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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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, coat dynamics are observed in cells lacking Vps1 (left, Fig.1) and compared against WT cells (right, Fig.1). Centroid tracking various endocytic proteins in yeast has shown that the centroid of the 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 into the cytoplasm. This jump is interpreted as loss of protein on the invagination on the membrane tube, to the fluorescent intensity at the base of the newly formed vesicle. Kymographs of Rvs167-GFP in Vps1 deleted cells however, show the same jump, indicating that vesicles are formed in the same position in Vps1 deletion cells as in WT cells. Vps1 deletion leads to a growth defect at 37C as has been shown before (supplementary Fig.1) Fig1a shows kymographs of coat protein Sla1 endogenously tagged at the N-terminus with eGFP in WT and *vps1*Δ cells. Inward movement of Sla1 in WT and vps1del cells are the same. In Fig.1b, the averaged centroid trajectory (ref2andrea)- henceforth centroid- of Sla1 endogenously tagged at the C-terminus with eGFP is tracked in 50 endocytic sites in vps1deletion and wild-type cells. Beginning of inward movement of the centroid is set as the initial position of averaged centroid. Inward movement of Sla1 centroid serves as a proxy for plasma membrane movement through the endocytic process (ref2andrea). Centroid movement

of Sla1-eGFP in wild-type cells shows a linear movement to about 150nm, and Sla1 movement in vps1del cells is the same. Position of the vesicle formed at the end of scission process is determined from the final position of the Rvs167 centroid (ref2andrea). Rvs167 centroid position in WT and vps1-del cells indicates that vesicle formation is the same as in WT cells.

Synaptojanins likely influence vesicle uncoating, but not scission dynamics.

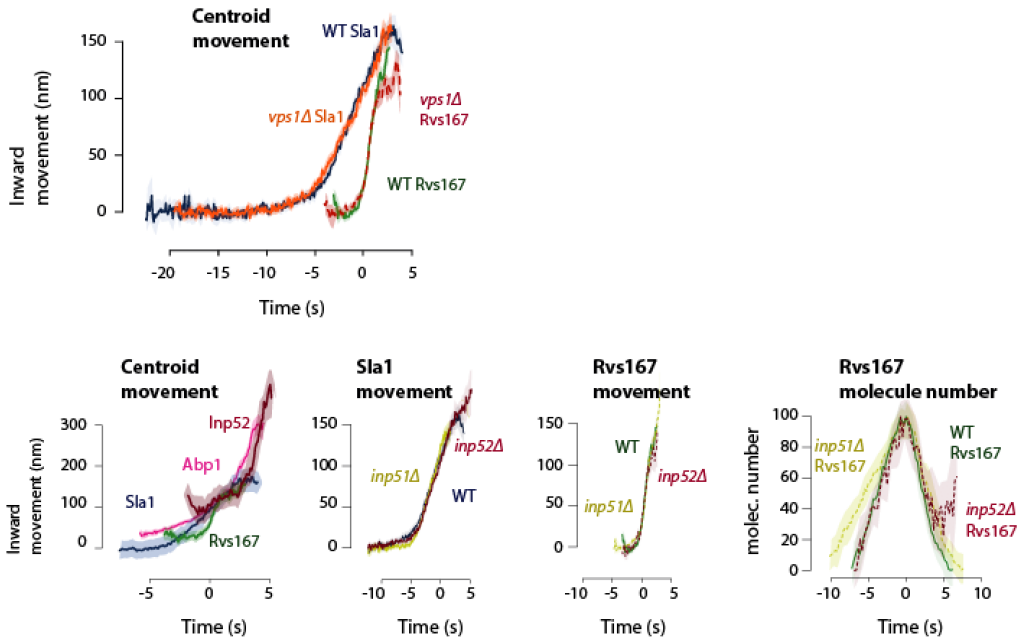
Liu et al (refliu) have proposed that an interaction between PiP2-hyrdolyzing Synaptojanins and BAR proteins could drive membrane scission. Here, Rvs BAR domains would form a scaffold on the membrane tube, preventing hydrolysis of underlying PIP2. Synaptojanin would arrive at inavaginated membranes, 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(refGolgi)- cellular localization is consistent with involvement in trans-Golgi signalling, and is taken to indicate lack of involvement in endocytosis, shown also in other studies (refother studies). 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 persistant after membrane scission. This delay in decrease of Sla1-GFP signal is consistent with delay in vesicle uncoating rather than membrane scissison. 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 inp51deleted cells, which could indicate a defect in recruiting proteins to endocytic sites, or in progression of endocytic invaginations. Since Sla1 movement is the same, we suggest a defect in the former rather than latter.

Rvs deletion reduces coat movement

The Rvs complex is known to influence scission: deletion reduces scission efficiency by 30% (ref1marko). Failed scission events are characterized by inward movement, followed by retraction of the coat protein Sla1 (ref1marko). Contribution of Rvs to the scission process, and therefore, an understanding of why its absence might cause a scission defect, is currently unclear. In the remaining 70% of successful invaginations, inward movement of the coat protein Sla1 also deviates from the wild-type. In Fig.1, the averaged centroid trajectory (ref2andrea)- henceforth centroid- of Sla1 endogenously tagged at the C-terminus with eGFP is tracked in 50 endocytic sites in rvs167deletion and wild-type cells. Beginning of inward movement is set as the initial position of averaged centroid. Inward movement of Sla1 centroid serves as a proxy for plasma membrane movement through the endocytic process (ref2andrea). Time alignment is established by tracking the centroid of a second protein, here m-Cherry tagged Actin binding protein Abp1. Simultaneous tracking of GFP-tagged protein of interest and m-Cherry tagged Abp1 allows us to align all other proteins to the Abp1 reference centroid (ref2andrea). Scission time, t=0, is established as the peak of the Abp1 fluorescence intensity, which in wild-type is concomitant with the peak of Rvs167 fluorescent intensity (ref2andrea, ref3wanda). Centroid movement of Sla1-eGFP in wild-type cells shows a linear movement to about 150nm. Sla1 centroid in rvs167deletion cells follows the wild-type centroid movement till about 60nm, after which movement slows down and scission occurs. That scission occurs at shorter invaginations lengths is confirmed by formation of smaller vesicles and shorter invagination lengths in rvs167deletion cells, quantified by Correlative light



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and electron microscopy (CLEM) (ref3wanda). Invagination lengths of 60nm is the time window for arrival of Rvs167 (ref3wanda), indicating that coat movement of endocytic sites in rvs167deletion cells progresses normally till the expected arrival of Rvs.

Discussion

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Methods and Materials

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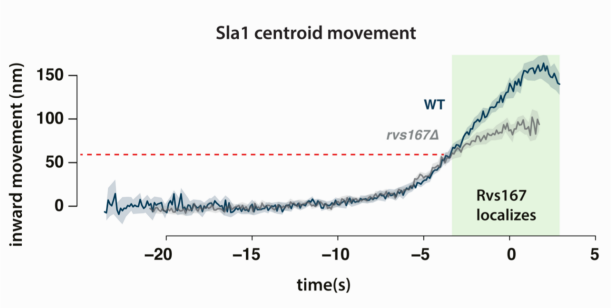

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