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MEETING REVIEW

Spheres of Hope, Packets of Doom: the Good and Bad of Outer Membrane Vesicles in Interspecies and Ecological Dynamics

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ABSTRACT Outer membrane vesicles (OMVs) are proteoliposome nanoparticles ubiquitously produced by Gram-negative bacteria. Typically bearing a composition similar to those of the outer membrane and periplasm of the cells from which they are derived, OMVs package an array of proteins, lipids, and nucleic acids. Once considered inconsequential by-products of bacterial growth, OMVs have since been demonstrated to mediate cellular stress relief, promote horizontal gene transfer and antimicrobial activity, and elicit metazoan inflammation. Recently, OMVs have gained appreciation as critical moderators of interorganismal dynamics. In this review, we focus on recent progress toward understanding the functions of OMVs with regard to symbiosis and ecological contexts, and we propose potential avenues for future OMV studies.

KEYWORDS host-microbe interactions, outer membrane vesicles

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We live in a world in which chemical cues produced by bacteria influence the cellular behavior of neighboring organisms (1). Identifying the modes for material exchange between organisms will inform our understanding of how long-term relationships are established and maintained. Gram-negative bacteria use diverse mechanisms to secrete compounds, ranging from simple systems (e.g., type I and V secretion) to multiprotein complexes (type III, IV, and VI secretion) (2). However, much less attention has been devoted to the role of outer membrane vesicles (OMVs) in interspecies interactions.

OMVs are nanoparticles composed of lipid bilayers originating from the outer membrane of Gram-negative bacteria (3), ranging in diameter from 20 to 200 nm (4). These spherical bodies are produced by all Gram-negative bacteria across a broad spectrum of conditions and environments (5–9). OMV biogenesis is constitutive, but the production rate and composition of OMVs are sensitive to stress and environmental fluctuations (10–14). First described half a century ago as membrane sacs that enable the excretion of cell wall material (15), OMVs are a generalized secretion system for complex combinations of compounds. In addition to material from the outer membrane and periplasm, OMVs frequently contain other materials in either the vesicle lumen or incorporated into the membrane bilayer, including cell wall components, nucleic acids, toxins, and labile carbon (16–20). Protected within a lipid bilayer from degradation, OMV contents may persist longer in extracellular environments than exposed macromolecules released through other means.

OMV content is diverse, enabling these abiotic spheroids to have a broad range of activities. They serve as effectors, agents of genetic exchange, or shared resources and can be deployed as a defense against competing organisms or as building blocks for

biofilms (7, 21–24). The contributions of OMVs to host colonization and disease have been characterized (18, 25–27). In contrast, the roles of OMVs in beneficial partnerships have been far less studied.

OMVs distribute materials between organisms, circumventing the need for direct cell-cell contact. In aqueous environments, these nanoparticles may migrate from the OMV producer to surprisingly distant sites within a host or ecosystem (28). For example, orally administered OMVs from *Bacteroides thetaiotaomicron* were capable of passing though the gut mucosal barrier in order to reach underlying epithelia and macrophages (29). Additionally, *Salmonella enterica* residing within the *Salmonella*-containing vacuole (SCV) of epithelial cells produces OMVs that escape the SCV, as well as the infected host cell, and enter neighboring cells (30). Delivery to other Gram-negative bacteria seems fairly permissive; OMVs can directly attach and fuse to the outer membrane of recipient cells (31–34). After attachment, the luminal contents of the OMVs are delivered into the periplasmic space of the recipient. OMVs enter eukaryotic cells through a variety of mechanisms: direct membrane fusion (28, 34, 35), lipid rafts (36), receptor-mediated endocytosis (19, 37), and caveolin-mediated endocytosis (38–42). These modes of entry potentially constrain the functions of OMVs as public goods in interdomain contexts.

Here, we evaluate the role of OMVs in mediating interspecies communication, cooperation, and conflict. We examine the benefits of OMV production for both the bacterial producer (symbiont in host contexts) and neighboring cells. Microbial symbionts compete with other symbionts and experience antagonistic host responses, and OMVs may help to reduce these burdens in mutualistic relationships, enabling microbes to attain a net benefit from their association with the host (43). We review recent findings regarding the physiological and ecological benefits of OMVs as molecular couriers in cooperative interactions and environmental settings, and we draw attention to potential lines of research on the beneficial role of OMVs.

BEING A GOOD NEIGHBOR: BENEFITS PROVIDED BY OMVs

As OMVs can be utilized by neighboring cells, they might be considered public goods (44). For instance, OMVs may facilitate cross-feeding, enabling the maintenance of complex communities in nutritionally dilute environments despite patchy resource distribution. OMVs from the cyanobacterium *Prochlorococcus* contain labile carbon that can be used as the sole carbon source for the heterotrophs *Alteromonas* and *Halomonas* (7). Similarly, gut-residing members of the *Bacteroides* genus distribute hydrolases and polysaccharide lyases via OMVs (45, 46); by making these enzymes publicly available, the microbiome can communally break down complex polysaccharides that are immutable to individual species. The exact benefit to the producer of providing these services in each of these circumstances is unclear, but it is assumed to provide a net benefit, such as a higher survival rate (44). To test this, the fitness of the OMV-producing *Prochlorococcus* sp. should be compared when grown alone (monoculture) or in coculture with heterotrophs from pelagic ecosystems. Specifically, varying the ratios of *Prochlorococcus* to consumer may more clearly illuminate the sociality of these interactions by displaying the relative effects of direct and indirect relationships between organisms.

In contrast, scavenging scarce resources has clear direct benefits for the OMV producer, as well as other "cheating" organisms. Metal ions are one such resource that OMVs may concentrate for microbial communities. Proteomic studies of OMVs from diverse bacterial species have revealed a number of metal-ion-binding proteins (47). Recent work by Lin et al. (48) elucidated a mechanism that enables *Pseudomonas aeruginosa* to access metal-rich OMVs via a type VI secretion system (T6SS). TseF is secreted by the T6SS and associates with the *Pseudomonas* quinolone signal (PQS) as well as *P. aeruginosa* receptors OprF and FptA. PQS has high affinity for iron, contributing to the sequestration of this ion in OMVs, which can then be utilized by *P. aeruginosa* under iron-limiting conditions. Thus, these proteins provide a means of scavenging and concentrating freely diffusing ions, offering a rich nutrient source to

organisms able to process them. In this manner, OMVs can modulate ecosystem production and diversity within these niches.

OMVs influence interactions between members of surface-associated polymicrobial communities, as well as regulate the ability to colonize different habitats, provide structure for growth, and promote dispersal. OMVs stimulate bacterial aggregation on substrates by trafficking quorum-sensing molecules (24, 33, 49, 50). For example, *Porphyromonas gingivalis* OMVs promote the aggregation of other species frequently found in the oral cavity, including *Staphylococcus aureus*, *Streptococcus*, *Actinomyces* spp., and even the opportunistic eukaryotic pathogen *Candida albicans* (51). In addition to providing stimulatory cues, bacteria may secrete OMVs to enhance and reinforce the physical structure of polymicrobial biofilms (21, 22, 50). Conversely, OMVs may enable bacterial cells to transition from sessile to planktonic lifestyles, as in the case of *Xylella fastidiosa* OMVs that inhibit attachment to plant cells (23). Ionescu et al. hypothesize that this confers a potential dispersal benefit to bacteria. Dispersal has been proposed be a social trait, potentially by reducing competition with nondispersing relatives (52). By mediating intraspecies and interspecies social behaviors on surfaces, OMVs are key modulators within complex communities.

Finally, OMVs are likely to play a role in life history transitions in eukaryotes. As the closest living relatives of animals, a group of free-living bacterivorous microbial eukaryotes called choanoflagellates have served as a key point of comparison for understanding animal origins (53–55) and the influence of bacteria on eukaryotic biology. The choanoflagellate *Salpingoeca rosetta* switches from a unicellular swimmer to a multicelled rosette upon exposure to lipid-based compounds produced by the marine *Bacteroidetes* organism *Algoriphagus machipongonensis* (56, 58). The morphogenic cues are highly hydrophobic sulfonolipids and are nearly insoluble in seawater, yet *A. machipongonensis* OMVs contain these bioactive compounds and directly fuse with unicellular *S. rosetta* (R. Alegado, A. Woznica, S. Cao, C. Beemelmanns, H. Turano, J. Clardy, and N. King, unpublished data). It remains unclear what benefit, if any, *A. machipongonensis* receives from *S. rosetta* as a result of this interaction; however, these data suggest that the bioactive compounds triggering multicellular development in *S. rosetta* may be perceived through OMVs. Thus, OMVs may be a general system for the trafficking of hydrophobic bioactive molecules, such as lipids. Indeed, OMVs are one of the only means by which bioactive lipids can be trafficked. Larval settlement and metamorphosis in several basal metazoans have been shown to require uncharacterized factors released into the water column and produced by benthic biofilms (59–63), and OMVs may be the vehicle by which signals are carried. We anticipate that additional cases of OMVs exerting influence on animal biology will be discovered.

ERECTING GOOD FENCES: OMVs AS SELF-PROTECTION

OMV biogenesis is constitutive in growing cells, yet the assumed metabolic cost of producing diverse OMVs as public goods would diminish the benefits for producers (64). OMV-producing cells could limit cheaters within their ecological niche by specifically targeting competitors with destructive OMVs while directing beneficial goods to mutualistic partners, but the most likely scenario is that these goods are concurrently delivered to both friends and foes.

Notably, bactericidal OMVs can act against both Gram-positive and Gram-negative cells (31). Determining whether bacteria produce OMVs that are more effective against closely related competitors would implicate a role in niche competition (65). The composition of stress-induced OMVs indicates that they are specialized to compete for key resources. For example, *Myxococcus xanthus* OMVs have significantly higher concentrations of alkaline phosphatase and other lytic enzymes than the *M. xanthus* cell envelope, suggesting that these particles lyse cellular prey and scavenge their phosphate (66, 67).

Conversely, OMVs may also serve as a defense mechanism for producers. These abiotic particles act as decoys for phage by increasing the available membrane surface area for attachment, reducing their potential infectivity (7, 67). Infection by T4 phages

TABLE 1 Examples of OMV cargo with roles in intercellular interactions

Class	Compound(s) (bacterial producer[s])[a]	Recipient(s)	Reference(s)
Proteinaceous	Cytolethal distending toxin (*Campylobacter jejuni*)	Human small intestine epithelial cell lines	18
	Bacteroides fragilis toxin 2 (*B. fragilis*)	Human colon epithelial cell lines	104
	CFTR inhibitory factor/Cif (*Pseudomonas aeruginosa*)	Human airway epithelial cell lines	28
	OmpU (*Vibrio fischeri*)	*Euprymna scolopes* phagocytic immune cells?	11
	Glycoside hydrolases and polysaccharide lyases that degrade dietary polysaccharides (*Bacteroides thetaiotaomicron*, *Bacteroides ovatus*)	*Bacteroidales* common in human intestinal microbiota (e.g., *Bacteroides vulgatus*)	45, 46
	Ef-Tu (*Xanthomonas campestris*)	*Arabidopsis thaliana* leaves	103
Carbohydrate	Polysaccharide A (*B. fragilis*)	Murine dendritic cells	29
	Peptidoglycan (*Helicobacter pylori*)	Human gastric epithelial cells	105
Nucleic acid	eDNA (*Pseudomonas aeruginosa*)	*P. aeruginosa* biofilms	106
	Virulence-conferring DNA (*Escherichia coli* O157:H7)	*E. coli* JM109, *Salmonella enterica* serovar Enteritidis	107
	Antibiotic resistance genes (*Neisseria gonorrhoeae*)	Penicillin-sensitive *N. gonorrhoeae*	108
	Small RNAs (*Vibrio cholerae*)	Unknown	109
	Small RNAs (*P. aeruginosa*)	Human bronchial epithelial cells	20
Small molecule	C_{16}-homoserine lactone (*Paracoccus denitrificans*)	Self	33
	Pseudomonas quorum signal (*P. aeruginosa*)	Self	49
	Labile carbon (*Prochlorococcus marinus*)	*Halomonas*, *Alteromonas*	7
Lipid	Rosette inducing factor 1 (*Algoriphagus machipongonensis*)	*Salpingoeca rosetta*	Alegado et al., unpublished

[a]CFTR, cystic fibrosis transmembrane conductance regulator; eDNA, extracellular DNA.

was shown to increase OMV vesiculation in *Escherichia coli* (68), and phage PHM-2 has been shown to bind purified *Prochlorococcus* OMVs (7). Li et al. recently proposed that OMV-mediated phage defense may be a resilience mechanism by maintaining functional diversity of the host microbiota (24). However, the extent to which OMV decoys benefit species unrelated to the OMV-producing cell is an open question, as phage attachment is highly species specific. If it were the case that OMV phage decoys conferred a survival advantage to related bacterial species, these abiotic particles would serve as a mechanism of kin discrimination. Together, these findings suggest that bacteria produce OMVs with either positive or negative effects on closely related cells, emphasizing the complex role that OMVs play in interorganismal dynamics.

In the context of interbacterial competition and host monitoring, OMVs play key protective roles for resident microbes (69–71). Supplementing *E. coli* or *Pseudomonas syringae* cultures with OMVs or a hypervesiculating mutant protected cells from a number of antimicrobial peptides that interact with negatively charged phospholipids, including polymyxin B, colistin, and melittin (67, 72). There is evidence that OMVs sequester these compounds, decreasing the effective concentration to which recipient bacterial cells are exposed. Likewise, OMVs may be capable of specifically sequestering other antagonistic molecules, such as antibodies, thereby modulating host immunity against resident microbes. OMV secretion may enhance virulence *in vivo* (73). Some pathogens preferentially package virulence factors in their OMVs (73–75). Other pathogenic bacteria secrete more OMVs than their nonpathogenic congeners (3, 76), evidence that increased production increases individual competitive advantage.

REACHING A DÉTENTE: ROLE OF OMVs IN STABILIZING COOPERATIVE RELATIONSHIPS

The ubiquitous and versatile nature of OMVs suggests that OMVs could act at the interface of host and microbe. These particles mediate a variety of interorganismal interactions (Table 1), likely dependent on recipient cell type and OMV composition (37, 77). Furthermore, OMVs have been shown to influence a number of homeostatic processes in animals, including cell proliferation (78), apoptosis (79), and autophagy (80). Despite the physiological and evolutionary significance of mutualisms (1), there is

a dearth of research on the functions of OMVs in mutualistic symbioses involving multicellular organisms.

Bacteroides fragilis is a prominent member of the human intestinal microbiota and has various capsular polysaccharides that it differentially displays. Some of these polysaccharides, such as polysaccharide A (PSA), are zwitterionic molecules that can be released into the intestinal lumen. PSA is taken up by host cells and shifts the host's immune system to a less inflammatory and regulatory T-cell-mediated state, which may ameliorate inflammatory disorders of the host (81–83). Immunoeffective PSA was detected in *B. fragilis* OMVs, implicating them as an *in vivo* delivery mechanism (29). This finding highlights the potential of OMVs to positively influence host health and the need for further exploration of OMVs isolated from microbial communities from different host sites. To that end, OMVs from probiotics, such as the *E. coli* Nissle 1917 strain, have recently been surveyed in an attempt to scour their contents for beneficial molecules (84), and they have been found to contain factors, such as the kinase-altering protein TcpC, that can positively affect intestinal health (85, 86).

The symbiosis between the marine bacterium *Vibrio fischeri* and the Hawaiian bobtail squid *Euprymna scolopes* is mediated by compounds produced by both partners. In the juvenile squid light organ, planktonic bacteria first attach and aggregate before proceeding through a series of chemical challenges produced by the host, ultimately colonizing the lumen of the deeper crypts of the light organ for the remainder of the squid's life (87). The development of this symbiosis drives physiological responses from the squid, such as migration of blood cells and reshaping of light organ tissue to more closely resemble that of an adult (87). Recently, nonproteinaceous contents from *V. fischeri* OMVs were found sufficient to stimulate a subset of these developmental responses, despite a lack of compounds known to stimulate light organ development (88). Examining hyper- and hypovesiculating mutants, as well as nonvesiculators, is complicated by pleiotropic consequences on other pathways, but these mutants may still provide insight into how OMVs shape symbioses.

IMPLICATIONS OF OMVs AS MATERIAL GOODS AND MISSILES IN MICROBIAL INTERACTIONS

Experimental approaches for investigating functions of OMVs. OMVs are constitutively released, yet they vary with environment and can promote survival for the bacterial producer (13, 67). Since OMVs benefit the producer, yet can exert a variety of effects on recipient organisms across spatial and temporal scales, they may be critical shapers of the economies between organisms and may act as currency or poison. Development of multispecies models as well as specialized host models (e.g., *ex vivo* studies and immunodeficient hosts) where bacteria reside in a niche with fewer host interactions will be valuable for untangling these complications. Although such models will lack a wild-type microbiota, these approaches may provide microbe-centric data that inform more natural situations.

Mechanisms underlying OMV-driven processes are difficult to unravel, due in part to the potential for OMVs to simultaneously and distinctly impact the host and other members of the microbiota. Proteomics has been frequently used to assess the functional capacity of OMVs, but additional omics approaches, particularly lipidomics, may reveal novel information about the OMV-cell interface. Recent work has demonstrated that lipid remodeling may occur prior to OMV formation (12, 13). This information can be incorporated into interaction networks that may reveal whether specific lipids are sorted into OMVs with characteristic functions as the producing cell responds to changing conditions. Synthetic liposomes that retain the chemical properties and topology of native membranes can then be employed to test biochemical necessity and sufficiency of compounds in OMV-cell interactions. OMVs from a variety of bacteria have broad effects on animal immune responses (reviewed in reference 89). Native and modified OMVs have been implemented as vaccines against pathogens, such as *Salmonella enterica* serovar Typhimurium and *Vibrio cholerae*, in animal models (26, 90, 91) and have reached clinical usage against *Neisseria meningitidis* (92–94). Due to the

size of OMVs, visualizing them *in situ* has generally required electron microscopy of fixed material, but improvements in superresolution techniques hold promise for tracking OMVs, their contents, and their interactions in living environments.

Accounting for OMVs in ecological community dynamics. Gram-negative bacteria have the capacity to release prodigious amounts of OMVs, but a precise measure of how much material is being released into the extracellular milieu has not been undertaken. Recent studies demonstrating high levels of OMVs in marine environments have illuminated the global nature of these abiotic environmental factors (7). Biller et al. raised the question of how these findings may affect our calculation of nutrient budgets across the biosphere (7). With recently improved estimates of microbial abundance, we can use *V. fischeri* as a model to estimate the mass of certain elements locked into OMVs to approximate the global elemental impact of OMVs. A culture of *V. fischeri* isolate ES114 grown in rich lysogenic broth with salt (LBS) saturates around 10^9 CFU · ml^{-1} and produces approximately 10 pg of OMV protein · ml^{-1} of culture in 24 h, a rate of 10^{-17} g of protein · $cell^{-1}$ · 24 h^{-1}. Although the growth rate of *V. fischeri* is likely higher when grown *in vitro* than for bacteria in the marine environment, it enables the following back of the envelope calculation. With the estimated 9.2×10^{29} prokaryotic cells (combining *Bacteria* and *Archaea*) in Earth's oceans (95) and applying a conservative estimate of 10% Gram-negative bacteria, we estimate that roughly ~1,000,000 metric tons of protein will be released in the ocean via OMVs every day. Assuming these proteins contain equal ratios of each of the 20 major amino acids, every 24 h, Gram-negative bacteria in the ocean release approximately 3.92×10^{11} g of carbon, 1.24×10^{11} g of nitrogen, and 1.96×10^{10} g of sulfur in OMV proteins alone. While this is a very rough estimate, it is interesting to note that it loosely matches an estimate of *Prochlorococcus* vesicle production (7). Although this is a small fraction compared to the oceanic reservoir of 4×10^{19} g of carbon (96) and is an overestimation of total mass being packaged into OMVs globally, even a fraction of this amount of organic material could alter marine carbon budgets in oligotrophic environments. Furthermore, the inclusion of nucleic acids, small molecules, and lipids packaged into OMVs would increase these values and present a more a sizable contribution to global elemental stores. While these estimates are based on simplistic assumptions, they illustrate the sheer potential of OMVs as a nutritional sink, or, if these materials are presented in bioaccessible forms, a nutritional bounty for organisms that can metabolize them, especially in highly competitive environments with varied access to nutrients.

Considering that isolation methods for viruses also result in enrichment of OMVs, estimates of viral abundance may be conflated with OMV abundance (97). This is a legitimate concern, as vesicles contain various amounts of DNA derived from bacterial, eukaryotic, and viral sources (98), which adds complexity to calculations describing the global impact of either group. Thus, to understand the ecological impacts of OMVs, we need to elucidate the mechanisms by which these particles are consumed, recycled, or otherwise turned over to release their molecular contents to the environment.

CONCLUDING REMARKS

OMVs have the capacity to facilitate beneficial outcomes across symbioses. In particular, OMVs shape metazoan microbiotas and promote interactions between partnered organisms by mediating physical proximity, temporal stability, and access to privileged niches within the host. On evolutionary time scales, public goods provided by OMVs may select for cooperative behavior, driving the establishment of mutualisms. Finally, we note that the preponderance of research has examined OMVs in the context of animal symbioses; to date, only a few studies have focused on OMV-plant interactions (23, 99–103). Extending the role of OMVs in the biology of other multicellular lineages is a budding area for future investigation.

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