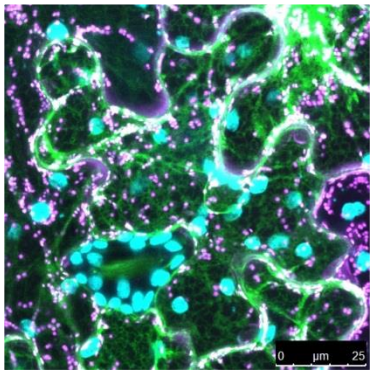
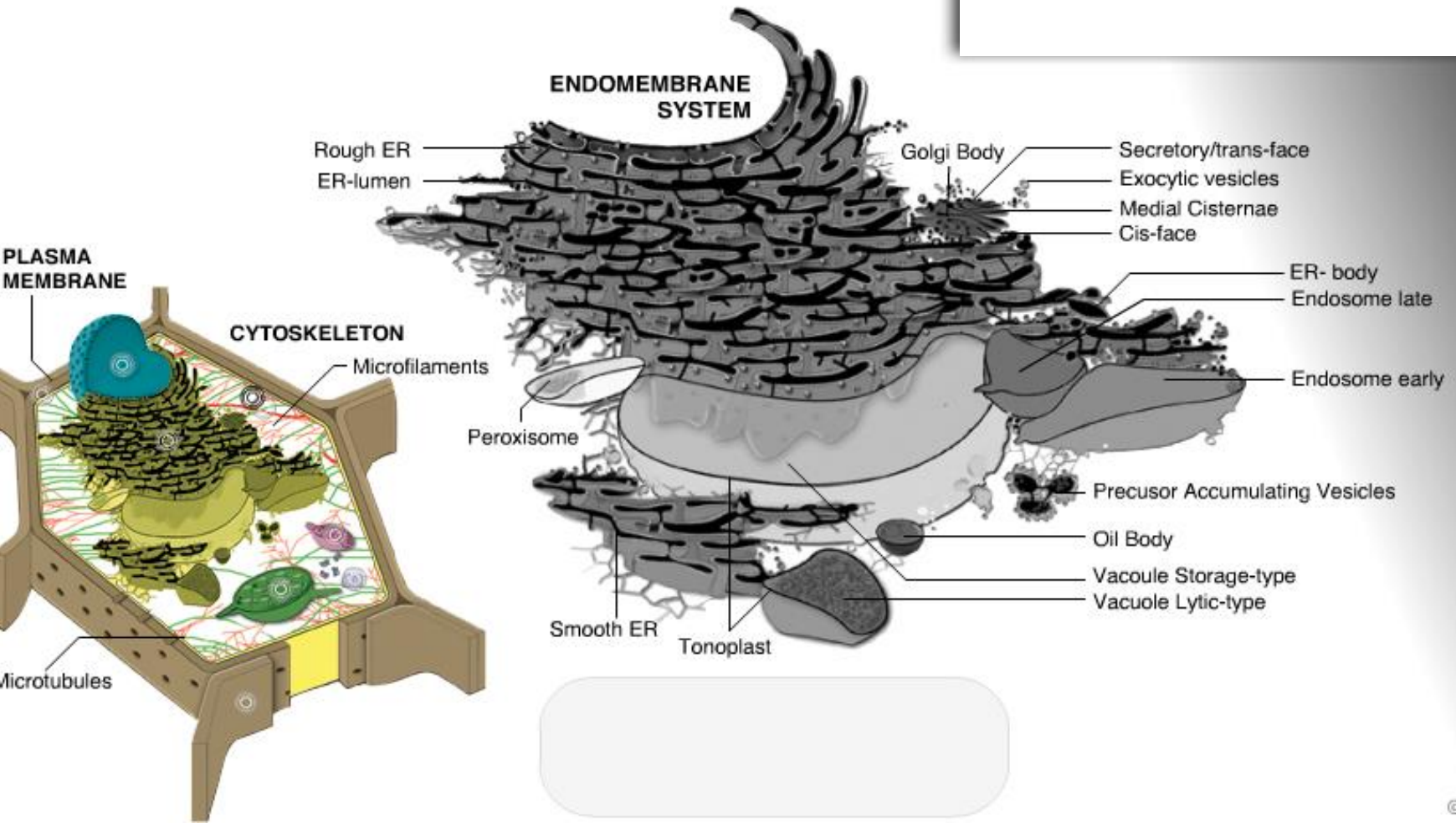
Plant Endomembrane Trafficking
–
Pathways and Regulators



Summer School 2015

Robatzek lab
Theoretical session

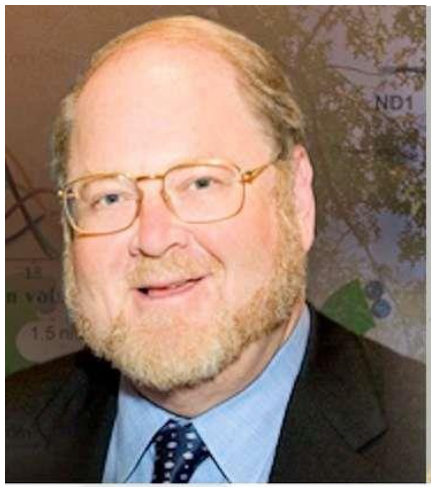
Michaela Kopischke

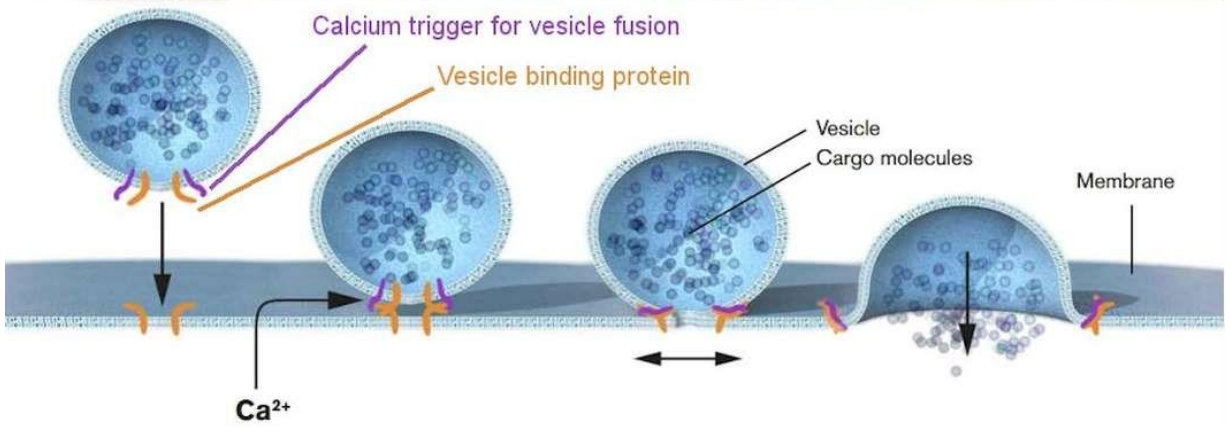


Nobel Prize in Physiology or Medicine 2013

[…] James E. Rothman, Randy W. Schekman and Thomas C. Südhof have elucidated […] fundamental processes in eukaryotic cells that […] ensure that molecular cargo is correctly destined. […] Vesicle transport and fusion operates, with the same general principles, in organisms as different as yeast and man. […] Without this exquisitely precise organization, the cell would lapse into chaos.

James E. Rothman Yale	Randy W. Schekman UC Berkeley	Thomas C. Suedhof Stanford





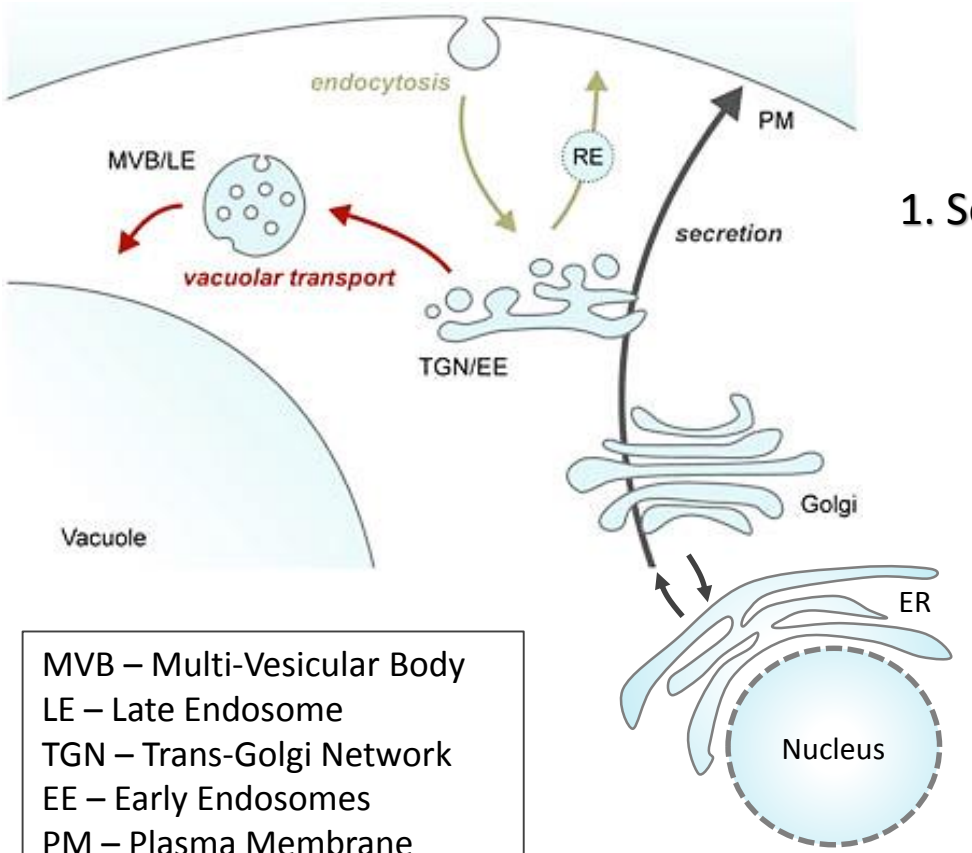
Juleen R. Zierath and Urban Lendahl
Karolinska Institutet

Endomembrane Trafficking Pathways

3. Endocytic Pathway

2. Vacuolar Transport Pathway

1. Secretory Pathway



MVB – Multi-Vesicular Body
LE – Late Endosome
TGN – Trans-Golgi Network
EE – Early Endosomes
PM – Plasma Membrane
ER – Endoplasmatic Reticulum

Figure adapted from “The Illuminated Plant Cell”

Plant Endosomal Compartments

Endosome Functions:

- Trafficking of biosynthetic and endocytic cargo
- intermediate compartments for storage, sorting and organelle targeting of material within the cell
- Required for key plant processes:
  - → Embry differentiation
  - → Guard cell movement
  - → Regulation of auxin transport
  - → Gravitropism
  - → Cell wall remodeling
  - → Defense response against pathogens
  - → Epidermis differentiation

Plant Endosomal Compartments

Endosome Functions:

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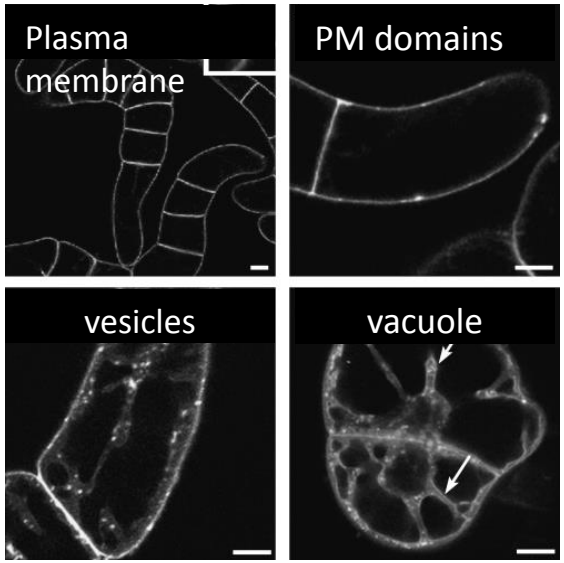
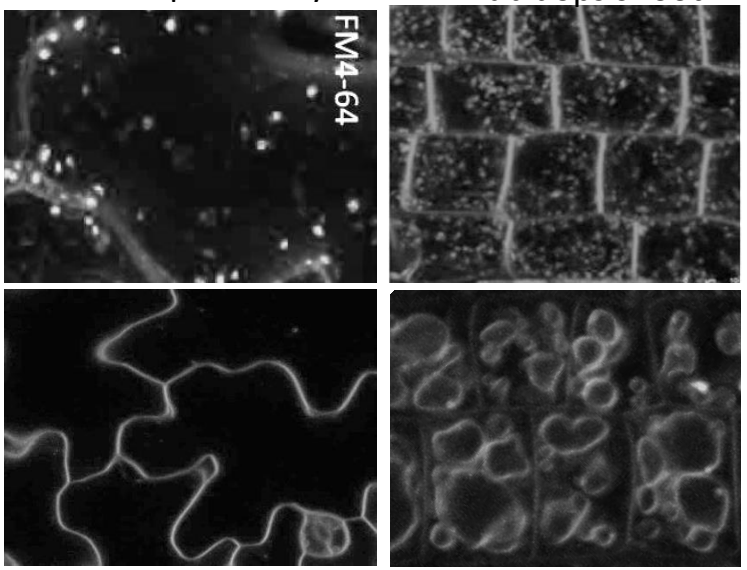
Endocytic tracer FM4-64

Tobacco BY-2 cells



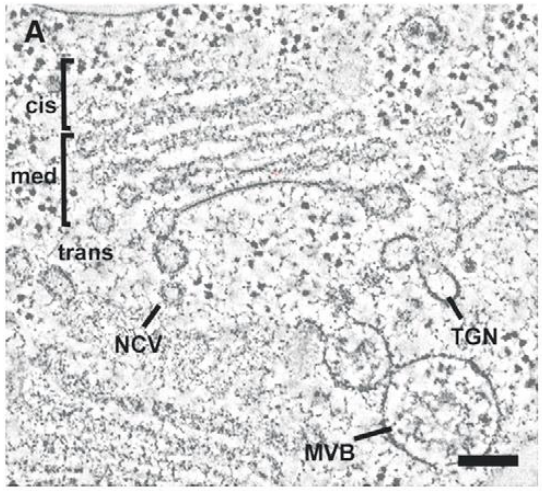
Arabidopsis cotelydon

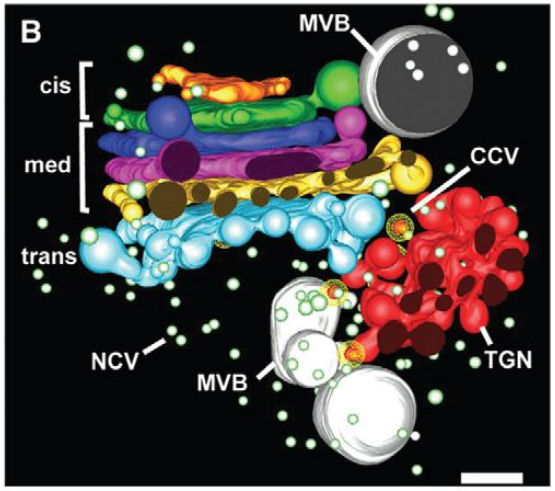
Arabidopsis root



Excitation: 488 nm
Emission: 580 – 640 nm

Plant Endosomal Compartments



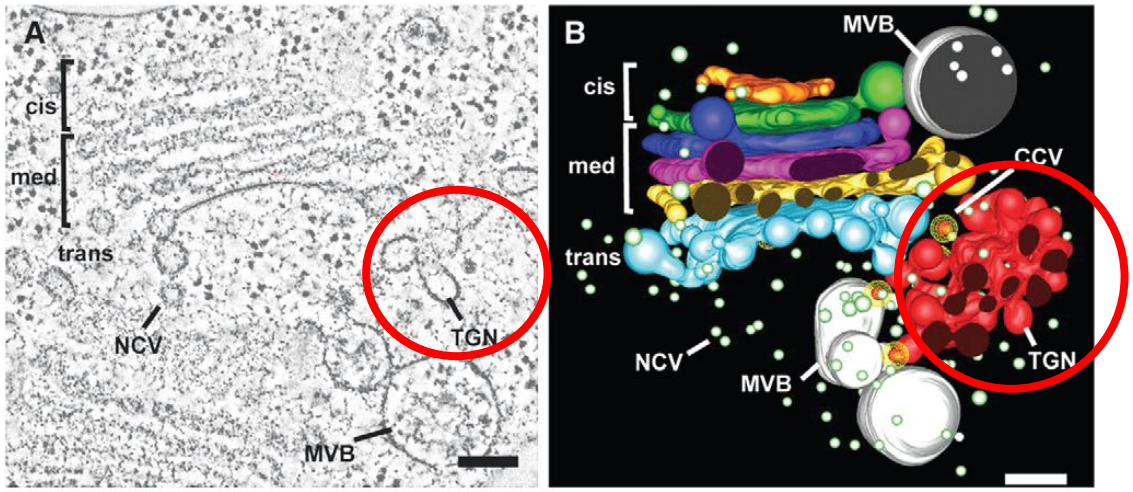


Early Endosomes: Trans-Golgi-Network (TGN)

Late Endosomes: Multi-Vesicular Bodies (MVBs)

Otegui et al., 2006, Plant Cell

Plant Endosomal Compartments



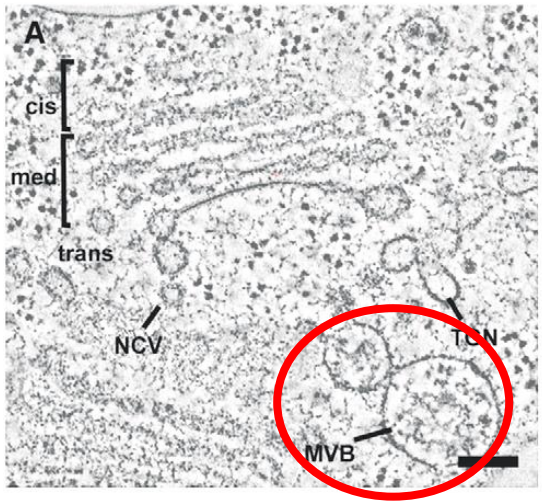
Early Endosomes: Trans-Golgi-Network (TGN)

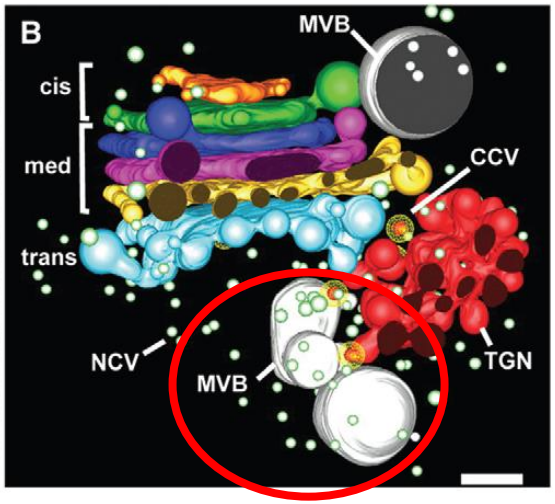
Late Endosomes: Multi-Vesicular Bodies (MVBs)

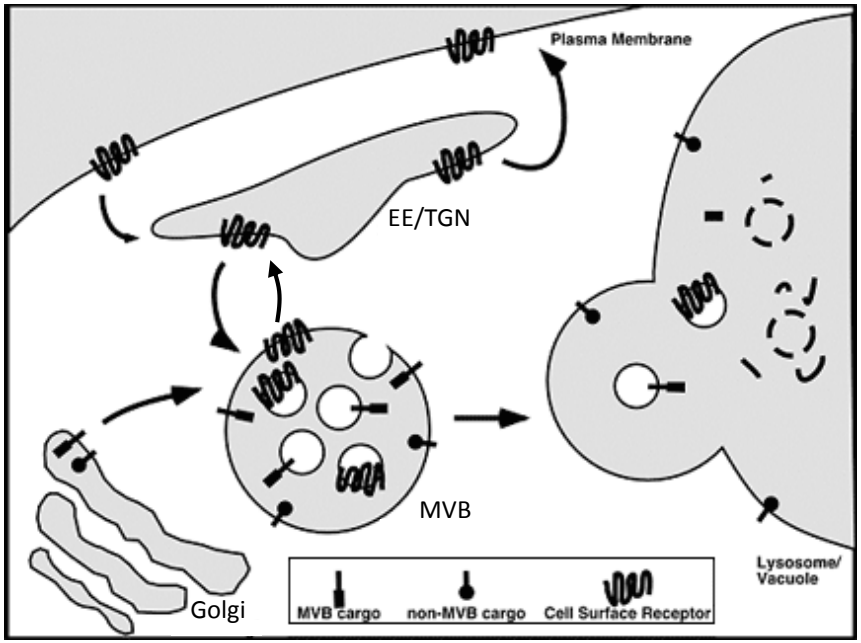
- 1st site for delivery of endocytosed cargo
- Rapidly labelled by FM4-64
- Central hub for biosynthetic and endocytic material
- Might contain specialised subdomains with distinct functions

Otegui et al., 2006, Plant Cell

Plant Endosomal Compartments







Late Endosomes: Multi-Vesicular Bodies (MVBs)

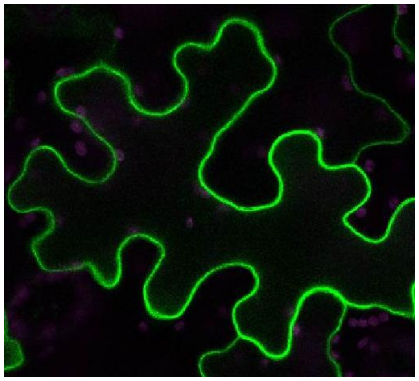
- Multivesicular structure
- Cargo in outer MVB membrane → delivery to tonoplast membrane (biosynthetic cargo or lipids) or recycling to EE
- Cargo in intraluminal vesicles → delivery to vacuolar lumen, destined for degradation
- Intraluminal vesicles generated through action of endosomal sorting complex required for transport (ESCRT)

adapted from David J. Katzmann lab homepage

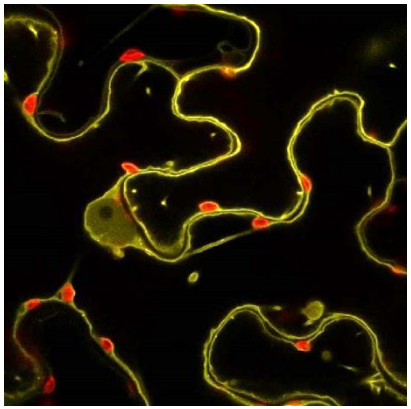
Otegui et al., 2006, Plant Cell

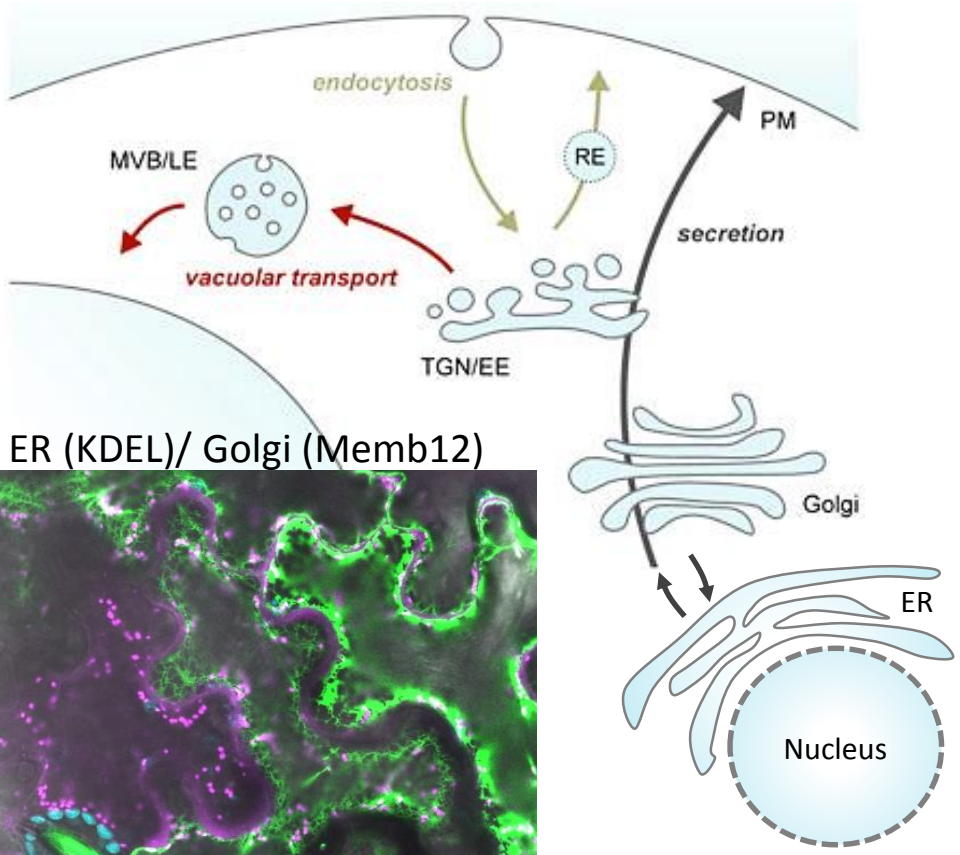
Endomembrane Trafficking Pathways

PM – FLS2



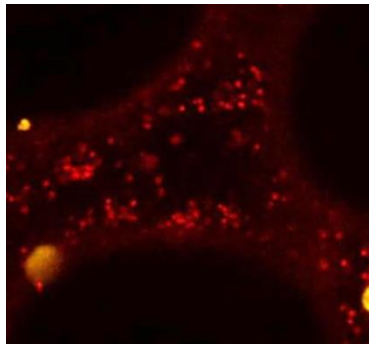
Vacuole – RabG3F



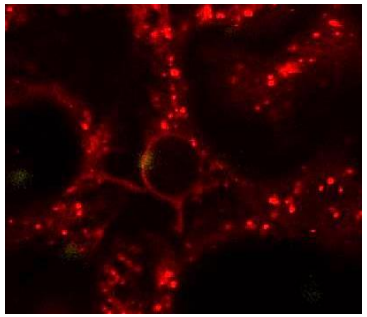


ER (KDEL)/ Golgi (Memb12)

TGN – Vha-A1



EE/LE – ARA7 (RabF2b)



LE/MVB – ARA6 (RabF1)

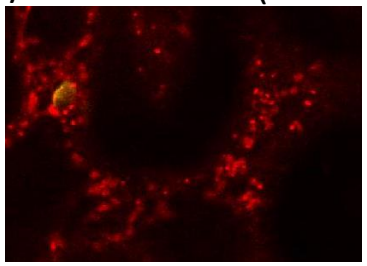
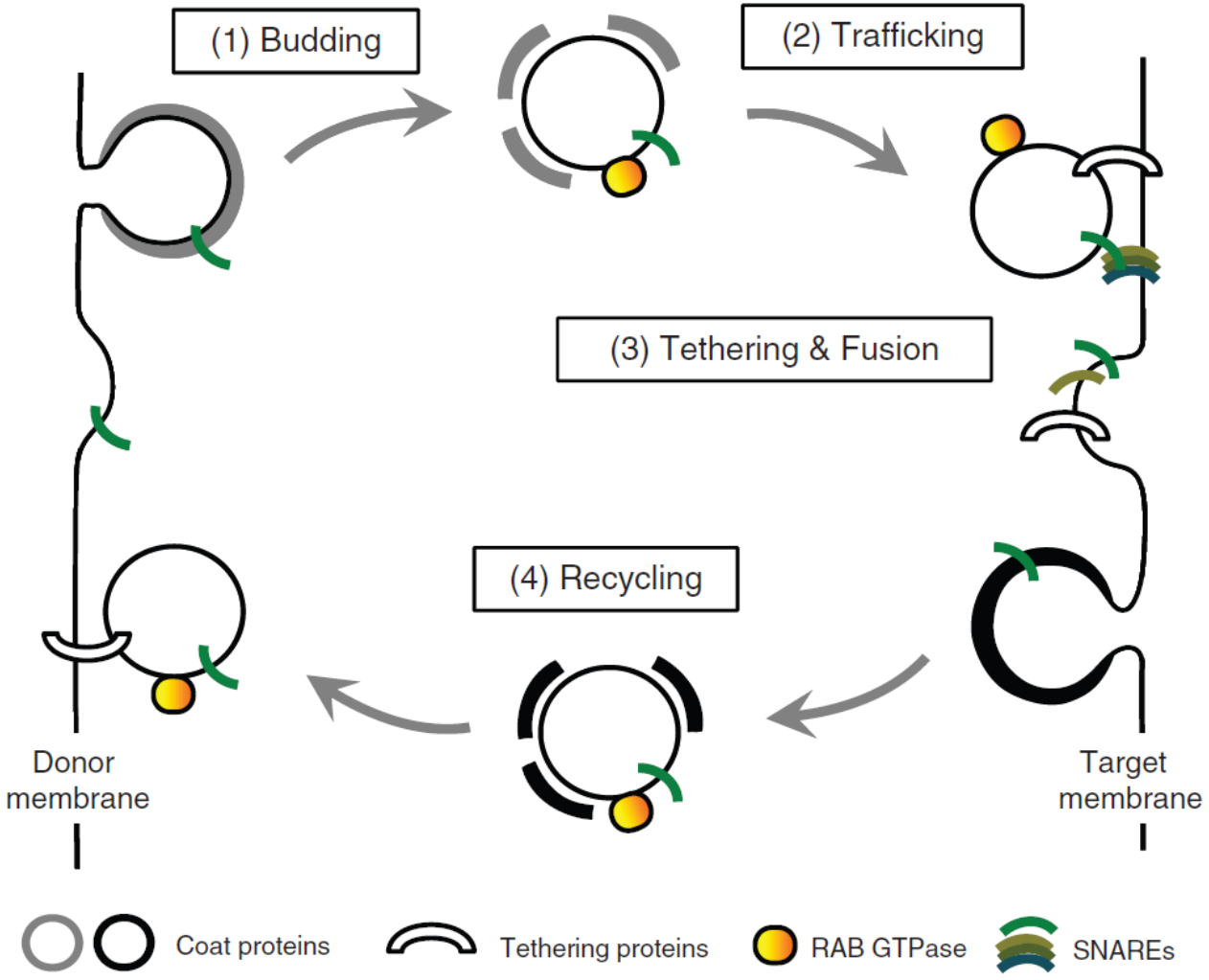


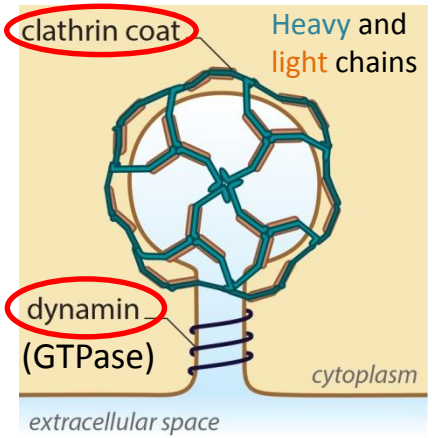
Figure adapted from "The Illuminated Plant Cell"

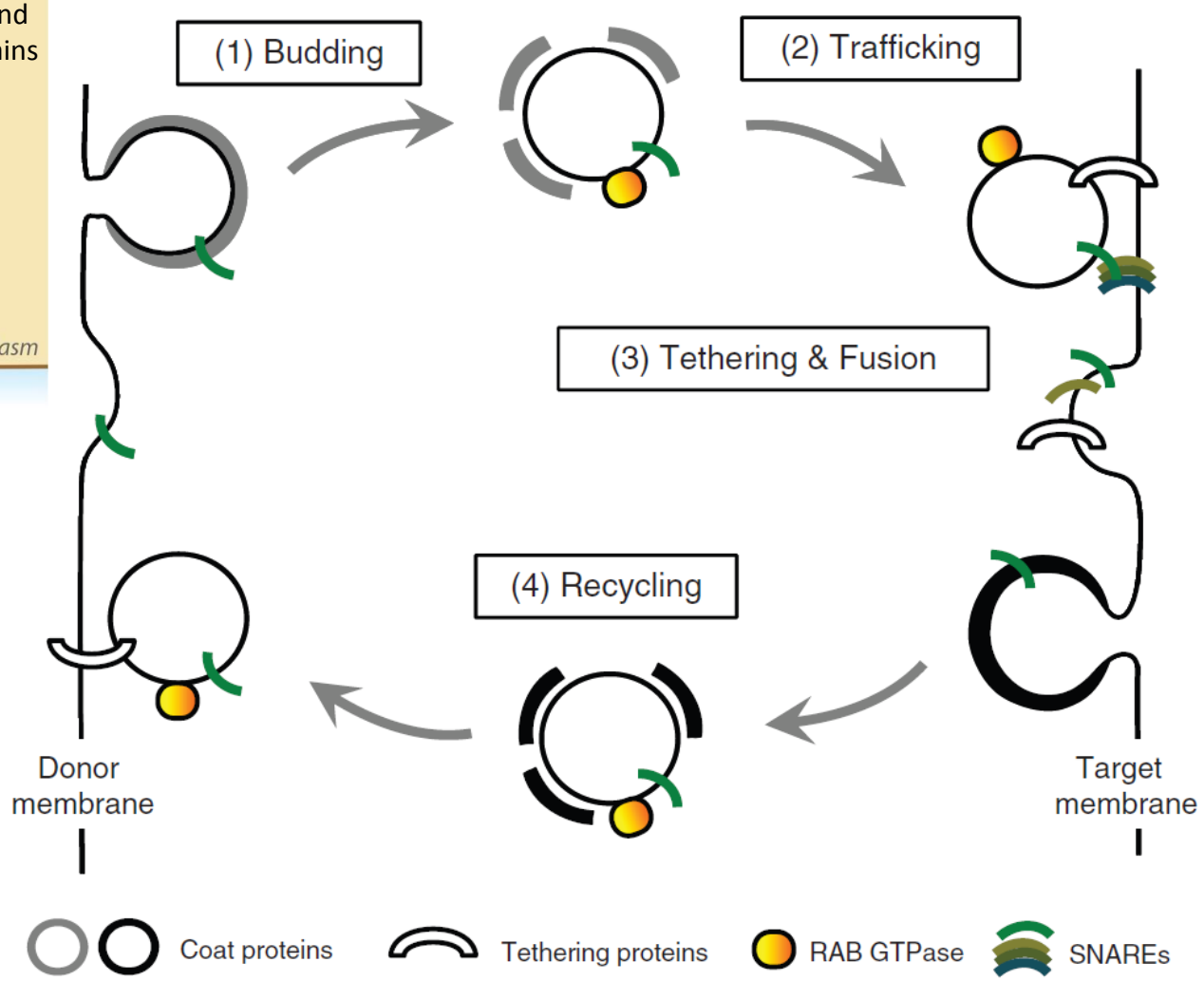
General Membrane Trafficking Machinery



Inada and Ueda 2014, PCP

General Membrane Trafficking Machinery





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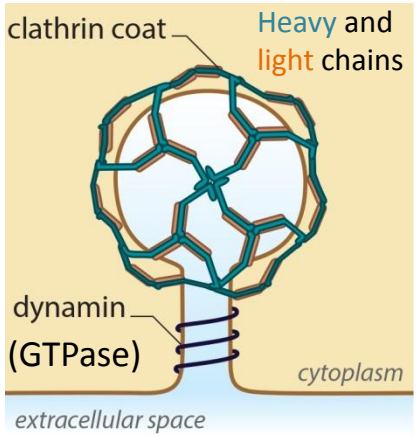
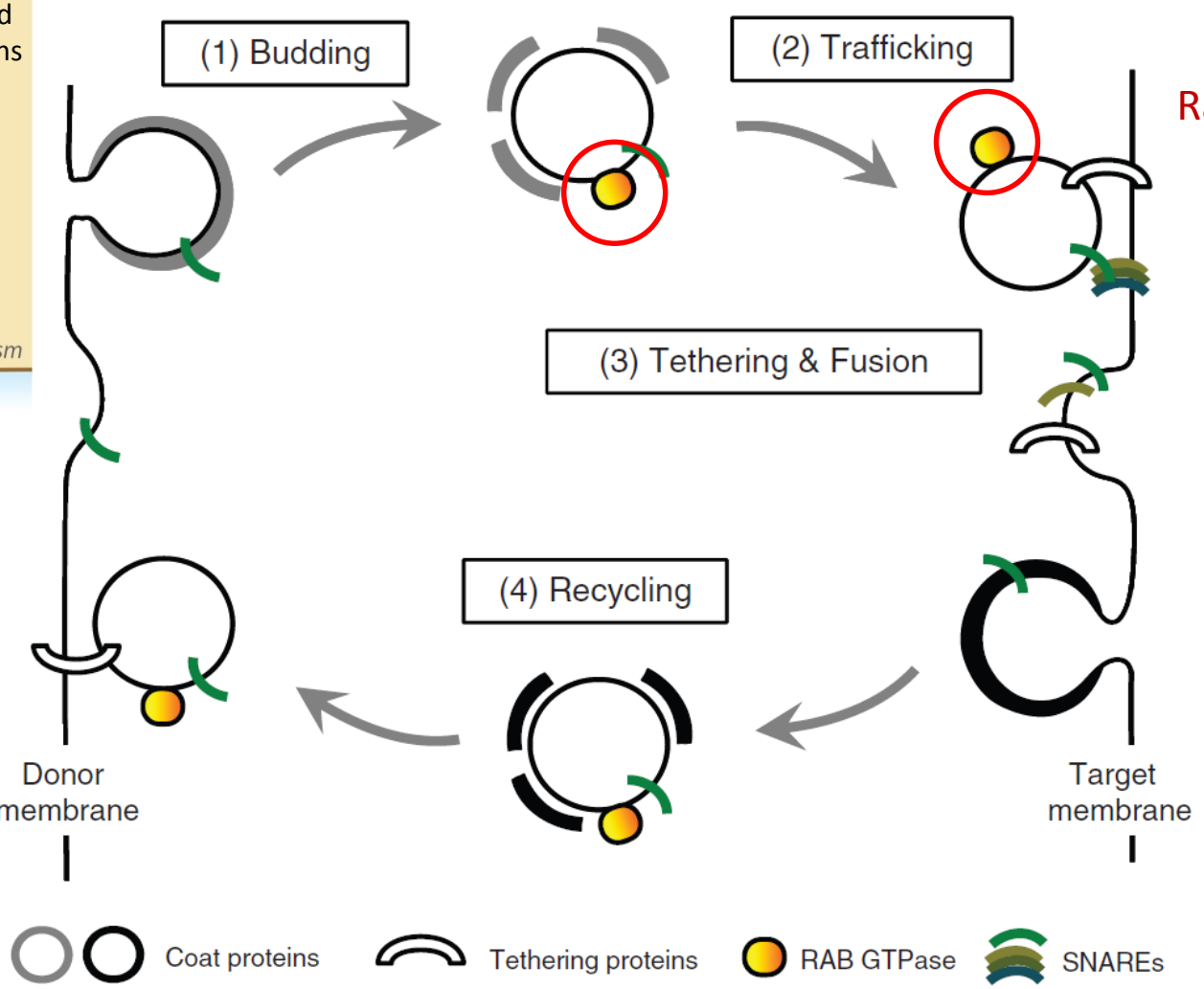
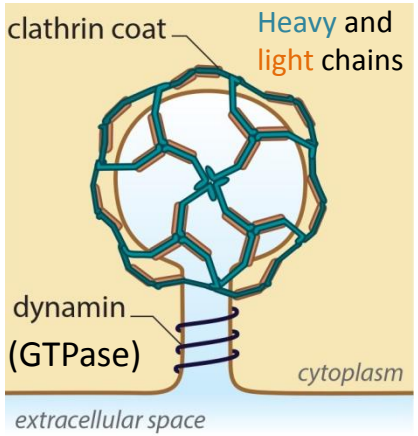


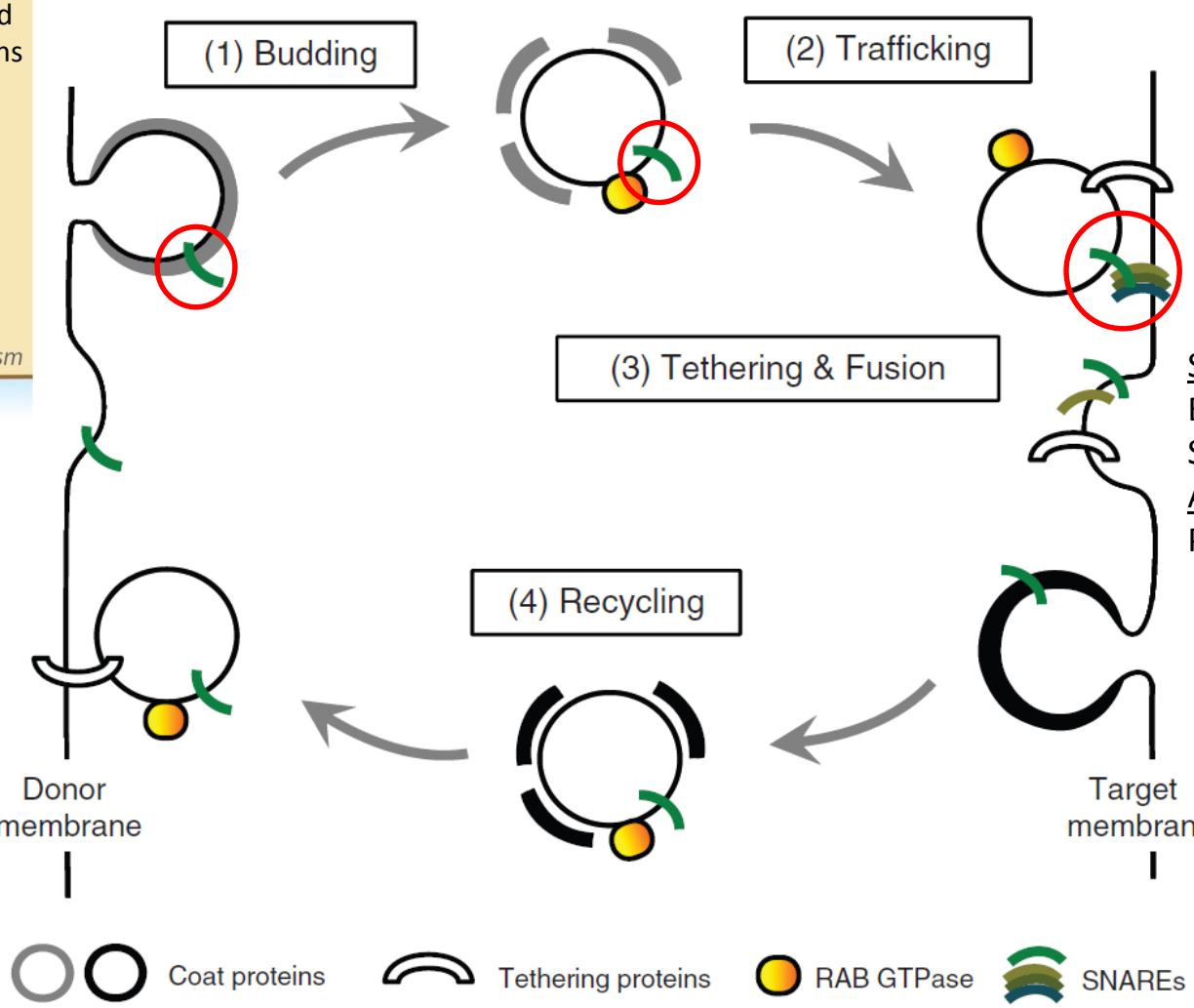


Rab GTPases

Inada and Ueda 2014, PCP

General Membrane Trafficking Machinery



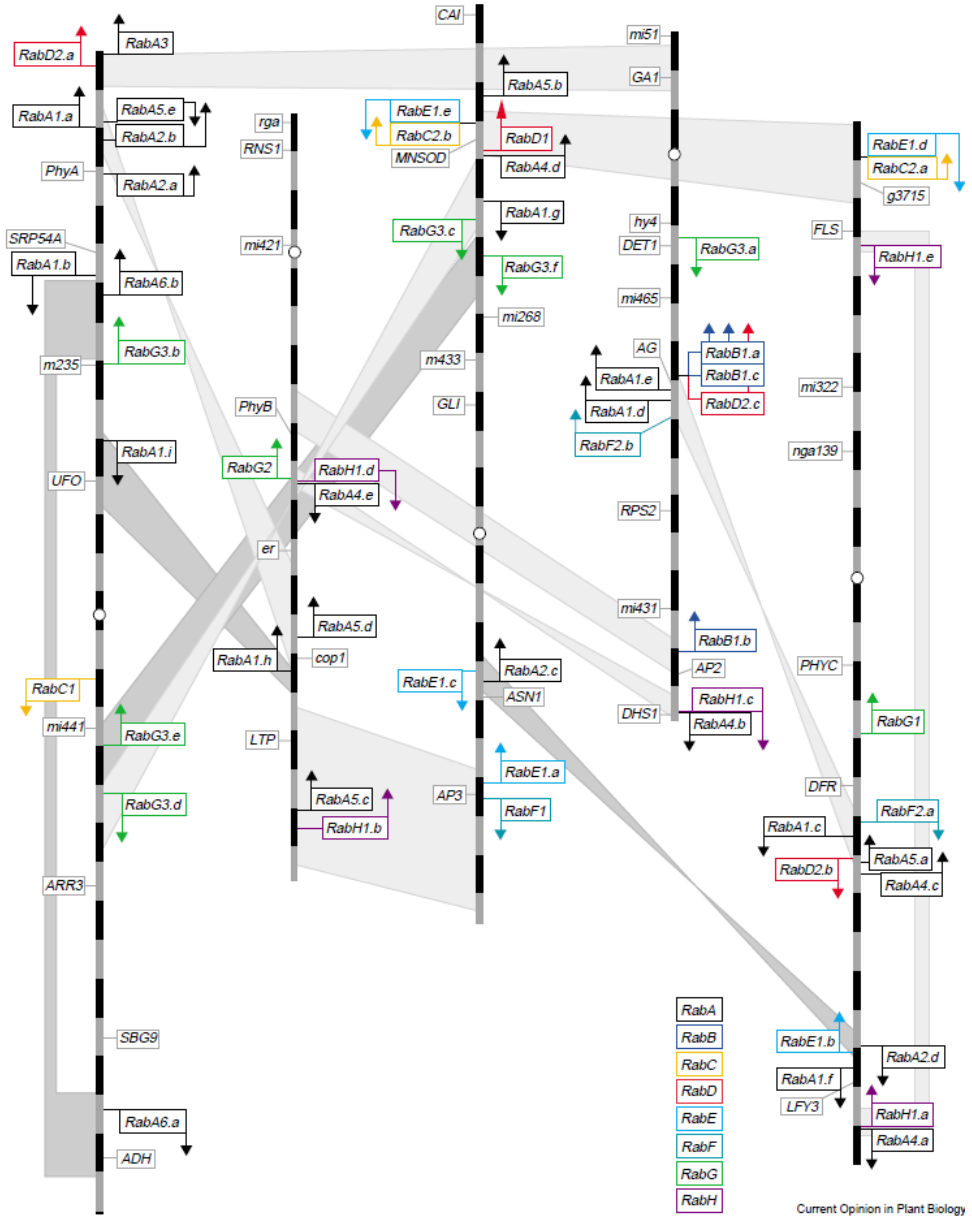


SNARE proteins

Soluble NSF (N-Ethylmaleimide-Sensitive Factor) Attachment Protein Receptor

Inada and Ueda 2014, PCP

Small Rab GTPases

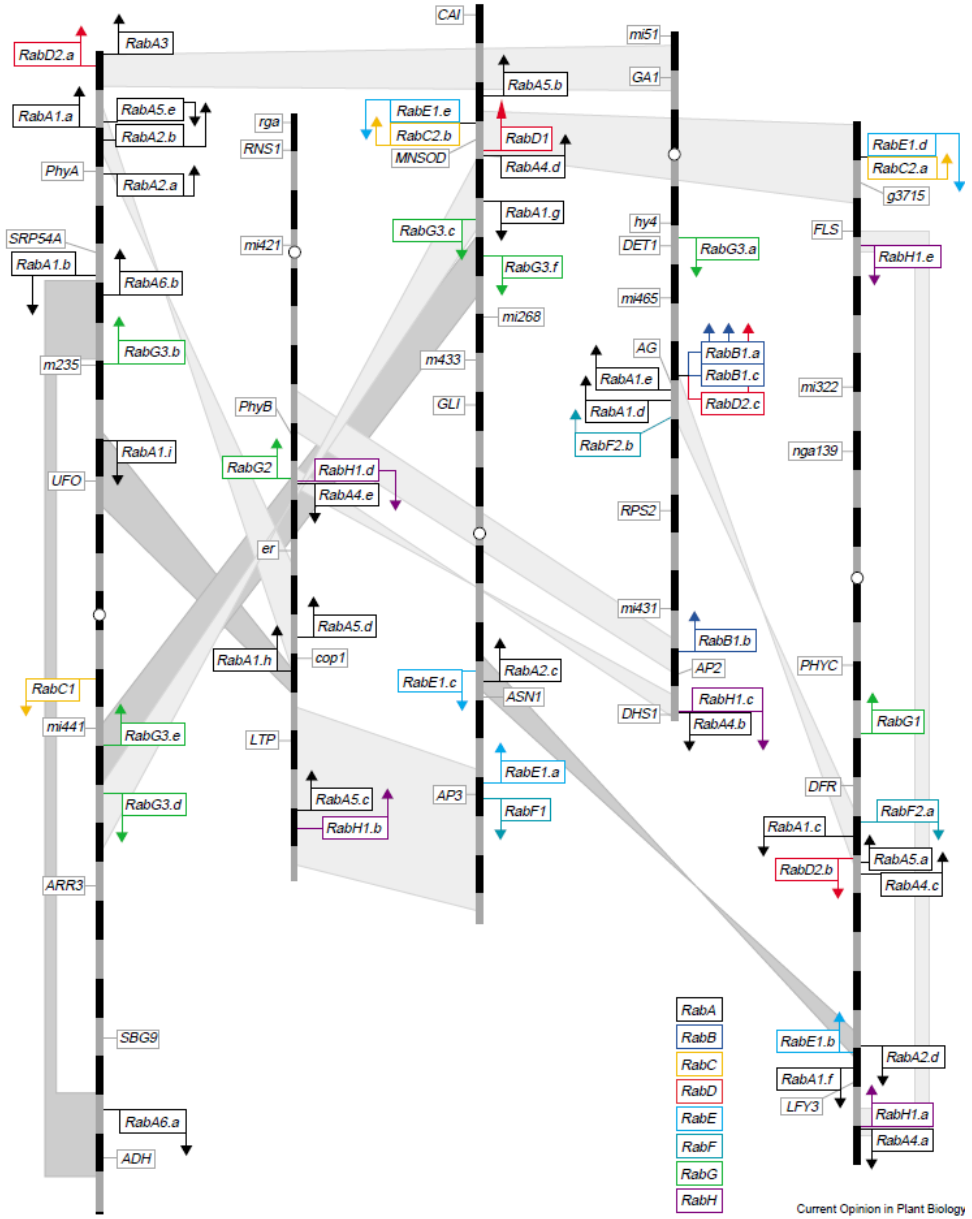


- Rab = “rat brain” proteins, low M_W
- Largest family of small GTPases (57 Arabidopsis, 52 Rice)
- Grouped into 8 clases (RabA-H):

Rab1/RabD	Rab2/RabB
Rab5/RabF	Rab6/RabH
Rab7/RabG	Rab8/RabE
Rab11/RabA	Rab18/RabC

Rutherford and Moore, 2002, COPB

Small Rab GTPases



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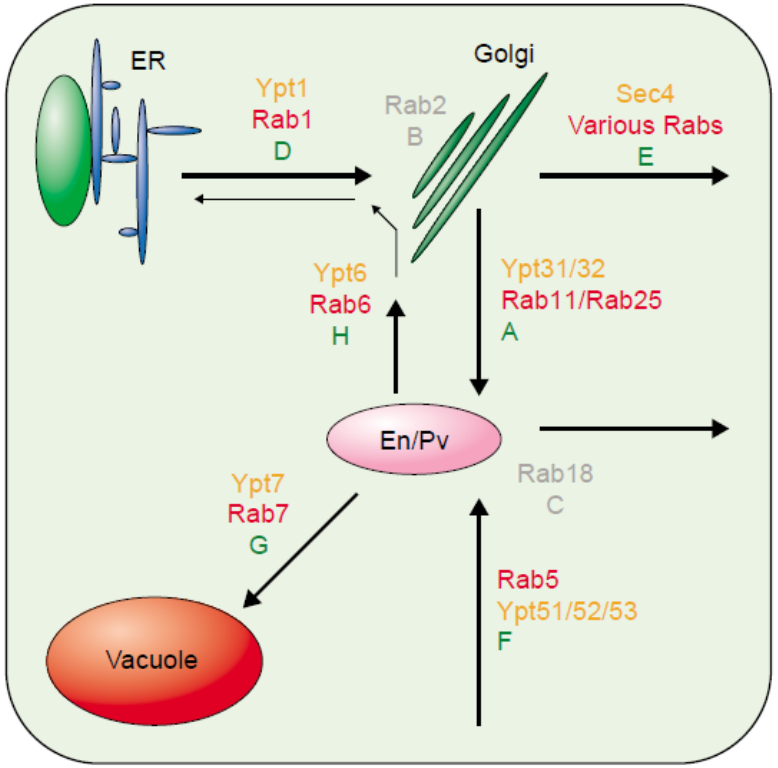
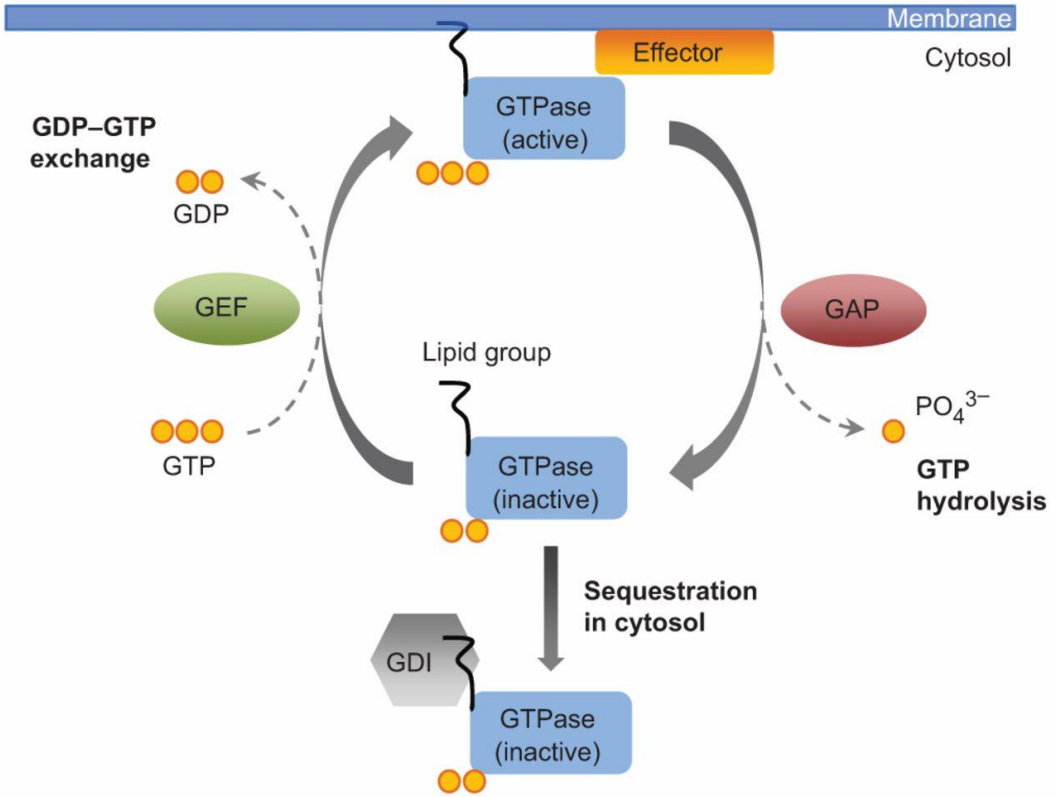


Rutherford and Moore, 2002, COPB

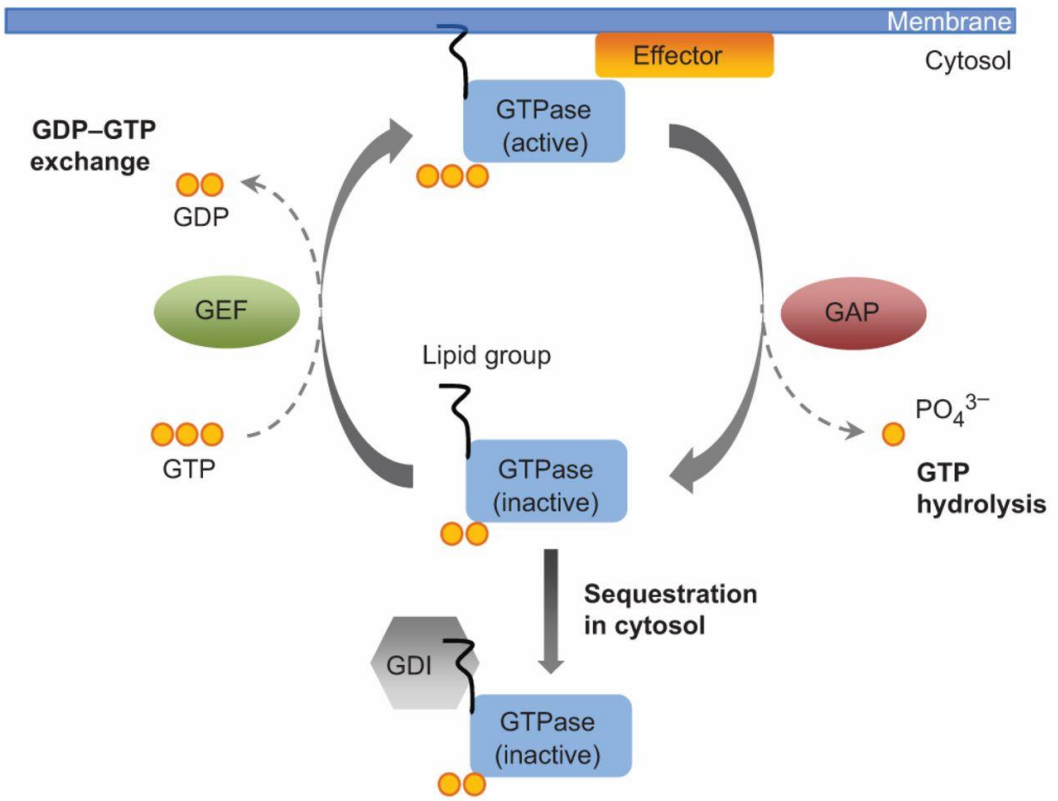
GDP-GTP exchange cycle of small GTPases



GAP – GTPase-activating proteins
GEF – guanin exchange factor
GDI – GDP-displacement inhibitor

Carla F. Bento et al., 2013

GDP-GTP exchange cycle of small GTPases



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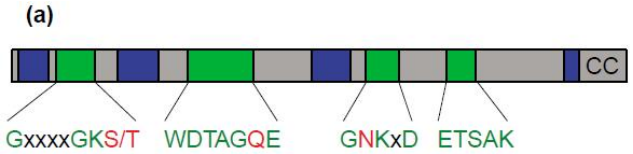
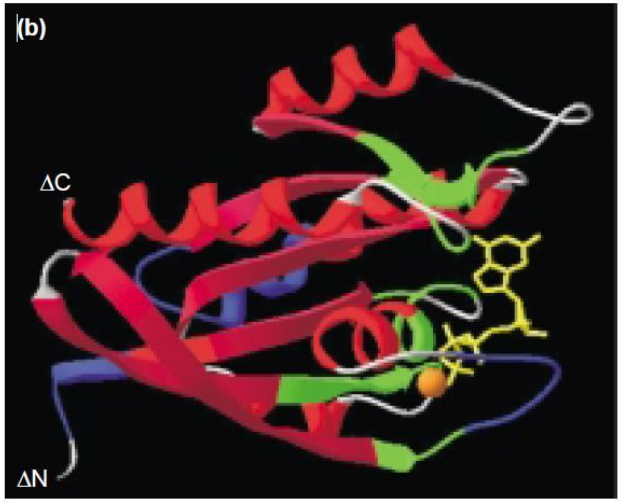


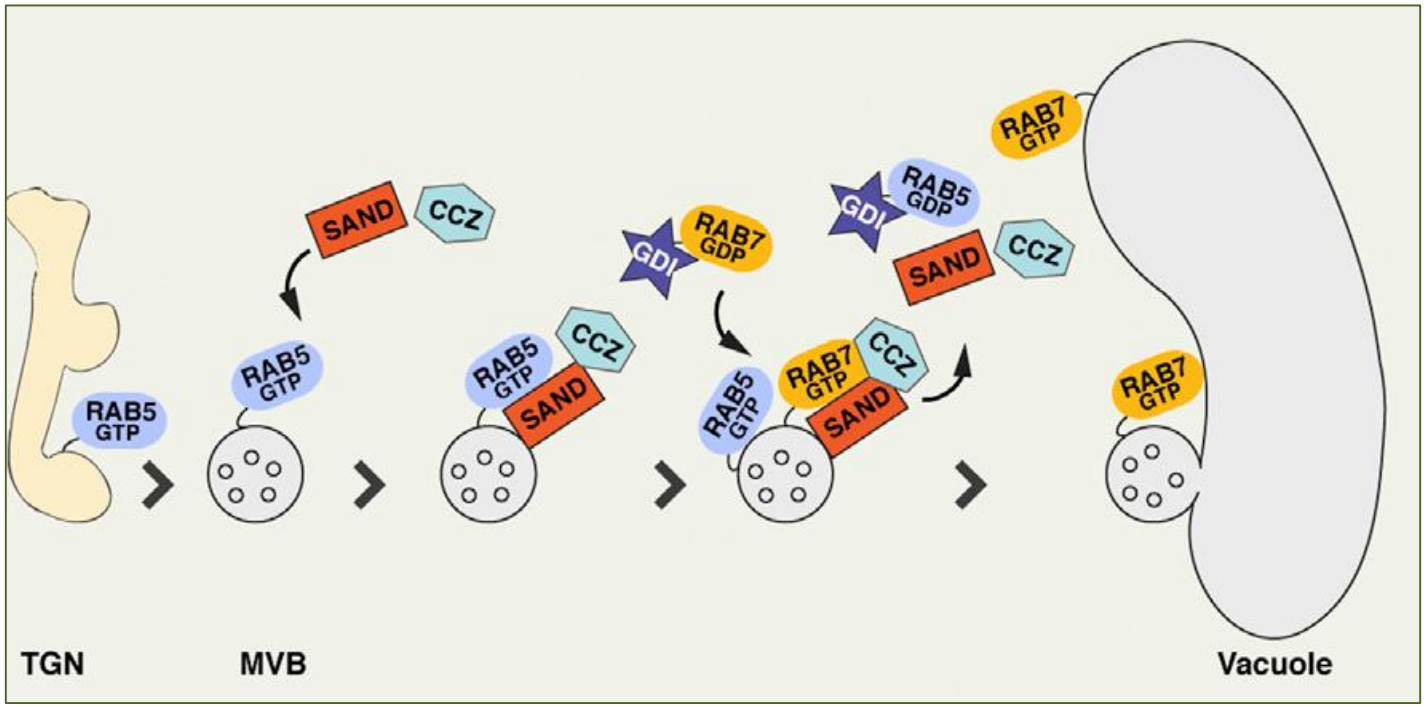


Dominant negative (DN): constitutively GDP-bound or inhibited GTP hydrolysis

Constitutively active (CA): constitutively GTP-bound

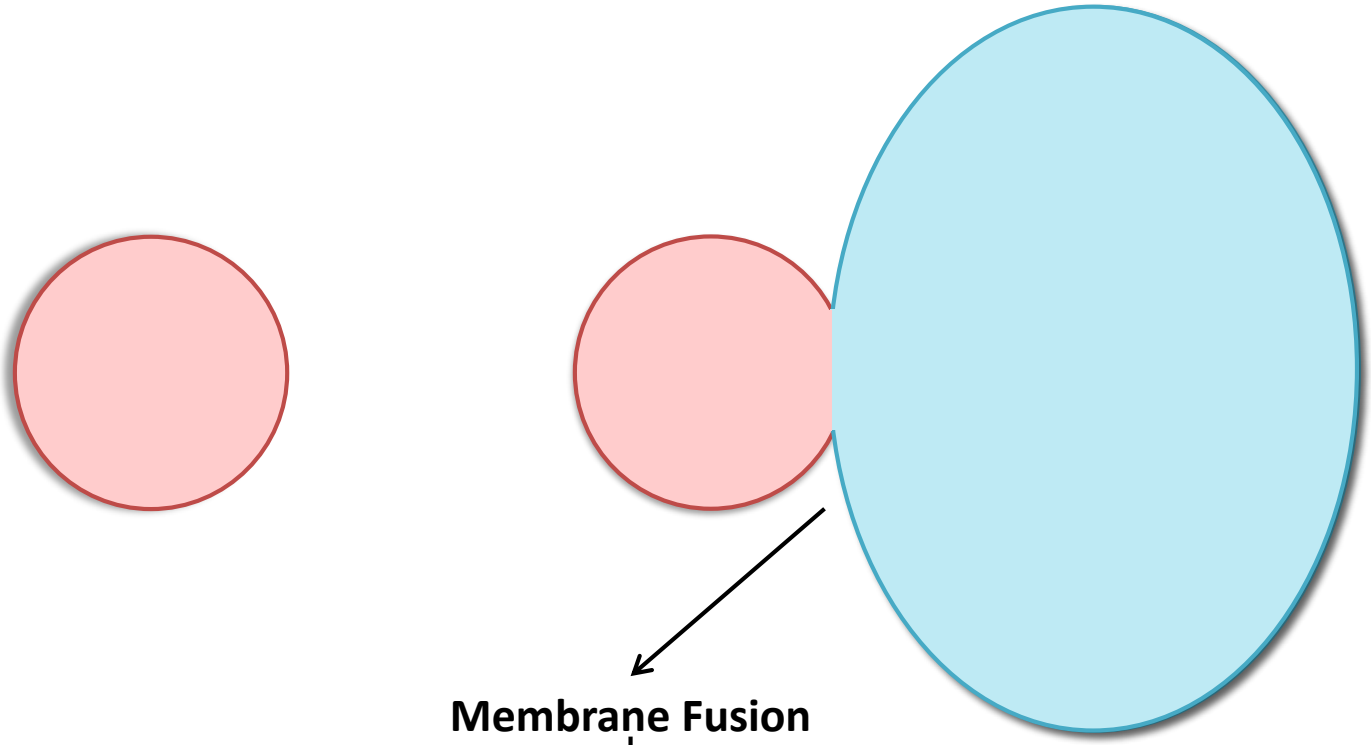
Carla F. Bento et al., 2013

Rab5 to Rab7 conversion on vacuolar trafficking pathway



Singh et al., 2014; Ebine et al., 2014; Cui et al., 2014

“SNARE – Ware”



Membrane Fusion

↓

energetically unfavorable

↓

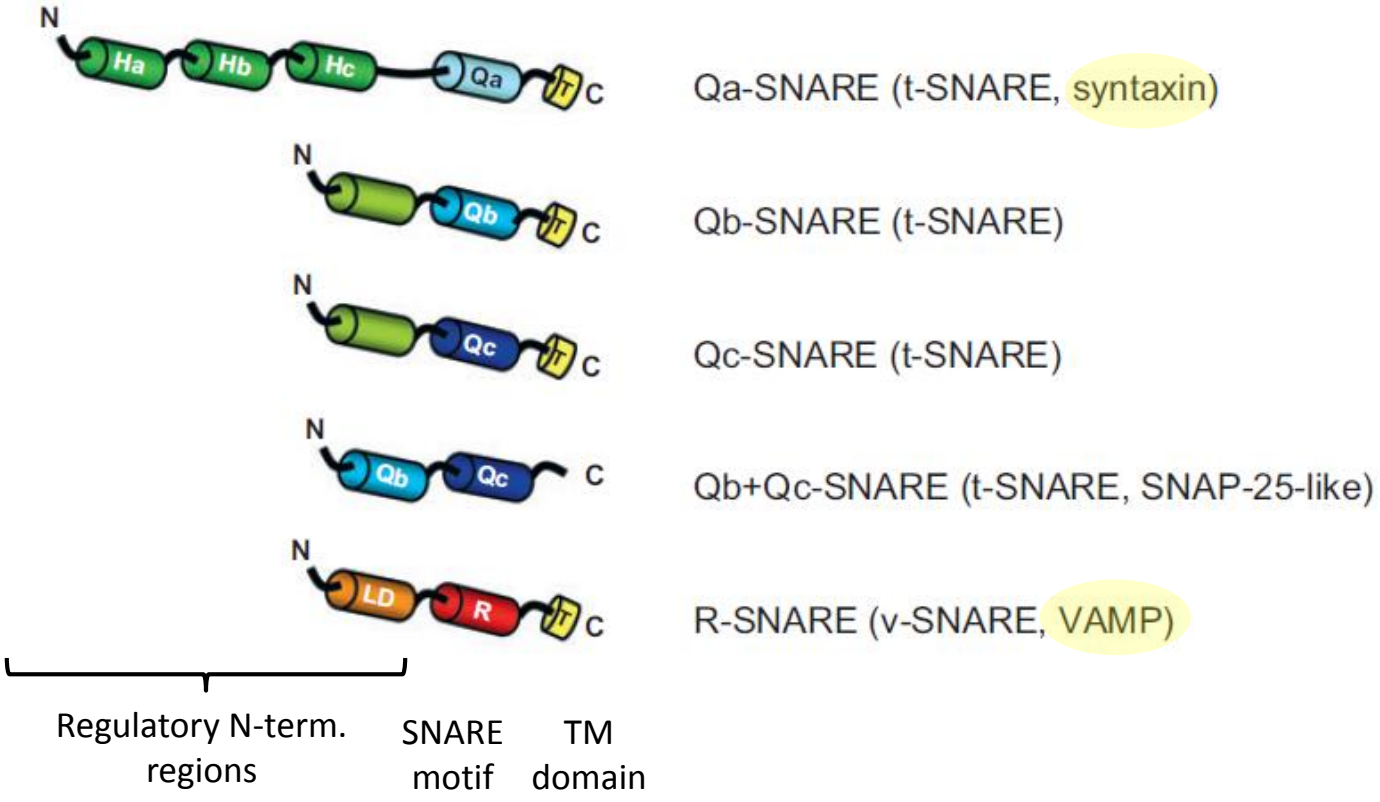
SNAREs as mediator

"SNARE – Ware"

Functional classification:

t-SNARE – at **t**arget membrane
v-SNARE – at **v**esicle membrane

Structural classification: 200 – 400 aa



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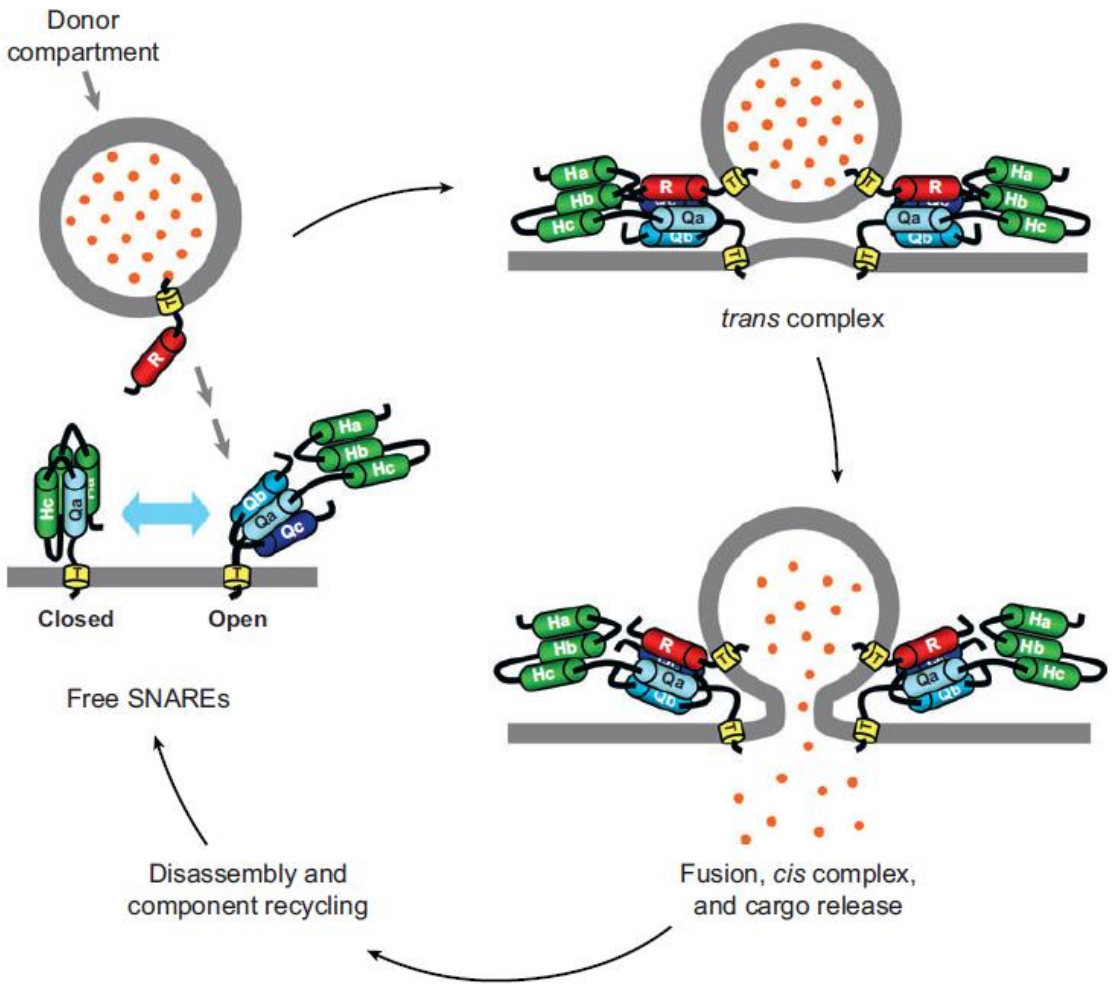
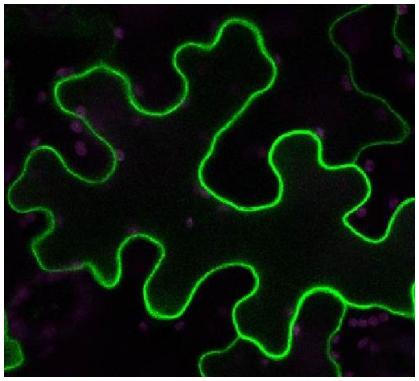
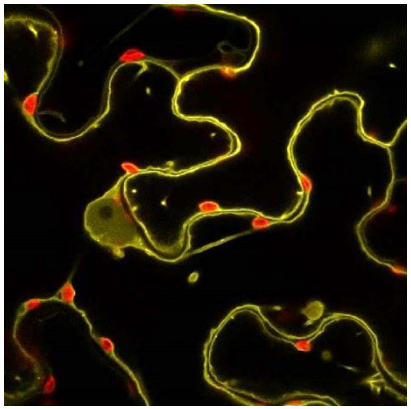

Donor compartment
R
Ha
Hb
Hc
Qa
Qb
Qc
Closed
Open
Free SNAREs
Disassembly and component recycling
trans complex
Fusion, cis complex, and cargo release

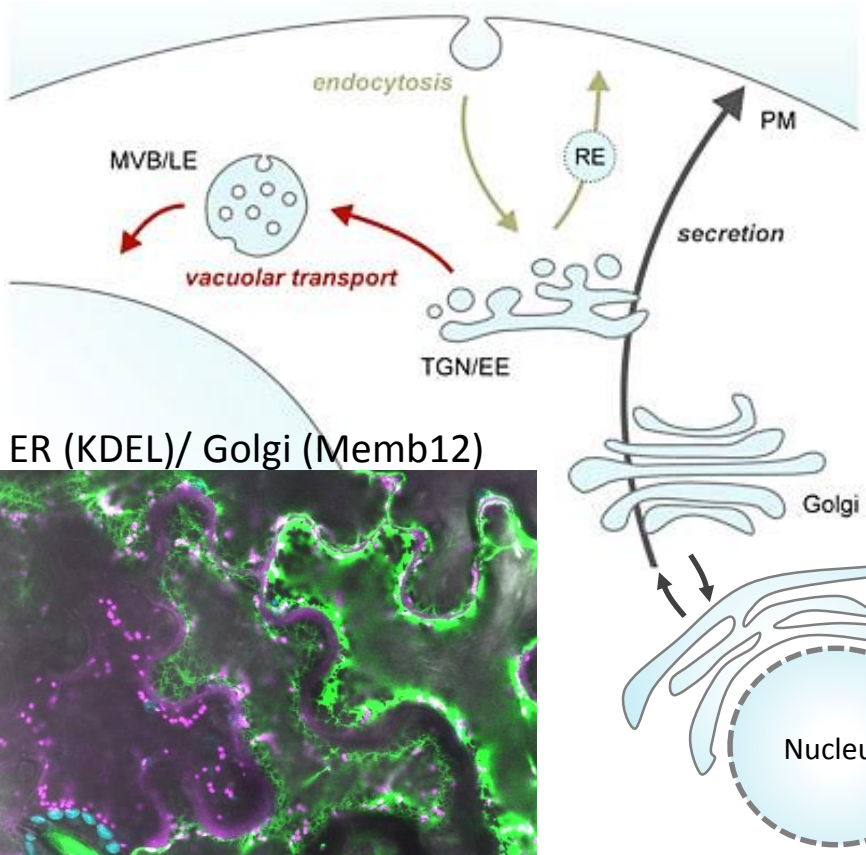
Endomembrane Marker Proteins

PM – FLS2



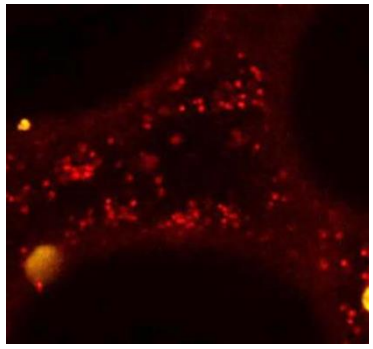
Vacuole – RabG3F



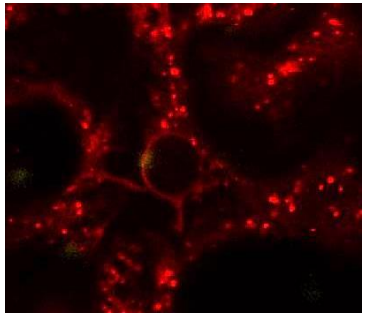


ER (KDEL)/ Golgi (Memb12)

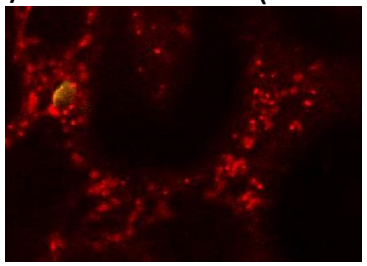
TGN – Vha-A1



EE/LE – ARA7 (RabF2b)



LE/MVB – ARA6 (RabF1)

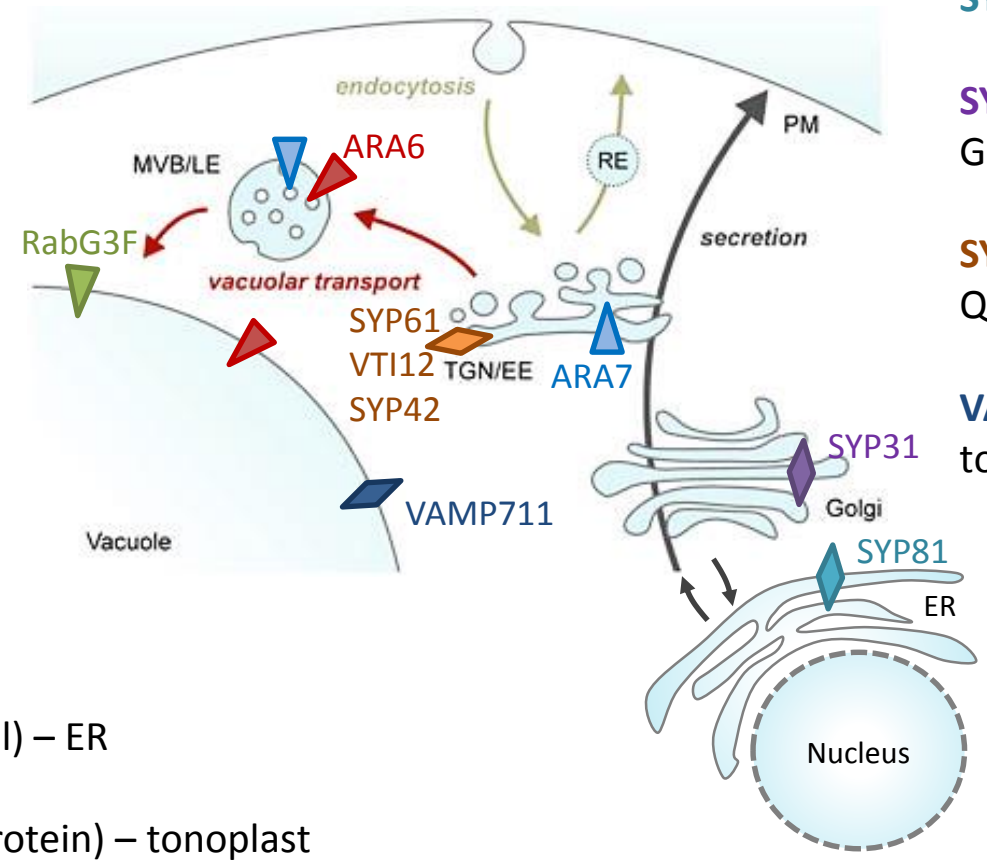


Endomembrane Marker Proteins

ARA7 (Rab5/ RabF2b) – TGN/MVB

ARA6 (Rab5/ RabF1) – MVB/ tonoplast

RabG3F (Rab7) – tonoplast



SYP81 – Qa-SNARE – ER

SYP31 – Qa-SNARE – Golgi

SYP61/ SYP42/ VTI12 – Q-SNAREs – TGN

VAMP711 – R-SNARE - tonoplast

Organelle Marker:
HDEL (ER-retention signal) – ER
Mannosidase – Golgi
TIP (tonoplast intrinsic protein) – tonoplast

FM4-64 – endocytic tracer

Endomembrane Marker Proteins

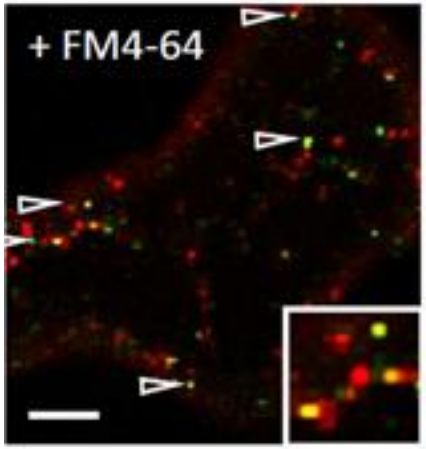
Markers:

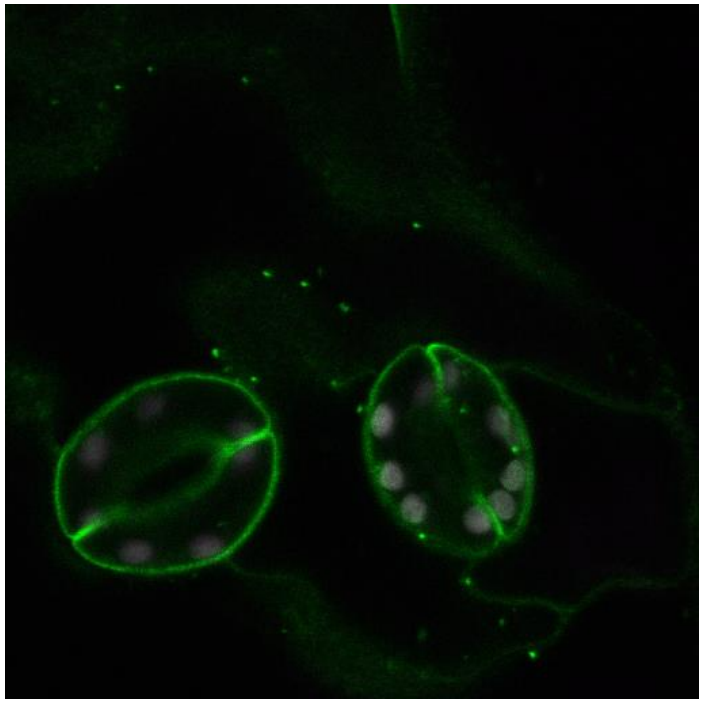
Compartment	Construct	Reference
ER: Endoplasmic reticulum	Sno[a] (EGFP)	Cutler et al., 2000
Golgi	Memb12-mCherry	Geldner et al., 2009
	ST-YFP	Zheng et al., 2004
TGN/EE: Trans-Golgi Network/Early Endosome	VHAa1-mRFP/-GFP	Dettmer et al., 2006
	CFP/mRFP-SYP61	Drakakaki et al., 2012; Robert et al., 2008
	YFP-Rab-A2	Chow et al., 2008
	VHA-a1-GFP/RFP	Dettmer et al., 2006
	SYP41-mRFP	Dettmer et al., 2006
	YFP-SCAMP1	Lam et al., 2007
	Ara7/RabF2b-mRFP	Ueda et al., 2004; Ebine et al., 2011
MVB/PVC/LE: Multivesicular body, prevacuolar compartment or late endosome	ARA6/RabF1-mRFP	Ueda et al., 2004; Ebine et al., 2011
Vacuole	VAMP711-YFP	Geldner et al., 2009
PM: Plasma Membrane	NPSN12	Geldner et al., 2009
Nucleoplasm	mRFP-H2B	He et al., 2015
Endocytic tracer that internalizes via endocytosis from the plasma membrane to the tonoplast.	FM4-64	Bolte et al., 2004; Jelínková et al., 2010

Practical: Tracing the Transport Route of FLS2

FLS2-GFP is internalised into vesicles upon flg22-treatment

FLS2-GFP vesicles are labelled by FM4-64





What is the nature of FLS2-GFP positive endosomes?

→ Co-localisation with:

SYP61 (TGN)
ARA7 (TGN/MVB)
ARA6 (MVB/tonoplast)

Beck et al., 2012, Plant Cell