﻿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 hypotheses 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 hypotheses of membrane scission are unlikely to sever the membrane in yeast, and that actin polymerization likely generates the forces required for scission.

Bitsikas, Vassilis, Ivan R Corrêa, and Benjamin J Nichols. 2014. ‘Clathrin-Independent Pathways Do Not Contribute Significantly to Endocytic Flux’. *ELife* 3 (September): e03970. https://doi.org/10.7554/eLife.03970.

Bui, Huyen T, Mary A Karren, Debjani Bhar, and Janet M Shaw. 2012. ‘A Novel Motif in the Yeast Mitochondrial Dynamin Dnm1 Is Essential for Adaptor Binding and Membrane Recruitment.’ *The Journal of Cell Biology* 199 (4): 613–22. https://doi.org/10.1083/jcb.201207079.

Cestra, G, L Castagnoli, L Dente, O Minenkova, A Petrelli, N Migone, U Hoffmüller, J Schneider-Mergener, and G Cesareni. 1999. ‘The SH3 Domains of Endophilin and Amphiphysin Bind to the Proline-Rich Region of Synaptojanin 1 at Distinct Sites That Display an Unconventional Binding Specificity.’ *The Journal of Biological Chemistry* 274 (45): 32001–7. https://doi.org/10.1074/JBC.274.45.32001.

Colwill, Karen, Deborah Field, Lynda Moore, James Friesen, and Brenda Andrews. 1999. ‘In Vivo Analysis of the Domains of Yeast Rvs167p Suggests Rvs167p Function Is Mediated Through Multiple Protein Interactions’. *Genetics* 152 (3): 881–93. http://www.genetics.org/content/152/3/881.

D’Hondt, Kathleen, Antje Heese-Peck, and Howard Riezman. 2000. ‘Protein and Lipid Requirements for Endocytosis’. *Annual Review of Genetics* 34 (1): 255–95. https://doi.org/10.1146/annurev.genet.34.1.255.

Farsad, K, N Ringstad, K Takei, S R Floyd, K Rose, and P De Camilli. 2001. ‘Generation of High Curvature Membranes Mediated by Direct Endophilin Bilayer Interactions.’ *The Journal of Cell Biology* 155 (2): 193–200. https://doi.org/10.1083/jcb.200107075.

Ferguson, Shawn M., Gabor Brasnjo, Mitsuko Hayashi, Markus Wölfel, Chiara Collesi, Silvia Giovedi, Andrea Raimondi, et al. 2007. ‘A Selective Activity-Dependent Requirement for Dynamin 1 in Synaptic Vesicle Endocytosis’. *Science* 316 (5824): 570–74. https://doi.org/10.1126/science.1140621.

Ferguson, Shawn M, Shawn Ferguson, Andrea Raimondi, Summer Paradise, Hongying Shen, Kumi Mesaki, Agnes Ferguson, et al. 2009. ‘Coordinated Actions of Actin and BAR Proteins Upstream of Dynamin at Endocytic Clathrin-Coated Pits’. *Developmental Cell* 17 (6): 811–22. https://doi.org/10.1016/j.devcel.2009.11.005.

Friesen, Helena, Christine Humphries, Yuen Ho, Oliver Schub, Karen Colwill, and Brenda Andrews. 2006. ‘Characterization of the Yeast Amphiphysins Rvs161p and Rvs167p Reveals Roles for the Rvs Heterodimer In Vivo’. *Molecular Biology of the Cell* 17 (3): 1306–21. https://doi.org/10.1091/mbc.E05-06-0476.

Galli, Valentina, Rafael Sebastian, Sandrine Moutel, Jason Ecard, Franck Perez, and Aurélien Roux. 2017. ‘Uncoupling of Dynamin Polymerization and GTPase Activity Revealed by the Conformation-Specific Nanobody Dynab’. *ELife* 6 (October): e25197. https://doi.org/10.7554/eLife.25197.

Goud Gadila, Shiva Kumar, Michelle Williams, Uma Saimani, Mariel Delgado Cruz, Pelin Makaraci, Sara Woodman, John C.W. Short, Hyoeun McDermott, and Kyoungtae Kim. 2017. ‘Yeast Dynamin Vps1 Associates with Clathrin to Facilitate Vesicular Trafficking and Controls Golgi Homeostasis’. *European Journal of Cell Biology* 96 (2): 182–97. https://doi.org/10.1016/J.EJCB.2017.02.004.

Grabs, D, V I Slepnev, Z Songyang, C David, M Lynch, L C Cantley, and P De Camilli. 1997. ‘The SH3 Domain of Amphiphysin Binds the Proline-Rich Domain of Dynamin at a Single Site That Defines a New SH3 Binding Consensus Sequence.’ *The Journal of Biological Chemistry* 272 (20): 13419–25. https://doi.org/10.1074/JBC.272.20.13419.

Grigliatti, Thomas A, Linda Hall, Raja Rosenbluth, and David T Suzuki. 1973. ‘Temperature-Sensitive Mutations in Drosophila Melanogaster XIV. A Selection of Immobile Adults \*’. *Molec. Gen. Genet* 120: 107–14. https://link.springer.com/content/pdf/10.1007%2FBF00267238.pdf.

Hoepfner, Dominic, Marlene van den Berg, Peter Philippsen, Henk F. Tabak, and Ewald H. Hettema. 2001. ‘A Role for Vps1p, Actin, and the Myo2p Motor in Peroxisome Abundance and Inheritance in *Saccharomyces Cerevisiae*’. *The Journal of Cell Biology* 155 (6): 979–90. https://doi.org/10.1083/jcb.200107028.

Kaksonen, Marko, and Aurélien Roux. 2018. ‘Mechanisms of Clathrin-Mediated Endocytosis’. *Nature Reviews Molecular Cell Biology* 19 (5): 313–26. https://doi.org/10.1038/nrm.2017.132.

Kaksonen, Marko, Yidi Sun, and David G. Drubin. 2003. ‘A Pathway for Association of Receptors, Adaptors, and Actin during Endocytic Internalization’. *Cell* 115 (4): 475–87. https://doi.org/10.1016/S0092-8674(03)00883-3.

Kaksonen, Marko, Christopher P. Toret, and David G. Drubin. 2005. ‘A Modular Design for the Clathrin- and Actin-Mediated Endocytosis Machinery’. *Cell* 123 (2): 305–20. https://doi.org/10.1016/j.cell.2005.09.024.

Kishimoto, T., Y. Sun, C. Buser, J. Liu, A. Michelot, and D. G. Drubin. 2011. ‘Determinants of Endocytic Membrane Geometry, Stability, and Scission’. *Proceedings of the National Academy of Sciences* 108 (44): E979–88. https://doi.org/10.1073/pnas.1113413108.

Kishimoto, Takuma, Yidi Sun, Christopher Buser, Jian Liu, Alphee Michelot, and David G. Drubin. 2011. ‘Determinants of Endocytic Membrane Geometry, Stability, and Scission’. *Proceedings of the National Academy of Sciences of the United States of America* 108 (44): E979–88. https://doi.org/10.1073/pnas.1113413108.

Kübler, E, and H Riezman. 1993. ‘Actin and Fimbrin Are Required for the Internalization Step of Endocytosis in Yeast.’ *The EMBO Journal* 12 (7): 2855–62. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC413538/.

Kukulski, Wanda, Martin Schorb, Marko Kaksonen, and John A. G. Briggs. 2012. ‘Plasma Membrane Reshaping during Endocytosis Is Revealed by Time-Resolved Electron Tomography’. *Cell* 150 (3): 508–20. https://doi.org/10.1016/j.cell.2012.05.046.

Lila, T, and D G Drubin. 1997. ‘Evidence for Physical and Functional Interactions among Two Saccharomyces Cerevisiae SH3 Domain Proteins, an Adenylyl Cyclase-Associated Protein and the Actin Cytoskeleton.’ *Molecular Biology of the Cell* 8 (2): 367–85. http://www.ncbi.nlm.nih.gov/pubmed/9190214.

Liu, Jian, Yidi Sun, David G. Drubin, and George F. Oster. 2009. ‘The Mechanochemistry of Endocytosis’. *PLOS Biol* 7 (9): e1000204. https://doi.org/10.1371/journal.pbio.1000204.

Madania, Ammar, Pascal Dumoulin, Sandrine Grava, Hiroko Kitamoto, Claudia Schärer-Brodbeck, Alexandre Soulard, Violaine Moreau, and Barbara Winsor. 1999. ‘The *Saccharomyces Cerevisiae* Homologue of Human Wiskott–Aldrich Syndrome Protein Las17p Interacts with the Arp2/3 Complex’. Edited by David Drubin. *Molecular Biology of the Cell* 10 (10): 3521–38. https://doi.org/10.1091/mbc.10.10.3521.

Meinecke, Michael, Emmanuel Boucrot, Gamze Camdere, Wai-Ching Hon, Rohit Mittal, and Harvey T McMahon. 2013. ‘Cooperative Recruitment of Dynamin and BIN/Amphiphysin/Rvs (BAR) Domain-Containing Proteins Leads to GTP-Dependent Membrane Scission.’ *The Journal of Biological Chemistry* 288 (9): 6651–61. https://doi.org/10.1074/jbc.M112.444869.

Moustaq, Laila, Iwona I Smaczynska-de Rooij, Sarah E Palmer, Christopher J Marklew, and Kathryn R Ayscough. 2016. ‘Insights into Dynamin-Associated Disorders through Analysis of Equivalent Mutations in the Yeast Dynamin Vps1.’ *Microbial Cell (Graz, Austria)* 3 (4): 147–58. https://doi.org/10.15698/mic2016.04.490.

Munn, A L, B J Stevenson, M I Geli, and H Riezman. 1995. ‘End5, End6, and End7: Mutations That Cause Actin Delocalization and Block the Internalization Step of Endocytosis in Saccharomyces Cerevisiae.’ *Molecular Biology of the Cell* 6 (12): 1721–42. https://doi.org/10.1091/MBC.6.12.1721.

Nannapaneni, Srikant, Daobing Wang, Sandhya Jain, Blake Schroeder, Chad Highfill, Lindsay Reustle, Delilah Pittsley, et al. 2010. ‘The Yeast Dynamin-like Protein Vps1:Vps1 Mutations Perturb the Internalization and the Motility of Endocytic Vesicles and Endosomes via Disorganization of the Actin Cytoskeleton’. *European Journal of Cell Biology* 89 (7): 499–508. https://doi.org/10.1016/j.ejcb.2010.02.002.

Pearse, B M. 1976. ‘Clathrin: A Unique Protein Associated with Intracellular Transfer of Membrane by Coated Vesicles.’ *Proceedings of the National Academy of Sciences of the United States of America* 73 (4): 1255–59. http://www.ncbi.nlm.nih.gov/pubmed/1063406.

Peters, Christopher, Tonie L Baars, Susanne Bühler, and Andreas Mayer. 2004. ‘Mutual Control of Membrane Fission and Fusion Proteins.’ *Cell* 119 (5): 667–78. https://doi.org/10.1016/j.cell.2004.11.023.

Picco, Andrea, Markus Mund, Jonas Ries, François Nédélec, and Marko Kaksonen. 2015. ‘Visualizing the Functional Architecture of the Endocytic Machinery’. *ELife*, February, e04535. https://doi.org/10.7554/eLife.04535.

ROTH, T F, and K R PORTER. 1964. ‘YOLK PROTEIN UPTAKE IN THE OOCYTE OF THE MOSQUITO AEDES AEGYPTI. L.’ *The Journal of Cell Biology* 20 (February): 313–32. http://www.ncbi.nlm.nih.gov/pubmed/14126875.

Rothman, Joel H., Christopher K. Raymond, Teresa Gilbert, Patrick J. O’Hara, and Tom H. Stevens. 1990. ‘A Putative GTP Binding Protein Homologous to Interferon-Inducible Mx Proteins Performs an Essential Function in Yeast Protein Sorting’. *Cell* 61 (6): 1063–74. https://doi.org/10.1016/0092-8674(90)90070-U.

Shupliakov, O, P Löw, D Grabs, H Gad, H Chen, C David, K Takei, P De Camilli, and L Brodin. 1997. ‘Synaptic Vesicle Endocytosis Impaired by Disruption of Dynamin-SH3 Domain Interactions.’ *Science (New York, N.Y.)* 276 (5310): 259–63. http://www.ncbi.nlm.nih.gov/pubmed/9092476.

Simunovic, Mijo, Jean Baptiste Manneville, Henri François Renard, Emma Evergren, Krishnan Raghunathan, Dhiraj Bhatia, Anne K. Kenworthy, et al. 2017. ‘Friction Mediates Scission of Tubular Membranes Scaffolded by BAR Proteins’. *Cell* 170 (1): 172-184.e11. https://doi.org/10.1016/j.cell.2017.05.047.

Sivadon, P, M Crouzet, and M Aigle. 1997. ‘Functional Assessment of the Yeast Rvs161 and Rvs167 Protein Domains.’ *FEBS Letters* 417 (1): 21–27. https://doi.org/10.1016/s0014-5793(97)01248-9.

Smaczynska-de Rooij, Iwona I., Ellen G. Allwood, Ritu Mishra, Wesley I. Booth, Soheil Aghamohammadzadeh, Martin W. Goldberg, and Kathryn R. Ayscough. 2012. ‘Yeast Dynamin Vps1 and Amphiphysin Rvs167 Function Together During Endocytosis’. *Traffic* 13 (2): 317–328. https://doi.org/10.1111/j.1600-0854.2011.01311.x.

Sweitzer, Sharon M, and Jenny E Hinshaw. 1998. ‘Dynamin Undergoes a GTP-Dependent Conformational Change Causing Vesiculation’. *Cell* 93 (6): 1021–29. https://doi.org/10.1016/S0092-8674(00)81207-6.

Takei, Kohji, Peter S. McPherson, Sandra L. Schmid, and Pietro De Camilli. 1995. ‘Tubular Membrane Invaginations Coated by Dynamin Rings Are Induced by GTP-ΓS in Nerve Terminals’. *Nature* 374 (6518): 186–90. https://doi.org/10.1038/374186a0.

Youn, Ji-Young, Helena Friesen, Takuma Kishimoto, William M. Henne, Christoph F. Kurat, Wei Ye, Derek F. Ceccarelli, et al. 2010. ‘Dissecting BAR Domain Function in the Yeast Amphiphysins Rvs161 and Rvs167 during Endocytosis’. *Molecular Biology of the Cell* 21 (17): 3054–69. https://doi.org/10.1091/mbc.E10-03-0181.

Yu, X. 2004. ‘The Yeast Dynamin-Related GTPase Vps1p Functions in the Organization of the Actin Cytoskeleton via Interaction with Sla1p’. *Journal of Cell Science* 117 (17): 3839–53. https://doi.org/10.1242/jcs.01239.

Zhang, Peijun, and Jenny E. Hinshaw. 2001. ‘Three-Dimensional Reconstruction of Dynamin in the Constricted State’. *Nature Cell Biology* 3 (10): 922–26. https://doi.org/10.1038/ncb1001-922.

Zhao, Wei-Dong, Edaeni Hamid, Wonchul Shin, Peter J Wen, Evan S Krystofiak, Seth A Villarreal, Hsueh-Cheng Chiang, Bechara Kachar, and Ling-Gang Wu. 2016. ‘Hemi-Fused Structure Mediates and Controls Fusion and Fission in Live Cells.’ *Nature* 534 (7608): 548–52. https://doi.org/10.1038/nature18598.