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Membrane vesicles and horizontal gene transfer in prokaryotes

Sara Domingues[1,2] and Kaare M Nielsen[3,4]

Membrane vesicles (MVs) are released from all living cells. MVs are lumen-containing spheres of lipid-bilayers derived from the cell surface. MVs are biologically active and contain various components, including genetic material. Both chromosomal and plasmid DNA, as well as different types of RNA have been detected in MVs. Vesicle-mediated transfer of genes coding for antibiotic resistance, virulence and metabolic traits has been reported in Gram-negative and Gram-positive bacteria and in Archaea. MVs can persist over time in natural environments. Here we review the characteristics of and the role of MVs in horizontal gene transfer (HGT) processes in prokaryotes.

Addresses
[1] Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal
[2] Center for Neuroscience and Cell Biology, 3004-517 Coimbra, Portugal
[3] Department of Life Sciences and Health, Oslo and Akershus University College, 0130 Oslo, Norway
[4] Genok-Center for Biosafety, SIVA Innovation Center, 9294 Tromsø, Norway

Corresponding authors: Domingues, Sara (saradomingues@ff.uc.pt), Nielsen, Kaare M (kaare.nielsen@hioa.no)

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Introduction

Living cells produce vesicles that are named differently accordingly to the taxonomic group that release them. Generally, they are called membrane vesicles (MVs) or extracellular vesicles (EVs); membrane vesicles in Archaea and Mycobacteria; membrane blebs, outer membrane blebs or outer membrane vesicles (OMVs) in Gram-negative bacteria; membrane vesicles in Gram-positive bacteria; exosomes, microvesicles or tolerosomes, among many other designations, in Eukarya (EVpedia; http://EVpedia.info).

Although originally considered a random outcome of cell lysis, it is now well established that MVs can be produced by secretion from living cells [1,2•]. Several models can explain the biogenesis (also called vesiculation) of MVs in processes that are not yet fully understood [2•,3•]. MVs are biologically active and a multitude of functions have been associated with these vesicles [1,2•,3•]. Here we review the characteristics of and the role of MVs in horizontal gene transfer (HGT) processes in prokaryotes.

Formation of membrane vesicles (MVs)

MVs are lumen-containing spheres of lipid-bilayers derived from the cell surface; occasionally they can be elongated or elliptical, especially during vesiculation [4,5]. Their diameter varies and ranges from 10 to 500 nm (Table 1). The amount and composition of vesicles vary even within the same species and population and depends on growth phase and environmental conditions [1,6,7]. For example, *Acinetobacter baumannii* ATCC19606 populations release small outer MVs in the early log-phase, large MVs in the early and mid-log phase, and medium sized vesicles called inner and outer membrane vesicles (IOMVs) comprising elements from the inner and outer membranes and a putative peptidoglycan layer, during the stationary phase [8].

MVs are secreted in a variety of environments, including planktonic and biofilm stages, within eukaryotic host cells, and when present in different growth media in the laboratory [6,7]. A conserved general mechanism for the biogenesis of MVs has not been identified so far, but several models have been proposed.

The most common MVs from Gram-negative bacteria are the OMVs, formed from the outer membrane (OM) of the cells. A multitude of mechanisms have been proposed to be involved in vesiculation [9–13]. The release of OMVs appear to occur after the OM budges out in areas where the OM is detached from the peptidoglycan layer; the amount of lipids, responsible for the fluidity and curvature of the OM, also influence the formation of the OMVs [2•].

The main question about the biogenesis of thick cell walled prokaryotes is how the MVs formed from the cell membrane escape the cell wall and are release in the environment. Until now there are three, non-mutually exclusive, proposed models. Briefly, turgor pressure from the cytoplasm may force the MVs through the pores of the cell wall; proteases might act to increase the size of cell wall pores; and/or MVs might reach the extracellular

Table 1

Size range of membrane vesicles released by prokaryotes

Prokaryote	Diameter (nm)	Reference
Gram-negative bacteria	10–500	[7,8]
Gram-positive bacteria	20–400	[3•]
Mycobacteria	50–300	[3•]
Archaea	50–230	[6,30]

environment after crossing the cell wall inside protein channels [3•].

The cell wall of archaea lacks peptidoglycan and different species have a high diversity in the composition of the cell envelope. The formation of MVs nevertheless seems to resemble that of Gram-positive bacteria. Protein-based studies suggested that MVs are released from the cell surface through the action of an endosomal sorting complex required for transport by protrusion of the membrane, followed by disassembly and consequent detachment of vesicles [6,14].

Content of membrane vesicles

In addition to the membrane components needed for the formation of the membrane sphere itself, MVs contain a range of other components such as inner-membrane, periplasmic and cytoplasmic components including proteins, polysaccharides and nucleic acids. The components may act as toxins, virulence factors, and in antibiotic degradation. Vesicles can also contain misfolded proteins or components related to the growth environment of the cells such as antibiotics and metal ions. MVs are predicted to have a role in stress response, cell-to-cell communication, nutrient acquisition, biofilm formation, in defence and in gene transfer resulting in acquisition of antibiotic resistance [1,2•,3•].

Relocation of genetic material in MVs

Although not yet completely clear, DNA can end up in MVs by different ways: via a cytoplasmic route, where the cytoplasmic content, including DNA, is trapped into IOMVs; via a periplasmic route, where the DNA has to relocate from the cytoplasm to the periplasmic space, followed by trapping in MVs; via an extracellular route, possibly due to broken MVs that re-annealed after release from the bacteria; or due to cell death [4,5,15–17]. Phages may also inject their DNA directly into MVs [18].

The mechanism(s) responsible for the presence of RNA in MVs is also not fully understood, though different options have been hypothesized: for instance protein synthesis close to the membrane site where vesiculation occurs resulting in trapping of mRNA together with the ribosomal proteins; and via the routes described for DNA [19,20].

It remains unclear if DNA/RNA is actively transported into particular types of MV or if the presence of genetic material in MV is mainly the outcome of random processes. A complication in the study of genetic material associated with MVs is to distinguish between surface associated nucleic acids (possible in a nuclease resistant state) [4,15,21,22] and nucleic acids present in the lumen [4,15,21–23] as a result of vesicle formation.

Studies of the content of genetic material in membrane vesicles

Genetic material has been reported to be present in MVs isolated from a variety of bacterial populations, including Gram-negative and Gram-positive bacteria, mycoplasma and archaea (Table 2). Genetic material up to 370 Kbp has been detected inside MVs, though smaller fragments are more frequent [24••,25,26••]. Several studies report the presence of DNA that can be chromosomal, plasmid

Table 2

Examples of genetic material present in membrane vesicles

Type of genetic material	Species	References
DNA		
Chromosomal	*Clostridium perfringens*, *Escherichia coli*, *Neisseria gonorrhoeae*, *Porphyromonas gingivalis*, *Prochlorococcus* sp., *Ruminococcus* spp., *Shewanella vesiculosa*	[5,18,21,23,24••,26••,28]
Plasmid	*Acinetobacter baumannii*, *A. baylyi*, *E. coli*, *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Thermococcales kodakaraensis*, *Thermococcus nautili*[a]	[4,15,16,22,23,26••,29••]
Viral	*E. coli*, *T. nautili*	[22,23]
Not specified	*Acholeplasma laidlawii*	[32]
RNA		
mRNA	*E. coli*, *P. gingivalis*, *Prochlorococcus*	[18,19,21]
rRNA	*E. coli*, *P. gingivalis*	[19,21]
sRNA	*E. coli*, *Vibrio cholerae*	[19,20]
tRNA	*E. coli*	[19]
Not specified	*N. gonorrhoeae*	[26••]

[a] Formerly *Thermococcus nautilus*.