

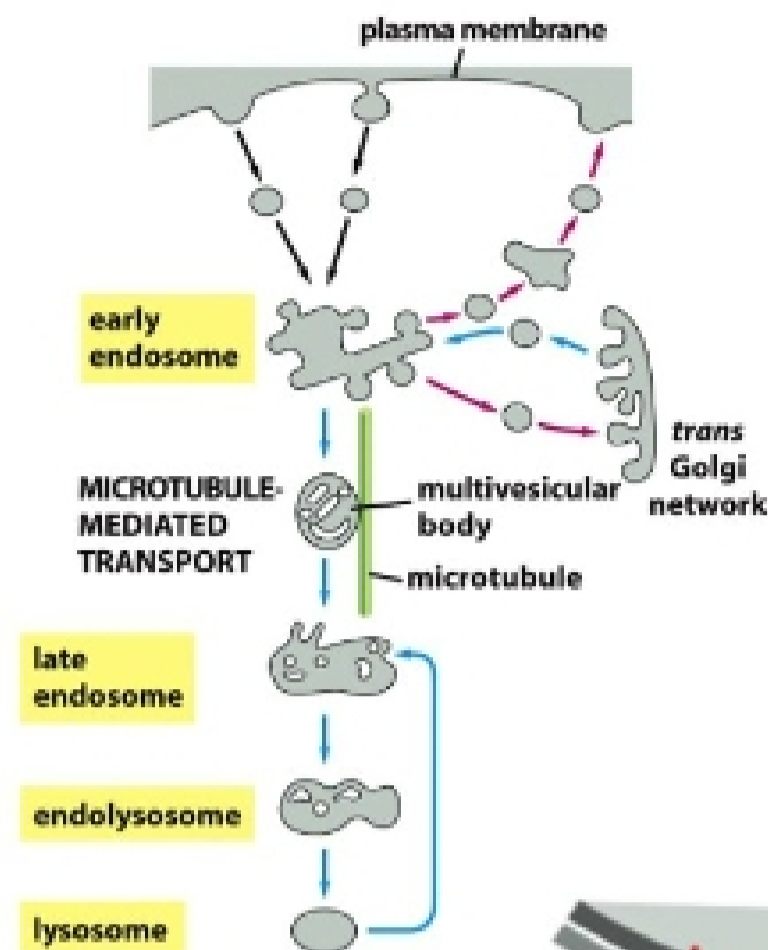
Lecture 10/31

Endosomes

- move through microtubules
- attach to target with tethering components
- don't move to cytoplasm through diffusion

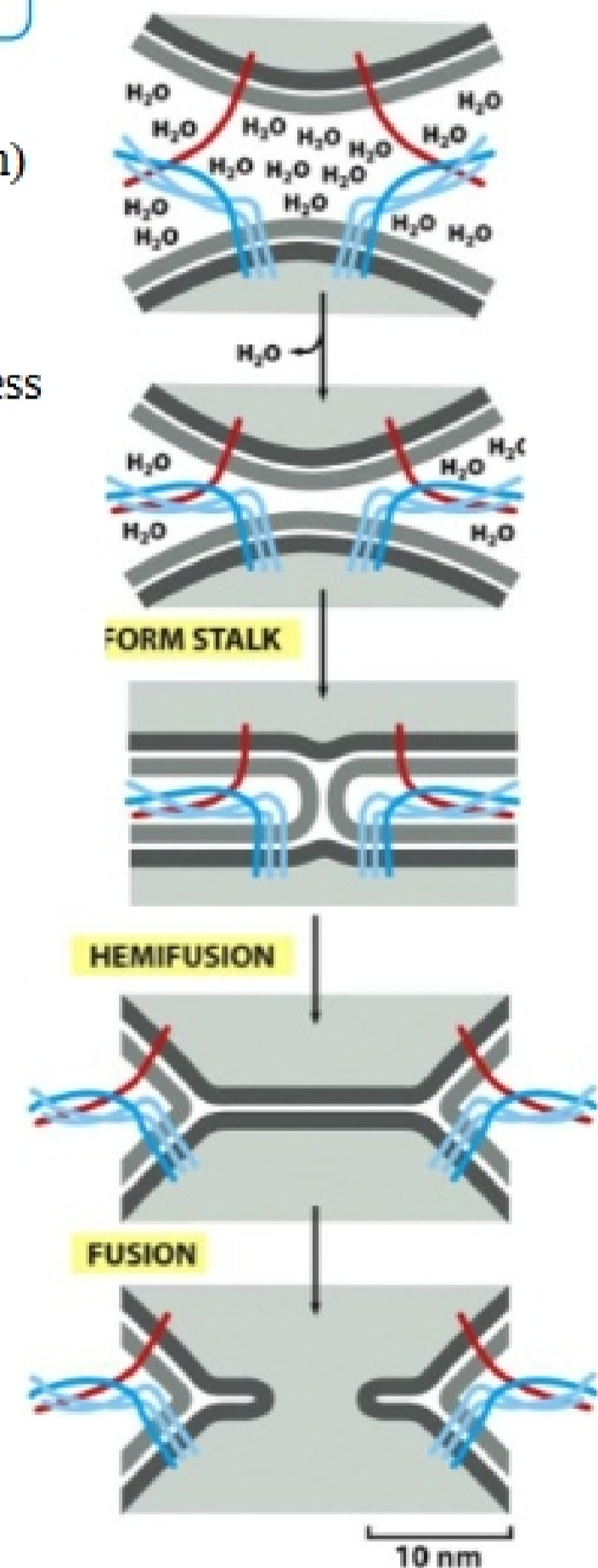
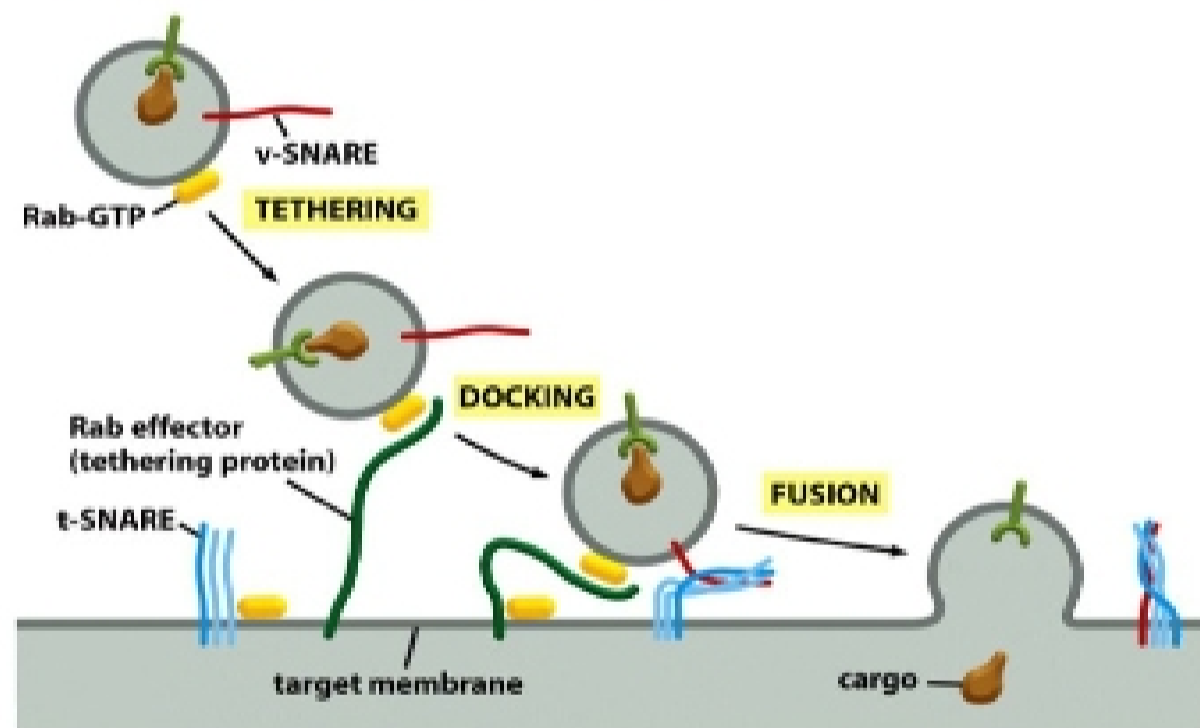
Tethering → docking → fusion

- endocytic vesicles to target membrane
- receptor mediated endocytosis into cytoplasm
- loses cost goes to early endosome

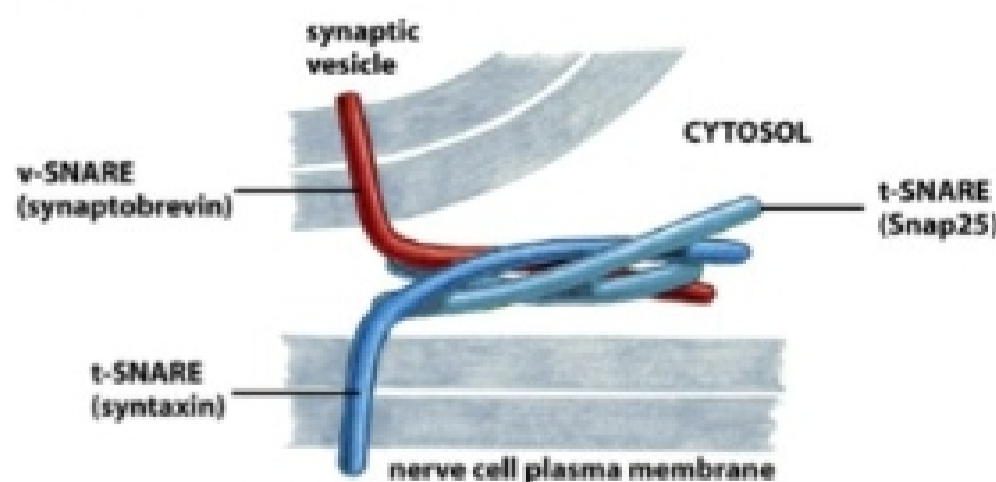


Process

- g protein changes conformation when GTP → GDP (Rab Protein)
- Rab-GTP binds to Rab effector filaments and protein tethers
- v-SNARE filaments proteins brought close enough to t-SNARE (1.5 nm) then twisting causes vesicle to merge with target
- v-SNARE and t-SNARE are spontaneous energy releasing process
- water molecules purged between membranes and they fuse
- Rab proteins and SNAREs specific to location
- Rab and SNARE recycle

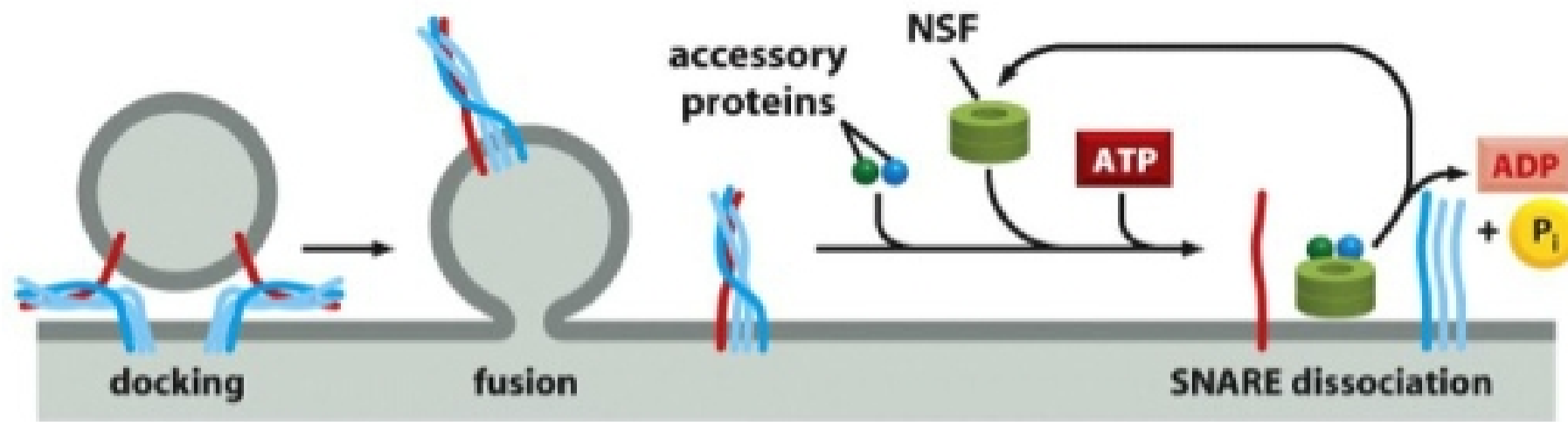


In neurotransmitter ...



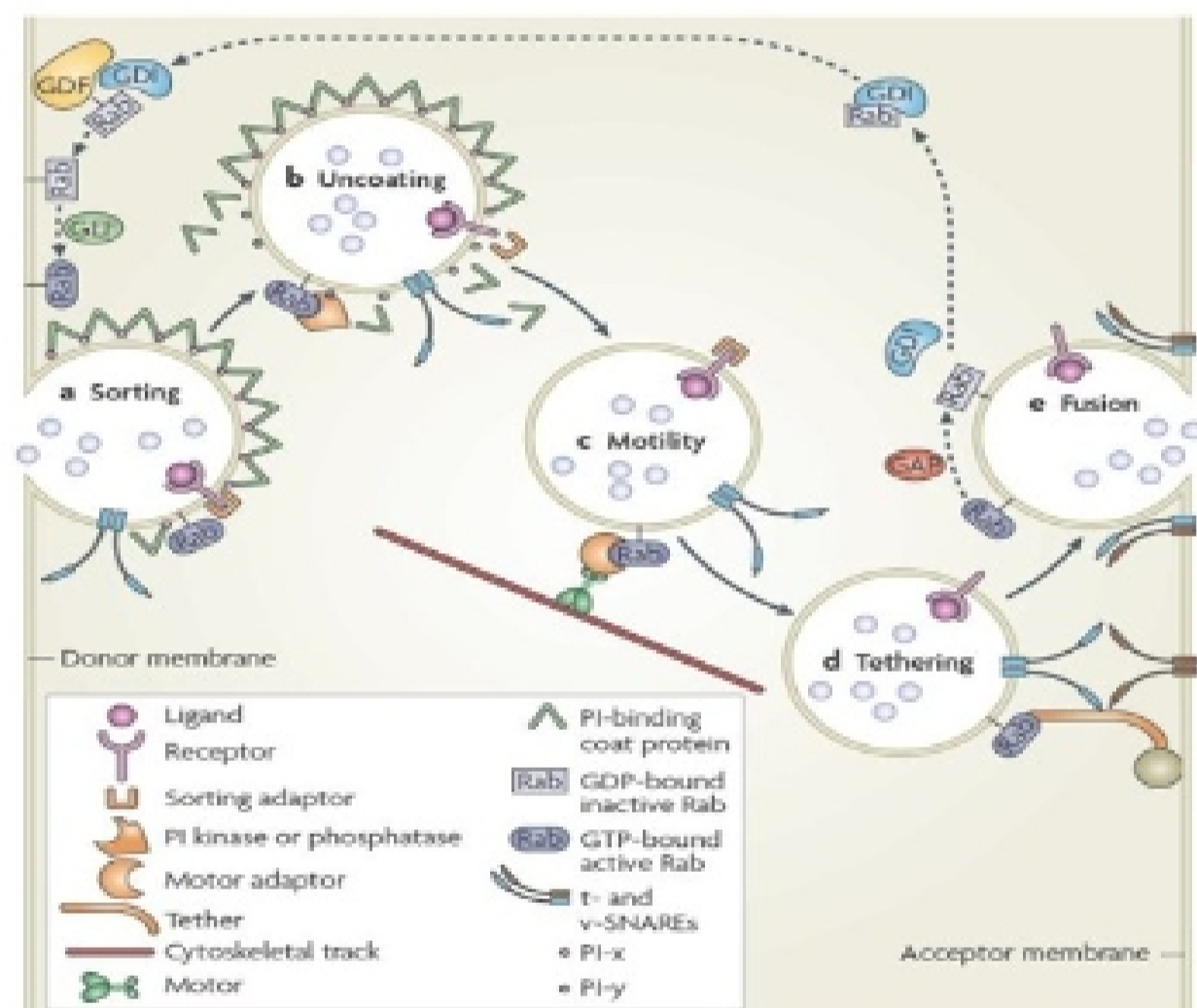
Docking, fusion, SNARE dissociation

- ATP hydrolysis separates v-SNAREs and t-SNAREs



Resolution

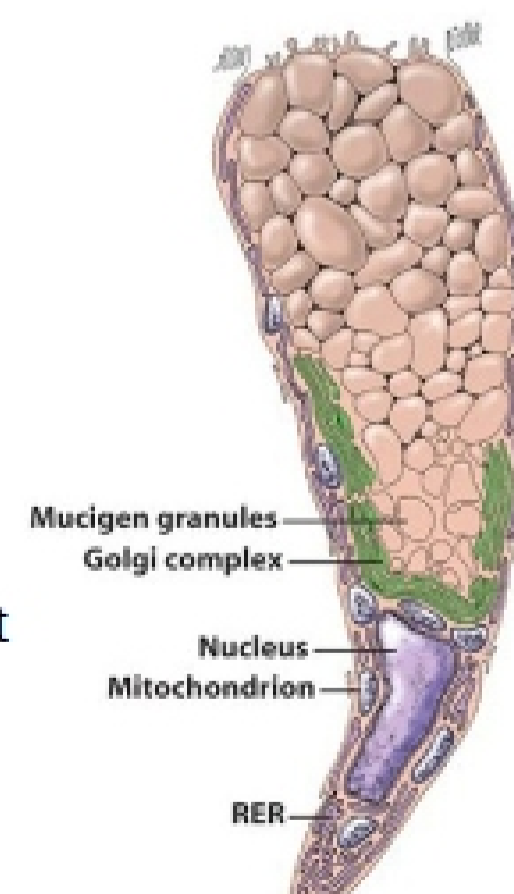
- coated vesicle (green $\wedge\wedge\wedge\wedge\wedge$) = clathrin
- HSP70 causes clathrin to fall off
- Rab-GTPase binds to motor protein and adaptor protein links the Rab protein
- Rab allows vesicle to move to target membrane
- Rab detaches from motor and binds to tethering protein
- v-SNAREs and t-SNAREs bind
- Rab recycles
- specificity comes from Rab



Sorting of Newly Synthesized Protein

Goblet cell (found in wall of intestine)

- secrete proteins with protective roles
- mucigen
 - complex of proteins and carbs and long chains of sugar
 - coats cell in small intestine to protect from digestive cells
 - minimize friction
- polarized cells
- each compartment is unique = what you find in each is different because of sorting
- secretion at top, fuses with plasma membrane to dump into intestine



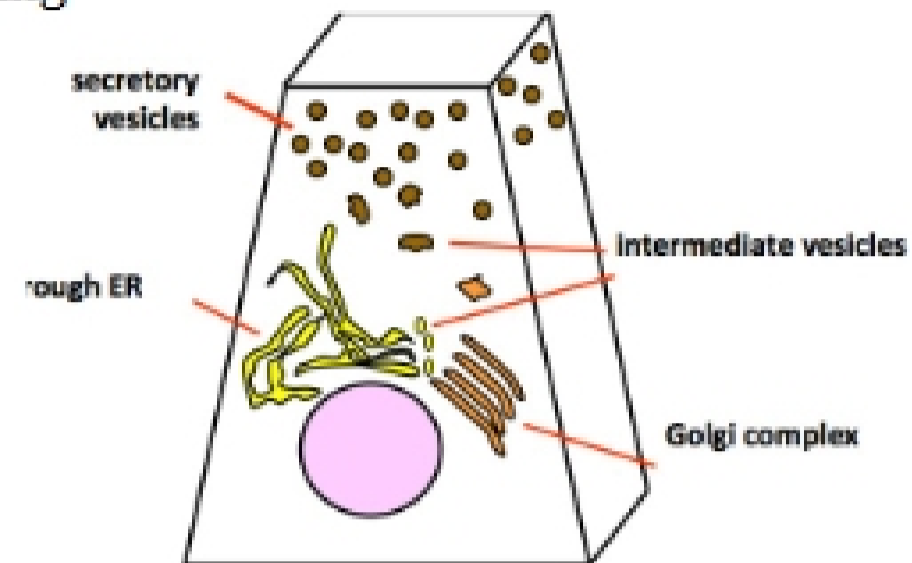
How does sorting work?

- George Palade (1974)

- sorting root of assembly
- Blobel (Palades student)
 - molecular mechanisms responsible for sorting

Sorting Newly-Produced Proteins: Co translational

- pancreatic model = best model for examining sorting
- assembly of secretory vesicles
- signal sequences
- integral membrane proteins
- core glycosylation
- sorting lysosomal enzymes



Pancreatic acinar cell

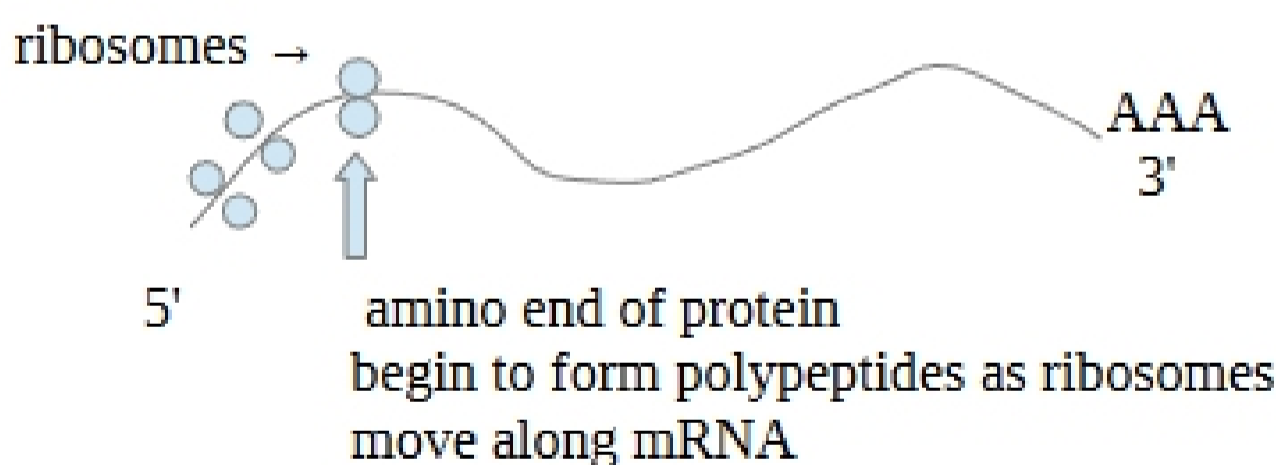
- alpha cells secrete glucagon
- beta cells secrete insulin
- acinar cells secrete digestive enzymes (released into duct tube) → delivered to intestine
- pancreatic duct
 - cells involved in secretory proteins
 - can take slices of pancreas and incubate in buffer then cells can still survive and produce secretory proteins
 - secretory side faces inside of the duct
- cells stimulated by peptide hormone for secretion

Protein Synthesis in Eukaryotes

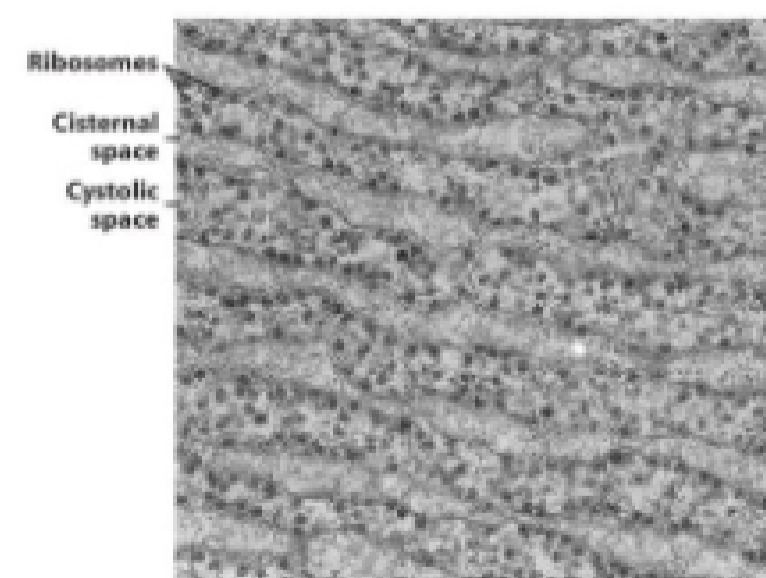
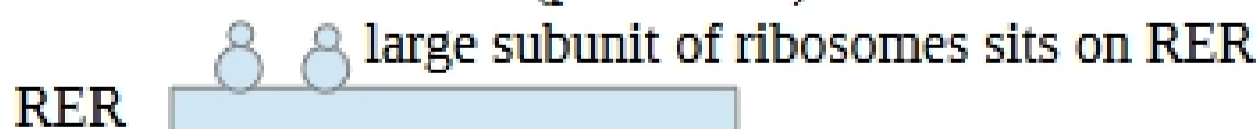
- free ribosomes (in cytoplasm)
- ER-bound ribosomes
- mitochondria and chloroplasts

Where are proteins that end up in secretory vesicles made?

- mRNA (free ribosomes)



ER-bound Ribosomes (picture →)



SER functions =

source of Ca^{++} , detoxification, lipid biosynthesis

Pulse – Chase Studies

- 1) slices of pig pancreas → put in erlenmeyer flask
- 2) incubated with radioactive amino acid → it incorporates into protein
- 3) traced the path of newly made proteins in cell