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Visualizing the Structural Progression of Clathrin Mediated Endocytosis with Fluorescence and Electron Microscopy

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Clathrin mediated endocytosis is a major route for the internalization of specific extracellular and membrane bound material in eukaryotic cells. Clathrin triskelia form into a polyhedral lattice of hexagons and pentagons that coat the endocytic site. At this site, the membrane forms a small (~100 nm) coated bud which is eventually cut from the plasma membrane to release an intracellular vesicle. Over 60 proteins are bound to the endocytic site and regulate the process. Adding complexity, there are multiple structural pathways that can create a clathrin coated vesicle.[1] Lattices can grow flat or highly curved and the curvature of a preformed lattice can increase over the course of endocytosis.[2-4] This is surprising because clathrin lattices are highly interwoven networks where each triskelion can bind up to nine other triskelia.[5] The molecular interactions controlling structural maturation and allowing lattice reorganization are unknown.

We have refined a suite of tools that allows us to investigate the developing architecture of clathrin coated pits. We use platinum replica electron microscopy to provide a high contrast view of the many different stages of clathrin lattices on the cytoplasmic side of the plasma membrane in mammalian cells.[6, 7] With this, we have observed thousands of clathrin structures in many different cell lines to examine the universal versus cell line specific behavior of clathrin structures. We combine this technique with super-resolution localization microscopy in a correlative method that pinpoints the location of specific proteins on clathrin structures with ~20 nm precision. This has allowed us to create an architectural map of protein organization in different stages of pit maturation.[8] We also use cryo-electron tomography to observe molecular-scale interactions at endocytic sites. The increased resolution of cryo-electron tomography has revealed disorder in the flat clathrin lattice in comparison to curved lattices leading us to propose that flat clathrin lattices are under strain. Together, these tools have allowed us to create a new model of clathrin mediated endocytosis.

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