

Micro 201:

Bernhardt Lecture 2 - The cell envelope and lipoprotein secretion

January 31, 2019

Overview:

In this lecture we will explore aspects of outer membrane assembly in Gram-negative bacteria. The outer membrane is the primary determinant of the intrinsic antibiotic resistance of Gram-negative bacteria. Studies of its assembly are therefore important so that we may learn new ways of defeating this formidable permeability barrier to allow antibiotics to penetrate Gram-negative cells. The outer membrane is unique because it is an asymmetric bilayer composed of lipopolysaccharide (LPS) molecules in the outer leaflet and phospholipids (PL) in the inner leaflet. LPS and PLs are synthesized at the inner face of the inner (cytoplasmic) membrane. How are these molecules transported across the envelope to build the outer membrane? How are outer membrane porins (β -barrels) inserted into the membrane? Lipidated proteins (lipoproteins) also need to be transported from the inner to the outer membrane. What is the machinery responsible for this? How does it work? All of these questions have occupied bacteriologists for some time. However, only very recently has much of the outer membrane assembly machinery been identified. We will discuss all aspects of outer membrane assembly throughout the lecture.

In the literature analysis, we will discuss the discovery of the lipoprotein transport machinery by Tokuda and co-workers. Their work highlights how biochemistry can be just as powerful as genetics when it comes to identifying new factors and interrogating their function. We will also discuss a recent paper from the Silhavy lab where they report that lipoproteins have unexpected topological forms. They can be surface exposed as well as periplasmic. The machinery that promotes lipoprotein exposure to the cell surface remains to be identified.

Papers for discussion:

Context:

The Lol system for lipoprotein transport was the first dedicated system for the delivery of outer membrane bound cargo to be identified. This paper details the beautiful biochemical approach that Tokuda and co-workers used to identify the first component of the system, LolA (called p20 in the paper). A series of papers follows this one in which similar approaches were used to identify the rest of the system. The second paper for discussion describes the surprising observation that lipoproteins in the outer membrane can be exposed to both side of the membrane.

1) S Matsuyama, T Tajima, H Tokuda, A novel periplasmic carrier protein involved in the sorting and transport of Escherichia coli lipoproteins destined for the outer membrane. EMBO J (1995) 14:3365-3372

Focus: Be prepared to discuss the details of the biochemical assays used. I will expect you to be able to draw diagrams on the board to explain the experimental protocol to the group.

Questions to ponder: In this paper the authors reconstitute lipoprotein release from the inner membrane. What are the remaining steps in transport? How would you attempt to reconstitute

them? LolA is a periplasmic protein that stimulates lipoprotein release from the inner membrane to the periplasmic fraction. How would you test if there are inner membrane proteins needed for this step too?

2) Charles E. Cowles, Yongfeng Li, Martin F. Semmelhack, Ileana M. Cristea, and Thomas J. Silhavy. The free and bound forms of Lpp occupy distinct subcellular locations in Escherichia coli. Mol Microbiol (2011) 79:1168-1181

Focus: Be prepared to discuss the details of the subcellular localization studies they used, especially the chemical modification methods.

General Review:

Henderson, Zimmerman, Crofts, Boll, Kuhns, Herrera, and Trent. The power of asymmetry: architecture and assembly of the gram-negative outer membrane lipid bilayer. Ann Rev Microbiol (2016) 70:255-278.