The Role of Seven-Transmembrane Domain MLO Proteins, Heterotrimeric G-Proteins, and Monomeric RAC/ROPs in Plant Defense

Justine Lorek, Ralph Panstruga, and Ralph Hückelhoven

Abstract MLO proteins are structurally reminiscent of G-protein-coupled receptors but act independently of heterotrimeric G-proteins as major susceptibility factors to powdery mildew fungi. In barley, monomeric RAC/ROPs, instead of heterotrimeric G-proteins, MLO-dependently modulate susceptibility to powdery mildew, which may involve functions in cytoskeleton remodeling. In contrast to the role of RAC/ROPs in barley susceptibility to powdery mildew, rice OsRAC1 exerts a central function in basal and effector-triggered immunity. In this context, a functional cooperation with the heterotrimeric G-protein subunit, Gα, and additional protein complexes with functions in plant immunity has been discovered. These polypeptides together modulate the oxidative burst and regulate the abundance of defense-associated messenger RNAs and defense proteins. This chapter highlights the interconnection of MLO, RAC/ROP, and heterotrimeric G-proteins in plant immunity.

1 Plant Defense Mechanisms

Plants are continuously exposed to a large range of pathogens with diverse life styles, but unlike animals, they are neither able to escape their enemies nor do they possess an adaptive immune system to protect themselves. Given that plants are resistant to the majority of ambient microbes, they obviously have evolved effective weapons to defeat their foes. Early defense responses in the battle against

J. Lorek and R. Panstruga (✉)
Department of Plant-Microbe Interactions, Max-Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, Köln 50829, Germany
e-mail: panstrug@mpiz-koeln.mpg.de

R. Hückelhoven
Technische Universität München, Lehrstuhl für Phytopathologie, Am Hochanger 2, Freising-Weihenstephan, 85350, Germany
e-mail: hueckelhoven@wzw.tum.de

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pathogens are triggered immediately after the first contact with a potential intruder. They rely on the recognition of pathogen-derived molecules, the so-called pathogen-associated molecular patterns (PAMPs), which are perceived via plasma membrane-localized pattern recognition receptors (PRRs). This type of pathogen resistance is referred to as PAMP-triggered immunity (PTI) (Chisholm et al. 2006; Jones and Dangl 2006).

PAMPs are highly conserved essential microbial molecules, including bacterial flagellin, lipopolysaccharides (LPS), and elongation factor Tu (EF-Tu), as well as chitin and β-glucan, which are cell wall components of fungi and oomycetes (Schwessinger and Zipfel 2008). A highly conserved flagellin-derived amino acid epitope, flg22, or in case of EF-Tu an 18 amino acid peptide, elf18, are sufficient to trigger PTI responses (Felix et al. 1999; Kunze et al. 2004). PRRs can be broadly grouped into two families, the receptor-like kinases (RLKs) and receptor-like proteins (RLPs), which lack a cytoplasmic kinase domain (Zipfel 2008). The best-studied plant PRRs are the *Arabidopsis thaliana* RLKs FLS2 (for bacterial flagellin or flg22) (Gómez-Gómez and Boller 2000), EFR (for bacterial EF-Tu or elf18) (Zipfel et al. 2006), CERK1 (Miya et al. 2007) and CEBiP (Kaku et al. 2006) (for fungal chitin), and the RLPs LeEix (for fungal xylanase EIX) (Ron and Avni 2004).

Following PAMP recognition, a plethora of defense responses is triggered to defeat the pathogen(s). Seconds to minutes after PAMP treatment, extracellular alkalinization, and ROS (reactive oxygen species) production occur. Intracellular signaling cascades involving Ca^{2+} fluxes and mitogen-activated protein kinases (MAPKs) lead to biosynthesis and extrusion of antimicrobial products such as PR (pathogenesis-related) proteins and low-molecular weight compounds (phytoalexins). Furthermore, the (1,3)-β-D polyglucan callose is locally deposited at the cell wall. The significance of many of these stereotypical stress responses to pathogen defense remains, however, largely elusive.

Some microbes have evolved strategies to overcome the PAMP-based defense system. Successful pathogens deliver a range of effector molecules that suppress PTI, thereby enabling host colonization (Chisholm et al. 2006; Jones and Dangl 2006; Bent and Mackey 2007; da Cunha et al. 2007). Bacteria transfer proteinaceous effectors via a dedicated delivery apparatus, the type III secretion system, which penetrates through the host cell wall and plasma membrane (Block et al. 2008). The molecular mechanisms of the delivery of fungal effectors into plant cells are still poorly understood (Ellis et al. 2006). Many fungi and oomycetes penetrate the plant cuticle and cell wall through mechanical and/or enzymatic means. Subsequently, intracellular infection structures (haustoria or infection hyphae) are formed, which are thought to serve as feeding organs for nutrient uptake as well as for effector delivery.

In response to the subversion of PTI, plants evolved a further layer of defense to recognize effectors either directly or indirectly by special immune receptors referred to as resistance (R) proteins. Typically, R proteins possess a characteristic nucleotide-binding site (NB) and a leucine-rich repeat (LRR) domain (Bent and Mackey 2007). The so-called effector-triggered immunity (ETI) conferred by R proteins is race-specific and historically known as gene-for-gene resistance

(Flor 1942). ETI shares common signaling and execution pathways with PTI, but generally the effector-based response is faster and usually results in localized programmed death of the attacked cell, which is also known as hypersensitive response (HR).

Recently, a number of defense execution components have been identified that contribute to the ability of the dicotyledonous reference plant *A thaliana* to resist penetration by the nonadapted powdery mildew fungus *Blumeria graminis* f. sp. *hordei* (*Bgh*), which is a natural pathogen of barley (Collins et al. 2003; Lipka et al. 2005; Stein et al. 2006; Kwon et al. 2008; Underwood and Somerville 2008). Two genetically separable pathways have been proposed to play a major role in pre-invasion resistance against powdery mildew fungi: one pathway comprises targeted vesicle-mediated and PEN1-dependent exocytosis. PEN1 is a syntaxin, also known as t-SNARE (*SOLUBLE N*-E-SENSITIVE FACTOR ATTACHMENT PROTEIN RECEPTOR), which participates in vesicle fusion events through formation of ternary SNARE complexes. In *A. thaliana*, PEN1, SNAP33 (SYNAPTOSOMAL-ASSOCIATED PROTEIN OF 33 kDa), and VAMP (VESICLE-ASSOCIATED MEMBRANE PROTEIN) 721/722 assemble into a ternary SNARE complex during antifungal defense. This SNARE complex is thought to mediate exocytotic delivery of toxic and/or cell wall-related cargo to the plant apoplast (Kwon et al. 2008). A second antimicrobial delivery system implicates the activity of the plasma membrane ABC transporter PEN3, which is proposed to export PEN2-generated toxic compounds contributing to penetration resistance (Stein et al. 2006). PEN2 is an unconventional myrosinase, associated with the surface of peroxisomes, presumably catalyzing the formation of toxic indole glucosinolate hydrolysis products (Lipka et al. 2005; Bednarek et al. 2009).

2 MLO: A Negative Modulator of Defense Against Powdery Mildew Fungi

Powdery mildew is a common fungal disease of many plant species. The disease has economical significance causing great yield losses in agriculture. In barley (*Hordeum vulgare*), an important crop plant, recessive mutations in the *MLO* (*MILDEW RESISTANCE LOCUS O*) gene confer durable broad-spectrum resistance to all known isolates of the barley powdery mildew fungus *B. graminis* f. sp. *hordei* (*Bgh*). For this reason, natural and induced *mlo* mutant alleles have been widely adopted in barley breeding programs (Büschges et al. 1997; Jørgensen 1992; Lyngkjaer et al. 2000). Naturally occurring broad-spectrum resistance to *Bgh* was first observed, in 1937, in Ethiopian barley landraces, which were later found to carry a mutation at the *MLO* locus (Jørgensen 1992; Piffanelli et al. 2004). For more than 60 years, *mlo*-based resistance was considered a barley-specific phenomenon. Recently, however, a requirement for MLO proteins in powdery mildew pathogenesis in the dicotyledonous plants *A. thaliana* (Consonni et al. 2006) and tomato (*Solanum lycopersicum*) was reported (Bai et al. 2008).

The *A. thaliana* genome encodes 15 proteins with extensive sequence similarity to barley MLO, which according to phylogenetic analysis can be grouped into four clades (Devoto et al. 2003; Chen et al. 2006b). Mutation of *AtMLO2* was found to confer only partial resistance to the adapted powdery mildew pathogen *Golovinomyces orontii*, since fungal invasion and subsequent conidiation in *Atmlo2* mutant plants were diminished but not completely eliminated as in case of barley *mlo* mutants (Consonni et al. 2006). *AtMLO2* belongs to a phylogenetic clade comprising two additional *MLO* genes, *AtMLO6* and *AtMLO12* (Chen et al. 2006b). Reminiscent of barley *mlo* mutants, a respective *Atmlo2 Atmlo6 Atmlo12* triple mutant was fully resistant to *G. orontii*. This finding indicates an unequal genetic redundancy among *AtMLO2*, *AtMLO6*, and *AtMLO12* regarding susceptibility against *G. orontii*, with a predominant role for *AtMLO2* in this context (Consonni et al. 2006).

Accumulating data indicate that MLO negatively affects PEN1- and PEN2/PEN3-dependent defense pathways during penetration resistance to powdery mildew fungi. In both barley and *Arabidopsis*, syntaxins (PEN1 or the barley ortholog ROR2) are required for *mlo*-based resistance, as *pen1* or *ror2* mutations in a *mlo*-resistant background restore wild-type-like entry rates of the respective powdery mildew pathogen (Freialdenhoven et al. 1996; Collins et al. 2003; Consonni et al. 2006). Moreover, also *Atmlo2 pen2* and *Atmlo2 pen3* double mutants exhibit wild-type levels of powdery mildew invasion, indicative of MLO acting as a negative modulator of the PEN2/PEN3-associated defense pathway (Consonni et al. 2006). Unlike *Atmlo2 pen1* plants, these double mutants in addition display a significant increase in powdery mildew conidiation, suggesting a role for PEN2/PEN3 in both pre- and postpenetration defenses in the context of *Atmlo2*-conditioned resistance.

Devoto et al. (1999) experimentally uncovered MLO as an integral plasma membrane-resident protein with seven transmembrane (TM) helices, an extracellularly located *N*-terminus, and a cytoplasmic *C*-terminus. The latter was subsequently found to harbor a calmodulin-binding domain (CaMBD) (Kim et al. 2002a,b). The CaMBD is conserved throughout the MLO family, suggesting that CaM binding is a general feature of MLO proteins. Mutations in the MLO-CaMBD lowered MLO-mediated susceptibility by 50%, indicating that CaM is either an activator of MLO function or a factor involved in signaling downstream of MLO (Kim et al. 2002a,b).

MLO proteins constitute the largest 7TM domain protein family in *A thaliana*. The sequence diversity, subcellular localization, and topology of MLO proteins are reminiscent of the G-protein-coupled receptor (GPCR) superfamily in metazoans (Devoto et al. 1999). In animals and fungi, GPCRs relay extracellular stimuli into intracellular signaling events by the activation of heterotrimeric G-proteins (see also chapter "Bioinformatics of Seven-Transmembrane Receptors in Plant Genomes"). To date, only sparse knowledge about potential plant GPCRs is available and although no significant sequence similarity between mammalian GPCRs and MLO proteins exists, these plant-specific 7TM domain proteins remain obvious receptor candidates for G-protein binding and signaling. Moreover, it is known that several human pathogens exploit host GPCRs for successful infection. Prominent

examples include the human immunodeficiency virus type 1 (HIV-1) and the bacterium *Stereptococcus pneumoniae*, which target GPCRs for host cell entry (Pease and Murphy 1998). Together, these facts raise the question whether MLO proteins might play a similar role during plant colonization by powdery mildew fungi. This topic as well as a putative involvement of MLO proteins in plant heterotrimeric G-protein signaling will be discussed in the present chapter. Furthermore, since the barley RAC/ROP protein HvRACB, a monomeric GTPase, operates in conferring susceptibility to *Bgh* in an MLO-dependent manner, the role of small GTPases during powdery mildew pathogenesis as well as in other defense-associated processes will be highlighted.

3 Plant Heterotrimeric G-Protein Signaling and Plant Defense

The canonical heterotrimeric G-protein signaling cascade is initiated upon cell surface perception of a ligand by the corresponding GPCR (Temple and Jones 2007). Like MLO proteins, GPCRs harbor 7TM domains and possess an extracellular amino- and intracellular carboxy-terminus. At the cytosolic face, GPCRs are associated with the G-protein, which consists of three distinct subunits, Gα, Gβ, and Gγ. The Gα subunit binds the guanine nucleotides GDP and GTP. In its GDP-bound state, the three subunits assemble to a heterotrimeric complex, which is associated to the GPCR. Extracellular binding of a cognate ligand to the receptor induces the exchange of GDP for GTP at the Gα subunit. In consequence, the heterotrimeric G-protein complex dissociates and Gα-GTP separates from the Gβγ dimer. Both, Gα-GTP and the Gβγ dimer detach from the receptor and can activate or inactivate downstream effectors. The intrinsic hydrolytic GTPase activity of Gα recovers the GDP-bound state, which promotes reassociation of the complex into its inactive form. Regulator of G-protein Signaling (RGS) proteins accelerate the GTPase activity of Gα to reinstate the inactive heterotrimeric complex (see also chapter "Plant G alpha Structure and Properties").

Based on the analysis of the complete genome sequences of the mono- and dicotyledonous reference plants rice (*Oryza sativa*) and *A thaliana*, there exist single copy genes for each of the Gα and Gβ subunits (*RGA1* and *RGB1* or *GPA1* and *AGB1*, respectively) and two genes encoding Gγ subunits (*RGG1* and *RGG2* or *AGG1* and *AGG2*, respectively). Thus, higher plants encode a much simpler repertoire of heterotrimeric G-protein components than other eukaryotes. However, plant G-protein subunits are involved in a wide range of processes including developmental events as well as responses to abiotic and biotic stresses (Perfus-Barbeoch et al. 2004; see also chapter "G proteins and plant innate immunity"). On the basis of pharmacological studies in cell cultures, a role for the heterotrimeric G-protein in plant defense has been originally proposed more than a decade ago (Legendre et al. 1992; Gelli et al. 1997; Beffa et al. 1995). Meanwhile, the involvement of the heterotrimeric G-protein complex in plant defense has been tested directly by the use of mutants, and the results of these studies are summarized and discussed in the following sections.

3.1 *Heterotrimeric G-Protein Signaling in Rice Defense Responses*

The first genetic evidence for an involvement of heterotrimeric G-proteins in defense mechanisms stems from research with the rice *dwarf1* (*d1*) mutant, lacking a functional Gα-encoding gene, *RGA1*, and its interaction with the rice blast fungus, *Magnaporthe oryzae* (previously *M. grisea*) (Suharsono et al. 2002; Lieberherr et al. 2005). Inoculation of *d1* mutants with an avirulent race of *M. oryzae* or treatment with a sphingolipid elicitor (SE) resulted in highly reduced defense responses, including diminished ROS production, lower accumulation of defense gene transcripts (*PR1* and *PBZ1*), as well as less HR-mediated cell death (Suharsono et al. 2002). Furthermore, expression of *RGA1* was induced by infection with the avirulent *M. oryzae* strain or upon treatment with SE (Suharsono et al. 2002). Likewise, in response to virulent rice blight bacteria, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), *d1* plants developed earlier and more severe disease symptoms and showed delayed accumulation of defense proteins, suggesting an involvement of the heterotrimeric G-protein also in defense responses to virulent pathogens (Komatsu et al. 2004). These results implicate the heterotrimeric G-protein α subunit as an important player in rice resistance to bacterial and fungal pathogens. However, inoculation of *d1* mutants with a virulent strain of *M. oryzae* caused disease symptoms that were indistinguishable from wild type (Suharsono et al. 2002). Furthermore, in *d1* mutant suspension-cultured cells treated with *N*-acetylchitooligosaccharide, the oligosaccharide elicitor chitin, the stimulation of defense responses such as extracellular alkalinization, ROS generation, phytoalexin accumulation, and induction of defense genes did not differ from that of wild-type cells (Tsukada et al. 2002). Taken together, these data indicate that the contribution of the heterotrimeric G-protein α subunit to defense signaling is pathogen- and elicitor-specific.

3.2 *Heterotrimeric G-Protein Signaling in Arabidopsis* Defense Responses

The involvement of heterotrimeric G-proteins in *Arabidopsis* defense responses has been documented mainly for necrotrophic pathogens. Mutants lacking a functional Gβ subunit, AGB1, showed increased susceptibility against the necrotrophic fungi *Plectosphaerella cucumerina*, *Alternaria brassicicola*, and *Fusarium oxysporum*, while Gα-deficient plants (*gpa1*) exhibited slightly enhanced resistance to these pathogens (Llorente et al. 2005; Trusov et al. 2006). The infection phenotype of double-knockout mutants lacking both subunits, Gα and Gβ, were indistinguishable from that of the single Gβ mutant (Trusov et al. 2006). This data strongly suggests that rather the Gβγ dimer, and not Gα, is the predominant factor

involved in the defense signaling pathway that is active against necrotrophic fungi in *Arabidopsis*.

A potential participation of Gγ1, but not Gγ2, along with Gβ in defense mechanisms was initially indicated by gene expression studies in *Arabidopsis* β-glucuronidase (GUS)-reporter lines infected with *A. brassicicola* and *F. oxysporum* (Trusov et al. 2007). These observations were corroborated by the infection phenotypes of the corresponding knockout mutants with these pathogens. While Gβ- and all tested Gγ1-deficient mutants showed increased susceptibility to these fungi and also exhibited reduced defense gene (*PDF1.2*) induction, plants lacking the Gγ2 subunit, AGG2, resembled the wild type (Trusov et al. 2007). Furthermore, Gβ- and Gγ1-deficient mutants showed reduced responses to methyl jasmonate, a signaling compound mainly involved in plant defense against necrotrophic pathogens, supporting the hypothesis that heterotrimeric G-proteins could play a role in jasmonate-mediated defense signaling (Trusov et al. 2006, 2007). In summary, these findings emphasize the requirement and importance of the Gβγ1 dimer for defense against necrotrophic fungi and preclude any significant role of the Gβγ2 dimer in this context. The slight increase in resistance observed for Gα-deficient mutants suggests that, with respect to plant defense, Gα acts by keeping the Gβγ1 attached to the inactive heterotrimeric complex (Llorente et al. 2005; Trusov et al. 2006, 2007). So far the *agb1 agg1* double-knockout mutant has not been tested regarding its infection phenotype to any pathogen, which would be an interesting addition to the present set of experiments.

Recent infection studies performed in our (J.L. and R.P.) laboratory implicate the Gβγ1 dimer also in defense against biotrophic powdery mildew fungi. Both *Gβ* and *Gγ1* knockout mutants exhibited slightly increased susceptibility to adapted as well as nonadapted powdery mildew fungi (*Golovinomyces orontii* and *Erysiphe pisi*, respectively; unpublished data). Surprisingly, the adapted pathogen *G. orontii* showed highly enhanced sporulation upon infection of knockout mutants lacking either the Gα, Gβ, Gγ1, or Gγ2 subunit or the RGS1-protein. This finding indicates a putative role of all heterotrimeric G-protein components in basal defense mechanisms that act following successful invasion by the fungus (unpublished data).

To investigate the involvement of heterotrimeric G-proteins in defense responses to bacteria, Trusov and colleagues challenged Gα and Gβ null mutants with compatible and incompatible strains of *Pseudomonas syringae* pv *tomato* (Trusov et al. 2006). In both cases, no difference between mutant and wild-type lines were observed, neither phenotypically nor with respect to the expression levels of the defense gene *PR1*, indicating that responses to *P. syringae* appear to be independent of heterotrimeric G-protein subunits. However, other data connect heterotrimeric G-protein signaling with bacterial PAMP perception and PTI. For instance, inhibition of stomatal opening by flg22 as part of PTI seems to implicate the Gα subunit, as Gα mutants showed impaired flg22-mediated stomatal closure (Zhang et al. 2008). Furthermore, it was recently postulated that the Gβ subunit is involved in ROS production triggered by the bacterial PAMPs flg22 and elf18. Additionally, Gβ seems to be required for elf18-mediated restriction of plant transformation via *Agrobacterium tumefaciens* (Ishikawa 2009).

In summary, a range of studies indicate a role of heterotrimeric G-proteins in plant defense in both monocotyledonous as well as dicotyledonous plants. Interestingly, rice and *Arabidopsis* Gα-deficient mutants displayed different pathogen responses. While in *A thaliana* the lack of the Gα subunit caused rather increased resistance to fungal pathogens, rice mutants exhibited reduced defense responses. Moreover, mutations in the Gα subunit induced different morphological phenotypes in both plant species, leading to dwarfism in rice, while in *A. thaliana* the mutation produced rather the opposite effect, with mutants being slightly larger than the wild type (Fujisawa et al. 1999; Ullah et al. 2003). These differences suggest that the G-protein subunits could have functionally diverged during evolution in monocots and dicots. The studies also indicate that in both plant clades the extent of heterotrimeric G-protein signaling in response to avirulent and virulent pathogens is pathogen- and/or elicitor-dependent.

4 MLO: A Putative Plant GPCR?

As outlined above, GPCRs are 7TM domain-containing proteins with an extracellularly localized *N*-terminus and a cytosolic *C*-terminus (Temple and Jones 2007). Approximately 1,000 GPCRs have been estimated to be encoded by mammalian genomes, whereas in plants only a few candidates for GPCRs have been identified. Given that even human GPCRs do not show extensive sequence conservation between subfamilies, searches on the basis of sequence homology alone could fail to detect plant GPCRs. Owing to this constraint, Moriyama et al. (2006) developed biocomputational tools by combining multiple protein classification methods, including alignment-free approaches, to identify the highly divergent GPCR candidates in plants (Kim et al. 2000; Moriyama et al. 2006; see also chapter "Bioinformatics of Seven-Transmembrane Receptors in Plant Genomes"). In a related approach, computational analysis of the entire virtual proteomes of the three model plant species, *A. thaliana*, *O. sativa*, and *Populus trichocarpa* were performed to identify plant protein sequences that most likely represent GPCRs (Gookin et al. 2008). Although there was some overlap between both studies, there were also considerable differences, emphasizing the importance of experimental verification of GPCR candidates based on functional studies.

At present, there are few proteins/protein families annotated as putative GPCRs in *Arabidopsis*. Among these candidates, only GCR1 shares extended (approximately 20%) sequence identity with known GPCRs, the cyclic AMP receptor, CAR1, found in *Dictyostelium discoideum* (slime mold), and the Class B Secretin family GPCRs (Josefsson and Rask 1997; Plakidou-Dymock et al. 1998). GCR1 physically interacts with the *Arabidopsis* Gα subunit, GPA1, but a ligand for GCR1 has not been identified (Pandey and Assmann 2004). Likewise, the *Arabidopsis* RGS1-protein is also predicted to represent a 7TM domain protein and has been shown to physically interact with the Gα subunit (Chen et al. 2006a). Lately, it has

been proposed that RGS1 acts together with the heterotrimeric G-protein complex as an extracellular glucose receptor (Jeffrey et al. 2008). Owing to seemingly erroneous biocomputational predictions, it has been proposed that the GCR2 protein functions as a GPCR for the phytohormone abscisic acid (ABA) (Liu et al. 2007), which lately has been challenged by several independent studies (Johnston et al. 2007; Gao et al. 2007; Guo et al. 2008). Recently, two novel GPCR-type G-proteins, named GTG1 and GTG2, were proposed as ABA receptors in *Arabidopsis* (Pandey et al. 2009). These newly discovered proteins combine dual functions, seemingly representing a new type of G-protein with classic GTP-binding and GTPase activity as well as operating as GPCRs that interact with the Gα subunit, GPA1, and specifically bind ABA.

The remaining GPCR candidates are represented by the plant-unique MLO proteins, which have a predicted 7TM domain topology that has been confirmed experimentally for barley MLO (Devoto et al. 1999). Loss-of-function mutations of the *MLO* gene confer resistance to pathogenic powdery mildew fungi in barley, *Arabidopsis* and tomato (Büschges et al. 1997; Consonni et al. 2006; Bai et al. 2008) (see *above*). A combined pharmacological and genetic study indicated, however, that powdery mildew susceptibility/disease resistance in barley is independent of heterotrimeric G-protein function (Kim et al. 2002b). In these experiments, the contribution of the HvGα subunit on susceptibility to the powdery mildew fungus *Bgh* was tested by transient expression of constitutive active and dominant negative HvGα variants in single barley leaf epidermal cells. None of these Gα variants did alter fungal entry rates in either susceptible wild-type *MLO* or resistant mutant *mlo* genotypes. Similarly, application of pharmacological G-protein activators did not change infection phenotypes. Taken together, these data provided first evidence that MLO proteins function independently of the heterotrimeric G-protein. Given that these results were based on transient expression of Gα variants and G-protein activators derived and known from studies in the animal but not the plant field, the findings of Kim et al. (2002b) have to be regarded with caution. Recently, our group (J.L. and R.P.), thus, chose a genetic approach using stable *Arabidopsis* knockout mutants lacking either the Gα, Gβ, Gγ1, or Gγ2 subunit, or the RGS1 protein to address the same question. These mutants exhibited susceptibility to *G. orontii* that was indistinguishable from wild-type, except for Gβ- and Gγ1-deficient mutants, which showed increased susceptibility to the powdery mildew pathogen, independently of the presence or absence of MLO. The findings suggest a role for these heterotrimeric G-protein components in antifungal defense mechanisms that are separate from MLO functions (unpublished data). Taken together, our data support the previous results from Kim et al. (2002b), indicating that susceptibility conferred by presence of MLO does not implicate heterotrimeric G-protein signaling, precluding a role of MLO as a GPCR in this context. However, since the biochemical core function of MLO proteins is still unknown, the possibility remains that members of the MLO family may turn out to operate as GPCRs in processes distinct from pathogen defense. Alternatively, MLO proteins may function as cell surface receptors via a signaling cascade that does not involve the heterotrimeric G-protein complex. It, nevertheless, remains also

possible that the function of MLO proteins is entirely unrelated to ligand binding and signal transduction.

5 Plant Rho-Like Proteins

In plants, small monomeric GTPases of the Rho-superfamily regulate the production of reactive oxygen species (ROS), Ca^{2+} fluxes, and cytoskeleton organization throughout plant development and during interactions with the environment. These processes are considered as key events in elicitor-triggered signal transduction and in the context of cell wall-associated defense mechanisms (Garcia-Brugger et al. 2006; Hückelhoven 2007). Intriguingly, in barley, MLO modulates local ROS production at the plant–pathogen interface, interacts with the cytoplasmic calcium sensor calmodulin in a Ca^{2+}-dependent manner, and affects actin cytoskeleton polarization during barley-powdery mildew interactions. These findings, thus, point to a possible link between Rho and MLO functions in powdery mildew susceptibility (Kim et al. 2002a,b; Hückelhoven and Kogel 2003; Opalski et al. 2005). The subclass of plant-specific Rho GTPases is called RAC or ROP (ROP: Rho of Plants) and constitutes a comparatively small protein family (Fu and Yang 2001). Winge et al. (2000) subdivided the 11 *Arabidopsis* RAC/ROP proteins into two major subgroups that can be distinguished by length due to an additional exon in group II. In contrast to *Arabidopsis*, grasses seem to express only six to nine RAC/ROP genes (Fu and Yang 2001; Christensen et al. 2003; Schultheiss et al. 2003, see chapter "ROP Evolution and ROPs in Grasses").

5.1 RAC/ROPs in Disease Resistance and Susceptibility

RAC/ROP proteins have been implicated in defense-related signal transduction, thus modulating the outcome of plant–pathogen interactions. Expression of a gene encoding a ROP-binding kinase that interacts with ROPs in vivo is locally activated when adapted or nonadapted pathogens attack *Arabidopsis* (Molendijk et al. 2008). It was also shown that a soybean RAC-like GTPase integrates into the microsomal membrane fraction following elicitation of the oxidative burst, suggesting that membrane localization of this RAC/ROP requires a biotic stress stimulus. Heterologous expression of constitutively activated GTP-bound (CA) or dominant negative (DN, GDP-bound or nucleotide-free) mutants of human HsRAC1 in soybean cells boosted or reduced, respectively, the oxidative burst in response to different elicitor preparations (Park et al. 2000). Vice versa, CA maize RAC proteins provoked ROS production in mammalian NIH 3 T3 cells (Hassanain et al. 2000). Additionally, DN OsRAC1 and antisense-mediated gene silencing of tobacco *NtRAC1* were able to suppress HR-mediated cell death in response to tobacco mosaic virus infection or to treatment with elicitor preparations in tobacco (Schiene et al. 2000; Moeder et al.

2005). However, a more detailed insight into the role of RAC/ROPs in interactions with microbes is only available for rice OsRAC1 and some barley HvRAC/ROPs as outlined below in detail.

5.1.1 RAC1 in Rice Disease Resistance

In the context of disease resistance, rice RAC1 is the best-characterized RAC/ROP protein (Table 1). Kawasaki et al. (1999) have shown that pathogen-triggered cell death in the *sl* lesion mimic mutant of rice could be modulated by the expression of CA or DN OsRAC1 in opposite directions: CA OsRAC1 supported cell death whereas DN OsRAC1 reduced cell death. CA OsRAC1 provoked the generation of ROS via a flavin-dependent oxidase, which was suggested to be a respiratory burst oxidase homolog (RBOH, see *below*). Subsequently, it was shown that expression of CA OsRAC1 was sufficient to confer resistance to virulent *M. oryzae*. Fungal invasion into transgenic CA OsRAC1-expressing rice plants was stopped coincident with the execution of an HR, which included the local generation of ROS. In contrast, DN OsRAC1 strongly suppressed race-specific resistance to avirulent *M. oryzae* but did not limit basal resistance to a virulent race (Ono et al. 2001). OsRAC1, thus, appeared to be a regulator of race-specific resistance to *M. oryzae*. Besides this, CA OsRAC1 supported basal resistance to virulent *X oryzae* pv. *oryzae* (Table 1). CA OsRAC1 could further complement loss of basal resistance to *M. oryza*e and *Xoo* in *OsRAR1* (*REQUIRED FOR MLA12-MEDIATED RESISTANCE*)-silenced rice RNA interference (RNAi) plants. Since the RAR1 zinc finger protein is considered to function as a cochaperone in race-specific immune complexes, the data support a function of OsRAC1 in ETI.

The biological effects of OsRAC1 in rice disease resistance described above are reminiscent of functions of Gα. Accordingly, expression of CA OsRAC1 in the rice *d1* mutant restored resistance to avirulent *M. oryzae*, execution of HR, defense gene expression, and ROS formation. This places OsRAC1 parallel to or downstream of Gα in resistance to *M. oryzae* (see also"Introduction" of the Chapter "Structure and function of ROPs and their GEFs"; Table 1) (Suharsono et al. 2002). Coimmunoprecipitation experiments showed association of OsRac1 with OsMAPK6, a mitogen-activated protein kinase activated during responses to pathogens or pathogen-derived elicitors (Lieberherr et al. 2005). In either *d1* or *OsRac1*-silenced cell lines treated with sphingolipid elicitor, OsMAPK6 protein levels and activity were reduced but mRNA accumulation was unaltered, suggesting posttranslational regulation of OsMPKA6 accumulation levels by Gα and OsRac1 (Table 1) (Lieberherr et al. 2005). Together, these results support a defense signaling cascade from the heterotrimeric G-protein α subunit via the small GTPase OsRac1 to OsMAPK6.

Consistent with a more general function of OsRAC1 in modulating defense-associated protein abundance, expression of CA OsRAC1 induced changes in the proteome of cultured rice cells that were similar to those induced by the sphingolipid elicitor. Among the upregulated proteins were many defense-related proteins, chaperones, proteases and protease inhibitors, phenylpropanoid biosynthesis

Table 1 Rice RAC/ROPs and interactors that operate in disease resistance

RAC/ROP Protein	RAC/ROP interactors[a] and function		Reference
	RAC/ROP interactor	Function	
OsRAC1		Signaling in cell death, reactive oxygen production, and race-specific resistance to *Magnaporthe oryzae*	Kawasaki et al. (1999); Ono et al. (2001)
		Signaling in basal resistance to *Xanthomonas oryzae* pv *oryzae*	Ono et al. (2001)
		Inhibits expression of the antioxidant metallothionein MT2b	Wong et al. (2004)
		Modulates host defense proteome similar to a sphingolipid elicitor	Fujiwara et al. (2006)
	Heterotrimeric G-protein Gα subunit	Acts upstream of RAC1 in race-specific resistance and elicitor signaling	Suharsono et al. (2002)
	MPK6	Is regulated by Gα and RAC1, complexes with active RAC1 and is involved in elicitor-activated gene expression	Lieberherr et al. (2005)
	RBOH-type NADPH oxidase	Is activated by active RAC1 via protein–protein interaction in planta and produces reactive oxygen species	Wong et al. (2004)
	Cinnamoyl-CoA reductase 1	Interacts with activated RAC1 and functions in biosynthesis of lignin	Kawasaki et al. (2006)
	RAR1	Is found in an immune complex with HSP90 and RAC1 and functions in PAMP-triggered immunity of rice. Additionally, OsRAC1 regulates RAR1 expression	Thao et al. (2007)
	HSP90	Is found in an immune complex with RAR1 and RAC1 and stabilizes the complex	Thao et al. (2007)
	RACK1	Complexes active RAC1, RAR1, and SGT1 and supports resistance to *M. oryzae* and reactive oxygen production	Nakashima et al. (2008)

[a]An interactor either interacts functionally or physically where indicated

enzymes, polyamine and ethylene-related proteins, redox proteins, and enzymes of the alcoholic fermentation pathway (Fujiwara et al. 2006). This strongly supports the view that OsRAC1 is a central node for the regulation of protein abundance in several pathways that are crucial for pathogen defense (Table 1).

However, what may actually stop invading pathogens from growth in CA OsRAC1-expressing rice plants? A truncated variant of the monolignol biosynthesis pathway enzyme cinnamoyl-CoA reductase was identified in a yeast two-hybrid assay to interact with CA OsRAC1 but not with DN OsRAC1. GTP-bound OsRAC1 interacted with cinnamoyl-CoA reductase in vitro and stimulated its enzymatic activity. Expression of CA OsRAC1 also elevated lignin contents in transgenic rice cell cultures and enhanced the activity of cinnamoyl-CoA reductase (Kawasaki et al. 2006). Together with the fact that CA OsRAC1 promotes ROS production, the data suggest that OsRAC1 orchestrates lignification of the plant cell wall (Table 1), which may be crucial for arresting invasive growth of *M. oryzae* in resistant plants (Schaffrath et al. 1995).

The role of OsRAC1 and related RAC/ROPs in ROS production was recently elucidated in more detail (Wong et al. 2004). OsRAC1 was found to interact with the *N*-terminal cytoplasmic extension of the plasma membrane-localized RBOH NADPH oxidase, which carries two potential calcium-binding EF-hand motifs. This interaction was demonstrated by in vitro pull-down experiments, yeast two-hybrid studies, and in vivo fluorescence resonance energy transfer (FRET) experiments. Depending on the presence of intact EF-hands, FRET efficiency dropped under high calcium concentrations, which indicates a role of calcium in controlling the OsRAC1-RBOH interaction. Transient coexpression of CA OsRAC1 and OsRBOHB in leaves of *Nicotiana benthamiana* resulted in enhanced ROS production when compared with the expression of each single protein, suggesting that both proteins synergistically contribute to ROS production. In contrast to OsRAC1, tobacco NtRAC5 attenuated an elicitor-activated burst and negatively regulated abundance of NtRBOHD (Morel et al. 2004). In humans, HsRAC is crucial for the activation of at least three types of RBOH-like NADPH oxidases that partially contribute to innate immunity and programmed cell death (Bedard and Krause 2007). Hence, NADPH oxidase activation by Rho-like GTPases is a conserved phenomenon in mammals and plants, although the structural basis for protein complex formation may differ in the two kingdoms (Table 1) (Kao et al. 2008).

OsRAC1 also directly interacts with OsRAR1 and the heat shock protein HSP90. Both are important components of *R* gene-mediated disease resistance (ETI). Interaction in vivo was supported by coimmunoprecipitation of OsRAC1 with RAR1, HSP90, and HSP70. CA OsRAC1-mediated boosting of elicitor responses was dependent on RAR1 and HSP90. OsRAC1 also regulates RAR1 expression at both the mRNA and the protein level (Table 1) (Thao et al. 2007). Most recently, RACK1 (RECEPTOR FOR ACTIVATED C-KINASE 1) was isolated via affinity chromatography using glutathione-*S* transferase (GST) epitope-tagged CA OsRAC1. RACK1 appears to form a protein complex by linking RBOH and OsRAC1 to RAR1 and SGT1 and, when overexpressed, it was sufficient to enhance resistance to virulent *M. oryzae* (Nakashima et al. 2008). The authors, thus,

suggested that RACK1 acts as a scaffolding protein in rice immune protein complexes (Table 1). In summary, OsRAC1 appears to be a key player in the rice PTI and ETI in the context of different plant–pathogen interactions.

5.1.2 RAC/ROPs in Barley Disease Resistance and Susceptibility

In contrast to the role of OsRAC1 in disease resistance of rice, the barley RAC/ROP protein HvRACB operates in susceptibility to the biotrophic barley powdery mildew fungus *B. graminis* f. sp. *hordei* (*Bgh*) (Table 2) (Schultheiss et al. 2002, 2003). Knockdown of HvRACB by RNAi in single epidermal cells, transformed via microprojectile-mediated gene delivery, rendered cells more resistant to fungal penetration. RNAi-mediated penetration resistance was not efficient in *ror1*-mutants, which are impaired in basal and nonspecific *mlo*-mediated resistance (Table 2). In contrast, expression of CA HvRACB supported fungal penetration success, whereas nonactivated wild-type HvRACB or closely related CA HvRACD had no effect. However, CA HvRACB did not break the highly effective *mlo*-mediated resistance. Together, this suggests that HvRACB modulates basal

Table 2 Barley RAC/ROPs and interactors that operate in disease resistance or susceptibility

RAC/ROP protein	RAC/ROP interactors[a] and function		Reference
	RAC/ROP interactor	Function	
HvRACB		Is required for full susceptibility to *Bgh*	Schultheiss et al. (2002, 2003)
		Supports entry by *Bgh*	Schultheiss et al. (2003, 2005)
		Functions in cell polarity and organization of actin microfilaments	Opalski et al. (2005)
	MLO	Is required for RACB function in susceptibility	Schultheiss et al. (2003)
	ROR1	Is required for RACB function in susceptibility and for resistance mediated by RACB RNAi	Schultheiss et al. (2002, 2003)
	RIC171	Interacts with RACB in planta and supports entry by *B. graminis*	Schultheiss et al. (2008)
HvRAC3		Supports entry by *B. graminis*	Schultheiss et al. (2003); Pathuri et al. (2008)
		Functions in cell polarity	Pathuri et al. (2008), Pathuri et al. unpublished
		CA Overexpression enhances cell size and susceptibility to *P.s.tabaci* in tobacco	Pathuri et al. unpublished
HvRAC1		Supports entry by *B. graminis*	Pathuri et al. (2008)
		Functions in cell polarity	Pathuri et al. (2008)
		Supports callose deposition and H_2O_2 production	Pathuri et al. (2008)
		Supports basal resistance to *M. oryzae*	Pathuri et al. (2008)

[a]An interactor either interacts functionally or physically where indicated

susceptibility of barley to *Bgh* in an MLO- and ROR1-dependent manner (Table 2) (Schultheiss et al. 2002, 2003).

The role of RAC/ROPs in dicot–microbe interactions is not yet understood. However, the ectopic expression of barley CA HvRACB or CA HvRAC3 in tobacco enhanced susceptibility to powdery mildew, and tobacco plants expressing CA HvRAC3 showed additional super-susceptibility to the bacterial pathogen *P syringae* pv. *tabaci* (Indira Pathuri and R.H. unpublished). Furthermore, an Arabidopsis *Rho-GTPase ACTIVATING PROTEIN* (*GAP*) T-DNA insertion allowed for accelerated fungal development and enhanced sporulation of powdery mildew (Christina Huesmann and R.H. unpublished). Together, this suggests an involvement of RAC/ROPs also in dicot susceptibility to various phytopathogens.

Since RAC/ROPs are key regulators of the cytoskeleton (see also chapter "ROP GTPases and the Cytoskeleton"), the role of HvRACB in filamentous F-actin organization under attack from *Bgh* was analyzed (Table 2) (Opalski et al. 2005). Knockdown of *HvRACB* led to more polarization of F-actin to the site of attempted penetration, which was correlated with enhanced resistance. In contrast, expression of CA HvRACB induced actin filament depolarization, supporting susceptibility. Together with the observation that virulent *Bgh* seemed to inhibit polarization of attacked cells in an MLO-dependent manner, this suggests that *Bgh* might target HvRACB to suppress polar plant defense, or to support haustorial establishment. CA HvRACB also partially inhibited polarization of *mlo* barley cells, however, without inducing susceptibility. Hence, HvRACB requires functional MLO in susceptibility, but can affect F-actin organization independently from MLO (Opalski et al. 2005).

Transgenic barley plants stably expressing CA HvRACB displayed enhanced susceptibility to powdery mildew. Additionally, CA HvRACB-expressing plants showed pleiotropic effects in root and shoot development as well as in water retention capacity, when cut off from water supply or when treated with abscisic acid. This suggests that HvRACB might have a physiological role in plant development and in biotic as well as abiotic stress responses (Schultheiss et al. 2005). In transient expression experiments, it was shown that other barley RAC/ROPs might fulfill HvRACB-redundant functions in susceptibility to *Bgh*. When stably expressed in barley, CA HvRAC1 and CA HvRAC3 exhibited similar effects on plant development as expression of CA HvRACB (Table 2). In particular, all three CA HvRAC/POPs abolished polarity in tip-growing root hairs (Pathuri et al. 2008). Additionally, transgenic barley plants expressing CA HvRACB or CA HvRAC1 showed significantly longer epidermal cells and aberrant development of stomata (Pathuri et al. 2008, 2009). Together, the data suggest that similar to what is known from *Arabidopsis* (Yalovsky et al. 2008), monocot RAC/ROPs have conserved functions in cell expansion and polarized tip growth. This supports the idea that virulent *Bgh* corrupts a plant tip growth program (see also chapter RAC/ROP GTPases in the "Regulation of Polarity and Polar Cell Growth") to establish a rapidly growing haustorium surrounded by a host-derived extrahaustorial membrane in intact epidermal cells of barley (Schultheiss et al. 2003; Opalski et al. 2005). This assumption was further corroborated by the observation of host-derived

actin rings, which can also be observed below the apical dome of tip-growing plant cells (Yalovsky et al. 2008), around the tip of emerging haustoria (Opalski et al. 2005).

Interestingly, similar to transgenic CA HvRACB barley lines, CA HvRAC1-expressing barley plants were super-susceptible to *Bgh*. This could be explained by enhanced success of fungal penetration. However, CA HvRAC1 barley plants displayed significantly more cells with whole cell hydrogen peroxide (H_2O_2) accumulation as visualized by 3,3′-diaminobenzidine (DAB) staining. This phenomenon was restricted to cells where *Bgh* failed to penetrate and can, thus, be considered as part of a secondary defense reaction. The same plants also reacted more frequently with localized callose deposition to attack by *Bgh* in cells that did not support resistance to fungal penetration (Table 2) (Pathuri et al. 2008). Hence, although barley RAC/ROPs function in conferring susceptibility, they might have an additional role in positively modulating cellular defense reactions, which is similar to the situation of OsRAC1 in rice. In accordance with this, CA HvRAC1-expressing barley plants showed enhanced basal resistance to *M. oryzae*, which could be explained by enhanced resistance to fungal penetration in the first attacked epidermal cell (Pathuri et al. 2008). Hence, both OsRAC1 and its closest relative in barley, HvRAC1, can support resistance to *M. oryzae* (Tables 1 and 2). However, CA OsRAC1 mediates fungus-induced HR whereas the CA HvRAC1 supports penetration resistance of living cells, which form localized cell wall appositions. This situation in barley is reminiscent of the role of MLO, which is required for penetration by *Bgh* but limits the penetration success of *M. oryzae* (Jarosch et al. 1999). These findings additionally support the above-mentioned functional link or partial redundancy of MLO and RAC/ROPs in barley. However, direct evidence for a cooperative function of MLO and RAC/ROPs is currently missing.

A possible link between RAC/ROPs and MLO might be the actin cytoskeleton (Opalski et al. 2005; Miklis et al. 2007). RAC/ROPs are well known as regulators of actin nucleation and dynamics. For instance, downstream of AtROP2, the Arabidopsis RIC proteins (RAC/ROP Interactive Cdc42/Rac Interactive Binding (CRIB)-Motif Containing Proteins), AtRIC1 and AtRIC4, regulate the establishment of spatial arrays of F-actin and microtubules during lobe and neck formation of interlocked epidermal pavement cells (Fu et al. 2005). A role of RAC/ROPs in actin nucleation is supported because RAC/ROPs interact with components of the actin-polymerizing WAVE complex, which is involved in epidermis development. Yeast two-hybrid experiments showed that AtROP2 interacts with PIR121/SRA1 subunits of this complex, suggesting that WAVE activity in plants may be regulated by RAC/ROPs (Basu et al. 2004). Recently, AtROP2 activation by the DOCK family protein SPIKE1, which has RAC/ROP-stimulating guanidine nucleotide exchange factor activity, has been evidenced. Hence, SPIKE1–ROP2–SRA1 signaling appears to operate during establishment of actin nucleation complexes (Basu et al. 2008). *Arabidopsis* AtICR1 (INTERACTOR OF CONSTITUTIVE ACTIVE ROPs 1) has been found to interact with both active RAC/ROPs and SEC3, which is associated with Rho in the exocyst complex in mammals (Lavy et al. 2007; Berken and Wittinghofer 2008). It also has been suggested that tobacco NtRAC1 controls

the activity of ACTIN DEPOLYMERIZING FACTOR NtADF1 during pollen tube growth. In analogy to mammalian systems, this might be facilitated via a RAC/ROP-activated kinase that phosphorylates NtADF1, leading to protein inactivation and subsequent actin polymerization. Because RAC/ROP activity is spatially and temporarily fine-tuned during pollen tube growth, this may contribute to the dynamics of F-actin throughout this morphogenetic process (Chen et al. 2003). Since *mlo*-resistance is partially compromised by overexpression of HvADF3, and because barley RAC/ROPs presumably inhibit the activity of HvADF3, it has been suggested that functional MLO in concert with RAC/ROPs inhibits F-actin reorganization for polar defense reactions or orchestrates actin dynamics during fungal entry (Opalski et al. 2005; Miklis et al. 2007).

In a targeted yeast two-hybrid assay, HvRACB was shown to interact with a 171 amino acid CRIB-motif-containing protein of barley designated HvRIC171. Interaction of HvRACB and HvRIC171 proteins was supported by bimolecular fluorescence complementation (BiFC), which indicated that HvRIC171 interacts with CA HvRACB but not with DN HvRACB in planta, and thus is likely involved in downstream effects of HvRACB-GTP (Schultheiss et al. 2008). Accordingly, similar to CA HvRACB, overexpression of HvRIC171 supported susceptibility to *Bgh*. In contrast, a presumably nonfunctional CRIB-containing HvRIC171-fragment of 46 amino acids bound CA HvRACB in planta but had a dominant negative effect on fungal penetration success when transiently expressed in barley epidermal cells (Table 2). A red fluorescing HvRIC171–DsRED fusion protein was recruited to the cell periphery by membrane-associated CA HvRACB, but not by DN HvRACB, and accumulated at sites of fungal penetration attempts. This suggests focal HvRACB activity at sites of attempted fungal penetration (Schultheiss et al. 2008). Further investigations have to show whether HvRIC171 interferes with F-actin organization or whether other barley RAC/ROP-interacting proteins could explain how *Bgh* corrupts RAC/ROPs for compatibility. Interestingly, type III effectors of bacterial pathogens target Rho family proteins of mammals. *Yersinia* outer protein effectors (YOPs) have GAP or guanine nucleotide dissociation inhibitor (GDI) functions (see also chapter "Regulatory and Cellular Functions of Plant RhoGAPs and RhoGDIs") or are Rho-cleaving cysteine proteases involved in actin reorganization for invasion of nonphagocytic cells (Gruenheid and Finlay 2003; Aepfelbacher et al. 2007). It remains to be seen whether in analogy, *Bgh* effectors target barley RAC/ROPs during powdery mildew pathogenesis.

5.2 ROPs and Lipid Rafts

Recently, it has been shown that a type I *Arabidopsis* RAC/ROP in an activity-dependent manner inserts into detergent-resistant membrane fractions, and that this recruitment is mediated via reversible *S*-acylation of a conserved cysteine residue (e.g., C156 in AtROP6) (Sorek et al. 2007). Together with earlier findings that further carboxy-terminal cysteine residues can be prenylated in type I RAC/ROPs

and/or acylated in type II RAC/ROPs (Lavy et al. 2002; Yalovsky et al. 2008; see also chapter "ROPs, Vesicle Trafficking and Lipid Modifications"), these data support that signaling downstream of RAC/ROPs may operate from specific lipid domains, which have been found to be enriched with other signaling proteins such as RLKs, NADPH oxidases, and syntaxins (Mongrand et al. 2004; Morel et al. 2004; Bhat and Panstruga 2005). A GFP-tagged version of CA AtROP6 was recently imaged at sites of attack from virulent powdery mildew on *Arabidopsis* supporting recruitment of RAC/ROPs into specialized membrane domains at intimate sites of fungal contact (Hoefle and Hückelhoven 2008). In this context, it is also noteworthy that truncated CA type I HvRACB or CA type II HvRAC3, in which presumably lipid-modified cysteine residues were removed, were dislocated from the plasma membrane and could no longer support fungal entry by *Bgh* (Schultheiss et al. 2003). This suggests that membrane or lipid raft association could be crucial for RAC/ROP function in susceptibility to *Bgh*.

6 Perspectives

OsRAC1 is linked to Gα functions and both are important in resistance to avirulent *M. oryzae* and virulent *X. oryzae*. OsRAC1 and Gα, thus, likely represent common elements of PTI and ETI in rice. In barley, RAC/ROPs rather than heterotrimeric G-proteins are modulators of MLO-mediated susceptibility to powdery mildew and of basal resistance to *M. oryzae,* which is also dependent on MLO. It remains, however, elusive how the pathogen recognition machinery connects to G-protein signaling. Despite the well-documented involvement of these proteins in interactions of grasses with pathogenic microbes and the conserved function of MLO in dicots, little is understood about the role of RAC/ROPs and heterotrimeric G-proteins and their interplay with MLO proteins in disease resistance of dicot plant species. Additional studies are, thus, required to shed light on the contribution of G-proteins in interactions of dicots with pathogenic organisms and on the potential role of heterotrimeric G-proteins in physiological functions of MLO. Additionally, the important question whether MLO and G-proteins might be direct or indirect targets of microbial effector molecules needs future clarification.

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