

# Regulation of membrane scission in yeast endocytosis

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**Abstract** This is not going to elife

## Introduction (Level 1 heading)

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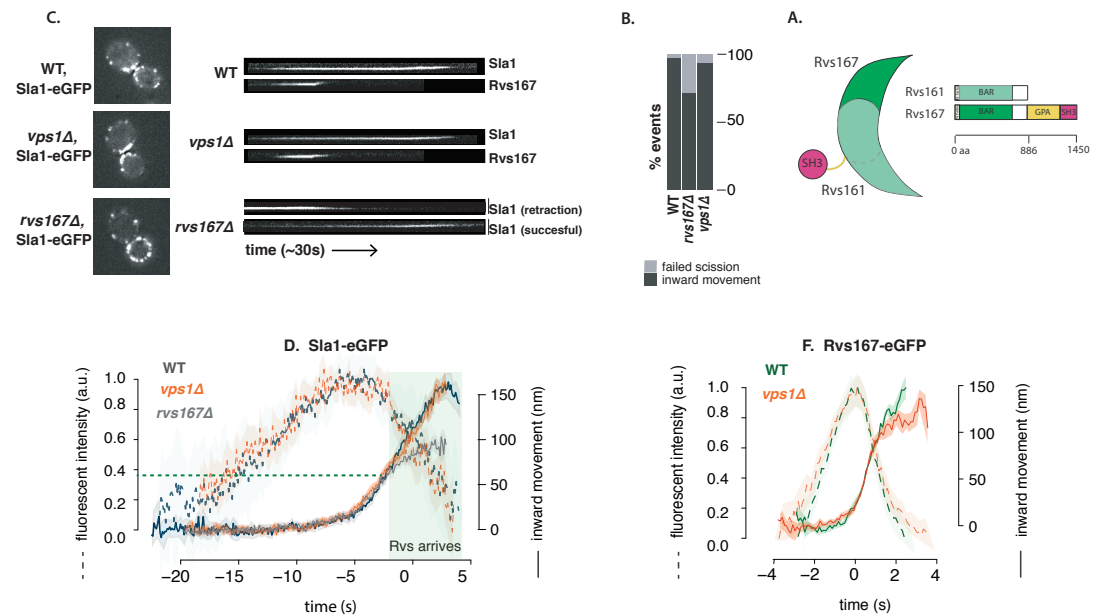
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## Results

**Vps1 does not influence coat or scission dynamics. Synaptojanins likely influence vesicle uncoating, but not scission dynamics.**

Endocytic membrane scission in mammalian cells is understood to be driven by constriction of the tubule neck by the Gtpase Dynamin (a bunch of dynamin papers). In yeast, it has been reported that the Dynamin-like protein Vps1 is recruited to endocytic sites (refAyscough). To test whether Vps1 influences scission, endocytic coat dynamics are observed in cells lacking Vps1 and compared against WT cells. Fig1a shows kymographs of coat protein Sla1 endogenously tagged at the N-terminus with eGFP in WT and *vps1Δ* cells. Dynamics of Sla1 in WT and *vps1Δ* cells are the same. In Fig.1b, the averaged centroid trajectory (ref2andrea)- henceforth centroid- of Sla1-eGFP is tracked in 50 endocytic sites in *vps1Δ* and wild-type cells. Inward movement of Sla1 centroid serves as a proxy for plasma membrane movement through the endocytic process (ref2andrea).  $y=0$  is set as the position of arrival of the protein complex, before inward movement begins. Centroid movement of Sla1-eGFP in wild-type cells shows a linear movement to about 150nm, and Sla1 movement in *vps1Δ* cells is the same.

Centroid tracking has shown that the number of yeast N-BAR protein Rvs167 peaks at the time of scission, and is followed by an rapid loss of fluorescent intensity, concomitant with a sharp jump of the centroid into the cytoplasm. This jump, also seen in Rvs167-GFP kymographs, is interpreted as loss of protein on the invagination on the membrane tube, causing an apparent spatial jump to the protein localized at the base of the newly formed vesicle. Kymographs of Rvs167-GFP in Vps1 deleted cells, show the same jump, indicating that vesicles are formed in the same position in Vps1



**Figure 1.** A half-columnwidth image using wrapfigure, to be used sparingly. Note that using a wrap figure before a sectional heading, near other floats or page boundaries is not recommended, as it may cause interesting layout issues. Use the optional argument to wrapfigure to control how many lines of text should be set half-width alongside it.

deletion cells as in WT cells. Hence, although Vps1 deletion leads to a growth defect at 37C (as has been shown before, supplementary Fig.1), lack of Vps1 protein does not influence the endocytic process.

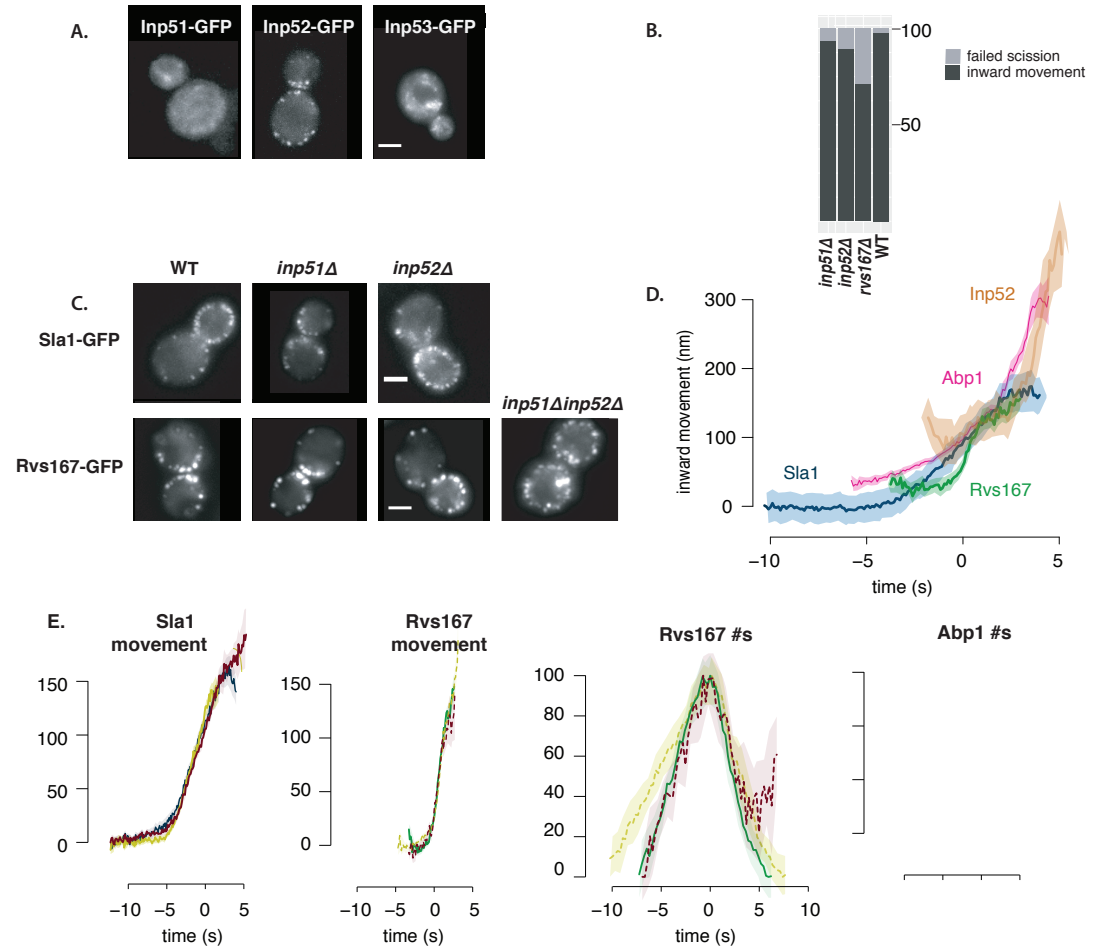
### Synaptojanins likely influence vesicle uncoating, but not scission dynamics.

As an alternate to forces from Dynamin constriction, Liu et al (refliu) have proposed that an interaction between PIP2-hydrolyzing Synaptojanins and BAR proteins could drive membrane scission. Here Rvs BAR domains would form a scaffold on the membrane tube, preventing hydrolysis of underlying PIP2. Synaptojanin would arrive at invaginated membranes, and hydrolyse unprotected PIP2. This generates a lipid boundary between BAR-protected PIP2 at the tube and hydrolyzed PIP2 at the bud tip. A line tension thus formed at the interphase between the two lipid types would then generate enough force to pinch off a vesicle.

Of the three Synaptojanin-like proteins in yeast- Inp51, Inp52 and Inp53- Inp51 exhibits a diffuse cytoplasmic signal. Inp53 localizes to patches within the cytoplasm- cellular localization is consistent with involvement in trans-Golgi signalling (refGolgi). Inp53 was not investigated further. Inp52 localizes to cortical actin patches that are endocytic sites. Two channel alignment shows that Inp52 patches arrive in the late scission stage, and localizes to the bud tip, consistent with a role in membrane scission.

Role of Inp51 and Inp52 are tested by following Sla1-GFP and Rvs167-GFP in cells with either Inp51, Inp52, or both deleted. Retraction events do not significantly increase compared to the WT in either *inp51del* or *inp52del* cells. Magnitude and speed of coat movement in *inp51del* is the same as the WT. In *inp52del* cells, coat movement also has the same magnitude and speed, but GFP signal is persistent after membrane scission. This delay in decrease of Sla1-GFP signal is consistent with delay in vesicle uncoating rather than membrane scission. Similarly, Rvs167 disassembly has a delay, while the assembly is similar to WT, indicating a delay in removing endocytic proteins from the newly formed vesicle. Assembly of Rvs167 has a delay in *inp51del* cells, which could indicate a defect in recruiting proteins to endocytic sites, or in progression of endocytic invaginations. Since

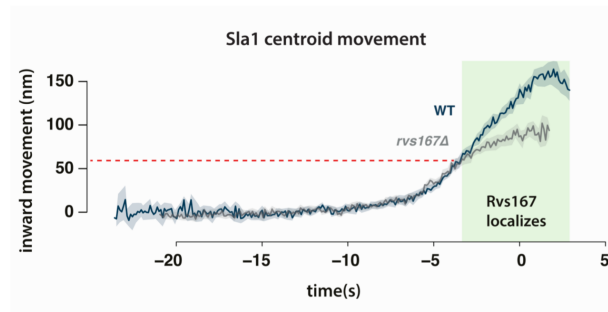
68 Sla1 movement is the same, we suggest a defect in the former rather than latter.



**Figure 2.** A half-columnwidth image using wrapfigure, to be used sparingly. Note that using a wrapfigure before a sectional heading, near other floats or page boundaries is not recommended, as it may cause interesting layout issues. Use the optional argument to wrapfigure to control how many lines of text should be set half-width alongside it.

## 69 Rvs deletion reduces coat movement

70 The Rvs complex itself is known to influence scission: deletion reduces scission efficiency by 30%  
 71 (ref1marko). Failed scission events are characterized by inward movement, followed by retraction  
 72 of the coat protein Sla1 (ref1marko). Contribution of Rvs to the scission process however, is  
 73 currently unclear. In the remaining 70% of successful invaginations, inward movement of the  
 74 coat protein Sla1 also deviates from the wild-type. In Fig.1, the averaged centroid trajectory  
 75 (ref2andrea) of Sla1-eGFP is tracked in rvs167deletion and wild-type cells. Time alignment is  
 76 established by tracking the centroid of a second protein, here m-Cherry tagged Actin binding  
 77 protein Abp1. Simultaneous tracking of GFP-tagged protein of interest and m-Cherry tagged  
 78 Abp1 allows us to align all other proteins to the Abp1 reference centroid (ref2andrea). Time=0, is  
 79 established as the peak of the Abp1 fluorescence intensity, which in wild-type is concomitant with  
 80 the peak of Rvs167 fluorescent intensity (ref2andrea, ref3wanda), and is time in which scission  
 81 occurs. Sla1 centroid in rvs167deletion cells follows the wild-type centroid movement till about  
 82 60nm, after which movement slows down and scission occurs. That scission occurs at shorter  
 83 invaginations lengths is confirmed by formation of smaller vesicles and shorter invagination lengths  
 84 in rvs167deletion cells, quantified by Correlative light and electron microscopy (CLEM) (ref3wanda).



**Figure 3.** A half-columnwidth image using wrapfigure, to be used sparingly. Note that using a wrapfigure before a sectional heading, near other floats or page boundaries is not recommended, as it may cause interesting layout issues. Use the optional argument to wrapfigure to control how many lines of text should be set half-width alongside it.

85 Invagination lengths of 60nm is the time window for arrival of Rvs167 (ref3wanda), indicating that  
 86 coat movement of endocytic sites in *rvs167*deletion cells progresses normally till the expected  
 87 arrival of Rvs.

### 88 **Rvs BAR domains recognize membrane curvature in-vivo**

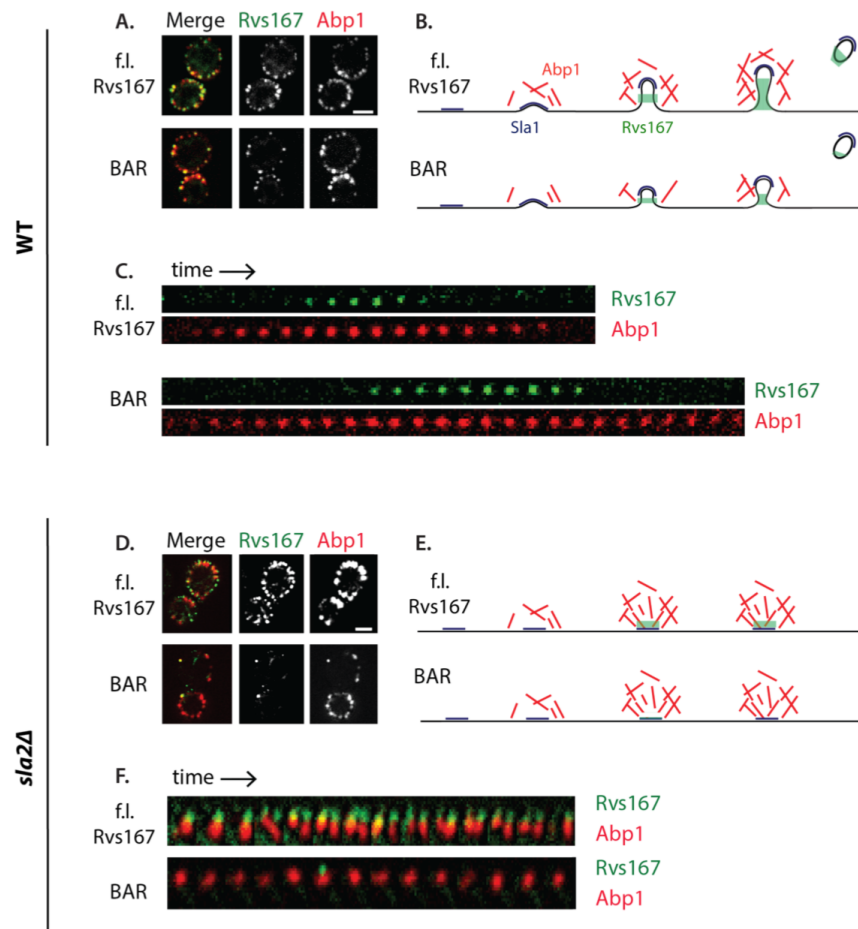
89 The curved tertiary structure and liposome binding assays of N-BAR domains have suggested  
 90 that they may have a preference for curved membrane that match their own intrinsic curvature.  
 91 Alternately, they may also impose their curvature on flat membrane and induce curvature formation.  
 92 In-vivo, the curvature interaction of Rvs167 has not been tested. In order to do so, we delete the SH3  
 93 domain of Rvs167 observe the localization of endogenously tagged Rvs167-eGFP and BAR-eGFP and  
 94 Abp1-mCherry in WT and *sla2*deletion cells. Sla2 acts as the molecular linker between forces exerted  
 95 by the actin network and the plasma membrane. Sla2deletion cells therefore have a polymerizing  
 96 actin network, but the membrane remains flat and endocytosis fails. In these cells, the full-length  
 97 Rvs167 protein co-localizes with Abp1-mCherry, indicating that it is recruited to endocytic sites.  
 98 BAR-eGFP localization is removed, except for rare transient patches that do not co-localize with  
 99 Abp1-mCherry.

### 100 **Rvs SH3 domains contribute to curvature independent localization**

101 We have shown that BAR domains need membrane curvature to localize. Full-length Rvs167,  
 102 however, is recruited to endocytic patches in *sla2*deletion cells. This indicates that a second  
 103 interaction, that is not the BAR-curvature dependent, recruits the protein to endocytic sites. This  
 104 interaction must come from the SH3 region, showing that Rvs localization is dependent on both  
 105 BAR as well as SH3 domain interactions. Absence of the SH3 domain also reduces total recruitment  
 106 of Rvs and Abp1 protein, giving the SH3 domain an important and surprising role in regulating the  
 107 late stage of endocytosis.

### 108 **SH3 domains are recruited by Myosin 5**

109 SH3 domains have been shown to interact with several proteins in the actin module of endocytosis:  
 110 Las17, type I myosins, and Vrp1 all have genetic or physical interactions with Rvs167 SH3 domains  
 111 (Lila and Drubin, 1997; Colwill et al., 1999; Madania et al., 1999; Liu et al., 2009). We tested the  
 112 interaction by studying the localization of full-length Rvs167 in cells with one of these proteins  
 113 deleted, and treated with Las17 to reproduce the situation in which BAR-curvature interaction  
 114 is removed. As seen in (some figure), deletion of neither Las17 nor Myo3 in combination with  
 115 LatA treatment does not remove the localization of Rvs167. Deletion of Vrp1 and Myo5, with LatA  
 116 treatment removes localization of Rvs167. Since Vrp1 is required for the recruitment of Myo5  
 117 (refMyo5), SH3 domains interact with Myo5 rather than Vrp1.



**Figure 4.** A half-columnwidth image using wrapfigure, to be used sparingly. Note that using a wrapfigure before a sectional heading, near other floats or page boundaries is not recommended, as it may cause interesting layout issues. Use the optional argument to wrapfigure to control how many lines of text should be set half-width alongside it.

118 what about the differences in myo5 and myo3 number... if the Rvs recruitment only  
119 slightly depended on myo3 we probably wouldnt see a difference

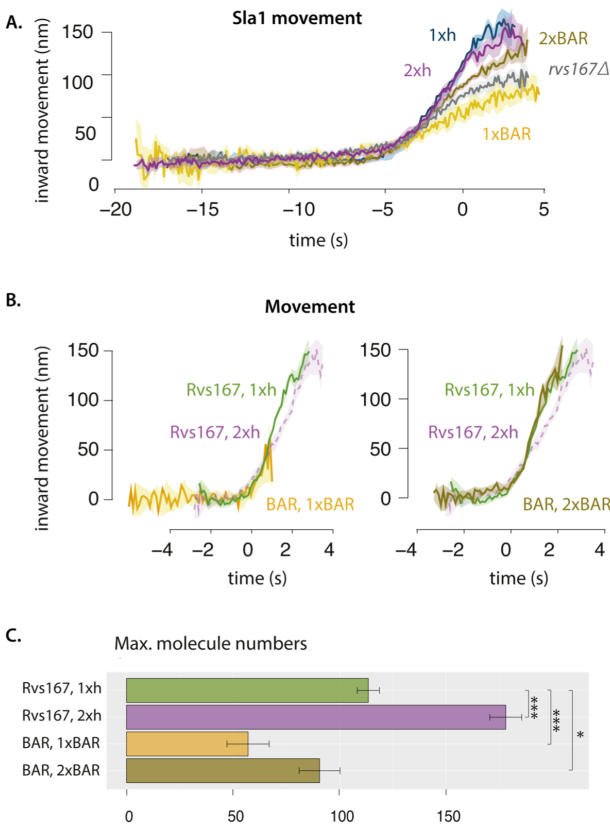
#### 120 **N-helix and GPA domains do not contribute to recruitment of Rvs or membrane** 121 **movement**

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#### 128 **Increased BAR domain recruitment corresponds to increased membrane move-** 129 **ment**

130 The decreased Sla1 movement in BAR cells can be explained by a loss of interaction of the SH3  
131 domain, or by reduced recruitment of the BAR domains. To check whether increasing the recruit-  
132 ment of the Rvs complex alone can rescue reduced Sla1 movement, the Rvs167 and Rvs161 ORF

133 was duplicated endogenously (ref Huber dude) in diploid and haploid yeast cells. In diploid cells,  
134 Rvs duplication results in either 4x copies of both Rvs genes, 2x copies (WT diploid) or 1x copies, in  
135 which one gene of Rvs167 and Rvs161 are deleted. We show that amount of Rvs167 recruited to  
136 sites increases linearly, without changing either the rate of movement or total movement before  
137 scission of Sla1. Similarly, in haploid cells, increasing the gene copy of Rvs167 and Rvs161 results in  
138 increased recruitment of Rvs167, without influencing the dynamics of Sla1. Expressing two instead  
139 of one copy of the Rvs167 BAR domain alone rescues the loss of Sla1 movement in the 1x copy  
140 of BAR domain alone, as well the inward jump of BAR-GFP itself. The loss of inward movement in  
141 1xBAR suggests that smaller vesicles are produced in these cells, confirmed by CLEM. This would in  
142 corollary indicate that the increased inward movement in 2xBAR produces WT-sized vesicles.



**Figure 5.** A half-columnwidth image using wrapfigure, to be used sparingly. Note that using a wrapfigure before a sectional heading, near other floats or page boundaries is not recommended, as it may cause interesting layout issues. Use the optional argument to wrapfigure to control how many lines of text should be set half-width alongside it.

143 **Discussion**

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## Methods and Materials

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