

Regulation of membrane scission in yeast endocytosis

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[‡]These authors also contributed equally to this work

Abstract This is not going to elife

Present address: [§]Department, Institute, Country; [¶]Department, Institute, Country

Introduction (Level 1 heading)

Thanks for using Overleaf to write your article. Your introduction goes here! Some examples of commonly used commands and features are listed below, to help you get started.

Here's a second paragraph to test paragraph indents. Lorem ipsum dolor sit amet, consectetur adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetur id, vulputate a, magna. Donec vehicula augue eu neque. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Mauris ut leo. Cras viverra metus rhoncus sem. Nulla et lectus vestibulum urna fringilla ultrices. Phasellus eu tellus sit amet tortor gravida placerat. Integer sapien est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget sem vel leo ultrices bibendum. Aenean faucibus. Morbi dolor nulla, malesuada eu, pulvinar at, mollis ac, nulla. Curabitur auctor semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum.

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 and scission dynamics are observed in cells lacking Vps1. Fig1a shows kymographs of coat protein Sla1 endogenously tagged at the N-terminus with eGFP in WT and vps1del cells. Lifetimes and 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 vps1 deletion 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.

Table 1. Automobile Land Speed Records (GR 5-10).

Speed (mph)	Driver	Car	Engine	Date
407.447	Craig Breedlove	Spirit of America	GE J47	8/5/63
413.199	Tom Green	Wingfoot Express	WE J46	10/2/64
434.22	Art Arfons	Green Monster	GE J79	10/5/64
468.719	Craig Breedlove	Spirit of America	GE J79	10/13/64
526.277	Craig Breedlove	Spirit of America	GE J79	10/15/65
536.712	Art Arfons	Green Monster	GE J79	10/27/65
555.127	Craig Breedlove	Spirit of America, Sonic 1	GE J79	11/2/65
576.553	Art Arfons	Green Monster	GE J79	11/7/65
600.601	Craig Breedlove	Spirit of America, Sonic 1	GE J79	11/15/65
622.407	Gary Gabelich	Blue Flame	Rocket	10/23/70
633.468	Richard Noble	Thrust 2	RR RG 146	10/4/83
763.035	Andy Green	Thrust SSC	RR Spey	10/15/97

Source: https://www.sedl.org/afterschool/toolkits/science/pdf/ast_sci_data_tables_sample.pdf

Table 1-source data 1. This is a description of a data source.

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 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.

Level 2 Heading

Nulla malesuada porttitor diam. Donec felis erat, congue non, volutpat at, tincidunt tristique, libero. Vivamus viverra fermentum felis. Donec nonummy pellentesque ante. Phasellus adipiscing semper elit. Proin fermentum massa ac quam. Sed diam turpis, molestie vitae, placerat a, molestie nec, leo. Maecenas lacinia. Nam ipsum ligula, eleifend at, accumsan nec, suscipit a, ipsum. Morbi blandit ligula feugiat magna. Nunc eleifend consequat lorem. Sed lacinia nulla vitae enim. Pellentesque tincidunt purus vel magna. Integer non enim. Praesent euismod nunc eu purus. Donec bibendum quam in tellus. Nullam cursus pulvinar lectus. Donec et mi. Nam vulputate metus eu enim.

70 Vestibulum pellentesque felis eu massa.

71 Level 3 Heading

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73 ultricies tellus, ac venenatis arcu wisi vel nisl. Vestibulum diam. Aliquam pellentesque, augue quis
74 sagittis posuere, turpis lacus congue quam, in hendrerit risus eros eget felis. Maecenas eget erat in
75 sapien mattis porttitor. Vestibulum porttitor. Nulla facilisi. Sed a turpis eu lacus commodo facilisis.
76 Morbi fringilla, wisi in dignissim interdum, justo lectus sagittis dui, et vehicula libero dui cursus
77 dui. Mauris tempor ligula sed lacus. Duis cursus enim ut augue. Cras ac magna. Cras nulla. Nulla
78 egestas. Curabitur a leo. Quisque egestas wisi eget nunc. Nam feugiat lacus vel est. Curabitur
79 consectetur.

80 Level 4 Heading

81 Sed commodo posuere pede. Mauris ut est. Ut quis purus. Sed ac odio. Sed vehicula hendrerit
82 sem. Duis non odio. Morbi ut dui. Sed accumsan risus eget odio. In hac habitasse platea dictumst.
83 Pellentesque non elit. Fusce sed justo eu urna porta tincidunt. Mauris felis odio, sollicitudin sed,
84 volutpat a, ornare ac, erat. Morbi quis dolor. Donec pellentesque, erat ac sagittis semper, nunc dui
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86 ad litora torquent per conubia nostra, per inceptos hymenaeos. Praesent sapien turpis, fermentum
87 vel, eleifend faucibus, vehicula eu, lacus.

88 Discussion

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92 Maecenas sed mi eget dui varius euismod. Phasellus aliquet volutpat odio. Vestibulum ante ipsum
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95 ac pretium ante justo a nulla. Curabitur tristique arcu eu metus. Vestibulum lectus. Proin mauris.
96 Proin eu nunc eu urna hendrerit faucibus. Aliquam auctor, pede consequat laoreet varius, eros
97 tellus scelerisque quam, pellentesque hendrerit ipsum dolor sed augue. Nulla nec lacus.

98 Methods and Materials

99 Guidelines can be included for standard research article sections, such as this one.

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101 libero. Vivamus viverra fermentum felis. Donec nonummy pellentesque ante. Phasellus adipiscing
102 semper elit. Proin fermentum massa ac quam. Sed diam turpis, molestie vitae, placerat a, molestie
103 nec, leo. Maecenas lacinia. Nam ipsum ligula, eleifend at, accumsan nec, suscipit a, ipsum.
104 Morbi blandit ligula feugiat magna. Nunc eleifend consequat lorem. Sed lacinia nulla vitae enim.
105 Pellentesque tincidunt purus vel magna. Integer non enim. Praesent euismod nunc eu purus.
106 Donec bibendum quam in tellus. Nullam cursus pulvinar lectus. Donec et mi. Nam vulputate metus
107 eu enim. Vestibulum pellentesque felis eu massa.

108 Some \LaTeX Examples

109 Use section and subsection commands to organize your document. \LaTeX handles all the formatting
110 and numbering automatically. Use ref and label commands for cross-references.

111 Figures and Tables

112 Use the table and tabular commands for basic tables — see [Table 1](#), for example.

113 You can upload a figure (JPEG, PNG or PDF) using the project menu. To include it in your
114 document, use the `\includegraphics` command as in the code for [Figure 3](#).

115 For a half-width figure or table with text wrapping around it, use

```
116 \begin{wrapfigure}{l}{.46\textwidth}  
117 \includegraphics[width=\hsize]{...}  
118 \caption{...}\label{...}  
119 \end{wrapfigure}
```

120 as in **Figure 1**. For tables:

```
121 \begin{wraptable}{l}{.46\textwidth}{  
122 \begin{tabular}{...}  
123 ...  
124 \end{tabular}}  
125 \caption{...}\label{...}  
126 \end{wraptable}
```

127 Be careful with these, though, as they may behave strangely near page boundaries, sectional
128 headings, or in the neighbourhood of lists or too many floats.

129 Labels for main videos can be added with `\video` e.g.

130 **Video 1**. This is a description of a main video.

131 **Video 2**. Another!

132 Labels for video supplements can be added within `figure` environments, after the `caption`,
133 using the `\videosupp` command: see **Figure 3–video 1** for an example.

134 If you use the following prefixes for your `\label`:

```
135 Figures fig:, e.g. \label{fig:view}  
136 Figure Supplements figsupp:, e.g. \label{figsupp:sf1}  
137 (we'll assume figsupp:sf1 is a figure supplement of fig:view in our example)  
138 Figure source data figdata:, e.g. \label{figdata:first}  
139 Videos video:, e.g. \label{video:mv1}  
140 Video supplements videosupp:, e.g. \label{videosupp:sv1}  
141 Tables tab:, e.g. \label{tab:example}  
142 Equations eq:, e.g. \label{eq:CLT}  
143 Boxes box:, e.g. \label{box:simple}
```

144 you can then use the convenience commands `\FIG{view}`, `\FIGSUPP[view]{sf1}`, `\TABLE{example}`,
145 `\EQ{CLT}`, `\BOX{simple}`, `\FIGDATA[view]{first}`, `\VIDEO{mv1}` and `\{VIDEOSUPP\}[view]{sv1}` *with-*
146 *out* the label prefixes, to generate cross-references **Figure 3**, **Figure 3–Figure Supplement 1**, **Table 1**,
147 **Equation 1**, **Box 1**, **Figure 3–source data 1**, **Video 1** and **Figure 3–video 1**. Alternatively, use `\autoref`
148 with the full label, e.g. **Appendix 1** (although this may not work correctly for figures and tables in
149 the appendices or boxes nor supplements at present).

150 Really wide figures or tables, that take up the entire page, including the gutter space: use
151 `\begin{fullwidth}... \end{fullwidth}` as in **Figure 2**. And sometimes you may want to use feature
152 boxes like **Box 1**.

153
154
155 **Figure 1**. A half-columnwidth image using
156 `wrapfigure`, to be used sparingly. Note that using a
157 `wrapfigure` before a sectional heading, near other
158 floats or page boundaries is not recommended, as
159 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.

Some filler text to sit alongside the half-width
figure. Lorem ipsum dolor sit amet, consectetur
adipiscing elit. Ut purus elit, vestibulum ut, plac-
erat ac, adipiscing vitae, felis. Curabitur dictum
gravida mauris. Nam arcu libero, nonummy eget,
consectetur id, vulputate a, magna. Donec vehic-
ula augue eu neque. Pellentesque habitant morbi

Figure 2. A very wide figure that takes up the entire page, including the gutter space.

Figure 2–Figure supplement 1. There is no limit on the number of Figure Supplements for any one primary figure. Each figure supplement should be clearly labelled, Figure 1–Figure Supplement 1, Figure 1–Figure Supplement 2, Figure 2–Figure Supplement 1 and so on, and have a short title (and optional legend). Figure Supplements should be referred to in the legend of the associated primary figure, and should also be listed at the end of the article text file.

160 tristique senectus et netus et malesuada fames
161 ac turpis egestas. Mauris ut leo. Cras viverra
162 metus rhoncus sem. Nulla et lectus vestibulum
163 urna fringilla ultrices. Phasellus eu tellus sit amet
164 tortor gravida placerat. Integer sapien est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget
165 sem vel leo ultrices bibendum. Aenean faucibus. Morbi dolor nulla, malesuada eu, pulvinar at,
166 mollis ac, nulla. Curabitur auctor semper nulla. Donec varius orci eget risus. Duis nibh mi, congue
167 eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum.
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171 mollis. Suspendisse ut massa. Cras nec ante. Pellentesque a nulla. Cum sociis natoque penatibus
172 et magnis dis parturient montes, nascetur ridiculus mus. Aliquam tincidunt urna. Nulla ullamcorper
173 vestibulum turpis. Pellentesque cursus luctus mauris.

174 Citations

175 LaTeX formats citations and references automatically using the bibliography records in your .bib
176 file, which you can edit via the project menu. Use the `\cite` command for an inline citation, like
177 `?`, and the `\citep` command for a citation in parentheses (`?`). The LaTeX template uses a slightly-
178 modified Vancouver bibliography style. If your manuscript is accepted, the eLife production team
179 will re-format the references into the final published form. *It is not necessary to attempt to format*
180 *the reference list yourself to mirror the final published form.* Please also remember to **delete the line**
181 `\nocite{*}` in the template just before `\bibliography{...}`; otherwise *all* entries from your .bib
182 file will be listed!

199 Mathematics

200 LaTeX is great at typesetting mathematics. Let X_1, X_2, \dots, X_n be a sequence of independent and
201 identically distributed random variables with $E[X_i] = \mu$ and $\text{Var}[X_i] = \sigma^2 < \infty$, and let

$$S_n = \frac{X_1 + X_2 + \dots + X_n}{n} = \frac{1}{n} \sum_i^n X_i \quad (1)$$

202 denote their mean. Then as n approaches infinity, the random variables $\sqrt{n}(S_n - \mu)$ converge in
203 distribution to a normal $\mathcal{N}(0, \sigma^2)$.

204 Nulla malesuada porttitor diam. Donec felis erat, congue non, volutpat at, tincidunt tristique,
205 libero. Vivamus viverra fermentum felis. Donec nonummy pellentesque ante. Phasellus adipiscing
206 semper elit. Proin fermentum massa ac quam. Sed diam turpis, molestie vitae, placerat a, molestie
207 nec, leo. Maecenas lacinia. Nam ipsum ligula, eleifend at, accumsan nec, suscipit a, ipsum.
208 Morbi blandit ligula feugiat magna. Nunc eleifend consequat lorem. Sed lacinia nulla vitae enim.
209 Pellentesque tincidunt purus vel magna. Integer non enim. Praesent euismod nunc eu purus.
210 Donec bibendum quam in tellus. Nullam cursus pulvinar lectus. Donec et mi. Nam vulputate metus
211 eu enim. Vestibulum pellentesque felis eu massa.

212 Other Chemistry Niceties

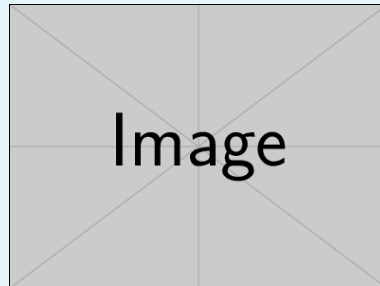
213 You can use commands from the `mhchem` and `siunitx` packages. For example: $\text{C}_{32}\text{H}_{64}\text{NO}_7\text{S}$; 5 μm ;
214 30 °C; $5 \times 10^{-17} \text{ M}$

183

Box 1. This is an example feature box

This is a feature box. It floats!

185



186

Box 1 Figure 1. 'Figure' and 'table' captions in feature boxes should be entered with `\featurefig` and `\featuretable`. They're not really floats.

187

188

189 Lorem ipsum dolor sit amet, consectetur adipiscing elit. Ut purus elit, vestibulum ut, placerat
190 ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget,
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197 eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit
198 amet orci dignissim rutrum.

Figure 3. A text-width example.

Figure 3–Figure supplement 1. Shorter caption for main text.

Figure 3–Figure supplement 2. This is another supplementary figure.

Figure 3–video 1. This is a description of a video supplement.

Figure 3–source data 1. This is a description of a data source.

Figure 3–source data 2. This is another description of a data source.

215 **Lists**

216 You can make lists with automatic numbering ...

- 217 1. Like this,
- 218 2. and like this.

219 ... or bullet points ...

- 220 • Like this,
- 221 • and like this.

222 ... or with words and descriptions ...

223 **Word** Definition

224 **Concept** Explanation

225 **Idea** Text

226 Some filler text, because empty templates look really poorly. Lorem ipsum dolor sit amet,
227 consectetur adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur
228 dictum gravida mauris. Nam arcu libero, nonummy eget, consectetur id, vulputate a, magna.
229 Donec vehicula augue eu neque. Pellentesque habitant morbi tristique senectus et netus et
230 malesuada fames ac turpis egestas. Mauris ut leo. Cras viverra metus rhoncus sem. Nulla et lectus
231 vestibulum urna fringilla ultrices. Phasellus eu tellus sit amet tortor gravida placerat. Integer sapien
232 est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget sem vel leo ultrices bibendum. Aenean
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234 nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam.
235 Duis eget orci sit amet orci dignissim rutrum.

236 **Acknowledgments**

237 Additional information can be given in the template, such as to not include funder information in
238 the acknowledgments section.

Firstly

Lorem ipsum dolor sit amet, consectetur adipiscing elit. Ut purus elit, vestibulum ut, placerat ac, adipiscing vitae, felis. Curabitur dictum gravida mauris. Nam arcu libero, nonummy eget, consectetur id, vulputate a, magna. Donec vehicula augue eu neque. Pellentesque habitant morbi tristique senectus et netus et malesuada fames ac turpis egestas. Mauris ut leo. Cras viverra metus rhoncus sem. Nulla et lectus vestibulum urna fringilla ultrices. Phasellus eu tellus sit amet tortor gravida placerat. Integer sapien est, iaculis in, pretium quis, viverra ac, nunc. Praesent eget sem vel leo ultrices bibendum. Aenean faucibus. Morbi dolor nulla, malesuada eu, pulvinar at, mollis ac, nulla. Curabitur auctor semper nulla. Donec varius orci eget risus. Duis nibh mi, congue eu, accumsan eleifend, sagittis quis, diam. Duis eget orci sit amet orci dignissim rutrum.

Appendix 1 Figure 1. This is a figure in the appendix

Secondly

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Appendix 1 Figure 2. This is a figure in the appendix

287 **Appendix 2**

288

290

Appendix 2 Figure 1. This is a figure in the appendix

Figure 2–Figure supplement 1. There is no limit on the number of Figure Supplements for any one primary figure. Each figure supplement should be clearly labelled, Figure 1–Figure Supplement 1, Figure 1–Figure Supplement 2, Figure 2–Figure Supplement 1 and so on, and have a short title (and optional legend). Figure Supplements should be referred to in the legend of the associated primary figure, and should also be listed at the end of the article text file.

Figure 3–Figure supplement 1. This is a supplementary figure's full caption, which will be used at the end of the manuscript.

Figure 3–Figure supplement 2. This is another supplementary figure.