

Study guide

Receptor Mediated Endocytosis

Cell Biology 2005

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Objectives

At the end of this unit, the student should be able to:

1. Compare and contrast major types of internalization events.
2. Categorize the variety of ligands that enter by receptor mediated endocytosis.
3. Describe necessary conditions and each step in the process of receptor mediated endocytosis.
 - a. Describe the mechanisms behind the operation of the clathrin coated pit.
 - b. What factors operate to keep the receptor in the pit and how is the pinching off of the vesicle regulated. (Adaptin, β -arrestins and dynamin)
 - c. Distinguish the functions and environment of the early endosome from those of the late endosome
 - d. Describe the routes of trafficking from the Golgi complex to the late endosomes
 - e. Demonstrate how a late endosome converts to a lysosome.
4. Identify each domain in the receptor mediated endocytosis pathway by its characteristic cytochemical or pH markers.

Competencies

Given a clinical scenario and the appropriate cytochemical markers (such as pH or other identifiers), the student should be able to deduce which domain is involved in an interrupted receptor-mediated endocytosis event.

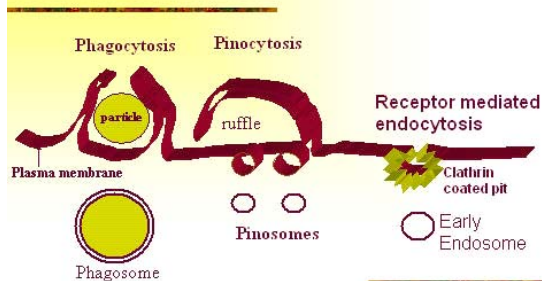
A Guide to studying this unit

Read pp 510--518. In particular, study the clinical scenario presented on page 512.

B. Lecture outline

I. How do cells internalize molecules and other cells? Compare and contrast major types of internalization events.

List the major types of internalization events.



In this unit, we will learn about a third set of structures: the endosomes. Endosomes are formed by receptor-mediated endocytosis. In this case, cells bring in proteins and other types of ligands attached to the plasma membrane via receptors

This figure diagrams the major internalization events. In the two views on the right, receptors are not needed for internalization. During phagocytosis, cells may simply internalize particles or cells, like bacteria (cell eating). In the second, called pinocytosis, cells internalize soluble material (cell drinking). In both types of internalization, the cells bring particles, cells or soluble material into the cell in a vacuole. When we study lysosomes, we learn that the vacuole formed in the cell by phagocytosis or pinocytosis often became a lysosome after hydrolases are brought to it and the pH was adjusted. The vacuoles formed are called phagosomes or macropinosomes.

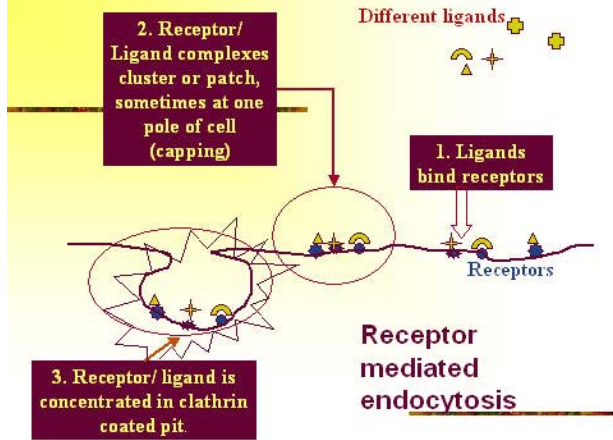
II. Categorize the variety of ligands that enter by receptor mediated endocytosis

Hormones and Growth Factors: Insulin, Epidermal Growth Factor, Growth Hormone, Thyroid stimulating hormone, Nerve Growth Factor, Calcitonin, Glucagon, Prolactin, Luteinizing Hormone, Thyroid hormone, Platelet Derived Growth Factor, Interferon, Catecholamines.

Toxins and lectins: Diphtheria Toxin, Pseudomonas toxin, Cholera toxin, Ricin, Concanavalin A.

Viruses: Rous sarcoma virus, Semliki forest virus, Vesicular stomatitis virus, Adenovirus
 Serum transport proteins and antibodies: Transferrin, Low density lipoprotein, Transcobalamin, Yolk proteins, IgE, Polymeric IgA, Maternal IgG, IgG, via Fc receptors

III. How does the process work? Describe each step in the process of receptor mediated endocytosis.



A. Step 1. Ligand binding

Receptors are brought to the plasma membrane by vesicles from the trans region of the Golgi complex. Receptors are transmembrane proteins. Review the definition of transmembrane proteins. Where and how are these receptor proteins inserted into the membrane? The receptors can move laterally in the membrane and collect in the specialized regions called clathrin coated pits. This is seen as Patching and capping.

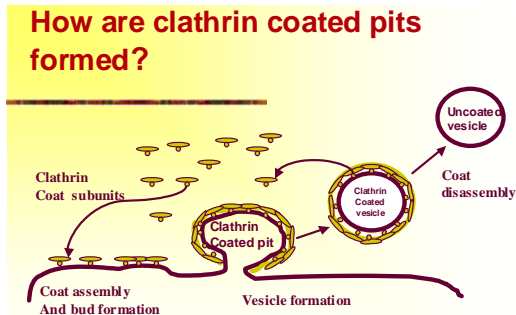
B. Step 2. Patching and capping

When the ligand binds to its specific receptor, the ligand-receptor complex accumulates in the coated pits. In many cells, these pits and complexes begin to concentrate in one area of a cell

C. Process can be used to bring in multiple ligands

D. Effect of temperature on the process: Warming promotes patching, capping, and endocytosis. May not be needed for binding, however.

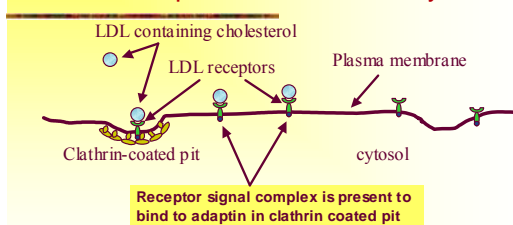
IV. Describe the mechanisms behind the selectivity of the clathrin coated pit



Recycled clathrin adds to membrane at the site of the pit. Invaginates into the cytoplasm (if temperature is 37 C.). Receptors are collected in pits. Pits pinch to form vesicles. Vesicles then lose clathrin coat.

Receptors are transmembrane proteins that may span the membrane. They have a signal sequence at the end of their cytoplasmic domain (carboxy terminus): Tyrosine-X- Arginine-Phenylalanine. Signal sequence binds to adaptin molecules in the clathrin coat. (Adaptor protein AP-2) . This stops and concentrates the receptor. It stays inside the pit. Signal sequence even stimulates more clathrin to accumulate. β Arrestins guide receptors to the pit and facilitate this binding.

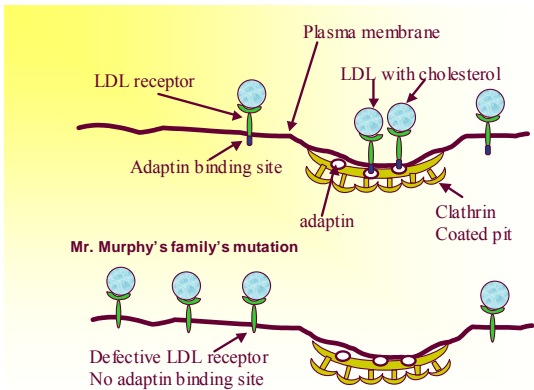
LDL receptors carry cholesterol into the cell via receptor mediated endocytosis



LDL receptor bind cholesterol and are, in turn, bound by adaptin to clathrin coated pits. This concentrates them and promotes the formation of clathrin coated vesicles.

Why is this important? Sequestration concentrates ligand to conserve intake of fluid. Also, serves as a gateway to entry.

There is a genetic defect in LDL receptors which prevents them from binding to Adaptin-2 . Thus, they do not enter clathrin coated pits and cannot be brought into the cell via receptor mediated endocytosis. The result is high serum cholesterol,



because LDL provides a critical mechanism for reducing cholesterol levels and getting it into the cells.

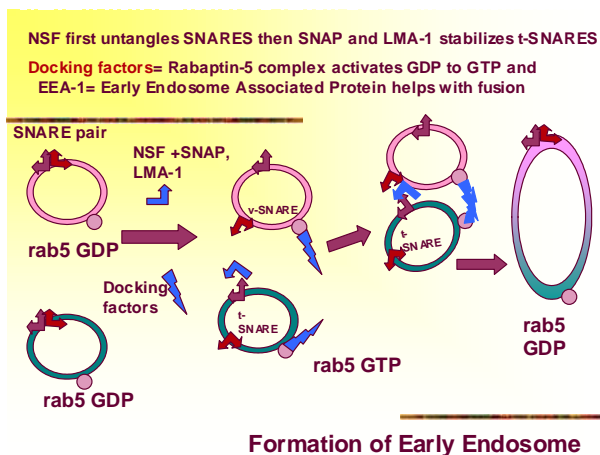
Hypercholesterolemia. There is a familial form that comes from a mutation in the LDL receptor. It binds cholesterol, but never lets it enter the cells. Mr. Murphy and 2/4 of his children had this disease. Also, father and 2/5 siblings had it. What kind of inheritance? Will cause heart attacks (early) and atherosclerosis.

V, Distinguish the functions and environment of the early endosome from those of the late endosome

A. How does the clathrin coated pit become a vesicle:

Dynamin, a GTPase becomes associated with the stem-like connection to the vesicle. GTP is hydrolyzed and this provides the energy needed for constriction of the connection and eventual disconnection, forming the clathrin-coated vesicle. The clathrin coat is then lost.

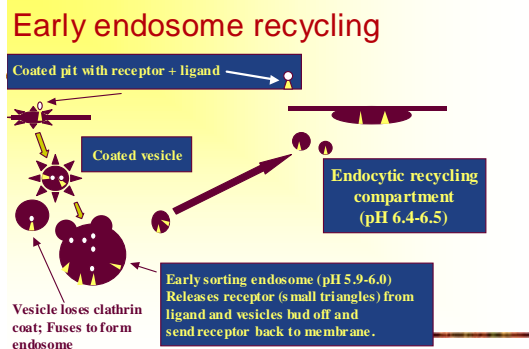
B. How are early endosomes formed?



To understand what happens to cholesterol, need to know about trafficking through the endosomes.

Vesicles lose clathrin coat and then fuse to form early endosome. (pH 5.9-6.0). In order to fuse, they carry a rab5 sorting signal linked to guanosine diphosphate (GDP). Also have “v-snares and t-snares”

C. Early endosome functions and characteristics.

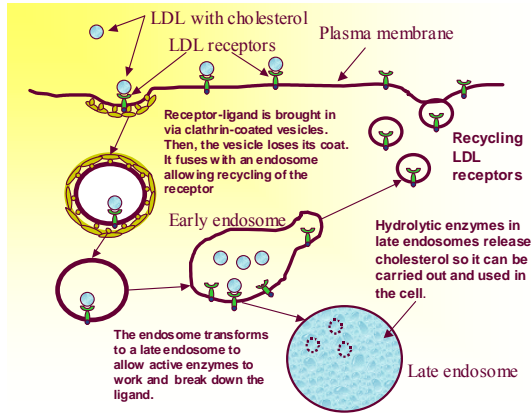


Early endosomes can release some receptors from their ligand. Low pH of 6 allows release. Receptors are then recycled back to the plasma membrane (pH >6). Then, early endosome may become a late endosome.

Thus, markers for early endosome compartment include: rab5-GDP, pH around 6.0, recycled receptors.

Apply this to LDL receptors.

Cartoon below shows that LDL receptors are an example of the class that are recycled. The LDL is released in the early endosome to allow return of the receptor and its re-use

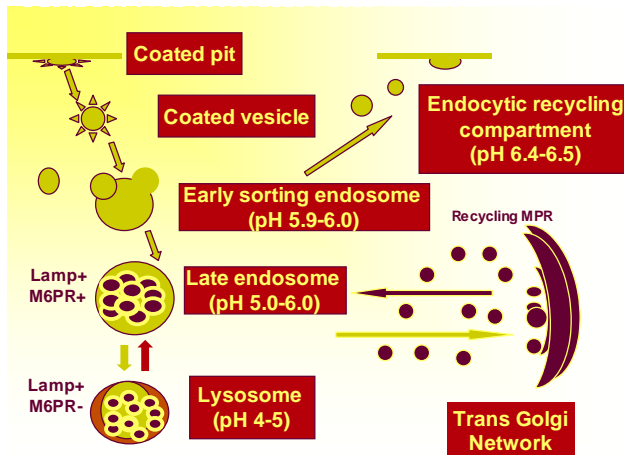


Early endosome transition to late endosome
Within a few minutes of membrane recycling, the early endosome becomes a late endosome. pH lowers further to 5.0-6.0; Rab sorting signal changes to rab7-GDP; Membrane rich; Distinguished by Lysobisphosphatidic acid (LBPA), a lipid; Communicates with the Golgi complex.

Cholesterol is released and eventually transported out of the late endosome to be used in membranes, steroid hormones, etc.

D. Late endosomes; Describe the routes of trafficking from the Golgi complex to the late endosomes.

Late endosomes are formed as the pH continues to drop to 5-6.0. Clathrin-coated vesicles from the Trans Golgi Network carry digestive enzymes to the late endosome and fuse with these structures, releasing their contents. The late endosome thus becomes a degradative body and also acquires the marker for mannose 6 phosphate receptor "MPR+." It changes its rab surface marker to rab7-GDP, probably to accommodate the new targeting vesicles with which it will fuse. This means that the late endosome can be identified by the presence of the rab7 GDP



Late endosomes include multivesicular bodies and contain whorls or vesicles of membranes inside. They also contain an unusual lipid which has become another marker for this stage. The lipid is called lysobisphosphatidic acid (LBPA) which has a larger head group than tail. Its structure enables it to be inserted into highly curved membranes, like the membrane whorls. It is believed that this allows retention and binding of specific molecules in the whorls by lipid-protein; lipid-lipid interactions. One type of molecule is cholesterol and it is believed that this is an important site for cholesterol accumulation.

Late endosomes function to degrade many proteins and lipids. They also are responsible for returning the MPR receptors back to the Trans Golgi network. They recycle these by budding off membranes that carry back the receptors and target the Trans Golgi membranes for fusion. After fusion, the MPR receptors are available to capture and sort new degradative enzymes for future trafficking to the late endosomes.

To summarize, markers for late endosomes include:

- rab7-GDP
- LBPA
- MPR+

E. Demonstrate how a late endosome converts to a lysosome

Finally, late endosomes may not be able to digest all the material that needs to be destroyed. Therefore, the next step is a fusion of late endosomes and lysosomes creating a hybrid organelle. The pH continues to

drop. Residual heavily glycosylated lysosomal associated membrane proteins (LAMPs) may thus be transmitted to lysosomes. LAMPs then become a marker for a late endosome or a lysosome. Since lysosomes do not have MPR receptors (they have all been sent to the Golgi), one could distinguish the lysosome and the late endosome on the basis of labeling for MPR. Thus, fusion between the late endosome and lysosomes begins after the MPR have been sent back to the Trans Golgi. The steps involved in forming late endosomes and lysosomes are drawn in the above cartoon. Note that lysosomes continue to communicate with late endosomes and may deliver important material back to this group of organelles. Lysosomes are considered the end product of endocytosis. Thus, lysosomes do not communicate directly with the Trans Golgi (and hence the plasma membrane). However, they could communicate with upstream structures by way of the retrograde transport to the late endosome.

To summarize, markers for lysosomes include: LAMP+, acid hydrolases, MPR negative, NPC1 (in normal cells) (Neiman Pick Complex protein).

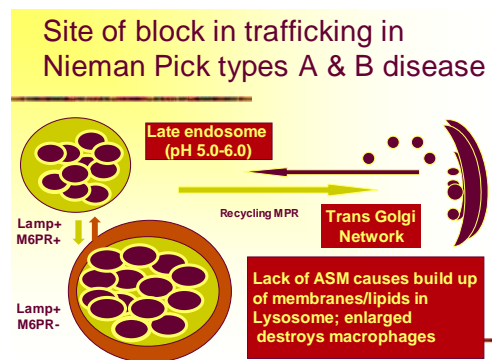
F. Importance of late endosome-lysosome compartments:

They regulate trafficking of critical nutrients. They also regulate cellular stores of different molecules, by enzymatically degrading them. If there is a failure in the trafficking or degradation, the lysosome will build up the product and eventually this will damage the cells. There may be rather wide-spread effects throughout the body.

V. Three examples of lysosomal storage diseases: Nieman Pick A, B, C

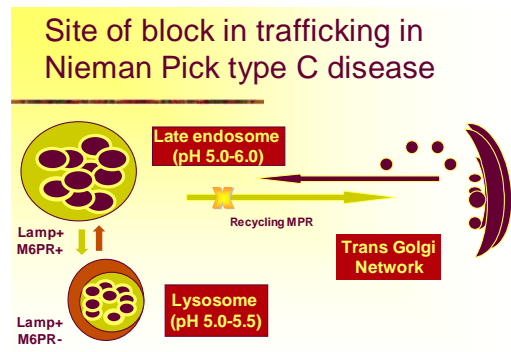
Neiman Pick Disease: good examples of genetic problems with late endosomal or lysosomal functions.

- Types A and B involve a deficiency in acid sphingomyelinase—(ASM)
 - Lack of ASM in lysosome will cause a lipid buildup.
 - Seen prominently in macrophages (cells that have a lot of lysosomes).
 - Lipid buildup eventually kills cells and damages organs, like spleen and liver.
- Type A is associated with neurological tissues and usually causes death within 2-3 years.
- Type B symptoms: enlarged spleen, respiratory problems, cardiovascular problems, can live into adulthood.
- Autosomal recessive



Nieman-Pick Type C (NPC) disorder: a problem with late endosome

- In the NPC disorder, there is a mutation in the NPC1 protein, which is needed for cholesterol transport.
- Cholesterol accumulates in late endosomes which also appear expanded. It is “stuck in traffic” and can’t get out of the endosomes.
- This also blocks retrograde transport of mannose 6 phosphate receptors to the Golgi complex.



Type C Niemann-Pick usually affects children of school age, but the disease may strike at any time from early infancy to adulthood. Always fatal. Some of the symptoms may include: Jaundice at (or shortly after) birth; An enlarged spleen and/or liver. Difficulty with upward and downward eye movements (Vertical Supranuclear Gaze Palsy). Slurred, irregular speech ("dysarthria") Learning difficulties and progressive intellectual decline ("dementia"); Sudden loss of muscle tone which may lead to falls ("cataplexy")

VI. What have we learned about receptor mediated recognition events?

Receptor must have recognition sites for ligand. Binding may activate second messengers.

Receptor must also have recognition site for clathrin coated pit (for adaptin). Clinical significance—hypercholesterolemia.

Endocytic vesicles must have specific rab5-GTP's to fuse and form early endosome.

Early endosome will recycle some receptors

Late endosome must have rab7 GDP recognition sites for fusion and communication with the Trans Golgi network.; Site for release of cholesterol—Clinically significant for Nieman Pick C

Site for recycling of Mannose 6 phosphate receptors.

Lysosomes are the final stop in the endocytic pathway.

Site for degradation of membranes, proteins, and lipids. Clinically significant for Nieman Pick A and B

Test yourself!! How much do you know about receptor mediated endocytosis?

- 1) List the major types of internalization events?
- 2) What types of ligands enter by receptor mediated endocytosis?
- 3) Describe each step in the process ?
- 4) Do coated pits accommodate only one ligand, or can more than one enter in the same pit?
- 5) What is the advantage of the patching and capping process to the cell?
- 6) What is the role of the clathrin around the coated pit?
- 7) What guides the ligand and receptor into the coated pit? What would happen if one mutated the signal sequence on the receptor molecule?
- 8) How does receptor mediated endocytosis reduce our serum levels of cholesterol?
- 9) What is the effect of temperature on the process?
- 10) How do early endosomes form? What is the Early Endocytic recycling pathway and how is it used?
- 11) What happens to the receptor in the endosome?
- 12) How do late endosomes form?
- 13) How do late endosomes become lysosomes ?

Sample question.

Ms. Jones brought her daughter to your ER because of persistent cough and fever. She was diagnosed with pneumonia and her spleen was enlarged. An analysis of her bone marrow cells showed abnormal large, foamy macrophages which were distinguished by LAMP +, mannose 6 phosphate negative (MPR-) bodies, which contained many membrane whorls. A defective enzyme involved in break down of lipids was the diagnosis. This defect was the result of a problem in the following domain:

- 1) Early endosome
- 2) Clathrin coated pits
- 3) Lysosomes
- 4) Trans Golgi complex
- 5) Rab5 GDP vesicles

Answer: 3

You suspect that your cardiac patient, Mr. Murphy, has hypercholesterolemia. You are waiting for blood tests from family members. In the meantime, your lab can take his blood cells and test trafficking of the LDL receptors. You use dual immunolabeling for LDL receptors and rab5 GDP sorting signal vesicles (pH 6.0) and find labeling for both constituents, but no contiguous labeling. In other words, LDL receptors are not in rab5 GDP vesicles. This confirms your hypothesis because.

- 1) LDL must enter by receptors stored and recycled in rab7 GDP coated vesicles
- 2) LDL receptors are defective and therefore not sorted to the plasma membrane
- 3) Trafficking of all vesicles containing mannose-6 phosphate receptors from the Golgi complex is blocked
- 4) There is a defect in adaptin carried by rab5 GDP vesicles.
- 5) Before they can get to rab5 GDP vesicles, LDL receptors must enter clathrin-coated pits.

Answer 5