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Cell biology of the plant-powdery mildew interaction

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Abstract

Powdery mildew fungi represent a paradigm for obligate biotrophic parasites, which only propagate in long-lasting intimate interactions with living host cells. These highly specialized phytopathogens induce re-organization of host cell architecture and physiology for their own demands. This likely includes the corruption of basal host cellular functions for successful fungal pathogenesis. Recent studies revealed secretory processes by both interaction partners as key incidents of the combat at the plant-fungus interface. The analysis of cellular events during plant-powdery mildew interactions may not only lead to a better understanding of plant pathological features, but may also foster novel discoveries in the area of plant cell biology.

Introduction

Powdery mildew is a widespread disease of many mono- and dicotyledonous plants that is caused by obligate biotrophic Ascomycetes of the order Erysiphales. Traditionally, the main thrust of powdery mildew research was devoted to disease resistance, especially in cereals such as wheat and barley. Accordingly, extensive genetic analyses during previous decades revealed major resistance genes that typically confer isolate-specific immunity in these species [1]. More recently, other host species, such as the dicotyledonous reference species *Arabidopsis thaliana* [2], and different aspects of this plant-pathogen interaction, such as fungal pathogenesis and the establishment of compatibility [3,4], gained increasing attention. This also applies to cell biological examination of plant-powdery mildew encounters, which are ideally suited for microscopic studies since the fungal pathogen exclusively colonizes the epidermal cell layer and thus host-pathogen contact sites remain readily accessible for microscopy (Figure 1). Moreover, owing to cell-autonomous nature of plant defence against powdery mildew invasion [5], the interaction is well suited for single cell analyses [6]. The obligate biotrophic pathogen accommodates dedicated infection structures, haustoria (Figure 2), inside colonized host cells. The associated perturbation of plant cellular structure and the dynamic responses of the host cell to this assault [7] bring up highly interesting cell biological questions. Consequently, during the last few years several excellent papers were published that highlight various cell biological aspects of the plant-powdery mildew interaction.

Early events: Fungal spore germination and appressorium formation

The asexual powdery mildew life cycle commences with the landing of conidiospores on the plant surface. Proteomic analyses of conidia from the barley pathogen *Blumeria graminis* f.sp. *hordei* (*Bgh*) revealed numerous proteins with functions in carbohydrate, lipid or protein metabolism, indicating that resting conidia are prepared for the catabolism of storage compounds as well as protein biosynthesis and folding [8,9]. Following spore germination, appressoria develop at the sites where fungal sporelings attempt to penetrate the host cell wall. Release of epicuticular alkanes (by a secreted *Bgh* lipase, Lip1), and/or very-long-chain aldehydes appears to play an important role in these early stages of pathogenesis, possibly for fungal adhesion to the leaf surface [10] or as an inducing cue for morphogenesis of powdery mildew infection structures [11].

The next step: The switch from surface growth to invasive fungal pathogenesis

Probably the most critical step in powdery mildew pathogenesesis is the successful invasion of the first epidermal cell. Effective host cell penetration in barley [12], Arabidopsis [13], tomato [14] and pea [15] requires presence of wild type variants of plant *Mildew Locus O* (*MLO*) genes, indicating a common molecular mechanism of powdery mildew pathogenesis in monocotyledonous and dicotyledonous plants. Recessively inherited loss-of-function *mlo* alleles mediate broad-spectrum powdery mildew resistance in these species that is effective at the pre-invasive stage, leading to early termination of pathogenesis. *MLO* genes code for a plant-specific type of integral membrane protein with yet unknown biochemical function [16]. They harbour a C-terminal cytoplasmic calmodulin binding domain that confers calcium-dependent calmodulin binding *in vitro* [17] and *in vivo* [18]. As revealed by *in planta* Fluorescence Resonance Energy Transfer (FRET) experiments, calmodulin binding to barley MLO increased transiently and locally at fungal penetration sites between 14 and 24 hours post inoculation, i.e. around the time when the switch occurs from surface to invasive growth [18]. Barley *MLO* and Arabidopsis *MLO2* were found to represent integral components of a conserved transcriptional regulon that functions in antifungal immunity [19]. This discovery assigns barley MLO and Arabidopsis MLO2 an authentic negative regulatory role in plant defence, suggesting that *mlo*-based resistance is not an accidental pleiotropic effect but likely the consequence of de-regulated (accelerated) Microbe-Associated Molecular Pattern (MAMP)-triggered plant immune responses. This notion is further supported by the fact that *mlo* resistance in both barley and Arabidopsis requires the same molecular components as basal (MAMP-triggered) defence [20]. For example, in both species *mlo* resistance relies on

members of the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) family (barley ROR2 and Arabidopsis PEN1, respectively) [13,21]. In further support of this concept it has now been found that *mlo* resistance in Arabidopsis is dependent on tryptophan-derived secondary metabolites such as the phytoalexin camalexin and indole glucosinolate derivatives [22], which are also crucial for resistance against non-adapted powdery mildew pathogens [23].

Recently, other members of the Arabidopsis MLO family were found to be involved in plant developmental processes. While Arabidopsis MLO4, a MLO family member predominantly expressed in epidermal cells of the root meristematic zone, is implicated in root thigmomorphogenesis [24], MLO7 co-functions with the receptor-like kinase FERONIA in pollen tube reception in the female gametophyte [25]. Notably, the *feronia* mutant in addition to its defect in fertilization exhibits marked powdery mildew resistance, which uncovers MLO family members and FERONIA as components that both function in either process, plant defence and pollen tube reception [25]. It remains, however, to be seen whether *MLO2* and *FERONIA* are components of the same genetic program and whether the respective mutants confer resistance *via* the same defence execution machinery.

The SNARE proteins PEN1/ROR2, which are required for resistance to penetration by powdery mildew fungi in Arabidopsis and barley, form ternary SNARE complexes with VAMP721/722 and SNAP33/34 [26,27]. Structure-function analysis of the PEN1 t-SNARE revealed that phosphorylation of N-terminal serine residues and amino acids of the linker region are required for full PEN1 defence activity *in planta* [28]. Before SNARE complex formation and vesicle fusion, vesicle tethering to the plasma membrane is a prerequisite for secretion of defensive vesicle cargo into the plant apoplast. The exocyst is an octomeric protein complex that mediates vesicle tethering to the plasma membrane before fusion [29]. The Arabidopsis exocyst component EXO70B2 interacts with SNAP33 in a yeast two-hybrid assay. However, Arabidopsis *exo70b2* mutants do not allow for enhanced fungal penetration but show an altered cell wall fortification (papilla) response when challenged by non-adapted *Bgh* [30]. Future studies are thus required to understand whether this results from redundancy of EXO70 subunits or whether resistance to powdery mildew is independent of EXO70 proteins.

Genetic evidence indicates a functional link between the t-SNARE ROR2 and the small GTPase ARFA1b/1c in barley. ARF1 is described to be crucial for vesicle budding from endomembranes, and ARFA1b/1c-labelled organelles are recruited to attempted *Bgh* penetration sites in barley epidermal cells (Figure 3). Both transient-induced gene silencing

(TIGS) of barley *ARFA1b/1c* and transient expression of ARFA1 mutant variants arrested in either the GDP- or GTP-bound status greatly enhanced the penetration success of *Bgh* on susceptible barley and restricted callose deposition in papillae [31]. However, this effect of dominant negative ARFA1b-GDP could not be observed in super-susceptible *ror2* barley. Expression of ARFA1b-GDP also prevented focal accumulation of yellow fluorescent protein (YFP)-tagged ROR2 at the sites of fungal attack. Since ARFA1b further co-localized with the endosomal marker ARA7, budding from endomembranes appears to be a prerequisite for ROR2-dependent focal accumulation of defensive compounds [31]. A function of ARF1 family members in nonhost resistance is also supported in grapevine, where the ARF1 pathway inhibitor brefeldin A limits penetration resistance to non-adapted powdery mildew. The same pathway might also support formation of the haustorial complex because brefeldin A also limited susceptibility to an adapted powdery mildew fungus [32]. Brefeldin A is known to affect retrograde trafficking of membranes from the Golgi apparatus to the endoplasmic reticulum, but it is not understood to what extent ARFA1b-like proteins are affected by this inhibitor. Similar dual functions were assigned to phosphoinositide 3-kinase dependent endosome trafficking when the corresponding inhibitor wortmaninn was applied [32]. Future genetic and live cell imaging approaches should shed more light on the question which particular branches of the multifaceted plant endomembrane trafficking pathways are recruited for penetration resistance against powdery mildew fungi.

Besides secretory processes that rely on endomembrane compartments and vesicle transport, membrane-localized ATP-binding cassette (ABC) transporters likewise contribute to the restriction of powdery mildew pathogenesis. In Arabidopsis, function of the PEN3/PDR8 transporter limits penetration by non-adapted powdery mildew fungi [33]. As PEN3 operates in the same pathway as PEN2, an unconventional myrosinase [23], and *PEN3* is co-expressed with *PEN2* and other genes required for the biosynthesis of indole glucosinolates [19], PEN3 is likely to extrude these toxic metabolites to the apoplast. Another member of the ABC transporter family is wheat LR34, a protein that confers resistance to multiple fungal pathogens (including powdery mildew fungi) in an allele-specific manner [34].

The fungal haustorium

Following successful host cell invasion, the accommodation of haustoria inside plant cells represents the next major step during powdery mildew pathogenesis. Haustoria remain separated from the plant cytoplasm since they are covered by the extrahaustorial membrane, a

host plasma membrane derivative that tightly surrounds invading fungal haustoria (Figure 2). The extrahaustorial membrane differs markedly in its molecular composition from the conventional host plasmalemma and was previously found to be devoid of a set of tested plant plasma membrane marker proteins [35,36]. Notably, the unusual Arabidopsis powdery mildew resistance protein RPW8.2 provides the first example of a host protein that is specifically targeted to the extrahaustorial membrane [37] (Figure 2). Confocal laser scanning microscopy and immunogold labelling of powdery mildew-infected, RPW8.2-YFP-expressing cells revealed accumulation of RPW8.2 at the extrahaustorial membrane of developing haustoria from 16 hours post inoculation onwards (Figure 2). Combined pharmacological and genetic analyses revealed that targeting of RPW8.2 to the extrahaustorial membrane is independent of salicylic acid biosynthesis and signalling, but dependent on actin cytoskeleton function. Though SA is not required for RPW8 recruitment to the extrahaustorial membrane, RPW8 resistance requires an ENHANCED DISEASE SUSCEPTBILITY1 (EDS1)-dependent salicylic acid amplification loop for its function [38]. Presence of RPW8.2 at the extrahaustorial membrane enhances hydrogen peroxide accumulation in and callosic encasement of the haustorial complex, two key events that strongly correlate with RPW8-mediated resistance [37-40]. Progressive encasement of haustorial complexes by a combination of callose and other cell wall polymers that extend from papillae was also observed in the case of interactions between Arabidopsis ecotype Col-0 (lacking RPW8) and the poorly adapted sow thistle powdery mildew pathogen, *Golovinomyces cichoracearum* UMSG1 [41], and between Arabidopsis Col-0 and the virulent powdery mildew pathogen, *G. orontii* [36,42]. However, the virulent powdery mildew species *G. cichoracearum* UCSC1 can largely suppress callosic encasements of haustoria in the absence of RPW8 [37], suggesting that this powdery mildew species is better adapted to Arabidopsis than the former two species.

The RPW8 family proteins are comprised of an N-terminal transmembrane and one to two coiled-coil domains [39], Apart from the structural similarity with regard to the coiled-coil domain they lack sequence similarity to prototypical resistance proteins of the nucleotide-binding leucine-rich repeat class [43]. Recently, the 14-3-3 lambda isoform was identified as a protein interacting with the C-terminus of RPW8.2. Knockdown of *14-3-3 lambda* compromised RPW8.2-dependent powdery mildew resistance, while its overexpression resulted in spontaneous leaf cell death and enhanced powdery mildew resistance [44].

Proteomic analyses of *Bgh* haustoria revealed the identity of a range of haustorial proteins, most of which function in metabolic pathways or energy production [9,45,46]. A few candidate effector proteins were also discovered in the haustorial proteome [9,46,47].

Ultrastructural examination of isolated haustorial complexes from *Golovinomyces orontii* revealed the presence of multivesicular compartments in the haustorial cytoplasm (Figure 4A), the paramural space of haustoria and in the extrahaustorial matrix (the zone between the extrahaustorial membrane and the fungal cell wall) [36]. Together with the finding that multivesicular bodies are also delivered from attacked host cells into papillae (Figure 4B,C) [42,48] these observations strengthen the notion that a dedicated secretory warfare takes place at the plant-fungus interface. It remains a major challenge for the future to uncover the cargo molecules transported by vesicles and multivesicular bodies on both the plant and fungal side.

Host cellular rearrangements during powdery mildew infection

The host cell architecture greatly changes for accommodation of the haustorial complex. This involves dynamic rearrangements of endomembrane compartments and the cytoskeleton (Figure 1) that are reminiscent of cellular reorganization events following a mechanical stimulus [49]. Plant RHO-like GTPases (RAC/ROPs) are considered major players in plant cytoskeleton organization. The barley RAC/ROP small GTPase RACB is a susceptibility factor required for the establishment of haustoria of *Bgh* in barley epidermal cells. Barley RACB regulates polar cell expansion in epidermal cells as well as filamentous actin re-organization upon fungal attack [50,51]. A RACB-interacting protein, RIC171, considered as downstream scaffolding protein supporting signalling from active RAC/ROP, conferred enhanced fungal entry (haustorium formation) into transformed cells of barley when over-expressed. Interestingly, RIC171 is recruited to the site of fungal attack and forms a complex with RACB at the site of pathogen entry, suggesting local formation of active RAC/ROP signalling complexes [52] (Figure 1E). Additionally, RACB interacts in barley with a novel microtubule-associated ROP GTPase activating protein, MAGAP1, which likely switches off RACB and hence negatively controls entry by *B. graminis* (Figure 1D). Microtubule organization greatly changes during fungal attack depending on whether the fungus succeeds in penetration or not. MAGAP1 appears also to regulate polarity of cortical microtubules. Hence, RAC/ROP-regulated microtubule dynamics may function in penetration resistance and cellular rearrangement for compatibility[53].

Later stages of powdery mildew pathogenesis

Post-penetration colonization involves the suppression of host cell death. ENHANCED DISEASE RESISTANCE 1 (EDR1) is a conserved protein kinase required for full susceptibility to powdery mildew in Arabidopsis. Loss of EDR1 leads to salicylic acid

signalling-dependent and late cell death-associated post-penetration resistance to *G. cichoracearum* [54]. Enhanced disease resistance of *edr1* mutants is not specific for powdery mildew [54], but correlates with an increased transcriptional response to powdery mildew inoculation [55]. Susceptibility to powdery mildew is re-established in an *edr1* mutant background by an additional mis-sense mutation in *KEG* (*KEEP ON GOING*), encoding a protein containing a functional RING finger E3 ubiquitin ligase, a kinase domain, ankyrin repeats and HERC2-like repeats. These HERC2-like repeats have now been shown to recruit EDR1 to an endomembrane compartment that was identified as trans-Golgi network/early endosome by co-localization with SYP61 [56]. Future work may now clarify whether endomembrane trafficking is a target of EDR1/KEG-controlled stress regulation or provides a platform for subcellular focusing of signalling complexes in pathogen response. A potential link between endomembrane-localized ARFA1b (see above) and EDR1/KEG1 is not yet established.

Autophagy is a catabolic process involved in the re-allocation of nutrient resources and in limiting pathogen-induced cell death. Both processes may be crucial for a compatible interaction with powdery mildew fungi. Accordingly, a set of Arabidopsis *atg* mutants (*atg2*, *atg5*, *atg7* and *atg10*) exhibits a pronounced powdery mildew-induced cell death response and enhanced disease resistance against *G. cichoracearum* [57]. *ATG* genes encode proteins that function in the biogenesis of autophagosomes, and accordingly *atg2* plants were shown to be defective in autophagosome formation. Notably, cell death in *atg2* plants only partially depends on salicylic acid, whereas effective defence to powdery mildew was largely dependent on this phytohormone [57]. It would thus be interesting to learn whether the pathogen-induced cell death response in *atg2* resembles a hypersensitive reaction or another type of cell death.

Post-penetration colonization by powdery mildew fungi is accompanied by the establishment of a nutrient sink at sites of infection [58]. This may result in formation of green islands of juvenile tissue at the site of colony formation, whereas the rest of the leaves shows symptoms of senescence [59]. A comprehensive study used laser-microdissection to identify genes that are locally expressed in Arabidopsis cells where *G. orontii* succeeded in colonization (samples taken at five days post inoculation; reflecting late changes in the host transcriptome). This procedure identified 67 transcription factors potentially involved in photosynthesis, abiotic stress responses, defence, auxin signalling, and the cell cycle. In particular MYB3R4 was identified as a transcription factor that is apparently required for pathogen induced endoreduplication and full susceptibility to *G. orontii* [60].

Endoreduplication together with alterations in carbohydrate metabolism might be used to meet the enhanced metabolic demands imposed by the fungus at the site of infection. Alcoholic fermentation genes are also often up-regulated in polyploid cells and in compatible interactions with biotrophs including powdery mildew fungi [61]. This up-regulation in fermentation was predicted to confer a metabolic advantage when hexose levels are high [61]. Interestingly, barley alcohol dehydrogenase appears to mediate susceptibility to *Bgh* [62], whilst the fungus itself lacks the pathway genes for alcoholic fermentation [63]. Another mechanism of susceptibility might be the transcriptional up-regulation of genes encoding transporters for carbohydrate supply across the host plasma membrane [58,60,64]. Accordingly, a novel type of sugar transporter gene called *SWEET12* shows elevated transcript levels in the compatible interaction of Arabidopsis and *G. cichoracearum* [65].

Conclusions

Novel discoveries in plant cell biology have been made on the basis of the powdery mildew-plant system due to the highly localized host responses upon infection by these obligate biotrophic phytopathogens. The induced site-specific host responses enable in some cases the detection of phenotypes that are otherwise masked by genetic redundancy. An example of this concept is the altered state of endoreduplication in the *myb3r4* single mutant [60], whereas double mutants were required to observe cell cycle-associated phenotypes under non-induced conditions [66]. Much of the recent progress in cell biological analyses of the plant-powdery interaction has been made in the area of secretion and vesicle trafficking. Prominent examples comprise the identification of a ternary SNARE complex for antifungal host defence [26-28], the detection of multivesicular bodies on both the plant and fungal side [36,42,48], as well as the characterization of regulators of vesicle transport [31] and cytoskeleton organization [50,53,67]. This emphasizes the presumed importance of these processes at all stages of plant-powdery mildew interactions. Novel tools for functional studies of powdery mildew genes, such as powdery mildew genome sequences [63] and host-induced gene silencing (HIGS) technology [68], have high potential for future detailed dissection of this combat. Likewise, new cell biological approaches such as advanced live imaging techniques [26,69] promise to disclose further secrets of the battle between plant and fungus and possibly reveal novel aspects of plant cell biology. Thus, the liaison between plant pathology and plant cell biology promises to remain a fruitful one.

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•• ARF GTPases are involved in vesicle budding, but their specific functions in plant pathogen responses are largely unknown. This study demonstrates that barley ARFA1b/1c is required for subcellular positioning of the t-SNARE ROR2 and for callose deposition at sites of attack from *Bgh*. Loss of ARFA1b/1c function strongly limits ROR2-dependent barley resistance to fungal penetration, suggesting that budding from endomembranes is a prerequisite for penetration resistance.

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• High quality transmission electron microscopy and immunogold labelling visualize the formation of the haustorial complex in a compatible interaction of Arabidopsis and *G. orontii*. Data suggest i.a. that, besides plants [42,48], powdery mildew fungi use multivesicular bodies and exosomes for secretion to the plant-parasite interface.

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•• The authors demonstrate for the first time that a host protein, the unusual resistance protein RPW8, is specifically enriched in the extrahaustorial membrane at the interface of Arabidopsis and *G. cichoracearum*. RPW8-loaded membrane compartments are delivered to the haustorial complex via an actin-dependent mechanism. RPW8 locally initiates oxidative defence and callosic encasement of haustoria. Genetic and pharmacological evidence suggests that targeting of RPW8 to the haustorial complex is pivotal for coordinated antifungal defence.

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Figure legends.

Figure 1. Cell biology of the barley-powdery mildew interaction.
A. GFP labelled Golgi bodies accumulate at the site of defended attack from *Bgh* (arrow) at 18 h after inoculation. Cytoplasm and nucleoplasm are labelled in red by soluble DsRED. Golgi bodies are marked by an in-frame-replacement of a fragment of barley Calreticulin 3 by GFP, (Ruth Eichmann, TU München, unpublished). Overlay of green, red and transmission channel.
B. Lipid material and vesicle-like structures stained by FM4-64 accumulate at the site of papilla formation and in surrounding cytoplasm during fungal attack.
C. Filamentous actin stained by Phalloidin-Alexa488 (in glow) polarizes to the site of interaction with *Bgh* at 22 h after inoculation [47]. The same fungus attempted twice to penetrate into a highly resistant barley *mlo* genotype. The first attempt (long arrow) is associated with apoplastic accumulation of phenolic substances, which gives rise to autofluorescence in the cell wall apposition, the horizontal anticlinal cell wall and a halo (in green). The consecutive penetration attempt (short arrow) just began close to the vertical anticlinal cell wall (not labelled), and defensive plant material is not yet visible.
D. Multichannel images of the same cell. Cortical microtubules labelled by RFP-MAGAP1 (in red, right panel) [51] focus towards the site of attack (arrow) from nonadapted *B. graminis* f.sp. *tritici* in barley. The cytoplasm (in green, left panel) is labelled by soluble GFP and also polarizes. The lower panel shows the overlay of both channels (left) and the transmission channel (right).
E. Multichannel images of the same cell. A yellow fluorescing complex of RACB (wild type) and RIC171 is visualized by Bimolecular Fluorescence Complementation (BiFC) [52]. The complex of RACB and RIC171 (in yellow; left panel) accumulates at the neck (arrowhead in all channels) of a haustorium (highlighted by the arrow in all channels), which is under formation in a susceptible barley genotype. The cytoplasm is labelled by soluble DsRED (2nd from left panel). The 2nd from right panel is a transmission micrograph; the right panel represents a maximum projection of 10 optical sections through the haustorium (overlay of all three channels).

Figure 2. RPW8.2-YFP accumulation around an *E. cichoracearum* haustorium.
Rosette leaves of a transgenic *Arabidopsis thaliana* (ecotype Col-0) line expressing *RPW8.2-YFP* from the *RPW8.2* promoter was challenged with *G. cichoracearum* UCSC1. Following staining with propidium iodide (to highlight fungal structures), the sample was imaged by

confocal laser scanning microscopy at two days post inoculation. The micrograph is a maximum projection of a Z-stack of 23 images (8.8 µm thick with a scanning interval of 0.4 µm). Note the greenish YFP label covering the surface of the haustorium (H), which delineates the extrahaustorial membrane. The micrograph is courtesy of Wenming Wang and Shunyuan Xiao. For further details see [37].

Figure 3. Accumulation of ARFA1b/1c-eGFP-labelled organelles at attempted *Bgh* entry sites.

Barley epidermal cells transiently expressing an ARFA1b/1c-eGFP fusion protein were challenged with *Bgh* conidiospores and cells imaged by confocal laser scanning microscopy at 12 hours post inoculation. Bright field image (**A**), GFP fluorescence (**B**) and overlay (**C**). Conidiospore, white arrowhead. Note the local accumulation of ARFA1b/1c-eGFP labelled organelles beneath the attempted fungal entry site (gray arrowhead). Size bar in (**C**), 20 µm. The micrographs are courtesy of Hans Thordal-Christensen. For further details see [31].

Figure 4. Multivesicular bodies in the plant-powdery mildew combat.

A. The transmission electron micrograph shows a section of an isolated *G. orontii* haustorium prepared by high pressure freezing and freeze substitution. Multivesicular bodies (white arrowheads) are amongst the most prominent organelles in the haustorial body (cytoplasm). Note the "tram line"-like haustorial plasma membrane in the right bottom corner, which together with the adjacent haustorial cell wall delimits the haustorial body from the extrahaustorial matrix. Scale bar, 1 µm. The micrograph is courtesy of Ulla Neumann. For further details see [36].

B. Host endoplasmic reticulum (ER) and paramural body (PB) at a cell wall apposition (CWA) beneath an appressorium of *Bgh* in *mlo*-barley at 20 h after inoculation. Note exosome-like vesicles within the apoplast/PB [48]. Arrows mark the limiting membrane of the PB, which is in continuum with the plant plasma membrane.

C. Entrapped membranes/exosome-like vesicles within a cell wall apposition in an epidermal cell of *mlo*-barley attacked by *Bgh* at 19 h after inoculation. Membranes are entrapped in the apoplast between the cell wall and callosic deposits. Callose is visualised by immunogold labelling [48].

Figure 1

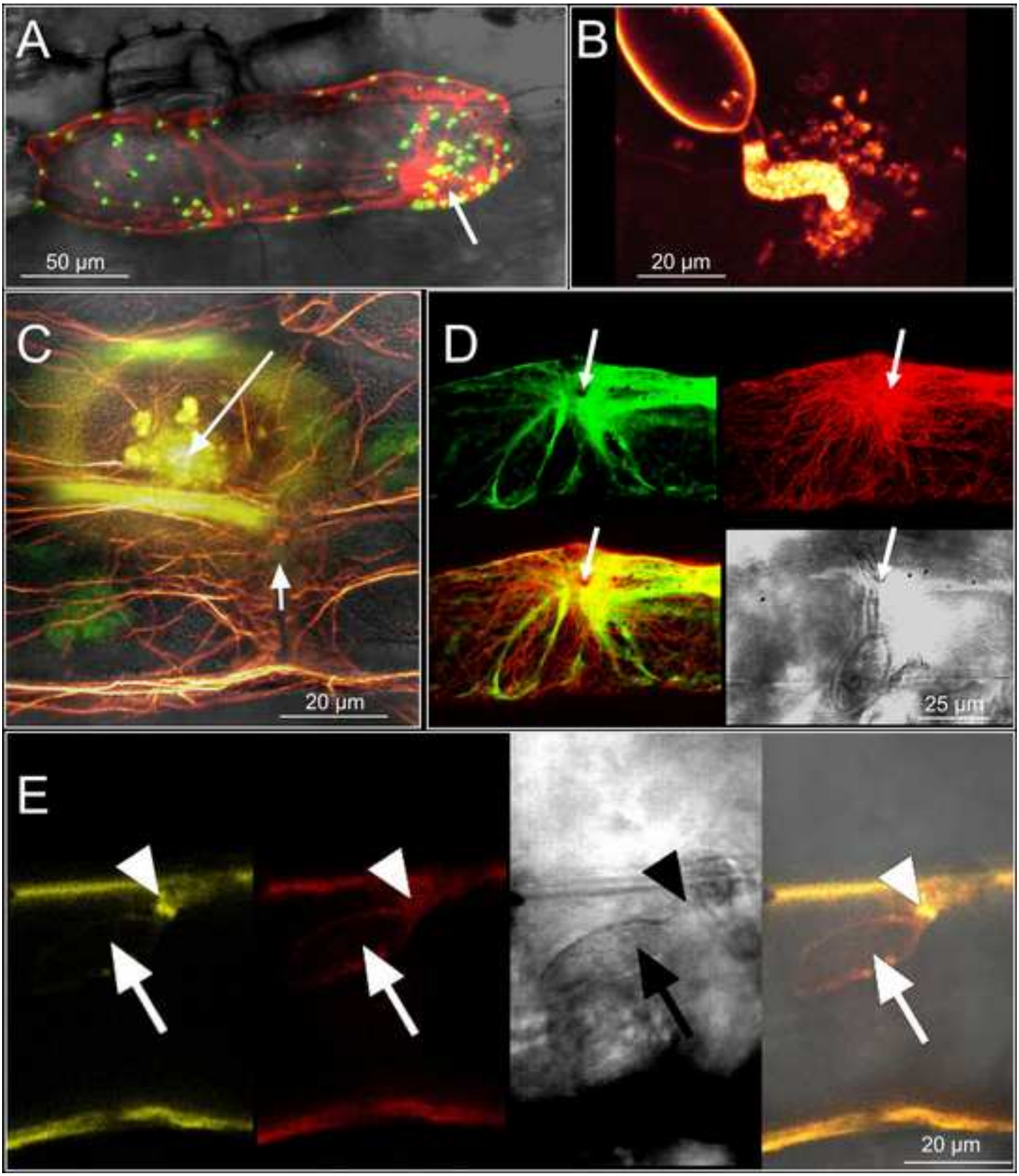

Figure 2

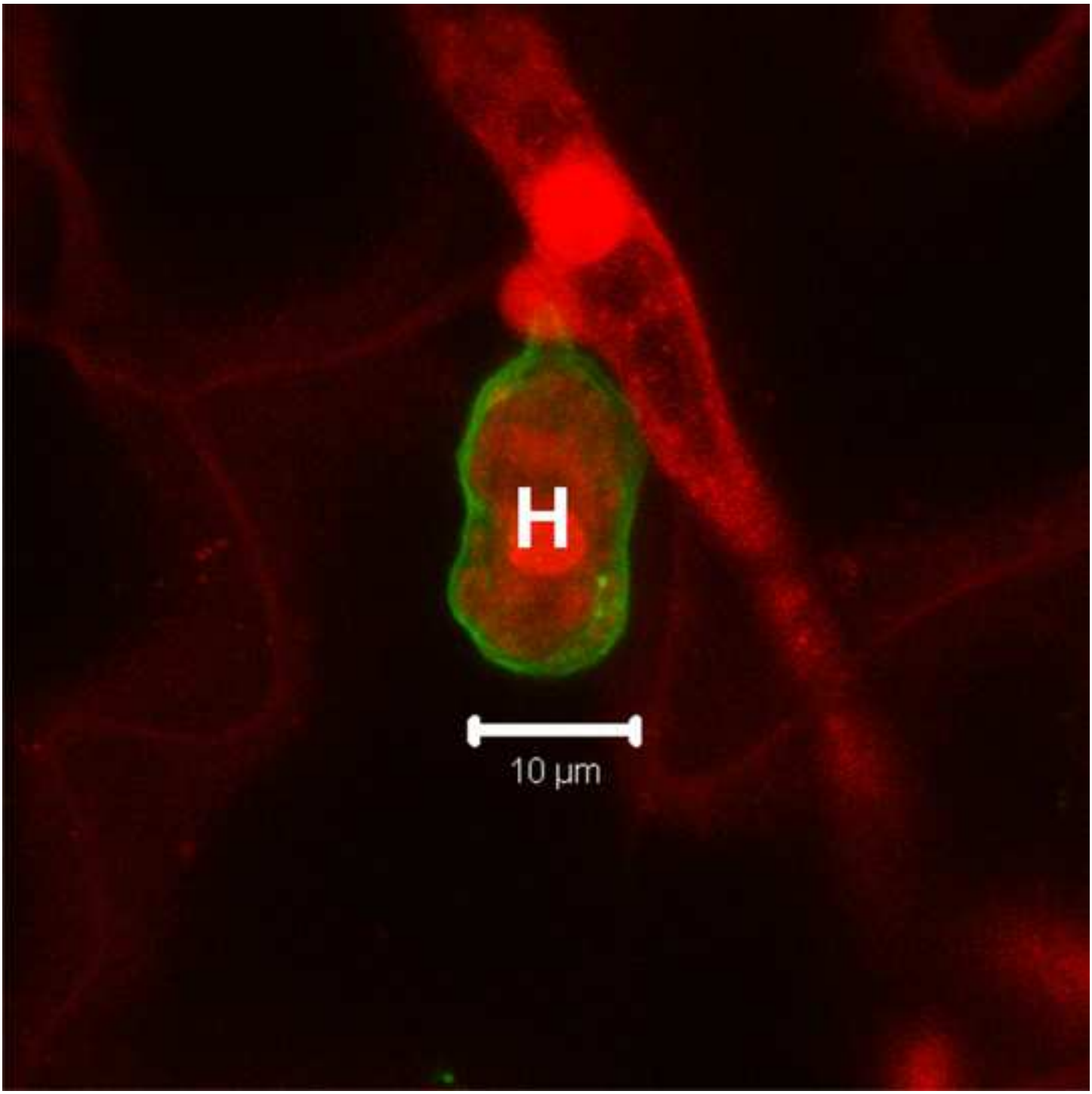

Figure 3

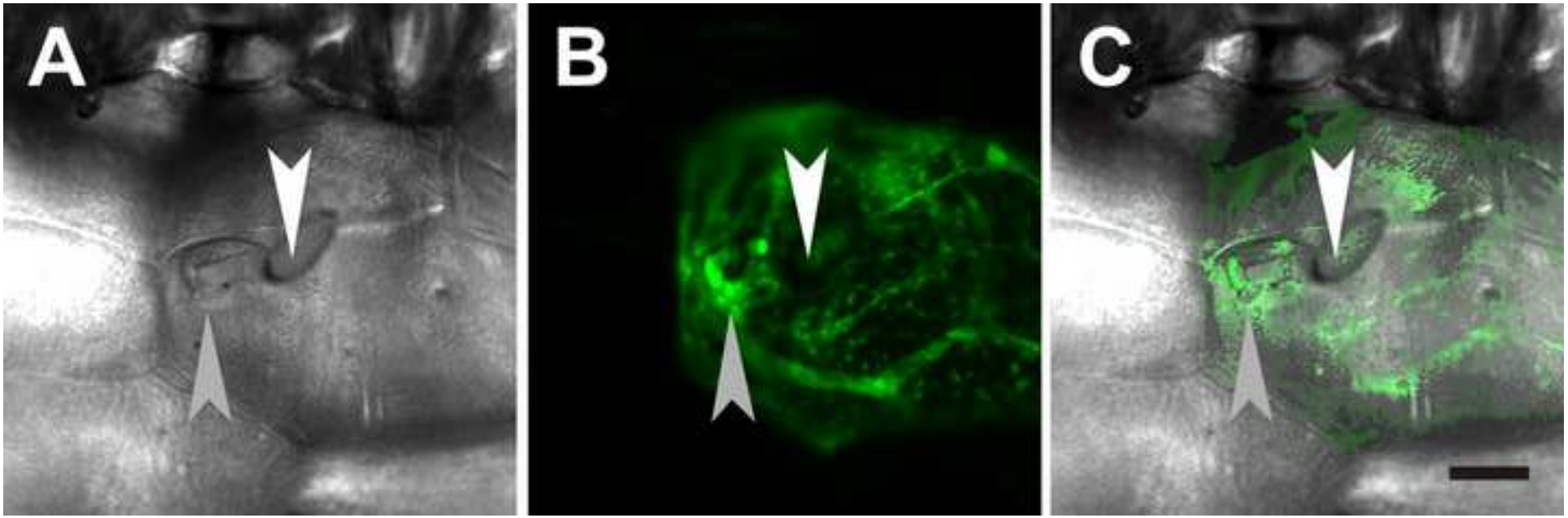

Figure 4