Ion and lipid signaling in apical growth—a dynamic machinery responding to extracellular cues

Rui Malhó*, Susana Serrazina, Laura Saavedra†, Fernando V. Dias and Reiaz Ul-Rehman*

BioISI – Biosystems & Integrative Sciences Institute, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

Apical cell growth seems to have independently evolved throughout the major lineages of life. To a certain extent, so does our body of knowledge on the mechanisms regulating this morphogenetic process. Studies on pollen tubes, root hairs, rhizoids, fungal hyphae, even nerve cells, have highlighted tissue and cell specificities but also common regulatory characteristics (e.g., ions, proteins, phospholipids) that our focused research sometimes failed to grasp. The working hypothesis to test how apical cell growth is established and maintained have thus been shaped by the model organism under study and the type of methods used to study them. The current picture is one of a dynamic and adaptative process, based on a spatial segregation of components that network to achieve growth and respond to environmental (extracellular) cues. Here, we explore some examples of our live imaging research, namely on cyclic nucleotide gated ion channels, lipid kinases and syntaxins involved in exocytosis. We discuss how their spatial distribution, activity and concentration suggest that the players regulating apical cell growth may display more mobility than previously thought. Furthermore, we speculate on the implications of such perspective in our understanding of the mechanisms regulating apical cell growth and their responses to extracellular cues.

Keywords: Ca\(^{2+}\), cyclic nucleotides, syntaxins, phosphoinositides, signaling, tip growth

Introduction

Apical tip growth is a form of cell extension common in all eukaryotes from rhizoids, pollen tubes, fungal hyphae to nerve cells. This growth form serves as a paradigm for cell polarity because cell extension is restricted to a narrow zone at the apex (Cheung and Wu, 2008). These cells are recognizably excellent models for cell research, particularly suitable for investigations on polarization, signal transduction, channel and ion flux activity, gene expression, cytoskeleton and wall structure, membrane dynamics and even cell-cell communication (Malhó et al., 2000; Onelli and Moscatelli, 2013; Sekereš et al., 2015).

As in any topic in science, advances in knowledge are dictated by current state of technology, the models under study and pre-existing ideas (typically represented in static diagrams such as the one represented in Figure 1). In the case of apical growth in plants, the outstanding technological advance registered in the past two decades, caused a perspective change from a morphological/structural approach to a dynamic/functional approach. When cell biology tools were predominantly used, researchers focused on larger cells that would grow straight and fast under in vitro conditions and would tolerate more harsh experimental conditions (e.g., microinjection, synthetic dyes, use of antibodies, and fixation methods). Lilium longiflorum was the paradigmatic example and underlying most experimental design was the hypothesis that if a molecule was important for tip growth, then
its concentration should be higher in the apex (Malhó et al., 2000). The development of molecular tools in parallel with non-invasive methods to study live cells, made Arabidopsis thaliana a more accessible model and diversified tip growth studies. With the burst of genomics, a wider range of species (e.g., Physcomitrella patens) became also more prone to functional studies. Simple traits like fast growth rate or straight growth direction no longer hold as “universal” physiological controls and data interpretation must consider cellular and tissue variability.

The diversity of species and characteristics of cells under study naturally generated a diversity of results (e.g., the natural growing environment of an Arabidopsis pollen tube is different from one of lily, from a root hair or from a moss rhizoid). Distinct asymmetric localization of components of the apical growth machinery (summarized in the diagram of Figure 1) were reported as part of new studies or reassessment of previous data. This, in turn, generated discrepancies, challenged old ideas and opened new perspectives. The architecture of the actin cytoskeleton (Vidali et al., 2009) and the secretory activity (Zonia and Munnik, 2008) are just two examples that challenged the “all-in-the-tip” concept and highlighted the importance of dynamics at the sub-apical region (Cheung and Wu, 2008; Sekereš et al., 2015). Here we explore the example of four different types of proteins recently studied by our group and which were found to be important for tip growth. In all four cases, we found that changes in growth pattern (e.g., redirectioning of growth axis, transient loss of polarity and recovery, oscillatory growth rates) were accompanied by protein delocalization. We discuss the implications of such findings.
Tip growing cells not only have to frequently adjust direction of growth axis but they also experience oscillations in growth rates. Several types of growth fluctuations (of asymmetric periodicity and intensity) have been observed and they seem to vary according to species and cell type. Whether these oscillations have any special physiological meaning or whether they are just a “built-in” characteristic is not clear. [Ca^{2+}] exhibits changes that correlated to such fluctuations of growth rates and reorientation of growth axis (Castanho Coelho and Malhó, 2006) so it is highly plausible that other components of the polarity mechanism (e.g., lipids and membrane-associated proteins) also exhibit changes in activity and/or localization. The timing of our observations (and the inherent physiological status of the cells) may thus influence our reports and help to explain some apparent discrepancies that exist in the literature.

**PIPK Kinases—Versatile and Key Transducers of “Signal to Form”**

In the last years PtdIns(4,5)P$_2$ and its synthesizing enzyme, phosphatidylinositol phosphate kinase (PIPK), have been intensively studied in plant cells, revealing a key role in the control of polar tip growth. Using fluorescence markers fused to the pleckstrin homology (PH) domain of the human PLCd1, PtdIns(4,5)P$_2$ was found to accumulate at the tip of growing apical cells (Kost et al., 1999; Dowd et al., 2006; Helling et al., 2006; Ischebeck et al., 2008; Sousa et al., 2008). Analysis of the PIPK members from Arabidopsis thaliana, Oryza sativa, and Physcomitrella patens showed that they share some regulatory features with animal PIPKs but also exert plant-specific modes of regulation (Saavedra et al., 2012). Deletion or overexpression of these lipid kinases were found to cause distinct phenotypes and perturbations in cellular processes such as cell wall deposition, endocytosis, and actin bundling (Ischebeck et al., 2008, 2010; Kusano et al., 2008; Sousa et al., 2008; Stenzel et al., 2008; Zhao et al., 2010; Saavedra et al., 2011). Interestingly, in actively growing pollen tubes, all the six PIPK isoforms (AtPIP5K10, AtPIP5K11, AtPIP5K2, AtPIP5K4, AtPIP5K5, and AtPIP5K6) were found to localize preferentially at the flanks of the tube apex and not superimposed with the highest PtdIns(4,5)P$_2$ concentration. However, it was also observed that the region displaying the highest protein fluorescent signal would change according to speed of growth and reorientation of the growth axis—slower growth resulting in delocalization from the flanks to the apex (e.g., Figure 6 of Ischebeck et al., 2008; Sousa et al., 2008). It has been suggested that distinct localization patterns of proteins may be the consequence of interactions with specific partners, which recruit them to different functional microdomains (Ischebeck et al., 2010). For example, PtdIns(4,5)P$_2$ could be channeled to targets via specific interactions PIPK-downstream effectors (Heilmann and Heilmann, 2015) resulting in differential cellular responses and phenotypes as observed upon deletion of AtPIP5Ks (Ischebeck et al., 2008, 2010; Kusano et al., 2008; Sousa et al., 2008).

Phosphatidylinositol phosphate kinases have membrane occupation and recognition nexus (MORN) motifs which are thought to be the plasma membrane localizing module (Kusano...
et al., 2008) but other modules were shown to be important for correct subcellular localization (Mikami et al., 2010; Stenzel et al., 2012). E.g., AtPIP5K5 and NtPIP5K6-1 require non-conserved linker (LIM) domain for correct localization in pollen tubes, as the deletion of the N-terminal and MORN domain did not affect their apical plasma membrane localization (Stenzel et al., 2012). It is thus possible that protein modules responsible for plasma membrane localization are distinct in each PIPK allowing a fine tuning that depends on differences in physiological and/or developmental status of cells, such as polarized and non-polarized. Full comprehension of the localization mechanisms will probably involve comparison of the function of the MORN, LIM, and kinase domains of every PIPKs in cells exhibiting the same stage in development.

Syntaxins—Spatial Targeting of Secretion and Membrane Recycling

Apical growth occurs by continuous vesicle secretion and delivery of new wall material. Therefore, the exact subcellular location of endocytic and exocytic domains is essential to determine cellular responses and reshape form. In pollen tubes, it was previously assumed that exocytosis events occur mostly at the extreme apex, where $[\text{Ca}^{2+}]$, is higher, while membrane recycling (endocytosis) would take place further back from the tip, at the flanks of the apex and/or at sub-apical regions (Picton and Steer, 1981; Castanho Coelho and Malhó, 2006). Recent data questioned this paradigm suggesting that the preferential location for fusion is on a limited membrane domain at the sub-apical flanks and not at the extreme apex (Bove et al., 2008; Zonia and Munnik, 2008). Similar observations have been made in root hairs (Ovecka et al., 2005). The hypothesis of two endocytic modes co-existing according to the growth conditions—a clathrin-dependent and an independent one—was raised (Camacho and Malhó, 2003; Monteiro et al., 2005) and experimental evidence to support it was obtained by Moscatelli et al. (2007) and McKenna et al. (2009).

We have also obtained evidence that two pollen-specific SNAREs (for soluble N-ethylmaleimide sensitive factor attachment protein receptor), syntaxins SYP125 and SYP124 (Silva et al., 2010; Ul-Rehman et al., 2011) have a complementary, not fully overlapping, dynamic distribution (Figure 2B). Similarly to PIPK proteins, the observed changes in protein accumulation at the plasma membrane might reflect specific interactions with unidentified targets (e.g., PIP2 and phosphatidic acid) which, under natural growth conditions of pollen tubes, could translate into discrete asymmetric secretory events and relate to the two endocytic modes. Indirect support for such hypothesis derives from our observations that in pollen tubes, $[\text{Ca}^{2+}]$, and membrane fusion exhibit frequent non-linear changes correlated to growth rates and reorientation of growth axis (Castanho Coelho and Malhó, 2006). Over-expression of PIP5K4 or Rab GTPases was also found to perturb SYT124 localization (Silva et al., 2010). The changes in SYT124 and SYT125 distribution observed upon growth modulation are thus likely to reflect membrane dynamics and a repositioning of the vesicle’s docking machinery upon intra- and extracellular cues. In nerve cells, syntaxins were reported to be involved in rapid and slow endocytosis (Xu et al., 2013) and could thus act as regulators of both endo- and exocytosis. VAMP726, another member of the SNARES family, was also shown to mediate fusion of endo- and exocytic compartments in pollen tube tip growth (Guo and McCubbin, 2012). A similar role has just been proposed for the exocyst (Jose et al., 2015), a complex known to be present in tip growing cells (Zárský et al., 2013).

Similarly to our CNGC observations, these findings highlight the importance of syntaxins in secretion and tip growth but must be interpreted considering that the localization reported reflects protein accumulation and not necessarily activity.

FAB Kinases- Dynamics in the Secretory Pathway

Most of the data now available relating apical polarity and growth focused on the role of plasma membrane (integral or associated) events. The plasma membrane and its links to the cell wall and the cytoskeleton are the obvious main targets for perceiving and transducing both intra- and extracellular cues. Notwithstanding, we have recently obtained data suggesting that PIPKfive/Fab1 kinases localized to the endomembrane compartment are also involved in the regulation of plasma membrane recycling events and thus in the maintenance of polarity (Serrazina et al., 2014).

In plants, the Fab1 phosphatidylinositol-3-monophosphate 5-kinases produce phosphatidylinositol (3,5)-bisphosphate [PtdIns(3,5)P$_2$], a phosphoinositide implicated in endomembrane trafficking and pH control in the vacuole (Dove et al., 2009; Bak et al., 2013). In pollen tubes, we found that AtFAB1B-GFP had a highly mobile reticulate-like distribution, distinct from γ-TIP (Serrazina et al., 2014) and decorating the sub-apical region in a manner similar to the actin cytoskeleton—a sort of V-shaped collar that adjusts and “accompanies” the reorientation of the growth axis (Vidali et al., 2009). This suggests that the protein is localized in protein storage vacuoles, endoplasmic reticulum and possibly trans-Golgi network mediating transport to and from the plasma membrane (Gary et al., 1998; Wang et al., 2011). Additionally, we found that FAB1 deletion resulted in lower internalization rates and perturbed secretion and deposition of new wall material (Serrazina et al., 2014). Analogous observations were made in root hairs (Hirano et al., 2011). FAB1 deletion was also found to impair acidification of the endomembrane compartment and to cause abnormal pollen tube diameter (Serrazina et al., 2014). The perturbation of proton gradients across membranes was reported to affect membrane curvature and vesiculization (Hope et al., 1989), protein sorting (Hurtado-Lorenzo et al., 2006), and ion fluxes, all of which are processes essential for tip growth.
Conclusion and Perspectives

The establishment and maintenance of apical polarity relies on a dynamic, mobile network of signaling mechanisms. Currently, our conceptual models focus mostly on apical and sub-apical localization (of ions, proteins, lipids), particularly at the plasma membrane (or associated with). Here we provided examples of four classes of proteins related to ion and lipid signaling which indicates that a careful analysis of localization, activity and mobility is required in order to fully assign their role in apical growth. This rationale can probably be extended to other equally relevant signaling components identified in these cells (e.g., Rop GTPases, Ca\(^{2+}\)-dependent protein kinases, actin and actin-binding proteins, etc; Figure 1; for a review see Onelli and Moscatelli, 2013). The results obtained with FAB kinases further suggest the importance of plasma membrane—endomembrane signaling raising new perspectives in the study of apical growth mechanisms. Signaling to other organelles and compartments is also likely to play key roles in the establishment of polarity.

We have discussed the hypothesis that, in nature, and upon a myriad of environmental (extracellular) cues, cellular responses involve flexible positioning of proteins, lipids and ion fluxes. Such dynamics may go partly unnoticed in the set-ups we devise for our experimental planning which are conditioned by the species under study, the technical approach, the pre-existing knowledge and, most importantly, by the requirements to test individual stimuli. Thus, transient gradients or peaks of activity/localization may or not be recorded (even dismissed) depending on the experimental set-up, cellular conditions and our biased previous background. Large single cell analysis and settings mimicking (or bearing in mind) the natural environment where cells develop, are required but may be difficult to implement. To compare and interpret results obtained with different experimental as well as plant systems will be a challenge, but one that must be tackled.

Acknowledgments

This work was supported by Fundação Ciência e Tecnologia (FCT/MCTES/PIDDAC, Portugal) with post-doc fellowship to LS (SFRH/BPD/81635/2011), and research funds to RM (PEst-OE/BI/A14046/2014; UID/MULTI/04046/2013).

References

Bak, G., Lee, E.-J., Lee, Y., Kato, M., Segami, S., Sze, H., et al. (2013). Rapid structural changes and acidification of guard cell vacuoles during stomatal closure require phosphatidylinositol 3,5-biphosphate. Plant Cell 25, 2202–2216. doi: 10.1105/tpc.113.110411

Bove, J., Vaillancourt, B., Kroeger, J., Hepler, P. K., Wiseman, P. W., and Geitmann, A. (2008). Magnitude and direction of vesicle dynamics in growing pollen tubes using spatiotemporal image correlation spectroscopy and fluorescence recovery after photobleaching. Plant Physiol. 147, 1646–1658. doi: 10.1104/pp.108.120212

Camacho, L., and Malhó, R. (2003). Endo-exocytosis in the pollen tube apex is differentially regulated by Ca\(^{2+}\) and GTPases. J. Exp. Bot. 54, 83–92. doi: 10.1093/jxb/erg043

Castanho Coelho, P., and Malhó, R. (2006). Correlative analysis of apical secretion and [Ca\(^{2+}\)]\(_{i}\) in pollen tube growth and reorientation. Plant Signal. Behav. 1, 152–157. doi: 10.4161/psh.1.3.2999

Cheung, A., and Wu, H. M. (2008). Structural and signaling networks for the polar cell growth machinery in pollen tubes. Annu. Rev. Plant Biol. 59, 547–572. doi: 10.1146/annurev.plant.59.032607.092921

Dove, S. K., Dong, K., Kobayashi, T., Williams, F. K., and Michell, R. H. (2009). Phosphatidylinositol 3,5-biphosphate and Fab1p/PIKfyve under PPIn endolysosome function. Biochem. J. 419, 1–13. doi: 10.1042/Bj20081950

Dowd, P. E., Courseo, S., Skipper, A. L., Kao, T.-H., and Gilroy, S. (2006). Petunia phospholipase C1 is involved in pollen tube growth. Plant Cell 18, 1438–1453. doi: 10.1105/tpc.106.041582

Fritsch, S., Wang, Y. F., Sladek, C., Poulsen, I. R., Romanowski, S. M., Schroeder, J. I., et al. (2007). A cyclic nucleotide-gated channel is essential for polarized tip growth of pollen. Proc. Natl. Acad. Sci. U.S.A. 104, 14531–14536. doi: 10.1073/pnas.0701781104

Gary, J. D., Wurmser, A. E., Bonangelino, C. J., Weisman, L. S., and Emr, S. D. (1998). Fab1p is essential for PtdIns(3)P 5-kinase activity and the maintenance of vacuolar size and membrane homeostasis. J. Cell Biol. 143, 65–79. doi: 10.1083/jcb.143.1.65

Gui, C. P., Dong, X., Liu, H. K., Huang, W. J., Zhang, D., Wang, S. J., et al. (2014). Overexpression of the tomato pollen receptor kinase LePRK1 rewrites pollen tube growth to a blebbing mode. Plant Cell 26, 3538–3555. doi: 10.1105/tpc.114.127351

Guo, F., and McCubbin, A. (2012). The pollen-specific R-SNARE/longin PIVAMP726 mediates fusion of endo- and exocytic compartments in pollen tube tip growth. J. Exp. Bot. 63, 3083–3095. doi: 10.1093/jxb/ers023

Heilmann, M., and Heilmann, I. (2015). Plant phosphoinositides-complex networks controlling growth and adaptation. Biochim. Biophys. Acta 1851, 759–769. doi: 10.1016/j.bbalip.2014.09.018

Helling, D., Possart, A., Cottier, S., Klahre, U., and Kost, B. (2006). Pollen tube tip growth depends on plasma membrane polarization mediated by tobacco PLC3 activity and endocytic membrane recycling. Plant Cell 18, 3519–3534. doi: 10.1105/tpc.106.047373

Hepler, P. K., Kunkel, J. G., Rounds, C. M., and Winship, L. J. (2012). Calcium entry into pollen tubes. Trends Plant Sci. 17, 32–38. doi: 10.1016/j.tplants.2011.10.007

Hirano, T., Matsuzewa, T., Takegawa, K., and Sato, M. H. (2011). Loss-of-function and gain-of-function mutations in FABA/B impair endomembrane homeostasis, conferring pleiotropic developmental abnormalities in Arabidopsis. Plant Physiol. 155, 797–807. doi: 10.1104/pp.110.167981

Hope, M. J., Redelmeier, T. E., Wong, K. F., Rodrigueza, W., and Cullis, P. R. (1989). Phospholipid asymmetry in large unilamellar vesicles induced by transmembrane pH gradients. Biochemistry 28, 4181–4187. doi: 10.1021/bi00436a009

Hurtado-Lorenzo, A., Skinner, M., El Annan, J., Futai, M., Sun-Wada, G. H., Bourgoin, S., et al. (2006). V-ATPase interacts with ARNO and Arf6 in early endosomes and regulates the protein degradative pathway. Nat. Cell Biol. 8, 124–136. doi: 10.1038/ncb1348

Ischebeck, T., Stenzel, I., and Heilmann, I. (2008). Type B phosphatidylinositol-4-phosphate 5-kinases mediate Arabidopsis and Nicotiana tabacum pollen tube growth by regulating apical pectin secretion. Plant Cell 20, 3312–3330. doi: 10.1105/tpc.108.059568

Ischebeck, T., Stenzel, I., Hempel, F., Jin, X., Mosblech, A., and Heilmann, I. (2010). Phosphatidylinositol-4,5-biphosphate influences Ni-Rac-mediated cell expansion in pollen tubes of Nicotiana tabacum. Plant J. 65, 453–468. doi: 10.1111/j.1365-313X.2010.04435.x

Jose, M., Tollis, S., Nair, D., Mitteau, R., Velours, C., Massoni-Laporte, A., et al. (2015). A quantitative imaging-based screen reveals the exocyst as a network hub connecting endocytosis and exocytosis. Mol. Biol. Cell 26, 2519–2534. doi: 10.1091/mbc.E14-11-1527

Kost, B., Lemichez, E., Spießhofer, P., Hong, Y., Tolias, K., Carpenter, C., et al. (1999). Rac homologues and compartmentalized phosphatidylinositol 4,5-biphosphate act in a common pathway to regulate polar pollen tube growth. J. Cell Biol. 145, 317–330. doi: 10.1083/jcb.145.2.317

Kusano, H., Tosterink, C., Vermeere, J. E., Tsuge, T., Shimada, H., Oka, A., et al. (2008). The Arabidopsis phosphatidylinositol 5-phosphate 5-kinase PIP5K3 is a key regulator of root hair tip growth. Plant Cell 20, 367–380. doi: 10.1105/tpc.107.056119
Lee, Y. J., Szumlanåski, A., Nielsen, E., and Yang, Z. (2008). Rho-GTPase-dependent filamentous actin dynamics coordinate vesicle targeting and exocytosis during tip growth. J. Cell Biol. 181, 1155–1168. doi: 10.1083/jcb.200801086

Malhó, R., Camacho, L., and Moutinho, A. (2000). Signaling pathways in pollen tube growth and reorientation. Annu. Bot. 85(Suppl. A), 59–68. doi: 10.1006/anbo.1999.0991

McKenna, S. T., Kunek, J. G., Bosch, M., Rounds, C. M., Vidali, L., Winship, L. J., et al. (2009). Exocytosis precedes and predicts the increase in growth in oscillating pollen tubes. Plant Cell 21, 3026–3040. doi: 10.1105/tpc.109.069260

Michaud, E., Lima, P. T., Borges, F., Silva, A. C., Portes, M. T., Carvalho, J. E., et al. (2011). Glutamate receptor-like genes form Ca$$^{2+}$$ channels in pollen tubes and are regulated by pistil D-serine. Science 332, 434–437. doi: 10.1126/science.1201101

Moscatelli, A., Ciampolini, F., Rodighiero, S., Onelli, E., Cresti, M., Santo, N., et al. (2007). Distinct endocytic pathways identified in tobacco pollen tubes using charged nanogold. J. Cell Sci. 120, 3804–3819. doi: 10.1242/jcs.012138

Onelli, E., and Moscatelli, A. (2013). Endocytic pathways and recycling in growing pollen tubes. Plants 2, 211–229. doi: 10.3390/plants2020211

Ovecka, M., Lang, I., Baluška, F., Ismail, A., Illes, P., and Lichtscheidl, I. (2005). Ion and lipid signaling in apical growth of plant cell polarity at different scales. J. Exp. Bot. 56, 1665–1674. doi: 10.1093/jxb/eri163

Picton, J. M., and Steer, M. W. (1981). Determination of secretory vesicle and reorientation through modulation of [Ca$$^{2+}$$], and membrane secretion. J. Exp. Bot. 32, 261–272.

Saavedra, L., Mikami, K., Saavedra, L., Hiwatashi, Y., Uji, T., Hasebe, M., and Sommarin, M. (2012). PIP kinases and sub-apical zones is involved in tip growth. BMC Plant Biol. 10:179. doi: 10.1186/1471-2229-10-179

Sousa, E., Kost, B., and Malhó, R. (2008). Arabidopsis phosphatidylinositol-4-monophosphate 5-kinase 4 regulates pollen tube growth and polarity by modulating membrane recycling. Plant Cell 20, 3050–3064. doi: 10.1105/tpc.108.058826

Spalding, E. P., and Harper, J. F. (2011). The ins and outs of cellular Ca$$^{2+}$$ transport. Curr. Opin. Plant Biol. 14, 715–720. doi: 10.1016/j.copbt.2011.08.001

Stenzel, I., Ischebeck, T., Konig, S., Holubowska, A., Sporysz, M., Hause, B., et al. (2008). The type B phosphatidylinositol-4-phosphate 5-kinase 3 is essential for root hair formation in Arabidopsis thaliana. Plant Cell 20, 124–141. doi: 10.1105/tpc.107.052852

Vidalî, L., Rounds, C. M., Hepler, P. K., and Bezanilla, M. (2009). Lifeact-mEGFP reveals a dynamic apical F-Actin network in tip growing plant cells. PLoS ONE 4:e5744. doi: 10.1371/journal.pone.0005744

Zonia, L., and Munnik, T. (2008). Vesicle trafficking dynamics and visualization of zones of exocytosis and endocytosis in tobacco pollen tubes. J. Exp. Bot. 59, 861–873. doi: 10.1093/jxb/ern007

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.