

Receptor-Mediated Endocytosis

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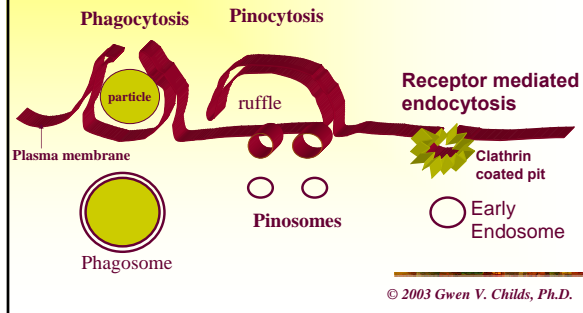
Cardiac emergency

- You are a cardiologist, asked to consult on the case of Mr. James Murphy, a 35 yo man who just had a heart attack.
- As you take his history, he is normal weight, but his cholesterol is 440 mg/dl.
- His father died of a heart condition at age 50. He had been adopted, so Mr. Murphy did not know his family history.
- You learn that his older sister, Jane, also had a heart attack (at age 45) but is recovering and being treated for high cholesterol. His younger brother also has high cholesterol (500 mg/dl).
- Two other younger siblings appear OK.
- Is this family history unusual?

Your response

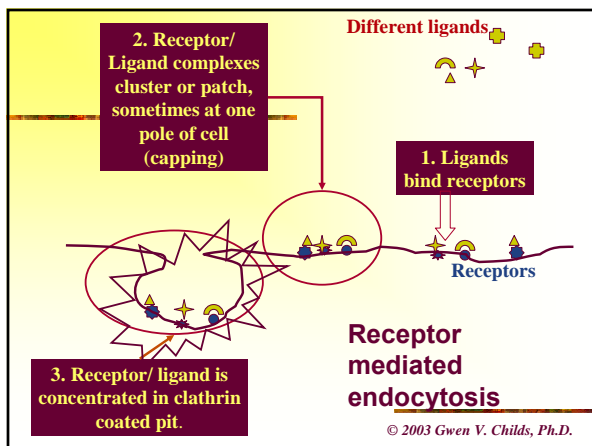
- You are concerned by the number of family members with cardiac problems and decide to test everyone.
- When cholesterol tests came back, your suspicions are confirmed:
 - Mrs. Joan Murphy, age 35, 160 mg/dl
 - Mary Murphy, age 10, 140 mg/dl
 - James Murphy Jr, age 6, 500 mg/dl
 - Brian Murphy, age 4, 150 mg/dl
 - John Murphy, age 2.5, 350 mg/dl
- Are these values unusual?
- To solve this case, you will need to know about receptor mediated endocytosis!

List the major types of internalization events.

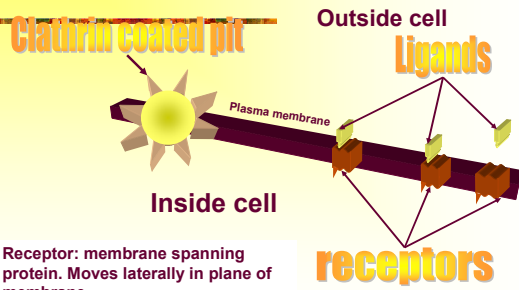


Types of molecules that enter by receptor mediated endocytosis:

- **Toxins**
- **Antibodies**
- **Viruses**
- **Hormones**
- **Growth factors**
- **Serum Transport proteins**
- **LDL (What is this protein?)**



Step 1: Receptor-ligand binding



Receptor: membrane spanning protein. Moves laterally in plane of membrane.

Ligand: binds receptor.

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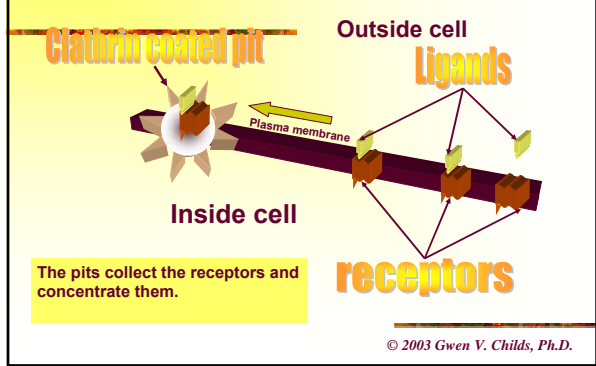
What is the significance of ligand-receptor binding?

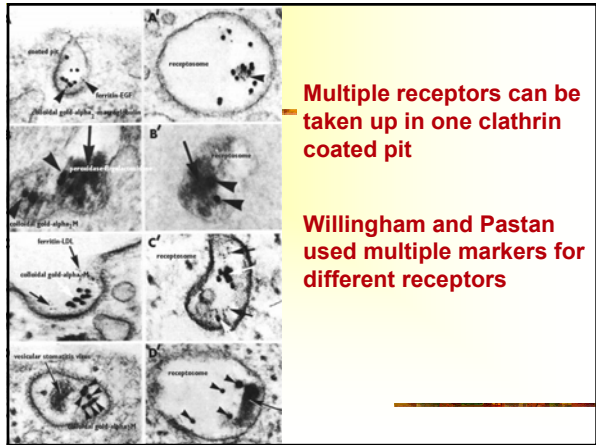
- **Receptor:** membrane-spanning protein with binding sites for ligand in extracellular domain.
- **Ligand binding:** causes rapid activation of second messengers in a cascade that eventually affects cell (stimulates, inhibits, etc.).
- One does not always need receptor mediated endocytosis to activate or inhibit a cell. Often, just the binding to the extracellular domain will activate the receptor mediated cascade.

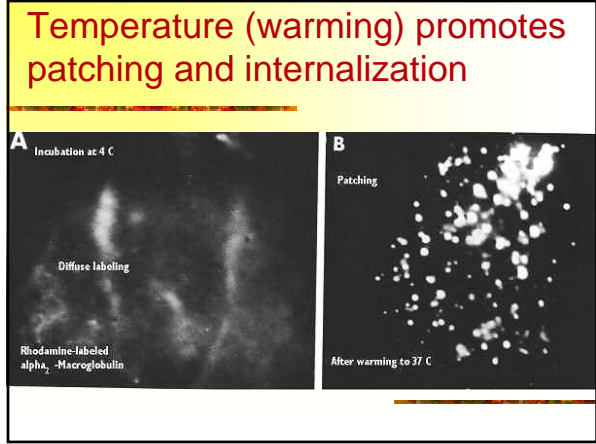
Why concentrate the receptor/ligand complex in patches, or at one pole of cell?

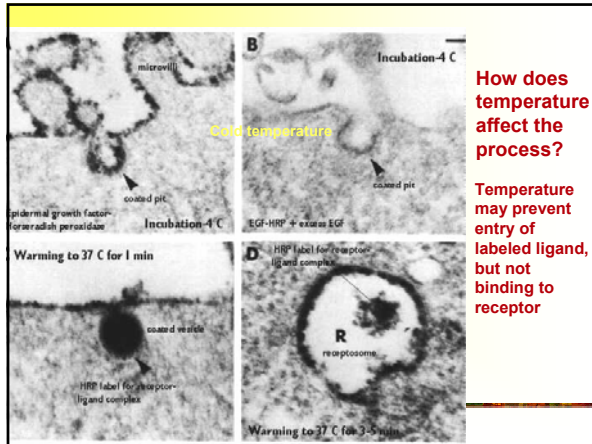
- Concentration allows regulated entry of fluids.
- If there wasn't some organization, there would be too much fluid entering.
- This makes more work for the cell.

Step 3: Receptor-ligand moves to clathrin-coated pits



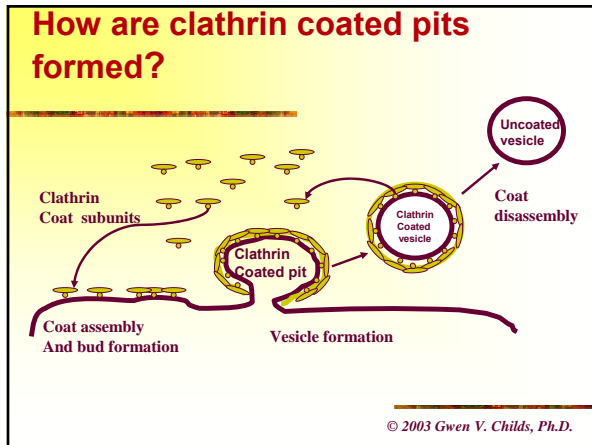


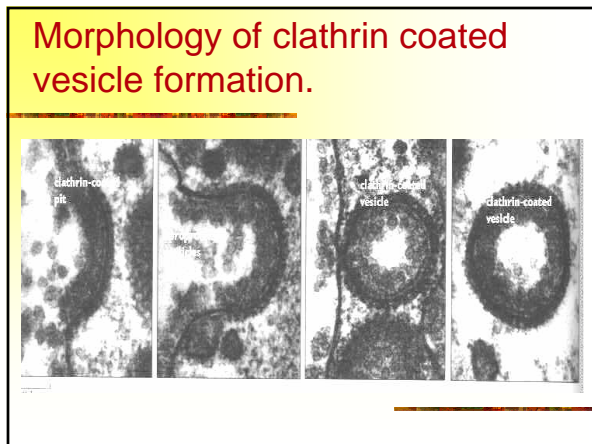




How does temperature affect the process?

Temperature may prevent entry of labeled ligand, but not binding to receptor





Gaidarov I, Santini, F, Warren, RA and Keen, JH Spatial control of coated-pit dynamics in living cells. Nature, Cell Biology 1: 1-7. 1999.

- Transfected cells with gene for clathrin linked to green fluorescent protein.
- Followed route of clathrin and its mobility in the membrane. Green fluorescent protein became the signal for clathrin.
- Looked at mobility and formation of coated pits.

Mobility of coated pits show they are organized.

- Time lapse photography suggests that coated pits appear, then disappear: constantly being reformed.
- When the early and later images were superimposed, the coated pits tended to reappear in the same place, as if they were anchored or somehow “organized”.
- It is believed that they are held in place by cytoskeletal system.

Gaidarov I, Santini, F, Warren, RA and Keen, JH

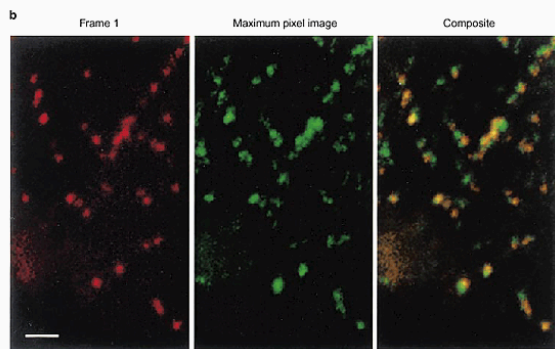


Photo taken for 1 sec (red) and then 30 consecutive photos- 18 sec intervals (green). Images superimposed show same linear pattern (yellow)

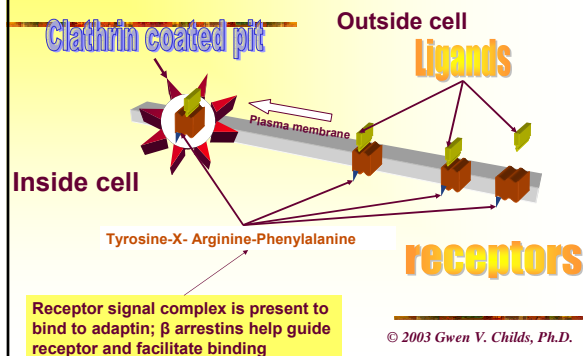
How do receptors know to enter via clathrin coated pit routes?

- Receptors are transmembrane proteins that may span the membrane.
- Proteins called "**β Arrestins**" assist in the guiding of the receptors to the clathrin-coated pits.
- Receptors 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). **β Arrestins** also facilitate this binding.
- This stops and concentrates the receptor. It stays inside the pit.
- Signal sequence even stimulates more clathrin to accumulate

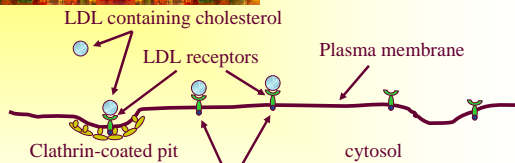
How the pit becomes a vesicle?

- The pit invaginates and eventually is bound to the plasma membrane by a narrow stem, sort of like an inverted goblet.
- Dynamin, a GTPase, becomes associated with the stem-like connection to the plasma membrane.
- Hydrolysis of GTP provides the energy needed for constriction and loss of this connection and ultimate formation of the clathrin-coated vesicle carrying the receptor and ligand as cargo.
- The clathrin coated vesicle then loses its coat and then fuses, by a specific sorting signal, with other vesicles to form the early endosome.

Step 2: Receptor-ligand concentration mediated by adaptin, arrestins, and signalling peptides



LDL receptors carry cholesterol into the cell via receptor mediated endocytosis

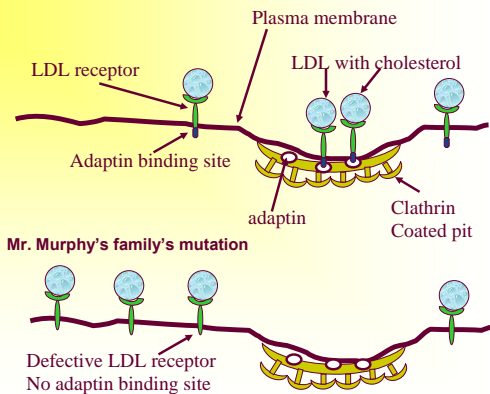


Receptor signal complex is present to bind to adaptin in clathrin coated pit

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

What happens when LDL receptors are defective?

- 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.

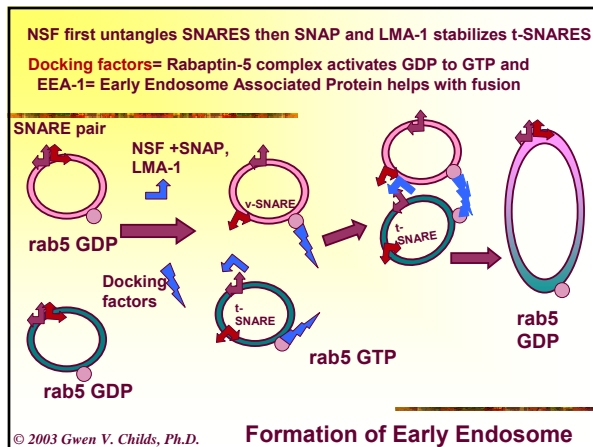


What happens if we can't bring cholesterol into our cells?

- **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, his father and 2/5 siblings had it. What kind of inheritance?**
- **Will cause heart attacks (early) and atherosclerosis.**

How do vesicles fuse to form early endosome?

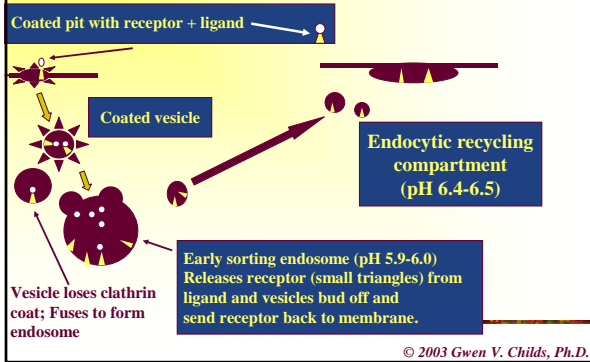
- **To understand what happens to cholesterol, need to know about trafficking through the endosomes.**
 - Vesicles lose clathrin coat and then fuse to form early endosome. (pH 5.9-6.0)
 - In order to fuse, they carry a rab5 sorting signal linked to guanosine diphosphate (GDP)
 - Also have "v-snares and t-snares"



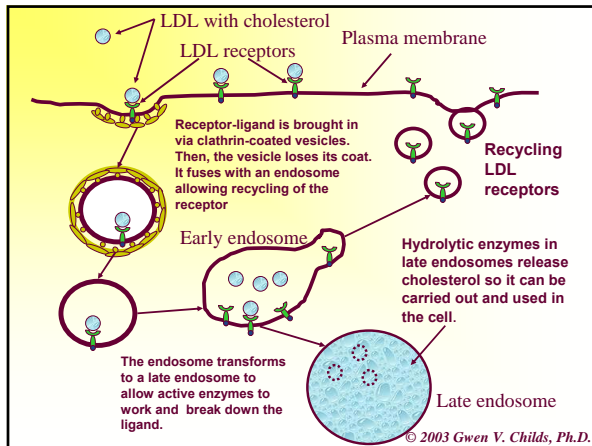
What happens to early endosome?

- Early endosome 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.

Early endosome recycling



<p>TFR monoclonal antibody localization of receptors to transferrin</p>	<p>Transferrin receptor is recycled</p>	<p>Are all receptors recycled?</p> <p>Varies with the receptor:</p> <ul style="list-style-type: none"> ➢ Transferrin receptor is recycled to surface (top). ➢ EGF receptor ends up in late endosome/ or lysosome and is degraded (bottom).
<p>EGFR monoclonal antibody localization of receptors to EGF</p>	<p>EGF receptor in lysosome</p>	



Next step: formation of the late endosome

- Within a few minutes of membrane recycling, the early endosome becomes a late endosome.
- Characteristics:
 - 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

Communication with Golgi Complex

- **Vesicles from trans Golgi C.:** deliver acid hydrolases
- Recall that acid hydrolases are sorted in Golgi complex, bound to mannose 6 phosphate receptors (M6PR).
- What type of body is forming at this point?

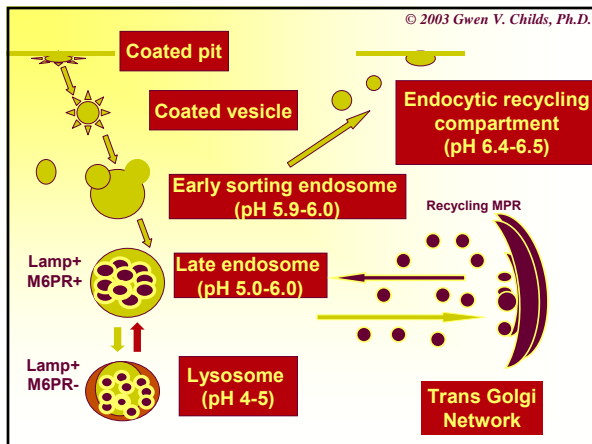
Late endosomes sort and degrade

- **Degradation**
 - many proteins, lipids, receptors that are not recycled.
 - Releases free cholesterol
- **Recycles to Golgi complex**
 - Receptors that are not degraded
 - Mannose 6 phosphate receptor back to Trans Golgi network.
- **Eventually fuses with lysosomes.**

Late endosomes fuse with lysosomes:

- pH continues to fall to 4-5
- Heavy degradation
- End point of endocytic pathway
 - receptors that are not recycled are degraded, along with the ligand
 - LAMP¹ positive bodies.
 - Not Mannose 6-Phosphate Receptor positive

¹. *Lysosomal associated membrane complex*



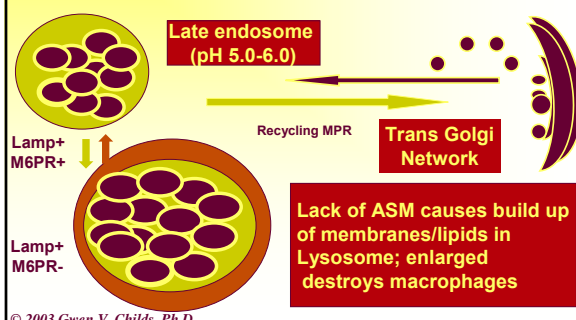
Why are these compartments so important?

- 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.

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

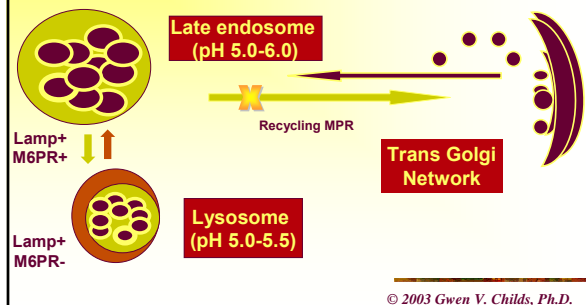
Site of block in trafficking in Nieman Pick types A & B disease



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.

Site of block in trafficking in Nieman Pick type C disease



Symptoms of patient with Nieman Pick Type C

- 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”)

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,

What have we learned about receptor mediated recognition events?

- 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 Neiman Pick A and B
