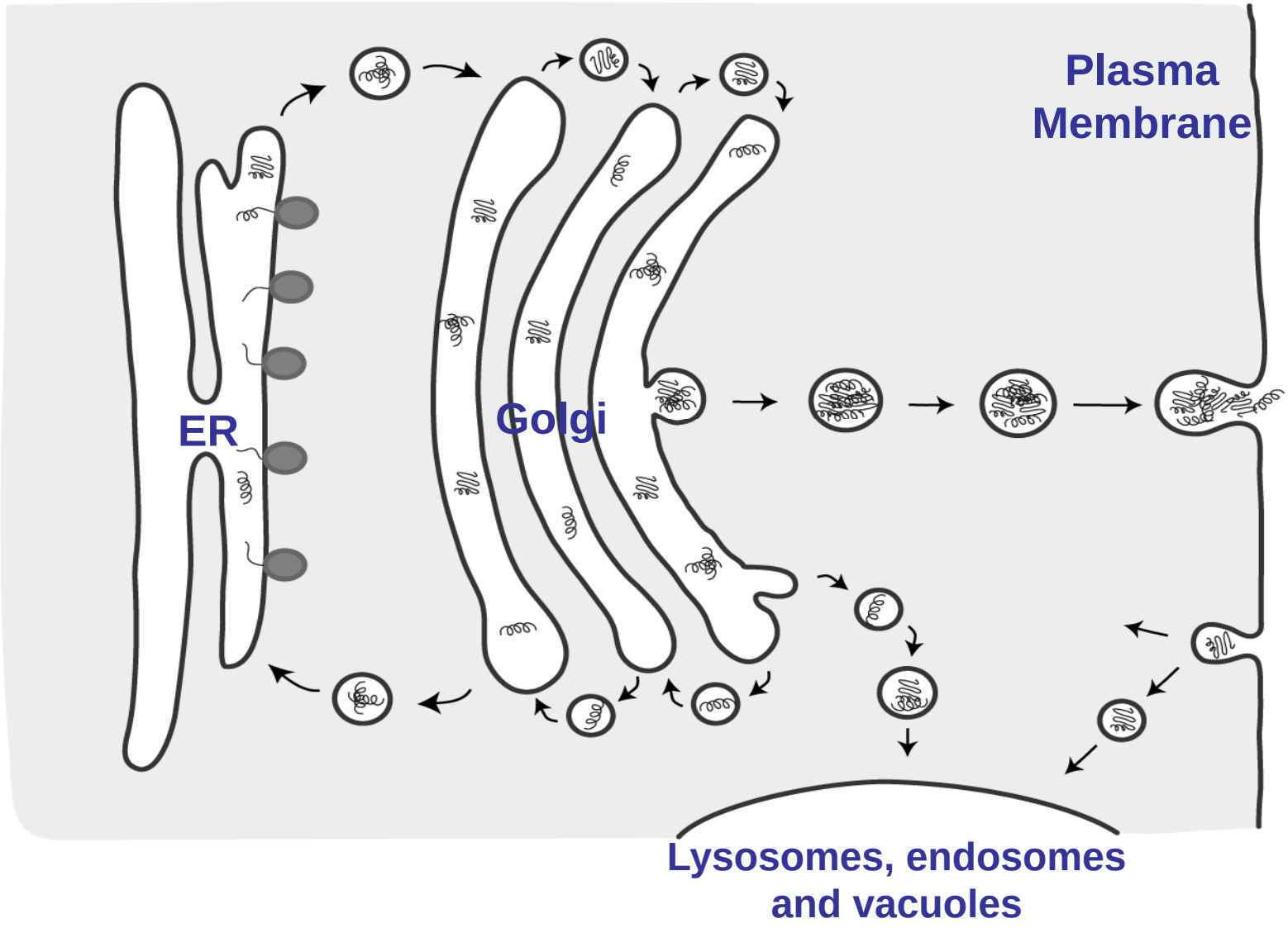
Molecular Architecture of the Exocyst Complex and its Function in Exocytosis

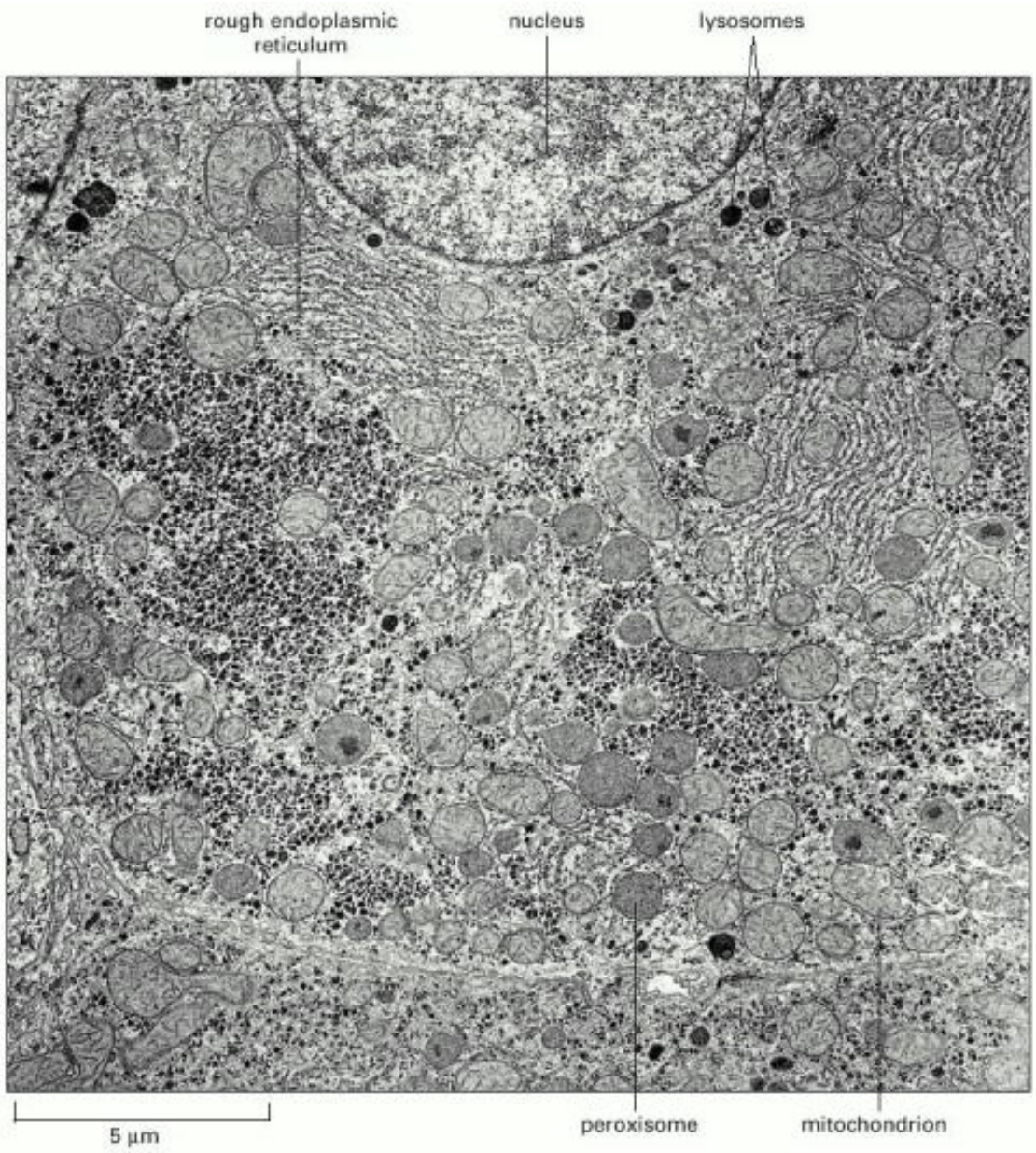
Mary Munson

University of Massachusetts Medical School, Worcester

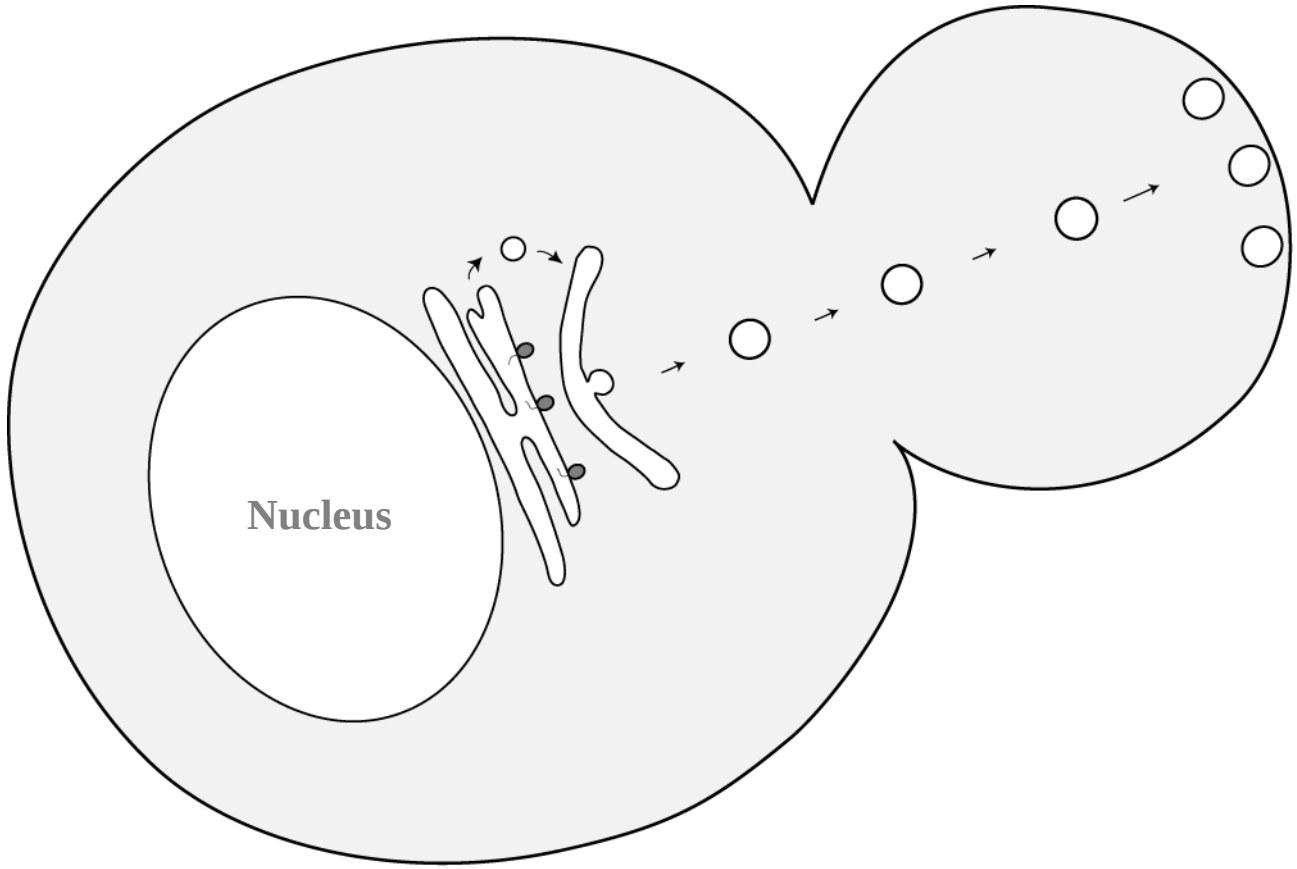
How is membrane trafficking controlled?



How does a vesicle deliver its contents to the correct target membrane?

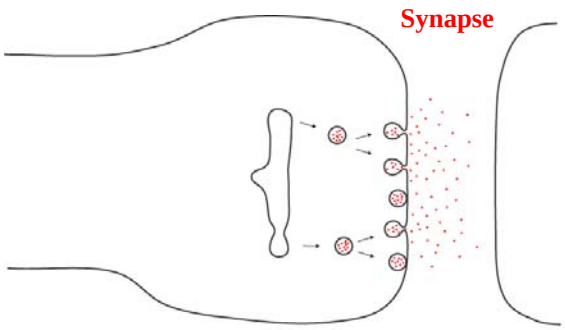


Exocytosis in yeast cells

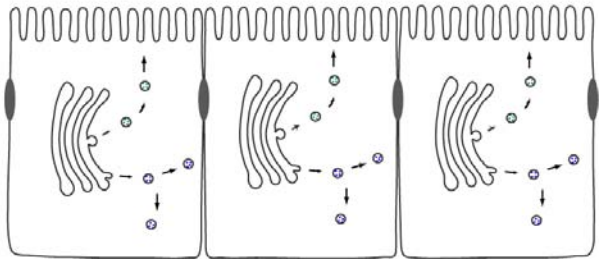


Trafficking mechanisms are highly conserved

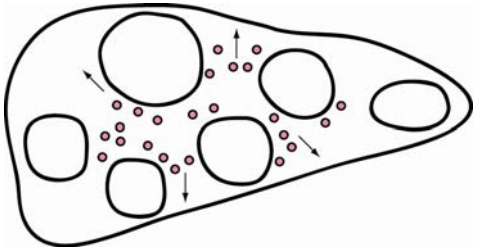
Neuron



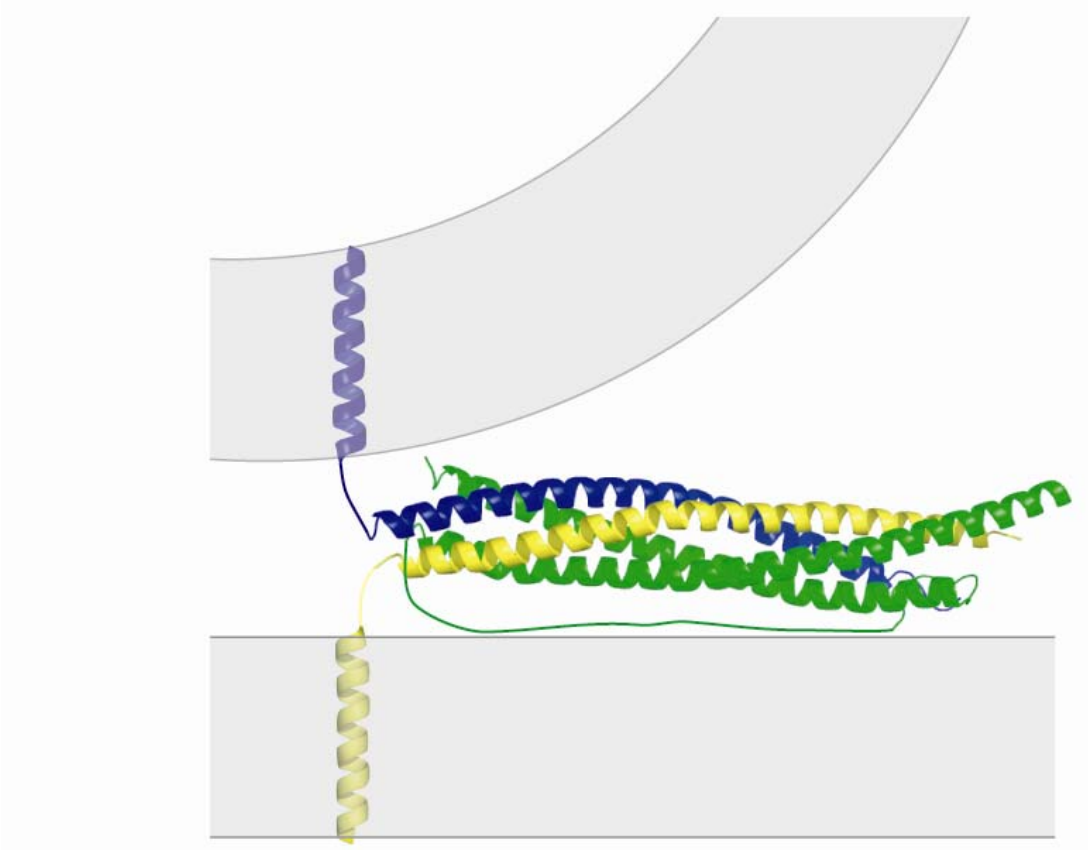
Polarized epithelial cell



Adipocyte



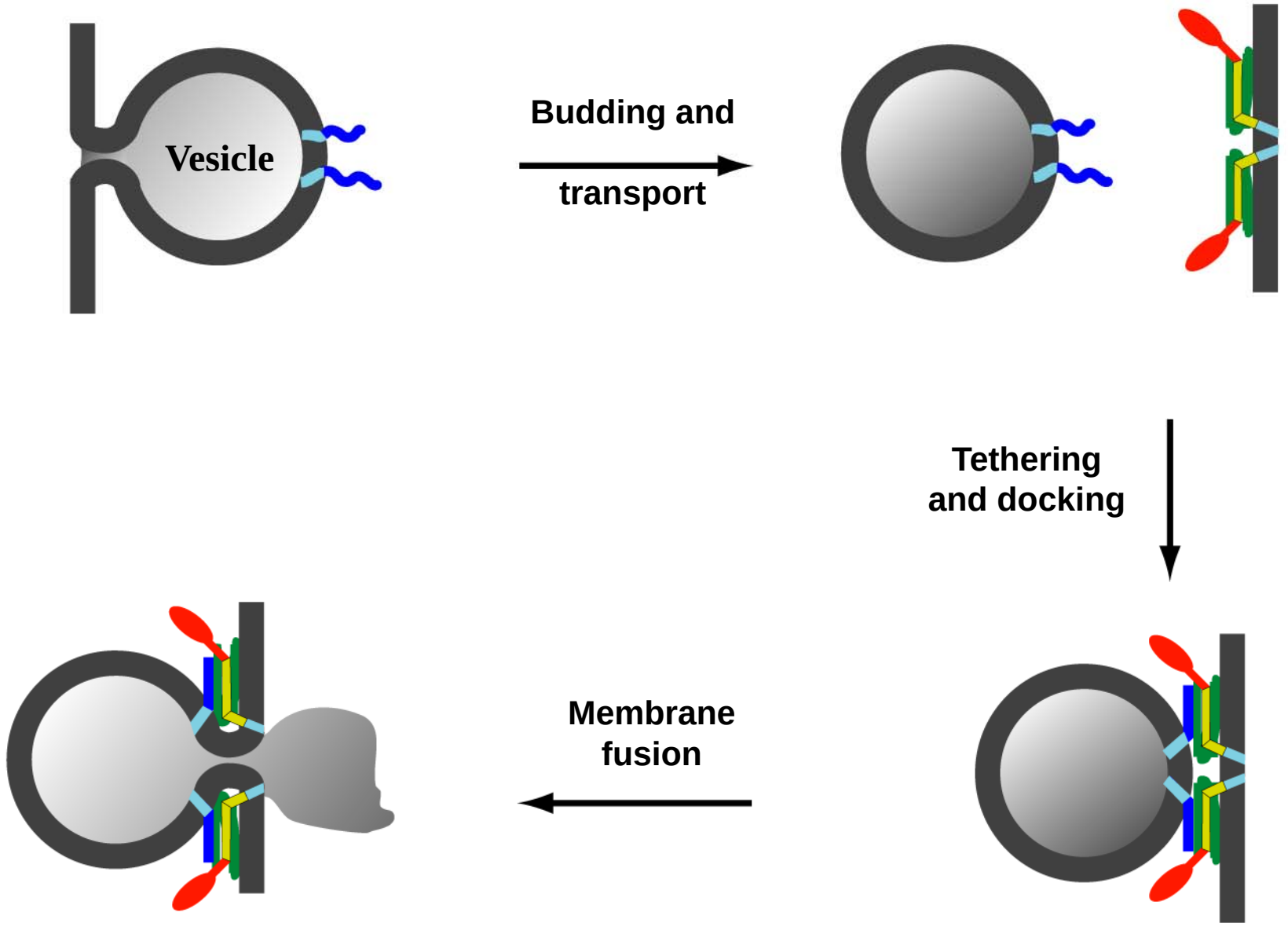
SNARE proteins form complexes for membrane fusion

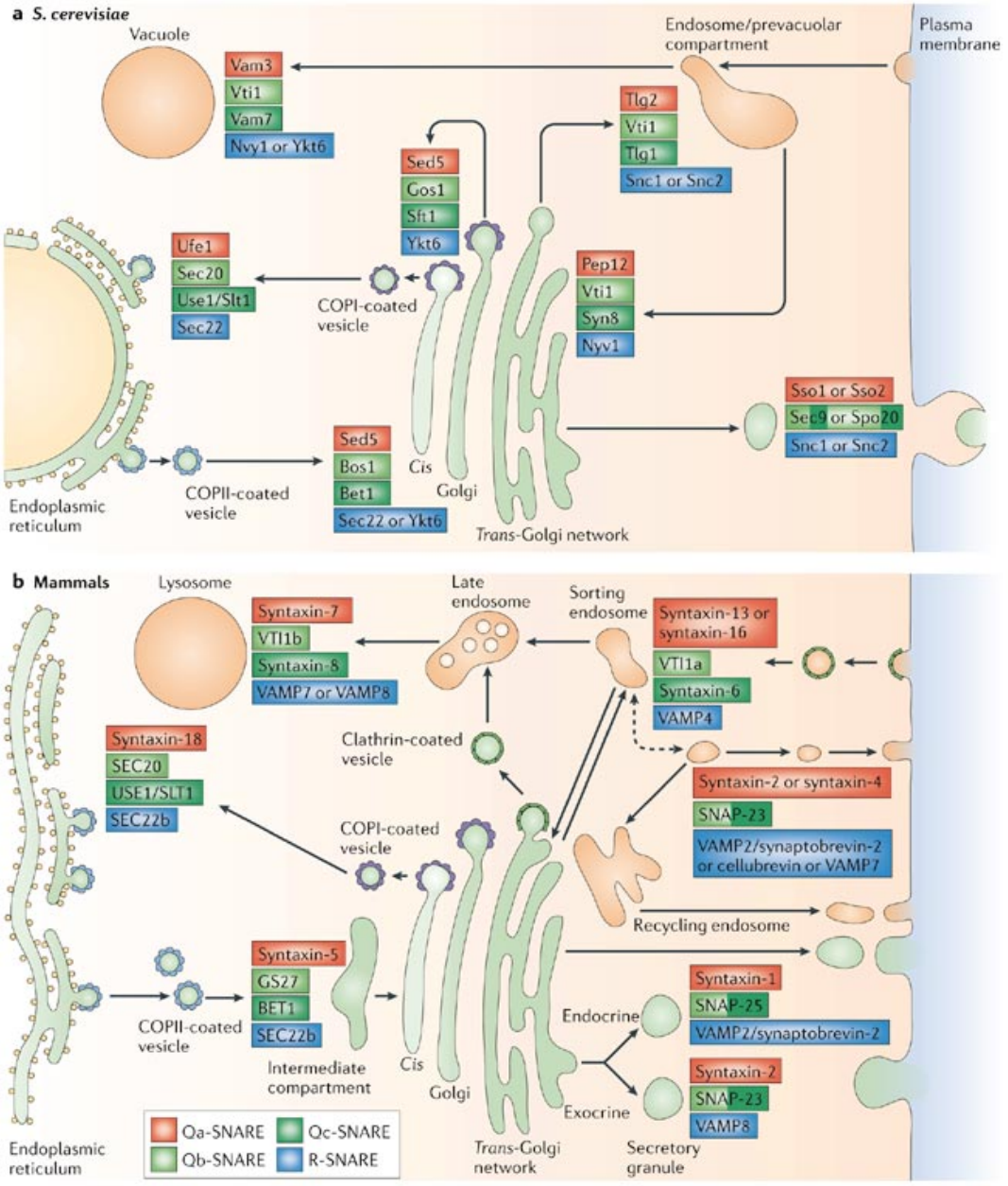


Neuronal SNARE complex

Sutton, et al. *Nature* (1998)

Do SNAREs regulate specificity?

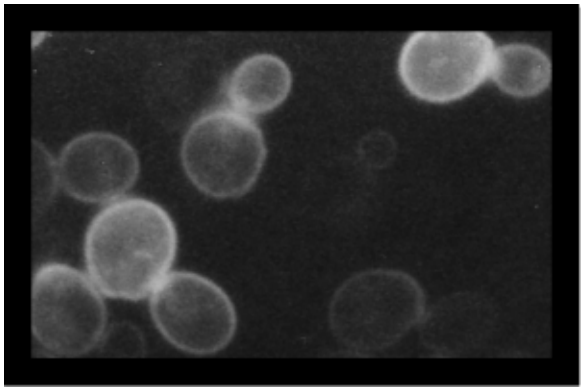


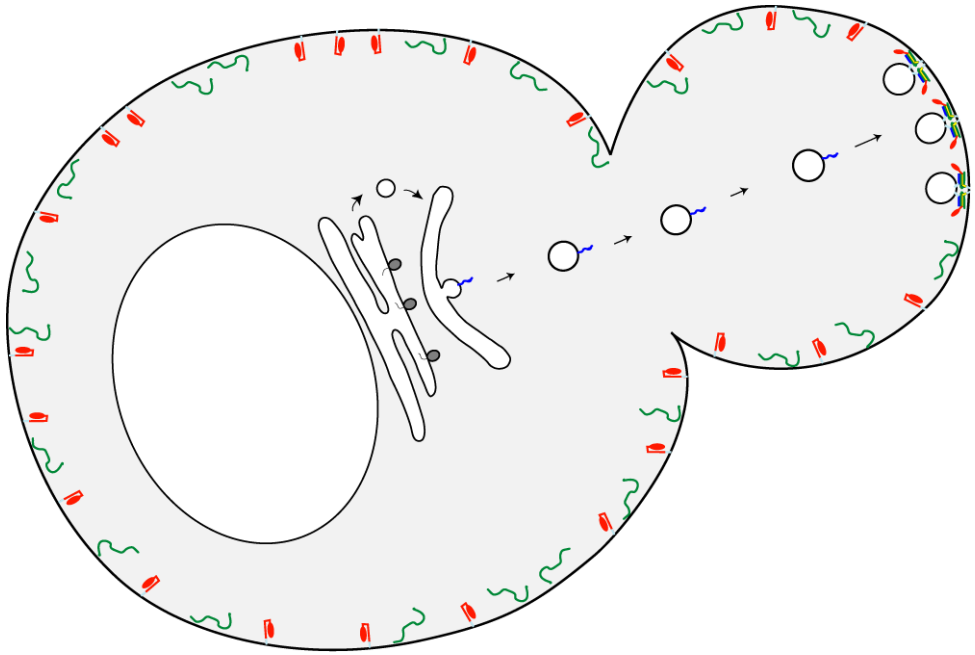


Jahn &Scheller
***Nat Rev Mol Cell Biol* (2006)**

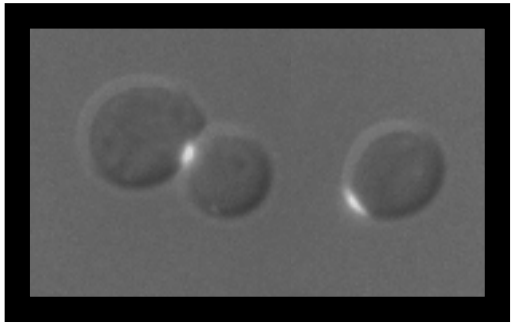
Growth and secretion are polarized, but t-SNAREs are not localized

GFP-Sso1

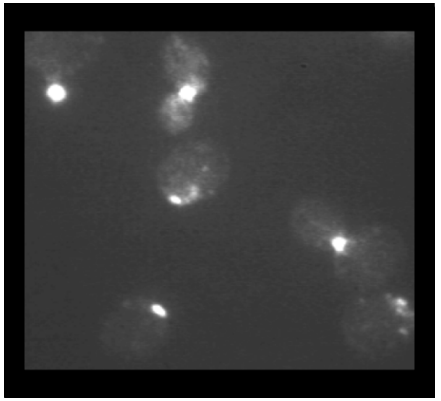




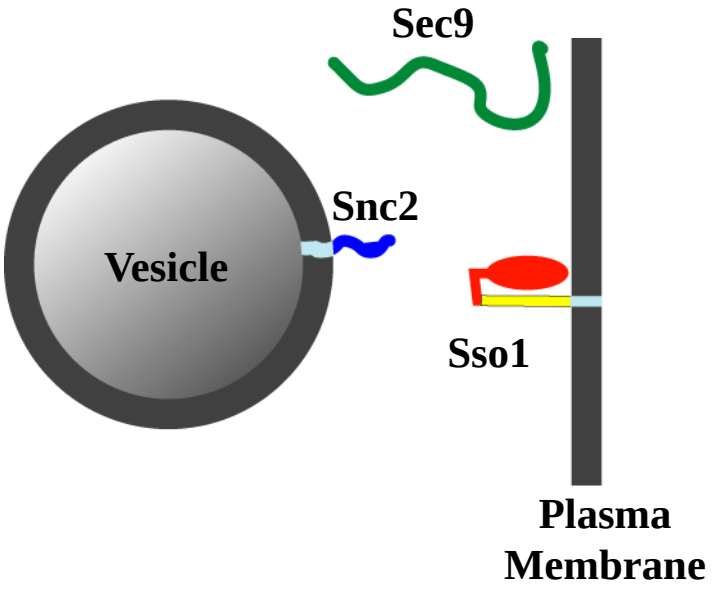
Sec6-GFP



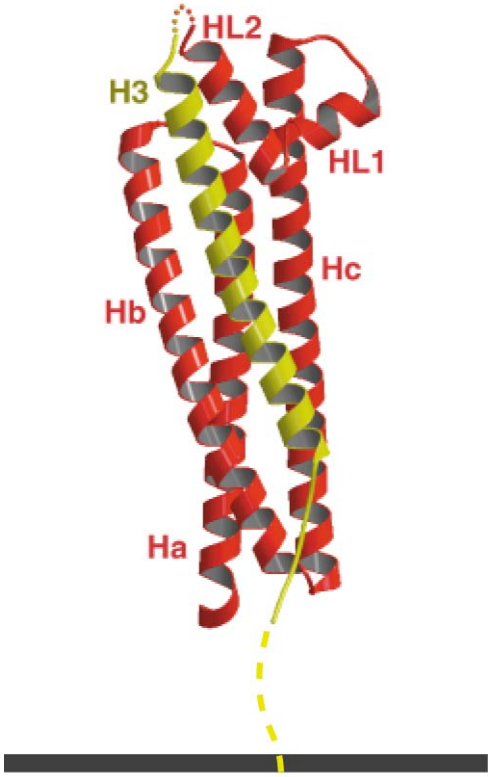
Sec4 IF



The N-terminal domain of Sso1 regulates SNARE complex assembly

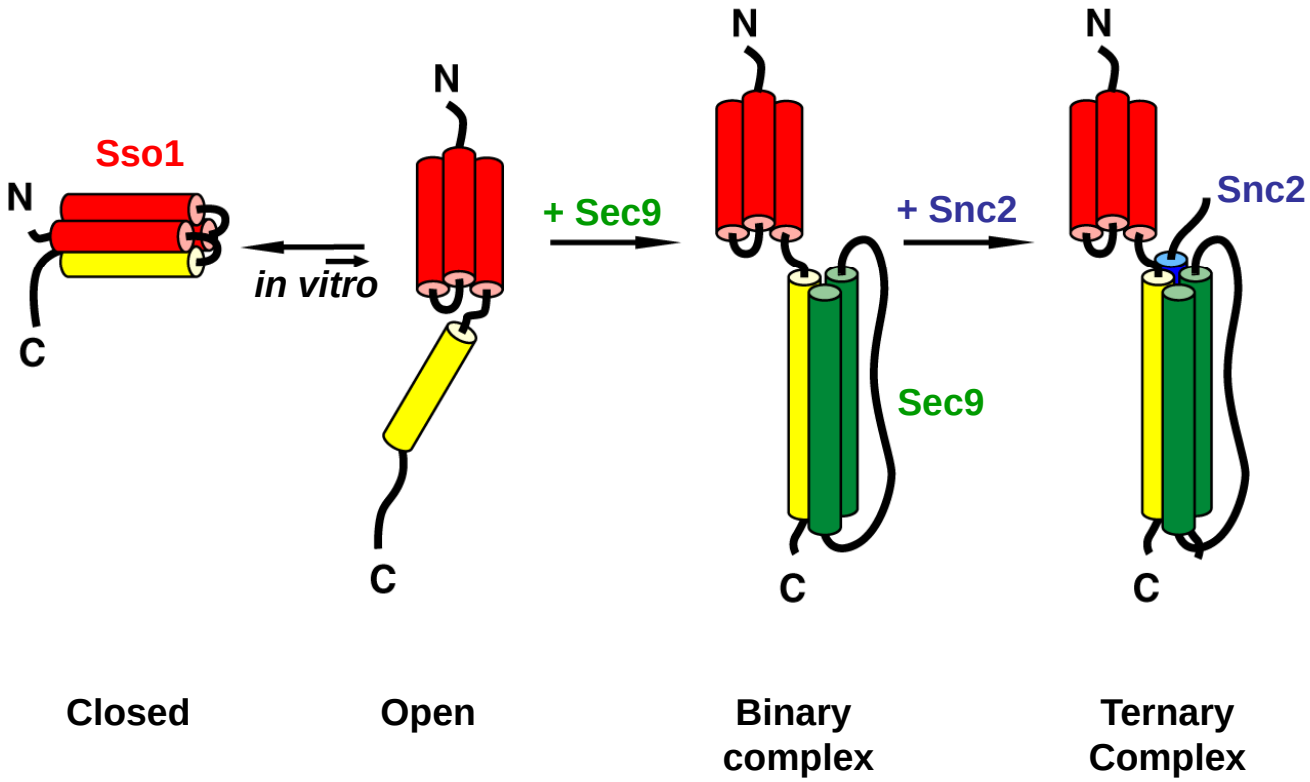


Sso1 – syntaxin
Sec9 – SNAP-25
Snc2 – VAMP

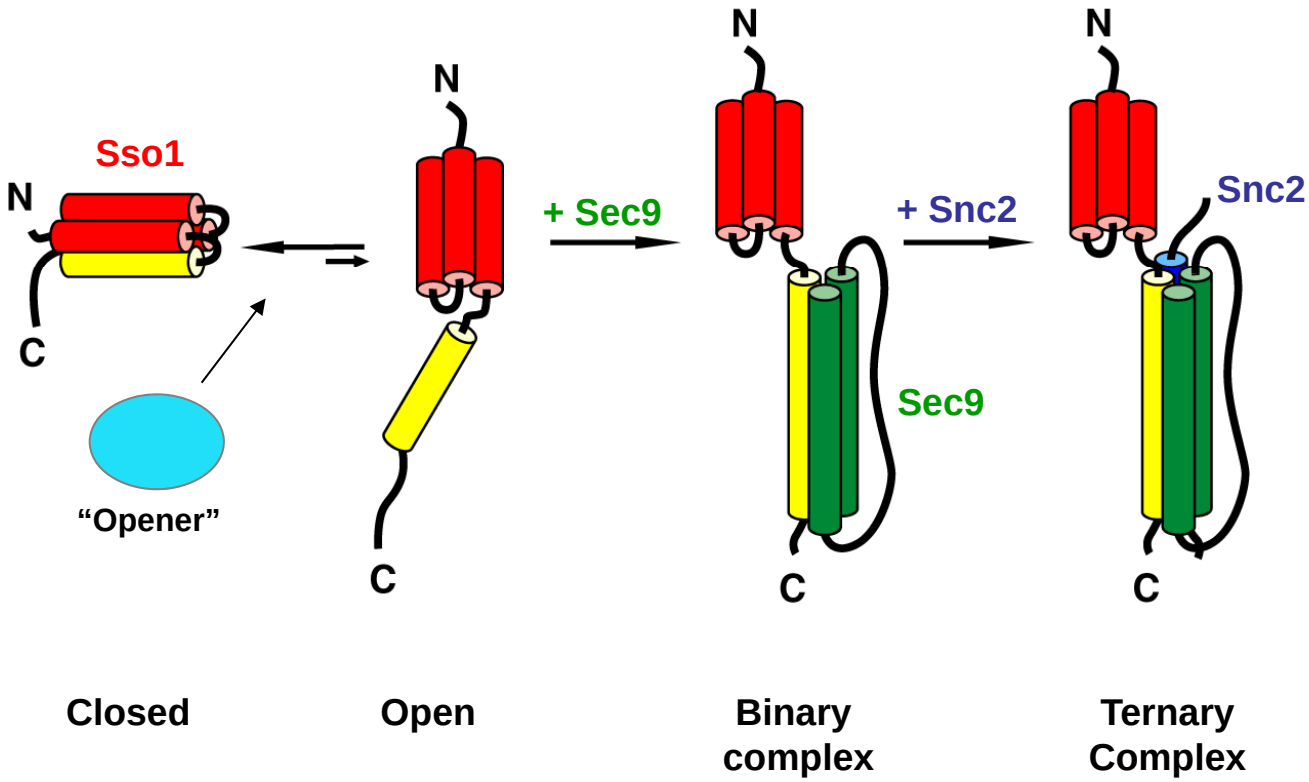


Sso1; Munson, et al.
Nature Str Biol (2000)

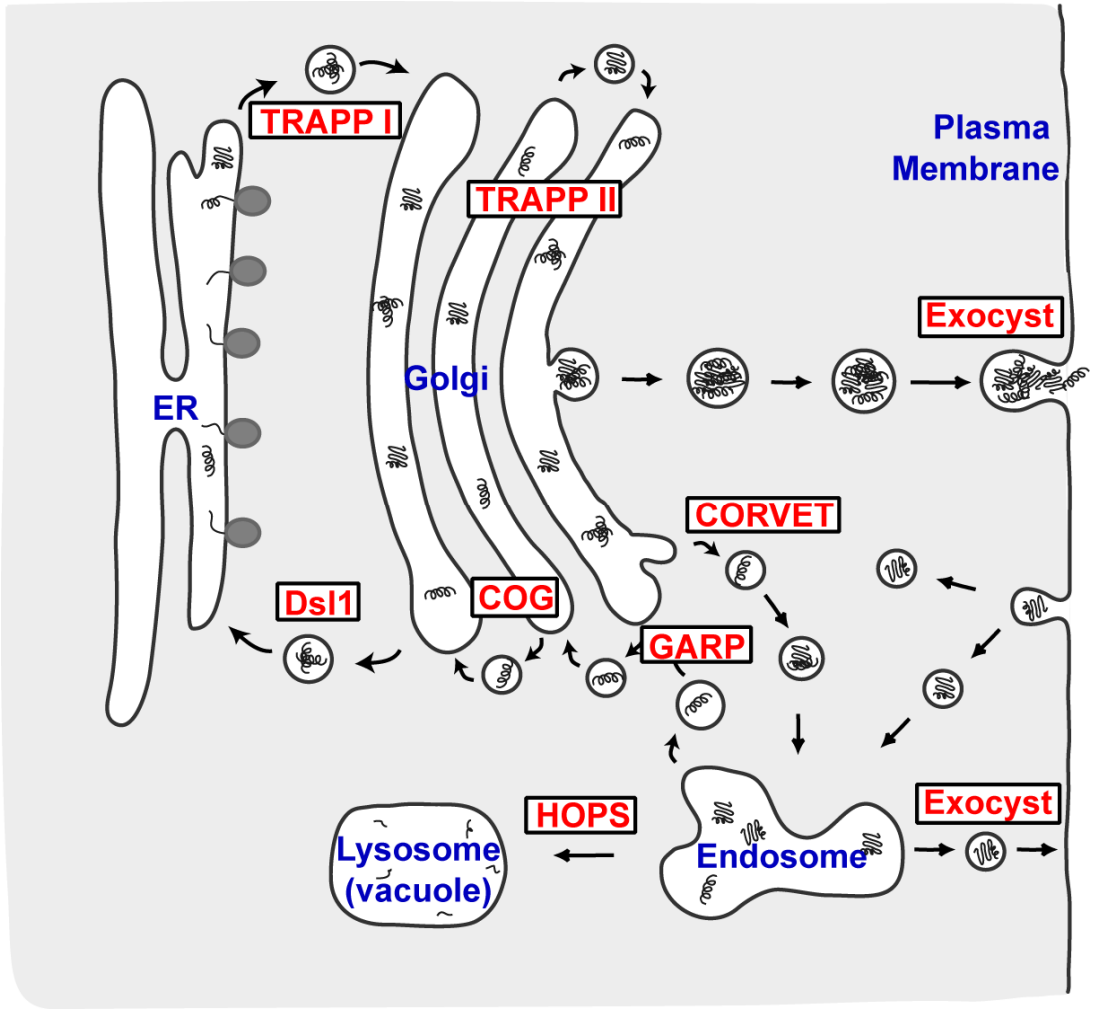
The closed conformation of Sso1 inhibits SNARE complex assembly



The closed conformation of Sso1 inhibits SNARE complex assembly

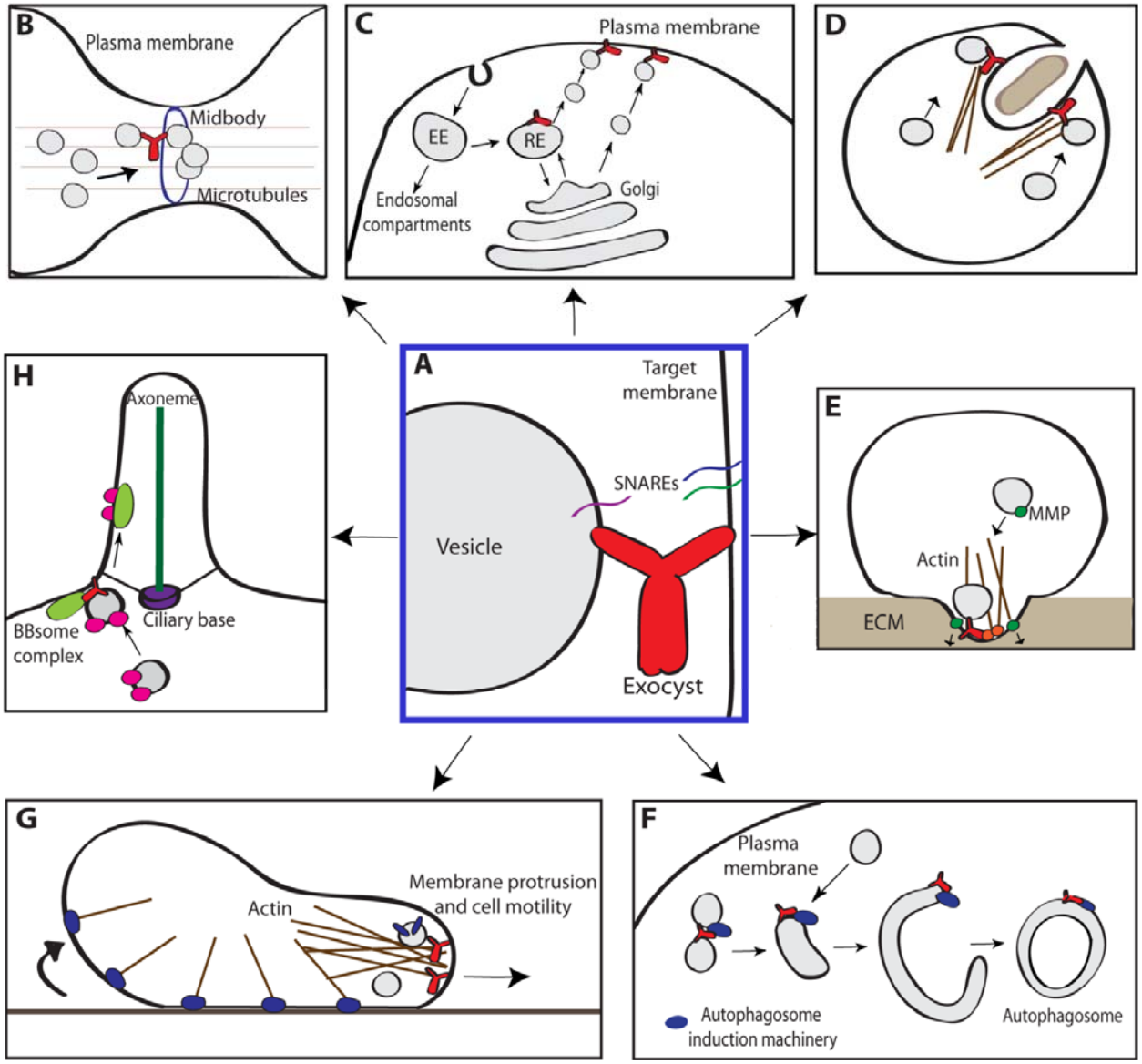


“Tethering” complexes



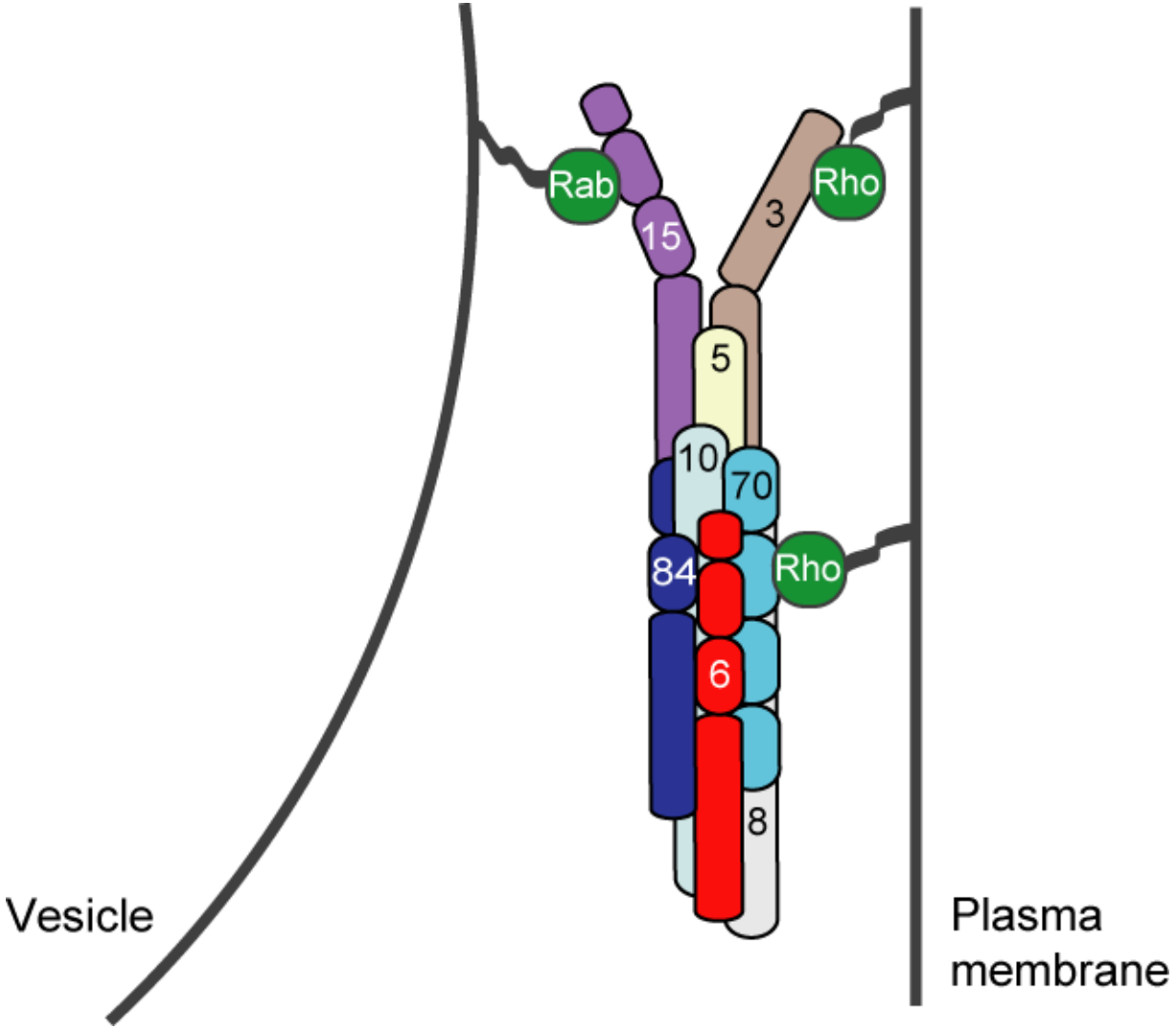
<u>Interact with specific Rabs and SNAREs to</u>:
Physically “tether” vesicle to target membrane?
Provide quality control to ensure correct vesicle and target membranes fuse?

Functional roles for the exocyst



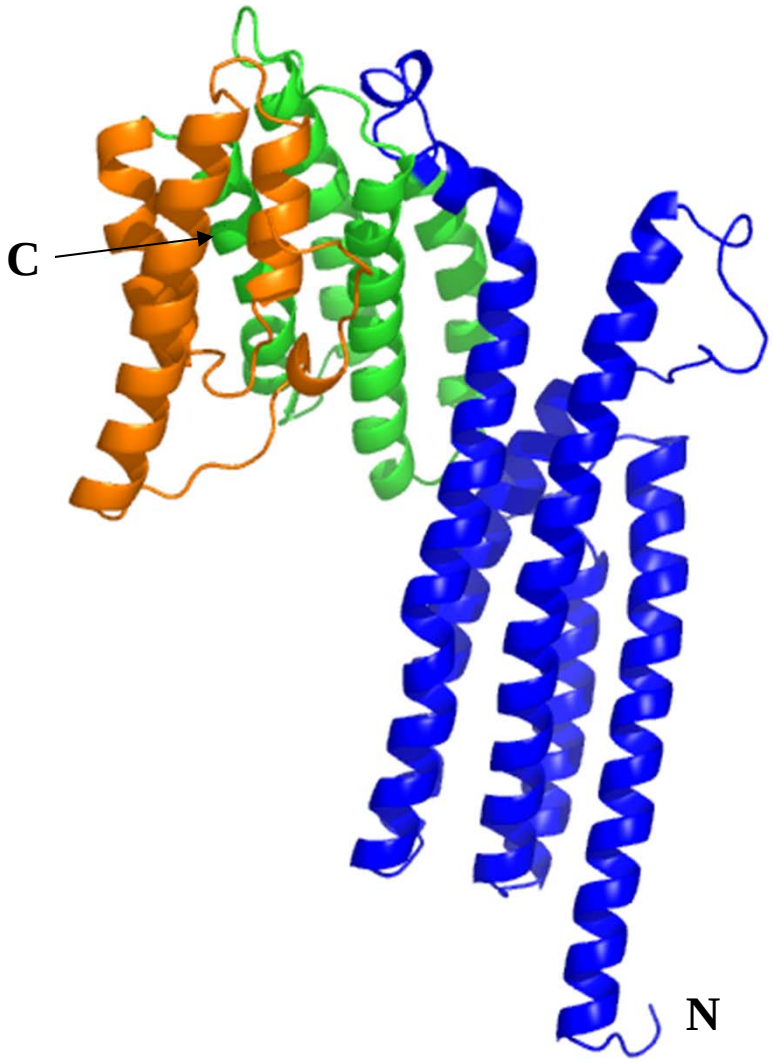
Sallah & Munson, submitted

Model of the yeast exocyst (Sec6/Sec8) complex



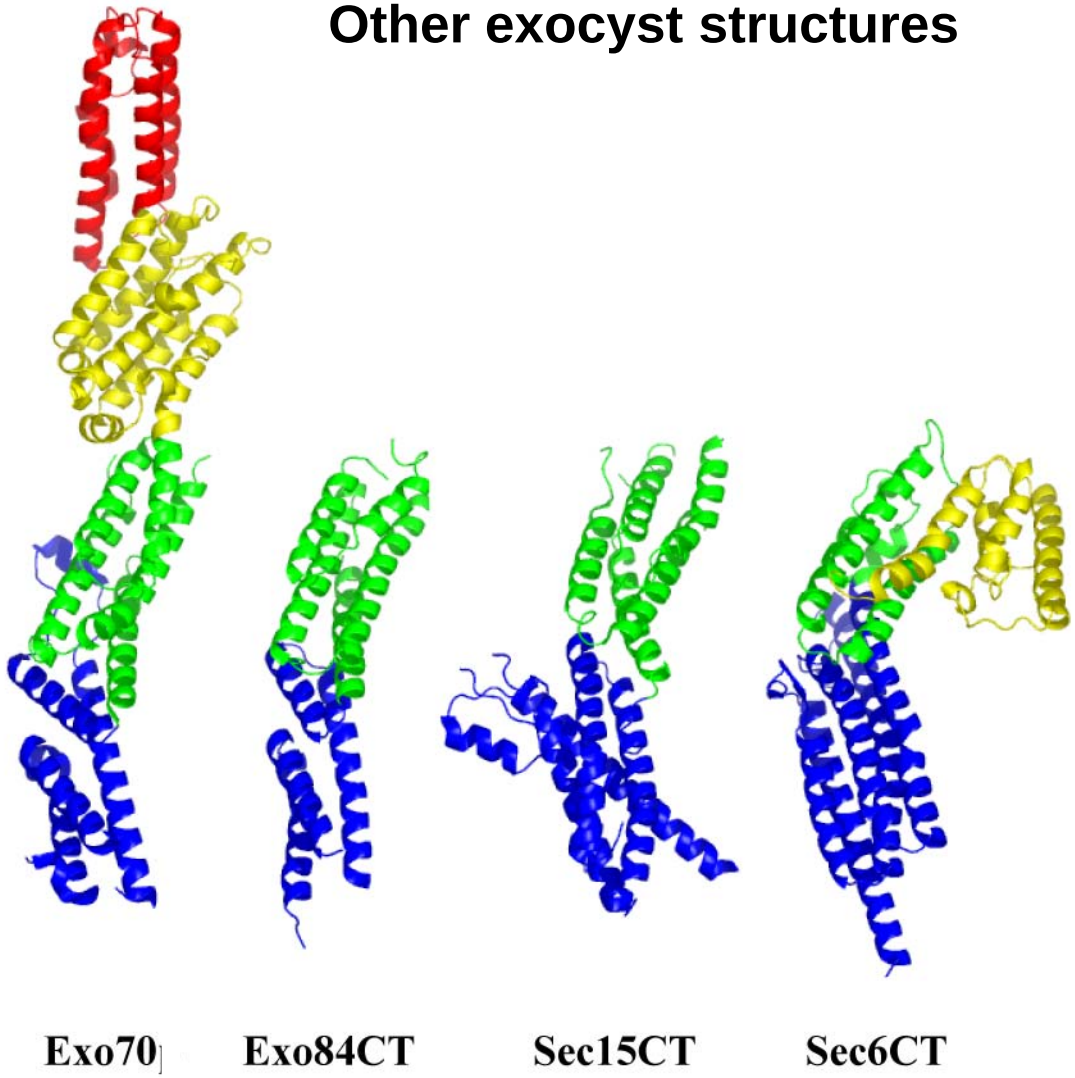
Munson & Novick, *Nat Str Mol Biol* 2006

Crystal structure of the Sec6 C-terminal domain



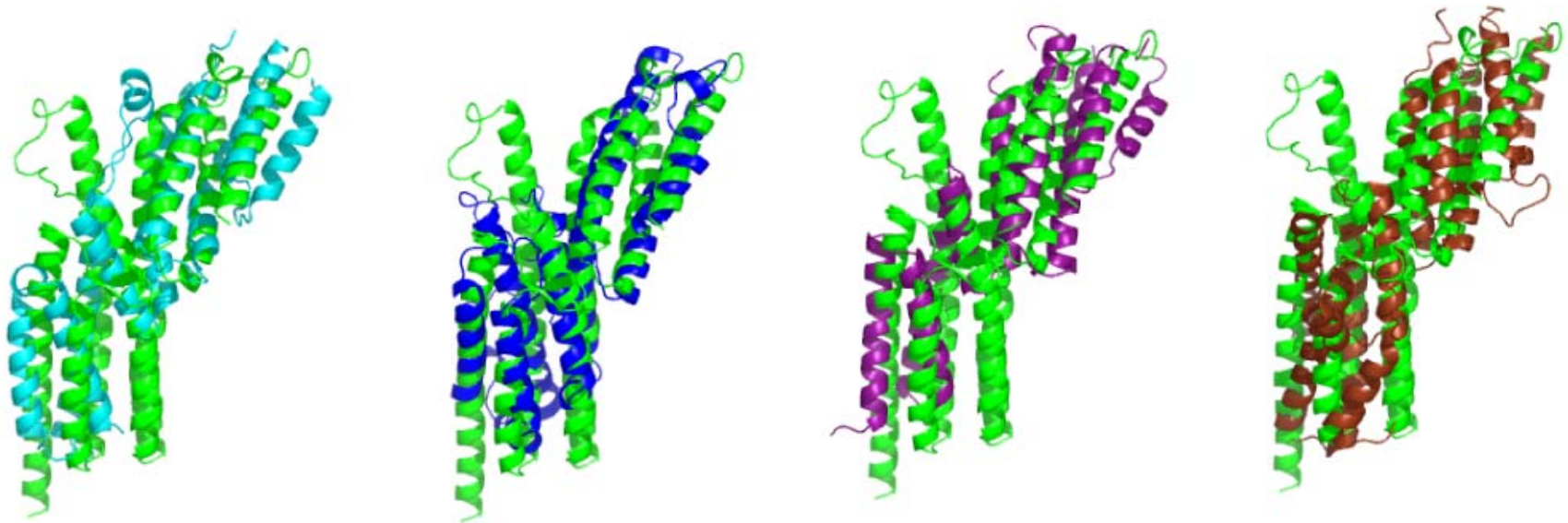
Sivaram, et al., *Nat Str Mol Biol* 2006

Other exocyst structures



Exo70/Exo84CT: Dong, et al. *Nat Str Mol Biol* 2005
Sec15CT: Wu, et al., *Nat Str Mol Biol* 2005
Sec6CT: Sivaram, et al., *Nat Str Mol Biol* 2006

Similarity between Sec6CT and the other exocyst structures

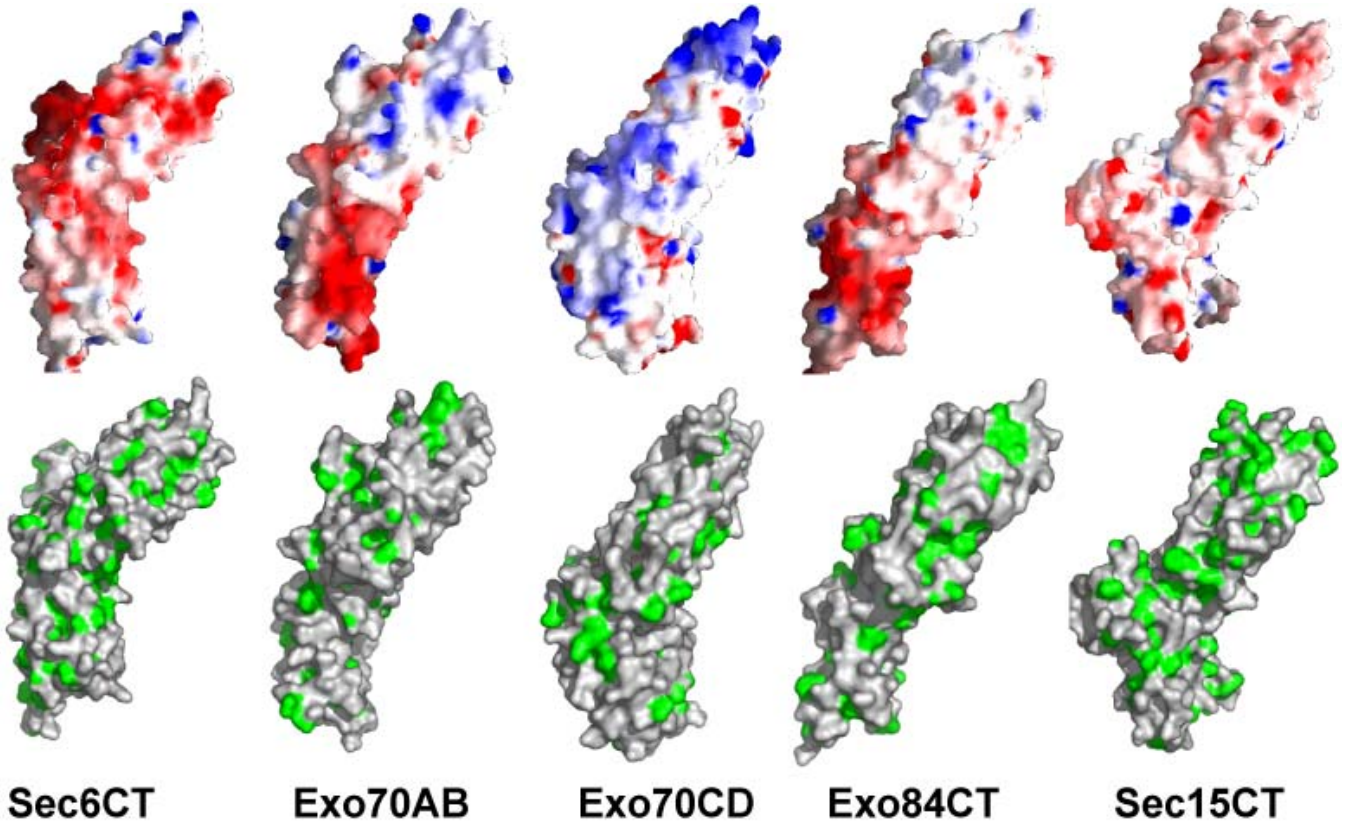


Sec6CT2 vs. Exo70AB vs. Exo70CD vs. Exo84CT vs. Sec15CT

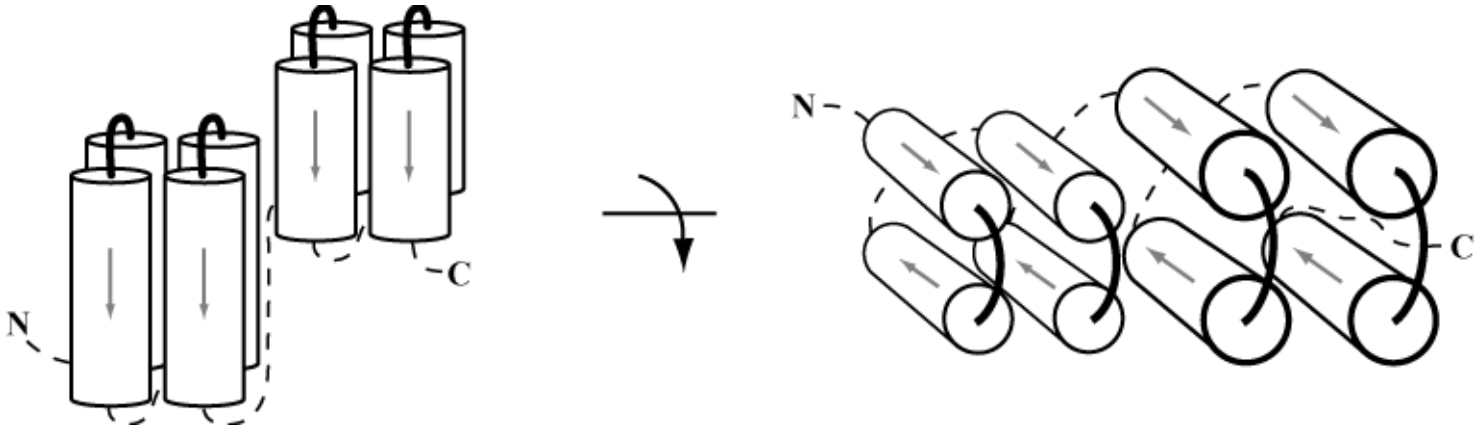
Differences between Sec6CT and the other exocyst structures

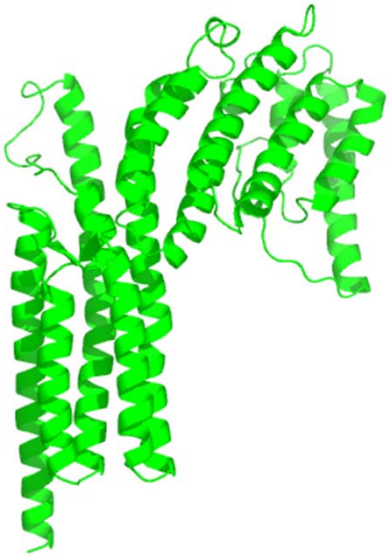
Electrostatic surface potential

Surface hydrophobics

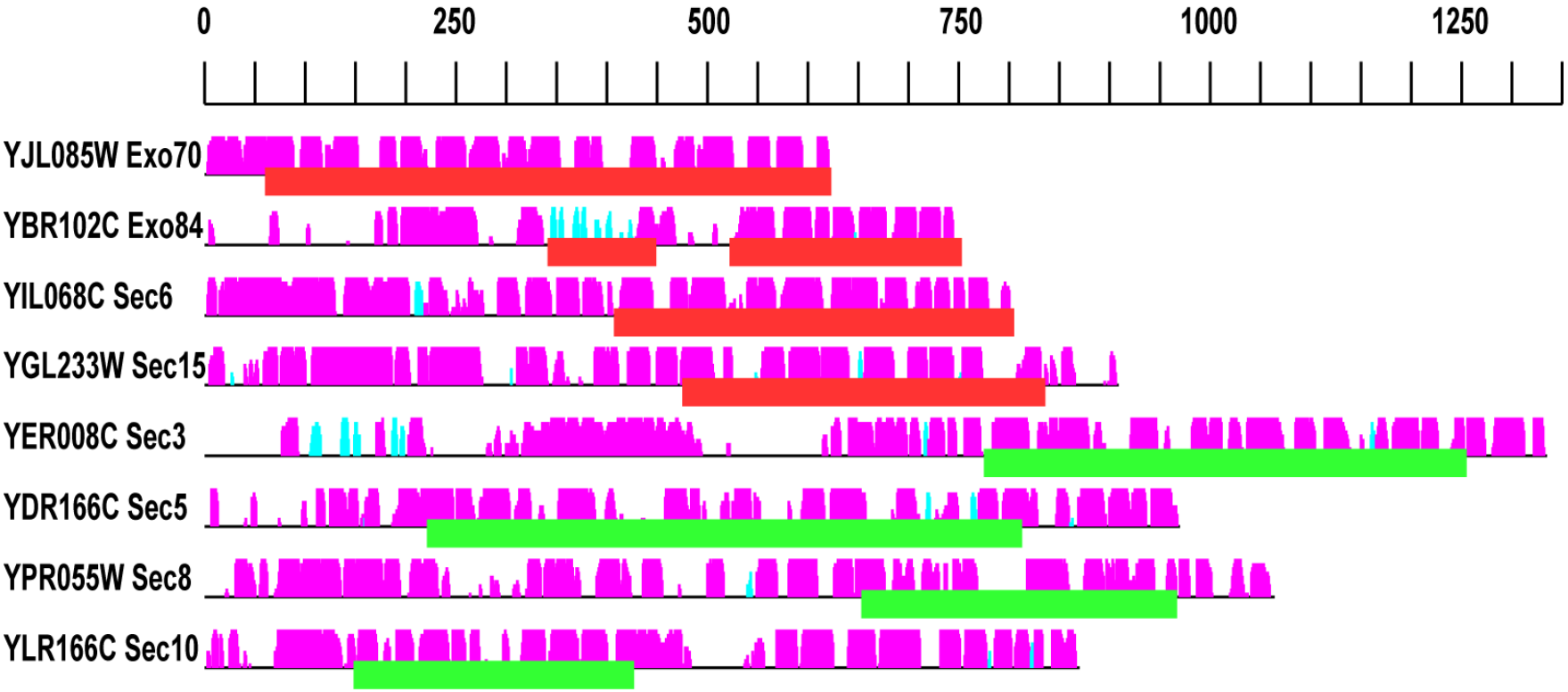


Divergent or convergent evolution?
Same helical bundle topology



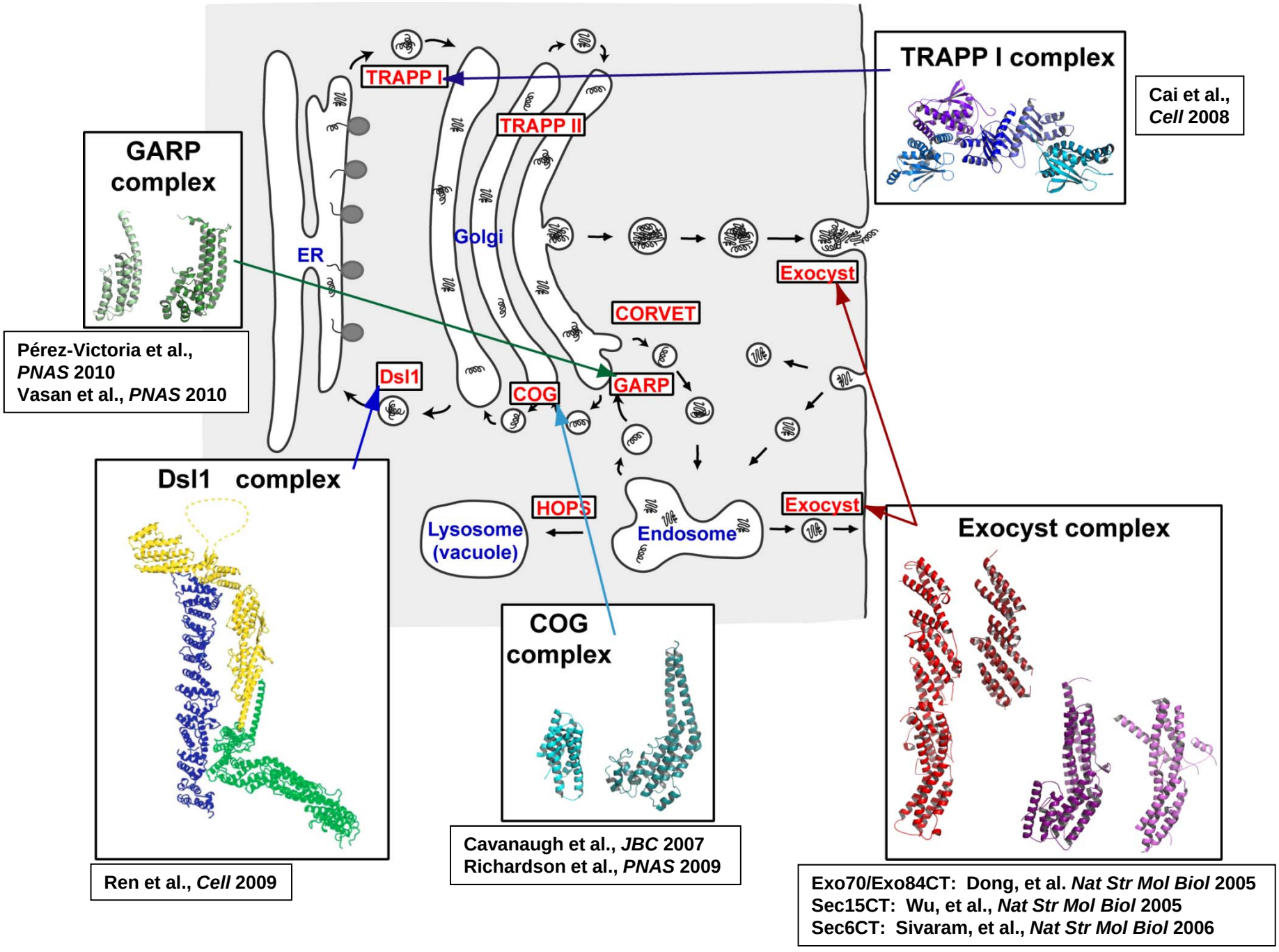


All of the exocyst subunits are predicted to have similar helical bundle structures

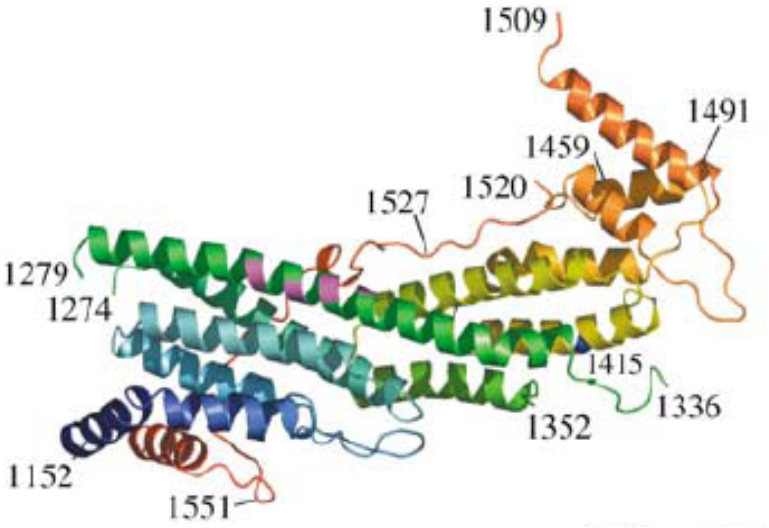


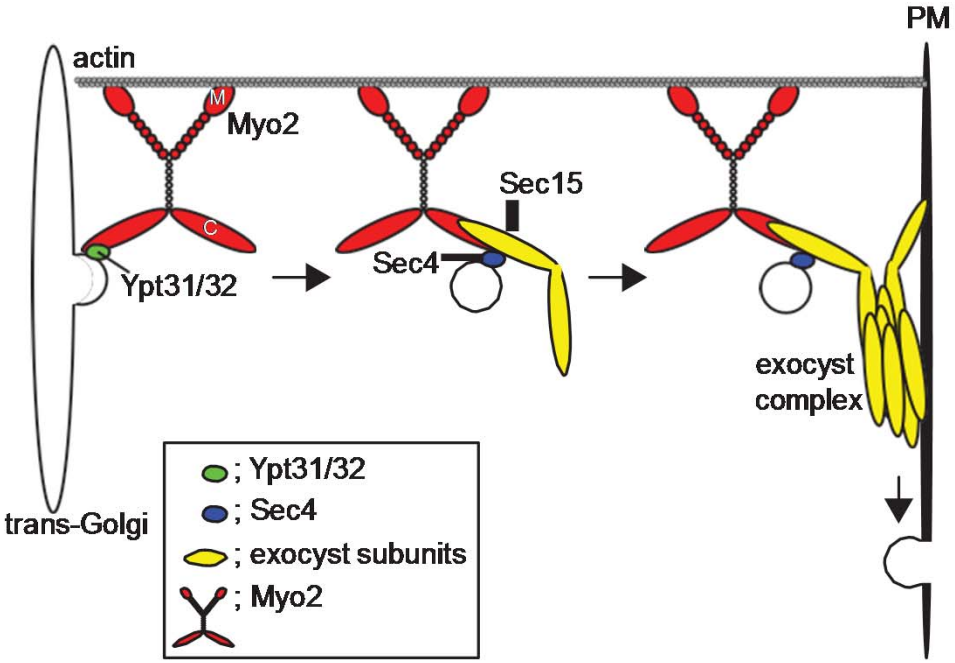
Croteau, Furgason, Devos & Munson,
PLoS ONE 2009

Similarity between the exocyst and other tethering complexes (CATCHR)



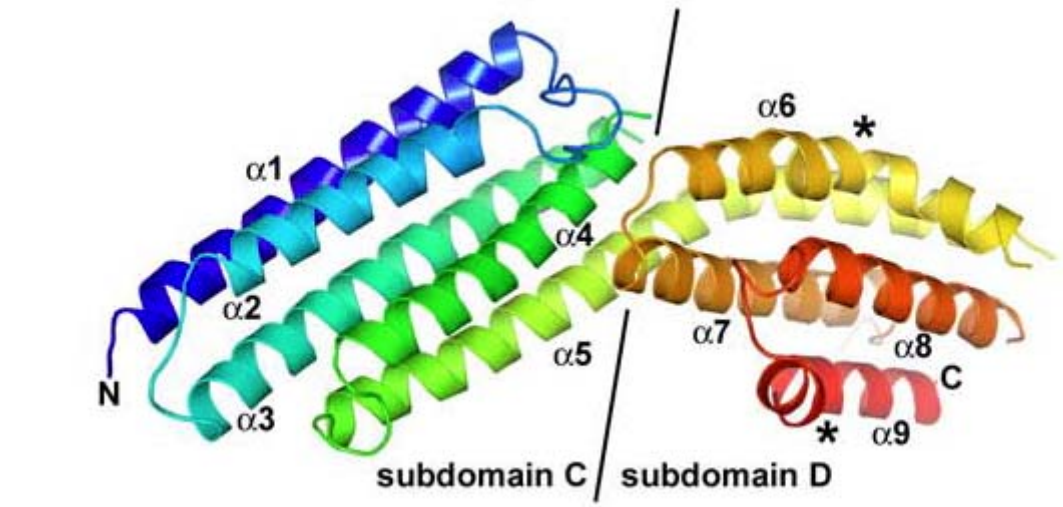
Similarity between the exocyst and Myo2 cargo-binding domain

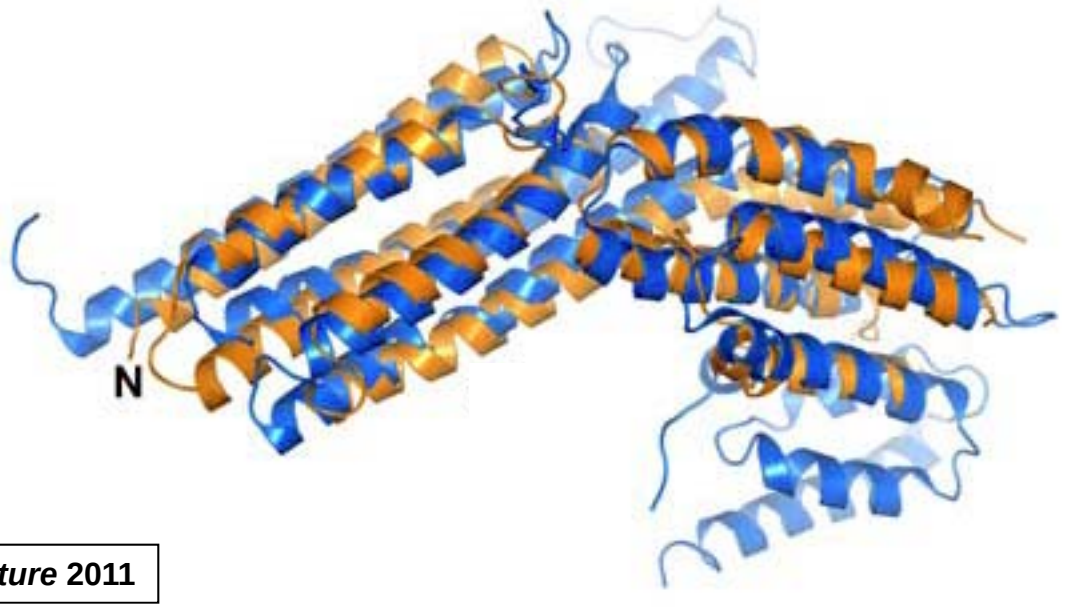




Pashkova, et al., *EMBO J* 2006
Jin, et al., *Dev Cell* 2012

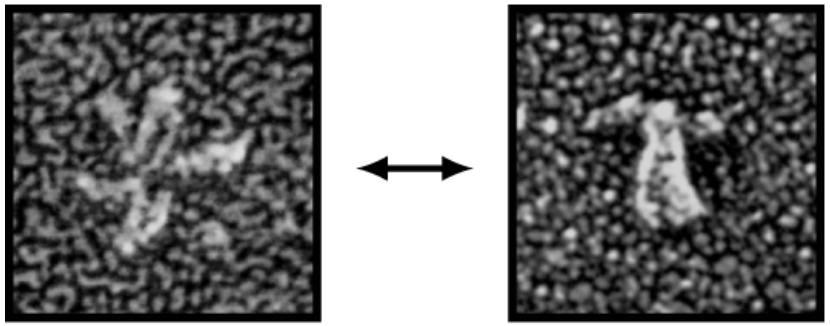
Similarity between the exocyst and Munc13, a neuronal SNARE regulator





Li et al., *Structure* 2011

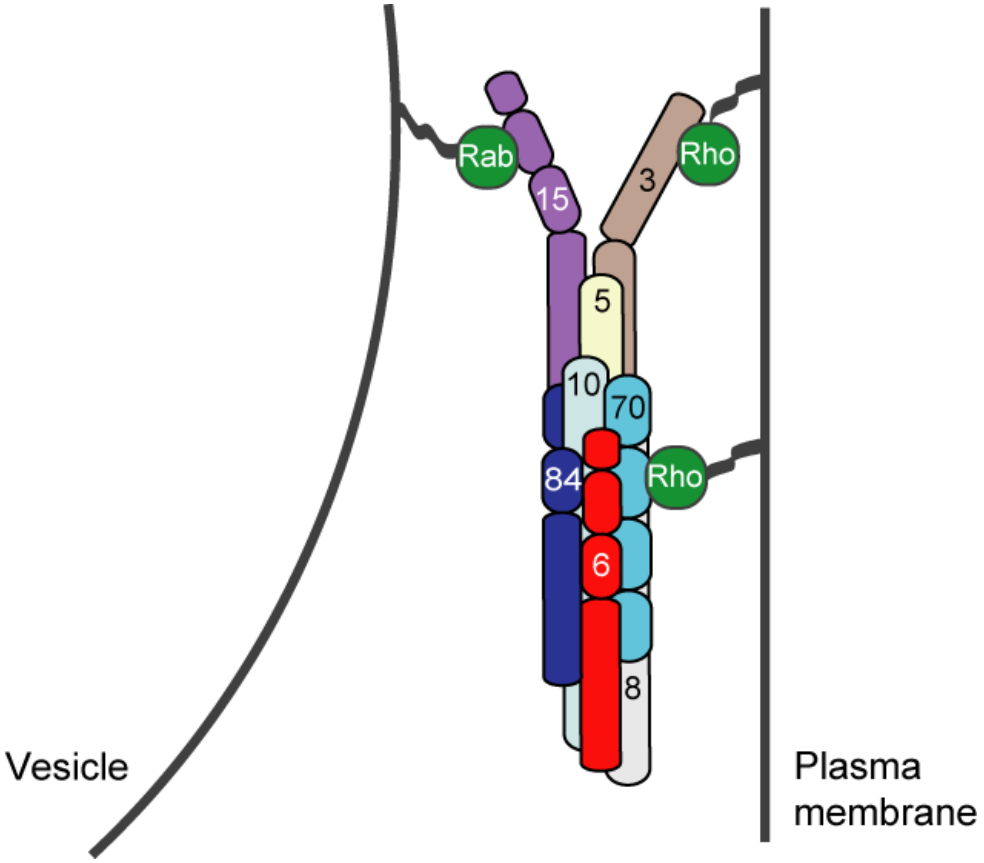
How does the exocyst assemble and does it tether secretory vesicles?



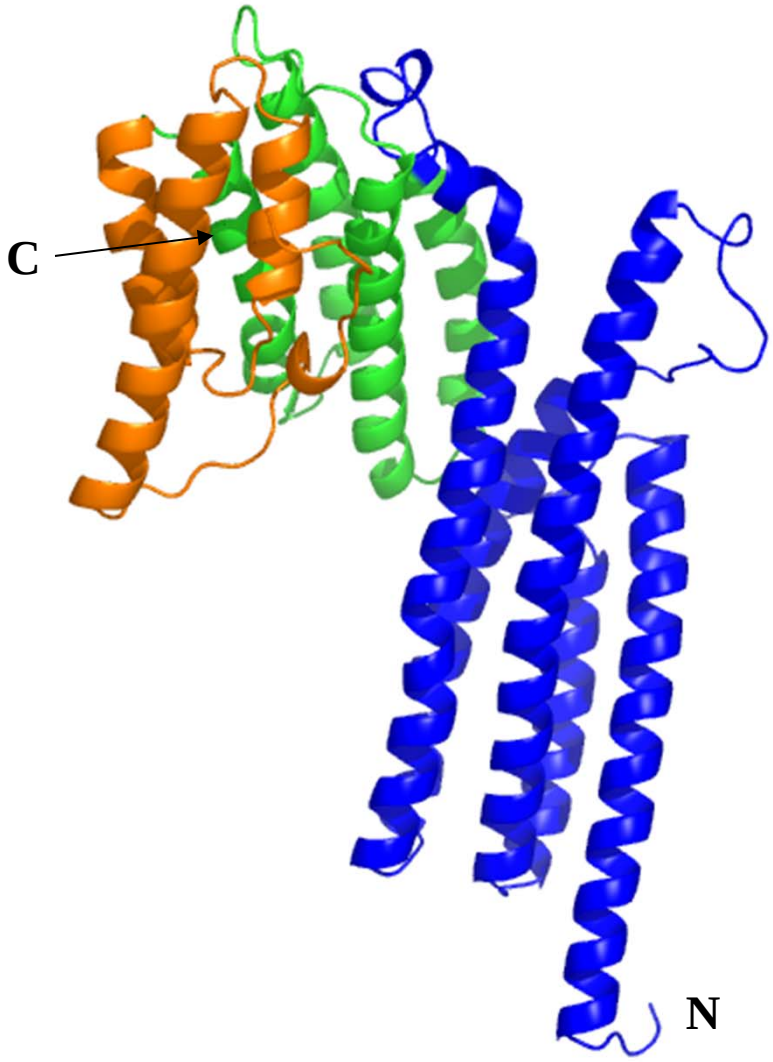
Unfixed

Glutaraldehyde fixed

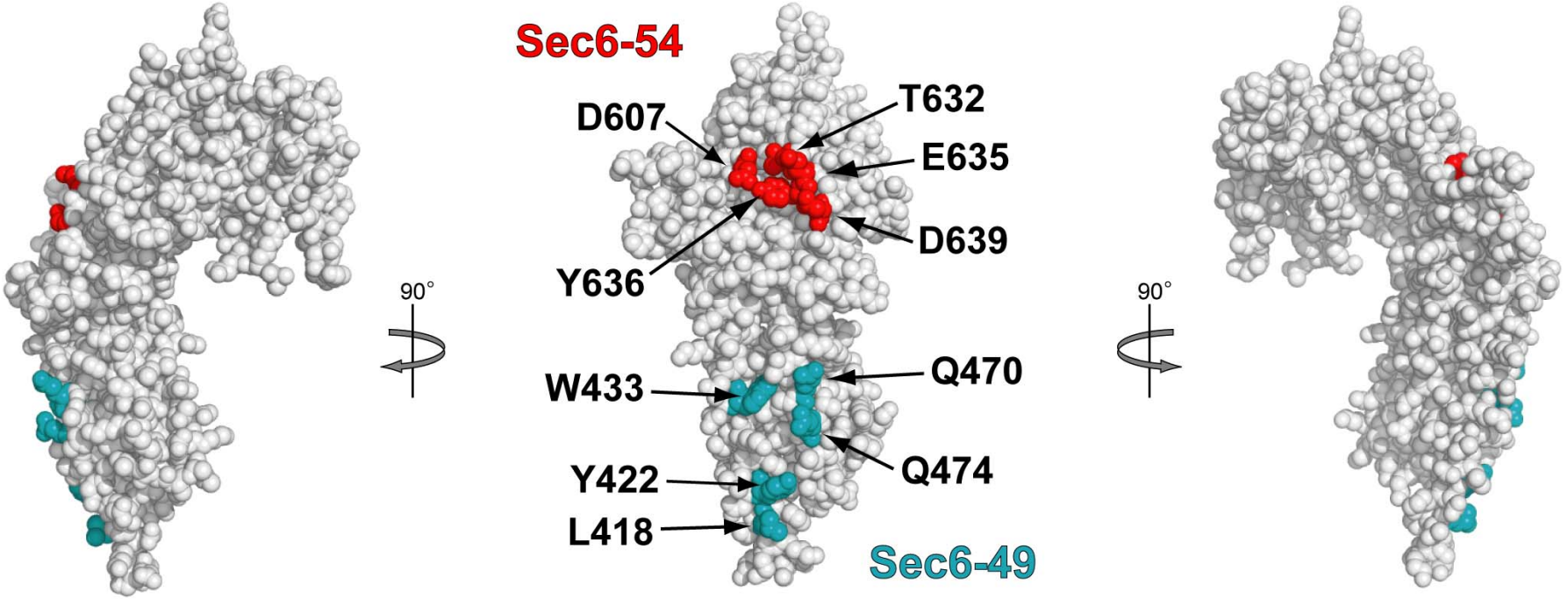
Hsu et al, *Neuron* (1998)



How does Sec6 bind to other partners?

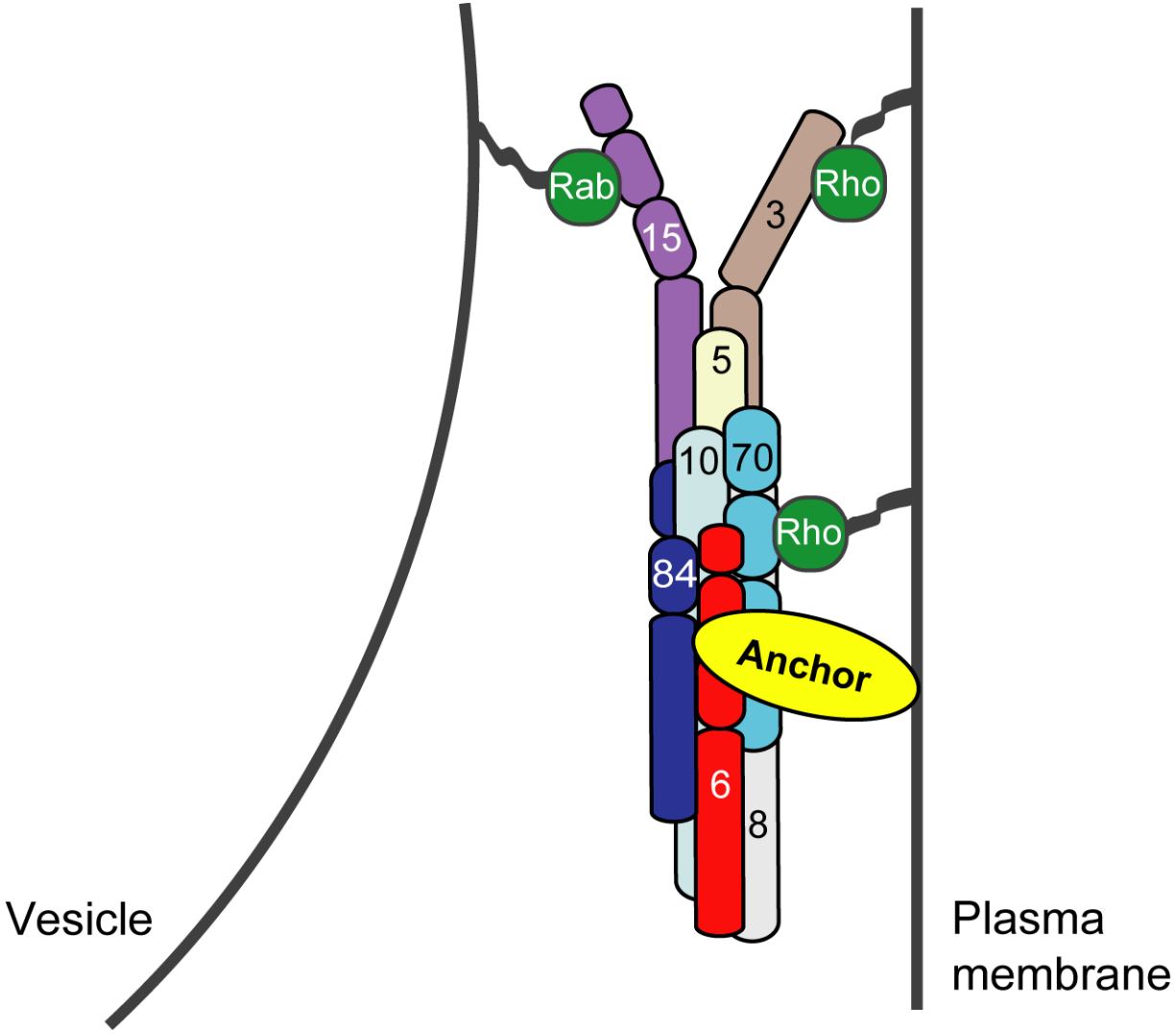


Conserved surface residues are required to anchor the exocyst at sites of secretion

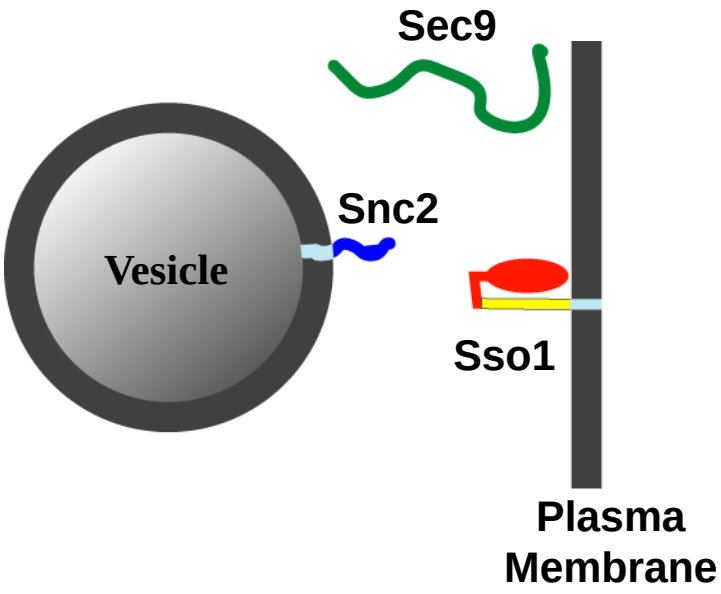


Songer & Munson, *MBC* 2009

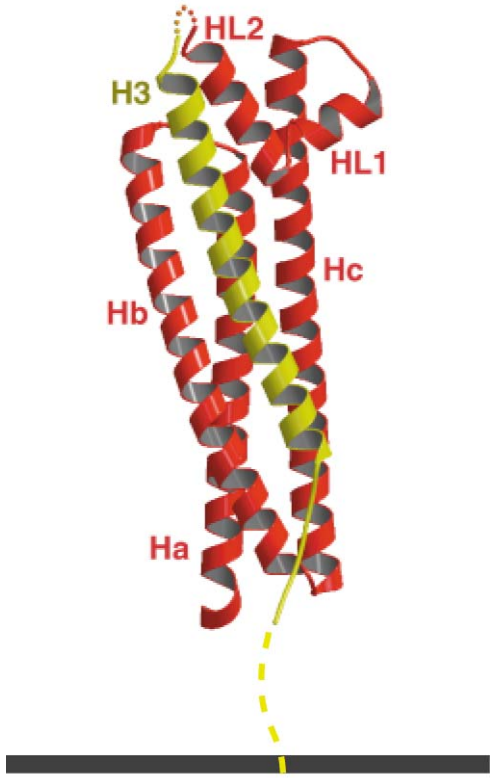
Conserved surface residues are required to anchor the exocyst at sites of secretion



Does Sec6 regulate SNARE complex assembly?

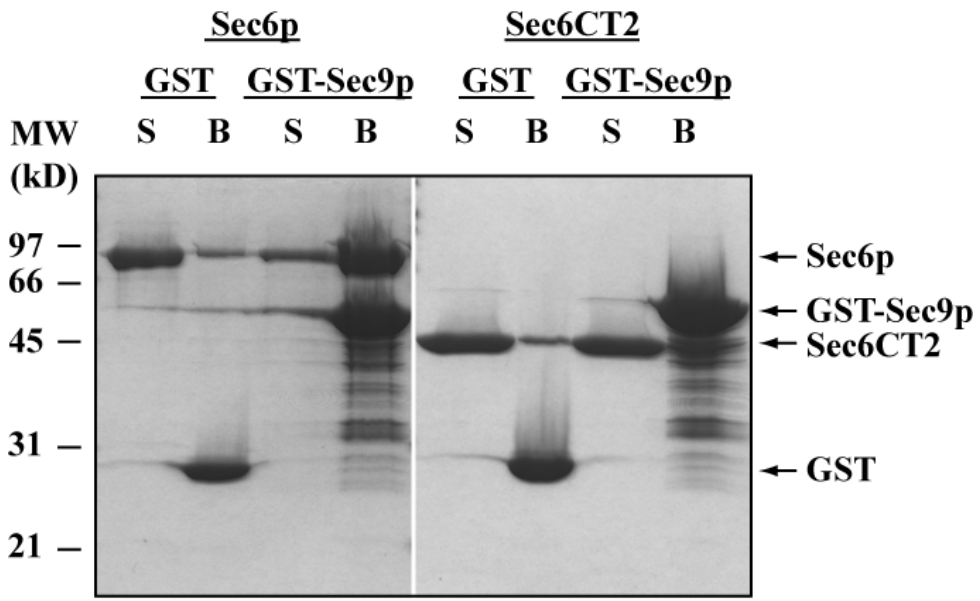


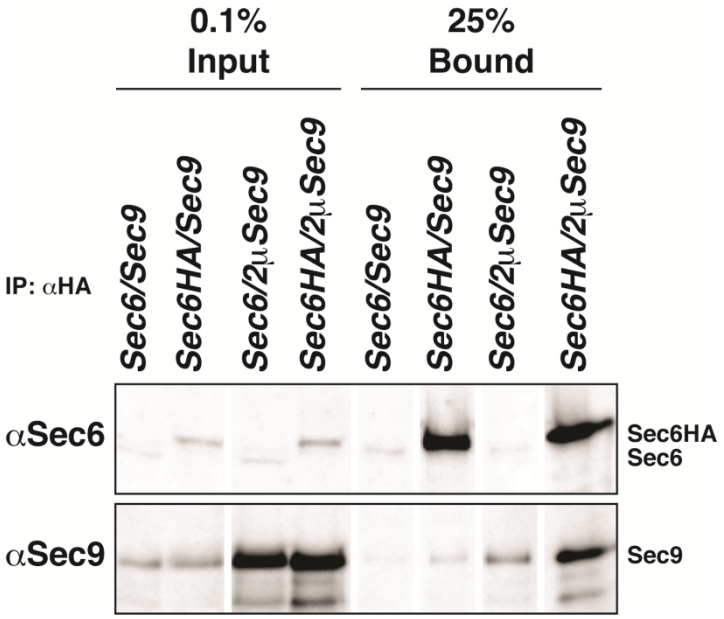
Sso1 – syntaxin
Sec9 – SNAP-25
Snc2 – VAMP

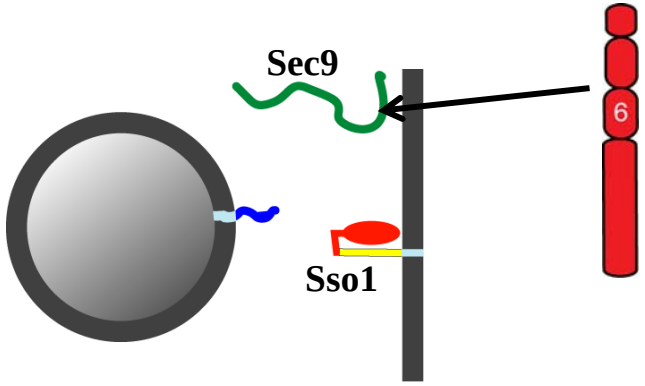


Sso1; Munson, et al.
Nature Str Biol (2000)

Sec6 binds to the t-SNARE Sec9 (SNAP-25); the C-terminal domain of Sec6 is not sufficient

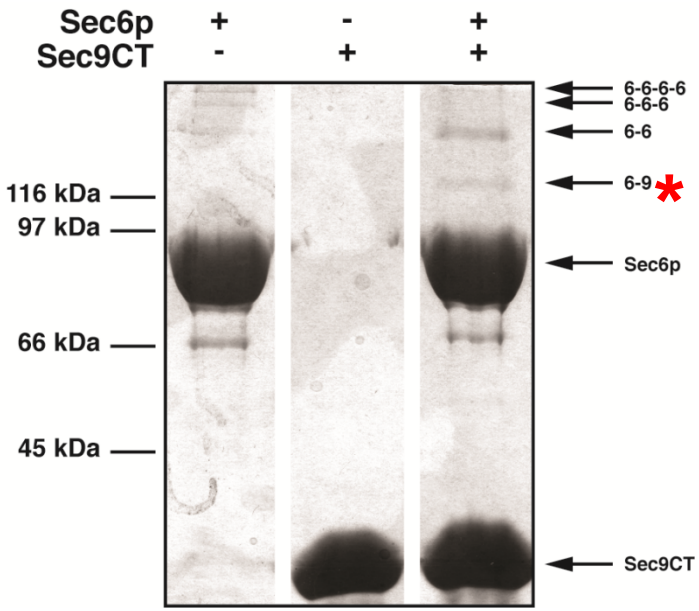




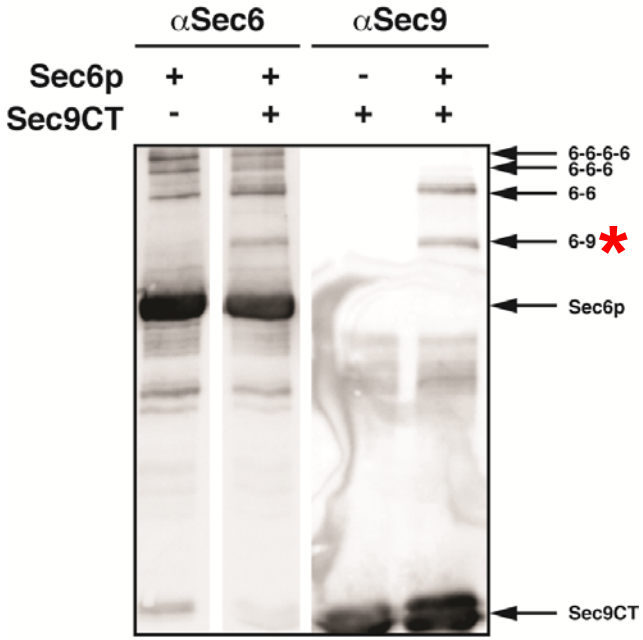


Sivaram, et al., *Biochemistry* 2005
Morgera et al. *MBC* 2011

Sec6 binds to the t-SNARE Sec9



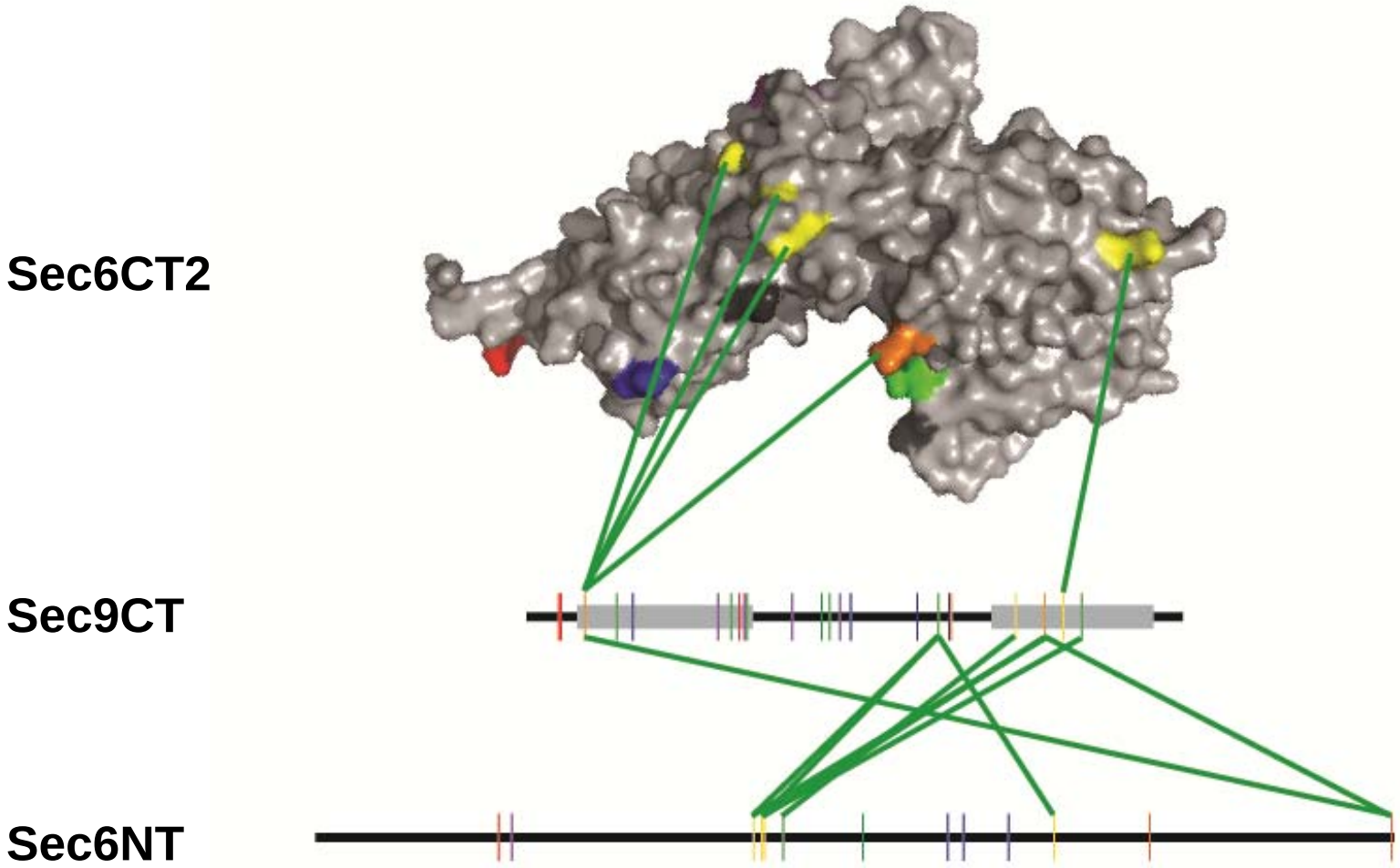
Coomassie stained



Western blots

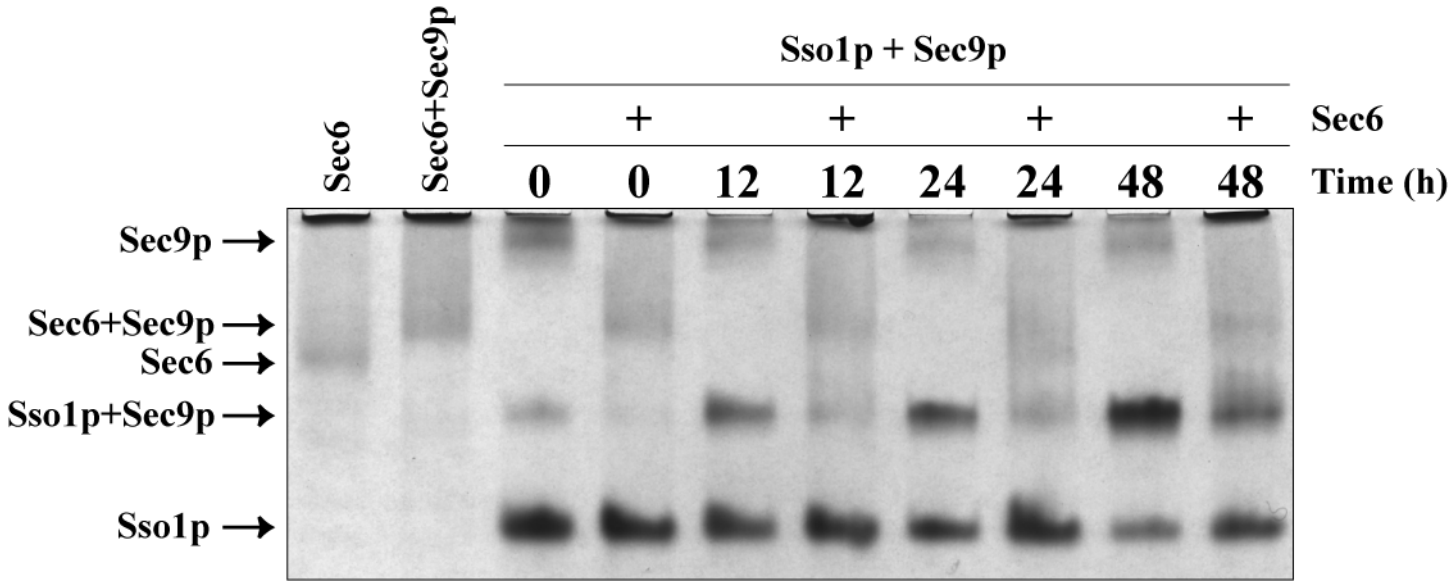
M. Dubuke

Sec6 binds to the t-SNARE Sec9



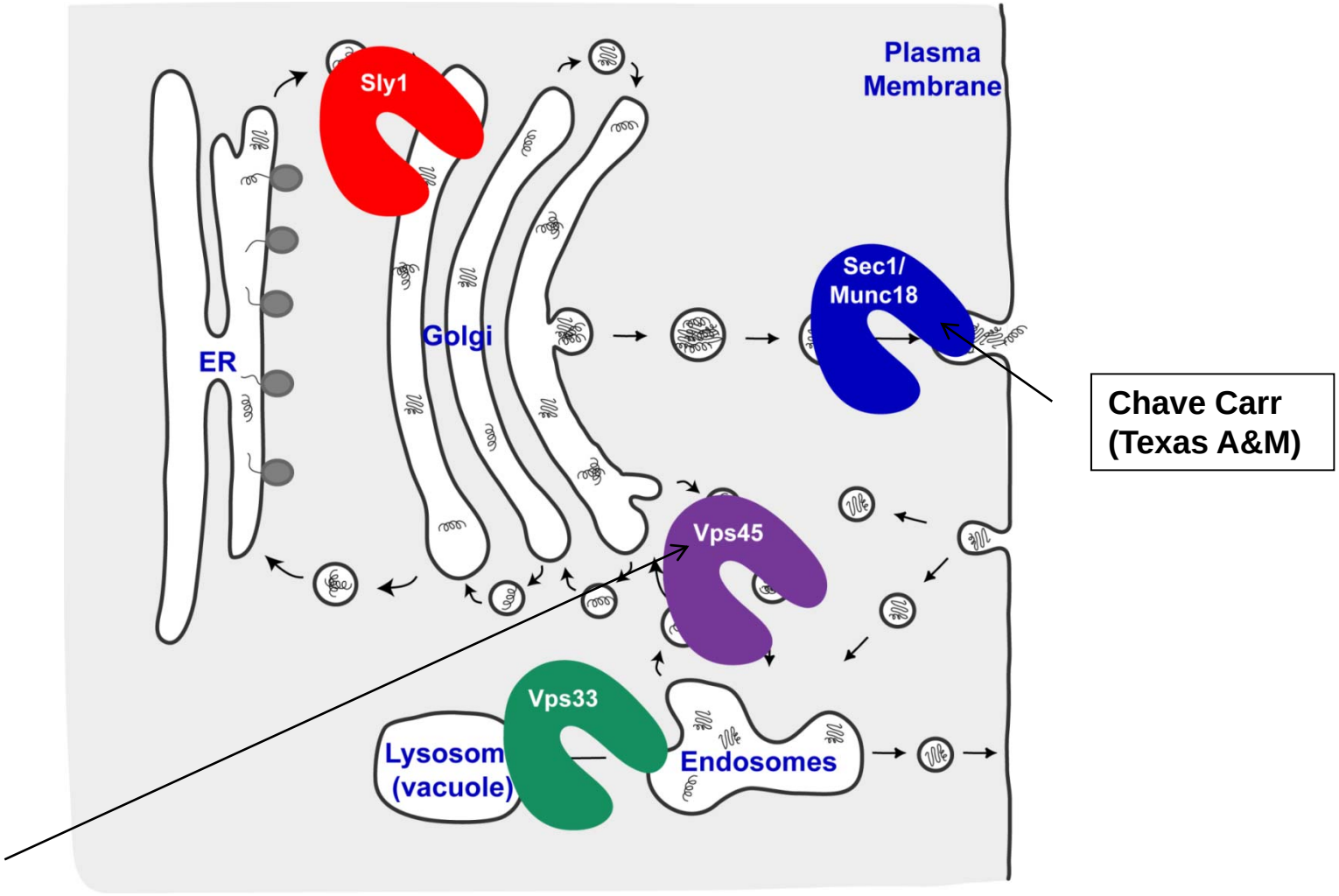
M. Dubuke,
S. Shaffer, S. Maniatis

Sec6 inhibits SNARE assembly



What releases the Sec6 inhibition?

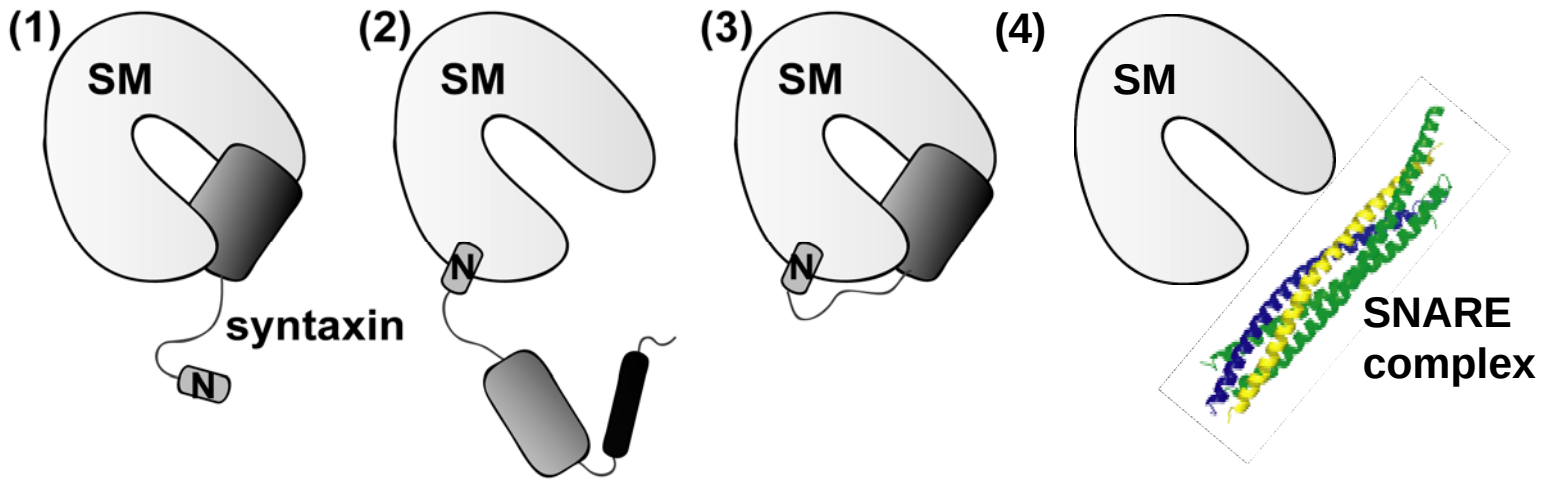
Sec1/Munc18 (SM) family of SNARE regulatory proteins



Chave Carr
(Texas A&M)

Furgason et al., *PNAS* 2009
with Nia Bryant (U Glasgow)

SM proteins bind SNAREs and SNARE complexes

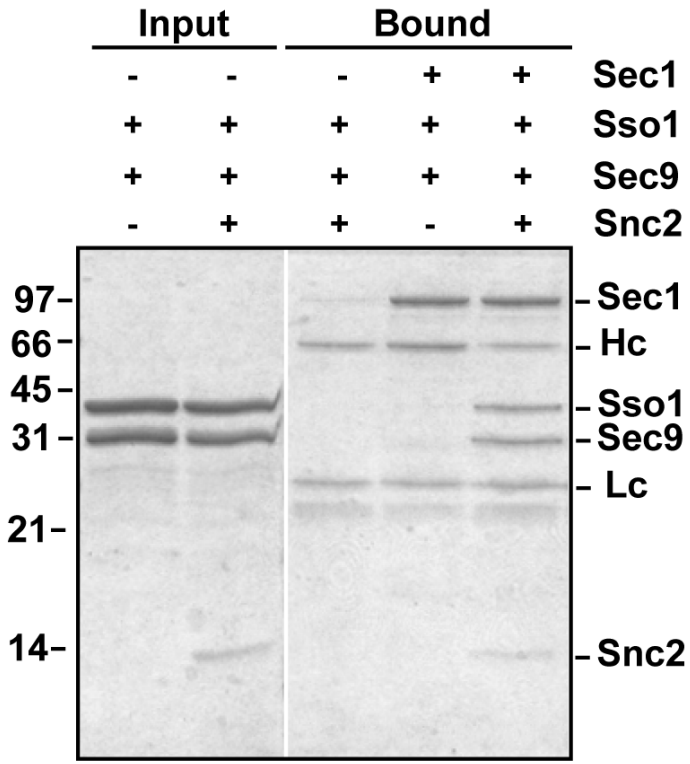


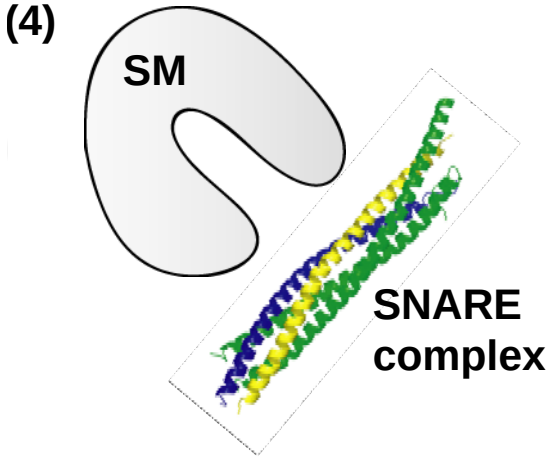
FUNCTION

Keeps syntaxin closed, to prevent inappropriate SNARE pairing? (inhibitory)	Opens syntaxin, to promote proper SNARE pairing? (stimulatory) or used for recruitment?	Both?	Regulates fusion?? or prevents disassembly?

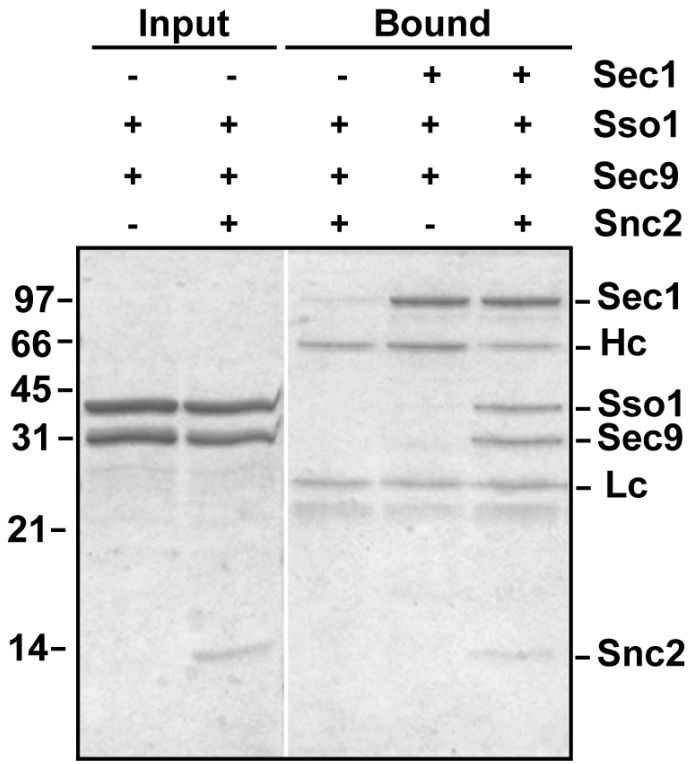
(interactions have also been detected with non-syntaxin SNAREs)

Yeast exocytic Sec1 binds SNARE complexes

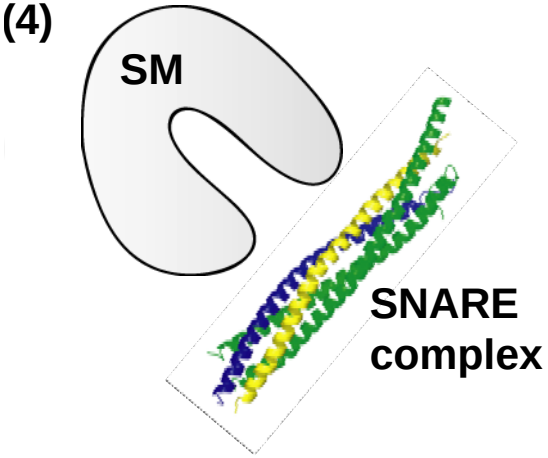




Yeast exocytic Sec1 binds SNARE complexes

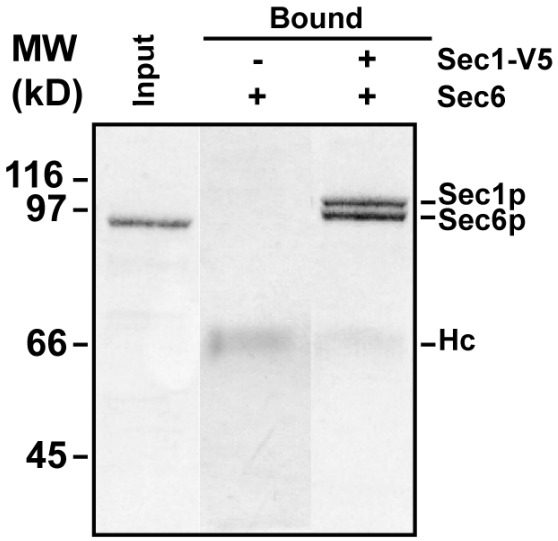


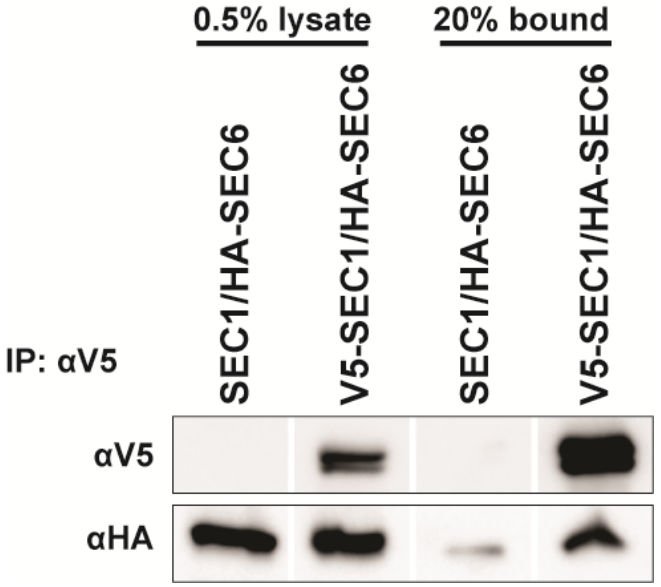
(4)



a. Sec1 binds SNARE complexes
b. Stimulates *in vitro* fusion of liposomes
c. Is mislocalized in exocyst mutants
d. Weak binding to exocyst complexes

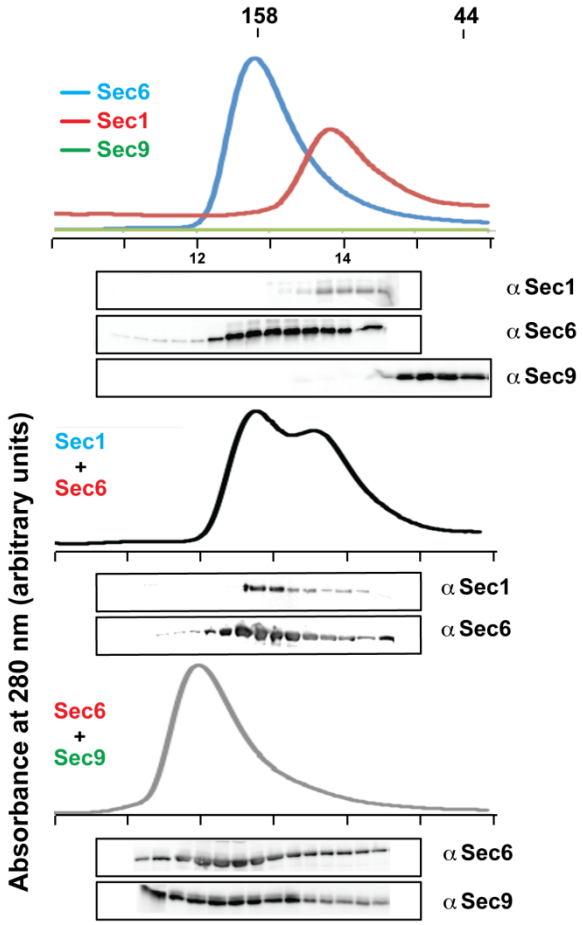
Sec6 interacts with the SNARE regulator Sec1





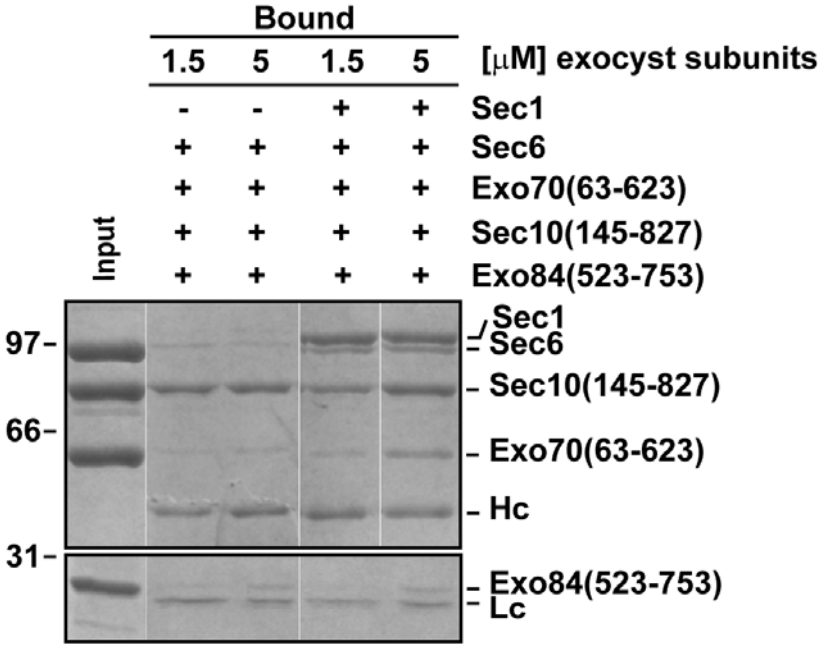
Morgera et al., *MBC* 2011

Sec1 competes with Sec9 for binding to Sec6



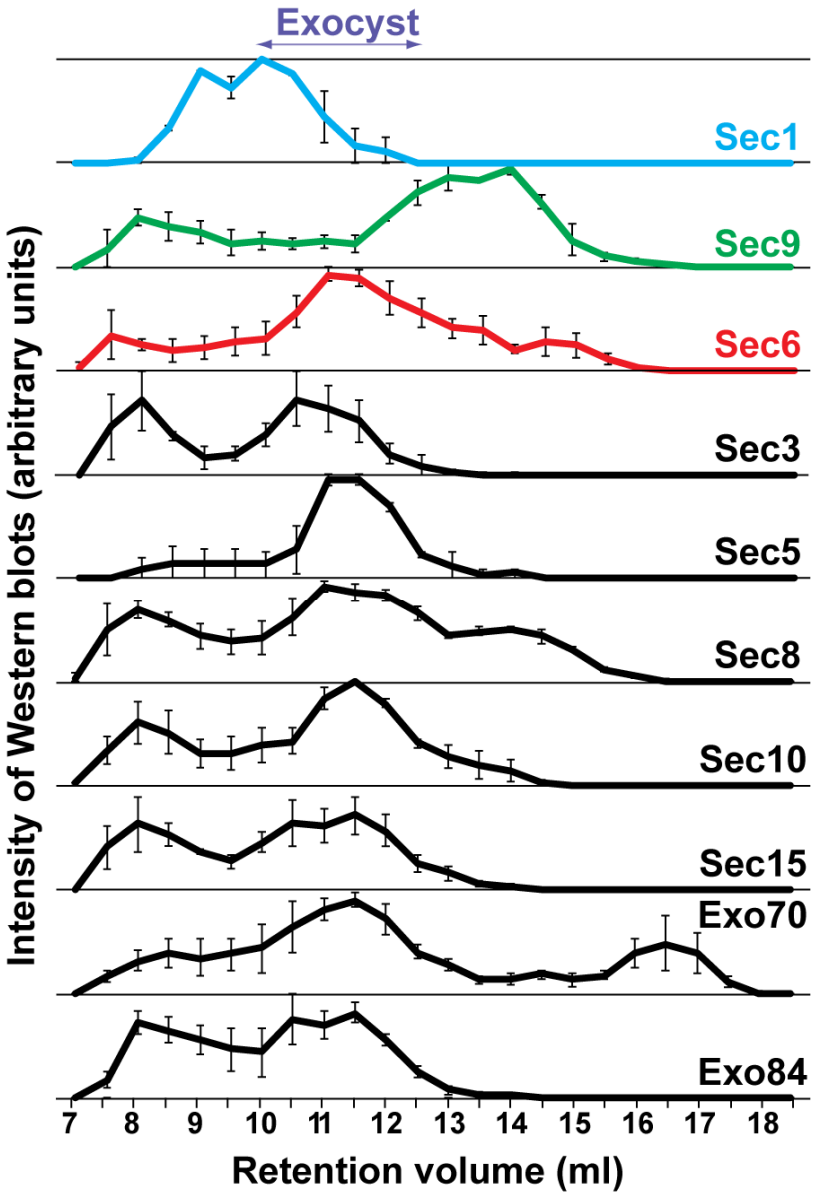
Morgera et al., *MBC* 2011

Sec1 binds to exocyst-bound Sec6; Sec9 competes



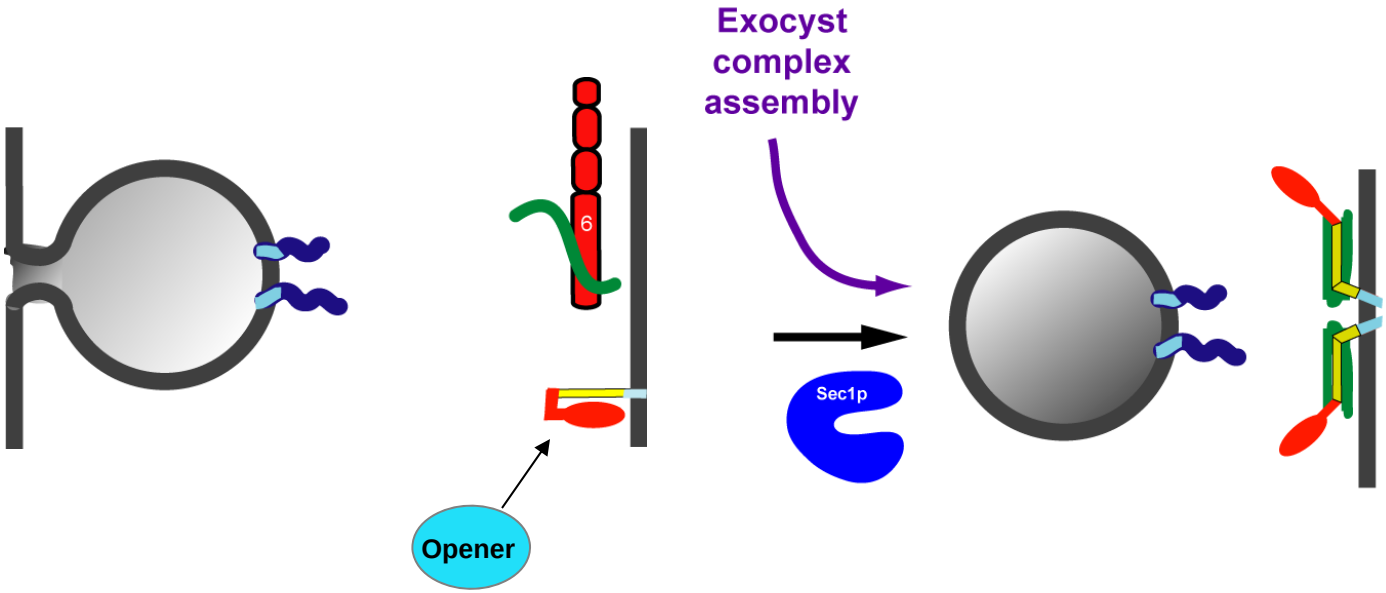
Morgera et al., *MBC* 2011

Gel filtration of yeast lysates

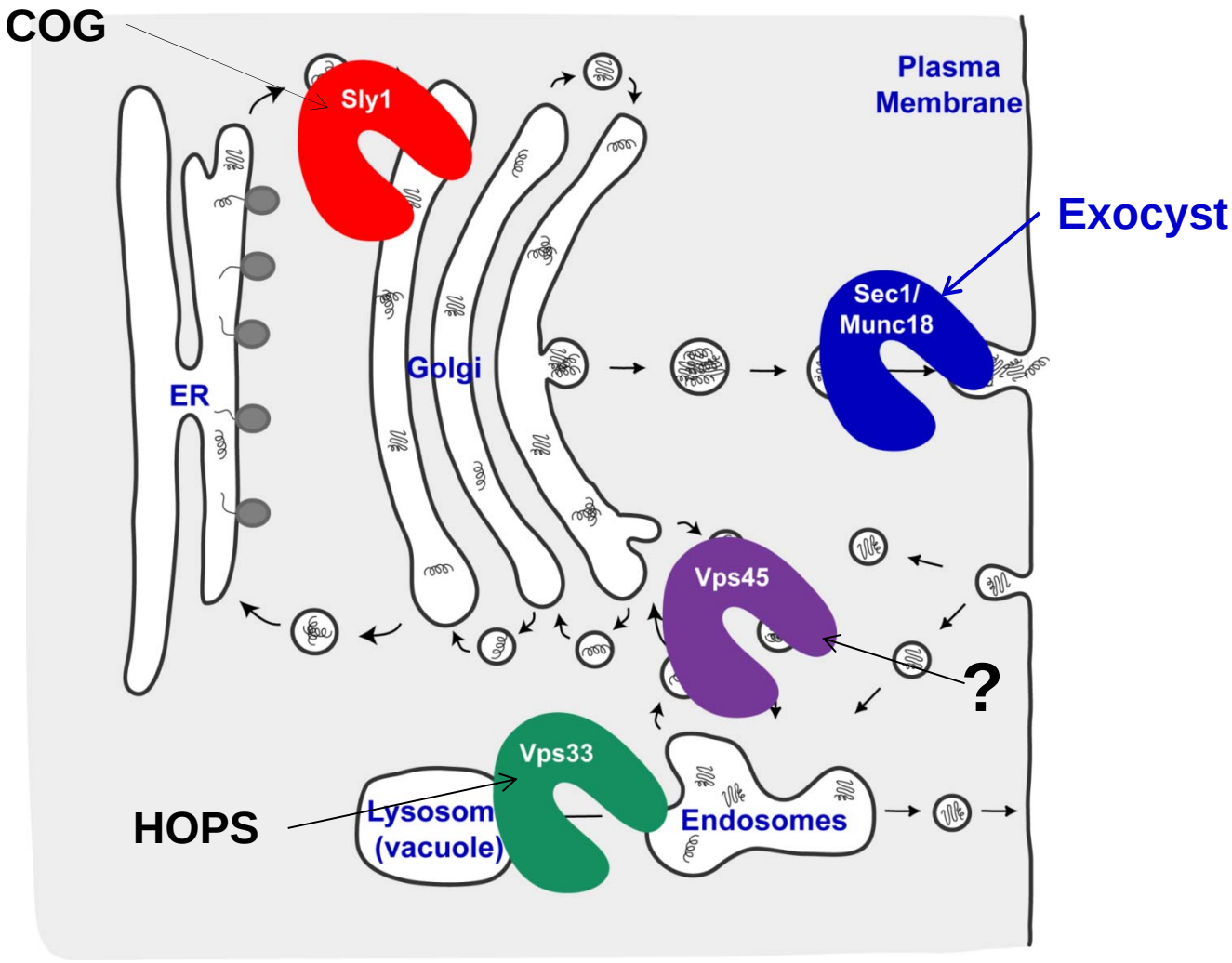


Michelle Dubuke
(Morgera et al., *MBC* 2011)

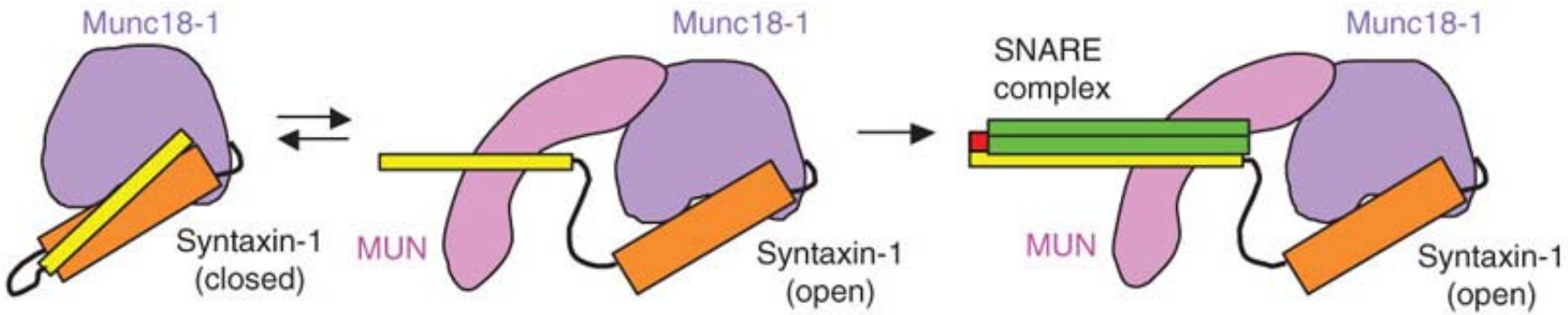
Sec6, Sec1 and the exocyst complex co-operate to regulate specific SNARE complex assembly



Every SM protein functions with a tethering complex?



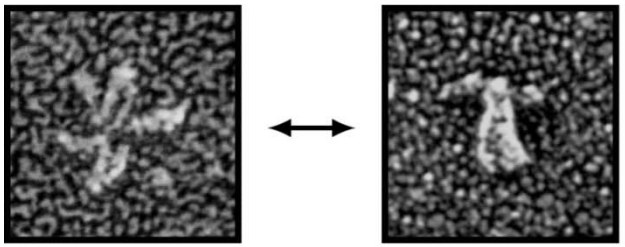
Munc13 can function to release Munc18's inhibition of Syntaxin

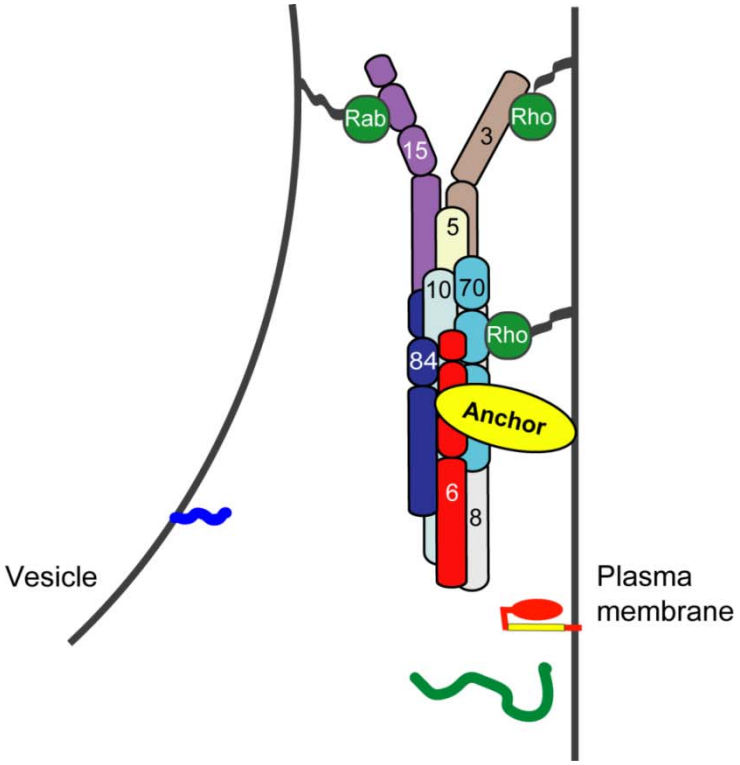


Could the exocyst-Sec1 complex be the opener?

Ma et al., *Nat Str Mol Biol* 2011

Conclusions





- Sec6 has a conserved helical bundle structure, similar to subunits from other tethering complexes
- Patches of conserved residues on Sec6 are necessary for anchoring the exocyst at sites of secretion
- Sec6 binds the plasma membrane SNARE Sec9, and the SNARE regulator Sec1 in order to regulate SNARE complex assembly

Acknowledgments

Michelle Dubuke
Caroline Duffy
Pallavi Gandhi
Miye Jacques
Francesca Morgera
Maggie Sallah
Kristine Zeeb

Previous lab members:

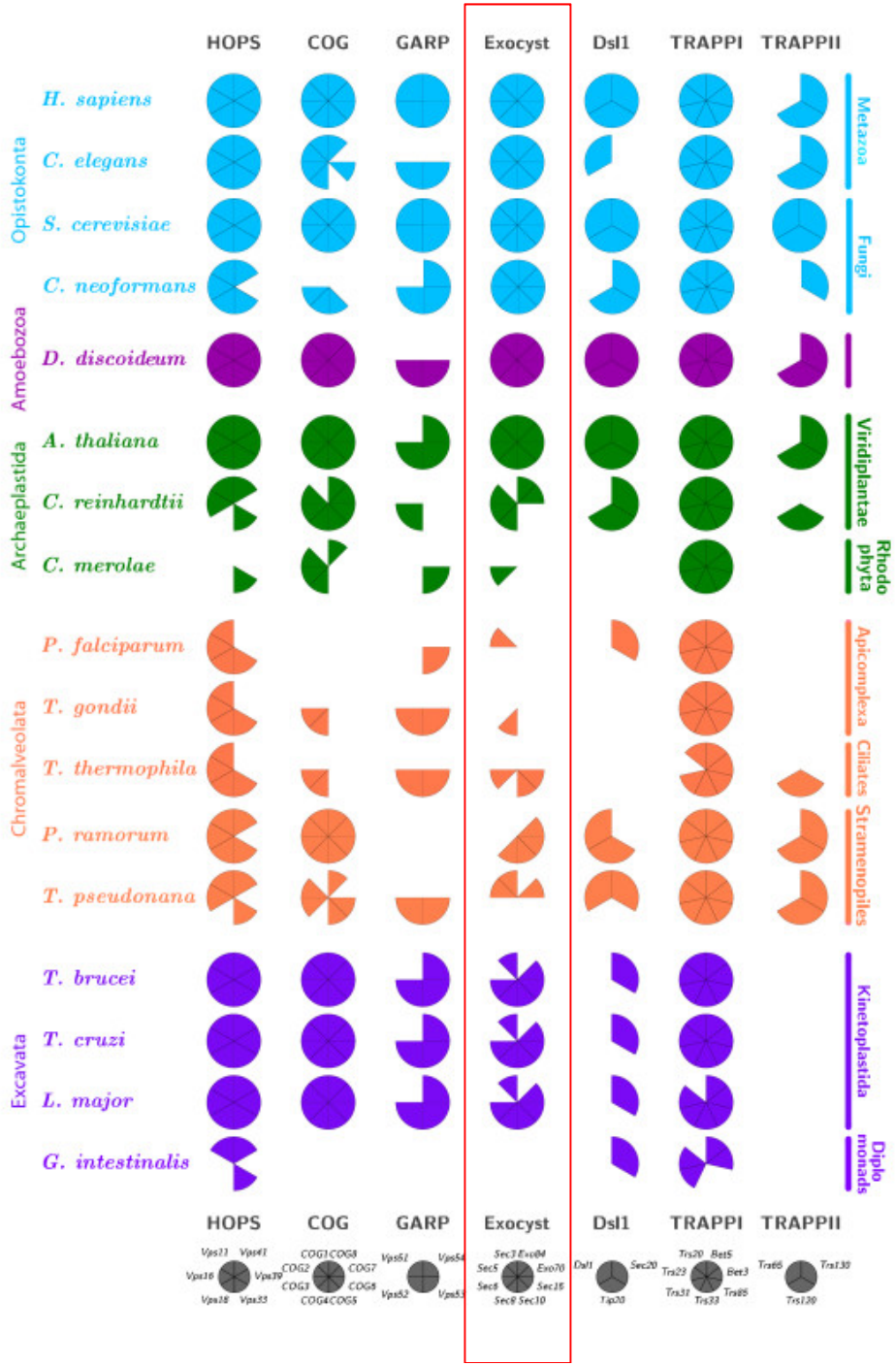
Dan Brewer
Melonnie Furgason
Sivaram Mylavarapu
Jen Songer

Clarissa Andre
Nikki Croteau
Amber Lachapelle
Jess Rocheleau
Beth Marek
Kevin O'Brien
Amanda Tichy
Jamie Towle
Ashleigh Wood
Leslie Guadron

NIH (NIGMS)

Chave Carr (Texas A&M)

Nia Bryant (U Glasgow)



Koumandou et al.,
***BMC Evol Biol* 2007**

“Structure” of the mammalian exocyst complex

Hsu et al, *Neuron* (1998)