**Abstract**

Endocytosis is an ancient pathway that regulates communication of the cell with its environment. During this process, the plasma membrane is deformed in a controlled sequence: a flat membrane forms an invagination that undergoes scission to produce a cargo-filled vesicle. Breaking the membrane invagination to form a vesicle is perhaps the most dramatic shape transition in this development. This has excited a large body of literature on the cause of membrane scission. Work on mammalian cells has converged on a scission mechanism based on membrane neck constriction by the GTPase dynamin. A clear understanding of what causes scission remains incomplete for the much simpler endocytic network in yeast cells.

In this thesis, I investigate the mechanism of membrane scission in *Saccharomyces cerevisiae* and the proteins involved by combining mutagenesis with live-cell imaging of fluorescently tagged proteins.

Endocytic sites are very stereotypic in yeast, recruiting about 50 proteins- most with mammalian homologues- in a highly specific sequence. These proteins can be assigned to separable modules based on their role in the endocytosis. Members of the coat module arrive when the membrane is still flat and form the template for invagination. Actin regulators arrive later and produce the forces required to pull up the membrane. Scission proteins arrive at the end of the timeline and regulate vesicle formation.

The yeast BAR domain complex Rvs is an important regulator of scission: in cells without Rvs, scission efficiency decreases by nearly 30%. The 70% of invaginations that undergo scission in the cells form smaller vesicles than usual. Rvs thus appears to regulate both timing and likelihood of scission, but it has not been clear how it does so, or how it gets recruited to membrane tubes in the first place.

I find that Rvs localization is timed by its BAR domain. The BAR domain senses a particular membrane shape, and Rvs is only recruited to endocytic sites once this shape is acquired. Surprisingly, localization efficiency and localization itself is affected by a second domain of Rvs167, the SH3 domain. This domain helps recruitment of Rvs and likely couples the actin network to vesicle scission, triggering disassembly of the actin network once scission occurs.

Several models have been proposed for what eventually causes scission. I test predictions of some of these models. I find that forces generated by dynamin and lipid hydrolysis do not drive vesicle formation. Scission timing is also independent of the number of BAR domains recruited to membrane tubes, so is not based on BAR concentration-dependent membrane rupture. This timing is instead regulated by the amount of actin at endocytic sites, and hence by the magnitude of forces generated on the membrane. There appears to be a threshold force over which the membrane reliably ruptures. The function of Rvs is to scaffold the membrane, and prevent scission before this force is generated, allowing reliable formation of vesicles.