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Calcium is an organizer of cell polarity in plants☆

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ABSTRACT

Cell polarity is a fundamental property of pro- and eukaryotic cells. It is necessary for coordination of cell division, cell morphogenesis and signaling processes. How polarity is generated and maintained is a complex issue governed by interconnected feed-back regulations between small GTPase signaling and membrane tension-based signaling that controls membrane trafficking, and cytoskeleton organization and dynamics. Here, we will review the potential role for calcium as a crucial signal that connects and coordinates the respective processes during polarization processes in plants. This article is part of a Special Issue entitled: 13th European Symposium on Calcium.

1. Introduction to polarity and plant development

At its simplest level, polarity can be defined as an asymmetric distribution of components along one or more axes, thereby breaking symmetry [1]. The most basic type of polarity involves establishing a single polar domain and can be found in a wide range of biological processes in unicellular as well as in multicellular organisms, ranging from asymmetric cell divisions, polarized axon growth, directional movement of motile cells, pollen tube and root hair formation in plants, zygote polarization in algae, etc. In each of these examples, the polarity is defined by the local accumulation of cellular components to one side of the cell.

In multicellular organisms, cells are often embedded in a three-dimensional tissue context, requiring additional dimensions of polarity. Hitherto, molecular markers exist defining apical and basal polarity (reflecting position along the embryonic axis), as well as inner- and outer-lateral polar domains (radial polarity) that can coexist within a single plant cell [2]. In addition to these four polar domains, specialized cell types have the capacity to develop additional polar features, superimposed on or across other polar domains, such as in the endodermis that develops casparian strips that encircle the cells [3] and root hairs that develop at a discrete positions in the outer-lateral domains of root epidermal cells [4]. Another complex manifestation of polarity in plants is seen during the morphogenesis of leaf epidermal cells in dicotyledons that is characterized by interdigitation of adjacent cells via the coordinated formation of lobes and indentations [5].

How plants can generate such complex polarity patterns remains poorly understood. Yet, several of the mechanisms underlying generation and maintenance of polarity become identified step by step. Two important cellular processes control polarity: 1) anisotropic membrane trafficking by local delivery or removal of specific membrane proteins and lipids, and 2) the polar organization and dynamics of the cytoskeleton. These cellular processes are believed to be controlled by signals derived from the local activity of small GTPases and from cellular mechanosensing mechanisms. These different aspects of polarity are tightly interconnected, making it difficult to assess their individual contribution to polarity and how they are coordinated to generate and maintain polarity.

In this review, we will focus on the mechanisms by which the second messenger Ca^{2+} is connected to each of the aforementioned polarity processes and signaling cascades and we elaborate on how Ca^{2+} could serve as a general coordinative and integrative signal for plant polarity, a principle that we propose not to be restricted to tip-growing cells (Fig. 1).

2. Calcium hallmarks polarity

Calcium is an elusive second messenger because it can be triggered by a wide range of signals and is transient in nature [6]. Therefore, it is commonly described in terms of Ca^{2+} signatures that can be very

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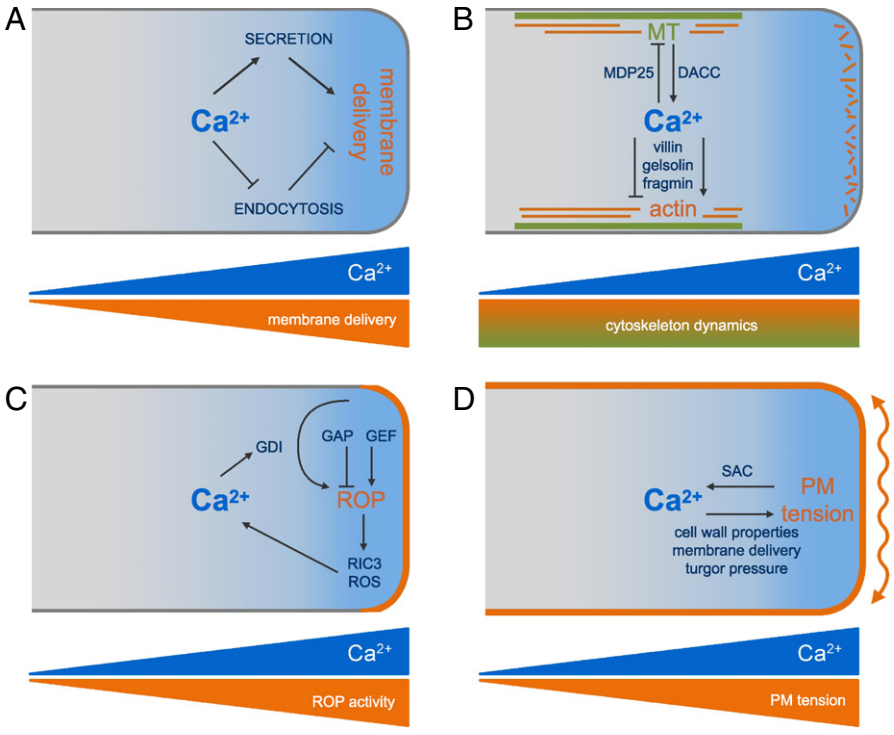

Fig. 1. Interconnection between Ca^{2+} and polarity components. A. A Ca^{2+} gradient spatially coordinates membrane trafficking. High Ca^{2+} concentrations to one side of a cell locally stimulate secretion while reducing (clathrin-mediated) endocytosis thereby locally promoting membrane delivery. The blue and orange color represent the gradients in Ca^{2+} and membrane delivery respectively. B. A Ca^{2+} gradient differentially controls cytoskeleton dynamics. Ca^{2+} controls F-actin dynamics and organization through Ca^{2+}-dependent actin regulating proteins of the villin/gelsolin/fragmin superfamily, regulating actin organization and dynamics. Via MICROTUBULE-DESTABILIZING PROTEIN25 (MDP25) Ca^{2+} can destabilize cortical microtubules (MT). In turn, MTs can stabilize Ca^{2+} signaling via controlling depolarization-activated Ca^{2+}channels (DACC). The blue color represents the Ca^{2+} gradient and the orange/green color the actin/MT dynamics respectively. C. Interaction between Ca^{2+} and ROP signaling to generate and maintain cell polarity. Polarized ROPs (orange gradient) locally stimulate Ca^{2+} entry through the ROP effector RIC3 and ROP-induced reactive oxygen species (ROS) production. ROP activity is positively or negatively regulated by GEFs or GAPs respectively. Polar localization of ROPs to the apex is maintained by GDI which in turn can be controlled by Ca^{2+}. The blue and orange color represent the gradients in Ca^{2+} and ROP activity respectively. D. Interplay between Ca^{2+} and PM tension. The plant cell wall and local differences in plasma membrane (PM) tension both contribute to cell polarization. Upon cellular growth, increased PM tension results in opening of stretch-activated Ca^{2+}channels (SACs) resulting in local Ca^{2+} influx. Ca^{2+} itself can control cell wall rigidity and membrane delivery and turgor pressure to regulate PM tension. The blue and orange color represent the Ca^{2+}gradient and locally high PM tension respectively.

local and short-lived, making it often difficult to detect reliably. Under these limitations, a sharp Ca^{2+} gradient can be detected in the tip of growing pollen tubes and root hairs, that is essential for polar growth [7,8]. In addition, local Ca^{2+} signaling is also central to polarity establishment in fucoid algal zygotes [9]. Importantly, manipulations that refocus Ca^{2+} signals are sufficient to reorient polarity of tip growth, not only in pollen tubes [10], but also in root hairs [11] demonstrating the potential of Ca^{2+} gradients as instructive signals for polarity. The role of Ca^{2+} as a regulator of polarity is mainly derived from studies on tip growth in pollen tubes, a cell type that is easily accessible, and expresses only a relatively small subset of the Ca^{2+} toolkit [12]. However, it must be noted that Ca^{2+} levels were also found to impact on apical–basal polarity in root cells [13], suggesting that the role of Ca^{2+} in plant polarity is not restricted to tip growth.

At least four distinct Ca^{2+} channels contribute to the Ca^{2+} gradient that exists at the pollen tube tip. The first, and most important Ca^{2+} channels for the tip-focused Ca^{2+} gradient, are stretch-activated Ca^{2+} channels (SAC) that open in response to plasma membrane strain, such as the strain associated with rapid growth [14]. However, the underlying molecular nature of these channels remains completely unknown. Secondly, members of the GLUTAMATE RECEPTOR-LIKE (GLR) family, AtGLR1.2 and AtGLR3.7 have been demonstrated to form Ca^{2+} channels in pollen tubes in response to D-Serine derived from the pistil to guide pollen tube growth [15]. Thirdly, several *CYCLIC NUCLEOTIDE GATED CATION CHANNEL* (*CNGC*) genes have been implicated in pollen tube growth [7]. Among them, CNGC18 displays a clear apical localization, with its strongest localization just behind the apex, a position that would allow to refocus the Ca^{2+} maximum in response to directional cues [16]. A fourth family of Ca^{2+} channeling proteins that could contribute to tip-focused Ca^{2+} gradients are the ANNEXINs, that can generate Ca^{2+} permeable channels in response to hydroxyl radicals [17]. Whereas these Ca^{2+} channels reflect mechanisms by which Ca^{2+} enters the cell through the plasma membrane, Ca^{2+} ATPases are continuously active to move Ca^{2+} from the cytosol into adjacent cellular compartments or the apoplast. These mechanisms can contribute to shaping the Ca^{2+} signal [7]. Several of these Ca^{2+} ATPases, such as ACA9, do not show polar localization [18], but are instead activated by local high Ca^{2+} concentrations, acting to dissipate Ca^{2+} signals.

Given that Ca^{2+} carries no structural information, the information embedded in Ca^{2+} gradients and temporal signatures needs to be decoded and translated into a cellular response. This can be achieved by a complex set of Ca^{2+} binding proteins (>250 in *Arabidopsis*) [19] that represent Ca^{2+} sensors with enzymatic activity (Ca^{2+} sensor responders; Ca^{2+}-dependent protein kinase/CDPK/CPK) or without enzymatic activity (Ca^{2+} sensor relays; calmodulin/CaM, calmodulin-like/CML, calcineurin B-like/CBL). Strong polarity defects in pollen tubes have thus far only been reported for overexpression of CDPKs [20], supporting the importance of local CDPK activity in directing pollen tube polarity [21]. Many known targets of CDPKs are ion channels, allowing to regulate osmotic pressure and membrane potential in the context of Ca^{2+} signals [6]. Moreover, it was recently found that CPK32 directly contributes to the tip-focused Ca^{2+} gradient by activating the Ca^{2+} permeability of CNGC18 [22]. Moreover, CNGC activity can be further modulated by interaction with active CaM [23], revealing a complex Ca^{2+}-dependent regulation of the Ca^{2+} channeling activities of CNGCs.

The complexity of Ca^{2+} signaling that is already needed for generating polarity within a simple pollen tube, suggests that the additional dimensions of polarity that exist in cells embedded in a tissue context will involve even more complex Ca^{2+} signaling. In the following paragraphs we will highlight the connections that exist between Ca^{2+} signaling and the cellular processes and signaling cascades that impact on polarity throughout the plant body.

3. Calcium coordinates the balance of exo- and endocytosis

The semi-fluid nature of the plasma membrane allows diffusion of membrane proteins and lipids, implying that maintenance of polarity requires spatially regulated membrane trafficking. Consistently, membrane trafficking is a crucial process to maintain tip growth in pollen [24,25], root hairs [26], as well as in apical–basal polarity in the plant body [27–29]. During tip growth, exocytosis (secretion and recycling) occurs predominantly in the tip, whereas endocytosis occurs subapically. Similarly, in apical polar domains of root cells, exo- and endocytosis are also spatially separated; exocytosis occurs in a super-apical domain, while endocytosis occurs mainly at the flanks of the respective polar domains [30].

In plants, the exocytosis-stimulating effect of Ca^{2+} could be mediated by annexins. These are Ca^{2+}-dependent phospholipid binding proteins that regulate secretion in animals [31], and possibly also in plants [32]. Consistently, overexpression of *ANNEXIN5* (*AnnAt5*) rendered *Arabidopsis* pollen partially resistant to brefeldin A (BFA) [33] (a fungal toxin which targets BARF GTPase guanine-nucleotide exchange factors, thereby inhibiting endosomal recycling [34] and late secretory and vacuolar trafficking [35]), and root hair growth is reduced in *annexin1* (*AnnAt1*) mutants [36]. However, the function of annexins in regulating Ca^{2+}-dependent secretion in plants remains to be demonstrated unequivocally. Importantly, it should be noted that the tip-focused Ca^{2+} gradient and the maximal growth phase of the pollen tube oscillate partially out of phase with each other [37,38], making a direct link between Ca^{2+} and secretion not very intuitive.

Clathrin-mediated endocytosis (CME) occurs mainly subapically in growing pollen tubes [25], as if excluded from the high Ca^{2+} concentrations found in the tip. Consistently, several components of the core CME machinery displayed a subapical localization in growing pollen [24,39]. Moreover, several subunits of a recently identified endocytic adaptor complex (T-Plate Complex; TPC) contain predicted Ca^{2+} binding EF-hand motifs [39], suggestive of a Ca^{2+} sensitive activity. Consistently, a treatment with the presumed intracellular Ca^{2+} store leak inducer, caffeine [40], could dislodge the TPC subunit, T-PLATE and Clathrin Light Chain2 from the cell plate during cytokinesis [41]. This is consistent with a model in which high Ca^{2+} concentrations act to inactivate CME. Calcium can also have an indirect effect on CME via phosphatidylinositides, at least in pollen tubes, where Ca^{2+} signals control the subapical localization of the $PI(4,5)P_2$-hydrolyzing enzyme NtPLC3 [42]. Interestingly, phosphatidylinositol bisphosphate ($PI(4,5)P_2$) is required for CME and polarity in pollen tubes [24] as well as root cells [43,44]. However the role of Ca^{2+} in regulating $PI(4,5)P_2$ homeostasis in root cells remains to be demonstrated.

Together, these findings suggest that Ca^{2+} could control polarity in different plant cell types through coordination of exo- and endocytosis (Fig. 1A).

4. Reciprocal interaction between Ca^{2+} signaling and the cytoskeleton

The actin filaments (F-actin) are critical elements in cell morphogenesis and directional growth. These dynamic structures control membrane trafficking, including correct delivery of secretory vesicles and regulation of vesicle docking and fusion [45], as well as actin regulation of endocytosis [46,47]. Interestingly, the actin filaments in pollen tubes occur in a polarized gradient overlapping with the tip-focused Ca^{2+} gradient [48] (Fig. 1B), an observation that can be mechanistically explained via the Ca^{2+}-dependence of actin organizing proteins (nucleation, bundling, and severing) such as members of the villin/gelsolin/fragmin superfamily [49], which also control directional elongation in other cell types [50].

Polarity can also be regulated via microtubules (MT) by providing structural rigidity and stiffness to the cell, imposing restrictions on cell expansion [51]. In addition, MTs were recently found to be part of a symmetry breaking system for secondary wall patterning in metaxylem cells [52]. Interestingly, Ca^{2+} can destabilize cortical MTs via MICROTUBULE-DESTABILIZING PROTEIN25 (MDP25) during directional elongation of hypocotyl cells [53]. Conversely, the MTs seem to contribute to the stability of Ca^{2+} signaling via control over the activity of depolarization-activated Ca^{2+} channels [54] (Fig. 1B).

This demonstrates that the intimate link between Ca^{2+} signaling and cytoskeleton dynamics is involved in sustaining and/or generating polarity in plants.

5. Ca^{2+} interdependence of ROP-based polarity signaling

Small GTPases of the Rho superfamily, such as Rac and Cdc42, play major roles in generating and stabilizing asymmetry [9,55,56]. Land plants have no Rac or Cdc42 in their genomes. Instead, they have a plant-specific subfamily dubbed 'Rho-like GTPase of Plants' or ROPs, that are closely associated with polarity in higher land plants [5], and recently also in the lower land plant *Physcomitrella patens* [57]. The polarization of Rho GTPase family members tightly interconnects via several effectors to other polarity-associated processes, including exo- and endocytosis and cytoskeleton organization [58].

ROP GTPases are active when bound to GTP and return to an inactive, GDP-bound state by hydrolysis of GTP to GDP. The exchange of GDP by GTP can be promoted by GEFs (guanine-nucleotide exchange factors), whereas inactivation is stimulated by GAPs (GTPase activating proteins) (Fig. 1C). Mechanisms that control GEFs and GAPs can thus be used for local regulation of ROP activity [59]. In addition, RhoGDIs (guanine nucleotide dissociation inhibitor) seem to act as conserved polarity facilitators of Rho GTPases via stimulation of Rho GTPase recycling (Fig. 1C). In yeast, the polarization of Cdc42 was proposed to be regulated by GDI-dependent Cdc42 removal from the plasma membrane, and subsequent rapid polar recycling [56]. A similar mechanism could also be active for ROP polarization, as a mutant in *AtRhoGDI1* (*supercentipede1*/*scn1*) shows ectopic ROP2 accumulation associated with multiple root hair initiation sites along a single trichoblast [60], and knock-down of *AtRhoGDI2a* showed strong pollen depolarization [61]. Two lines of evidence suggest that this GDI-mediated mechanism is under control of Ca^{2+}. Firstly, the strength of pollen tube depolarization in *AtRhoGDI2a* knock-downs was found to be dependent on the Ca^{2+} concentration in the germination medium [61]. Secondly, it was shown that the phosphorylation status of conserved CPK3 phosphorylation sites in AtRhoGDI1, impacts on ROP-regulated polarization processes during pavement cell morphogenesis [62]. Although the direct involvement of Ca^{2+} in this process remains to be demonstrated unequivocally, it represents an interesting hypothesis on how Ca^{2+} can feed into ROP polarity.

While these data suggest that ROP polarity can be Ca^{2+} regulated, ROPs can in turn control Ca^{2+} signaling in pollen tubes, via ROP-interactive CRIB-containing protein 3 (RIC3)-regulated Ca^{2+} influx at the apex of pollen tubes [63] (Fig. 1C). Moreover, ROPs can also directly activate reactive oxygen species (ROS) production via respiratory burst oxidase homologues (RBOHs) [64], to activate Ca^{2+} channels that contribute to the tip-focused Ca^{2+} gradient in root hairs [65]. Interestingly, whereas the fungus *Piriformospora indica* can activate ROP-dependent actin remodeling, ROP signaling is not required for Ca^{2+} signals that are induced during infection [66].

In biological systems, polarization mechanisms involve coupled feed-back mechanisms that are optimized for robust symmetry breaking [56]. The reciprocal regulation between Ca^{2+} and ROP signaling could thus represent such a feed-back mechanism that underlies symmetry breaking in plants.

6. Mechano-sensitive polarity signaling involves Ca^{2+}

Until recently, the cell wall was mostly regarded as a passive capsule that protects the cell against the high turgor pressure. However, it is becoming more and more clear that the interaction between the cell and its encapsulating cell wall has important implications for polarity (Fig. 1D).

On the one hand, the cell wall contributes directly to the maintenance of polarity by restricting lateral diffusion rates of plasma membrane proteins [67,68]. On the other hand, local differences in the strength or elasticity of the cell wall in conjunction with the turgor pressure, as well as the anchorage of the plasma membrane to the cell wall can generate local differences in membrane tension, thereby directly impacting on polarity [69]. Although the underlying mechanism is not well understood, local differences in plasma membrane tension can be directly translated in polarity instructing Ca^{2+} signals via stretch-activated Ca^{2+} channels in the plasma membrane. This mechanism is believed to be relevant for tip growth processes where the fast growth generates a significant strain on the plasma membrane [7].

In addition, Ca^{2+} seems to contribute to several determinants of plasma membrane strain. At the level of the cell wall, Ca^{2+} can change the mechanical properties of de-esterified pectin, via cross-linking [70]. On the other hand, Ca^{2+} signaling could modify the cell wall via control over secretion of e.g. cell wall modifying enzymes [71], as indicated by the tremendous anisotropy in cell wall composition observed in root hairs and pollen tubes [72]. In addition Ca^{2+} itself can reduce the plasma membrane tension via modulation of membrane trafficking (cf. above) and via regulation of ion channel activities that modify the turgor pressure [6]. Thus, Ca^{2+} provides an interesting signal through which such mechanical signals can be translated in changes in polarity.

7. Conclusions and perspectives

Polarity in plants is a complex issue involving complex membrane trafficking and dynamic cytoskeleton reorganization in the context of an interconnected signaling network of small GTPases and physical cues. Although most of our knowledge derives from studies in pollen tubes and root hairs, which are examples of extremely polarized cells, it is slowly becoming more and more clear that cellular processes and signaling networks that govern polarity in tip growth are probably also at work in generating and maintaining polarity in other polarized cells and tissues.

The second messenger Ca^{2+} is commonly accepted as a core regulator of polarity in tip growth. Here, we illustrated the potential of Ca^{2+} as a general regulator of polarity, through highlighting the intimate connections between Ca^{2+} and polarity-driving processes and signaling cascades that are active throughout plant development. The lack of reports demonstrating Ca^{2+} gradients coinciding with other polarity-hallmarking events, argues against our generalization of Ca^{2+} as ubiquitous polarity-instructing signal. Yet, we believe that the reports thus far lack the necessary spatio-temporal resolution and sensitivity to visualize such Ca^{2+} signals in plant cells within a complex tissue context. Consistent with this notion is that the use of an ultrasensitive genetically encoded Ca^{2+} indicator, yellow cameleon (YCnano), has only recently allowed to visualize a wave of Ca^{2+} signals moving from the root to the shoot upon application of salt stress through the cortical cell file [73]. Similarly, a BRET-based GFP-aequorin (G5A) Ca^{2+} reporter was recently tested in plants, also visualizing a similar salt-induced mobile Ca^{2+} signal with high sensitivity [74]. This suggests that implementation of alternative genetically encoded Ca^{2+} indicators in plants, could provide the plant community with an unprecedented sensitivity and resolution to revisit this question.

Other difficulties associated with assessing the role of Ca^{2+} signaling in polarity are directly related to the Ca^{2+} signaling toolkit of plants. On the one hand, gene function can be masked by extensive functional redundancy among Ca^{2+} channels and decoders, as they are often encoded in large multi-gene families [19,75]. On the other hand, through divergence in evolution, plants lack well-characterized Ca^{2+} channels present in animals, such as L-type voltage-dependent Ca^{2+} channels, transient receptor potential (TRP) channels, inositol triphosphate receptors (IP_3R) and ryanodine receptors [75]. This implies that we cannot simply use Ca^{2+} channel blockers or agonists that were developed to target specific animal Ca^{2+} channels, to dissect the involvement of related Ca^{2+} channels in plants. While several important types of Ca^{2+} channels can be characterized electrophysiologically in plant cells, the molecular nature of the channels is often not known. Prominently among them are the stretch-activated Ca^{2+} channels that contribute to the Ca^{2+} gradient in pollen tubes. This fundamental gap in plant Ca^{2+} signaling, precludes the thorough evaluation of Ca^{2+} signaling in plant polarity. Recently, a completely new family of Ca^{2+} channels involved in osmosensing has been identified in plants [76], opening up new avenues for Ca^{2+} research and polarity.

Conflict of interest

The authors declare no conflict of interest.

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