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

Li Wang, E. Scott Seeley, William Wickner and Alexey J. Merz (2002)

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Presented by Ruby Kish
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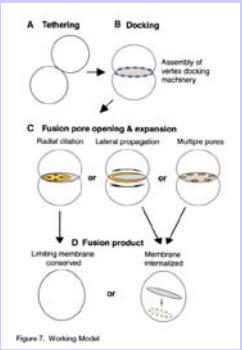
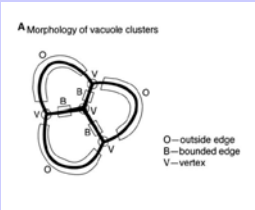
Overview



- 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

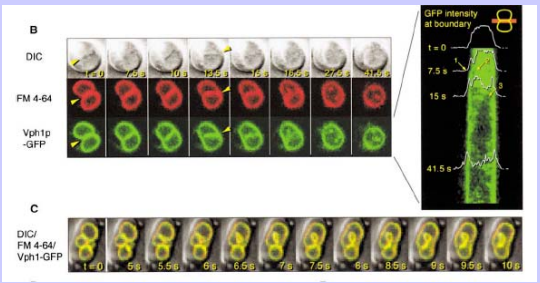


- Internalization of border membranes during fusion would mean that fusion occurs at vertices
- Vertices are the area where two boundary membranes or an outside and boundary membrane meet
- Outer membranes fuse, boundary membranes internalized

Proteins involved in yeast vacuole fusion

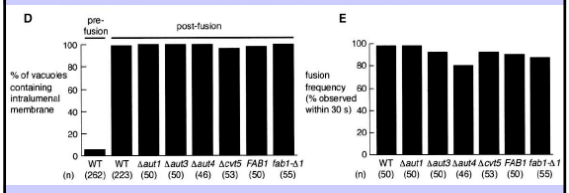
Protein		Function
HOPs Complex	Vps39p*, Vps33p	Includes GEF*
t-SNARE	Vam3p	Tethering
Rab	Ypt7p	GTPase
Protein phosphatase I	Glc7p	Fusion
Alkaline Phosphatase	Vac8p	Fusion
Controls		
Vacuolar ATPase	Vph1p, Vma11p	
Vacuolar Marker	Pho8p	

Evidence for fusion at vertices



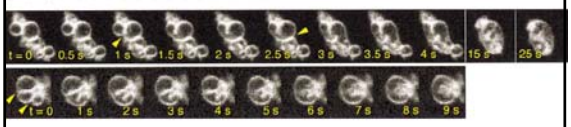
1. Vertex starts off as concave, eventually relaxing, becoming flat
2. At vertex boundary membrane becomes mobile
3. There is a drop in fluorescence at the boundary region

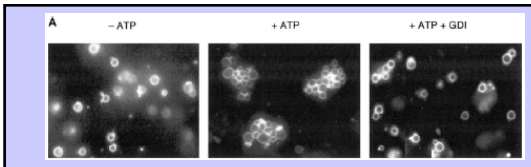
Intralumenal membranes are a direct result of fusion and not due to endocytosis

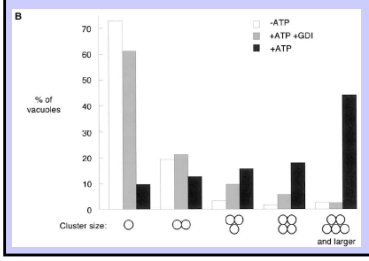


- Only 5% of wildtype vacuoles show intralumenal membranes pre-fusion
- Mutants defective in two endocytotic pathways, as well as mutant defective in vacuole membrane turnover still contain intralumenal membranes post-fusion

Purified vacuoles fuse with the same frequency as vacuoles *in vivo* and also appear to fuse at vertices and create intralumenal membranes

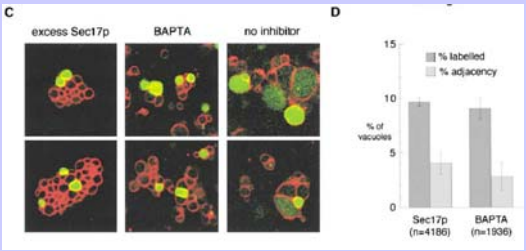




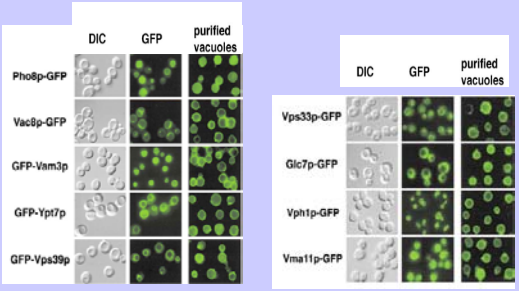


Vacuole docking is ATP dependent

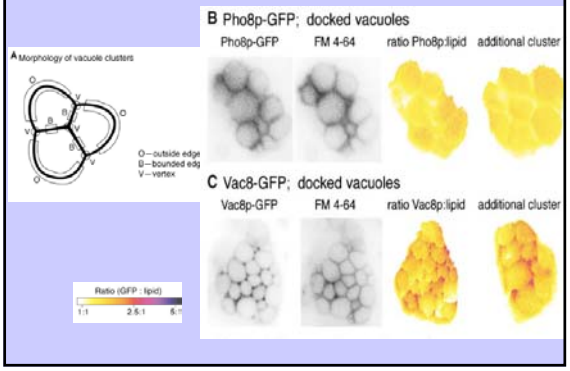
Docked Vacuoles lack pores



GFP-labelled Proteins don't alter Vacuole Morphology



Protein distribution on docked Vacuoles



Protein Localization at Docking Vertices is Regulated and Selective

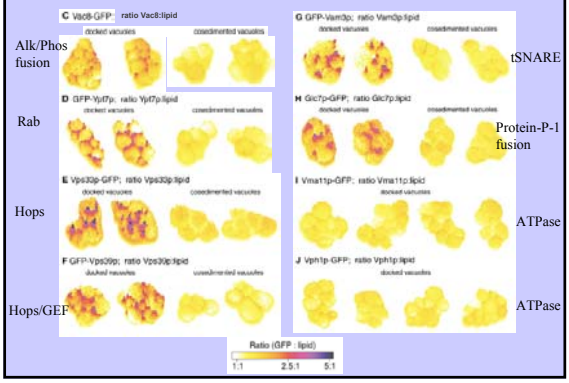
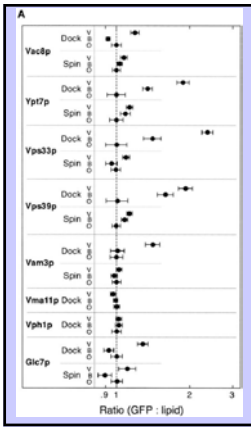


Table 1. Relative Abundance Levels of GFP-Tagged Proteins on Vacuole Membranes and Their Enrichment at Interfaces of Docked Vacuoles

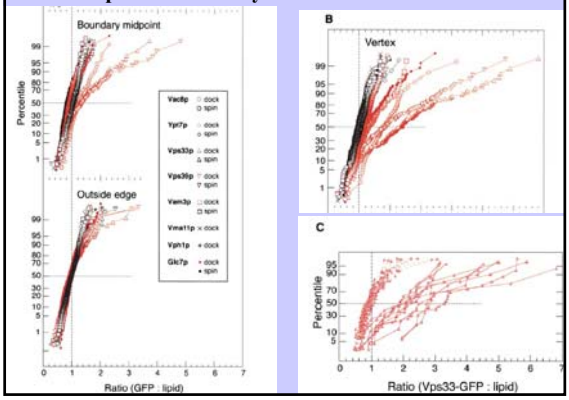
GFP-Tagged Proteins		Relative Abundance	Enrichment at Docking Site
Vacuolar marker	Pho8p	N.D.	–
Vacuole inheritance	Vac8p	150	+
t-SNARE	Vam3p	40	+
HOPS complex	Vps39p	33.3	+
	Vps33p	20	+
GTPase	Ypt7p	400	+
Protein phosphatase I	Glc7p	67	+
Vacuolar ATPase	Vph1p	200	–
	Vma11p	50	–



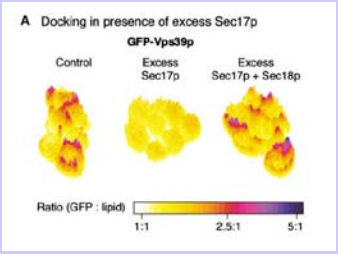
Mean protein:lipid ratios

-proteins on docked vacuoles localizes to vertices

Morphometric Analysis of Protein Localization

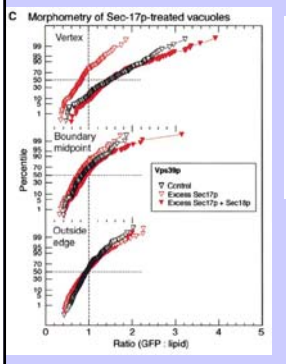


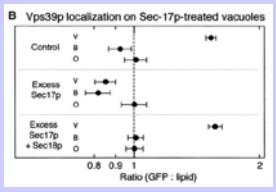
Proteins localize to vertex after tethering



•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

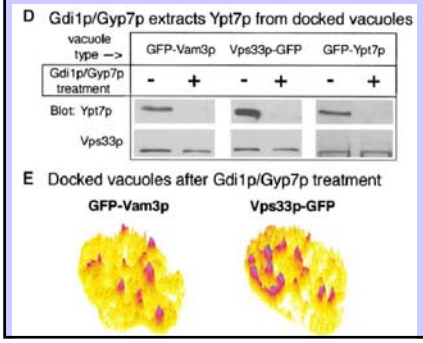




•Ratio of GFP:lipid decreased in Sec17p treated vacuoles

•Addition of Sec18p + Sec17p restores wildtype phenotype

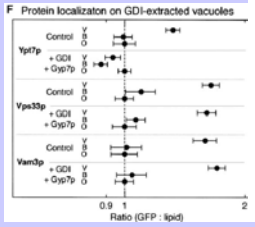
Removal of Rab/Ypt7p from Docked Vacuoles doesn't alter Protein Localization

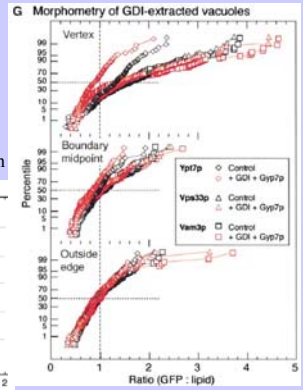


•GDI and Gyp7p (GAP) dephosphorylated and remove Ypt7p (Rab) from membrane

•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

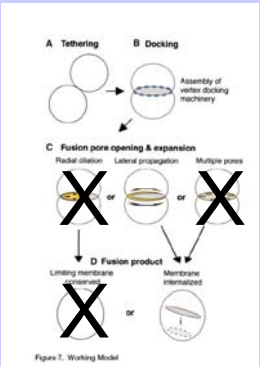


Figure 7. Working Model

•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