Lysosomes and endocytic pathways

9/27/2012

Phyllis Hanson

General principles
Properties of lysosomes
Delivery of enzymes to lysosomes
Endocytic uptake – clathrin, others
Endocytic pathways – recycling vs. degradation

- Vesicle formation & composition controlled by coat proteins and interacting factors
- Vesicle targeting and fusion controlled by rab proteins, docking/tethering factors, and specific SNARE combinations
- Anterograde and retrograde pathways
Characteristics of Lysosomes

- Major site of intracellular degradation; contain many hydrolytic enzymes
- Acidic (pH 5); maintained by a proton pump
- Semi-permeable membranes due to presence of multiple transporters
- Protease resistant membranes that contain special membrane proteins

How are newly synthesized enzymes targeted to lysosomes?

- In mammalian cells, mannose-6-phosphate is a sorting signal for lysosomal hydrolases
- Modification of N-linked oligosaccharide in cis-Golgi
- M-6-P is recognized by a receptor in trans-Golgi network

Pathways for internalization at the cell surface

**Figure 1** Multiple portals of entry into the mammalian cell. The endocytic pathways differ with regard to the size of the endocytic vehicle, the nature of the cargo (ligands, receptors and lipids) and the mechanism of vesicle formation.

Conner & Schmid, 2003
Clathrin mediated endocytosis

* predominant endocytic pathway

* membrane and fluid uptake, responsible for most receptor mediated endocytosis

* 2-3% of cell surface occupied by clathrin coated pits

* lifetime of coated pit estimated to be ~1 minute before pinching off as coated vesicle
For closed polygonal structure, always need 12 pentagons

Hexagon #s vary: brain CCVs: ~75 nm, with 20 hexagons; fibroblast CCVs: ~120 nm with 60 hexagons

Clathrin coated vesicle cycle

- Adaptor protein(s) bind to membranes and cargo
- Adaptor protein(s) recruit clathrin, create nascent vesicle
- Clathrin and adaptor proteins are sufficient to form lattices and buds on liposomes, but cooperate with other “accessory” proteins in vivo
- “Accessory” proteins regulate coat assembly, membrane fission, and clathrin coat disassembly
Structure of the AP-2 ‘adaptin’

Signals for adaptor-dependent receptor internalization

- LDL receptor: tyrosine containing motif Yxxφ
- Other tyrosine containing motifs: FxNPxY
- Dileucine containing motif
- Phosphorylated serine-rich domains, especially at C-terminus of GPCRs
- Motifs involving ligand-induced phosphorylation
- Motifs involving ubiquitination
  - Constitutive recycling requires constitutively exposed internalization signal; triggered internalization controlled by transiently added internalization signal
AP-2 was first discovered, but is not the only adaptor at the PM

Experiment:
Use siRNAs to knock-down AP-2 µ2 subunit or clathrin heavy chain

Study effect of these manipulations on coated pit abundance and endocytic trafficking in HeLa cells


Coated pit abundance

AP-2 depletion has selective effects

… conclude that there must be other adaptor molecules involved in internalization of EGFR

Candidates include proteins that can bind plasma membrane lipids, clathrin, and cargo, such as: epsin, Hip1, Dab2, ARH
Dynamin: GTPase essential for membrane fission

• Self-assembles into tubular polymers at vesicle neck, these undergo conformational change to promote scission
• Also involved in localized actin dynamics
Watching clathrin mediated endocytosis in living cells

- Use clathrin-RFP and TIRF to follow clathrin coated vesicles
- Simultaneous imaging of GFP-dynamin or GFP-actin
- Shows sequence of events in real time
  - Clathrin pit appears
  - Dynamin is recruited
  - Clathrin starts to leaves the PM
  - Dynamin goes away, actin is recruited
  - Everything vanishes

Merrifield, 2002 (and more -including cargo- in 2005, 2011 papers)
Other pathways for internalization from cell surface

- Caveolae
- Macropinocytosis
- Phagocytosis
- Other clathrin-independent mechanisms

Caveolae (= “little caves”) on the plasma membrane
Caveolar endocytosis

- Minor pathway compared to clathrin mediated endocytosis
- Internalizes membranes enriched in lipid rafts
- Pathway used by GPI-anchored proteins, toxins, viruses
- Internalized caveolae travel to caveosome, ER, Golgi, endosome
- Pathway requires dynamin, actin, and probably others
- Key difference from clathrin pathway is that caveolar “coat” does not disassemble, instead contents diffuse out or dissociate
Macropinocytosis

- Associated with membrane ruffling
- Induced in cells following growth factor stimulation
- Role for Rho-family GTPase and actin-driven formation of membrane protrusions
- Formation of macropinosome follows fusion of membrane protrusions with plasma membrane
Macropinocytosis increases fluid uptake with no change in (clathrin-dependent) receptor endocytosis

Fluorescent dextran uptake (fluid phase marker)  Transferrin uptake (marker of clathrin mediated endocytosis)

**FIGURE 3**
Points of regulation of macropinocytosis: (1) the initiation and formation of ruffles; (2) closure of ruffles at their outermost margins to form vesicles; (3) removal of F-actin from the macropinosome; (4) interaction with other endocytic compartments; and (5) disposal by recycling to the cell surface, as in HeLa cells, or merger with a different endocytic compartment, as in macrophages and dendritic cells.
Phagocytosis

- particle triggered engulfment of large structures such as bacteria, yeast, remnants of dead cells, arterial fat deposits
- best in professional “phagocytes”, e.g. macrophages, etc.

Example: Fc receptors on macrophages are activated by antibodies bound to surface antigens on bacteria, and trigger a signalling cascade that activates the small GTPases Cdc42 and Rac to induce actin assembly and formation of cell-surface extensions that zipper up around and engulf the pathogen.
After formation, phagosomes typically mature in parallel with endocytic pathway (time to lysosome similar)

Overview of the endocytic pathway
Intracellular itineraries of receptors

- Different receptors have different pathways once inside cell
  - recycling to plasma membrane is default
  - delivery into lysosome requires signal
- Examples
  - LDL receptor
  - Transferrin receptor
  - EGF receptor

**pH controls receptor-ligand interactions**

*Figure 33-13* Progressive decrease in luminal pH facilitates protein sorting in the endosomal compartment. Interactions of many cargo molecules with their receptors are pH dependent; dissociation places ligands in the luminal space, whereas receptors remain associated with membrane. Geometric considerations, as well as sorting motifs on the receptors, facilitate sorting of membrane from internal volume. Unoccupied receptors whose ligands, such as LDL, have dissociated under the relatively mild acidic conditions encountered in early endosomes are efficiently recycled back to the cell surface. Iron carried by transferrin (Tfn) dissociates at a pH of approximately 6, but apoferritin remains bound to and recycles with its receptor. Mannose-6-phosphate receptors (MPRs) carry their ligands to late endosomes before dissociation at lower pH and recycling back to the TGN. EGF remains bound, and both ligand and receptor (EGFR) are delivered to and degraded in lysosomes. PM, plasma membrane.
LDL (low density lipoprotein particle) receptor: receptor recycles, cargo is degraded

- LDL receptors bind LDL particles
- Coated pit forms
- Endocytosis
- Lysosome
- Hydrolytic enzymes degrade LDL cargo
- Recycling endosome
- Budding off transport vesicles
- Return of LDL receptors to plasma membrane

Cell gets amino acids, cholesterol, fatty acids from degraded LDL

Transferrin receptor: receptor and transferrin recycle, Fe$^{3+}$ internalized

- Prototypical recycling receptor
- $t_{1/2}$ for recycling ~ 16 min
- Similar to kinetics of bulk lipid recycling
- Receptor recycles 100+ x during lifetime
EGF receptor: receptor, EGF degraded

- Accumulates in coated pits only after ligand binding
- Internalization requires active kinase domain
- Receptor and ligand both delivered to lysosomes & degraded
- Results in receptor down-regulation

Hanson & Cashikar 2012
How are lumenal vesicles made?

Endosome maturation: what defines different endosomes?
Rab proteins define different endosomes (and other) organelles

~60 Rab GTPases in the human genome
Rab GTPase Cycle

Example: Rab conversion between early and late endosomes

Rink et al., 2005
Pulse-chase analysis showing colocalization of rab5 and rab7 with fluorescent cargo (LDL)

Molecular mechanisms of conversion?

Clues in rab5 effectors … affinity chromatography to find things that bind GST-rab5 in GTP bound state

Rink et al. now show that these include class C VPS complex, which in turn has GEF activity for rab7. Also propose that rab7 may have rab5 GAP as an effector.