BACTERIAL PHYSIOLOGY

Bacterial secretion into vesicles

Five distinct secretion pathways are used to transport proteins across the Gram-negative bacterial double membrane. This is in addition to three transport routes across the inner membrane. Even so, *Escherichia coli*, the best-studied bacterium, can still spring a surprise on microbiologists. Reporting in *Cell*, Wai *et al.* describe a new mechanism of secretion in which a toxin is exported in vesicles. Vesicle or bleb formation is nothing new — but this is the first time vesicle-mediated secretion has been given clear physiological relevance.

Common laboratory strains of *E. coli* can produce ClyA, a cytotoxin that is secreted into the bacterial periplasm but acts on mammalian cells. How does the toxin access its target site? Using immunofluorescence, Wai *et al.* showed that ClyA is surface-exposed. Closer examination with electron microscopy and atomic force microscopy revealed a multitude of small outer-membrane vesicles (OMVs) approximately 50–200 nM in diameter surrounding the bacteria. Analysing the protein content of these vesicles proved that they were derived from the outer membrane. Strains of *E. coli* that do not produce ClyA still produced OMVs. Reassuringly, pathogenic *E. coli* and *Salmonella enterica* serovar Typhi strains that produce ClyA also localized the toxin into OMVs, so this process isn't restricted to laboratory-adapted strains. The OMVs typically had ring-like pores, which were shown to be assemblies of ClyA protein.

Toxicity assays showed that ClyA present in OMVs was at least 8 times more toxic than purified ClyA. This striking result prompted the authors to examine ClyA multimerization, and cross-linking experiments confirmed that periplasmic ClyA is monomeric compared with multimeric ClyA present in OMVs. Further, removing the disulphide-bond oxidation machinery from *E.coli* dramatically increased the action of ClyA. The authors couldn't detect proteins that participate in disulphide-bond formation in OMVs containing ClyA, raising the possibility that the redox status of ClyA contributes to oligomerization and toxicity. OMVs seem to contain a subset of periplasmic proteins, which means that *E.coli* could have a crude protein-sorting mechanism that selects which proteins are exported in OMVs.

OMVs are formed by a whole variety of bacterial species, including commensal *Bacteroides* species and pathogenic *Neisseria* species. If bacteria can sort periplasmic proteins into vesicles, the challenge will be to find out how sorting is accomplished, how it is regulated and whether this process can be used as a model for eukaryotic vesicle trafficking.

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References and links

ORIGINAL RESEARCH PAPER Wai *et al.* Vesicle-mediated export and assembly of pore-forming oligomers of the enterobacterial ClyA cytotoxin. Cell **115**, 25–35 (2003)

FURTHER READING Miller, S. I. *et al.* Bacterial vesicle formation as a mechanism of protein transfer to animals. Cell **115**, 2–3 (2003)

WEB SITE

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http://www.molbiol.umu.se/forskning/uhlin/