﻿Introduction

Clathrin-mediated endocytosis involves the reshaping a flat plasma into a (tubular) invagination that eventually forms a vesicle. Forces that drive the transition from invagination to spherical vesicle in mammalian cells are provided by conformation changes of the GTPase Dynamin. Dynamin is now known to act in concert with the crescent-shaped N-BAR proteins Endophilin and Amphiphysin (ref. Dynamin papers). Yeast dynamin Vps1 has been proposed to interact with endocytic proteins, although the its role in the scission process is still debated (references). In yeast cells, what causes membrane scission is thus unclear, although the yeast N-BAR protein complex Rvs has been identified as an important component of the scission module (ref Marko, ANdrea). The Amphiphysin and Endophilin homologue in yeast is the heterodimeric complex Rvs composed of Rvs161 and Rvs167 (Friesen et al., 2006, Youn et al., 2010). Deletion of Rvs 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 a cresent-shaped structure, Rvs167 has a Glycine-Proline-Alanine rich (GPA) region and a C-terminal SH3 domain. Rvs161 and Rvs167 N-BAR domains are 42\% similar, and 21\% identical, but are not interchangeable (Sivadon, Crouzet and Aigle, 1997). The GPA region is thought to act as a linker with no known other function, while loss of the SH3 domain affects budding pattern and actin morphology (ref). Most Rvs deletion phenotypes can however, be rescued by expression of the BAR domain alone (Sivadon, Crouzet and Aigle, 1997), suggesting that the BAR domains are the main functional unit of the Rvs complex. In keeping with this theory, Rvs has been shown to tubulate liposomes in vitro (Youn et al., 2010). The Rvs complex arrives at endocytic sites in the last stage of the endocytosis, and disassembles rapidly at the time of membrane scission (Picco et al., 2015), consistent with a role in membrane 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.

We used quantitative live-cell imaging and genetic manipulation in *Saccharomyces cerevisiae* to investigate the influence of Rvs and several Rvs interacting proteins that have been suggested to have a role in scission. We found that arrival of Rvs to endocytic sites is timed by interaction of its BAR domain with a specific membrane curvature. The Rvs167 SH3 domain affects localization efficiency of the Rvs complex and also influences invagination dynamics. This indicates that both BAR and SH3 domains are important for the role of Rvs as a regulator of scission. We tested current models of membrane scission, and find that deleting yeast synaptojanins or dynamin does not change scission dynamics. Interfacial forces at lipid boundaries are therefore unlikely to be sufficient for scission, and forces exerted by dynamin are not required. Furthermore, invagination length is insensitive to overexpression of Rvs, suggesting that the recently proposed mechanism of BAR-induced protein friction on the membrane is not likely to drive scission. We propose that recruitment of Rvs BAR domains prevents scission and allows invaginations to grow by stabilizing them. We also propose that vesicle formation is dependent on forces exerted by a different module of the endocytic pathway, the actin network. Preventing premature membrane scission via BAR interaction could allow invaginations to grow to a particular length and accumulate enough forces within the actin network to reliably cut the membrane.