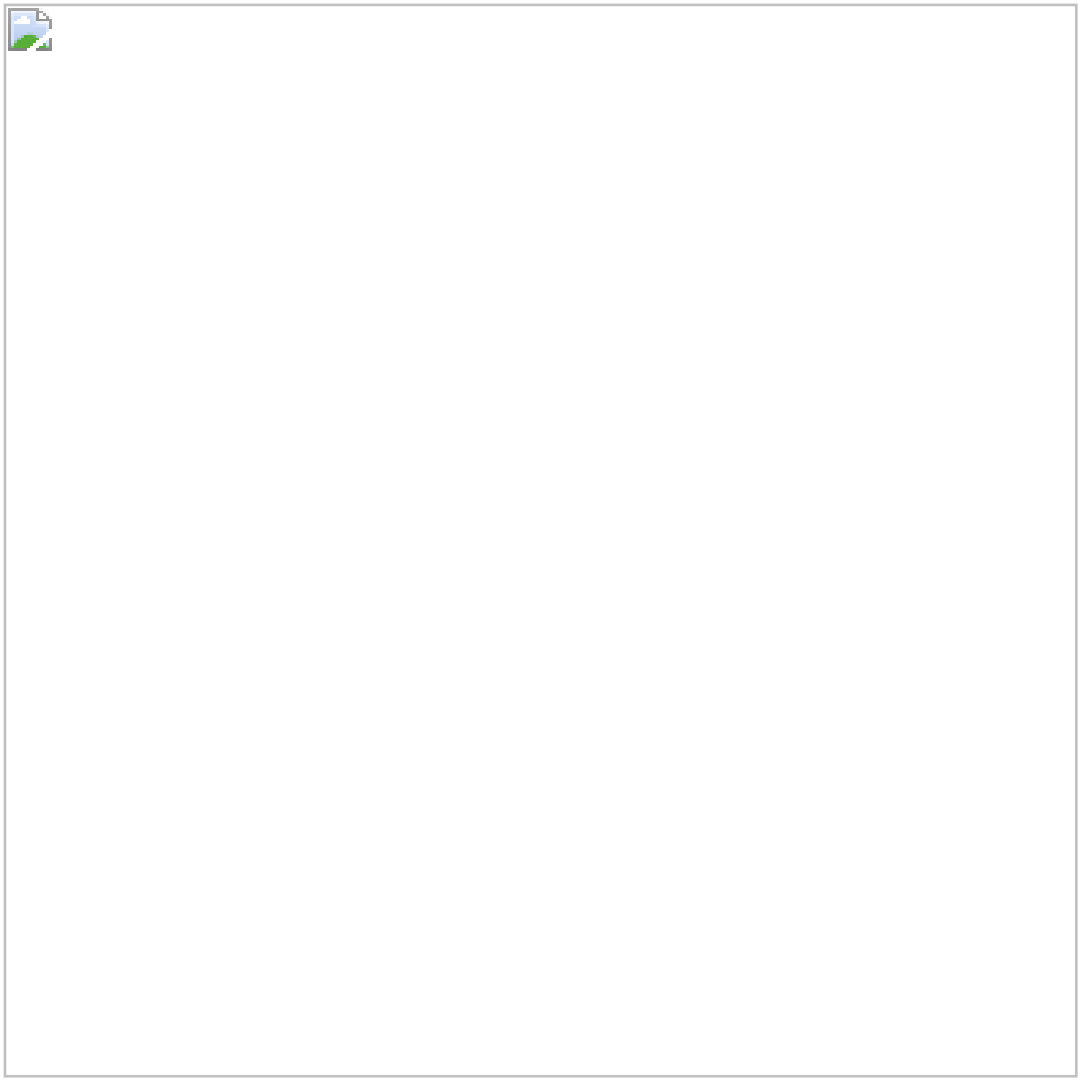

Biotrophy at Its Best: Novel Findings and Unsolved Mysteries of the Arabidopsis-Powdery Mildew Pathosystem

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Biotrophy at Its Best: Novel Findings and Unsolved Mysteries of the Arabidopsis-Powdery Mildew Pathosystem

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It is generally accepted in plant-microbe interactions research that disease is the exception rather than a common outcome of pathogen attack. However, in nature, plants with symptoms that signify colonization by obligate biotrophic powdery mildew fungi are omnipresent. The pervasiveness of the disease and the fact that many economically important plants are prone to infection by powdery mildew fungi drives research on this interaction. The competence of powdery mildew fungi to establish and maintain true biotrophic relationships renders the interaction a paramount example of a pathogenic plant-microbe biotrophy. However, molecular details underlying the interaction are in many respects still a mystery. Since its introduction in 1990, the Arabidopsis-powdery mildew pathosystem has become a popular model to study molecular processes governing powdery mildew infection. Due to the many advantages that the host Arabidopsis offers in terms of molecular and genetic tools this pathosystem has great capacity to answer some of the questions of how biotrophic pathogens overcome plant defense and establish a persistent interaction that nourishes the invader while in parallel maintaining viability of the plant host.

1. INTRODUCTION

Powdery mildew (PM) is a widespread fungal disease of great agricultural and economic importance (Bélanger et al., 2002; Glawe, 2008). The disease is caused by Ascomycetes of the order Erysiphales and is characterized by the appearance of white "powdery" symptoms on the surface of aboveground plant organs. The white powder represents the combination of fungal mycelium and asexual propagation structures (conidiophores and conidia). In total, more than 400 PM species are able to colonize nearly 10,000 plant species (Takamatsu, 2004). These comprise many economically relevant crop and ornamental plants, including grain-producing species (e.g. barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*)), legumes (e.g. pea (*Pisum sativum*)), fruit-producing plants (e.g. apple (*Malus domestica*) and tomato (*Solanum lycopersicum*)) and roses (*Rosa hybrida*) (Linde et al., 2006; Attanayake et al., 2010; Dean et al., 2012). While some PM species can infect a broad range of plants, others have a very narrow host spectrum. PMs are plant parasites that exhibit an obligate biotrophic lifestyle, i.e. they require living plant tissue for development and propagation (Panstruga and Schulze-Lefert, 2002). Most species grow epiphytically, and intracellular haustoria, dedicated hyphal projections, are the only fungal structures present within plant tissue. Thus, plant-PM encounters can be easily studied by light and fluorescence microscopy, and the disease has become a paradigm for the interaction between plants and biotrophic plant parasites (Micali et al., 2008; Hückelhoven and Panstruga, 2011). This is also reflected by the fact that the PMs are considered as one of the "top 10 fungal pathogens in molecular plant pathology" (Dean et al., 2012).

Four PM species are known to be able to complete their asexual life cycle on Arabidopsis (*Arabidopsis thaliana*): *Erysiphe cruciferarum* (Koch and Slusarenko, 1990), *Golovinomyces* (syn. *Erysiphe*) *cichoracearum* (*Gc*) (isolate UCSC1; Adam and Somerville, 1996), *Golovinomyces* (syn. *Erysiphe*) *orontii* (*Go*) (Plotnikova et al., 1998), and the tomato PM pathogen *Oidium neolycopersici* (Bai et al., 2008). These four PM species differ in some morphological characteristics such as the size of the conidia, the shape of appressoria and haustoria, and the number of conidiophores per colony (Micali et al., 2008). Despite their principal capacity to colonize Arabidopsis, not all Arabidopsis ecotypes are equally susceptible to these virulent PMs. A survey based on 360 Arabidopsis ecotypes with two of the above-mentioned PM species (*Go* UCSC1 und *E. cruciferarum* UEA1) revealed differential phenotypes with respect to PM colonization. Although the majority of accessions were susceptible to both species, 147 exhibited resistance to at least one of them, with 84 accessions showing species-specific resistance (Adam et al., 1999).

Recently described PM isolates recovered from common sow thistle (*Sonchus oleraceus*; designated *Gc* UMSG1) and tobacco (*Nicotiana tabacum*; designated *Gc* SICAU1) are only partially adapted to Arabidopsis. These isolates show considerable host

cell penetration rates but either fail to complete their life cycle (*Gc* UMSG1) or show only little sporulation (*Gc* SlCAU1) on Col-0 wild type plants. Thus, the two isolates are able to overcome pre-invasion resistance but are presumably limited at later steps in the infection by post-invasion resistance mechanisms (Wen et al., 2011; Zhang et al., 2015a). Together with the four above-mentioned virulent species and even less adapted species isolated from other plant hosts (e.g. *Erysiphe pisi*, colonizing several legumes including pea, and *Blumeria graminis* f.sp. *hordei* (*Bgh*), the barley PM pathogen), these PMs cover a broad range of host adaptation levels. They therefore offer the opportunity to study different mechanisms of plant immunity such as basal defense and nonhost resistance (NHR).

In this chapter, we update our previous synopsis of the Arabidopsis-PM pathosystem (Micali et al., 2008) by highlighting new findings and incorporating novel developments. We portray the fungal life cycle, describe the first and second line of plant defense, elaborate on NHR and susceptibility factors, illustrate the role of intracellular trafficking and phytohormone-based defense signaling, explain the host transcriptional response and finally take a look on the fungal side of the interaction (Figure 1). Key genes (and corresponding AGI codes) mentioned in the text whose mutation, silencing or overexpression results in an altered PM-related phenotype are described in Supplemental Table 1.

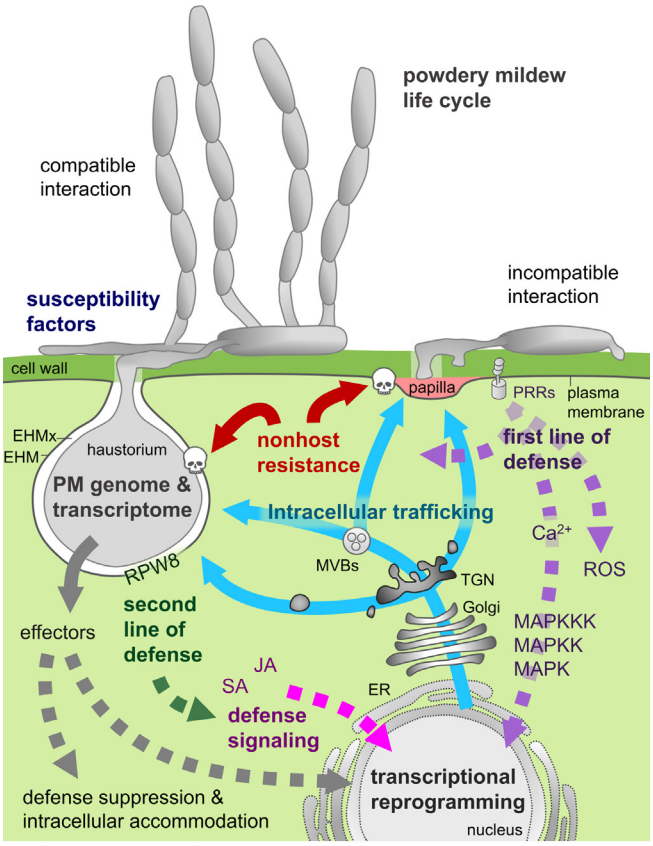

Figure 1. Graphical overview of the topics discussed in this article.

EHM, extrahaustorial membrane; EHMx, extrahaustorial matrix; ER, endoplasmic reticulum; JA, jasmonic acid; MAPK(KK/KKK), mitogen-activated protein kinase (kinase kinase/kinase kinase kinase); MVBs, multivesicular bodies; PRRs, pattern recognition receptors; RPW8, RESISTANCE TO POWDERY MILDEW 8 protein; ROS, reactive oxygen species; SA, salicylic acid; TGN, *trans*-Golgi network. See text for further explanations.

2. POWDERY MILDEW LIFE CYCLE AND HAUSTORIUM STRUCTURE

2.1 The fungal life cycle

As mentioned above, most PM fungi grow epiphytically on their respective host plants. Only single PM species of the genera *Leveillula* and *Phyllactinia* are an exception, as they propagate (*L. taurica*) or form haustoria (*P. guttata*) endophytically in the leaf mesophyll tissue after entering through stomata (Boesewinkel, 1980). In natural environments, PM conidiospores (mitotic, asexual spores) are mostly distributed by wind or animals. Under laboratory conditions, inoculations are performed by brushing, leaf-to-leaf transfer or dusting of spores from infected material onto healthy plants (Micali et al., 2008). Once situated on a plant leaf or stem, the PM spore develops a short germ tube (Figure 2), and approximately six hours post inoculation (hpi), the appressorium, a thickened infection structure, forms at the tip of this hypha. At least in the case of *Bgh* the appressorium builds up high pressure in order to breach the plant cuticle and cell wall (Pryce-Jones et al., 1999). Unlike in many other plant-pathogenic fungi, cell wall-degrading enzymes seem to play a minor role in host cell invasion, as *Bgh* has a comparatively low number of genes encoding such carbohydrate-active enzymes (CAZymes; Spanu et al., 2010). After successful cell wall penetration, the fungus enters the host cell without disrupting the host plasma membrane and the haustorium, a specialized hyphal feeding structure with protrusions for surface enlargement, is formed (12-14 hpi; Figure 2). Haustorium development involves the formation of the extrahaustorial membrane (EHM), which separates plant and fungal structures. The haustorium represents the major interaction site between the fungus and the host plant, and it is supposed to be the hub for effector secretion and nutrient uptake (reviewed in O'Connell and Panstruga, 2006). Supposedly once the haustorium is established, the fungus gains the nutrients necessary for its epiphytic growth. This becomes visible as secondary hyphae forming the PM colony (from ca. 24-48 hpi onwards; Figure 2). Further, the secondary hyphae form new appressoria and penetrate nearby cells. The cycle concludes by the formation of conidiophores, specialized hyphae giving rise to new conidiospores (3-7 days post inoculation (dpi); Figure 2). Sporulation of the two preferentially studied Arabidopsis-infecting PMs, *Gc* UCSC1 and *Go*, becomes macroscopically visible at 7 to 10 dpi (see Figure 6).

In temperate climates, PM fungi have to overwinter periods during which the host plant is either not present (annual plants) or defoliates (perennial plants). To cope with such conditions, the fungal pathogen can engage in sexual reproduction based on two compatible mating types. This process gives rise to endurable ascospores (meiospores) enclosed in asci, emerging from fruiting bodies (cleistothecia or chasmothecia). These structures form in the mesophyll and are visible as black-brownish spots on leaves

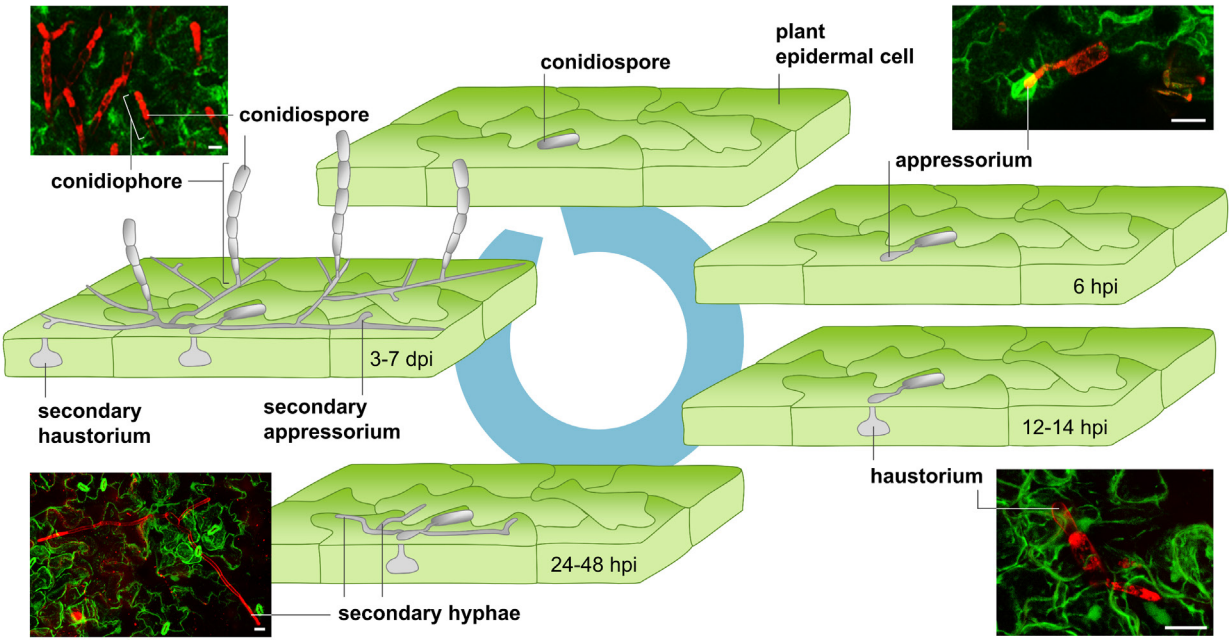

Figure 2. Asexual life cycle of *G. orontii* in association with Arabidopsis.

The central part of the figure illustrates schematically the key steps of the life cycle, while the micrographs show the actual fungal infection structures. The confocal laser scanning micrographs were obtained from transgenic Col-0 plants stably expressing yellow cameleon inoculated with *Go*. Fungal infection structures were stained with FM4-64 (shown in red) while green fluorescence is representative of cytosolic yellow cameleon fluorescence. Bars: 20 µm.

in fall. The ascospores mature within the ascus and are able to persist for longer periods outside the host plant. Meta-analysis of genomic data, however, suggests that at least in the case of grass PMs sexual reproduction is a comparatively rare event (Hacquard et al., 2013; Wicker et al., 2013). To our knowledge, the formation of fruiting bodies has not been demonstrated on Arabidopsis.

2.2 The haustorium

The PM haustorium is the only fungal structure that resides within the plant, namely inside plant epidermal cells (with the exception of *L. taurica* and *P. guttata*; see section 2.1). As mentioned above, this structure likely represents the main interaction site between the plant and the fungus (O'Connell and Panstruga, 2006). However, four layers separate the haustorial cytoplasm from the plant cytoplasm: the haustorial plasma membrane, the fungal cell wall, the extrahaustorial matrix (EHMx), and the EHM. The EHM is a plant-derived membrane surrounding the haustorium. Despite the continuity of the EHM with the host plasma membrane, its composition is, however, distinct from the latter (Koh et al., 2005; O'Connell and Panstruga, 2006; Micali et al., 2011). The EHM attaches to the haustorial neck, the contact site of the haustorium and the plant cell wall, which separates the EHMx from the apoplast (Gil and Gay, 1977). The EHMx forms the transition zone between plant and fungus and is supposed to enable both nutrient uptake and effector delivery (Bushnell, 1972).

Mature *Go* haustoria are typically ca. 16 µm wide and 10 µm long elliptic bodies with finger-like projections coiled around the main body (Figure 3A-B; Micali et al., 2011). They contain a single nucleus and numerous mitochondria. In addition, the haustorial cytoplasm and the EHMx comprise a high number of vesicles, potentially due to fusion of multi-vesicular bodies (MVBs) with the plasma membrane resulting in the release of cargo vesicles into the EHMx (exosomes; Figure 3A). On the plant side, the endoplasmic reticulum (ER) and plant MVBs locate close to the EHM (Micali et al., 2011).

Mature haustoria are often fully or partially encapsulated by encasements and cell wall appositions enclosing the EHM and EHMx, even during the compatible interaction between *Go* and Arabidopsis (Figure 3C). In fact, 20-55 % of *Go* haustoria are encased to different degrees. These encapsulations depend on the age of the haustorium and contain β-1,3-polyglucans (e.g. callose), xyloglucans, rhamnogalacturonans, and arabinogalactan proteins. Deposition starts at the haustorial neck and gradually encloses the maturing haustorium (Meyer et al., 2009; Micali et al., 2011). Compared with papillae (see section 3.3), which represent multi-layered focal cell wall reinforcements (Naumann et al., 2013), encasements seem to comprise a uniform single layer surrounding the haustoria (Micali et al., 2011). Although they typically contain callose, the formation of these encapsulations is independent from the pathogen-induced callose synthase GLUCAN SYNTHASE-LIKE 5/POWDERY MILDEW RESISTANT 4 (GSL5/PMR4: At4g03550), suggesting that in the absence of the enzyme other cell wall polymers replace the β-1,3-polyglucan

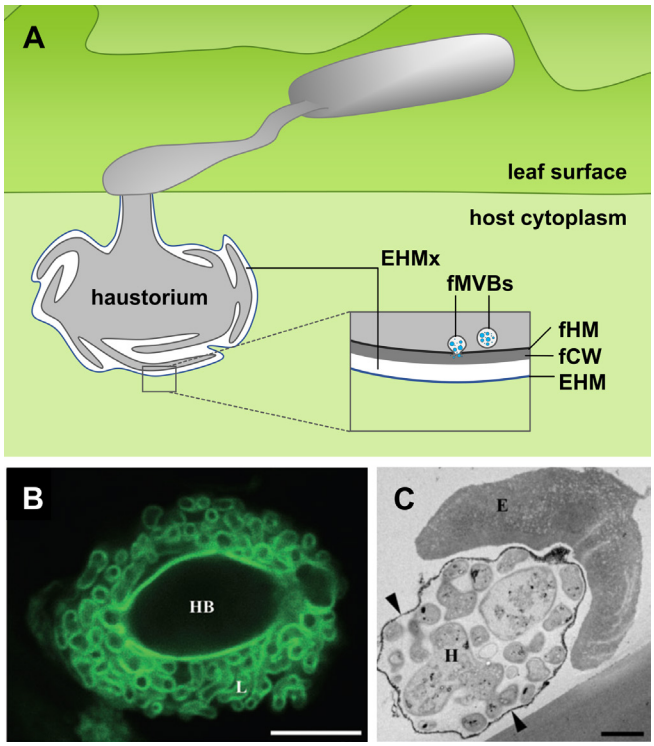

Figure 3. The PM haustorium.

The fungal haustorium forms within cells of the leaf epidermis after penetration. **A.** Scheme of a PM haustorium (grey) separated from the plant cytoplasm by fungal haustorial membrane (fHM), fungal cell wall (fCW), extrahaustorial matrix (EHMx) and extrahaustorial membrane (EHM). The inset depicts the proposed exocytosis of fungal multivesicular bodies (fMVBs) **B.** Wheat germ agglutinin staining of chitin in an isolated haustorium of *Go*. The confocal laser scanning micrograph shows a mature haustorium body (HB) with numerous haustorial lobes (L). **C.** Partial callose encasement of an isolated *Go* haustorium. The electron-opaque EHM (arrowheads) surrounds the haustorium (H) but not the callose-containing encasement (E). Bars: **B** 5 µm; **C** 2 µm. Panels **B** and **C** reproduced with permission from (Micali et al., 2011) (Copyright by John Wiley & Sons (Cellular Microbiology)).

(Meyer et al., 2009). The hypothesis that encasements indicate incomplete adaption of *Go* to Arabidopsis is supported by the fact that they are absent in interactions with *Gc* (Koh et al., 2005; Meyer et al., 2009). Moreover, haustoria of *Bgh* are encapsulated in leaves of the nonhost plant Arabidopsis, but not in leaves of its host plant barley, indicating that *Bgh* effectively suppresses the encasement of haustoria in a suitable host (Meyer et al., 2009).

3. FIRST LINE OF DEFENSE

3.1 MAMP-triggered responses

The first barriers PM pathogens encounter during infection are the cuticle and epicuticular waxes overlying the plant cell wall (Malinovsky et al., 2014). As mentioned above (see section 2.1), PMs presumably employ mainly hydrostatic pressure to penetrate this preformed perimeter of epidermal cells. Accordingly, the plant can sense the pathogen in several ways. Firstly, the pressure exerted on the plant cell might activate plant mechanosensors (Bhat et al., 2005; Ellinger and Voigt, 2014a). Secondly, damage-associated molecular patterns (DAMPs) released by the breakdown of the plant cell wall, or microbe-associated molecular patterns (MAMPs) released by the fungus, can be detected by pattern recognition receptors (PRRs) and activate immune signaling (Boller and Felix, 2009).

The carbohydrate polymer chitin is a major constituent of fungal cell walls and when exogenously applied to Arabidopsis activates MAMP-triggered immune responses. Chitin is perceived by the membrane-localized PRRs CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1: At3g21630) (Miya et al., 2007) and the LYSIN MOTIF RECEPTOR-LIKE KINASEs 4/5 (LYK4/5: At2g23770/At2g33580) (Cao et al., 2014). Application of MAMPs, including chitin, leads to the accumulation of defense-related proteins and the deposition of callose at seemingly random locations in treated tissues (Gómez-Gómez et al., 1999; Luna et al., 2011; Underwood and Somerville, 2013). This phenomenon is similar to the localized formation of papillae (see section 3.3) and suggests that MAMP-induced PRR activation alone can trigger the establishment of papilla-like structures. The adapted PM *Gc* shows increased sporulation on *cerk1* mutants in comparison to wild type plants, which suggests that signaling through CERK1 contributes to basal resistance to the PM disease (Wan et al., 2008). It is presently unknown whether *lyk4* or *lyk5* mutants are more susceptible to PM as well. Presumably, plants can perceive further PM-derived MAMPs. However, the only other known molecule from a PM pathogen that activates defense gene expression and decreases fungal growth in various cereals after application is a soluble carbohydrate elicitor isolated from conidia of the wheat PM pathogen, *Blumeria graminis* f.sp. *tritici* (*Bgt*) (Schweizer et al., 2000).

3.2 Papilla formation

Plant cell responses to the early detection events mentioned above include polarization of cellular organelles and rearrangement of cytoskeletal elements (microtubules and actin filaments) below the attack site (Schmelzer, 2002; Hückelhoven and Panstruga, 2011). Underneath the attempted penetration site defense-related proteins focally accumulate (Assaad et al., 2004; Bhat et al., 2005; Kwon et al., 2008b; Meyer et al., 2009; Kwaaitaal et al., 2010). Furthermore, the plasma membrane is altered locally and gains lipid raft-like properties (Bhat et al., 2005). Both virulent and non-virulent PM fungi induce the formation of a small, dome-like structure called papilla below the incipient fungal appressorium (Collins et al., 2003; Assaad et al., 2004; Koh et al., 2005). Among other components such as membranous vesicles, the papilla contains callose (Figure 4), silicon, reactive oxygen species (ROS) and phenolic compounds. The resulting structure is believed to reinforce the cell wall to prevent fungal invasion (Zeyen et al., 2002). This hypothesis is supported by a correlation between the timing of papilla formation and PM resistance. Mutation of the target membrane SOLUBLE N-ETHYLMALEMIDE-SENSITIVE FACTOR ATTACHMENT PROTEIN RECEPTOR (t-SNARE) PENETRATION

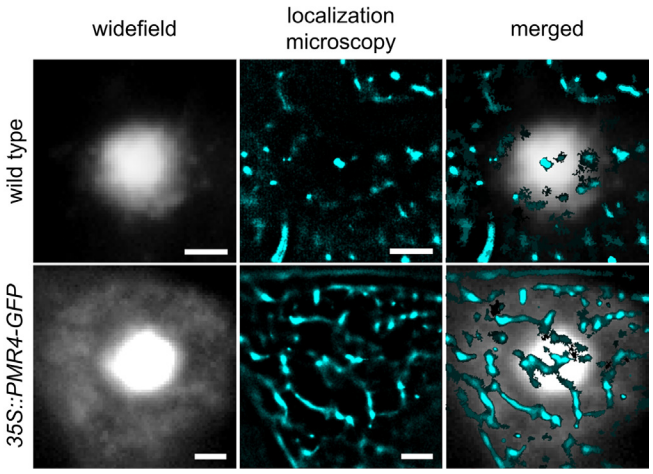

Figure 4. Nanoscale resolution of callose polymer fibrils in pathogen-induced cell wall papillae.

Three-week-old Arabidopsis wild type and pathogen-resistant *PMR4-GFP* overexpressing lines (*P35S::PMR4-GFP*) were inoculated with the adapted powdery mildew *Gc*. Localization microscopy (dSTORM: direct stochastical optical reconstruction microscopy) of aniline blue-stained callose polymer fibrils in pathogen-induced papillae at sites of attempted fungal penetration at 12 hpi in rosette leaves. Scale bars = 2 µm. Unpublished micrograph, courtesy of Christian A. Voigt and Dennis Eggert.

(PEN)1/SYNTAXIN OF PLANTS (SYP)121 (At3g11820) and the vesicle-associated SNAREs VESICLE-ASSOCIATED MEMBRANE PROTEINs (VAMPs)721/722 (At1g04750/At2g33120) delays papilla formation and increases *Bgh* penetration success (Assaad et al., 2004; Kwon et al., 2008b; Böhlenius et al., 2010). The ubiquitin ligase ARABIDOPSIS TOXICOS EN LAVADURA 31 *(*ATL31: At5g27420), which is a regulator of responses to changes in the cellular carbon/nitrogen ratio, interacts with PEN1 in co-immunoprecipitation experiments. Transient expression assays in *Nicotiana benthamiana* leaves inoculated with *Bgh* show accumulation of ubiquitination activity deficient ATL31^{C143S}-GFP around papillae and in vesicle-like structures near papillae, while fluorescence is undetectable for ATL31-GFP. Furthermore, overexpression of ATL31 in Arabidopsis *pen1*-1 mutants results in enhanced penetration resistance to *Bgh* and faster formation of papillae (Maekawa et al., 2014). The same observation was made for *RAB GTPASE HOMOLOG 4c (RABA4c:* At5g47960*)* overexpression lines (Ellinger et al., 2014), suggesting that only the timely formation of papillae can restrict invasion by PM fungi.

3.3 Callose deposition

Accumulation of the β-1,3 polyglucan callose, as the major constituent of papillae, is a generic response to pathogen challenge (Ellinger and Voigt, 2014b). Together with the β-1,4 polyglucan cellulose, callose generates a three-dimensional network of ~250 nm fibrils, which can provide protection against cell wall hydrolysis by fungal enzymes (Figure 4; Eggert et al., 2014). Callose could not be unambiguously linked to resistance for a long time, and work on a mutant of the *GSL5/PMR4* gene initially even suggested that loss of papillary callose reduces sporulation of adapted PMs (Jacobs et al., 2003; Nishimura et al., 2003). However, the inhibition of post-invasive fungal growth in *pmr4* mutants relies on hyper-induced salicylic acid (SA) responses upon PM attack. When the *pmr4* knockout mutation is combined with a further mutation that leads to a loss of SA biosynthesis or signaling the increased resistance is compromised (Nishimura et al., 2003). Except for the loss of callose, papillae of *pmr4* mutant plants have a similar appearance as papillae of wild type plants (Nishimura et al., 2003). While loss of callose in the *pmr4* mutant has only limited impact on penetration resistance (Jacobs et al., 2003; Ellinger et al., 2013), increased callose deposition after PM attack caused by *PMR4* overexpression results in full penetration resistance to both *Gc* and *Bgh*. The latter effect seems to correlate with structural differences of papillae in *PMR4* overexpression lines compared to the wild type, as the transgenic lines show larger cores of callose-dense deposits, whereas wild type papillae display a more diffuse structure (Naumann et al., 2013). Together these findings indicate that additional papillary components support the contribution of callose to prevent fungal penetration (Ellinger et al., 2013). The PMR4-GFP fusion protein focally accumulates at the PM attack site and its presence coincides with the occurrence of callose deposits. The callose accumulations in the *PMR4-GFP* overexpression line are not only enlarged, but also deposited in a layer facing the fungus on top of the cellulose microfibrilar network (Eggert et al., 2014). The increase in the proportion of callose presumably protects the cellulose component of papillae from enzymatic digestion (Eggert et al., 2014). In contrast to the increased post-penetration resistance in *pmr4* mutants, the increase in resistance caused by *PMR4-GFP* overexpression is independent from SA- or jasmonic acid (JA)-mediated defense (Ellinger et al., 2013).

Similar to what was reported for barley (Böhlenius et al., 2010), ADP ribosylation factor-GTP exchange factor (ARF-GEF)-mediated vesicle trafficking is essential for callose accumulation in papillae in Arabidopsis (see section 7; Nielsen et al., 2012). This ARF-GEF-dependence indicates that either PMR4 accumulation at fungal attack sites, the delivery of callose precursors, and/or the callose deposition process itself involves vesicle-mediated transport processes. PMR4 interacts with and acts as an effector of the small GTPase of the Ras (rat sarcoma) superfamily, RABA4c (Ellinger et al., 2014). *RABA4c* expression is transiently upregulated prior to callose deposition in response to biotic stress. Knockouts of *RABA4c* exhibit a delayed increase of callose synthase activity, slightly reduced numbers of callose deposits, and slightly increased *Gc* penetration rates. By contrast, overexpression of *RABA4c* results in full penetration resistance to *Gc* and hastens and increases callose deposition. Both effects depend on the presence of PMR4 and RABA4c GTPase activity. The RABA4c localization to membranes is independent on the prenylation of its C-terminal CaaX motif (‘C’ cysteine, ‘a’ aliphatic amino acid, ‘X’ variable amino acid). A C-terminal RABA4c-mCitrine fusion lacking this lipid modification still localizes to membranes, though solely when PMR4 is present, which supports the finding that both proteins physically interact *in planta* (Ellinger et al., 2014). Rab (Ras-related in brain) GTPases play major roles in virtually all vesicle trafficking processes in eukaryotic cells. To what extent PMR4 localization to the plasma membrane or to fo-

cal accumulation sites depends on RABA4c-dependent vesicle trafficking pathways is unknown.

3.4 Extracellular deposition of proteins into papillae

The discovery that components of a SNARE protein complex are involved in penetration resistance suggests that these proteins directly control vesicle fusion at the PM attack site (Collins et al., 2003; Kwon et al., 2008a; Kwon et al., 2008b). After vesicle fusion and cargo release, SNARE proteins are usually recycled and stay on the cytosolic side of the plasma membrane (Kwon et al., 2008a). Surprisingly, in case of the focal accumulation of SNARE proteins at attempted fungal entry sites this is not the case. Instead, fluorescent fusions of PEN1, SOLUBLE N-ETHYLMALEIMIDE-SENSITIVE FACTOR ADAPTOR PROTEIN (SNAP)33 (At5g61210; a t-SNARE) and the ATP-binding cassette (ABC) transporter PEN3 (At1g59870) accumulate within papillae and haustorial encasements and therefore end up in the extracellular (apoplastic) space. Within cell wall appositions, GFP-PEN1 co-localizes with the lipophilic fluorescent tracer of endosomes, FM4-64, indicating that membrane material co-accumulates with these proteins in papillae and haustorial encasements (Meyer et al., 2009; Nielsen et al., 2012). As demonstrated by electron microscopy and co-localization with the Rab-like GTPase MVB marker ARA6/RABF1-GFP (At3g54840), MVBs focally accumulate at pathogen attack sites (see Figure 7). It is therefore conceivable that MVBs contribute to extracellular deposition of otherwise intracellularly localized proteins (An et al., 2006; Meyer et al., 2009; Nielsen et al., 2012). According to this hypothesis, vesicles containing PEN1, SNAP33 and PEN3 may sort and incorporate into the lumen of MVBs after endocytosis. These MVBs might subsequently fuse with the plasma membrane, which could explain extracellular protein delivery (Meyer et al., 2009). The extent and significance of the extracellular deposition of otherwise intracellular proteins for PM resistance is currently unknown.

3.5 Silicon-mediated resistance

Silicon (Si) contributes to PM resistance of cereals and various other plant species (Fauteux et al., 2005). Accordantly, watering Arabidopsis plants with silicon results in a lower PM disease incidence although Arabidopsis lacks dedicated Si transporters (Ghanmi et al., 2004). Accumulation of insoluble Si at PM attack sites led to the hypothesis of Si acting as a simple physical barrier (Bélanger et al., 2002). However, not in every case presence of insoluble Si correlates with increased resistance to fungal penetration. Consequently, a physiological or biochemical role in mediating cellular resistance has been postulated (Bélanger et al., 2003). While Si fertilization alone has a minor effect on transcript abundance, *Gc* inoculation of Si fertilized plants versus *Gc* inoculation of non-Si supplemented plants attenuated the magnitude of PM-induced down-regulation of genes by more than 25 % (Fauteux et al., 2006). As many of these PM-repressed genes are related to primary metabolism, the Si-mediated reduced downregulation might indicate stress alleviation. Consequently, Si feeding potentially facilitates a more efficient response to PM infection (Fauteux et al., 2006). This hypothesis is further corroborated by transgenic Arabidopsis plants stably expressing the wheat Si transporter *Ta*Lsi1. These plants have increased Si levels and concomitantly further enhanced PM resistance in the presence of Si compared to wild type plants (Vivancos et al., 2015).

4. SECOND LINE OF DEFENSE: RPW8-MEDIATED BROAD-SPECTRUM RESISTANCE

In many plant species that can be colonized by PM fungi, dedicated dominantly or semi-dominantly inherited resistance (*R*) genes provide isolate-specific protection as a second line of defense against the disease (Chelkowski et al., 2003; Bai et al., 2005; Marone et al., 2013). These types of genes typically encode canonical nucleotide binding site-leucine-rich repeat (NB-LRR/NLR) proteins (Takken and Goverse, 2012). *R* genes occur typically in multiple allelic forms within plant populations. These polymorphic variants are effective against particular pathogen isolates encoding effectors that are recognized by the respective R proteins ("gene-for-gene relationship"; Flor, 1971). It is thought that plant R proteins either directly or indirectly associate with cognate effector proteins to trigger a boosted defense output that often culminates in a hypersensitive response (HR) associated with local host cell death, thereby restricting pathogen proliferation (Dangl and Jones, 2001).

Notably, prototypical *R* genes that are effective against PM fungi have not been found in Arabidopsis yet. Instead, a polymorphic genetic locus, *RESISTANCE TO POWDERY MILDEW 8* (*RPW8*), harboring two unconventional non-NB-LRR type PM resistance genes, is a major source of resistance in Arabidopsis. The *RPW8* locus has a complex arrangement that differs between Arabidopsis accessions. In the resistant ecotype Ms-0 the *RPW8* locus harbors five gene copies that encode small sequence-related basic proteins with a predicted N-terminal transmembrane domain and one or two C-terminal coiled-coil domains. Of these, two paralogs (tandemly arranged *RPW8.1* and *RPW8.2*) contribute to effective resistance against PM, while other paralogs (*HR1*, *HR2* and *HR3*) of the *RPW8* locus are inactive in this respect (Xiao et al., 2001). Phylogenetic analysis on the basis of syntenic loci in the Arabidopsis relatives *Arabidopsis lyrata*, *Brassica rapa* and *Brassica oleracea* suggests that *RPW8.1* and *RPW8.2* likely evolved from a *HR3*-like ancestor gene through a series of gene duplication events and subsequent diversification by positive selection (Xiao et al., 2004).

The PM resistance-conferring *RPW8* locus, which was originally described in the accession Ms-0 (Xiao et al., 1997), shows a widespread distribution in Arabidopsis populations. Most PM resistant accessions contain a "functional" version of *RPW8.1* and/or *RPW8.2*. The locus is thus a major source of natural PM resistance in Arabidopsis (Orgil et al., 2007; Göllner et al., 2008). Notably, the Col-0 reference accession lacks functional copies of *RPW8.1*/*RPW8.2* and is thus susceptible to all known PM species that are capable to colonize Arabidopsis plants (Xiao et al., 2001). Resistance mediated by *RPW8* occurs after the establishment of haustoria and is typically associated with the accumulation of hydrogen peroxide and localized host cell death, although these responses exhibit some degree of plasticity in different ecotypes

(Xiao et al., 2001; Göllner et al., 2008). *RPW8.1* and *RPW8.2* operate through a SA-dependent positive feedback loop, which also promotes transcript accumulation of the two genes (Xiao et al., 2003). Consistently, *RPW8*-mediated PM resistance requires components of SA signaling (*ENHANCED DISEASE SUSCEPTIBILITY (EDS)1* (At3g48090) and *EDS5* (At4g39030), *PHYTOALEXIN-DEFICIENT (PAD)4* (At3g52430), *ARABIDOPSIS NON-EXPRESSER OF PR GENES (NPR)1:* At1g64280)) that also play a role in basal defense (Xiao et al., 2005). Overexpression or ectopic expression of RPW8 proteins leads to enhanced resistance against diverse biotrophic pathogens (cauliflower mosaic virus and the oomycete *Hyaloperonospora arabidopsidis*), but more pronounced susceptibility to necrotrophic pathogens (*Alternaria* and *Botrytis* ssp.; Wang et al., 2007; Ma et al., 2014).

Although RPW8 function in the context of PM infection is rather evident, information on the protein and its *in planta* activity is limited. Yeast two-hybrid assays identified 14-3-3λ (AT5g10450) and the PHYTOCHROME-ASSOCIATED PROTEIN PHOSPHATASE TYPE 2C (PAPP2C: At1g22280) as potential RPW8.2 interactors (Yang et al., 2009; Wang et al., 2012). While genetic evidence suggests that the 14-3-3 protein is a positive regulator of RPW8 function, PAPP2C seems to be a negative regulator of cell death and PM resistance. Notably, following PM attack RPW8.2 accumulates at the EHM (Figure 5; Wang et al., 2009). In fact, RPW8.2 was the first protein described to localize to this specialized membrane compartment. At the EHM, RPW8.2 activates defense signaling *via* SA and promotes the localized accumulation of hydrogen peroxide and encasement of the haustorial complex (Wang et al., 2009). Targeting of RPW8.2 to the EHM occurs independently of SA accumulation, but requires actin function and involves transport on secretory vesicles (Wang et al., 2009; Kim et al., 2014). Interestingly, ectopic expression of RPW8.1-YFP or RPW8.2-YFP from the respective native promoters, mutually exchanged promoters, or the constitutive viral 35S promoter, results in distinct localization patterns of the proteins and differential resistance phenotypes against PMs. Precise spatiotemporal expression thus appears to be a prerequisite for proper RPW8.2 function (see section 7; Figure 5; Wang et al., 2010; Ma et al., 2014).

To obtain a better understanding of the RPW8.2 protein domains that contribute to its subcellular localization and defense activity, Wang and co-workers functionally analyzed more than one hundred RPW8.2 variants regarding their trafficking and defense properties (Wang et al., 2013). This study revealed single amino acid residues that are critical for the antifungal activity and the induction of cell death. It also uncovered two short stretches rich in basic amino acids that together with the predicted N-terminal transmembrane domain define a core targeting signal for the EHM. This region, which comprises 60 amino acids in total, is necessary and sufficient for localization of RPW8.2 to the EHM. Based on the mis-localization of some RPW8.2 mutant variants to the nucleus and/or plastidic stromules the authors propose the existence of a dedicated membrane trafficking pathway towards the EHM (Wang et al., 2013). Notably, the two short basic stretches that contribute to EHM localization apparently also play a role in nucleocytoplasmic trafficking of RPW8.2, suggesting that a portion of the RPW8.2 pool might have a function in the nucleus (Huang et al., 2014). Overexpression of non-functional, yet EHM-targeted RPW8.2 versions can exert a dominant-negative effect

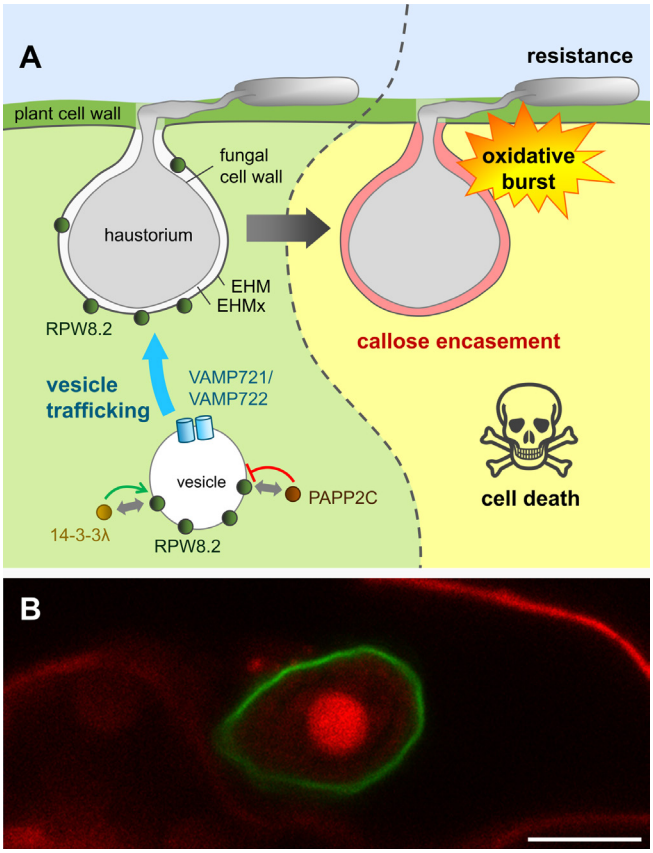

Figure 5. RPW8 localizes at the EHM and contributes to cell death upon PM infection.

A. Scheme depicting RPW8.2 function. Left: RPW8.2 interactors and RPW8.2 deposition at the EHM. Right: RPW8.2-triggered oxidative burst and callose-encasement of haustoria correlates with subsequent host cell death. **B.** Confocal laser scanning micrograph of GFP-labelled RPW8.2 (green) in the EHM. Red, propidium iodide stained plant and fungal structures. Bar = 10 µm. Unpublished micrograph, courtesy of Wenming Wang and Shunyuan Xiao.

on functional RPW8.2, thereby compromising RPW8.2-mediated PM resistance. Such dominant-negative RPW8.2 variants also affect basal defense against PM and result in an enhanced disease susceptibility (*eds*) phenotype, suggesting the existence of further EHM-localized factors that contribute to basal levels of post-penetration resistance in Arabidopsis (Zhang et al., 2015b).

Widespread presence of a locus that confers broad-spectrum PM resistance (*RPW8*) might explain why no canonical cytoplasmic NB-LRR type R proteins against this disease evolved in Arabidopsis. Although such genes are seemingly lacking in natural Arabidopsis populations, a heterologously expressed R protein from monocotyledonous barley can confer isolate-specific PM resistance in Arabidopsis. Transgenic expression of the barley MILDEW RESISTANCE LOCUS A1 (MLA1) coiled-coil NB-LRR-type resistance protein in a partially immunocompromised mutant background (*pen2 pad4 senescence-associated gene 101* (*sag101*: At5g14930)) results in isolate-specific resistance

against the matching barley PM *Bgh* (see section 9.3; Maekawa et al., 2012)). This remarkable finding suggests that the signaling machinery acting downstream of MLA1 activation is conserved between monocotyledonous and dicotyledonous plant species, two lineages that diverged ca. 200 million years ago. MLA1 function in resistance responses towards PM in Arabidopsis does not require SA, JA or ethylene (ET). As in barley, in Arabidopsis MLA1 exhibits nucleocytoplasmic partitioning and its activation upon PM inoculation results in pronounced and sustained transcriptional reprogramming (Maekawa et al., 2012).

5. NONHOST RESISTANCE

As mentioned, PM species either can have a wide or narrow host range or might even be specialized to a single host plant species. For example, *Go* is virulent on Arabidopsis, on other Brassicaceae species, as well as on Solanaceae and Cucurbitaceae species, but does not infect Rosaceae or Asteraceae (Plotnikova et al., 1998). In contrast to *Go*, the pea PM pathogen *E. pisi* and the barley PM *Bgh* are not able to cause disease on Arabidopsis, as they show little penetration success and no completion of their asexual life cycle. Consequently, they do not give rise to any visible epiphytic colonization and symptom formation (Lipka et al., 2005). This is essentially due to NHR of plants against pathogens, which by definition is resistance of an entire plant species against all genetic variants of a microbial species (Lipka et al., 2005; Nürnberger and Lipka, 2005). Mechanistically, NHR seems to be equivalent to basal defense or innate immunity and supposedly relies mainly on preformed defenses and MAMP-triggered immune responses (Thordal-Christensen, 2003; Nürnberger and Lipka, 2005). Accordingly, components involved in NHR often not only contribute to defense against non-adapted, but also against adapted pathogens. In the case of filamentous phytopathogens, NHR can be subdivided in pre- and post-invasive resistance. Pre-invasive NHR restricts the penetration of fungal and oomycete pathogens, including PMs, whereas post-invasive NHR eventuates if the non-adapted pathogen succeeds in host cell entry, and frequently results in an HR associated with local host cell death. This response is mainly effective against biotrophic pathogens as it deprives the invader of nutrients (Glazebrook, 2005).

Several main components from two distinct pathways of pre-invasive NHR have been identified so far. The first pathway relies on PEN1, which is believed to mediate exocytosis of potentially harmful cargo upon pathogen attack by forming ternary SNARE complexes with SNAP33 and VAMP721/722 (see section 7; Kwon et al., 2008b; Kwaaitaal et al., 2010). The second pathway includes PEN2 (At2g44490) and PEN3. PEN2 is a tail-anchored β-thioglucoside glucohydrolase that synthesizes indole glucosinolates from a tryptophan-derived precursor (Bednarek et al., 2009; Clay et al., 2009; Fuchs et al., 2015). It contains a carboxy terminal tail anchor that targets the protein to peroxisomal and outer mitochondrial membranes. Requirement of the CYTOCHROME P450 monooxygenase CYP81F2 (At5g57220) for PEN2-mediated resistance to PM penetration indicates its involvement in pathogen-induced production of 4-substituted indol-3-ylmethyl-glucosinolate (I3G) substrates of PEN2 (Bednarek et al., 2009; Clay et al., 2009). Consistently, CYP81F2-RFP localizes to the ER membrane, focally accumulating at sites of *Bgh* attack. This suggests that PEN2 substrate production occurs in close proximity to PEN2-decorated mitochondrial subpopulations that are recruited to sites of attempted fungal invasion (Fuchs et al., 2015). The products of PEN2 are thought to be exported by the PEN3 ABC transporter (Stein et al., 2006). Mutants of *PEN3* result in pathogen-inducible, PEN2-dependent over-accumulation of an indole compound (4-*O*-β-D-glucosyl-indol-3-yl formamide) in leaves. This suggests that PEN3 is involved in the transport of this indole or a precursor during pathogen defense (Lu et al., 2015). PEN3 interacts with calmodulin (CAM)7 (At3g43810) and *cam7* mutant plants are more susceptible to the non-adapted fungal pathogens *Bgh* and the Asian soybean rust fungus *Phakopsora pachyrhizi*, demonstrating that CAM7 and therefore Ca^{2+} sensing/transmission of Ca^{2+} signals is an important factor of NHR in Arabidopsis (Campe et al., 2015). Fluorophore-tagged PEN1, PEN2 and PEN3 focally accumulate at PM penetration sites (Assaad et al., 2004; Lipka et al., 2005; Stein et al., 2006). Recruitment of PEN1 and PEN3 fusions with GFP to infection sites can be triggered by MAMPs, but distinct mechanisms contribute to the transport of the two proteins (Underwood and Somerville, 2013). Interestingly, PEN1 and PEN3 accumulate in the apoplast at sites of papilla formation (see section 3.4; Meyer et al., 2009; Underwood and Somerville, 2013). Importantly, although the *pen* and *cam7* mutants allow increased host cell entry, subsequent host cell death due to post-penetration NHR restricts infection success.

Key components of post-invasive NHR are EDS1, PAD4 and SAG101, all of which are required, to different degrees, for full resistance against various pathogens (Feys et al., 2005; Wiermer et al., 2005; Rietz et al., 2011; Wagner et al., 2013). Single mutations in *EDS1*, *SAG101* and *PAD4*, and the respective double mutants in combination with *pen2* are insufficient to allow sporulation of *Bgh* and *E. pisi* on Arabidopsis. However, the pre- and post-invasive NHR deficient mutant *pen2 pad4 sag101* enables these non-adapted pathogens to form secondary hyphae. *E. pisi* even causes macroscopically visible PM symptoms resulting from moderate conidiation, and *Bgh* occasionally forms conidiospores (Lipka et al., 2005).

Besides extracellular papilla formation (see section 3.2), a prominent aspect of pre-invasive NHR is the focal accumulation of various cellular components and organelles towards sites of attempted pathogen invasion. These structures include secretory vesicles, peroxisomes, mitochondria and the ER (Koh et al., 2005; Böhlenius et al., 2010). In Arabidopsis these rearrangements further comprise the accumulation of proteins with defense functions, e.g. the PEN proteins (Assaad et al., 2004; Lipka et al., 2005; Stein et al., 2006) and the callose synthase PMR4/GSL5 (Ellinger et al., 2013), at *Bgh* attack sites. This focal aggregation requires reorganization of the actin cytoskeleton towards sites of fungal ingress, emphasizing the central role of actin-based transport processes in pre-invasive NHR and plant antifungal immunity in general (see section 7; Takemoto et al., 2006; Underwood and Somerville, 2008; Feechan et al., 2011; Underwood and Somerville, 2013; Yang et al., 2014).

Another component of the NHR to PM is the Arabidopsis phospholipase Dδ (PLDδ: At4g35790), which is involved in the biosynthesis of phosphatidic acid (Wang, 2004). Phosphatidic

acid can serve as a precursor for membrane phospholipids or as a signaling molecule and may play a role in plant defense, as its levels increase after MAMP perception or recognition of various pathogen effectors (van der Luit et al., 2000; de Jong et al., 2004; Andersson et al., 2006; Kirik and Mudgett, 2009). A PLDδ fusion with GFP accumulates around papillae at sites of attempted *Bgh* penetration. Additionally, the *pldδ* mutant allows increased cell entry by *Bgh* and *E. pisi*, and shows delayed up-regulation of early MAMP-responsive genes after chitin treatment (Pinosa et al., 2013).

Finally, the phytohormone abscisic acid (ABA) seems to be involved in NHR against PMs. The NAC transcription factor (TF) ARABIDOPSIS THALIANA ACTIVATING FACTOR1 (ATAF1: At1g01720) contributes to defense against *Bgh*. Loss of ATAF1 partially compromises *Bgh* penetration resistance, which correlates with the induction of ABA biosynthesis and transcript accumulation of ABA-responsive genes (Jensen et al., 2007; Jensen et al., 2008). By contrast, endogenous ABA levels are decreased after inoculation of wild type plants with *Bgh*. ATAF1-dependent suppression of ABA levels after pathogen challenge suggests that ATAF1 acts as attenuator of ABA signaling in order to mediate efficient penetration resistance against *Bgh* (Jensen et al., 2008).

6. SUSCEPTIBILITY FACTORS

As discussed in the previous chapters, the strictly biotrophic PM fungi have to overcome plant defense responses in order to complete their life cycle (Panstruga, 2003). Furthermore, they need to exploit the host cell's infrastructure to establish the haustorium, their presumed feeding structure (Mendgen and Hahn, 2002; Schulze-Lefert and Panstruga, 2003). During compatible interactions, specific host genes, operationally termed compatibility or susceptibility factors, are found to be crucial for successful pathogenesis by a specific pathogen, and lack of these factors results in resistance to this pathogen (Vogel and Somerville, 2000; Panstruga, 2003; Lapin and Van den Ackerveken, 2013). Two independent forward genetic screens, performed in the late 1990s, identified several PM compatibility factors (Frye and Innes, 1998; Vogel and Somerville, 2000).

6.1 *EDR* genes

Transcriptional activation of *PATHOGENESIS-RELATED* (*PR*) genes is one hallmark of induced defense (reviewed in Loake and Grant, 2007). During a genetic screen aimed to identify novel elements of plant defense, three mutants with enhanced disease resistance (*edr1* (At1g08720), *edr2* (At4g19040) and *edr3* (At3g60190)) that do not express *PR1* (At2g14610) upon inoculation with *Gc* were isolated (Frye and Innes, 1998; Frye et al., 2001; Tang and Innes, 2002; Tang et al., 2005a; Tang et al., 2005b; Tang et al., 2006). Interestingly, all three mutants show characteristics of "late-acting" resistance (i.e., at 5 to 8 dpi), which is associated with accelerated mesophyll cell death leading to macroscopic patches of lesions and either drastically reduced or absent sporulation. Genetic epistasis analysis revealed that *edr*-mediated resistance is SA-dependent and JA-independent (Frye and Innes, 1998; Tang et al., 2005b, 2006).

EDR1 encodes a mitogen-activated protein kinase kinase kinase (MAPKKK) that negatively regulates plant disease resistance (Frye et al., 2001). The *edr1* mutant displays enhanced cell death during infection with the adapted PM pathogen *Gc* and in response to drought stress (Frye et al., 2001; Tang et al., 2005b; Tang et al., 2005a). Cell death associated with *edr1* resistance requires the E3 ubiquitin ligases ATL1 (At1g04360) and KEEP ON GOING (KEG: At5g13530). Both E3 proteins are inhibited by interaction with EDR1, and the cell death phenotypes associated with *edr1* are suppressed upon their depletion, indicating that EDR1 acts as a negative regulator of programmed cell death (Serrano et al., 2014). KEG possibly recruits EDR1 to the *trans*-Golgi network (TGN) and in turn EDR1 regulates E3 ligase activity of KEG to further suppress cell death (see section 7; Gu and Innes, 2011; Liu and Stone, 2013). Overexpression of *ATL1* causes extensive cell death, which depends on its E3 ligase activity. Strikingly, knockdown of *ATL1* expression does not only interfere with *edr1*-mediated cell death, but causes hypersusceptibility to PM infection, demonstrating that ATL1 is a positive regulator of pathogen-induced cell death (Serrano et al., 2014). A further link of EDR1 to suppression of cell death is provided by its inhibitory interaction with mitogen-activated protein kinase kinase (MKK)4 (At1g51660) and MKK5 (At3g21220) that are part of the MAPK cascade fine-tuning plant immunity (see section 8.1; Zhao et al., 2014).

EDR2 encodes a mitochondrial protein with a pleckstrin homology domain and a steroidogenic acute regulatory protein-related lipid transfer (START) motif. Both EDR1 and EDR2 function in a common genetic pathway as evidenced by the *edr1 edr2* double mutant, showing resistance phenotypes that are indistinguishable from the respective single mutants (Tang et al., 2005b). In addition, *edr1* and *edr2* both display enhanced senescence in response to ET. Interestingly, mutations in the aminotransferase *AGD2-LIKE DEFENSE RESPONSE PROTEIN1* (*ALD1*: At2g13810) suppress *edr2*-mediated phenotypes including PM resistance, programmed cell death and ET-induced senescence, but not the *edr1 edr2* double mutant phenotype (Nie et al., 2011). This raises the question how EDR1 and EDR2 activities are coordinated during the regulation of defense, cell death and ET-induced senescence.

Different from *EDR1* and *EDR2*, *EDR3* seems to function in a separate pathway, since *edr3* does not display an early senescence phenotype. *EDR3* encodes a dynamin-like protein localized partially to mitochondria. Despite the absence of a constitutive cell death phenotype in Arabidopsis, the mammalian counterpart of EDR3 plays a role in regulating mitochondrial dynamics associated with programmed cell death (Tang et al., 2006).

Recently, a fourth *EDR* gene, *EDR4* (At5g05190), with unknown protein function and preferential localization of the gene product at the plasma membrane and endosomal compartments, has been isolated. Like previously identified EDRs, *EDR4* is involved in negative regulation of SA-dependent PM resistance (Wu et al., 2015). EDR4 functions in the same pathway as EDR1 and EDR2 and interacts with EDR1, recruiting it to fungal penetration sites. The shared phenotypic features of *edr* mutants suggest a general link between SA-mediated resistance, mitochondrial function and programmed cell death (Ausubel, 2005).

6.2 *PMR* genes

In a genetic screen with the aim to identify susceptibility factors involved in interactions between Arabidopsis and the PM pathogen *Gc*, six *powdery mildew resistant* mutants, *pmr1* to *pmr6*, were isolated. Four of the corresponding genes, namely *PMR2* (At1g11310), *PMR4/GSL5* (see section 2.2, 3.3 and 5), *PMR5* (At5g58600) and *PMR6* (At3g54920), have been cloned and to some extent functionally characterized (Vogel and Somerville, 2000; Vogel et al., 2002; Jacobs et al., 2003; Nishimura et al., 2003; Vogel et al., 2004; Consonni et al., 2006). The *pmr2* mutant is defective in *MILDEW RESISTANCE LOCUS O* (*MLO*)*2* (At1g11310), which encodes an integral membrane protein of unknown function (see section 6.3). *PMR5* belongs to a large plant-specific gene family of unknown function and *PMR6* encodes a glycosyl-phosphatidyl-inositol (GPI)-anchored pectate lyase-like protein (Vogel et al., 2002; Jacobs et al., 2003; Nishimura et al., 2003; Vogel et al., 2004; Consonni et al., 2006).

The latter *pmr* mutants, *pmr5* and *pmr6*, are believed to impact cell wall integrity, further stressing the contribution of the cell wall to PM resistance. The Arabidopsis *pmr5* mutant exhibits resistance to the adapted PM fungi *Gc* and *Go*, and enrichment of pectin as well as reduced pectin modification occurs in the cell walls of *pmr5* plants (Vogel et al., 2004). In addition, PMR5 contributes to PEN2-mediated pre-invasion resistance to the non-adapted fungus *Magnaporthe oryzae*. The *pen2 pmr5* double mutant shows enhanced penetration success of *M. oryzae* (Maeda et al., 2009), indicating that PMR5 is involved in host and nonhost resistance and emphasizing the importance of cell wall integrity for both types of resistance. PMR6 localizes at the plant cell wall, where it might degrade pectin. In line with this assumption, the *pmr6* mutant displays increased pectin and uronic acid contents. Like *pmr5*, the *pmr6* mutant is resistant to *Gc* and *Go,* which is in both cases independent of SA, ET and JA signaling (Vogel et al., 2002). The *pmr5 pmr6* double mutant shows increased resistance compared to the respective single mutants, suggesting that the two genes may function separately during plant defense. Furthermore, PMR5 and PMR6 are involved in the regulation of ploidy in mesophyll cells underlying the fungal feeding sites (see section 9.4; Chandran et al., 2013).

6.3 *MLO* genes

Arabidopsis *MLO* susceptibility genes were isolated and characterized based on their sequence similarity to barley *Mlo* (Consonni et al., 2006) and identified as the *pmr2* mutant in the above-mentioned forward genetic screen (Vogel and Somerville, 2000). According to phylogenetic analyses, there are 15 *MLO* genes distributed into five clades in Arabidopsis, of which *MLO2*, *MLO6* (At1g61560) and *MLO12* (At2g39200) belong to the same clade (Devoto et al., 2003; Acevedo-Garcia et al., 2014). *mlo2* mutants display reduced penetration success and less sporulation after infection with the adapted PM fungus *Go* (Consonni et al., 2006). Interestingly, *MLO2* controls penetration success of PM fungi together with *MLO6* and *MLO12*. While the *mlo6* and *mlo12* single and double mutants do not show any resistance phenotype, they gradually increase resistance of *mlo2* if combined in double and triple mutant combinations, with the *mlo2 mlo6 mlo12* triple mutant being fully resistant (Figure 6; Consonni et al., 2006). *MLO* genes encode evolutionary ancient integral membrane proteins with seven transmembrane domains and unknown biochemical activity (Devoto et al., 2003; Kusch et al., 2016). Besides Arabidopsis and barley, mutation of closely related *MLO* genes in tomato, pea and further plants render these host species resistant to PM infection, indicating a similar function of the respective proteins (Bai et al., 2008; Humphry et al., 2011).

Figure 6. Macroscopic infection phenotypes of Col-0 and the *mlo2 mlo6 mlo12* mutant.

Five-week-old wild type (Col-0) and *mlo2 mlo6 mlo12* plants (in Col-0 genetic background) were inoculated with *Go* and photographs were taken one week after inoculation.

Similar to NHR, *mlo2*-mediated PM resistance does not depend on major phytohormone signaling pathways such as those relying on JA, ET or SA (Consonni et al., 2006). By contrast, all three *PEN* genes are required for *mlo2*-mediated resistance to PM (Consonni et al., 2006). These findings suggest that *mlo*-mediated resistance and NHR may share overlapping pathways in plant defense (Humphry et al., 2006). Besides the PEN proteins, CYP79B2 (At4g39950) and CYP79B3 (At2g22330), two cytochrome monooxygenases that catalyze the entry step towards the production of diverse indolic metabolites, including the Arabidopsis-specific phytoalexin camalexin and indole glucosinolates, are required for *mlo2*-mediated resistance. In contrast to CYP79B2 and CYP79B3, another cytochrome P450 monooxygenase, PAD3 (At3g26830), which catalyzes the final step in camalexin biosynthesis, only plays a minor role in *mlo2*-mediated resistance (Consonni et al., 2010).

7. INTRACELLULAR TRAFFICKING

The pathogen-triggered rearrangement of cellular components correlates with the formation of papillae (see section 3.2) and a major radial reorganization of actin filaments underneath attempted PM entry sites (Kobayashi et al., 1997; Takemoto et al., 2006). Pharmacological treatment of leaves with inhibitors

of actin filament polymerization (cytochalasins and latrunculin B) and myosin (BDM (2,3-butanedione monoxime) and NEM (*N*-ethyl-maleimide)) results in reduced recruitment of organelles and vesicles towards the site of fungal attack and decreased PM penetration resistance (Figure 7; Kobayashi et al., 1997; Yun et al., 2003; Yang et al., 2014). Conversely, silencing of genes coding for subclass I actin depolymerization factors (ADFs) increases resistance against *Go* and results in enhanced filament bundling during early Go infection (Inada et al., 2016). Together these findings suggest that intact actin microfilaments and myosin motors are required for successful defense. In fact, single mutants of *MYOSIN XI* genes (*xi-1-1* (At1g17580), *xi-2-1*, *xi-2-2* (At5g43900), *xi-i-1*, *xi-i-2* (At4g33200), *xi-k-1*, *xi-k-2* (At5g20490)), one triple mutant (*xi-1-1*, *xi-2-1*, *xi-k-2*) and one quadruple mutant (*xi-1-1*, *xi-2-1*, *xi-i-1*, *xi-k-2*) exhibit higher penetration frequencies compared to Col-0 wild type upon *Bgh* inoculation. Furthermore, upon challenge with *Gc*, the quadruple mutant shows increased fungal growth and hyphal branches at 3 dpi and more conidiophores at 7 dpi compared to Col-0 wild type (Yang et al., 2014). Collectively, these findings indicate that transport activities along the actin cytoskeleton might be crucial for pre- and possibly post-invasive defense against PMs.

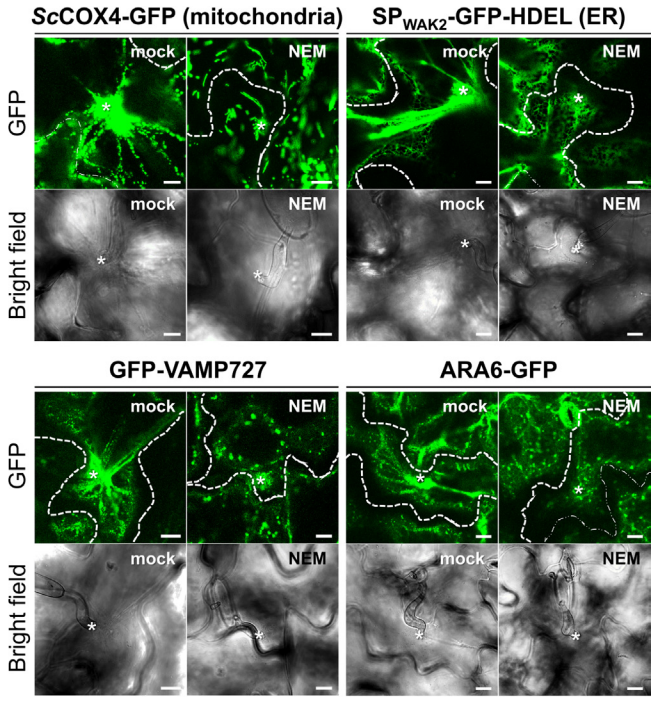

Figure 7. Myosin inhibition affects the recruitment of organelles and endomembrane compartments to PM attack sites.

Leaves stably expressing GFP fusions of (i) *Saccharomyces cerevisiae* cytochrome c oxidase IV (*Pd35S::ScCOX4-GFP*; a mitochondrial marker; Nelson et al., 2007), (ii) the signal peptide of WALL-ASSOCIATED KINASE 2 together with the ER retention signal HDEL (*Pd35S::SP$_{AtWAK2}$-GFP-HDEL*; an ER marker; Nelson et al., 2007), (iii) the Rab5 GTPase ARA6/RAB1F (*PARA6::ARA6-GFP*; an endomembrane vesicle marker; Goh et al., 2007), and (iv) the v-SNARE VAMP727 (*PVAMP727::GFP-VAMP727*; an endomembrane vesicle marker; Ebine et al., 2008) were infiltrated with water (mock) or 1 mM *N*-ethylmaleimide (NEM; a myosin inhibitor) and 1 h later inoculated with *Bgh*. Infected epidermal cells (indicated by dashed lines) were examined by confocal microscopy ca. 16 hpi. Projections of z-stacks are shown. Asterisks indicate the *Bgh* penetration site. Bar = 10 µm. Unpublished micrographs, courtesy of Yangdou Wei.

SNARE proteins mediate fusion events between vesicular and target membranes. Based on the presence of a critical arginine or glutamine residue in the center of the SNARE domain, this family is divided into R- or Q-SNARE proteins, respectively, where the latter can be further subdivided into Qa-, Qb- or Qc-SNAREs (Collins et al., 2003; reviewed in Lipka et al., 2007). PEN1 (Qa-SNARE), SNAP33 (Qb+Qc-SNARE) and VAMP721/722 (R-SNARE) form a ternary SNARE complex that focally accumulates at fungal penetration sites. This complex is required for the timely assembly of papillae and most likely for the release of pathogen-induced vesicle cargo (see section 3.1; Assaad et al., 2004; Kwon et al., 2008b; Kwaaitaal et al., 2010). In addition to these SNARE proteins, the TGN-localized Qa-SNAREs of the SYP4 family, which are plant orthologs of the syntaxin 16 in animals and yeast *Tlg2* (t-SNARE affecting a late Golgi compartment), seem to be required in PM disease resistance responses. Double mutant *syp42* (At4g02195) *syp43* (At3g05710) plants show increased secondary hyphae formation compared to the Col-0 wild type after inoculation with the non-adapted PM *E. pisi*, while *Go* infection is unaltered (Uemura et al., 2012). Interestingly, mRFP-VAMP722 partially colocalizes with GFP-tagged SYP43, but not with Venus-SYP61 (At1g28490), another TGN marker. In addition, GFP-SYP43 localizes between the TGN cisternae (labeled with Venus-SYP61) and compartments labeled with mRFP-VAMP722 (Uemura et al., 2012). Furthermore, the TGN-localized KEG ubiquitin ligase, which interacts with EDR1 (see section 6.1) and regulates transport of membrane-associated proteins to the vacuole, is degraded following the maturation of *Gc* haustoria (Gu and Innes, 2012). These observations suggest that KEG might be a plausible virulence target of the PM fungus. Together, these findings highlight the importance of the TGN during PM infection.

The ARF-GEF inhibitor brefeldin A (BFA) has been widely used to study the impact of membrane trafficking in PM interactions. For example, treatment with BFA hampers penetration resistance to *Bgh* in Col-0 leaves (Nielsen et al., 2012). Additionally, BFA-treated leaves of a *pen1* transgenic line expressing GFP-PEN1 show reduced accumulation of the fusion protein and callose at the sites of attempted fungal penetration. As strong mutants of the well-studied BFA-sensitive ARF-GEF GNOM (At1g13980) are dwarfed and therefore not suitable for detailed analysis, Nielsen and co-workers generated transheterozygote plants, carrying two different mutated alleles of GNOM (gnom$^{B409/emb30-1}$). These partially complement the respective nonfunctional domains of the ARF-GEF dimer. *Bgh* infection of gnom$^{B409/emb30-1}$ plants reveal an increase in fungal penetration and a delay in callose deposition and papillary GFP-PEN1 accumulation, thus mimicking BFA treatment (Nielsen et al., 2012). Together these findings suggest that BFA-sensitive GNOM regulates sorting of material to be transported to the papilla, including PEN1 (Nielsen et al., 2012). Notably, BFA treatment of the above-mentioned myosin quadruple knockout mutant (*xi-1-1 xi-2-1 xi-1 xi-k-2*) results in retention of GFP-PEN1 at the plasma membrane, which contrasts its accumulation in BFA bodies in Col-0 epidermal cells. Additionally, accumulation of GFP-PEN1,

callose and autofluorescent material at attempted penetration sites is reduced in the myosin quadruple mutant upon *Bgh* infection (Yang et al., 2014). This experimental outcome implies that members of the myosin XI family are involved in subcellular trafficking pathways that modulate penetration resistance to PM.

As previously mentioned (see section 4), the R protein RPW8.2 localizes to the EHM in cells attacked by the adapted PM pathogens *Gc* and *Go* (Wang et al., 2009; Micali et al., 2011). Localization studies using RPW8.2-YFP under the control of its native promoter in transgenic *Go*-infected Col-0 plants revealed that accumulation of RPW8.2 occurs around mature haustoria that have been partially or completely encased (Figure 5; Micali et al., 2011). Immunogold labeling of RPW8.2-YFP in plants infected with *Gc* supports localization at the EHM, which is reduced after treatment with the actin polymerization inhibitor cytochalasin E (Wang et al., 2009). Overexpression of *ADF6* (At2g31200) in Col-0 plants causes the same response, indicating that intact actin microfilaments are required for successful recruitment of RPW8.2 to the EHM. By contrast, treatment with oryzalin, a microtubule polymerization inhibitor, does not affect the localization of the resistance protein (Wang et al., 2009). Furthermore, immunogold labeling experiments showed the presence of RPW8.2 in vesicle-like endomembrane compartments on the cytoplasmic side of the callose encasement of the haustorial complex (Wang et al., 2009). A recent study revealed that the same RPW8.2-containing vesicles co-localize with the R-SNARE proteins VAMP721 and VAMP722. While in the absence of VAMP721 trafficking of RPW8.2 to the EHM is delayed, lack of VAMP722 has a less drastic impact. Reduced EHM targeting efficiency of RPW8.2-YFP in the tested mutants correlates with enhanced *Go* sporulation (Kim et al., 2014). Moreover, delivery of RPW8.2 to the EHM is independent of SA signaling and PEN1 function, implying that VAMP721/722 vesicles are required for pre-invasive and post-invasive vesicle trafficking pathways in defense against PMs (Wang et al., 2009; Kim et al., 2014).

Host membrane trafficking plays a central role during defense against PM fungi and in other plant-microbe interactions (Dörmann et al., 2014; Inada and Ueda, 2014; Leborgne-Castel and Bouhidel, 2014; Teh and Hofius, 2014). Therefore, it is not surprising that pathogens including PMs may attempt to interfere with this pathway. Consistent with this notion, the *Bgh* effector candidate BEC4 interacts with a member of the ARF-GTPase activating protein (ARF-GAP) family in barley (Schmidt et al., 2014). The Arabidopsis ortholog of this protein is AGD5 (At5g54310). Interestingly, *agd5* mutant alleles show considerably elevated *E. pisi*, but unaltered *Go* entry rates. Whether more PM effectors target the host trafficking machinery will be an object of further investigations.

8. PHYTOHORMONE-RELATED DEFENSE SIGNALING

While the first line of plant defense against fungal pathogens largely relies on cell surface-mediated defense signaling initiated by recognition of MAMPs (see section 3), secondary (e.g. post-penetration) defense responses are often induced by the SA or JA/ET phytohormone signaling pathways.

8.1 Salicylic acid-mediated resistance

As for other biotrophic interactions, SA-mediated defense signaling plays a pivotal role in Arabidopsis defense against adapted PM fungi: SA-dependent gene expression and immune responses increase in Arabidopsis leaves upon infection with PMs and contribute to restriction of colony expansion and reproduction of the fungi (Zimmerli et al., 2004; Chandran et al., 2009). Consequently, many mutants with defects in SA biosynthesis, accumulation and signaling exhibit enhanced susceptibility (hypersusceptibility) to *Go* and *Gc* (Dewdney et al., 2000; Chandran et al., 2009; Zhang et al., 2015a). Likewise, interference with SA accumulation by transgenic expression of NahG, a *Pseudomonas* salicylate hydroxylase that degrades SA, increases susceptibility against *Gc* isolates (Ederli et al., 2015; Zhang et al., 2015a). Despite these genetic indications for an involvement of SA in defense against PM infection, there is currently only limited direct evidence for increased SA levels during PM infection (Fabro et al., 2008).

As the final steps of SA biosynthesis in the leaf take place in chloroplasts (Strawn et al., 2007), export of the hormone is required for elevated cytosolic and nuclear SA levels. Consequently, loss-of-function mutation of DP-E2F-like 1 (DEL1: At3g48160), a transcriptional repressor of the gene encoding the plastidic SA exporter EDS5/SALICYLIC ACID INDUCTION DEFICIENT (SID)1 (At4g39030), results in enhanced SA-dependent resistance against *Go* (Chandran et al., 2014). This phenotype correlates with elevated basal SA levels and increased transcript abundance of SA-responsive genes in the *del1* mutant (Chandran et al., 2014). Strikingly, DEL1 also promotes cell proliferation by repressing genes involved in the induction of endoreduplication (see section 9.4; Vlieghe et al.; Lammens et al., 2008). Together these findings suggest that DEL1-mediated control of SA levels regulates the balance between growth and immunity in developing leaves. The translation of distinct SA levels into specific defense responses occurs by the action of NPR proteins in Arabidopsis (reviewed in Pajerowska-Mukhtar et al., 2013; Seyfferth and Tsuda, 2014; Yan and Dong, 2014). The outcome of SA-mediated signaling depends on subcellular SA levels and the abundance of active NPR1 in the nucleus. Its paralogs, the SA receptors NPR3 (At5g45110) and NPR4 (At4g19660), cooperatively fine-tune NPR1 degradation by their competitive SA concentration-dependent interaction with nuclear NPR1. Moderately elevated SA levels, as present during systemic acquired resistance (SAR), allow the accumulation of active NPR1 protein in the nucleus. Subsequent interaction of NPR1 with TGA transcription factors (binding to a TGACG nucleotide motif) promotes gene expression of SA-responsive genes and induces SA-mediated defense. The relevance of NPR1 for the Arabidopsis-PM interaction is substantiated by identification of NPR1 as a protein-protein interaction network hub during *Go* infection (Jiang et al., 2016). This NPR1 interaction network includes the TGA-interacting GLUTAREDOXIN 480 (GRX480/ROXY19: At1g28480) involved in regulating SA/JA antagonism (Zander et al., 2012) and several TGA transcription factors (TGA1: At5g65210, TGA2: At5g06950, TGA3: At1g22070, TGA7: At1g77920) that can further regulate the expression of defense-related genes. Genes that show SA-dependent transcript accumulation during PM infection encode proteins involved in redox regulation, vacuolar transport, secre-

tion, and signaling-relevant processes such as Ca^{2+} homeostasis and SA/JA cross talk (Chandran et al., 2009).

ROP GTPases (Rho (RAS homologue) of plants) are molecular switches and key regulators of immunity (Kawano et al., 2014). Mutation of the Arabidopsis ROP-GAPs *ROPGAP1* (At5g22400) and *ROPGAP4* (At3g11490), trapping their (yet unidentified) target ROPs in the active state, results in enhanced susceptibility to *E. cruciferarum* (Hoefle et al., 2011; Huesmann et al., 2011). Accordantly, expression of an inactive (dominant negative) ROP6 ($rop6^{dn}$; At4g35020) variant, unable to interact with downstream effectors, results in reduced penetration by *Go.* This correlates with an increased transcript abundance of SA-responsive genes, such as *PR1*, and elevated SA-mediated defense responses. However, *Go* resistance of the $rop6^{dn}$ transgenic plants is uncoupled from SA signaling (Poraty-Gavra et al., 2013). Nevertheless, these results, together with previous findings in barley (Hoefle et al., 2011; Scheler et al., 2016), suggest a positive role of active ROPs in mediating susceptibility to adapted PMs.

A number of proteins whose deficiency leads to enhanced PM resistance, such as EDR1 to 4, LESION INITIATION 2 (LIN2: At1g03475), and GSL5/PMR4 are associated with the repression of SA-mediated defense. This is indicated by requirement of SA for the increased disease resistance phenotypes of the respective mutants (see section 6; Vorwerk et al., 2007; Zhang et al., 2007; Wawrzynska et al., 2010; Guo et al., 2013; Wu et al., 2015). The MAPKKK EDR1, for example, negatively regulates SA-dependent defense responses and cell death. In consequence, *edr1* mutants are constitutively primed for SA-inducible defense which might occur *via* the regulation of the MAPKs MPK3 (At3g45640) and MPK6 (At2g43790) (Beckers et al., 2009). The role of EDR1 in the control of SA signaling probably relies on its interaction with MKK4 and MKK5, the upstream MAPKKs activating MPK3 and MPK6 (Zhao et al., 2014). EDR1 negatively affects MKK4 and MKK5 levels, presumably resulting in repression of the MKK4/MKK5-MPK3/MPK6 cascade involved in the induction of SA signaling (Zhao et al., 2014; Wu et al., 2015). Mutations in *MKK4*, *MKK5* or *MPK3* (but not in *MPK6*) suppress the *edr1* phenotype, indicating a requirement of these kinases for *edr1*-mediated PM resistance. The same holds true for *edr4,* which is in line with the need of EDR4 for the relocation of EDR1 to the PM penetration site (see section 6.1; Wu et al., 2015). Strikingly, overexpression of MKK4 or MKK5 causes *edr1*-like resistance and PM-induced cell death, pointing to an additional role for the MKK4/MKK5-MPK3/MPK6 kinase cascade in SA-induced cell death, parallel to its contribution to SA-regulated gene expression (Zhao et al., 2014).

NPR3-mediated NPR1 degradation at high SA levels promotes the onset of cell death. However, while no PM phenotype has been reported for *npr3* (Liu et al., 2005), *npr1* and *npr4* mutant plants are more susceptible to *Gc* (Reuber et al., 1998; Liu et al., 2005; Humphry et al., 2010). Thus, it remains elusive to which extent NPR3-mediated cell death contributes to defense against PMs. In addition to NPR3, also NPR4 adds to pathogen-triggered cell death (Pajerowska-Mukhtar et al., 2013; Kumar, 2014; Yan and Dong, 2014). A role for SA-mediated cell death responses in PM defense is supported by correlation of enhanced SA signaling with increased PM-induced cell death in several of the above mentioned resistant mutants (Guo et al., 2013).

Mutants impaired in autophagy further corroborate a role of cell death in PM resistance, as some of them display early leaf senescence and spontaneous cell death, which in several cases coincides with increased PM resistance (Yoshimoto et al., 2009; Wang et al., 2011b; Wang et al., 2011a). Autophagy targets organelles and cytosolic proteins for vacuolar/lysosome-mediated degradation (Liu and Bassham, 2012). The mutant of *AUTOPHAGY-RELATED 2 (ATG2:* At3g19190), impaired in the early steps of autophagosome biogenesis, exhibits severe PM-induced cell death and increased resistance when challenged with *Gc,* while susceptibility towards *Go* is unaltered (Yoshimoto et al., 2009; Wang et al., 2011b; Wang et al., 2011a). PM resistance of *atg2* plants depends on SA signaling, while cell death is partially independent of SA. In conclusion, autophagy contributes to suppression of cell death and defense response to PM fungi; however, the mechanisms by which autophagy controls these processes are yet unknown (Wang et al., 2011a; Wang et al., 2011b).

8.2 Contribution of JA and ET signaling to PM resistance

SA-mediated defense appears to act mainly against biotrophic pathogens, while the JA and ET pathways are preferentially linked to resistance against necrotrophic parasites (Thomma et al., 2001; Glazebrook, 2005). Induced systemic resistance (ISR) confers JA/ET-mediated protection of shoot tissues *via* root-to-shoot signaling. ISR is initiated by interactions with beneficial microbes such as arbuscular mycorrhiza or plant growth-promoting rhizobacteria in the root and has proven effective against necrotrophs and herbivores (reviewed in Pieterse et al., 2012). Despite the fact that PM fungi are obligate biotrophs, root colonization with the putative plant growth-promoting basidiomycete *Piriformospora indica* reduces *Go* conidiation in a JA signaling-dependent manner (Stein et al., 2008). This finding suggests that besides SA also JA contributes to resistance against PM fungi (Figure 8). Accordingly, *Bgh* inoculation of Arabidopsis induces expression of genes that are controlled by the JA/ET signaling pathways (Zimmerli et al., 2004). By contrast, although endogenous JA levels are enhanced during the formation of haustoria by *Gc*, this does not result in transcript accumulation of JA/ET-responsive genes (Reuber et al., 1998; Nishimura et al., 2003; Zimmerli et al., 2004; Glazebrook, 2005; Fabro et al., 2008). Nevertheless, constitutive or ectopic activation of the JA/ET pathway due to elevated JA/ET levels in the mutant of *CELLULOSE SYNTHASE 3/CONSTITUTIVE EXPRESSION OF VSP 1* (*CESA3/CEV1*: At5g05170) or treatment of Col-0 with methyl-JA enhances resistance against *Gc*. This effect depends on the JA receptor component CORONATINE-INSENSITIVE PROTEIN 1 (COI1: At2g39940; Ellis et al., 2002a; Ellis et al., 2002b; Zimmerli et al., 2004). In conclusion, the findings suggest that, although elicitation of JA/ET-mediated defense signaling seems to be restricted to incompatible PM-host interactions, JA/ET-induced defense responses are effective against virulent PM fungi if stimulated constitutively, artificially or systemically, despite the biotrophic nature of the interaction. Consequently, JA/ET signaling must either be suppressed or failed to be elicited by adapted PMs during a successful infection (Figure 8; reviewed in Antico et al., 2012).

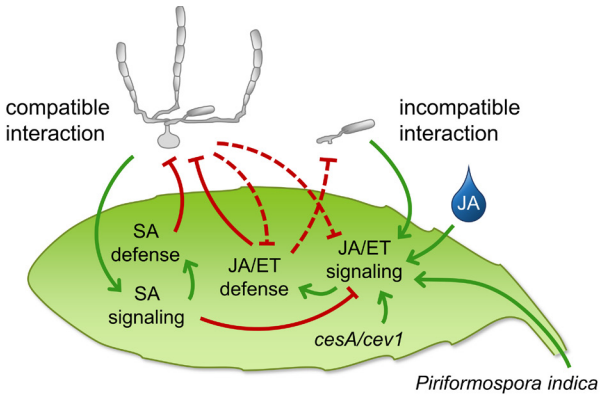

Figure 8. Integration of phytohormone signaling in defense against PM fungi.

Although only incompatible PM-host interactions elicit JA/ET-mediated defense, JA/ET-induced defense responses are effective against virulent PM fungi if stimulated constitutively (*cesA/cev1*), artificially (JA treatment) or systemically (*Piriformospora indica* root colonization). These findings suggest that virulent fungi suppress JA/ET signaling during compatible interactions. This suppression might involve the antagonistic action of SA signaling. Solid lines indicate experimentally supported impacts, while dashed lines indicate speculative connections.

9. HOST TRANSCRIPTIONAL REPROGRAMMING

Transcriptional changes in response to PM inoculation reflect a combination of both activation of defense after recognition of the pathogen and host cell manipulation by the fungal invader. Arabidopsis responds to PM attack with the differential regulation of defense-related genes. While many genes are induced in both host and nonhost interactions, changes in gene expression occur more rapidly and are often more pronounced in nonhost interactions than in host interactions, indicating that virulent fungi might suppress gene expression related to basal defense (Zimmerli et al., 2004). A large subset of the PM-responsive genes are TFs. These induced or repressed TFs further transcriptionally regulate secondary up- or downregulated genes and thus enable the coordinated expression of genes in fine-tuned expression networks (Zimmerli et al., 2004; Fabro et al., 2008; Chandran et al., 2009; Chandran et al., 2010; Christiansen et al., 2011).

9.1 WRKY transcription factors contribute to defense regulation

Among the genes that show altered transcript accumulation in response to *Go,* members of the plant-specific WRKY (single amino acid letter code for tryptophan-arginine-lysine-tyrosine) TFs represent the most prominent TF family (Chandran et al., 2009). WRKY TFs are key regulators of pathogen-triggered changes in gene expression that act as transcriptional activators or repressors in various homo- and heterodimer combinations. They function up- and downstream of hormone signaling pathways, are involved in the antagonistic control of SA and JA/ET signaling pathways and can be regulated by MAPKs (reviewed in Bakshi and Oelmüller, 2014; Buscaill and Rivas, 2014; Caarls et al., 2015). The involvement of WRKYs in defense of Arabidopsis against PMs is indicated by transcriptional changes of WRKY-encoding genes in response to *Go* and an enrichment of WRKY-targeted W-box *cis*-regulatory elements in promoters of genes differentially transcribed upon PM challenge (Chandran et al., 2009). Furthermore, expression of WRKY TFs is enhanced in PM resistant *edr1* plants relative to the wild type in response to *Gc*, and genes whose promoters contain W-boxes are likewise enriched in this dataset. As PM resistance in *edr1* plants depends on the MPKK4/5-MPK3/6 cascade, the expression of WRKY TFs might be regulated *via* this pathway (Christiansen et al., 2011).

In barley, the MLA10 NB-LRR interacts with the *Bgh* AVR_{A10} (avirulence A10) effector and induces transcriptional changes by inhibition of *Hv*WRKY1 and *Hv*WRKY2. Both TFs supposedly act as transcriptional repressors of genes involved in basal defense and effector-triggered immunity (ETI; Shen et al., 2007). Similarly, the closely related Arabidopsis TFs *WRKY18* (At4g31800) and *WRKY40* (At1g80840), whose transcription is rapidly induced during PM infection, negatively regulate defense against *Go*. Together these results indicate functional conservation of the defense-repressive role of this WRKY sub-family (Shen et al., 2007). Altered pathogen-induced transcriptional reprogramming in the *Go*-resistant *wrky18 wrky40* double mutant corroborates the negative impact of WRKY18/40 on defense-related gene expression (Pandey et al., 2010; Schön et al., 2013). Chromatin immunoprecipitation (ChIP) experiments revealed binding of WRKY40 to W-box containing promoter regions of *EDS1*, the AP2 (apetala 2)-type TF gene *REDOX RESPONSIVE TRANSCRIPTION FACTOR 1* (*RRTF1*: At4g34410) and to *JASMONATE-ZIM-DOMAIN PROTEIN 8* (*JAZ8*: At1g30135), a member of the JA-signaling repressor gene family (Pandey et al., 2010). Thus, WRKY18/40 TFs seem to repress the transcription of positive defense regulators such as EDS1, and positively modulate JA-signaling (Pandey et al., 2010; Schön et al., 2013). Although the regulatory role of *Hv*WRKY1/2 and WRKY18/40 is conserved between barley and Arabidopsis, an Arabidopsis R protein interfering with WRKY18/40 function remains to be identified. The conservation of MLA1 functionality and induction of MLA-dependent defense gene expression in response to *Bgh* might indicate the existence of a respective MLA analog in Arabidopsis (see section 4; Maekawa et al., 2012). In contrast to WRKY18 and WRKY40, WRKY70 (At3g56400) contributes to resistance of Arabidopsis to *Gc* and inactivation of the respective gene results in increased susceptibility to this pathogen (Li et al., 2006). *WRKY70* overexpression coincides with a partially NPR1-dependent suppression of JA responsive genes, indicating a role of this TF in the control of SA/JA crosstalk (Li et al., 2006; Caarls et al., 2015).

9.2 Hormone signaling-induced transcriptional reprogramming during defense

SA signaling contributes to gene expression during the Arabidopsis-PM interaction (see section 8.1). This involves *Go*-induced expression of genes related to Ca^{2+} signaling and genes coding for redox regulators that contribute to NPR1 activation (Chandran et al., 2009). Transcript accumulation of SA-responsive

TFs, SA biosynthesis genes (*ISOCHORISMATE SYNTHASE 1* (*ICS1*)/*SID2*: At1g74710) and SA-responsive pathogenesis-related genes such as *PR1* emphasizes the predominant contribution of SA signaling to PM-induced gene expression. In line with this notion, SA signaling-dependent resistance of the *wrky18 wrky40* double mutant correlates with massive *Go*-induced transcriptional reprogramming (Pandey et al., 2010; Schön et al., 2013). The *edr1* mutation, which enhances SA-dependent PM resistance, affects accumulation of defense-related transcripts in response to *Gc,* including transcripts encoding WRKY and AP2/ET-response element binding factor (ERF) TFs (Christiansen et al., 2011). Furthermore, genes encoding proteins associated with ROS production and the endomembrane system are induced in infected *edr1* plants. PM-induced enrichment of the latter together with transcripts associated with secretion suggests that the secretory pathway may play an important role in *edr1*-mediated immunity (Christiansen et al., 2011). This assumption is in agreement with the relocalization of EDR1 from the ER to the plant-fungal interface during *Gc* infection (Christiansen et al., 2011; Wu et al., 2015).

TFs of the AP2/ERF family particularly regulate genes related to JA/ET signaling. Besides WRKY TFs, AP2/ERF TFs and transcripts associated with AP2/ERF response elements (GCC-boxes) are over-represented amongst genes upregulated during PM infection in the *edr1* mutant (Christiansen et al., 2011). Arabidopsis ERF6 (At4g17490) and ERF104 (At5g61600) are phosphorylated by MPK6 and/or MPK3, indicating a regulation of these ERFs by defense-related MAPK cascades (Bethke et al., 2009; Meng and Zhang, 2013; Tsuda and Somssich, 2015). Furthermore, *ERF1* (At3g23240) and *ERF2* (At5g47220) transcripts accumulate upon *Go* infection (Chandran et al., 2009). A role of ERF1 in defense against PMs is in line with the finding that *ERF1* overexpression results in enhanced resistance to *Go* (Gu et al., 2002; Chandran et al., 2009). *OCTADECANOID-RESPONSIVE ARABIDOPSIS AP2/ERF 59* (*ORA59*: At1g06160), a master regulator of ERF-controlled JA/ET signaling, has been identified, together with other ET/JA-responsive genes, as differentially regulated in SA-deficient mutants *versus* wild type plants upon *Go* infection. This suggests that ORA59 modulates the crosstalk between SA and JA/ET signaling during PM-induced defense responses (Chandran et al., 2009). In agreement with this finding, ORA59 was identified as a major target for SA antagonism (Zander et al., 2012; Van der Does et al., 2013; Zander et al., 2014; reviewed in Caarls et al., 2015). A dominant allele of *SIGNAL RESPONSIVE1* (*SR1*: At2g22300), encoding a calmodulin-binding TF that regulates ET-induced senescence by binding to the *ETHYLENE INSENSITIVE3* (*EIN3*: At3g20770) promoter, has been identified as a gain-of-function suppressor of *edr2*-mediated resistance to *Gc* (Nie et al., 2012). SR1 binds to the promoter of *NON-RACE-SPECIFIC DISEASE RESISTANCE1* (*NDR1*: At5g06320). NDR1, a membrane-associated protein, contributes to plant immunity mediated by several coiled-coil NB-LRRs and SAR (Century et al., 1997; Shapiro and Zhang, 2001; Zhang and Shapiro, 2002). A *sr1* null mutant conditions resistance to *Gc*, while an additional mutation in *ndr1* suppresses this phenotype. Together these data suggest that SR1 plays a criticial role in PM resistance, possibly by regulating *EIN3* and *NDR1* expression (Nie et al., 2012).

Integrative analysis of protein interaction networks and transcriptomics during *Go* infection revealed a negative correlation between development-related and defense-related genes/proteins (Jiang et al., 2016). While many defense-related genes are induced after *Go* infection, the majority of genes linked to development are downregulated. Interestingly, auxin-related genes are overrepresented among nodes connecting the defense and development sub-networks. Together these findings emphasize that defense is triggered at the expense of developmental programs and that regulation of this trade-off involves auxin (Jiang et al., 2016).

9.3 Attuned transcriptional regulation coordinates defense

Despite its compatible nature, the Arabidopsis-*Gc* interaction elicits expression of genes related to NHR (Chandran et al., 2010). Remarkably, in compatible interactions, neither activation of NHR genes nor of SA-induced defense are sufficient to confer resistance (Chandran et al., 2009; Chandran et al., 2010). The PEN1/SNAP33/VAMP722 and the PEN2/PEN3 pathways are important determinants of NHR and are additionally required for *mlo2*-mediated immunity (see section 4 and 6.3). In line with their pivotal role in defense, *PEN1, PEN2, PEN3, SNAP33* and *MLO2* share a substantial amount of coexpressed genes, with the majority of transcripts accumulating in response to biotic stresses and MAMP treatment (Humphry et al., 2010). Notably, many transcripts of this regulon accumulate in *Bgh*-inoculated Arabidopsis plants ectopically expressing the barley MLA1 R protein. This implies a substantial overlap of MLA1-dependent transcriptional regulation and basal resistance against PM fungi (Humphry et al., 2010; Maekawa et al., 2012).

Consistent with the role of PEN2 and PEN3 in indole glucosinolate biosynthesis and secretion, many of the coexpressed genes are associated with the glucosinolate pathway. One example is the gene encoding MYELOBLASTOSIS (MYB)51 TF (At1g18570), a major regulator of defense-related expression of glucosinolate biosynthesis genes (Humphry et al., 2010). Transcriptomic evaluation of the PM resistant *wrky18 wrky40* double mutant revealed that WRKY18 and WRKY40 suppress crucial biosynthesis genes of the indolic phytoalexin camalexin (Pandey et al., 2010). Accordingly, increased expression of camalexin and indole glucosinolate biosynthesis genes after pathogen challenge in *wrky18 wrky40* plants correlates with the enhanced accumulation of the phytoalexin camalexin and 4MI3G (4-methoxyindol-3-ylmethyl-glucosinolate), an indole glucosinolate intermediate relevant for PM resistance, in this mutant (Pandey et al., 2010; Schön et al., 2013). Loss of function of the crucial 4MI3G biosynthesis gene *CYP81F2* suppresses *wrky18 wrky40*-mediated inhibition of host cell entry, indicating that the indolic metabolite is required for penetration resistance of the double mutant against *Go* (Schön et al., 2013).

The group of genes coexpresed with *PEN3* further showed a significant overrepresentation of components involved in Ca^{2+} signaling such as calcium/calmodulin-dependent protein kinases (CCaMKs; Humphry et al., 2010; Campe et al., 2015). Consistently, components of the Ca^{2+} homeostasis and signaling machinery such as *CAM9/CALMODULIN-LIKE* (*CML*)*9* (At3g51920) and calreticulin-encoding genes are rapidly induced after *Go* inoculation, and *CML38* (At1g76650) is constitutively upregulated in the highly resistant *wrky18 wrky40* mutant (Chandran et al, 2009; Chandran et al., 2010, Pandey et al., 2010).

In conclusion, coexpression of genes encoding proteins involved in secondary metabolite biosynthesis (such as *PEN2* or cytochrome P450s like *CYP83B1* (At4g31500)) together with genes whose products mediate exocytosis/extrusion (SNAREs, exocyst subunits and ABC transporters like *PEN3*) suggests that production and secretion of antimicrobial compounds is transcriptionally attuned. Additional coregulation of receptor-like kinase genes, transcripts of Ca^{2+} signaling components and the heterotrimeric G-protein β and g subunits *AGB1* (At4g34460) and *AGG1* (At3g63420) indicates that also recognition and signaling components are coexpressed with key components of antifungal defense (Humphry et al., 2010; Lorek et al., 2013). Enhanced host cell entry by *Go* and *E. pisi* of Gβ-deficient mutants emphasizes the importance of second messenger signaling *via* heterotrimeric G-protein components for PM resistance (Lorek et al., 2013). Finally, identification of defense-related TFs in the regulon indicates that recognition of microbes, response initiation, and defense execution are transcriptionally coordinated to enable an efficient immune output.

9.4 Host transcriptional changes indicate an adaption to the accommodation of the biotrophic pathogen

Besides defense-related transcriptional changes induced by microbe recognition, host gene expression is potentially impacted by the action of PM effectors to promote fungal accommodation. Consequently, adaptation of the host metabolism to the presence of the pathogen has been reported (reviewed in Wildermuth, 2010). Laser microdissection-assisted site-specific profiling of transcript abundance during late *Go* infection stages (5 dpi) suggests a suppression of photosynthesis and points to a carbon source-to-sink transition in PM-infected cells (Figure 9; Chandran et al., 2009; Chandran et al., 2010). The PM-triggered induction of genes associated with sugar metabolism and hexose transporters associated with sink organs further reinforces this idea. The respective proteins might contribute to the availability of carbohydrates at infection sites, and consistently their elevated expression levels may reflect an increased demand for hexoses by the fungus (Fabro et al., 2008; Chandran et al., 2009; Chandran et al., 2010). Increased transcript abundance of genes related to respiration, including glycolysis, the tricarboxylic acid cycle, and the mitochondrial electron transport chain further strengthen the notion of an adaptation to the elevated energy consumption by the infected tissue (Fabro et al., 2008; Chandran et al., 2010).

Adjustment of the plant host metabolism to support the growth of the biotrophic pathogen is consistent with an increased ploidy level of the mesophyll cells underlying infected epidermal cells at later stages of compatible interactions (Figure 9; Chandran et al., 2010; Chandran et al., 2013). This correlates with the accumulation of *PLANT UBX DOMAIN-CONTAINING PROTEIN 2* (*PUX2*: At2g01650) transcripts after *Go* infection (Chandran et al., 2009). Strikingly, the onset of *PUX2* induction at 5 dpi overlaps with the occurrence of endoreduplication in mesophyll cells, and corresponds with fungal growth and reproduction (Chandran et al., 2013). The resulting polyploidy might compensate for the increased metabolic activity resulting from the nutritional demands of the fungus. This is supported by decreased spore formation coinciding with reduced basal ploidy in *pux2*, and thus identifies endoreduplication as a potential determinant of susceptibility to PM (Chandran et al., 2010; Chandran et al., 2013). The presence of UBX (ubiquitin regulatory X) and PUB (peptide:N-glycanase/UBA or UBX-containing proteins) domains in PUX2 suggests that, like other proteins with similar domain structures, it might act as a regulatory cofactor of CELL DIVISION CONTROL PROTEIN 48 (CDC48: At3g09840). Indeed, this AAA-ATPase (ATPase associated with diverse cellular activities) interacts with PUX2 *in vitro* (Rancour et al., 2004). As CDC48 complexes contribute to cell cycle progression, its interaction with PUX2 might regulate cell ploidy (Rancour et al., 2004; Madsen et al., 2009; Yamanaka et al., 2012; Gallois et al., 2013). MYB3R4 (At5g11510), a cell cycle control-associated MYB3R TF activating G2/M progression, is locally induced 5 dpi with *Go*. As genome duplication is a controlled process that occurs during mitosis, it is conceivable that MYB3R4

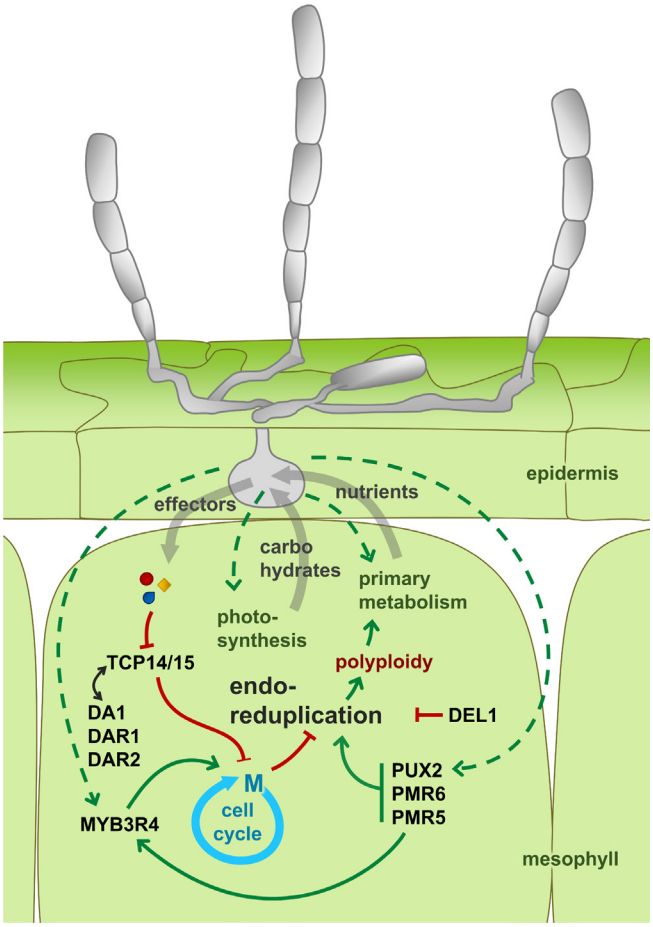

Figure 9. Proposed model of endoreduplication in PM pathogenesis.

The scheme depicts regulators and mechanisms involved in the control of endoreduplication and consequences of PM-induced mesophyll polyploidy. A PM (grey)-colonized leaf epidermal cell and an underlying mesophyll cell are shown. Grey arrows indicate the proposed translocation of components between cells. Solid lines indicate proven regulatory impacts and dashed lines indicate speculative regulatory impacts. M = mitosis.

is required for PM-induced polyploidy, which is supported by the phenotype of the *myb3r4* mutant (Haga et al., 2007; Chandran et al., 2010; Chandran et al., 2013). Similar to *pux2, myb3r4* mutants exhibit reduced PM conidiophore formation. The negative impact on basal cell ploidy levels and increased resistance of *pux2* is further phenocopied by *pmr6*. By contrast, reduced fungal reproduction associated with *pmr5* does not impact basal ploidy levels but correlates with a suppression of the PM-induced increase in ploidy (Chandran et al., 2013). Analysis of *pmr5* microarray data reveals an enrichment of MYB3R TF binding elements among cell cycle regulation-related genes showing altered expression in the mutant. This suggests that PMR5 acts upstream of a MYB3R TF to control PM-induced ploidy (Chandran et al., 2013).

A critical role of elevated ploidy for fungal virulence is further strengthened by the identification of the TEOSINTE BRANCHED/CYCLOIDEA/PROLIFERATING CELL FACTOR (TCP)13 (At3g02150), TCP14 (At3g47620) and TCP15 (At1g69690) basic helix-loop-helix (bHLH) TFs as common targets of several PM, oomycete and bacterial effectors (Weßling et al., 2014). TCP14 and TCP15 repress endoreduplication by directly regulating the expression of cell-cycle genes (Peng et al., 2015). Mutation of TCP13, TCP14, and to a lesser extent of TCP15, results in increased susceptibility towards *Go* (Weßling et al., 2014). A link to ubiquitin-mediated regulation of ploidy is provided by the ubiquitin receptors DA1 (At1g19270; “Dà” is Chinese for “large”), DA-RELATED (DAR)1, and DAR2 (At2g39830), which interact with and modulate the stability of TCP14/15 to regulate endoreduplication (Peng et al., 2015). Remarkably, DEL1, known to repress genes required for the onset of endoreduplication (Vlieghe et al., 2005; Lammens et al., 2008), does not impact the *Go*-induced increase in mesophyll ploidy when mutated or overexpressed (Chandran et al., 2014). Instead, microarray analyses of *del1* plants revealed an induction of basal defense gene expression compared to wild type (see section 8.1). The identification of effector targets involved in adaptation of the host metabolism (Weßling et al., 2014) marks one of the first steps towards elucidation of pathogen-induced reprogramming of the plant transcriptome. Further characterization of the mechanisms by which the fungus enforces adjustment of the plant cellular program to promote its intracellular accommodation will be an important aspect of future research.

10. POWDERY MILDEW GENOMES AND TRANSCRIPTOMES

PM fungi have sizeable genomes, which are about four times larger than those of most other ascomycetes (average ascomycete genome size: 36.9 Mbp; Mohanta and Bae, 2015). The genome of *Go*, for example, is approximately 160 Mbp in size (Spanu et al., 2010). By contrast, the number of coding genes in the PM genomes is comparatively low (average number of ascomycete coding genes: 11,129; Mohanta and Bae, 2015). Only ca. 6,500 genes each have been annotated in the *Bgh* and *Bgt* genomes, and ca. 7,100 genes (on the basis of assembled transcript contigs) are expressed in *Go* haustoria (Spanu et al., 2010; Weßling et al., 2012; Wicker et al., 2013; Kusch et al., 2014). The biological reason for the surprisingly low gene number most likely lies in the biotrophic life style: due to the close association of parasite and host, the fungus acquires its nutrients from the plant. As a result, the need for the maintenance of many complex biosynthesis pathways is low, whereas the requirement to control the host cell by secreted effectors is high (Spanu et al., 2010). Associated with this unusual ratio of genome size to gene number is the presence of numerous nested retrotransposons that cover most of the PM genomes. These retrotransposons are physically closely associated with effector protein-encoding genes and are therefore thought to be involved in the rapid evolutionary adaptation of PMs (Hacquard et al., 2013).

In a transcriptomic approach using a cDNA library obtained from mature *Go* haustoria extracted from heavily infected Arabidopsis leaves, protein-coding genes for translation and protein turnover were recognized to be most abundant (Weßling et al., 2012). This is in line with the finding that haustoria contain an abundance of cytoplasmic and endoplasmic reticulum-connected ribosomes, pointing at high levels of protein biosynthesis (Micali et al., 2011). Genes associated with mycelium development were also found to be highly represented in the *Go* haustorial transcriptome. By contrast, the transcript levels of sugar and amino acid transporters are comparatively low (Weßling et al., 2012). A substantial proportion of the transcripts are predicted to encode secreted effector proteins: 115 *Go* effector candidates (OECs) were discovered in the transcriptome of isolated haustoria (Weßling et al., 2012; Weßling et al., 2014). 84 of these OECs were subject of a comprehensive protein interaction study with a subset of Arabidopsis host proteins. In this work, identification of an interspecies effector convergence network revealed common effector target proteins (hubs) for Arabidopsis pathogens from three kingdoms of life, i.e. *Go* (a PM fungus), *H. arabidopsidis* (an oomycete), and *P. syringae* (a bacterium; Weßling et al., 2014). Interestingly, mutants of many of the respective host target genes show altered disease phenotypes (towards either increased resistance or higher susceptibility). This effector convergence suggests that biotrophic pathogens from different kingdoms manipulate the same host plant processes (Weßling et al., 2014). Among the common effector targets, proteins involved in cell cycle regulation/plant development are highly represented, e.g. the TFs TCP13, TCP14, and TCP19 (At5g51910). Since the TF MYB3R4 seems to be involved in PM-induced increase in polyploidy (see section 9.4; Chandran et al., 2010), these findings may indicate that the manipulation of the host cell cycle is crucial for the *Go* infection process and that of a range of other pathogens as well (Weßling et al., 2014).

11. OUTLOOK

For the success of an obligate biotrophic plant pathogen it is critical to avoid and/or suppress plant defense and manipulate the host to support its accommodation, nutrition and development. As discussed above in detail, current research on the Arabidopsis-PM interaction provides insights into factors that render a compatible interaction successful for the fungus. Published and forthcoming sequences of PM fungal genomes (Bindschedler et al., 2016), identification of PM effectors together with critical host targets (Weßling et al., 2014), adaptation of the plant metabolism to the presence of the pathogen (Chandran et al., 2009; Chandran et al., 2010; Jiang et al., 2016), and modulation of plant de-

velopment (Chandran et al., 2010; Jiang et al., 2016) contribute to identification of determinants of this biotrophic relationship. Recent combined analysis of protein-protein interaction networks and transcriptomics of *Go-* and *Botrytis cinerea*-infected Arabidopsis provides further insights into networks that are crucial for the biotrophic PM interaction in comparison to necrotrophic interactions (Jiang et al., 2016). Future integration of similar data sets on further biotrophic plant-microbe interactions will provide next steps towards identification of common determinants of biotrophy and potentially allow identification of host components that can be targeted to increase resistance against important biotrophic pathogens.

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