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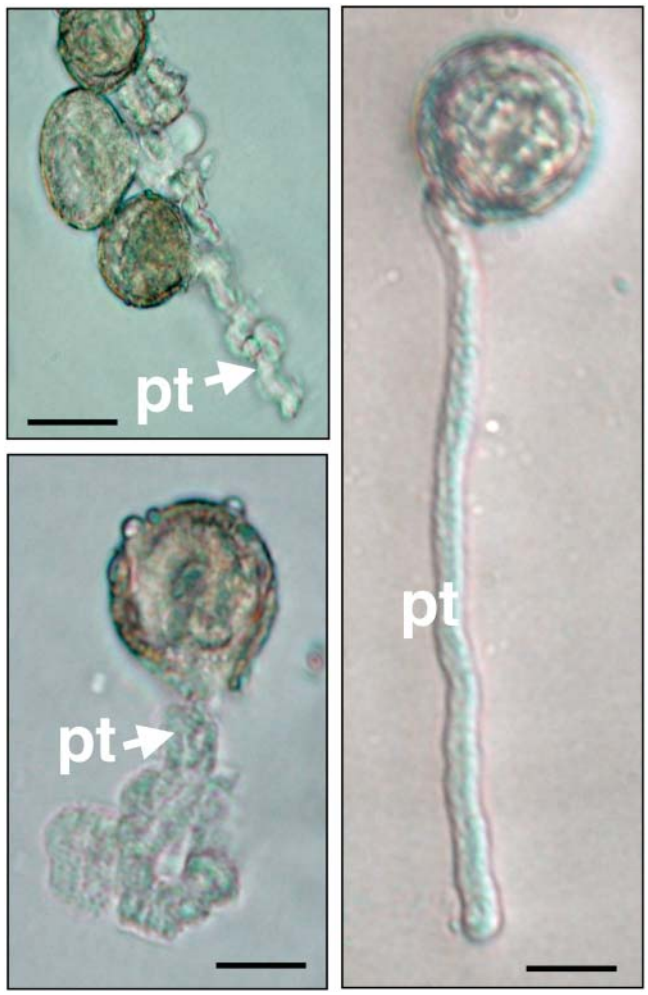
VANGUARD1—At the Forefront of Pollen Tube Growth

Pollen tube growth is one of the most fascinating—and essential—phenomena in the life cycle of flowering plants. After a compatible interaction between pollen grains and the stigma surface, the pollen germinates and forms the pollen tube, which grows through the stigma, style, and transmitting tract to deliver the sperm cells to the ovule. Pollen tube growth occurs only at the extreme apex of the tube through the mechanism of polarized tip growth (reviewed in Hepler et al., 2001). Other systems that use polarized tip growth include plant root hairs, fern and moss protonemata, and fungal hyphae. In each of these cases, tip growth involves responses to external signals that guide the direction of growth toward a specific goal. In the case of pollen tubes, growth is directed precisely to the egg to achieve the ultimate goal of fertilization. Our understanding of the mechanisms of pollen tube growth and navigation is increasing, but critical gaps remain. Calcium ions and protons play key roles in the control of pollen tube growth, but their precise roles, as well as how their concentrations are regulated and how they are connected to various signaling proteins, remain to be determined.

KEY FEATURES OF POLLEN TUBE GROWTH

After germinating on the stigmatic surface, the pollen grain forms a tube that elongates exclusively at the apex (reviewed in Franklin-Tong, 1999; Lennon and Lord, 2000; Holdaway-Clarke and Hepler, 2003). Callose plugs are laid down at regular intervals behind the growing tip, and the region adjacent to the plug becomes vacuolated, which serves to maintain a region of concentrated cytoplasm, containing organelles and the sperm nuclei, near the growing tip. The extreme end of the growing tip consists of a highly dynamic clear zone that contains vesicles and cell wall precursors. Activity in this zone involves continual biosynthesis of cell wall and plasma membrane and turnover of cytoskeletal components as the tube elongates and interactions with the surrounding tissues that guide growth toward the ovule (reviewed in Feijó et al., 2004).

One of the key features of growing pollen tubes is a steep tip-focused Ca^{2+} gradient. The gradient is thought to be maintained by influx of extracellular Ca^{2+} through Ca^{2+} channels active only at the extreme end of the growing tip and dissipation of Ca^{2+} away from the tip where Ca^{2+} channels are not active (Franklin-Tong, 1999). Pollen tube growth follows an oscillatory pattern, wherein regular bursts of rapid growth rate



Growth of *vgd1* Pollen Tubes.

Arabidopsis *vgd1* mutant pollen tubes grown in vitro exhibit retarded growth and unusual shapes (top left) and usually burst open (bottom left) compared with normal wild-type pollen tubes (right).

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are followed by brief quiescent periods. Work with rapidly growing pollen tubes from *Lilium longiflorum* (Easter lily) has shown that the internal free Ca^{2+} concentration and Ca^{2+} influx at the growing tip oscillate out of phase with growth rate; the peak internal calcium concentration lagging 1 to 4 s and Ca^{2+} influx lagging 11 to 12 s behind peak growth rates (Holdaway-Clarke et al., 1997; Messerli et al., 2000). By analyzing irregularities in the oscillations, Holdaway-Clarke et al. (1997) concluded that oscillations in Ca^{2+} influx do not appear to drive tip growth, but rather Ca^{2+} influx lags behind growth rate such that the growth rate at a given point determines the magnitude of Ca^{2+} influx ~11 s later. Nonetheless, maintenance of the dynamic Ca^{2+} gradient is essential for pollen tube growth and seems to involve complex interactions among ROP GTPases, the actin cytoskeleton, and Ca^{2+} at the level of intracellular signaling (Li et al., 1999; Gu et al., 2003).

There is also evidence that oscillatory behavior of protons is important for pollen tube growth, as high concentrations of protons in the tip region (i.e., low pH) are correlated with the most rapid growth rates (Feijó et al., 1999). In addition, it has recently been shown that there is a gradient in the concentration of γ-amino butyric acid in pollen tubes that is important in regulating growth and guidance toward the ovule (Palanivelu et al., 2003). How these intracellular gradients associated with tip growth are linked to extracellular activities is an intriguing and largely unexplored area in the field of pollen tube growth.

Growing pollen tubes must maintain a delicate balance between loosening of the cell wall to allow for rapid cell elongation and maintaining sufficient rigidity to withstand the considerable turgor pressure within the growing tip. Ca^{2+} and protons are both intimately linked to dynamic properties of the cell wall, principally through their influence on pectin, a heterogeneous polymer of polygalacturonic acids that is a major component of cell walls. Ca^{2+} ions bind to anionic acid moieties of nonesterified homogalacturonan molecules in pectin, creating cross links between molecules that provide increased rigidity to the pectin polymer and cell wall (reviewed in Vorwerk et al., 2004). On the other hand, protons promote the activity of endopolygalacturonases, which act to loosen cell walls by depolymerizing pectin molecules.

ROLE OF PECTIN METHYLESTERASES AND *VANGUARD1*

Pectin is thought to be secreted in the methylesterified state into the tip region of growing pollen tubes (Goldberg et al., 1996). Pectin methylesterases (PMEs) are considered to be key regulators of cell wall rigidity, as they catalyze the demethylesterification of homogalacturonans, thereby exposing the anionic sites where Ca^{2+} can bind. The cell wall at the growing tip of pollen tubes is highly pectinaceous, so the degree of Ca^{2+} cross-linking—and thus the activity and localization of PMEs—may be of particular importance in controlling pollen tube growth.

In this issue of *The Plant Cell*, **Jiang et al. (pages 584–596)** report a significant advance in our understanding of the mechanics of pollen tube growth. Previous reports have provided evidence that PMEs function in pollen tubes and that the pectin methylation state, both in pollen tubes and surrounding female tissues, is an important parameter in controlling pollen tube growth. For example, PMEs have been isolated from willow (Futamura et al., 2000) and maize (Wakeley et al., 1998) that are expressed specifically in mature pollen grains. However, functional characterization of a PME and genetic evidence of its role in pollen tube growth has been lacking. The authors show that *VANGUARD1* (*VGD1*) encodes a pollen-specific PME that is required for the normal growth of pollen tubes in vivo and provide solid genetic and biochemical evidence that pectin demethylesterification catalyzed by VGD1 is indeed a critical component of pollen tube growth.

The authors isolated the *vgd1* mutant in a screen for reduced fertility from *Dissociation* (*Ds*) transposon insertion lines of Arabidopsis. Homozygous *vgd1* mutants produced smaller siliques with fewer seeds than the wild type, which was associated with a single *Ds* insertion affecting only the male gametophytic function. Further analysis showed that pollen was able to germinate, but growth of *vdg1* pollen tubes through the transmitting tract of the style was retarded. Pollen tubes were morphologically normal when grown on stigmatic tissue, and guidance toward the ovule was unaffected, but pollen tubes grown in vitro (on an agar-sucrose medium) were misshapen, grew more slowly than wild-type pollen tubes, and more than 90% of them burst open, indicating structural instability (see figure).

VGD1 was cloned and found to encode a PME-homologous protein containing a secretion-related transmembrane domain, a PME inhibitor-homologous domain, and a PME-homologous domain. The authors next assayed PME activity in crude pollen extracts and found the activity associated with *vgd1* pollen reduced to 82% of that measured in wild-type pollen. This suggests that VGD1 has PME activity in vivo, but it is likely not the only PME enzyme active in pollen. Comparison with the Arabidopsis genome revealed two other genes encoding PME-homologous proteins with >50% similarity to *VDG1*, one of which is located at the same locus as *VGD1* along with a third gene encoding a smaller protein with lower homology to *VGD1*.

DUAL ROLE FOR VGD1?

Depending on enzyme characteristics and conditions in the medium, PMEs may catalyze demethylesterification in a linear fashion or at random points along a pectin chain. These two types of reactions produce different products that may have distinct functions (Micheli, 2001). The linear reaction produces the anionic moieties that can interact with Ca^{2+} to create the pectate gel that provides increased rigidity to the cell wall, whereas random demethylesterification releases protons that contribute to the loosening of cell walls by promoting the activity of endopolygalacturonases. Historically, it was believed that acidic PMEs generally catalyze random demethylesterification and basic PMEs catalyze the linear reaction, but there is evidence that at least

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some PME isoforms can act randomly or linearly depending on environmental conditions (Micheli, 2001).

VGD1 has a predicted isoelectric point of a basic PME (corresponding to pH 8.9), and *vgd1* mutant pollen tubes with decreased PME activity burst when grown in vitro, both of which suggest that the enzyme functions within the pollen tube cell wall to strengthen the growing pollen tube (i.e., through linear demethylesterification). However, it is also possible that the enzyme catalyzes random demethylesterification, for example, under conditions of low pH. Oscillations in pH at the growing tip might even cause oscillations in the activity of VGD1 between random and linear demethylesterification to promote wall loosening during periods of peak growth rate rapidly followed by brief periods of wall strengthening, respectively. Although there are no data to support this idea, it is consistent with the observation that pH oscillates in the tip region in phase with growth (lowest pH coinciding with peak growth rate; Feijó et al., 1999). VGD1 might also be secreted outside the pollen tube tip and act within the extracellular matrix of the female tissues in the stigma, style, and transmitting tract. If conditions here were favorable for linear demethylesterification, VGD1 might act to facilitate loosening of the cell wall in these tissues to promote penetration by the growing tube.

Jiang et al. show that VGD1, and likely other pollen-specific PMEs, are essential for controlling the rapid growth rate of elongating pollen tubes. It may be that the family of pollen-specific *PME* genes in Arabidopsis (as well as other plants) encodes enzymes with distinct activities (e.g., wall loosening versus wall strengthening) in the growing pollen tube or that VGD1 and other PMEs have more than one type of activity, depending on pH and/or other conditions. Future research will need to pinpoint the precise location of action and in vivo catalytic function of each of these enzymes (e.g., random versus linear demethylesterification) as well as the parameters that control their localization and activity, including intracellular and intercellular signaling events, such as ROP GTPase and calcium signaling, and signaling related to navigation of the growing tube toward the ovule.

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