Regulation of ROP GTPase Signalling at the Gene Expression Level: A Review

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Abstract: Research of plant RHO-type (ROP) GTPases has considerably accelerated during the last few years. Now it is clear that these small proteins play central roles as signalling molecules during many basic cellular processes including cell shape determination, polar growth and responses to hormones, stress factors or pathogens. ROP GTPases have the potential to interact with a plethora of regulators and effectors that finally determines their signalling specificity. These proteins, similarly to the ROP GTPases themselves, are coded by small gene families increasing the complexity of the regulation. The comparison of gene expression patterns of the individual members of these gene families may help to reveal potential signalling chains with biological relevance. In this review previous observation on the differential expression of ROP GTPase genes in various organs and during various developmental processes is summarized. In addition, an inventory of presently known ROP GTPases-interactor proteins is provided with brief descriptions and with correlative comparison of gene expression patterns based on available microarray data.

Keywords: Expression pattern, RHO GTPase, ROP regulator, ROP effector, microarray data, protein interaction.

INTRODUCTION

Small (20-40 kD) RAS-family GTP-binding proteins are key components of various regulatory networks in eukaryotic cells [1]. These proteins, with intrinsic GTPase activity, cycle between GTP- and GDP-bound forms and their nucleotide-bound state strictly determines their cellular activities. The structurally very similar RAS-type GTPases can be divided into several subclasses (RAS, RAN, RAB, ARF, RHO) based primarily on their structure and cellular function [2]. The family of RHO-type GTPases is known to be involved especially in the regulation of the cytoskeleton, reactive oxygen generation and gene expression [3, 4].

RHO GTPases are well conserved among all eukaryotes considering their overall structure and cellular roles as well [5]. However, due to the early split of viridiplantae from the animal-fungal-amoebozoa lineage, RHO ("RHO-of-plant" or ROP) GTPases of plants occupy an unique position in the family. In addition to their somewhat divergent primary structure, the upstream as well as downstream signalling steps, associated with ROP GTPase activity, also have plant-specific features [5, 6].

Investigations of ROP GTPases have attained considerable interest during the last years. ROP function was found to be associated with key cellular events in plants including the establishment of cell polarity, polar growth and cell expansion, vesicular trafficking, cell wall synthesis, hormonal signalling, and production of reactive oxygen species in response to pathogen attack (for recent reviews see [7, 8]).

The increasing number of ROP-interacting upstream regulatory and downstream effector proteins (see Fig. **1** for the scheme of ROP-mediated signalling) delineates complex regulatory networks involved in the above processes [6]. Furthermore, the cycling of RHO family proteins between the inactive GDP- and active GTP-bound forms is not only dependent on protein-protein interactions [9] but is also coupled to intracellular translocation events [10, 11]. Therefore the post-translational regulation of the activity of ROP GTPases is of primary significance concerning their signal transduction potential.

However, as all components of ROP-mediated signalling pathways are coded by small gene families, the importance of transcriptional regulation can not be ruled out, especially in establishing the specificity of signalling components in certain developmental or biological processes [12].

Here we evaluate and correlate available gene expression data to highlight the diversity as well as the specificity of ROP GTPase signalling networks in plants.

PROTEINS INVOLVED IN ROP-MEDIATED SIGNALLING AND THEIR EXPRESSION

Differential Expression of ROP GTPases

Arabidopsis thaliana (L) Heynh. has a family of 11 ROP GTPases, all of them with a very similar overall structure (for reviews [13, 14]; for the used nomenclature see [14]). It is not surprising therefore that the regulation of specific developmental events by specific isoforms requires cell type or tissue-specific expression of the corresponding ROP genes. For example, AtROP1 is expressed only in pollen and flowers [15], whereas AtROP7 is expressed exclusively in roots, stems and hypocotyls [15] and its expression is downregulated following ethylene treatment [16]. Recently, the promoter of AtROP7 was analyzed in transgenic Arabidopsis plants and was found to limit reporter gene expression to the xylem during the late stages of xylem differentiation [17]. AtROP7 was also implicated in secondary xylem formation by an *in silico* gene expression analysis approach [18]. At-

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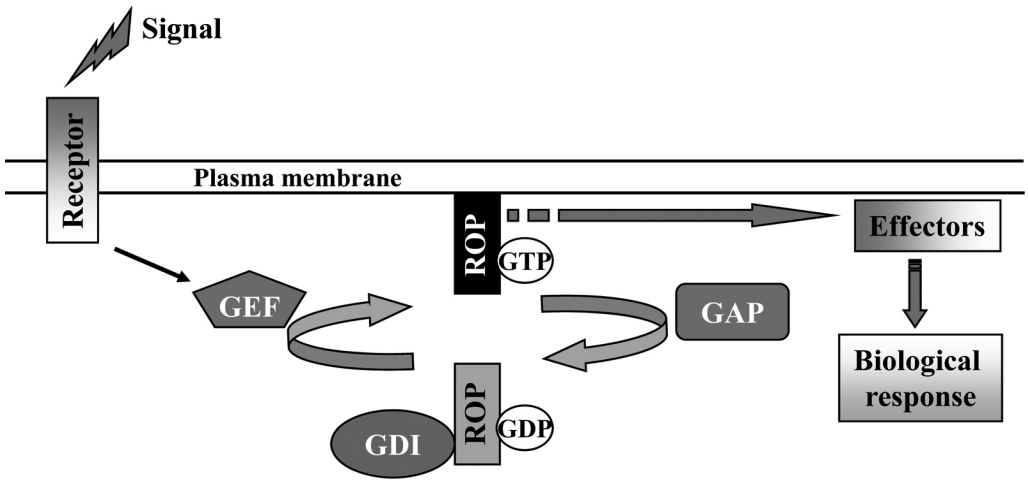

Fig. (1). Schematic drawing of the ROP GTPase cycle and related signalling steps. Various signals may activate receptors, most likely receptor-like kinases, that initiate ROP GTPase-mediated signalling through the activation of ROP GEFs. ROP GEFs facilitate GDP-to-GTP exchange and therefore activate ROPs. Active and membrane- associated ROPs mediate the signal towards various effectors (see text for details) which further transfer the signal for cellular targets evoking the biological response. GAPs and GDIs take part in the inactivation of ROPs by accelerating GTP hydrolysis and by stabilizing the ROP in GDP-bound state as well as sequestering the protein from the membrane, respectively.

ROP4 is ubiquitously expressed in all Arabidopsis organs [15, 19] while AtROP6 is absent from closed flowers and siliques but it is expressed in open flowers and in vegetative tissues [19]. Moreover, it is present at a much higher level in guard cells than in the surrounding epithelial cells [20].

There are many indications on developmental stage-specific gene expression of ROP GTPases in various other plant species in addition to Arabidopsis. In *Lotus japonicus L.*, one of the two ROP GTPases expressed in root nodules exhibited a strong increase in its transcript level during root nodule development [21]. Three *Medicago sativa* L. ROP GTPase genes are, although active throughout the plant, differentially expressed during somatic embryogenesis and in response to Rhizobial infection of roots [22]. One of the members of the cotton (*Gossypium hirsutum* L.) ROP family, GsRAC13, is specifically expressed in cotton fibres undergoing transition from primary to secondary cell wall synthesis, while another one, GhRAC1, is specifically enhanced in the elongation phase of fibre development and declines thereafter [23]. In addition, this gene is abundantly expressed in other rapidly growing cotton tissues. The *Zinnia elegans* Jacq. ZeRAC2 mRNA molecules were demonstrated to accumulate preferentially in xylem parenchyma and tracheal element precursor cells [24].

In *Brassica napus* L., the expression pattern of five ROP GTPase genes has been investigated in various organs as well as during androgenesis [25]. In addition to the pollen-specific expression of BnROP5, the contrasting expression level of BnROP5 and BnROP9 during embryogenic cell formation from microspores could be established.

In grape (*Vitis vinifera* L.), differential expression of the seven investigated ROP genes during grape development as well as in response to various growth regulators in suspension cultured cells has been established [26].

As monocots are considered, a survey of ROP-mRNA abundance in maize, based on multiplex reverse transcriptase-polymerase chain reaction and a massively parallel signature sequencing database, showed significant spatial and temporal overlap of the nine (maize has only nine ROP genes) transcripts, with high levels of all mRNA in tissues in which cells are actively dividing and expanding [27]. However, only a subset of maize ROPs is highly expressed in mature leaves and pollen [27]. Rice ROP genes have also been shown to be regulated during anther development and in various organs [28]. OsRACB gene expression has been shown to be strongly up-regulated by salt stress in roots, but not in shoots and leaves [29]. Promoter analysis verified salt inducibility and revealed that the promoter can also be activated by salicylic acid but not by abscisic acid [29].

The above examples clearly indicate that the signalling specificity of a given ROP GTPase is strongly dependent on its cell- and tissue-specific expression.

However, not only ROP GTPases but their numerous interacting protein partners can be transcriptionally regulated increasing the signalling specificity of ROP GTPase-related networks. Although information on ROP regulators and effectors continuously increases, the data on their gene expression pattern are still limited. Below we provide an up to date inventory of ROP regulators and effectors with a short functional description and the available protein-protein interaction as well as gene expression data. The detailed functional characterization of these proteins has already been described in several excellent reviews recently [5-8, 30].

The Regulators of ROP GTPase Activity

The GTPase cycle of RHO GTPases is facilitated by three types of proteins: the nucleotide exchange factors (GEFs), the GTPase activating proteins (GAPs) and the guanine nucleotide dissociation inhibitors (GDIs) (for a review see [9]). These regulatory proteins are also the products of small gene families in Arabidopsis (for reviews [5, 6], Table **1**).

Table 1. ROP regulators and their experimentally indicated interactions with ROPs (only Arabidopsis genes are given, even if the interaction of homologous proteins was detected in other species; Nt *Nicotiana tabacum*, At *Arabidopsis thaliana*)

Annotation	Name	AGI	Affy ID	Interaction	References
ROP GTPase activating protein	GAP1	At4g03100	255410_at	AtROP1	[31]
	GAP2	At5g22400	249933_at	AtROP1	[31]
	GAP3	At2g46710	266324_at	AtROP1	[31]
	GAP4	At3g11490	259287_at	AtROP2	[32]
	GAP5	At1g08340	261809_at		
	GAP6	At2g27440	265666_at		
ROP GDP-dissociation inhibitor protein	GDI1	At3g07880	258637_at	AtROP4	[33]
	GDI2a	At1g62450	265115_at	NtRAC5	[34]
	GDI2b	At1g12070	264395_at		
ROP guanyl-nucleotide exchange factor	GEF1	At4g38430	252975_s_at	AtROP1, 4	[35, 36]
	GEF2	At1g01700	261590_at	AtROP4	
	GEF3	At4g00460	255757_at		
	GEF4	At2g45890	266913_at		
	GEF5	At5g05940	250756_at		
	GEF6	At3g55660	251778_at		
	GEF7	At5g02010	251080_at		
	GEF8	At3g24620	258129_at	AtROP4	[37]
	GEF9	At4g13240	254757_at	AtROP1	[36]
	GEF10	At5g19560	245945_at		
	GEF11	At1g52240	259836_at		
	GEF12	At1g79860	260161_at		
	GEF13	At3g16130	258330_at		
	GEF14	At1g31650	246576_at	AtROP1	[36]
	SPIKE1	At4g16340	245492_at	AtROP2, 3, 4, 5, 8, 10, 11	[38, 39]

In the Arabidopsis genome three genes encoding ROP GDI-like proteins can be found [6]. The approximately 22 kDa proteins contain highly conserved amino acids in their isoprene binding pocket and exhibits 29% to 37% similarity to known mammalian homologues [40]. Most RHO GTPases are post-translationally modified by isoprenylation of the cysteine residue within a C-terminal CAAX box [11]. This lipid modification is responsible for the association of RHO GTPases with appropriate membrane regions where they exert their function [41]. RHOGDI proteins interact with and are able to remove prenylated RHO GTPases from membranes and form cytoplasmic heterodimers with them [41]. In this way, they negatively regulate the signalling activity of RHO GTPases.

Using the yeast two-hybrid system and *in vitro* assays, it could be demonstrated that AtRHOGDI1 specifically interacts with both AtROP4 and AtROP6 [40]. AtRHOGDI1 expression was found to be ubiquitous in the Arabidopsis organs [40]. Co-expression of the AtRHOGDI1 gene with the NtRAC1 gene in transfected tobacco protoplasts repressed NtRAC1-dependent auxin-responsive promoter activation, indicating that these proteins indeed function as negative regulators of ROP GTPase signalling *in planta* [42]. Altered ("more cytoplasmic") intracellular localization of the GTPase if co-expressed with the GDI gene was also observed [42].

AtRHOGDI1 was also implicated in the spatial regulation of root hair growth and was hypothesized to control the activity of the NADPH-oxidase (RHD2/AtrbohC) complex in this context [33].

Nicotiana tabacum L. Nt-RHOGDI2 has been shown to be highly and specifically expressed in elongating pollen tubes where it regulates the polar localization of the Nt-

RAC5 GTPase and pollen tube expansion [34]. Although Nt-RHOGDI1 has also been shown to interact with Nt-RAC5, it is not expressed in flower or pollen where Nt-RAC5 is expressed at the highest level, but both proteins are expressed in the shoot apex and young leaves where they may have functional interaction [43].

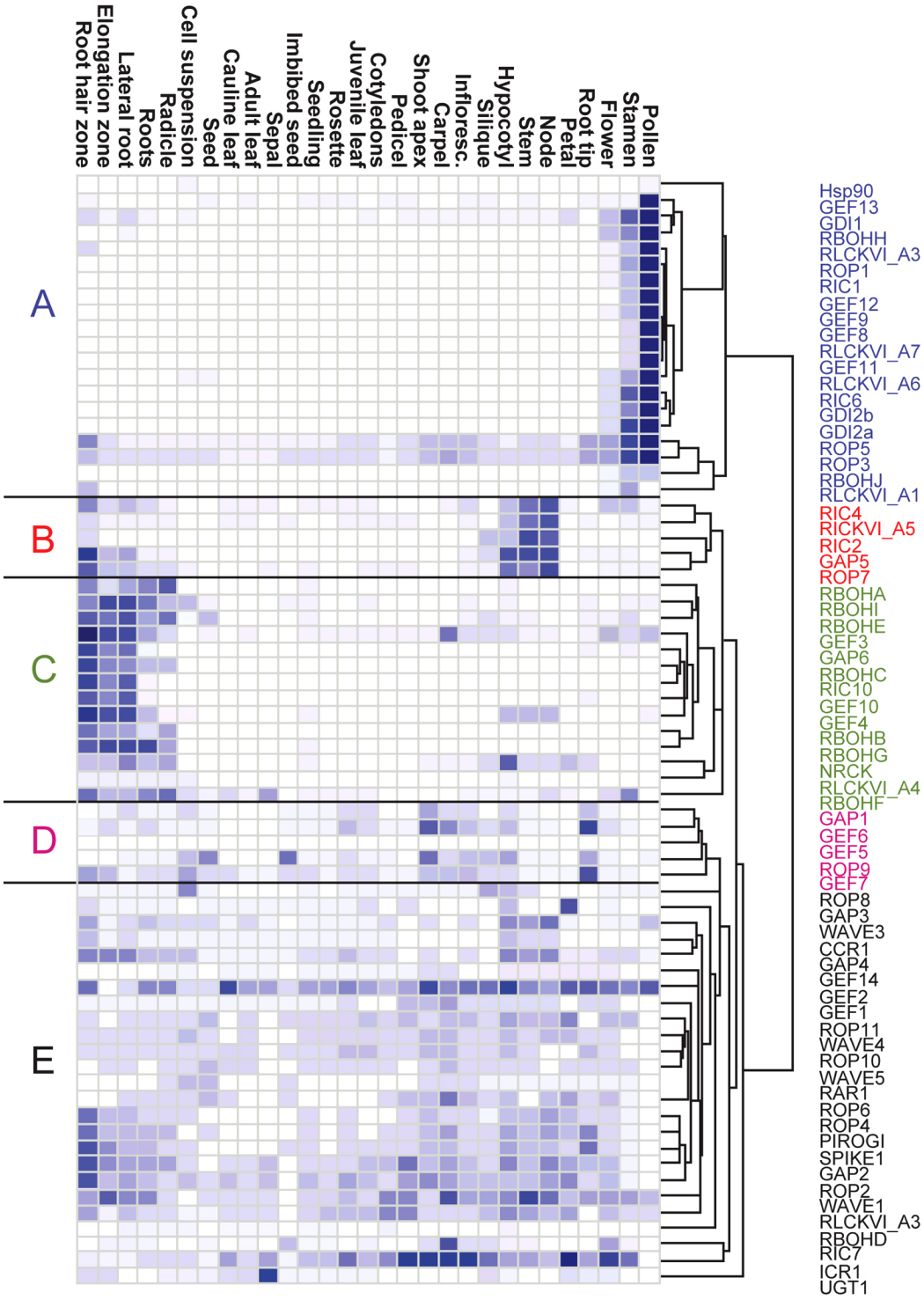

Fig. (2). Expression pattern of ROPs and their known/hypothesized regulators and effectors. Relative gene expression is shown based on microarray data stored in the Genevestigator [75, 76] database and analyzed by a hierarchical clustering algorithm of the Genvestigator tool. Darker blue color means higher relative expression. Main clusters (A-E) are separated by lines and the corresponding genes are highlighted by the same color.

The Arabidopsis genome encodes six GAP proteins. GAP proteins accelerate the hydrolysis of RHO-bound GTP and therefore promote the inactivation of RHO GTPases [44]. All Arabidopsis GAPs contain the conserved CRIB (CDC42/RAC-interactive binding) motif playing a role in binding the GTPase [31].

Point and deletion mutants in the CRIB region reduced the intrinsic GAP activity *in vitro*, documenting a critical role of this protein interaction motif in plant GAP activity [31]. In tobacco pollen tubes, Nt-RHOGAP1 has been shown to be localized subapically and hypothesized to restrict Nt-RAC5 activity to the apical region [45] in concert with Nt-RHOGDI2 [46]. A negative feedback loop including At-ROPGAP4 and AtROP2 was revealed in Arabidopsis seedlings subjected to oxygen deprivation. It was shown that ROPGAP4 transcription is increased due to the active At-ROP2-dependent production of H_2O_2 [32].

Guanine nucleotide exchange factors (GEFs) catalyze the dissociation of GDP from the inactive RHO proteins and allow the binding of GTP, which induces a conformational change that permits interaction with downstream effectors [9, 12]. Due to this activity, GEFs are primarily responsible for linking cell-surface receptors to RHO protein activation in metazoa [12]. Plants do not contain homologues of DH (Dbl-homology) and PH (pleckstrin homology) domain-containing GEF proteins characteristic for animal cells. However, recently, a specific family of plant proteins, carrying the PRONE- (plant-specific ROP nucleotide exchanger) domain exhibiting GEF-activity, has been revealed [35, 36, 47]. Five out of the 14 PRONE-containing Arabidopsis GEFs are specifically expressed or enriched in pollens [48]. These proteins have a specific conserved C-terminus implicated in the regulation of their activity [48]. Among the other ROPGEF genes there are two which are root specific while the others are expressed in vegetative organs as determined by RT-PCR analysis [49] in agreement with microarray data (see Fig. **2**).

A distinct protein with ROPGEF activity was also identified as SPIKE1 in Arabidopsis [39, 49]. This protein, homologous to the human DOCK family RHOGEFs [50], was shown to control actin-dependent morphogenesis through the heteromeric WAVE and ARP2/3 complexes [39, 49].

Effectors Downstream of ROP GTPases

Exchange of the hydrolyzed GDP for GTP results in a conformational change of GTPases, unmasking structural domains by which they can bind to their target proteins, the effectors. Many effectors of yeast and animal RHO-type GTPases are well known [51]. RHO and CDC42/RAC GTPases bind different effectors and are involved in specific but interlinked signalling cascades in animal cells. In plants, a single group of RHO-type GTPases, the ROP proteins, has to fulfil all of RHO functions, and it can even be hypothesized that certain roles of the missing mitogen-activated RAS proteins are also played by ROPs in plants [30].

In spite of the high level of structural similarity of plant ROP GTPases to mammalian CDC42/RAC proteins, there are considerable differences as their possible effectors are concerned [51]. One of the most characteristic differences is that in plants no protein kinases carrying the CRIB-motif and directly activated by ROP GTPases could be identified. In animal cells, mitogenic signals are mediated by RAS that activate the RAF-MEKK-MAPK protein kinase cascade, while CDC42 and RAC activate another specific class of protein kinases, the members of the p21-activated kinase (PAK) family, containing the CDC42/RAC interactive binding (CRIB) motif [62, 63]. While the RAS-RAF-MEKK-MAPK pathway may be specific to metazoa, PAK kinases regulated by CDC42 are present also in yeast; with MAP kinase cascades among their effectors [64].

The lack of RAS from plants as well as the significance of animal and yeast CDC42/RAC proteins in protein kinase signalling strongly support the hypothesis that ROPs are also linked to protein kinase cascades in plants. In rice, OsRAC1 was shown to be required for OsMAPK6 protein accumulation and activity in response to elicitor treatment, indicating the role of OsRAC1, and potentially other ROPs, in the activation of MAP-kinase cascades [65]. In the same study it was also proved that the constitutively active OsRAC1 protein occurs in the same protein complex together with OsMAPK6.

Recently, the direct interaction of plant ROP GTPases with receptor-like cytoplasmic kinases (RLCK class VI) has been reported in Arabidopsis [59], and could also be detected in alfalfa (Dorjgotov D and Fehér A, unpublished). This kinase family has 13 members belonging to two groups in Arabidospsis [66]. The Arabidopsis RLCK-VI genes are rather ubiquitously expressed in plant development, but some members are strongly expressed in the pollen [59, 66]. In addition, the expression of one of the ROP-interacting RLCK VI kinases was shown to be predominantly associated with vasculature and upregulated in leaves exposed to pathogens [59].

A further kinase designated as cysteine-rich receptor kinase (NCRK) belonging to a distinct kinase family has also been shown to interact with ROPs [59]. None of these plant-specific ROP-interacting kinases has any characteristic domain or motif that could be correlated with their ability to bind ROP GTPases.

AtROP5 (AtRAC2) was found to physically associate with a phosphatidylinositol monophosphate kinase (PtdIns P-K) activity [67]. This observation indicates that lipid kinases can also be potential ROP effectors in plants similarly as in animal cells [68], although the direct interaction of this type of kinases with ROPs needs to be experimentally verified.

Plants have a family of small CRIB-containing proteins called RICs (RHO-interacting CRIB-motif containing proteins) [53]. RICs are represented by eleven members in Arabidopsis and are highly divergent in sequence outside of the CRIB region. Overexpression of various RIC proteins in pollen tubes causes distinct phenotypes indicating specific functions for the individual RICs [53]. That RICs are indeed downstream targets of ROP GTPases could be the best demonstrated during the establishment of epidermal cell shape in Arabidopsis by the concerted action of AtROP2/4, AtRIC1 and 4 [52]. RIC proteins have been implicated in actin polymerization, microtubule bundling, Ca^{2+} fluxes during polar growth, cell expansion and morphogenesis [54, 69, 70]. The majority of RIC genes (RIC1,2,4,7,9) is constitutively expressed in various parts of Arabidopsis plants; only RIC3, RIC5, and RIC6 are flower specific [54]. The RIC10

Table 2. ROP effectors and their experimentally indicated interactions with ROPs. ROPs (only Arabidopsis genes are given, even if the interaction of homologous proteins was detected in other species; Os *Oryza sativa.* At *Arabidopsis thaliana*)

Annotation	Name	AGI	Affy ID	Interaction	Reference
ROP-interactive CRIB motif-containing protein	RIC1	At2g33460	255837_at	At ROP1, 2, 4	[52, 53]
	RIC2	At1g27380	264495_at		
	RIC3	At1g04450		At ROP1	[53, 54]
	RIC4	At5g16490	250120_at	At ROP1, 2, 4	[52-54]
	RIC5	At3g23380		At ROP1	[53]
	RIC6	At2g20430	257397_at		
	RIC7	At4g28560	253769_at		
	RIC8	At1g03982			
	RIC9	At1g61795			
	RIC10	At4g04900	255307_at		
	RIC11	At4g21745		At ROP11	
KLUNKER-like	PIROGI	At5g18410	250041_at	AtROP2	[55]
Cinnamoyl-CoA reductase	CCR1	At1g15950	261792_at	OsRAC1	[56]
UDP-glucosyl transferase	UGT1	At1g05560	263184_at	AtROP1	[57]
Respiratory burst oxidase	ATRBOHA	At5g07390	250629_at	OsRAC2, 5, 7	[58]
	ATRBOHB	At1g09090	264647_at	OsRAC1, 2, 5, 7	[58]
	ATRBOHC	At5g51060	248486_at	OsRAC2, 6, 7	[58]
	ATRBOHD	At5g47910	248719_at	OsRAC2, 3, 6, 7	[58]
	ATRBOHE	At1g19230	256011_at	n.t.	
	ATRBOHF	At1g64060	262344_at	n.t.	
	ATRBOHG	At4g25090	254092_at	n.t.	
	ATRBOHH	At5g60010	247647_at	n.t.	
	ATRBOHI	At4g11230	254912_at	n.t.	
	ATRBOHJ	At3g45810	252544_at	n.t.	
Wiskott-Aldrich syndrome protein-family	WAVE1/SCAR1	At2g34150	256721_at	AtROP5, 8, 11	[38]
	WAVE2/SCAR4	At1g29170		AtROP2, 5, 7, 8, 11	[38]
	WAVE3/SCAR4	At5g01730	251105_at	AtROP7, 8, 11	[38]
	WAVE4/SCAR2	At2g38440	265275_at	AtROP5, 8, 11	[38]
	WAVE5/SCAR-like	At4g18600	254621_at	AtROP8	[38]
Receptor-like cytoplasmic protein kinase	AtRLCKVI_A1	At5g57670	247872_at 247920_at		
	AtRLCKVI_A2	At2g18890	266946_at		
	AtRLCKVI_A3	At5g65530	247170_at		
	AtRLCKVI_A4	At5g10520	250443_at	AtROP4	[59]
	AtRLCKVI_A5	At5g35960	249676_at		
	AtRLCKVI_A6	At3g05140	259350_at	AtROP4	[59]
	AtRLCKVI_A7	At5g18910	249950_at		

(Table 2). Contd…..

Annotation	Name	AGI	Affy ID	Interaction	Reference
Novel cystein-rich protein kinase	NRCK	At2g28250	265545_at	AtROP4, 11	[59]
Tropomyosin-related protein	ICR1	At1g17140	262538_at	AtROP6, 10	[60]
Required for Mla12 resistance	RAR1	AT5G51700	248379_at	OsRAC1	[61]
Heat shock protein 90	Hsp90	At5g52640	248332_at	OsRAC1	[61]

transcript appeared to be more abundant in leaves and roots but was expressed weakly in flowers, inflorescences, stems, and siliques [53].

In the absence of any recognizable enzyme activity of these very small (116-224 amino acids) proteins, it is very likely that they serve as adaptors to link ROP GTPases to effectors [6]. The nature of these RIC-dependent effectors still remains to be elucidated.

In addition to RICs, there are further plant-specific downstream targets of ROP GTPases. A recently discovered interacting partner of the active AtROP1 GTPase, ICR1 (interactor of constitutive active ROPs 1; [60]), may also serve as an adaptor protein linking ROP GTPase signalling to the exocyst complex involved in exocytosis-related vesicle trafficking and membrane fusions.

The Arabidopsis UDP-glucose transferase (UGT1) directly binds AtROP1 and *via* this interaction ROP1 may indirectly regulate callose synthase activity and the formation of the new cell wall during cytokinesis as UGT1 transfers UDP-glucose to the catalytic subunit of callose synthase [57]. Another plant-specific ROP effector, cinnamoyl-CoA reductase, has also a role in cell wall formation, namely, it catalyses the NADPH-dependent synthesis of lignin monomers [57].

In rice (*Oryza sativa* L.), the OsRAC1 GTPase has been found to form complex(es) with the RAR1 (required for Mla12 resistance) protein as well as the heat-shock proteins, HSP90 and HSP70, during the pathogen-triggered innate immunity response of the plant [61].

There are only few known RHO effectors that are more-or less conserved between plant and animal cells. Two ROP-interactor proteins, PIROG1 and AtWAVE, may belong to protein complexes that are distantly related to animal WAVE complexes implicated in actin polymerization [38, 55]. Five WAVE (or SCAR) genes might be implicated in ROP signalling [55]. All AtWAVE/SCAR genes have been found to be expressed in most organs with some exceptions and with some contradictions among various analyses [38, 71].

In mammalian cells, RAC is a regulator of the NADPH oxidase complex, a specialized enzyme of phagocytic cells that generates oxygen radicals to kill internalized microorganisms [4]. A cytoplasm-derived component of the plasma membrane-bound oxidase complex, p67phox, directly binds to RAC1 and RAC2 [72]. In plants, several experimental observations indicate a similar involvement of ROP GTPases into the oxidative burst-caused cell death as a pathogen-defence function [73]. The link between ROP GTPase-dependent signalling and NADPH oxidase-dependent ROS generation has also been demonstrated during root development [33, 74]. The direct interaction of rice (*Oryza sativa* L.) ROP GTPases and the conserved N-terminal region of OsRBOHA-D proteins, the Arabidopsis homologues of the gp91phox catalytic subunit of the NADPH-oxidase complex, has only recently been proved [58], but the family of these proteins is larger compromising 10 members. All *OsRBOH* genes, with the exception of *OsRBOHD* and *OsRBOHH*, are constitutively expressed in roots, shoots, leaves and calli [58].

Correlative Expression of ROP Signalling-Related Genes

Since the above protein families have many members which are not well characterized, it is very difficult to predict functional relationships among them. One possible way to clarify the picture is to investigate and compare gene expression patterns in order to restrict the predictions to those members which may occur within the same cells/tissues/organs.

To examine the potential relationships between ROP GTPases, their regulators and effectors, a hierarchical clustering analysis of microarray expression data was performed using the Genvestigator database and analysis tools [75, 76]. Tables **1** and **2** show the proteins/genes used for the comparison highlighting also the possibility for protein-protein interaction indicated by *in vitro* and/or *in vivo* experiments. Fig. (**2**) shows that many of the investigated genes are expressed in several organs/tissues, making it difficult to predict functional interactions among them. However, several proteins of these families show a preferential expression in only a small subset of organs/tissues and the correlation of the relative expression pattern of several putative partners is evident.

On Fig. (**2**) it is shown that five main expression clusters (A-E) could be observed. These clusters include genes with preferential or more abundant expression in the pollen and flower (clusterA), stem, node and hypocotyl (clusterB), root (clusterC), shoot and root tip, flower and silique (clusterD), and all over the plant (clusterE). Cluster E contains the highest number of genes with relatively ubiquitous expression, although preferential expression in some organs/tissues can be observed for individual genes even within this family.

The most obvious is the strong, and more or less specific, expression of a subset of genes in the pollen, including genes for ROP1,3,5 (with ROP3 and 5 expressed in many other

tissues as well); GEF8,9,11,12,13; GDI1,2A,2B (with GDI1 also expressed at a relatively high level in vegetative tissues, especially in the root); RIC1,6; RLCK_A3,6,7; RBOHH,J. Pollen-specific expression ROP1,3,5 GTpases is well established [19] and the specific expression of a subclass of ROP-GEFs in the pollen has also been confirmed by RT-PCR analysis [48]. Abundance of these transcripts in the pollen is mainly related to the establishment of polarity during pollen tube growth [46, 77, 78]. The representation of the various families with several members indicates the significance of the process the success of which is ensured by parallel pathways.

The root-enhanced expression of several other members is also clear and well defined. Interestingly, many NADPH complex subunits (RBOHA,B,C,E,F,G,I) belong to this cluster. The NADPH oxidase system has been implicated in root growth as the AtRBOHC-deficient *rhd2* mutants of Arabidospsis have short root hairs and stunted roots as consequences of defective ROS production, Ca^{2+} uptake and consequently cell expansion [79]. ROS formation in the root hairs is dependent on AtROP2, and this is impaired in the *rhd2* mutant, indicating a functional relationship between AtROP2 and AtRBOHC [70]. Although ROP2 [80], ROP4 and 6 [81] GTPases have been shown to play role in root hair growth, they exhibit a more ubiquitous expression pattern (Fig. **2**). Their root specific interacting partners are not known, but PIROGI, SPIKE1, GAP2 and WAVE1 have the most similar expression pattern while RIC10, GAP6, GEF3,4 and 10 might be suggested as further candidates based on their preferential expression in the root hair zone (Fig. **2**). RLCK_VIA5, RIC4, ROP7, GAP5, RIC2, are also expressed in the root hairs but they form a specific group due to their high relative expression in stems, nodes and hypocotyls as well (Fig. **2**). WAVE3, CCR1 and GAP4 are also expressed at a relatively high level in these organs although belong to group E with a more ubiquitous expression pattern. ROP9, GEF5,6,7 and GAP1 exhibit relatively high expression in root and shoot tips in addition to other tissues. Therefore they may be important for meristem formation and maintenance.

ICR1 has also been shown to affect root meristem formation [60], although it shows stronger accumulation in the flower (petal, sepal) and the shoot apex (Fig. **2**).

As it can be seen on Tables **1**, **2** and Fig. (**2**), only few potential interactions indicated by the characteristic gene expression clusters has already been experimentally validated such as the interaction of the preferentially pollen expressed AtROP1 with RIC1,4 and GEF9 as well as NtRAC5 (ROP5) with GDI2a, in Arabidopsis and tobacco, respectively. In contrast, the functional interaction of ROPs and ROP partners with distinct expression patterns could also be demonstrated for example in epidermal cells (AtROP2,4 and AtRIC1,4 [52]) where these proteins are expressed only at a low level in comparison to other organs (Fig. **2**).

CONCLUSIONS

RHO/ROP GTPase-dependent signalling pathways are complex, overlapping, interlinked and are built up by signalling modules including ROP GTPases themselves, the regulators of their GTPase cycle and downstream effectors, all coded by gene families. Signalling specificity is exerted at various levels including posttranslational modifications affecting intracellular localization and biochemical activity as well as the regulation of gene expression. *In vitro* as well as *in vivo* protein-protein interaction analysis often insufficient to reveal the biological specificity and relevance of the interaction as one protein might potentially interact with a high number of upstream regulators as well as downstream targets. Gene expression analysis may serve as a complementary approach to indicate the biological specificity and functional potential of the predicted interactions.

Obviously, the relative gene expression patterns do not allow the unambiguous prediction of potential interactions. However, there are several studies which indicate the co-evolution of interacting proteins and members of signalling networks preserving similar expression pattern of the involved genes in order to ensure the presence of the partners in proper ratios [82-85]. Clustering based on gene expression may help to establish unique sets of signalling partners associated with cell types and/or signalling pathways. However, to have a clear picture a better resolution of gene expression/protein accumulation data would be required.

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