ROS Regulation of Polar Growth in Plant Cells[1][OPEN]

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Root hair cells and pollen tubes, like fungal hyphae, possess a typical tip or polar cell expansion with growth limited to the apical dome. Cell expansion needs to be carefully regulated to produce a correct shape and size. Polar cell growth is sustained by oscillatory feedback loops comprising three main components that together play an important role regulating this process. One of the main components are reactive oxygen species (ROS) that, together with calcium ions (Ca^{2+}) and pH, sustain polar growth over time. Apoplastic ROS homeostasis controlled by NADPH oxidases as well as by secreted type III peroxidases has a great impact on cell wall properties during cell expansion. Polar growth needs to balance a focused secretion of new materials in an extending but still rigid cell wall in order to contain turgor pressure. In this review, we discuss the gaps in our understanding of how ROS impact on the oscillatory Ca^{2+} and pH signatures that, coordinately, allow root hair cells and pollen tubes to expand in a controlled manner to several hundred times their original size toward specific signals.

Polar growth occurs in root hair cells and pollen tubes, whereas coordinated multidirectional growth occurs in various other plant cells, such as puzzle-shaped pavement cells, trichomes, and stomata. Root hairs are single cells that absorb water and nutrients from the soil and have important roles in plant-microbe interactions, such as the legume-*Rhizobium* symbiosis, which involves nodule formation and N_2 fixation (Oldroyd, 2001; Oldroyd and Dixon, 2014). Pollen tubes are cells that carry sperm nuclei to the ovule, thus facilitating fertilization and seed production. Polar growth is guided by specific external signals, such as nutrient gradients and small attractant peptides. For instance, the LURE1 Cys-rich peptide, which is secreted by synergid cells, and two groups of plasma membrane receptor-like kinases with extracellular Leu-rich repeats and an intracellular kinase domain (namely MALE DISCOVERED1-MDIS1-INTERACTING RECEPTOR-LIKE KINASE1/2 and POLLEN-SPECIFIC RECEPTOR-LIKE KINASE6 [PRK6]) within the growing pollen tube are involved in pollen tube guidance (Takeuchi and Higashiyama, 2016; Wang et al., 2016). Both types of receptor kinases bind to LURE1, triggering downstream responses, such as activation of the core tip growth machinery (e.g. ROPGEF8, ROPGEF9, ROPGEF12, and ROPGEF13) and RHO OF PLANTS1 (ROP1), in the case of PRK6 (Takeuchi and Higashiyama, 2016). By contrast, little is known about the molecular mechanisms that guide the polar growth of root hairs. Root hair shape, cell length, and density are strongly modulated by environmental signals, such as the gradients of low-mobility mineral nutrients (e.g. phosphorus, iron, and manganese) or scarce elements (e.g. vanadium and boron) in the soil (Yi et al., 2010; Martín-Rejano et al., 2011; Niu et al., 2014; Lin et al., 2015). Other signals, such as water status, carbon monoxide, and carbon dioxide levels, also affect root hair development (Guo et al., 2009; Niu et al., 2011; Kwasniewski et al., 2016). Polar growth also is responsive to endogenous signals, such as ethylene and auxin (for review, see Lee and Cho, 2013; Velasquez et al., 2016). For instance, auxin activates the class I basic helix-loop-helix transcription factor ROOT HAIR DEFECTIVE6-LIKE4 (RSL4), thus modulating downstream gene expression to trigger root hair cell expansion (Yi et al., 2010; Datta et al., 2015).

During polar cell expansion, the apical zone is characterized by a gradient of cytoplasmic Ca^{2+} ions ($_{cyt}Ca^{2+}$) and apoplastic reactive oxygen species ($_{apo}$ROS) production. High levels of $_{cyt}Ca^{2+}$ in the tip zone trigger $_{apo}$ROS production, in a reaction catalyzed by NADPH oxidases (NOXs). Furthermore, high levels of reactive oxygen species (ROS) transiently elevate the concentration of $_{cyt}Ca^{2+}$ (Duan et al., 2014) by an unknown mechanism. NOXC (Bibikova et al., 1998; Foreman et al., 2003; Monshausen et al., 2007, 2008) and NOXH/NOXJ (Wu et al., 2010; Boisson-Dernier et al., 2013) were proposed previously to connect $_{apo}$ROS production with the transient activation of plasma membrane Ca^{2+} channels (CaCs; Table I) in growing root hairs and growing pollen tubes, respectively. A ROS burst is crucial for pollen tube rupture and sperm release (Duan et al., 2014). Oscillatory growth also is linked to changes in pH that are mostly regulated by membrane H^+-ATPases (AHA; Falhof et al., 2016) and by cation (H^+)/anion (OH^-)-permeable channels and

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Table I. *Selected examples of putative plasma membrane CaCs present in Arabidopsis and expressed preferentially in polar-expanding cells (pollen tubes or root hairs)*

Protein	Ion selectivity	Tissue expression	Assumed function-Mutant Phenotype	References
Cyclic Nucleotide Gated Channels (CNGC) are stimulated by the binding to cyclid Adenosin Mono Phosphate (cAMPc) or to cyclid Guanosin Mono Phosphate (cGMP). CNGC have six transmembrane domains with a pore situated between 5 and 6. In the C-terminus have a regulatory sequence where the cyclic nucleotide-binding is partially overlapping with a calmodulin-bind suggesting an interaction between these domains (Tian et al., 2006; López-Bucio et al., 2013). Unfortunately, there are no specific pharmacological inhibitors described for these channels so far.				
CNGC7	Ca^{2+}, K^+	Pollen and phloem	Male reproductive fertility. Pollen tube tip growth.	(Tunc-Ozdemir et al., 2013)
CNGC8	Ca^{2+}, K^+	Flower and stamen		
CNGC14	unknown	Root and root hair	Ca^{2+} dependent gravitropic responsiveness of roots	(Shih et al., 2015)
CNGC10	Ca^{2+}, K^+, Mg^{2+}	Sperm cell, pollen, and root	Light modulated development. Salt stress Ca^{2+} dependent gravitropic responsiveness of roots	(Li et al., 2005; Christopher et al., 2007; Guo et al., 2008; Urquhart et al., 2011)
CNGC16	Ca^{2+}	Pollen and phloem	Stress tolerance in pollen development	(Tunc-Ozdemir et al., 2013)
CNGC18	Ca^{2+}, K^+	Pollen and pollen tube	Pollen tube growth. Pollen tube guidance to ovules	(Chang et al., 2007; Frietsch et al., 2007; Gao et al. 2014; Gao et al. 2016)
Glutamate-Like Receptors (GLR) are non-selective cation channels. In plants, GLRs have four transmembrane domains as well as the LBD (Ligand Binding Domain) and they are proposed to form *in vivo* tetramers. Asn, Gly (Glycine) and Ser (Serine) are the best agonists of Glu (Glutamate), which binds to LBD and open GLRs to the cation transport. In *Arabidopsis thaliana* there are 20 putative GLRs (Roy et al., 2008) and several of them are highly expressed in pollen grains. The inhibitor CNQX (*6-cyano-7-nitroquinoxaline-2,3-dione*) acts as antagonist of Glu, blocking its union site (Michard et al., 2009) and is able to phenocopy *glr1.2-1* mutant (Song et al., 2004).				
GLR1.2	Ca^{2+}	Pollen and leaf	Pollen development/ Deformed pollen tips and tubes.	(Michard et al., 2011)
GLR3.7	Ca^{2+}	Root phloem and flower	Pollen development/pollen tubes grew slower	(Michard et al., 2011)
Voltage Gated Channels. In this category are included the depolarisation-activated Ca^{2+} channel TPC1 and Annexins (ANN). TPC1 is proposed to be localized mostly in the vacuole membrane (Dadacz-Narloch et al. 2013) although some previous reports also showed their localization in the plasma membrane (Hashimoto et al. 2004; Wang et al. 2005). TPC1 has two pores, each consisting of six transmembrane domains (S1-12) and in the linker region between the two pores (between S6 and S7) there are two EF-hand motifs required to enable channel responses at physiological changes in Ca^{2+} concentration (Palmgren, 2001; Sondergaard et al., 2004). Recently, it as shown under salt stress root tips of *Arabidopsis* plants triggered a Ca^{2+} wave, dependent on the function of TPC1, that spread to the aerial parts of the plant suggesting that TPC1 is crucial for transmitting a long distance Ca^{2+} mediated signal (Hedrich and Marten, 2011). On the other hand, ANN are Ca^{2+} and phospholipid-binding proteins that in plants and animals have a highly similar protein structure. ANN have core domains compose by four-eight homologous repeats of approximately 70 amino acids that contain a conserved Ca^{2+} and membrane-binding motif. When $Ca^{2+}{}_{cyt}$ increase, they relocate to the plasma membrane and there some ANN are capable to form putative voltage-gated channels. In *A. thaliana* the ANN family has eight members (ANN1-8). ANN1 is a Ca^{2+} -permeable transporter sensitive to ROS in roots providing a molecular link between reactive oxygen species and ${}_{cyt}Ca^{2+}$ (Laohavisit et al., 2012). Also, ANN1 shows peroxidase activity (Konopka-Postupolska et al., 2009; Fuglsang et al., 2014). Still, it is completely unknown the molecular mechanism by which ANN1 is able to either directly transport Ca^{2+} or indirectly, trigger a Ca^{2+} mediated response to certain stimuli (e.g. ROS).				
TPC1	non-selective	Root, root hair, guard cell, shoot and cotyledon	Propagation of salt-stress signal	(Hedrich and Marten, 2011; Dadacz-Narloch et al. 2013)
ANN1	Ca^{2+}	Root and root hair	Plant defense and Stress tolerance. Short root hairs	(Huh et al., 2010; Laohavisit et al., 2012; Richards et al., 2014)
Osmosensitive Gated Ca2+ Permeable Channels (CSC1 and OsCA1) and related ***M*echano*s*ensitive *c*hannels (Msc)**. The two best examples of Ca2+ permeable channels in plant cells are the osmosensors channels OSCA1, for reduced hyper*OS*molality induced [CA^{2+}] increase 1, and CSC1, for Calcium permeable Stress-gated cation Channel *1*. In *Arabidopsis thaliana* they are 15 members of the OSCA family (Yuan et al., 2014). OSCA1 is a hyperosmolalilty-gated non selective cation channel that permeates Ca2+ ions (Yuan et al., 2014). CSC1 is permeable to Ca^{2+} and it is gated by physical signals like hyperosmotic stress (Hou et al., 2014). The closest candidates of Ca^{2+} permeable channels CSC1 and OSCA1 are the *M*id1-Complementing *A*ctivity (MCA) family of mechanosensing channels that perceive touch and osmotic signals (Kurusu et al., 2012). MSL proteins (MscS-like, homologs of bacterial Mechanosensitive channel of Small conductance, MscS) interact with the plasma membrane and this is crucial for respond to changes in membrane tension (Kurusu et al., 2013). To date MSL8 is the first mechanosensing channel linked to pollen tube growth (Hamilton et al. 2015).				
MCA1	Ca^{2+}	Cotyledon, stem, leaf and root	Not reported yet	(Nakano et al., 2011)
MCA2	Ca^{2+}	Cotyledon, leaf and root	Touch-related root responses	(Nakano et al., 2011; Nakano et al., 2014)
OSCA1	Ca^{2+}	Leaf, flower, root, and guard cell	Osmo-stress tolerance	(Yuan et al., 2014)
CSC1 (OSCA1.2)	Ca^{2+}	Root, carpel, pistil ovule	Hyperosmotic-Stress tolerance	(Hou et al., 2014)
OSCA1.5	unknown	Root, root hair and ovule	Not reported yet	
OSCA2.4	unknown	Root, root hair, guard cell and leaf	Not reported yet	
MSL8	Cl^-	Pollen grain and pollen tube	Regulates osmotic forces during pollen hydration and germination	(Hamilton et al. 2015)

antiporters (Ca^{2+}/H^+ exchangers). AHA2 is highly expressed in growing root hairs, while the AHA presumed to be responsible for pumping H^+ out of pollen tubes remains to be identified. AHA directly regulates apoplastic pH ($_{apo}$pH), which impacts the enzymatic machinery that modifies wall components during cell expansion. Polar growth involves the deformation and stretching of the existing primary wall in the apical zone, which is accompanied by the secretion of new cell wall materials (Altartouri and Geitmann, 2015). In addition, in vitro pollen tube tip growth and root hair expansion are coupled to the Ca^{2+} gradient that directs polar secretion, the arrangement of the actin cytoskeleton, the movement of organelles, and the biochemical

activity of necessary enzymes (Sanders et al., 2002; Fan et al., 2004; Rounds and Bezanilla 2013; Huang et al., 2015). Oscillations in ROS, Ca^{2+}, and pH are coupled to transient cell wall loosening to allow turgor-driven localized cell expansion (Braidwood et al., 2014; Spartz et al., 2014; Wolf and Höfte, 2014). In root hairs, the maxima of these oscillatory fluctuations in $_{apo}$ROS concentration and apoplastic/cytoplasmic pH precede cell growth peaks by 7 to 8 s, while $_{cyt}Ca^{2+}$ oscillations lag oscillations in cell growth by approximately 5 to 6 s (Monshausen et al., 2007, 2008). In pollen tubes, the oscillations in $_{cyt}Ca^{2+}$ concentration are delayed by approximately 11 s relative to cell expansion rates (Pierson et al., 1994). Thus, polar cell growth is preceded, and perhaps transiently repressed, by high levels of $_{cyt}Ca^{2+}$ and, subsequently, high $_{apo}$ROS concentrations and a more alkaline $_{apo}$pH.

Studies using molecular biosensors that detect rapid changes in the concentrations of several small molecules have emerged recently and provided insight into the molecular mechanism underlying tip growth (Uslu and Grossmann, 2016). Examples of these include biosensors that detect concentrations of ROS (Belousov et al., 2006; Costa et al., 2010), Ca^{2+} (Horikawa et al., 2010; Bardgett et al., 2014; Keinath et al., 2015), and H^+ (Monshausen et al., 2007, 2011; Gjetting et al., 2012) at high spatiotemporal resolution in real time in different subcellular compartments. Specifically, a dynamic view of cytoplasmic reactive oxygen species ($_{cyt}$ROS) oscillations during growth is provided by a genetically encoded HyPer sensor and by roGFP (for redox-sensitive GFP) coupled to OXIDANT RECEPTOR PEROXIDASE1 (Orp1), both of which are sensitive to hydrogen peroxide (H_2O_2; Mishina et al., 2013). HyPer consists of a circularly permuted yellow fluorescent protein (cpYFP) molecule coupled to a regulatory domain of the *Escherichia coli* H_2O_2 sensor OxyR. When exposed to H_2O_2, the excitation peak of cpYFP shifts from 420 to 500 nm, while the emission peak remains unchanged (at 516 nm; Belousov et al., 2006), thus allowing this probe to be used as a ratiometric biosensor (Costa et al., 2010; Hernández-Barrera et al., 2015). Importantly, HyPer and various other biosensors (e.g. the Ca^{2+} sensor R-GECO1) are sensitive to cellular pH, whereas others, such as roGFP2, are not. R-GECO1 has been engineered to contain dithiol/disulfide switches that act on a few Cys residues in the protein. As the oxidized and reduced forms of R-GECO1 have characteristic fluorescent spectra, this probe can be used ratiometrically to report on the thiol-disulfide status of the cell (Schwarzländer et al., 2008). When roGFP2 is fused directly to the human glutaredoxin Grx1 (Grx1-roGFP2), it detects changes in glutathione redox potential (Yu et al., 2013), while an Orp1-roGFP2 fusion can be used to monitor H_2O_2 (Gutscher et al., 2009). These probes may provide standardized response kinetics based on defined reaction mechanisms that are independent of the local intracellular environment of the sensor (Wagner et al., 2015).

It remains unclear how oscillatory gradients of $_{cyt}Ca^{2+}$, ROS, and cytoplasmic/apoplastic pH gradients are maintained during polar growth in pollen tubes and root hairs (Wudick and Feijó, 2014). Figure 1 shows possible links between ROS and other molecules in the cell surface-plasma membrane space of the growing tip. Below, we discuss recent exciting developments in our understanding of how ROS control polar growth in root hairs and pollen tubes. For discussions of the roles of ROS, Ca^{2+}, and pH signaling in plant development and physiology, we refer the reader to other recent reviews (Swanson et al., 2011; Kurusu et al., 2013; Gilroy et al., 2014; Haruta et al., 2015; Kärkönen and Kuchitsu, 2015).

$_{apo}$ROS HOMEOSTASIS IS REGULATED BY PLASMA MEMBRANE NOXS AND APOPLASTIC TYPE III SECRETED PEROXIDASES

Although ROS are produced in several intracellular compartments (e.g. chloroplasts, mitochondria, and peroxisomes; Foyer and Noctor, 2003; Overmyer et al., 2003), we will focus exclusively on the $_{apo}$ROS that are generated on the extracellular face of the plasma membrane. While the term ROS includes a variety of small molecules, we will concentrate our attention on the most discussed, including the hydroxyl radical ($^{\bullet}OH$), the superoxide radical ($O_2^{-\bullet}$), H_2O_2, and singlet oxygen. Singlet oxygen is an excited state molecule with a short half-life (100 ns) that can travel only a short distance in cells (less than 100 nm; Niedre et al., 2002). Consequently, it reacts directly with molecules in close proximity to its site of production (Triantaphylidès and Havaux, 2009). $O_2^{-\bullet}$ is a highly unstable ion that is unable to diffuse through membranes. By contrast, H_2O_2 is relatively stable (half-life of approximately 1 ms) and moves through membranes via aquaporins. H_2O_2 reacts selectively with Cys residues in signaling proteins (e.g. peroxiredoxins, thioredoxins, and Cys-rich receptor-like kinases; Paulsen and Carroll, 2010), and its concentrations are tightly controlled by antioxidant systems. These features make H_2O_2 a good signaling candidate among the various ROS.

Although multiple enzymes (e.g. oxalate oxidases, amine oxidases, lipoxygenases, and quinone reductases) contribute to the production and accumulation of $_{apo}$ROS (Passardi et al., 2004; Cona et al., 2006), most $_{apo}$ROS are produced by NOX proteins, which also are known as RBOHs (for respiratory burst oxidase homologs). In addition to NOX, we also consider the apoplastic type III peroxidases ($_{apo}PER_{III}$) in this review, since, as discussed below, these enzymes may influence the pool of $_{apo}$ROS. Other reactive oxidizing species, such as nitric oxide, nitric dioxide, dinitrogen tetroxide, and nitrous acid, also may play roles in tip growth. Nitric oxide is a well-studied signal that has an important effect on polar growth (Lombardo and Lamattina, 2012; Domingos et al., 2015). However, the focus of our discussion will be on the roles of ROS.

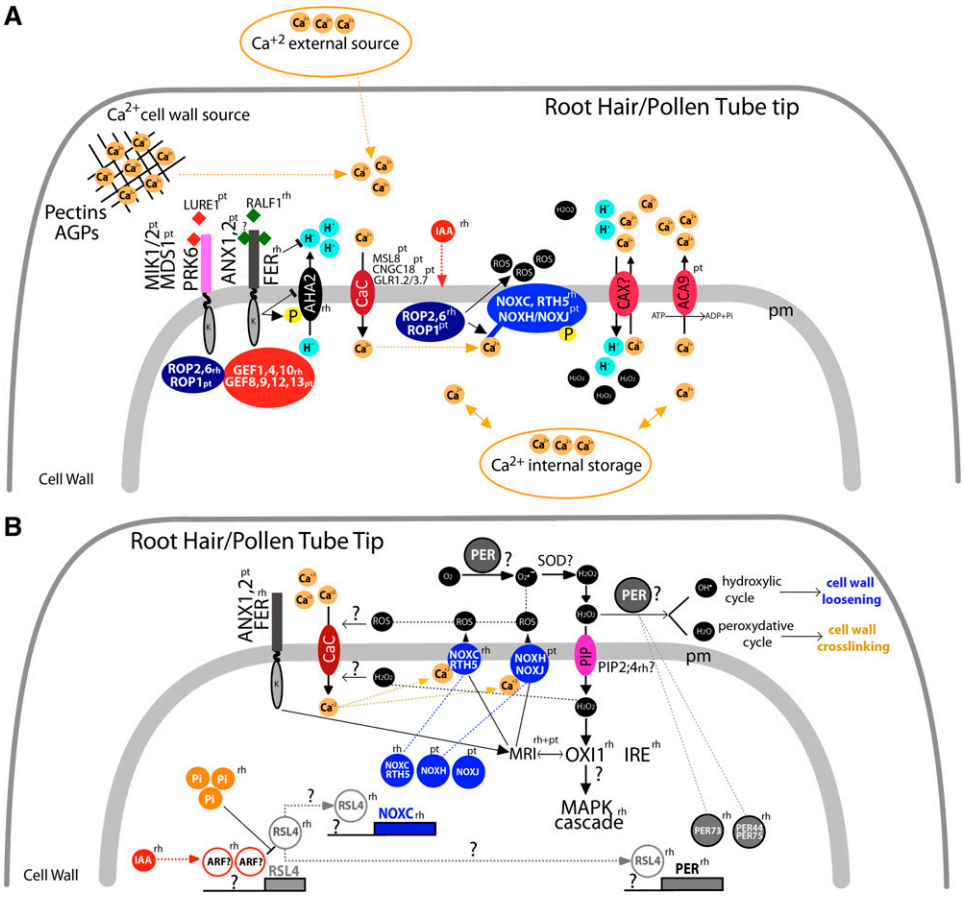

Figure 1. Proposed scheme to describe how ROS are interconnected with Ca^{2+}-pH signaling components in polar growing cells. A, Ca^{2+} ions are taken up by the cell from external sources or stored in the cell wall and released by changes in $_{apo}$pH controlled mainly by plasma membrane AHA. In addition, Ca^{2+} can be stored in subcellular compartments (e.g. vacuoles and ER-Golgi) and released into the cytosol. Still poorly characterized, plasma membrane CaCs transport free Ca^{2+} ions from the apoplast into the cytoplasm (Table I). To maintain physiologically low concentrations of $_{cyt}Ca^{2+}$, ACAs transport Ca^{2+} ions back to the apoplast. In addition, the H^+/Ca^{2+} antiporter activity of CAX translocates Ca^{2+} back to the apoplast and, simultaneously, imports H^+ into the cytoplasm. $_{apo}$ROS production is catalyzed by the NOX proteins, NOXC and RTH5 (in maize) in root hairs and NOXH/NOXJ in pollen tubes. These NOX proteins are regulated by complex partners, including Ca^{2+} ions, ROPs, and kinases. NOX produces apoplastic ion superoxide (O_2^-), which mostly is converted by superoxide dismutases (SODs) to H_2O_2. In root cells, the CrRLK1L kinase FER, which contains a malectin-like extracellular domain, binds to RALF1 and inhibits growth. In growing root hairs, FER forms a complex with the guanine nucleotide-exchange factor ROP-GEF1, and possibly with other ROP-GEFs (such as ROP-GEF4 or ROP-GEF10), to recruit and then activate the plant RHO GTPase ROP2. In pollen tubes, ANX1 and ANX2 are the CrRLK1L proteins that regulate ROS-linked cell growth. In addition, pollen tube guidance to the ovule relies on two groups of Leu-rich receptor-like kinases (MDS1-MIK1/2 and PRK6) binding to the Cys-rich peptide LURE1 (see text for details) and downstream ROPGEFs (ROPGEF8, ROPGEF9, ROPGEF12, and ROPGEF13) and ROP1 (at least demonstrated for PRK6). Then, ROP1 possibly targets the N-terminal domain of NOXH/NOXJ to trigger ROS production. In root hairs, spatially active ROP2 triggers ROS production, since it is able to bind directly to the N terminus of NOXC and enhance its enzymatic activity. Auxin (as indole acetic acid [IAA]) enhances ROP2 activity to generate ROS by triggering NOXC activation. B, In root hairs, high auxin levels (IAA), possibly attained through the activation of unknown ARF proteins, up-regulate the expression of the transcription factor basic helix-loop-helix RSL4 (in addition to activating ROP2) and promote ROS production. It is proposed here that RSL4 controls (directly or indirectly) the transcription of NOXC/RTH5 and NOXH/NOXJ as well as secreted type III peroxidases (PER) in root hairs. By contrast, high levels of Pi act as a repressor of RSL4 expression. High concentrations of $_{apo}$ROS enhance or reduce the stiffness of the cell wall, thereby either promoting or restricting cellular extension. PER uses H_2O_2 as an oxidant to convert cell wall phenolic compounds and structural proteins into free radicals that can subsequently come together to form covalent linkages (in the peroxidative cycle), thus restricting growth. Also, $_{apo}H_2O_2$ and oxygen (O_2) can be used to generate hydroxyl radicals (in the hydroxylic cycle), including $^{\bullet}$OH, which mediates the nonenzymatic cleavage of polysaccharides. This would induce transient relaxation of the cell wall, allowing tip growth. ROS also could promote Ca^{2+} transport by triggering the activation of CaCs (Table I) by unknown mechanisms, possibly in the apoplast, cytoplasm, or on both sides. Not all ROS remains in the apoplast, since some of the H_2O_2 may be transported back to the cytoplasm by aquaporins (plasma membrane intrinsic proteins [PIPs]) and trigger a signal cascade response (e.g. OXI1-mediated MAPK cascade). OXI1, an AGC2 kinase, could activate MARIS

Plant NOXs are complex plasma membrane proteins with six transmembrane domains, an N-terminal regulatory domain that includes a ROP GTPase-binding region, two EF hands, and several potential phosphorylation sites (Takeda et al., 2008) as well as a C-terminal FAD/NADP(H)-binding domain. All NOXs produce apoplastic ion superoxide, which is mostly converted chemically or enzymatically by superoxide dismutases to oxygen and H_2O_2. H_2O_2 production and transport need to be fine-tuned with high precision. Arabidopsis (*Arabidopsis thaliana*) NOX proteins (AtNOXA–AtNOXJ) have been implicated in ROS signaling linked to several developmental and abiotic/biotic stress responses (Torres and Dangl, 2005; Müller et al., 2009; Dubiella et al., 2013; Lee et al., 2013; Xie et al., 2014). Two NOXs (AtNOXH and AtNOXJ) have been implicated in pollen tube tip expansion and fertilization (Boisson-Dernier et al., 2013; Kaya et al., 2014; Lassig et al., 2014) and one NOX (AtNOXC, named ROOT HAIR DEFECTIVE [RHD2]) in root hair tip growth (Fig 1; Foreman et al., 2003; Takeda et al., 2008). Recently, a monocot NOX named RTH5 (from maize [*Zea mays*]), with high sequence similarity to AtNOXH and AtNOXJ, was identified as an important player in the transition between root hair bulge formation and tip growth (Nestler et al., 2014).

NOX proteins contain several EF hand motifs in their N-terminal domains, and they are activated by Ca^{2+} and by Ca^{2+}-mediated protein phosphorylation. Both modifications (Ca^{2+} binding and phosphorylation) have synergistic effects on NOX activity, although phosphorylation seems to be a prerequisite for Ca^{2+}-mediated NOX activation (Wong et al., 2007; Kimura et al., 2012; Takahashi et al., 2012). Experiments carried out in HEK293T (human embryonic kidney) cells showed that induced mutations in the EF hand domains of NOXC and in NOXH/NOXJ proteins partially or completely suppressed ROS production (Kaya et al., 2014). Ser residues that are crucial for activity have been identified as phosphorylation sites in the N-terminal regions of several NOXs, including Ser-318/Ser-322 in NOXC (Kobayashi et al., 2007; Nühse et al., 2007; Takeda et al., 2008; Sirichandra et al., 2009; Dubiella et al., 2013; Gao et al., 2014). In NOXH and NOXJ sequences, Ser-318 also is conserved and Ser-322 is replaced with a potential Thr phosphosite. This suggests the existence of a putative common activation mechanism mediated by Ca^{2+} and the phosphorylation of both NOXC and NOXH/NOXJ in growing pollen tubes and root hairs.

Little is known about the transcriptional regulation of NOXC and NOXH/NOXJ during polar growth. In root hairs, RSL4, which is downstream of the morphogenetic RHD6/RSL1 program, is thought to be a master regulator of growth (Datta et al., 2015). RSL4 is activated by low concentrations of inorganic phosphate (Pi; an environmental signal) and high concentrations of endogenous auxin, by an unknown mechanism (Yi et al., 2010; Datta et al., 2015). RSL4 controls the expression of root hair-specific genes, including several that encode cell wall proteins (e.g. extensins [EXT] and Pro-rich proteins [PRPs]) and cell wall enzymes (e.g. $_{apo}PER_{III}$, expansins, xyloglucan-endotransglucosylase/hydrolase [XTH], and pectate lyases). In addition, it is possible that RSL4 directly or indirectly activates the transcription of NOXC during root hair growth. It is tempting to speculate that auxin and low-Pi conditions both up-regulate RSL4 expression to trigger ROS-linked polar growth in root hairs (Fig. 1B). However, the molecular links between auxin-mediated regulation of root hair growth and RSL4 activation (Yi et al., 2010; Datta et al., 2015) and between *RSL4* expression and ROS production remain to be elucidated. Furthermore, the overexpression of RSL proteins in *Brachypodium* spp., rice (*Oryza sativa*), and wheat (*Triticum aestivum*) promotes root hair growth, and MpRSL1 in the liverwort *Marchantia polymorpha* (Proust et al., 2016) and PpRSL1 and PpRSL2 in the moss *Physcomitrella patens* (Jang and Dolan, 2011) are regulated by auxin and trigger cell expansion. Consequently, the auxin-RSL module is thought to have been conserved during plant evolution as a master regulator of polar growth and may have been derived from the first land plants that lived almost 500 million years ago (Pires et al., 2013; Proust et al., 2016). It is unclear if the auxin-RSL module controlled polar cell expansion by triggering ROS production in the early plant lineages. In pollen tubes, no transcription factor has hitherto been described as a central regulator of growth linked to NOXH/NOXJ-mediated ROS production.

In addition to NOX proteins, $_{apo}PER_{III}$ has a direct impact on $_{apo}ROS$ levels (Fig. 1B) and can be positively modulated by Ca^{2+} (Plieth and Vollbehr, 2012). Depending on the substrate, peroxidases can perform peroxidative, hydroxylic, and oxygen reduction (Passardi et al., 2004). In the first reaction, $_{apo}PER_{III}$ use H_2O_2 as an oxidant to convert cell wall phenolic compounds and structural proteins (e.g. extensins) to free radicals that can subsequently come together to form covalent linkages. In the hydroxylic cycle, apoplastic hydrogen peroxide ($_{apo}H_2O_2$) and oxygen are converted by $_{apo}PER_{III}$ into radicals, including $^{\bullet}OH$, that cleave polysaccharides in a nonenzymatic fashion (Dunand

Figure 1. (*Continued.*)
(MRI), a receptor-like cytoplasmic kinase present in growing root hairs and pollen tubes. MRI would be a downstream component of FER in root hairs and of ANX1/ANX2 in pollen tubes. Other root hair-specific AGC kinases in the VIII subfamily are IRE and AGC2-1. Solid arrows indicate a signaling pathway, a downstream step, or transport across the plasma membrane; solid lines indicate a close relationship between proteins or ions. P, Phosphorylated site; pm, plasma membrane; pt, pollen tubes; rh, root hairs.

et al., 2007). In addition, $_{apo}PER_{III}$ generates $O_2^{-\bullet}$ from oxygen reduction that can subsequently be dismutated to $_{apo}H_2O_2$. Overall, $_{apo}PER_{III}$ either augments or reduces the pool of $_{apo}H_2O_2$ (Francoz et al., 2015). An increase in $_{apo}H_2O_2$ concentration leads to rigidification of the cell wall, triggering an immediate cessation of cell expansion (Monshausen et al., 2007). Thus, some of these $_{apo}PER_{III}$ proteins could act to cross-link, at least transiently, specific cell wall components.

It is also important to highlight that cell wall chemistry is quite different in root hairs and in pollen tube cells (for details, see Gu and Nielsen, 2013). In root hairs, the walls are composed mostly of (xylo)glucans, pectins, and *O*-glycoproteins (Galway et al., 2011; Velasquez et al., 2011; Peña et al., 2012), while in pollen tubes, the walls are enriched in pectins and also contain glycoproteins and xyloglucans/cellulose (Dardelle et al., 2010). Cell wall deficiencies in any of these polymers inhibit polar cell elongation in root hairs and pollen tubes, indicating that these polymers operate together to modulate controlled expansion (Bernhardt and Tierney, 2000; Favery et al., 2001; Pang et al., 2010; Ringli, 2010; Park et al., 2011; Velasquez et al., 2011; Zabotina et al., 2012; Wang et al., 2014). Specifically, Hyp-rich glycoproteins of the EXT type (Velasquez et al., 2011) and PRP (Bernhardt and Tierney, 2000) are Tyr cross-linked by an unidentified $_{apo}PER_{III}$ to facilitate the formation of a cell wall glycoprotein network (Cannon et al., 2008). This was suggested to occur in growing root hairs (Velasquez et al., 2015). By contrast, the $_{apo}ROS$ produced by the activity of $_{apo}PER_{III}$ (in hydroxylic cycles) would enhance polysaccharide cleavage and act as a wall-loosening agent that promotes growth (Fry, 1998; Schopfer et al., 2002; Dunand et al., 2007; Macpherson et al., 2008) independently of Ca^{2+} signaling. Recently, two PER proteins (PER44 and PER75) were found to be repressed at the transcriptional level under low-gravity conditions. Indeed, the root hairs of the corresponding transfer DNA mutants (*per44* and *per75*) exhibited tip rupturing and other defects under normal growth conditions (Kwon et al., 2015). Also, the PER inhibitor salicylhydroxamic acid drastically represses root hair growth. Both *per* mutants and salicylhydroxamic acid treatment cause root hairs to burst, possibly because the highly relaxed cell wall at the root hair tip cannot withstand the turgor pressure (Kwon et al., 2015). So far, no *per* mutants have been reported to have defective polar growth in pollen tubes, probably due to the high degree of genetic redundancy between the 71 encoded $_{apo}PER_{III}$ proteins in Arabidopsis (Francoz et al., 2015). Some $_{apo}PER_{III}$ proteins appear to harden the cell wall structure, while others impact cell wall loosening during plant development (Passardi et al., 2006; Jin et al., 2011; Pedreira et al., 2011; Herrero et al., 2013; Kunieda et al., 2013; Lee et al., 2013; Shigeto et al., 2013; Manzano et al., 2014). It is unclear how these two opposite effects on cell wall polymers that are mediated by $_{apo}PER_{III}$ are coordinated during the oscillatory growth cycles of these cells.

$_{apo}ROS$ in the form of H_2O_2 (and other reactive species) could have a direct impact on the redox state of Cys-rich proteins, such as Cys-rich receptor-like kinases (CRKs; Chen, 2001). CRKs represent one of the largest groups of receptor-like kinases (RLKs), with 44 members in Arabidopsis (Wrzaczek et al., 2010). The extracellular domain of CRK comprises three well-conserved Cys residues (C-X_8-C-X_2-C) that form disulfide bridges and are potential targets for thiol-redox regulation. CRK may sense extracellular ROS and amplify the initial ROS signal. Recently, a large-scale broad phenotyping assessment of a complete *crk* transfer DNA insertion line collection showed that CRK controls several aspects of plant development (Bourdais et al., 2015), although direct evidence that links CRK to polar growth remains to be presented.

It is possible that polar growth processes are regulated by mechanisms involving the oscillatory changes in pH, which are controlled by the activation/deactivation of H^+ pumps (AHA), H^+/OH^--permeable channels, and calcium-exchanger (CAX) protein-type antiporters. Oscillating apoplastic H^+ fluxes produce transient alkaline or more acidic environments on the growing tip apoplast that would activate or repress the activity of the peroxidative or oxidative cycle of $_{apo}PER_{III}$. In addition, the activation/inactivation of many other cell wall-modifying enzymes, such as expansins (e.g. EXP7, EXP12, and EXP18 in root hairs and maize ZmEXPB1 and ZmEXPB11 in pollen tubes), pectin methyl esterases (e.g. PME1 and PME48 in pollen tubes), pectate lyases (e.g. ROOT HAIR-SPECIFIC14 in root hairs and PECTATE LYASE-LIKE in pollen tubes), and XTH (e.g. XTH12 and XTH26 in root hairs and XTH29 and XTH30 in pollen), is thought to trigger either localized wall rigidification or cell wall loosening. The regulation of polar growth by the pH microenvironment is consistent with the observed total cessation of growth in alkaline medium (pH 8) and root hair bursting, resulting from uncontrolled growth coupled to cell wall breakdown, on acidic medium (pH 4.5; Monshausen et al., 2007).

Specifically, PMEs, which demethylesterify carboxyl residues on the pectin backbone, affect not only cell wall viscosity but also Ca^{2+} dynamics. PME activity renders carboxyl residues present in pectins capable of binding to Ca^{2+} ions, which results in pectin cross-linking and likely cell wall stiffening at the pollen tube tip (Bosch and Helper, 2005; Palin and Geitmann, 2012). On the other hand, PME inhibitor proteins (e.g. PMEI-2) block this activity and retard deesterification reactions, thereby preventing excessive cross-linking of pectins in the pollen tube tip (Röckel et al., 2008). The coregulation of PME-PMEI interactions has been characterized in several biological processes (e.g. plant pathogen interactions and inflorescence meristem development; Wolf et al., 2009). Such interactions also are involved in regulating pollen tube growth, but evidence for similar interactions in root hair cells is lacking.

$_{apo}$ROS IS CHANNELED INTO THE CYTOPLASM TO TRIGGER DOWNSTREAM RESPONSES

Although the ROS associated with polar growth are produced in the apoplast by NOX and other enzymes, most of the ROS occurs in the cytoplasm, close to the growing tip, during polar growth (Monshausen et al., 2007). It is not known how the ROS signal is transmitted to the cytoplasm or how the different subcellular compartments that contribute to the $_{cyt}$ROS pool are connected. It is postulated that $_{apo}H_2O_2$ is actively transported into the cytoplasm by a subgroup of plasma membrane aquaporins of the PIP type (Dynowski et al., 2008; Hooijmaijers et al., 2012). Recently, it was shown that AQUAPORIN8 is able to translocate $_{apo}H_2O_2$ into the hyphae of the plant pathogen *Botrytis cinerea* (An et al., 2016). In addition, the in vivo influx of $_{apo}H_2O_2$ triggered by PIP1;4 in Arabidopsis was recently shown to occur in plant cells during pathogen infection and pathogen-associated molecular pattern recognition (Tian et al., 2016). The transport of H_2O_2 by several AtPIP proteins, including PIP2;4 and PIP2;7, which are highly expressed in root hairs, was demonstrated in uptake and/or toxicity H_2O_2 assays in heterologous systems (e.g. yeast cells). In addition, a *pip2;4* mutant was reported to have longer root hairs than the wild type when grown under Pi starvation or under normal Pi conditions (Lin et al., 2011). Still, loss-of-function mutants for these PIPs remain to be characterized with regard to ROS levels at the root hair/pollen tube growing tip, and an in vivo function in ROS transport during the growth of these cells has yet to be demonstrated. ROS production also could originate from several enzymes located in intracellular compartments, such as chloroplasts, vacuoles, mitochondria, and the endoplasmic reticulum (ER).

It is unclear how transiently elevated $_{cyt}$ROS concentrations are transduced in a downstream signaling response. OXIDATIVE STRESS INDUCIBLE1 (OXI1), which belongs to AGC kinase (for protein kinase A/protein kinase G/protein kinase C) family, was shown to be required for ROS-mediated events in root hair elongation. Thus, OXI1 (also named as AGC2-1; At3g25250) appears to connect oxidative burst signals with downstream responses (Anthony et al., 2004; Rentel et al., 2004). Recently, it was postulated that OXI1 activates MRI, a receptor-like cytoplasmic kinase of the VIII subfamily. MRI present in growing root hairs and pollen tubes is postulated to act downstream of the *Catharanthus roseus* RLKs FERONIA (FER) and ANXUR1 (ANX1)/ANX2 (Boisson-Dernier et al., 2015). Recently, it was shown that MRI interacts with OXI1, and in an vitro assay it was phosphorylated by OXI1 (Liao et al., 2016). In leaves, OXI1, which relays ROS signals, phosphorylates MITOGEN-ACTIVATED PROTEIN KINASE3 (MAPK3), MAPK4, and MAPK6, which activate genes involved in the ROS response (Moon et al., 2003). The OXI1 kinase activity on MAPKs is induced by H_2O_2, although the molecular mechanism underlying root hair growth is still unclear (Rentel et al., 2004). Several mutants of the MAPK signaling cascade exhibit defects in root hair growth (Nakagami et al., 2006; Qiu et al., 2008; Beck et al., 2010), suggesting that several components of this signaling cascade are positive regulators of root hair tip growth. By contrast, the MAPK6 mutant (*mapk6wb/ir*) developed extra-long root hairs, suggesting that MAPK6 is a negative regulator of root hair elongation (López-Bucio et al., 2014). It is uncertain whether these components of the MAPK cascade are linked to $_{cyt}H_2O_2$-OXI1 activation, as reported for wounding, cell wall damage, or pathogen attack. In addition, other signals, such as Ca^{2+} ions, hormones (e.g. auxin and ethylene), or nutrient status (e.g. low Pi), also could be linked to a MAPK-mediated response. INCOMPLETE ROOT HAIR ELONGATION (IRE; Oyama et al., 2002), which is an AGC kinase (like OXI1), also promotes root hair growth. It is unclear how all of these components are interconnected to orchestrate polar growth in root hairs.

DOES A ROS-INDEPENDENT, pH-DEPENDENT MECHANISM UNDERLIE POLAR CELL EXPANSION?

Analysis of growth and surface pH in real time established that growth accelerates after apoplastic acidification and slows upon alkalinization. An alkaline environment (approximately pH 8) stopped tip growth in root hairs, whereas an acidic medium (approximately pH 4.5) triggered uncontrolled expansion of root hairs and cell bursting (Monshausen et al., 2007). Surprisingly, root hairs of the *nox*C mutant (*rhd2-1*; Takeda et al., 2008) were rescued by increasing the extracellular pH from 5 to 6. This mutant still showed reduced $_{cyt}$ROS levels but a normal tip-focused Ca^{2+} gradient (Monshausen et al., 2007). This finding suggests that physiological levels of ROS are not absolutely critical for root hair development or for gating the Ca^{2+} channels needed to generate the tip-focused gradient. It is unclear why a shift in pH to a more neutral environment is able to restore Ca^{2+} signatures and polar growth in a ROS-deficient mutant. The $_{apo}$pH is thought to be regulated by proton fluxes generated by AHA, which also would regulate the release of Ca^{2+} ions stored in the cell wall (Fig. 1A). AHA, as a member of the P-type superfamily of ATPases, transports H^+ out of the cell and regulates the membrane potential and intracellular pH homeostasis (Haruta et al., 2015). Of the 11 functional AHA proteins present in Arabidopsis (Palmgren, 2001; Haruta et al., 2015), AHA1 and AHA2 are the most highly expressed isoforms. Accordingly, *aha2* mutants showed reduced cell elongation in hypocotyls. A number of environmental factors that regulate plant growth target the autoinhibitory domain of AHA, and several RLKs that phosphorylate specific sites of AHA have been identified (Falhof et al., 2016). Some of them are able to activate or repress AHA2 (Fuglsang et al., 2014; Haruta et al., 2014). The *C. roseus* RECEPTOR-LIKE KINASE1-LIKE (CrRLK1L) kinase FER binds to RAPID ALKALINIZATION FACTOR1

(RALF1). Upon binding, FER-RALF1 inhibits root growth by phosphorylating Ser-899 in AHA2 by an unknown kinase (Haruta et al., 2014). FER forms a protein complex with the guanine nucleotide-exchange factor ROP-GEF1 (ROP-GEF4 or ROP-GEF10 may be additional components of the complex) to recruit and activate the plant RHO GTPase ROP2 (Duan et al., 2010). The active form of ROP2 (and possibly ROP6) enhances the enzymatic activity of NOXC (Foreman et al., 2003; Takeda et al., 2008; Oda et al., 2010). In a similar way, it is expected that the CrRLK1L proteins present in growing pollen tubes, ANX1 and ANX2, recruit ROP1 by an unknown ROPGEF, with any of the ROPGEFs that interact with the PRK6 receptor (i.e. ROPGEF8, ROPGEF9, ROPGEF12, or ROPGEF13) being the best candidates. Then, ROP1 would bind to NOXH/NOXJ and promote ROS production in growing pollen tubes (Fig. 1A). In addition, active ROP1 and ROP2 proteins that are targeted to the plasma membrane of tip-growing cells have a direct impact on actin dynamics and vesicle trafficking. ROP-interactive CRIB motif-containing proteins control pollen tube growth by regulating F-actin dynamics (Gu et al., 2003, 2005), and similar effector components might regulate actin homeostasis and vesicle trafficking in growing root hair tips. A more general CrRLK1L-NOX-GEF-ROP signaling module was proposed recently as a conserved system that controls cell expansion throughout the plant (Nissen et al., 2016). Another member of the CrRLK1L family, CAP1 (for $_{cyt}Ca^{2+}$-associated protein kinase), was found recently to regulate root hair growth by maintaining $_{cyt}Ca^{2+}$ gradients (Bai et al., 2014). Although CAP1 is localized in the root hair vacuolar membrane, it is responsible for maintaining an alkaline $_{cyt}$pH by modulating the cytoplasmic NH_4^+ levels. CAP1 may sense the cytoplasmic NH_4^+ status and regulate its homeostasis by modulating the $_{cyt}Ca^{2+}$ and $_{cyt}$pH gradients (Bai et al., 2014).

Ca^{2+} TRANSPORT AND HOMEOSTASIS AFFECT POLAR GROWTH

Like ROS, Ca^{2+} ions act as secondary messengers in various signaling transduction processes, including polar growth. Ca^{2+} ions can be transported from external sources into the cell or can be stored in the cell wall polymers, mostly in negatively charged pectins and arabinogalactan proteins (Tian et al., 2006; Lamport and Várnai, 2013). Changes to a more acidic pH environment that are controlled mainly by plasma membrane AHAs could produce a significant release of Ca^{2+} ions within the apoplast space. Also, Ca^{2+} can be made available in the cytosol from subcellular compartments (e.g. vacuoles, ER, and Golgi). To date, little is known about the plant plasma membrane CaCs that account for the focused $_{cyt}Ca^{2+}$ gradient in the tip of polar growing cells, although more information is available for pollen tubes than for root hair cells (Table I). This could be explained by redundancies of multiple CaC members or as a consequence of the lethal nature of the corresponding CaC mutants. In addition, it is possible that CaCs act as nonselective Ca^{2+}-permeable channels.

Five types of Ca^{2+}-transporting systems have been characterized to date in plant cells (Table I): osmosensitive gated Ca^{2+}-permeable channels (CSC1 and OsCA1) and the related mechanosensitive channels; the cyclic nucleotide-gated channel (CNGC) family; glutamate like-receptor (GLR) genes; and voltage-gated channels (TWO PORE CHANNEL11 and annexins [ANNs]). Specific members of these families are expressed in root hairs or pollen tubes. Indeed, loss-of-function lines for some of these proteins display defects in pollen tube germination and reproduction and several other pleiotropic phenotypes (Table I; Konrad et al., 2011; Hou et al., 2014; Richards et al., 2014; Yuan et al., 2014). One of the best examples of the biological roles of CaCs was recently reported for CNGC18, GLR1.2/GLR3.7, and MSL8 channels. Based on careful characterization using mutant analysis, in vivo fertilization experiments, Ca^{2+} imaging, and electrophysiology, CNGC18 was clearly shown to be the main CaC involved in pollen tube guidance to the ovule (Gao et al., 2014, 2016). Previously, it was proposed that GLR1.2 and GLR3.7 are important components of the Ca^{2+} transport system in growing pollen tubes (Michard et al., 2011). These two pollen GLRs are activated specifically by D-Ser, and the enzyme SERINE RACEMASE1 (which converts L-Ser into D-Ser) also was localized close to the entry point of ovules (the micropyle). In the case of the MscS-like protein MSL8, a homolog of the bacterial mechanosensitive channel of small conductance (MscS) is required to maintain an optimal osmotic potential to ensure the cellular integrity required to drive germination during pollen germination and tube growth (Hamilton et al., 2015). This shows that several CaCs may act in the same cell type in response to different stimuli or in the same biological process, but at different steps.

Active efflux pumping of Ca^{2+} is a requirement to restore low concentrations of $_{cyt}Ca^{2+}$ (around 100–200 nM) after the gradient has triggered a signaling event. The removal of Ca^{2+} from the cytosol to the apoplast as well as to the endomembrane system requires active transport against its electrochemical gradient. Autoinhibitory P-type IIB Ca^{2+}-ATPases (ACAs) as well as the Ca^{2+}/H^+ antiporters of CAX proteins are the best candidates to catalyze this movement. In addition, both ACA and CAX as well as ECA proteins (for P-type IIA Ca^{2+}-ATPases) also all are present in the endomembrane system, so they can contribute to balancing the pool of $_{cyt}Ca^{2+}$ after a signal transduction event. Of the 10 ACA pumps present in Arabidopsis, only ACA9 was shown to be targeted to the pollen tube plasma membrane, where it had a tremendous impact on Ca^{2+} homeostasis and seed production (Schiøtt et al., 2004), while ACA13, which is expressed in the papilla cells of the stigma, exported Ca^{2+} to support compatible pollen grain germination (Iwano et al., 2014). No ACA has been reported thus far to act on root hair cells. In the

case of the CAX antiporters, the counter ion (H^+) movement provides the driving force for Ca^{2+} transport. While several members of CAX are encoded in the Arabidopsis genome, only CAX4 and CAX9 are highly expressed in pollen tubes (Pina et al., 2005; Wang et al., 2008), suggesting that their main role is in tube growth; however, their molecular characterization is pending. So far, no CAX members have been reported to exist in root hairs.

SUMMARY AND FUTURE QUESTIONS

Recent studies provide insight into ROS as signaling molecules that have a great impact on the regulation of polar cell expansion. Apoplastic/cytoplasmic ROS homeostasis is tightly linked to Ca^{2+} transport and pH regulation and also to changes in cell wall dynamics during polar growth. Nonetheless, several links are missing between the signaling function of ROS and the molecular mechanisms that trigger this developmental process in response to internal and external signals. For example, the mechanism by which changes in $_{apo}$pH bypass low levels of $_{apo}$ROS (e.g. in the *noxC rhd2* mutant) to trigger and sustain root hair growth remains to be determined. An important goal of future studies is to identify unknown components that are controlled by pH and to establish how these relate to $_{cyt}Ca^{2+}$ gradients. Furthermore, the molecular identity of the CaC(s) involved in polar cell growth and the mechanism by which ROS or other signals activate these channels remain to be established. Several CaC candidates are listed in Table I. It is likely that more than one type of CaC is active in these polar growing cells. It has been proposed that the ROS-mediated activation of several CaCs is triggered by signals in the apoplast (Foreman et al., 2003; Takeda et al., 2008; Duan et al., 2014), although the underlying mechanism is unclear. The H_2O_2-responsive Ca^{2+}-permeable K^+ channel SHAKER-LIKE STELAR K^+ OUTWARD RECTIFIER (Garcia-Mata et al., 2010) and the ROS-stimulated ANN1 (Laohavisit et al., 2012) may function in tip growth. Based on current evidence, it cannot be discounted that ROS also activates CaCs from the cytoplasmic side. Another important aspect of polar growth that remains unclear is how ROS signals are transduced to a downstream signaling cascade. There is evidence that MRI, OXI1 (and possibly other AGC kinases), and the MAPK cascade are involved in the downstream responses to ROS; however, it remains unknown how these components are interconnected. pH homeostasis also plays an important role in cellular expansion, but it is unclear how $_{apo}$pH regulates Ca^{2+} fluxes, how H_2O_2 and other ions are transported, and what signals and activators trigger H^+ pumping specifically in growing root hairs and pollen tubes. It is possible that ANX1/ANX2 in pollen tubes and FER in root hairs activate/repress the activity of AHA2 (and other AHA proteins) in response to specific signals (e.g. low Pi, high levels of auxin, and RALF peptide binding). Root hairs and pollen tube cells are excellent model systems for dissecting the cellular effectors required for polar growth. These systems are highly amenable to both genetic studies and sophisticated imaging approaches. Deciphering these processes is not only of scientific interest but potentially has important agronomic implications in developing more efficient crops in nutrient-deficient soils and enhancing their seed production.

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