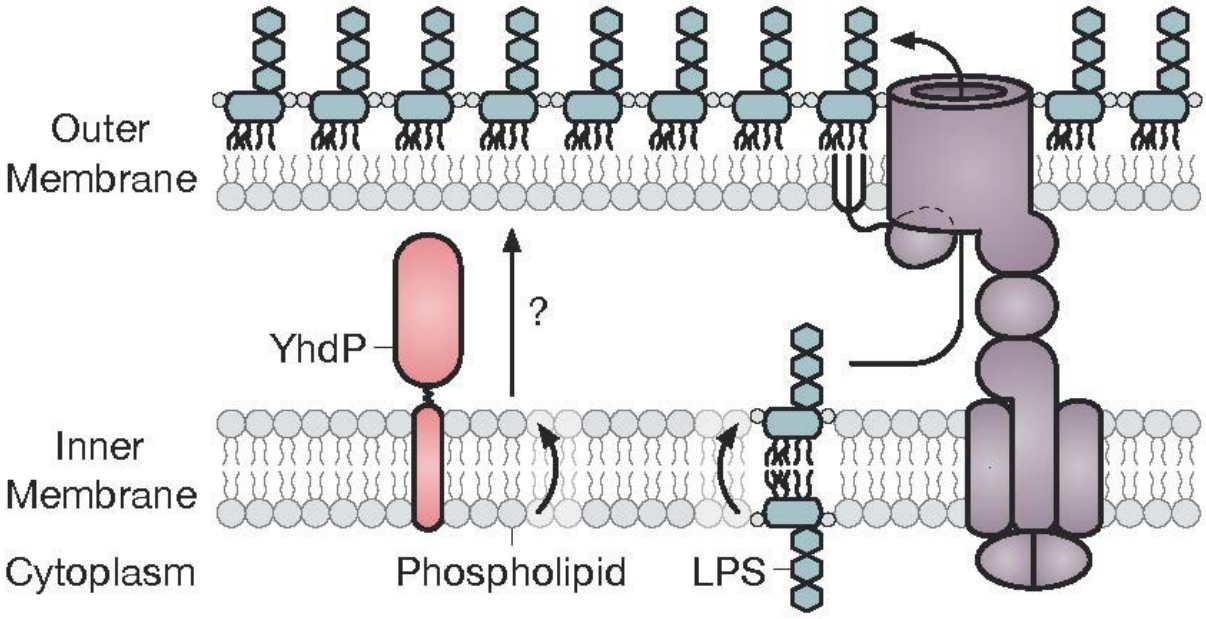
Researchers find key to piercing harmful bacteria's armor

November 9 2020, by Caitlin Sedwick



In Gram-negative bacteria, LPS and phospholipids are manufactured at the inner bacterial membrane and must be delivered across the cell wall to the outer membrane. The manufacture and delivery of LPS to the outer bacterial membrane is carefully balanced against phospholipid levels because imbalances can be lethal to the cell. Credit: Princeton University

Bacteria are single-celled organisms that are essential to human health, both in our environment and inside our own bodies. However, certain bacterial species can make us sick.

When a physician suspects an illness of bacterial origin, they will perform diagnostic tests to identify what bacterial species is causing disease so that a course of treatment can be devised. One of these tests is called the Gram stain, after Hans Christian Gram, who developed the technique in the 1880s. Gram discovered that certain bacterial species, the so-called 'Gram-negative' bacteria, shrug off a purple dye he was using to help visualize the microbes under his microscope. Scientists eventually discovered that Gram-negative bacteria resist dye uptake because they are enveloped in what is, essentially, a microbial suit of armor: their vulnerable cell membrane is protected by a layer of tightly packed sugars called the cell wall, and on top of that, a specialized outer membrane.

"Understanding how bacteria build this barrier is an important step in engineering strategies to circumvent it," said Thomas Silhavy, the Warner-Lambert Parke-Davis Professor of Molecular Biology, and the senior author on two new papers investigating the outer membrane, one in the journal *Proceedings of the National Academy of Sciences* and the other in the journal Trends in Microbiology.

One of the main components of the outer membrane is a unique molecule called lipopolysaccharide (LPS), which covers the surface of the cell. "LPS helps to increase the mechanical strength of the Gram-negative cell envelope and it also forms a surface coating that prevents toxic molecules, including certain antibiotics, from entering the cell," said Randi Guest, a postdoctoral research associate in the Silhavy lab, a lecturer in molecular biology, and the lead author of the Trends article.

LPS is a famously potent toxin that can cause severe illness when it is released from the bacterial outer membrane or secreted by the cell.

"The amount of LPS produced by the cell is carefully controlled, as too little LPS may lead to cell rupture, while too much LPS, especially if not

properly assembled, is toxic," said Guest. "We reviewed studies of three essential membrane proteins that monitor not only LPS biosynthesis inside the cell, but also transport to, and proper assembly at the cell surface."

As Guest and colleagues discuss in their article, the construction of the bacterial outer membrane represents a complex problem for bacteria because potentially dangerous LPS, made inside the cell, must be transported across the cell wall to reach the outer membrane. In addition, these processes must be balanced against the manufacture and transport of the other components of the membrane, which in Gram-negative bacteria is mainly made up of a class of molecules called phospholipids.

"One long-standing mystery in the field is how phospholipids are transported to the outer membrane," said Silhavy. One idea is that phospholipids can flow passively back and forth between the bacterium's inner cell membrane and its outer membrane at zones of contact, but this idea is highly controversial. New research from Silhavy's group provides support for the idea that a passive mode of transport does exist.

Jackie Grimm, then a graduate student in Silhavy's lab, together with Handuo Shi, a graduate student in KC Huang's laboratory at Stanford, led an effort to identify proteins involved in trafficking phospholipids between the inner and outer membranes. For their studies, the colleagues used bacteria that have a mutation that increases the rate at which phospholipids flow from the inner membrane to the outer membrane. When they are deprived of nutrients, these bacteria experience shrinkage and rupture of the inner membrane, followed by cell death, because they are unable to make new phospholipids for the inner membrane to replace those lost to the outer membrane. The authors introduced additional mutations into these bacteria, then looked for genes which, when mutated, affect how quickly the bacteria die after nutrient withdrawal.

"We used next-generation sequencing to screen for genes involved in this process and found that disruption of the gene *yhdP* slowed phospholipid transport," said Silhavy.

Although their data indicate that the protein encoded by *yhdP* is involved in phospholipid transport between the inner cell membrane and the outer membrane, Grimm, Shi and their colleagues noted that it's not yet clear how YhdP protein works to affect this process. A potential clue might be found in its predicted similarity to other proteins whose function is already known. One of these is a mammalian protein that forms a channel that transports phospholipids across membranes.

"This suggests that YhdP might form a hydrophobic channel between the inner and outer membrane through which phospholipids flow," noted Silhavy.

"Silhavy and colleagues provide the strongest data to date towards identifying how phospholipids are transported between membranes in bacteria, an elusive question for decades in our field," said M. Stephen Trent, Distinguished Professor of Infectious Diseases at the University of Georgia, who was not involved in the work. "They make a strong argument with genetics and biophysics that a protein of unknown function, YhdP, affects a rapid transport process for phospholipids between membranes. It will be really interesting to learn YhdP's role in phospholipid transport in the future."

More information: Jacqueline Grimm et al, The inner membrane protein YhdP modulates the rate of anterograde phospholipid flow in Escherichia coli, *Proceedings of the National Academy of Sciences* (2020). DOI: 10.1073/pnas.2015556117

Provided by Princeton University