

On your mark, get set, GROW! LePRK2–LAT52 interactions regulate pollen tube growth

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Recent discoveries show that LAT52 and LePRK2, two pollen-specific proteins, interact in what might be an autocrine signaling system. This exciting finding indicates that successful fertilization requires ligand–receptor kinase signals that regulate pollen-tube growth. The stage is now set to identify other components of this pathway and to explore their connections with the many signals exchanged between pollen and pistil.

A molecular courtship ritual between pollen and pistil ensures that fertilization in flowering plants is appropriate and successful. The first question to be settled is whether pollen and pistil, brought together by wind or insects, represent a suitable pairing. Once a mate has been identified, compatible signaling molecules play a key role, guiding the rapidly elongating pollen tube along its precisely defined journey to the ovule (Fig. 1).

In *Brassica*, the signal that inhibits germination of self-pollen is mediated by a ligand–receptor kinase interaction [1]. The stigma-expressed *S* locus (self-incompatibility) receptor kinase (SRK) binds a small pollen-expressed cysteine-rich protein (SCR) in an *S* haplotype-specific interaction [2,3]. This interaction initiates a signal transduction cascade that inhibits hydration, and consequently growth of self-pollen, while allowing pollen from non-self to grow [1].

Unlike the inhibitory kinases involved in *Brassica* self-incompatibility, pollen-expressed receptor kinases can stimulate pollen development and pollen tube growth in compatible pollinations. These include pollen receptor kinase 1 (PRK1, [4]) from petunia and three pollen-specific tomato kinases (LePRK1, LePRK2 and LePRK3 [5,6]). These pollen receptor-like kinases (RLK) all have extracellular domains with five or six leucine-rich repeat motifs (LRR) and an intracellular kinase domain. LePRK1 and LePRK2 encode active kinases that are concentrated at the pollen tube plasma membrane [5]. Intriguingly, LePRK2 phosphorylation is reduced when pollen fractions are treated with stigma, but not leaf, extracts [5].

The LePRK ligand hunt

To find LePRK ligands, Weihua Tang and colleagues conducted a yeast two-hybrid screen using the extracellular domains of LePRKs to search for interacting proteins encoded by a pollen cDNA library [7]. Although two-hybrid screens demand that interacting proteins meet in the

yeast nucleus, many potential ligands for the LePRKs were identified that were known to be secreted or had N-terminal secretion signals. Interacting proteins fell into four known classes: small cysteine-rich proteins, cell wall remodeling enzymes, a LRR-containing protein, and a protein containing EF-hand calcium-binding motifs; several proteins were also identified that displayed no resemblance to proteins of known function [7].

The small cysteine-rich proteins caught the immediate attention of Sheila McCormick's group: these proteins share structural features with SCR, and they included LAT52, a protein discovered by the McCormick group in the late 1980's [8]. LAT52 was cloned four times when the extracellular domain of LePRK2 (but not LePRK1 or

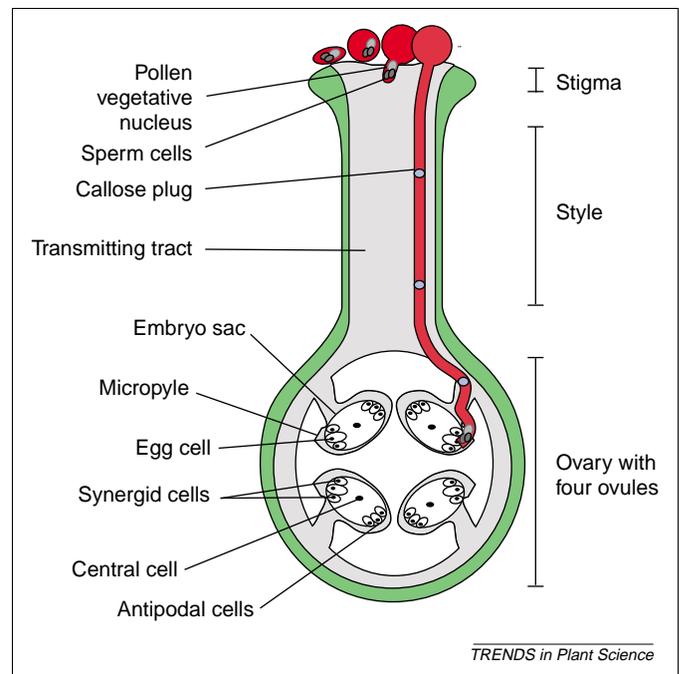


Fig. 1. The path of the pollen tube. When pollen grains land on a compatible stigma they hydrate (depicted as a transition from an oval to a round shape) and become metabolically active. Pollen tube germination follows within minutes; tube growth is polar and extremely rapid, occurring by deposition of new cellular components at the tip. The pollen tube penetrates the stigma cell wall and enters the style, growing in the rich extracellular matrix of the transmitting tract. As the pollen tube grows, callose plugs are periodically deposited that segregate the active tip from older parts of the tube cell. Finally, the pollen tube reaches the ovary where it is guided to the micropyle of an ovule. The two synergids, the egg and central cell, and three antipodal cells comprise the embryo sac, or female gametophyte. To achieve fertilization, the pollen tube bursts within one of two synergid cells, delivering one sperm to fertilize the egg, and another to fertilize the central cell, producing the embryo and endosperm, respectively.

LePRK3) was used as the two-hybrid bait. *LAT52* encodes a secreted protein and the McCormick laboratory has developed several reagents, including antibodies and transgenic plants, to study its function [9].

By hunting for interactions in a pollen library, the focus was limited to proteins that might participate in autocrine signaling. Many cells talk to themselves; the classic example is the T-cell, which produces Il-2 to induce self-proliferation [10]. Cell migration can also rely on self-signaling: When an adhesion molecule on the surface of neurons undergoes proteolysis, a soluble fragment interacts with integrins on the same cell and facilitates cell migration [11].

LePRK2–LAT52 interactions occur *in vivo* and are regulated by pollen tube development

An antibody against LePRK2 precipitated LAT52 from a mature pollen extract, whereas pre-immune serum did not [7]. By contrast, antibodies against LePRK1 and LePRK3 precipitated LAT52 in only trace amounts (anti-LePRK1) or not at all (anti-LePRK3). The extracellular domain of LePRK2 was sufficient for co-immunoprecipitation of LAT52 from mature pollen protein extracts, indicating that the kinase domain is not required for binding – a finding that differs from the observed requirement for an active intracellular kinase domain in the interaction between the LRR–RLK CLAVATA1 (CLV1) and its ligand, CLV3 [12].

Interestingly, although purified LePRK2 was able to bind LAT52 extracted from mature pollen grains, a similar interaction could not be demonstrated using extracts derived from *in vitro* germinated pollen tubes. To further define this difference, the McCormick group characterized the LAT52 molecules purified from mature pollen. The purified soluble fraction lacked LePRK2, yet LAT52 was found in a complex that was much larger than its apparent molecular weight of ~20 kDa. If the complex was purified from pollen tubes grown *in vitro* or if extracts were heated, the mass of this complex decreased (Fig. 2). Taken together, these results suggest that LAT52 is a member of a heat-sensitive complex that is altered during pollen tube germination and is required for binding of LAT52 to LePRK2 (Fig. 2).

LePRK2 is phosphorylated when associated with pollen membranes, and this modification is reduced when pollen membrane fractions are treated with stigma, but not leaf, extracts [5]. This suggests that LePRK2 interacts with a factor expressed specifically by the stigma, potentially a component that takes the place of LAT52 upon pollen tube germination.

A role for LePRK2–LAT52 in pollen tube germination and growth

Expressing antisense *LAT52* from its own promoter in transgenic tomato plants results in pollen tubes that fail to exit the style and have a short and twisted growth pattern [9]. *In vitro*, the mutant phenotype was more severe and pollen failed to hydrate and germinate. The results presented by Tang *et al.* now indicate that LAT52 might have an essential role in activating a signaling cascade via interaction with LePRK2 [7]. It is possible that

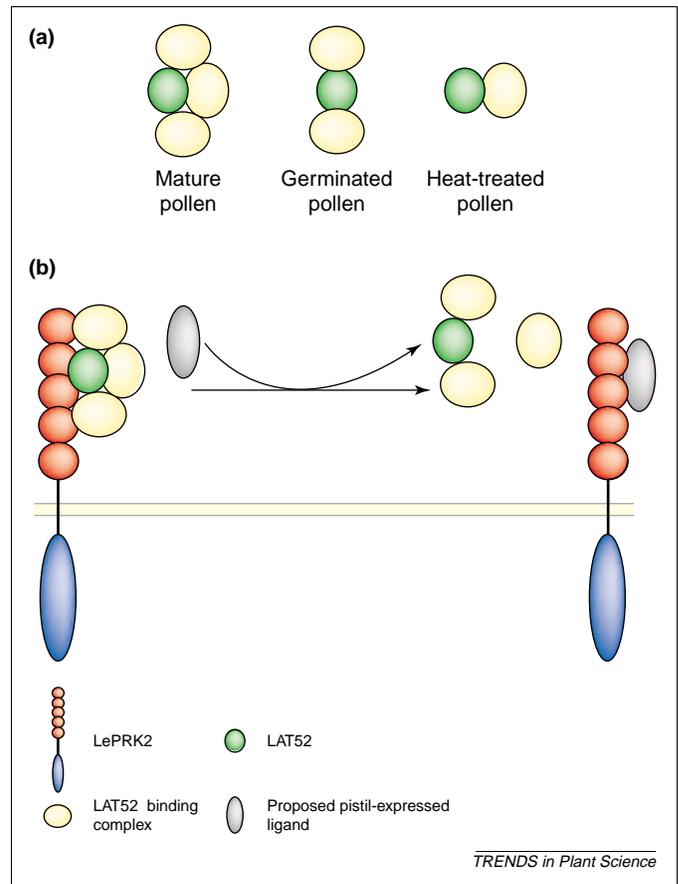


Fig. 2. Regulation of LePRK2–LAT52 binding. (a) In conditions that favor LePRK2 interaction, LAT52 was found in a large protein complex (>100 kDa). The size of this complex was smaller when LAT52 was isolated from germinated pollen tubes (50–100 kDa) or when mature pollen grain extracts were heated to 70 °C (<50 kDa), conditions that disrupted LePRK2 binding. (b) The interaction between LePRK2 and LAT52 is disrupted by pollen tube germination. One possibility, depicted here, is that a pistil-expressed LePRK2 ligand replaces LAT52. It is predicted that this ligand switch would result in changes in LePRK2 signaling activity necessary to regulate proper pollen tube growth. The five leucine-rich repeat motifs of LePRK2 are depicted as red circles and the kinase domain as a blue oval.

dissociation of LAT52 and LePRK2, or binding of LePRK2 by other proteins, serves as a checkpoint for pollen tube growth. As Tang *et al.*, point out, interactions between LePRK2 and its ligand(s) might represent an autocrine pollen signaling system that plays a vital role in regulating the initiation and maintenance of pollen tube growth [7].

There is much to be done to test these ideas. The isolation of LePRK2 mutants that phenocopy the loss of LAT52 function would support the idea that these two proteins function together to regulate pollen tube germination, growth or guidance. Similarly, biochemical characterization of LePRK2 kinase activity and the structure of the LePRK2–LAT52 complex will determine whether LAT52 binding changes LePRK2 signaling, either by repression or activation of kinase activity, indicating that LAT52 is indeed a ligand. It will also be important to address whether other proteins that interact with LePRK2 function as ligands. There is evidence that they might:

- LePRK2 reaches its maximum expression after pollen tube germination [5] – a time when it does not interact with LAT52 [7].

- Several other proteins were identified in the two-hybrid screen [7].
- LePRK phosphorylation is reduced by stigma extracts [5] – an effect that is not mediated by LAT52, which is expressed only in pollen.

Unraveling the mechanism of the LAT52–LePRK2 signaling system and its role in tomato pollen tube germination and growth will serve as a model for reproductive signaling in many plant species.

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References

- 1 Dixit, R. and Nasrallah, J.B. (2001) Recognizing self in the self-incompatibility response. *Plant Physiol.* 125, 105–108
- 2 Kachroo, A. *et al.* (2001) Allele-specific receptor-ligand interactions in *Brassica* self-incompatibility. *Science* 293, 1824–1826
- 3 Takayama, S. *et al.* (2001) Direct ligand–receptor complex interaction controls *Brassica* self-incompatibility. *Nature* 413, 534–538
- 4 Mu, J.H. *et al.* (1994) Characterization of a pollen-expressed receptor-like kinase gene of *Petunia inflata* and the activity of its encoded kinase. *Plant Cell* 6, 709–721
- 5 Muschietti, J. *et al.* (1998) Pollen tube localization implies a role in pollen–pistil interactions for the tomato receptor-like protein kinases LePRK1 and LePRK2. *Plant Cell* 10, 319–330
- 6 Kim, H.U. *et al.* (2002) New pollen-specific receptor kinases identified in tomato, maize and *Arabidopsis*: the tomato kinases show overlapping but distinct localization patterns on pollen tubes. *Plant Mol. Biol.* 50, 1–16
- 7 Tang, W. *et al.* (2002) A cysteine-rich extracellular protein, LAT52, interacts with the extracellular domain of the pollen receptor kinase LePRK2. *Plant Cell* 14, 2277–2287
- 8 Twell, D. *et al.* (1989) Isolation and expression of an anther-specific gene from tomato. *Mol. Gen. Genet.* 217, 240–245
- 9 Muschietti, J. *et al.* (1994) LAT52 protein is essential for tomato pollen development: pollen expressing antisense LAT52 RNA hydrates and germinates abnormally and cannot achieve fertilization. *Plant J.* 6, 321–338
- 10 Morgan, D.A. *et al.* (1976) Selective *in vitro* growth of T lymphocytes from normal human bone marrows. *Science* 193, 1007–1008
- 11 Mechttersheimer, S. *et al.* (2001) Ectodomain shedding of L1 adhesion molecule promotes cell migration by autocrine binding to integrins. *J. Cell Biol.* 155, 661–673
- 12 Trotochaud, A.E. *et al.* (2000) CLAVATA3, a multimeric ligand for the CLAVATA1 receptor-kinase. *Science* 289, 613–617

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What drives plant stress genes?

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Currently, there is a lot of interest in the plant stress response. Using large-scale genomics approaches, more and more genes are being identified that are involved in or even regulate this complex process. The recent boost in expression profile analyses for several plant stress responses has enabled the identification of new promoter elements as important factors in establishing the expression regulatory network controlling plant stress response.

Plants as sessile organisms are constantly exposed to changes in environmental conditions. When these changes are rapid and extreme, plants generally react with some form of stress response. Stress is not necessarily a problem for plants because they have evolved effective mechanisms to avoid or reduce the possible damage caused by stress. The response to changes in environment can be rapid, depending on the type of stress. This is accomplished partly by activation of inactive transcription factors such as bZIP proteins [1] or by preferential translation of pre-made mRNAs [2]. In addition, new transcripts are made and within a few hours a steady level of stress adaptation has been reached. How this stress response is controlled at

the transcriptional level is still the subject of several studies. It is clear that the response of plants to stress varies from species to species and from stress to stress. Although there are common stress genes responding to various abiotic or biotic stresses, such as the genes involved in the detoxification of reactive oxygen species, there are also stress-specific response genes [3].

In general, the transcriptional regulation of genes is directly controlled by a network of transcription factors and transcription factor binding sites (TFBS). TFBS are DNA elements that are often located in the regions directly upstream of protein coding sequences, but sometimes are located at more distant sites or even in introns. Binding of transcription factors to TFBS can be facilitated by activator proteins that promote conformational changes or secondary modifications to activate transcription factors or that promote accessibility of TFBS. By contrast, there are suppressor proteins that compete with transcription factors for binding to the binding sites or to other transcription factors, or which are able to inactivate transcription factors. The presence or absence of transcription factors, activators and suppressors regulating transcription of target genes often involves a whole cascade of signalling events determined by tissue type, developmental stage or environmental condition [4]. There

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