF4/19 has been reported to inhibit pollen germination [18]. Qu and colleagues constructed *ralf4/19* double mutants to show that their pollen grains spontaneously burst upon germination and are completely sterile. An independent study by Grossniklaus and colleagues showed that transgenic Arabidopsis lines silenced for RALF4/19 displayed similar phenotypes, albeit weaker than the *ralf4/19* double mutants [19]. Qu and colleagues show that both BUPS1/2 and ANX1/2 can directly bind RALF4/19 with comparable affinity, but not RALF23, which is a ligand for FER [17]. Further study show that BUPS1/2 interact with ANX1/2 through the ectodomain, indicating that BUPS1/2 and ANX1/2 form a receptor complex to perceive RALF4/19.

The aforementioned findings elegantly illustrate how the Buddha traps the Monkey King (sperm cells) under the mountain (pollen tube). The readers may wonder where is the Tangseng's hand (hands) lifting BUPS in the story of plant fertilization. The authors envisioned that specific RALF peptides produced by ovules maybe the long sought-after Tangseng's hand, which de-represses

BUPS1/2 to allow pollen tube burst in the ovule. An examination of transcriptome data uncovered 9 ovule-expressed RALFs. The authors tested synthesized peptides to show that one of the ovule-specific RALFs, RALF34, but not RALF4 and RALF23, can trigger pollen tube rupture at a nanomolar concentration, providing strong evidence that RALF34 is a hand of Tangseng. Unlike the *ralf4/9* double mutant, the *ralf34* shows wild-type fertilization phenotype, suggesting that other female-derived RLAFs may have overlapping function with RLAF34, a possibility remains to be tested.

This and other studies raise several important questions. Data presented in this study suggest that BUPS1/2 and ANX1/2 form a receptor complex for antagonistic ligands [17]. While RALF4/19 inhibit pollen tube rupture, RALF34 triggers it. The authors show that RALF34 can compete with RALF4/19 for BUPS1/2-binding, suggesting that RALF34 triggers rupture by displacing RALF4/19 from BUPS-ANX complex. Given the similarity of RALFs in their amino acid sequences, it remains to be elucidated how a RALF34-bound receptor complex allows pollen rupture whereas a RALF4/19-bound receptor complex inhibits it. One possibility is that the same receptor can form different receptor complexes by associating with distinct components or co-receptors, and that slightly different ligands are perceived by different receptor complexes, which produce opposite signal outputs. Indeed, stomatal patterning and inflorescence development are differentially controlled by different ERECTA receptor complexes and EPF peptides [20]. In addition, Grossniklaus and colleagues showed that RALF4/19 also bind at the surface of pollen tube several LEUCINE-RICH REPEAT EXTENSION (LRX) proteins, which are known to regulate cell wall development, and suggested that the interaction mediates the inhibition of pollen tube growth [19]. Whether LRX proteins are also involved in pollen tube rupture remains to be tested.

In sum, this exciting study fills a major gap in our understanding of plant fertilization and illustrates a precise spatiotemporal regulation mechanism underlying pollen tube integrity control. Before pollen tube reception, RALF4/9 bind the BUPS-ANX receptor complex to maintain the pollen tube integrity. After pollen tube reception, RLAF34 competes with RALF4/9 for the BUPS-ANX receptor complex and triggers the pollen tube rupture to release the sperm cell for fertilization. The findings open up a new avenue to Buddha's script in the field of plant reproduction research.

## Conflict of interest

The authors declare that they have no conflict of interest.

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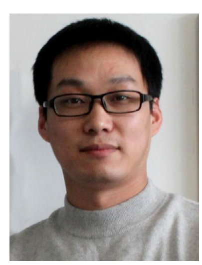

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