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Functional Advantages Conferred by Extracellular Prokaryotic Membrane Vesicles

Andrew J. Manning and **Meta J. Kuehn**
Department of Biochemistry, Duke University Medical Center, 221 Nanaline Duke Bldg., Box 3711 Biochemistry, Durham, NC 27710

Abstract

The absence of subcellular organelles is a characteristic typically used to distinguish prokaryotic from eukaryotic cells. But recent discoveries do not support this dogma. Over the past 50 years, researchers have begun to appreciate and characterize Gram-negative bacterial outer membrane derived vesicles and Gram-positive and archaeal membrane vesicles. These extracellular, membrane-bound organelles can perform a variety of functions, including binding and delivery of DNA, transport of virulence factors, protection of the cell from outer membrane targeting antimicrobials, and ridding the cell of toxic envelope proteins. Here we review the contributions of these extracellular organelles to prokaryotic physiology and compare these with the contributions of the bacterial interior membrane bound organelles responsible for harvesting light energy and for generating magnetic crystals of heavy metals. Understanding the roles of these multifunctional extracellular vesicle organelles as microbial tools will help us to better realize the diverse interactions that occur in our polymicrobial world.

Keywords

Outer Membrane Vesicles; Transformation; Virulence Factor Transmission; Antimicrobial Protection; Biofilm; Prokaryotic Envelope

Introduction

Adaptive evolution has generated a number of tools that bacteria can use for survival in what were once claimed inhospitable environmental niches. With the advent of acutely sensitive technologies, the scientific community has discovered that there is rarely a niche absent some sort of bacterial life. Until recently, scientists assumed that as single celled organisms, bacteria live a selfish and lonely lifestyle, acting solely on behalf of their own survival. Now, altruistic characteristics of bacterial life have been observed, and, in some cases, altruistic behavior was found to be necessary for survival of an entire, complex bacterial community (Kreft, 2004). In order to function altruistically, there must also be a method by which bacteria sense their exterior world and can communicate with each other. This has been described as quorum sensing, a method of communication through small molecule signaling resulting in a remarkably sensitive system allowing bacteria to be aware of the

meta.kuehn@duke.edu, 919-684-2545 Phone, 919-684-8885 FAX.

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composition of their surroundings (Waters and Bassler, 2005). Therefore, we should consider that bacteria have adapted and evolved to survive in a constantly changing and complex environment by generating an arsenal of extracellular “tools”, and that such tools could have either selfish uses, or could impact a greater community. Additionally, a single tool could be developed to have multiple uses. In this review, we will discuss bacterial extracellular vesicles as a tool with multiple functions, and we will compare and contrast these with intracellular bacterial organelles.

Vesicle origins and production

Outer membrane vesicles (OMVs) were originally described as constitutively-produced membranous spheres generated from Gram-negative bacteria (Knox et al., 1966). OMVs have been found to be ~10–300 nm in diameter and composed of outer membrane lipids and proteins as well as soluble, lumenal content from the periplasm (Beveridge, 1999; Kulp and Kuehn, 2010). Proteomic studies frequently identify some cytoplasmic proteins as well as inner membrane proteins associated with OMVs (Ferrari et al., 2006; Lee et al., 2008), although it remains unclear whether these are true OMV components or contamination of the preparations.

Recently, it has been appreciated that membrane vesicles (MVs) are also produced by the other prokaryotes, Gram-positive bacteria and archaea. Gram-positive MVs have recently been characterized from *Bacillus subtilis* and *Staphylococcus aureus* (Gurung et al., 2011; Rivera et al., 2010). Gram-positive MVs are cytoplasmic membrane-derived, ~50–150 nm in diameter, and proteomics studies show they are composed of mostly cytosolic protein, cytoplasmic membrane-associated protein, as well as some secreted protein (Gurung et al., 2011).

The cell wall of Archaea differs substantially from that of bacteria, typically consisting of an S-layer of peptide or glycopeptides surrounding the bacteria. Regardless of the difference in composition, archaeal species have been discovered to constitutively produce MVs which are approximately ~90–230 nm in diameter. Electron tomography studies of *Sulfolobus* have shown MVs budding from the membrane of the bacterium (Ellen et al., 2009). Electron micrographic studies have shown that *Thermococcales* MVs are ~50–150 nm in diameter (Soler et al., 2008). For *Ignicoccus*, MVs have been described to exist in the periplasm and possibly transport key factors between the cytoplasmic membrane and the outer layer, which in *Ignicoccus,* is actually not an S-layer (Rachel et al., 2002).

For Gram-negative OMVs, several models of biogenesis have been hypothesized based on architectural features of the envelope and vesiculation mutants (Beveridge, 1999; Deatherage et al., 2009; Kulp and Kuehn, 2010). In-depth genetic studies are underway to reveal other biosynthetic pathways critical to OMV production (Kulp, Schmid and Kuehn, unpublished data). Evidence that OMV production is altered in response to both intracellular and extracellular stimuli suggests that there are multiple regulatory pathways, and possibly multiple mechanisms, that serve to induce or reduce OMV production (Baumgarten et al., 2012; Baumgarten et al., 2011; Grenier et al., 1995; Manning and Kuehn, 2011; Mashburn-Warren et al., 2008; McBroom and Kuehn, 2007)(Fig 1). In contrast to OMVs, the

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mechanics and regulation of MV production by Gram-positive bacteria have not yet been addressed in a systematic way. Since other reviews have emphasized the understanding of vesiculation mechanisms, this review will focus on the shared and unique functional characteristics of prokaryotic OMVs and MVs (Table 1).

Vesicle-mediated genetic transformation

The acquisition of foreign DNA is one of the major ways by which bacteria can quickly adapt to a new environment. Shared genetic material can confer survival mechanisms such as antibiotic resistance enzymes, colonization structures such as pili, or toxic features, such as proteases and toxins (Baquero, 2009). Natural competency and conjugation by the type IV secretion system are the best studied mechanisms governing bacterial DNA transfer (Toussaint and Chandler, 2012). However, as discussed below, several groups have determined that OMVs and MVs can carry DNA and transfer the DNA from the vesicle to the host cell via what is assumed to be a fusion reaction.

Vesicle-associated DNA has been found to be both bound to the OMV surface and packaged inside the vesicles. Surface associated DNA may be bound via electrostatic interactions with LPS (Dorward and Garon, 1990). In several cases, DNA was determined to be on the interior of the OMVs/MVs using DNAse protection assays, however the mechanism by which DNA is packaged as vesicle cargo remains unknown (Dorward and Garon, 1989; Dorward et al., 1989; Soler et al., 2011). From a study of OMVs purified from many different Gram-negative and Gram-positive species, Dorward et al concluded that only Gram-negatives produce OMVs with lumenal DNA (Dorward and Garon, 1990). For *Haemophilus influenzae*, OMV-associated DNA is associated with its competence state (Concino and Goodgal, 1982).

Yaron et al, found *E.coli* O157:H7 OMVs carried both chromosomal and plasmid DNA, and co-incubation of the OMVs with various hosts, including *E. coli* JM109 and *Salmonella* serovar *Enteritidis*, resulted in their transformation (Yaron et al., 2000). OMV-mediated transformation is assumed to be through the fusion and consequent transfer of the DNA from the vesicle lumen to the host cell. Interestingly, *Pseudomonas aeruginosa* OMVs were found to contain DNase-resistant plasmid DNA in their lumen as well as have the ability to take up exogenous DNA, however, despite evidence of OMV-cell fusion, these OMVs were not able to transform bacteria (Renelli et al., 2004).

In studies of the archaeon *Thermococcales*, DNA was also found to be associated with MVs (Soler et al., 2011; Soler et al., 2008). A subsequent study demonstrated that DNA contained within the MVs produced by one strain of *T. kodakaraensis* can transform other strains of *T. kodakaraensis* with a shuttle plasmid (Gaudin et al., 2012). This is the first evidence of a MV-mediated mechanism for lateral gene transfer in archaea. Since the DNA within the *Thermococcales* MVs is protected from thermodenaturation, it was suggested that this mode of transfer may be a very stable manner to transport DNA in extreme temperatures (Soler et al., 2008).

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Virulence factor transport

Virulence factors play key roles in the relationship between pathogens and host organisms by enabling bacteria to colonize and survive in a host and thereby cause infection. Although many of these factors are typically secreted solubly by one of several bacterial secretion systems (Gerlach and Hensel, 2007; Hayes et al., 2010; Henderson et al., 2004; Jani and Cotter, 2010; Johnson et al., 2006; Juhas et al., 2008; Records, 2011; Wallden et al., 2010), a number of important toxins and host-interacting factors are now known to be associated with vesicles and use vesicles as a mechanism of entry into host cells. As complexes that contain adhesive and proinflammatory components, OMVs have been shown to impact both the entry mechanism and the host response to the virulence factor. Since these topics have been recently reviewed in the literature (Ellis and Kuehn, 2010; Macdonald and Kuehn, 2012) they will be only briefly summarized here.

Vesicle-mediated virulence factor association and delivery have been described for both important Gram-positive and Gram-negative human pathogens, including enterotoxigenic *Escherichia coli* (ETEC), *P. aeruginosa, Helicobacter pylori, Aggregatibacter actinomycetemcomitans, Vibrio cholerae, N. meningitidis, S. aureus* and *Bacillus anthracis* (Chatterjee and Chaudhuri, 2011; Chutkan and Kuehn, 2011; Gurung et al., 2011; Kadurugamuwa and Beveridge, 1995; Mullaney e t al., 2009; Ricci et al., 2005; Rivera et al., 2010; Rompikuntal et al., 2012; van der Ley and van den Dobbelsteen, 2011). Like DNA, virulence factors may be internally packaged as soluble cargo or externally associated to the vesicles. OMV association and activity of heat-labile enterotoxin (LT) of ETEC has been well-studied. LT binds specifically to a portion of LPS which is a highly abundant component of the OMV surface, and this interaction impacts both the toxicity and proinflammatory response of epithelial cells to LT (Chutkan and Kuehn, 2011; Horstman and Kuehn, 2000, 2002; Kesty et al., 2004; Mudrak et al., 2009). Proteomics and animal studies suggest that the MVs produced by *S. aureus* MVs contain toxic lumenal proteins (Gurung et al., 2011). These MVs serve as a delivery mechanism that bind cholesterol rich domains of eukaryotic membranes and deliver lumenal proteins that cause cell death. *B. anthracis* has also been shown to produce MVs that contain biologically active anthrax toxin (Rivera et al., 2010).

An important facet of OMV physiology is their immunoreactivity. Vesicles trigger proinflammatory immune responses which are likely to impact pathogenesis (Ellis and Kuehn, 2010; Shah et al., 2012). Due to the fact that OMVs carry virulence factors as well as common cell surface antigens and natural adjuvant (endotoxin), they are also effective as protective vaccines (Collins, 2011; Ellis and Kuehn, 2010; Moshiri et al., 2012). Engineering of OMV content to include protective antigens and reduce endotoxicity has made them potentially useful and versatile vaccine vectors (Chen et al., 2010; Kesty and Kuehn, 2004; Muralinath et al., 2010; Roier et al., 2012).

Protection from internal stress

Upon stressful environmental conditions such a s heat shock or treatment with denaturants, the periplasmic space of Gram-negative bacteria can become filled with misfolded envelope

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proteins. Typically, proteases assist in degrading misfolded protein, and over-expressed proteins that become insoluble are sometimes sequestered into periplasmic inclusion bodies (Carrio and Villaverde, 2002). However, in cases where these mechanisms are insufficient or are not functioning properly, export of accumulated protein via outer membrane vesiculation appears to be an alternative (McBroom and Kuehn, 2007; Tashiro et al., 2009). As this space is confined by the crosslinking of the envelope, the accumulation of misfolded protein might cause physical stress that could generate an outward force on the outer membrane, or signaling by accumulated protein could induce vesiculation (Kulp and Kuehn, 2010)(Fig 1). OMV production as a relief for envelope stress has only been studied in Gram-negative bacteria, however, the principle may also be applicable to archaea since they also possess a double membrane barrier, and potentially also to cytosolic stress relief for Gram-positive bacteria.

The link between OMVs and envelope stress was first observed by McBroom et al in 2006. In a screen for random *E. coli* transposon mutants with vesiculation phenotypes, σ^E envelope stress response pathway genes were enriched (McBroom et al., 2006). Follow-up studies (McBroom and Kuehn, 2007) demonstrated that the absence of the major σ^E-regulated periplasmic chaperone/protease DegP caused increased OMV production. Vesiculation could also be induced by overproduction of σ^E-activating polypeptides, which was found to be enriched cargo in the resulting OMVs. Furthermore, overexpressed non–σ^E-activating proteins were also inducers of OMV production, but these were un-enriched as OMV cargo. Finally, it was noted that hyper-vesiculating strains survived better upon treatment with denaturant and upon expression of a toxic envelope protein, suggesting that OMV production is an effective release for toxic envelope products. These data suggest that OMVs can be loaded with specific cargo that is misfolded and/or in high abundance in the periplasmic space, and that vesiculation can be a regulated response to toxic stress caused by the misfolded or overabundant envelope protein.

Consistent with these results, a more recent study demonstrated that the absence of the major periplasmic chaperone/protease, MucD, in *P. aeruginosa* (which corresponds to DegP of *E. coli*), caused increased vesiculation, and that the OMVs had increased protein concentration compared to WT OMVs (Tashiro et al., 2009). Furthermore, induced expression of MucD resulted in a reduction of OMV production, in effect because the periplasm was “clean” of misfolded protein (Tashiro et al., 2009). This reduction in OMV production was consistent with results using low levels of DegP overexpression in *E. coli* (McBroom and Kuehn, 2007). As these phenotypes were independent of the production of *Pseudomonas* quinolone signal (PQS), which was previously suggested as the molecule governing OMV production in *P. aeruginosa* (Mashburn-Warren et al., 2008), it suggests that there are multiple pathways to regulate OMV production in this species.

Decoys for antimicrobial attack

As the components that comprise the bacterial outer membrane are generally the same as those in OMVs, it stands to reason that these extracellular blebs interact with outer membrane-directed factors in the extracellular milieu. Early studies implicated a plausible role for OMV production in response to drugs and the adsorptive capability of OMVs. In

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1991, a study demonstrated that OMVs could adsorb antibacterial components of the human serum (Grenier and Belanger, 1991). Later, a study of the effects of chlorhexidine on the Gram-negative *Porphyromonas gingivalis* showed OMVs could protect the bacteria by adsorption of the compound (Grenier et al., 1995). As described below, two recent studies have followed up the idea of OMVs as cellular decoys (Baumgarten et al., 2012; Manning and Kuehn, 2011). MVs have not yet been identified to have antimicrobial defense capacities.

Baumgarten et al found that treatments of *Pseudomonas putida* with environmental stressors such as long chain alcohols, metal chelators, osmotic shock, and heat shock all resulted in an increase of vesiculation and the production of OMVs with differential protein cargo that was dependent on treatment (Baumgarten et al., 2012). Similarly, sub-inhibitory concentrations of polymyxin B, an outer-membrane acting antimicrobial peptide (AMP), induced vesiculation in both laboratory and pathogenic strains of *E. coli* with no significant increase in cell death or release of periplasmic markers (Manning and Kuehn, 2011). Both polymyxin B and the chemically related polymyxin E, could be adsorbed by OMVs (Manning and Kuehn, 2011), and OMV-bound AMPs were completely inactivated (Manning and Kuehn, unpublished data). This data supported the theory that OMVs not only act to adsorb detrimental environmental factors but are also induced in response these same factors. In these cases, the stresses targeted the outer membrane. Antibiotics that act internally were not observed to stimulate vesiculation significantly (Manning and Kuehn, 2011).

Bacteriophage represent another ubiquitous antimicrobial agent found in nearly all environmental niches, and phage, like AMPs, bind to the outer membrane. Therefore, it was hypothesized that OMV “decoys” would also protect bacteria from phage infection (Manning and Kuehn, 2011). Lytic phage typically have two stages of host cell identification, a primary transient interaction that originally identifies a host typically by LPS interactions, and a secondary irreversible interaction that occurs once a host factor is recognized on the bacterial cell surface (Miller et al., 2003). Phage lethality results from their ability to gain entry into the bacterial cytoplasm and take control of the replication machinery. Bacteria have developed numerous phage resistance mechanisms in an ongoing arms race between the two organisms. These include outer membrane modification of either the LPS or other surface factor, as well as an interior mechanism using the CRISPR system which generates immunity to the DNA of invading phage (Barrangou and Horvath, 2012). OMV production can now be added to this list of resistance mechanisms. *E. coli* phage T4 and *E. coli*-derived OMV were found to interact, resulting in an irreversible loss of phage potency (Manning and Kuehn, 2011).Together, these studies support the concept that that OMVs can act as regulated decoys to protect Gram-negative bacteria from antimicrobials directed at their outer membrane.

Cross-species interactions

The typical laboratory scenario of a single bacterial species living in isolation in a particular environment is convenient for studying individual bacterial species, however, this is not the typical scenario in real world environmental niches. The studies discussed below demonstrate the impact of OMVs from one species on another, often showing that OMVs

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can be bacteriolytic. The results suggest that OMVs could benefit bacterial survival by acting as scavengers to access, bind, transport, and ultimately deliver nutrients by a fusion mechanism to recipient bacteria.

In a broad-spectrum survey of OMV activity, OMVs produced by a diverse set of Gram-negative strains and species were found to be bactericidal for a variety of Gram-negative and Gram-positive bacteria (Li et al., 1998). The lethal activity was proposed to be due to digestion of the cell wall by OMV-associated autolysins delivered upon contact with the recipient cell (Kadurugamuwa and Beveridge, 1996; Li et al., 1996). This theory was supported by biochemical determination of autolysin in the OMVs, and electron micrograph evidence for both fusion of OMVs with cells and the appearance of cell envelope damage at the point of contact (for Gram-positive cells) or fusion (for Gram-negative cells.)

Lysobacter spXL1 is known for secreting multiple bacteriolytic enzymes (L1–L5) into the extracellular space, and it was found that at least one of its bacteriolytic enzymes is secreted by OMVs and that OMV association affected its activity (Vasilyeva et al., 2008). Treatment with soluble L5 endopeptidase had little effect on the Gram-negative species *Erwinia marcescens*, whereas L5 enclosed within OMVs caused lysis. Gram-positive bacteria also lysed when treated with OMVs containing L5. This study suggests that there is a synergistic or fusion reaction that results in the delivery of the endopeptidase to its site of action. As discussed earlier, other studies have shown the capability of OMVs to transfer DNA between bacteria, thus their ability to transfer other molecules is reasonable.

Another example of an OMV-associated extracellular prot ease is one that is localized to the surface of OMVs produced by *Pseudomonas fragi*. The presence of the protease was previously found to correlate with the appearance of membranous blebs on the exterior of *P. fragi* (Thompson et al., 1985). Thompson et al used immunoelectron microscopy to identify the location of this protease, and they concluded that the protease was indeed excreted and attached to the outer membrane at sites of vesiculation (Thompson et al., 1985).

Although archaea are not known to be pathogenic, toxic proteins associated with archaeal MVs are proposed to help them attain a growth advantage in an environmental niche (Prangishvili et al., 2000). *Sulfolobus acidocaldarius* have been shown to produce antimicrobial proteins called sulfolobicins that are similar to the bacteriocins. Typically, bacteriocins are secreted directly in to the extracellular milieu, however, sulfolobicins are associated with the MVs produced by *S. acidocaldarius* (Prangishvili et al., 2000).

Community living

Many bacteria form a protective, multi-cellular form known as the biofilm, and several studies have implicated roles for OMVs in these structures. Biofilms are a very important stage of the bacterial lifecycle for both pathogens and environmental species, and the development and maintenance of bacterial biofilms have been the subject of increasing numbers of investigations in recent years, particularly because they promote antibiotic resistance. For instance, the ability of *P. aeruginosa* to maintain an infection in the lungs of cystic fibrosis patients has been discovered to require the generation of a biofilm (Govan and Deretic, 1996; Schooling et al., 2009; Yu et al., 2012). Bacterial biofilms generally

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consist of tightly packed bacteria within a n extracellular matrix (ECM) produced by the cells which consists of sugars and proteins, resulting in an aggregate that is difficult to penetrate and disrupt. Nutrients must be shared within the biofilm so that the internal bacteria that do not have access to nutrients do not starve.

A recent study has shown that low OMV producing strains have a reduced ability to generate robust biofilms (Baumgarten et al., 2012). Although this suggests that OMVs could play a significant role in the formation and maintenance of the bacterial biofilm, relatively few studies have been able to elucidate their exact role. OMVs could provide several functions to a biofilm: as a nutrient delivery mechanism or as an adherent substance that contributes to the ECM.

Studies by Schooling et al showed that OMVs within biofilms of *P. aeruginosa* interact with DNA, a common component of the ECM (Schooling and Beveridge, 2006; Schooling et al., 2009). Whereas DNA is typically found in the lumen of OMVs produced by planktonically grown cells, for biofilm-derived OMVs, it is found on their exterior. It was further elucidated that association with OMVs changed the chemical properties of the DNA, suggesting that the interaction between the DNA and OMVs generated an even more stable construct than either alone. They concluded that the OMVs play a structural role in the generation of the ECM and help stabilize the biofilm.

Yonezawa et al, studying *Helicobater pylori*, found that OMVs were a major component of the biofilm formed by strain TK1402 (Yonezawa et al., 2009). Using biofilm thickness as an output for the robustness of biofilms, the study identified that, under conditions that induce vesiculation, biofilm formation is increased. When the media was altered to reduce vesiculation, biofilm formation also reduced, and when OMV fractions were used to supplement these poor biofilm conditions, biofilms became much more robust. These results strongly implicate a role for OMVs in biofilm development and stability.

Internal bacterial membrane-bound organelles

Despite typical dogma, interior membrane-bound compartments do exist in bacteria, although these interior vesicles are used for very specific purposes and are uncommon amongst laboratory bacteria. In general, such prokaryotic organelles can be categorized into two major classes based on the composition of their enclosing membrane. In the first class, the organelles are separated from the cytosol by a lipid monolayer or protein shell and will not be discussed further here. The second class is characterized by the lipid bilayer surrounding the organelle. Two general types of these interior membrane bound organelles are called magnetosomes and photosynthetic membranes, and we found it interesting that they share some properties with extracellular vesicles.

Magnetosomes are lipid bilayer-enclosed intracellular compartments that house crystals of the magnetic mineral magnetite (Fe_3O_4) or greigite (Fe_3S_4) (Komeili, 2011; Murat et al., 2010). These prokaryotic compartments are found in magnetic bacteria that constitute a phylogenetically diverse group of bacteria that use geomagnetic field lines to find their preferred redox conditions (Komeili, 2011; Murat et al., 2010). Recent work in proteomics, genomics, and cryotomography of the magnetosome have elucidated a specialized protein

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sorting pathway for proteins involved in their generation, as well as the genes responsible for the generation and maintenance of the magnetosome (Komeili, 2011). Electron tomographic studies have recently shown that the lipid bilayer, which is derived from the inner membrane of the bacteria, is not detached, thus creating a nearly complete membranous sphere enclosing the magnetite that maintains its connection with the inner membrane (Murat et al., 2010). This arrangement, along with further protein scaffolding, results in the linear formation of multiple magnetosomes, despite the attraction of the magnetite crystals to each other. Like OMVs, these membrane-bound organelles provide multiple services to the bacterium. Magnetosomes not only orient the bacteria, but also serve as a source of biomineralization of the iron in the environment that could be used in future times of iron starvation (Komeili, 2011; Murat et al., 2010).

The photosynthetic organelles responsible for deriving energy from light are perhaps the most studied prokaryotic membrane-bound intracellular compartments. They fall into three distinct categories, classified by the characteristics of each, as well as the bacteria in which they are found. Purple photosynthetic bacteria have chromatophores contained within the intracytoplasmic membrane, cyanobacteria have thykaloid membrane compartments, and green photosynthetic bacteria have chlorosome compartments. All three of these types of photosynthetic membranes act to generate energy most efficiently, by increasing the available light-exposed surface area, as well as by increasing the number of protein complexes available to complete the necessary reaction, all while providing an ideal environment within the cell for the reaction to occur. These different types of photosynthetic membrane differ in the manner in which they are formed and maintained.

Some of these prokaryotic intracellular organelle functional characteristics, light absorption and biomineralization, have been attributed to OMVs as well. In a recent study by Song et al, *V. cholerae* OMVs were reported t o adsorb some of the detrimental effects of UV light on bacteria (Song and Wai, 2009). In some extremophilic bacteria that live in harsh environments, biomineralization has been attributed to their OMVs (Matlakowska and Sklodowska, 2009). It was shown that OMVs were on the surface of black shale and it was proposed that they contributed to the bioweathering of black shale by bacteria (Matlakowska et al., 2012). These OMVs had the capability to adsorb P, Mg, Si, Al, and Ca from the black shale and were a major component of the bacterial biofilm generated on the surface of the black shale.

Conclusions

Bacteria and archaea have evolved to survive in a variety of harsh conditions and therefore have the ability to manage widely diverse environmental stressors. They have developed a “toolbox” to quickly respond to stress, displaying a remarkable capacity to adapt to a variety of different niches using both biochemical and genetic means. In this review, we have covered the many roles of the bacterial OMVs as well as of the less studied Gram-positive and archaeal MVs (Table 1). It has been over 50 years since OMVs were discovered, but their contributions to prokaryotic physiology continue to be discovered. Their wide capabilities converge on a rather simple theme: a membranous bleb produced from the outer surface can be a multifunctional tool for the cell. This adaptive tool may have initially

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evolved for a singular purpose, which may be why it remains a constitutive process observed for an enormous variety prokaryotes. However, it has also developed, over time, to provide a variety of specific beneficial functions. Here, we focused on how vesicles benefit prokaryotic life in native, complex environments. We also have found that we can learn functional parallels from characterization of the structurally related intracellular vesicle organelles of prokaryotes. As technologies develop and we better understand polymicrobial interactions, even more roles for prokaryotic vesicles are likely to be uncovered.

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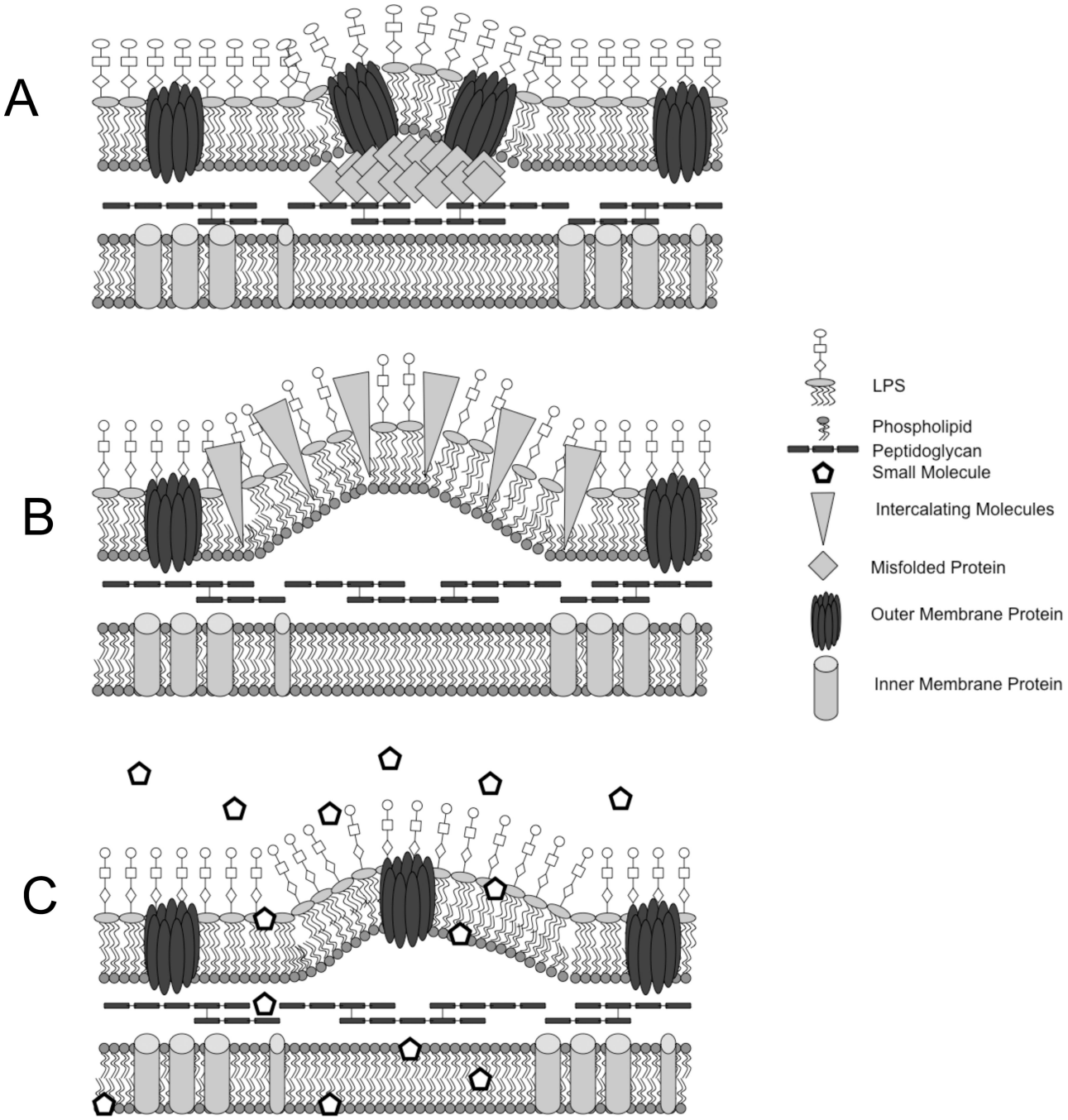

Figure 1. Possible Mechanisms of OMV Induction

Three possible models to explain how OMVs form in response to different stimuli. **A**) The accumulation of misfolded or overexpressed protein in the periplasm may result in a physical force on the outer membrane, causing the envelope to separate, the outer membrane to bulge, and eventually lead to the release of the OMV. **B**) External agents that act at the outer membrane could intercalate into the outer membrane, and disturb the packing of the outermost membrane. This disruption may lead to a change in the curvature of the membrane generating a bulge that eventually could bud off completely. **C**) A naturally

secreted small molecule which can pass through the cell envelope could result in a change in the regulation of genes, causing an increase or decrease in levels of OMVs.

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Table 1

Summary of OMV and MV functions for Gram-negative bacteria, Gram-positive bacteria, and archaea.

Function	Species	Reference
Transport DNA/Transform	*P. aeruginosa*	(Renelli et al., 2004)
	N. gonorrhoeae	(Dorward et al., 1989)
	B. burgdorferi	(Dorward and Garon, 1989)
	A. tumefaciens	(Dorward and Garon, 1990)
	E. coli	(Dorward and Garon, 1990)
	H. influenzae	(Dorward and Garon, 1990)
	M. osloensis	(Dorward and Garon, 1990)
	S. typhimurium	(Dorward and Garon, 1990)
	S. marscens	(Dorward and Garon, 1990)
	S. dysenteriae	(Dorward and Garon, 1990)
	S. flexneri	(Dorward and Garon, 1990)
	Y. pestis	(Dorward and Garon, 1990)
	Thermococcales	(Soler et al., 2011; Soler et al., 2008)
	T. kodakaraensis	(Gaudin et al., 2012)
Transport of Toxic Factor to Eukaryote	*P. aeruginosa*	(Kadurugamuwa and Beveridge, 1997)
	A. actinomycetemcomitans	(Rompikuntal et al., 2012)
	V. cholerae	(Chatterjee and Chaudhuri, 2011)
	N. meningitidis	(van der Ley and van den Dobbelsteen, 2011) (Gurung et al., 2011)
	S. aureus	(Rivera et al., 2010)
	B. anthracis	
Respond to Envelope Stress	*E. coli*	(McBroom et al., 2006; McBroom and Kuehn, 2007)
	S. typhimurium	(McBroom and Kuehn, 2007)
	P. aeruginosa	(Tashiro et al., 2009)
Protect Against Antimicrobial Agent	*E. coli*	(Manning and Kuehn, 2011)
	P. gingivalis	(Grenier et al., 1995)
	P. aeruginosa	(Kadurugamuwa et al., 1993)
	P. putida	(Baumgarten et al., 2011)
Transport of Toxic Factor to Prokaryote	*P. aeruginosa*	(Kadurugamuwa and Beveridge, 1996; Li et al., 1996, 1998)
	S. acidocaldarius	(Prangishvili et al., 2000)
	E. coli	(Li et al., 1998)
	S. pullorum	(Li et al., 1998)
	S. arizonae	(Li et al., 1998)
	S. cholerae-suis	(Li et al., 1998)
	E. agglomerans	(Li et al., 1998)
	P. vulgaris	(Li et al., 1998)
	S. marcescens	(Li et al., 1998)
	K. pneumoniae	(Li et al., 1998)
	S. flexneri	(Li et al., 1998)
	C. freundii	(Li et al., 1998)
	M. morganii	(Li et al., 1998)
	P. trifolii	(Li et al., 1998)
	Lysobacter spXL1	(Li et al., 1998)
	P. fragi	(Vasilyeva et al., 2008)
		(Thompson et al., 1985)
Produce/Maintain Biofilm	*P. aeruginosa*	(Schooling and Beveridge, 2006; Schooling et al., 2009)
	H. pylori	(Yonezawa et al., 2009; Yonezawa et al., 2011)
Other Functions:	Mixed Culture	(Matlakowska et al., 2012)
Biomineralization	*V. cholerae*	(Song and Wai, 2009)
Adsorb UV	*X. nematophilus*	(Khandelwal and Banerjee-Bhatnagar, 2003)
Insecticidal Activity	*S. putrefaciens*	(Gorby et al., 2008)
Redox-Reactivity		