Plant Cell Monogr (3)
R. Malhó: The Pollen Tube
DOI 10.1007/7089_041/Published online: 26 January 2006

The Pollen Tube: A Model System for Cell and Molecular Biology Studies

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Abstract Pollen tubes, the active male gametophytes of seed plants, are the vectors carrying the male sperm cells to the egg cell of the female gametophyte in the ovules of seed plants. Unlike most plant cells in which growth occurs by modification of the existing wall and the insertion of new material throughout its surface, pollen tubes extend strictly at their apex, undergoing a specialized type of growth called tip growth. Consequently, these cells exhibit a highly asymmetric functional behaviour in processes such as ion fluxes, secretion, wall assembly and cytoskeletal arrangements. This spatial segregation is very attractive for cell biology studies. But the pollen tube can also be regarded as a single haploid cell carrying the sperm cells and thus of great interest for genetical and molecular studies. Last, but not least, pollen is easy to germinate under in vitro conditions, where tubes can grow extremely rapid, making it accessible to application of a wide range of technologies. Therefore, it stands as an ideal system for cell and molecular studies. Here I review some of the basic concepts of pollen tube growth (which are thoroughly discussed in subsequent chapters), address current paradigms and how these are likely to be challenged by recent data that stress how dynamic these cells are.

1 Introduction

The pollen grain, upon germination on a receptive stigma, develops a pollen tube that grows through the pistil towards the ovule while carrying the sperm cells to the embryo sac. The two main functions of the pollen tube are then to elongate and to interpret the guidance cues from the female tissue. Despite this apparent simplicity and the large amount of data already available, many questions remain to be answered.

Pollen tubes are thought to derive from the haustoria by which the primitive microgametophytes fed on the host sporophyte. The initial steps of pollen germination consist of an extensive hydration process that will permit metabolism to resume. During this phase the volume of organelles increases proportionally (Malhó and Pais 1992). The entrance of water is likely to be driven by ion fluxes (Hepler et al., this volume), namely K^+ influx (Feijó et al. 1995) increasing the turgor pressure. A high turgor pressure will preferably stretch the plasma membrane at the germination pore of the grain because in this region an additional

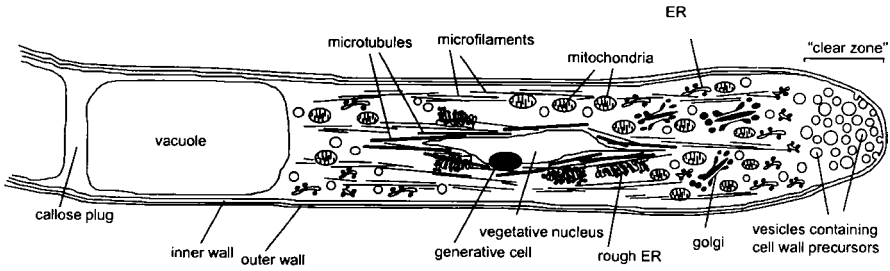

Fig. 1 Diagram depicting the intracellular organization of a pollen tube. The "four zones" classic zonation of a pollen tube is illustrated: apical or clear zone, sub-apical, nuclear and vacuolar. Reproduced from Franklin-Tong (1999). Copyrighted by the American Society of Plant Biologists. Reprinted with permission

exine wall is lacking. Stretch-activated channels (Sze et al., this volume) may open and cause a local depolarisation of the plasma membrane, a cation influx, and a local increase in $[Ca^{2+}]_c$ generating a positive feed-back mechanism.

The elongation of the emergent pollen tube is accomplished through a form of cell extension common in all eukaryotes from fungal hyphae to nerve cells: tip growth. This growth form serves as a paradigm for cell polarity because cell extension is restricted to a narrow zone at the apex. Although some differences exist between species, a general model for pollen tube ultrastructure considers four cytological domains (Cresti et al. 1977; Fig. 1): an apical or "clear zone", devoided or large organelles and packed with golgi vesicles that fuse with the apex delivering wall precursors (Malhó et al. 2005; Geitmann and Steer, this volume); a sub-apical region with a typical cytoskeletal arrangement (Yokota and Shimmen, this volume and Cai and Cresti, this volume) and rich in mitochondria, dictyosomes and endoplasmic reticulum; a nuclear zone where the vegetative nuclei and the sperm cells move; a vacuolar zone that enlarges as tube grows. The continued growth of the pollen tube causes the regular formation of cytoplasmic interruptions of callose ("callose plugs"; Heslop-Harrison, 1987). These plugs isolate the older vacuolated parts of the tubes and confine the cytoplasm to the front regions of the cell. This led to the suggestion, never properly confirmed, that the volume of cytoplasmic material remains constant over the whole process of growth until the mycropile (Malhó et al. 1992). Sanders and Lord (1992) took the implications of this suggestion a step further and proposed that pollen tubes should be viewed not as a growing cell but as a "moving" one (Johnson and Lord, this volume).

2
A Signaling Network Spatially Segregated

Signaling is an integral component in the establishment and maintenance of cellular identity. Tip-growing cells and pollen tubes, in particular, have often

been considered an ideal system to investigate signal transduction mechanisms partly because of the characteristics above mentioned. A direct proof that this is more than a nice sentence to put in introductions and abstracts is the fact that so many signalling pathways have been identified and a role assigned in germination and tip growth; ions (Ca^{2+}, H^+, K^+, Cl^-), calmodulin (CaM), phosphoinositides, phospholipids, protein kinases, cyclic nucleotides, 14-3-3 proteins and GTPases (Malhó and Camacho 2004; Hepler et al., this volume–Zárský et al., this volume). These constitute a large and complex web of signaling networks that intersect at different levels (Fig. 2) such as the con-

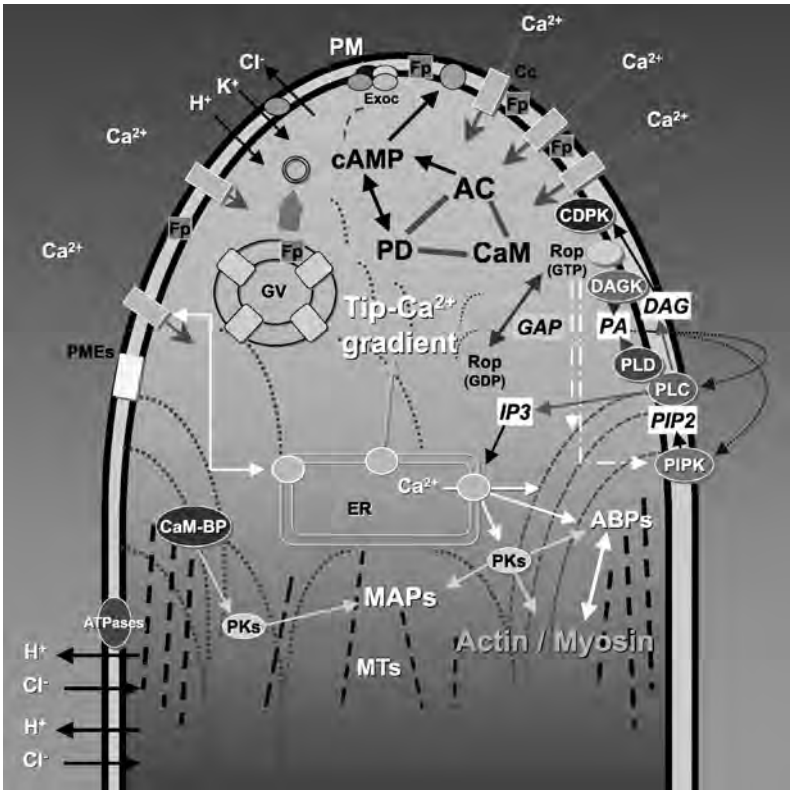

Fig. 2 Apical region of a growing pollen tube illustrating the main signalling transduction pathways and their components. A close interaction between the different pathways foresees the existence of a highly complex, signalling loop in this region, capable to interpret simultaneous (and possibly contradictory) extracellular cues. ABPs, actin-binding proteins; AC, Adenylyl cyclase; CaM-BP, Calmodulin-binding protein; Cc, Ca^{2+} channels; DAG, diacylglycerol; DAGK, DAG kinase; Exoc, Exocyst; Fp, Fusogenic protein (SNAREs and/or anexins); GAP, Rop GTPase activating protein; GV, Golgi vesicle; IP3, Inositol 1,4,5 triphosphate; PD, Phosphodiesterase; PIP2, phosphatidylinositol-(4,5)-bis phosphate; PIPK, phosphatidylinositol kinase; PLC, phospholipase C; PLD, phospholipase D; PM, plasma membrane; PMEs, pectin-methyl-esterases; R, IP3 receptor

trol of vesicle targeting/fusion, the physical state of the actin cytoskeleton, cell wall assembly and extracellular communication (Yokota and Shimmen, this volume–Johnson and Lord, this volume).

2.1 Ca^{2+}, Central Regulator or Follower?

Cytosolic free calcium ($[Ca^{2+}]_c$) is a key element in the regulation of pollen tube elongation and guidance. A tip focused $[Ca^{2+}]_c$ gradient has been imaged with a high 1–3 μM Ca^{2+} concentration in the tip region and a low 0.2–0.3 μM Ca^{2+} concentration in the subapical and basal part of the tube (see detailed discussion in Hepler et al., this volume). Disruption of this gradient leads to inhibition of tube growth and available data indicates its involvement in the control of cytoplasmic streaming (Zárský et al., this volume, and Yokota and Shimmen, this volume), cell wall assembly (Geitmann and Steer, this volume), membrane trafficking and secretion (Hwang and Yang, this volume; Malhó et al. 2005), self-incompatibility (De Graaf et al., this volume) and tube guidance (Johnson and Lord, this volume, and Higashiyama and Inatsugi, this volume).

However, the mechanisms which enforce and regulate the $[Ca^{2+}]_c$ gradient at the tube apex are still controversial (Hepler et al., this volume–Hwang and Yang, this volume). Apical influx of extracellular Ca^{2+} is required but there is an apparent discrepancy between internal Ca^{2+} measurements and external Ca^{2+} fluxes (Hepler et al., this volume) that suggest the existence of primary mechanisms to regulate the ion dynamics. The cell wall and/or internal stores (Malhó and Camacho 2004; Hepler et al., this volume and Sze et al., this volume) were suggested to play an important role. At the molecular level, GTPases have been proposed to act in this process as major signalling switches (Camacho and Malhó 2003; Zheng and Yang 2000) and a detailed discussion of this issue can be found in Chapter V. Phosphoinositides and signalling phospholipids are also emerging as powerful modulators of Ca^{2+}-mediated signals and crucial for the establishment and maintenance of tip growth. The role of these molecules can be found in Chapter VI.

2.2 Crosstalk of Signalling Pathways

CaM is a Ca^{2+} sensor known to modulate the activity of many proteins. In living pollen tubes CaM seems to distribute evenly (Moutinho et al. 1998) but a higher concentration of CaM-target molecules, possibly cytoskeletal elements, was suggested to exist in the sub-apical region. The actin distribution observed in the sub-apical region of pollen tubes (Hepler et al., this volume and Yokota and Shimmen, this volume) resembles the V-shaped col-

lar reported for CaM binding (Moutinho et al. 1998) and thus an interaction between CaM and actin has been hypothesized. This interaction could be dependent on the levels of phosphatidylinositol (4,5)-bisphosphate (Desrivières et al. 2002), thus linking CaM to the phosphoinositide signaling pathway (Zárský, this volume).

Although CaM distributes evenly, Rato et al. (2004) found that CaM activity is higher in the apex of growing tubes and the area of higher activity superimposes to a considerable degree with the tip-focused $[Ca^{2+}]_c$ gradient. Furthermore, it was found that CaM activity oscillates with a period similar to $[Ca^{2+}]_c$ (40–80 sec). We have also shown, as with the manipulation of $[Ca^{2+}]_c$ in the apex (Malhó and Trewavas 1996), that a decrease in CaM levels in one side of the apical dome led to growth axis reorientation to the opposite side. This clearly involves CaM in the molecular events that control pollen tube guidance (Hepler et al., this volume; Johnson and Lord, this volume). CaM might also participate in a feed-back regulation of Ca^{2+} stores (Sze et al., this volume). CaM can achieve regulation of Ca^{2+} stores and $Ins(1,4,5)P_3$ receptors (reviewed in Malhó and Camacho 2004) suggesting that CaM may allow both feedback control of membrane receptors and integration of inputs from other signaling pathways.

In addition to a role for Ca^{2+} in the control of CaM activation, Rato et al. (2004) provided evidence that a cAMP signalling pathway is involved. A cAMP-dependent signalling pathway in pollen was recently shown (Moutinho et al. 2001) and cAMP levels were found to be approximately uniform in the pollen tube cytosol but showing transient increases in the apical region upon reorientation and apical perturbations. CaM thus emerges as a strong candidate to integrate signals between Ca^{2+} and cAMP signalling pathways. Rato et al. (2004) found also that pharmacological modulation of cAMP levels caused equivalent changes in CaM activity suggesting that the activation of downstream targets of cAMP is involved in the regulation of CaM activity, possibly through $[Ca^{2+}]_c$.

The actin cytoskeleton and the secretory apparatus are putative candidates for a cross-regulation between signalling pathways. A growing body of evidence implicates CaM as an important receptor linking changes in Ca^{2+} with cytoskeletal function (Yokota and Shimmen, this volume) and Rato et al. (2004) found that a decrease in CaM levels on one side of the apical dome results in a decrease of secretory activity and reorientation. Diminishing cAMP levels mimicked this effect while an increase of cAMP (which augments CaM activity) promoted secretion. These data further support the claim for a close relationship between Ca^{2+} – CaM and intracellular cAMP in the control of pollen tube growth. Phosphoinositides and phospholipids have also been reported to modulate the actin cytoskeleton and apical secretion. Monteiro et al. (2005a, 2005b) reported that PIP_2, $Ins(1,4,5)P_3$ and phosphatidic acid (PA) regulate tip growth through a multiple pathway system involving coordinated regulation of $[Ca^{2+}]_c$, endo/exocytosis