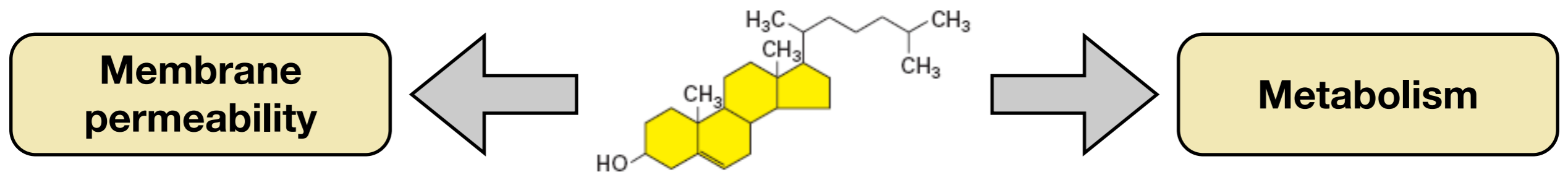# Cellular control of cholesterol

Peter Takizawa
Department of Cell Biology

- Brief overview of cholesterol's biological role

- Regulation of cholesterol synthesis

- Dietary and cellular uptake of cholesterol

Cholesterol is critical for cell function and human physiology.

**Membrane permeability**

Metabolism

You hear a lot of bad things about cholesterol; it's important to remember the good things and why it's biologically important. Cholesterol has many important functions, including its role in membrane permeability, especially the cell membrane, and its role in metabolism.

# Cholesterol reduces permeability of lipid membranes.
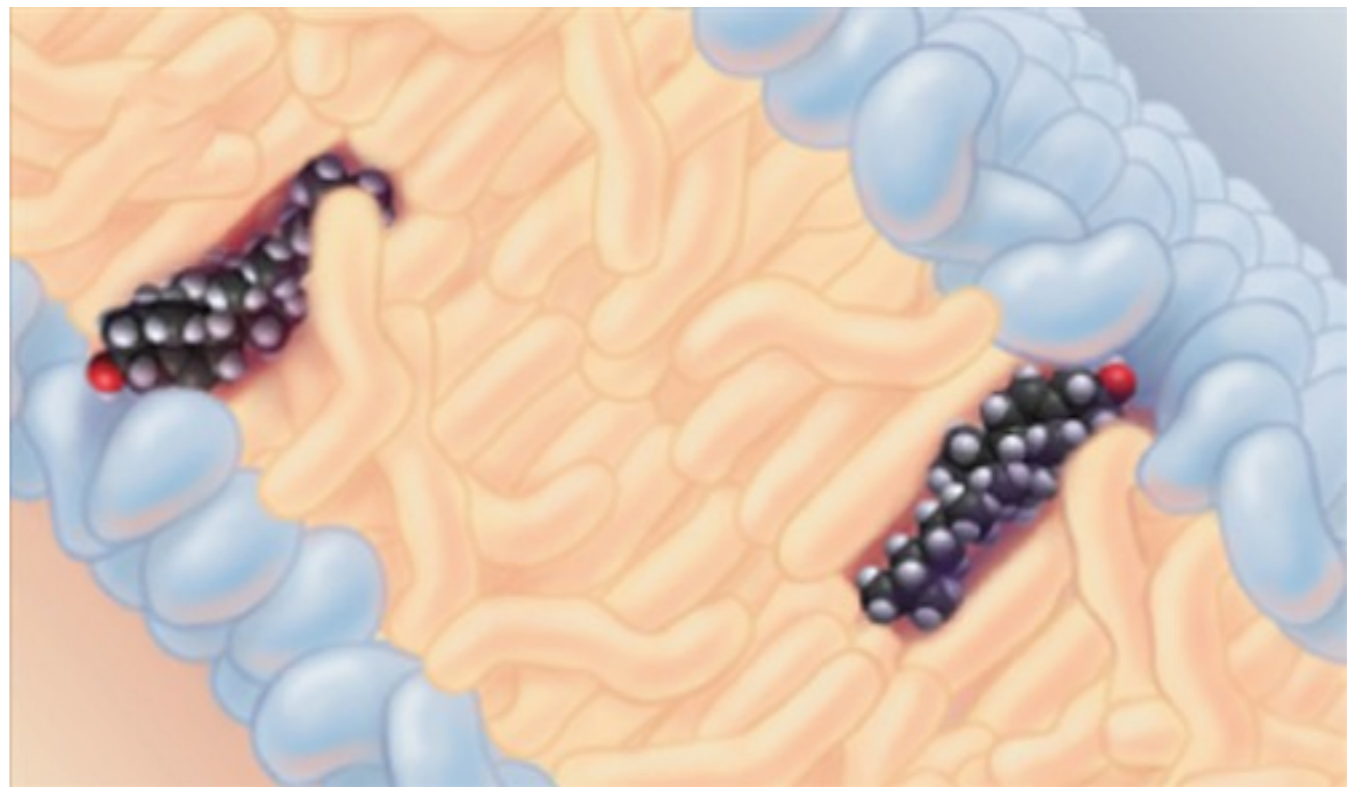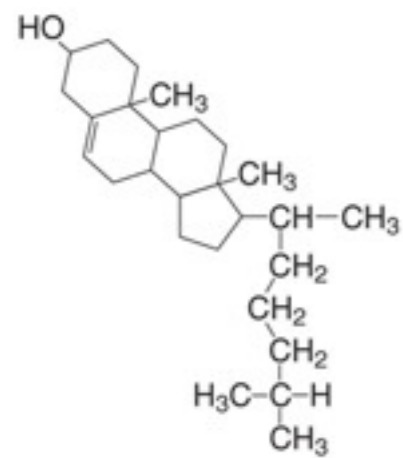
Cholesterol plays a critical role in the function of the cell membrane which has the highest concentration of cholesterol with around 25–30% of lipid in the cell membrane being cholesterol. Cholesterol plays has a role in membrane fluidity but it's most important function is in reducing the permeability of the cell membrane. Cholesterol helps to restrict the passage of molecules by increasing the packing of phospholipids. Cholesterol can fit into spaces between phospholipids and prevent water–soluble molecules from diffusing across the membrane. THe hydrophilic hydroxyl group of cholesterol interacts with aqueous environment, whereas the large hydrophobic domain fits between C–tails of lipids.

Cholesterol is the central component in several metabolic pathways.

Bile synthesis ← → **Steroid synthesis**
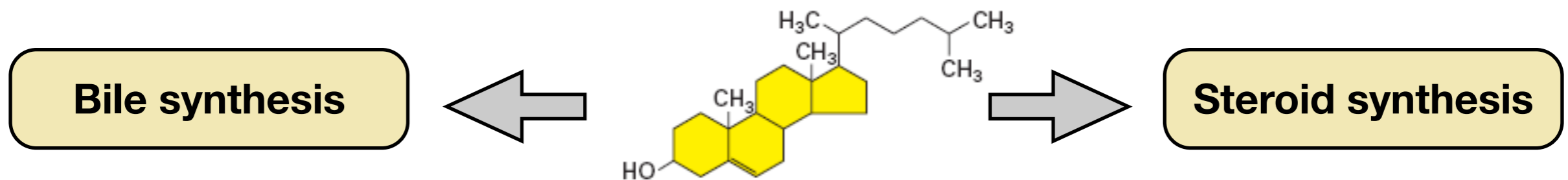
The second important role for cholesterol is as a building block for many other biologically important molecules. Cholesterol is the starting point for the synthesis of bile salts that help digest and solubilize lipids and fats and convert them to a form that can be absorbed by cells in the small intestine. Cholesterol is also the starting point for the synthesis of all steroid hormones, including cortisol, estrogen and testosterone.

Cells synthesize cholesterol and take up cholesterol from external environment.
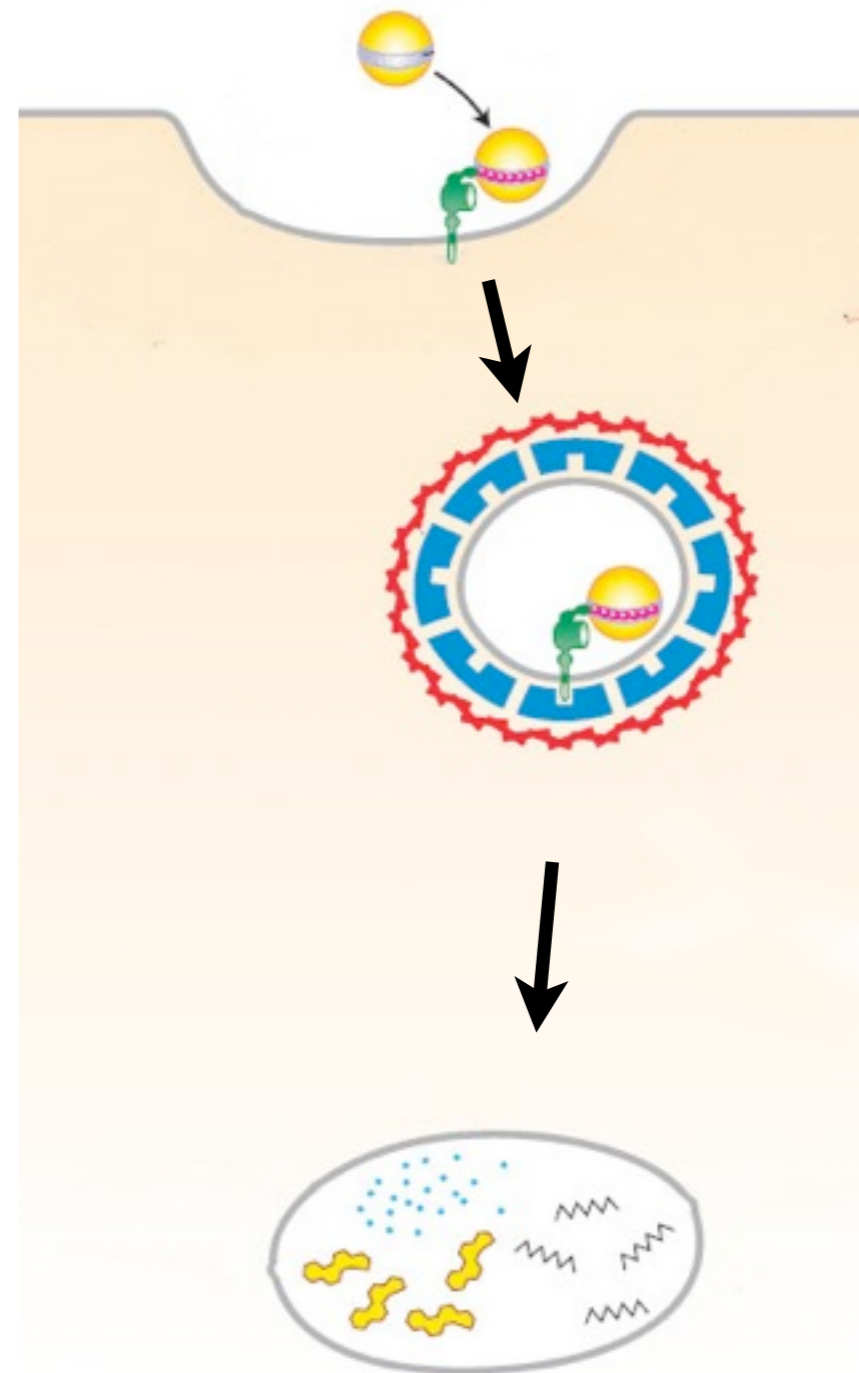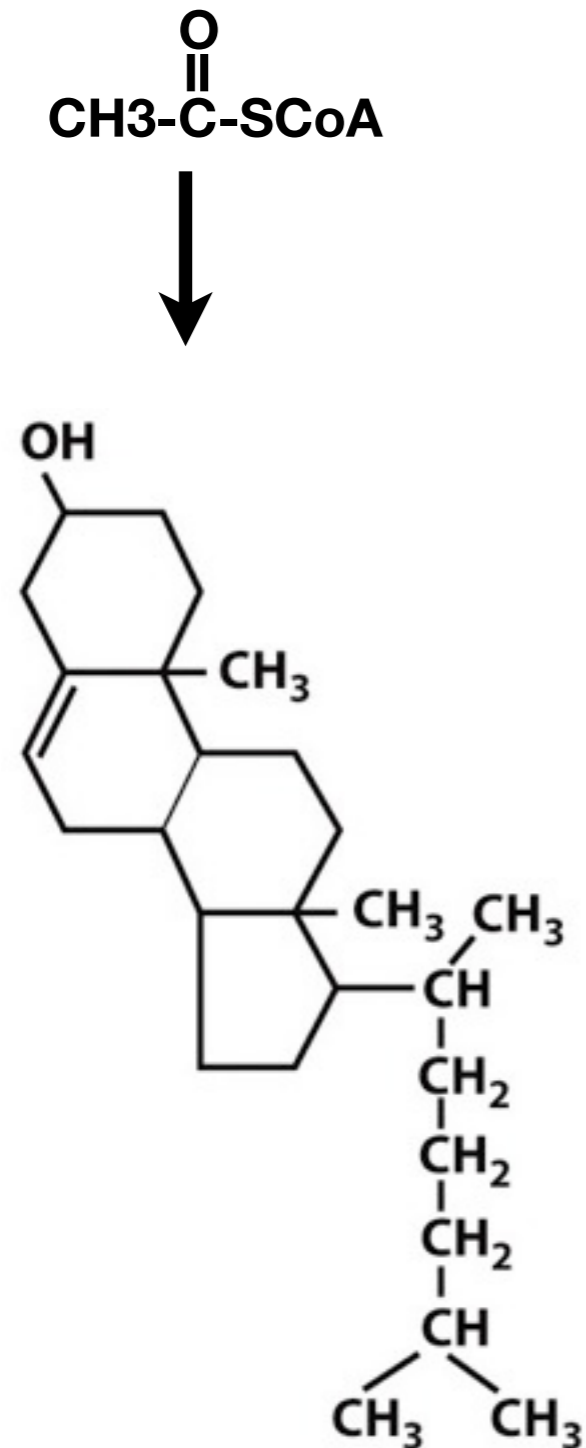
Cells obtain cholesterol from two sources. They can synthesize cholesterol starting from acetyl–CoA or they can take cholesterol up from the surrounding environment. This is the cholesterol that comes from the food we eat. Cells can take up cholesterol from the blood stream by receptor–mediated endocytosis and then process that cholesterol for cellular use. Both mechanisms contribute to total cellular cholesterol. The activities of the two pathways are coordinated. For example, inhibiting the synthesis of cholesterol stimulates the uptake of cholesterol from the blood via endocytosis. This interaction between synthesis and endocytosis is one approach to lower plasma cholesterol concentration.

Regulation of cholesterol synthesis

Cholesterol is made in the ER and delivered by the secretory pathway to plasma membrane and organelles.
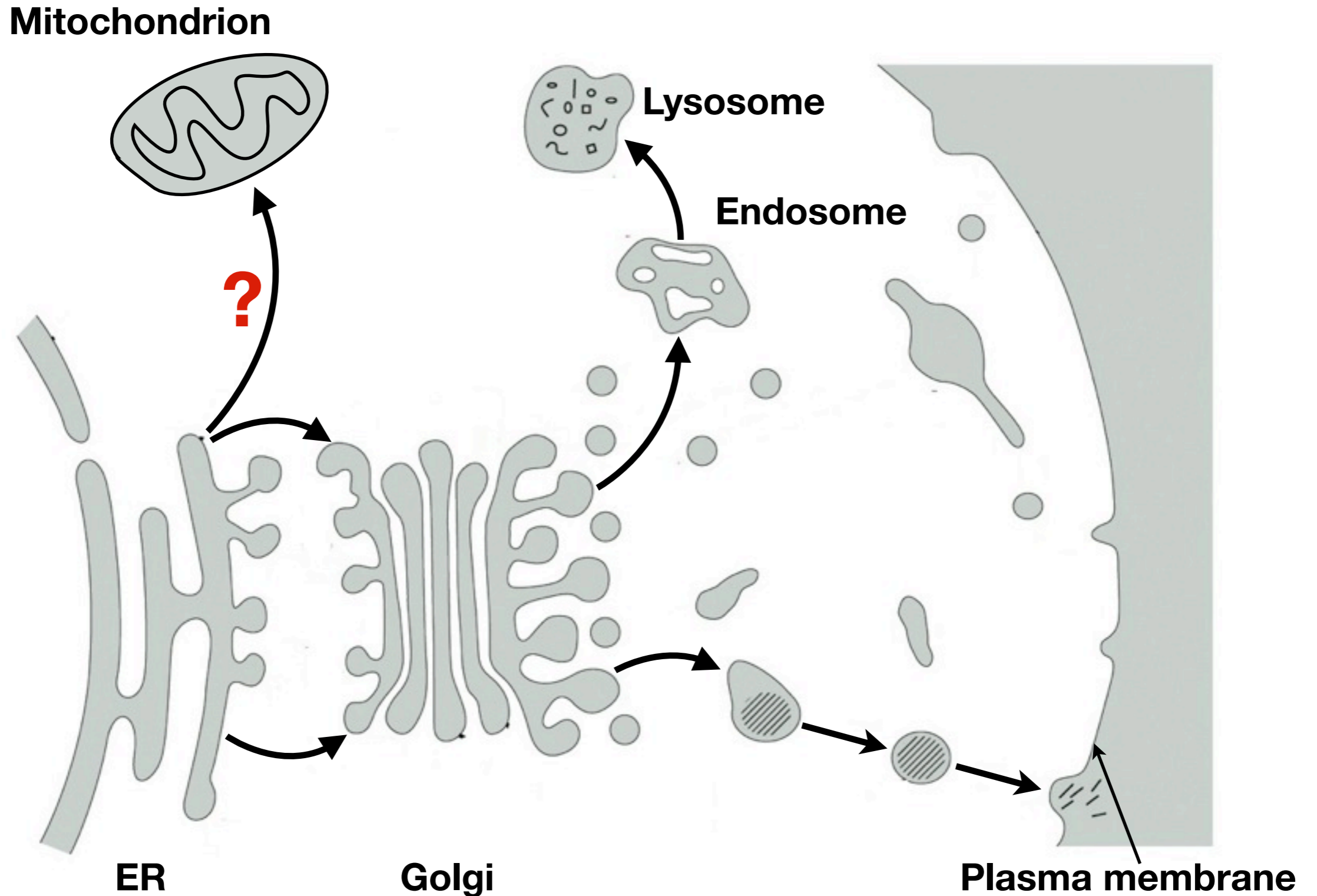
Cholesterol in synthesized in the ER and from there it gets transported to a variety of intracellular organelles. The cholesterol content of ER is very low, so most synthesized cholesterol is moved to other destinations. One destination is the plasma membrane where cholesterol is important for the structure and function of the cell membrane. But cholesterol is also sent to other organelles, including mitochondria. One interesting question is how cholesterol is moved to different parts of the cell. For those organelles that are part of the secretory pathway (e.g. Golgi, lysosomes) vesicular transport can deliver cholesterol. However, mitochondria are not part of the secretory pathway and they also need cholesterol.

Lipid transfer proteins shuttle cholesterol from ER to other membrane compartments.
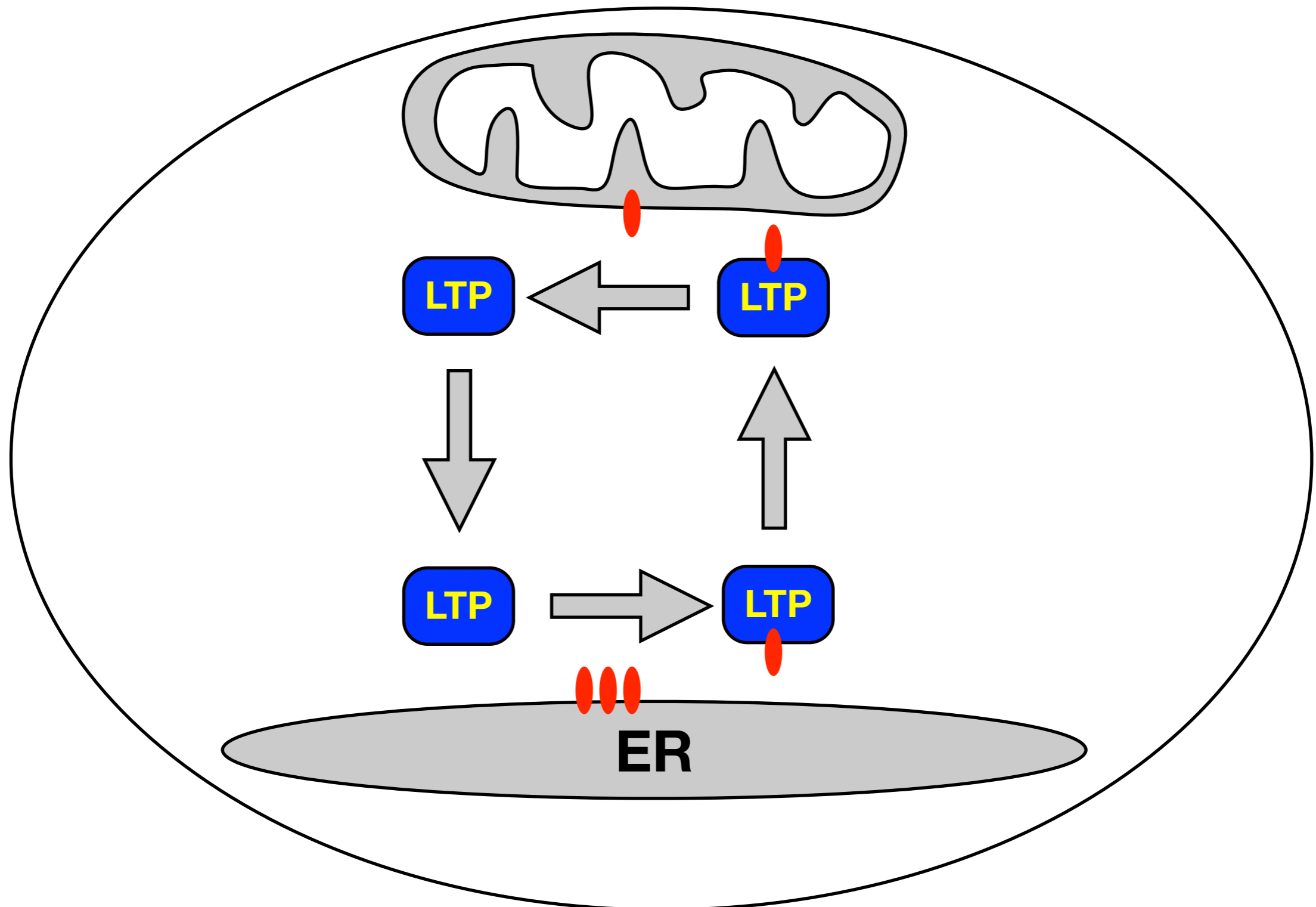

For this reason cells express a set of proteins called lipid transfer proteins that ferry lipids and cholesterol between organelles. These proteins ferry cholesterol from the ER to different intracellular destinations bypassing the secretory pathway. Lipid transfer proteins interact with membranes and bind specific lipids, in this case cholesterol. The proteins diffuse to target membrane, the mitochondrial membrane, and release into the membrane.

# Cytosolic and ER enzymes synthesize cholesterol from acetyl-CoA.
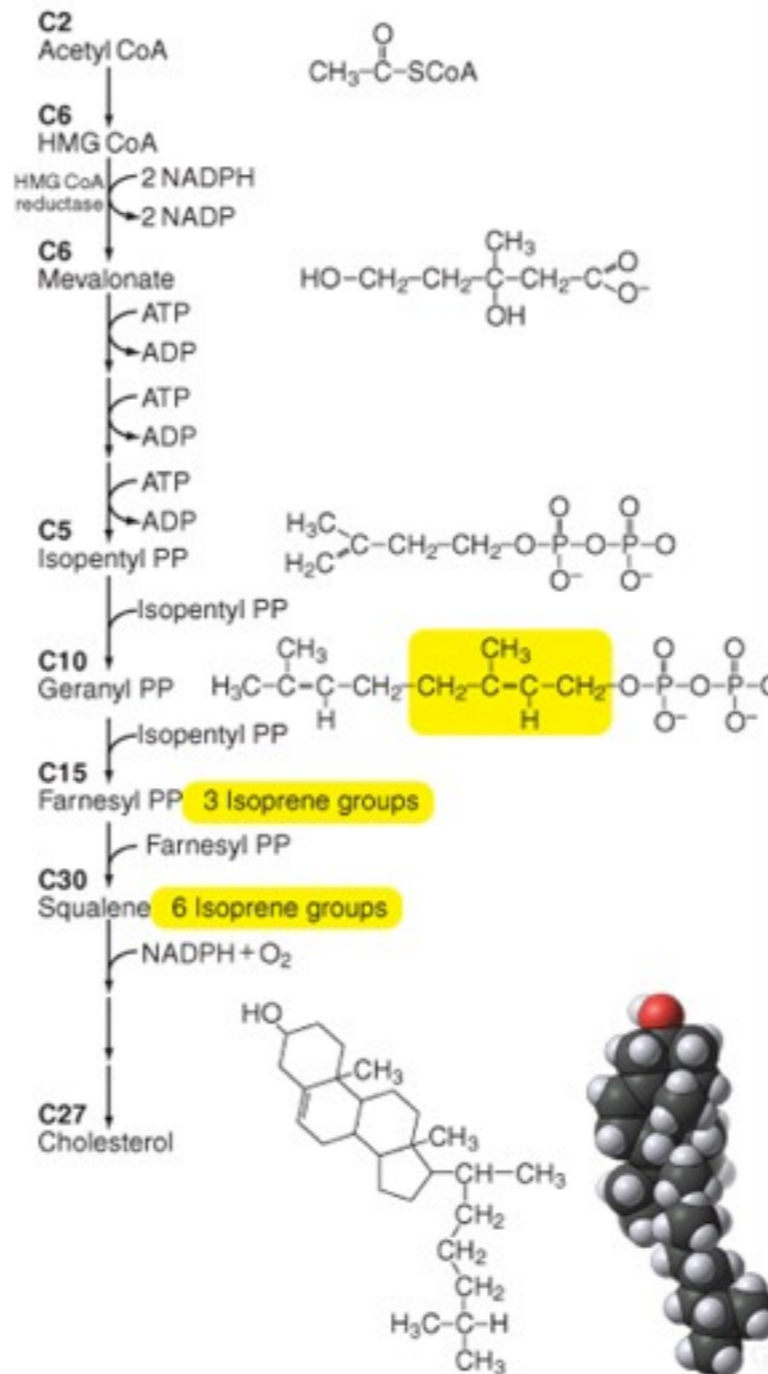
Cholesterol synthesis is a multistep process, a few of which are shown here. What's remarkable is that all the carbons in cholesterol come from one molecule: acetate in the form of acetyl–CoA. As mentioned most of the reactions take place on the outer leaflet of the ER, but a few of the initial steps occur in the cytosol. One critical step is the conversion of HMG–CoA to mevalonate. This is the rate limiting step in the synthesis of cholesterol. The reaction is catalyzed by an enzyme called HMG CoA reductase. The reason rate–limiting steps gain a lot of attention is that they are inviting targets to slow the rate of the entire reaction.

HMG-CoA reductase catalyzes the rate-limiting step in the synthesis of cholesterol.
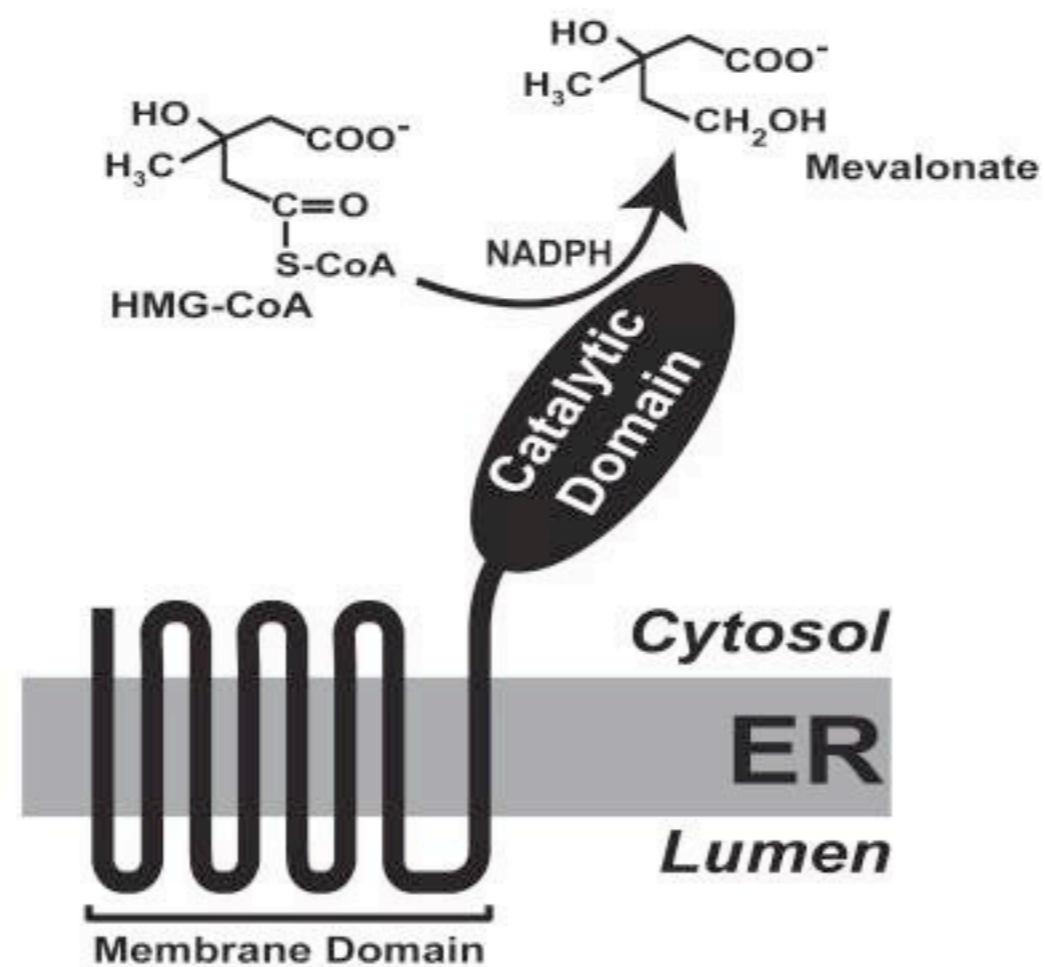

HMG–CoA reductase is a membrane protein with multiple transmembrane domains and catalytic domain that faces the cytosol. This domain converts HMG–CoA into mevalonate. The membrane domains are not simply a way of anchoring HMG–CoA in the ER but they play an important regulatory role. The membrane domains are sterol–sensing domains, as they interact with sterols and change conformation. This will be important for how the cell down regulates HMG–CoA reductase when cholesterol levels are high. One class of inhibitors of HMG–CoA reductase, the statins, function as molecular mimics of HMG–CoA and compete for binding to HMG–CoA reductase. The statins thereby inhibit the synthesis of mevalonate and slow the rate of cholesterol synthesis. To account for the reduction of cholesterol synthesis, cells increase the rate of uptake of cholesterol from outside thereby lowering the amount of cholesterol in the blood.

# Elevated levels of cholesterol target HMG-CoA reductase for proteolysis.
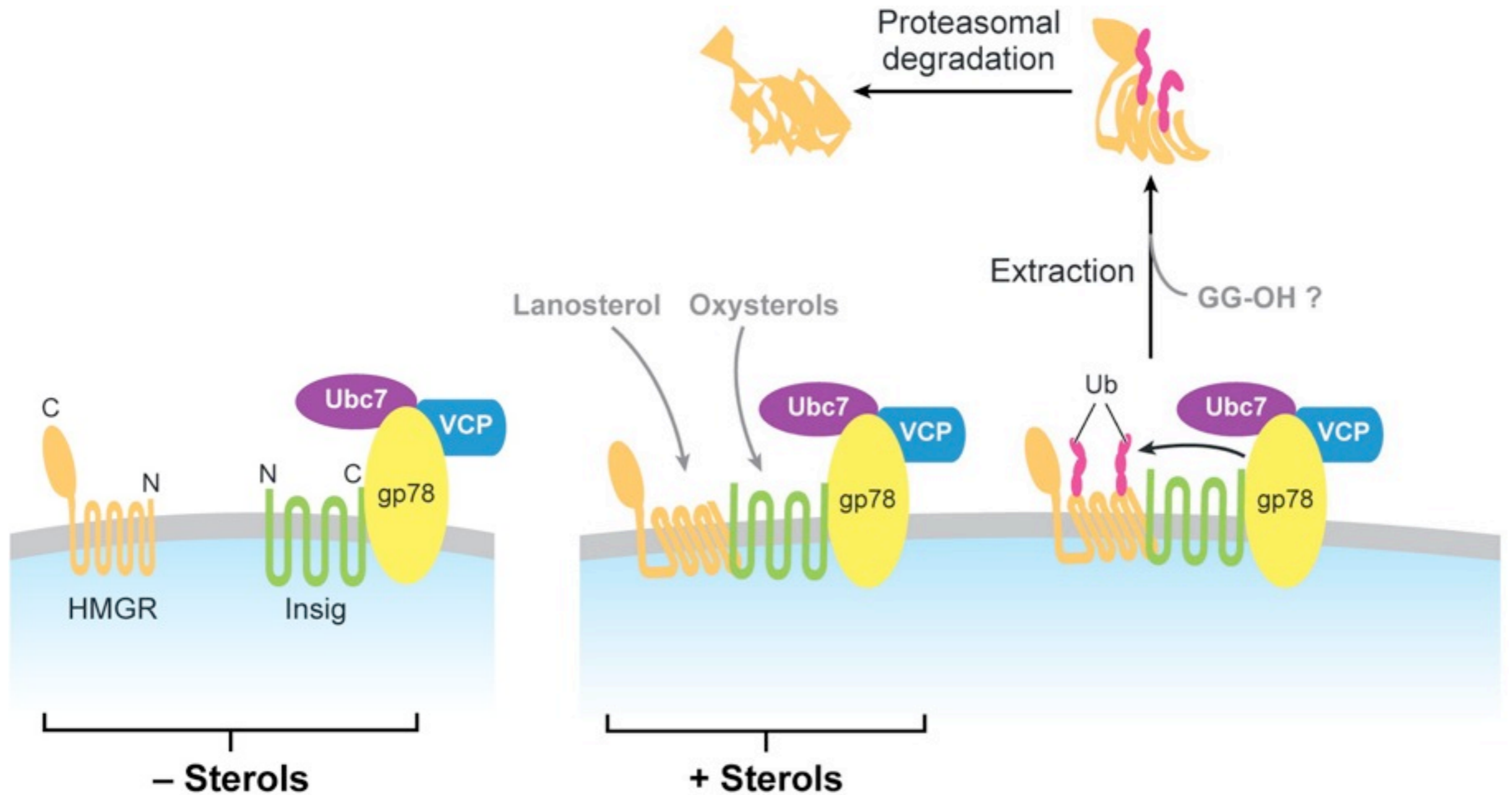
Cells also decrease HMG–CoA reductase activity when cholesterol levels are high. The transmembrane domains in HMG–CoA reductase are sterol sensing domains. They interact with an intermediary product in the cholesterol biosynthesis pathway, lanosterol. When cholesterol levels are high, lanosterol accumulates in the membrane of the ER and binds the transmembrane domains of HMG–CoA reductase. Upon binding lanosterol, the transmembrane domains undergo a conformational change that allows them to bind to Insig. Insig in the ER membrane is associated with a set of proteins that will lead to the degradation of HMG–CoA reductase. One of these proteins, gp78, is a ubiquitin ligase that transfers ubiquitin onto HMG–CoA reductase. The protein VCP is an ATPase that by an unknown mechanism extracts HMG–CoA reductase from the ER and allows it to interact with the proteosome for digestion. When cholesterol levels are low, HMG–CoA reductase does not interact with Insig.

Cellular cholesterol levels regulate expression of enzymes in cholesterol synthesis pathway.
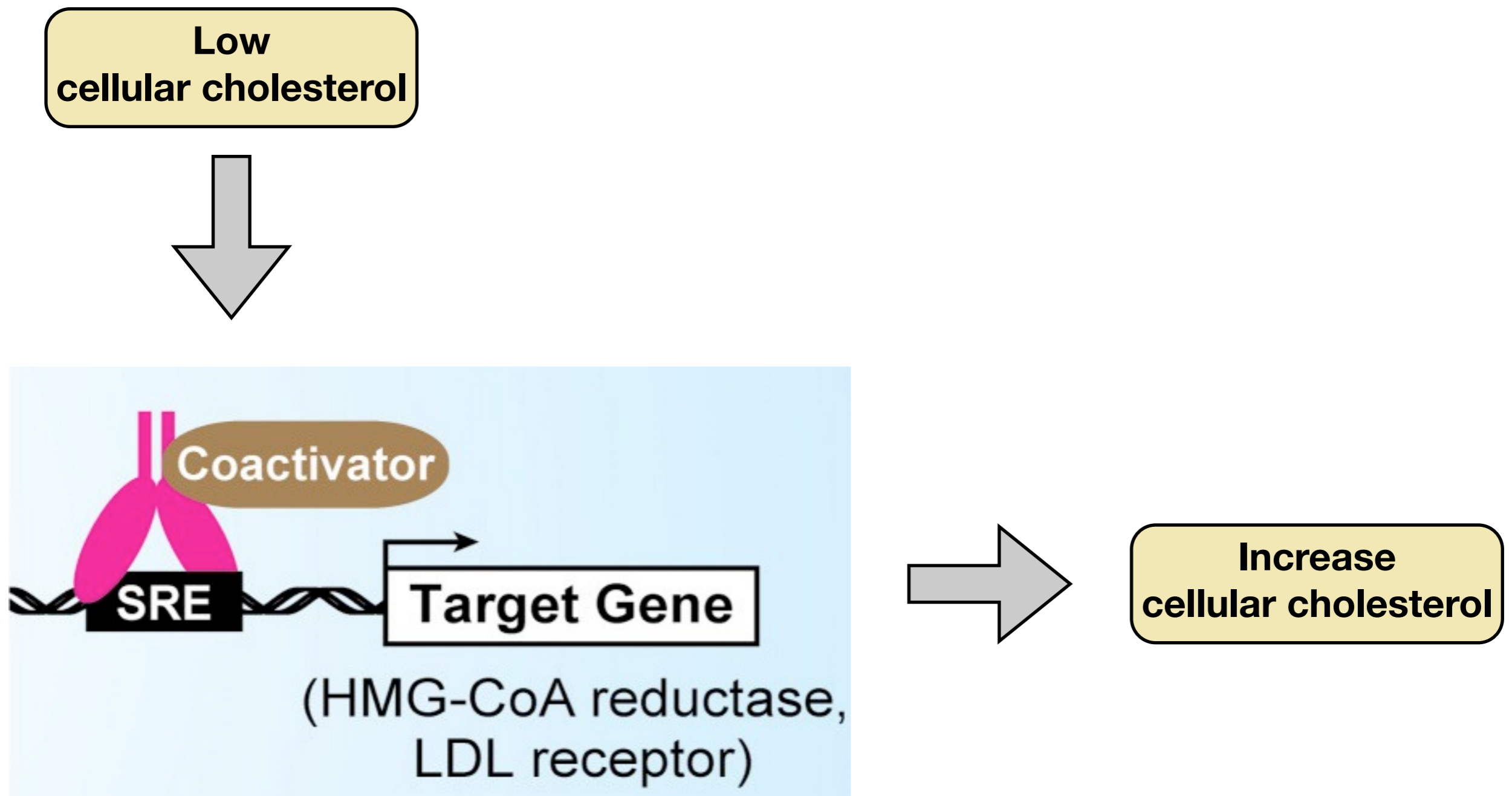

When cells are faced with low cholesterol levels, they respond by increasing the gene expression of proteins that stimulate biosynthesis of cholesterol, such as HMG-CoA reductase, and proteins that increase the uptake up cholesterol from the external environment, LDL receptor. These genes contain a common upstream regulatory element called the sterol response element that binds a transcriptional activator, called the sterol response element binding protein. When bound to the element, the binding proteins turns on transcription of downstream gene. So, a key question is how the cell regulates the binding of the binding protein to the response element and how that regulation is sensitive to cholesterol levels.

Sterol response element binding protein localizes to the ER in the presence of cholesterol.
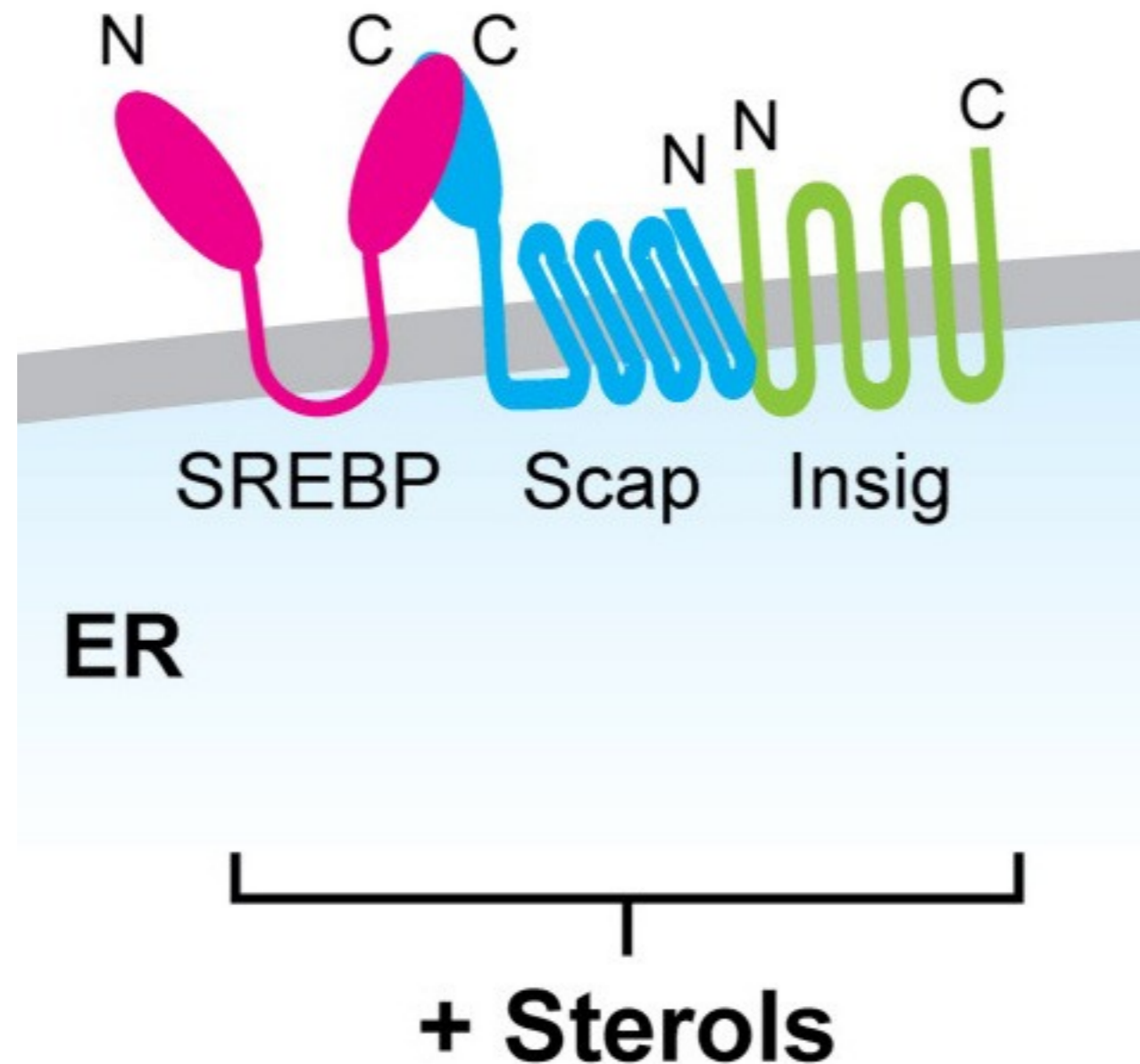

In the presence of high cholesterol, the SREBP is localized to the ER membrane as a transmembrane protein. The protein is held in the ER through an interaction with Scap, which is a sterol sensing protein. The transmembrane domains of Scap associate with cholesterol and assume a conformation that allows them to bind to Insig. Insig anchors Scap and SREBP in the ER and prevents them from escaping the ER via vesicular transport. So, when cholesterol levels are high, the trimeric complex forms and SREBP is kept in the ER. Clearly, in this state SREBP cannot enter the nucleus to turn on expression of cholesterol synthesis genes.

Low levels of cholesterol allow SRBEPs to leave the ER and traffic to the Golgi.
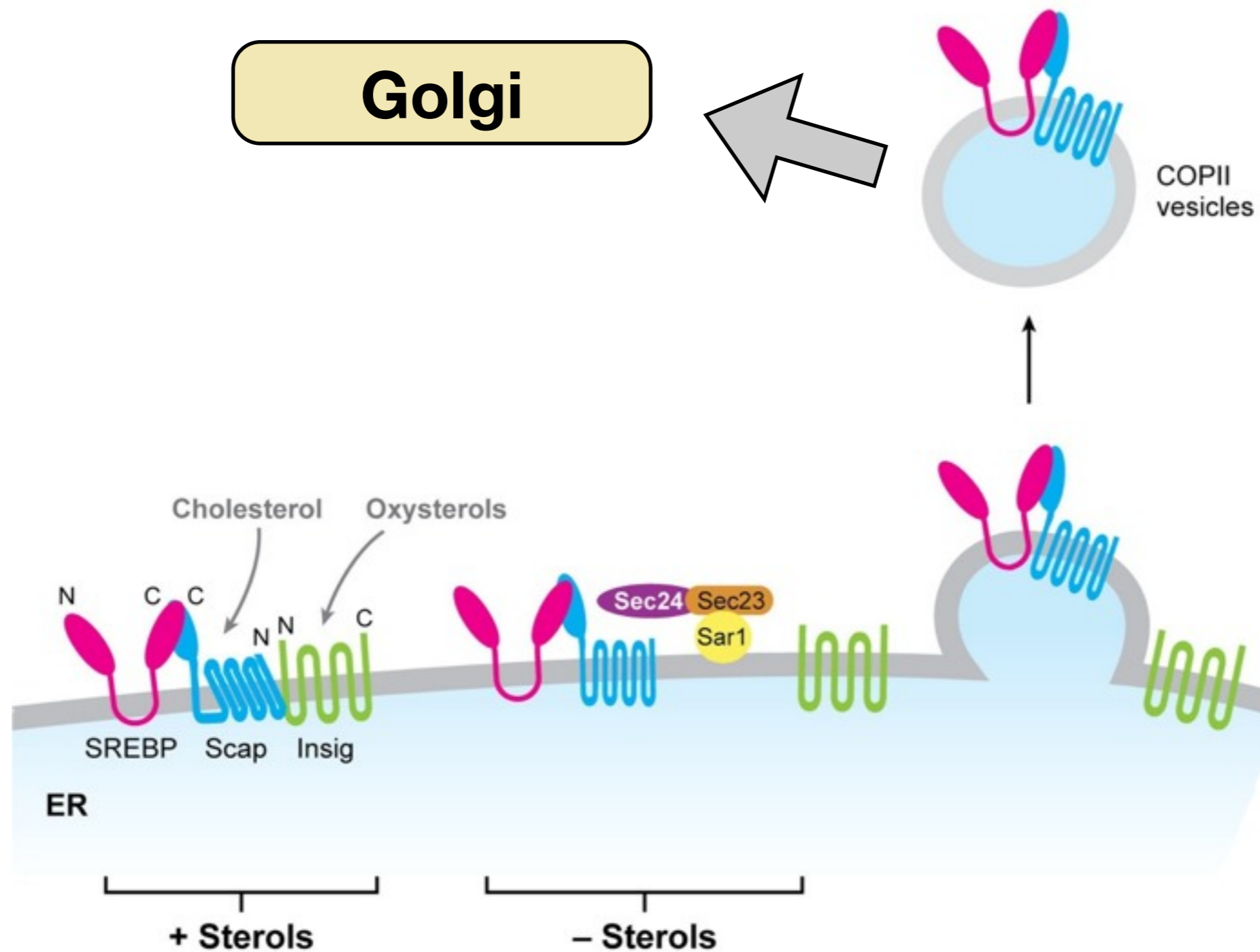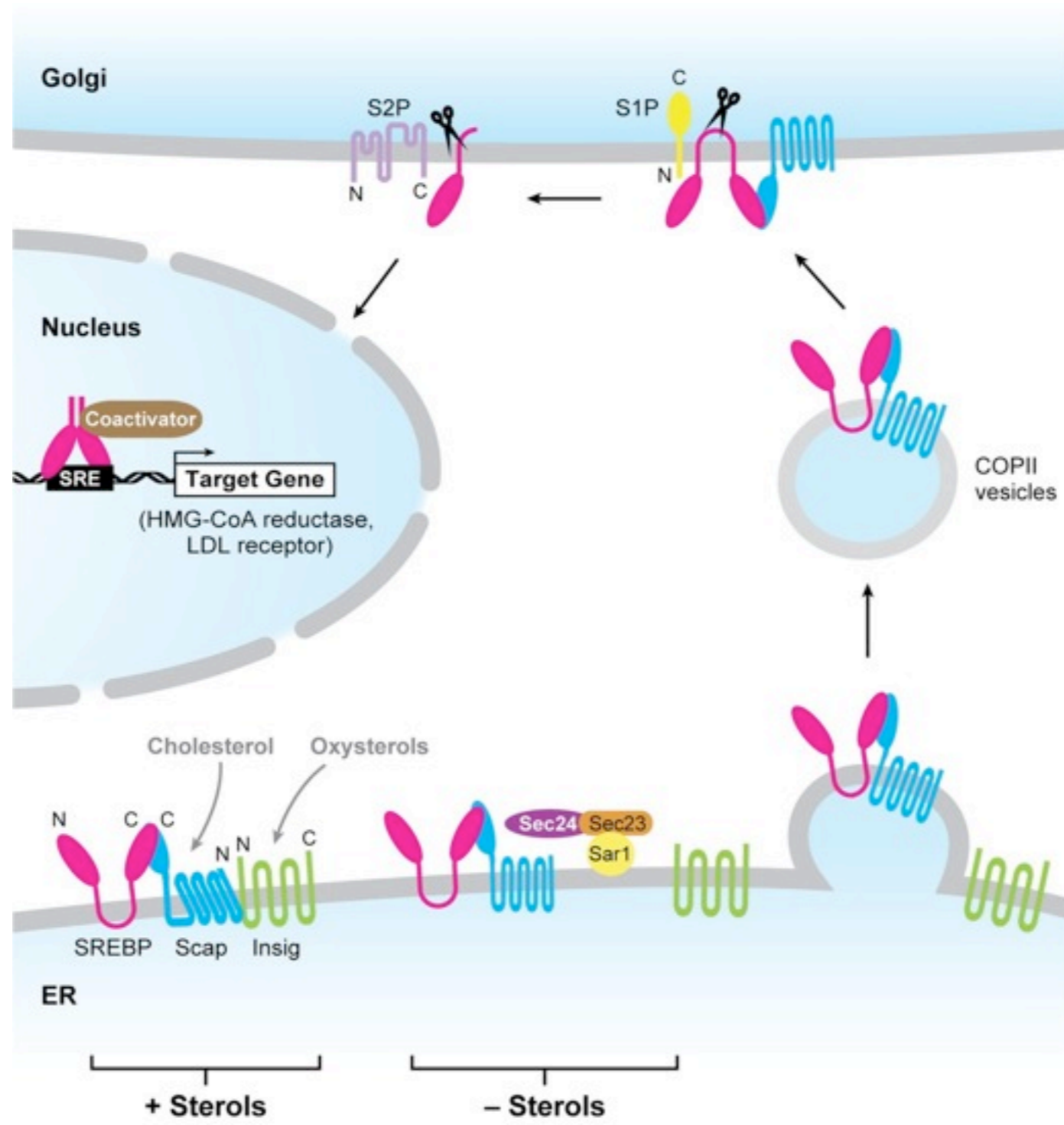

When cholesterol levels fall, the transmembrane domains of Scap are no longer associated with cholesterol and undergo a conformational change that dissociates Insig. No longer bound to Insig, the SREBP–Scap are free to enter COP II coated vesicles that bud from the ER and traffic to and fuse with the Golgi. So, the net effect of low cholesterol is to allow SREBP to move to the Golgi.

Proteases in Golgi release transcription factor that activates gene expression.
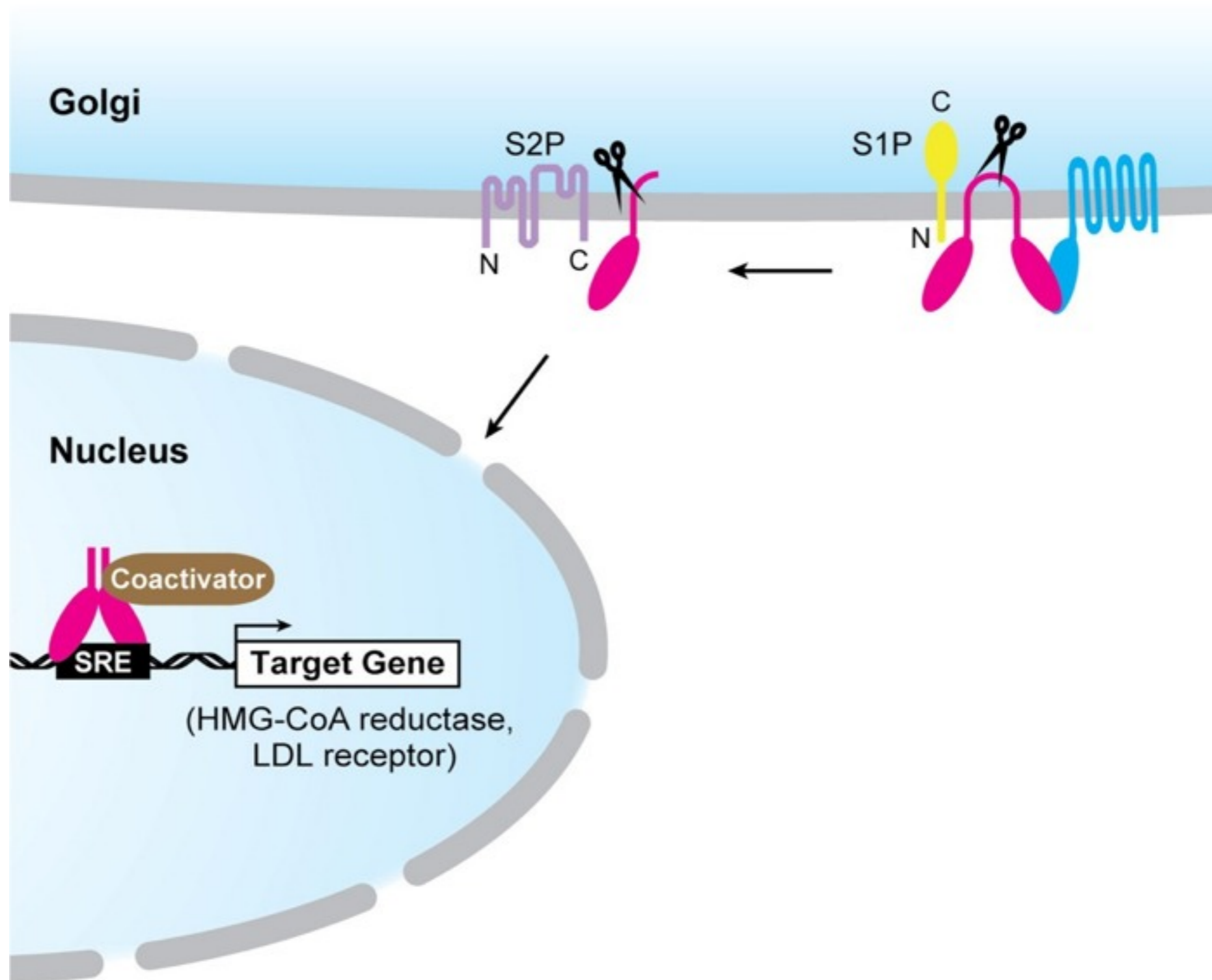

Two proteases await the arrival of SREBP called site 1 and site 2 protease. Site 1 protease cleaves SREBP in the lumenal loop, yielding two single transmembrane proteins. Site 2 protease cleaves SREBP within the transmembrane domain, allowing SREBP to escape from the Golgi membrane and enter the cytosol. SREBP contains an NLS that mediates its import into the nucleus where it can bind SRE and activate gene expression.

Golgi

S2P

S1P

N C

N C

Nucleus

Coactivator

SRE   Target Gene

(HMG-CoA reductase,
LDL receptor)

COPII
vesicles

Cholesterol   Oxysterols

Sec24 Sec23

Sar1

N   C C

N   N   C

SREBP   Scap   Insig

ER

+ Sterols   – Sterols

Uptake of dietary cholesterol

Plasma lipoproteins carry cholesterol and other lipids throughout the body.

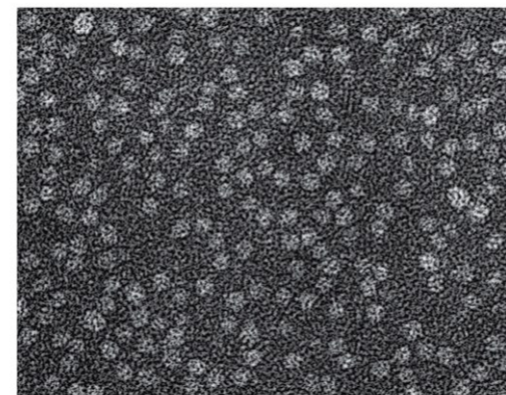| Major Class of Serum Lipoproteins						
		Composition (wt%)				
Lipoprotein	Density (g/ml)	Protein	Phospholipids	Free Cholesterol	Cholesteryl esters	Triacylclyerols
Chylomicrons	<1.006	2	9	1	3	85
VLDL	0.95-1.006	10	18	7	12	50
LDL	1.006-1.063	23	20	8	37	10
HDL	1.063-1.210	55	24	2	15	4
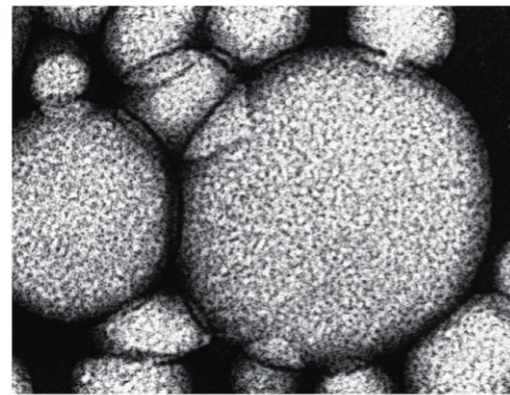
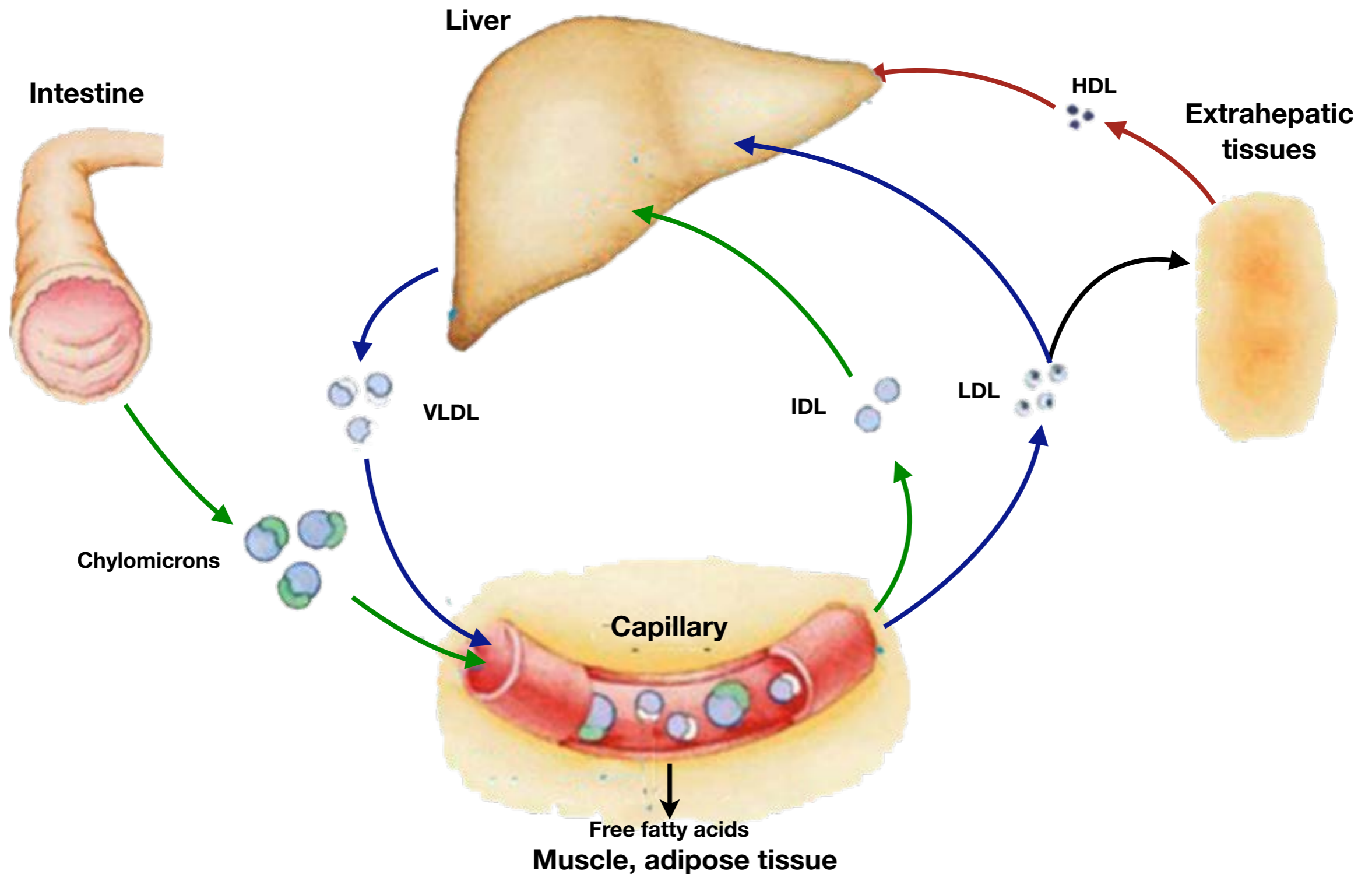**Chylomicrons (×60,000)**
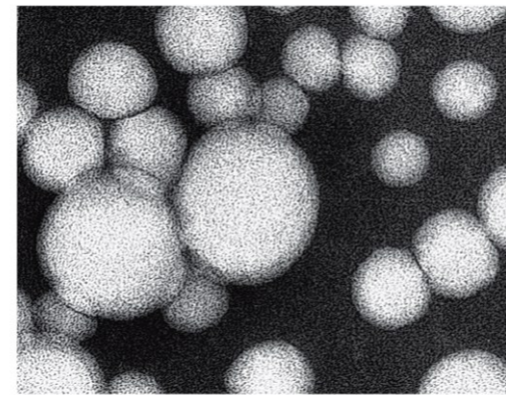
VLDL (×180,000)
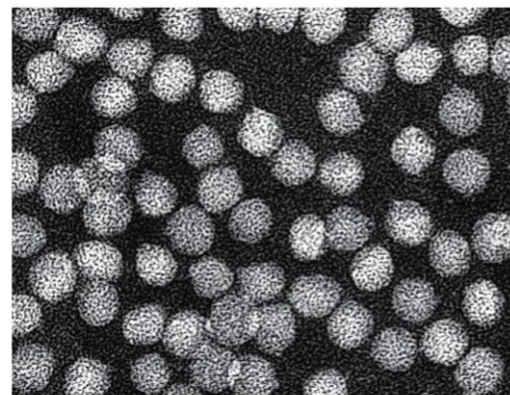
LDL (×180,000)


HDL (×180,000)

Cholesterol makes its way through the body in a set of different lipoprotein complexes. There are four major lipoprotein complexes with different sizes and different compositions of phospholipids, cholesterol and triglycerides. Note that LDL has the highest percentage of cholesterol of all the lipoproteins. In contrast, HDL has a relatively lower percentage of lipid compared to the other lipoproteins. LDL is often referred to as bad cholesterol and HDL as good cholesterol but both have the same cholesterol just different relative amounts.

# Dietary cholesterol is transported from the small intestine to the liver and then delivered to cells.

Now we'll look at how cholesterol is moves through the body in these different lipoprotein complexes. After being absorbed by the intestine, cholesterol is packaged into chylomicrons that enter the lymphatic system and then the blood stream. As chylomicrons pass through the capillaries of muscle and adipose tissue, enzymes in the walls of the capillaries extract triglycerides from the chylomicrons. The triglycerides are used by muscle or stored in adipose tissue. After removing triglyceride,s the lipoprotein is called a chylomicron remnant. The lipoprotein then travels to the liver where hepatocytes in the liver endocytose the remnant and extract the lipids, cholesterol and triglycerides. These are repackaged into lipoproteins called VLDL that contain a mix of lipids, cholesterol and triglycerides. In the blood, the muscle and adipose tissue remove much of the triglycerides from the VLDL, converting it to LDL. LDL is the lipoprotein that cells can internalize to obtain cholesterol. When they contain too much lipid and cholesterol, they package the excess into HDL. The liver can take up HDL and excrete the excess cholesterol in bile or repackage it for delivery to other cells.

# Enterocytes absorb and process cholesterol and release it in chylomicrons.
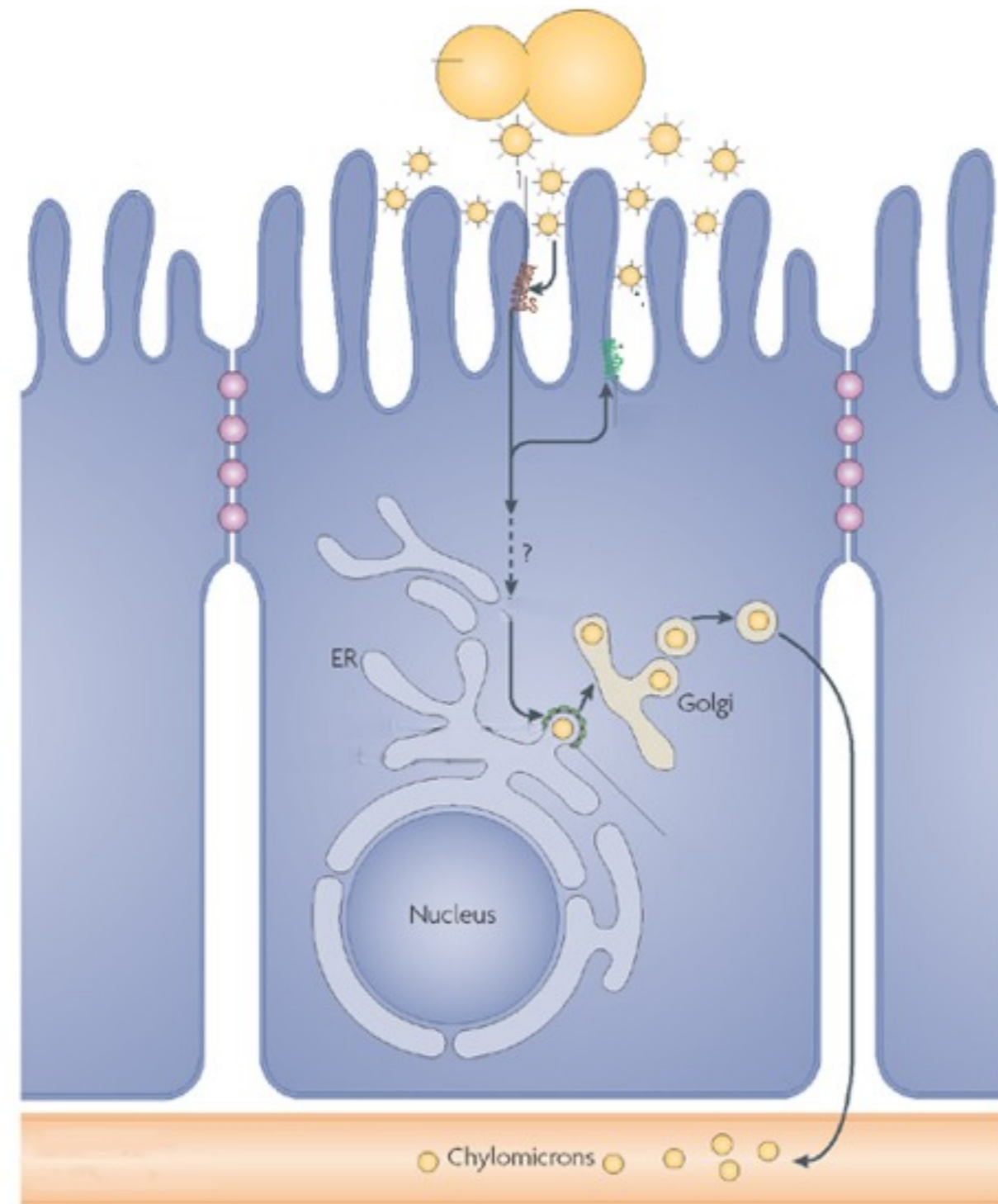
The epithelial cells that line the small intestine absorb cholesterol from the food we eat. The first step in this process is to breakdown the fat into smaller particles from which lipids and cholesterol can be extracted. Fats are digested by lipases and solubilized by bile from the liver. The combination of lipases and bile converts fat into small miscelles that contain a mixture of lipids, triglycerides and cholesterol. Lipids and cholesterol were originally thought to diffuse through the plasma membrane of enterocytes but there is now evidence that protein channels facilitate the passage across the plasma membrane. Inside the cell, cholesterol is delivered to the ER where the cell begins assembly of chylomicrons. Chylomicrons increase in size as the pass through the secretory pathway and are eventually secreted into the basolateral space. Because chylomicrons are so large, they cannot enter the blood stream through blood vessels but instead enter the lymphatic system. This is crucial for the processing of chylomicrons because blood from the intestine flows directly to the liver, bypassing the muscle and adipose tissue that utilize the triglycerides in chylomicrons. By entering the lymphatic system, chylomicrons reenter the blood via the thoracic duct and are then exposed to adipose and muscle tissue before reaching the liver.

Lipid transporters facilitate cholesterol and lipid entry into the cell.
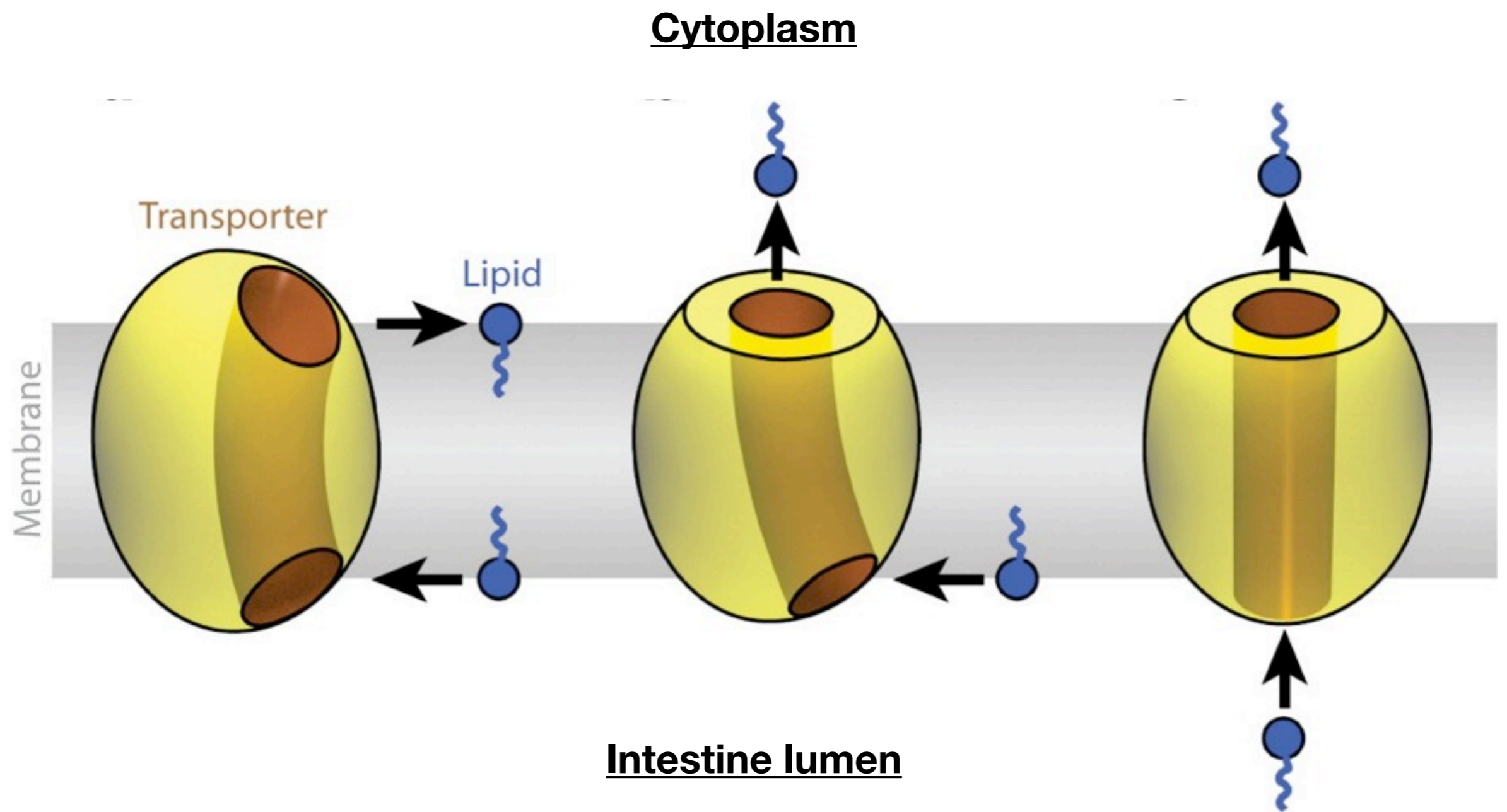
As mentioned, lipids and cholesterol are thought to enter enterocytes through proteins in the plasma membrane. These proteins appear to form channels in the plasma membrane but the exact mechanism by which lipids and cholesterol move from one side the other is not clear. One mechanism is that cholesterol diffuses into the outer leaflet of the plasma membrane and then use the channel to flip to the inner leaflet or enter the cytosol to be bound by a carrier protein. An alternative mechanism is that cholesterol simply traffics through the channel without entering the plasma membrane.
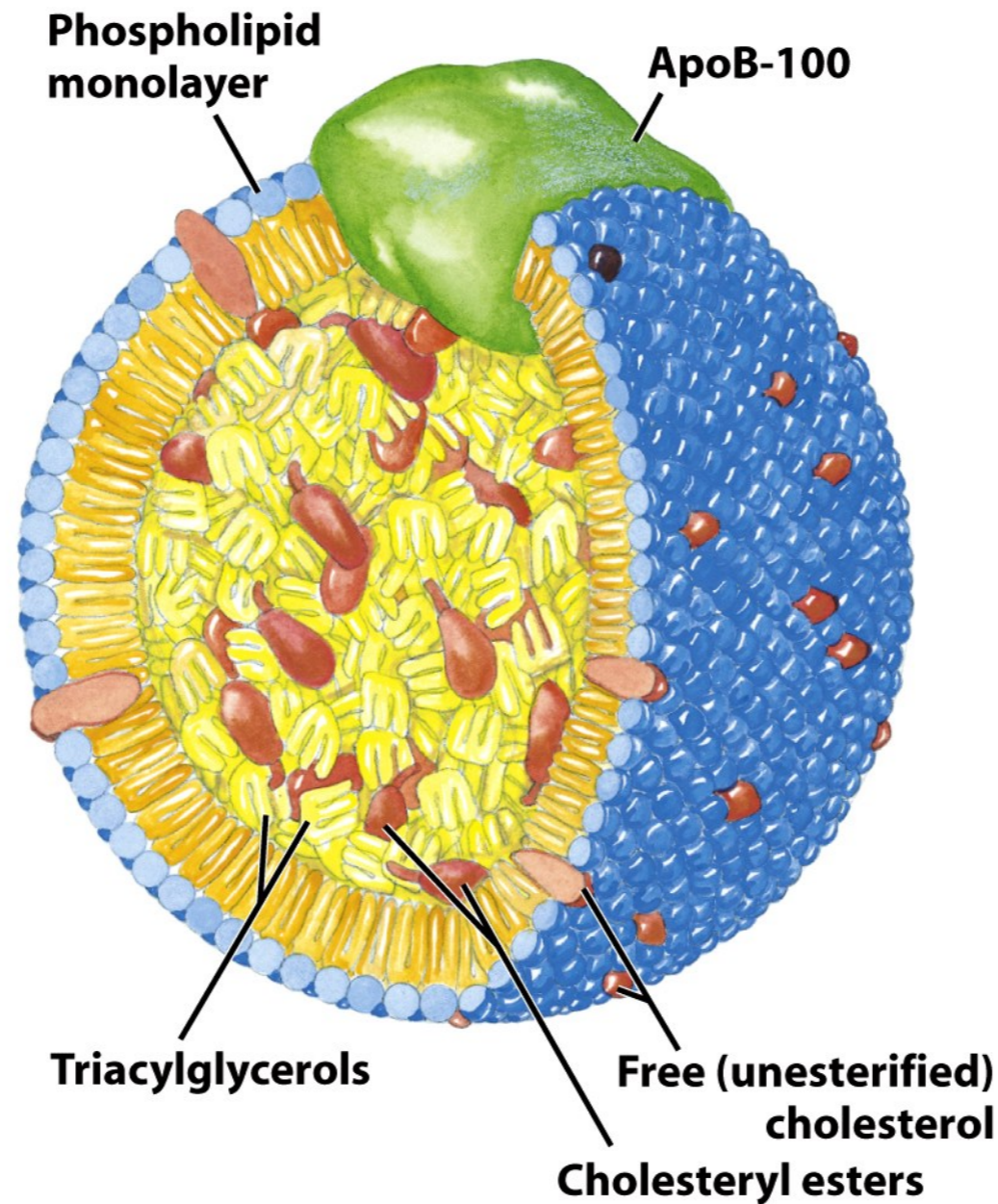
Niemann-Pick Like Protein is critical for intestinal absorption of cholesterol.
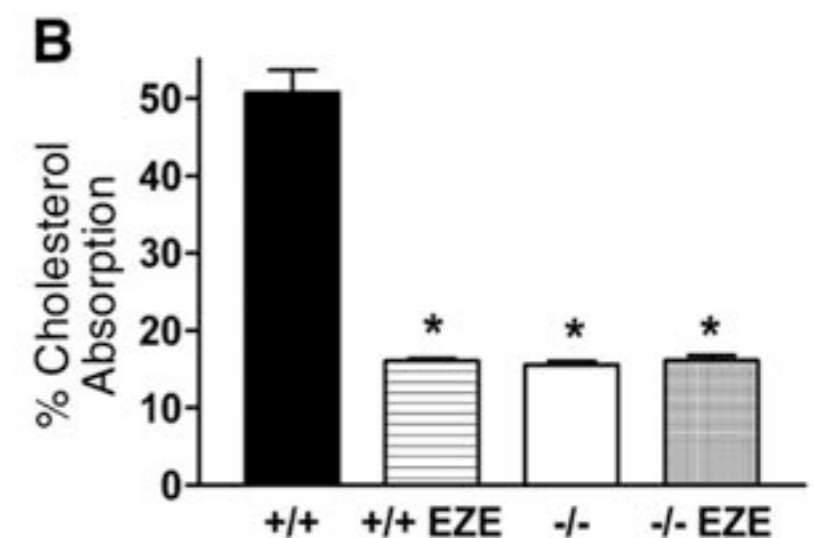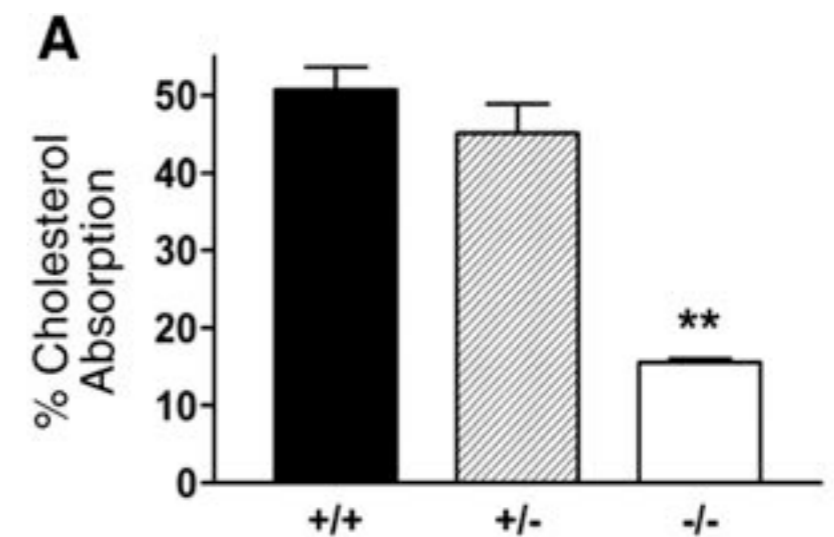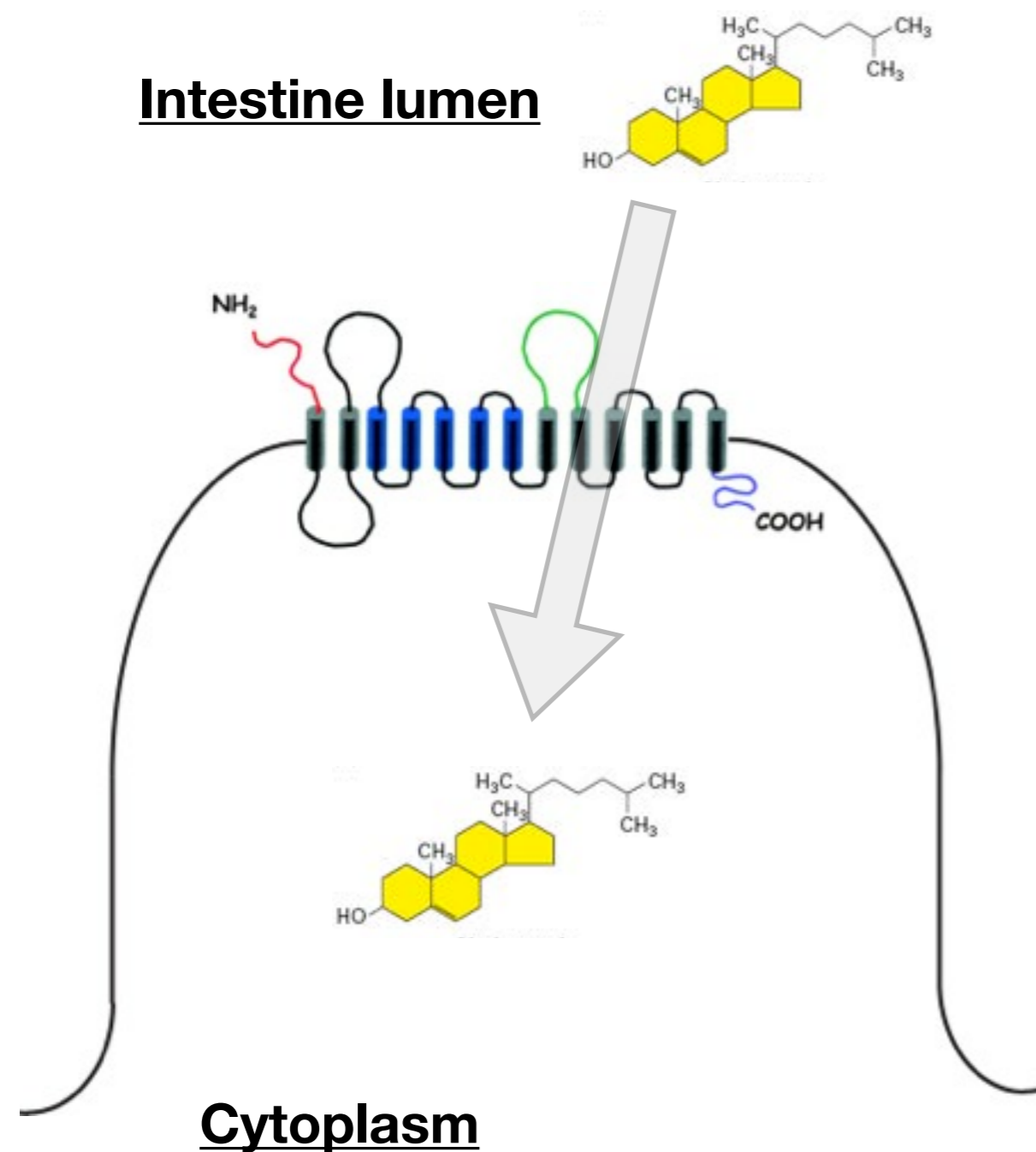
The channel responsible for allowing cholesterol to enter enterocytes has been identified, Niemann–Pick Like protein. It's a protein with 13 transmembrane domains that localizes to the plasma membrane of enterocytes. Some of the transmembrane domains interact with cholesterol. Importantly, mice, in which the both genes for Niemann–Pick Like protein were knocked out, show much lower cholesterol absorption across the intestine. Interestingly, the knock out animals show a similar reduction in cholesterol absorption as wild type animals treated with ezetimibe. Ezetimibe is currently in use as a cholesterol–lowering drug and recent work shows that ezetimibe binds directly to Niemann–Pick Like protein, suggesting that its mechanism of action is to inhibit the activity of this cholesterol transporter.

Cellular uptake and processing of LDL

Cells take up cholesterol by endocytosis of LDL from the blood stream.

Phospholipid monolayer

ApoB-100

Triacylglycerols

Free (unesterified) cholesterol

Cholesteryl esters

This cartoon shows the structure of LDLs. The center contains mixture of triglycerides and cholesterol esters. The outer shell contains a monolayer of phospholipid. Note that membranes contain a phospholipid bilayer because the interact with an hydrophilic environment on their outside and inside. In contrast the inner surface of lipoprotein is hydrophobic so a monolayer allows the hydrophobic tails to interact with the core whereas the hydrophilic head groups interact with the aqueous environment. Apoprotein associates with LDL to increase its solubility. Cells contain receptors that bind Apoprotein.

The LDL receptor binds LDLs and is taken up by clathrin-mediated endocytosis.
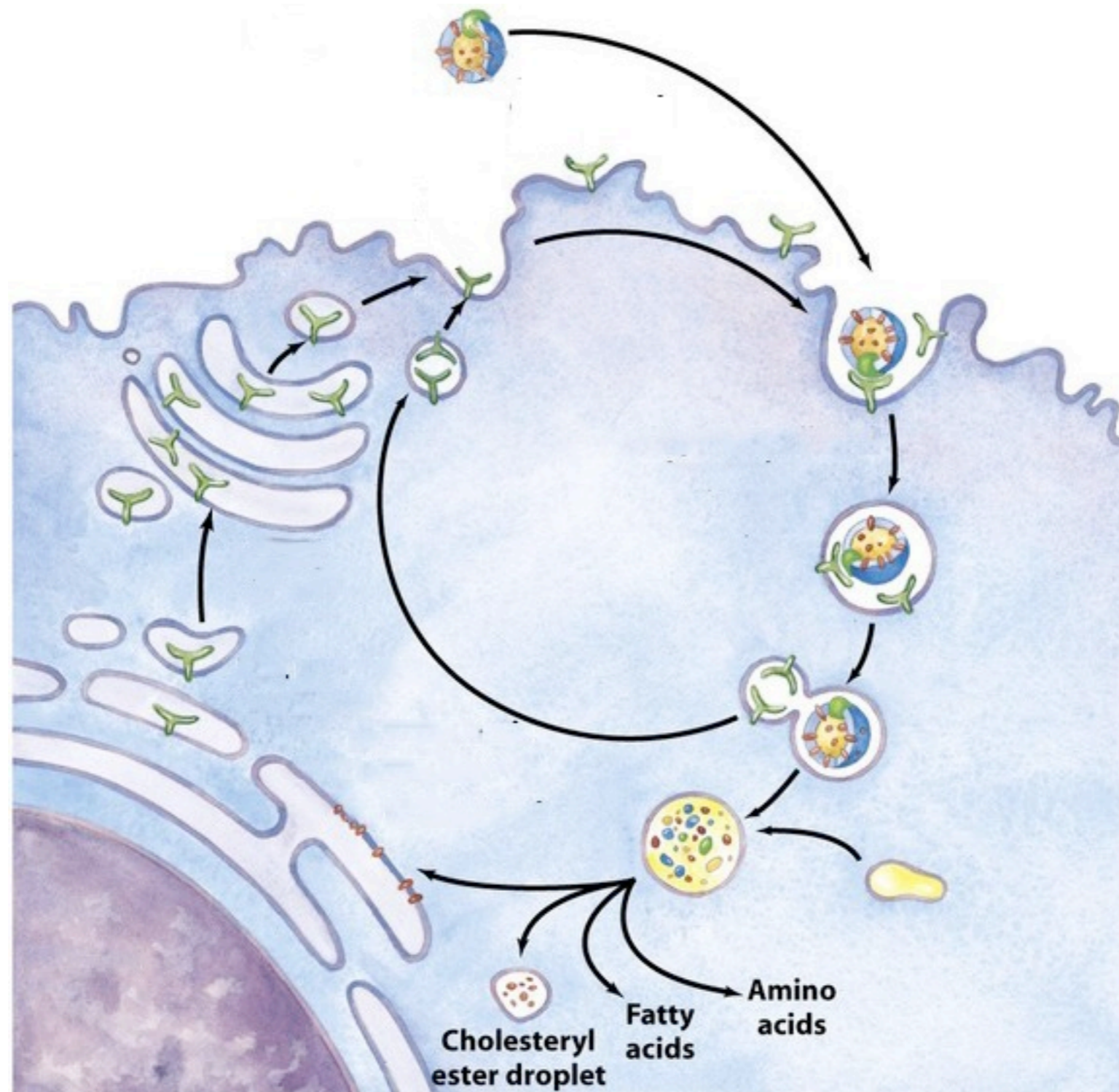

The uptake of LDL into cells is classic example of receptor–mediated endocytosis. Cells express LDL receptor on their plasma membrane. The receptor binds to sites on Apoprotein in LDL. Bound receptors cluster in coated pits and are then endocytosed by clathrin. The endocytic vesicles acidify to become endosomes and the low pH causes a conformational change in the LDL receptor which releases LDL. The LDL receptor is sorted into vesicles that return the receptor to the cell membrane. The remaining endosome fuses with the lysosome where proteases and lipases can digest the lipoprotein. The component parts are then targeted to their appropriate subcellular location. For cholesterol, it most likely returns to the ER but could also be trafficked to mitochondria for synthesis of steroids. Cells can efficiently remove LDL from serum if they express and adequate amount of LDL receptor on their cell surface and can endocytose LDL receptor. Several genetic mutations in the receptor reduce its numbers at the cell membrane, leading to excess serum LDL and hypercholesterolemia.

PCSK9 binds LDL receptor and targets it to lysosomes, reducing cells' ability to take up LDL.
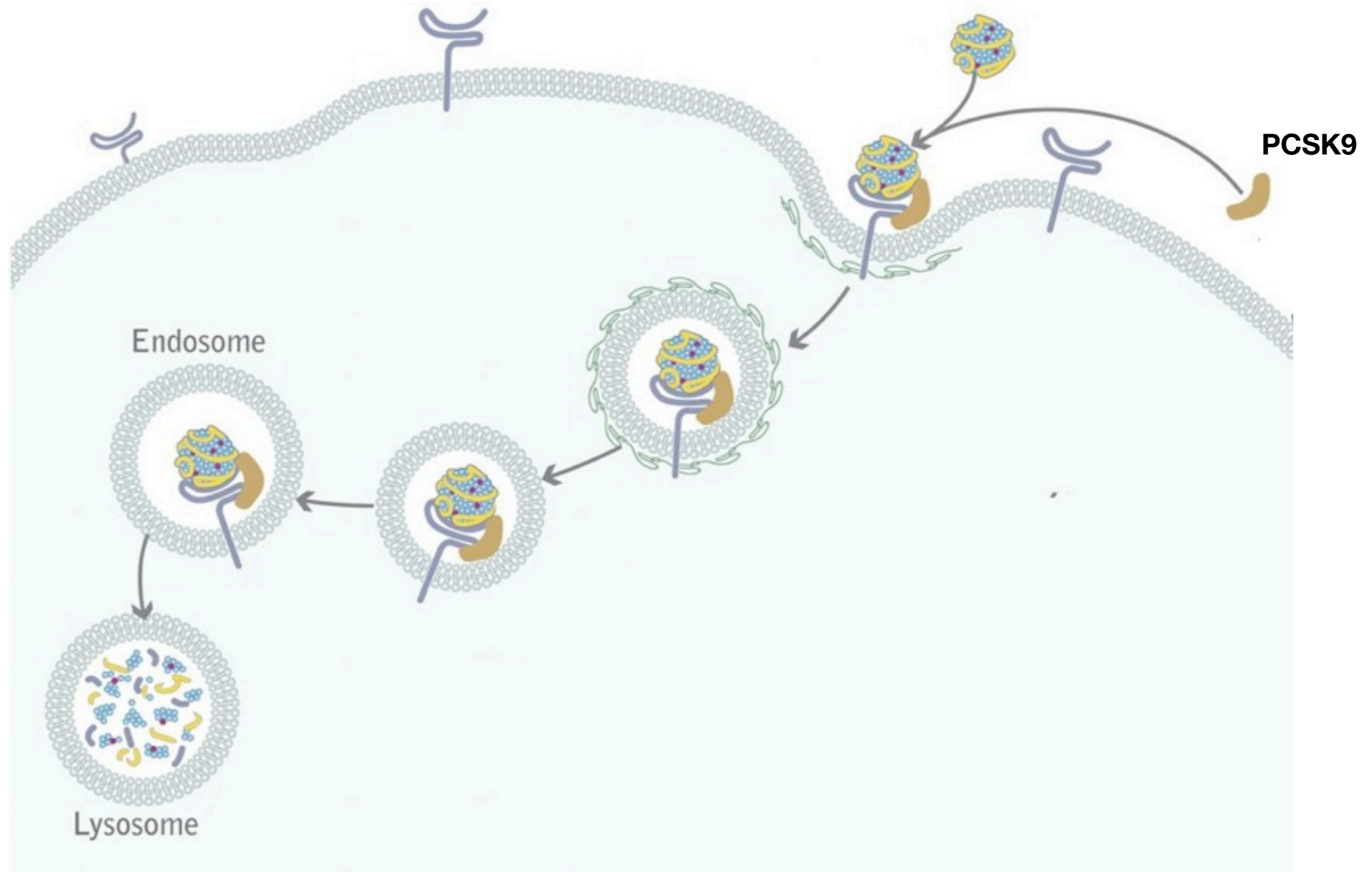

Our understanding of how cells take up LDL was radically altered by the discovery of a protein called PCSK9. PCSK9 or proprotein convertase subtilisin/kexin type 9 is an extracellular protein that binds the LDL receptor. In the endosome, PCSK9 binds tightly to the LDL receptor and prevents it from releasing LDL. Consequently, both LDL and receptor are delivered to the lysosome where they are degraded. The effect of PCSK9 is to reduce the number of LDL receptors on the cell membrane, decreasing a cell's ability to take up LDL.

Expression of PCSK9 is under control of sterol-response element binding protein (SREBP).
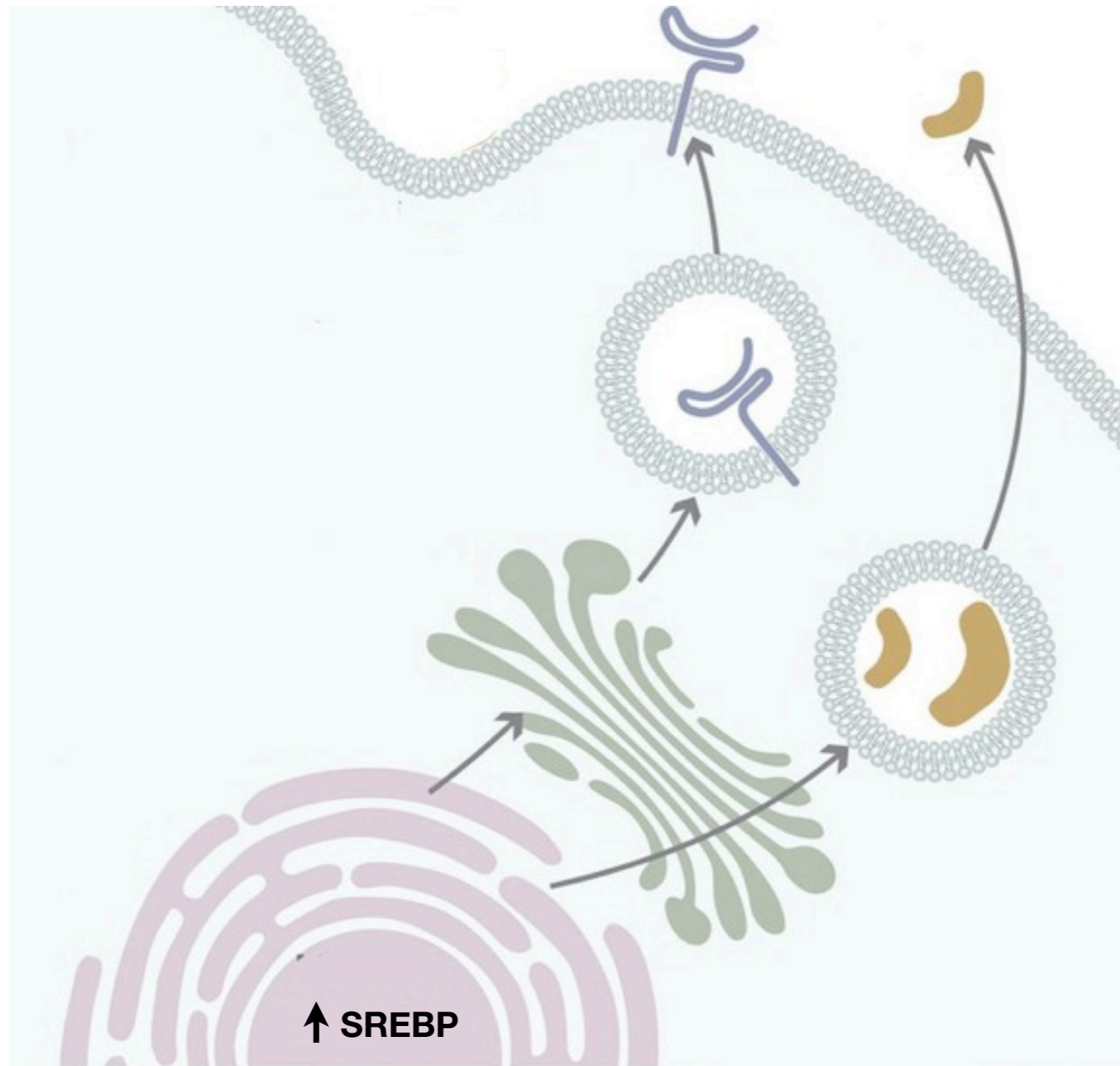

↑ SREBP

One of the challenges of PCSK9 is that the gene which encodes PCSK9 is regulated by the sterol–response element. That means when cells activate expression of LDL via the SREBP, they also increase the expression of PCSK9. One of the desired effects of statins is to increase the expression of LDL receptor through activation of the SRE. The elevated levels of LDL receptor allow greater uptake of LDL, reducing plasma LDL levels. Statins, however, also increase the expression of PSCK9 which could potentially reduce the number of LDL receptors on the cell membrane by targeting them to the lysosome.

Antibodies to PCSK9 prevent its binding to the LDL receptor and reduce serum LDL levels.
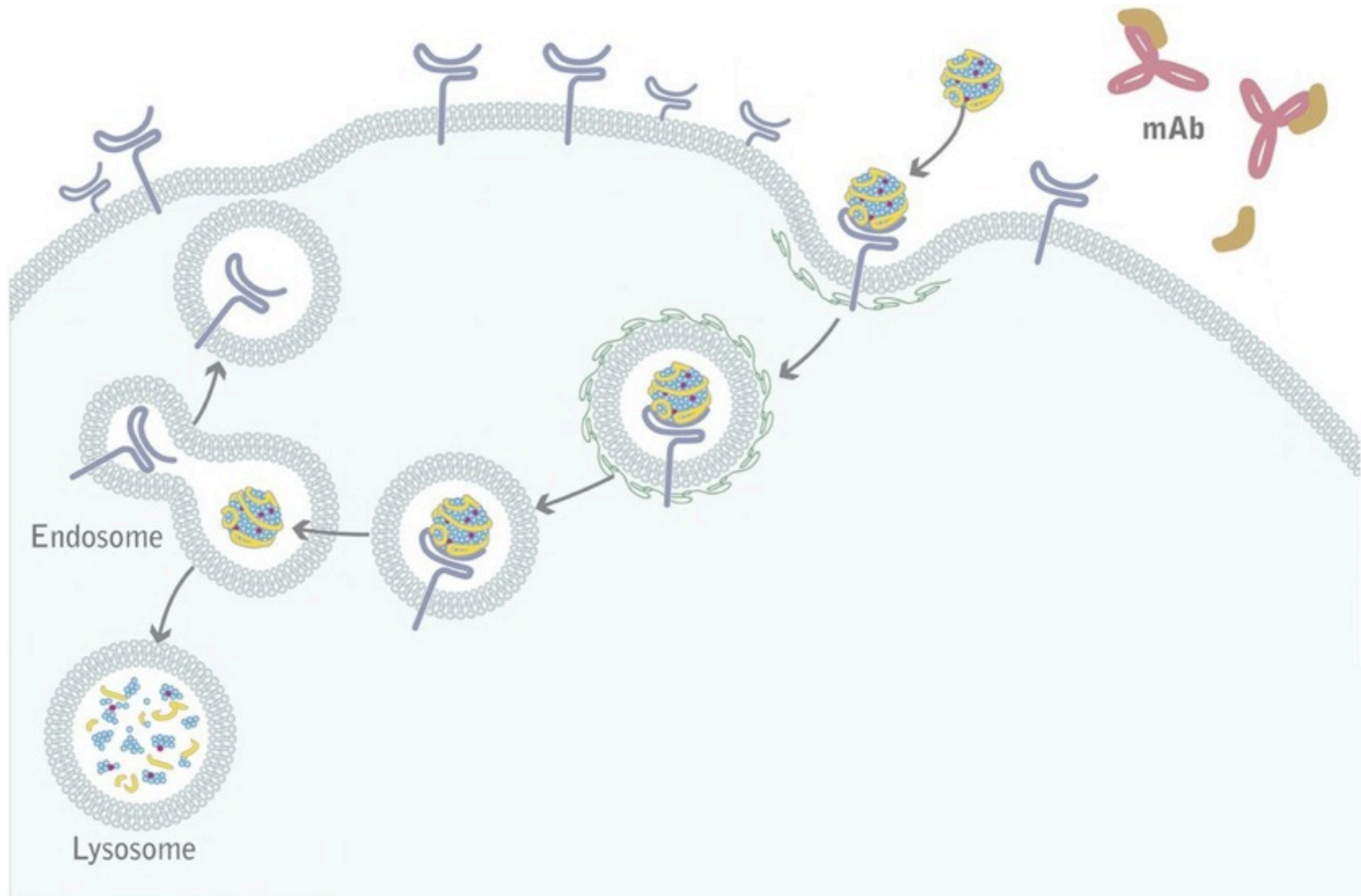

To decrease the levels of PCSK9, several pharmaceutical companies have developed antibodies to PCSK9 that prevent it from interacting with the LDL receptor. Some of these antibodies are in clinical trials.

Membrane and soluble proteins mediate cholesterol export from lysosomes.
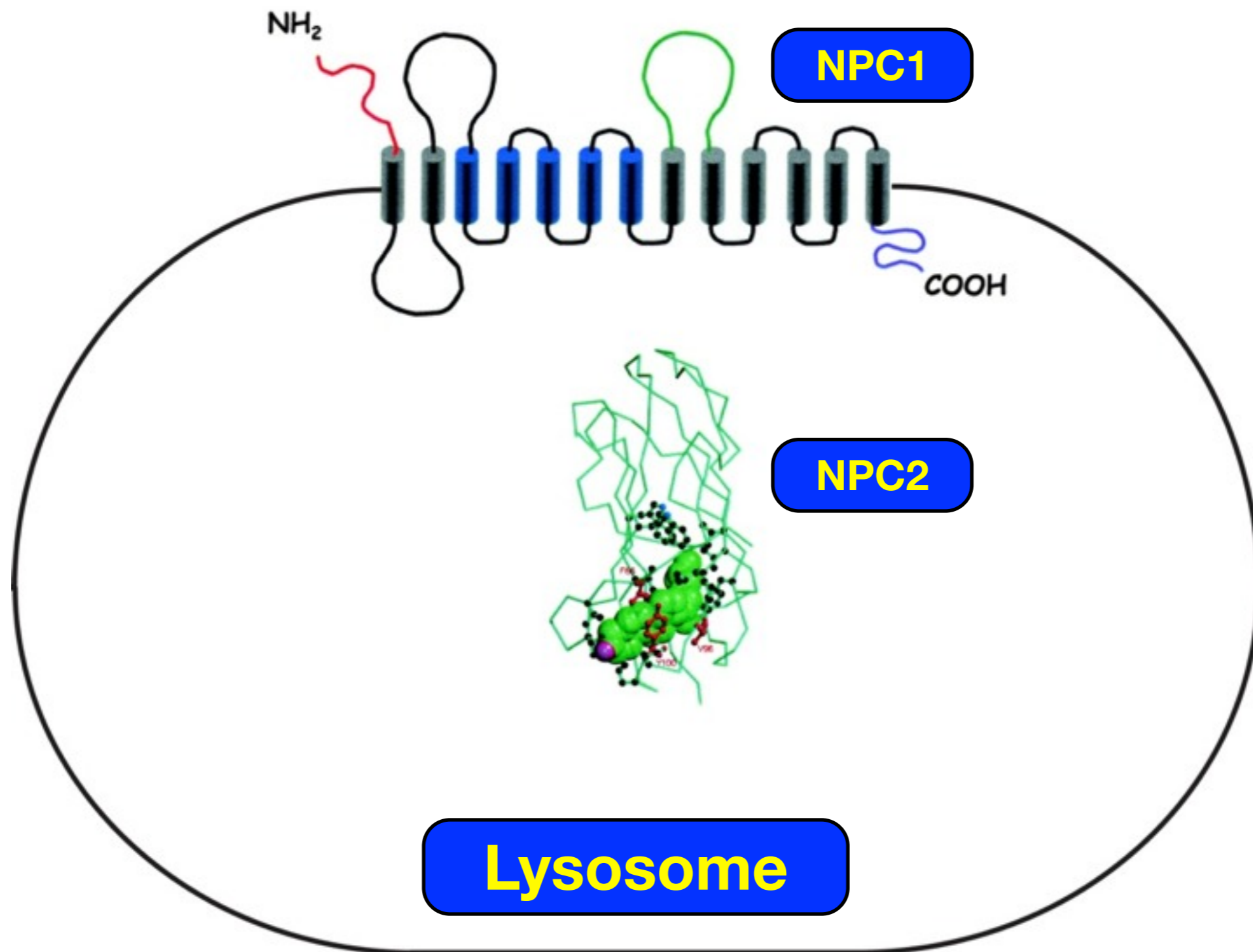
Once LDL is delivered to the lysosome and lipases and proteases have processed LDL into individual molecules, the cholesterol needs to be moved from the lysosome to the ER. The Niemann–Pick proteins mediate exit of cholesterol from lysosomes. NPC1 is a transmembrane protein that resides in the membranes of lysosomes and late endosomes. NPC2 is soluble protein within the lysosome and endosome that binds cholesterol. The exact mechanism of how these proteins export cholesterol is unclear but one model is that NPC2 carries cholesterol to NPC1 and NPC1 mediates its transport across the membrane similar to NPC–like protein in enterocytes. Once out of the lysosome, cholesterol can be delivered to the ER or mitochondria by lipid–transport proteins.

Mutations in NPC1 and NPC2 lead to enlargement of spleen and liver and neurodegeneration.
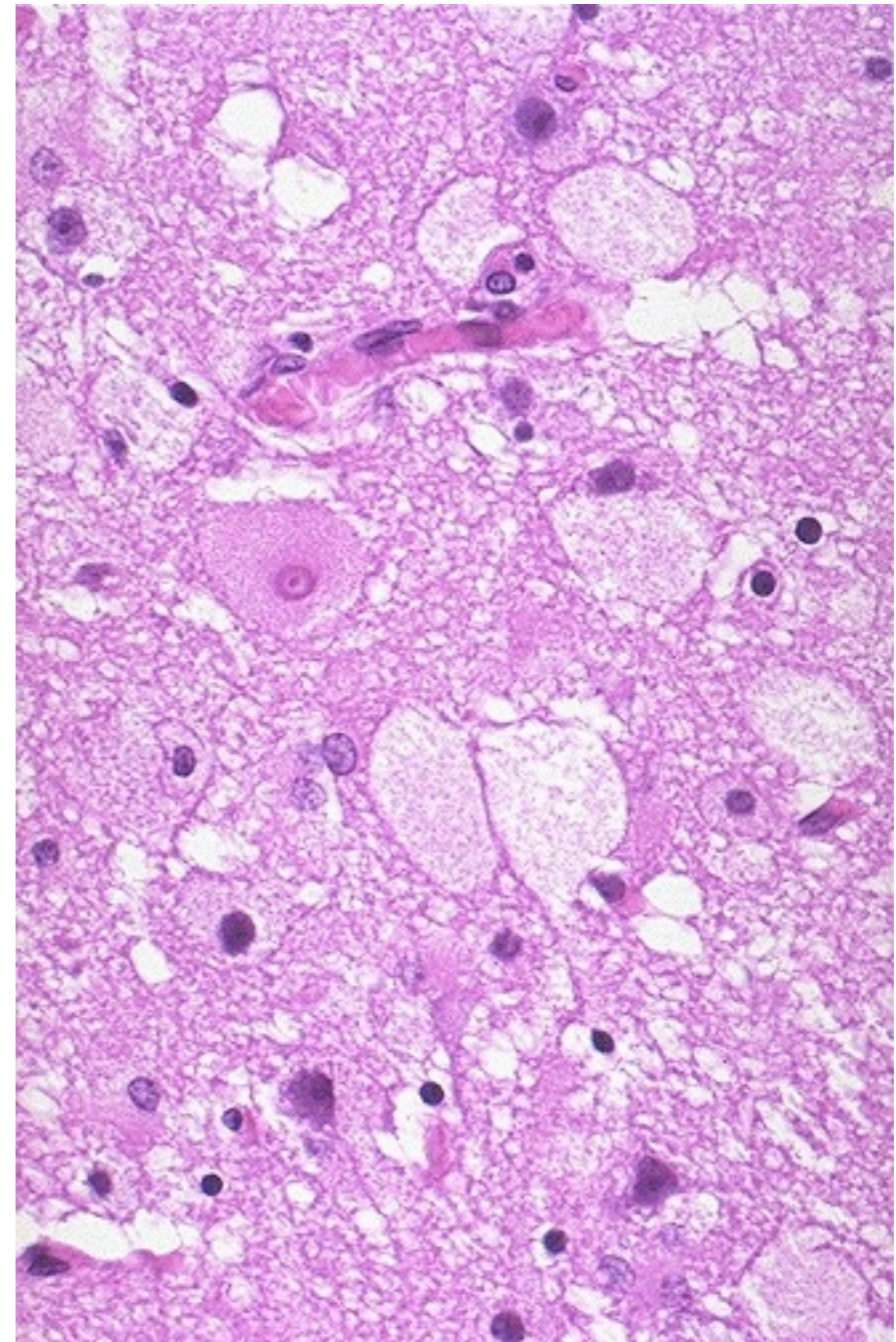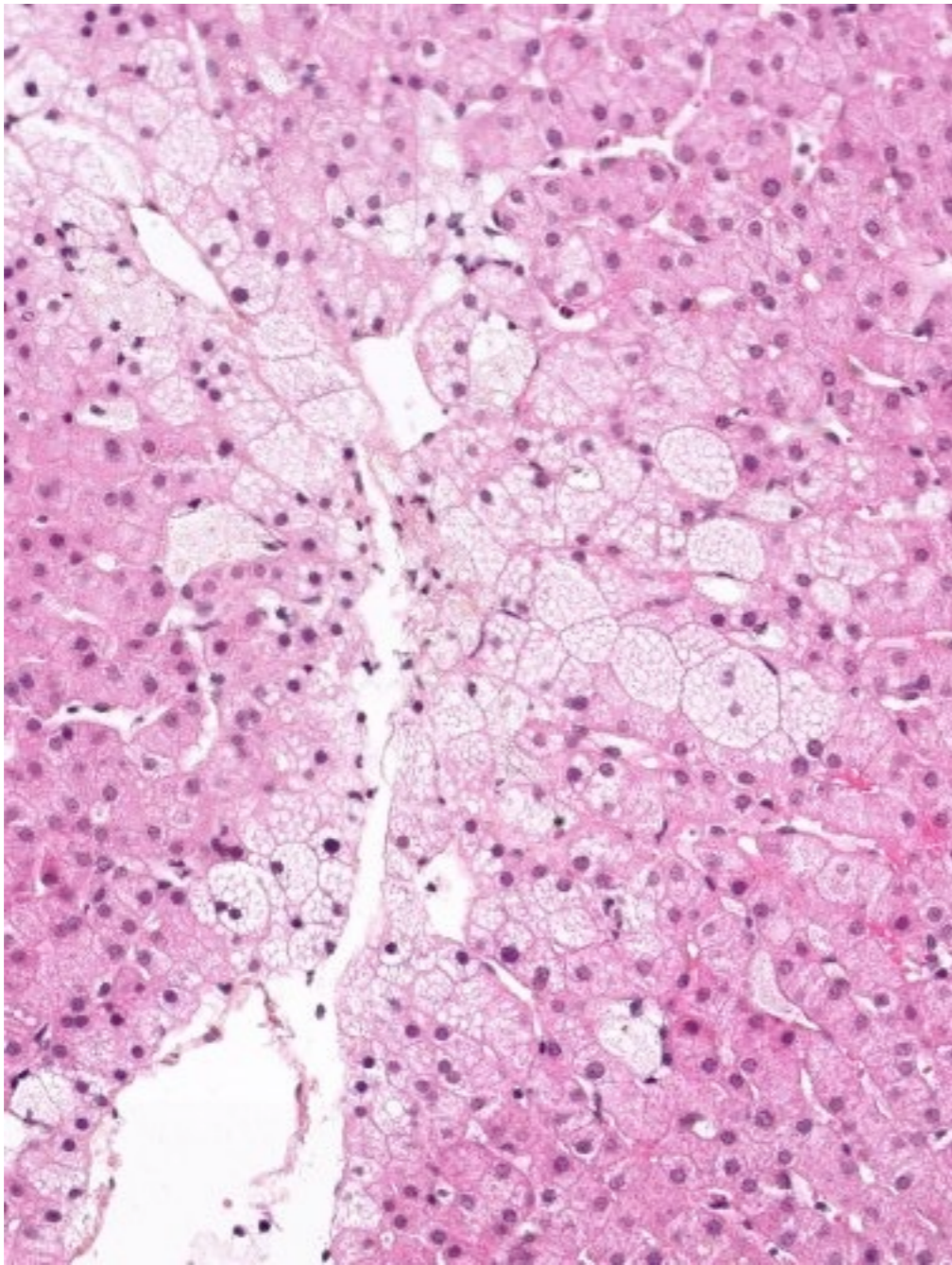
Mutations in Niemann–Pick proteins prevent the release of cholesterol from lysosomes, leading to the accumulation of cholesterol in lysosomes. The lysosomes become enlarged and begin to fill the cytoplasm of cells. Soon, the viability of cells is compromised. The liver and spleen are strongly affected by mutations in Niemann–Pick genes, but the main clinical manifestation in neurological as the activity of neurons is reduced as the cell fills with lysosomes.

Uptake and processing of excess serum LDL

Macrophages engulf oxidized LDL via scavenger receptors.

Lysosome
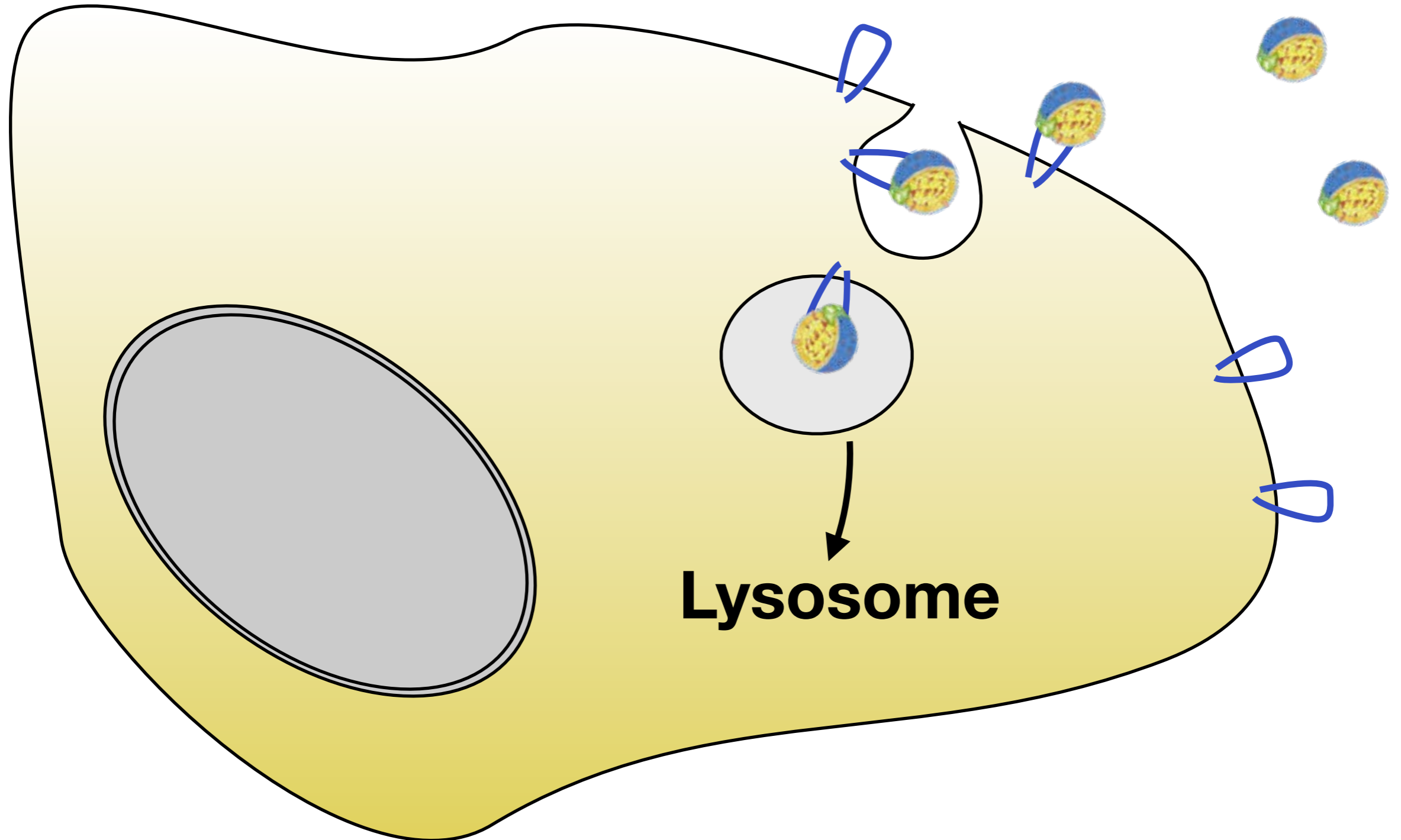
In addition, to the LDL receptor, some cells have another receptor to take up LDL: the scavenger receptor. The scavenger receptor is expressed primarily on macrophages and recognizes a range of substances in addition to LDL. The scavenger receptor binds to oxidized LDL and the receptor–LDL complex are endocytosed and then targeted to the lysosome for degradation.

Accumulation of LDL in macrophages leads to development of foam cells.

capillary

Foam cells
macrophages with residual
bodies filled with lipid
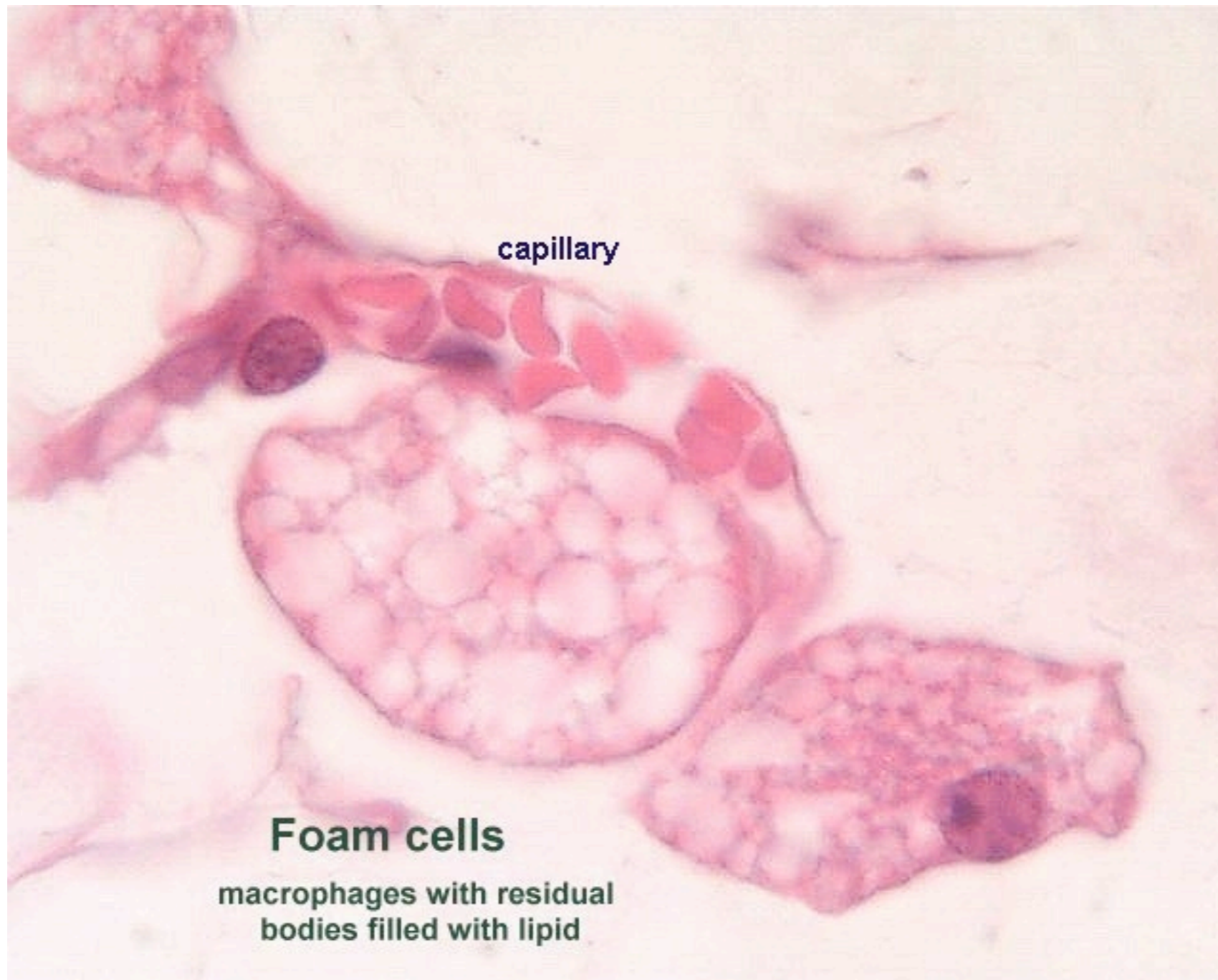
If macrophages endocytose a lot of LDL, cholesterol accumulates within the macrophages and their cytoplasm becomes filled with lipid droplets, giving the cells a foamy appearance. In isolation, these cells are not dangerous, but....

Accumulation of foam cells in artery wall is an early event in atherosclerosis.

Artery Lumen
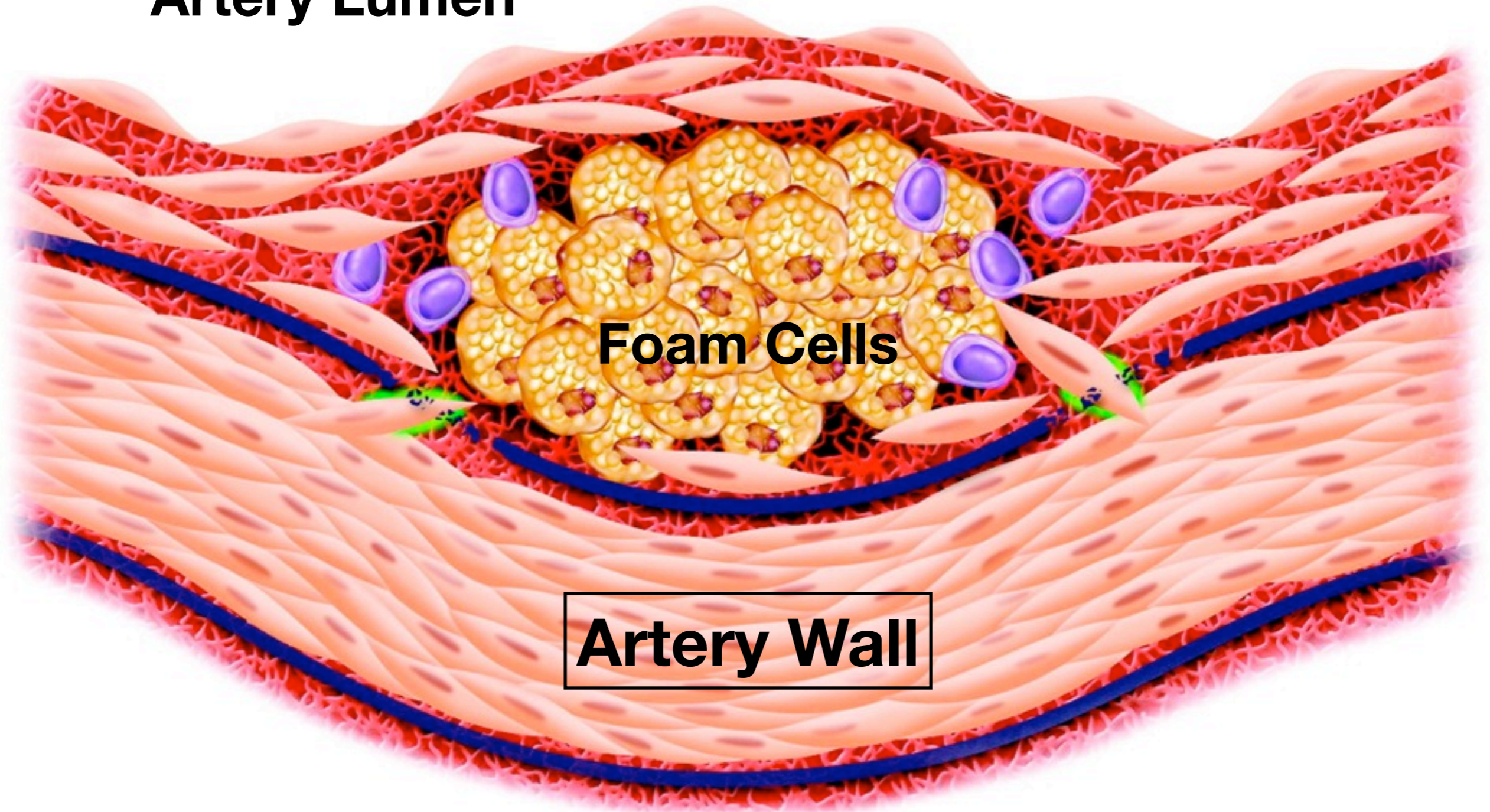
Foam Cells

Artery Wall

....foam cells can also accumulate in the wall of large blood vessels, leading to the formation of an atheroma. Atheromas are dangerous because they are precursors to the development of atherosclerosis.

Take home points...

- Cells synthesize cholesterol and take up cholesterol from serum and both processes regulated by the amount of cellular cholesterol.

- Cholesterol moves through the body in different lipoprotein complexes that differ in size and density.

- Transporters move cholesterol across cell membrane and lysosome membrane.

- LDL receptor is normally recycled unless bound to PCSK9

- Macrophages take up excess LDL and develop into foam cells.