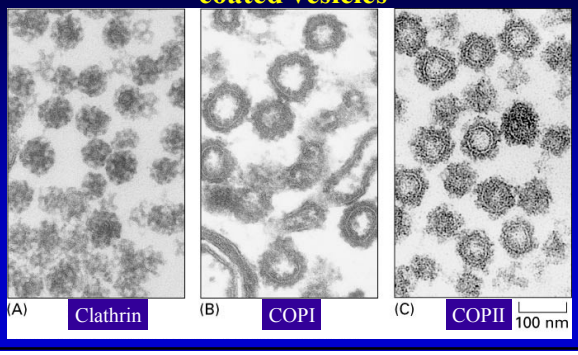
The birth of coat proteins

The COP coat and trafficking in the ER-Golgi system

- Early stages of the secretory pathway
- Exiting the endoplasmic reticulum: COPII assembly
- Cycling back to the endoplasmic reticulum: COPI assembly

Three most characterized coated vesicles: clathrin, COPI and COPII-coated vesicles



Early stages of the secretory pathway

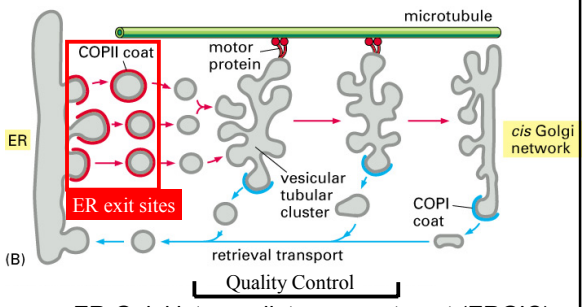
The ER-cis Golgi system

The ER is an organelle that coordinates the synthesis, folding, export and degradation of nascent protein cargo

Exit from the endoplasmic reticulum to the Golgi

GFP VSV-G
40 C - ER
32 C - PM

QuickTime™ and a Sorenson Video 3 decompressor are needed to see this picture.



ER-Golgi intermediate compartment (ERGIC) or transitional endoplasmic reticulum

Which proteins exit the ER and which stay there?

Proteins that EXIT:

- Membrane proteins destined to other membranous organelles, e.g., the Golgi the plasma membrane, and secreted proteins.
- The ultimate goal of the ER is to insure that only mature, functional cargo is directed to downstream compartments, or the cell surfac.

Which proteins exit the ER and which stay there?

Proteins that STAY:

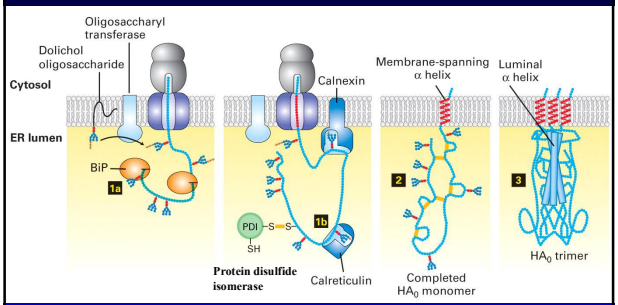
Chaperones, e.g., Bip calnexin, controlling protein translocation and folding

Enzymes (PDI) catalyze thiol-disulfide interchange.

Heat shock proteins, that use ATP binding and hydrolysis to promote folding

Lectins (sugar-binding proteins) that monitor the folding of newly-borned glycoproteins.

ER proteins involved in folding and assembly of membrane proteins



How ER localization of membrane and soluble proteins is determined?

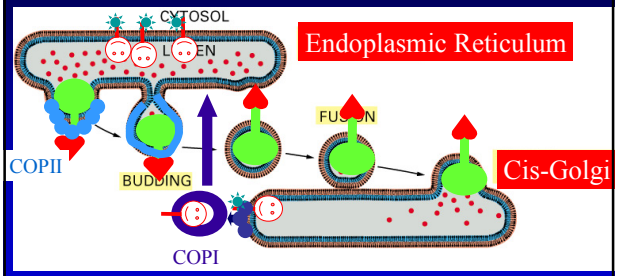
The action of prevention of passive loss of ER resident proteins by retention and the active selection of cargo exit is mediated by

COPI and COPII coat proteins

Exiting the endoplasmic reticulum:

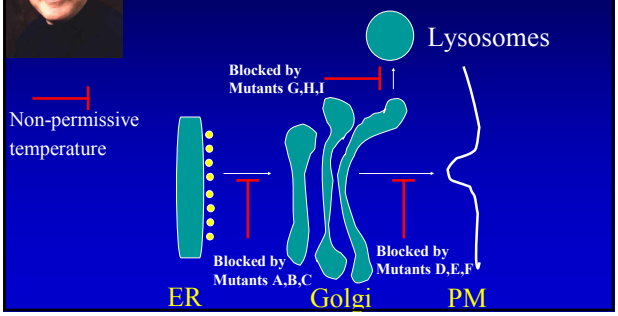
COPII coat assembly

Sorting in the endoplasmic reticulum - cis-Golgi systems

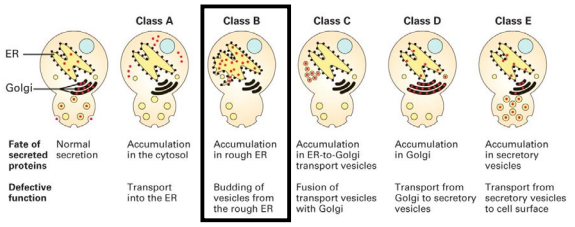


The genetic approach for studying vesicular transport

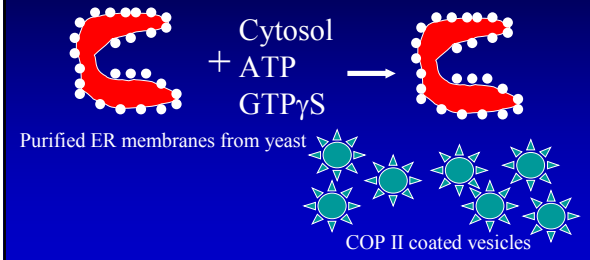
Randy Schekman at Berkeley University discovered the yeast temperature sensitive *sec* mutants



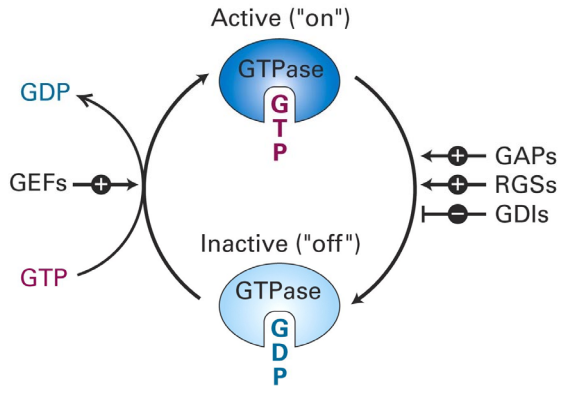
Yeast cells with mutations in genes encoding for COPII proteins are class B *sec* mutants



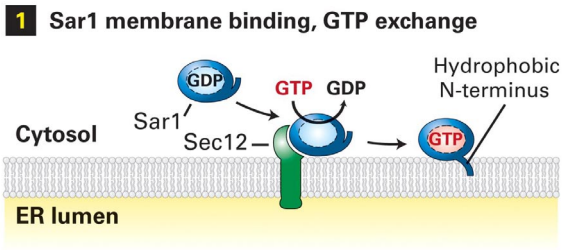
COPIIcoated vesicles can be produced in vitro in a cell-free system

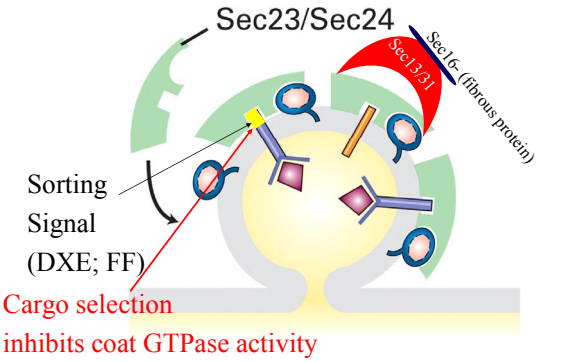


Biochemical analysis revealed coat protein composition

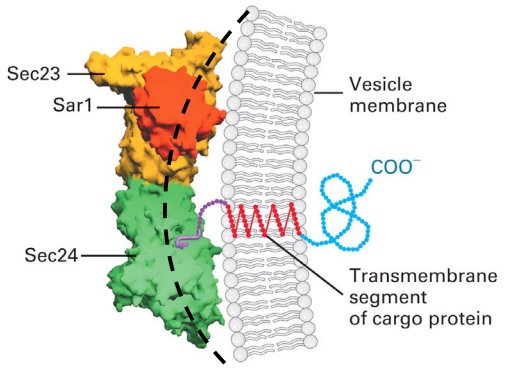


COP II assembly is initiated by the recruitment Of a small GTPase - Sar1

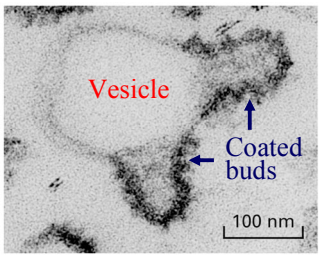




Sec 23/24 structure induces membrane bending

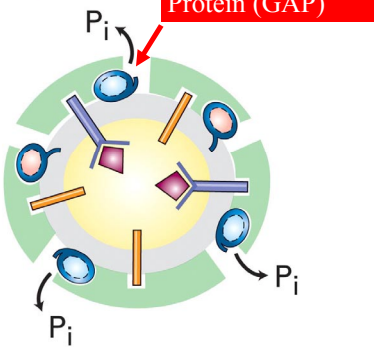


Coat assembly curves membrane surfaces *in vitro*

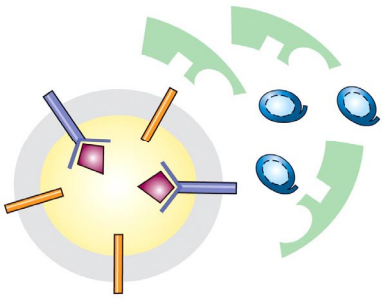


Phospholipid vesicles + purified COPII = highly curved coated buds

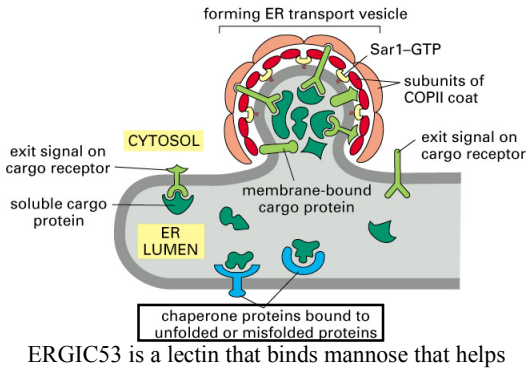
3 GTP hydrolysis



4 Coat disassembly



Uncoated vesicle



ERGIC53 is a lectin that binds mannose that helps to pack several secreted proteins, including Factor V and Factor VIII proteins

Cell. 1998 Apr 3;93(1):61-70. Related Articles, Links

Mutations in the ER-Golgi intermediate compartment protein ERGIC-53 cause combined deficiency of coagulation factors V and VIII.

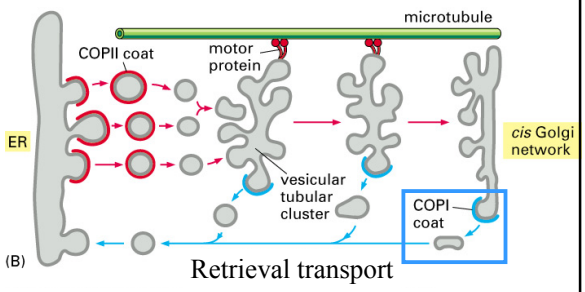
Nichols WC, Seligsohn U, Zivelin A, Terry VH, Hertel CE, Wheatley MA, Moussalli MJ, Hauri HP, Ciavarella N, Kaufman RJ, Ginsburg D.

Department of Internal Medicine, University of Michigan, Ann Arbor 48109-0650, USA.

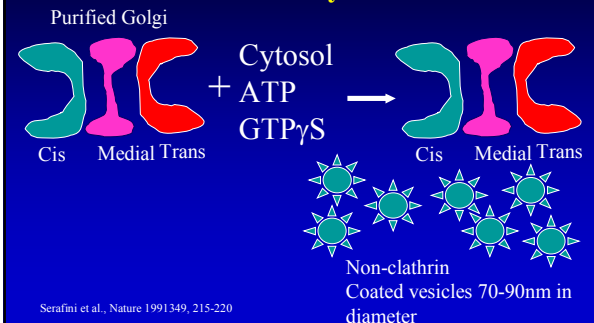
Combined deficiency of factors V and VIII is an autosomal recessive bleeding disorder resulting from alterations in an unknown gene on chromosome 18q, distinct from the factor V and factor VIII genes. ERGIC-53, a component of the ER-Golgi intermediate compartment, was mapped to a YAC and BAC contig containing the critical region for the combined factors V and VIII deficiency gene. DNA sequence analysis identified two different mutations, accounting for all affected individuals in nine families studied. Immunofluorescence and Western analysis of immortalized lymphocytes from patients homozygous for either of the two mutations demonstrate complete lack of expression of the mutated gene in these cells. These findings suggest that ERGIC-53 may function as a molecular chaperone for the transport from ER to Golgi of a specific subset of secreted proteins, including coagulation factors V and VIII.

Cycling back to the ER:

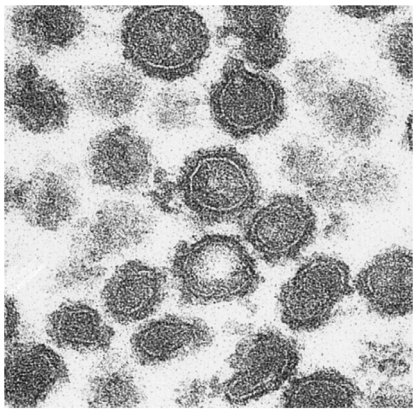
COPI (coatomer) coat assembly



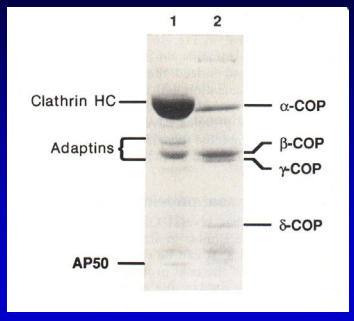
Golgi-derived non-clathrin coated vesicles can be produced in vitro in a cell-free system



Serafini et al., Nature 1991349, 215-220



SDS-PAGE analysis of COP I vesicles

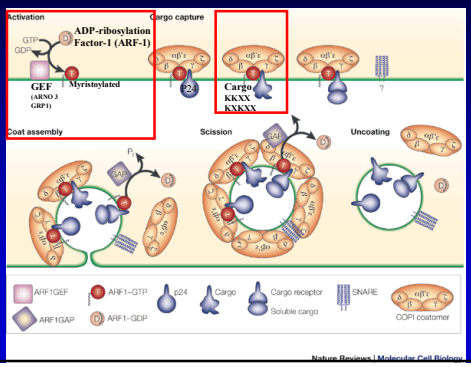


Seven COP coat components : α, β, ε, δ, β' γ, and Arf-1f

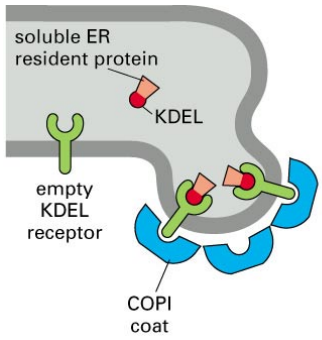
COPI recruitment

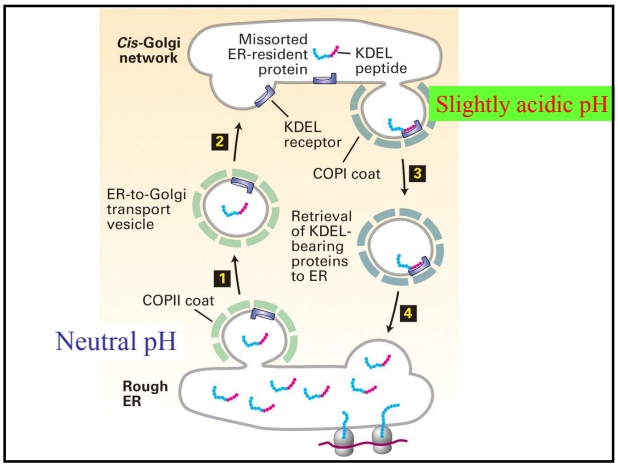
- Mediated by ARF1.
- The GEF of ARF-1 is an important factor that determines the site of coat assembly
- Prompted by weak association with head-groups of phosphatidic acid, and phosphoinositides
- Prompted by interactions with the cytoplasmic tails of cargo proteins recruited into the bud

Key steps in the formation of COPI-coated vesicles



How ER luminal (soluble) proteins are located at the ER? - the KDEL signal





SUMMARY

- Small GTPases belonging to the ras superfamily initiate coat assembly.
- Phosphoinositide-based domains on the cytoplasmic surface mediate initial steps in coat assembly
- Sorting signal in cargo proteins stabilize the assembly process, resulting in selectivity in cargo entry into the coated bud.
- GAP activity causes coat dissociation

?

- How the GEFs of ARF1 or Sar1 recognize the correct membrane compartment?
- What is the biochemical nature of the assembly microdomain (What factors regulate coat assembly)?
- What prompts the GAP activity?