Superresolution imaging of endocytosis in yeast

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Keywords: Superresolution microscopy, Single-Molecule Localization Microscopy, Clathrin-Mediated Endocytosis, Saccharomyces cerevisiae

Clathrin-mediated endocytosis is a highly intricate cellular process, which in yeast involves the ordered recruitment and disassembly of around 60 proteins. Diffraction-limited live-cell microscopy has lead to tremendous insight into composition and dynamics of the endocytic machinery. Electron microscopy on the other hand offers nanometer resolution, but lacks molecular specificity. Thus, the structural organization of most endocytic proteins in situ is largely unknown.

We employ localization microscopy (PALM/STORM) to study endocytic structures in Saccharomyces cerevisiae. This method is ideal to study static structures, which is why cells are typically fixed during sample preparation, leading to the loss of temporal resolution. This is mirrored in considerable heterogeneity among the imaged endocytic sites, which complicates their interpretation. By blocking actin polymerization using latrunculin A, we arrest the endocytic sites in comparable states, allowing us to validate previously observed structures. Our current efforts focus on the coat and actin assembly preceding scission. Here, we were able to reveal subdiffraction features of the endocytic coat protein Sla1, which is involved in the regulation of actin polymerization. We show that Sla1 assembles in a ring shape independent of membrane curvature. We also visualized Abp1, which binds throughout polymerized actin at endocytic sites. By imaging a high number of endocytic sites, we attempt to describe the dynamic organization of the endocytic actin structures.

Extending the approach by dual-color imaging, we are pursuing to obtain a comprehensive structural picture of the endocytic machinery in yeast.