Gram-negative bacteria such as *Escherichia coli* have a multi-layered envelope that is delimited by two membranes. Their inner membrane, which surrounds the cytoplasm, is a phospholipid bilayer, while their outer membrane, which surrounds the aqueous periplasm, is asymmetrically built with phospholipids in its inner leaflet and lipopolysaccharides (LPS) in its outer leaflet. Since phospholipids and LPS are synthesized at the inner membrane, they must be transported across the cell envelope to build the outer membrane. For decades, we have known that the multi-protein Lpt system transports LPS, but how phospholipids are transported to the outer membrane has been elusive. I will present our recent work on the AsmA-like protein family. We propose that three functionally redundant AsmA-like paralogs are required for the biogenesis of the outer membrane because they transport phospholipids across the periplasm. We propose that these proteins, like the Lpt system, form inter-membrane bridges that provide a continuous hydrophobic groove that shields acyl chains from the aqueous periplasmic compartment as they travel to the outer membrane. These proteins are thought to be the ancestors of inter-organelle lipid transporters in eukaryotic cells.

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**Noon, Monday, March 4, 2024**  
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