New Phytologist

Review

Research review

Cell-autonomous defense, re-organization and trafficking of membranes in plant–microbe interactions

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Received: *25 March 2014*
Accepted: *16 July 2014*

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New Phytologist (2014) **204:** 815–822
doi: 10.1111/nph.12978

Key words: effector, exocyst, membrane domains, membrane heterogeneity, microtubules, plastids, ROP GTPase, syntaxin.

Summary

Plant cells dynamically change their architecture and molecular composition following encounters with beneficial or parasitic microbes, a process referred to as host cell reprogramming. Cell-autonomous defense reactions are typically polarized to the plant cell periphery underneath microbial contact sites, including *de novo* cell wall biosynthesis. Alternatively, host cell reprogramming converges in the biogenesis of membrane-enveloped compartments for accommodation of beneficial bacteria or invasive infection structures of filamentous microbes. Recent advances have revealed that, in response to microbial encounters, plasma membrane symmetry is broken, membrane tethering and SNARE complexes are recruited, lipid composition changes and plasma membrane-to-cytoskeleton signaling is activated, either for pre-invasive defense or for microbial entry. We provide a critical appraisal on recent studies with a focus on how plant cells re-structure membranes and the associated cytoskeleton in interactions with microbial pathogens, nitrogen-fixing rhizobia and mycorrhiza fungi.

Introduction

When plant cells come into contact with pathogenic microbes, nonself-recognition by the plant immune system triggers innate immune responses. One class of nonself molecules is called microbe/pathogen-associated molecular patterns (MAMPs/PAMPs) and represents epitopes that are often evolutionarily conserved in microbes but absent from plants. These molecules are detected on the host cell surface by plasma membrane (PM)-resident pattern recognition receptors, thereby initiating M/PAMP-induced immune responses. Another class of immune receptors (resistance proteins) is mainly defined by the intracellular nucleotide-binding domain and leucine-rich repeat-containing protein family. These intracellular receptors detect either the action or structure of polymorphic pathogen effectors that are delivered inside host cells during pathogenesis. Successful pathogen reproduction on a host plant demands suppression of M/PAMP-triggered immune responses by pathogen effectors and necessitates that none of the intracellular effectors is recognized by intracellular immune receptors. Pathogen effectors may also control host cell functions for accommodation of parasitic infection structures and maintain host cell viability. In mutualistic endosymbiosis, recognition of microbe-derived molecules specific to these endosymbionts triggers host cell reprogramming, which prepares the cell for microbial colonization. In both, pathogenic and mutualistic interactions, microbial recognition, signal transduction and cellular responses rely on membrane-associated processes. Plants have to establish membrane asymmetry and novel cellular compartments for focal defense at microbe contact sites or for local support of microbial invasion. This involves lipid signaling, the formation of plasma membrane micro-domains and *de novo* biogenesis of unique membrane compartments. Vesicle trafficking and cytoskeleton rearrangements are furthermore required to implement the cellular re-organization program. This review focuses on recent advances in our understanding on how host membrane signaling, membrane dynamics and the cortical

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cytoskeleton are re-organized at a cellular level during interactions with microbes.

Subcellular heterogeneity of plasma membranes

Host cell infections by rhizobia, arbuscular mycorrhiza fungi and haustorium-forming pathogenic fungi and oomycetes are characterized by a significant structural and morphological rearrangement of the host PM leading to the formation of the peribacteroid membrane (PBM), the periarbuscular membrane (PAM) and the extrahaustorial membrane (EHM), collectively referred to as perimicrobial membrane compartments (PMCs). They continuously enclose the invading microbes throughout their life cycles within the host. Formation of these structures requires a re-organization of the plant cell wall at attempted entry sites and/or bacterial liberation into the host cell, a massive expansion of the existing lipid bilayers and changes in protein composition to match their physiological needs. During mutualistic interactions, the initial steps of infection droplet formation during root nodule symbiosis and the transition from the hyphal trunk to a highly branched arbuscule are accompanied by a reduction in cell wall thickness and degree of organization, respectively (Rich *et al.*, 2014). By contrast, parasitic fungal attack is often characterized by the formation of ring-like or cone-shaped cell wall appositions called papillae (Assaad *et al.*, 2004; Underwood & Somerville, 2013). Despite these opposing processes, a common set of membrane trafficking proteins such as syntaxins (Assaad *et al.*, 2004) and exocytotic VESICLE-ASSOCIATED MEMBRANE PROTEINS (VAMPs) (Kwon *et al.*, 2008b; Ivanov *et al.*, 2012) have been shown to be generally required for host cell responses towards invading microbes in plants (see 'Cell-autonomous and vesicle-mediated responses' below). Vesicle trafficking from the Golgi apparatus/trans Golgi network (TGN) allows host cells to target proteins and lipids to infection sites and the newly forming PMCs and thereby to define novel membrane domains. Prominent examples for targeted delivery are the syntaxin PEN1/SYP121 and the ATP-binding cassette transporter PEN3/PDR8 that focally accumulate at sites, where powdery mildew fungi attempt penetration (Assaad *et al.*, 2004; Underwood & Somerville, 2013). During symbiotic interactions, a similar focal targeting to infection sites has been described, amongst others, for the *Medicago truncatula* BLUE COPPER-BINDING PROTEIN1 and SYMBIOTIC REMORIN 1 that localize to the hyphal trunk and rhizobial liberation sites, respectively (Pumplin & Harrison, 2009; Lefebvre *et al.*, 2010). Throughout the interaction, PMCs further differentiate and specialize, a process that is accompanied by significant changes in their membrane proteomes (Wienkoop & Saalbach, 2003). But how can proteins specifically locate to PMC membranes although these membranes are in an apparent continuum with the PM? Proteins may either contain specific targeting signals as shown for Arabidopsis RESISTANCE TO POWDERY MILDEW8.2 (RPW8.2) (Wang *et al.*, 2013) and/or their respective promoters are spatiotemporally regulated as demonstrated for the *M. truncatula* PHOSPHATE TRANSPORTER 4 (MtPT4) (Pumplin *et al.*, 2012). Opposite to BLUE COPPER-BINDING PROTEIN1 that associates with the peripheral PM of arbuscule-containing cells and the perihyphal membrane surrounding the hyphal trunk but is not present on the PAM, MtPT4 exclusively localizes to the PAM but is excluded from the peripheral PM and the perihyphal membrane (Pumplin & Harrison, 2009; Pumplin *et al.*, 2012). However, no target signal was identified that could explain this specific localization. Instead, Pumplin *et al.* (2012) demonstrated that polar secretion of PAM-localized MtPT4 is achieved by preferred fusion of bulk secretory vesicles with the developing PAM rather than with the peripheral PM and strictly depends on a tight spatiotemporal control of promoter activity.

Although the *MtPT4* promoter is only activated in a specific cell in the presence of the arbuscular mycorrhiza fungus, RPW8.2 is constitutively expressed at basal levels in epidermal and mesophyll cells but is strongly induced during powdery mildew infection. In line with results described for MtPT4 such spatiotemporal regulation of *RPW8.2* expression results in a predominant secretion of the protein to the EHM in an actin-dependent manner (Wang *et al.*, 2009). After exit from the *trans*-Golgi, RPW8.2 is carried mainly on VAMP722 vesicles to the EHM of young haustoria (Kim *et al.*, 2014). Furthermore, targeting of RPW8.2 is mediated by its transmembrane domain and two short EHM targeting signals containing an R/K-R/K-x-R/K motif (Wang *et al.*, 2013). The exact function of these motifs remains to be studied.

Some proteins, such as Remorins, also label a variety of membrane compartments in living plant cells, although they lack transmembrane domains. Remorins can accumulate on perimicrobial membranes such as the PBM (SYMBIOTIC REMORIN 1; Lefebvre *et al.*, 2010) or the EHM (REM1.3; Lu *et al.*, 2012; Bozkurt *et al.*, 2014). Interestingly, PMC localization of these proteins is independent of the spatiotemporal regulation of their endogenous promoters as ectopic expression resulted in the same localization (Lu *et al.*, 2012; Toth *et al.*, 2012; Bozkurt *et al.*, 2014). As the Golgi-derived secretory vesicles do not only shuttle proteins but also lipids, it can be hypothesized that targeting of soluble proteins to these PMCs may be mediated by post-translational lipidation of the proteins and/or direct interactions with intrinsic membrane proteins (Konrad *et al.*, 2014).

After development of PMCs, their specific protein composition can only be maintained by physical borders restricting lateral diffusion of transmembrane proteins from the PM to the PMC and *vice versa*. Existence of such barriers is supported by findings that ectopic expression of transmembrane proteins such as MtPT1 and MtPT4 did not result in a diffusion of these proteins from the peripheral PM to the PAM (Pumplin *et al.*, 2012). Furthermore, a constitutively expressed aquaporin also remained excluded from the EHM and was unable to cross the haustorial neck region (Wang *et al.*, 2013). It can be hypothesized that *de novo* synthesized host cell wall material such as papillae that form during pathogen infection or are triggered by M/PAMPs, as well as cell-wall depositions along the hyphal trunk before arbuscule formation, support such functions, for example by tethering PM-resident proteins to *de novo* formed cell wall constituents and restricting their lateral diffusion. Cortical microtubules may provide further barriers for lateral diffusion of membrane-associated proteins (see

'Plasma membrane-to-cytoskeleton communication', Fig. 2 below and Yang & Lavagi, 2012).

In summary, local defense and the formation of PMCs during symbiotic and pathogenic interactions involves core proteins for membrane fusion. Endomembrane dynamics deliver specific proteins and lipids to the site of infection and PMC formation. Formation and maintenance of PMCs is partially cytoskeleton-dependent. Therefore, we survey these aspects more specifically.

Cell-autonomous and vesicle-mediated responses

Earlier cell biological and pharmacological studies demonstrated a rapid re-organization of the cytoskeleton and organelles in single attacked host cells, oriented towards and surrounding pathogen and symbiont contact sites. These cell-autonomous responses have been described as pathogen-induced cell polarization (Kwon *et al.*, 2008a). A combination of experimental approaches implicated exocytosis to be mediated by ternary SNARE complex formation between PM-localized PEN1/SYP121 syntaxin, endosome-resident VAMP721/722 proteins and the adaptor SNAP33 and to be an important mechanism of pre-invasive resistance to *Blumeria graminis* and *Erysiphe pisi* (Fig. 1); (Kwon *et al.*, 2008b). However, the detection of PEN1, SNAP33 and membrane material entrapped in papillae suggested that exosome biogenesis/release might act as an additional secretory mechanism in pre-invasive defense (Fig. 1); (Meyer *et al.*, 2009). VAMP722 was shown to partly co-localize with the syntaxins SYP41, SYP42 and SYP43 that reside on the *trans* side of the TGN (Uemura *et al.*, 2012). This and an elevated entry rate of *E. pisi* on *syp42 syp43* double mutants indicate that the biogenesis of VAMP722 endosomes initiates at the TGN. The TGN might therefore supply at least some of the cargo for extracellular PEN1-mediated and RPW8.2-dependent post-invasive defense (Fig. 1); (Uemura *et al.*, 2012). The finding that the same vesicles are needed for PEN1/SYP121-dependent exocytosis at the PM (Kwon *et al.*, 2008b) and for transport of RPW8.2 resistance protein to the EHM (Kim *et al.*, 2014) implies the existence of a bifurcated VAMP722 trafficking pathway with at least partial cargo selectivity for extracellular pre-invasive defense and post-invasive resistance at the EHM (Fig. 1).

Polar PEN3 recruitment at the host–pathogen interface, but not PEN1, is an actin-dependent process (Underwood & Somerville, 2013). The Arabidopsis ARF-GEF (ADP ribosylation factor GTP exchange factor) GNOM was shown to be required for constitutive recycling of PM-resident PEN1 to endosomes in the absence of pathogen, for the accumulation of extracellular PEN1, callose and membrane material in pathogen-induced papillae, and for full pre-invasive resistance to *B. graminis* (Fig. 1; Nielsen *et al.*, 2012). Additionally, the Arabidopsis Rab5-like GTPase ARA6, a marker

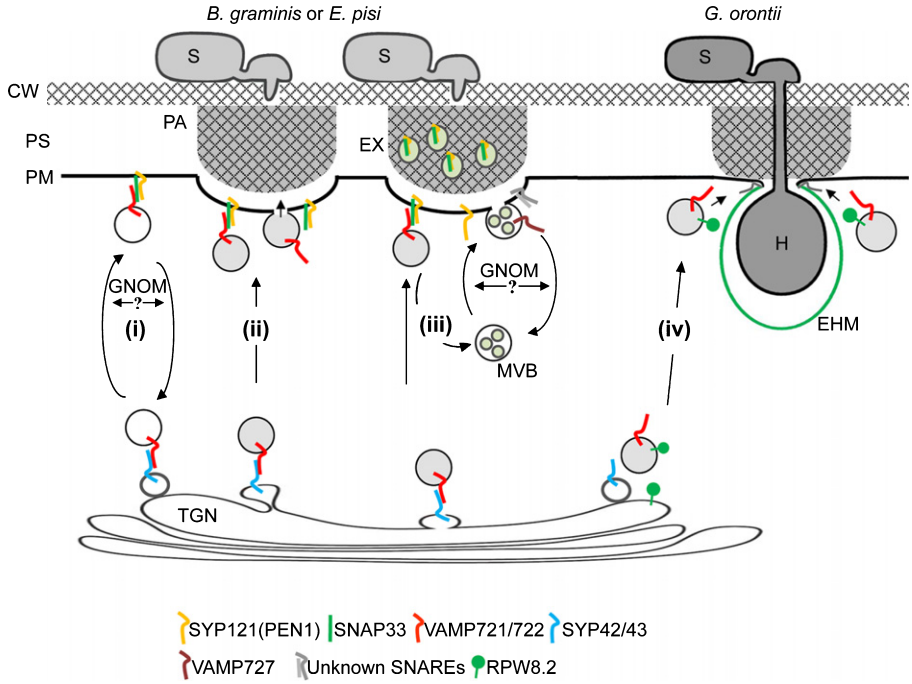

Fig. 1 Vesicle-mediated pre- and post-invasive defense responses at powdery mildew contact sites. Four simplified vesicle-mediated pathways at the plasma membrane (PM) and pathogen contact sites are schematically represented in (i) to (iv). Pathway (i) illustrates in healthy plants continuous cycling of VAMP721/722 endosomes between the trans-Golgi network (TGN) and the PM. The pre-invasive defense pathway (ii) likely involves PEN1 syntaxin-dependent discharge of VAMP721/722 vesicle cargo at the PM in the paramural space (PS) in response to attack of germinated spores (S) of the nonadapted powdery mildews *Blumeria graminis* or *Erysiphe pisi*. The pre-invasive defense pathway (iii) likely involves the fusion of VAMP727-containing multivesicular bodies (MVB) with the PM and entrapment of PEN1-containing exosomes (EX) in the newly formed cell wall material of papillae (PA). The targets of the ARF guanine-nucleotide exchange factor GNOM are unknown. In the post-invasive defense pathway (iv) to the host-adapted powdery mildew *G. orontii* the Arabidopsis resistance protein RPW8.2 is carried by VAMP721/VAMP722 vesicles to the extrahaustorial membrane (EHM) surrounding a haustorium (H). The white- and grey-colored VAMP721/722 endosomes (black circles) denote the dynamic change of their cargo in healthy plants and upon powdery mildew attack, respectively. CW, preformed host cell wall.

of multivesicular bodies (MVBs), accumulates underneath attempted *B. graminis* entry sites and mediates PEN1-dependent membrane fusion between the PM and MVBs (Ebine *et al.*, 2011). This supports the idea of an MVB-derived exosome biogenesis and transport of extracellular PEN1 exosomes and callose, or callose synthase. However, ARA6-regulated and PEN1-dependent membrane fusion at the PM involves SNARE complex formation with endosome-resident VAMP727 (Ebine *et al.*, 2011). Collectively, this points to the existence of two mechanistically distinct secretory processes with partly shared components: (1) PEN1 ternary SNARE complex-dependent exocytosis mediating discharge of vesicle cargo into the apoplastic space and (2) release of PEN1-containing exosomes at the PM after recycling of the syntaxin from the PM to MVBs (Fig. 1). In light of the crucial role vesicle-trafficking has in plant defense, one might expect that powdery mildews evolved effectors to sabotage this process. Indeed, a *B. graminis* effector candidate, designated BEC4, was shown to interact with a plant-encoded ARF-GTPase activating factor (ARF-GAP) as potential host target (Schmidt *et al.*, 2014). Enhanced invasive growth of nonadapted *E. pisi* on the ARF-GAP mutant *Atadg5* is consistent with the idea of BEC4-mediated manipulation of ARF-GAP activity to suppress pre-invasive defense.

Other SNARE proteins have been shown to be crucial for the establishment of mutualistic interactions. The SNAREs SYP32-1 (Sed5) and SYP71 of *Lotus japonicus* are required for nodule formation and function (Mai *et al.*, 2006; Hakoyama *et al.*, 2012). In addition, *M. truncatula* SYP132 localizes to the infection threads, infection droplet structures and the symbiosome membrane (Catalano *et al.*, 2007; Limpens *et al.*, 2009). Interestingly, the *M. truncatula* SNAREs SYP22 and VTI11 did not label mature symbiosomes, indicating that this peribacteroid compartment does not share similarities with vacuoles or prevacuolar compartments (Limpens *et al.*, 2009). However, these markers are recruited to the PBM upon nodule senescence (Limpens *et al.*, 2009). This is accompanied by the formation of a lytic symbiosome compartment that resembles a vacuole. During mycorrhization, knock-down of the Q-SNARE LjVTI12 had a mild impact on arbuscule formation (Lota *et al.*, 2013). By contrast, knock-out mutants of VAMP72 exhibit dramatic alterations in fungal colonization (Ivanov *et al.*, 2012). In line with this, VAMP72-labelled vesicles accumulated at the contact site of *Gigaspora gigantea* with *M. truncatula* root epidermal cells and labelled segments of the perifungal membrane (Genre *et al.*, 2012). It will be exciting to learn how the vesicles are correctly targeted and how their cargos are selected for defense or the formation of PMCs.

Function of tethering complexes

Besides SNARE complexes, exocyst complexes were suggested to play a role in the accommodation of arbuscules (Genre *et al.*, 2012). For example, ectopically expressed Arabidopsis exocyst subunit Exo84b accumulated at the tip of growing intracellular hyphae of *Gigaspora gigantea* in *M. trunculata.* The exocyst is an octameric protein tethering complex that acts by establishing contact of membranes before SNARE complexes drive membrane fusion. Pioneering work in yeast has shown that tethering proteins form complexes capable of participating in one or more intracellular trafficking pathway and that they are able to interact with several of the main components of the cellular trafficking machinery (Heider & Munson, 2012).

Arabidopsis mutants of exocyst subunit Exo70B2 showed defects in the formation of papillae in response to penetration attempts of nonadapted *B. graminis* (Pecenkova *et al.*, 2011). A candidate gene approach identified the putative exocyst subunit EXO70F-like in barley as a component required for pre-invasive resistance to *B. graminis* (Ostertag *et al.*, 2013). Moreover, the same study identified COG1 and COG3, two subunits of the conserved oligomeric Golgi complex, another octameric tethering complex, which in yeast functions in retrograde trafficking, to be required for protein secretion and pre-invasive resistance. Tethering complexes are expected to contribute to defense responses by functioning in the secretion of antimicrobial compounds. Additionally, these complexes could mediate the transport of PM-resident proteins involved in immune signaling. In support of this possibility, Arabidopsis Exo70B2 is required for early responses triggered by various M/PAMPs, suggesting its involvement in signaling triggered by pattern recognition receptors. The reduced responsiveness to M/PAMPs may explain the enhanced susceptibility of *exo70B2* mutants to *Pseudomonas syringae* (Stegmann *et al.*, 2012). A similar scenario might hold true for *Exo70H1* because mutant *exo70H1* plants are more susceptible to *P. syringae*, too (Pecenkova *et al.*, 2011). However, it remains unknown whether the defects in the formation of papillae are linked to the reduced responsiveness to M/PAMPs observed in *exo70B2*.

Recent data opened a third potential function for the exocyst tethering complex. Exo70B1, the nearest homolog of Exo70B2, was reported to function in autophagy-related transport to the vacuole (Kulich *et al.*, 2013). In addition to the colocalization of Exo70B1-positive compartments to the autophagic marker Atg8F, *exo70B1* mutant plants displayed anthocyanin accumulation defects. Loss of *Exo70B1* function also results in spontaneous cell death at developmental stages shortly before flowering (Stegmann *et al.*, 2014). Spontaneous lesions are reminiscent of those described for many autophagy mutants, supporting a role in this process. Of note, *exo70B1* plants also show a reduced responsiveness to the bacterial flagellin-derived M/PAMP flg22 (Stegmann *et al.*, 2012) and are more susceptible to *P. syringae* at developmental stages before cell death is observed (Stegmann *et al.*, 2014). By contrast, plants were less susceptible to the biotrophic oomycete *Hyaloperonospora arabidopsidis* and displayed enhanced cell death in response to the nonadapted oomycete *Phytophthora infestans* (Stegmann *et al.*, 2014). It needs to be clarified whether there is a mechanistic link between the deregulation of autophagy, cell death and the reduced M/PAMP-triggered signaling.

Plasma membrane-to-cytoskeleton communication

The plant cortical cytoskeleton is considered as an important factor for controlled vesicle delivery at the PM. However, it is poorly understood how PM-associated signaling events translate into a rearrangement of cytoskeleton arrays and how cytoskeleton-

associated signaling regulates membrane traffic. Protein–protein interaction studies suggested a role of barley PM-associated RAC/ROP monomeric GTPase and RAC/ROP-interacting proteins in cytoskeleton organization during fungal invasion. The barley RAC/ROP protein RACB is required for full susceptibility to haustorial invasion by *B. graminis* and thus a potential virulence effector target (Hoefle *et al.*, 2011). Hoefle *et al.* (2011) identified a new isoform of ROP-GTPase activating proteins, the microtubule-associated ROP-GAP1 (MAGAP1) of barley, which directly interacts with RACB. ROP-GAPs deactivate RAC/ROPs by promoting hydrolysis of RAC/ROP-bound GTP. MAGAP1 limits fungal entry and may thus antagonize RACB by feedback from MTs. Barley cells, which resist fungal entry, strongly differ in MT organization from those that accommodate haustoria (Fig. 2) and MAGAP1 supports the organization and polarity of MT-arrays in interactions with *B. graminis* (Hoefle *et al.*, 2011). RACB further binds RBK1 (ROP binding kinase 1), a receptor-like cytoplasmic kinase. RBK1 activity might be stimulated at the PM by activated RACB. Silencing of *RBK1* in barley epidermal cells destabilizes MTs and supports haustorium formation. Hence, RBK1 restricts fungal entry, which could result from a negative feedback from activated RBK1 on the susceptibility factor RACB (Huesmann *et al.*, 2012) (Fig. 2). In Arabidopsis, RACB-homologous RAC/ROPs (ROP2, ROP4, ROP6, ROP11) have the ability to influence the organization or depletion of microtubules via downstream scaffolds and executer proteins. *Vice versa*, microtubules function in plasma membrane heterogeneity by providing a diffusion barrier for membrane-associated proteins such as RAC/ROPs leading to partial self-organization of membrane asymmetry (Fig. 2); (Yang & Lavagi, 2012). A dominant negative form of ROP6 caused developmental defects, alterations of F-actin and MT arrays and reduced susceptibility to the adapted powdery mildew *Golovinomyces orontii*. This suggests that wild-type ROP6 is needed for effective reproduction of the pathogen. Expression of dominant negative ROP6 also led to salicylic acid-mediated defense, but this was not responsible for reduced pathogenic success of *G. orontii* (Poraty-Gavra *et al.*, 2013).

RAC/ROPS are emerging as players in interactions with beneficial microbes, too. Arabidopsis ROP1 and ROP6 were reported to be involved in F-actin bundling in interactions with the root endophyte *Piriformospora indica* (Venus & Oelmüller, 2013). *Lotus japonicas* NOD factor receptor NFR5 associates with ROP6, which is required for normal development of infection threads preceding root nodule formation (Ke *et al.*, 2012). In addition, silencing of the *M. truncatula ROP9* resulted in an impaired nodulation whereas it increased arbuscular mycorrhization (Kiirika *et al.*, 2012). In *Phaseolus vulgaris*, NOD factors can provoke F-actin re-organization in root hairs, apparently at sites of infection thread initiation (Zepeda *et al.*, 2014). Possibly, receptor-mediated activation of RAC/ROPs regulates cytoskeleton organization. Arabidopsis M/PAMP-activated EFR receptor complex triggers an increase in density of filamentous F-actin in Arabidopsis epidermal cells. This resembles the effect of mutations in actin depolymerizing factor 4, which is among others involved in callose deposition in response to *P. syringae* (Henty-Ridilla *et al.*, 2014). However, evidence is lacking for Arabidopsis M/PAMP-triggered immunity to be linked to RAC/ROP signaling.

Together, RAC/ROP proteins appear to provide a missing link between recognition events at the plasma membrane and re-organization of the cytoskeleton in immunity and microbial invasion. It will be interesting to see whether ROPs and cytoskeleton–PM communication are involved in the initiation and maintenance of membrane identity of PMCs (see 'Subcellular heterogeneity of plasma membranes' above).

The role of plastidial lipids

Organellar membrane lipids are involved in the plant response to microbial attack and in interactions with beneficial microorganisms. This includes the generation of lipid-derived signals, the proliferation of lipids for new membrane compartments or compositional changes of lipid bilayers at the subcellular level. The chloroplast is among the first cellular compartments showing

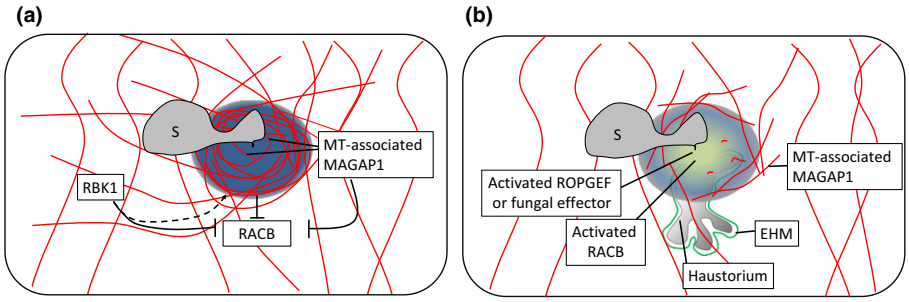

Fig. 2 Schematic representation of cortical microtubule (MT) organization and hypothetical RAC/ROP activity at sites of attack from the barley powdery mildew fungus. In cells, which resist fungal invasion (a), a dense meshwork of MTs (red) is organized in concentric arrays at the outer epidermal cell cortex and polarized to the site of attempted penetration. MT-associated MAGAP1 works in spatial control of RAC/ROP activity by negative feedback from MTs (red) to plasma membrane-associated RAC/ROPs. MTs and MAGAP1 might further restrict lateral diffusion of active RACB to sites of fungal attack. Together this results in a membrane domain of little RACB activity (dark blue) in which cell-wall associated defense is successful. RBK1 further stabilizes MTs by a mechanism, likely involving negative feedback from activated RAC/ROPs. In cells accessible for fungal invasion and haustorium accommodation (b), RACB might be activated by a ROP-specific guanine nucleotide exchange factor (ROPGEF) or a fungal virulence effector in particular membrane domains (light green). RACB activity leads to loosening of MT arrays allowing for ingrowth of the haustorial complex. MT depletion further limits negative feedback of RAC/ROP signaling from MTs and allows for lateral diffusion of membrane-associated RAC/ROPs. EHM, extrahaustorial membrane; S, fungal spore (conidium).

physiological responses after pathogen attack (Fig. 3). During infection, chlorophyll and chloroplast lipids are broken down by pheophytinase, phospholipases and galactolipases accompanied with the release of phytol and fatty acids. Phytol is employed for the synthesis of the antioxidant tocopherol (vitamin E), and phytol and fatty acids are converted together with fatty acids into fatty acid phytyl esters, which are transiently deposited in the chloroplasts. Interestingly, the *chilling sensitive1* mutant of Arabidopsis carrying a mutation in a resistance gene-like *Chs1* reveals a strong increase in tocopherol and fatty acid phytyl esters at low temperatures (Zbierzak *et al.*, 2013).

Different breakdown products of chloroplast lipids serve as signaling compounds during infection (Fig. 3). Chloroplast-derived linolenic acid (18:3) is the precursor for oxo-phytodienoic acid synthesis in the lipoxygenase pathway. Oxo-phytodienoic acid is converted into jasmonic acid during pathogen infection. Oleic acid (18:1) is a lipid-derived signal of biotic stress responses that acts via nitric oxide (Mandal *et al.*, 2012). Azelaic acid, a 9-carbon dicarboxylic acid, was implicated in systemic acquired resistance (Zoeller *et al.*, 2012). Azelaic acid is derived from fatty acids carrying a double bond at position 9 (18:3, 18:2, 18:1) by chemical fragmentation, and is a general oxidative stress signal (Zoeller *et al.*, 2012). Furthermore, dehydroabietanal is an isoprenoid-derived mobile signal during systemic acquired resistance (Chaturvedi *et al.*, 2012).

During nodulation, host cells need to be synthesize large amounts of lipids for the establishment of the PBM. The galactolipid digalactosyldiacylglycerol (DGDG) is produced in the root plastids and is transported to extraplastidial membranes, including the PBM, where it makes up a considerable proportion of the lipid bilayer (Wewer *et al.*, 2014); (Fig. 3). DGDG is phosphorus-free and therefore, it can replace phospholipids saving plant phosphate resources.

During mycorrhization by *Rhizophagus irregularis,* arbuscule-harboring host cells contain a high number of plastids, which extend into tubular structures closely associated with the arbuscules. Some of these plastidial tubuli resemble stromules, thin extensions containing the two envelope membranes and stroma. This close connection led to the conclusion that host cell plastids and arbuscules interact at a metabolic level. Indeed, plastid-derived lipids are crucial for fungal colonization (Fig. 3). For example, carotenoids are processed by carotenoid cleavage dioxygenases into shorter (C13-C20) isoprenoids with hormonal (abscisic acid) or signaling functions (strigolactones, mycorradicin) during plant infections with mycorrhizal fungi (Walter *et al.*, 2010). Interestingly, a variant of RPW8.2 (see 'Subcellular heterogeneity of plasma membranes' above and Fig. 1), which is transported to the EHM, was also found to be associated with a peristromule membrane compartment (Wang *et al.*, 2013). This membrane seems to be connected with the EHM further highlighting the close interactions between plastids and fungal invaders.

Compatibility of lipid composition between the fungal and host plasma membrane

The question of how the host cell can distinguish between pathogenic and symbiotic infections is still not fully understood. It is likely that the protein and lipid composition of the invading

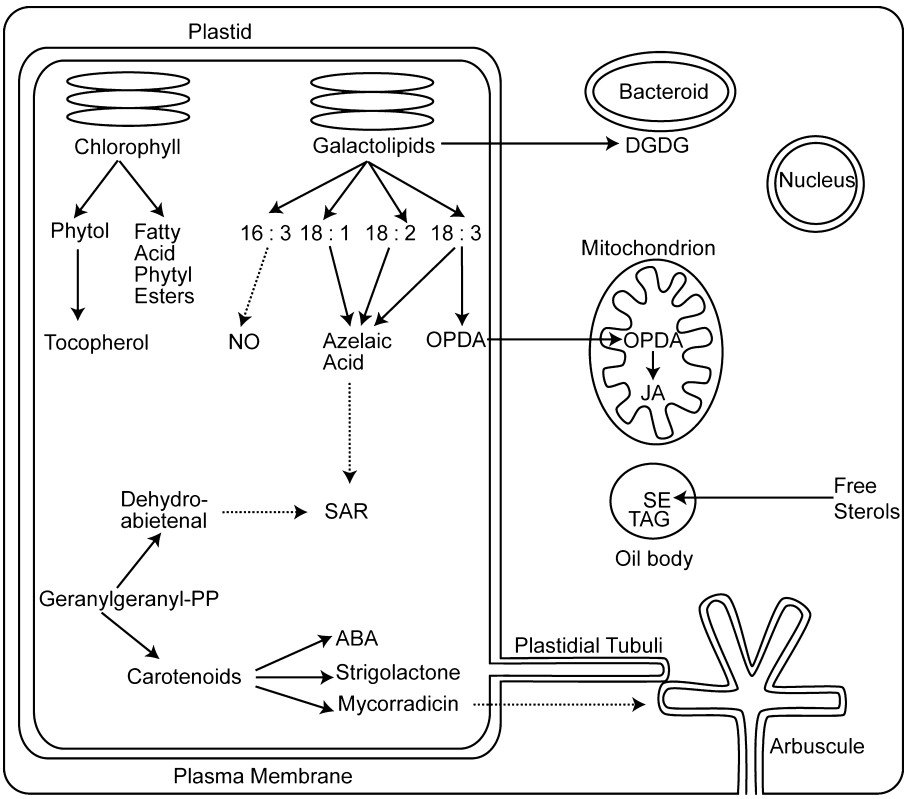

Fig. 3 Organelle-derived lipid responses in the host plant cell. Infection of the plant cell with different pathogens or symbionts results in numerous lipid-derived responses, in particular involving plastidial lipid metabolisms. Solid arrows, metabolic routes; dotted arrows, functional actions. Fatty acids are abbreviated as X:Y with X, number of carbon atoms and Y, number of double bonds. ABA, abscisic acid; DGDG, digalactosyldiacylglycerol; JA, jasmonic acid; NO, nitric oxide; OPDA, oxophytodienoic acid; SAR, systemic acquired resistance; SE, sterol ester; TAG, triacylglycerol.

microbe contributes to triggering or suppressing the plant immune response. In this context, it is interesting to note that pathogenic fungi contain the M/PAMP ergosterol in their PM. Symbiotic fungi, for example *Rhizophagus irregularis*, however, are devoid of ergosterol, but instead produce plant-like sterols. Furthermore, *R. irregularis* changes its membrane lipid composition during mycorrhization, as the synthesis of unusual phospholipids is suppressed in intraradical hyphae. These adaptation processes might be important in establishing a functional membrane for exchange of nutrients and signals and to avoid the plant immune response (Wewer *et al.*, 2014). In addition, the PM lipid composition of the plant cell is adjusted during infection. Lipid turnover results in the deposition of fatty acids in the form of triacylglycerol in oil bodies in the cytosol. Furthermore, sterol esters accumulate during abiotic stress (Zbierzak *et al.*, 2013) and during plant–pathogen interactions. Sterol esters are involved in the regulation of the free sterol contents in the PM. Interestingly, a block in sterol ester synthesis accompanied by an increase in free sterols correlates with an enhanced nonhost resistance response (Kopischke *et al.*, 2013).

Conclusions

Cell autonomous defense, microbe-triggered formation of new apoplastic compartments and of PMC host membranes require massive re-organization of plant membrane systems and the host cytoskeleton. Therefore, membranes of unique identity have to be generated. Membrane identity is given by protein and lipid compositions that are initiated and maintained by tight spatio-temporal control of biosynthesis, transport and delivery. For perimicrobial host membranes, which are continuous with the PM, additional barriers for lateral diffusion of lipids and proteins are postulated. Recent studies provided protein markers for microbe-associated membrane domains and first insight into how plants organize membrane herterogeneity, lipid patterns and vesicle delivery at the plant–microbe interface. Not only is better understanding of the role of lipids and molecular scaffolds in the formation of membrane domains needed, but also elucidation of how gene expression is connected to the dynamics and identity of membranes at the cellular level. It will be exciting to learn how microbes interfere with host membrane identity during plant invasion.

Acknowledgements

We apologize to those authors who contributed to the field but were not cited due to limitation of space and the number of references. Experimental work of the authors was supported by grants from the German Research Foundation priority program SPP1212 'Microbial Reprogramming of Plant Cell Development' to P.D., T.O., P.S-L., M.T. and R.H.

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