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Review

How pollen tubes grow

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Abstract

Sexual reproduction of flowering plants depends on delivery of the sperm to the egg, which occurs through a long, polarized projection of a pollen cell, called the pollen tube. The pollen tube grows exclusively at its tip, and this growth is distinguished by very fast rates and reaches extended lengths. Thus, one of the most fascinating aspects of pollen biology is the question of how enough cell wall material is produced to accommodate such rapid extension of pollen tube, and how the cell wall deposition and structure are regulated to allow for rapid changes in the direction of growth. This review discusses recent advances in our understanding of the mechanism of pollen tube growth, focusing on such basic cellular processes as control of cell shape and growth by a network of cell wall-modifying enzymes, molecular motor-mediated vesicular transport, and intracellular signaling by localized gradients of second messengers.

Keywords: Pollen tube growth; Pectin methylesterases; Molecular motors; Rab and Rop/Rac GTPases

Introduction: pollen and pollen tube

Sexual reproduction of flowering plants requires delivery of the sperm to the egg. The process begins with deposition of the pollen grain, containing the male gametes and delivered by an insect, wind or other means, on the female stigmatic tissue. If the pollen–stigma interaction is compatible, the pollen grain hydrates and germinates shortly following landing on the stigma. During germination, a defined area in the pollen plasma membrane – the tip growth domain to which post-Golgi vesicles are targeted and fused, promoting directional growth – is established, and the pollen tube elongation begins, often reaching astounding rates of growth. For example, the maize pollen tube can grow as fast as 1 cm/h and extend to about 1 ft in length within 24 h (Barnabas and Fridvalszky, 1984).

During the elongation process, the pollen cytoplasm, vegetative nucleus and sperm cells are transported within the tube, which grows through intercellular spaces in the pistil. To serve as a conduit through which the fertilizing sperm cells can travel, the tube plots its course through the transmitting tissues, eventually reaching the eggs within the ovary (Johnson and Preuss, 2002; Kim et al., 2003; Palanivelu and Preuss, 2000; Ray et al., 1997). Molecular cues, such as the transmitting tissue-specific (TTS) protein in tobacco (*Nicotiana tabacum*) (Cheung et al., 1995) or gamma-aminobutyric acid (GABA) in *Arabidopsis* (Palanivelu et al., 2003), are located within the pistil tissues and guide the tube to deliver the sperm cells to the embryo sac for fertilization (Cheung et al., 1995; Johnson and Preuss, 2002; Lord and Russell, 2002). Additionally, the female tissues provide pollen tube-attracting signals as has been shown using mutants with delayed development of the embryo sac, that fail to attract nascent pollen tubes (Shimizu and Okada, 2000).

Over decade ago, two major biochemical mechanisms driving the pollen tube elongation were discovered: a steep calcium gradient within the pollen tube tip and the contribution of actin microfilaments to the elongation process. Pollen tubes exhibit a sharp, tip-focused intracellular calcium gradient that drives and orients their apical growth. While plant cells generally maintain a cytosolic calcium concentration of approximately 100 nM, during pollen tube growth a much higher concentration of several micromolar calcium is

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found in the apical few micrometers of the growing tip (see Holdaway-Clarke and Hepler, 2003 for review; Malhó et al., 1994). Apical calcium concentration in the nascent lily (*Lilium longiflorum*) pollen tubes, for instance, is estimated at 3–5 μM, while the rest of the tube retains the normal cellular calcium levels (~100 nM). Interestingly, this steep apical gradient returns to the basal cellular level within ~20 μm from the tip apex (Miller et al., 1992; Obermeyer and Weisenseel, 1991; Pierson et al., 1994; Rathore et al., 1991). Several studies have shown, that calcium gradient dissipation by various methods, including BAPTA buffer injections, mild thermal shock, calcium channels blockers and others, lead to the inhibition of the pollen tube growth (Li et al., 1996; Pierson et al., 1994; Rathore et al., 1991). Also, artificial generation of a focused elevated internal calcium level through localized photolysis of caged calcium has altered tip growth directionality, again supporting the notion of the necessity of the calcium gradient for the pollen tube growth (Malhó and Trewavas, 1996). The gradient high-point is in the immediate vicinity of the tip apex, and it appears to be derived, at least to some extent, from the influx of extracellular calcium through stretch-responsive channels activated by deformations in the nascent tip wall (Holdaway-Clarke et al., 1997; Pierson et al., 1994, 1996). However, although calcium-permeable channels are likely to contribute to the gradient formation and control, the specific molecular mechanisms of this process remain largely unknown (Dutta and Robinson, 2004).

Actin is essential for the polarized tip growth (Geitmana et al., 2000; Vidali and Hepler, 2001; Vidali et al., 2001). Together with myosin motors, actin microfilaments support vesicular transport and other crucial processes critical for the tube growth, since inhibition of actin polymerization by drugs, such as latrunculin B and cytochalasin B, effectively blocks pollen tube elongation (Gibbon et al., 1999; Miller et al., 1999). Although the abundance of the longitudinal actin filaments within the pollen tube is beyond doubt, it remains unclear which specific form(s) or population(s) of the F-actin are directly involved in the tip growth.

The role of microtubules in pollen tube growth, on the other hand, has been quite puzzling, since in two species of the *Nicotiana* family, tobacco and *Nicotiana alata*, and in *Endymion nonscriptus*, pharmacological disruption of microtubules by oryzalin or colchicine had no effect on pollen tube elongation (Åström et al., 1995; Heslop-Harrison et al., 1988; Laitiainen et al., 2002). Conversely, in Norway spruce (*Picea abies*), oryzalin or colchicine treatments partially blocked pollen germination and growth, generating swollen tips and shorter tubes (Anderhag et al., 2000). Also, microtubule inhibitors colchicine and propham inhibited pollen tube growth in another *Nicotiana* species, *Nicotiana sylvestris* (Joos et al., 1994).

The functions of the actin microfilaments and their myosin motors and the roles of ionic gradients, mainly that of calcium, in the pollen tube growth, the mechanisms of the pollen chemotaxis to the ovule, and the identity and action of pistil-guiding signals have been the subjects of numerous excellent reviews (Bedinger, 1992; Feijo et al., 2001; Hepler et al., 2001; Higashiyama et al., 2003; Johnson and Preuss, 2002; Mascarenhas, 1993; McCormick and Yang, 2005; Palanivelu and Preuss, 2000; Taylor and Hepler, 1997). Conversely, less attention was paid to recent significant advances in our understanding of the roles of (i) the cell wall-modifying enzymes pectin methylesterases (PMEs) and cellulose synthases, (ii) small GTPases involved in the regulation of traffic of membrane vesicles and dynamics of actin microfilaments, (iii) microtubule-associated molecular motors dyneins and kinesins, and (iv) second messengers, such as the calcium/phospholipid system and cAMP. This review discusses our up-to-date knowledge of these aspects of the pollen tube growth.

Cell wall-modifying enzymes: PMEs and cellulose synthases

Pectin methylesterases (PMEs)

In most plant species, the pollen tube cell wall consists of two layers, the inner sheath of callose and outer coating containing mainly pectin with cellulose and hemicellulose. The pollen tube grows exclusively at its tip, where the newly synthesized cell wall is continually forming (Taylor and Hepler, 1997). A single pectin layer, lacking callose or cellulose, forms the tip cell wall (Ferguson et al., 1998) and provides this area with sufficient rigor to maintain the cellular integrity, on the one hand, and with plasticity to allow directional tube growth, on the other (Steer and Steer, 1989).

During the tube elongation process, pectins are polymerized, as well as methyl esterified and modified with side chains within the Golgi apparatus, and transported in the Golgi-derived vesicles to the pollen tip (Li et al., 1995; Staehelin and Moore, 1995; Sterling et al., 2001). Following vesicle discharge, homogalacturonan – a linear polymer composed of 1,4-α-D-galacturonic acid residues, which represent the major component of pectin – is gradually deesterified by pectin methylesterases (PMEs). The deesterification of the polygalacturonic acid chain converts the methoxyl groups into carboxyl groups, exposing the acidic residues which then are cross-linked by calcium ions, creating a new layer of pectin (Catoire et al., 1998). The key role of PMEs during cell wall formation requires tight regulation of this enzymatic activity, which is achieved most likely through the properties of the surrounding medium, with pH being one of the key parameters. Specifically, the localized reduction in pH, due to the deesterification process, may promote cell wall relaxation by stimulating the activity of several cell wall-loosening hydrolases, such as polygalacturonases and pectate lyases (Ren and Kermode, 2000; Wen et al., 1999). The interplay between these opposing effects – cell wall stiffening by the PME action and cross-linking by calcium versus cell wall loosening by different hydrolases – likely regulates the directional growth of the pollen tube tip (Bordenave, 1996; Catoire et al., 1998; Moustacas et al., 1991).

PMEs represent a large family of plant enzymes originally discovered and characterized in the kiwi fruit (Balestrieri et al., 1990; Camardella et al., 2000; Giovane et al., 1995). For