Vesicular Transport (Day 10)

Vesicles must be selective. From ER to Golgi, a vesicle must not carry cargo supposed to remain in ER, and must not fuse with any other organelle.

Mechanisms of Membrane Transport and Compartment Identity

- constant transport b/w membrane-enclosed compartments
- each compartment maintains special identity in form of unique combination of molecular markers
- cells achive this by tightly controlling the membrane components that are packaged into departing transport vesicles

Coated Vesicles

Most transport vesicles form from specialised, coated regions of membranes.

↓

bud off as coated vesicles (distinctive cage of proteins coating their cytosolic surface)

↓

before vesicles fuse with a memb., they shed off their coat so that the membrane surfaces of the vesicle compartment can interact directly and fuse

Membrane coat forms two main functions:

a) inner coat layer that concentrates specific memb.

proteins in a specialised patch giving rise to vesicle memb.

b) outer-coat layer that assembles into a curved basket-like lattice that shapes the vesicle.

Clathrin Coat Assembly and Disassembly

- major component of clathrin-coated vesicles is clathrin, which forms outer layer of coat
- clathrin → heavy chain
  → light chain
- 3 heavy chains and 3 light chains assemble into triskelion

  ↓

  assemble into basketlike framework

- clathrin assembly induces formation of coated buds (pits on plasma membrane)

  ↓

  pinch off to become clathrin-coated vesicles

  ↓

  adaptor proteins form a discrete inner layer of coat, positioned between clathrin cage & cytosolic face of memb.

  ↓

  bind to various transmembrane protein cargoes and transmembrane receptors that capture soluble cargo molecules inside the membrane (cargo receptors)

  ↓

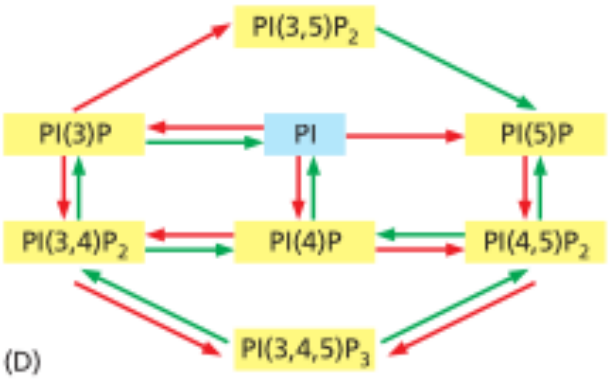
  specific set of molecules packaged into clathrin coated vesicles

Assembly of adaptor proteins on the membrane is tightly controlled, e.g, AP2

When AP2 binds to a specific phosphatidylinositol
↓
it requires a different conformation that exposes binding sites for cargo receptors in membrane
↓
simultaneous binding of AP2 to cargo receptors enhances binding of AP2 to membrane

Phosphoinositides mark organelles & membrane domains

- inositol phospholipids ~10% of total phospholipids in memb.
  ↳ regulatory func.
- undergo rapid cycles of phosphorylation and dephosphorylation → produce types of phosphoinositides (PIPs)
- interconversion of PI and PIPs highly compartmentalised



(D)

Cytoplasmic proteins regulate pinching off & uncoating of coated vesicles

As a clathrin-coated bud grows, soluble cytoplasmic proteins including dynamin regulate pinching off membrane to release fully formed clathrin-coated vesicle

Monomeric GTPases control coat assembly

- coat recruitment GTPases

ARF
- responsible for assembly of COPI & clathrin coats at Golgi memb.

Sar I
- responsible for assembly of COPII coats at ER memb.

Rab 7
- initiates assembly of retromer coats at endosome membrane.

- usually found in cytosol as inactive, GDP-bound state

Budding of COPII coated vesicles

When COPII coated vesicle is set to bud from ER membrane

↓

Sar1 GEF embedded in ER memb. binds to cytosolic Sar I

↓

Sar1 releases GDP

↓

binds GTP as soon as GDP is released, because conc (GTP) ↑ in cytosol

↓

GTP-bound Sar1 exposes amphiphilic helix which inserts into lipid bilayer cytoplasmic leaflet of ER memb.

↓

Budding initiated

Coat Recruitment GTPases function in coat disassembly

Coats must disassemble after vesicle buds off.
(otherwise vesicle cannot fuse with membrane)

⇓

Budding vesicles incorporate proteins to initiate disassembly only after vesicle has fully formed
↳ triggered by coat-recruitment GTPases

How?

Hydrolysis of GTP bound to GTPase → conformational change → hydrophobic tail pops out of memb → vesicle's coat disassembles

↓

Rate at which GTPase hydrolyses GTP → time reqd. to disassemble coat.

Cop II coats disassemble using SarI, and a fully formed vesicle is formed only when bud formation is faster than timed disassembly process.
Once a vesicle pinches off, GTP hydrolysis releases SarI, but vesicle is stabilised by cooperative interactions, hence coat remains until it reaches target membrain

For COPI-coated vesicles, the curvature of the vesicle membrane serves as a trigger to begin uncoating.

Rab proteins guide transport vesicles to their target memb.

- transport vesicles must be highly accurate in recognising the target memb. they fuse with
- specificity in targeting is ensured because all Transport vesicles display surface markers & target membranes display complement receptors

Two types of markers: Rab proteins (direct vesicle to specific points on correct target memb.) & SNARE proteins (enable fusion of lipid bilayers)

Rab proteins

- monomeric GTPases
- family contains many proteins, associated with one or more membrane-enclosed organelles of secretory or endocytic pathway — each of these organelles have at least one Rab on its cytosolic surface

- Rab proteins cycle between a membrane and the cytosol and regulate the reversible assembly of protein complexes on membrane

- In their GDP-bound state, they are inactive and bound to another protein (GDP dissociation inhibitor) that keeps them soluble in cytosol
- Membrane-bound RAB GEFs activate Rab proteins by catalysing exchange of GDP for GTP
- Once in GTP bound state, Rab's lipid anchors insert into membrane where Rab binds to Rab effectors

↓

Rab effectors engage SNARE proteins → guides and docks it on correct membrane

The assembly of Rab proteins and their effectors on an organelle membrane can be cooperative and results in the formation of large, specialized membrane patches that define the identity of that organelle.

One Rab protein can be replaced by a different Rab protein, and this can change the identity of its associated organelle. This is accomplished by one Rab protein selectively recruiting and activating a different Rab protein whose complement of effectors includes proteins that inactivate the first Rab protein and thereby disassemble its associated membrane patch. Such ordered recruitment of sequentially acting Rab proteins is called a Rab cascade (Figure 13–19). Over time, for example, Rab5-associated membrane patches are replaced by Rab7-associated membrane patches on endosomal membranes. This converts an early endosome, marked by Rab5, into a late endosome, marked by Rab7. Because the set of Rab effectors recruited by Rab7 is different from that recruited by Rab5, this change reprograms the compartment including the incoming and outgoing traffic and repositions the organelle away from the plasma membrane toward the cell interior. All of the cargo contained in the early endosome that has not been recycled to the plasma membrane is now part of a late endosome. This process is also referred to as endosome maturation. The self-amplifying nature of the Rab-associated membrane patches renders the process of endosome maturation unidirectional and irreversible.

SNARE proteins

- fusion protein responsible for fusion of membranes by removal of water molecules from hydrophilic surface of membrane (unfavourable process)
- 35 different SNAREs, each associated with a special organelle
- complementary sets — v-SNAREs on vesicle membrane &
  t-SNAREs on target membrane
  - ↳ highly specific
    - ↳ specificity during fusion
- need to be pried apart before they can work together again