

## Vesicular traffic ER to Golgi and Beyond



## Secretory pathway – way to the surface



- Synthesis of proteins and lipids with translocation to ER or insertion into ER membranes
- Modifications
  - Proteins – co-translational and post-translational in rough ER and Golgi
  - Lipids – mostly posttranslational glycosylation
- Delivery to destination place
  - Outside
  - Cell membrane or membranes of the organelles

## Concerns about delivery



- Has to be specific for an organelle
  - Different proteins have different destinations
- Has to preserve identity of the organelle
  - Has to be free of components “leaking” from ER or Golgi

## Steps in vesicular transport

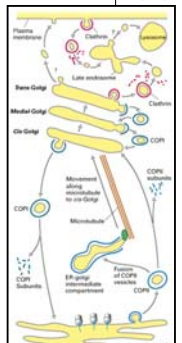


- Cargo selection – “what goes where”
  - Recruitment and sorting
- Container formation
  - Vesicle formation and pinching
  - Addressing
- Targeting (docking) and fusion with the destination compartment
  - Delivery to the proper address

## Destinations Remember about recycling too



- ER → Golgi
- Golgi → surface (exocytosis)
  - Constitutive
  - Regulated
- Golgi → lysosomes
- Golgi → endosomes → lysosomes
- Golgi back to ER
- Within Golgi
- Surface → endosomes → lysosomes (endocytosis)



## Major players



- Cargo selection
  - Recruitment signals
  - Sorting motifs
- Container - vesicles
  - Address - coat proteins specific for different destinations
- Small GTPases - energy
  - Regulate timing and fidelity
- Docking complex

## Cargo selection

- Budding vesicle contains only proteins destined for transport to particular destination
- That means that resident components of organelle of origin (ER or Golgi) as well as proteins going to different destinations have to be excluded !!!



## Cargo selection

- Recruitment signals on cargo proteins
  - "To go or not to go"
  - Short peptide sequences in protein sequence
  - Only proteins that have recruitment signals will go, the rest will stay in the ER or Golgi (because both ER and Golgi have their own proteins such as modification enzymes and they do not want to lose them)



## Cargo selection

- Sorting motifs
  - Where to go
  - Diacidic motif linked to tyrosine – for transport from ER to Golgi

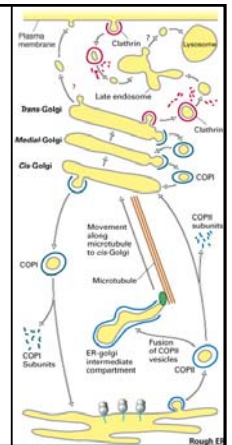
Aspartic acid – X- glutamic acid linked to tyrosine  
Tyrosine does not have to be near

VSV-G	TM-18aa-YTD <sup>+</sup> EWRLGK
CFTR (NBD1)	TM-212aa-YD <sup>+</sup> YLLD-287aaTM
GLUT4	TM-26aa-YLGFPE <sup>+</sup> D
LDLR (prox. Yxxx)	TM-172aa-YGKTT <sup>+</sup> EE <sup>+</sup> NCHN-219aa
CI-M6PR	TM-26aa-YKAV <sup>+</sup> EE <sup>+</sup> DEEN-42aa
E-cadherin	TM-85aa-YISLL <sup>+</sup> EE <sup>+</sup> SDS-43aa
EGFR	TM-85aa-YKLL <sup>+</sup> EE <sup>+</sup> VDF-87aa
ASGPR H1	NTK <sup>+</sup> YD <sup>+</sup> GL <sup>+</sup> CH <sup>+</sup> EE <sup>+</sup> ES-26aaTM
NGFR	TM-45aa-YISL <sup>+</sup> PFK <sup>+</sup> EE <sup>+</sup> LLNG-7aa
THR	15aa-YT <sup>+</sup> SLA <sup>+</sup> CD <sup>+</sup> EE <sup>+</sup> DTY-26aaTM



## Coat proteins

- 3 types of "coat" = destination
  - COPII – from rough ER to Golgi
  - Clathrin – from Golgi to the surface via endosome and from surface to endosome → lysosome
  - COPI – recycling in Golgi and from Golgi back to ER



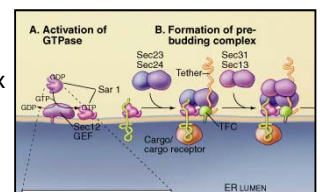
## Vesicle formation and budding

- ER to Golgi
  - Mediated by COPII
  - Catalyzed by GTPase Sar1



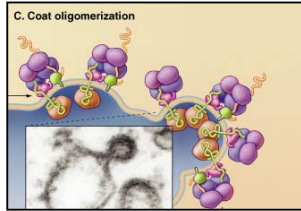
## Coat assembly and budding

- Activation of GTPase
- Active Sar1 recruits cargo through recognition of sorting motifs
- Formation of prebudding complex



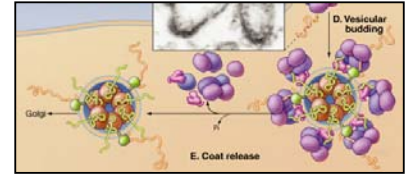
## Coat assembly and budding

- Addition vesicular ID (where to go because cargo is inside, sorting motifs no longer visible)
- Coat assembly
- Membrane invagination → bud formation



## Coat assembly and budding

- Vesicle pinching and release
- Coat depolymerization – vesicular ID (destination address exposed)
- Vesicle is moved to final destination and docked



## What has to be taken care of during delivery

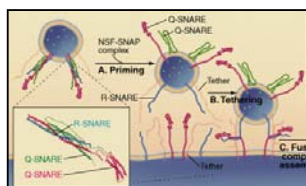
- Fidelity
  - Recognition signals both on vesicle and destination organelle
- Vesicular ID
  - Must be included during vesicle formation
- Homing device
  - Docking complex on the destination membrane

## Delivery of intracellular vesicles

- Similar for all vesicles regardless of coat
- Fusion occurs after depolymerization of the coats
- Two steps
  - Targeting to appropriate place
  - Fusion

## Vesicle targeting

- Depolymerization of coat uncovers vesicular ID = tethering factors and (vesicular) V-SNARE proteins on the vesicular membrane
- Targeting components have to be a part of a vesicle

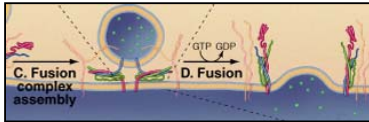


## Tethering factors

- Have long extended **coil-coiled domains**
- Bind to similar domains on docking complex
- In COPII mediated targeting
  - P115 on the vesicle
  - GM130/GRASP65 on Golgi

## Vesicle targeting

- V-SNARE targets the vesicle to its correct membrane fusion partner T-SNARE (target)
  - The specificity of the interaction ascertains proper targeting

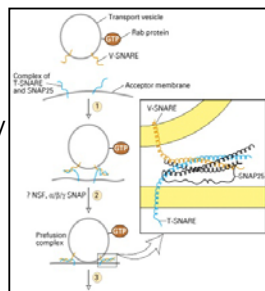


## Vesicle docking

- V and T SNARE work by interactions between arginines (R) and glutamines (Q)
  - Therefore they can be called R and Q SNARES
- Cis-SNARE pairing
  - 4 helix structure of coil-coiled domains

## Regulated fusion of vesicles with cell membrane

- Usually the signal for fusion is increased concentration of intracellular calcium
- **Synaptotagmin** is the key  $Ca^{2+}$  sensing protein



## Calcium dependence of fusion process

- $Ca^{2+}$  can come from outside of the cells through voltage dependent calcium channels or from intracellular calcium stores (in response to membrane depolarization in excitable cells)
- $Ca^{2+}$  ions activate synaptotagmin that starts fusion (exocytosis)
- Extra  $Ca^{2+}$  ions are rapidly removed from cytosol by  $Ca^{2+}$  ATPases (pumps)

## Fusion

- After docking the vesicle waits for signal to fuse with the target membrane
- Membranes merge
- Following fusion the SNARE complexes are disassembled

## Fusion

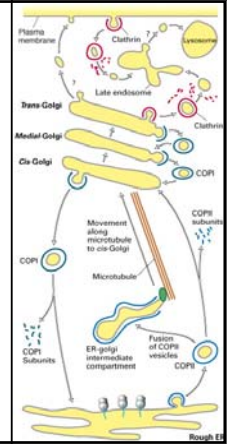
- If the protein was inside ER it is secreted (inside of ER becomes the outside of the cell)
- If the protein was inserted in the ER membrane it is now in the destination membrane
- The sidedness of the membrane is preserved through the entire process
  - Parts of protein that faced cytosol are still facing cytosol
  - Parts that faced inside of ER are now on the outside of the cell or facing inside of the organelle

## Progression from ER to Golgi



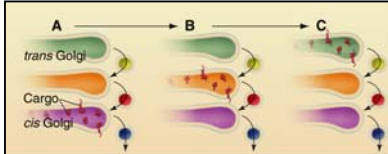
## Movement through Golgi stack

- Cis → medial → trans
- Cisternal progression (maturation) with subsequent recycling of Golgi enzymes



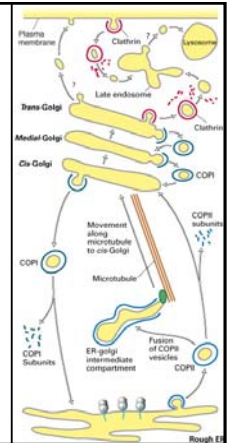
## Recycling within Golgi and back to ER

- Golgi enzymes are retained in Golgi by specific sequences localized within membrane domains and retrograde transport



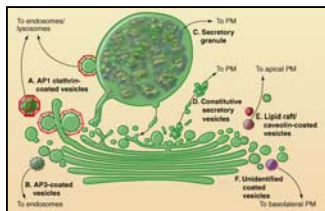
## Recycling within Golgi and back to ER

- COPI mediated
- Polymerize and depolymerize similar to COPII
- Incorporation into COPI coated vesicles is based on dilysine sorting motif
  - KKxxCOOH

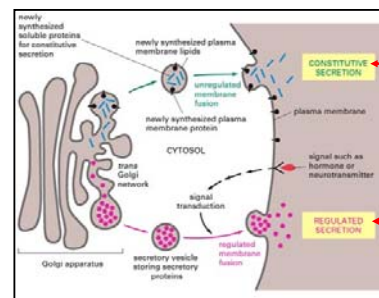


## Divergence of cargo at the trans-Golgi network

- Now from Golgi there are many choices...
- Sorting based on
  - Protein motifs
  - Physical properties such as aggregation
  - Geometric consideration



## Transport to plasma membrane - exocytosis



## Constitutive secretion

- “Unregulated”, constant
- Whatever was not retained goes to the surface
- No signal necessary
- No known coat proteins have been identified
- Membrane tubules rather than vesicles carry cargo to the surface



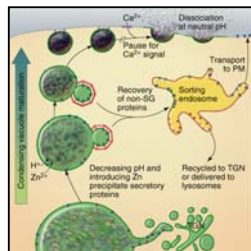
## Regulated secretion

- For proteins that are not needed all the time
  - Hormones
  - Transmitters
  - Cytokines



## Regulated secretion

- Sorting controlled by selective protein aggregation with selectogranins in trans-Golgi
- Vesicle waits for a signal to merge with the membrane



## Protein sorting in polarized cells

- Proteins are directly sorted to apical and basolateral surfaces of the polarized cells
- or
- Apical membrane proteins are endocytosed and moved across the cell (transcytosis)



## Transcytosis

