Communication is key: extracellular vesicles as mediators of infection and defence during host–microbe interactions in animals and plants

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One sentence summary: Within the context of animal extracellular vesicles (EVs) and their interactions, recent developments in the growing fields of plant and fungal EVs suggest complex cross-kingdom communication with microbes for infection/protection.

Editor: Bart Thomma

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INTRODUCTION

The endomembrane system was discovered in the mid-1940s, aided by newly developed cell fractionation and electron microscopy (EM) techniques, leading the way to understand the traditional secretory pathway with the help of pancreatic exocrine cells (Palade 1975). In the meantime, insights were made into intercellular communication as well as with the extracellular environment, which is crucial in many cellular processes including cell survival, differentiation, proliferation and apoptosis. An understanding of this form of communication has led to the establishment of the role of extracellular vesicles (EVs) as mediators of such communication by facilitating the exchange of growth factors, enzymes, cytokines and various other signalling molecules. As far back as 1946, EVs were first reported as a precipitable factor. In the later coagulation research of Peter Wolf, this was called ‘platelet dust’ (Wolf...
Vesicles as thermodynamic entities

All living cells vesiculate, allowing for intracellular and extracellular compartmentalization and the evolutionary fitness this entails. However, the integral role of vesiculation in cellular life has emerged gradually. Following the formalization and universal adoption of cell theory throughout the 18th and 19th centuries, the initial conception of a dynamic and polymorphous cell membrane dates to suggestions made by late 19th century scientists such as S. Quincke, who posited that fluid fats must be their cell membrane, and barrier properties (Danielli and Davson 1935), and cytoplasmic) proteins and nucleic acids. This content can vary according to growth conditions (Dauros Singoreño et al. 2017).

Secretion of EVs by fungi and plants was noted in the 1960s. Hyphae of true fungi (Eumycota) were shown to secrete vesicles, termed lomasomes, that looked and behaved a lot like MVs (Moore and McAlear 1961). MVs were later shown and correctly identified in meristem cells of carrot (Daucus carota) cell suspension cultures (Halperin and Jensen 1967). Similar to the earlier study in fungi, MVs were noted to fuse with the plasma membrane, releasing their contents into the cell wall. This review will discuss the progress that has been made since these pioneering studies to better understand EV biogenesis and function in plants and fungi and their relationship to cross-kingdom interactions.

Intra- and extracellular vesicles

Despite much fundamental research, the roles of vesicles in cellular communication remained obscure until the late 20th century, with most work focusing on intracellular vesicle communication. Through the Nobel prize-winning work of Randy Schekman, James Rothman and Thomas Südhof, it was discovered that intracellular vesicles of eukaryotes comprise a fundamental part of the endomembrane system, trafficking cargo between the nuclear envelope, endoplasmic reticulum (ER), Golgi and plasmalemma (Kaiser and Schekman 1990; Hata, Slaughter and Sudhoff 1993; Sollner et al. 1993). As such, specialized vesicles, such as lysosomes, endosomes and autophagosomes, are often categorized as separate organelles within this system (Harris 1986). Many of these complex sorting pathways are now broadly described, at least in model organisms (Nebenfuhr 2002; Hu et al. 2015; Palmisano and Melendez 2019).

Comparably, the EVs of eukaryotes have not until recently enjoyed the same limelight, while carrying no less complexity in terms of trafficking pathways. Indeed, it is tempting to speculate that when considering the ability for EVs to engage in cross-kingdom communication, it may ultimately be found that EVs represent a greater diversity of messages than their evolutionarily conserved intracellular counterparts. Despite initial neglect, EVs of animals and all other kingdoms are now relatively well studied. Discussing all varieties of protist and prokaryotic EVs is beyond the scope of this review, with each deserving its own dedicated space. Instead, the focus of this review shall be to compare and contrast the three multicellular eukaryotic kingdoms of animals, plants and fungi and explore their interactions.

EXTRACELLULAR VESICLES

EVs in humans and animals as a paradigm

Since the 1940s it has been known that human plasma contains a subcellular component facilitating fibrin formation (Chargaff and West 1946; O’Brien 1955). Later, through the use of EM, it
was possible to show that these subcellular factors comprised microscopic vesicles, originally termed ‘platelet dust’, nowadays EVs, and that they possessed procoagulant activity, similar to that provided by intact platelets (Wolf 1967).

More recently and since the formation of the International Society for Extracellular Vesicles (Araldi et al. 2012) the interest in EVs has grown exponentially (Srivastava et al. 2020). Progressing from an initial interest in their procoagulant properties, they were found to play roles in inflammation (Freyssinet et al. 1999; Nieuwland and Sturk 2002), and the circulating EVs in blood were found to be derived from a range of cells including platelets, erythrocytes, lymphocytes, granulocytes, monocytes and endothelial cells. Many pathogens also release EVs as a decoy function to prevent the deposition of complement or to activate and consume complement in the surroundings as was found with the unicellular protozoan parasite, Trypanosoma cruzi (Cestari et al. 2012). Furthermore, the infection process, certainly for intracellular pathogens, stimulates release of EVs from host cells. As well as playing evasive strategies for example as decoys (Inal et al. 2013b), pathogens may opportunistically utilize host EVs to acquire complement inhibitors (Cestari et al. 2012; Inal, Ansa-Addo and Lange 2013a). The decoy function of EVs is not unique to animal cells as bacteria produce MVs for interception of bacteriophages (Toyofuku, Nomura and Eberl 2019). These bacterial MVs also carry enzymes that can degrade antibiotics (Schwechheimer and Kuehn 2015). Furthermore, just as outer membrane vesicles (OMVs) from Porphyromonas gingivalis may assist with the interaction of other periodontal bacterial pathogens with eukaryotic host cells (Kamaguchi et al. 2003), we found this to also be so with the intestinal parasite Giardia intestinalis whose EVs aided attachment to intestinal epithelial cells (Evans-Osses et al. 2017). EVs from protozoan parasites, such as T. cruzi shuttle genetic information between parasites and host cells. Fungal EVs meanwhile are rich in enzymes able to degrade the cell wall that likely explains their route across the cell wall, a similar problem to that faced by MVs from Gram-positive bacteria as well as several virulence factors as described later.

Properties and mechanism of release of mEVs (microvesicles) and sEVs (apoptotic bodies)

According to MISEV2018 (Thery et al. 2018) EVs comprise the small sEVs and medium mEVs as well as large EVs (sEVs or apoptotic cell-derived EVs). mEVs are phospholipid-rich, microscopic vesicles formed by exocytic budding of the plasma membrane (Fig. 1). During EV formation, the lipid asymmetry of the lipid bilayer, which comprises phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC) and sphingomyelin (SM) is lost, resulting in an outer leaflet that is rich in negatively charged phospholipids. Whilst the neutral phospholipid PC and SM are primarily located on the outer leaflet of the lipid bilayer, the negatively charged PS and PE are located to the inner leaflet. This asymmetrical distribution of phospholipids in the plasma membrane is actively maintained by various enzymes, including aminophospholipid translocase (APT, flipase) or floppase (Sims and Wiedmer 2001), but also scramblase, calpain and gelosin (the latter present only in platelets) (Piccin, Murphy and Smith 2007). The lipid asymmetry is maintained by these enzymes allowing membrane phospholipids to move to the outer leaflet whilst the aminophospholipids are simultaneously redirected to the inner leaflet of the bilayer (Piccin, Murphy and Smith 2007). When cells become activated or during early apoptosis the ability to maintain this asymmetric distribution of the lipid bilayer is lost. Negatively charged phospholipids such as PS and PE are then exposed at the membrane surface. When intracellular concentrations of calcium rise for example during activation of cells (Stratton et al. 2015), infection by intracellular pathogens, or sublytic deposition of calcium ionophore or of complement proteins as a membrane attack complex, then the steady state is changed resulting in PS expression on the membrane surface (Fox et al. 1990; Connor et al. 1992; Diaz and Schroit 1996).

The intracellular mechanism(s) leading to mEV biogenesis are not fully elucidated, but the process does seem to be dependent on an underlying stimulus. There may even be multiple biogenesis pathways depending on the stimulus, and mEV release may occur through either activation of cell death, whether apoptotic or necrotic (Ardoine and Pieske 2008). The signals that induce cell activation/apoptosis, include chemical stimuli, such as cytokines, endotoxin and thrombin, or physical stimuli, such as hypoxia or shear stress (Vanwijk et al. 2002), the latter typically being important in mEV release from platelets (Gasser et al. 2003). Other triggers would include complement membrane attack complex C5b-9, with or without antibodies, phorbolesters, calcium ionophore (A23187), adenosine diphosphate, adrenaline and microbial peptides such as formyl-methionyl-leucylphenylalanine (Gasser et al. 2003).

Cellular activation of platelets leads to mEV formation (Fig. 1) through a rise in cytosolic calcium and the concomitant activation of calpain and protein kinases, which causes cytoskeletal rearrangement, membrane blebbing and mEV formation (Wiedmer and Sims 1991; Yano et al. 1994; Miyazaki et al. 1996). mEVs may also be released in vitro by depriving cells of growth factor or through complement activation (Hamilton et al. 1990; Jimenez et al. 2003).

In apoptosis, IEV (or apoptotic body) release is associated with membrane blebbing, which involves a redistribution of cellular contents, likely due to changes in volume-induced stress during cell death perhaps related to volume stress that occurs as cells die. ROCK-1 (Rho associated kinase 1), an effector of Rho GTPases, is essential for apoptotic membrane blebbing, although not all cells bleb, and is activated during mEV biogenesis (Distler et al. 2005); indeed blebbing itself can differ during the different stages of apoptosis. In the terminal phases of apoptosis mEV release seems most likely to occur and this is likely to coincide with cell fragmentation and apoptotic body formation, which represents collapsed cells undergoing nuclear fragmentation. Differences in the mechanism of mEV formation are likely to depend on whether the cells are undergoing cell activation or apoptosis and such differences may consequently lead to variations in mEV size and macromolecular cargo (protein and RNA), which may also lead to functional differences.

sEVs are generated through exocytosis

As for mEVs, sEVs play roles in maintaining normal cellular physiology as well as in disease pathology (Vlassov et al. 2012). In terms of biogenesis, sEVs have an endocytic origin. During endocytosis an early endosome is formed. This may then either follow a degradative pathway, upon fusion with lysosomes, or undergo intraluminal budding to generate ILVs within an MVB. Upon fusion of the MVB with the plasma membrane, its cargo of ILVs is released as sEVs (Fig. 1). There are two separate pathways that result in the formation of ILVs. For the inward budding process and cleavage of bud necks of the MVB limiting membranes,
Figure 1. Biogenesis of microvesicles (mEVs), ILVs, exosomes (sEVs) and apoptotic bodies (sEVs) in animals. (A) mEVs are shed from the plasma membrane and shown in larger scale as a result of increased $[Ca^{2+}]$, cytoskeletal disruption and loss of lipid asymmetry. (B) sEVs are formed by intraluminal budding of late endosomes/MVBs and released upon their fusion with the plasma membrane. TSG101 is a protein involved in ILV biogenesis. (C) sEVs (apoptotic bodies) are released from the cell surface during apoptosis. Although evidence suggests mEV biogenesis, sEVs are more commonly generated in fungi and plants. Cellular structures are not drawn to scale.

components of endosomal sorting complex required for transport (ESCRT) are involved (van Dommelen et al. 2012). In the second pathway known as the ESCRT-independent exosomal pathway, SMase results in the hydrolysis of sphingomyelin. This generates the cone shaped ceramide, which is believed to result in an immediate negative curvature on the cytosolic leaflet of the endosomal membrane. In turn this induces the inward budding into the endosome and formation of the ILVs (Hurley et al. 2010).

sEVs have a density in sucrose from 1.13 to 1.19 g cm$^{-3}$ and as for mEVs share marker proteins with their parental cell (Inal et al. 2013b; Raposo and Stoorvogel 2013). Amongst characteristic marker proteins, distinctive for sEVs, and present in high abundance (Conde-Vancells et al. 2008; Subra et al. 2010), are heat shock proteins (Hsp90 and Hsc70), fusion proteins and membrane transport proteins (GTPases, annexins and flotillin), proteins involved in ILV biogenesis (TSG101 and Alix) and a range of tetraspanins (CD9, CD63, CD81 and CD82).

Given the generation of EVs during exocytosis (sEVs) or blebbing of membranes (mEVs), their origin can be tracked by cell-specific protein markers. The rules governing the incorporation of different proteins into EVs are not known. These EVs also carry antigens expressed on the surface of the mother cell (Lynch and Ludlam 2007). It is this anionic phospholipid surface that then mediates many of the biological functions of mEVs in animals including the binding of coagulation factors as well as the expression of functional molecules such as selectins or tissue factor.

**IV biogenesis in filamentous microbes**

Understanding of EVs in other multicellular eukaryotes has lagged behind and it was not until this millennium that a general awareness of fungal and plant EVs has emerged (An, van Bel and Hückelhoven 2007; Rodrigues et al. 2007). Clear documentation of mEVs biogenesis in fungi is lacking. However, an EM study of protoplasts from Aspergillus nidulans first documented vesicles budding from the fungal plasma membrane (Gibson and Peberdy 1972). Further work on fungal protoplasts of *Aspergillus fumigatus* recently showed that specific EVs are generated via plasma membrane budding similar to mEV production in animals (Rizzo et al. 2020). The authors mentioned that the fungal cell wall might preclude the observation of vesicles budding from the plasma membrane reminiscent of mEVs biogenesis in fungi.

Conversely, definitive proof does exist for sEVs biogenesis from MVB in multicellular eukaryotes other than animals. The powdery mildew pathogen Golovinomyces orontii produces MVBs that fuse with the plasma membrane to release sEVs (Table 1) (Micali et al. 2011). The oomycete that caused the Irish potato famine, Phytophthora infestans, and the rice blast fungus Magnaporthe oryzae deliver effectors into the cytoplasm of their hosts via unconventional protein secretion pathways (Giraldo et al. 2013; Liu et al. 2014). Upon penetration of the rice epidermis, M. oryzae initially forms invasive hyphae (IH) that secrete apoplastic effectors via conventional secretion. IH also form biotrophic...
## Table 1. Evidence for involvement of extracellular vesicles in controlling biological processes.

| Kingdom | Biological process/organism | Structure | Compatible reaction | Incompatible reaction, defence | References |
|---------|-----------------------------|-----------|---------------------|-------------------------------|------------|
| Plant   | Flower fertilization         | Pollen grain | MVBs, EVs; Exo70A1<sup>a</sup> | Autophagy                      | Goring (2018) |
| Plant   | Barley (interaction with Bgh<sup>b</sup>) | Haustorium | MVBs, ‘mEVs’, ‘autophagy’<sup>c</sup> | HR, MVBs | An et al. (2006a) |
| Plant   | Barley (interaction with Bgh<sup>b</sup>) | Haustorium | MVBs, ‘Autophagy’<sup>d</sup> | sEVs; PEN1, HvEXO70F<sup>e</sup> | An et al. (2006b) |
| Plant   | Arabidopsis (penetration resistance) | Penetration sites | sEVs, tetraspanin, sRNAs | | An, van Bel and Hückelhoven (2007); Oster tag et al. (2013) |
| Plant   | Barley (Ramularia interaction) | | ROR1, ROR2<sup>f</sup> | | McGrann et al. (2014) |
| Fungus  | Golovinomyces orontii | Haustorium | MVBs, sEVs | | Micali et al. (2011) |
| Fungus  | Blumeria graminis f. sp. hordei | Appressorium, haustorium | MVBs | | An et al. (2006a) |
| Fungus  | Magnaporthe grisea (host penetration) | Appressorium | Tetraspanin (‘sEVs’) | | Clergeot et al. (2001) |
| Fungus  | Magnaporthe oryzae (host colonization) | BIC<sup>g</sup> | Autophagy, unconventional secretion | | Sun et al. (2018) |
| Protist | Histoplasma capsulatum | Receptor-mediated endocytosis | Prevention of phagocytosis, apoptosis of host cell macrophage | | Garfoot and Rappleye (2016) |
| Animal  | Homo sapiens, natural killer cells and cytotoxic T-cells | Attack complex | EV release of perforins and granzymes | | Schmidt, Tramsen and Lehrnbecher (2017); Di Pace et al. (2020); Del Vecchio et al. (2021) |

<sup>a</sup>MVB, multivesicular body; EV, extracellular vesicle; molecular component involved in secretion is listed.

<sup>b</sup>Bgh, Blumeria graminis f. sp. hordei.

<sup>c</sup>In quotations: The authors suggest compartments/processes based on microscopic evidence; microvesicles (mEVs) may form at the extrahaustorial membrane, MVBs and vesicles were found in the central vacuole.

<sup>d</sup>In quotations: Published suggested cellular process.

<sup>e</sup>Examples of molecular components involved in penetration resistance employing exosomes (sEVs).

<sup>f</sup>Penetration resistance may have trade-offs regarding resistance against pathogens other than powdery mildew fungi.

<sup>g</sup>BIC, biotrophic interfacial complex.
interfacial complexes (BICs) that accumulate cytoplasmic effectors via unconventional secretion.

Molecular mechanisms of fungal EV formation

Secretory regulators and Snf7p, which are involved in MVB formation, influence the composition and release of EVs in yeast (Oliveira et al. 2010b; Russell et al. 2012). MVB formation is also dependent on the ESCRT complex. The ESCRT machinery determines the size, abundance and composition of EVs (Zhao et al. 2019). EV cargo enriched in cell wall remodelling enzymes protects against antifungal compounds (Zarnowski et al. 2018; Zhao et al. 2019). Vps20 and Snf7 are among the constituents of the ESCRT-III complex that cleaves off ILVs (Babst et al. 2002; Oliveira et al. 2013). Membrane curvature and budding of vesicles is dependent on APTs that contribute to lipid asymmetry (Farge et al. 1999). The P4-ATPase Drs2p is an APT involved in endo/exocytic pathways (Gall et al. 2002; Liu et al. 2008). Similarly, APT1 of Cryptococcus neoformans contributes to polysaccharide secretion via EVs and pathogenesis as well as the intracellular membrane architecture (Rizzo et al. 2014; Rizzo et al. 2018). A genetic screen in Saccharomyces cerevisiae resulted in identification of snf7Δ, vps20Δ and drs2A as oxalate-sensitive mutants (Cheng et al. 2007). While these three genes are clearly involved in ILV and sEV formation, it remains to be determined whether they alter transport of oxalate to the vacuole, out of the cell or both. Besides, deletion of a putative phospholipid-translocating scramblase of Cryptococcus gattii increased the size and altered the composition of EVs; intracellular vesicles and membranes were affected as polysaccharide secretion and capsule formation were enhanced (Reis et al. 2019).

A loose consensus has developed that not only do fungi indeed release sEVs via an endosomal/exosomal, MVB-like mechanism, but also through at least one other independent process, analogous to mEV membrane budding (Oliveira et al. 2010b; Huang et al. 2012; Oliveira et al. 2013; Rodrigues et al. 2014; Bleackley et al. 2019). Experiments in C. neoformans showed that mutants lacking Golgi reassembly and stacking protein (GRAsp) and the autophagy regulator Atg7 produce only sEVs, with authors suggesting these to be produced via unconventional secretion that bypasses autophagosomal and ESCRT/MVB pathways (Peres da Silva et al. 2018). Similar work in S. cerevisiae showed that while ESCRT proteins helped determine protein composition, they were not essential for EV release (Oliveira et al. 2010b).

EV biogenesis in plants

Definitive information on mEV biogenesis in plants is lacking, although plant cells infected with fungus produce membrane evaginations (An et al. 2006b) into the extracellular matrix that is in contact with the invading pathogen, reminiscent of mEVs in animals (Fig. 2). In the absence of biomarkers, mEV biogenesis in plants remains speculative.

As in other eukaryotes, production of EVs in plants depends on the secretory pathway and involves the exocyst complex (Vukasinovic and Zarsky 2016; Picco et al. 2017) consisting of eight subunits (Safavian et al. 2015). Vesicle fusion is facilitated with the help of vesicle (v)-SNARE and target (t)-SNARE complexes. Vesicle secretion includes canonical and unconventional secretion pathways, the latter of which results in the release of sEVs and mEVs (Inal et al. 2013b). Extracellular fluids were collected from imbibed sunflower seeds to demonstrate the existence of vesicles with a diameter of 50–200 nm that contain a lectin and a Rab11 GTPase (Regente et al. 2009). Further analysis of imbibed sunflower seeds demonstrated unconventional secretion of the Helja lectin (Regente et al. 2012). EVs from sunflower seedlings were enriched in cell wall remodelling enzymes and defence proteins (Regente et al. 2017). Strikingly, PMRS5 involved in pectin methyl esterification and susceptibility to penetration by powdery mildew pathogens (Vogel et al. 2004; Chiniquy et al. 2019) was associated with EVs (de la Canal and Pinedo 2018). Cell wall remodelling activities may allow EVs to pass through the cell wall and mediate or restrict other forms of transport or pathogen ingress. EVs from apoplastic fluids of Arabidopsis thaliana leaves were enriched in proteins involved in biotic and abiotic stress responses (Rutter and Innes 2017). Analysis of the xylem sap of tomato showed that the majority of proteins were not part of the canonical secretion pathway, suggesting the existence of unconventional secretion pathways (de Lamo et al. 2018). Sphingolipids were enriched in EVs from apoplastic fluids of A. thaliana leaves relative to whole leaf extracts (Liu et al. 2020). The majority of EV sphingolipids was composed of glycosyl inositol phosphoceramides and this negatively changed sphingolipid was less abundant in leaves of the TETRASPANIN 8 (tet8) mutant relative to wild-type plants.

The predominant pathway of EV biogenesis in plants is via MVBs, and evidence for differences between tetraspanin (TET8)- and t-SNARE (VEN1)-positive putative sEVs were reported in A. thaliana (He et al. 2021); TET8- and PEN1-positive sEVs fractionate differently and differ in vesicular content. Additionally, exocyst-positive organelle (EXPO)-derived EVs were reported (Wang et al. 2010a); these putative sEVs were reportedly endosome-derived but not related to MVBs.

Developmental control of vesicle secretion and sorting in plants

Plants constitutively secrete EVs (Regente et al. 2009; Rutter and Innes 2017) but also respond to biotic cues (Rutter and Innes 2017; Goring 2018). A well-documented developmental event controlled by stimulus-dependent vesicle trafficking is flower fertilization (Goring 2018). When self-incompatible pollen lands on a stigma, the S-locus protein 11/S cysteine-rich ligand is transferred from the pollen coat to the stigmatic papilla cell carrying the corresponding S receptor kinase (Watanabe, Suwabe 2022, Vol. 46, No. 1

Figure 2. Microscopic evidence for mEV (microvesicle) formation in haustorium containing epidermal cells of powdery mildew (Blumeria graminis f. sp. hordei) infected susceptible barley cultivar Fallas (f) at 20-21 h postinoculation. (A) Schematic representation of cellular structures and compartments. (B) An MVB (arrow) near a haustorium. Arrowheads point to evaginations or protrusions of the extrahaustorial membrane; mEVs are formed by such evaginations or protrusions. ATG, appressorial germ tube; CV, central vacuole; CW, cell wall; CWA, cell wall apposition; EC, epidermal cell; HB, haustorial body; HN, haustorial neck; bar, 200 nm; from An et al. (2006b) with modifications.
and Suzuki 2012). The signal transduction pathway downstream of this molecular recognition event results in phosphorylation and activation of ARC1, an E3 ubiquitin ligase that targets the exocyst component Exo70A1 for degradation (Katashiba and Nasrallah 2014). Consequently, MVBs are targeted to the vacuole, accumulating in autophagic bodies (Table 1) (Safavian and Goring 2013; Goring 2018). Conversely, when compatible pollen lands on the stigmatic surface, small local calcium waves are initiated that precede pollen hydration, tube germination and penetration (Iwano et al. 2015). MVBs rapidly fuse with the plasma membrane to release sEVs into the stigmatic cell wall (Table 1) (Elleman and Dickinson 1996; Safavian and Goring 2013). As a result, the stigmatic cell wall in contact with the pollen grain expands in preparation for pollen penetration (Elleman and Dickinson 1996). As plant sEVs are enriched for aquaporins and cell wall degrading enzymes (Regente et al. 2017; Rutter and Innes 2017), their secretion probably contributes to pollen hydration, tube germination and penetration.

Cellular uptake of EVs

There are four mechanisms by which EVs can interact with recipient cells (Fig. 3). These are (i) fusion, (ii) surface protein interaction, triggering signal transduction in the target cell, (iii) activation of an EV-bound surface protein and (iv) endocytosis.

Membrane fusion (Fig. 3A) is likely to be mediated by a prior interaction of surface proteins between EV and target cell. Adhesion proteins for example on endothelial progenitor cell-derived mEVs are thought to interact with fusion proteins on recipient endothelial cells to facilitate fusion (Hargett and Bauer 2013). Such fusion may also lead to the transfer of surface receptor proteins, resulting in particular cellular responses (Mause and Weber 2010; Meckes and Raab-Traub 2011). EVs and cells may also simply interact via receptor–ligand interactions (Fig. 3B), triggering signal transduction in the target cell but with no fusion or uptake. Another example of protein interactions without EV fusion or uptake (Fig. 3C) would be that following activation of an EV-bound transforming growth factor β (TGF-β), bound in a latent complex on the EV surface, by plasmin or integrin, releasing it to interact with its cognate receptor on the target cell. In terms of endocytosis, EVs may be taken up by ligand-mediated endocytosis or macropinocytosis (Costa Verdera et al. 2017).

Uptake of EVs by fungal and plant cells

It was demonstrated that 60–80 nm liposomes could penetrate the fungal cell wall with a predicted pore size of ~6 nm, suggesting that the cell wall is more dynamic than previously thought, with flexible viscoelastic properties permitting bi-directional vesicle traffic from and to cells (Walker et al. 2018). No mechanistic information on vesicle fusion/uptake in fungal cells is currently available, although TEM evidence suggests that the hydrophobic polyene antibiotic amphotericin B did promote liposome uptake and fusion in Candida albicans. The fungal pathogen Sclerotinia sclerotiorum rapidly internalizes EVs from the host plant sunflower (Regente et al. 2017). As a result, hyphal growth inhibition and abnormalities occurred as well as cell death. Botrytis cinerea, a pathogen related to S. sclerotiorum, was shown to internalize sEVs of Arabidopsis thaliana containing small RNA (sRNA) to target fungal genes involved in virulence and secretion (Cai et al. 2018). It is not understood how uptake of plant EVs by fungal spores occurs (Regente et al. 2017).

Uptake of EVs by plant cells remains even more mysterious. However, uptake of garlic-derived nanovesicles by liver cells was shown to involve the interaction between the transmembrane glycoprotein heterodimer CD98 and a mannose-binding lectin (Song et al. 2020). This finding suggests that similar interactions between glycoproteins and lectins could play a role in EV uptake by plant and fungal cells.

Walking through walls: EV release and uptake in bacteria, fungi and plants

Unlike animal cells, bacteria, fungi and plants contain cell walls that may interfere with secretion, delivery and uptake of EVs. Despite the perceived physical restrictions of cell walls, it is now appreciated that all organisms with cell walls are able to produce and, in the case of fungi, take up EVs. Although the model organism S. cerevisiae has extensively been used to study secretion, fungal EVs were first observed experimentally in the opportunistic pathogen C. neoformans (Takeo et al. 1973). These early freeze-dried EM studies, replicated in C. albicans (Anderson, Mihalkik and Soll 1990) and S. cerevisiae (Osumi 1998), depict various vesicular structures penetrating and emerging from the cell wall. To date, EVs have been identified in many clinically relevant genera, including Histoplasma, Paracoccidioides, Sporothrix, Candida, Malassezia, Aspergillus and Fusarium (Bielska and May 2019).

Much attention has been given to the problem of how vesicles traverse thick cell walls, such as those found in fungi, mycobacteria, Gram-positive bacteria and plants. With regards to fungi, speculations have ranged from turgor pressure forcing vesicles through the wall, to enzymatic cell-wall modification, as well as transit through channels, allowing vesicles to ‘walk through
the wall’ (Brown et al. 2015). While some of these conjectures await corroboration, a number of experiments have shown a way through. First, degradative and remodelling enzymes have indeed been recurrently found in a range of fungal EVs (Albuquerque et al. 2008; Zhao et al. 2019; Karkowska-Kuleta et al. 2020). Second, cell-wall pore size on the surface of S. cerevisiae has been shown to fluctuate between 50 and up to 400 nm when under stress (de Souza Pereira and Geibel 1999), suggesting a gating method. Factors impacting pore size include osmotic changes (Garcia-Rubio et al. 2019), oxidative stress (de Souza Pereira and Geibel 1999) and stage in the cell cycle (Gow and Hube 2012). Moreover, it is likely that EVs, themselves morphologically dynamic, pass through pores much smaller than their spherically idealized diameter (Brown et al. 2015). In C. neoformans melanization was shown to decrease porosity, causing vesicles to accumulate between the plasma membrane and cell wall (Jacobson and Ikeda 2005).

While much of this research has been done on budding yeast and C. neoformans, the implications are far-reaching in the latter fungus has a filamentous stage and cell walls of all fungi have similar composition, consisting of mannoproteins, β-glucans and chitin (Brown et al. 2015). Although cell wall composition differs in oomycetes in that they do not produce chitin, their pore sizes of cell walls are equally tiny being impermeable to molecules with diameters in excess of 2–3 nm (Money 1990). Estimated pore sizes of plant cell walls are 5–7 nm in diameter based on the permeability of globular proteins of 36–67 kDa (Fry 2017). However, it has been appreciated that plant cell walls are dynamic (Greve and Labavitch 1991; Rose et al. 1998) and plant cells have been shown to secrete much larger molecules (Fry 2017). It is well possible that the dynamic cell wall through interaction with EVs facilitates their passage through the assistance of cell wall modifying enzymes as outlined in this treatise.

**Fungal EVs as virulence factors**

Since mutants with impaired EV secretion exhibit reduced fitness, and application of additional EVs increases infectivity, there is strong evidence associating fungal EVs with virulence (Panepinto et al. 2009; Huang et al. 2012; Wolf et al. 2015). A diverse range of macromolecules are featured in fungal EVs, with roles in virulence, signalling, scaffolding and metabolism, including proteins, lipids, nucleic acids, glycans, pigments and sterols (Kitajma 2000; Bleackley et al. 2019). Moreover, the EV profile can vary according to environmental conditions, such as the relative availability of nutrients (Cleare et al. 2020), host immune response (Vargas et al. 2015) and potentially quorum sensing (Padder, Prasad and Shah 2018). Indeed, EVs derived from C. albicans biofilm comprise a single population with ESCRT proteins implicated in their biogenesis, whereas planktonic EVs are more polydisperse in size with a bimodal distribution, indicating distinct subpopulations (Zarnowski et al. 2018). Such data resembles recent work in model bacterial organism *Pseudomonas aeruginosa*, showing quorum-dependent biofilm EVs to differ in profile from planktonic EVs (Cooke et al. 2019), thus highlighting the evolutionary conserved relationship between EVs and biofilm.

Fungal EVs may be internalized by host immune cells via endocytic pathways (Fig. 3D). Fungal EVs provoke strong animal immune responses in vitro and in vivo, offering the potential for mycosis vaccines (Freitas et al. 2019). Common EV-associated immunogens include cell-surface PAMPs such as membrane-bound glycan moieties. In particular those of *Paracoccidioides brasiliensis* and *P. lutzii* have been characterized as being recognized by C-type lectin receptors (CLR) found on the surface of macrophages and dendritic cells (Peres da Silva et al. 2015a). Similarly, lipid components of the cell wall present in EVs, such as glucosylceramide, have been shown to bind IgG2a monoclonal antibodies (Toledo et al. 2001).

As with much early immunological work, the story of inflammatory mediators remains somewhat convoluted, however, some consistency has been shown across fungal species with EVs isolated from *Aspergillus flavus* (Brauer et al. 2020), *Trichophyton interdigitale* (Bittencourt et al. 2018) and *Paracoccidioides brasiliensis* (da Silva et al. 2016) all inducing macrophage polarization to M1 in vitro. Acute-phase pro-inflammatory tumour necrosis factor α (TNF-α) also appears to be broadly released from professional antigen presenting cells when in the presence of EVs from *C. albicans*, *Malassezia* spp., *T. interdigitale* and *Sporothrix brasiliensis* (Campos et al. 2015; Bielska and May 2019). Much of the work in this area (Freitas et al. 2019) suggests a nuanced interplay between immunostimulatory and immunosuppressive effects, with elevated nitrous oxide (NO) and the cytokines IL4, IL10, IL12, TGF-β, IL6, IL12 and IFNγ featuring frequently.

For example, there has been ongoing debate as to whether EVs act deleteriously on the host immune system or otherwise provide beneficial challenge. In the well-studied case of opportunistic fungal pathogen *C. neoformans*, EVs harbour the capsular antigen glucuronoxylomannan (GXM), which can suppress monocytes, neutrophils and T lymphocytes (Monari, Bistoni and Vecchiarelli 2006) and has been shown to confer cytotoxic effect directly to macrophages via the Fas/FasL pathway (Villena et al. 2008). These EVs induce macrophages to produce anti-inflammatory TGF-β and IL-10 in vitro, while conversely stimulating via TNF-α (Oliveira et al. 2010a). In the search for fungal EV-based vaccines, it has been shown that *C. neoformans* mutants lacking wild-type GXM fail to generate a protective immune response in a murine vaccination model, whereas GXM-containing EVs stimulated resistance to infection in a *Galleria mellonella* model (Colombo et al. 2019). Based on these observations, it was suggested the host-protective effects of EVs may outweigh pathological effects (Freitas et al. 2019). However, the evidence is thus far insufficient to guide clinical practice. Similarly, *Malassezia sympodialis* releases allergenic EVs, which induce high levels of both the pro-inflammatory TNF-α and the anti-inflammatory IL-4 and are consequently associated with a possible dual immunoregulatory function in atopic eczema (Gehrmann et al. 2011).

In a vivid example of cross-kingdom communication, evidence indicates that *C. neoformans* EVs enhance brain infection by facilitating the crossing of the blood–brain barrier and mediate antifungal action of the host by inducing cytokines (Huang et al. 2012). Bolstering the view that EVs can act as immunological effectors at great distance, recent work shows that EVs isolated from virulent strains of *C. gattii* are readily taken up by macrophages, stimulating the rapid growth of less-virulent, intracellular, non-outbreak fungal cells that would otherwise be degraded (Bielska et al. 2018).

**Be quiet! EVs and cross-kingdom RNA silencing**

For reasons outlined in the previous section, EVs are uniquely positioned as vectors for cross-kingdom RNA interference (RNAi) dissemination by providing protection from enzymatic degradation and opportunities for cell targeting (Fire et al. 1998; Cheng et al. 2014). Phrased inversely, RNA may not get very far, particularly outside host cells, without encapsulation by EVs. This view has been challenged, as extracellular RNAs are also stabilized by
RNA-binding proteins (RBPs), such as nucleophosmin 1 (NPM1) and argonaute2 (AGO2) (Wang et al. 2010b; Zhao et al. 2019). Others point out that such RBPs are often associated with loading of RNA into EVs, and so may be supportive, rather than alternative (Leidal et al. 2020; Xu et al. 2020).

RNAi and EV biogenesis are suggested to be linked, based on work in animals showing that depleting ESCRT proteins to block MVB formation results in impaired miRNA silencing and loss of the cytoplasmic foci known as P-bodies, where many of the RNA-induced silencing complex (RISC) proteins necessary for silencing are localized (Lee et al. 2009). Microarray and bioinformatic analysis of RNA extracted from primary T lymphoblast sEVs revealed a common sequence motif, named the EXOmotif, found only in vesicular sRNA. Importantly, mutagenesis of this motif inhibited packaging into sEVs and introduction of this motif into non-consensus miRNAs stimulated sEV release (Villarroya-Beltri et al. 2013). However, miRNAs without an EXO motif are also found in EVs, so further proteins have been sought, with the Y-box protein 1 (YBX1) being identified in tetraspanin (CD63)-positive sEVs and subsequently implicated in EXO-independent secretion (Shurtleff et al. 2016), although others (Jeppesen et al. 2019) were unable to reproduce this finding. While genetic screenings have largely been overlooked as interrogative tools for elucidating RNA packaging in EVs, an innovative CRISPR-Cas9 miRNA barcoding strategy was applied to corroborate established EV-supporting genes, such as Rab27a and sphingomyelinase, and identify novel contributors, specifically the role of the Wnt signalling pathway (Lu et al. 2018). How translatable this work is to other kingdoms of life remains to be seen.

**Role of EVs in the virulence of phytopathogenic fungi**

Little is known about the role of phytopathogenic EVs in fungal virulence. EVs isolated from in the axenically grown wheat pathogen *Zymoseptoria tritici* contained relatively few carbohydrate-active hydrolytic enzymes, proteases and effectors relative to conventionally secreted proteins (Hill and Solomon 2020). Nevertheless, the cotton pathogen *Fusarium oxysporum* f. sp. *vasinfectum* releases EVs in liquid cultures that contain a purple pigment and trigger a phytotoxic response when infiltrated into leaves (Bleackley et al. 2019). It was also mentioned in this article that *M. oryzae* delivers effectors via vesicles (Giraldo et al. 2013).

During plant-pathogen interactions, sRNAs are exchanged to execute cross-kingdom/organism RNA interference (Fig. 4). The fungal pathogen *Botrytis cinerea*, for instance, delivers sRNAs to silence corresponding host target genes involved in immunity (Weiberg et al. 2013). Conversely, the host plant *A. thaliana* generates sRNAs that target fungal genes involved in vesicle trafficking to reduce pathogen virulence (Cai et al. 2018). These plant-derived sRNAs are found in a subpopulation of EVs, i.e. tetraspanin-containing sEVs (Table 1). These putative sEVs contain the RNA-binding proteins argonaute1 (AGO1), RNA helicases (RH) and annexins (ANN) to facilitate sRNA loading and/or stabilization (He et al. 2021). Whereas AGO1, RH11 and RH37 selectively bind to EV-enriched sRNAs, ANN1 and ANN2 bind sRNAs nonspecifically. The rh11 rh37 and ann1 ann2 double mutants of *A. thaliana* were hypersusceptible to *B. cinerea*. Plant EVs were recently shown to contain ‘tinyRNAs’ 10–17 nucleotides (nt) in length, and the presence of 21–24 nt long sRNA in EVs was challenged (Cai et al. 2018; Baldrich et al. 2019). Some miRNAs and secondary siRNAs are enriched in the apoplastic fluid but not in EVs, suggesting EV-independent sRNAs secretion pathways (Baldrich et al. 2019). EVs accumulating at the haustorial interface may well contribute to fungal EV-mediated RNAi (Bozkurt and Kamoun 2020).

**Use of RNAi for plant biotechnology**

Host-induced gene silencing (HIGS) has been developed as a novel alternative crop protection strategy against pathogens and pests (Koch and Kogel 2014). Recently, EVs from transgenic *A. thaliana* expressing noncoding dsRNA have been shown to concomitantly silence two fungal cytochrome P450 genes and inhibit growth in *Fusarium graminearum*, both in vitro and in planta; notably, ESCRT-III mutants were further shown to be HIGS impaired (Koch et al. 2020).

**Vesicle trafficking during host–pathogen interactions**

When fungal spores first land on plant surfaces, they produce adhesives to avoid displacement as a result of the physical environment or microbial competition (Tucker and Talbot 2001). In the case of rust fungi, thigmotropism is sufficient in the absence of chemical sensing for germ tube and appressorium formation (Hoch et al. 1987). Other fungal pathogens, like *Colletotrichum gloeosporioides*, depend on host chemicals, e.g. wax components, for appressorium formation (Podila, Rogers and Kolattukudy 1993). However, little is known about the exchange of information via plant and fungal secretion pathways until the stage of pathogen penetration of host cells.

The powdery mildew fungus *Blumeria graminis* f. sp. hordei (*Bgh*) produces a primary germ tube after attachment to the barley leaf surface that penetrates the cuticle. This penetration event triggers *H₂O₂* accumulation and formation of a papilla containing host cell wall appositions (Huckelhoven and Kogel 2003). However, this early event does not determine the outcome of this host–pathogen interaction. Instead, it is the host...
response to a second appressorial germ tube that determines whether the interaction will be compatible or incompatible (Collins et al. 2003).

**Resistance of plants against penetration by pathogens**

Resistance against powdery mildew fungi is associated with an apoplastic immune response of epidermal cells, which generates papillae underneath fungal contact sites through appositions between the cell wall and the plasma membrane (An et al. 2006b). These papillae contain callose, a β-1,3-glucan plant cell wall polymer, generated by the enzyme PMR4/GSL5 (Jacobs et al. 2003; Nishimura et al. 2003). Callose deposition was monitored in a mutant screen for nonhost resistance of *A. thaliana* against the nonadapted pathogen *Bgh*, resulting in identification of the syntaxin or t-SNARE YFP121/PEN1; its ortholog *Required for mlo-specified resistance2 (ROR2)* contributes to basal penetration resistance of barley against the same pathogen (Collins et al. 2003). Plasma membrane-localized syntaxin ROR2 and its interacting partner HvSNAP34 are thought to facilitate exocytosis or homotypic fusion of vesicles (Pickett and Edwardson 2006). Vesicle fusion is driven by complex formation of t-SNAREs and v-SNAREs through vesicle-associated membrane proteins (VAMPs). PEN1 and SNAP33 form a SNARE complex with an overaccumulation of H$_2$O$_2$ (Kumar 2001). Barley *mlo* mutants are also more susceptible to the head blight pathogen *Fusarium graminearum* (Jansen et al. 2005). In field trials and seedling assays, *mlo* alleles increased the severity of Ramularia leaf spot disease caused by the pathogen *Ramularia collo-cygni* (McGrann et al. 2014). Disease symptoms were reduced in *mlo5 ror1* and *mlo5 ror2* double mutants but fungal biomass remained as high as in *mlo* single mutants, indicating that *ROR* genes regulate the transition from endophytic to necrotrophic colonization. Conversely, *mlo5* mutants did not affect *Fusarium* spp. and *R. collo-cygni* colonization compared with corresponding wild-type cultivars in independent field trials (Hofer et al. 2015), suggesting that environmental conditions may have an influence on this trade-off. Collectively, these findings suggest that host EV release may mediate resistance or susceptibility depending on particular pathogen penetration and colonization strategies. Vesicle secretion may have different consequences depending on the pathogen that is being encountered by a specific host. In response to powdery mildews, EVs may protect the host against pathogen ingress. Other pathogens, however, may be stimulated to infect and colonize when receiving information from the host in form of secreted vesicles. The cargo of EVs may differ depending on whether a deterring or stimulating activity exists, but this has not yet been investigated. Environmental factors may influence the crosstalk that exists between hosts and pathogens under different field settings. Much needs to be explored regarding the details of vesicle trafficking affecting different outcomes.

**Evidence for a role of the exocyst in innate plant immunity**

Other Exo70 genes than the ones mentioned earlier are involved in defence against microbial pathogens. Exo70B2 was identified as a target of the plant U-box-type ubiquitin ligase 22 (PUB22), which together with PUB23 and PUB24 down-regulates pathogen-associated molecular pattern (PAMP)-triggered immunity (Trujillo et al. 2008). Accordingly, *exo70B2* mutants were hyper-susceptible to the virulent bacterial pathogen *Pseudomonas syringae* pv. *tomato* (Pst) DC3000 and obligate biotrophic downy mildew oomycete pathogen *Hyaloperonospora arabidopsidis* (Steigmann et al. 2012). *Exo70B1*, on the other hand, is involved in pathogen-specific immune responses; *exo70B1* mutants were lesion mimics with increased susceptibility to *Pst* DC3000 but elevated resistance against *H. arabidopsidis*.
Vesicle secretion in phytopathogens during host penetration and colonization

Appressoria and haustoria of *B. grisea* generate MVBs (Table 1) (An et al. 2006b). The 'punchless' mutant of *M. grisea* is able to generate appressoria but unable to penetrate rice leaves (Clergeot et al. 2001). The inactivated *PLS1* gene encodes a tetraspanin, which is a known marker for sEVs (Inal et al. 2013b). It would be desirable to determine the production of sEVs in the wild type and the 'punchless' mutant of *M. grisea* (Table 1).

Uncoated and coated vesicles were observed in epidermal invasion by infection vesicles of *Colletotrichum lindemuthianum* (Mengden and Deising 1993), suggesting membrane recycling and vesicle secretion at the initial cellular contacts between the invading pathogen and its bean (*Phaseolus vulgaris*) host. Later stages of colonization by *M. oryzae* involve vesicle trafficking. Upon penetration of epidermal cells, the fungus forms IH that secrete apoplastic effectors like Bas4 via the conventional secretion pathway. IH form BICs in a newly infected rice cells. BICs accumulate cytoplasmic effectors like Pwl2 that are destined for translocation into host cells via an unconventional secretion pathway; this type of secretion is dependent on exocyst and t-SNARE complexes (Giraldo et al. 2013). Autophagy was recently shown to maintain the biotrophic phase of *M. oryzae*; *aim1* mutants were impaired in maintaining BICs as evidenced by loss of Pwl2 expression and cytoplasmic accumulation of the apoplastic effector Bas4 (Table 1) (Sun et al. 2018).

Plant-pathogen communication with EVs

The abundance of EVs collected from apoplastic fluids of *A. thaliana* leaves increased after infection with *Pst DC3000* or treatment with salicylic acid (Rutter and Innes 2017). The protein composition of these EVs suggests that they probably represent sEVs that are enriched for PEN1.

Apoplastic fluids from bean leaves induce excision of a 106 kb genomic island from the chromosome of *P. syringae* pv. *phaseolicola* containing the effector gene *avrRph8* (Godfrey et al. 2011). The generated circular episome suppresses expression of *avrRph8*, thus preventing its recognition by the *P. vulgaris* receptor encoded by the corresponding R3 gene (Pitman et al. 2005). It remains to be determined which apoplastic molecules or EVs may be involved in bacterial recognition and generation of this stealth episome.

Viral infection and spread in plants differs from animals (Gray and Banerjee 1999). Most plant viruses are insect-borne. For instance, phloem-feeding insects will deliver viruses into host cells, from which systemic spread occurs through plasmodesmata from cell to cell via the symplasmic route. This can occur via viral ribonucleoprotein complexes or entire virions (Niehl and Heinlein 2011). An exception to this rule was recently published, demonstrating that turnip mosaic virus can be secreted via sEVs in form of double-stranded RNA or viral replication complexes (Movahed et al. 2019). The onset of viral secretion occurs at the ER with assembly of replication complexes into vesicles that bypass the Golgi apparatus to reach MVBs (Wang et al. 2010a). Viral delivery into the apoplast can explain the presence of replication vesicles in xylem vessels that spread viral infection even after girdling of the stem (Wan et al. 2015). sEVs are also essential for release of rice dwarf virus from insect vector cells through fusion of MV and the plasma membrane (Wei, Hibino and Omura 2009).

Animal EVs during attack

Unlike plants, the immune system of animals relies on phagocytosis (Stotz et al. 2003). This may be one of the reasons why EVs do not function as defence compartments in animals as they do in plants during the abovementioned penetration resistance. Despite little comparison of fungal pathogenesis of plants with that of animals in the scientific literature, there are obvious differences.

First, penetration or uptake by fungal pathogens of host plant and animal cells reflects the differing challenges posed. Whilst penetration of plant cells may require mechanical pressure, proteolytic degradation or enzymatic degradation of cell walls as well as entry through stomata, animal pathogens such as *Histo- plasma capsulatum* are taken up by receptor-mediated endocytosis (Table 1) (Garfoot and Rappleye 2016). Second, in animal (vertebrate) species, the immune system stimulates inflammation, attracting cells to control infection. As plants cannot recruit cellular immune effectors, an invading hypha interacts with a solitary plant cell. In the case of biotrophic pathogens, such infection thus triggers programmed cell death of the challenged plant cell, in an attempt to stave off infection. With no cell-mediated immunity, plant EVs with antifungal properties, released into the apoplastic space, take up this role (Regente et al. 2017; Cai et al. 2018). In animals, there are many EV-mediated interactions between host and pathogen (Inal, Ansa-Addo and Lange 2013a). Natural killer (NK) cells kill virally infected cells by inducing apoptosis; they also kill fungi directly (Table 1) (Schmidt, Tramsen and Lehrnbecher 2017) as well as cancer cells. Released perforins allow granzymes to enter the infected cell inducing apoptosis; this also occurs upon interaction of death receptor ligands with death receptors such as Fas. This killing mechanism (although research has focused on cancer cells) can also be mediated by EVs released from the NK cell (Di Pace et al. 2020). EVs derived from another innate immune cell, the cytotoxic CD8+ T-cell, may also mediate cell death as well as activate other immune cells (Table 1) (Del Vecchio et al. 2021).

CONCLUDING REMARKS

EVs contribute to cellular functions of all living organisms (Fig. 5). It is therefore hypothesized that EVs are conserved and have been produced by the earliest living cells. This thermodynamically underpinned biophysical process is thought to have evolved over time to incorporate complex regulatory control to prevent random fusion events and allow selective packaging and
Prokaryotic EVs are not the topic of this article, but production of OMVs by Gram-negative species and the more recently identified cytoplasmic membrane vesicles (CMVs) of Gram-positives suggest that the last common ancestor of all three domains of life produced EVs (Gill, Catchpole and Forterre 2019; Toyofuku, Nomura and Eberl 2019). In addition to the biogenesis of OMVs through blebbing of the outer membrane, Gram-negative bacteria generate outer-inner MVs (OMVs) and explosive OMVs (EOMVs) after bacteriophage-induced explosive lysis (Toyofuku, Nomura and Eberl 2019). These OMVs and EOMVs contain cytoplasmic macromolecules. Similarly, CMVs of Gram-negative bacteria also contain cytoplasmic components because they do not contain an outer membrane and periplasmic space. Prokaryotic vesiculation is shown to be involved in generalized secretion, virulence and membrane remodelling, as well as specific envelope stress responses, biofilm development, horizontal gene transfer, phage receptor transfer and extracellular scaffolding (Kulp and Kuehn 2010; Manning and Kuehn 2011; Guerrero-Mandujano et al. 2017).

Some archaeal genomes encode ESCRT III proteins and/or Vps4 ATPases to produce MEVs (Ellen et al. 2009). Based on this primordial ESCRT complex (Spang et al. 2015), it is parsimonious to propose that MEVs are evolutionarily old and plasma membrane blebbing is a more ancient mechanism of EV production. Of note, bacterial MV production is also based on membrane blebbing. In contrast, MVBs and sEVs are suggested to be a more recent evolutionary invention of eukaryotes because the machinery required for generating MVBs and sEVs is more complex, requiring ESCRT I-III, and not yet present in archaea. In addition, tetraspanins, which are found in sEVs, are limited to all eukaryotic cells and not found in archaea (Huang et al. 2005). Membrane blebbing, biogenesis of EVs, is a hallmark of apoptosis found only in animals (Kutscher and Schramm 2017). Although programmed cell death does occur in other eukaryotes, all morphological and molecular hallmarks like caspases are only present in animals (Koonin and Aravind 2002). It is therefore suggested that sEVs recently developed in the animal kingdom (Fig. 5). Of note, EVs can contain entire organelles (Lieberthal and Levine 1996); this does not happen in mEVs.

In eukaryotes, the array of uses and manner in which EVs are produced and processed has expanded further to include complex immunomodulatory interactions as well as widespread RNA silencing via miRNAs, siRNAs, amongst others. This appears to be conserved across all four kingdoms. Fungal EVs have been shown to be immune-modulatory in animals. In animal cells, EVs are implicated in modulation of both the pre-metastatic niche and cancer microenvironment. Fungal and plant cell walls may restrict movement of mEVs and sEVs. The pathways leading to their biogenesis may therefore be deemphasized when cell walls with small apparent pore sizes exist. However, when cell wall degradation occurs, production of mEVs may become important (An et al. 2006b; Rizzo et al. 2020).

Table 1 adds to a final summary of published findings and interpretations related to plant and microbial interactions. Vesicular secretion contributes to compatible interactions and pollen hydration as the first step during flower fertilization. In contrast, incompatible interactions between host plants and powdery mildew fungi are dependent on the secretion of sEVs for penetration resistance. Microscopic and genetic evidence suggest that autophagy occurs during plant-pathogen interactions. Fungal autophagy is specifically needed for prolonged compatible interactions with M. oryzae. At the same time, fungal secretion appears to be essential for fungal infection and colonization of the host. Vesicle trafficking may therefore be complex and serve dual needs. The same may be happening in host plants to moderate the immune response while combating pathogens. Trade-offs exist during defence against different pathogens and there may be an environmental influence on these trade-offs. Secretion of EVs by host plants may therefore not always be directed for defence but also facilitate compatible interactions. Plant developmental and immune reactions may therefore have similarities after all.

ACKNOWLEDGEMENTS

Ralph Hückelhoven kindly provided an EM image and a cartoon to generate one of the figures for this article. Thanks are expressed to Nick Talbot (Sainsbury Laboratory) and Dawn Arnold (UWE Bristol) for inspirational discussions at BSPP meeting in 2019. John Labavitch (UC Davis) is posthumously acknowledged for his expertise and insights into the dynamics of plant cell walls and their variable pore sizes.

FUNDING

BBSRC-Newton Fund (Grant Ref: BB/R019819/1) and BBSRC-FOF (Grant Ref: BB/V01725X/1) provided support for this work to HUS. JI was part funded by Industrial Academic Partnership Pathways project 612224 from the REA FP7 (project No. LSC09R83474). DB is funded by a University of Hertfordshire Quality-related Research (QR) funded PhD studentship. The University of Hertfordshire also provided salaries for JI and HUS.

CONFLICT OF INTEREST. None declared.
REFERENCES

Albuquerque PC, Nakayasu ES, Rodrigues ML et al. Vesicular transport in Histoplasma capsulatum: an effective mechanism for trans-cell wall transfer of proteins and lipids in ascomycetes. Cell Microbiol 2008;10:1695–710.

Allain V, Bourgaux C, Couvreur P. Self-assembled nucleolipids: from supramolecular structure to soft nucleic acid and drug delivery devices. Nucleic Acids Res 2012;40:1891–903.

An Q, Ehlers K, Kogel KH et al. Multivesicular compartments proliferate in susceptible and resistant MLA12-barley leaves in response to infection by the biotrophic powdery mildew fungus. Neu Phytol 2006b;172:563–76.

An Q, Huckelhoven R, Kogel KH et al. Multivesicular bodies participate in a cell wall-associated defence response in barley leaves attacked by the pathogenic powdery mildew fungus. Cell Microbiol 2006a;8:1009–19.

An Q, van Bel AJE, Hu¨ckelhoven R. Do plant cells secrete exosomes derived from multivesicular bodies? Plant Signal Behav 2007;2:4–7.

Anderson J, Mihalik R, Soll DR. Ultrastructure and antigenicity of the unique cell wall pimple of the Candida opaque phenotype. J Bacteriol 1990;172:224–35.

Araldi E, Kramer-Albers EM, Hoen EN et al. International Society for Extracellular Vesicles: first annual meeting, April 17–21, 2012: ISEV-2012. J Extracell Vesicles 2012;1:19995.

Ardoin SP, Pisetsky DS. The role of cell death in the pathogenesis of the ascomycete Cryptococcus neoformans. Nat Rev Microbiol 2006a;4:271–82.

An Q, van Bel AJE, Hu¨ckelhoven R. Do plant cells secrete exosomes derived from multivesicular bodies? Plant Signal Behav 2007;2:4–7.

Bielska E, Sisquella MA, Aldeieg M et al. Pathogen-derived extracellular vesicles mediate virulence in the fatal human pathogen Cryptococcus gattii. Nat Commun 2018;9:1556.

Babst M, Katzmann DJ, Estepe-Sabal RJ et al. Escrt-III: an endosome-associated heterooligomeric protein complex required for mvb sorting. Dev Cell 2002;3:271–82.

Baldrich P, Rutter BD, Karimi HZ et al. Plant extracellular vesicles contain diverse small RNA species and are enriched in 10- to 17-nucleotide “tiny” RNAs. Plant Cell 2019;31:315–24.

Bielska E, May RC. Extracellular vesicles of human pathogenic fungi. Curr Opin Microbiol 2019;52:90–9.

Bielska E, Sisquella MA, Aldeieg M et al. Pathogen-derived extracellular vesicles mediate virulence in the fatal human pathogen Cryptococcus gattii. Nat Commun 2018;9:1556.

Bitencourt TA, Rezende CP, Quaresemin NR et al. Extracellular vesicles from the dermatophyte Trichophyton interdigitale modulate macrophage and keratinocyte functions. Front Immunol 2018;9:2343.

Bleckley MR, Samuel M, Garcia-Ceron D et al. Extracellular vesicles from the cotton pathogen Fusarium oxysporum f. sp. vas- infectum Induce a phytotoxic response in plants. Front Plant Sci 2019;10:1610.

Bohlenius H, Morch SM, Godfrey D et al. The multivesicular body-localized GTPase ARFA1b/1c is important for callose deposition and RO2 syntaxin-dependent preinvasive basal defense in barley. Plant Cell 2010;22:3831–44.

Bozkurt TO, Kamoun S. The plant–pathogen haustorial interface at a glance. J Cell Sci 2020;133:jcs237958.

Brauer VS, Pessoni AM, Bitencourt TA et al. Extracellular vesicles from Aspergillus flavus induce M1 polarization. mSphere 2020;5:e00190–120.

Brown L, Wolf JM, Prados-Rosas R et al. Through the wall: extracellular vesicles in Gram-positive bacteria, mycobacteria and fungi. Nat Rev Microbiol 2015;13:620–30.

Budin I, Bruckner BJ, Szostak JW. Formation of protocell-like vesicles in a thermal diffusion column. J Am Chem Soc 2009;131:9628–9.

Cai Q, Qiao L, Wang M et al. Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. Science 2018;360:1126–9.

Campos JH, Soares RP, Ribeiro K et al. Extracellular vesicles: role in inflammatory responses and potential uses in vaccination in cancer and infectious diseases. J Immunol Res 2015;2015:832057.

Cestari I, Ansa-Addo E, Declindo P et al. Trypanosoma cruzi immune evasion mediated by host cell-derived microvesicles. J Immunol 2012;188:1942–52.

Chargaff E, West R. The biological significance of the thromboplastic protein of blood. J Biol Chem 1946;166:189–97.

Chen IA, Walde P. From self-assembled vesicles to protocells. Cold Spring Harb Perspect Biol 2010;2:a002170.

Cheng L, Sharples RA, Siciluna BJ et al. Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. J Extracell Vesicles 2014;3:23743.

Cheng V, Stotz HU, Hippchen K et al. Genome-wide screen for oxalate-sensitive mutants of Saccharomyces cerevisiae. Appl Environ Microbiol 2007;73:5919–27.

Chiniquy D, Underwood W, Corwin J et al. PM5R, an acetylation protein at the intersection of pectin biosynthesis and defense against fungal pathogens. Plant J 2019;100:1022–35.

Cleave LG, Zamith D, Heyman HM et al. Media matters! Alterations in the loading and release of Histoplasma capsulatum extracellular vesicles in response to different nutritional milieus. Cell Microbiol 2020;22:e13217.

Cleregeot PH, Gourmès M, Cots J et al. PLS1, a gene encoding a tetraspanin-like protein, is required for penetration of rice leaf by the fungal pathogen Magnaporthe grisea. Proc Natl Acad Sci 2001;98:6963–8.

Collins NC, Thordal-Christensen H, Lipka V et al. SNARE-protein-mediated disease resistance at the plant cell wall. Nature 2003;425:973–7.

Colombo AC, Rela A, Normile T et al. Cryptococcus neoformans gluconoxyloolaminan and sterylglucoside are required for host protection in an animal vaccination model. mBio 2019;10:e02909–18.

Conde-Vancells J, Rodriguez-Suarez E, Embade N et al. Characterization and comprehensive proteome profiling of exosomes secreted by hepatocytes. J Proteome Res 2008;7:5157–66.

Conn CE, Drummond CJ. Nanostructured bicontinuous cubic lipid self-assembly materials as matrices for protein encapsulation. Soft Matter 2013;9:3449–64.

Connor J, Pak CH, Zwaal RF et al. Bidirectional transbilayer movement of phospholipid analogs in human red blood cells: evidence for an ATP-dependent and protein-mediated process. J Biol Chem 1992;267:19412–7.

Cooke AC, Nello AV, Ernst RK et al. Analysis of Pseudomonas aeruginosa biofilm membrane vesicles supports multiple mechanisms of biogenesis. mBio 2019;10:e0212275.

Costa Verdera H, Gitz-Francois JJ, Schifflers RM et al. Cellular uptake of extracellular vesicles is mediated by clathrin-independent endocytosis and macroinocytosis. J Control Release 2017;266:100–8.

Danielli JF, Davson H. A contribution to the theory of permeability of thin films. J Cell Comp Physiol 1935;5:495–508.

da Silva TA, Roque-Barreira MC, Casadevall A et al. Extracellular vesicles from Paracoccidioides brasiliensis induced M1 polarization in vitro. Sci Rep 2016;6:35867.
Douro� Singorenko P, Chang V, Whitcombe A et al. Isolation of membrane vesicles from prokaryotes: a technical and biological comparison reveals heterogeneity. J Extracell Vesicles 2017;6:1324731.

de la Canal L, Pinedo M. Extracellular vesicles: a missing component in plant cell wall remodeling. J Exp Bot 2018;69:4655–8.

de Lamo FJ, Constantin ME, Fresno DH et al. Xylem sap proteomics reveals distinct differences between R gene- and endophyte-mediated resistance against Fusarium wilt disease in tomato. Front Microbiol 2018;9:2977.

Del Vecchio F, Martinez-Rodriguez V, Schukking M et al. Professional killers: the role of extracellular vesicles in the reciprocal interactions between natural killer, CD8+ cytotoxic T-cells and tumour cells. J Extracell Vesicles 2021;10:e12075.

de Souza de Souza Pereira R, Geibel J. Direct observation of oxidative stress on the cell wall of Saccharomyces cerevisiae strains with atomic force microscopy. Mol Cell Biochem 1999;201:17–24.

Diaz C, Schroit AJ. Role of translocases in the generation of phosphatidylserine asymmetry. J Membr Biol 1996;151:1–9.

Di Pace AL, Tumino N, Bessi F et al. Characterization of human NK cell-derived exosomes: role of DNAM1 receptor in exosome-mediated cytotoxicity against tumor. Cancers 2020;12:661.

Distler JH, Huber LC, Hueber AJ et al. The release of microparticles by apoptotic cells and their effects on macrophages. Apoptosis 2005;10:731–41.

Dobereiner HG, Kas J, Noppl D et al. Budding and fission of vesicles. Biophys J 1993;65:1396–403.

Elleman CJ, Dickinson HG. Identification of pollen components regulating pollination-specific responses in the stigmatic papillae of Brassica oleracea. New Phytol 1996;133:197–205.

Ellen AF, Albers SV, Huibers W et al. Proteomic analysis of secreted membrane vesicles of archaeal Sulfolobus species reveals the presence of endosome sorting complex components. Extremophiles 2009;13:67–79.

Errington J. L-form bacteria, cell walls and the origins of life. Open Biol 2013;3:120143.

Evans-Osses I, Mojoli A, Munguio-Tortajada M et al. Microvesicles released from Giardia intestinalis disturb host-pathogen response in vitro. Eur J Cell Biol 2017;96:131–42.

Farge E, Ojcius DM, Subtil A et al. Enhancement of endocytosis due to aminophospholipid transport across the plasma membrane of living cells. Am J Physiol 1999;276:C725–33.

Fire A, Xu S, Montgomery MK et al. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 1998;391:806–11.

Fox JE, Austin CD, Boyles JK et al. Role of the membrane skeleton in preventing the shedding of procoagulant-rich microvesicles from the platelet plasma membrane. J Cell Biol 1990;111:843–93.

Freitas MS, Bonato VLD, Pessoni AM et al. Fungal extracellular vesicles as potential targets for immune interventions. mSphere 2019;4:e00747–19.

Freyssinet JM, Toti F, Hugel B et al. Apoptosis in vascular disease. Thromb Haemost 1999;82:727–35.

Fricke H. The electric capacity of suspensions with special reference to blood. J Gen Physiol 1925;9:137–52.

Fry SC. Cell walls. In: Thomas B, Murray BG, Murphy DJ (eds). Encyclopedia of Applied Plant Sciences. 2nd edn. Amsterdam: Elsevier, 2017;161–73.

Gall WE, Geething NC, Hua Z et al. Drs2p-dependent formation of exocytic clathrin-coated vesicles in vivo. Curr Biol 2002;12:1623–7.

Garcia-Rubio R, de Oliveira HC, Rivera J et al. The fungal cell wall: Candida, Cryptococcus, and Aspergillus species. Front Microbiol 2019;10:2993.

Garfoot AL, Rappleye CA. Histoplasma capsulatum surmounts obstacles to intracellular pathogenesis. FEBS J 2016;283:619–33.

Gasser O, Hess C, Miot S et al. Characterisation and properties of ectosomes released by human polymorphonuclear neutrophils. Exp Cell Res 2003;285:243–57.

Gehrmann U, Qazi KR, Johansson C et al. Nanovesicles from Malassezia sympodialis and host exosomes induce cytokine responses: novel mechanisms for host–microbe interactions in atopic eczema. PLoS One 2011;6:e21480.

Giibbon RK, Peberdy JF. Fine structure of protoplasts of Aspergillus nidulans. J Gen Microbiol 1972;72:529–38.

Gill S, Catchpole R, Forterre P. Extracellular membrane vesicles in the three domains of life and beyond. FEMS Microbiol Rev 2019;43:273–306.

Giraldo MC, Dagdas YF, Gupta YK et al. Two distinct secretion systems facilitate tissue invasion by the rice blast fungus Magnaporthe oryzae. Nat Commun 2013;4:1996.

Godfrey SA, Lovell HC, Mansfield JW et al. The stealth episome: suppression of gene expression on the excised genomic island PPHGI-1 from Pseudomonas syringae pv. phaseolicola. PLoS Pathog 2011;7:e1002010.

Goring DR. Exocytosis, exosomes, and autophagy in the regulation of Brassicaceae pollen–stigma interactions. J Exp Bot 2018;69:69–78.

Gorter E, Grendel F. On bimolecular layers of lipoid on the chromatic surface of the onion. Nature 1925;116:439–43.

Gow NA, Hube B. Importance of the Candida albicans cell wall during commensalism and infection. Curr Opin Microbiol 2012;15:406–12.

Gray SM, Banerjee N. Mechanisms of arthropod transmission of plant and animal viruses. Microbiol Mol Biol Rev 1999;63:128–48.

Greve LC, Labavitch JM. Cell wall metabolism in ripening fruit. V. Analysis of cell wall synthesis in ripening tomato pericarp tissue using a d-[U-13C]glucose tracer and gas chromatography–mass spectrometry. Plant Physiol 1991;97:1456–61.

Guerrero-Mandujano A, Hernández-Cortez C, Ibarra JA et al. Isolation of extracellular vesicles from the gall of Dracunculus medinensis. Exp Parasitol 2012;129:221–8.

Hall DG, Pethica BA. Thermodynamics of micelle formation. In: Schick MJ (ed). Nonionic Surfactants. New York: Marcel Dekker, 1967, 516–67.

Halperin W, Jensen WA. Ultrastructural changes during growth and embryogenesis in carrot cell cultures. J Ultrastruct Res 1967;18:428–43.

Hamilton KK, Hattori R, Esmon CT et al. Complement proteins C5b-9 induce vesiculation of the endothelial plasma membrane and expose catalytic surface for assembly of the prothrombinase enzyme complex. J Biol Chem 1990;265:3809–14.

Hargett LA, Bauer NN. On the origin of microparticles: from “platelet dust” to mediators of intercellular communication. Pulm Circ 2013;3:329–40.

Harris N. Organization of the endomembrane system. Ann Rev Plant Physiol 1986;37:73–92.

Hata Y, Slaughter CA, Sudhof TC. Synaptic vesicle fusion complex contains unc-18 homologue bound to syntaxin. Nature 1993;366:347–51.
He B, Cai Q, Qiao L et al. RNA-binding proteins contribute to small RNA loading in plant extracellular vesicles. Nat Plants 2021;7:342–52.

Hertwig O. The Cell: Outlines of General Anatomy and Physiology. London: Swan Sonnenschein, 1895.

Hill EH, Solomon PS. Extracellular vesicles from the apoplastic fungal wheat pathogen Zymoseptoria tritici. Fungal Biol Biotechnol 2020;7:13.

Hill TL. Thermodynamics of Small Systems. New York: W.A. Benjamin, 1964.

Hoch HC, Staples RC, Whitehead B et al. Signaling for growth orientation and cell differentiation by surface topography in uromycyes. Science 1987;235:1659–62.

Hofer K, Linkmeyer A, Teixtor K et al. MILDEW LOCUS O mutation does not affect resistance to grain infections with Fusarium spp. and Ramularia collo-cygni. Phytopathology 2015;105:1214–9.

Hu YB, Dammer EB, Ren RJ et al. The endosomal–lysosomal system: from acidification and cargo sorting to neurodegeneration. Transl Neurodegener 2015;4:18.

Huang C, Quinn D, Sadovsky Y et al. Formation and size distribution of self-assembled vesicles. Proc Natl Acad Sci 2017;114:2910–5.

Huang C, Yuan S, Dong M et al. The phylogenetic analysis of tetraspanins projects the evolution of cell–cell interactions from unicellular to multicellular organisms. Genomics 2005;86:674–84.

Huang SH, Wu CH, Chang YC et al. Cryptococcus neoforms-derived microvesicles enhance the pathogenesis of fungal brain infection. PLoS One 2012;7:e48570.

Huckelhoven R, Kogel KH. Reactive oxygen intermediates in trichodiene synthase gene disrupted in barley and wheat spikes inoculated with wild-type and fungal wheat pathogen Zymoseptoria tritici. Transl Neurodegener 2014;3:47–85.

Karkowska-Kuleta J, Kulig K, Karnas E et al. Characteristics of extracellular vesicles released by the pathogenic yeast-like fungi Candia glabrata, Candia parapsilosis and Candia tropicalis. Cells 2020;9:1722.

Katahisa H, Nasrallah JB. Self-incompatibility in Brassicaceae crops: lessons for interspecific incompatibility. Breed Sci 2014;64:23–37.

Kitajma Y. Structural and biochemical characteristics of pathogenic fungus: cell walls, lipids and dimorphism, and action modes of antifungal agents. Nippon Ishinkin Gakkai Zasshi 2000;41:211–7.

Koch A, Kogel KH. New wind in the sails: improving the agronomic value of crop plants through RNAi-mediated gene silencing. Plant Biotechnol J 2014;12:821–31.

Koch A, Schlemmer T, Höfle L et al. Host-induced gene silencing involves transfer of dsRNA-derived siRNA via extravascular extracellular vesicles. bioRxiv 2020.

Koonin EV, Aravind L. Origin and evolution of eukaryotic apoptosis: the bacterial connection. Cell Death Differ 2002;9:394–404.

Kulich I, Pecenkova T, Sekeres J et al. Arabidopsis exocyst subcomplex containing subunit EXO70B1 is involved in autophagy-related transport to the vacuole. Traffic 2013;14:1155–65.

Kulp A, Kuehn MJ. Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annu Rev Microbiol 2010;64:163–84.

Kumar J-H. A compromised Mlo pathway affects the response of barley to the necrotrophic fungus Bipolaris sorokiniana (teleomorph: c ochliobolus sativus) and its toxins. Phytopathology 2001;91:127–33.

Kusch S, Panstruga R. mlo-based resistance: an apparently universal “weapon” to defeat powdery mildew disease. Mol Plant Microbe Interact 2017;30:179–89.

Kutscher LM, Shaham S. Non-apoptotic cell death in animal development. Cell Death Differ 2017;24:1326–36.

Kwok C, Neu C, Pajonk S et al. Co-option of a default secretory pathway for plant immune responses. Nature 2008;451:835–40.

Leidal AM, Huang HH, Marsh T et al. The LC3-conjugation machinery specifies the loading of RNA-binding proteins into extracellular vesicles. Nat Cell Biol 2020;22:187–99.

Lieberthal W, Levine JS. Mechanisms of apoptosis and its potential role in renal tubular epithelial cell injury. Am J Physiol 1996;271:F477–88.

Lipka V, Dittgen J, Bednarek P et al. Pre- and postinvasion defenses both contribute to nonhost resistance in Arabidopsis. Science 2005;310:1180–3.

Liu K, Surendhran K, Nothwehr SF et al. P4-ATPase require-ment for AP-1/clathrin function in protein transport from the trans-Golgi network and early endosomes. Mol Biol Cell 2008;19:3526–35.

Liu NJ, Wang N, Bao JJ et al. Lipidomic analysis reveals the importance of GPCs in Arabidopsis leaf extracellular vesicles. Mol Plant 2020;13:1523–32.
Liu T, Song T, Zhang X et al. Unconventionally secreted effectors of two filamentous pathogens target plant salicylate biosynthesis. Nat Commun 2014;5:4686.

Lu A, Wawro P, Morgens DW et al. Genome-wide interrogation of extracellular vesicle biology using barcoded miRNAs. eLife 2018;7:e41460.

Lynch SF, Ludlam CA. Plasma microparticles and vascular disorders. Br J Haematol 2007;137:36–48.

Manning AJ, Kuehn MJ. Contribution of bacterial outer membrane vesicles to innate bacterial defense. BMC Microbiol 2011;11:258.

Markvoort AJ, Marrink SJ. Lipid acrobatics in the membrane fusion arena. Curr Top Membr 2011;68:259–94.

Mause SF, Weber C. Microparticles: protagonists of a novel communication network for intercellular information exchange. Circ Res 2010;107:1047–57.

McGrann GR, Stavrinides A, Russell J et al. A trade off between mlo resistance to powdery mildew and increased susceptibility of barley to a newly important disease, Ramularia leaf spot. J Exp Bot 2014;65:1025–37.

Mec克斯 DGJ, Raab-Traub N. Microvesicles and viral infection. J Virol 2011;85:12844–54.

Mengden K, Deising H. Infection structures of fungal plant pathogens: a cytological and physiological evaluation. New Phytol 1993;124:193–213.

Meyer D, Pajonk S, Micali C et al. Extracellular transport and integration of plant secretory proteins into pathogen-induced cell wall compartments. Plant J 2009;57:986–99.

Micali CO, Neumann U, Grunewald D et al. Biogenesis of a specialized plant–fungal interface during host cell internalization of Golovinomyces orontii haustoria. Cell Microbiol 2011;13:210–26.

Miyazaki Y, Nomura S, Miyake T et al. High shear stress can initiate both platelet aggregation and shedding of procoagulant containing microparticles. Blood 1996;88:3456–64.

Monari C, Bistoni F, Vecchiarelli A. Glucuronoxylomannan exhibits potent immunosuppressive properties. FEMS Yeast Res 2006;6:537–42.

Money NP. Measurement of pore size in the hyphal cell wall of Achlya bisexualis. Exp Mycol 1990;14:234–42.

Moore RT, McAlear JH. Fine structure of mycota. 5. Lomasomes: components are released into the extracellular space by vesicles in infected leaves. Plant Physiol 2019;180:1375–88.

Nebenfuhr A. Vesicle traffic in the endomembrane system: a tale of COPs, Rabs and SNAREs. Curr Opin Plant Biol 2002;5:507–12.

Niehl A, Heinlein M. Cellular pathways for viral transport through plasmodesmata. Protoplasma 2011;248:75–99.

Nielsen ME, Feechan A, Bohlenius H et al. Arabidopsis ARF-GRP exchange factor, GNOM, mediates transport required for innate immunity and foci accumulation of syntaxin PEN1. Proc Natl Acad Sci 2012;109:11443–8.

Niewland R, Sturk A. Platelet-derived microparticles. In: Michelson AD (ed). Platelets. London: Academic Press, Elsevier Science, 2002;255–65.

Nishimura MT, Stein M, Hou BH et al. Loss of a callose synthase results in salicylic acid-dependent disease resistance. Science 2003;301:969–72.

Nolte ’t Hoen E, Cremer T, Gallo RC et al. Extracellular vesicles and viruses: are they close relatives? Proc Natl Acad Sci 2016;113:9155–61.

O’Brien JR. The platelet-like activity of serum. Br J Haematol 1955;1:223–8.

Oliveira DL, Freire-de-Lima CG, Nosanchuk JD et al. Extracellular vesicles from Cryptococcus neoformans modulate macrophage functions. Infect Immun 2010a;78:1601–9.

Oliveira DL, Nakayasu ES, Joffe LS et al. Characterization of yeast extracellular vesicles: evidence for the participation of different pathways of cellular traffic in vesicle biogenesis. PLoS One 2010b;5:e11113.

Oliveira DL, Rizzo J, Joffe LS et al. Where do they come from and where do they go: candidates for regulating extracellular vesicle formation in fungi. Int J Mol Sci 2013;14:9581–603.

Ostertag M, Stammer J, Douchkov D et al. The conserved oligomeric Golgi complex is involved in penetration resistance of barley to the barley powdery mildew fungus. Mol Plant Pathol 2013;14:230–40.

Osumi M. The ultrastructure of yeast: cell wall structure and formation. Micron 1998;29:207–33.

Padder SA, Prasad R, Shah AH. Quorum sensing: a less known mode of communication among fungi. Microbiol Res 2018;210:51–8.

Palade G. Intracellular aspects of the process of protein synthesis. Science 1975;189:347–58.

Palmisano NJ, Melendez A. Autophagy in C. elegans development. Dev Biol 2019;447:103–25.

Panepinto J, Komperda K, Frases S et al. Sec6-dependent sorting of fungal extracellular exosomes and laccase of Cryptococcus neoformans. Mol Microbiol 2009;71:1165–76.

Pecenkova T, Hala M, Kulich I et al. The role for the exocyst complex subunits Exo70B2 and Exo70H1 in the plant-pathogen interaction. J Exp Bot 2011;62:2107–16.

Pecenkova T, Sabol P, Kulich I et al. Constitutive negative regulation of R proteins in Arabidopsis also via autophagy related pathway? Front Plant Sci 2016;7:260.

Peres da Silva R, Heiss C, Black I et al. Extracellular vesicles from Paracoccidioides pathogenic species transport polysaccharide and expose ligands for DC-SIGN receptors. Sci Rep 2015a;5:14213.

Peres da Silva R, Martins ST, Rizzo J et al. Golgi reassembly and stacking protein (GRASP) participates in vesicle-mediated RNA export in Cryptococcus neoformans. Genes 2018;9:400.

Peres da Silva R, Puccia R, Rodrigues ML et al. Extracellular vesicle-mediated export of fungal RNA. Sci Rep 2015b;5:7763.

Piccin A, Murphy W, Smith OP. Circulating microparticles: pathophysiology and clinical implications. Blood Rev 2007;21:157–71.

Picco A, Rastorza-Azarate I, Specht T et al. The in vivo architecture of the exocyst provides structural basis for exocytosis. Cell 2017;168:400–12.

Pickett JA, Edwardson JM. Compound exocytosis: mechanisms and functional significance. Traffic 2006;7:109–16.

Pitman AR, Jackson RW, Mansfield JW et al. Exposure to host resistance mechanisms drives evolution of bacterial virulence in plants. Curr Biol 2005;15:2230–5.

Podilla GK, Rogers LM, Kolattukudy PE. Chemical signals from avocado surface wax trigger germination and appressorium formation in Colletotrichum gloeosporioides. Plant Physiol 1993;103:267–72.

Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013;200:373–83.

Regente M, Corti-Monzon G, Maldonado AM et al. Vesicular fractions of sunflower apoplastic fluids are associated with potential exosome marker proteins. FEBS Lett 2009;583:3363–6.
Regente M, Pinedo M, Elizalde M et al. Apoplastic exosome-like vesicles: a new way of protein secretion in plants? Plant Signal Behav 2012;7:544–6.

Regente M, Pinedo M, San Clemente H et al. Plant extracellular vesicles are incorporated by a fungal pathogen and inhibit its growth. J Exp Bot 2017;68:5485–95.

Reis FCG, Borges BS, Jozefowicz LJ et al. A novel protocol for the isolation of fungal extracellular vesicles reveals the participation of a putative scramblase in polysaccharide export and capsule construction in Cryptococcus gattii. mSphere 2019;4:e00080–19.

Rizzo J, Chaze T, Miranda K et al. Characterization of extracellular vesicles produced by Aspergillus fumigatus protoplasts. mSphere 2020;5 https://doi.org/10.1128/mSphere.00476-20.

Rizzo J, Colombo AC, Zamith-Miranda D et al. The putative flipase Apt1 is required for intracellular membrane architecture and biosynthesis of polysaccharide and lipids in Cryptococcus neoformans. Biochim Biophys Acta 2018;1865:532–41.

Rizzo J, Oliveira DL, Joffe LS et al. Role of the Apt1 protein in polysaccharide secretion by Cryptococcus neoformans. Eukaryot Cell 2014;13:715–26.

Rodrigues ML, Nakayasu ES, Almeida IC et al. The impact of proteomics on the understanding of functions and biogenesis of fungal extracellular vesicles. J Proteomics 2014;97:177–86.

Rodrigues ML, Nimrichter L, Oliveira DL et al. Vesicular polysaccharide export in Cryptococcus neoformans is an eukaryotic solution to the problem of fungal trans-cell wall transport. Eukaryot Cell 2007;6:48–59.

Rose JK, Hadfield KA, Labavitch JM et al. Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. Plant Physiol 1998;117:345–61.

Russell MRG, Shideler T, Nickerson DP et al. Class E compartments form in response to ESCRT dysfunction in yeast due to hyperactivity of the Vps21 Rab GTPase. J Cell Sci 2012;125:5208–20.

Rutter BD, Innes RW. Extracellular vesicles isolated from the leaf apoplast carry stress-response proteins. Plant Physiol 2017;173:728–41.

Safavian D, Goring DR. Secretory activity is rapidly induced in stigmatic papillae by compatible pollen, but inhibited for self-incompatible pollen in the Brassicaceae. PLoS One 2013;8:e84286.

Safavian D, Zayed Y, Indriolo E et al. RNA silencing of exocyst genes in the stigma impairs the acceptance of compatible pollen in Arabidopsis. Plant Physiol 2015;169:2526–38.

Schmidt S, Tramsen L, Lehrnbecher T. Natural killer cells in antifungal immunity. Front Immunol 2017;8:1623.

Schwechheimer C, Kuehn MJ. Outer-membrane vesicles from Gram-negative bacteria: bio genesis and functions. Nat Rev Microbiol 2015;13:605–19.

Shurtleff MJ, Temocie-Diaz MM, Karfilis KV et al. Y-box protein 1 is required to sort microRNAs into exosomes in cells and in a cell-free reaction. eLife 2016;5:e19276.

Sims PJ, Wiedmer T. Unraveling the mysteries of phospholipid scrambling. Thromb Haemost 2001;86:266–75.

Sollner T, Whiteheart SW, Brunner M et al. SNAP receptors implicated in vesicle targeting and fusion. Nature 1993;362:218–24.

Song H, Canup BSB, Ngo VL et al. Internalization of garlic-derived nanovesicles on liver cells is triggered by interaction with CD98. ACS Omega 2020;5:23118–28.

Spang A, Saw JH, Jorgensen SL et al. Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature 2015;521:173–9.

Srivastava A, Amreddy N, Pareek V et al. Progress in extracellular vesicle biology and their application in cancer medicine. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2020;12:e1621.

Stegmann M, Anderson RG, Ichimura K et al. The ubiquitin ligase PUB22 targets a subunit of the exocyst complex required for PAMP-triggered responses in Arabidopsis. Plant Cell 2012;24:4703–16.

Stegmann M, Anderson RG, Westphal L et al. The exocyst subunit Exo70B1 is involved in the immune response of Arabidopsis thaliana to different pathogens and cell death. Plant Signal Behav 2013;8:e27421.

Stein M, Dittgen J, Sanchez-Rodriguez C et al. Arabidopsis PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. Plant Cell 2006;18:731–46.

Stutz HU, Augustin R, Khalturin K et al. Novel approaches for the analysis of immune reactions in Tunicate and Cnidarian model organisms. In: The New Panorama of Animal Evolution. Sofia: Pensoft Publishers, 2003.

Stratton D, Moore C, Zheng I et al. Prostate cancer cells stimulated by calcium-mediated activation of protein kinase C undergo a refractory period before re-releasing calcium-bearing microvesicles. Biochem Biophys Res Comm 2015;460:511–7.

Subra C, Grand D, Laulagnier K et al. Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins. J Lipid Res 2010;51:2105–20.

Sun G, Elowsky C, Li G et al. TOR-autophagy branch signaling via Imp1 dictates plant–microbe biotrophic interface longevity. Pls Genet 2018;14:e1007814.

Szathmáry E, Santos M, Fernando C. Evolutionary potential and requirements for minimal protocells. Top Curr Chem 2005;259:167–211.

Takoe K, Usaka I, Uehira K et al. Fine structure of Cryptococcus neoformans grown in vitro as observed by freeze-etching. J Bacteriol 1973;113:1442–8.

Tanford C. The Hydrophobic Effect: Formation of Micelles and Biological Membranes. New York: John Wiley and Sons, 1973.

Thery C, Witwer KW, Aikawa E et al. Minimal information for studies of extracellular vesicles 2018 (MISVE2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesicles 2018;7:1535750.

Toledo MS, Suzuki E, Levery SB et al. Characterization of monoclonal antibody MEST-2 specific to glucosylceramide of fungi and plants. Glyobiology 2001;11:105–12.

Toyofuku M, Nomura N, Eberl L. Types and origins of bacterial membrane vesicles. Nat Rev Microbiol 2019;17:13–24.

Trujillo M, Ichimura K, Cassais C et al. Negative regulation of PAMP-triggered immunity by an E3 ubiquitin ligase triplet in Arabidopsis. Curr Biol 2008;18:1396–401.

Tucker SL, Talbot NJ. Surface attachment and pre-penetration stage development by plant pathogenic fungi. Annu Rev Phytopathol 2001;39:385–417.

van Dommelen SM, Vader P, Lakhal S et al. Microvesicles and exosomes: opportunities for cell-derived membrane vesicles in drug delivery. J Control Release 2012;161:635–44.

Vanhijk MJ, Svedas E, Boer K et al. Isolated microparticles, but not whole plasma, from women with preeclampsia impair endothelium-dependent relaxation in isolated myometrial arteries from healthy pregnant women. Am J Obstet Gynecol 2002;187:1686–93.
Vargas G, Rocha JD, Oliveira DL et al. Compositional and immunobiological analyses of extracellular vesicles released by Candida albicans. Cell Microbiol 2015;17:389–407.

Villarroya-Beltri C, Gutierrez-Vazquez C, Sanchez-Cabo F et al. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. Nat Commun 2013;4:2980.

Villena SN, Pinheiro RO, Pinheiro CS et al. Capsular polysaccharides galactoxylomannan and glucuronoxylomannan from Cryptococcus neoformans induce macrophage apoptosis mediated by Fas ligand. Cell Microbiol 2008;10:1274–85.

Vlassov AV, Magdaleno S, Setterquist R et al. Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochim Biophys Acta 2012;1820:940–8.

Vogel JP, Raab TK, Somerville CR et al. Mutations in PMR5 result in powdery mildew resistance and altered cell wall composition. Plant J 2004;40:968–78.

Vukasinovic N, Zarsky V. Tethering complexes in the Arabidopsis endomembrane system. Front Cell Dev Biol 2016;4:46.

Walker L, Sood P, Lenardon MD et al. The viscoelastic properties of the fungal cell wall allow traffic of AmBisome as intact liposome vesicles. mBio 2018;9:e02383–17.

Wan J, Cabanillas DG, Zheng H et al. Turnip mosaic virus moves systemically through both phloem and xylem as membrane-associated complexes. Plant Physiol 2015;167:1374–84.

Wang J, Ding Y, Wang J et al. EXPO, an exocyst-positive organelle distinct from multivesicular endosomes and autophagosomes, mediates cytosol to cell wall exocytosis in Arabidopsis and tobacco cells. Plant Cell 2010a;22:4009–30.

Wang K, Zhang S, Weber J et al. Export of microRNAs and microRNA-protective protein by mammalian cells. Nucleic Acids Res 2010b;38:7248–59.

Watanabe M, Suwabe K, Suzuki G. Molecular genetics, physiology and biology of self-incompatibility in Brassicaceae. Proc Jpn Acad Ser B 2012;88:519–35.

Wei T, Hibino H, Omura T. Release of Rice dwarf virus from insect vector cells involves secretory exosomes derived from multivesicular bodies. Commun Integr Biol 2009;2:324–6.

Weber A, Wang M, Lin FM et al. Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science 2013;342:118–23.

Wiedmer T, Sims PJ. Participation of protein kinases in complement C5b-9-induced shedding of platelet plasma membrane vesicles. Blood 1991;78:2880–6.

Wolf JM, Espadas J, Luque-Garcia J et al. Lipid biosynthetic genes affect Candida albicans extracellular vesicle morphology, cargo, and immunostimulatory properties. Eukaryot Cell 2015;14:745–54.

Wolf P. The nature and significance of platelet products in human plasma. Br J Haematol 1967;13:269–88.

Xu YF, Xu X, Gin A et al. SRSF1 regulates exosome microRNA enrichment in human cancer cells. Cell Commun Signal 2020;18:130.

Yano Y, Kambayashi J, Shiba E et al. The role of protein phosphorylation and cytoskeletal reorganization in microparticle formation from the platelet plasma membrane. Biochem J 1994;299:303–8.

Yoshimoto K, Jikumaru Y, Kamiya Y et al. Autophagy negatively regulates cell death by controlling NPR1-dependent salicylic acid signaling during senescence and the innate immune response in Arabidopsis. Plant Cell 2009;21:2914–27.

Zarnowski R, Sanchez H, Covelli AS et al. Candida albicans biofilm-induced vesicles confer drug resistance through matrix biogenesis. PLoS Biol 2018;16:e2006872.

Zhao K, Bleackley M, Chisanga D et al. Extracellular vesicles secreted by Saccharomyces cerevisiae are involved in cell wall remodelling. Commun Biol 2019;2:305.