Sandra Schmid: Insights into Clathrin-Mediated Endocytosis

by Mary Bates, PhD, Science Writer
http://marybatessciencewriter.wordpress.com/

Sandra Schmid, who holds the Cecil H. Green Distinguished Chair in Cellular and Molecular Biology at the University of Texas Southwestern Medical Center, gave the final talk of the morning. Schmid began her remarks by explaining that the plasma membrane is more than just a physical barrier that protects the inside of the cell; it is also a sophisticated communication device that governs the cell's interactions with its environment and other cells. Cells use endocytic membrane trafficking to control and regulate signaling receptors on the cell surface, remodel the cell membrane, and control the concentration of transporters in response to changes in the extracellular environment.

The major pathway for endocytosis in cells is clathrin-mediated endocytosis (CME). CME involves the assembly of the protein clathrin and adaptor complexes onto the cytosolic surface of the plasma membrane to form clathrin-coated pits (CCPs). These coated pits invaginate and pinch off to form clathrin-coated vesicles (CCVs) that carry their cargo into the cell.

Schmid described how she used total internal reflection fluorescence (TIRF) microscopy to watch CME directly in cells with fluorescently labeled clathrin. She observed a great deal of heterogeneity, both in the intensity of the CCPs and in their lifetimes, with some pinching off very quickly and others lasting longer. Schmid identified three distinct subpopulations: two that are very short-lived (5-20 seconds) and one longer-lived subpopulation with lifetimes anywhere from 30 seconds to several minutes. The short-lived species turned out to be abortive CCPs where the coats disassembled without the uptake of membrane and cargo, whereas the longer-lived species were productive events that went to completion.

Lorinda Smith, Madeline Singer and Bo Foreman at the Wiley reception

Schmid suggested a working model: there might be an endocytosis checkpoint that's gating coated pit maturation and regulating the early stages in vesicle formation. To investigate it, she developed a more sensitive method of detecting CCPs in cells that was more accurate at a low signal to noise ratio. The problem was the method was too sensitive, so Schmid put her data through a thresholding process that would distinguish between sporadic clathrin assemblies and legitimate CCPs. The results indicated that legitimate CCPs are defined by a regulated, multi-step maturation process.

Schmid then presented data suggesting one of the responders to this proposed endocytic checkpoint is the GTPase dynamin, which is best-known for its role in fission at late stages of vesicle formation. Schmid found dynamin plays a dual role in CME: it is recruited early to CCPs, and when the pits are deeply invaginated there is a burst of dynamin assembly to drive membrane fission. She also found dynamin is not recruited to all coated pits. The coated pits to which dynamin is recruited undergo this multistep maturation process, but the coated pits where no dynamin was detected are all much shorter-lived. This suggests that early dynamin recruitment to these coated pits is necessary for them to proceed along through the maturation process.

Another important component in Schmid's working model are the endocytic accessory factors, which interact with the appendage (or ear) domain of the AP2 alpha adaptor protein. Schmid discovered that interactions between the alpha appendage domain and certain endocytic accessory factors are necessary to create the curvature of CCVs. Her research suggests some of the late regulatory events in CME do not occur without the recruitment of endocytic accessory factors to coated pits.

The endocytic accessory factors recruited to alpha appendage domains have varied functions: they are involved in generating membrane proteins, cargo selection, recruitment of actin, and they serve as scaffolding molecules. Currently, Schmid's lab is analyzing all the accessory factors using the assays she's developed to understand which factors are required for each process. As she continues to model CCP maturation, Schmid hopes to be able to see which stages in this multistep process are regulated by different endocytic accessory factors.