Crosstalk between ROP GTPase signaling and plant hormones

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Highlight

Discussions regarding the interplay between ROP GTPase signaling and phytohormones offer insights into the orchestration of cell-autonomous and cell-non-autonomous mechanisms in plant development.

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Abstract

Rho of Plants (ROPs) constitute a plant-specific subset of small guanine nucleotide-binding proteins within the Cdc42/Rho/Rac family. These versatile proteins regulate diverse cellular processes, including cell growth, cell division, cell morphogenesis, organ development, and stress responses. In recent years, the dynamic cellular and subcellular behaviors orchestrated by ROPs have unveiled a notable connection to hormone-mediated organ development and physiological responses, thereby expanding our knowledge of the functions and regulatory mechanisms of this signaling pathway. This article delineates advancements in understanding the interplay between plant hormones and the ROP signaling cascade, centering primarily on the connections with auxin and abscisic acid pathways, alongside preliminary discoveries in cytokinin, brassinosteroid, and salicylic acid responses. It endeavors to shed light on the intricate, coordinated mechanisms bridging cell-level and tissue-level signals that underlie plant cell behavior, organ development, and physiological processes, and highlight future research prospects and challenges in this rapidly developing field.

Keywords: Rho of plants, GTPase, plant hormone, auxin, abscisic acid

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Introduction

Rho of Plants (ROPs) are plant homologs of the Cdc42/Rho/Rac family of small guanine nucleotide (GTP) binding proteins (GTPases) (Feiguelman *et al.*, 2018). They exist in two primary forms determined by their binding status with GTP or GDP: an active GTP-binding form and an inactive GDP-binding form (Fu and Yang, 2001; Kost, 2008; Shichrur and Yalovsky, 2006). Guanine nucleotide exchange factor (GEF), GTPase-activating protein (GAP), and Guanine nucleotide dissociation inhibitor (GDI) are three key regulatory factors that control the transition of ROP between its active and inactive states (Figure 1A) (Feiguelman *et al.*, 2018). RopGEFs promote the conversion of ROP-GDP to ROP-GTP. ROP-related GAPs enhance the GTP hydrolysis activity of ROPs, thus converting ROP-GTP to ROP-GDP. RopGDIs dissociate ROPs from the membrane, negatively regulating membrane localization and activation of ROPs. Plant ROPs comprise two subgroups (Fowler, 2010; Winge *et al.*, 2000; Zheng and Yang, 2000). Type-I ROPs are present in all land plants and are featured by a carboxyl-terminal prenylation motif that mediates membrane attachment. Their evolutionary origin dates back to early streptophyte lineage and coincides with the innovation of multicellularity in plants (Figure 1B) (Mulvey and Dolan, 2023b). Type-II ROPs are found only in seed plants and are believed to have evolved from type-I ROPs as a result of acquiring additional sequences that disrupt the prenylation motif (Fowler, 2010; Winge *et al.*, 2000; Zheng and Yang, 2000). Consequently, type-II ROPs are anchored to the membrane through S-acylation in the new carboxyl-terminal GC-CG box (Lavy *et al.*, 2002; Lavy and Yalovsky, 2006). Despite these differences, the catalytic GTPase domains of type-I and type-II ROPs are highly conserved. The functional divergence between them may largely result from differential transcriptional regulation and membrane targeting mechanisms (Yalovsky, 2015).

ROPs are best known for their crucial roles in regulating cell morphogenesis and organization and have been shown to trigger cytoskeleton-associated downstream signaling by activating various effectors (Feiguelman *et al.*, 2018; Li *et al.*, 2023; Ou and Yi, 2022). Present studies of ROP signaling mainly focus on simple cellular systems such as pollen tubes (Li *et al.*, 2023), root hairs (Mendrinna and Persson, 2015), leaf epidermal cells (Lin and Yang, 2020), and secondary cell walls in the xylem (Xu *et al.*, 2022a). These have collectively revealed ROP-dependent mechanisms in polarized cell growth, cell division, and cell morphogenesis (Li *et al.*, 2023; Muller, 2023; Oda and Fukuda, 2013; Ou and Yi, 2022; Yi and Goshima, 2022). The function of ROP signaling in regulating cell growth and morphology is conserved in basal land plants (Bao *et al.*, 2022; Bascom *et al.*, 2019; Burkart *et al.*, 2015; Cheng *et al.*, 2020; Hiwatashi *et al.*, 2019; Ito *et al.*, 2014; Le Bail *et al.*, 2019; Mulvey and Dolan, 2023a; Orr *et al.*, 2020; Rong *et al.*, 2022; Ruan *et al.*, 2023; Yi and Goshima, 2020). As these fields are beyond the scope of this review, we refer the readers to the following reviews for exciting progress in these areas (Cheung and Wu, 2008; Craddock *et al.*, 2012; Feiguelman *et al.*, 2018;

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Honkanen and Dolan, 2016; Kost, 2008; Li *et al.*, 2023; Lin *et al.*, 2015; Lin and Yang, 2020; Liu *et al.*, 2021; Mendrinna and Persson, 2015; Muller, 2023; Nibau *et al.*, 2006; Oda and Fukuda, 2013; Ou and Yi, 2022; Pan *et al.*, 2023; Qin and Dong, 2015; Smokvarska *et al.*, 2021; Yalovsky, 2015; Yang and Lavagi, 2012). It is noteworthy that ROPs also control many developmental processes including seed dormancy and germination, embryo development, seedling growth, and the development of organs such as roots, inflorescence stems, leaves, and petals (Chen *et al.*, 2011; Chen *et al.*, 2012; Huang *et al.*, 2014; Lavy *et al.*, 2007; Li *et al.*, 2001; Li *et al.*, 2005; Li *et al.*, 2008; Li *et al.*, 2018; Lin *et al.*, 2012; Liu *et al.*, 2023a; Liu *et al.*, 2017; Nagawa *et al.*, 2012; Nibau *et al.*, 2013; Poraty-Gavra *et al.*, 2013; Ren *et al.*, 2017; Ren *et al.*, 2016; Tao *et al.*, 2002; Zhao *et al.*, 2015), although the underlying mechanisms in these processes are not fully understood.

Compared to the ROP pathway, plant hormones are recognized as central driving factors regulating organ development and morphogenesis. The emergence of terrestrial plants coincided with the complexity of multicellular tissues and an enhanced capacity to adapt to terrestrial environments. Therefore, it is conceivable that this process is closely linked to the origin and evolution of hormone signaling pathways (Blazquez *et al.*, 2020; Bowman *et al.*, 2017; Rensing *et al.*, 2008). Indeed, major hormone pathways involved in developmental regulation, such as auxin and cytokinin (CK) pathways, have emerged before the land plant ancestor (Figure 1B) (Carrillo-Carrasco *et al.*, 2023; Powell and Heyl, 2023). CK and abscisic acid (ABA) signaling pathways are among the earliest phytohormones whose occurrence correlates with the emergence of type-I ROPs and the transition from unicellularity to multicellularity (Mulvey and Dolan, 2023b; Powell and Heyl, 2023; Sun *et al.*, 2020). Other pathways mediated by jasmonic acid (JA) (Blazquez *et al.*, 2020; Wang *et al.*, 2015), salicylic acid (SA) (Jia *et al.*, 2023; Wang *et al.*, 2015), gibberellin (GA) (Blazquez *et al.*, 2020; Wang *et al.*, 2015), and brassinosteroid (BR) (Kim and Russinova, 2020; Wang *et al.*, 2015), appear to be established around the time of land colonization by plants or later, potentially in adaption to stress pressures and the need for complex organ construction in terrestrial environments.

Alterations in developmental patterns necessitate the coordination of changes in cell structure and tissue organization and the establishment of intercellular communication modules. Classical hormone responses involve hormone synthesis, transport, receptor perception, signal transduction, and ultimately changes in gene expression levels (Blazquez *et al.*, 2020; McSteen and Zhao, 2008). Notably, hormone signaling, such as auxin, can also stimulate rapid responses independent of its transcriptional activity (Dubey *et al.*, 2021; Fiedler and Friml, 2023). ROP, as a membrane signaling switch regulating cellular behavior, has been found to interact with hormone signals, such as auxin (Lin *et al.*, 2015; Pan *et al.*, 2015; Wu *et al.*, 2011), ABA (Hsu *et al.*, 2021), CK (Li *et al.*, 2013; Liu *et al.*, 2023a), and BR (Zhang *et al.*, 2022). The interplay between ROPs and hormone signaling is gradually being unveiled, initiating a new era in understanding cell-autonomous regulation mechanisms and intercellular communication that collectively determine organogenesis. Nevertheless,

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this emerging field still faces challenges and lacks a unified model, partly because ROP and hormone pathways function in different ways: ROPs typically influence calcium signaling, cytoskeleton, and intracellular trafficking to regulate cell morphology and behaviors in a cell-autonomous manner (Feiguelman *et al.*, 2018; Li *et al.*, 2023; Muller, 2023; Ou and Yi, 2022), while hormones control cell fate and cell differentiation through transcriptional regulation non-autonomously (Blazquez *et al.*, 2020). In this review, we present updates on our understanding of the crosstalk between ROP signaling and hormone pathways and highlight directions and challenges for further studies in this exciting area.

Discovery of ROPs and their interaction with hormones

Genes encoding plant ROPs were cloned in the 1990s (Winge *et al.*, 1997; Winge *et al.*, 2000; Yang and Watson, 1993) and were initially found to regulate pollen tube growth (Kost *et al.*, 1999; Lin *et al.*, 1996; Lin and Yang, 1997). Soon after the discovery, ROPs were found broadly expressed in complex organs and were required for organ development (Li *et al.*, 2001; Li *et al.*, 1998). Some phenotypes are reminiscent of effects induced by hormone treatment (Li *et al.*, 2001). Since then, an increasing number of studies have revealed reciprocal regulation between the ROP pathway and multiple hormone signals, such as auxin (Tao *et al.*, 2005; Tao *et al.*, 2002), ABA (Lemichez *et al.*, 2001; Zheng *et al.*, 2002), CK (Li *et al.*, 2013), ethylene (Zermiani *et al.*, 2015), SA (Rong *et al.*, 2016), and BR (Zhang *et al.*, 2022) (Table 1). The best-studied examples are ROP-auxin interactions during the development of the interdigitated pavement cells in leaves and the roles of ROPs in ABA-regulated processes such as stomatal closure, seed dormancy, and root growth inhibition. In the following, we discuss the current understanding of ROP signaling and hormone crosstalk by focusing on auxin and ABA. We provide updates in these fields and also discuss recent findings related to other hormones and developmental processes. These discussions may offer insight into the mechanism that integrates cell-autonomous and cell-non-autonomous signaling pathways for establishing complex organs in plants.

Crosstalk between ROP signaling and auxin pathways

Auxin perception, transport, and function

As the first discovered plant hormone, auxin regulates nearly all processes of plant growth and development (Friml, 2022), including the establishment of the body axis and primordial formation in early embryos (Verma *et al.*, 2021), the formation and maintenance of root and shoot meristems (Pernisova and Vernoux, 2021; Roychoudhry and Kepinski, 2022), and gravitropic and phototropic growth (Han *et al.*, 2021; Li *et al.*, 2022b). At the single-cell level, auxin promotes cell wall expansion and cell growth (Du *et al.*, 2020). Two classes of receptors mediate auxin perception: through binding with auxin, intracellular receptors TIR1/AFB family F-box proteins target the Aux/IAA transcription repressors for degradation, leading to transcriptional expression of auxin-

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responsive genes (Leyser, 2018; Weijers and Wagner, 2016); extracellular receptor ABP1 functions together with the membrane receptor-like kinase TMK1 to mediate transcription-independent signal transduction (Napier, 2021; Xu *et al.*, 2014). Besides perception, the distribution of auxin is critical for its function. Auxin transport is regulated by the PIN family efflux transporters and AUX1/LAX influx carriers (Hammes *et al.*, 2022). Their polar localization on the plasma membrane generates auxin gradient in tissues and differential downstream transcriptional responses, therefore leading to organ initiation and development (Hajny *et al.*, 2022).

Principal modes of ROP-auxin interactions

ROP-auxin interactions are found at both transcriptional and non-transcription levels (Figure 2). In 2002, based on the similarity of phenotypes in transgenic plants overexpressing tobacco NtRAC1 (a homolog of ROPs) to those in auxin-related defects, Tao et al. found that ROPs could activate auxin responses (Tao *et al.*, 2002) and the activation process depends on the degradation of Aux/IAA repressors (Tao *et al.*, 2005). Later studies show that PIN transporters are the primary targets of ROPs (Figure 2). For instance, the ROP effector ICR1 positively regulates exocytosis and polar localization of PINs (Hazak *et al.*, 2010; Lavy *et al.*, 2007; Li *et al.*, 2008). During pavement cell morphogenesis and root development, ROP2/4/6 inhibits the endocytosis of PIN proteins. These processes are mediated by ROP effectors RIC4 and RIC1 which directly modulate and organize filamentous actin (F-actin) and microtubule (MT) networks (Chen *et al.*, 2012; Fu *et al.*, 2005; Fu *et al.*, 2002; Fu *et al.*, 2009; Nagawa *et al.*, 2012; Xu *et al.*, 2010), in a similar way as they do in canonical ROP pathway found in tip-growing cells (Feiguelman *et al.*, 2018; Li *et al.*, 2023; Ou and Yi, 2022). Perturbation of the function of ROP activators, including RopGEF7 and RopGEF1, alters PIN localization and auxin responses (Chen *et al.*, 2011; Liu *et al.*, 2017). RopGEF7 can bind ROP3 and likely activates it during embryo and seedling development as well as in root gravitropic growth (Huang *et al.*, 2014). These findings indicate that canonical ROP pathways commonly regulate membrane trafficking of auxin transporters to modulate auxin-dependent processes.

The regulation of the ROP pathway by auxin signaling can occur at multiple levels (Figure 2). Firstly, auxin can promote the expression of various components of the ROP pathway genes, such as ROP3/6/9 (Huang *et al.*, 2014; Nibau *et al.*, 2013; Poraty-Gavra *et al.*, 2013), RopGEF7 (Chen *et al.*, 2011), and ROP effector proteins ICR1 and RICs (Choi *et al.*, 2013; Hazak *et al.*, 2010). Secondly, auxin can regulate the stability of ROP pathway proteins. For instance, ICR1 is rapidly degraded by the TIR1/AFB pathway at the auxin maxima, thus forming a negative feedback regulation (Hazak *et al.*, 2010; Hazak *et al.*, 2014). Additionally, auxin can rapidly activate ROPs independent of transcriptional regulation, thereby regulating cell morphology and growth development (see below) (Xu *et al.*, 2014; Xu *et al.*, 2010).

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Auxin-ABP1/ABL1/2-TMK1 pathway

The activating effect of auxin on ROP was discovered as early as twenty years ago (Tao *et al.*, 2002). However, whether auxin activates ROP through transcriptional regulation or non-transcriptional regulation has only been recently resolved. Early evidence suggested that auxin might activate ROP through non-transcriptional mechanisms. Firstly, auxin can rapidly activate ROP2/6 within 30 seconds (Xu *et al.*, 2010). Transcriptional regulation mediated by auxin typically takes several minutes, a timeframe that seems insufficient to meet the demands of such rapid responses (Badescu and Napier, 2006). Secondly, the activation of ROP depends on ABP1 (Xu *et al.*, 2010). ABP1 is an auxin receptor located in the endoplasmic reticulum and apoplast and has been found to function extracellularly for rapid auxin responses (Napier, 2021). Additionally, ABP1 can form an auxin-dependent complex with the receptor-like kinase TMK1 (Xu *et al.*, 2014). Despite Gao et al. finding that *abp1* single mutants have no impact on auxin response and organ development (Gao *et al.*, 2015) and previously reported defects are attributed to background mutations in other genes (Dai *et al.*, 2015; Enders *et al.*, 2015; Michalko *et al.*, 2015), raising questions about ABP1 as an extracellular receptor for auxin, the latest research has demonstrated that ABL1/2 are co-receptors of TMK1 for auxin perception and function partially redundantly with ABP1 in the apoplast, confirming that the ABP1/ABL1/2-TMK1 complex drives rapid auxin response on the plasma membrane (Yu *et al.*, 2023).

ABP1-mediated rapid responses can activate ROP2/6, which, in turn, feedback regulates the localization of PIN proteins, influencing auxin distribution and the auxin response pathway (Figure 2). In pavement cells, activated ROP2 and ROP6 are localized to the lobe and indentation regions, respectively (Fu *et al.*, 2005; Fu *et al.*, 2009). ROP6, by activating the effector RIC1, promotes microtubule organization in the indentation region, mediating plasma membrane invagination (Fu *et al.*, 2009). ROP2 facilitates lobe expansion by activating the effector RIC4 for F-actin assembly and concurrently inhibiting RIC1 (Fu *et al.*, 2005). Interestingly, PIN proteins are polarly localized to the lobe region, not at the indentation region (Figure 2A). This localization is achieved through ABP1-ROP2/4 and actin-mediated inhibition of PIN1 endocytosis (Nagawa *et al.*, 2012; Xu *et al.*, 2010). In root development, auxin and ABP1 similarly inhibit the endocytosis of PIN1/2, promoting auxin efflux to establish the correct auxin gradient (Paciorek *et al.*, 2005; Robert *et al.*, 2010). The inhibition of PIN1/2 endocytosis by auxin depends on ROP6 and the effector RIC1 but not on ROP2 (Chen *et al.*, 2012; Choi *et al.*, 2013). Therefore, despite the involvement of different ROPs and effector factors in regulation, the feedback mechanism involving auxin-dependent ROP activation and the inhibition of PIN endocytosis may be universal (Pan *et al.*, 2015). A similar mechanism has recently been discovered in cotton fiber cells (Xi *et al.*, 2023). In pavement cells, does auxin form a local concentration gradient through PIN, thereby influencing cell morphology? As a small molecule, auxin can rapidly diffuse in the apoplast, making it unlikely to distribute differentially in a small area. How

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does a global signal then selectively activate ROP2/4 and ROP6 in distinct regions? The involvement of a differential sensing ability of auxin in these regions seems unlikely, given that the distribution of TMK1 in the lobe and indentation regions does not exhibit significant differences (Xu *et al.*, 2014). Instead, the ROP2/4 and ROP6 pathways reciprocally inhibit each other and probably generate an interdigitated pattern through self-organization (Fu *et al.*, 2005; Lin *et al.*, 2015; Xu *et al.*, 2011).

The mechanism by which TMK1 activates ROP is currently unclear. One promising possibility is that TMK1 facilitates the clustering of ROPs into lipid nanodomains (Pan *et al.*, 2023; Smokvarska *et al.*, 2021). Membrane lipid nanodomains or lipid rafts are cholesterol and sphingolipid-enriched regions of the membrane (Li *et al.*, 2024). They provide a platform to concentrate membrane receptors and signaling components, thus nicely fulfilling the needs for polar localization and activation of ROPs. Indeed, auxin induces lipid ordering in the indentation region in pavement cells, leading to the formation of nanoclusters of TMK1 and ROP6 (Pan *et al.*, 2020). This process depends on cholesterol synthesis, S-acylation of ROP6, and TMK1/4. In the root, auxin similarly stimulates ROP6 nanoclustering and requires the interaction between the polybasic tail of ROP6 and phosphatidylserine (Platre *et al.*, 2019). Although the direct link between Auxin-ABP1-TMK1 and ROPs is still unknown, emerging evidence suggests the involvement of RopGEFs. In the root, the inhibition of PIN2 endocytosis by ROP6-RIC1 is directly regulated by the guanine exchange factor SPK1 (Lin *et al.*, 2012). As the receptor-like kinase FER directly interacts with RopGEF during the auxin-induced root hair formation and activates ROPs (Duan *et al.*, 2010) and FER and TMK1 both belong to the family of receptor-like kinases (Dievart *et al.*, 2020), TMK1 might function similarly in pavement cells. The Arabidopsis genome encodes fourteen RopGEFs and one SPK1-GEF (Feiguelman *et al.*, 2018; Fowler, 2010). The development of root hairs requires at least three RopGEF proteins RopGEF1/4/10 (Duan *et al.*, 2010; Huang *et al.*, 2013). Further research is needed to clarify which RopGEFs are regulated by TMK1 and whether TMK1 directly interacts with and activates RopGEFs (Feher and Lajko, 2015; Miyawaki and Yang, 2014). Recently, the membrane-associated protein kinase MAKR2 was found to mutually inhibit the auxin-TMK1 pathway during root gravitropic growth, regulating the ROP6-mediated asymmetric distribution of PIN2 (Marques-Bueno *et al.*, 2021). There might be other regulatory factors in the auxin-ABP1/ABL1/2-TMK1-ROP pathway. The formation of ROP nanoclusters has been reported in various cells such as root hairs (Fiona Fuchs *et al.*, 2021), pollen tubes (Fratini *et al.*, 2021), moss protonemal cells (Ruan *et al.*, 2023), and in response to osmotic stress and other physiological processes (Smokvarska *et al.*, 2023; Smokvarska *et al.*, 2020). It is possible that hormone and other membrane signal-triggered clustering of ROPs is a common mechanism in regulating ROP signaling and involves receptor-like kinase-mediated activation (Pan *et al.*, 2023).

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Interaction of the auxin-ROP pathway with other signals

In addition to the pathways mentioned above, the crosstalk between the auxin-ROP pathway involves interactions with other signaling molecules (Figure 2B). For instance, auxin can promote the generation of reactive oxygen species (ROS) to regulate root hair growth. This process relies on the FER-RopGEF1/4/10-ROP pathway (Duan *et al.*, 2010; Huang *et al.*, 2013). Further research is warranted to investigate whether auxin functions through an extracellular pathway to regulate FER activity. Root hairs typically arise from local protrusions formed at the base by the outermost root epidermal cells (Vissenberg *et al.*, 2020). The initiation of root hairs is determined by ROP2/4/6 (Gendre *et al.*, 2019; Jones *et al.*, 2002; Molendijk *et al.*, 2001). Genetic analyses indicate that auxin synergistically regulates ROP localization in root hair cells with ethylene signaling (Fischer *et al.*, 2006). Ethylene treatment in auxin-related mutants promotes ectopic root hair formation, further supporting the potential crosstalk between auxin and ethylene signaling (Kiefer *et al.*, 2015). In the inhibition of root growth, ethylene functions upstream of auxin synthesis, transport, and the TIR1/AFB-Aux/IAA pathway, but does not require the ABP1-ROP6-RIC1 pathway (Wang *et al.*, 2018). Cell growth and organ development mediated by the auxin-ROP pathway are regulated by a kinase cascade composed of MKK3-MPK1-RBK1 (Enders *et al.*, 2017), of which the cysteine-rich receptor-like kinase RBK1 directly phosphorylates ROP4/6 and potentially inhibits its activity (Molendijk *et al.*, 2008). Nitric oxide has been reported to inhibit root growth. It functions to promote the S-acylation and membrane localization of ROP2 and inhibit PIN1 abundance in an ROP2-dependent manner (Kenesi *et al.*, 2023). The ROP effector ICR1 recruits the calcium-binding protein CMI1 to microtubules, mediating auxin-dependent calcium signaling response and root growth (Hazak *et al.*, 2019). In addition to regulating the cytoskeleton and calcium signaling, ROP can also mediate auxin-dependent TOR activation. ROP2/4/6 directly binds to TOR and promotes auxin-stimulated TOR phosphorylation and activation, therefore driving translation reinitiation and cell proliferation (Li *et al.*, 2017; Schepetilnikov *et al.*, 2017). Notably, in animals, Rac1 similarly binds mTOR and regulates its membrane localization and activity (Saci *et al.*, 2011). This function appears to be specific to Rac but not played by other groups of the Rho superfamily in animals. Moreover, although Cdc42, Rho, and Rac all regulate actin organization, they are distinctly involved in generating filopodia, stress fiber, and lamellipodia (Hall, 1998). Because ROPs represent the only group of the Rho superfamily in plants (Zheng and Yang, 2000), whether the reported non-canonical functions of ROPs and their interactions with auxin signaling are common or specific to each member or subgroup remains to be addressed.

Cell type-specific auxin-ROP signaling

The interaction between auxin and the ROP pathway is a common phenomenon, but the cell-specific mechanisms are still under investigation. In maize subsidiary mother cells, ROP2/9 regulates cell polarity-dependent asymmetric division (Humphries *et al.*, 2011). The polarization of the

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subsidiary mother cell is regulated by auxin and correlates with changes in the localization of PIN proteins (Livanos *et al.*, 2015). Therefore, auxin and the ROP pathway may coordinate in establishing polarity before the division of subsidiary mother cells. During root hair development in Arabidopsis, the nucleus undergoes two consecutive migrations in different directions. This process is regulated by auxin and the ROP pathway. However, genetic analysis suggests they may function independently (Nakamura *et al.*, 2018). The auxin-insensitive mutant *arx1* exhibits enhanced ROP2 levels and genetically interacts with ROP2 in regulating trichome branching, suggesting a crosstalk between the auxin pathway and ROP in regulating trichoblast cell morphology (Liu *et al.*, 2023b). Some ROPs may regulate development by inhibiting, rather than activating, auxin responses. For instance, the knockdown of *rop9* enhances the effects of auxin in promoting lateral root formation and inhibiting primary root growth (Nibau *et al.*, 2013), although contradictory results were obtained in another study using *rop9* mutants (Choi *et al.*, 2014).

In the liverwort *Marchantia polymorpha*, auxin is synthesized in the meristematic tissue and regulates thallus development (Eklund *et al.*, 2015). The expression of the auxin synthesis gene YUC2 and auxin-regulated thallus formation and dormancy are regulated by MpROP (Rong *et al.*, 2022). These observations suggest that the interaction between auxin and the ROP pathway has already evolved in the early development of terrestrial plant organs. In the moss *Physcomitrium patens*, auxin can promote the differentiation of caulonemal cells (Thelander *et al.*, 2018). During caulonemal cell growth, the ROP effector PpRIC localizes to the apical membrane and the nucleus (Ntefidou *et al.*, 2023). Nuclear-localized PpRIC has an inhibitory effect on auxin-induced caulonemal cell differentiation (Figure 3), indicating a negative regulatory role of the ROP pathway in auxin response in moss (Ntefidou *et al.*, 2023). Similar to PpROP4 (Cheng *et al.*, 2020; Yi and Goshima, 2020), the moss PIN homolog PpPINA localizes to the apical tip of caulonemal cells (Viaene *et al.*, 2014), consistent with lower auxin response signals in apical cells (Thelander *et al.*, 2019). However, the mechanism by which PpRIC inhibits auxin response remains unknown, as the loss of PpRIC does not affect the expression of auxin-responsive genes or the localization of PpPINA, and auxin does not affect the transcription and localization of PpRIC (Ntefidou *et al.*, 2023). Because cell length is not affected in *Ppric* mutants or overexpression lines, PpRIC appears to be an atypical ROP effector that does not participate in cell growth but plays an important role in cell differentiation. Interestingly, the polar localization of PpPINA is negatively and positively regulated by actin and MT, respectively (Tang *et al.*, 2023). There might be other ROP effectors that control auxin gradients through cytoskeletons and membrane trafficking (Figure 3). In Arabidopsis root hairs, PIN2 exhibits a localization similar to moss PIN proteins (Tang *et al.*, 2023). However, unlike PIN, the auxin influx carrier AUX1 does not localize in root hairs although it is essential for root hair growth (Jones *et al.*, 2009). Mutations and overexpression of auxin transporters further indicate the necessity of polar transport of auxin for root hair growth (Velasquez *et al.*, 2016). Similar to root hairs (Pitts *et al.*,

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1998), pollen tube growth is positively regulated by auxin (Chen and Zhao, 2008; Gao *et al.*, 2019; Wu *et al.*, 2008). In Arabidopsis, only PIN8 is expressed in pollen tubes and is found to localize at the endoplasmic reticulum (Ding *et al.*, 2012). Pollen tubes may have acquired the ability to store auxin in the endoplasmic reticulum and release it in demand since they cannot obtain auxin from other cells. Nevertheless, the interaction between ROP and auxin signals in tip-growing cells remains poorly understood (Pan *et al.*, 2015). Apart from the FER-RopGEF-ROP pathway (Duan *et al.*, 2010), it is likely that additional molecules play a role in auxin-ROP signaling.

Crosstalk between ROP signaling and ABA pathways

ABA core signaling pathway

ABA is another important hormone that regulates plant growth, development, and physiology (Chen *et al.*, 2020) and plays a crucial role in abiotic stress responses (Waadt *et al.*, 2022). ABA treatment usually promotes seed maturation and dormancy (Ali *et al.*, 2022) and induces stomatal closure (Hsu *et al.*, 2021; Munemasa *et al.*, 2015). In development, ABA typically exerts inhibitory effects on plant growth, although positive regulatory roles have also been noted (Humplik *et al.*, 2017). The core signaling pathway of ABA involves PYLs-PP2C-SnRK2s (Cutler *et al.*, 2010). PYLs serve as intracellular receptors for ABA, inhibiting the activity of the phosphatase PP2C. PP2C, in turn, dephosphorylates and inhibits the activation of SnRK2s. In the presence of ABA, the inhibitory effect of PYLs on PP2C is relieved, leading to the activation of SnRK2s and expression of responsive genes as well as non-transcriptional signaling events (Chen *et al.*, 2020; Humplik *et al.*, 2017).

ROP-ABA interactions in regulating stomata closure

As ABA regulates stomatal closure through organizing the F-actin network (Eun and Lee, 1997; Kim *et al.*, 1995) and ROPs are key regulators of actin assembly (Fu *et al.*, 2002), the interaction between ROP signaling and ABA was initially studied in stomata (Figure 4A and Table 1). In 2001, Lemichez et al. demonstrated that ABA inactivates AtRAC1/AtROP6, thus promoting F-actin disassembly and stomatal closure (Lemichez *et al.*, 2001). This process requires the PP2C member ABI1. The following studies provide more evidence in supporting ROP-ABA crosstalk and have revealed that ROPs mainly act as a negative regulator of ABA signaling. For instance, Arabidopsis ROP9 and ROP10 inhibit ABA-mediated stomatal closure, seed dormancy, and root growth inhibition (Choi *et al.*, 2014; Zheng *et al.*, 2002). ROP11 has a similar function to ROP10 but genetically acts in parallel pathways (Li *et al.*, 2012a). ABA-mediated stomatal closure is also negatively regulated by ROP2 (Hwang *et al.*, 2011). In light response, light triggers the translocation of ROP2 and its effector RIC7 to the plasma membrane and promotes stomatal opening (Jeon *et al.*, 2008). In this process, ROP2 plays an inhibitory role in stomatal opening as opposed to its function in ABA signaling (Hwang *et al.*, 2011; Jeon *et al.*, 2008). Why does ROP2 negatively regulate both the closure and opening of stomata? Under light, actin filaments are radially oriented to facilitate stomatal

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opening; darkness or ABA treatment disrupts actin organization, leading to stomatal closure (Eun and Lee, 1997; Kim *et al.*, 1995). One possible explanation is that the actin network must be balanced for sufficient strength and plasticity during stomatal opening. The assembly and arrangement of F-actin induced by ROPs promote the stomatal opening, however, excess assembly and high stability of F-actin can inhibit stomatal opening.

Mechanisms of ROP regulation on ABA signaling

In Arabidopsis, the active form of ROP11 directly binds to ABI1/2 in vitro and releases the inhibition of ABA and PYLs on ABI1/2 phosphatase activity (Li *et al.*, 2012b; Yu *et al.*, 2012). Therefore, PP2C may be an important direct target of ROPs. RopGEF1 has been shown to bind to the receptor-like kinase FER and activate ROPs (Duan *et al.*, 2010). FER and various RopGEFs, such as RopGEF1/2/4/10, all have negative regulatory functions in ABA-mediated responses, such as seedling de-greening, root growth inhibition, and seed dormancy (Li and Liu, 2012; Li *et al.*, 2018; Li *et al.*, 2016; Yu *et al.*, 2012; Zhao *et al.*, 2015). Hence, the entire FER-RopGEF-ROP pathway may be involved in ABA responses. Consistent with this notion, RALF, a ligand for FER, like ABA, can inhibit root growth (Haruta *et al.*, 2014). At the molecular level, both RALF and ABA promote the phosphorylation of FER. The effect induced by ABA is caused by the inactivation of PP2C/ABI2 which dephosphorylates FER (Chen *et al.*, 2016). These findings indicate that FER integrates RALF and ABA signaling pathways through its phosphorylation levels to regulate ROP activity (Chen *et al.*, 2016). ROPs may also inhibit ABA signaling by acting on effectors. Loss of function of *ric1* enhances the inhibition of seed germination, later root formation, and primary root elongation by ABA (Choi *et al.*, 2013). The direct mechanism of action of ROP effectors remains to be studied. In *ric1* mutants, the expression of ABA-responsive genes is upregulated (Choi *et al.*, 2013). Transcriptional regulation may be involved (Figure 4B). In support of this notion, ABA-mediated transcriptional responses are also significantly altered in *rop10* mutants (Xin *et al.*, 2005).

Mechanisms of ABA regulation on ROP signaling

The primary effect of ABA on the ROP pathway is to promote the inactivation of ROPs (Figure 4). Early biochemical and genetic analyses have shown that the active forms of ROP significantly decrease upon ABA treatment (Lemichez *et al.*, 2001) and overexpressing constitutively-active ROPs could suppress ABA responses (Hwang *et al.*, 2011). The inactivation of ROP is associated with changes in its localization and the downregulation of RopGEF protein stability. For example, ABA can induce the translocation of ROP11 from the plasma membrane to the nucleus, thereby relieving its inhibition of ABA signaling (Li *et al.*, 2012a). ABA treatment promotes the translocation of RopGEF1/2 to granular structures associated with multivesicular bodies, suggesting that ABA induces RopGEF degradation through the vacuolar pathway (Li *et al.*, 2016; Zhao *et al.*, 2015). Interestingly, RopGEF2 also localizes to mitochondria, and ROP2/6/10 can recruit it to the cell

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membrane, thus preventing its degradation (Zhao *et al.*, 2015). The recruitment of RopGEF2 to the plasma membrane by ROPs seems to require its N-terminal sequence. Unlike RopGEF2, the N-terminus of RopGEF1 is phosphorylated by CPK kinases, which promotes the degradation of RopGEF1 (Li *et al.*, 2018). Despite the regulation of protein localization and stability, ABA may inhibit the ROP pathway through transcriptional regulation (Figure 4B and Table 1). In mosses, ABA has inhibitory effects on protonemal cell growth and the expression of ROPs and RopGEFs (Beier *et al.*, 2023). In Arabidopsis, ABA treatment leads to the downregulation of ROP9 and ROP10 expression (Nibau *et al.*, 2013; Zheng *et al.*, 2002). The *rop9* RNAi mutant is insensitive to ABA, suggesting that ROP9, unlike ROP10, functions as a positive regulator of ABA (Nibau *et al.*, 2013). Similarly, the expression of *Camellia sinensis* CsRAC1 is positively regulated by ABA. Overexpression of CsRAC1 can enhance the inhibitory effect of ABA on seed germination (Xu *et al.*, 2022b). In Arabidopsis, the ABA response factor HDA15 and the transcription factor MYB96 form a complex that deacetylates histones in the promoters of ROP6/10/11, thereby inhibiting their expression (Lee and Seo, 2019; Seo *et al.*, 2009). Interestingly, the deacylation of the ROP6 promoter is also regulated by HDA6 during pavement cell development (Du *et al.*, 2024). HDA6 was originally identified as a negative factor in auxin-responsive transgene expression but does not affect the expression of endogenous auxin-inducible genes (Murfett *et al.*, 2001). Since the expression of at least some ROP-related genes, such as RopGEF2 (Zhao *et al.*, 2015) and ROP11 (Li *et al.*, 2012a), is not affected by ABA (Figure 4B), whether transcriptional regulation of the ROP pathway by ABA is a universal mechanism remains to be further explored.

Interactions between the ROP pathway and other hormone signals

In early studies, developmental phenotypes resulting from dysregulation of ROP activity exhibited similarities to the effects of BR treatment, suggesting an interaction between BR signaling and the ROP pathway (Li *et al.*, 2001). Subsequent research revealed that BR promotes the polar localization of ROP2 and the redistribution of PIN2, thereby enhancing root gravitropic growth (Li *et al.*, 2005). This process requires the involvement of F-actin. Therefore, BR may regulate the distribution of auxin by regulating ROPs and PINs similar to auxin. In pavement cells, ROP2 and ROP6 are localized at the lobe and indentation regions, respectively, to facilitate puzzle-shape morphogenesis (Fu *et al.*, 2005; Fu *et al.*, 2009). BIN2 kinase, one of the core components in BR signaling, phosphorylates ROP-related GAP proteins PHGAP1/2 in the indentation region and promotes their protein stability (Figure 2A), leading to the inactivation of ROP2 (Lauster *et al.*, 2022; Zhang *et al.*, 2022). In the lobe region, BR inactivates BIN2, thus promoting the degradation of PHGAP1/2 and the activation of ROP2 to promote lobe expansion (Zhang *et al.*, 2022). Through mutant screening, Li et al. found that CK signaling mutants exhibited abnormal pavement cell morphogenesis (Li *et al.*, 2013). Genetic analysis indicates that ROP2/4 acts downstream of CK

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signaling and their activity is inhibited by CK (Li *et al.*, 2013). Recently, rice OsRopGEF10-OsRAC3 was found to inhibit CK signaling during crown root development. They directly bind to OsAHP1/2, sequester it to the cell membrane, and prevent it from activating downstream transcription factors in the nucleus, thereby negatively regulating CK responses (Liu *et al.*, 2023a). OsRopGEF10 also promotes the expression of OsRR6 to inhibit CK signaling (Liu *et al.*, 2023a). Interestingly, the expression and activity of OsRAC3 are positively regulated by auxin, indicating a crosstalk between auxin and CK signaling. In pollen tubes, high concentrations of SA inhibit tip growth. This effect is attributed to the increase in ROP activity caused by the inhibition of ROP-related GAP REN1 by SA (Rong *et al.*, 2016). The inhibitory effect of SA on pollen tube growth does not rely on its receptors but rather involves endocytic pathways (Rong *et al.*, 2016). In wheat *Triticum aestivum*, TaROP10 negatively regulates stripe rust defense responses (Shi *et al.*, 2021). Its expression is suppressed by SA and ABA, suggesting a negative feedback regulation between the ROP pathway and SA responses. In Arabidopsis, the inactivation of ROP6 also negatively influences SA synthesis and the expression of SA-responsive genes (Poraty-Gavra *et al.*, 2013). These studies indicate that the interaction between ROP signaling and hormones such as BR, CK, and SA is a common phenomenon (Table 1). Further exploration of the roles of ROP in hormonal responses will be an important direction for understanding this versatile molecular switch.

Concluding remarks and future perspectives

Similar to yeast Cdc42, ROPs play a crucial role in polarity establishment in single cells such as pollen tubes and root hairs (Li *et al.*, 2023; Muller, 2023; Ou and Yi, 2022). However, as complex organs emerged in land plants, it is not surprising that ROP-regulated signaling has been increasingly utilized during tissue and organ development (Li *et al.*, 2001). Particularly, ROP-related genes have significantly expanded in seed plants, accompanied by a unique type-II ROP subfamily (Brembu *et al.*, 2006; Fowler, 2010; Winge *et al.*, 2000). How do ROPs adapt to multicellularity and regulate tissue and organ development? Increasing evidence indicates that ROP signaling interacts with a variety of hormonal pathways, thus linking cellular dynamics to a broader developmental context involving cell-cell communication and transcriptional regulation. Currently, it is well accepted that ROP signaling undergoes self-organization and determines cell polarity, growth direction, and division directions at the single-cell level (Lin *et al.*, 2015; Pan *et al.*, 2023; Smokvarska *et al.*, 2021; Yang and Lavagi, 2012; Yi and Goshima, 2022). In contrast, hormone signals, such as auxin, regulate the fate and behavior of cell populations through intercellular communication at the tissue level (Bhatia and Heisler, 2018; Hajny *et al.*, 2022; Leyser, 2011). There is a clear gap in our understanding of organismal development between the cell and tissue levels. Exploring the mechanisms underlying ROP-hormone crosstalk would possibly help bridge this gap.

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Although research on the interactions between plant hormones and the ROP signaling pathway is still incomplete, some studies, such as those related to pavement cells (Figure 2A), have revealed certain patterns and provided a paradigm: auxin rapidly activates ROPs through a non-canonical ABP1/ABL1/2-TMK1 pathway; ROPs, in turn, act on cytoskeleton networks through effectors to inhibit the endocytosis of PIN proteins, therefore regulating the auxin gradient and auxin-dependent morphogenesis (Chen *et al.*, 2015; Lin *et al.*, 2015; Liu *et al.*, 2021). This mechanism is likely conserved in root growth regulation across species (Dubey *et al.*, 2021; Xi *et al.*, 2023). The auxin-ROP interaction network should be not limited to this simple model. For example, recent studies show that mechanical stress in the epidermis of cotyledons can be sensed by FER, which activates ROP6 along with RopGEF14 to regulate pavement cell interdigitation (Lin *et al.*, 2022; Tang *et al.*, 2022). FER and its interaction with RopGEFs have been found to participate in auxin-induced root hair formation (Duan *et al.*, 2010; Huang *et al.*, 2013). Auxin may regulate ROP activity through other membrane signaling components. Furthermore, the expression of many ROP pathway-related genes is regulated by auxin (Figure 2B). Auxin-responsive genes typically use cis-regulatory elements for transcriptional regulation (Weijers and Wagner, 2016). Some of the ROP-related genes have been identified to contain potential auxin response elements in their promoters (Li *et al.*, 2022a; Nibau *et al.*, 2013; Poraty-Gavra *et al.*, 2013), implying that transcriptional regulation of ROP signaling components by auxin might be another important mechanism in auxin-ROP crosstalk. An in-depth analysis of the function of cis-regulatory elements in ROP-related genes may help understand whether auxin and other hormones play a direct role in the transcriptional regulation of ROP signaling. In addition to controlling PIN localization, ROPs can also interact with translation regulatory factors, such as TOR proteins, to regulate gene expression (Li *et al.*, 2017; Schepetilnikov *et al.*, 2017). In other hormone response processes, ROPs directly interact with signal transduction factors, such as the ABA pathway components ABI1/2 (Li *et al.*, 2012b) and the rice CK pathway components OsAHP1/2 (Liu *et al.*, 2023a). Further identification of ROP-interacting molecules involved in hormone response is key to deciphering the crosstalk between these two types of signaling pathways. Particularly, in recent years, the functions of ROPs in cell division and stress responses have emerged (Engelhardt *et al.*, 2020; Ganotra *et al.*, 2023; Kawano *et al.*, 2014; Waadt *et al.*, 2022; Yi and Goshima, 2022). There should be more components in CK and ABA pathways and ROP signaling that interact with each other and await to be discovered.

Currently, studying ROP-hormone crosstalk in a multicellular context is still challenging. Firstly, hormone signals typically act on cell populations, with longer response times, while the molecular machinery of the ROP pathway often operates in single or a few cells, with relatively faster response kinetics. To unravel the fine interaction mechanisms between hormone signals and the ROP pathway, long-term time-lapse imaging of cell populations at the tissue level with high spatiotemporal resolution is needed. This requires the use of brighter and more stable fluorescent markers, the

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optimization of sample preparation, and the precise recognition of cell identity. In higher plants, it is possible to overcome these problems by studying tissues that are easy to manipulate and image, such as leaf epidermal cells (Liu *et al.*, 2021), using recently developed bright and stable fluorescent proteins, such as mNeonGreen and StayGold (Hirano *et al.*, 2022; Shaner *et al.*, 2013), and applying low phototoxicity fluorescence imaging techniques such as light-sheet microscopy (Ovecka *et al.*, 2022). Secondly, exploring how the ROP pathway and hormone signals interact at the transcriptional regulation level may need transcriptomics analysis with higher spatiotemporal resolution. Single-cell sequencing technology developed in recent years holds promise in this regard (Shaw *et al.*, 2021). Additionally, using new plant models such as mosses and liverworts is another option. Mosses and liverworts have relatively simple tissue structures and typically consist of single layers or a few layers of cells, making them ideal for visualizing organ development at the single-cell level (Naramoto *et al.*, 2022). The main components in ROP and hormone signaling pathways are present in the moss and liverwort genomes (Blazquez *et al.*, 2020; Eklund *et al.*, 2010; Fowler, 2010; Guillory and Bonhomme, 2021; Wang *et al.*, 2015). Their functional studies have received widespread attention in recent years (Flores-Sandoval *et al.*, 2023; Ou and Yi, 2022). Investigating ROP-hormone crosstalk in basal land plants may provide valuable insights into the principles underpinning multicellular morphogenesis.

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Acknowledgments

We apologize to authors whose original publications may not be cited due to space limitations.

Author contributions

H.T., R.L., and P.Y. prepared the figures and tables and wrote the manuscript.

Conflict of interest

The authors declare no conflict of interest.

Funding

This work is supported by Graduate Education and Teaching Reform Research Project (Grant No. GSSCU2023058) and Youth Science and Technology Leading Talent Cultivation Project from Sichuan University (Grant No. 0082604151432), and National Natural Science Foundation of China (Grant No. 32270776) to P.Y.

Data availability

This paper does not include experimental data.

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Figure 1. Category and evolutionary origin of ROPs. (A) Domain organization of type-I and type-II ROPs and the cycling of ROPs between the GTP-bound and GDP-bound forms. Both groups comprise a GTPase domain (G domain), a polybasic region (PBR), and a carboxyl terminus. The carboxyl terminus of type-I ROPs is featured by a CAAX motif wherein C is an invariant cysteine, A represents an aliphatic amino acid, and X is a non-specific residue. The carboxyl terminus of type-II ROPs has a glycine (G) and cysteine (C)-containing GC-CG motif. Guanine nucleotide exchange factor (GEF) converts GDP-bound ROPs to the GTP-bound form. GTPase-activating protein (GAP) stimulates the GTPase activity of ROPs and facilitates the conversion of ROP-GTP to ROP-GDP. Guanine nucleotide dissociation inhibitor (GDI) extracts ROPs from the membrane to cytosol. (B) Evolutionary origin of type-I and type-II ROPs. The putative origins of major phytohormone signaling pathways and developmental patterns are shown to indicate their phylogenetic relationship with ROPs. The origin of phytohormone is based on the following literature: auxin (AUX) (Carrillo-Carrasco *et al.*, 2023), cytokinin (CK) (Powell and Heyl, 2023), abscisic acid (ABA) (Sun *et al.*, 2020), salicylic acid (SA) (Jia *et al.*, 2023; Wang *et al.*, 2015), jasmonic acid (JA) (Blazquez *et al.*, 2020; Wang *et al.*, 2015), gibberellin (GA) (Blazquez *et al.*, 2020; Wang *et al.*, 2015), and brassinosteroid (BR) (Kim and Russinova, 2020; Wang *et al.*, 2015). *Note that BR signaling might have been established early in *Klebsormidiophyceae* and charophytes because major components except BKI1 are present in these lineages.

Figure 2. Crosstalk between ROP signaling and auxin pathways in flowering plants. (A) ROP signaling and auxin pathways in pavement cell morphogenesis. Note that BR has also been recently shown to participate in activating ROP2 through downregulating PHGAP1/2 stability. (B) ROP signaling and auxin pathways in other developmental processes. Most of the presented network is derived from studies in root growth and root hair development. Stars indicate that redundant paralogs are likely involved and are not shown. Transcriptionally regulated factors are highlighted in the dashed oval. The lack of a direct interaction or regulatory mechanism is indicated with dashed lines.

Figure 3. Interactions between ROP signaling and auxin in the moss *Physcomitrium patens*. The life cycle of *P. patens* is dominated by the development of filamentous protonemata and leafy shoots termed gametophores (not shown). Protonemata are generated through tip growth and comprise two types of tissues, namely caulonemata and chloronemata. Caulonemal cells have fewer and smaller chloroplasts than chloronemal cells and could differentiate into chloronemal cells over time during development. The transition from caulonemal cells to chloronemal cells is inhibited by auxin. The dashed box shows localization patterns of PpROP, PpRIC, and PpPIN in tip-growing protonema cells. Auxin exhibits a gradient down to the tip due to the polar localization of PpPIN. The ROP effector PpRIC is localized at the growing tip and displays a tip-to-base gradient in the nucleus. Nuclear PpRIC can inhibit auxin-induced caulonemal cell differentiation. Localization of PpPIN depends on microtubule (MT) and actin. This process might be regulated by ROP signaling through uncharacterized effectors.

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Figure 4. Crosstalk between ROP signaling and ABA pathways. (A) ROP signaling and ABA pathways in stomatal closure and opening. Some of the regulatory nodes such as FER-RopGEFs are also functional in other developmental processes. (B) ROP signaling and ABA pathways in other developmental processes. Most of the presented network is derived from studies in seed dormancy, root growth inhibition, and seedling development. Stars indicate that redundant paralogs are likely involved and are not shown. Transcriptionally regulated factors are highlighted in the dashed ovals. The lack of a direct interaction or regulatory mechanism is indicated with dashed lines.

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Table 1. ROP-related genes involved in hormone responses.

Gene	Species	Process	Function	Reference
Auxin signaling				
Ntrac1	*Nicotiana tabacum*	(a) seedling growth; (b) root development; (c) root hair initiation and morphology.	(a) activated by auxin; (b) promotes Aux/IAA degradation and auxin-responsive gene expression.	(Tao *et al.*, 2005; Tao *et al.*, 2002)
rop2	*Arabidopsis*	morphology of pavement cells, trichome, and other cells.	(a) activated by auxin-ABP1/ABL1/2-TMK1; (b) induces lobe expansion by promoting RIC4-mediated F-actin assembly and PIN1 localization at the lobe membrane; (c) inhibits RIC1-triggered MT organization; (d) inhibits clathrin-mediated PIN1 internalization.	(Fu *et al.*, 2005; Fu *et al.*, 2002; Nagawa *et al.*, 2012; Xu *et al.*, 2014; Xu *et al.*, 2010; Yu *et al.*, 2023)
rop3	*Arabidopsis*	(a) embryo development; (b) root gravitropism; (c) hypocotyl elongation; (d) cotyledon development.	(a) expression induced by auxin; (b) promotes membrane localization of PIN1/3 but not PIN2 or AUX1; (c) promotes auxin-responsive gene expression.	(Huang *et al.*, 2014)
rop4	*Arabidopsis*	pavement cell morphogenesis.	(a) activated by auxin-ABP1/ABL1/2-TMK1; (b) induces lobe expansion by promoting RIC4-mediated F-actin assembly and PIN1 localization at the lobe membrane; (c) inhibits RIC1-triggered MT organization; (d) inhibits clathrin-mediated PIN1 internalization.	(Fu *et al.*, 2005; Nagawa *et al.*, 2012; Xu *et al.*, 2014; Xu *et al.*, 2010; Yu *et al.*, 2023)
rop6	*Arabidopsis*	(a) pavement cell morphogenesis; (b) root gravitropism; (c) vascular tissue patterning; (d)	(a) expression induced by auxin; (b) activated by auxin-ABP1/ABL1/2-TMK1; (c) promotes pavement cell	(Chen *et al.*, 2012; Fu *et al.*, 2009; Lin *et al.*, 2012; Poraty-Gavra *et al.*, 2013; Xu *et al.*,

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		lateral root initiation; (e) inflorescence development.	indentation by regulating RIC1-triggered MT organization; (d) inhibits clathrin-mediated endocytosis of PIN1/2 in pavement cells; (e) inhibits PIN2 internalization via RIC1 and actin in roots.	2014; Xu *et al.*, 2010; Yu *et al.*, 2023)
rop9	*Arabidopsis*	(a) primary root growth; (b) embryo development.	(a) expression induced by auxin; (b) inhibits or promotes auxin-responsive gene expression.	(Choi *et al.*, 2014; Nibau *et al.*, 2013)
ropgef1	*Arabidopsis*	(a) root hair growth; (b) embryo development; (c) root gravitropic growth; (d) primary root growth.	(a) may be involved in auxin-induced root hair growth; (b) promotes correct localization of PIN2/7 and AUX1; (c) promotes auxin-responsive gene expression; (d) promotes actin assembly.	(Duan *et al.*, 2010; Liu *et al.*, 2017)
ropgef4	*Arabidopsis*	root hair growth.	interacts with FER and may be involved in auxin-induced root hair growth by activating ROP2/6.	(Duan *et al.*, 2010; Huang *et al.*, 2013)
ropgef7	*Arabidopsis*	meristem maintenance in embryo and seedling roots.	(a) expression induced by auxin; (b) promotes PIN1 localization; (c) promotes auxin-responsive gene expression; (d) binds ROP3 and activate ROPs.	(Chen *et al.*, 2011)
ropgef10	*Arabidopsis*	root hair initiation.	interacts with FER and may be involved in auxin-induced root hair initiation by activating ROP2/6.	(Duan *et al.*, 2010; Huang *et al.*, 2013)
spk1	*Arabidopsis*	(a) primary root growth; (b) lateral root initiation; (c) root gravitropic growth.	(a) mediates auxin-induced ROP6 activation; (b) inhibits PIN2 internalization; (c) promotes auxin-responsive gene expression.	(Lin *et al.*, 2012)

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ric1	*Arabidopsis*	(a) pavement cell morphogenesis; (b) root growth; (c) lateral root formation.	(a) expression induced by auxin; (b) acts as a ROP6 effector to promote MT ordering in pavement cells; (c) inhibits clathrin-mediated endocytosis of PIN1/2 in pavement cells; (d) inhibits PIN2 internalization via actin in roots (e) promotes auxin-responsive gene expression.	(Chen *et al.*, 2012; Choi *et al.*, 2013; Fu *et al.*, 2005; Fu *et al.*, 2009; Lin *et al.*, 2012; Xu *et al.*, 2014; Xu *et al.*, 2010)
ric4	*Arabidopsis*	pavement cell morphogenesis.	(a) acts as a ROP2 effector to promote F-actin assembly; (b) inhibits PIN1 internalization.	(Fu *et al.*, 2005; Nagawa *et al.*, 2012; Xu *et al.*, 2014)
icr1	*Arabidopsis*	(a) embryo development; (b) root development; (c) lateral root initiation.	(a) expression induced by auxin treatment; (b) promotes membrane localization of PIN1/2 likely via exocytosis and recycling; (c) degraded by high auxin activity.	(Hazak *et al.*, 2010; Hazak *et al.*, 2014; Lavy *et al.*, 2007; Li *et al.*, 2008)
fer	*Arabidopsis*	root hair initiation and growth.	(a) promotes auxin-induced root hair growth by activating ROPs via interacting with RopGEF1/4/10.	(Duan *et al.*, 2010; Huang *et al.*, 2013)
makr2	*Arabidopsis*	(a) primary root growth; (b) root gravitropic growth.	(a) promotes asymmetric PIN2 localization; (b) acts genetically upstream of TMK1 and ROP6 to inhibit their function; (c) inhibited by auxin-TMK1-mediated phosphorylation and dissociation from plasma membrane.	(Marques-Bueno *et al.*, 2021)
rbk1	*Arabidopsis*	(a) root elongation; (b) cotyledon expansion.	inhibits auxin signaling by phosphorylating ROP4/6 and likely inactivating them downstream of MKK3/MPK1.	(Enders *et al.*, 2017; Molendijk *et al.*, 2008)

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cmi1	*Arabidopsis*	(a) primary root growth; (b) root hair growth; (c) hypocotyl elongation; (d) lateral root formation.	(a) expression induced by auxin; (b) recruited to MTs by ICR1 in Ca^{2+} dependent manner; (c) promotes auxin-induced Ca^{2+} patterning; (d) inhibits auxin-induced root growth retardation; (e) inhibits auxin-responsive gene expression.	(Hazak *et al.*, 2019)
tor	*Arabidopsis*	(a) shoot development; (b) root development; (c) leaf organogenesis.	(a) activated by auxin and ROP2; (b) phosphorylates E2Fa/b to promote translation reinitiation and cell proliferation.	(Li *et al.*, 2017; Schepetilnikov *et al.*, 2017)
hda6	*Arabidopsis*	pavement cell morphogenesis.	(a) suppresses the expression of auxin-responsive transgene reporters via transgene silencing; (b) inhibits histone acylation of ROP6 promoter and ROP6 expression.	(Du *et al.*, 2024; Murfett *et al.*, 2001)
Ghrop6	*Gossypium hirsutum*	cotton fiber development.	inhibits GhPIN3 localization via GhRIC1.	(Xi *et al.*, 2023)
Osropgef10	*Oryza sativa*	crown root development.	(a) expression induced by auxin; (b) interacts with OsRAC3 to inhibit CK signaling.	(Liu *et al.*, 2023a)
Osrac3	*Oryza sativa*	crown root development.	(a) expression induced by auxin; (b) activated by auxin; (c) sequesters OsAHP1/2 to the plasma membrane to inhibit CK signaling.	(Liu *et al.*, 2023a)
Mprop	*Marchantia polymorpha*	(a) rhizoid growth; (b) meristem notch formation; (c) gemma dormancy.	(a) promotes auxin synthesis gene expression; (b) promotes cell division orientation.	(Rong *et al.*, 2022)
Ppric	*Physcomitrium patens*	caulonemal cell differentiation.	(a) localizes at the plasma membrane and in the nucleus; (b) nuclear PpRIC	(Ntefidou *et al.*, 2023)

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			inhibits auxin-induced caulonemal cell differentiation.	
ABA signaling				
rop2	*Arabidopsis*	stomatal opening and closure.	(a) translocates to plasma membrane under light; (b) inhibits light-induced stomatal opening likely via activating RIC7; (c) translocates into the cytosol upon ABA treatment; (d) inhibits ABA- and CO_2-induced stomatal closure likely via regulating endocytosis.	(Hwang *et al.*, 2011; Jeon *et al.*, 2008)
rop6	*Arabidopsis*	stomatal closure.	(a) inactivated by ABA likely via ABI1; (b) promotes actin assembly and organization.	(Lemichez *et al.*, 2001)
rop9	*Arabidopsis*	(a) seed dormancy; (b) root elongation.	(a) expression inhibited by ABA; (b) promotes or inhibits ABA-responsive gene expression.	(Choi *et al.*, 2014; Nibau *et al.*, 2013)
rop10	*Arabidopsis*	(a) root elongation; (b) stomatal closure; (c) seed germination.	(a) expression inhibited by ABA; (b) inhibits ABA-responsive gene expression.	(Choi *et al.*, 2014; Xin *et al.*, 2005; Zheng *et al.*, 2002)
rop11	*Arabidopsis*	(a) seed germination; (b) seedling growth; (c) stomatal closure; (d) primary root growth.	(a) translocates into the nucleus upon ABA treatment; (b) binds to ABI1 and prevents it from being inactivated by ABA; (c) inhibits ABA-responsive gene expression.	(Li *et al.*, 2012a; Li *et al.*, 2012b; Yu *et al.*, 2012)
ropgef1	*Arabidopsis*	(a) stomatal closure; (b) primary root growth; (c) seed germination; (d) seedling development.	(a) inhibits ABA-induced stomatal closure by activating ROP11; (b) translocates to cytosol for degradation upon ABA treatment; (c) inhibits ABA response likely by interacting with ABI1; (d) phosphorylated by	(Li and Liu, 2012; Li *et al.*, 2018; Li *et al.*, 2016; Yu *et al.*, 2012)

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			CPK4 and likely CPK3/6/11 for degradation.	
ropgef2	*Arabidopsis*	(a) seed germination; (b) seedling development.	(a) associates with mitochondria and recruited to plasma membrane by ROP2/6/10; (b) degraded upon ABA treatment.	(Zhao *et al.*, 2015)
ropgef4	*Arabidopsis*	(a) stomatal closure; (b) primary root growth.	(a) inhibits ABA-induced stomatal closure by activating ROP11; (b) may be degraded upon ABA treatment.	(Li and Liu, 2012; Li *et al.*, 2016; Yu *et al.*, 2012)
ropgef10	*Arabidopsis*	(a) stomatal closure; (b) primary root growth.	(a) inhibits ABA-induced stomatal closure by activating ROP11; (b) may be degraded upon ABA treatment.	(Li and Liu, 2012; Li *et al.*, 2016; Yu *et al.*, 2012)
ric1	*Arabidopsis*	(a) seed germination; (b) root growth; (c) lateral root formation.	(a) expression induced by ABA; (b) inhibits ABA-responsive gene expression.	(Choi *et al.*, 2013)
ric7	*Arabidopsis*	stomatal opening.	(a) translocates to plasma membrane by light; (b) inhibits light-induced stomatal opening.	(Jeon *et al.*, 2008)
fer	*Arabidopsis*	(a) seedling development; (b) primary root growth; (c) stomatal closure.	(a) expression inhibited by ABA; (b) inhibits ABA-induced phenotypes likely via regulating RopGEF1/4/10-ROP11; (c) increased phosphorylation level by RALF and ABA treatment and dephosphorylated by ABI2.	(Chen *et al.*, 2016; Duan *et al.*, 2010; Yu *et al.*, 2012)
hda15	*Arabidopsis*	(a) seed germination; (b) drought tolerance.	(a) expression induced by ABA; (b) binds to MYB96 and inhibits the expression of ROP6/10/11 by deacylating H3/H4 in the promoter.	(Lee and Seo, 2019)
myb96	*Arabidopsis*	(a) seed germination; (b)	(a) expression induced by ABA; (b)	(Lee and Seo, 2019; Seo *et al.*,

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		drought tolerance.	binds to HDA15 and inhibits the expression of ROP6/10/11 by deacylating H3/H4 in the promoter.	2009)
Ppropgef1/2/3/4/5/6	*Physcomitrium patens*	(a) tip cell growth; (b) cell morphology.	expression inhibited by ABA treatment.	(Beier *et al.*, 2023)
Pprop1/2/3/4	*Physcomitrium patens*	(a) tip cell growth; (b) cell morphology.	expression inhibited by ABA treatment.	(Beier *et al.*, 2023)
Csrac1	*Camellia sinensis*	seed germination.	(a) expression inhibited by ABA treatment; (b) inhibits the inhibitory effect of ABA on seed germination.	(Xu *et al.*, 2022b)
SA signaling				
rop6	*Arabidopsis*	pathogen response.	(a) inhibits SA-responsive gene expression; (b) inhibits SA synthesis; (c) inhibits pathogen entry.	(Poraty-Gavra *et al.*, 2013)
ren1	*Arabidopsis*	pollen tube growth.	abundance at pollen tube tip inhibited by SA but increased by methylated SA, resulting in hyperactivation and inactivation of ROPs, respectively.	(Rong *et al.*, 2016)
Tarop10	*Triticum aestivum*	pathogen response.	(a) expression inhibited by SA; (b) interacts with TaTrxh9 and negatively regulates resistance against the stripe rust.	(Shi *et al.*, 2021)
BR signaling				
rop2	*Arabidopsis*	root gravitropic growth.	(a) expression and localization enhanced by BR treatment; (b) may promote BR-induced gravitropic growth via positively regulating PIN2 expression; (c)	(Li *et al.*, 2005)
phgap1/2	*Arabidopsis*	pavement cell morphogenesis.	(a) stabilized by BIN2 phosphorylation at the indentation region and inactivates ROP2; (b) degraded at the	(Lauster *et al.*, 2022; Zhang *et al.*, 2022)

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			lobe region due to the inhibition of BIN2 by BR, leading to activation of ROP2.	
CK signaling				
rop2/4	*Arabidopsis*	pavement cell morphogenesis.	activity decreased by ARR20, a positive regulator in CK signaling pathway.	(Li *et al.*, 2013)
Osropgef10	*Oryza sativa*	crown root development.	(a) expression induced by CK; (b) binds to OsAHP1/2 and recruits them to the plasma membrane; (c) promotes OsRR6 expression; (d) inhibits OsHK1 and OsRR22 expression.	(Liu *et al.*, 2023a)
Osrac3	*Oryza sativa*	crown root development.	(a) expression induced by CK; (b) binds to OsAHP1/2 and recruits them to the plasma membrane.	(Liu *et al.*, 2023a)

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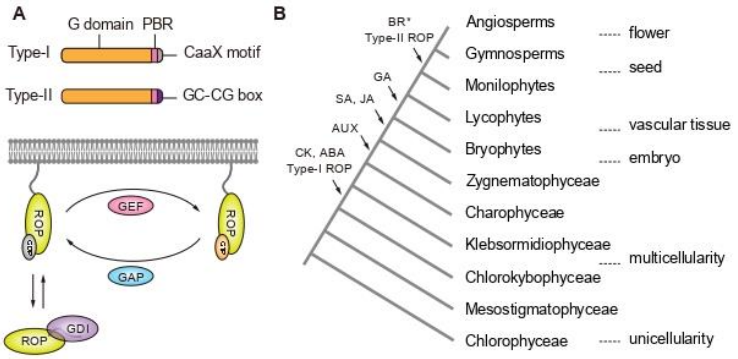

Figure 1. Category and evolutionary origin of ROPs. (A) Domain organization of type-I and type-II ROPs and the cycling of ROPs between the GTP-bound and GDP-bound forms. Both groups comprise a GTPase domain (G domain), a polybasic region (PBR), and a carboxyl terminus. The carboxyl terminus of type-I ROPs is featured by a CAAX motif wherein C is an invariant cysteine, A represents an aliphatic amino acid, and X is a non-specific residue. The carboxyl terminus of type-II ROPs has a glycine (G) and cysteine (C)-containing GC-CG motif. Guanine nucleotide exchange factor (GEF) converts GDP-bound ROPs to the GTP-bound form. GTPase-activating protein (GAP) stimulates the GTPase activity of ROPs and facilitates the conversion of ROP-GTP to ROP-GDP. Guanine nucleotide dissociation inhibitor (GDI) extracts ROPs from the membrane to cytosol. (B) Evolutionary origin of type-I and type-II ROPs. The putative origins of major phytohormone signaling pathways and developmental patterns are shown to indicate their phylogenetic relationship with ROPs. The origin of phytohormone is based on the following literature: auxin (AUX) (Carrillo-Carrasco et al., 2023), cytokinin (CK) (Powell and Heyl, 2023), abscisic acid (ABA) (Sun et al., 2020), salicylic acid (SA) (Jia et al., 2023; Wang et al., 2015), jasmonic acid (JA) (Blazquez et al., 2020; Wang et al., 2015), gibberellin (GA) (Blazquez et al., 2020; Wang et al., 2015), and brassinosteroid (BR) (Kim and Russinova, 2020; Wang et al., 2015). *Note that BR signaling might have been established early in Klebsormidiophyceae and charophytes because major components except BKI1 are present in these lineages.

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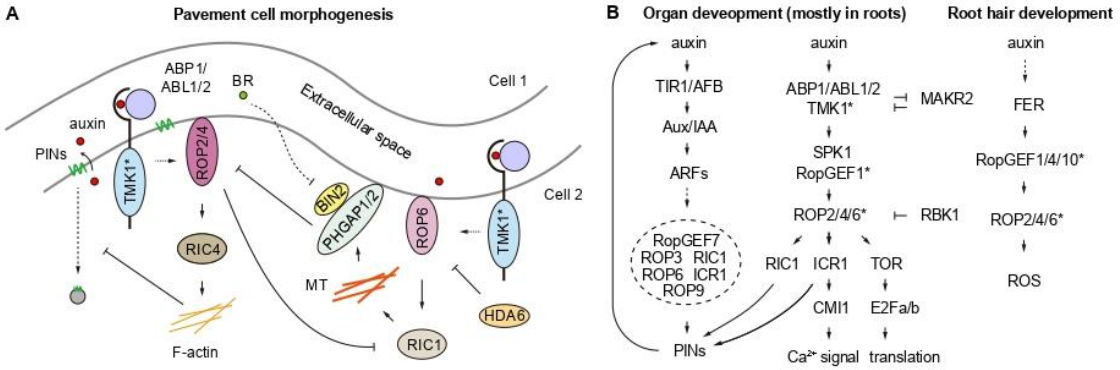

Figure 2. Crosstalk between ROP signaling and auxin pathways in flowering plants. (A) ROP signaling and auxin pathways in pavement cell morphogenesis. Note that BR has also been recently shown to participate in activating ROP2 through downregulating PHGAP1/2 stability. (B) ROP signaling and auxin pathways in other developmental processes. Most of the presented network is derived from studies in root growth and root hair development. Stars indicate that redundant paralogs are likely involved and are not shown. Transcriptionally regulated factors are highlighted in the dashed oval. The lack of a direct interaction or regulatory mechanism is indicated with dashed lines.

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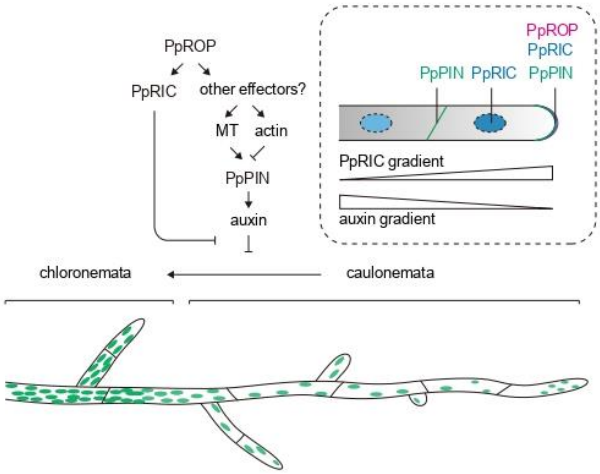

Figure 3. Interactions between ROP signaling and auxin in the moss *Physcomitrium patens*. The life cycle of P. patens is dominated by the development of filamentous protonemata and leafy shoots termed gametophores (not shown). Protonemata are generated through tip growth and comprise two types of tissues, namely caulonemata and chloronemata. Caulonemal cells have fewer and smaller chloroplasts than chloronemal cells and could differentiate into chloronemal cells over time during development. The transition from caulonemal cells to chloronemal cells is inhibited by auxin. The dashed box shows localization patterns of PpROP, PpRIC, and PpPIN in tip-growing protonema cells. Auxin exhibits a gradient down to the tip due to the polar localization of PpPIN. The ROP effector PpRIC is localized at the growing tip and displays a tip-to-base gradient in the nucleus. Nuclear PpRIC can inhibit auxin-induced caulonemal cell differentiation. Localization of PpPIN depends on microtubule (MT) and actin. This process might be regulated by ROP signaling through uncharacterized effectors.

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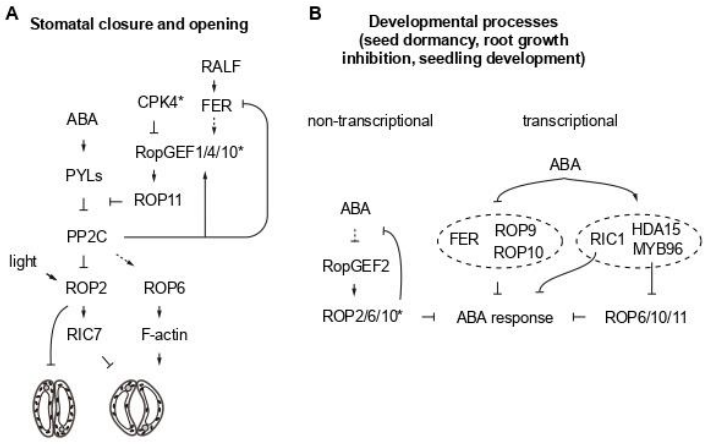

Figure 4. Crosstalk between ROP signaling and ABA pathways. (A) ROP signaling and ABA pathways in stomatal closure and opening. Some of the regulatory nodes such as FER-RopGEFs are also functional in other developmental processes. (B) ROP signaling and ABA pathways in other developmental processes. Most of the presented network is derived from studies in seed dormancy, root growth inhibition, and seedling development. Stars indicate that redundant paralogs are likely involved and are not shown. Transcriptionally regulated factors are highlighted in the dashed ovals. The lack of a direct interaction or regulatory mechanism is indicated with dashed lines.

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