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Extracellular Vesicles in Cross Kingdom Communication

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SUMMARY

Communication between plant cells and interacting pathogens is crucial for establishment of relationship between them. This requires the secretion and uptake of functional molecules to and from the extracellular environment and is essential for the survival of both plants and their pathogens. Emerging evidence has shown that both plant and microbial EVs play important roles in cross-kingdom molecular exchange between hosts and interacting microbes to modulate host immunity and pathogen virulence.

INTRODUCTION

Numerous plant pathogens, including bacteria, fungi, viruses and nematodes, are responsible for causing pleothera of plant diseases, which reduce the yield and the quality of agricultural production worldwide every year. Plants and pathogens secrete multitudes of molecules into the extracellular environment for cross-border communication, which plays a crucial role in plant defense and pathogen virulence. Such communication between plants and pathogens requires the transport of molecules, *viz.,* antimicrobial metabolites from hosts to pathogens or effector proteins and toxins from pathogens to hosts across cellular boundaries. Recently, it has been identified that RNA molecules, particularly regulatory small RNAs (sRNAs) are found to travel in between interacting organisms to induce gene silencing in trans, thus playing an important role in plant-pathogen interactions. Among such interactions one potential pathway of cell-to-cell communication is through extracellular vesicles (EVs).

Plant extracellular vesicles:

EVs are lipid bilayer–bound spheres that contain a diverse variety of enclosed bioactive cargos, such as proteins, nucleic acids, and metabolites from the secreting cells, traverse the extracellular environment and enter interacting organisms to play significant roles in regulating host-pathogen interactions (Cai *et al*., 2021).

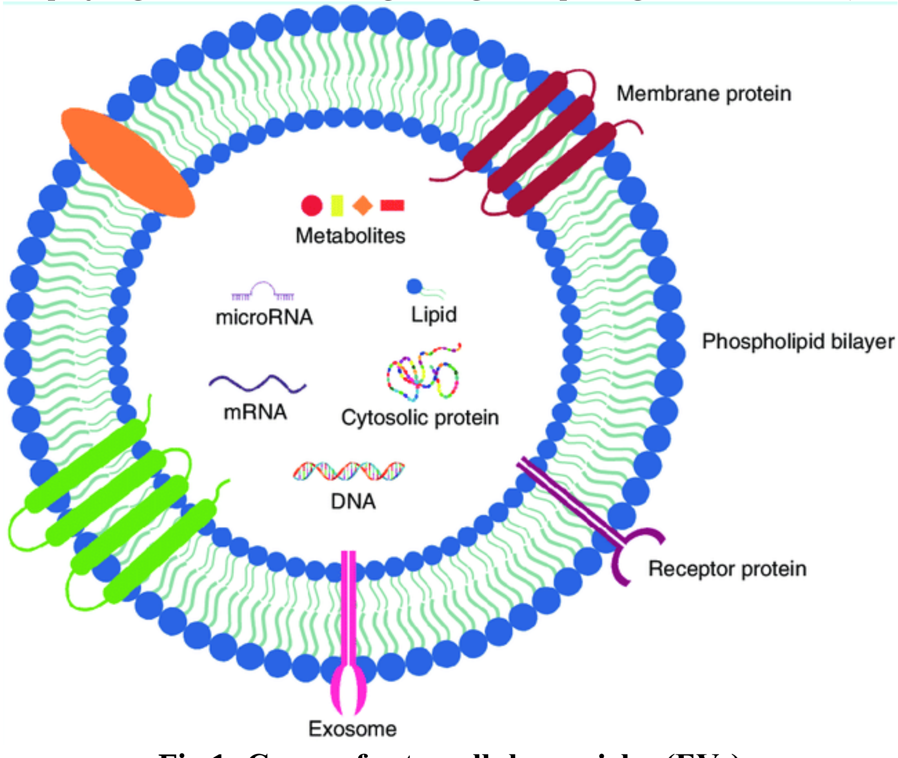

Fig.1: Cargo of extracellular vesicles (EVs)

Plant EVs were initially found in carrot cell cultures through electron microscopy in 1967. Since then, EVs have been observed in extracellular fluids of leaves, roots and imbibing seeds. However, studies on the specific characteristics and the biochemical contents have shown that plant EVs have specific protein markers containing a distinct set of RNAs, proteins and metabolites, which are likely to have an regulatory functions in recipient cells and interacting organisms (Cai *et al*., 2021). Some important plant EVs are enlisted below,

a. Tetraspanin-Positive Extracellular Vesicles - Exosomes : In plant systems, Arabidopsis encodes Extracellular vesicles belonging to TETRASPANIN (TET) family. Expression analysis revealed that two closely related tetraspanin genes, TET8 and TET9, were found to be highly induced by infection with the pathogen *Botrytis cinerea* suggesting that they are involved in plant defense responses (Cai *et al*., 2021).

b. Exocyst-Positive Organelle-Derived Extracellular Vesicles: Another class of plant EVs is derived from exocyst-positive organelles (EXPOs). The EXPO is a novel organelle identified by expressing an Arabidopsis homolog of the exocyst protein Exo70E2 in Arabidopsis and tobacco (*Nicotiana tabacum*) suspension cells (Cai *et al*., 2021).

c. Penetration 1-Positive Extracellular Vesicles: At a lower ultracentrifugation speed (40,000 × g) plant EVs were isolated from Arabidopsis leaf apoplastic fluid. Proteomic analysis of these EVs revealed that contain proteins involved in biotic and abiotic stress responses, including plant-specific Penetration 1 (PEN1). PEN1 was initially identified as a plasma membrane-associated syntaxin (Cai *et al*., 2021).

Extracellular Vesicles in Plant Defence:

Plant-derived EVs play also important roles in cell-to-cell communication with microbes. Several studies have shown that immune stress stimulates EV secretion from plant cells (Rutter & Innes, 2017). Both infection with *P. syringae* pv *tomato* DC3000 bacteria and activation of immune signaling in response to the defense hormone salicylic acid increases EV abundance in Arabidopsis extracellular fluids (Cai *et al*., 2018). An obvious function of EVs at the plant-pathogen interface is to serve as mechanical protection against invading pathogens. Indeed, plant resistance to pathogen entry is linked to the polarized immune response. This pathway functions by concentrating EVs in the extracellular space beneath attempted sites of pathogen penetration, resulting in the formation of so-called papillae (Kwon *et al*., 2008a).

Extracellular vesicles in cross-kingdom small RNA trafficking and plant immunity:

Plant EV secretion was found to be increased by pathogen infection, suggesting that EVs play important roles in plant immunity and because of the material transport nature of EVs, plants and many interacting pathogens have evolved to utilize EVs to exchange functional molecules, including proteins, RNAs and metabolites, between host cells and interacting organisms.

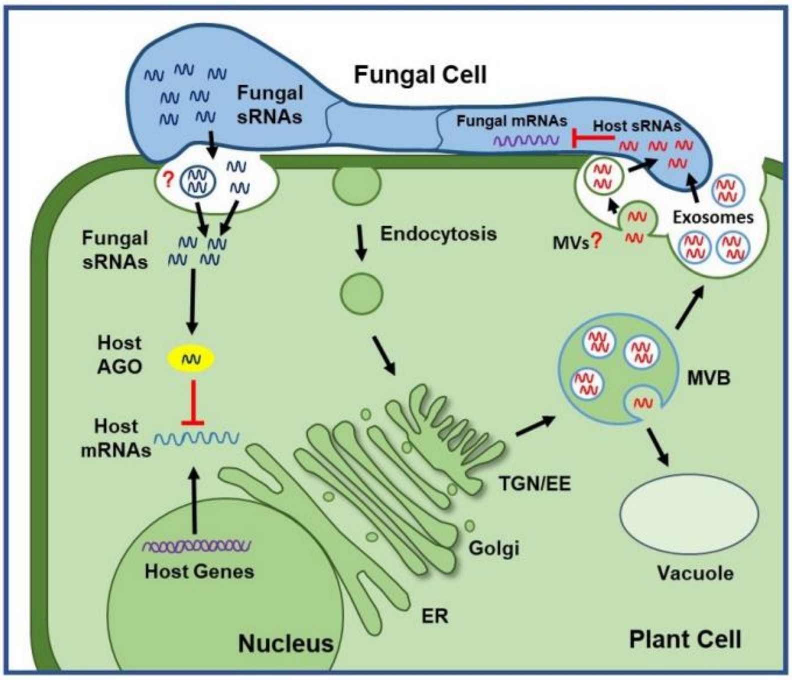

Fig.2: Cross-kingdom RNAi between plants and fungal pathogens

sRNA-mediated RNA interference (RNAi) is a conserved gene-silencing mechanism in eukaryotes to regulate endogenous and exogenous gene expression. RNAi has been co-opted via a genetic modification to resist various crop pests and pathogens. Recent studies have found that sRNAs can move across the cellular boundaries between plant and animal hosts and their interacting pathogens and parasites, thus triggering gene silencing in trans, a cross-kingdom RNAi process. Arabidopsis delivers a particular set of miRNAs and small interfering RNAs (siRNAs), including phased secondary siRNAs (phasiRNAs), into interacting *B. cinerea* cells, inducing the silencing of fungal genes that are involved in pathogenicity(Cai *et al*., 2021).

CONCLUSION

Both plants and plant pathogens release EVs containing a wide variety of defence-related cargoes but there is only limited and fragmentary knowledge on the involvement of EVs in plant–pathogen interactions. The precise functions of EVs remain to be clearly elucidated specifically related to the plant immune response, defining their composition and documenting their function. Such studies are essential for understanding the functions of EVs and would greatly facilitate the development of improved disease management practices for sustainable agriculture.

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