

Plant Reproduction: GABA Gradient, Guidance and Growth Dispatch

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How a pollen tube manages to navigate through the female tissues during plant reproduction has been a mystery. A new analysis of an *Arabidopsis* mutant has provided the strongest evidence yet that a GABA gradient may be a critical signal for correct targeting of the pollen tube.

In animals, the amino acid γ -amino butyric acid, GABA for short, is well known for its role as an inhibitory neurotransmitter [1]. In recent years, GABA has also been found to play excitatory roles in developing nervous systems [2,3]. In plants, GABA may be involved in development, as well as defense and other stress responses [4–6]. Palanivelu *et al.* [7] have now reported strong evidence that a gradient of GABA is critical for the normal growth and guidance of a pollen tube in the female tissues to achieve the accurate targeting of the pollen tube for the delivery of sperm cells.

During sexual reproduction of flowering plants, pollen grains are deposited onto the surface of the female structure called the stigma (Figure 1). Proper interaction of the pollen grain and the stigma results in pollen tube germination. The newly formed pollen tube is able to penetrate the surface of the stigma cells and grow inside the female tissues. The growing pollen tube provides a passage for the sperm cells to reach and eventually fuse with the egg cell and another cell called the central cell, which are deeply buried inside the ovary, in the female tissues of the ovule (Figure 1). So for the sperm cells to reach the egg and central cell, the pollen tube must correctly navigate through several female tissues.

How this navigation is accomplished has been a mystery. Genetic studies suggested that the ovule (Figure 1) may send an attractive signal [8,9]. In mutants where the diploid tissues of the ovule appear normal but the female gametophytes develop abnormally, pollen tube guidance is severely affected. This implies that the haploid female gametophyte has a critical role in pollen tube guidance. Ablation experiments indicated that the synergid cells at the opening called the micropyle [10] are required for attracting the pollen tube, although the nature of the attractive signal was not established. Because mutants defective in the development of the diploid ovule tissues also have abnormal female gametophytes, it is not clear whether the diploid tissues have a separate role in pollen tube guidance.

Other studies support the idea that the pollen tube itself also plays a critical role in determining its growth

pathway. The properties of one mutant in particular, *pop2*, suggest that a single gene may act on both the male and female sides of this interaction [11]. The homozygous *pop2* mutant is self-sterile. Examination of pollen and ovule development revealed no abnormality, suggesting that the defect is in the interaction between the two. Indeed, the mutant pollen tube can germinate and penetrate the stigma, but it wanders aimlessly afterwards, even when it comes close to the egg cell by chance (Figure 2). Pollen tube guidance is thus abnormal in this mutant.

To understand how *POP2* affects pollen tube guidance, Palanivelu *et al.* [7] isolated the gene by positional cloning. This revealed that *POP2* encodes a transaminase which had previously been shown to behave as a GABA transaminase *in vitro* [12]. Indeed, biochemical tests indicated that *pop2* mutant tissues have more than 100-fold greater GABA levels than wild-type tissues, whereas the levels of other amino acids are not affected. Further evidence for the elevation of GABA being the cause of the fertility defect in the *pop2* mutant came from the analysis of the wild-type and three different *pop2* mutant alleles. Even within the same plant, flowers on the branches accumulate less GABA and have higher fertility than those on the main stem.

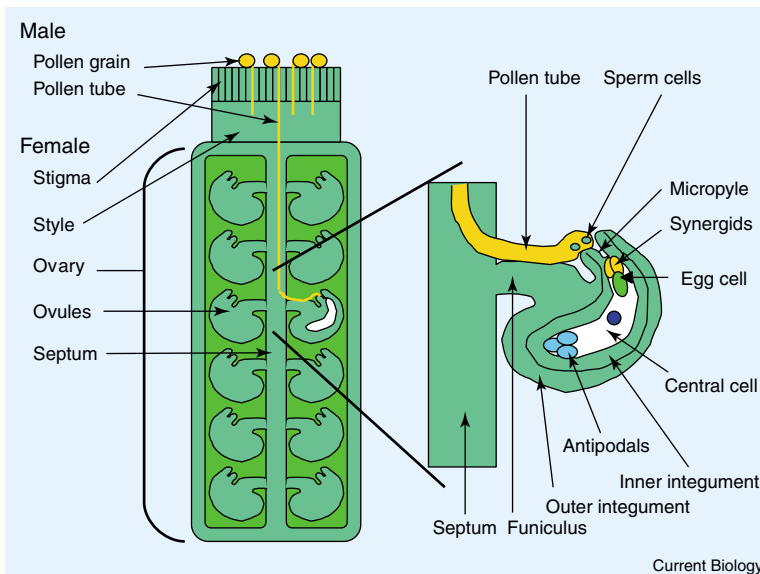
How can the levels of GABA affect pollen tube guidance? A plausible idea is that a gradient of GABA might provide the positional cue for attracting pollen tubes to the correct final target. To test this hypothesis, Palanivelu *et al.* [7] measured the levels of GABA in different parts of the female reproductive organ, along the path of pollen tube growth. In the wild-type pistil, from the stigma, with which pollen interacts initially, to the style and septum, and finally to the inner integument, the part of the ovule that encloses the egg and central cells, the GABA levels gradually increase from 20 to 60 μM , then to 160 μM , and finally to 500 μM . In contrast, the levels of GABA in the *pop2* mutant stigma, style, and septum were found to be abnormally high, at levels from 1200 to 1900 μM , and even higher (24,000 μM) in the inner integument.

Thus there is a gradient of GABA levels along the path of pollen tube growth, and the *pop2* mutation results in a great elevation of GABA levels and the virtual elimination of the gradient along much of this path. How does *POP2* affect the differential levels of GABA in different female tissues? Immunolocalization experiments of the *POP2* protein revealed that it is readily detected in tissues — outer integument — that have relatively low levels of GABA, but barely or not at all in tissues — inner integument — with high GABA levels. In the *pop2* mutant ovules, both the inner and outer integuments have high levels of GABA.

The fact that a GABA gradient exists along the path of pollen tube growth is consistent with the GABA gradient being an important positional cue. Does the *pop2* mutant phenotype support the idea of pollen tube

Figure 1. The *Arabidopsis* female structure at the time of pollination.

On the left is a pistil, with the stigma at the top, a short style, and a long ovary, which contains a number of ovules. On top of the stigma are several pollen grains, the pollen tubes of which have germinated and grown into the pistil; one of the pollen tubes has grown through the septum and reached the ovule. On the right is an enlarged view of an ovule with the most distal portion of a pollen tube, which contains two sperm cells. The ovule is connected to the septum by the funiculus. The diploid tissues of the ovule include the outer and inner integuments, which envelope the haploid female gametophyte. Seven cells make up the female gametophyte: the egg cell and two synergids situated near the micropyle, the three antipodals at the other end, and the central cell with a large diploid nucleus.



guidance by a GABA gradient? Careful examination showed that the rate of pollen tube growth from stigma to septum is similar in wild-type and the *pop2* mutant ovaries, suggesting that the early portion of the gradient is not essential for proper pollen tube growth. In contrast, after the *pop2* pollen tubes exit the septum tissue, they grow in a way that seems to be undirected. Even when the pollen tube tip comes close to the micropyle by chance, it continues to march on, oblivious of the nearby micropyle (Figure 2). These observations are consistent with an important role for a GABA gradient along the later portion of the pollen tube path, because the cells around the micropyle normally have the highest GABA levels, whereas in the *pop2* mutant, ovules and funiculus also have very high levels.

These results suggest that POP2 function is important in female tissues to establish a GABA gradient. Reciprocal crosses, however, indicate that the *pop2* mutant is male and female fertile when crossed with the wild type. In particular, wild-type pollen tubes were found to efficiently target the micropyle in the *pop2* mutant ovary. Palanivelu *et al.* [7] suggest that, when a wild-type pollen tube grows through *pop2* female tissues, the POP2 activity in the pollen tube is able to reduce GABA levels, allowing the pollen tube to sense the higher levels further along the normal path. The fact that *pop2* pollen tubes can also reach the micropyle in a wild-type ovary, which has a normal GABA gradient, indicates that POP2 activity in the pollen is not critical when the female environment is normal.

In addition to their abnormal guidance, *pop2* pollen tubes exhibit growth arrest in a *pop2* ovary, suggesting that GABA may also regulate pollen tube growth. Consistently, GABA was found to promote pollen tube growth *in vitro* at low to medium levels, but inhibit pollen tube growth at high levels. Moreover, *pop2* pollen tubes are sensitive to the inhibition by GABA at lower levels, even those that still promote wild-type pollen tubes. It is reasonable to propose that wild-type pollen can reduce GABA to a level that does not inhibit

growth in both normal and *pop2* pistils, whereas a *pop2* pollen tube cannot reduce the GABA level and is inhibited by the high GABA levels in the *pop2* pistil after the exit from the septum. Furthermore, *in vitro* tests were not able to demonstrate a role for GABA as the attractive signal for pollen tube growth, indicating that other molecules are needed.

Regardless of whether the GABA gradient indeed provides a signal for pollen tube guidance or high levels of GABA inhibit pollen tube growth *in vivo*, or both, it is not known how GABA acts to cause such end results. In animal neural systems, GABA binds to surface receptors, in some cases — the so-called ‘ionotropic’ receptors — regulating the activities of ion channels [13]. The *Arabidopsis* genome sequence has not yet revealed any homologs of such animal GABA receptors. Animals also have G-protein-coupled, ‘metabotropic’ GABA receptors [13]; homologs of G proteins and G-protein-coupled receptors have been identified in plants, although their direct interactions have not been demonstrated [14,15]. The only identified *Arabidopsis* gene for a canonical heterotrimeric G protein α subunit, *GPA1*, is expressed in the pollen tube [16], although it is not known whether it plays a role in pollen tube growth or guidance.

It is known, nevertheless, that pollen tube growth involves highly dynamic extracellular and intracellular Ca^{2+} gradient and pH changes [17]. The synthesis of GABA in plant cells is closely associated with Ca^{2+} and H^+ levels [4], suggesting a possible mechanism to couple GABA level with the regulation of pollen tube growth. In addition, pollen tube growth consumes a lot of energy, and many mitochondria are present at the growing region of the tube [18]. The synthesis of GABA followed by the reaction catalyzed by POP2 is an alternative way to generate succinate, which is then used by the Krebs cycle for energy production. The *pop2* mutation might block the flow of GABA to succinate, perhaps reducing energy production in the pollen.

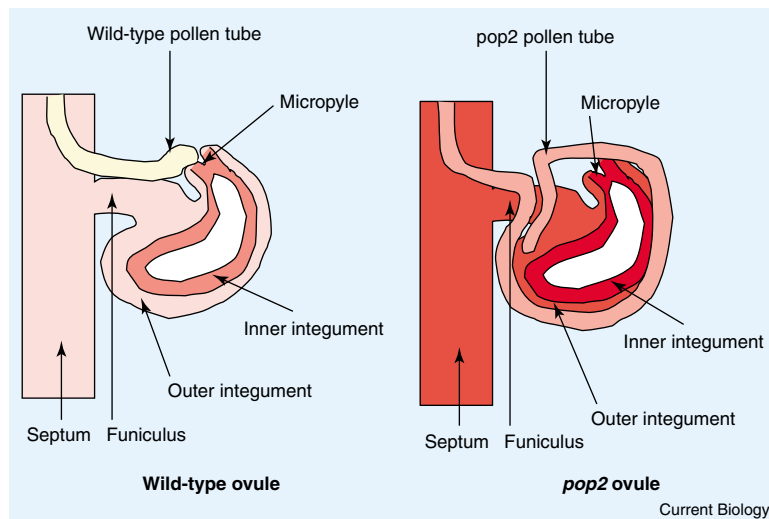


Figure 2. GABA levels in wild-type and *pop2* mutant ovules.

A wild-type ovule is shown on the left, and a *pop2* ovule on the right. In the wild-type ovule, the inner integument (medium shade) has a much higher level of GABA than other tissues of the ovule or the septum (light shade). The pollen tube grows towards the micropyle. In the *pop2* mutant ovule, the levels of GABA in the entire ovule, including the funiculus and outer integument (medium shade), are very high, and that in the inner integument is even higher (dark shade). The *pop2* pollen tube fails to grow toward the micropyle. When it is near the micropyle, it does not stop, but rather continues beyond the correct final destination.

In conclusion, the new genetic and molecular studies of the *Arabidopsis POP2* gene [7] clearly demonstrate a role for normal levels of GABA in plant reproduction, particularly pollen tube growth and/or guidance. The available molecular data suggest that the mechanism for GABA activity in pollen tube growth and guidance is likely to be different from those controlling animal neuronal synaptic activities of GABA.

References

- Petroff, O.A. (2002). GABA and glutamate in the human brain. *Neuroscientist* 8, 562-573.
- Owen, D.F. and Kriegstein, A.R. (2002). Is there more to GABA than synaptic inhibition? *Nature Rev. Neurosci.* 3, 715-727.
- Ben-Ari, Y. (2002). Excitatory actions of GABA during development: the nature of the nurture. *Nature Rev. Neurosci.* 3, 728-739.
- Bown, A.W. and Shelp, B.J. (1997). The metabolism and functions of γ -aminobutyric acid. *Plant Physiol.* 115, 1-5.
- Kathiresan, A., Miranda, J., Chinnappa, C.C. and Reid, D.M. (1998). Gamma-Aminobutyric acid promotes stem elongation in *Stellaria longipes*: the role of ethylene. *Plant Growth Reg.* 26, 131-137.
- Shelp, B.J., Bown, A.W. and McLean, L.D. (1999). Metabolism and functions of gamma-aminobutyric acid. *Trends Plant Sci.* 4, 446-452.
- Palanivelu, R., Brass, L., Edlund, A.F. and Preuss, D. (2003). Pollen tube growth and guidance is regulated by *POP2*, an *Arabidopsis* gene that controls GABA levels. *Cell* 114, 47-59.
- Hulskamp, M., Schneitz, K. and Pruitt, R.E. (1995). Genetic evidence for a long-range activity that directs pollen tube guidance in *Arabidopsis*. *Plant Cell* 7, 57-64.
- Ray, S.M., Park, S.S. and Ray, A. (1997). Pollen tube guidance by the female gametophyte. *Development* 124, 2489-2498.
- Higashiyama, T., Yabe, S., Sasaki, N., Nishimura, Y., Miyagishima, S., Kuroiwa, H. and Kuroiwa, T. (2001). Pollen tube attraction by the synergid cell. *Science* 293, 1480-1483.
- Wilhelmi, L.K. and Preuss, D. (1996). Self-sterility in *Arabidopsis* due to defective pollen tube guidance. *Science* 274, 1535-1537.
- Van Cauwenberghe, O.R., Makhmoudova, A., McLean, M.D., Clark, S.M. and Shelp, B.J. (2002). Plant pyruvate-dependent gamma-aminobutyrate transaminase: Identification of an *Arabidopsis* cDNA and its expression in *Escherichia coli*. *Can. J. Bot.* 80, 933-941.
- Watanabe, M., Maemura, K., Kanbara, K., Tamayama, T. and Hayasaki, H. (2002). GABA and GABA receptors in the central nervous system and other organs. *Int. Rev. Cytol.* 213, 1-47.
- Ma, H. (1994). GTP-binding regulatory proteins in plants: New members of an old family. *Plant Mol. Biol.* 26, 1611-1636.
- Colucci, G., Apone, F., Alyeshmerni, N., Chalmers, D. and Chrispeels, M.J. (2002). *GCR1*, the putative *Arabidopsis* G protein-coupled receptor gene is cell cycle-regulated, and its overexpression abolishes seed dormancy and shortens time to flowering. *Proc. Natl. Acad. Sci. U.S.A.* 99, 4736-4741.
- Weiss, C.A., Huang, H. and Ma, H. (1993). Immunolocalization of the G protein α subunit encoded by the *GPA1* gene in *Arabidopsis*. *Plant Cell* 5, 1513-1528.

- Holdaway-Clarke, T.L., Weddle, N.M., Kim, S., Robi, A., Parris, C., Kunkel, J.G. and Hepler, P.K. (2003). Effect of extracellular calcium, pH and borate on growth oscillations in *Lilium formosanum* pollen tubes. *J. Exp. Bot.* 54, 65-72.
- Mascarenhas, J.P. (1993). Molecular mechanisms of pollen tube growth and differentiation. *Plant Cell* 5, 1303-1314.