AUTOMATED SUPERRESOLUTION MICROSCOPY REVEALS THE DYNAMIC STRUCTURAL ORGANIZATION OF THE ENDOCYTIC MACHINERY

Markus Mund, Joran Deschamps, Jan v.d. Beek, Marko Kaksonen, Jonas Ries
European Molecular Biology Laboratory (EMBL)
Meyerhofstr. 1, 69117 Heidelberg, Germany
E-mail: ries@embl.de

KEY WORDS: Single-molecule localization microscopy, PALM, STORM, endocytosis, structural cell biology.

Clathrin-mediated endocytosis is a highly intricate cellular process, which involves the ordered recruitment and disassembly of around 60 proteins. Because of the limited resolution of live-cell microscopy and the lack of molecular specificity in electron microscopy, the structural organization of endocytic proteins is largely unknown.

We use automated high-throughput single-molecule localization microscopy to determine this crucial missing information on protein localizations within the endocytic machinery and focus on S. cerevisiae as a model organism [1]. Currently, live-cell superresolution microscopy is still too limited in terms of spatial and temporal resolution, the number of frames that can be acquired and the number of proteins that can be studied simultaneously. Thus, our approach is to image specific proteins in a large number of unsynchronized fixed endocytic sites in dual-color in combination with a reference protein. With the help of this reference protein we assign an exact time point to each site and integrate data for many different proteins into one common coordinate system. The aim is to acquire a time-resolved localization map of the entire endocytic machinery (Figure 1), which would provide us with a comprehensive structural picture of endocytosis in yeast.

In yeast endocytosis, polymerizing actin provides the force to invaginate the membrane with a high efficiency and remarkable temporal and spatial regularity. We directly visualize the structural relation between polymerized actin and actin interacting proteins to ultimately understand how actin polymerization is regulated in situ. We discovered that several actin nucleation promoting factors and coat proteins show a striking ring-shaped organization [2] even prior to actin polymerization. This lateral pre-patterning of the endocytic site provides an elegant explanation for an efficient force generation by the actin machinery and force transfer to the membrane.


Figure 1: Left: dual-color diffraction limited image of endocytic proteins in yeast. Right: Dynamic 3-color reconstruction of the endocytic machinery showing the membrane (green), actin (red) and an actin nucleation promoting factor (blue). Scale bar 100 nm.