Researchers break through the wall in bacterial membrane vesicle research

September 7 2017

Many bacteria release membrane vesicles, which are nanoscale spheres consisting of a cellular membrane containing biomolecules. Membrane vesicles can transport DNA and proteins, and are involved in bacterial interactions. They have potential applications in nanotechnology and biomedicine, including cancer treatment. However, the formation of membrane vesicles by bacteria is currently not well understood. In particular, the release mechanism of membrane vesicles of Gram-positive bacteria, which have a very thick cell wall, has remained an enigma.

In a collaborative effort, Japanese and Swiss researchers investigated membrane vesicle formation in the model Gram-positive bacterium Bacillus subtilis. The team was able to visualize the release of membrane vesicles by using live cell imaging techniques and state-of-the-art electron cryotomography. Live cell imaging allows researchers to follow the fate of cells during membrane vesicle formation in real time, while electron cryotomography can provide high-resolution three-dimensional structures of cells in a near-native state.

Live cell imaging has showed that cells releasing membrane vesicles died but retained their cell structure. Electron cryotomography further demonstrated that small holes form in the cell wall through which the cellular membrane protrudes and forms vesicles. A series of experiments indicated that the holes in the cell wall were formed by an endolysin, an enzyme that is typically used by bacteria-infecting viruses (phages) to break the host cell wall to induce their release into the environment.

"This is one of the first examples clearly showing how membrane vesicles are formed in Gram-positive bacteria," says first author Masanori Toyofuku of the University of Tsukuba and the University of Zurich. "We found that membrane material is extruded through a hole in the cell wall, forms a vesicle, and then eventually detaches from the cell."

The live cell imaging experiments also indicated that membrane vesicle formation by a cell can trigger vesicle formation in neighboring bacteria because the released endolysin damages the cell wall of nearby cells. Endolysin has previously been shown to induce membrane vesicle formation in Gram-negative bacteria. The team's findings therefore emphasize the importance of endolysins in membrane vesicle production in bacteria, suggesting a universal formation mechanism. It has been hypothesized that membrane vesicle production is important for neutralizing environmental agents such as antimicrobial peptides or bacteriophages that target the membranes of bacteria.

"Considering the high abundance of phages in the environment and other mechanisms that disrupt the cell wall, cell lysis may play a major role in membrane vesicle formation in nature," says Leo Eberl of the University of Zurich. "It would be interesting to elucidate the proportions of vesicles in the environment that originate from cell lysis and alternative mechanisms."

The knowledge that endolysins induce the formation of membrane vesicles in bacteria may advance their use in nanotechnology and biomedicine. "We envisage that mass production of membrane vesicles may be achieved through development of bacterial strains with engineered endolysin production," explains Nobuhiko Nomura of the University of Tsukuba.

More information: Masanori Toyofuku et al, Prophage-triggered

membrane vesicle formation through peptidoglycan damage in Bacillus subtilis, *Nature Communications* (2017). DOI: 10.1038/s41467-017-00492-w

Provided by University of Tsukuba