

Small GTPases: Rho of Plant (ROP) in Development and Stress Signaling

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ABSTRACT

The small GTPase superfamily is comprised of four families (ROP, ARF, RAB and RAN) in the plant kingdom. The members of this superfamily show diversity in structure and function, despite possessing a conserved G-domain for GTP-binding and hydrolysis. ROP (Rho of Plants) has emerged as plant-specific GTPases to orchestrate unique cellular function such as polarity establishment, stress and plant hormonal signaling. The prominent role in their functional capability is evident from their varied expressional regulation in different developmental and stress conditions. The functional activity of small GTPases is tuned by both positive (guanine exchange factor, GEF) and negative regulators (Guanine Dissociation Inhibitor, GDI and GTPase activating protein, GAP), which favors the ON and OFF state of small GTPases, respectively. The expressional variation of regulators as well as their spatial and temporal expression pattern determine the activity and hence turn-on the switch of small GTPases. In this report, we are exploring the expressional and functional analysis of ROP class of small GTPases in plants under stress and developmental signaling pathways.

Keywords: abiotic and biotic stress, guanosine triphosphatases, RAC/ROP, signal transduction

Abbreviations: **ABI**, ABA insensitive; **ARF**, ADP ribosylation factor; **GAP**, GTPase-activating protein; **GDI**, guanine dissociation inhibitor; **GEF**, guanine exchange factor; **GTPase**, guanosine triphosphatase; **RAB**, ras superfamily of monomeric G proteins; **RAC**, rho family GTPase; **RAN**, ras-related nuclear protein; **ROP**, rho of plant

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INTRODUCTION

The development of animal and plant systems possesses contrasting features because of the sessile and plastic nature of plants. Hence, plants need sophisticated signaling switches to adapt to the constantly changing environment. Small guanosine triphosphatases, (GTPases) have emerged as important molecular switches in all eukaryotes including animal and plant signal transduction pathways. Small GTPases are a group of GTP binding proteins constituting a superclass in the eukaryotes. The small GTPases regulate numerous cellular processes by transition between the active GTP-bound and inactive GDP-bound conformations. Small GTPases, often referred, Ras-like GTPases is further classified as eight sub-families namely Ras, Rho, Rab, Arf, Ran, Rad, Rap and Rheb (Takai 2001). Each member of these families is implicated in a specific or in a combined cellular function, which can sometime be overlapping. The



Fig. 1 The cartoon shows the OFF and ON state of small GTPase protein based on GDP and GTP bound form and other regulators controlling the transition between the two states.

architecture of small GTPase is characterized by a conserved GTP-binding (G-domain) and a divergent effector domain (Yang 2002; Berken 2006; Nibau 2006). Small GTPases are monomeric in nature and their size ranges from 20 to 30 kDa (Paduch 2001). In animals, Ras-like GTPases acts as versatile cytoplasmic signaling molecule controlling the gene expression to regulate cellular proliferation and differentiation.

GTPase switching mechanism

The three-dimensional structure of GTPase as a complex with their regulators has revealed their regulatory mechanism. Since they act as transducers in cell signaling, their function is controlled by upstream activators and downstream effector proteins. These regulatory proteins help in maintaining a balance between the active and inactive form of these small GTPases. The active or ON form is typically the GTP-bound state wherein it can further transduce the signal to downstream effectors. The inactive or OFF form is supported by GDP-bound state wherein it can accept signal from upstream activating proteins for a whole round of progressive cycling mechanism. This is also the theme behind the temporal regulation of cellular activities regulated by small GTPases (Yang 2002).

Regulation of GTPase activity

The activity of small GTPase is controlled by three different classes of proteins namely; Guanine Exchange Factors (GEFs), GTPase Activating Proteins (GAPs) and Guanine Dissociation Inhibitors (GDIs). GEFs promote the active or 'ON' state by a nucleotide exchange mechanism by replacing GDP with GTP, while GAPs and GDIs helps in reverting back to inactive or OFF state of small GTPases by dissociating GTP (Fig. 1). Most small GTPases exist in either a membrane associated or cytoplasmic state. Regulatory GEFs provide a clutch in controlling the activity of these GTPases by being available in a membrane-associated state. All of the small GTPases also possess an intrinsic GTPase activity wherein the hydrolytic activity leads to inactive OFF state. However, this activity is enhanced by GAP (GTPase Activating Protein). GDI has a negative control over the activated form since it sequesters the active membrane bound state to inactive cytoplasmic state. These interactions have enabled these small molecules to generate functional diversity and have created novel function in different phyla (Berken 2006).

Plant-specific small GTPases

Ras and Rho are the crucial signaling proteins known to transmit extracellular signals in animals. Even though the Ras-family of GTPase is completely absent in plant kingdom (Zheng and Yang 2000), the genomes of *Arabidopsis* and rice have revealed that plant kingdom has a few heterotrimeric G proteins, which is in sharp contrast to animal system, are enabled by wide array of G-proteins coupled receptors (GPCRs) for its signaling mechanisms. In animals, Ras and Rho GTPases are activated in response to various stimuli thereby serving as signaling nodal points. Rab and Arf proteins are implicated in vesicular transport acting as coordinators of intracellular trafficking. Given that Ras is absent in plant kingdom, Rac or Rop (Rho of plants) has emerged as a sole GTPase protein involved in signaling pathways in plants. Both the names, Rac and Rop refer to extended Rho family of small GTPase in plants due to its sequence similarity. The genome sequence analyses have revealed 11 ROP/RACs in *Arabidopsis*, 7 in rice and 9 in maize (Christensen 2003). The presence of multiple numbers of ROP genes in plants indicates their functional versatility and redundancy.

REGULATION OF SIGNALING EVENTS DURING PLANT GROWTH AND DEVELOPMENT

Polar growth

The most striking role of plant-specific RAC/ROPs is in the control of polarity establishment in pollen tubes and root hairs (Nibau 2006). The polarity development is a resulting phenomenon of actin cytoskeleton fine-tuning through multiple cellular pathways. The best characterized among them is the cytosolic Ca^{2+} gradient, which is effected by altered

RAC/ROP expression in polarized pollen tube growth. Convincing evidences from null, constitutively active (CA), and dominant negative (DN) mutants have shown the involvement of ROP GTPases in polar growth of pollen tubes and root hairs (Berken 2006). The negative regulation of AtROP1 and AtROP5 leads to inhibition of growth in the pollen tube while the ectopic expression on the overexpression mutants leads to depolarization suggesting their crucial role in polarization of pollen tube growth (Berken 2006). Furthermore, it has been shown that ROP GTPases temporally control the polar growth by fine modulation of downstream effector pathways such as F-actin assembly and tip-focused Ca^{2+} gradient formation (Hwang 2005). ROPs mediated polar root-tip growth was demonstrated in *Arabidopsis* root meristem and trichoblasts with plasma membrane localized ROP GTPases functioning in Ca^{2+} gradient pathways (Molendijk 2001). ROPs are strategically located at the tip of growing apex cells and their disruption in AtROP1 and AtROP5 leads to growth inhibition. Their importance in sexual reproduction is evident from the defective seed set in plants expressing multiple copies of AtROP9 (AtRAC7) due to impaired pollen tube growth (Cheung 2003). Similar mechanisms are likely to be involved in controlling the polar growth of unspecialized epidermal cells in leaves that follow a diffused growth pattern (Berken 2006).

It is apparent that ROPs should be involved in many aspects of cellular development owing to their impact on cytoskeletal components and Ca^{2+} fluxes. AtROP1 has been found to activate its downstream effector, RIC3 (ROP-interactive CRIB, Cdc 42/Rac interactive binding motif-containing protein 3) inducing accumulation of Ca^{2+} in the tip for favoring actin disassembly. In an alternative effector mechanism, AtROP1 also activates RIC4 to promote F-actin assembly. The ability to counteract assembly and disassembly at the same time by interacting with specific effector allows AtROP1 protein to modulate actin dynamics with the polar growth of the pollen tube. It has been found that RIC4 membrane localization parallels the pollen tube growth dynamics, wherein ROP activation synchronizes with the F-actin assembly mediated by RIC4 (Hwang 2005). This ROP1 oscillation is apparently ahead of Ca^{2+} accumulation regulated by RIC3 suggesting the temporal and spatial regulation of AtROP activity to fine-tune the growing tip cells. In yet another novel pathway, a tobacco NtRAC1 modulates NtADF1 (Actin Depolymerization Factor) activity by specifically phosphorylating Ser-6 residue of ADF to impact actin dynamics (Chen 2003).

In an interesting finding, AtROP2 and AtROP6 were found to be antagonistic ROP GTPases in controlling cytoplasmic auxin signaling mechanism wherein the spatial activation of ROP2 by auxin through ABP1 (Auxin Binding Protein1) occurs to interdigitate lobes and indentations during the growth of leaf pavement cells (PCs) in *Arabidopsis* (Xu 2010). ROP2 activation leads to RIC4 activation at lobe sites of the cell to promote F-actin assembly requiring for outgrowth of lobes. ROP2 also mediates PIN1 (PIN-FORMED1) localization to the apical region. Auxin transported by localized PIN1 activates ROP2 establishing a feedback loop to sustain the lobe outgrowth. The same PIN1 transported auxin also activates ROP6 after diffusing through the cell wall that in turn activates RIC1, which can regulate microtubule framework by binding to it and restrict growth locally.

The role of AtROPs in establishing polarity during embryonic development is also evident when up regulation of ROP activity leads to defective establishment of root-shoot axis. This is in fact due to the poorly regulated PIN1 localization since endocytic vesicles are responsible for PIN1 localization. Moreover, role of AtROPs in membrane trafficking was also evident from the genetic analysis of constitutively active AtROP10, these plants exhibited inhibited endocytosis and membrane recycling (Nibau 2006). Thus, RAC/ROP regulate cytoskeleton dynamics and vesicle trafficking, which is necessary for normal plant growth and

development.

RAC/ROP in hormone signaling

The most significant advances related to ROP functions have emerged by studies showing that ROPs have roles in regulating plant responses towards hormones, such as auxin and ABA. Besides strong evidences for the positive regulation of auxin signaling, RAC/ROPs seem to negatively regulate ABA (Abscisic Acid) signaling. AtRAC1 (AtROP3) has been found to be the central component of ABA-mediated stomatal closure. ABA treatment leads to inactivation of AtRAC1 leading to disruption of actin cytoskeleton in the stomata, which is found to be the limiting step of stomatal closure phenomenon (Lemichez 2001). The same inactivation by over-expressing DN forms in *abi1-1* (ABA-insensitive) mutants overcomes the ABA insensitivity. These evidences suggest that RAC/ROP has negative effect on ABA signaling. In another study, AtROP10 has been found to regulate canonical ABA responses such as stomatal closure, control of seed dormancy and germination besides root growth suppression (Zheng 2002). These effects have been shown to occur by the modulation of MAPKs (Mitogen activated Protein Kinases) and transcription factors that are responsible for ABA responses (Berken 2006). In a recent study, a cross-talk mechanism was reported wherein the FERONIA kinase negatively regulates the ABA response under increased levels of ABA which is however earlier reported to be a positive regulator of auxin response under the absence of ABA conditions. In this detailed mechanistic-pathway, it has been shown that *AtROP11/AtRAC10* if activated by its upstream positive regulators like GEF1, GEF4 and GEF10, physically interacts with ABI2 phosphatase, a downstream target to negatively modulate the ABA response. The disruption of this module with mutant analysis has all shown ABA-hypersensitive responses. More importantly, *AtROP10* and *AtROP11* with its high similarity in terms of amino acid sequences have been found to be functionally dissimilar. Although they both show ABA-hypersensitivity with mutants, *AtROP10* did not interact with ABI2 in interaction assays indicating it works in different pathway to bring about the same response (Yu *et al.* 2012). ROP family members participate in numerous distinct signaling pathways controlling plant growth, development, and stress response. The signaling ability of constitutively active ROP2 transgenic *Arabidopsis* plants were found to be hampered in several developmental processes regulated by different hormones, including auxin, brassinosteroid, and ABA (Li *et al.* 2001). Exogenous application of phytohormones such as ABA and auxin on constitutively active and dominant negative ROP2 transgenic plants resulted in altered responses (Li *et al.* 2001). Previous studies on RAC/ROP associated auxin signaling events suggested the existence of important molecular response like ubiquitin/26S proteasome mediated proteolysis. RAC/ROPs are activated by auxin, which in turn promotes the 26S-mediated degradation of AUX/IAA repressors leading to transcription of auxin-responsive genes. RAC/ROPs function to assemble active mega-nuclear protein complexes to degrade the suitable proteins acting on the auxin-signaling pathway (Nibau 2006). A different study in tobacco confirmed activation of auxin mediated gene expression through Rac/Rop GTPase, where NtRac1 was found to be regulating auxin signaling, which ultimately affect the downstream responsive genes (Tao *et al.* 2002).

RAC/ROPs in stress signaling

Since plant specific-Rho (ROP), which binds to guanosine triphosphate is the only ubiquitous signaling protein in plant; their important role in physiological response such as stress signaling in plants is obvious. The role of RAC/ROPs in stress signaling mechanisms have been established in many plant species that primarily regulates the oxidative environment of the cell. Several studies on rice showed that

elicitor induction leads to activation of *OsRAC1* leading to the production of NADPH oxidase, which is primary response protein in plant defense against pathogen attacks. When constitutively active and dominant negative forms of *OsRAC1* were introduced into wild type and lesion mimic mutant of rice were analyzed for H₂O₂ production in transformed cell cultures, it was shown that *OsRAC1* acts as positive regulators of ROS (Reactive Oxygen Species) production mediating pathogen resistance (Kawasaki 1999). Moreover, *OsRAC1* was found to mediate lignin biosynthesis by promoting cinnamoyl CoA reductase activation leading to improved defense responses. In yet another defense control, *OsRAC1* also acts to suppress metallothionein expression, one of the major ROS scavengers. In contrary to this, *NtRAC5* showed down regulation of NADPH oxidase expression while responding to elicitors like cryptogein (Morel 2004). The increased expression of RAC/ROP due to loss-of-function of GAP, ROPGAP4 (Rho like small GTPase Activating Protein 4) lead to increased alcohol dehydrogenase (ADH) expression while responding to oxygen deprivation but decreased tolerance to stress. Moreover, they function as molecular rheostat under the conditions of oxygen deprivation (Nibau 2006).

Small GTPase mediated H₂O₂ production is one of the earlier finding to assert it has role in secondary cell wall development in cotton species (Nibau 2006). It is well known that ROS has emerged to be secondary messengers in many signaling processes such as root hair development to regulate polar Ca²⁺ gradient signaling (Berken 2006). *Arabidopsis* root hair defective mutants (RHD2) were found to be impaired in a NADPH oxidase leading to improper spatial control of ROS production and high Ca²⁺ concentration at the tip (Nibau 2006). When ROS/hydroxyl radicals were exogenously applied on the surface of *rhd2*, it led to depolarized growth indicating the importance of spatial polarized control of ROS accumulation (Nibau 2006). Furthermore, AtROP2 was found to be accumulating in Rho-GDI mutant responsible for the same ectopic ROS production. This showed that RAC/ROP act upstream of NADPH oxidase to co-ordinate spatial ROS production thereby establishing required actin dynamics for polar growth. AtROP1 to 6 belongs to the group IV of the ROP gene family (Christensen *et al.* 2003). Most of the members of this group were found to be involved in the ROS production, which can induce programmed cell death. For instance, ROPGAP4 (Rho like small GTPase activating protein 4) in *Arabidopsis* functions as a regulator of ROP-mediated signal transduction rheostat. ROPGAP4 helps to control the production of H₂O₂, which is required for the expression of beneficial genes and to prevent cell death due to H₂O₂ accumulation. This kind of negative feedback regulation by ROPGAP4 results into the oxygen deprivation tolerance and might boost the productivity of crops that undergo transient submergence due to floods (Baxter-Burrell *et al.* 2002). Further study of the orthologs of this group in rice has identified a novel gene *OsRacB*, which was predicted to be an accessory regulatory component in salt stress response. *OsRacB* transcript level was found to be elevated in the roots after salt treatment. Interestingly, *OsRacB* overexpressing transgenic plants were found to be tolerant to salinity conditions but loss-of-function plants did not show the sensitive phenotype and were growing just like control plants (Luo *et al.* 2006). More studies in different plant species have also confirmed the involvement of small GTP binding protein in adaptation to these stresses. In *M. crystallinum* expression and GTP binding activity of a plant specific Rab5 family protein gets strongly affected under salt stress conditions (Bolte *et al.* 2000).

The expression of *OsRab7* proteins was differentially regulated under ABA, cold, dehydration, and salt stress treatments (Nahm *et al.* 2003). The *Rab7* gene in *Arabidopsis* was found to be highly expressive throughout biotic and abiotic stress (Gorvin and Levine 2000; Mazel *et al.* 2004). Further molecular characterization of *AtRab7* suggested it to be a positive regulator of salt and osmotic stress and was

observed to accumulate less reactive oxygen species during salt stress (Mazel *et al.* 2004). Similar observation was also reported in tobacco, where a *Pennisetum glaucum* *PgRab7* gene showed enhanced tolerance to drought and salt stress (Agarwal *et al.* 2008).

Effector proteins for small GTPase in plants

The search for animal and fungi homologs of effector proteins in plants did not yield any major convincing interacting partners. Instead, plants seem to have a set of effector proteins unique to them as in the case of ROP effectors. CRIB (Cdc42/Rac-Interactive Binding) motif is usually found in animal WASP (Wiskott-Aldrich Syndrome Protein) and p21^{PAK} that are major Cdc42/Rac effector proteins that act with GTP-bound form of Rac-GTPases. The major group of this class in plants is referred as *ROP* Interactive *CRIB* motif containing proteins (*RICs*). CRIB motif is responsible for interaction with RAC/ROP GTPases for RICs. Plant CRIB motif residues are conserved despite being diverged in other residues, which contribute to variation. Interestingly, CRIB motif is also present in upstream regulators such as RopGAP proteins. The *Arabidopsis* genome is found to contain 11 RICs, which are divergent among themselves indicating the regulation of many downstream signaling pathways (Nagawa 2010).

Recently, receptor-like cytoplasmic kinases (RCLKs) and cysteine-rich receptor kinases have been found to interact with ROP GTPases. In another study, *OsRAC1* is found to directly bind to respiratory burst oxidase homolog (Rboh), a catalytic subunit of NADPH oxidase necessary for ROS production (Wong *et al.* 2007).

Upstream regulators of small GTPase

In animals Rop-GAPs are a class of regulators specifically interacting with the activated GTP-bound ROP GTPases and promote GTP hydrolysis upon binding (Berken 2006). Plant Rop-GAPs have a GAP domain similar to animals containing a conserved arginine finger to stabilize the transition state during hydrolysis. Besides this conserved domain, most Rop-GAPs display a conserved upstream proline rich region (PRRs) and CRIB motif near the N-terminus. Interestingly, the CRIB motif in Rop-GAPs is not identified so far in other Rho-family GAPs but is present in Cdc42 and Rac effectors (Berken 2006). The CRIB domain is likely to contribute to the specificity of Rop-GAPs to interact with specific ROPs.

The most significant control of ROP activity comes from the regulators to fine-tune the spatial signals coming from the external environment. Hence, it is important that these upstream regulator components should be positioned to co-ordinate these signals. Rho-GEFs links most of the upstream signals to ROP mediated signaling pathways. *Arabidopsis* genome revealed the presence of 14 RhoGEFs identified through yeast two-hybrid screening. The same study identified a unique domain within the plant-specific Rop-GEFs, PRONE (Plant-Specific Rop Nucleotide Exchanger) that can form tight complex with nucleotide-dissociated ROPs (Berken 2006). Overexpression of RopGEF1 in pollen tube leads to growth depolarization, a phenotype that mimics RAC/ROP up regulation (Gu 2006). A pollen-specific, kinase partner protein (KPP, a ROPGEF homolog) in tomato is shown to interact with pollen specific receptor kinases, LePRK1 and LePRK2 aiding in pollen tube germination and growth. It shows similar phenotype when overexpressed leading to depolarized growth (Kaothien 2005).

GDI is third class of regulatory molecules that influence the activated state of Rho GTPases by sequestering them and changing their subcellular localization from membrane to cytosol thereby negatively regulating their activation on the membrane. When Rho GDIs are overexpressed, they sequester the GFP-tagged ROPs from the plasma membrane in protoplasts and pollen tubes by directly affecting the activation status. This spatial regulation has pro-

vided a model for polarized growth in pollen tubes (Berken 2006).

THE IMPORTANCE OF SUBCELLULAR LOCATION IN ROP SIGNALING

The most intriguing feature of signaling mechanism mediated by Rho is their subcellular localization to a specific domain of plasma membrane. Various proteins including ROPs, involved in the control of cell polarity, growth and morphogenesis are localized to specific plasma membrane domains (Kost 1999; Fu 2002; Jones 2002; Gu 2005, 2006).

Since RAC/ROP GTPases can relay the signals only if localized in the proper membrane environment, their upstream regulators and downstream effectors should properly co-localize within this membrane environment. This also provides targeting mechanisms to control this signaling process. In case of depolarized pollen tube growth, the localization of RAC/ROPs is extended to a broader membrane domain where the ectopic expansion occurs. Therefore, proper expansion means that suitable targeting mechanisms are in place to control the RAC/ROP activation and further polarization of cell growth. This localized-activation also makes the regulators to exert differential control by interacting with RAC/ROP GTPases and thereby recruiting to either activated or inactivated functional site. In NtRAC5, a mutation abolishes its interaction with NtRhoGDI2 leading to mis-localized GTPase state on the pollen tube suggesting its ability to depolarize pollen tube growth (Klahre 2006).

Another study demonstrated the spatiotemporal dynamic role of Rho GTPase required for pollen tube growth and oscillation. Pollen specific ROP1 together with other related ROPs are localized to the apical region of the plasma membrane and regulate downstream pathways promoting polarized growth and oscillation in pollen tube (Ju 2005). In the case of Rho monomeric GTPases in *Arabidopsis*, specific ROPs are known to control root hair and tip growth. GFP localization studies confirmed the presence of Rop2 GTPase throughout the tip region and to the site of hair formation (Jones 2002). A different study provided convincing evidences for the dynamic localization of Rho GTPases in vacuole development and plant cell growth. The accretion and localization of ROP GTPases in the tonoplast experience dynamic changes, which results into the development of young vacuole into large central vacuole during cell division in pea tapetum (Gu *et al.* 2001).

Lipid modification is another level of spatial regulation in RAC/ROP GTPases. RAC/ROP GTPase can be divided into two major class, type I and type II based on the C-terminal structural elements. Class I GTPases has characteristic motif with CaaL pattern ('a' represents aliphatic amino acid) and they are believed to be prenylated while an additional intron in the same region makes class II RAC/ROP by enabling this to be palmitoylated instead of prenylation. Class I RAC/ROPs seem to be distributed between cytosol and membrane by presumable interaction with GDI while palmitoylation in class II RAC/ROP prevents interaction with GDI. Class II RAC/ROPs is found to be associated with plasmalemma in a palmitoylation-dependent manner. Differences in lipid modification have enabled RAC/ROPs to be differentially localized in membrane microenvironments called detergent-resistant microdomains (DRMs), and hence provide another layer of control in regulating the activity of GTPases upon requirement (Nibau *et al.* 2006).

CONCLUSION AND FUTURE PERSPECTIVES

The small GTPase constitute a large and diverse gene family, which regulates multiple physiological as well as developmental pathways in plants. Plants represent mainly four classes of small GTPases such as Rab, Rho, Arf and Ran. Ras GTPases were not detected in plants, which is possibly due to lack of Tyr kinase receptors, which are the upstream regulators of Ras signaling in animals. A lot have been known about the signaling pathways of RHOs in ani-

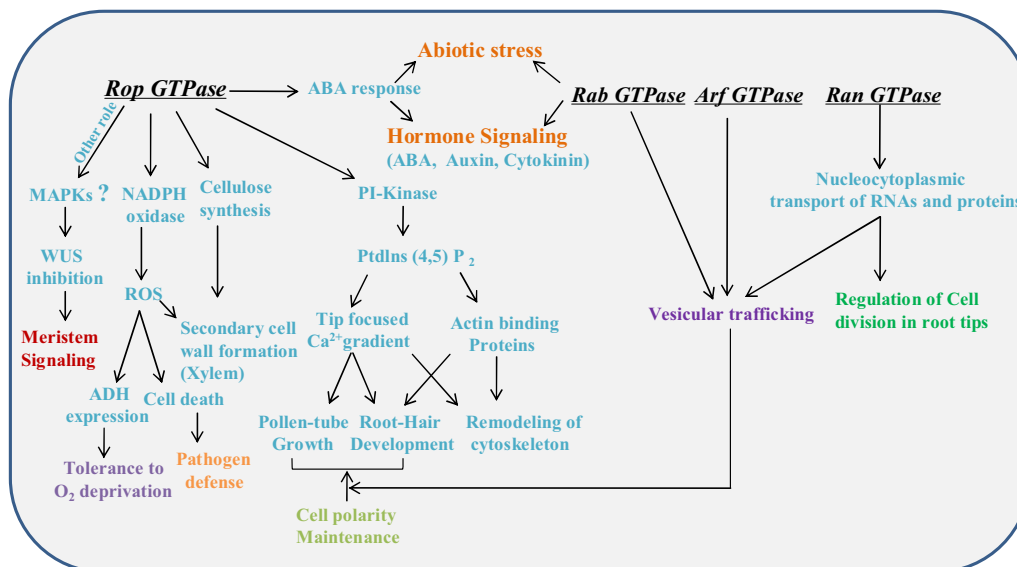


Fig. 2 Overview of small GTPase mediated regulation in plants. The known signaling mechanism of the Rop, Ran, Arf, Rab GTPase and their hypothetical activity are indicated in this model. The question mark indicates the hypothetical aspect of MAPKs in Rop signaling pathway. The crosstalk between different pathways can be seen, as in, for example, the cell polarity, the actin cytoskeleton pathways, abiotic stress and hormone signaling. In addition, Rop regulated reactive oxygen species, has been hypothesized to control three different processes including pathogen defense, secondary cell wall formation and tolerance to O₂ deprivation. Both Rop and Rab GTPase are known to be regulating several abiotic as well as hormonal responses. Ran GTPase is known to be involved in the nucleocytoplasmic transport of RNAs and proteins. ROS, reactive oxygen species; MAPKs, mitogen activated protein kinases; PI, phosphatidylinositol; PtdIns (4,5) P₂, phosphatidylinositol (4,5)-bisphosphate; WUS, WUSCHEL protein.

imals, but the mechanism of how they transduce signals to regulate developmental processes in plants is not understood well. Although, a number of instances in plants suggest, a similar functions for the plant small GTPases like in animals. Many regulators of small GTPases such as GAP and GEF families have also been identified in plants indicating the conservation of small GTPase mediated signaling pathways throughout evolution. Conversely, in plants the conserved function such as regulation of actin cytoskeleton and cellular signaling pathways possess some exclusive difference in GTPase mediated regulatory mechanism than animal. Plant RAC/ROP GTPases are set apart in different subfamilies based on their site of localization. RAC/ROPs are linked upstream to heterotrimeric G proteins and coupled receptors suggesting that they might be involved in numerous signaling pathways. Many recent studies have established that ROPs perceive multiple signals. In the **Fig. 2**, multiple roles of small GTPases in plants have been discussed to be involved in several physiological processes like hormone regulation, pollen development, and in response to both biotic and abiotic stresses etc. RAC/ROPs are strategically placed to perceive multiple signals and bring together diverse mechanisms that regulate transcription, protein synthesis and degradation. The regulation of crucial cellular pathways by RAC/ROPs put cell into an optimal state to resist continuous challenges that it experience during growth, differentiation, development and interactions with the environment (**Fig. 2**). The connection of small GTP binding protein in vesicle transport, endomembrane system, abiotic and biotic stresses should be investigated at the molecular level. Further studies are required to elucidate the detail mechanistic involvement of small GTPase and their activator (GEFs) and inhibitor proteins (GAPs) in specific physiological as well as developmental processes in plants.

ACKNOWLEDGEMENTS

The work in G.K.P' lab is supported by research and development grant from Delhi University and the University Grants Commission (UGC), India.

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