Minireview

Small GTPase 'Rop': molecular switch for plant defense responses

Ganesh K. Agrawal[a,b,c,*], Hitoshi Iwahashi[d], Randeep Rakwal[b,d,*]

[a] *Research Laboratory for Agricultural Biotechnology and Biochemistry (RLABB), GPO Box 8207, Kathmandu, Nepal*
[b] *Bio-Resource Research and Development Company Pvt. Ltd. (BIRD), GPO Box 8207, Kathmandu, Nepal*
[c] *Plant Functional Genomics Laboratory (PFGL), National Institute of Agrobiological Sciences (NIAS), Kannondai 2-1-2, Tsukuba, Ibaraki 305-8602, Japan*
[d] *Molecular and Microbial Ecology Research Group, Institute for Biological Resources and Functions, National Institute of Advanced Industrial Science and Technology (AIST), Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan*

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Abstract The conserved Rho family of GTPases (Rho, Rac, and Cdc42) in fungi and mammals has emerged as a key regulator of diverse cellular activities, such as cytoskeletal rearrangements, programmed cell death, stress-induced signaling, and cell growth and differentiation. In plants, a unique class of Rho-like proteins, most closely related to mammalian Rac, has only been found and termed 'Rop' (Rho-related GTPase from plant [Li et al. (1998) Plant Physiol. 118, 407–417; Yang (2002) Plant Cell 14, S375–S388]). ROPs have been implicated in regulating various plant cellular responses including defense against pathogens. It has been shown that ROPs, like mammalian Rac, trigger hydrogen peroxide production and hence the 'oxidative burst', a crucial component associated with the cell death, most likely via activation of nicotinamide adenine dinucleotide phosphate oxidase in both monocotyledonous and dicotyledonous species. Recent studies have established that ROPs also function as a molecular switch for defense signaling pathway(s) linked with disease resistance. As discerning the defense pathway remains one of the priority research areas in the field of plant biology, this review is therefore particularly focused on recent progresses that have been made towards understanding the plant defense responses mediated by ROPs.

Key words: Defense pathway; Barley; Nicotinamide adenine dinucleotide phosphate oxidase; Reactive oxygen species; Tobacco; *Oryza sativa*; *OsRac1*

1. Introduction

Rho GTPases belong to the Ras superfamily of small GTPases, and have been categorized into three related subfamilies known as Rho, Rac, and Cdc42, based on their sequence homology and cellular functions [1,2]. Members of the Rho GTPase family act as key regulators in yeast and mammalian cells of diverse processes such as organization of the actin cytoskeleton and cell polarity development, cell wall synthesis, hydrogen peroxide (H_2O_2) production, cytokinesis, cell cycle progression and differentiation [3–5]. Interestingly, some lower eukaryotes lack certain subfamilies of the Rho GTPase family. *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe* has no Rac orthologs, whereas both the Rho and Cdc42 are absent from *Dictyostelium discoideum* [5]. In plants, no obvious orthologs of the Rho family have so far been identified. Plants however do possess a unique subfamily of the Rho family, termed Rop (Rho-related GTPase from plant) that manifest a slightly higher overall similarity with mammalian Rac [6–11]. ROP is activated by an upstream signal and like mammals thought to involve at least three regulators (Fig. 1), the guanine nucleotide exchange factors (GEFs), the GTPase-activating proteins (GAPs), and the guanine nucleotide dissociation inhibitors (GDIs) [9–12]. The guanosine diphosphate (GDP)-bound inactive form of ROP is converted into its active guanosine triphosphate (GTP)-bound form by GEF that interacts with one or more downstream cellular

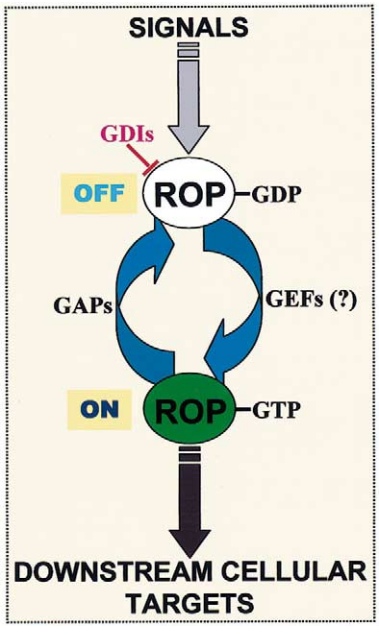

Fig. 1. Activation of ROP. ROPs relay extracellular signals to downstream cellular targets through the active GTP-bound form of ROPs. The GTPase switch is activated by GEFs, where GTP replaces GDP. GAPs are involved in deactivation of the GTPase switch by stimulating intrinsic GTPase activity. GDIs prevent the activation process by removing GDP-bound ROP GTPase.

*Corresponding author. Fax: (81)-29-861 6066.
E-mail addresses: gkagrawal@onebox.com (G.K. Agrawal), rjunko@nifty.com (R. Rakwal).

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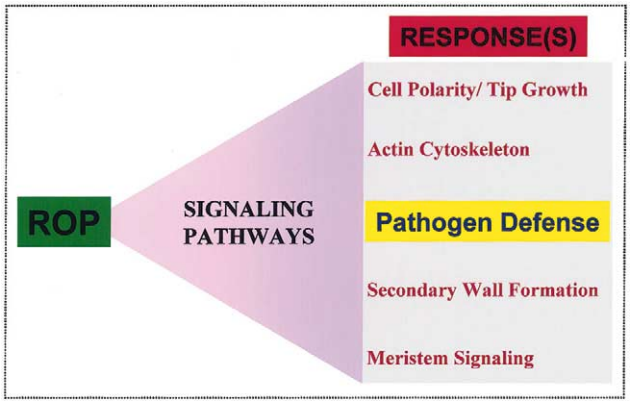

Fig. 2. ROP-mediated signaling pathways leading to diverse cellular responses.

target proteins and produces a variety of cellular responses. The active GTP form is inactivated by the hydrolysis of GTP to GDP. This hydrolysis occurs through either the intrinsic ability of the GTP form or though association with GAP. GDI negatively regulates the GDP form. The GAP and GDI homologs have been identified but GEF remains to be discovered [9–11].

ROPs are emerging as an important switch in plant signal transduction (for reviews see [9,10]). Accumulating evidence suggests that ROPs are involved in mediating multiple signaling pathways leading to a diverse array of cellular responses (summarized in Fig. 2). These responses except the pathogen defense have been described in several recent review articles [8–11,13]. Recent studies have clearly demonstrated that ROPs are involved in mediating the plant defense response pathways, including disease resistance. Rice (*Oryza sativa* L.) has been studied in detail in this regard [14–16], in addition to the work conducted in soybean [17], tobacco [18,19], maize [20] and barley [21]. Rice (*O. sativa* ssp. japonica-type cv. Nipponbare and indica-type) is an excellent model plant among the monocotyledonous (monocot) cereal crop species [22–26]. The purpose of this minireview is to summarize and discuss the current progress made towards understanding the role/function of ROPs in plant self-defense mechanisms, and their possible implication in providing improved crop resistance.

2. Alignment and phylogenetic analysis of ROPs

To date numerous ROPs have been deposited in the database, with many more expected to be identified in the near future from various plant species. As we have focused primarily on the plant defense, ROPs shown to be involved in defense/stress responses (including the oxidative burst) were selected for sequence comparison and their analysis. It should be mentioned that most of the ROP genes/proteins studied with respect to defense responses have been termed as 'Racs' in the literature [14,18,21], and for clarity we have used the same gene/protein name(s) in this review. *OsRac1*, the most well-characterized ROP gene with respect to disease resistance was taken as a reference member. The deduced amino acid sequence of OsRac1 in alignments (using BLAST X interrogation, NCBI) with homologous sequences from other monocot and dicotyledonous (dicot) species, and related mammalian Rho, Rac and Cdc42 reference family members, is shown in Fig. 3. ROPs share greater than 69% similarity with each other and at least 43% similarity with the mammalian Rho family members at the amino acid level. Looking at the sequence alignment, the presence of conserved GTPase (I and III), effector (II), and GDP/GTP-binding domains (IV and VI), Rho insert region (V), and the membrane localization signal (MLS, VII) are quite apparent, which is in line with the previously assigned domains [9]. Although all these domains are highly conserved among plant ROPs, there are distinct differences in the amino acid residues of effector domain, and the Rho insert region is highly variable with the mammalian Rho family members (Fig. 3). These unique features in the effector domains are consistent with the observation that plants apparently possess few homologs of mammalian Rho effectors [9]. Moreover, all ROPs contain two putative serine/threonine phosphorylation sites (SYR and SSK, marked by yellow arrows), which were suggested to be targets of the receptor-like kinases in an earlier study [27]. Previously, based on the C-terminal region of ROPs and the variability of the MLS, ROPs were divided into four groups [28]. Among the ROP proteins presented here, three groups are apparently based on the MLS, CXX (OsRac1, OsRac2, and ZmRop7), CXXS/A (ZmRacC, AtRac7, OsRac3, and ZmRacA), and CXXL (all other ROPs) that is similar to the mammalian Rho family members (Fig. 3). The MLS, as the name suggests, is important for localization of the ROP proteins to the various membrane systems. The cysteine residue (indicated by a red arrow) is the site where the isoprenyl moiety is covalently attached and likely to allow ROP protein to be anchored to the membranes [29–31].

In rice, the OsRac1, which carries the CXX-type MLS, was convincingly demonstrated to localize to the plasma membrane of rice protoplasts [15]. Replacement of the cysteine residue (C of CFA, Fig. 3) with a serine caused loss of membrane localization, indicating that a cysteine residue at the C-terminal motif is essential for the membrane localization. Another example comes from *Arabidopsis*, where deletion of the C-terminal motif also resulted in loss of membrane local-

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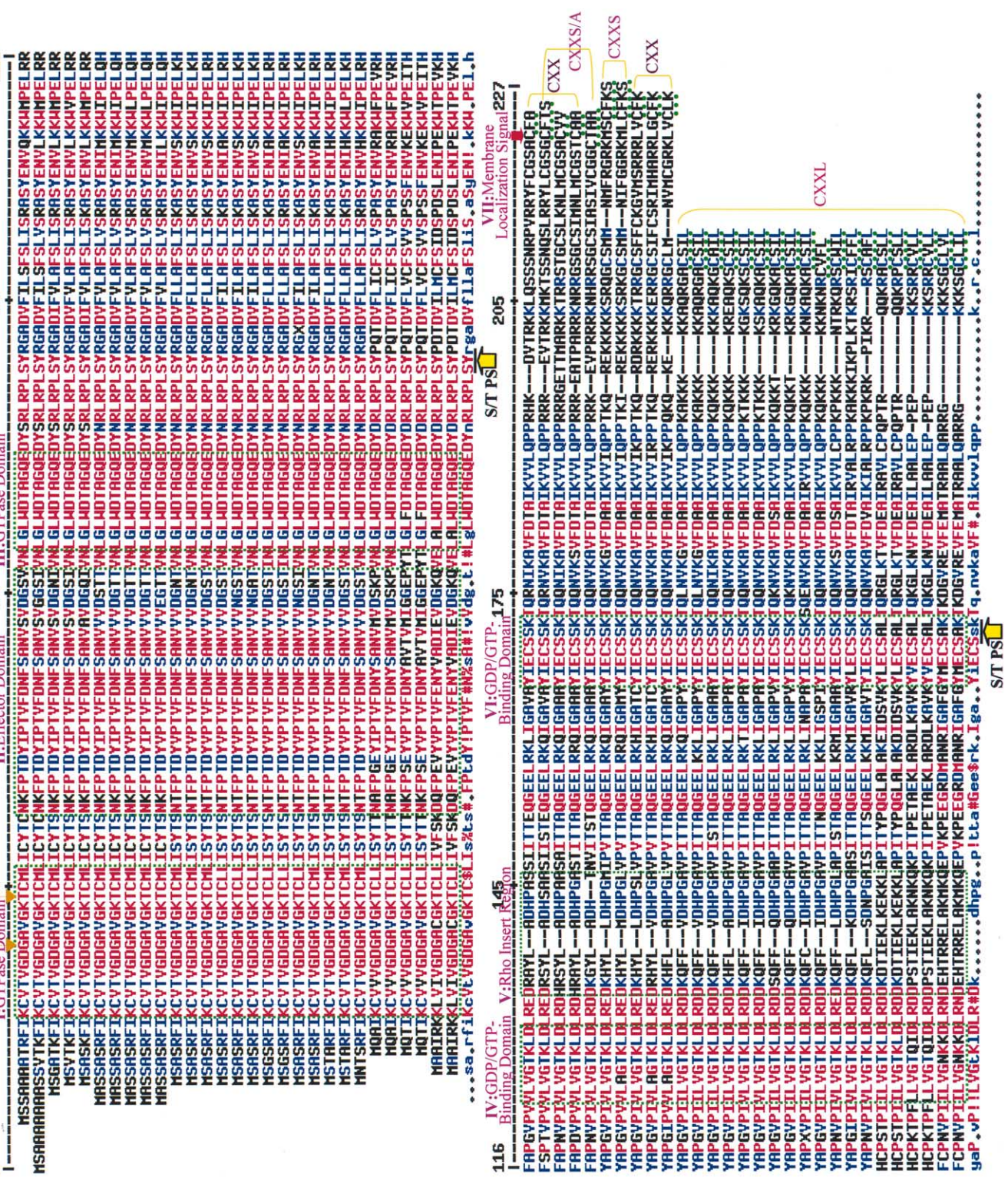
Fig. 3. Full-length ROP protein sequences from various plant species in comparison with the mammalian Rho family members. Abbreviations for species are: *O. sativa*, Os; *Zea mays*, Zm; *Arabidopsis thaliana*, At; *Nicotiana tabacum*, Nt; *Hordeum vulgare*, Hv; *Lotus japonica*, Lj; *Beta vulgaris*, Bv; *Medicago trunculata*, Mt; *Cicer arietinum*, Ca; *M. sativa*, Ms; *Gossypium hirsutum*, Gh; *Homo sapiens*, Hs; and *Mus musculata*, Mm. Accession numbers are given in parentheses besides individual proteins. Alignment and homology of amino acid (aa) sequence was done using the MultAlin 5.4.1 (INRA) and CLUSTAL W (1.81) programs at ExPASy (www server), against sequences in the GenBank and EMBL DNA database. The high-, low- and neutral-consensus aa residues are depicted in red, blue and black colors, respectively. Highly conserved aa residues appear in high-consensus color and as an uppercase letter in the consensus line. Distinct functional domains (I–VII, broken green lines) are shown. The glycine (G) and threonine (T) residues marked by arrows in the domain I were changed to valine (V) and asparagine (N), respectively to make a constitutively active and dominant negative form of the OsRac1 gene. Red arrow marks the cysteine residue in the C-terminal motif, CXXL. The putative serine/threonine phosphorylation sites (S/T PS), SYR and SSK, are marked (yellow arrows). The well-characterized monocot OsRac1, and the Rho family members are also marked, along with the distinct MLSs.


G→V T→N
I:GTPase Domain
II:Effector Domain
III:GTPase Domain
IV:GDP/GTP Binding Domain
V:Rho Insert Region
VI:GDP/GTP Binding Domain
VII:Membrane Localization Signal
S/T PS
CXX
CXXS/A
CXXS
CXX
CXXL
OsRac1(AB029508)
ZmRacC(AF126054)
OsRac2(AB029509)
ZmRop7(AJ278664)
AtRac7(AF079484)
OsRac3(AB029510)
ZmRacA(AF126052)
OsRop4(AF380335)
ZmRop4(AJ278666)
NtRac4(AJ496228)
OsRacB(AF250327)
HvRacB(AJ344223)
OsRacD(AF218381)
LjRac1(Z73961)
NtRac(AJ250174)
BvRac1(Z49131)
NtRop3(AF498357)
CaRac1(AB024996)
HsRac1(AJ251210)
AtRac5(AF115472)
OsRop5(AF376055)
AtRac2(BAB08242)
GhRac9(S57326)
HsRac2(NM002872)
MmRac2(A60134)
HsCdc42(BC018266)
MmCdc42(U37720)
HsRhoA(AF498970)
MmRhoA(AF178958)
Consensus

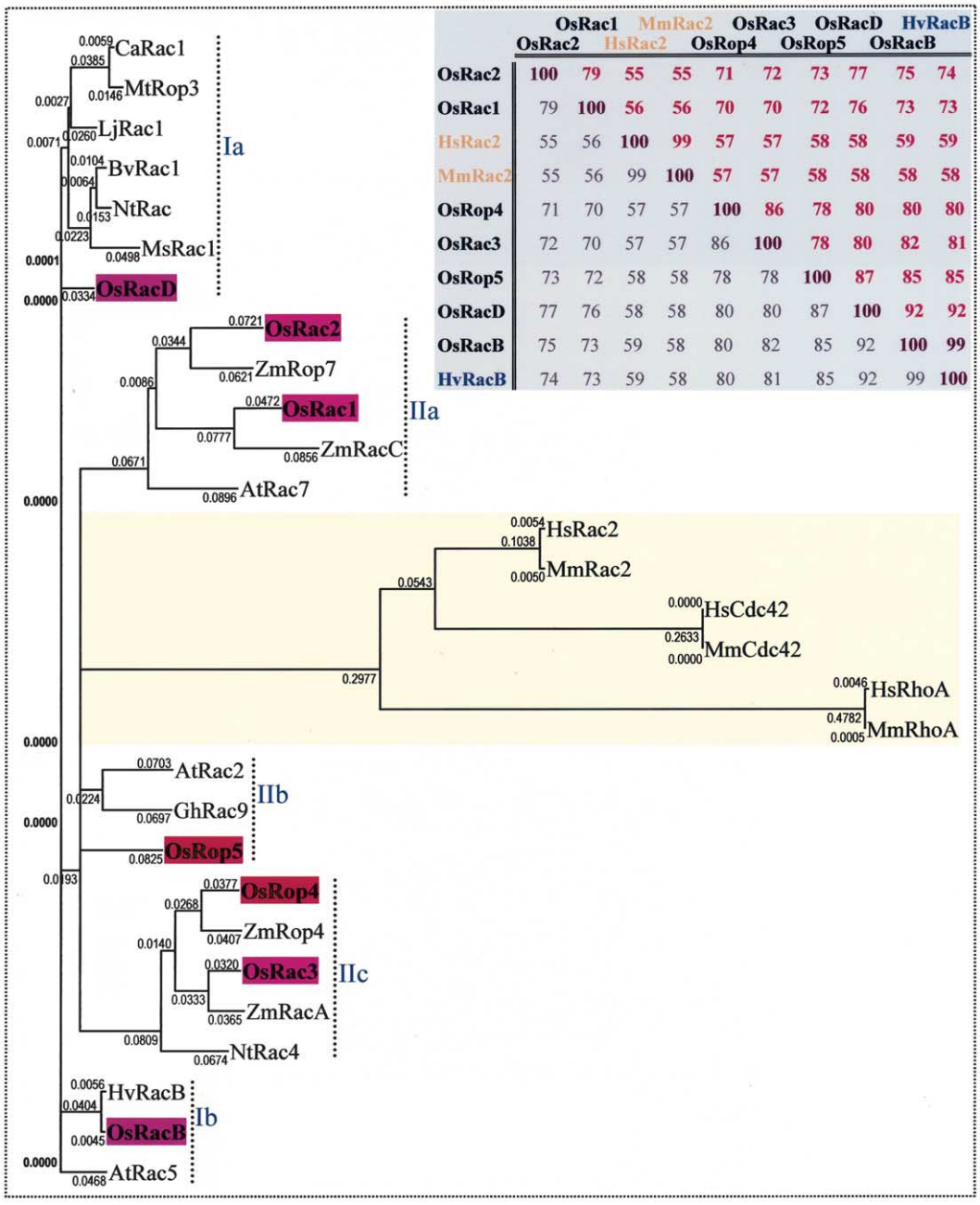


Fig. 4. Phylogenetic tree of Rop and Rho family member proteins reveals distinct groups and subgroups. The phylogenetic tree was constructed by the NJ (neighbor-joining) method using the Genetyx program (SDC Software Development, Tokyo, Japan). Bold letters indicate the rice ROP proteins (highlighted in bold and colored in shades of red), which are classified in groups I and II. The groups I and II can be subdivided into subgroups, Ia and Ib, and IIa–c, respectively (see text for details). The Rho family members are highlighted in yellow. Inset: Percentage similarity among rice ROP proteins in comparison with its mammalian Rac homologs, HsRac2 and MmRac2, and the barley HvRacB characterized for its role in defense/stress.

ization [32]. So what is the importance of this plasma membrane localization? One such significance can be attributed to the fact that ROP proteins also have a function in activating the plant nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which is most likely to be localized at the plasma membrane, thereby transducing external stimuli to downstream cellular targets, via the oxidative burst [9,10].

A phylogenetic tree constructed based on the above full-length sequences of Rop and Rho family proteins revealed that ROPs are distinct from the three subfamilies of Rho GTPases, Rac, Cdc42 and Rho (Fig. 4). Two groups have also been previously proposed for ROP proteins in *Arabidopsis*, one based on the presence of a C-terminal motif, CXXL, and another lacking this motif, but retaining a cysteine-containing motif [7]. Looking at our phylogram, two major groups of ROPs can be distinguished, groups I and II. The group I ROPs are characterized by the presence of the CXXL motif at the C-terminal, and can be further subdivided into two subgroups Ia and Ib, containing both the dicot and monocot ROP proteins. Group II ROPs, which show slightly variability in the C-terminal motif, can be subdivided into three subgroups, IIa (CXX and CXXS), IIb (CXXL), and

IIc (CXX and CXXS). Previously, AtRac2 along with GhRac9 was proposed to be a separate subgroup among the group I ROPs [7]. Our current classification shows that these two ROP proteins with a recently identified OsRop5 protein that also has the CXXL motif may comprise a separate subgroup among group II ROPs. Moreover, the known rice ROP proteins can be broadly subdivided into five subgroups (indicated by different shades of red, Fig. 4). Previously based on a phylogenetic analysis, it was proposed that *Arabidopsis* ROPs belong to as many as six distinct subgroups [6,7]. Inset of Fig. 4 shows the sequence similarity among the ROPs that have been characterized with respect to defense response, in particular the rice OsRac1 and the barley HvRacB. The percentage similarity between the mammalian homologs of OsRac1, HsRac2 and MmRac2 is also depicted for comparison.

3. Reactive oxygen species (ROS) production – conservation between mammals and plants

One of the mechanisms by which ROS are produced is through the activation of a membrane-associated NADPH oxidase [33,34]. The NADPH oxidase complex is comprised of multiple membrane-associated (cytochrome b_{558} consisting of the gp91^{phox} and p22^{phox} subunits) and cytosolic components (Rac, p67^{phox}, p47^{phox}, p40^{phox}). The multicomponent enzyme catalyzes the reduction of oxygen to generate superoxide anion ($^{\bullet}O_2^-$) that subsequently dismutes to H_2O_2 and results in the formation of other ROS. Mammalian Rac2 (HsRac2) has been known as a key regulatory component of the NADPH oxidase. The idea that most of the components of the NADPH oxidase, if not all, may have structural and functional conservation between mammals and plants has come to light from several studies. Antibodies raised against the components of the mammalian NADPH oxidase complex were shown to cross-react with proteins of the same molecular weight in several plant species [35–37]. Furthermore, functional homologs of gp91^{phox} have also been found in several plants including rice, *Arabidopsis*, tobacco and tomato, however no homologs of two other regulatory subunits p47^{phox} and p67^{phox} have yet been reported [6,19,38–40]. No report on p47^{phox} and p67^{phox} homologs in plants may reflect recruitment of novel proteins by plant NADPH oxidase. This assumption gets support from the fact that there is a lack of similarity between the ROP insert region and its mammalian counterpart Rac insert region that is known to interact with p67^{phox} [10,19]. Alternatively, the NADPH oxidase complex may be regulated in a p67^{phox}-independent manner.

Based on the above findings, it is believed that NADPH oxidase-like complex is involved in generating ROS in plants. In tomato, it has been shown that a Rac2-specific antibody detects a 21-kDa tomato protein that could be translocated to microsomal membranes in response to elicitor treatments [37]. Based on immunological studies with human Rac2 protein, the possible involvement of small G-proteins in the regulation of elicitor-induced oxidative burst was also suggested in tobacco cell suspension cultures [41]. Moreover, identification of a rice homolog (*OsRac1*) of human Rac and functional dissection using its constitutive active and dominant negative forms were found to activate and suppress, respectively, the H_2O_2 production in both the cultured rice cells and the leaf of transgenic rice plants [14]. Interestingly, the observed H_2O_2 production was inhibited by diphenylene iodonium, an inhibitor of NADPH oxidase. Similar results were seen when mutant forms of a cotton ROP (*GhRac13*) and human *HsRac1* were manipulated in the heterologous *Arabidopsis* and soybean cultured cells, respectively [17,42]. Furthermore like *HsRac1*, introduction of dominant positive versions of maize Rac (*ZmRac*) genes into mammalian cells resulted in production of superoxide and other ROS [20]. A more direct evidence for ROP involvement in H_2O_2 production comes from identification and characterization of *Arabidopsis* mutant defective in the *RopGAP4* gene, a ROP deactivator [43]. These independent findings clearly indicate a remarkable functional conservation between plant ROPs and mammalian Rac in controlling the ROS production. Hence it can be suggested that ROP regulates ROS production most likely via a NADPH oxidase, similar to that of mammals.

4. ROP involvement in plant defense responses

ROS have emerged as important components of cell signaling in addition to their roles in programmed cell death, a characteristic feature of the plant self-defense mechanisms(s) [44,45]. However, the ROS production and its direct link with the cell death is ill-defined, compared to the relatively well-described cell death pathways in mammals, often referred to as apoptosis (for review see [46,47]). The rapid production of ROS is observed in plants when they are infected by non-host or avirulent strains of pathogens, which triggers hypersensitive response (HR) in the infected area, and thereby kills pathogens in the infected cells. Identification of ROPs and their role in ROS production led to the assumption that ROPs mediate the plant defense response pathways. In an impressive piece of work in rice, the constitutive active form of *OsRac1* was shown to cause HR-like responses and resistance against a virulent race of rice blast fungus (*Magnaporthe grisea*, race 007) and bacterial blight (*Xanthomonas oryzae* pv. *oryzae*, race 1) [15]. Such constitutive expression also enhanced the production of phytoalexin, momilactone A, and altered the expression of defense/stress-related genes, D9 (terpenoid cyclase) and POX 22.3 (peroxidase). On the other hand, the dominant negative form of *OsRac1* in plants suppressed the HR induced by the avirulent race of blast fungus (*M. grisea*, race 031). In these experiments, a good correlation between the ROS production and HR was established. These results clearly suggest a general role of OsRac1 in disease resistance of rice.

Most recently, the same group attempted to link the heterotrimeric GTP-binding protein (G-proteins) with OsRac1 [16]. The G-proteins, whose complex is made up of Gα, Gβ, and Gγ subunits, is known to transduce diverse signals from plasma membrane receptors to the cell interior, and thereby controlling a wide range of cellular activities, including pathogen defense and tolerance to stress [48,49]. Considerable pharmacological and other evidence indicate that plant G-proteins are also crucial for sensing and responding to environmental and hormonal signals. A rice dwarf mutant is a best example for hormonal signals where deletion within the coding sequence of Gα protein renders the mutant unable to respond to plant growth hormones [50]. To address the role of G-protein in association with OsRac1 in disease resistance in rice, Suharsono et al. used mutants (called *d1*, dwarf 1) of the single copy *Gα* gene of rice [16]. It was found that *d1* mutants had reduced resistance to *M. grisea* infection and delayed pathogen-

esis-related (PR) gene (*PBZ1* and *PR1*) expressions. Moreover, in *d1* mutant cell cultures, a strong suppression of H_2O_2 production and delayed expression in *PBZ1* upon sphingolipid elicitor (SE) treatment were also found. Additionally, *Gα* mRNA accumulation was induced by an avirulent race of *M. grisea* in leaf and by treatment with SE in both leaf and cell cultures. The *Gα* mRNA induction upon rice blast infection was found to be specifically localized to the infected region of the leaf. Based on these results it was suggested that Gα is involved in *R* gene-mediated disease resistance in rice at least in rice blast interactions. Now, to know whether *OsRac1* restores the resistance of *d1* mutants, the constitutive active form of *OsRac1* was introduced into the *d1* mutants. Recovery of resistance to rice blast and restoration of H_2O_2 production and *PR* gene expression in the transgenic plants and cell cultures indicate that OsRac1 operates downstream of Gα. Whether *OsRac1* is required by all *R* genes for blast resistance remains to be tested, because more than 13 *R* genes corresponding to various races of the rice blast fungus are known [15].

Two other reports in barley and tobacco further demonstrate the involvement of ROP proteins in plant defense responses. In barley, an RNAi approach was taken to study the role of HvRacB in resistance to the barley powdery mildew fungus (*Blumeria graminis* f. sp. *hordei*) [21]. Transgenic plants co-suppressing *HvRacB* showed reduced fungal haustorium establishment in a cell-autonomous and genotype-specific manner, but not cell death of host cells, indicating that HvRacB negatively regulates the defense pathway. It was also suggested that HvRacB functions in a similar fashion as Mlo based on the fact that delivery of *HvRacB*-dsRNAi into epidermal cells induced resistance with a similar efficiency as *Mlo*-dsRNAi [51]. The Mlo protein is a membrane spanning protein reminiscent of a G-protein coupled receptor [52]. In animals such proteins are known to interact with G-proteins and/or small GTP-binding proteins via different cytoplasmic domains [53]. As HvRacB and Mlo are required for fungal entry in barley epidermal cells, it was speculated that these two proteins might be linked functionally [21]. In tobacco, transgenic plants expressing heterologous *Medicago sativa*, *MsRac1* gene, in sense orientation resulted in development of brown necrotic lesions and subsequently cell death, whereas in antisense orientation caused no clear formation of necrotic lesions or any other visible defense reactions upon treatment with yeast elicitor [18]. These results indicate that MsRac1 plays an important role in establishment of plant defense reactions in tobacco. In all, these studies clearly stress the growing importance of ROP proteins in mediating plant defense response pathways.

5. ROP-mediated defense response: an emerging model

Based on accumulating evidence, a model illustrating the ROP-mediated signaling pathways leading to defense responses is presented in Fig. 5. Rice is the one so far best-characterized with respect to disease resistance against *M. grisea* and SE [14–16]. Evidence on OsRac1 demonstrates that it functions as a positive regulator for defense pathway, and Gα protein is its upstream component. It is believed that pathogen/elicitor-derived signals are likely to be received by as yet unknown receptor(s), and transmitted to OsRac1 through Gα. Activation of OsRac1 triggers ROS production via a NADPH oxidase complex, altered gene expression, and phytoalexin production in order to confer appropriate resistance against *M. grisea*. Using cell cultures, it was suggested that a functional Gα along with OsRac1 are required for full accumulation of *PBZ1* [16]. This is based on the result that the *d1* cell cultures expressing the constitutively active *OsRac1* though restoring the H_2O_2 production to a level close to that of the wild-type cell culture upon SE treatment show very weak constitutive expression of the *PBZ1* gene [16]. This leads us to the supposition that OsRac1 activation by Gα or their

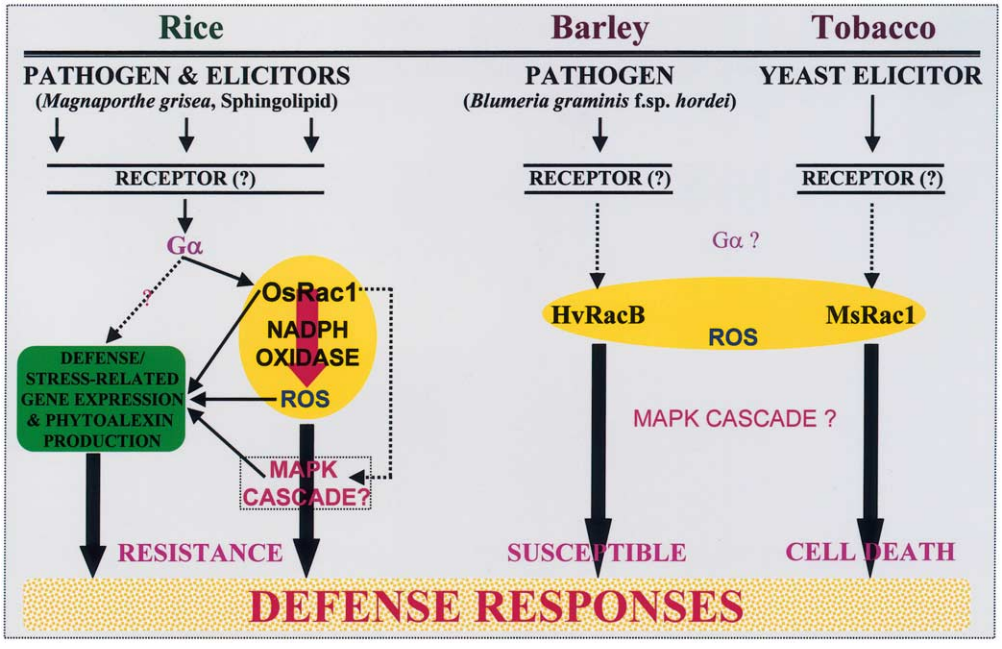


Fig. 5. ROP involvement in plant defense responses. The emerging pathways in well-characterized rice along with the relatively less-studied barley and tobacco leading to defense responses are schematically illustrated. For details see text.

interaction might be activating another defense pathway in addition to the pathway leading to H_2O_2 production that is required for full activation of the *PBZ1* gene. It is also likely that Gα might interact with other unknown components to activate a pathway(s) (shown by broken line, Fig. 5) responsible for activation of *PBZ1* or other *PR* genes that remain to be investigated. Furthermore, initial results on OsRac1 also point towards separation of the pathways committed for H_2O_2 production and activation of *PBZ1* downstream of OsRac1, though it still needs to be substantiated in both the cell cultures and plant.

We also propose that mitogen-activated protein kinase (MAPK) cascade might be one of the components of the defense pathway mediated by OsRac1 given the fact that in yeast and mammalian cells the Rho family GTPase-mediated pathways include a MAPK cascade [12,54,55]. Our proposed idea is also based on mounting evidence that MAPK cascade is an integral part of a signaling pathway including the defense pathway(s), and serves as a link in various ways between the upstream signals and the downstream effectors [56,57]. MAPK cascade is composed of at least three MAPKs (MAPKKK–MAPKK–MAPK) where they phosphorylate and activate each other and thereby control multicellular functions, including cell death, cell cycle and resistance to pathogens [56–58]. There could be at least two possible scenarios for MAPK activation by OsRac1. In one scenario, OsRac1 may form multimolecular complexes comprising a MAPK cascade with the help of scaffold protein(s), and hence can affect the activity and localization of this complex and can coordinately regulate multiple pathways without interacting physically with individual component of the complex. In another scenario, OsRac1 may interact with one of the components of the multimolecular complex to control particular response. The best example of such scenarios is the pheromone- and nitrogen starvation-induced signaling in yeast, where Cdc42p plays an essential role in controlling these pathways by assembling different multimolecular complexes [2]. In addition to this, the mammalian Rac and Cdc42 proteins are also good examples known to control a cascade leading to activation of c-Jun amino-terminal kinases (JNKs)/stress-activated MAPKs (SAPKs) [54,55]. Furthermore, as rice MAPKs induced by ROS (H_2O_2) have recently been identified [57], it is thought that ROS produced due to activation of NADPH oxidase by OsRac1 may itself activate a MAPK cascade associated with disease resistance.

On the other hand, genetic manipulation of HvRacB and MsRac1 in barley and tobacco, respectively, reveals that these ROP proteins are the components of the defense pathway. No other components up- or downstream of these ROPs have yet been reported. Nevertheless, we believe that a pathway similar to rice might be operating in barley and tobacco considering the conservation of most of the signaling pathways across plant species and higher eukaryotes [25]. However, it might be possible that the mode of regulation and the action of the component involved in the signaling pathway may differ. Therefore, it would be interesting to investigate whether Gα and MAPK operate up- and downstream of a pathway, respectively, mediated by HvRacB or MsRac1. Interestingly, the functional dissection of HvRacB unveils that HvRacB, like Mlo, plays a negative role to barley powdery mildew fungus [21,51], whereas loss of OsRac1 or Mlo function in rice causes hypersusceptibility to the fungal parasite *M. grisea* [15,59]. Phylogenetically, the HvRacB forms a separate group from OsRac1, and this may be one possible reason behind the functional difference. Functional analysis of the OsRacB gene, which falls in the same group with HvRacB, will certainly help in resolving this issue. Moreover, preliminary evidence on MsRac1 suggests a positive role to the yeast elicitor, resulting in cell death. However, its role in causing resistance/susceptibility to pathogens is not yet known. It is important to emphasize that as a transgenic approach was taken to uncover the function of ROP proteins, their specificity remains unknown, and hence a definitive conclusion must await loss-of-function mutants.

6. Conclusion and future perspectives

Recent progress that has been made towards understanding the role of ROP proteins in plant self-defense mechanisms illustrates ROP involvement in regulation of the ROS production and the defense response. The present findings also suggest that a single ROP may coordinately regulate multiple defense signaling pathways to mount an appropriate response against pathogen assault. Therefore there is a possibility that disease-resistant plants can be produced by introduction of a single molecular switch 'ROP' in the resistance signaling pathways. These initial studies are highly encouraging and provide several new challenges. An immediate challenge will be to find the loss-of-function mutants of the ROP genes. So far, numerous ROP proteins from different plants have been isolated but no ROP mutants are currently known except for the *Arabidopsis RopGAP4*. One possible reason might be the functional redundancy of ROP proteins, as several closely related ROPs are known to exist in each plant species. For example, at least 10, 11, 33 ROP proteins are known in rice, *Arabidopsis*, and *Lotus japonicus*, respectively. Another is to find components taking part in the ROP-mediated defense response pathways. Furthermore, as ROP proteins are known to modulate signaling pathways other than related to pathogen defense, functional analyses (expression analysis, manipulation of constitutively active/dominant negative forms, cellular localization, etc.) of each ROP gene will be needed to identify which gene has a role in defense response. For this purpose rice (and even *Arabidopsis*) could be an excellent system, as its genome information is now available and the mutant population induced by tissue culture and *Ac*/*Ds* system has been created for functional analysis using forward and reverse genetic approaches [60,61], considering the progress achieved in it. Finally, unraveling the role/function of ROPs in rice may help in establishing a biological model in this immensely socio-economically important crop, and that could be exploited to improve other cereal crops and plants in general, as the effect of ROP has already been demonstrated to be functionally conserved.

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