﻿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; ROTH and PORTER 1964; Pearse 1976). Over 50 different proteins are involved in reshaping a flat plasma membrane into an invagination that eventually forms the vesicle (Kaksonen and Roux 2018). Forces that drive the transition from invagination to spherical vesicle in multicellular eukaryotes are provided by the GTPase Dynamin (Grigliatti et al. 1973; Takei et al. 1995; Galli et al. 2017; Ferguson et al. 2007; Sweitzer and Hinshaw 1998). Dynamin is now known to interact via its proline-rich-domain with SH3 domains of crescent-shaped N-BAR proteins like Endophilin and Amphiphysin (Grabs et al. 1997; Cestra et al. 1999; Meinecke et al. 2013; Farsad et al. 2001; Ferguson et al. 2009). Conformation changes of Dynamin recruited to N-BAR molecules cause constriction of the underlying invaginated membrane, resulting in vesicle formation (Zhao et al. 2016; Zhang and Hinshaw 2001; Shupliakov et al. 1997).

In yeast, CME is the only pathway for uptake of cargo, and involves a similar membrane transformation as in other eukaryotes. 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 (Kaksonen and Roux 2018). Actin nucleation and polymerization drives the formation of tubular invaginations in yeast (Kübler and Riezman 1993; Kaksonen, Sun, and Drubin 2003). The role of Dynamin in this process has been debated: yeast dynamin-like protein Vps1 has a major role in the Golgi and other membrane trafficking pathways (Rothman et al. 1990; Peters et al. 2004; Hoepfner et al. 2001), and been proposed to interact with endocytic proteins (Nannapaneni et al. 2010; Yu 2004; Smaczynska-de Rooij et al. 2012). Its contribution to CME is however, still debated (Goud Gadila et al. 2017; Takuma Kishimoto et al. 2011). 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(T. Kishimoto et al. 2011; Kaksonen, Toret, and Drubin 2005; D’Hondt, Heese-Peck, and Riezman 2000; Munn et al. 1995) . The two Rvs proteins are homologues of N-BAR proteins 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 (Kukulski et al. 2012; Kaksonen, Toret, and Drubin 2005). Apart from the 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 (Sivadon, Crouzet, and Aigle 1997). 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 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 sites (Picco et al. 2015; Kukulski et al. 2012). 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 Vps1 lacks a canonical BAR-protein binding site (Bui et al. 2012; Moustaq et al. 2016), it may be recruited via a different mechanism and induce scission. Liu et al., proposed that Synaptojanins may selectively hydrolyze lipids at endocytic sites, causing line tension between two lipid types that results in scission (Liu et al. 2009). Protein friction along the membrane invagination has been proposed as a mechanism by which scission may occur (Simunovic et al. 2017). We used quantitative live-cell imaging and genetic manipulation in *Saccharomyces cerevisiae* to test these theories and investigate the function of Rvs in endocytosis. We found that Rvs is recruited to endocytic sites by both BAR and SH3 domains. Of several potential actin-interacting binding partners of the SH3 domains such as Myo3, Myo5, Vrp1, Abp1 (Lila and Drubin 1997; Liu et al. 2009; Colwill et al. 1999; Madania et al. 1999). 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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