MCDB/CHEM 103/203; BMSE 233

Molecular Trafficking and Signaling

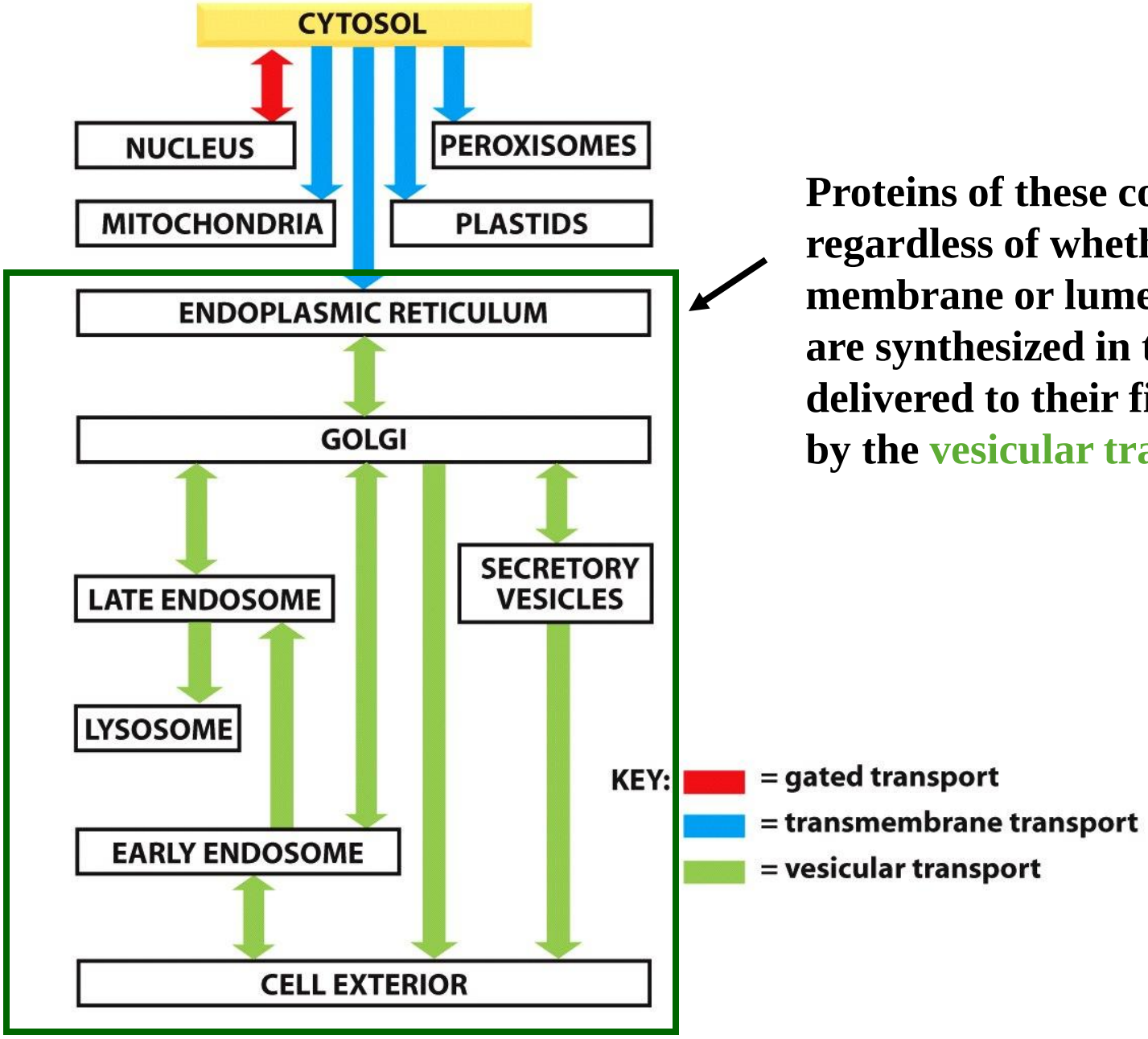
Lecture 17

Associated Textbook Reading:

pp. 754-766

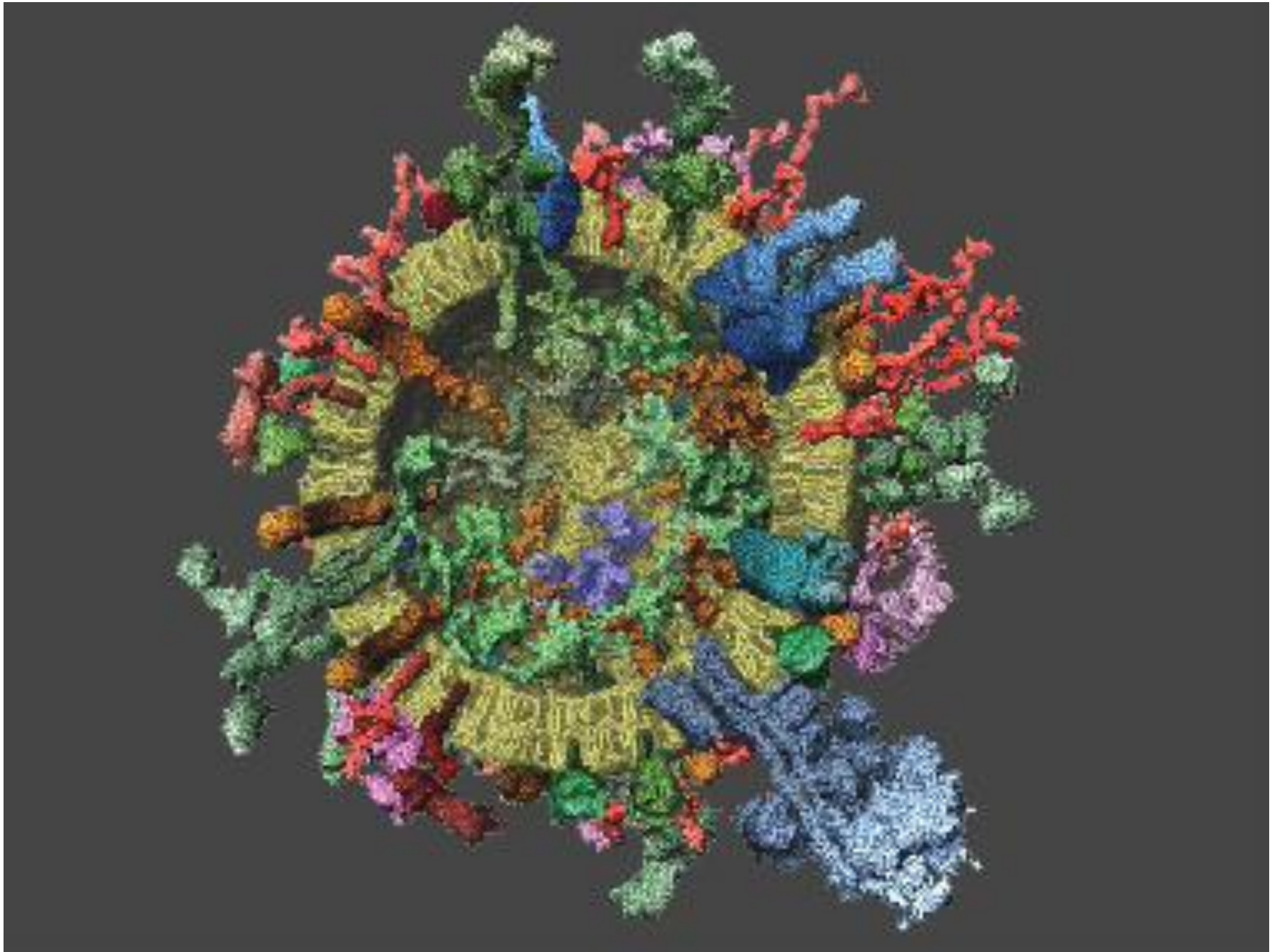
Secretory, Endocytic, and Sorting Pathways:

Mechanisms of Transport Between the ER, Golgi, Lysosomes, and the Cell Surface



Proteins of these compartments, regardless of whether they are membrane or lumenal proteins, are synthesized in the ER and delivered to their final destinations by the vesicular transport pathway.

Example of a Vesicle: A Synaptic Vesicle (movie)



Budding from the donor compartment

Movement along the cytoskeleton structure

Tethering & docking to the target compartment

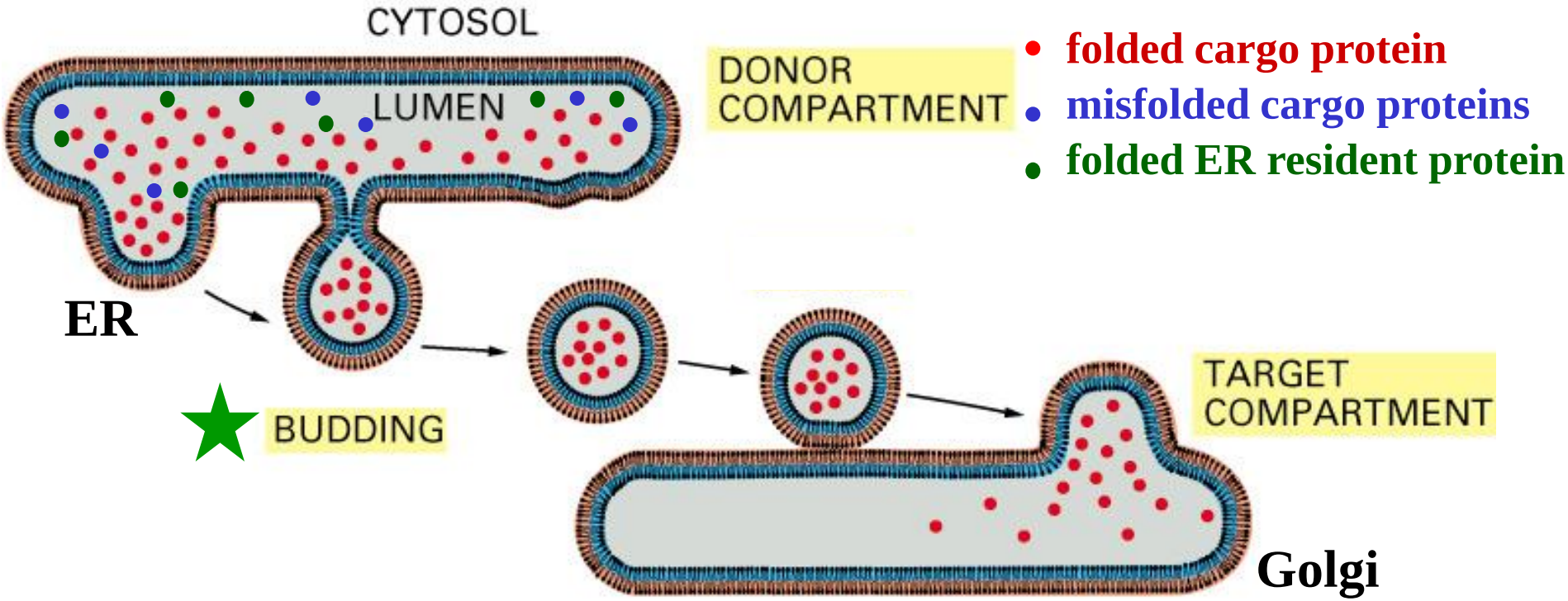
Fusion to the target compartment

Budding of Transport Carriers (Vesicles) from the Donor Compartment

A part of the donor membrane becomes a transport carrier via membrane deformation & fission –a vesicle

Some cargo proteins are selected and recruited into the vesicle, yet others are excluded

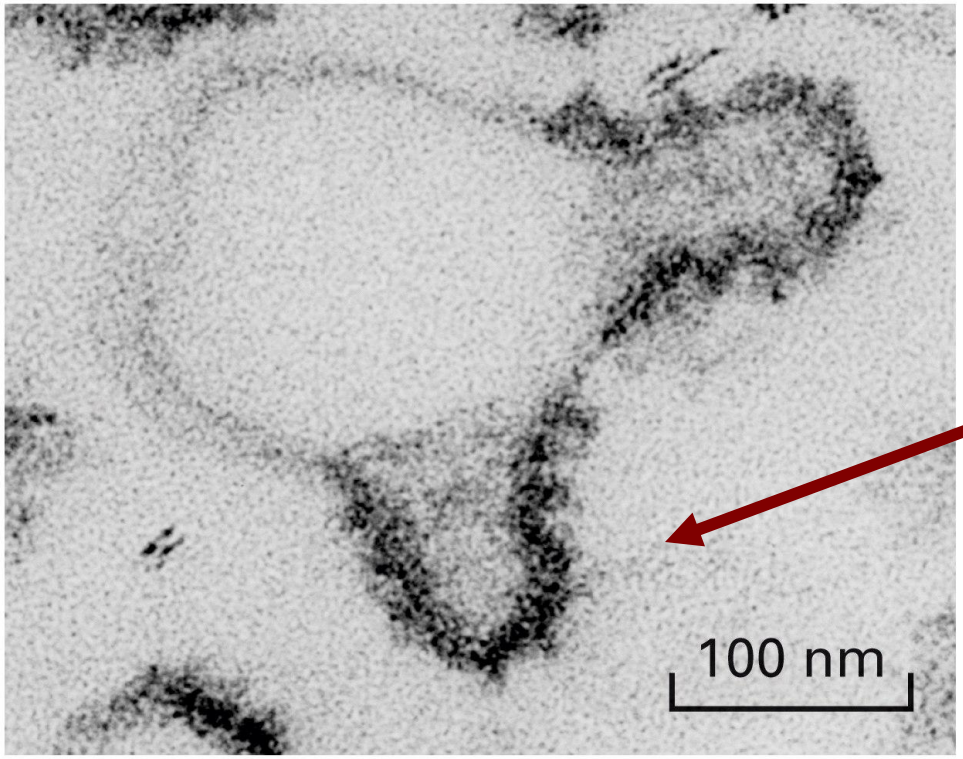
ER-to-Golgi transport cargo selection



Budding: Coat Proteins and Small GTPases

Coat Proteins

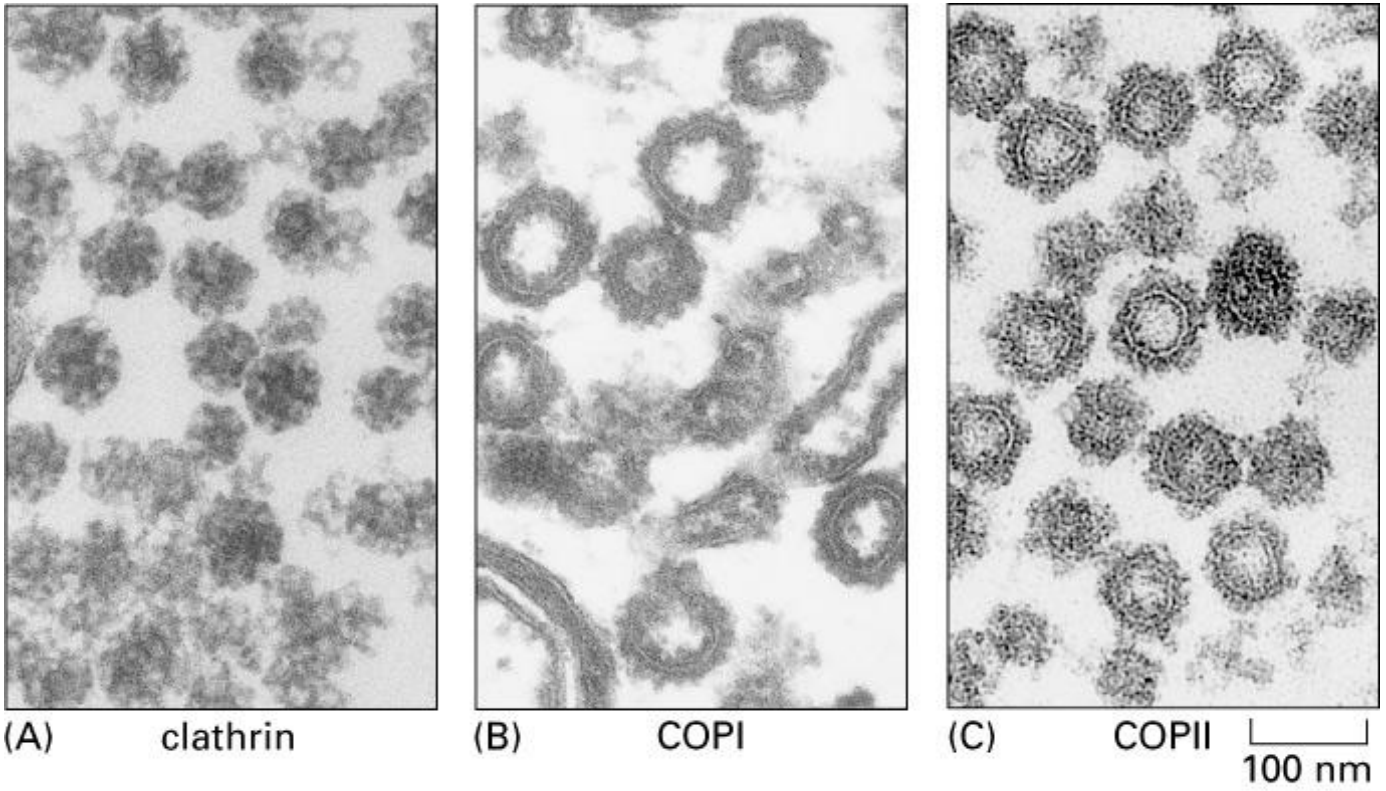
Coat proteins form a complex on the cytoplasmic side of the donor membrane.
Coat proteins promote the formation of a carrier by facilitating membrane deformation.
Coat proteins facilitate the cargo selection by binding (directly or indirectly) with specific transport signals found on cargo molecules.



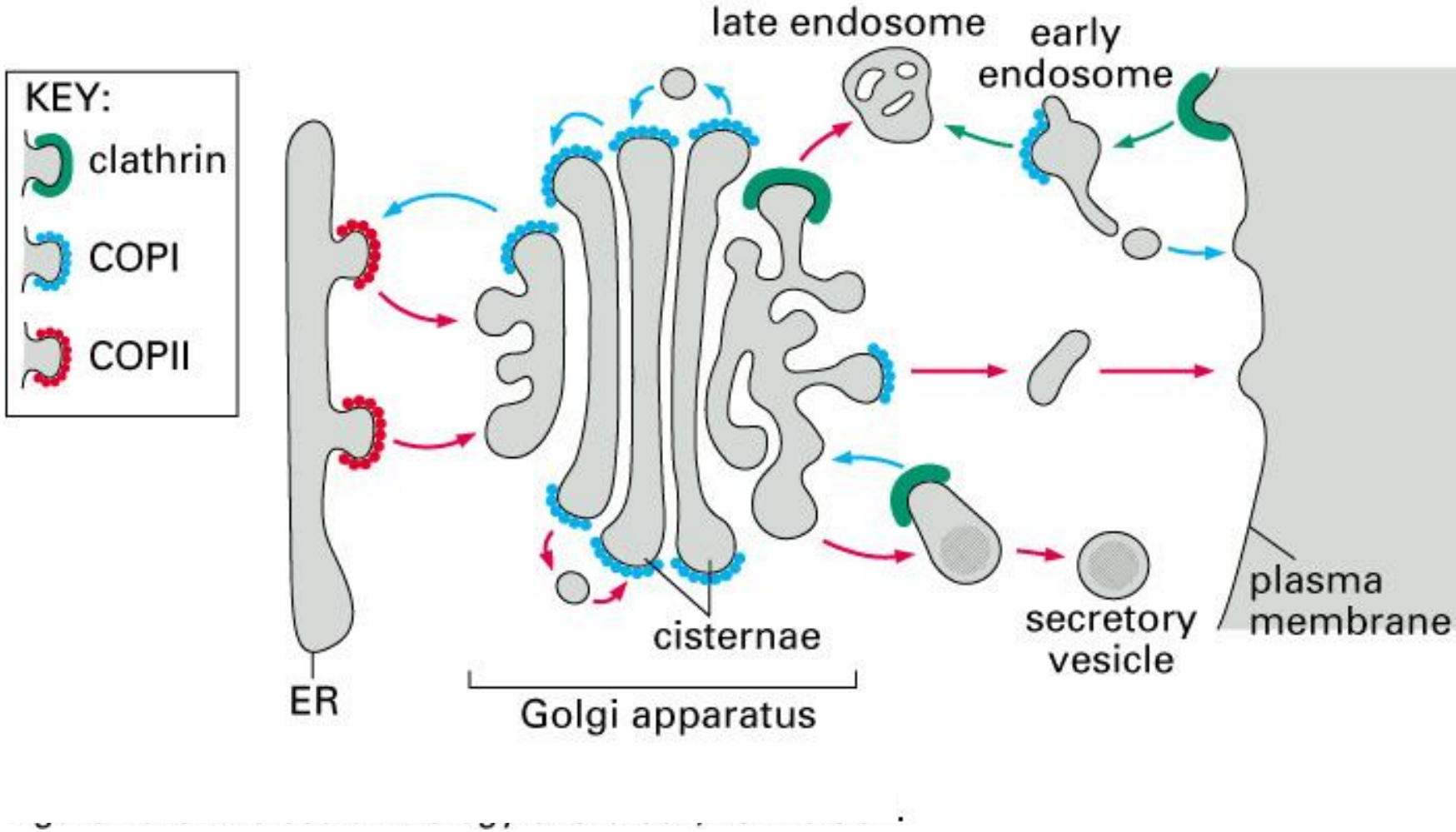
In-vitro budding reaction using isolated ER membrane vesicles.

An electron-dense coat associated with the budding vesicles. Since the coats are protein-rich, they appear electron-dense.

Types of Coat Protein Complexes in a Eukaryotic Cell:
Three Examples



Different Coats Are Involved in Different Transport Steps



COPII: ER to Golgi

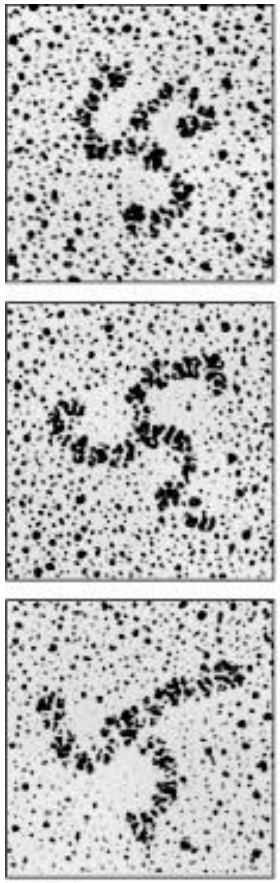
COPI: Golgi to ER, within Golgi, some transport initiated from endosomes

Clathrin: Transport initiated from Golgi (e.g. Golgi-to-endosome), plasma membrane (e.g. internalization), and endosomes.

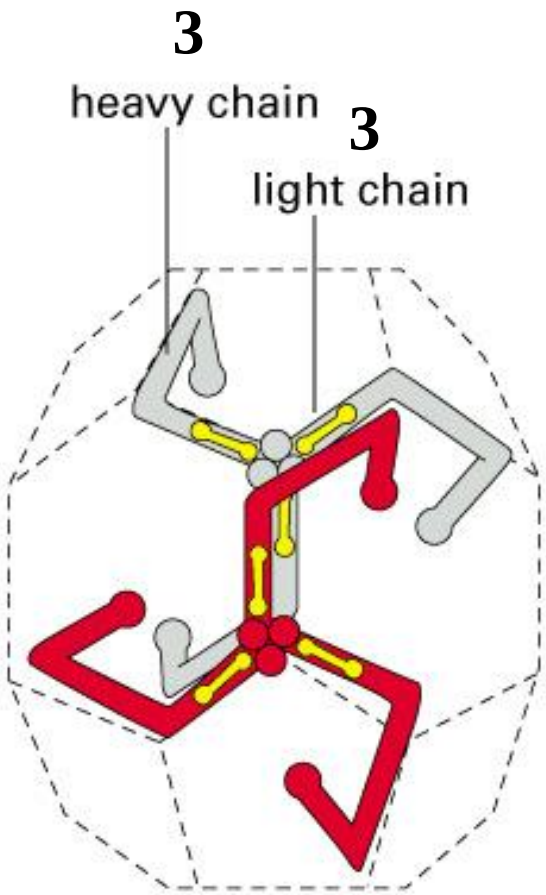
Triskelion Organization of Clathrin

Triskelions (3 heavy + 3 light chains) assemble into a polygonal lattice

triskelion

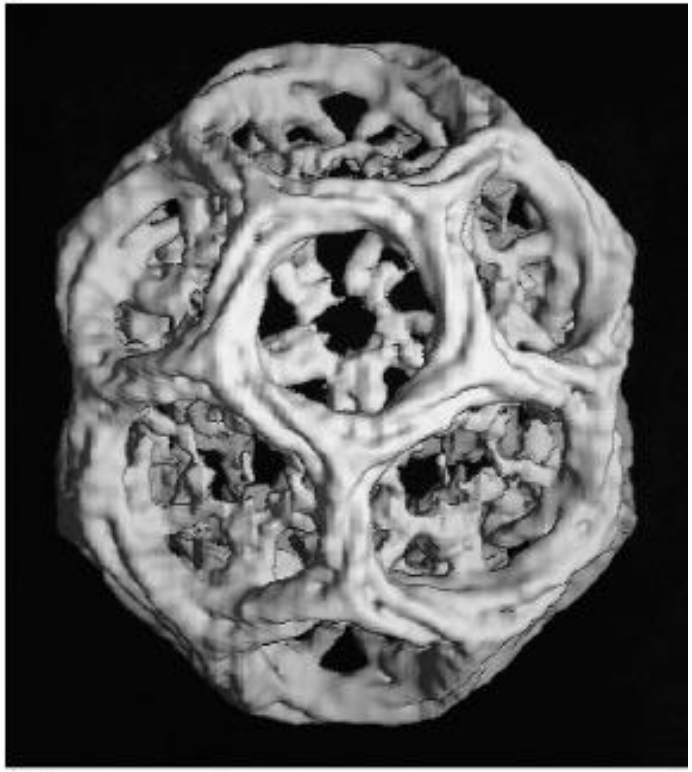


(A)



(B)

pentagons and hexagons



(C)

50 nm

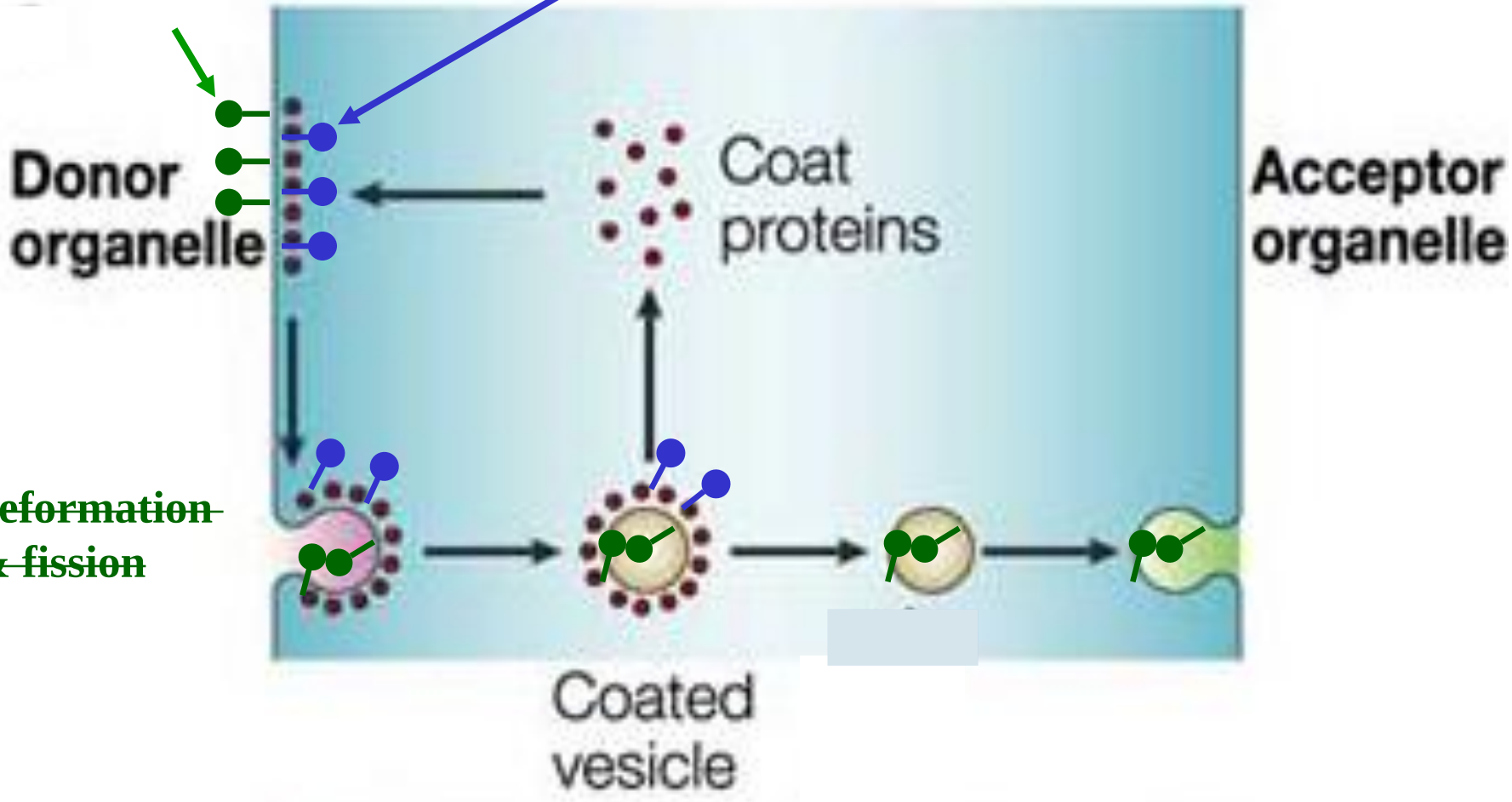
Vesicle Formation and Budding: Clathrin Vesicles (movie)

Post-Budding: I: Disassembly of Coat Protein Complexes After Budding

The coat proteins are shed off shortly after the transport carriers bud off the donor membrane toallow the subsequent tethering/docking/fusion of the transport carrierswith the target membrane (also for the purpose of recycling).

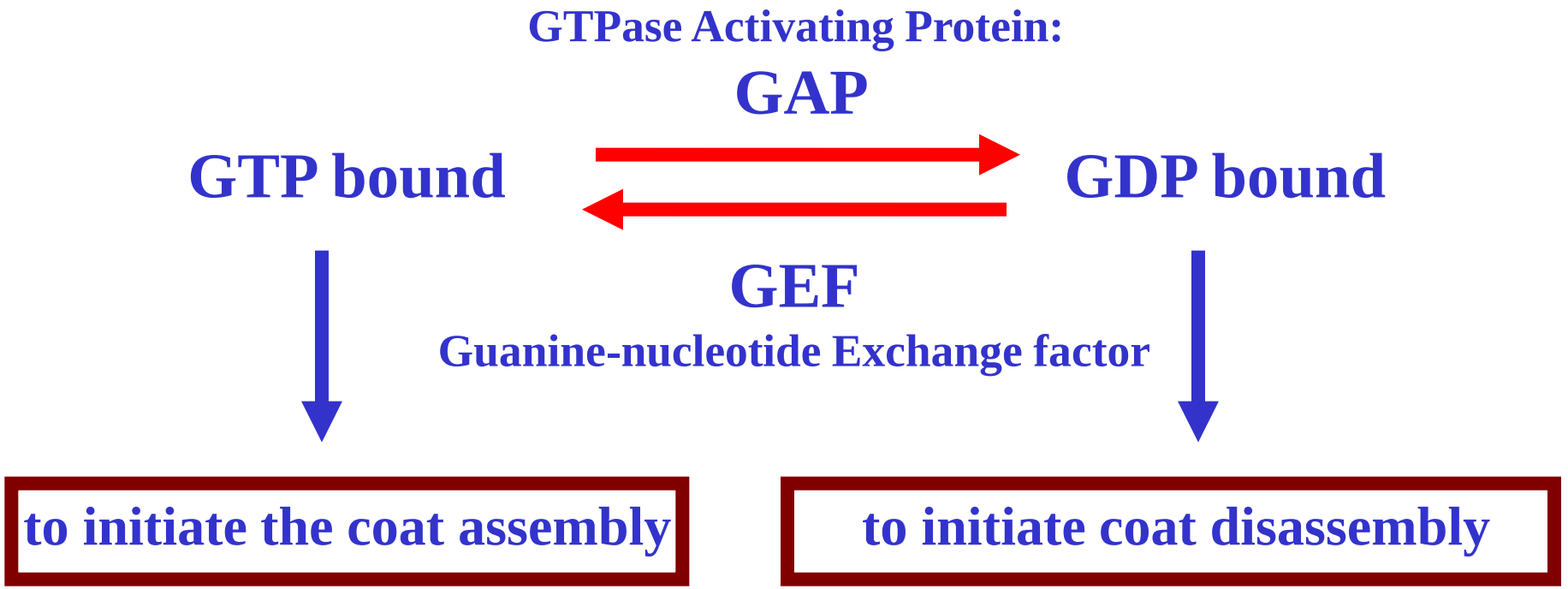
cargo proteins (to be discussed later)

There are other transport machinery components required in budding.



~~**deformation & fission**~~

Small GTPases Act as a "Molecular Clock" to Control the Assembly & Disassembly of a Coat Complex



Effector of a small GTPase: A protein or protein complex that binds the GTP form of a small GTPase, and performs specific functions downstream of that GTPase

Coat proteins can be considered as effectors of small GTPases

How Do a Small GTPase and a Coat Work Together to Control Budding?

Budding from the ER as an example:
Sar1 (GTPase) controls the COPII (coat) formation at the ER

1. Recruitment and activation of Sar1 GTPase at the ER membrane

2. Sar1-GTP initiates the COPII coat assembly to form a budding carrier

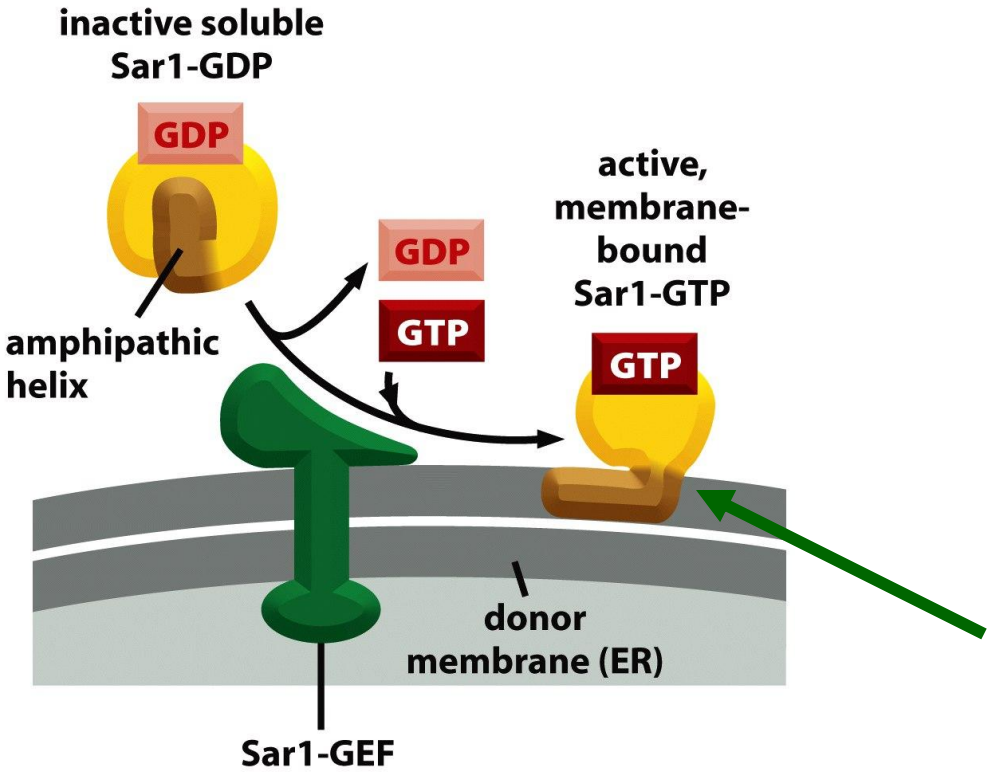
3. COPII components also select specific cargo proteins to be packed into carriers

4. After budding, GTP hydrolysis of Sar1 initiates the COPII disassembly

Recruitment and Activation of Sar1 GTPase at (and only at) the ER Membrane

Sar1 has a hydrophobic tail which determines whether Sar1 is soluble or membrane-bound. The exposure of the tail depends on whether Sar1 binds to GTP or GDP

Sar1-GDP is found in the cytosol (the tail is hidden)
Sar1-GTP is on the ER membrane (the tail is exposed)

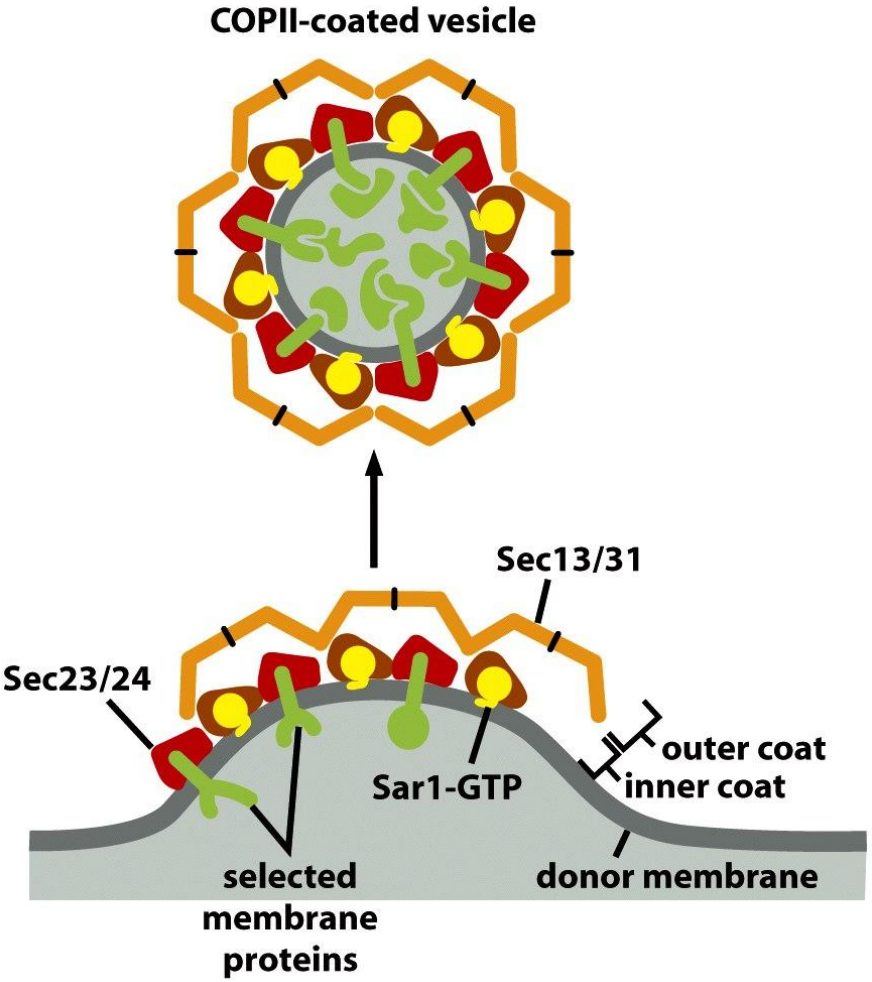


Sar1-GDP is converted to Sar1-GTP by a Sar1-GEF, Sec12, which is an ER resident integral membrane protein

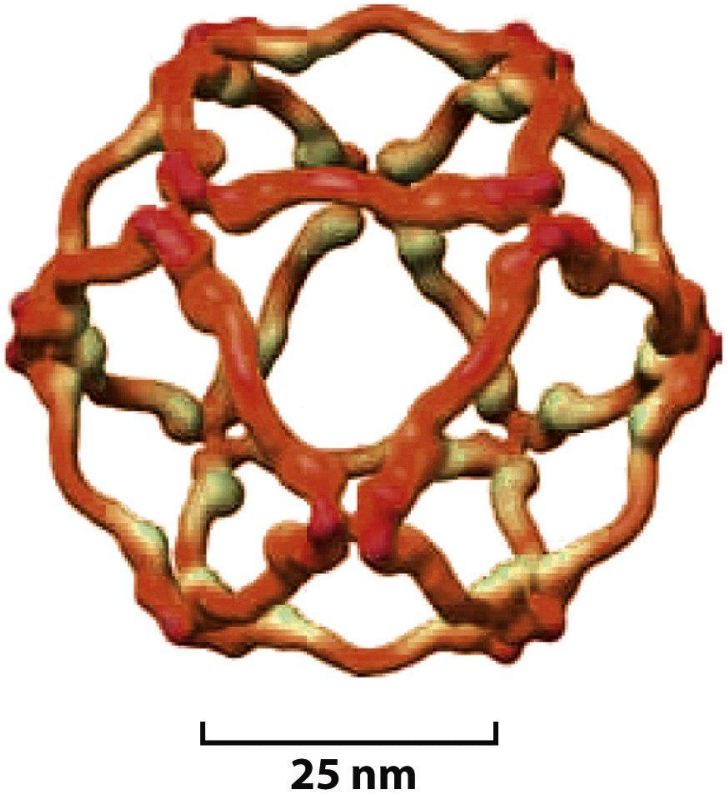
Because Sec12 is an ER integral membrane protein, Sar1 can only be recruited and activated on the ER membrane

ER-Localized Sar1-GTP Initiates the COPII Coat Assembly by Interacting With COPII Coat Components

COPII components: Sec23/Sec24 complex, Sec13/Sec31complex, Sec16
Sar1-GTP recruits the Sec23/24 complex which in turn recruits the Sec13/31 complex



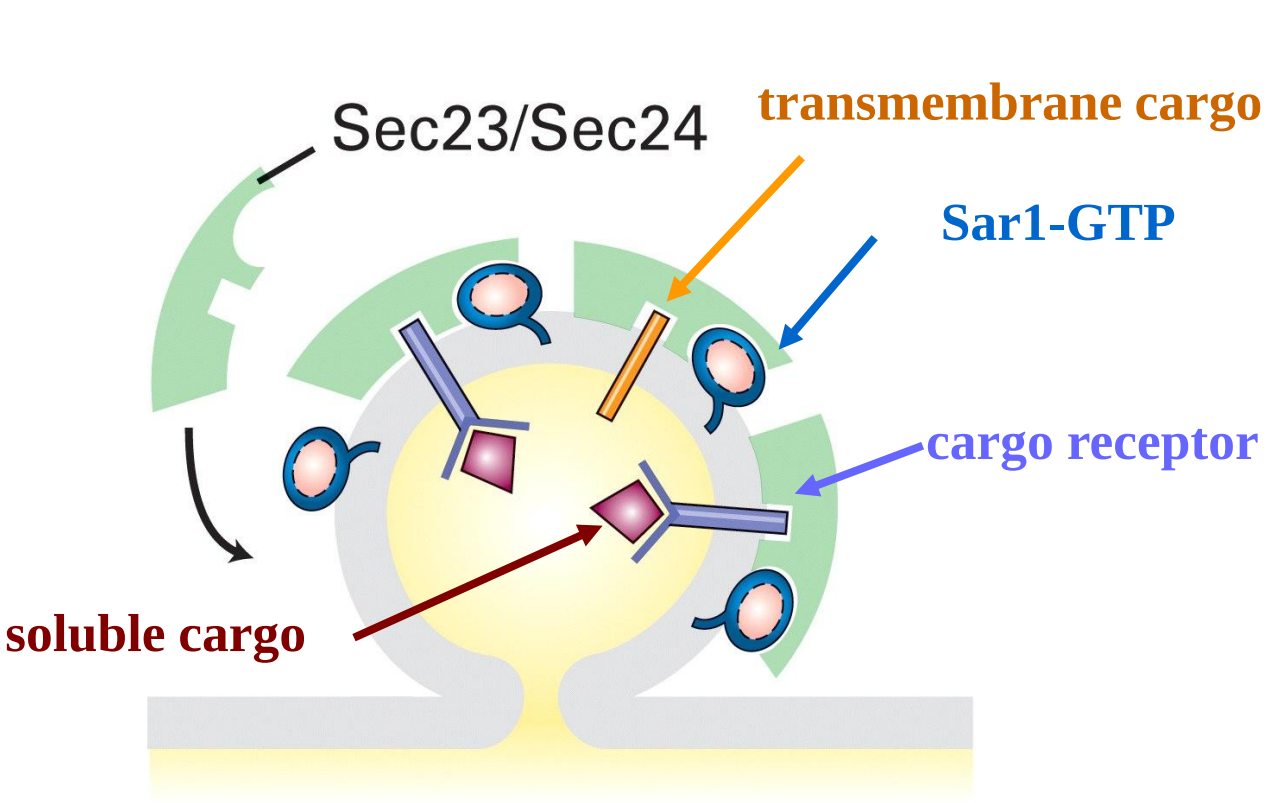
Sec13/31 alone can assemble into a cage to enclose a COPII vesicle

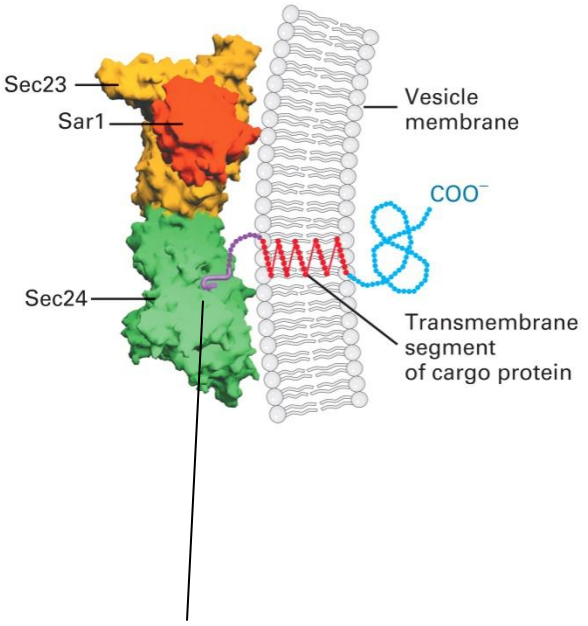


What is the function of Sec23/24 if Sec13/31 alone can assemble into a COPII cage?

COPII Components Also Select Specific Cargo Proteins to be Packed into Vesicles

Sec24 is the major cargo – interacting protein





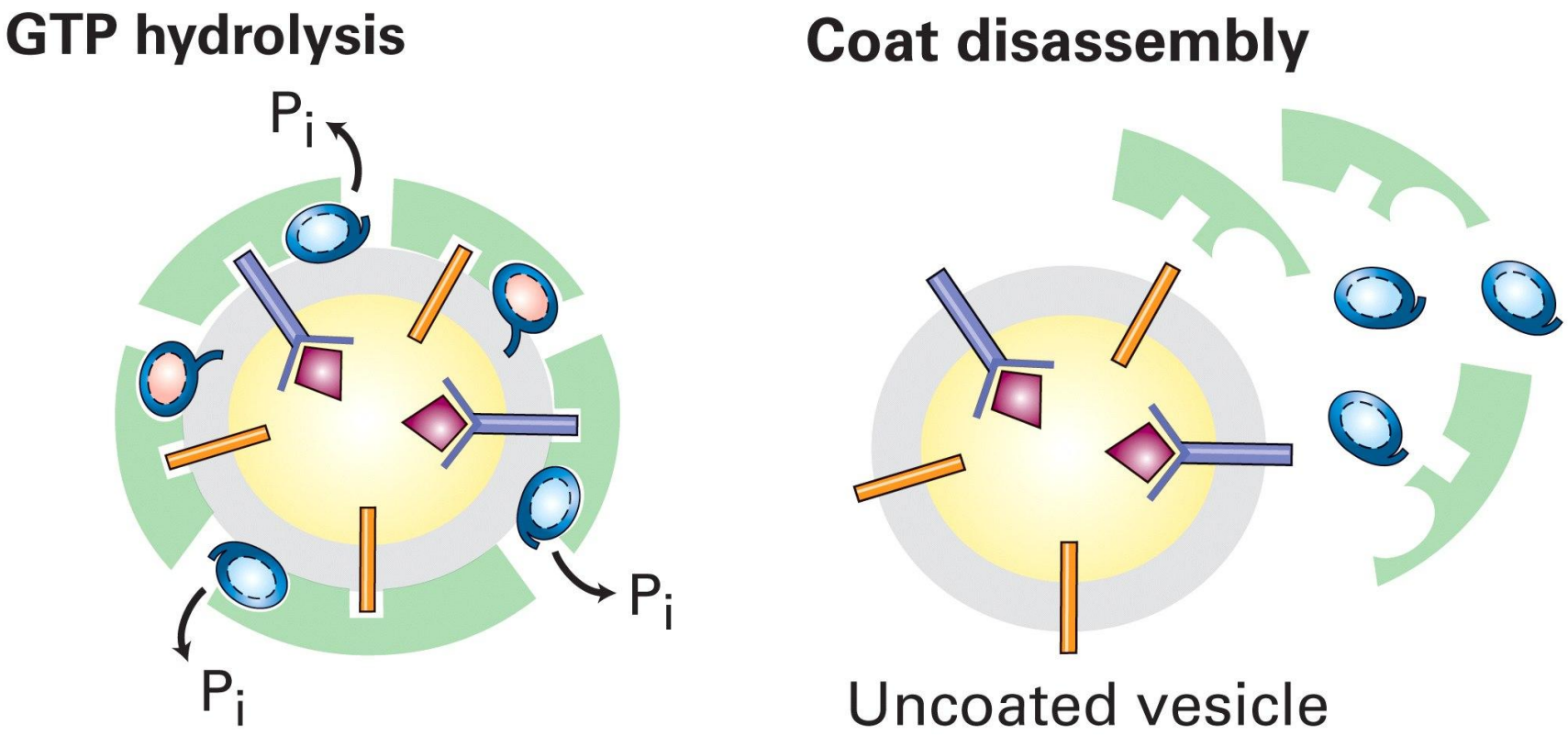
Note the different ways to recruit transmembrane and lumenal (soluble) cargo

GTP Hydrolysis Initiates the Disassembly of the COPII Coat After Budding

Sec23 is a GAP for Sar1

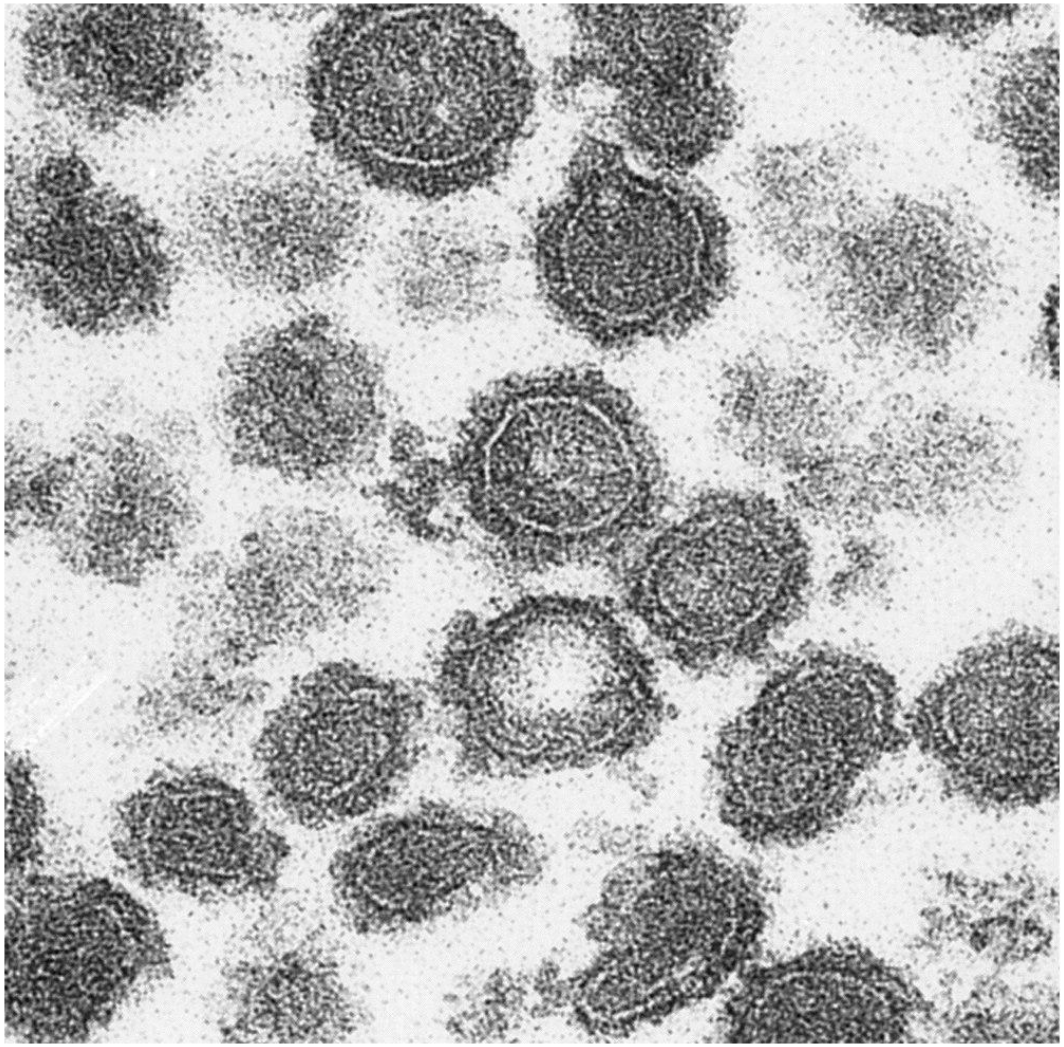
After the budding of a carrier, Sec23 promotes the GTP hydrolysis of Sar1-GTP

Sar1GDP is released from the carrier, which induces the COPII coat disassembly

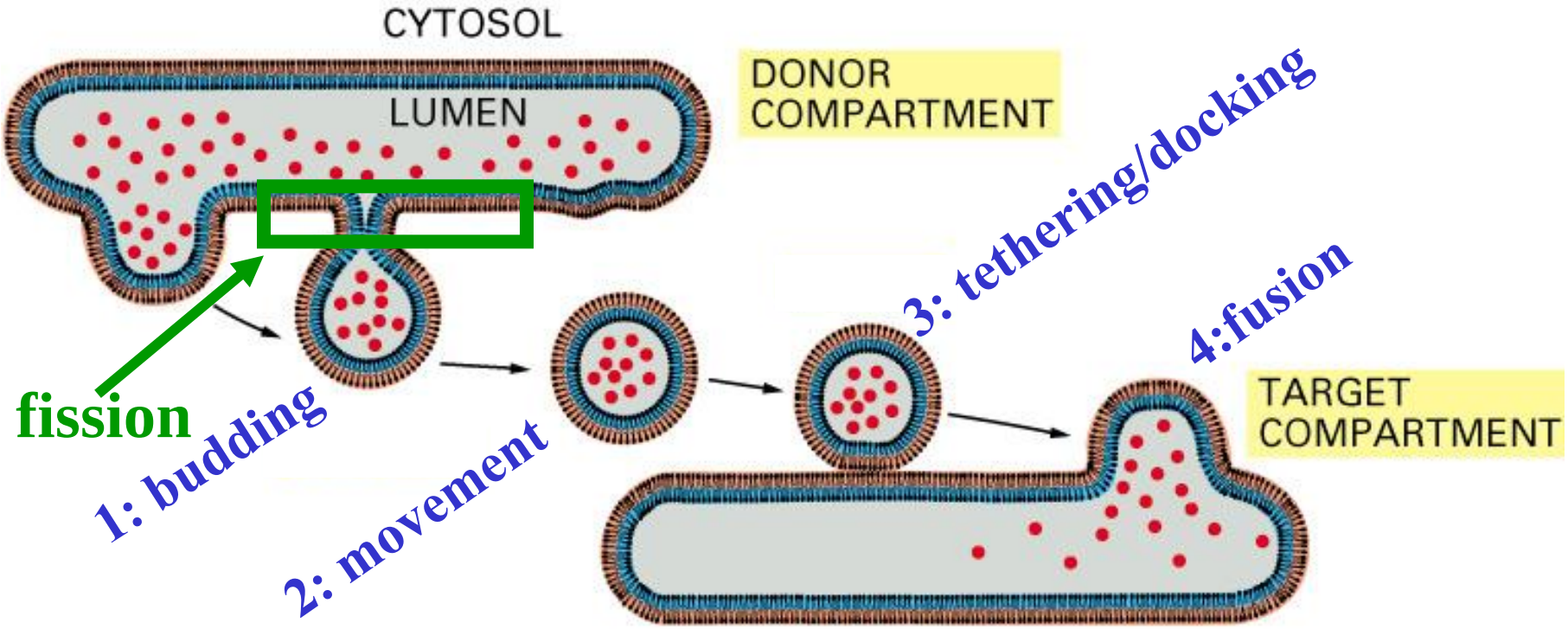


Sar1 GTPase acts as a timer for COPII coat assembly/disassembly

What Would Happen to the ER-to-Golgi Transport in the Presence of a Non-Hydrolyzable GTP such as GppNp in a Cell-Free Transport Assay?



Post-Budding Considerations
II: Fission of Transport Carriers From the Donor Membrane

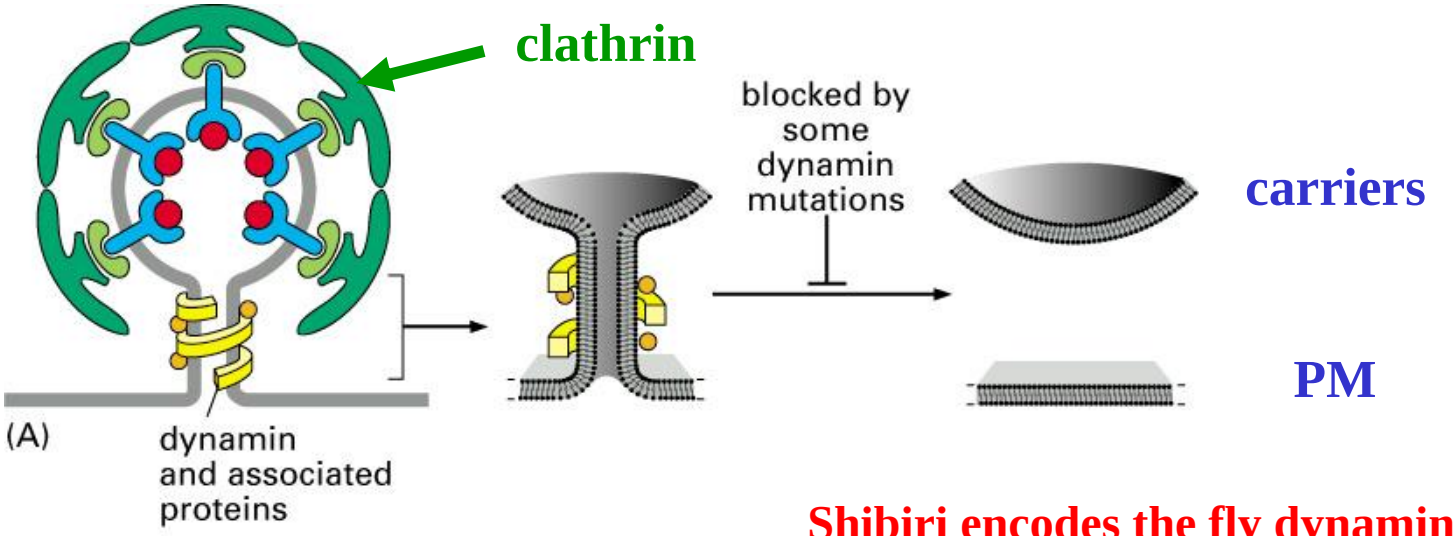


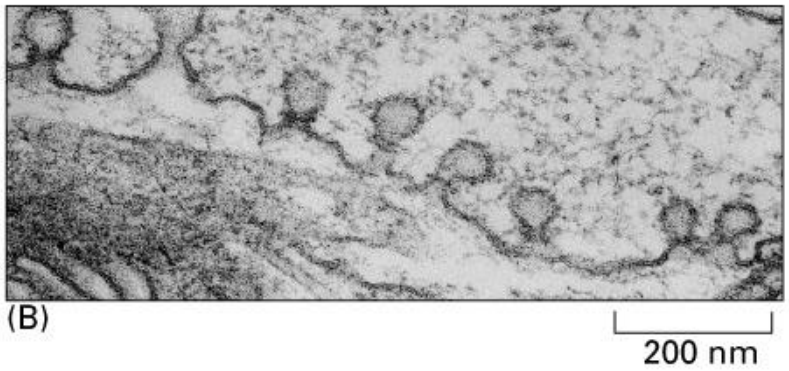
Fission of clathrin-coated carriers is best characterized.
The dynamin family of proteins appears to play a role in this process.
The fission of COPI and II vesicles are less understood

Fission of Clathrin-Coated Vesicles From the Plasma Membrane

Dynamin is a GTPase and it forms a ring around the neck of a forming clathrin carrier

Dynamin then recruits other proteins to the neck. These proteins work together to constrict the neck until the vesicle pinches off from the donor membrane. This pinching off correlates with the hydrolysis of GTP





Shibiri encodes the fly dynamin protein. A temperature sensitive shibiri mutant with defective GTP hydrolysis at above 38 ˚C blocks the internalization & recycling of synaptic vesicles at the nerve terminus. As a consequence, neuronal transmission is inhibited and mutant flies become paralyzed at high temperature.

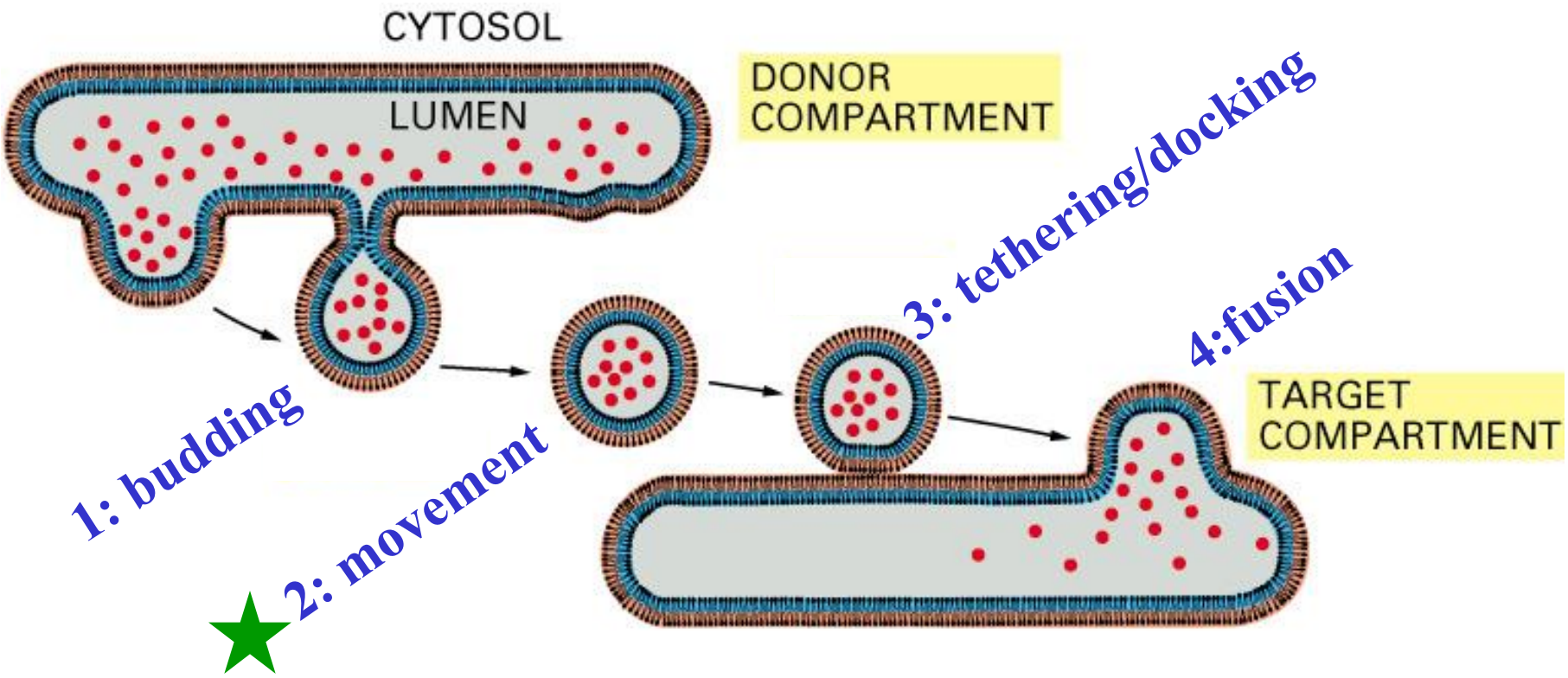
Budding from the donor compartment

Movement along the cytoskeleton structure

Tethering & docking to the target compartment

Fusion to the target compartment

Movement of Transport Carriers to Target Compartments



Once budded off, the transport carriers move toward the target compartment along the cytoskeleton in a directional manner with the help of motor proteins

Motor proteins utilize ATP hydrolysis to move cargo molecules (e.g. proteins, vesicles or carriers, or even organelles) along the cytoskeleton.

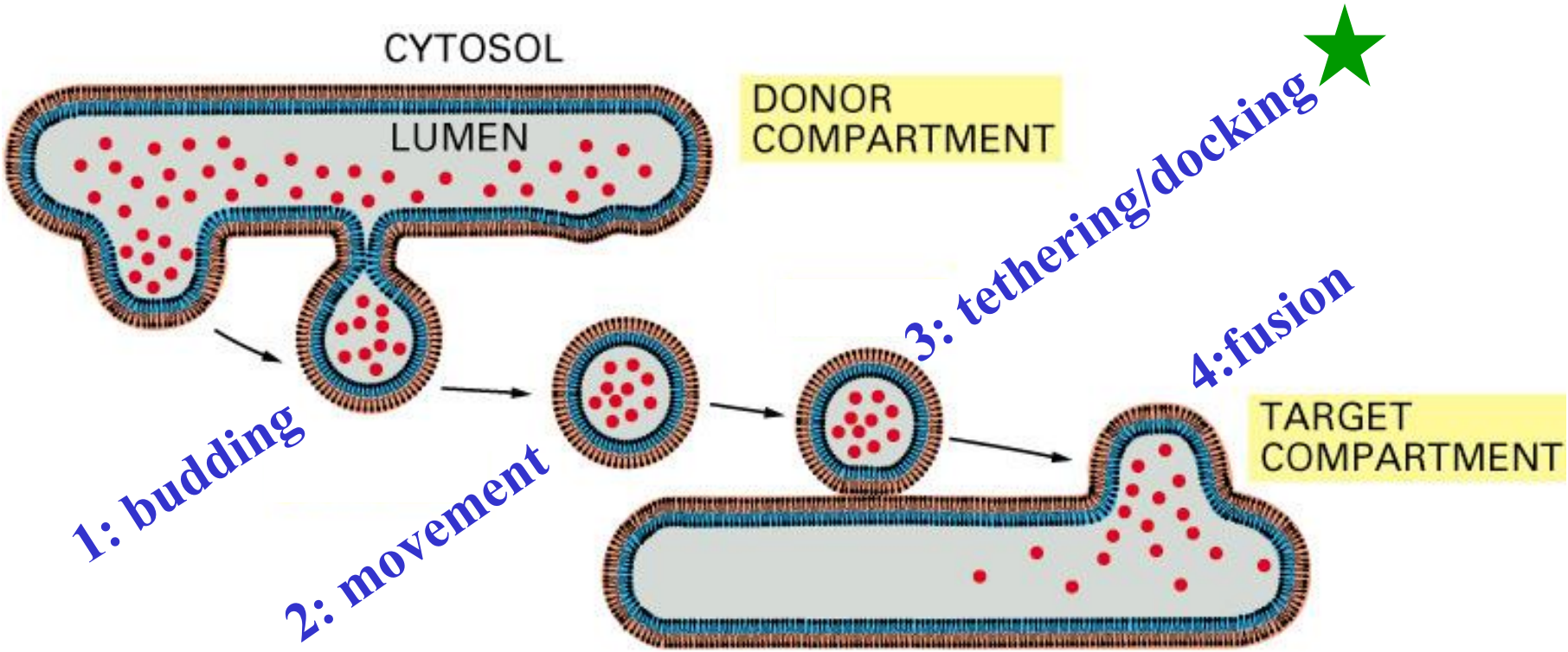
Budding from the donor compartment

Movement along the cytoskeleton structure

Tethering & docking to the target compartment

Fusion to the target compartment

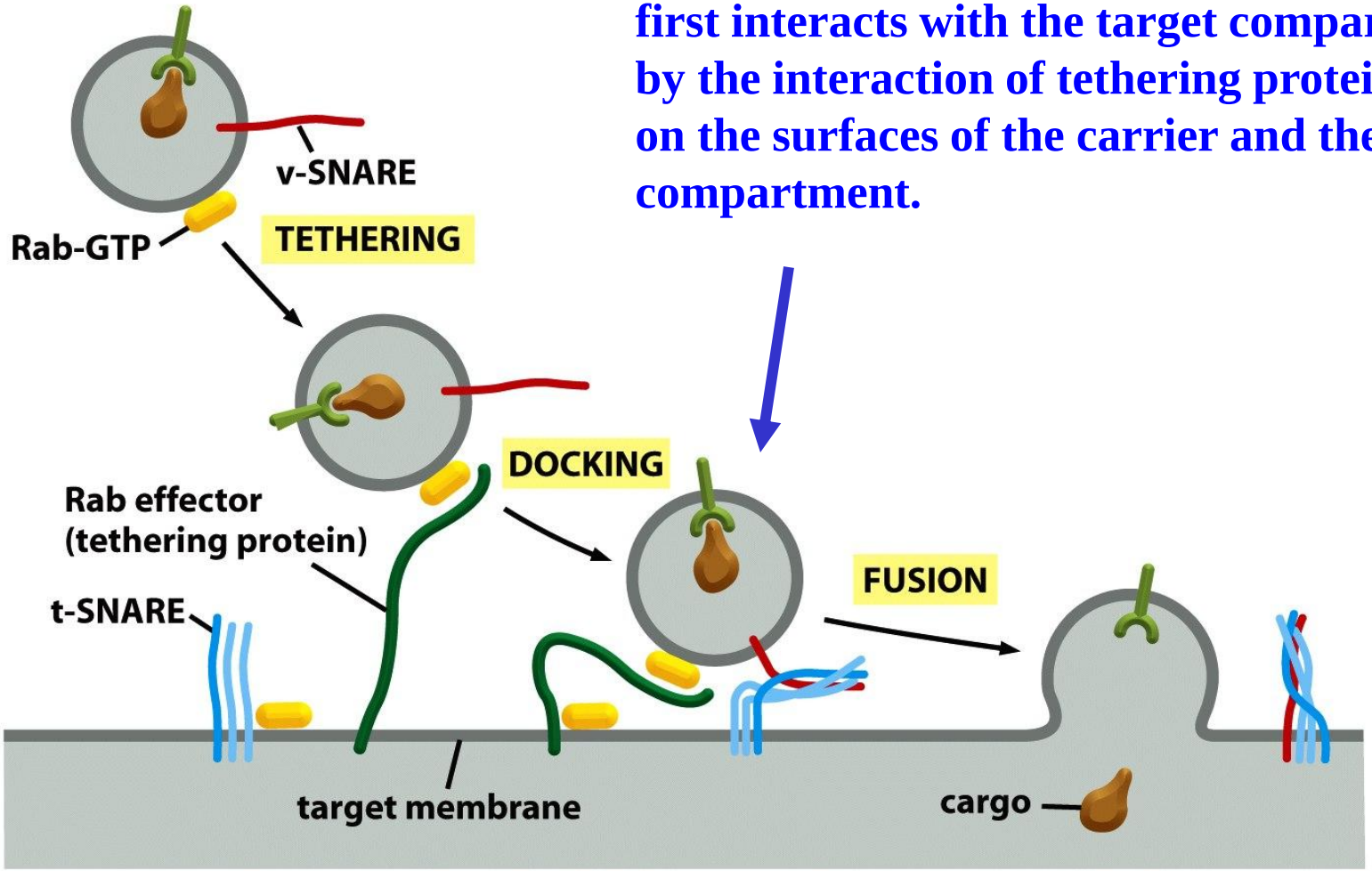
Tethering and Docking of Transport Carriers on Target Compartments



When the transport carrier moves near the target compartment, it tethers and docks to the target compartment to prepare for fusion.

Tethering and Docking

Tethering/docking: A transport vesicle/carrier first interacts with the target compartment by the interaction of tethering proteins found on the surfaces of the carrier and the target compartment.



Tethering Proteins: Rab GTPases and Rab Effectors

Rab is A GTPase found on the transport carrier, the target membrane, or both.

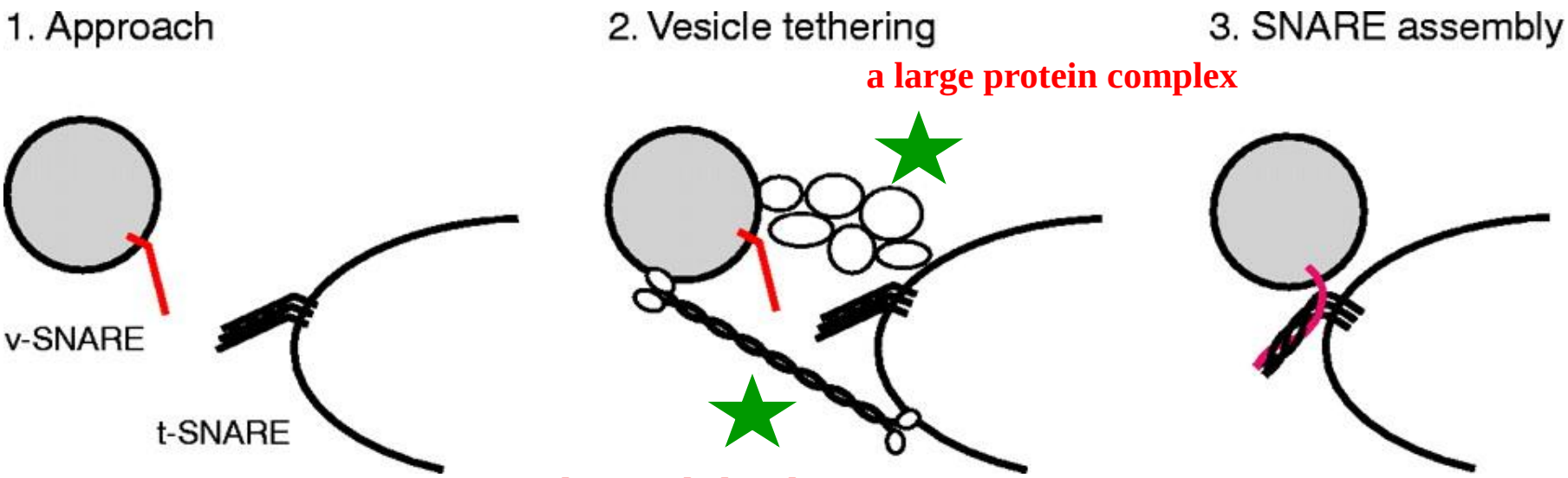
The GTP-bound form of Rab promotes the tethering and docking of transport carriers to their target membrane by recruiting other tethering factors.

By tethering and docking transport carriers to the target membrane, Rabs facilitate the formation of the *trans*-SNARE complex and thus membrane fusion (to be discussed later).

How Does an Activated Rab Protein Promote Tethering/Docking?

Two common types of Rab effectors mediate the interaction between carriers and the target membrane during tethering/docking.

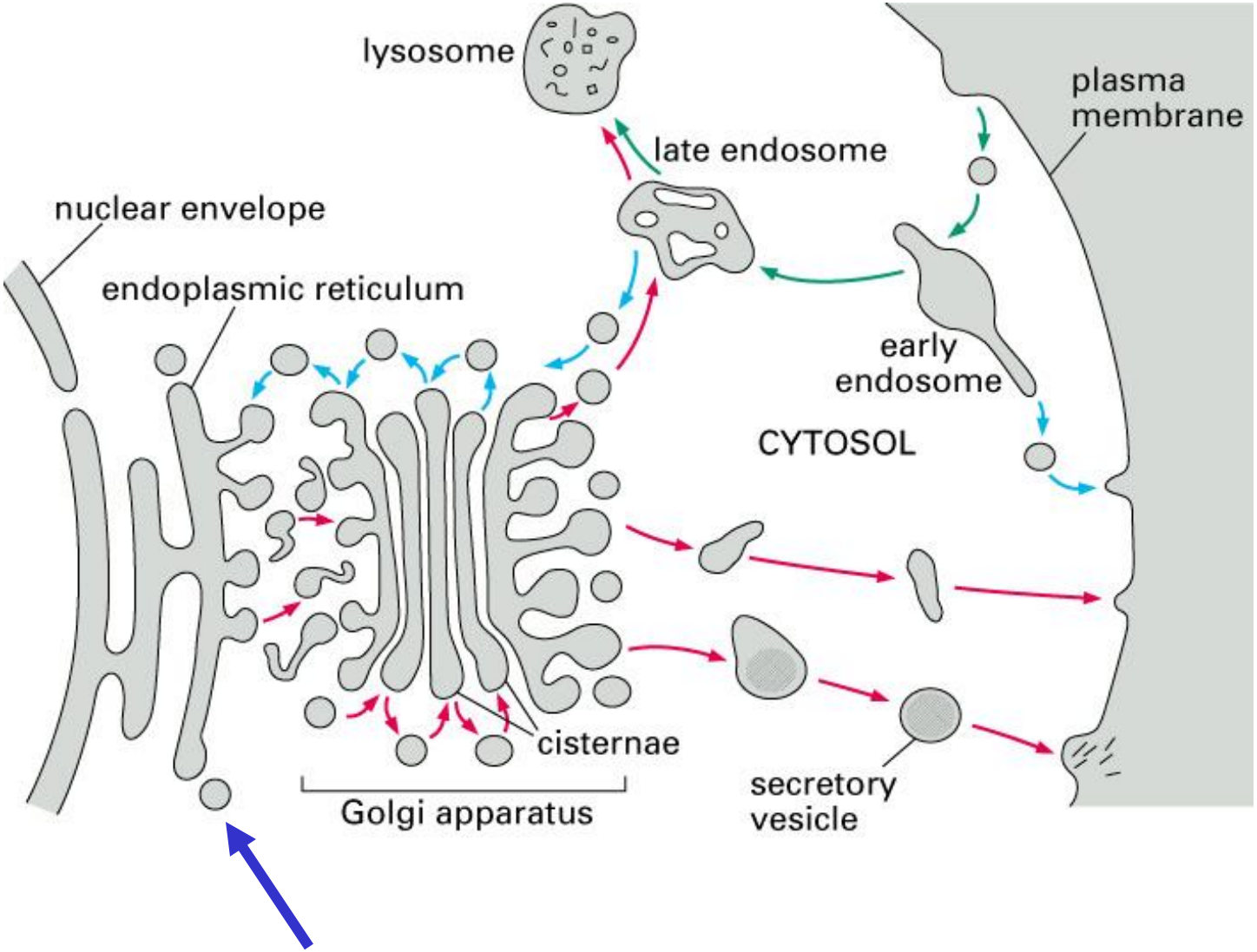
(tethering/docking)



a large protein complex

a long coiled-coil protein

Specificity of Membrane Tethering/Docking



Which compartment am I going to fuse with?

How do Rab proteins confer the specificity of membrane tethering and docking?

Tethering proteins: Rab GTPases and Rab effectors

Rab is A GTPase found on the transport carrier, the target membrane, or both

Its GTP active form promotes the tethering and docking of transport carriers to their target membrane by recruiting other tethering factors

By tethering and docking transport carriers to the target membrane, Rabs facilitate the formation of the *trans*-SNARE complex and thus the membrane fusion (to be discussed later)

Since different Rab proteins reside at different locations and each Rab recruits specific effectors, the interaction between a Rab and its effectors increases the specificity of membrane tethering/docking

How to Recycle a Rab Protein…

Note the similarities and the differences between Sar1 and Rab

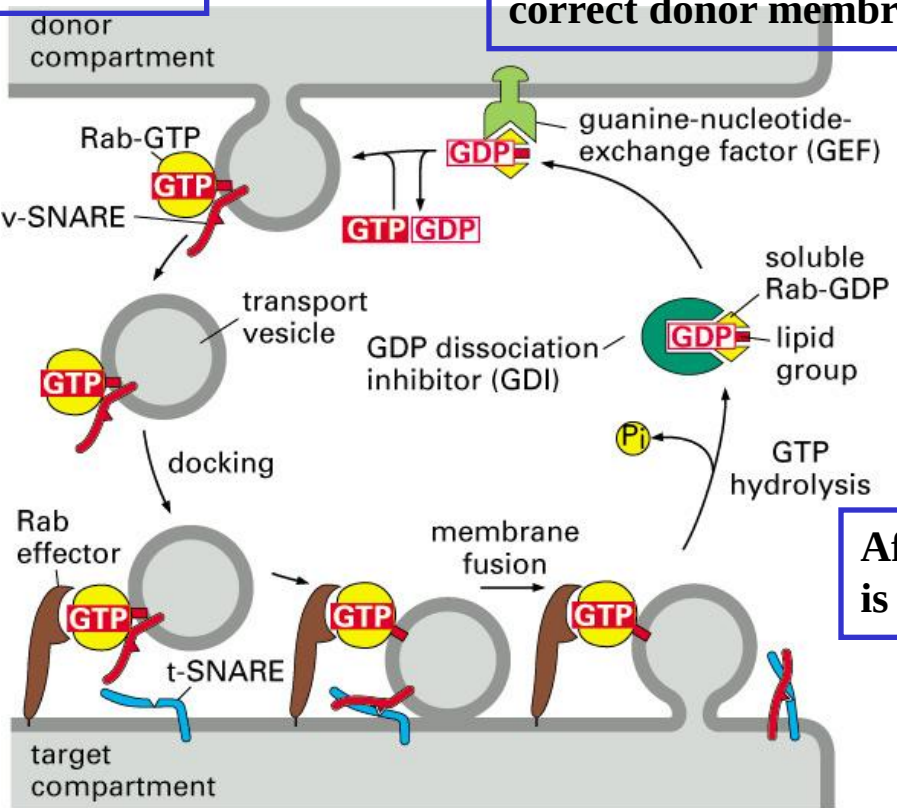
the lipid group is exposed to tether the RabGTP to the donor membrane tightly

to provide the compartment specificity of Rab

GTP binding causes a conformational change of Rab

A GEF on the donor membrane converts RabGDP to RabGTP (this only occurs on the correct donor membrane)

RabGTP and v-SNARE are packed into the transport carrier



Since the lipid-group of RabGDP is hidden, RabGDP recycles to the cytosol via the help of RabGDI

to prepare the vesicle docking & fusion

After the fusion, RabGTP is hydrolyzed to RabGDP.

RabGTP on the transport carrier interacts with its effectors on the target membrane.

to tether and dock the transport carrier, and to increase the specificity of fusion

The Rab-mediated docking allows the v-SNARE to bind to its cognate t-SNARE

to facilitate the fusion process

Rab Proteins and Their Effectors Have Multiple Functions Besides Tethering / Docking

Tethering/docking of transport carriers

Membrane fusion

Formation of transport carriers

Movement of transport carriers

There is one Sar1, six Arfs, and more than 60 Rabs!

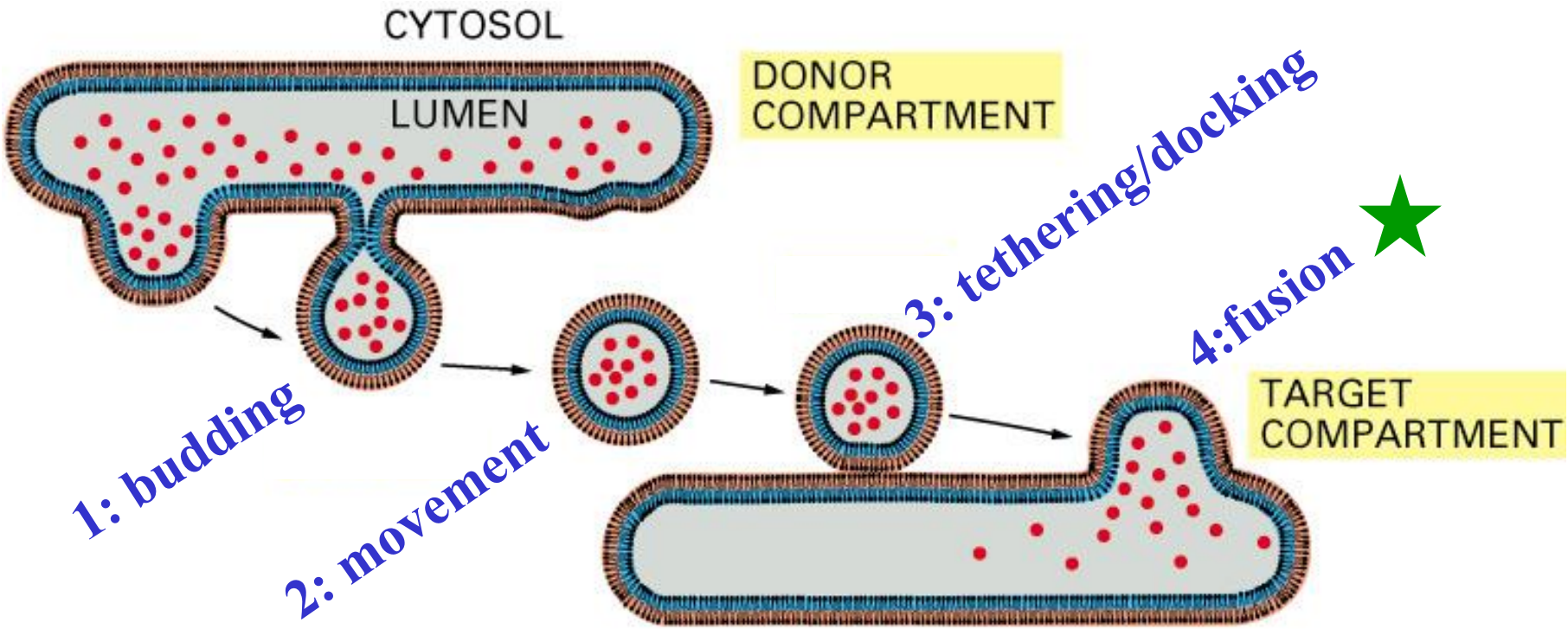
Budding from the donor compartment

Movement along the cytoskeleton structure

Tethering & docking to the target compartment

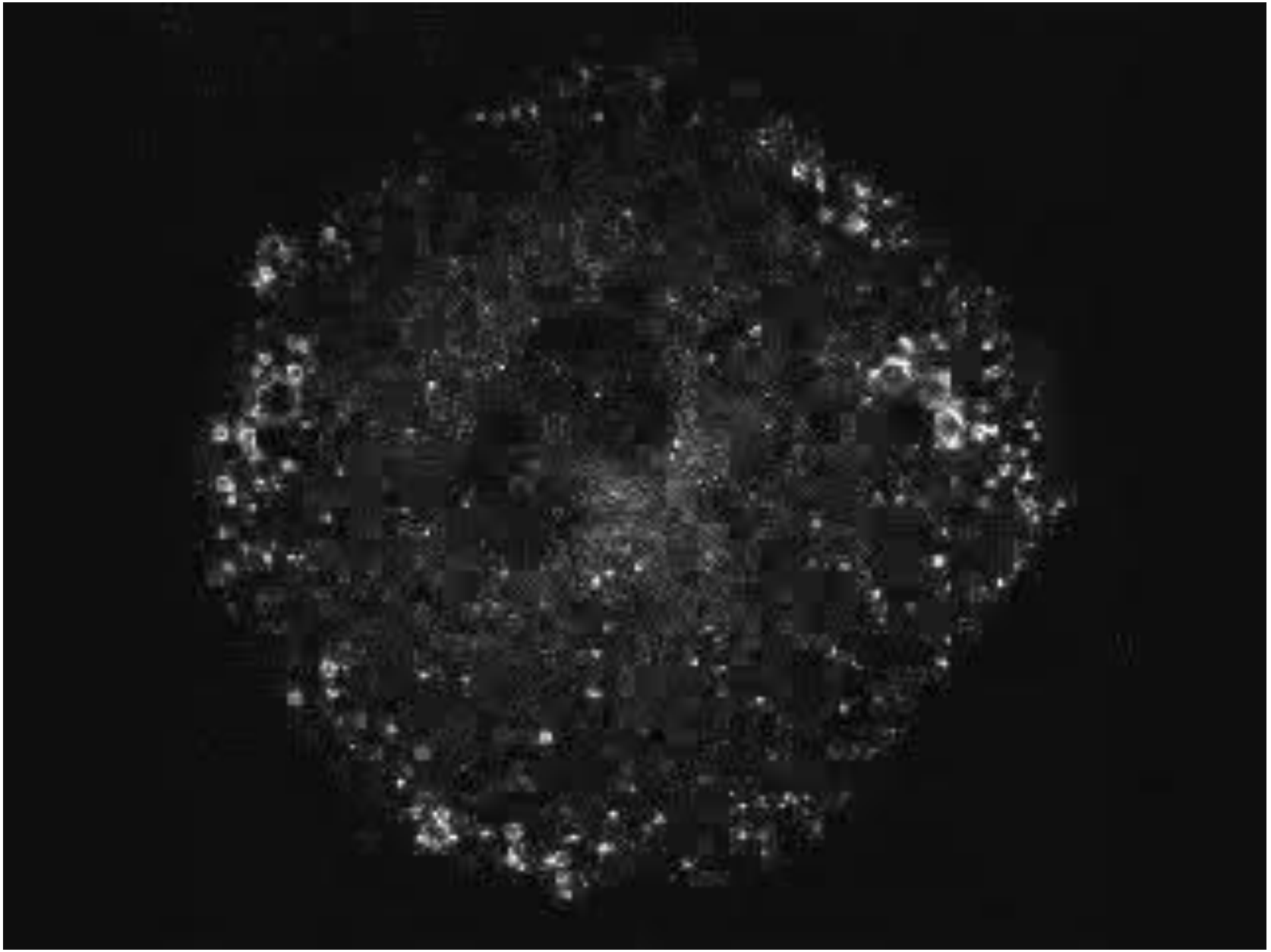
Fusion to the target compartment

Fusion of Transport Carriers with the Target Compartment

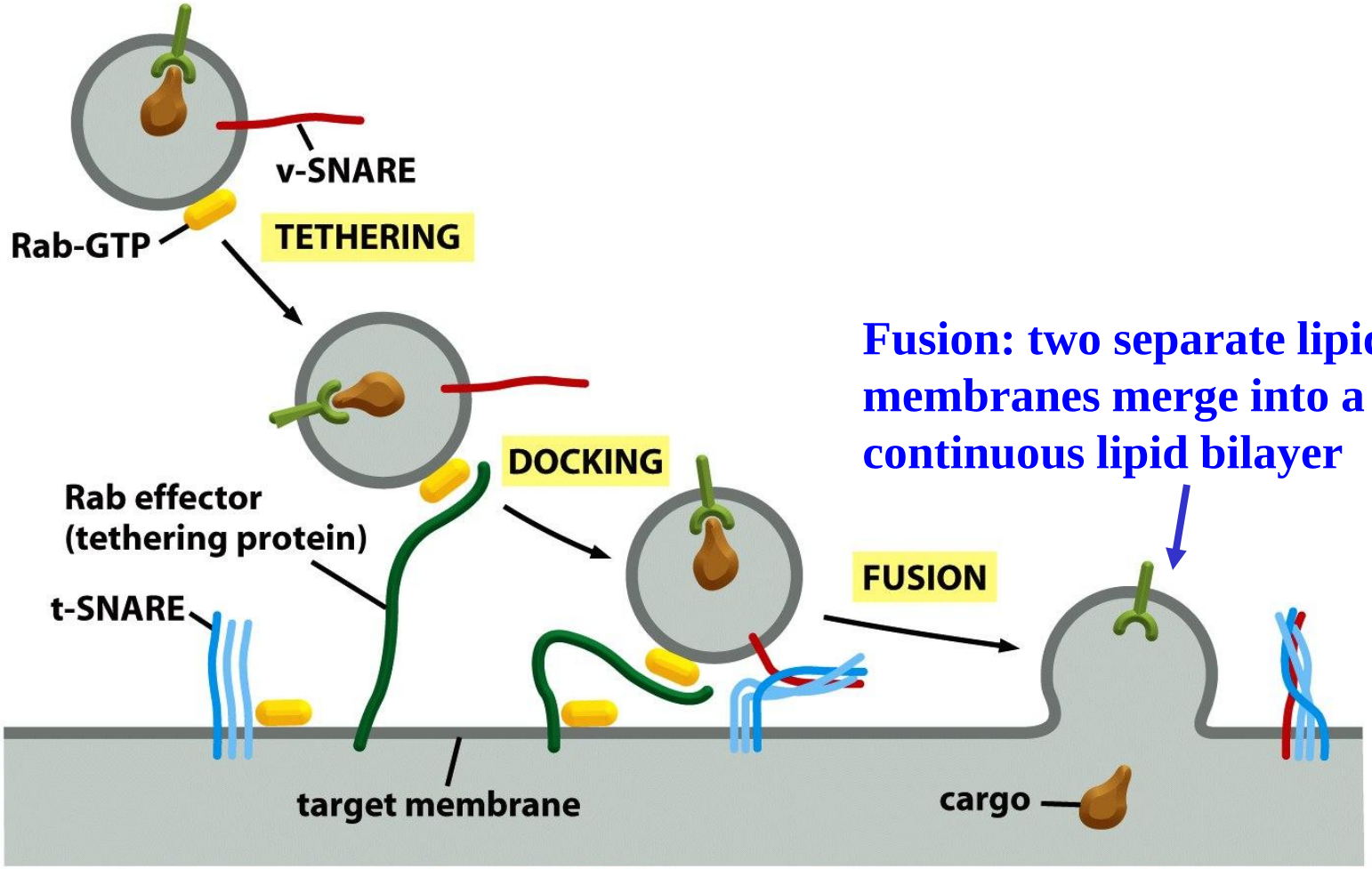


After fusion, the cargo molecules are now inside the target compartment

Vesicle Fusion movie (endosome example)



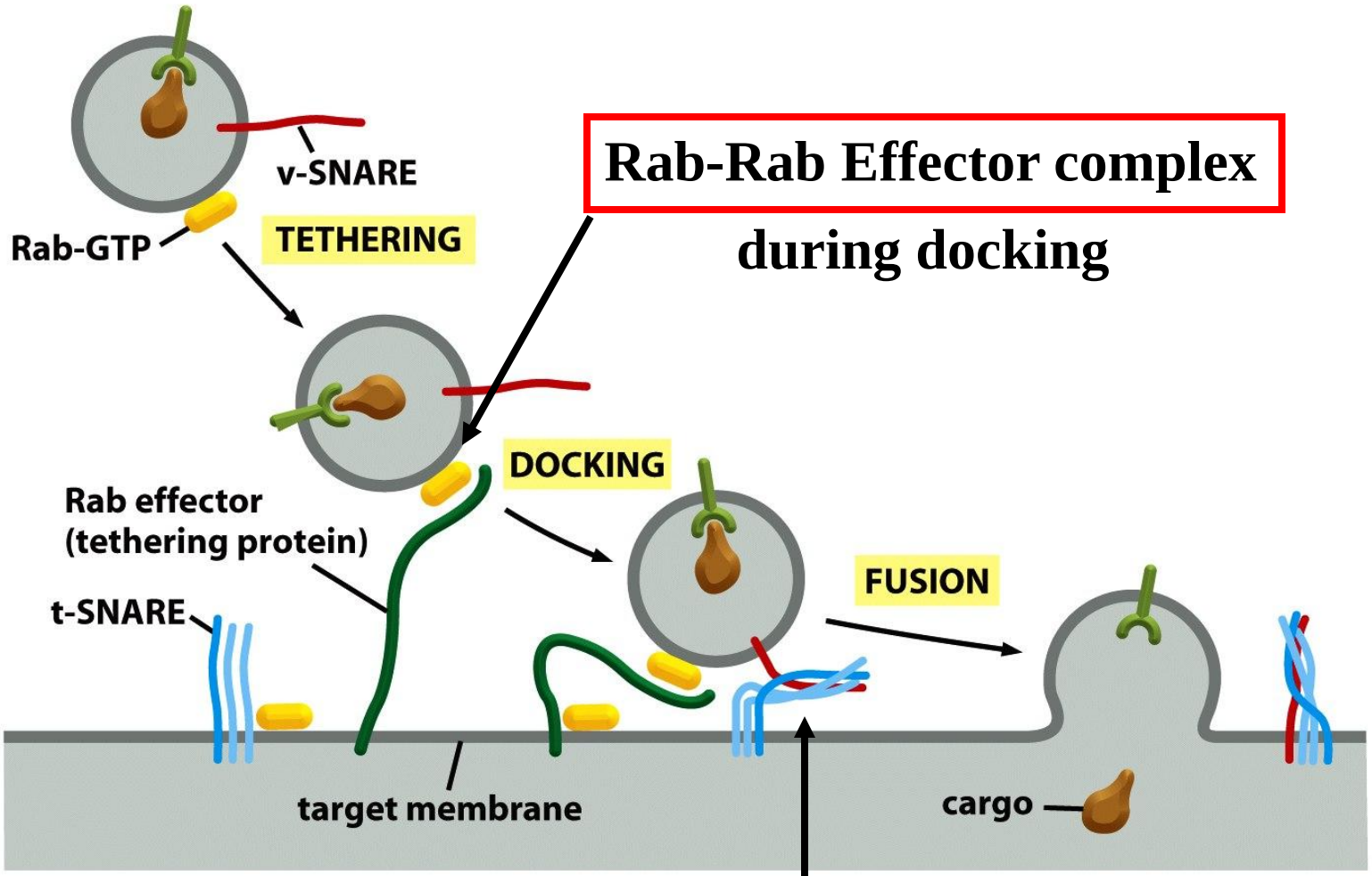
Carrier/Vesicle Fusion



Fusion: two separate lipid membranes merge into a single continuous lipid bilayer

How is fusion mediated? (SNARE proteins)

Specificity of Membrane Tethering/Docking and Fusion



Both contribute to the specificity of membrane transport.

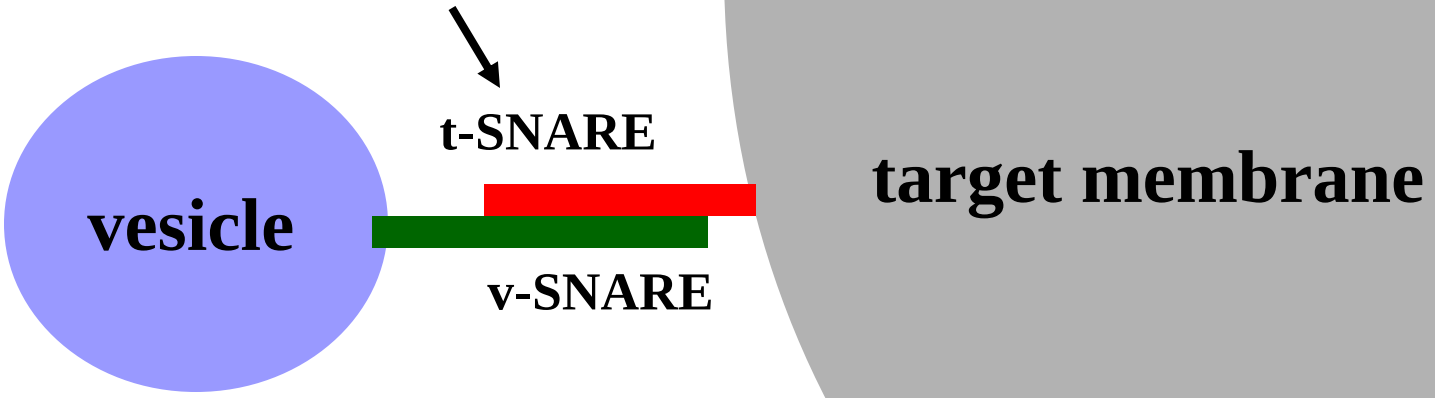
SNARE proteins are integral or lipid-anchored membrane proteins with the bulk of the protein in the cytoplasm.

SNARE proteins display compartment-specific localization.

Two types of SNARE proteins:
v-SNARE (associated with vesicles, packed into vesicle during the budding)
t-SNARE (associated with the target membrane)

The cytoplasmic domain of each SNARE protein contains a SNARE motif.
The SNARE motifs of paired v- and t-SNAREs form a *trans*-SNARE complex to promote membrane fusion.

a *trans*-SNARE complex (consists of 4 SNARE motifs)
(i.e. complex formed via v- and t-SNAREs at different membranes)
For simplicity, only one v- and one t-SNARE are shown below.



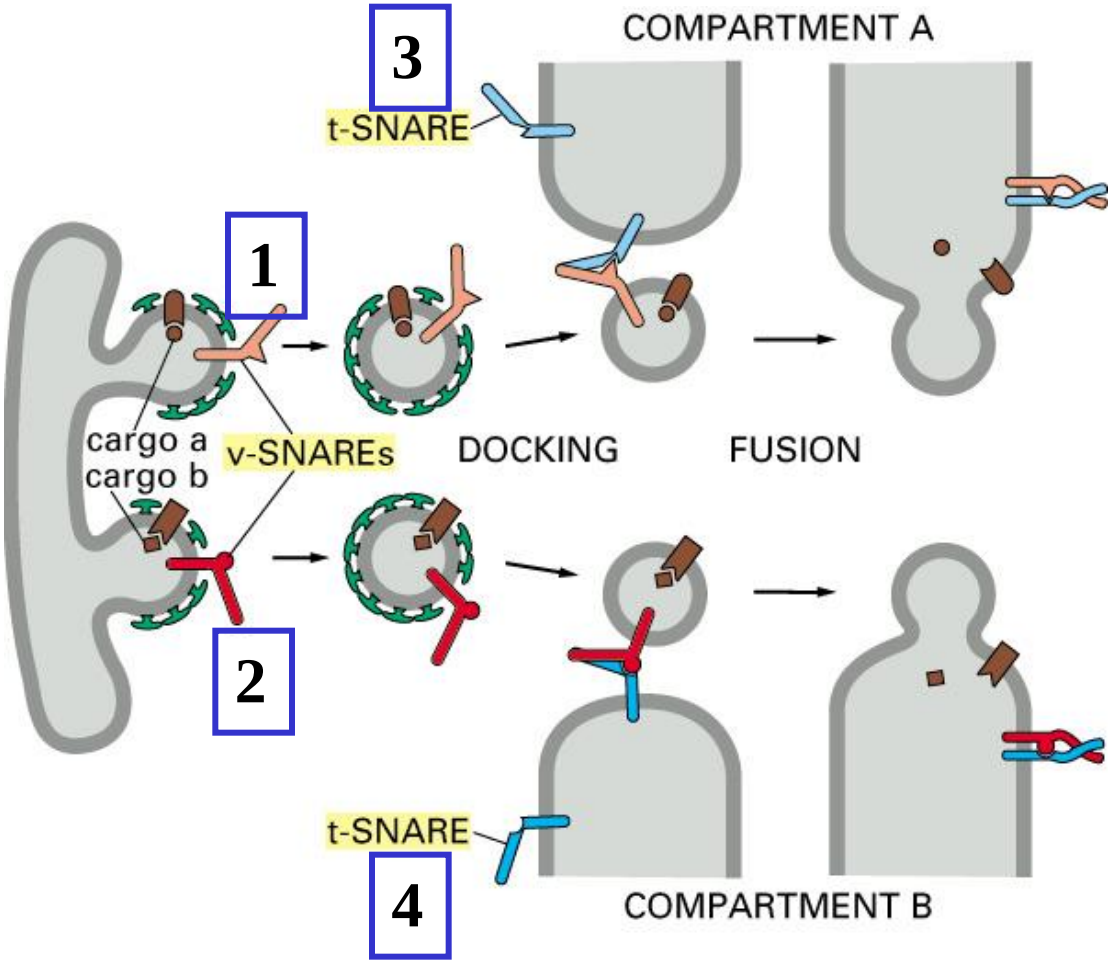
Essential Roles of SNARE Proteins in Membrane Fusion

to ensure specificity of membrane fusion: e.g. to make sure that COPII carriers only fuse with the Golgi compartment
(both Rab and SNARE proteins control the transport specificity)

to catalyze the fusion of transport carriers with the target membrane

How Do *trans*-SNARE Complexes Provide Fusion Specificity?

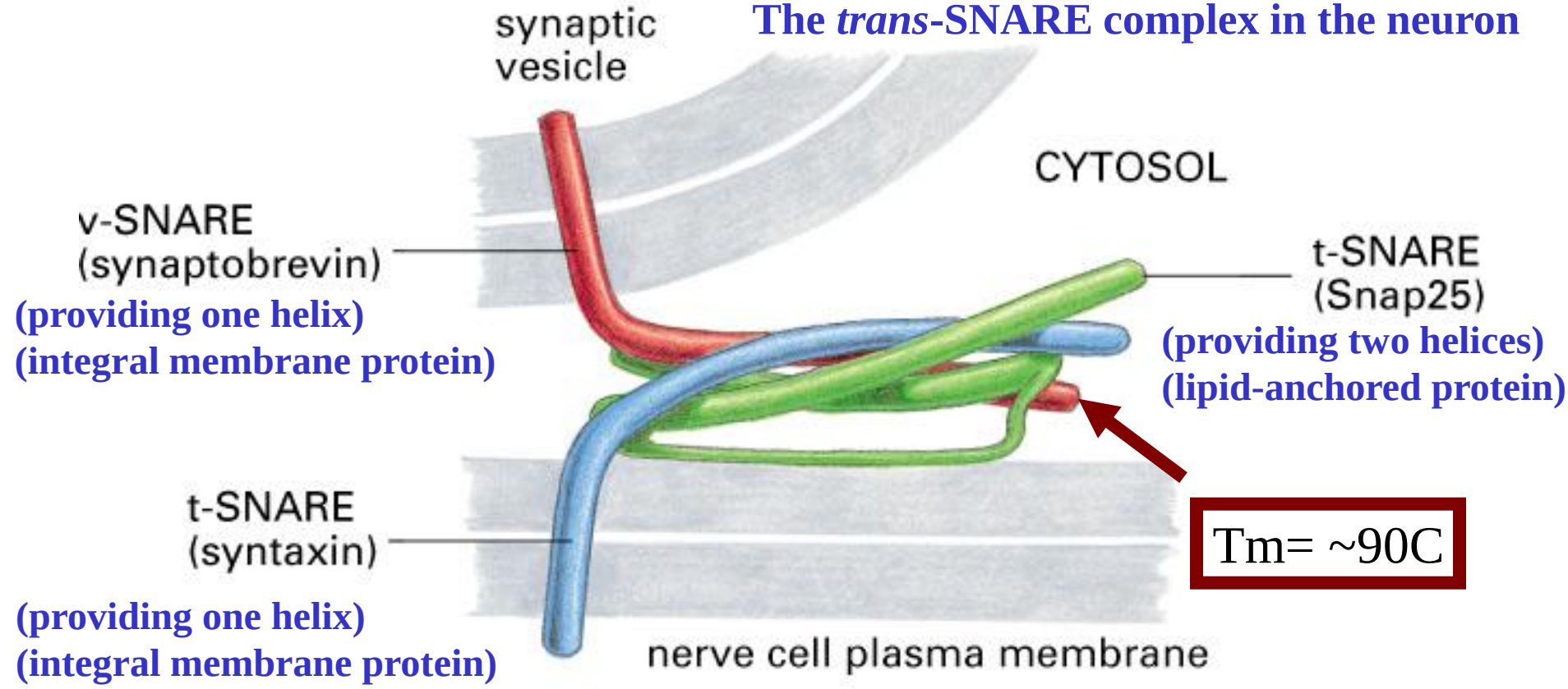
1. **Only complementary v- and t-SNARE proteins can pair to form a complex in vivo. For example (1, 3) or (2,4), but not (1, 4) or (2,3)**
2. **SNARE proteins display compartment-specific localization. Thus, v-SNARE 1 is packed into the carrier destined to compartment A, where t-SNARE 3 resides.**



How Does the Formation of a *trans*-SNARE Complex Drive Fusion?

A *trans*-SNARE complex is a four-helix bundle (4 SNARE motifs) composed of 1 v-SNARE and 2-3 t-SNAREs.

The *trans*-SNARE complex in the neuron

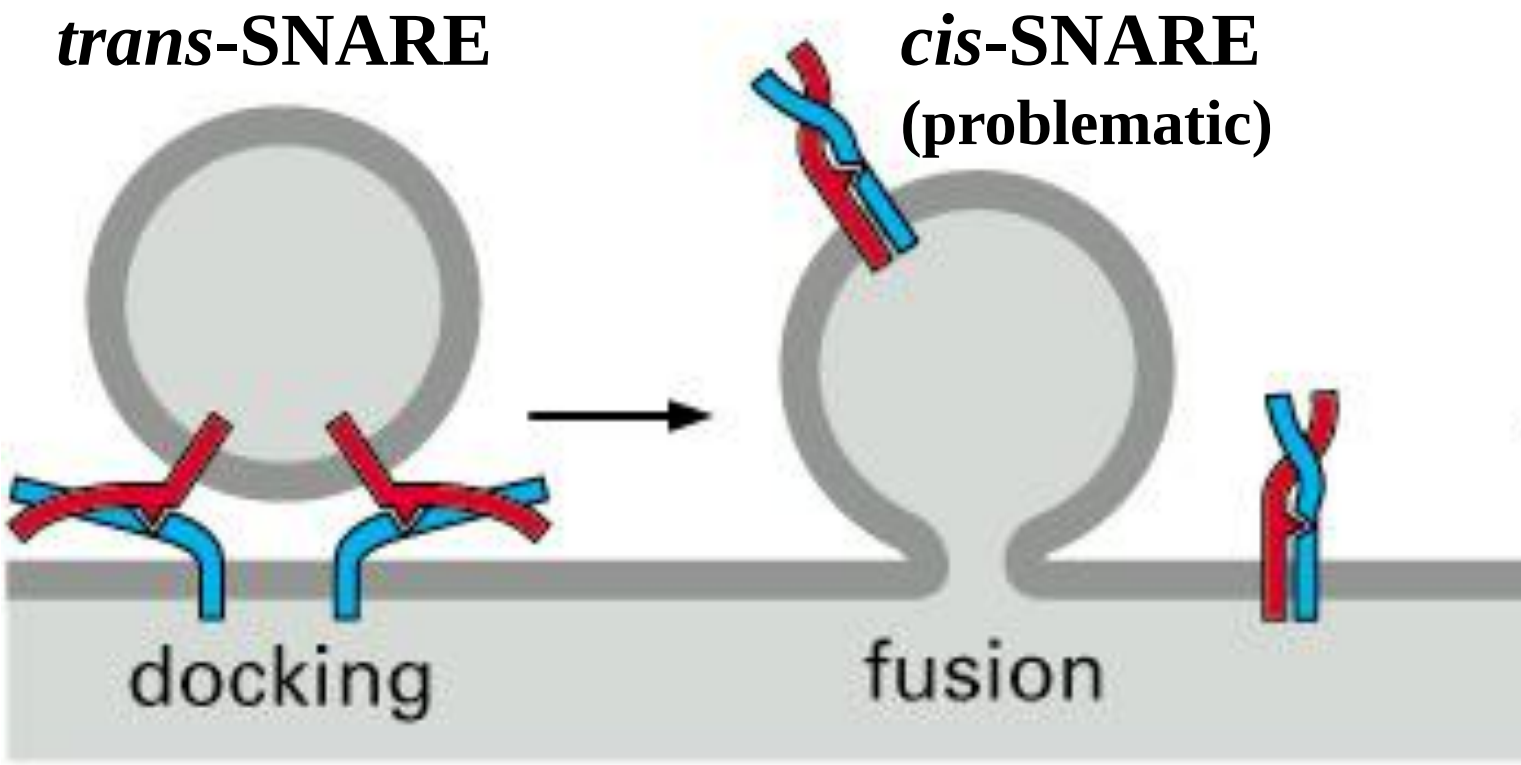


***trans*-SNARE is a highly stable complex** → **Energy can be used to promote membrane fusion**

How to Recycle SNAREs?

After fusion, the *trans*-SNARE complex becomes a *cis*-SNARE complex, which in principle is also very stable

For simplicity, only one v- and one t-SNARE are shown

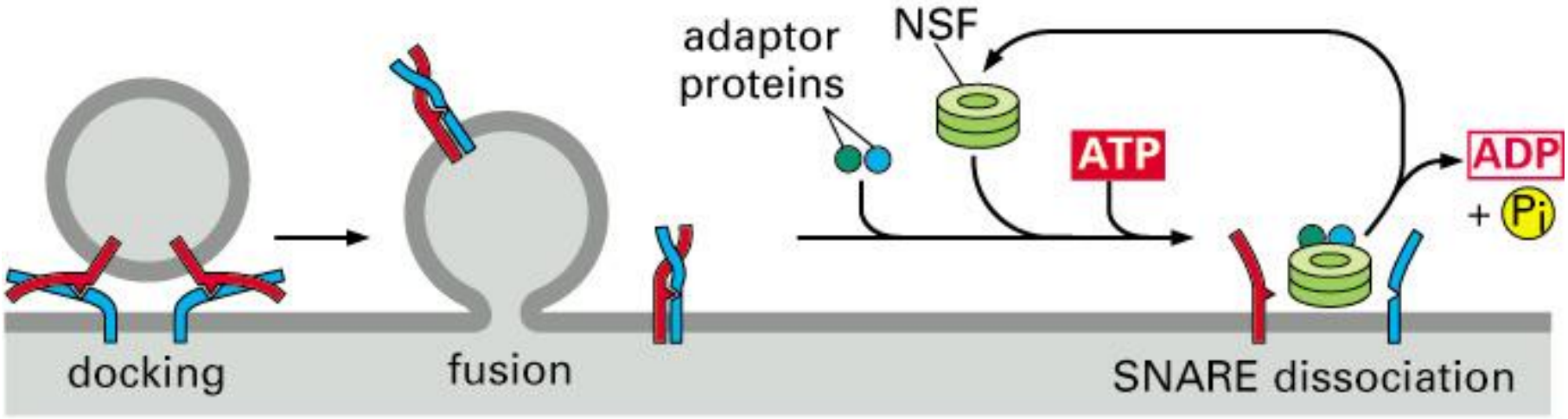


If not taken care of, these v- and t-SNARE proteins can only be used once

NSF Utilizes ATP to Pry Apart the *cis*-SNARE Complex

NSF: N-ethylmaleimide-sensitive fusion protein

After fusion, NSF binds to the *cis*-SNARE complex via adaptor proteins, and hydrolyzes ATP, thus using energy, to separate the SNARE proteins.



SNAREs are recycled after each round of fusion
(recycling is a general principle in cell biology, e.g. Ran, SRP, Sar1, ….)

The Essential Roles of Small GTPases on Membrane Trafficking