Exchanging missives and missiles: the roles of extracellular vesicles in plant–pathogen interactions

Petra C. Boevink

The James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK
Correspondence: petra.boevink@hutton.ac.uk

Extracellular vesicles (EVs) are secreted by organisms from all forms of life. In the mammalian field they are intensively studied due to their importance in disease and potential for therapeutic use. However, there has been little research in plants and thus the paper by Regente et al. (2017) is a valuable addition to a small but hopefully growing body of data. The authors conducted proteomic analysis on purified sunflower EVs and demonstrated that they are enriched in defence-related proteins. They found that fungal spores treated with fresh EV preparations are damaged and show reduced growth.

In addition to being a means to unconventionally secrete proteins to the apoplast, extracellular vesicles (EVs) are presumably being secreted by plant cells for communication with neighbouring plant cells and for interaction with microbes and other organisms. Indeed, it is conceivable that the latter might be more important as it could be argued that plasmodesmata can take care of the bulk of protein and RNA exchange between neighbouring plant cells. On the other hand biology loves complexity and redundancy.

There are several potential pathways for the production of EVs and different classes of EVs are recognized, such as microvesicles and exosomes (Mulcahy et al., 2014). Exosomes are described in the mammalian literature as originating from multivesicular bodies (MVBs). Some of the potential pathways for EV production and uptake are outlined in Box 1. However, another potential source has been identified in plants: the exocyst-positive organelle (EXPO; Wang et al., 2010), which is probably equivalent to specialized secretory autophagosomes identified in yeast (Bruns et al., 2011). In the Arabidopsis EV proteome published by Rutter and Innes (2017), the RPM1 INTERACTING PROTEIN 4 (RIN4) was detected and this protein was also identified in a proteome published by Rutter and Innes (2017) and Rutter and Innes (2005). The EV proteome, which are well known to be involved in pathogen recognition and plant defence (Lannoo and Van Damme, 2014) and in mammalian systems have been shown to stimulate uptake by immune cells (e.g. Jack et al., 2005). The EV proteome contains both proteins that are characteristic of defence receptors and proteins that are targeted to each other’s cells. What determines the specificity and direction of travel during EV exchange? It would be inefficient if a source cell reabsorbed its own EVs and potentially dangerous for a plant cell to absorb EVs containing damaging molecules or enzymes that have been secreted to attack pathogen cells. The most straightforward mechanism for generating specificity would be to decorate the EV membranes with targeting proteins, glycoproteins or other molecules creating a system similar to the mechanisms of vesicle targeting within the cell. The targeting proteins would interact with proteins or other identifiers on the target cells and perhaps even stimulate uptake. Human cancer cell exosome uptake was reduced to 43% after the exosomes had been treated with protease K, and treatment of the cells with protease K also reduced exosome uptake, by 32% (Escrevente et al., 2011). Uptake was also reduced when cells were treated with an excess of monosaccharides, indicating the importance of glycoprotein recognition. Regente et al. (2017) identified mannose-binding lectins in the EV proteome, which are well known to be involved in pathogen recognition and plant defence. We should not forget that some, or perhaps many, exosomes are thought simply to degrade in the apoplast to allow the release of cytoplasmic proteins through unconventional

© The Author 2017. Published by Oxford University Press on behalf of the Society for Experimental Biology. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
secretion. Are there specific EV populations that are differentially fated – to either degrade or fuse with the target cell? Does the intimate plant–pathogen or plant–symbiont interface zone have specific properties that favour uptake over degradation?

Plant EVs on the attack

In Regente et al. (2017) fungal spores showed signs of membrane disruption after exposure to fresh sunflower EV preparations by uptake of propidium iodide and Evans Blue dyes.
and some apparent rupture. Following exposure to FM4-64-labelled EVs, the FM4-64 dye accumulated inside fungal spores rather than at the spore plasma membrane. This indicates that the EVs are being endocytosed as opposed to fusing with the spore plasma membrane. Regente et al. (2017) tested artificial vesicles and found no effect on spore vitality but at this stage it is difficult to guess how equivalent they were to EVs. Labelling of the artificial vesicles with FM4-64 would show whether they were taken up by the spores. If EVs interact with target membranes through proteins or other molecules on their surfaces as for mammalian exosomes (Escrevente et al., 2011), and this interaction is required for uptake, then the artificial vesicles may not interact appropriately for uptake. Treatment of plant EVs with proteases to remove surface proteins as has been done for mammalian exosomes would indicate whether the system in plants also depends on surface proteins.

The treatment of fungal spores by Regente et al. (2017) with a concentrated preparation of EVs was likely to be beyond anything the spores would experience in nature. However, at the point of contact between pathogen and host, at haustoria for example, the local concentration of EVs may be quite high. If spore disruption was not caused by direct membrane effects then it must be a result of the actions of the proteins or compounds that the EVs were carrying. The exosome proteomes described by Rutter and Innes (2017) and Regente et al. (2017) were rich in defence proteins including those involved in the myrosinase–glucosinolate system, which produces toxic compounds.

Just as EVs have great potential as therapeutic agents in the medical field, the characterization of plant EVs that target pathogens could lead to novel crop protection strategies. If the proteins and glycoproteins present on pathogen-targeting EVs are found to be specific this could potentially allow the production (either enhanced in planta or in a culture system) of EVs carrying highly effective biocides or RNAs that are specifically taken up by economically important pathogens or pests. Host-induced gene silencing is a method shown to provide crop protection (Koch et al., 2013) that is almost certainly dependent on EV delivery of RNA.

**The fight back**

In addition to plants producing EVs to attack potential pathogens the reverse is also likely – that pathogens produce EVs to control and attack plants. Pathogens, symbionts and pests have all been shown to produce effectors to control the responses of their host plants. Some of these effectors have been shown to enter host cells and many others are assumed to do so; these are classed as cytoplasmic effectors (as opposed to apoplastic effectors, which function outside the host cell). Bacterial pathogens have specific structures such as the type 3 secretion system to deliver their effectors but no such structures have been identified for eukaryotic pathogens. Giraldo et al. (2013) demonstrated that cytoplasmic effectors are non-conventionally secreted by Magnaporthe oryzae and we recently showed that an oomycete RXLR effector was secreted through an unconventional route (Wang et al., 2017). These studies open up the possibility that these effectors are secreted in association with EVs. Can we detect effectors in EV preparations? Are pathogen-derived EVs endocytosed by host cells? How are they specifically targeted to host cells? How do cytoplasmic effectors reach their ultimate destinations within host cells? This is currently a hot topic in plant pathology. One curious feature to note is that the RXLR effector Pi04314, and indeed all characterized RXLR class effectors, possess signal peptides. Exosomes are described as being generated by invagination of the MVB membrane which results in engulfment of cytoplasmic content. Microvesicles are described as budding directly from the plasma membrane, and thus also engulf cytoplasmic content. Signal peptide-containing proteins, however, will have entered the endoplasmic reticulum co-translationally, so what is their route to unconventional secretion? The review by Ding et al. (2012) summarizes evidence for unconventional secretion of both cytoplasmic proteins and ER contents. They include in their diagrams how autophagosomes and the related EXPOs may be derived from the ER. Further evidence for this is summarized by Zhuang et al. (2016). This would presumably result in signal peptide-positive effectors being associated with the exterior of EXPO-derived exosomes, unless there is invagination of the interior of the two membranes or engulfment of ER-derived vesicles by the developing EXPO.

A further exciting area of study will be characterization of RNA populations in pathogen– and plant-derived EVs. The delivery of small RNAs to control gene expression within the host or mRNAs to ensure the production by the host of specific proteins helpful to the establishment of infection are both possible through EVs as they can protect the vulnerable RNAs from the apoplastic environment. Botrytis cinerea has been shown to produce RNA that are targeted to host cells (Weiberg et al., 2013). Rutter and Innes (2017) also cite mRNA movement from the parasitic plant Cuscuta to host cells but plasmodesmata are formed between Cuscuta and their hosts. Thus mRNAs may move symplastically, though of course this does not rule out an exchange of EVs between Cuscuta and its host.

**Conclusions**

The proteomics of plant EVs has only been published by Rutter and Innes (2017) and Regente et al. (2017). Although it is early days and robust proteomics requires large amounts of repetition, careful and consistent data processing and high-quality reference genomes, results to date provide some logical candidate components, as mentioned above. There is considerable scope for proteomics of plant EV populations produced in response to different conditions and stimuli and from a range of species. Proteomics of trypsin-treated EVs would allow us to see which proteins are associated with the exterior of the EVs.

The analysis of EVs involved in plant–pathogen interactions is only just beginning but their potential for increasing...
our understanding of the exchanges determining disease outcomes and the potential for the development of new strategies to combat disease in economically important crops will ensure the rapid expansion of this field.

Key words: Antifungal, apoplast, exosomes, extracellular vesicles (EVs), fungal growth, fungal spores, growth inhibition, intercellular communication, plant defence, proteomic analysis.

References

Bruns C, McCaffery JM, Curwin AJ, Duran JM, Malhotra V. 2011. Biogenesis of a novel compartment for autophagosome-mediated unconventional protein secretion. The Journal of Cell Biology 195, 979–992.

Ding Y, Wang J, Wang J, Stierhof YD, Robinson DG, Jiang L. 2012. Unconventional protein secretion. Trends in Plant Science 17, 606–615.

Escrevente C, Keller S, Altevogt P, Costa J. 2011. Interaction and uptake of exosomes by ovarian cancer cells. BMC Cancer 11, 108.

Giraldo MC, Dagdas YF, Gupta YK, et al. 2013. Two distinct secretion systems facilitate tissue invasion by the rice blast fungus Magnaporthe oryzae. Nature Communications 4, 996.

Jack DL, Lee ME, Turner MW, Klein NJ, Read RC. 2005. Mannose-binding lectin enhances phagocytosis and killing of Neisseria meningitidis by human macrophages. Journal of Leukocyte Biology 77, 328–336.

Koch A, Kumar N, Weber L, Keller H, Imani J, Kogel KH. 2013. Host-induced gene silencing of cytochrome P450 lanosterol C14 alpha-demethylase-encoding genes confers strong resistance to Fusarium species. Proceedings of The National Academy of Sciences, USA 110, 19324–19329.

Lannoo N, Van Damme EJ. 2014. Lectin domains at the frontiers of plant defense. Frontiers in Plant Science 5, 397.

Mulcahy LA, Pink RC, Carter DRF. 2014. Routes and mechanisms of extracellular vesicle uptake. Journal of Extracellular Vesicles 3, 24641.

Regente M, Pinedo M, San Clemente H, Balliau T, Jamet E, de la Canal L. 2017. Plant extracellular vesicles are incorporated by a fungal pathogen and inhibit its growth. Journal of Experimental Botany 68, 5485–5495.

Rutter BD, Innes RW. 2017. Extracellular vesicles isolated from the leaf apoplast carry stress-response proteins. Plant Physiology 173, 728–741.

Sabol P, Kulich I, Žárský V. 2017. RIN4 recruits the exocyst subunit EXO70B1 to the plasma membrane. Journal of Experimental Botany 68, 3253–3265.

Wang J, Ding Y, Wang J, Hillmer S, Miao Y, Lo SW, Wang X, Robinson DG, Jiang L. 2010. EXPO, an exocyst-positive organelle distinct from multivesicular endosomes and autophagosomes, mediates cytosol to cell wall exocytosis in Arabidopsis and tobacco cells. The Plant Cell 22, 4009–4030.

Wang S, Boevink PC, Welsh L, Zhang R, Whisson SC, Birch PRJ. 2017. Delivery of cytoplasmic and apoplastic effectors from Phytophthora infestans haustoria by distinct secretion pathways. New Phytopathologist 216, 205–215.

Weiberg A, Wang M, Lin FM, Zhao H, Zhang Z, Kaloshian I, Huang HD, Jin H. 2013. Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science 342, 118–123.

Zhuang X, Chung KP, Jiang L. 2016. Origin of the Autophagosomal Membrane in Plants. Frontiers in Plant Science 7, 1655.