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Previews

GABA, a New Player in the Plant Mating Game

Pollen tubes are guided through female tissues to deliver sperms to the embryo sac. A recent study reveals a GABA gradient along the pollen tube path, which, together with guidance defects in GABA-overaccumulating mutants, implies a role for GABA in regulating pollen tube growth and guidance.

In flowering plants, mating involves the delivery of two sperms by the male gametophyte pollen to the embryo sac (female gametophyte) in the ovule. Among numerous obstacles to successful mating, the most significant is a physical barrier of multiple tissues between the male and female gametophytes (Figure 1). Pollen grains landing on the stigma surface initiate polar growth to form a tip-growing tube, which penetrates the stigma, moves through the style and the transmitting track, emerges from the septum, and is guided along the funiculus before targeting the embryo sac. How the pollen tube finds its way to the ovule is a question that has fascinated biologists for centuries (Lord, 2003). Obviously, it must communicate with the female tissues along this tortuous path. Over the past decade, several female signals have been implicated in early parts of this path, including lipids, glycoproteins, small polypeptides, and pectin polysaccharides (Lord and Russell, 2002). But nothing is known about signaling events that occur near the ovule.

Now, elegant work, published in the July 11 issue of *Cell*, from Preuss's group has implicated GABA (γ-amino butyric acid) in the regulation of pollen tube growth and guidance (Palanivelu et al., 2003). GABA is a ubiquitous glutamate derivative with diverse functions, ranging from neuron development in animals to defense in plants to nutrient storage in microbes. First discovered as a neurotransmitter, GABA has been shown to be multifunctional in neuron development, with functions including trophic effects on neurite outgrowth and migration (Owens and Kriegstein, 2002). Pollen tube growth/guidance and neuron outgrowth/guidance are analogous in many ways; both are tip-growing systems involving conserved regulatory mechanisms (e.g., Rho GTPase and calcium), and both require adhesion, attraction, and repulsion for guidance (Palanivelu and Preuss, 2000; Zheng and Yang, 2000). GABA seems to add another level to this analogy.

Insights into the role of GABA in pollination were gained from the studies of *POP2* encoding a GABA-degrading transaminase. *pop2* mutant tubes grow normally up to the septum but fail to target the *pop2* ovule. Interestingly, this defect is associated with GABA overflooding in the pollen tube and the ovule. Importantly GABA accumulates in the pistil as a gradient along the pollen tube path, with the highest level in a subset of inner integument cells adjacent to the micropyle—the site of pollen tube entry into the embryo sac. Thus, GABA could be the first ovule-derived signal implicated in the final stage of pollen tube guidance.

This report also provides some insights into the mechanism for GABA gradient formation. GABA seems to be produced in a subset of the inner integument cells adjacent to the micropyle. GABA diffusion from the source could generate the ovule-to-septum gradient. However, diffusion alone seems unable to explain the observed GABA gradient along the whole pollen tube path because lengths of the path for tubes pollinating the stigma-end ovules drastically differ from those reaching the bottom of the pistil (Figure 1). GABA degradation mediated by intracellular POP2 helps to maintain the septum-to-stigma gradient, further disputing a simple diffusion mechanism. Polar transport could allow GABA gradient formation in a complex spatial manner (Figure 1), similar to the polar transport of auxin (indole acetic acid), a well-characterized example of small molecules that form a gradient in plants (Ottenschlager et al., 2003).

The authors made a fascinating observation that only *pop2* mutant tubes, but not WT tubes, fail to target the *pop2* mutant ovule. Since *pop2* tubes have a 4-fold increase in GABA, this observation implies that POP2-mediated GABA removal is necessary for pollen tubes to respond to increasing GABA levels. Signal attenuation is a common strategy used for the perception of a gradient signal. For example, chemotropic response to cAMP in *Dictystostelium* cells also requires cAMP degradation. Since POP2 is localized in the cytoplasm and, thus, most likely degrades internalized GABA in pollen tubes, GABA could be perceived by a cytoplasmic receptor. In neurons, however, all GABA receptors are localized to the plasma membrane. If GABA is perceived by a PM-localized receptor, the internalized or cytoplasmic GABA must be recycled to the cell surface or mediate a positive-feedback regulation of GABA signaling.

This report also demonstrates an important role for GABA in pollen tube growth regulation. GABA levels (20–100 μM) matching those in the stigma, style, and septum promoted cultured pollen tube growth. This finding may provide some new insights into the mechanism of pollination. First, it can explain why in vivo tubes grow much faster than cultured tubes, a phenomenon known for a long time, but lacking a molecular explanation. Second, the GABA gradient may help to speed up tube growth on its way to the ovule. Growth acceleration along its path together with guidance signaling could ensure that each tube efficiently reaches its target. A role for this gradient in regulating growth, but not guidance, can also explain why both WT and *pop2* pollen tubes reach the septum normally in the *pop2* pistil, where the septum-to-stigma gradient is disrupted because of GABA overflooding.

How does GABA regulate growth? It could function as a signal to promote polarized growth or provide nutrient sources, similar to the action of TTS (a tobacco glycoprotein that stimulates and attracts pollen tubes) (Wu et al., 1995). TTS can be deglycosylated by pollen tubes

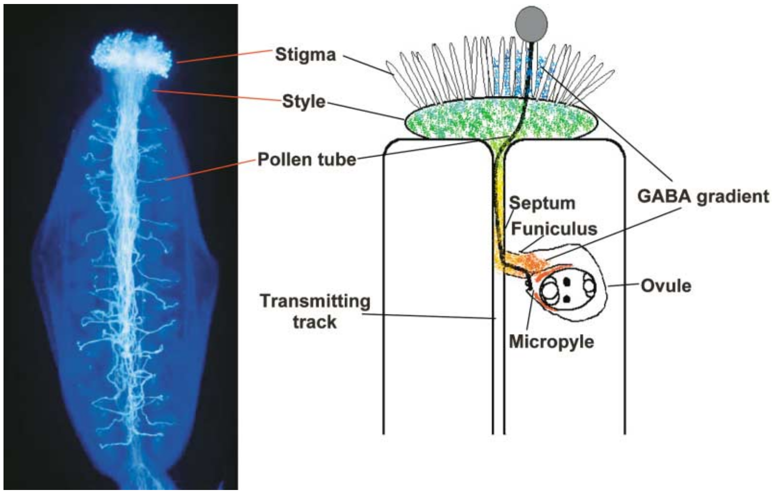

Figure 1. GABA Gradient and Pollen Tube Guidance

(A) A stained pistil showing that each ovule is targeted by one of the numerous pollen tubes extending from the stigma surface. Pollen tubes (white) were stained with aniline blue, while the unstained pistil is shown as the blue background.

(B) A diagram depicting a GABA gradient from the ovule to the stigma. Color-coding indicates the gradient (red, high GABA; yellow and green, medium GABA; blue, low GABA).

and exhibits a glycosylation gradient in the style. While TTS is specific to the style and only provides a carbon source, GABA appears to have a broader growth stimulatory role, as it can do so in the stigma, style, and septum. GABA also has the potential to serve as a source of both carbon and nitrogen.

An interesting question is whether there are common mechanisms underlying how various signals can regulate both growth and guidance in different systems. Growth stimulation and attraction could be coupled by a single mechanism when the stimulatory molecule forms a gradient, as do GABA and TTS. All cells capable of guidance are tip-growing cells; presumably, the region capable of sensing growth and guidance signals is also restricted to the tip. As such, spatial distribution of a gradient of extracellular signals could determine growth direction.

This report has identified an exciting new player in the plant mating game, but the role of GABA is far from clear, given the complexity of the system. Recent studies from Lord's group show that SCA (stigma/stylar cysteine-rich adhesin) acts together with pectin to mediate pollen tube adhesion and may also facilitate pollen tube attraction (Lord, 2003). Similarly, GABA also seems to require partners, as locally applied GABA alone is insufficient to attract pollen tubes. Other ovule-derived signals, e.g., diffusible signals from synergid cells, are clearly required for guidance to the ovule (Higashiyama et al., 2001). Therefore, multiple coordinating signals ensure successful targeting of one ovule by one pollen tube. GABA could also act in a redundant pathway, as shown for GABA in neuron development (Owens and Kriegstein, 2002). It will be interesting to see the effect of eliminating GABA gradients on pollen tube growth and guidance.

In short, this report significantly advances our understanding of the mystery behind plant mating and expands the role of GABA as a ubiquitous regulatory molecule. This work also raises more questions than it answers concerning the role of GABA in pollination. What is the precise role of GABA in pollen tube growth and guidance? How is the GABA gradient established? What other factors work together with GABA to attract pollen tubes? What is the receptor(s) for GABA? How does GABA signaling impact intracellular signaling machinery controlling polarized growth, like Rho GTPase pathways and calcium?

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Selected Reading

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