Vacuole Fusion at a Ring of Vertex Docking Sites Leaves Membrane Fragments within the Organelle

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Presented by Ruby Kish & Karen Swanson
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Overview

- Previous models show boundary membrane dilating, spreading to the external membrane, boundary membrane is conserved
- Proposed model suggests that membrane is internalized and degraded

Morphology of Vacuole fusion

- Internalization of border membranes during fusion would mean that fusion occurs at vertices
- Vertices are the area where two boundary membranes or an outside and boundary membrane meet
- Outer membranes fuse, boundary membranes internalized

Proteins involved in yeast vacuole fusion

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOPS Complex</td>
<td>Vps39p*, Vps33p</td>
</tr>
<tr>
<td>t-SNARE</td>
<td>Van3p</td>
</tr>
<tr>
<td>Rab</td>
<td>Ypt7p</td>
</tr>
<tr>
<td>Protein phosphatase I</td>
<td>Glc7p</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>Vac8p</td>
</tr>
</tbody>
</table>

Controls

- Vacuolar ATPase
- Vph1p, Vma11p
- Vacuolar Marker
- Pho8p

Evidence for fusion at vertices

1. Vertex starts off as concave, eventually relaxing, becoming flat
2. At vertex boundary membrane becomes mobile
3. There is a drop in fluorescence at the boundary region

Intraluminal membranes are a direct result of fusion and not due to endocytosis

- Only 5% of wildtype vacuoles show intraluminal membranes pre-fusion
- Mutants defective in two endocytotic pathways, as well as mutant defective in vacuole membrane turnover still contain intraluminal membranes post-fusion
Purified vacuoles fuse with the same frequency as vacuoles in vivo and also appear to fuse at vertices and create intralumenal membranes.

Docked Vacuoles lack pores

GFP-labelled Proteins don’t alter Vacuole Morphology

Protein distribution on docked Vacuoles

Protein Localization at Docking Vertices is Regulated and Selective
**Morphometric Analysis of Protein Localization**

**Proteins localize to vertex after tethering**

- Addition of excess Sec17p stops the fusion reaction at an early stage and prevents protein accumulation at the vertex.
- Addition of Sec18p binds to Sec17p removing the inhibitory effect on fusion and results in protein accumulation at the vertex.

**Removal of Rab/Ypt7p from Docked Vacuoles doesn’t alter Protein Localization**

- GDI and Gyp3p (GAP) dephosphorylated and remove Ypt7p (Rab) from membrane.

**Table 1. Relative Abundance Levels of GFP-Tagged Proteins on Vacuole Membranes and Their Enrichment at Interfaces of Docked Vacuoles**

<table>
<thead>
<tr>
<th>GFP-Tagged Proteins</th>
<th>Relative Abundance</th>
<th>Enrichment at Docking Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuolar marker</td>
<td>Photlp</td>
<td>N.D.</td>
</tr>
<tr>
<td>Vacuole inheritance</td>
<td>Vac8lp</td>
<td>150</td>
</tr>
<tr>
<td>t-SNARE</td>
<td>Vam3p</td>
<td>40</td>
</tr>
<tr>
<td>COP complex</td>
<td>Vps33p</td>
<td>30.3</td>
</tr>
<tr>
<td>GTPase</td>
<td>Ypt7p</td>
<td>20</td>
</tr>
<tr>
<td>Protein phosphatase I</td>
<td>Gbl7p</td>
<td>67</td>
</tr>
<tr>
<td>Vacuolar ATPase</td>
<td>Vph1p</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>Vma11p</td>
<td>60</td>
</tr>
</tbody>
</table>

Mean protein:lipid ratios

- proteins on docked vacuoles localizes to vertices

- Ratio of GFP:lipid decreased in Sec17p treated vacuoles
- Addition of Sec18p + Sec17p restores wildtype phenotype
• There is more efficient Ypt7p extraction at the vertices and boundaries
• There is no change in localization of Vps33p and Vam3p after Ypt7p extraction

Conclusions

• Vacuoles fuse at vertices
  Appearance of intralumenal membrane
  Pores do not form prior to fusion

• Rab, SNAREs and Rab effectors concentrate at the vertices and catalyze reactions that lead to fusion