﻿Introduction

Clathrin-mediated endocytosis (CME) is the major endocytic process by which cargo from the cell exterior is incorporated into a Clathrin-coated vesicle that is then transported into the cell interior (Bitsikas, Corrêa, and Nichols 2014; Heuser and Reese 1973). Over 50 different proteins are involved in reshaping a flat plasma membrane into an invagination that eventually forms a vesicle (Kaksonen and Roux 2018). Forces that drive the transition from invagination to spherical vesicle in mammalian cells are provided by the GTPase Dynamin. Dynamin is now known to interact via its proline-rich-domains with SH3 domains of crescent-shaped N-BAR proteins like Endophilin and Amphiphysin. Conformation changes of Dynamin molecules recruited to an N-BAR scaffold cause constriction of the underlying invaginated membrane, resulting in vesicle formation.

In yeast, CME is the only pathway for uptake of cargo, and comprises of a similar process as in mammalian cells. Most mammalian CME proteins have homologues in yeast: these proteins drive the establishment of endocytic sites, form the mechanical link between membrane and actin proteins, Actin nucleation and polymerization then drives the formation tubular invaginations, and eventually cargo-filled vesicles are formed (Marko Aurelieven review). The role of Dynamin in this process has been debated: yeast dynamin Vps1 has a major role the golgi trafficking pathway, and been proposed to interact with endocytic proteins. Its importance in CME vesicle scission is however, still debated (references). In yeast cells, what causes membrane scission is thus unclear, although the yeast N-BAR Rvs complex (a heterodimeric complex of the proteins Rvs161 and Rvs167) has been identified as an important component of the scission module (ref Marko, ANdrea). The two Rvs proteins are yeast homologues of Amphiphysin and Endophilin (Friesen et al., 2006, Youn et al., 2010). Deletion of Rvs167 reduces scission efficiency by nearly 30\% and reduces the invagination lengths at which scission occurs (wanda). Apart from a canonical N-BAR domain which forms the crescent-shaped structure, Rvs167 has a Glycine-Proline-Alanine rich (GPA) region and a C-terminal SH3 domain (Sivadon, Crouzet and Aigle, 1997). The GPA region is thought to act as a linker with no other known function, while loss of the SH3 domain affects budding pattern and actin morphology Most Rvs deletion phenotypes can be rescued by expression of the BAR domains alone (Sivadon, Crouzet and Aigle, 1997), suggesting that the BAR domains are the main functional unit of the Rvs complex.

The Rvs complex can tubulate liposomes in vitro, indicating that the BAR domains can impose curvature on membranes (Youn et al., 2010). However, Rvs arrives at endocytic sites when membrane tubes are already formed: curvature sensing rather than generation is the likely interaction of the complex with endocytic invaginations. Rvs molecules arrive at endocytic sites about 4 seconds before scission, and disassemble rapidly at the time of scission (Picco et al., 2015), consistent with a role in scission. While it is shown to be involved in the last stages of endocytosis, a mechanistic understanding of the influence of Rvs on scission remains incomplete.

Several scission models have been proposed that allow a major role for Rvs and are tested in this work. Although the yeast Dynamin Vps lacks a canonical BAR domain-binding site, it may be recruited via a different mechanism and induce scission (ref). Liu et al., proposed that Synaptojanins may selectively hydrolyze lipids at endocytic sites, causing line tension between two lipid types that results in scission. Protein friction along the membrane invagination has been proposed as a mechanism by which scission may occur. We used quantitative live-cell imaging and genetic manipulation in *Saccharomyces cerevisiae* to test these theories and investigate the influence of Rvs in endocytic scission. We found that Rvs is recruited to endocytic sites by both BAR and SH3 domains. Of several likely actin-interacting binding partners of the SH3 domains such as Myo3, Myo5, Vrp1, Abp1 (references) we found that type I myosin Myo3 interacts with Rvs SH3 domains. Our data also suggests that the aforementioned theories of membrane scission are unlikely to sever the membrane in yeast, and that actin polymerization likely generates the forces required for scission.

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