A model for how the Golgi works and transport vesicle tethering at the Golgi

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All Eukaryotes have a Golgi

- Glycosylation enzymes
- Rabs, tethers and SNAREs
- Most eukaryotes use stacked structures

3D Tomogram of the Golgi

Insulin secreting HIT cell: rapid frozen, freeze substituted, cut into 400nm sections

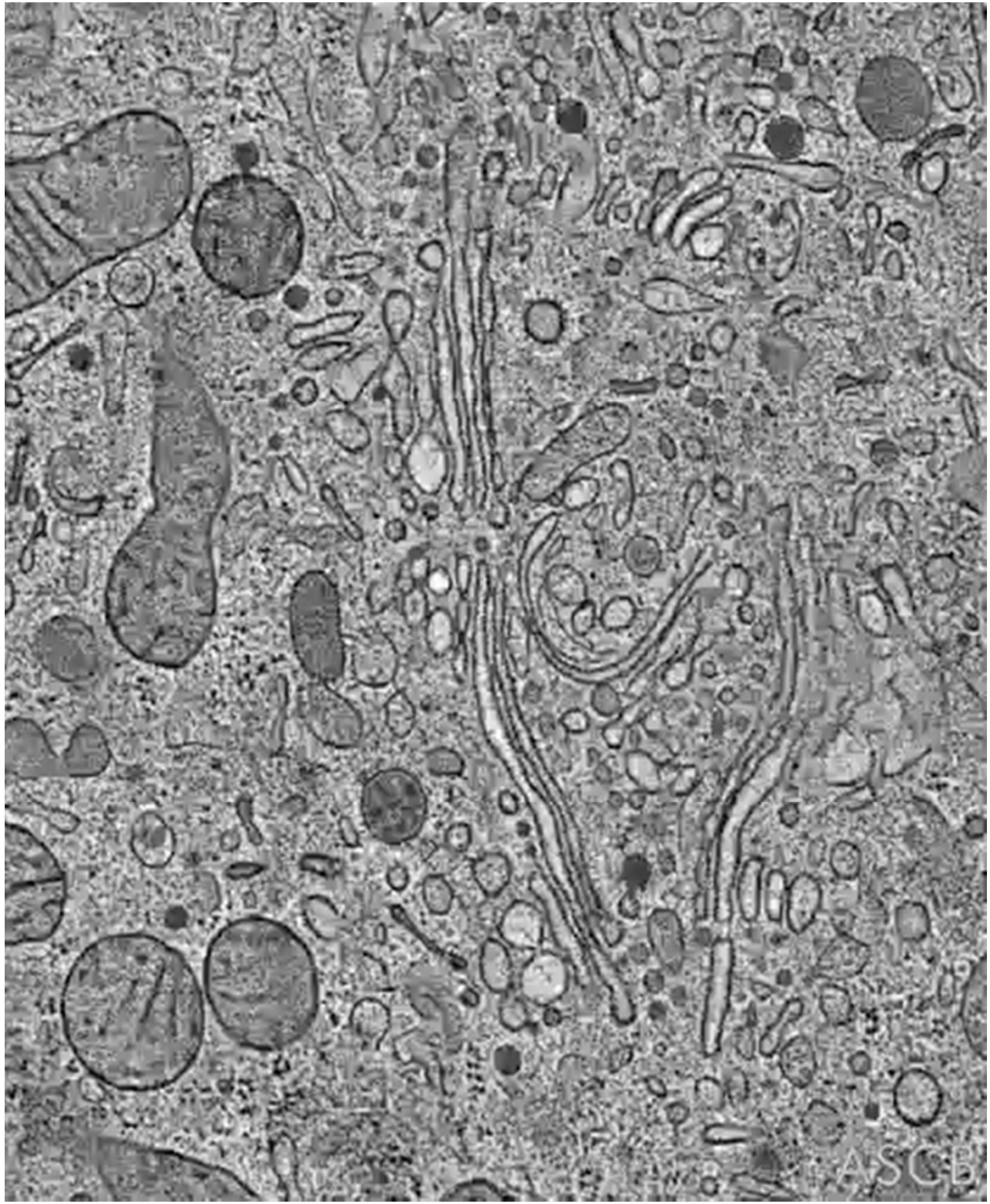
Specimen is tilted 120°, with high voltage EM images captured every 1.5°; process is repeated with 90° rotation

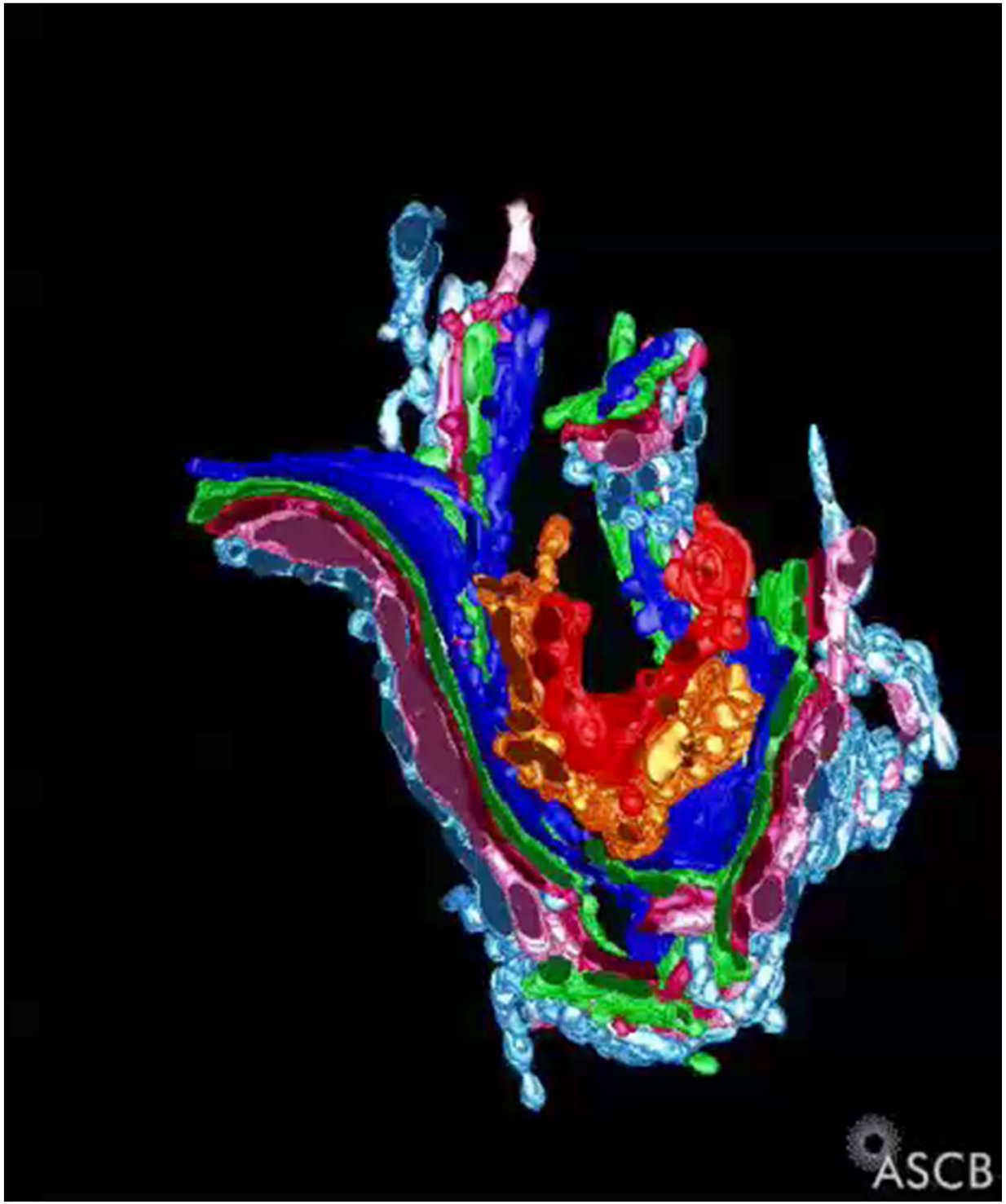
Computationally reconstructed in 3D

Resolution = 6-7 nm

Brad Marsh, Kathryn Howell, Dick McIntosh

University of Colorado Health Science Center (Aurora) and Boulder;
University of Queensland at Brisbane


ASCB


ASCB

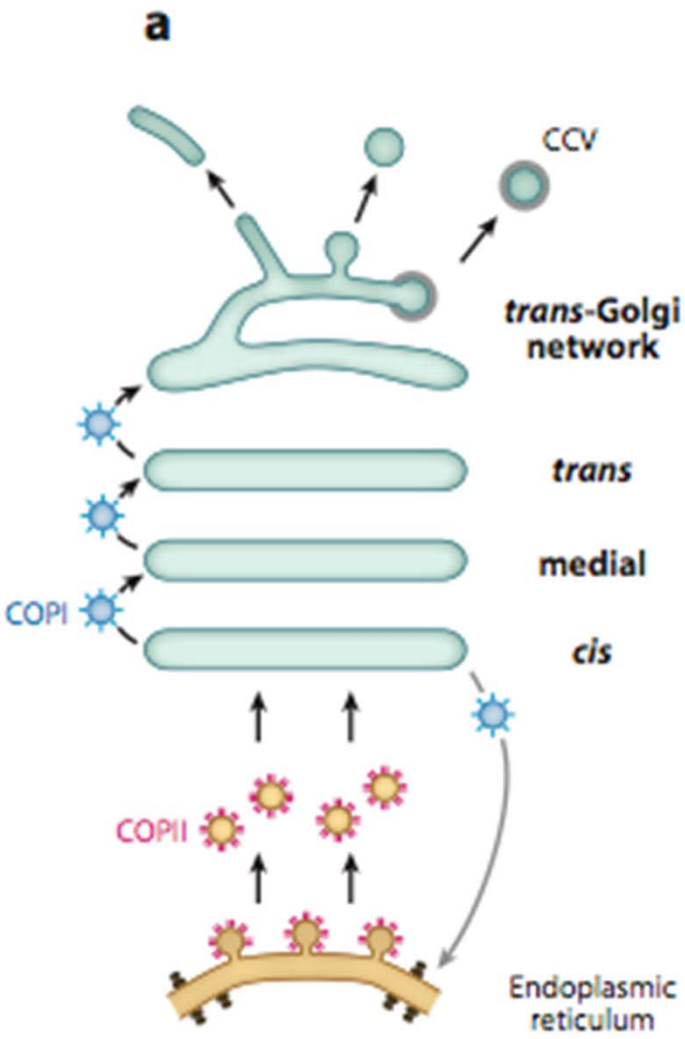
Of fundamental interest:

How are these compartments generated and maintained?

How do proteins move through this compartment?

How do transport pathways acquire directionality?

Two models for transport through the Golgi complex

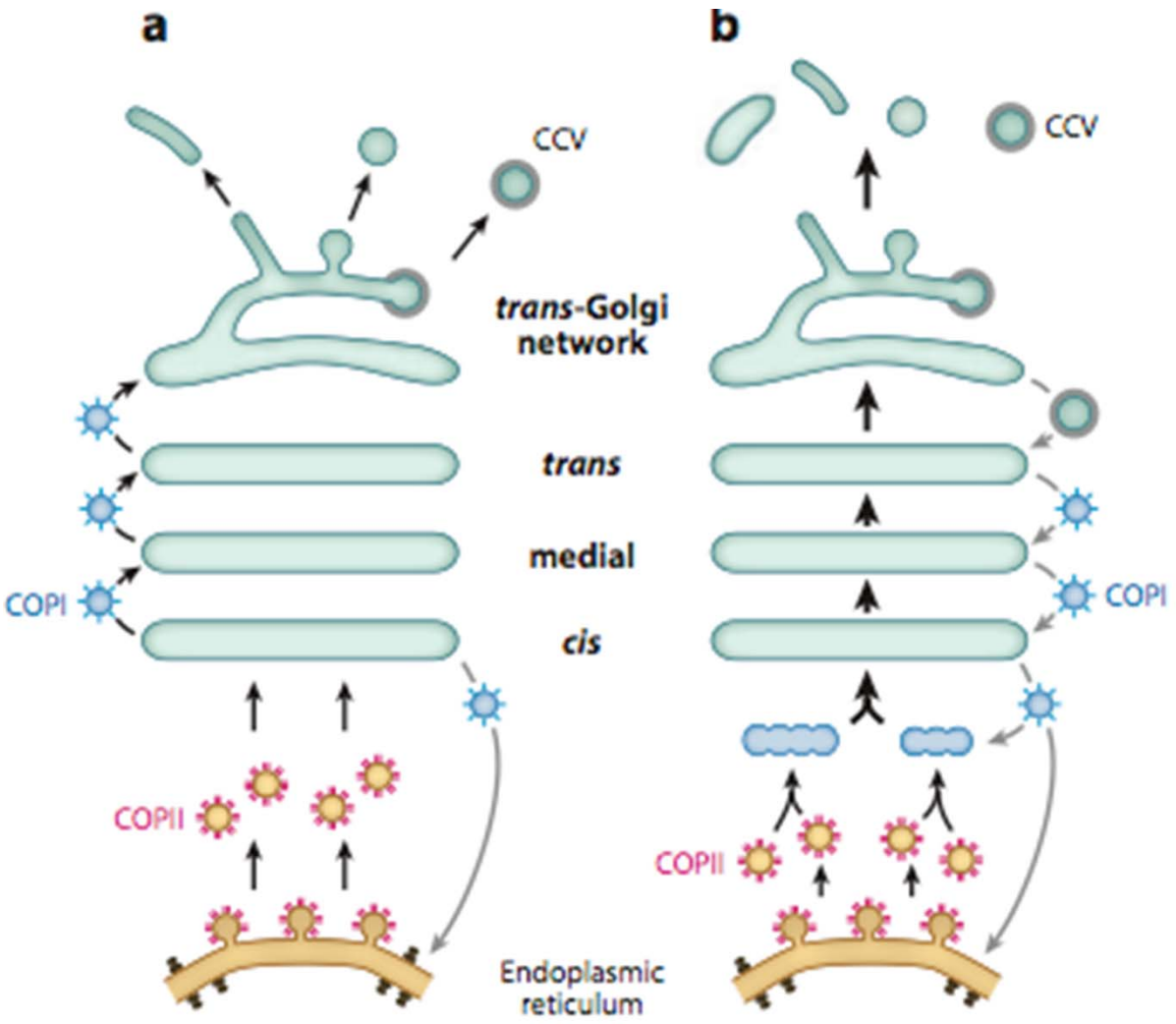


Model #1

Stable compartment; vesicles carry **CARGO**

Ben Glick & Aki Nakano (2009) Ann. Rev. Cell Dev. Biol. 25:113

Two models for transport through the Golgi complex



Model #2

Maturing compartment; vesicles carry **ENZYMES**

Ben Glick & Aki Nakano (2009) Ann. Rev. Cell Dev. Biol. 25:113

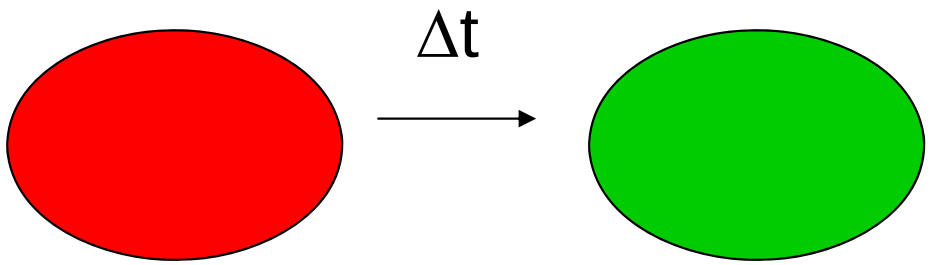
Distinction: What's in the vesicles?

A. Stable model, vesicles contain cargo

B. Maturation model, vesicles contain enzymes

Data support both models

1. S. cerevisiae Golgi is not stacked in high glucose; maturation has been seen in live cell video
   (Glick, Nakano, Novick)



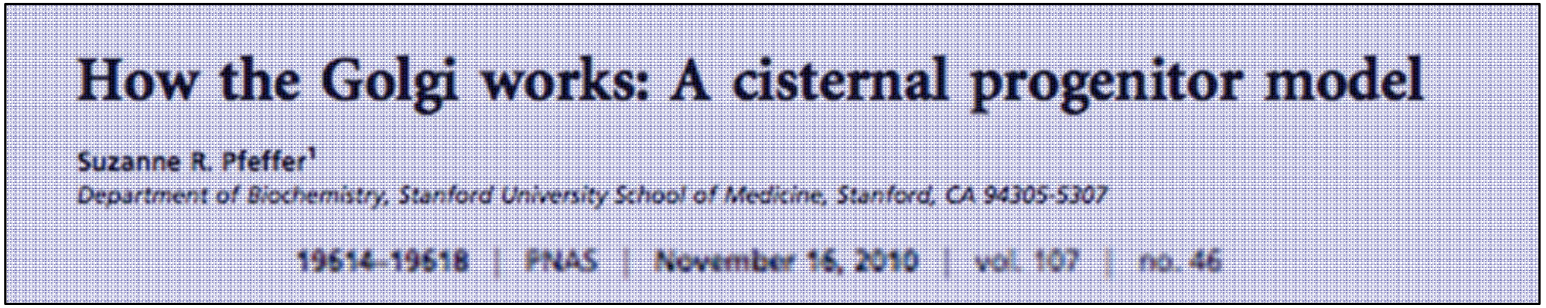
Data support both models

- vesicles contain cargo and in most studies, not enzymes

So how would this work for a stacked Golgi?

- Large cargo (collagen) moves through mammalian stack without leaving cisternae (Luini)
- Small tubules can sometimes be seen between cisternae (maybe they don't mature?) (Luini, Marsh)
- Export kinetics don't fit with maturation (Lippincott-Schwartz)
- **The Golgi stack appears very stably connected**

Rab GTPase Cascades can help explain “apparent” maturation and pathway directionality

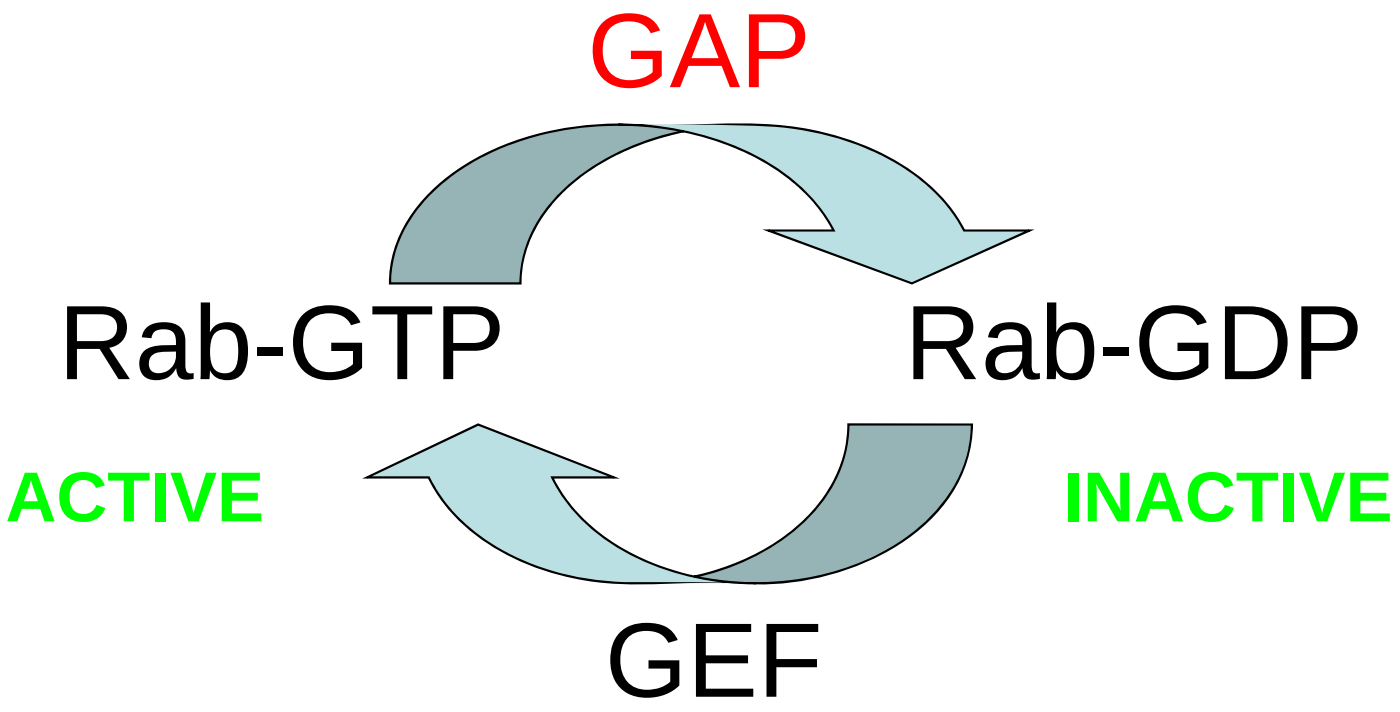


How the Golgi works: A cisternal progenitor model

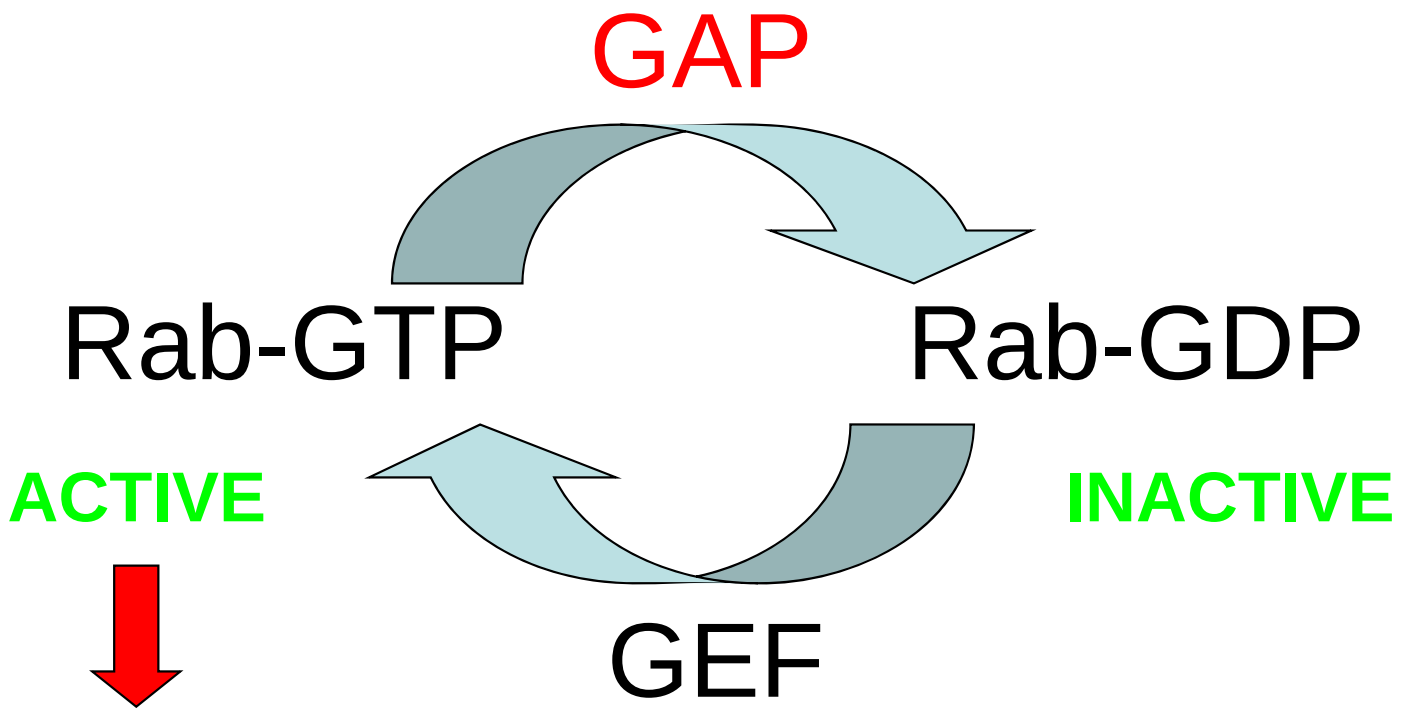
Suzanne R. Pfeffer[1]
Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305-5307

19614–19618 | PNAS | November 16, 2010 | vol. 107 | no. 46

Rabs interconvert between nucleotide states



Rabs interconvert between nucleotide states



“effector proteins”

Rab GTPases

Cargo collection/vesicle formation

Vesicle motility

Vesicle docking

Vesicle fusion

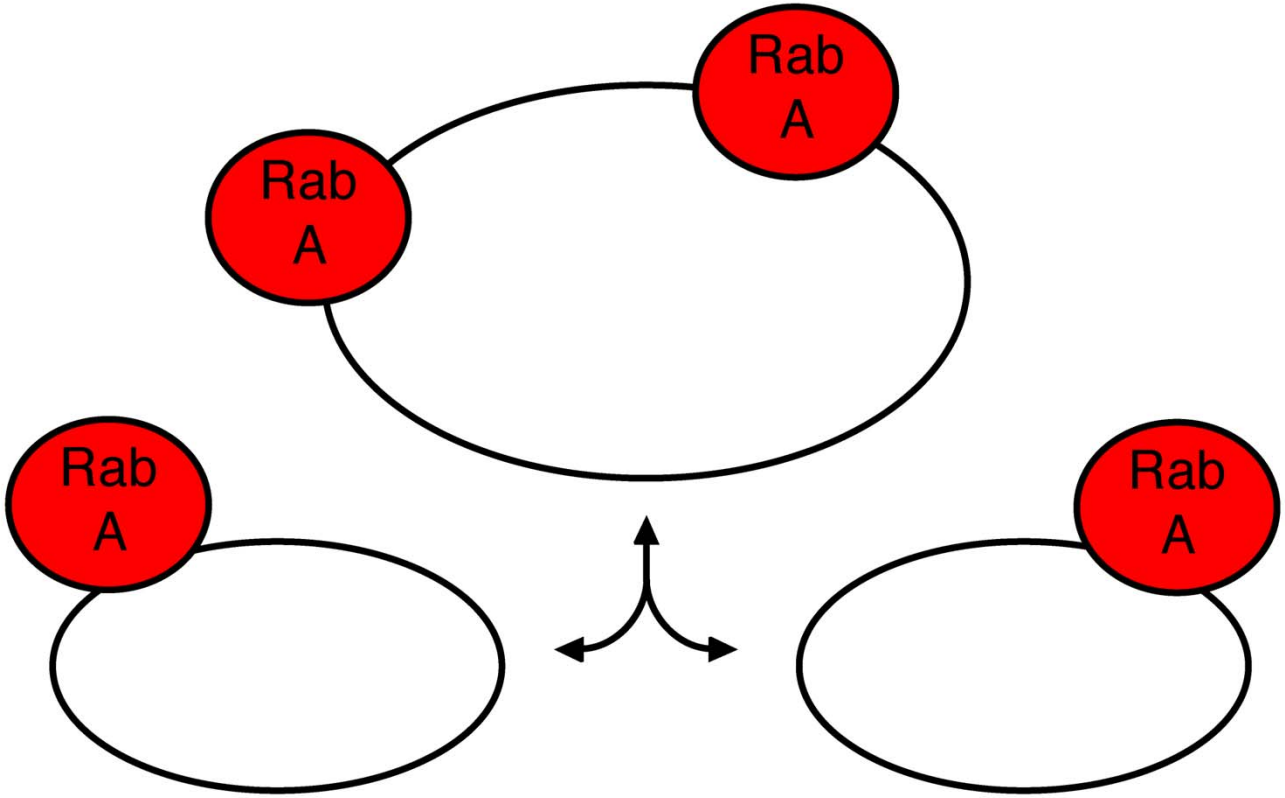
Catalyze formation of function-specifying membrane-microdomains

Rab GTPases template directionality

RabA → RabB → RabC

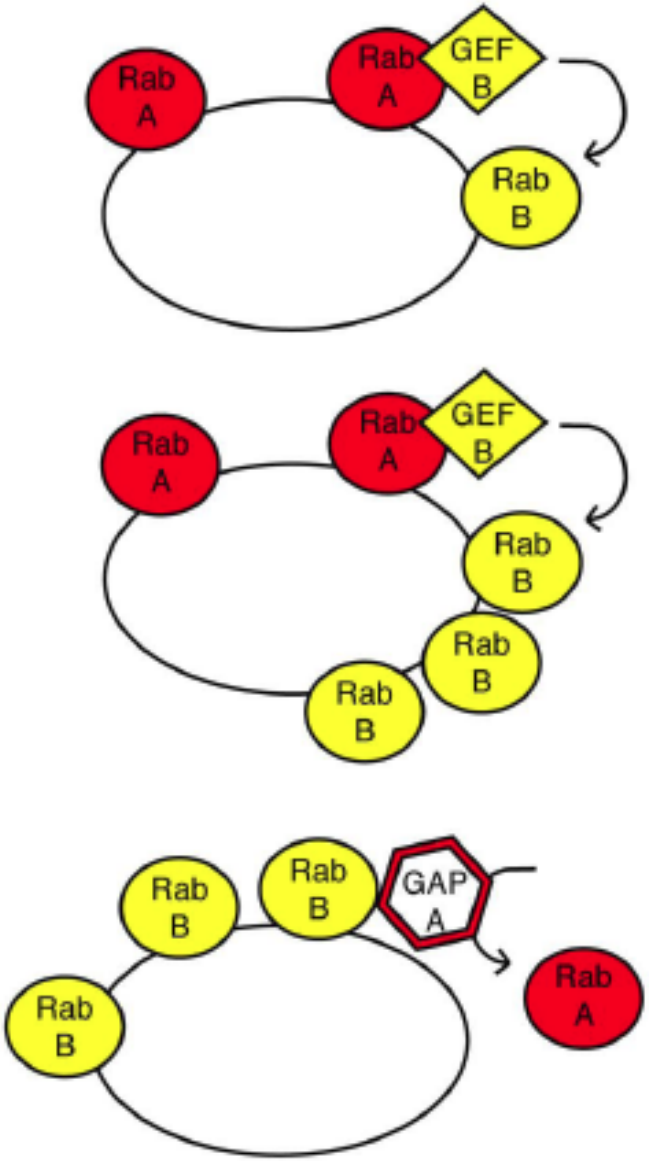
“Rab cascade” or “Rab conversion” (Novick, Zerial)

Cellular membranes undergo **Rab regulated-** fission and homotypic fusion



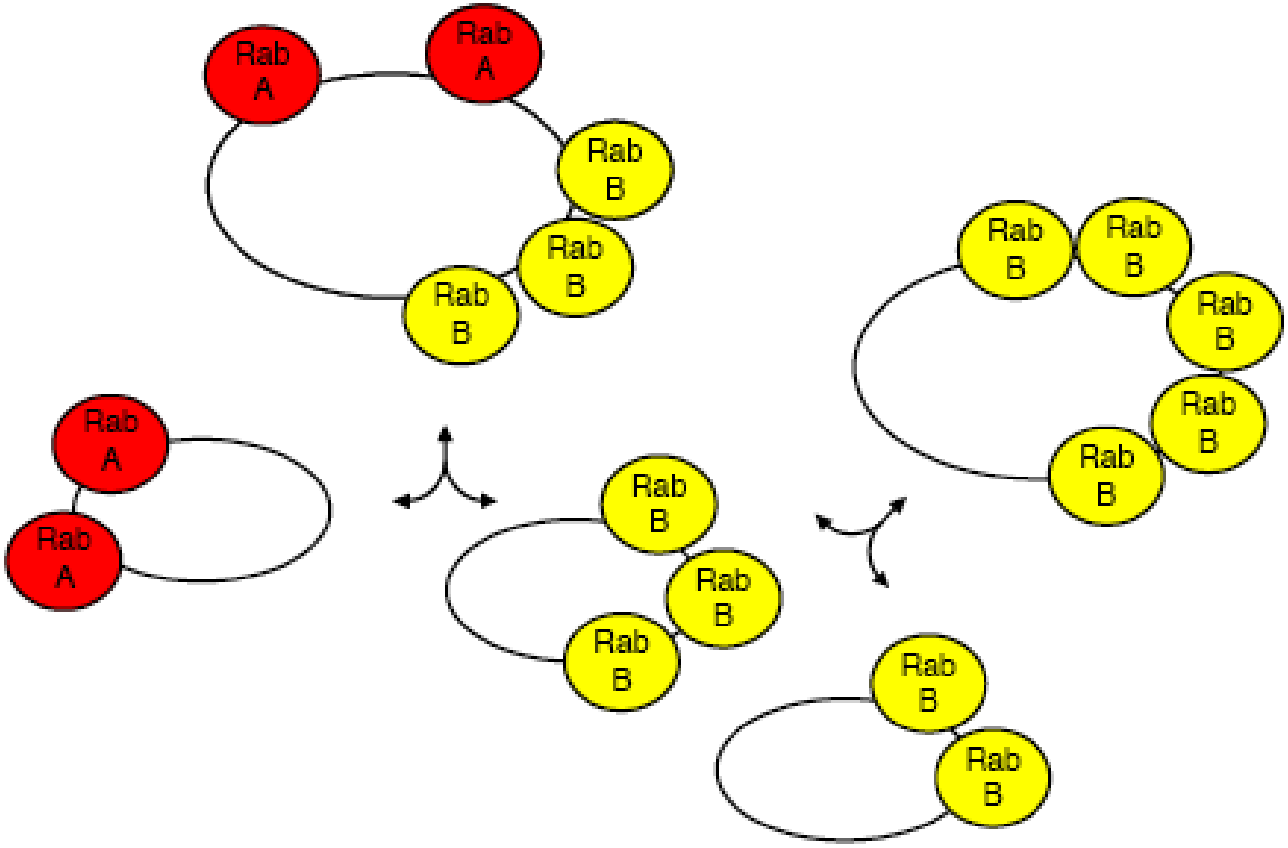
Rabs recruit tethering factors and SNAREs for fusion

Rab ‘conversion’ of compartment identity



Novick, Zerial

Fission and fusion can recreate unique compartments



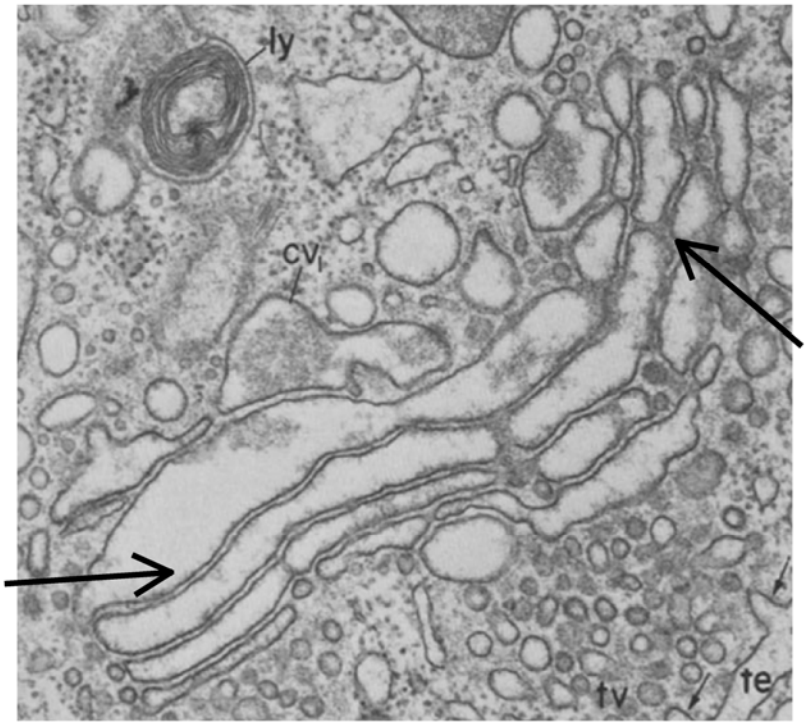
A Rab GAP cascade defines the boundary between two Rab GTPases on the secretory pathway

Félix E. Rivera-Molina[a] and Peter J. Novick[b,1]

"Our live cell imaging studies are generally consistent with the cisternal maturation model….However, close examination suggests that other processes may contribute as well. **Golgi compartments were seen to be dynamic, undergoing a certain amount of fission and fusion. In some cases (30%), a Ypt32p compartment appeared to fuse to a Ypt1p compartment to yield a mixed compartment or a mixed compartment appeared to undergo segregation and fission to yield separate compartments**…"

(2009) Proc. Natl. Acad. Sci. 106, 14408

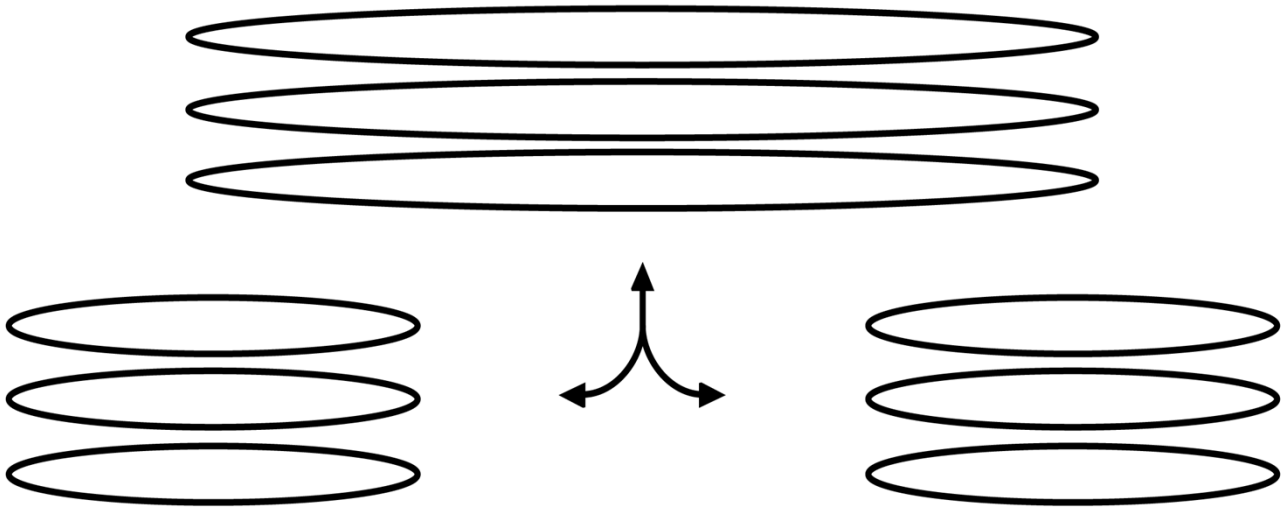
The Golgi is not continuous



close membrane apposition

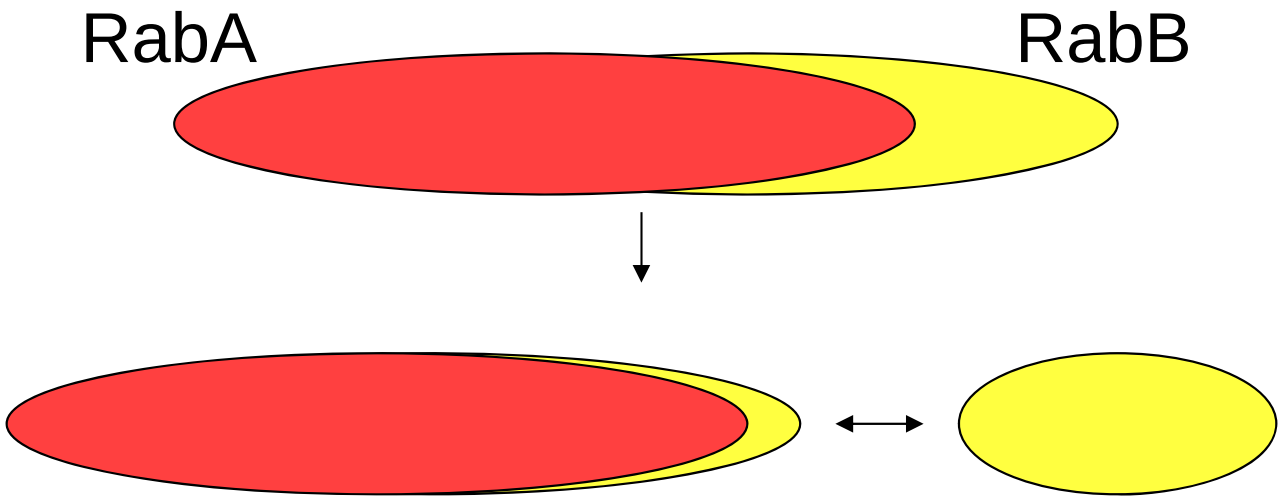
cisternal discontinuity

The Golgi stack also undergoes fission and homotypic fusion



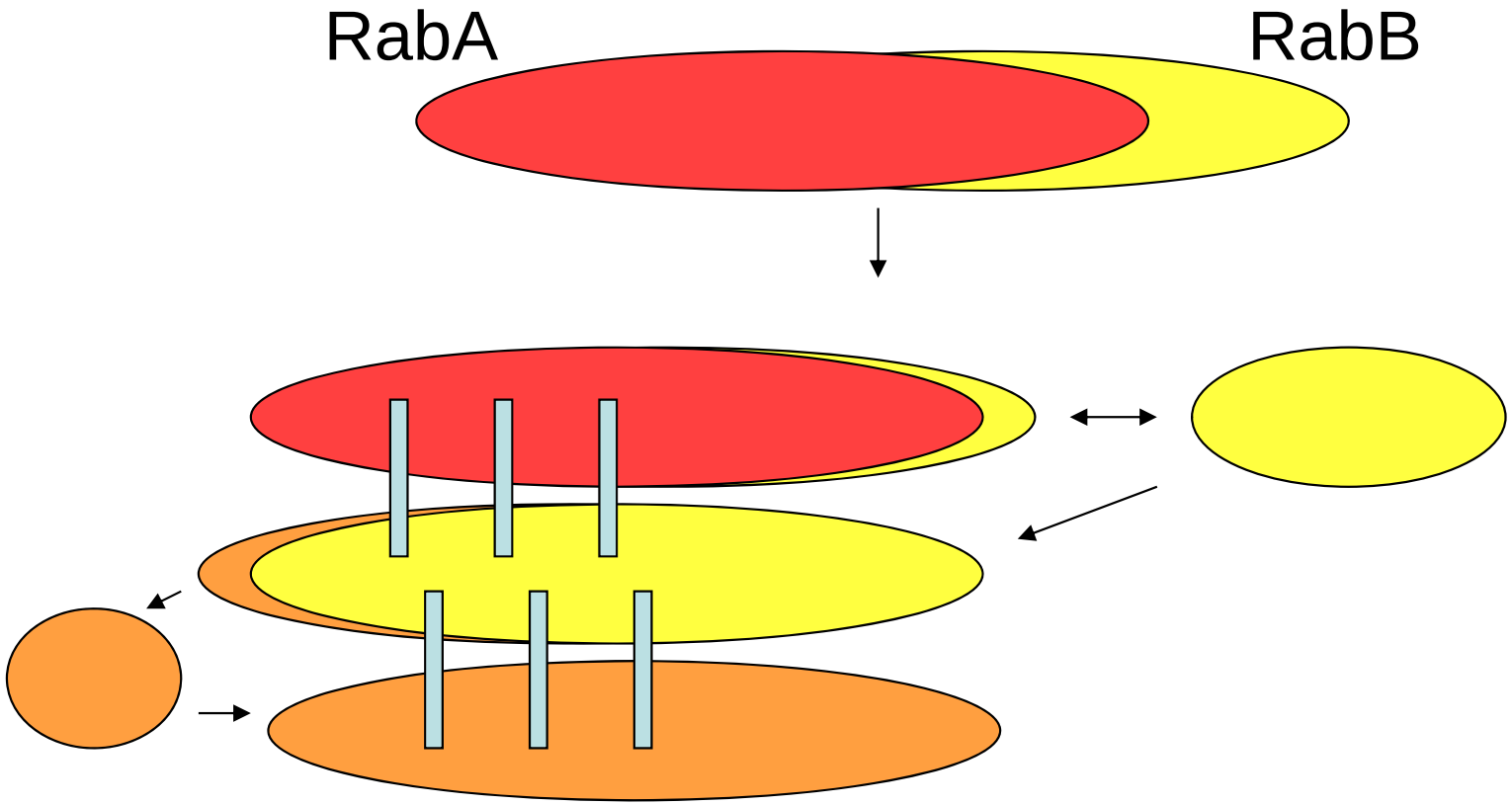
Fission and fusion are likely much more common than we think: simple microtubule depolymerization generates Golgi mini-stacks that are readily reversed on drug washout

Cisternal Progenitor Model for Golgi stack formation

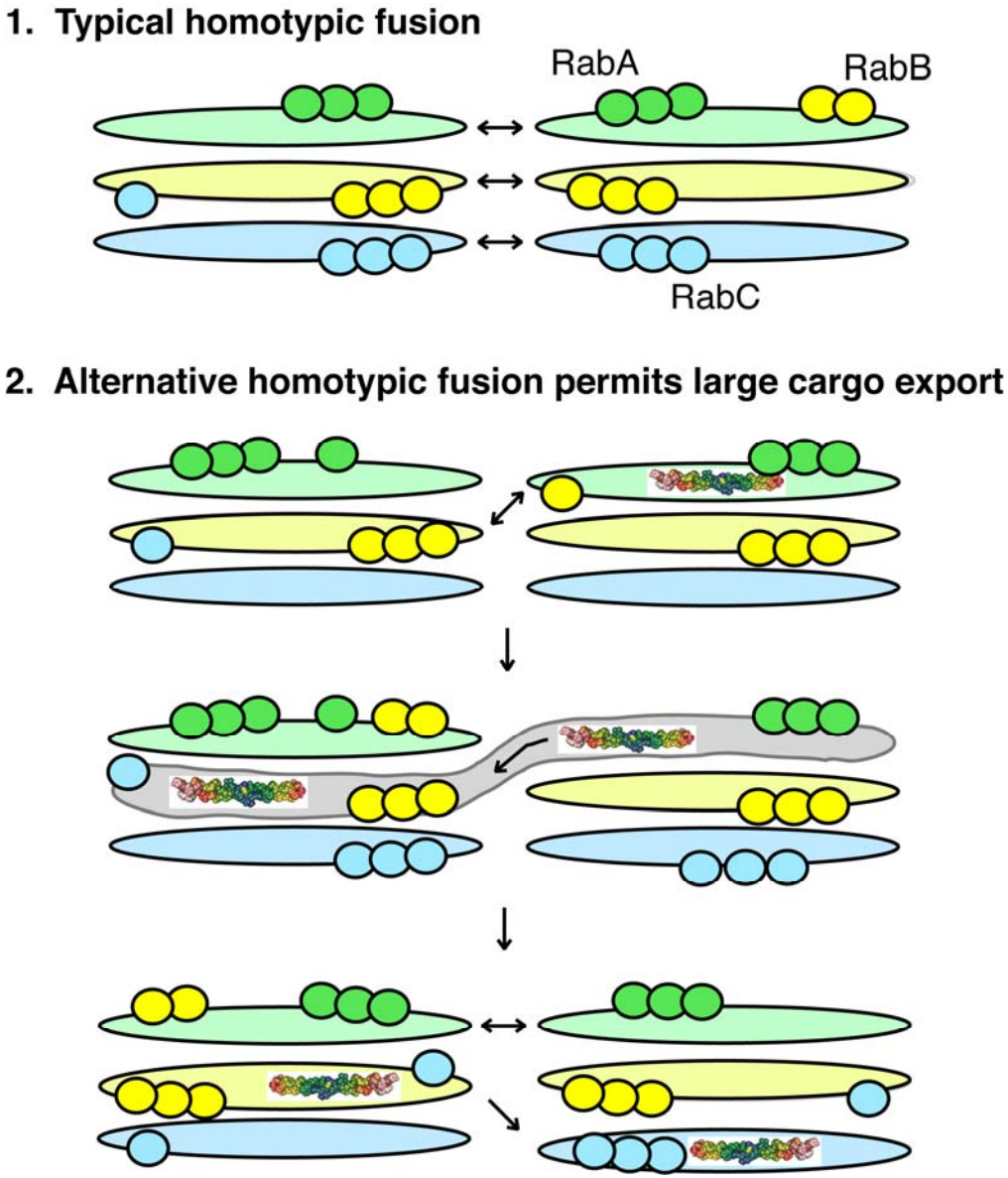


Each domain has SNARE proteins and tethers to mediate HOMOTYPIC fusion

Cisternal Progenitor Model for Golgi stack formation



Local Homotypic Fusion Permits Large Cargoes to Move across the stack



Golgin Proteins

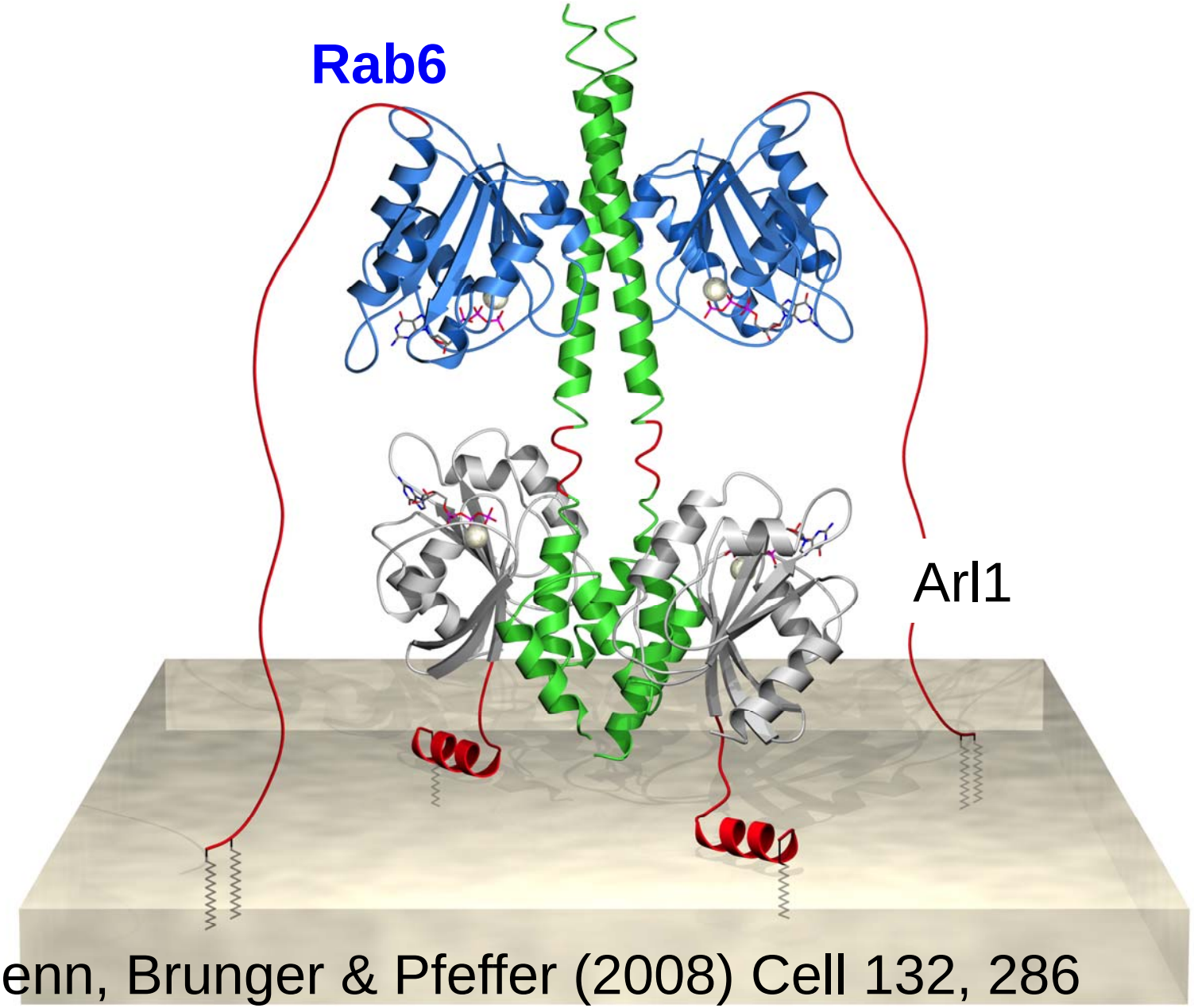
Long, coiled coil proteins needed for Golgi structure maintenance and function

“Golgi coiled coil protein of 185K”
GCC185

GCC185

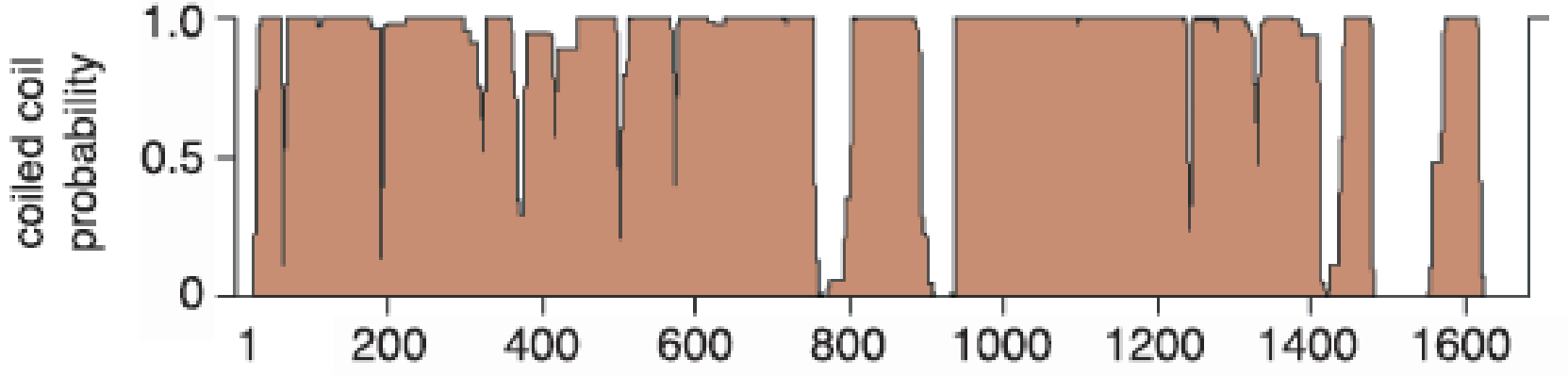
- Needed for normal Golgi structure
- Also functions to tether Rab9+ transport vesicles inbound to the Golgi from late endosomes
- C-terminally anchored at the trans Golgi by Rab6 and Arl1 GTPases

Adjacent GTPase binding sites on GCC185 localize the protein to the trans Golgi network

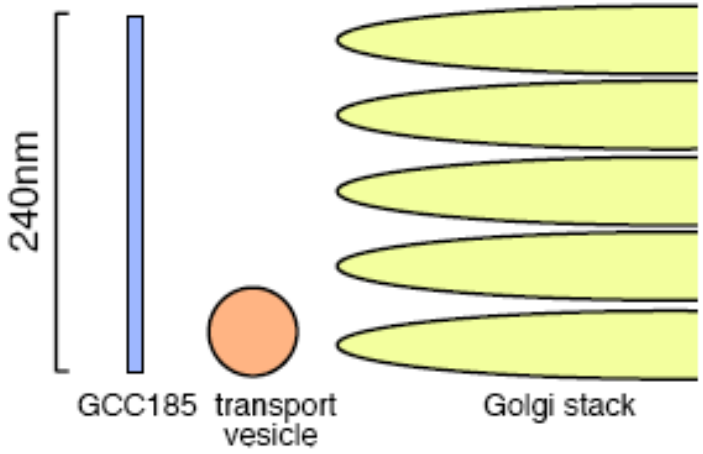


Burguete, Fenn, Brunger & Pfeffer (2008) Cell 132, 286

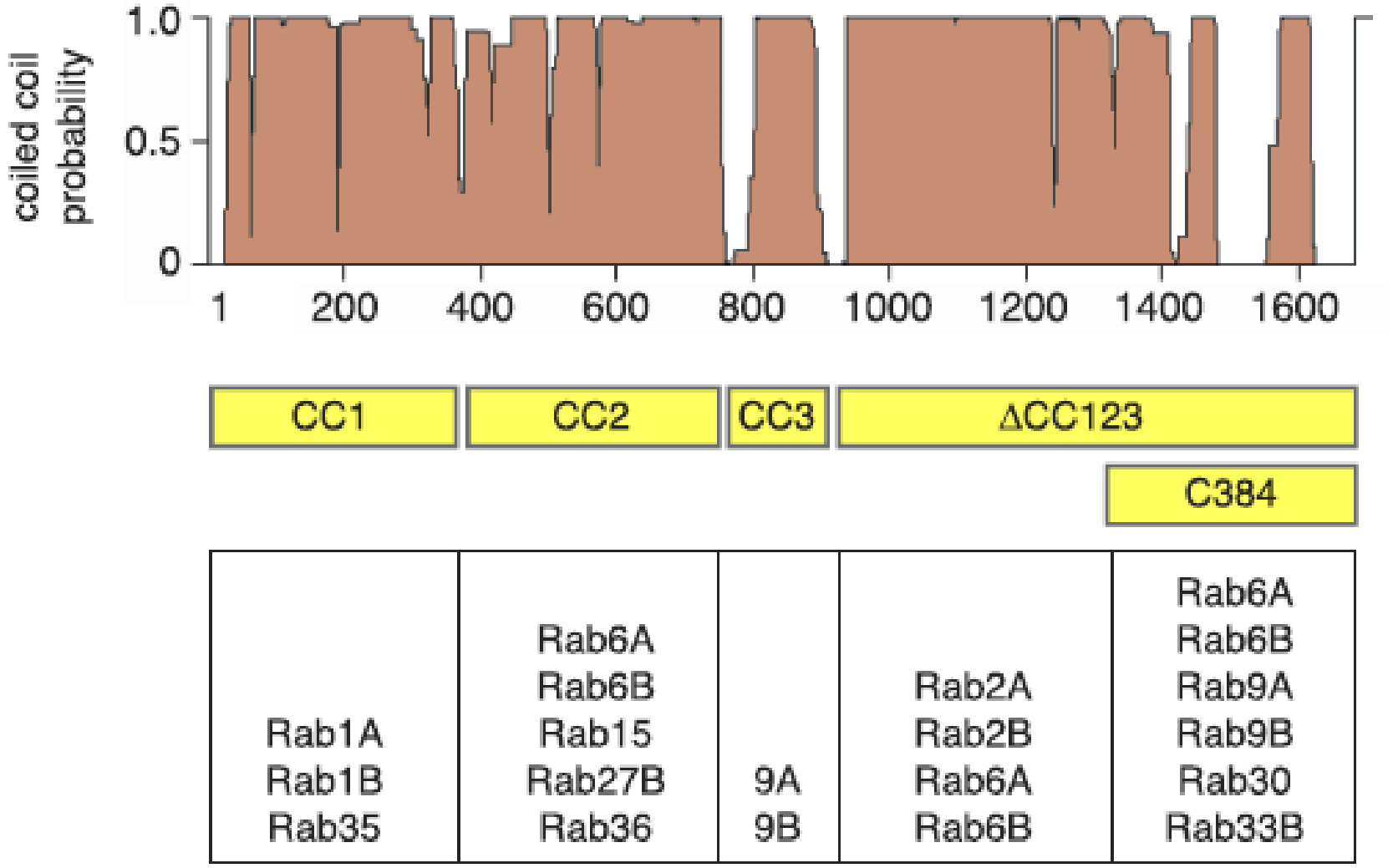
GCC185 is predicted to be almost all coiled coil



If fully extended, this protein could reach 240nm--this is the entire width of the Golgi stack!



GCC185 binds 14 different Rabs across its entire length!

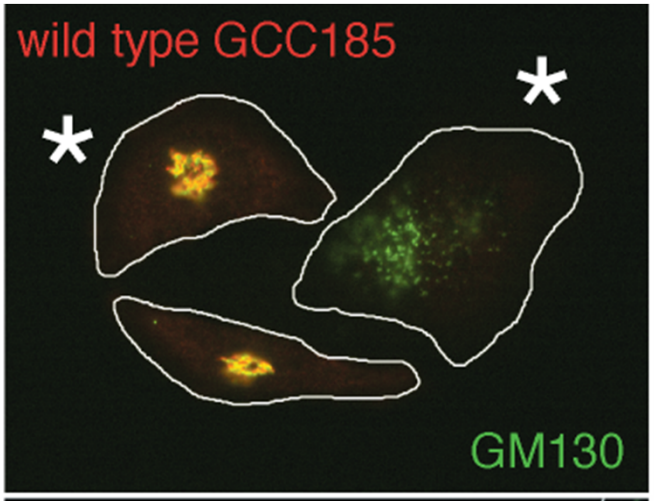


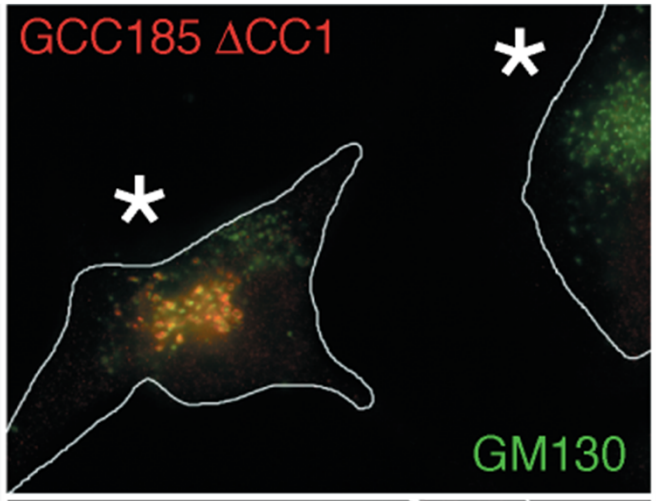
Hayes, Brown et al. (2009) Mol. Biol. Cell

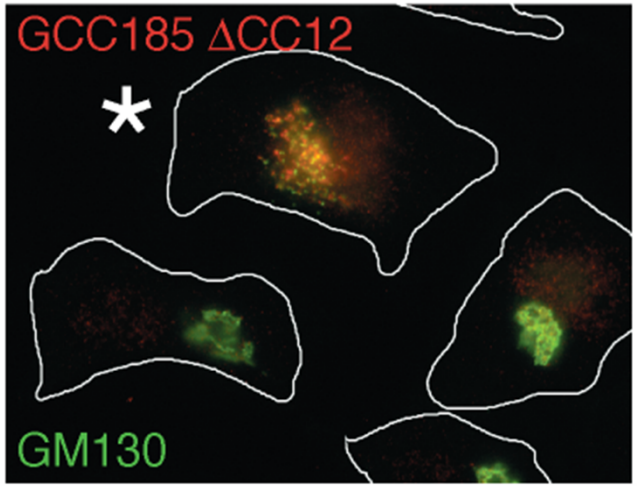
Are all these binding sites needed for GCC185 function?

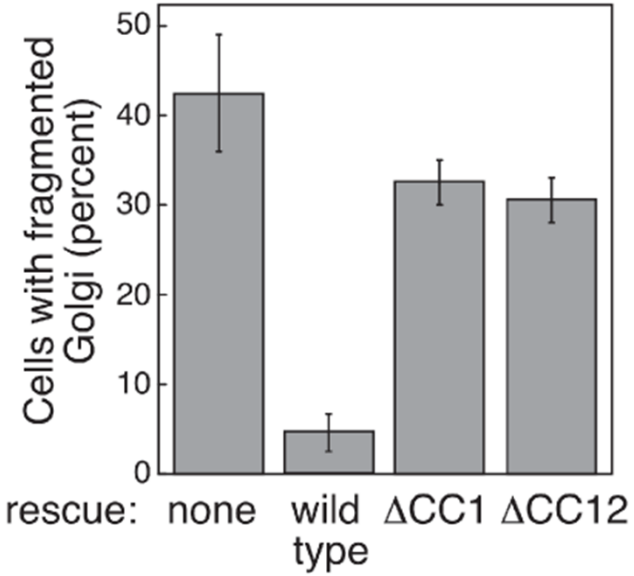
1. siRNA deplete GCC185 from cells
2. Transfect in deletion/mutant “rescue” constructs
3. Score cells for Golgi and transport vesicle phenotypes

GCC185's N-terminus is required for normal Golgi morphology



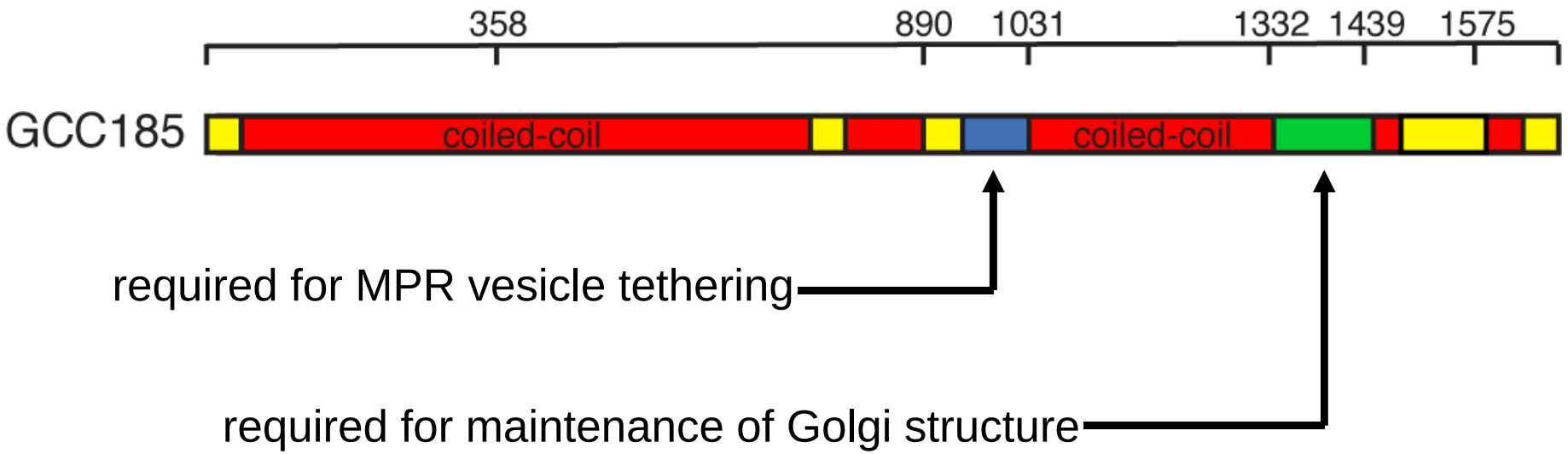






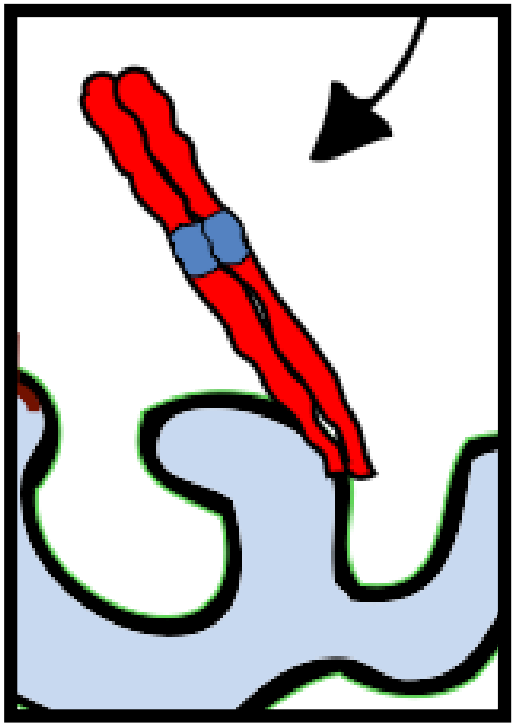
Garret Hayes

Two distinct domains mediate vesicle docking or structure maintenance

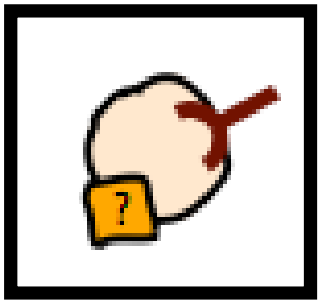


Frank Brown et al. (2011) J. Cell Biol.

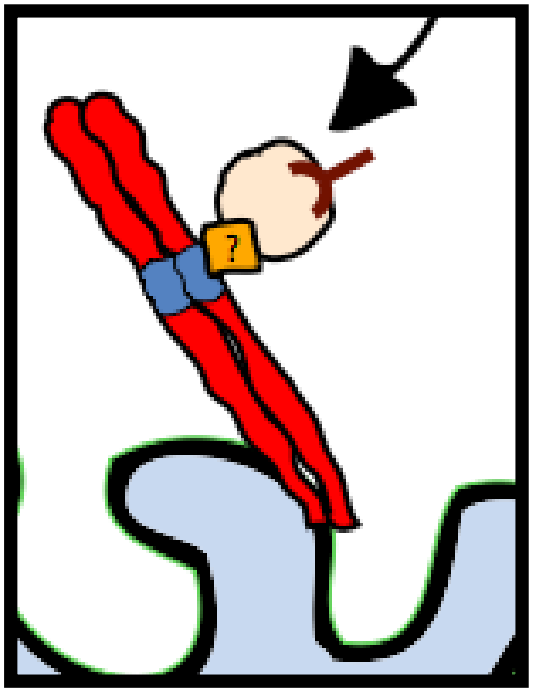
What component of MPR transport vesicles is recognized by GCC185?



- Identified a domain required for vesicle tethering

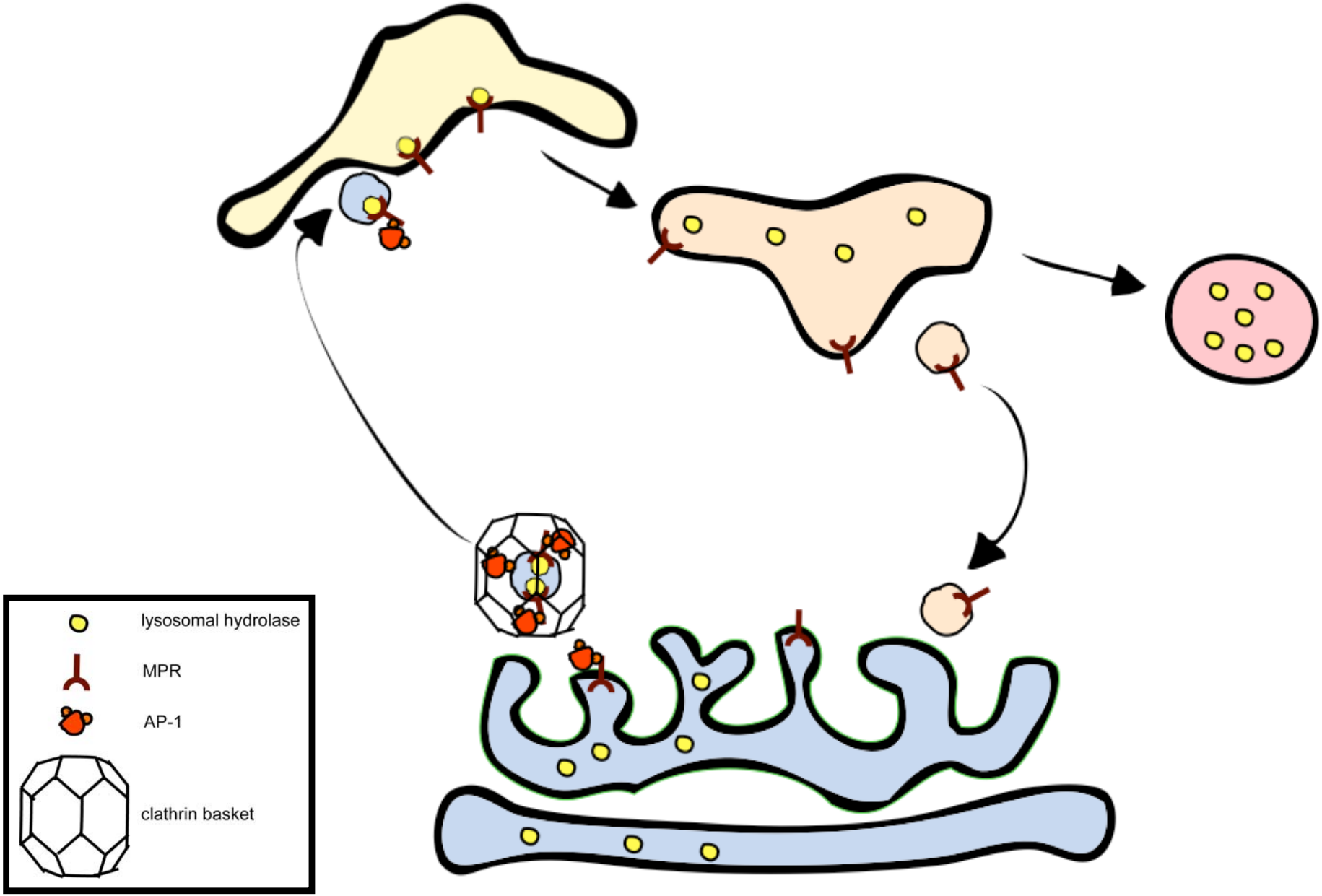


- Composition of incoming transport vesicles is not known

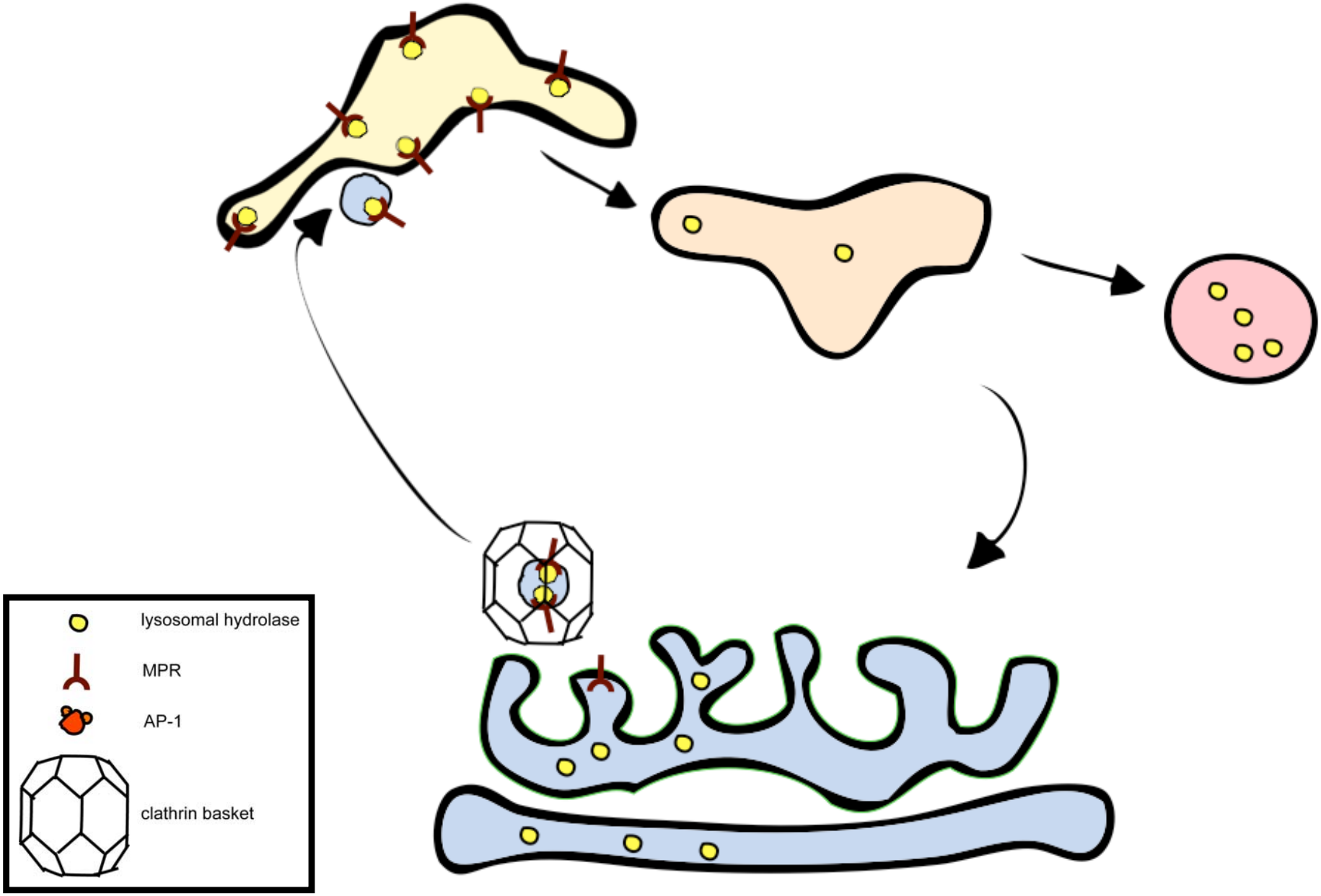


- Affinity chromatography could identify proteins mediating vesicle tethering

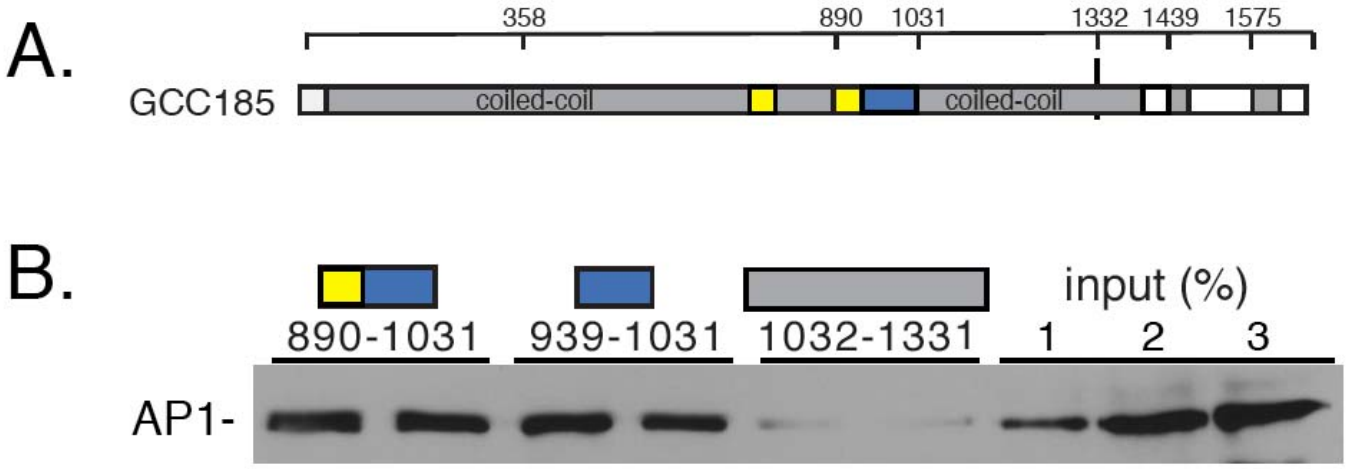
AP-1 functions in MPR exit from Golgi



AP-1 depletion accumulates MPRs in early endosomes



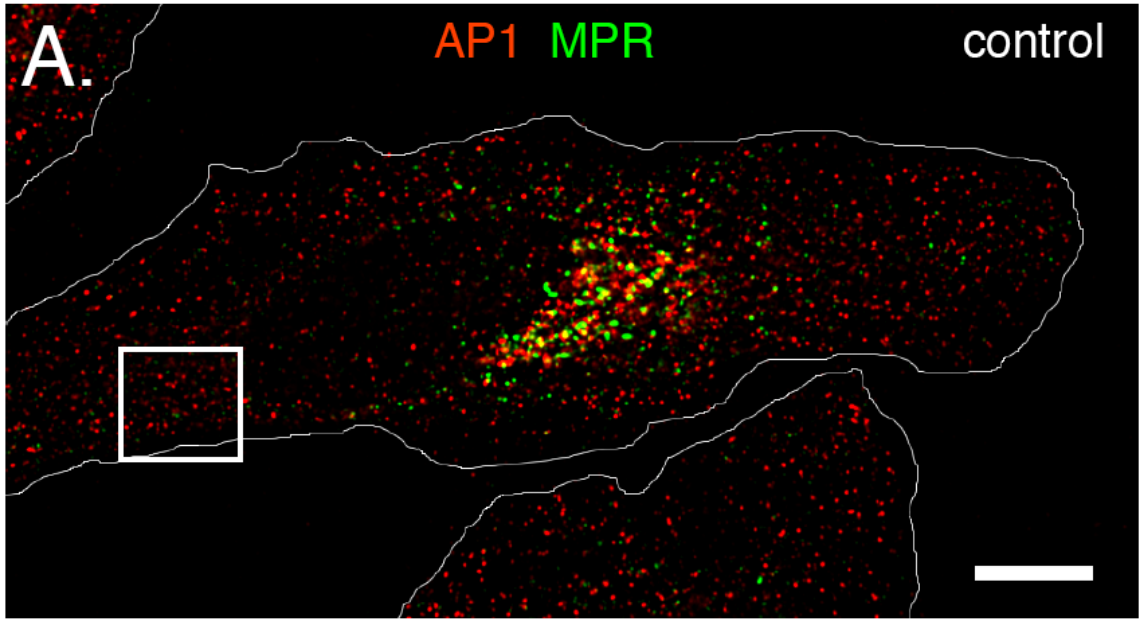
The domain needed for vesicle tethering binds the AP-1 clathrin adaptor

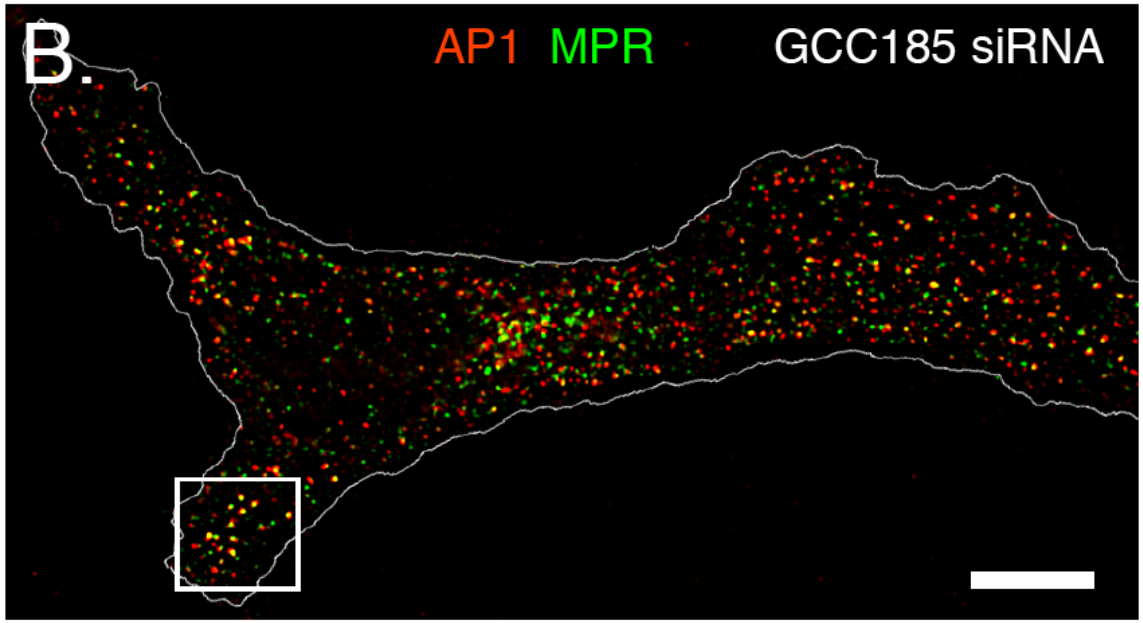


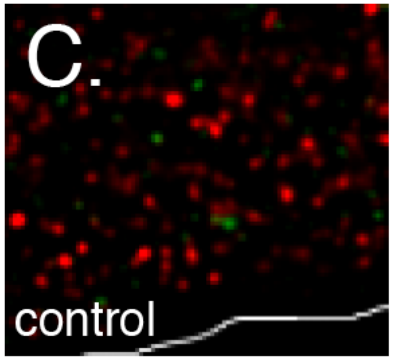
Predictions

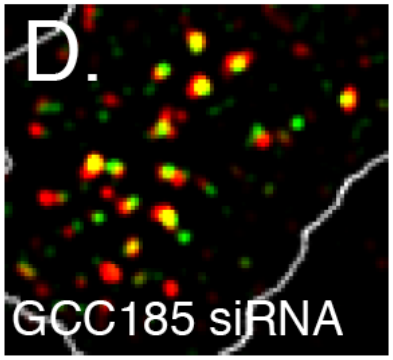
1. If AP1 decorates the vesicles that dock via GCC185, AP-1 vesicles should accumulate in cells depleted of GCC185
2. GCC185 interaction with AP1 should not be used to localize AP1 at the Golgi

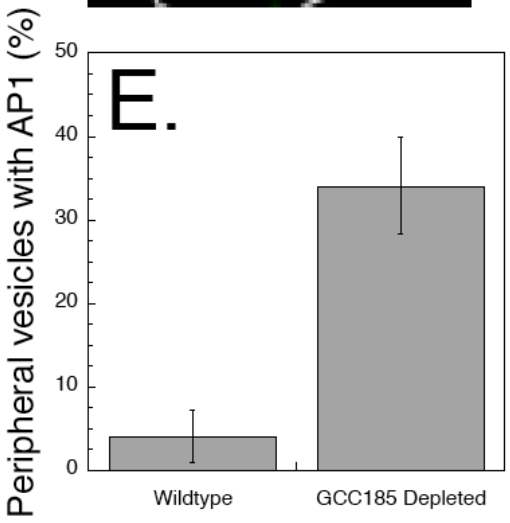
AP-1 decorates transport vesicles that accumulate in cells depleted of GCC185



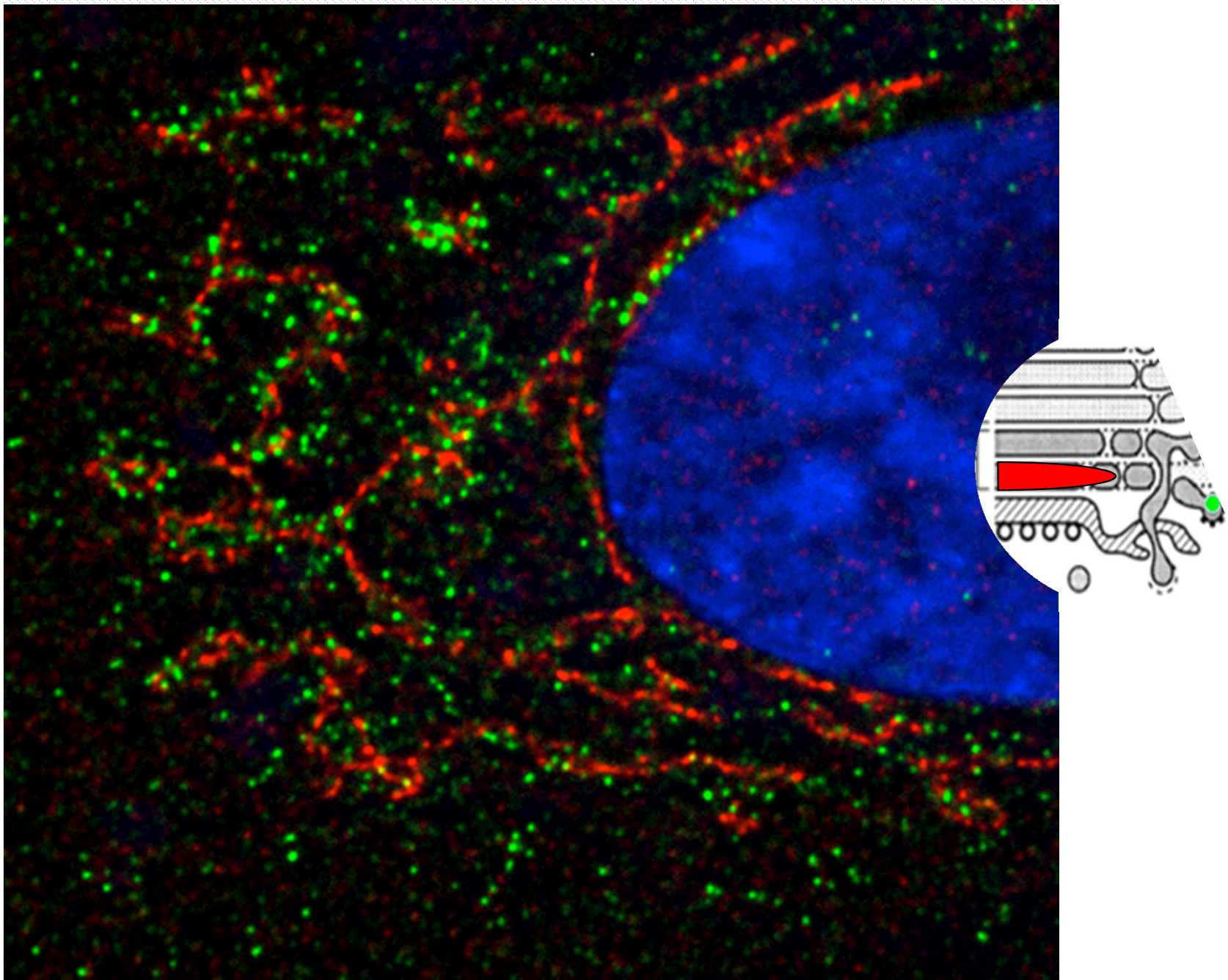




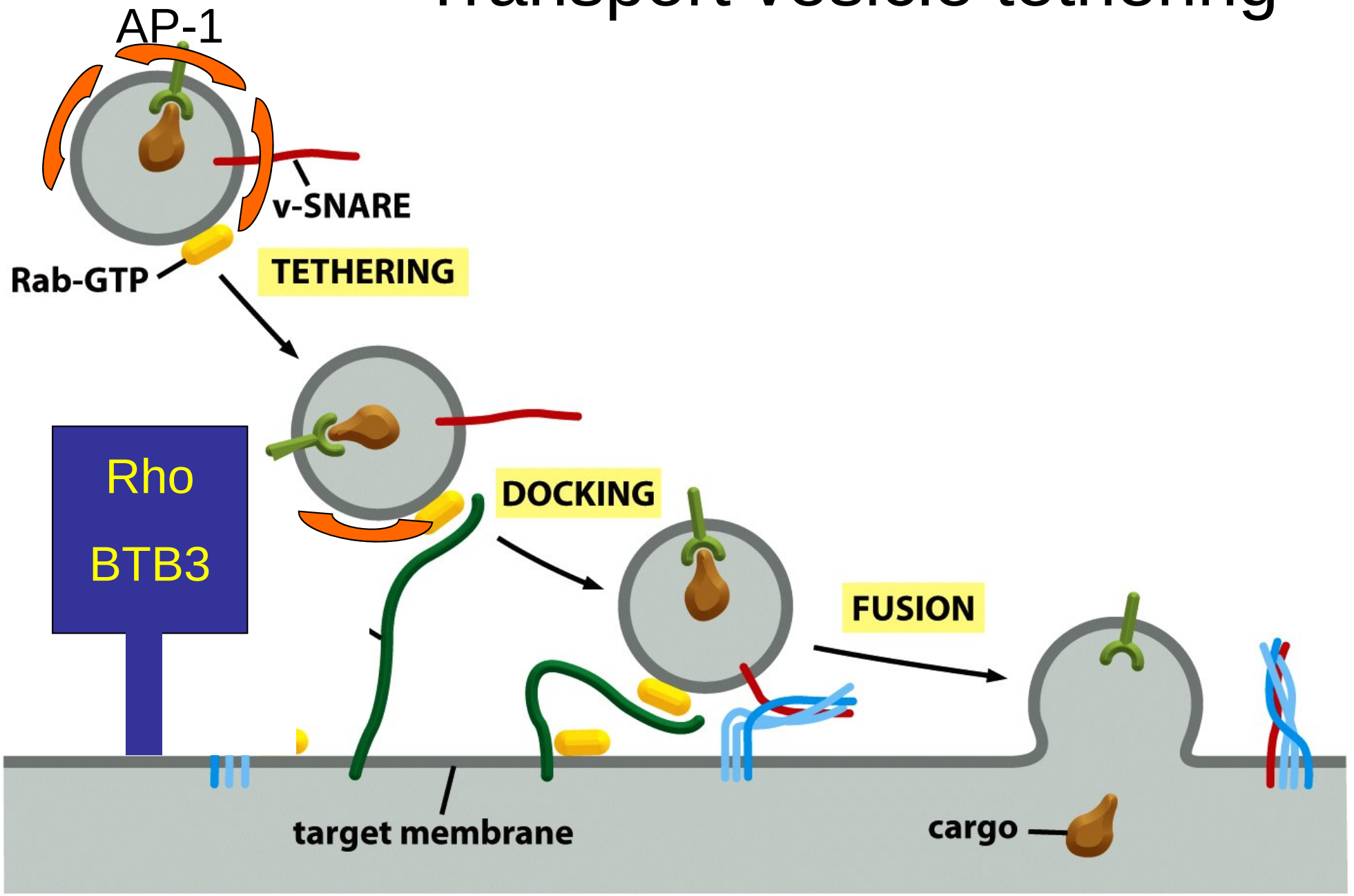




GCC185 and AP-1 decorate distinct trans Golgi membrane domains



Transport vesicle tethering



New findings:

1. Retrograde Rab9-transport vesicles are AP-1 decorated
2. AP-1 coat components interact with tethers thus are still present at the target membrane

Cisternal Progenitor Model

- Stable Golgi compartments may be established by Rab GTPases and ordered by Rab cascades
- Golgi stacks likely undergo continual fusion and fission (requiring motors); large cargoes are accommodated.
- Golgins are important for Golgi stack assembly and catalyze vesicle tethering by binding multiple Rabs and other proteins to drive vesicle docking and fusion and also homotypic docking and fusion

Garret Hayes, Frank Brown, Ryan Nottingham

Ganesh Pusapa

Alondra Burgue