Research in Microbiology 163 (2012) 607–618

www.elsevier.com/locate/resmic

Offense and defense: microbial membrane vesicles play both ways

Ian A. MacDonald [a], Meta J. Kuehn [b,*]

[a] Department of Molecular Genetics and Microbiology, 221 Nanaline Duke, Box 3711 Biochemistry, Duke University Medical Center, Durham, NC 27710, USA
[b] Department of Biochemistry, 220A Nanaline Duke, Box 3711 Biochemistry, Duke University Medical Center, Durham, NC 27710, USA

Received 24 August 2012; accepted 24 October 2012
Available online 30 October 2012

Abstract

Microbes have evolved over millennia to become adapted and specialized to the environments that they occupy. These environments may include water or soil, extreme environments such as hydrothermal vents, and can even include a host organism. To become adapted to these locations, microbes have evolved specific tools to mediate interactions with the environment. One such tool that prokaryotes have evolved includes the production of membrane vesicles (MVs). MVs are 10–300 nm spherical blebs derived from the outermost membrane and have known functions in protein secretion, immune activation and suppression, stress response, attachment, internalization and virulence. In this review, we consider the highly conserved role of membrane vesicles derived from Gram-negative, Gram-positive and archaeal species as a mechanism to facilitate intermicrobial and microbe-host interaction. We examine both the offensive and defensive capabilities of MVs in regard to the interaction of MVs with both host and microbial cells in their environment.

Keywords: OMV; MV; Bacterial pathogenesis; Archaea; Biofilm; Antimicrobial resistance; Envelope stress response

1. Introduction

Microbial life has evolved over the millennia to survive and thrive in a wide variety of environments across the globe. Many diverse species live in a temperate environment, with some, like bacterial pathogens, specialized to colonize host organisms. Other organisms, like archaea, occupy the extreme environments of the world. Regardless of the location, microbes have needed to evolve tools to cope with and grow within a changing environment. One such tool that has evolved to facilitate microbe–microbe, microbe–host and microbe–environment interactions is the production of membrane vesicles (MVs). MVs are typically 10–300 nm spherical blebs that originate from the outermost cell membrane, although their size range can vary depending on the species. MVs are distinct from membranous blebs produced during cell lysis as they are produced as a regulated, selective secretion event (Ferrari et al., 2006; McBroom et al., 2006; Mug-Opstelten and Witholt, 1978; Zhou et al., 1998). The MV secretion mechanism functions to disseminate virulence factors including toxins and degradative enzymes into the extracellular milieu. Once released, MVs have been demonstrated to function offensively as a virulence factor delivery mechanism, as well as defensively, to aid in the colonization of a host and the survival of an organism in a hostile environment. This review will highlight the evolutionarily conserved nature of MVs derived from Gram-negative, Gram-positive and archaeal species and present how membrane vesicles facilitate microbial interactions with cells and factors in the environment.

2. Microbial membrane vesicle formation

2.1. Background and history

For nearly five decades, the study of MV formation by prokaryotes has focused on Gram-negative bacteria. Since the

* Corresponding author. Tel.: +1 919 684 2545; fax: +1 919 684 8885.
E-mail addresses: Ian.macdonald@duke.edu (I.A. MacDonald), Meta.kuehn@duke.edu (M.J. Kuehn).

0923-2508/$ - see front matter
http://dx.doi.org/10.1016/j.resmic.2012.10.020

1960's, observations have been made regarding spherical membrane blebs present in electron micrograph images of Gram-negative bacteria (Birdsell and Cota-Robles, 1967; Knox et al., 1966). The content, composition and purpose of these structures were largely unknown. It was often assumed that the observed membranous structures were cell debris fragments caused by lysis. Since that time, the production and presence of MVs have been ubiquitously identified for a variety of Gram-negative species, including environmental, laboratory, as well as clinical and pathogenic isolates (Ellis and Kuehn, 2010; Kuehn and Kesty, 2005; Unal et al., 2011).

More recently, attention has turned to determining if MV production is an evolutionarily conserved process that also occurs for Gram-positive and archaeal species. Gram-positive bacteria and archaea notably lack a second lipid bilayer which was the site of MV production in Gram-negative bacteria. Despite the obvious physical differences in cell wall and membrane structure, spherical membrane blebs have been observed in the culture supernatant of Gram-positive bacteria and archaea, (Deatherage and Cookson, 2012; Ellen et al., 2010; Lee et al., 2009; Rivera et al., 2010; Soler et al., 2008).

Because the motif of secretion of the outermost membrane is evolutionarily conserved, MVs are expected to hold great importance for prokaryotic survival. By examining the mechanics of MV formation and regulation and the roles of MVs in intracellular interactions, we hope to gain insight into how MVs benefit the microbes. This review will serve to briefly highlight conserved aspects of MV production and focus more closely on their role as molecular tools to perform offensive and defensive tasks for a variety of prokaryotic microbes.

2.2. Gram-negative OMVs

MVs produced by Gram-negative bacteria are typically 10–300 nm spherical blebs derived from the outer membrane (OM) and therefore are termed "OMVs." The Gram-negative bacterial OM is distinct from the inner, cytoplasmic membrane (IM) and is separated from the IM by the periplasmic space and a thin layer of peptidoglycan (PG). OM is composed of phospholipids and endotoxin (or lipopolysaccharide, LPS) as the lipid constituent of the inner and outer leaflets, respectively, in addition to integral OM proteins (OMPs) and lipid-anchored lipoproteins. As derivatives of the OM, OMVs contain these OM components as well as soluble content inside and attached to their outer surface of the vesicles. The contents of the vesicle lumen are derived from the periplasm between the IM and OM. Additionally, proteins and macromolecules have been found associated with LPS on the external surface of OMVs (Horstman and Kuehn, 2000; Schooling and Beveridge, 2006; Schooling et al., 2009). Much recent research has focused on OMV proteins that could play a role in bacterial pathogenesis, including toxins, degradative enzymes, and other virulence factors, and these will be discussed in depth later in this review.

Several studies have provided important evidence supporting the hypothesis that OMV production represents a pathway for the secretion of specific proteins. Proteomic analysis has revealed that the protein profiles of OMVs are distinct from those of the cellular subfractions (Choi et al., 2011; Lee et al., 2007, 2008). It was also demonstrated by McBroom et al., that particular lumenal cargo are enriched in OMVs as compared to the periplasm (McBroom and Kuehn, 2007). Also, particular LPS subtypes are enriched in OMVs as compared to the OM and these can participate in OMV cargo selection (Haurat et al., 2011; Kadurugamuwa and Beveridge, 1995; Kadurugamuwa et al., 1993). These data form the basis of the theory that OMVs are a secretion pathway, rather than the random product of the loss of bacterial integrity or lysis.

Elucidating the mechanism(s) for OMV formation has been the focus of more recent efforts. As past publications have discussed mechanisms of Gram-negative OMV formation in detail (Deatherage et al., 2009; Kuehn and Kesty, 2005; Kulp and Kuehn, 2010), we will only highlight the conserved mechanistic elements.

Since OMVs originate from the bacterial envelope, it was logical for initial investigations of OMV formation to focus on the contributions of envelope components (OM, IM, and PG). Membrane-anchored lipoproteins covalently and non-covalently link the PG to the two membrane bilayers, giving the cell rigidity. It is commonly hypothesized that in order for OMVs to form, a localized depletion of these linkages is necessary. Indeed, strains lacking the genes (e.g. *tol-pal*, *lpp*, or *ompA*) or residues critical for the non-covalent and covalent OM–PG linkages exhibited increased OMV production (Bernadac et al., 1998; Deatherage et al., 2009; Moon et al., 2012; Walburger et al., 2002). These data confirmed that the loss of PG linkages to the OM can lead to OMV shedding; however, whether regulated depletion or breaking of those links occurs in wild-type cells remains an unanswered question in the field.

In addition to the role of interactions between envelope components, the role of cell surface-linked sugars in OMV formation has been investigated. In the Gram-negative opportunistic pathogen *Pseudomonas aeruginosa*, two distinct forms of LPS are present in the OM (Kadurugamuwa and Beveridge, 1995; Kadurugamuwa et al., 1993). The O-specific antigen, or B-band LPS, is composed of many tandem sugar repeats that vary depending on the specific strain serotype and is highly charged. The common antigen, or A-band LPS, is composed of a limited number of rhamnose sugar repeats and is uncharged. Beveridge and coworkers observed that a majority, if not all, of the LPS in naturally-produced OMVs was B-band LPS (Kadurugamuwa and Beveridge, 1995; Kadurugamuwa et al., 1993). It was proposed that the charged nature of B-band LPS created repulsion forces in the membrane facilitating OM blebbing. In contrast, when they analyzed OMVs isolated from *P. aeruginosa* treated with the cell well perturbing agent, gentamicin, both A- and B-band LPS were present. This supported the model that native OMV budding sites are enriched in B-band LPS. Further support for the correlation of B-band LPS and OMV production was made by Sabra