Membranes: Other Considerations

There are many functions and factors relating to cell membranes that don't fit into broad categories. Those items will be the focus of this section.

Endocytosis

Besides transporter proteins and ion channels, another common way for materials to get into cells is by the process of endocytosis. Endocytosis is an alternate form of active transport for getting materials into cells. Some of these processes, such as phagocytosis, are able to import much larger particles than would be possible via a transporter protein. Like transporter proteins, endocytosis uses energy for the purpose (though it is not as visible as with protein transporters), but unlike protein transporters, the process is not nearly as specific for individual molecules.

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As a result, the process usually involves the importation of many different molecules each time it occurs. The list of compounds entering cells in this way includes LDLs and their

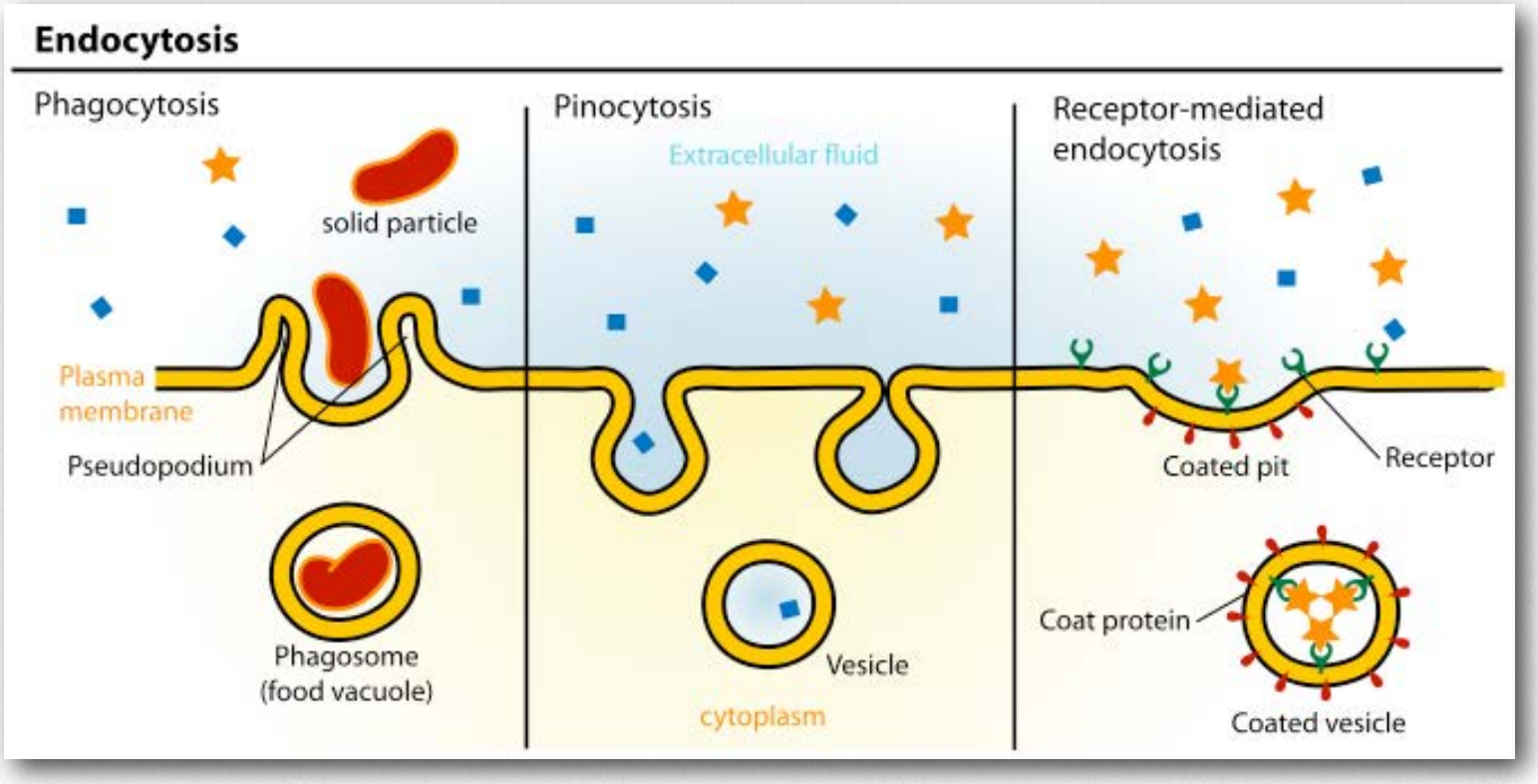

Figure 3.53 - Three types of endocytosis

lipid contents, but it also include things like iron (packaged in transferrin), vitamins, hormones, proteins, and even some viruses sneak in by this means. There are three types of endocytosis we will consider (Figure 3.53).

Receptor mediated endocytosis

The process of receptor mediated endocytosis is a relatively specific means of bringing molecules into cells because it requires the incoming material to be somehow associated with a specific cell surface receptor. In the example of Figure 3.53, the receptor is the cellular LDL receptor. Clathrin-coated invaginations, as

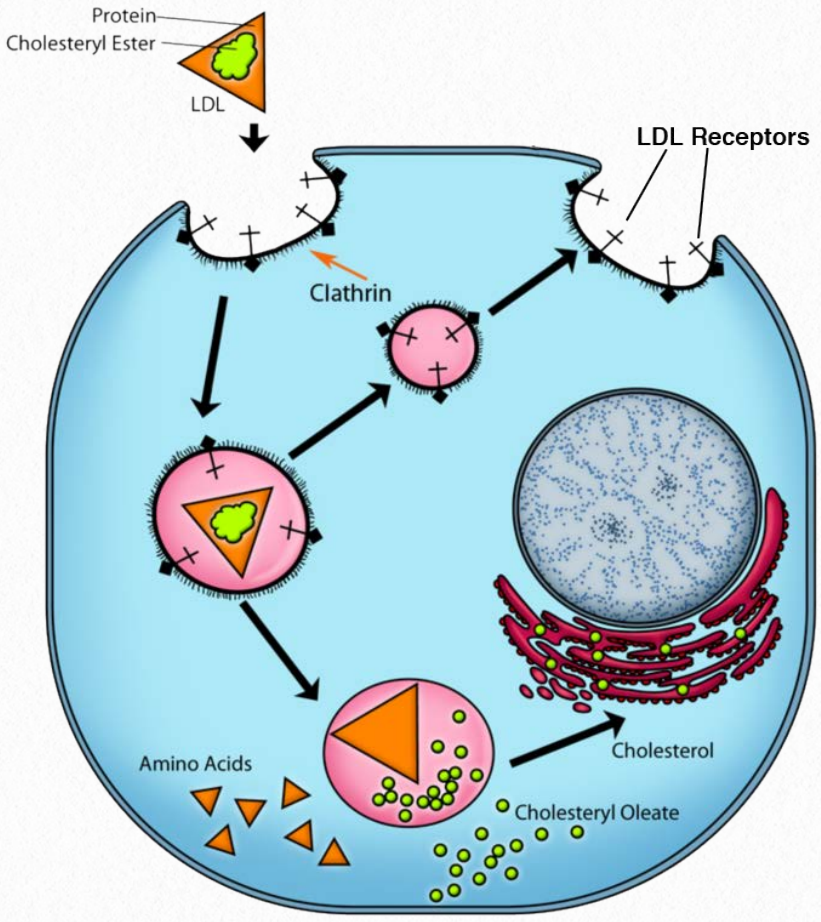

Figure 3.54 - Overview of clathrin-based receptor mediated endocytosis

Image by Aleia Kim

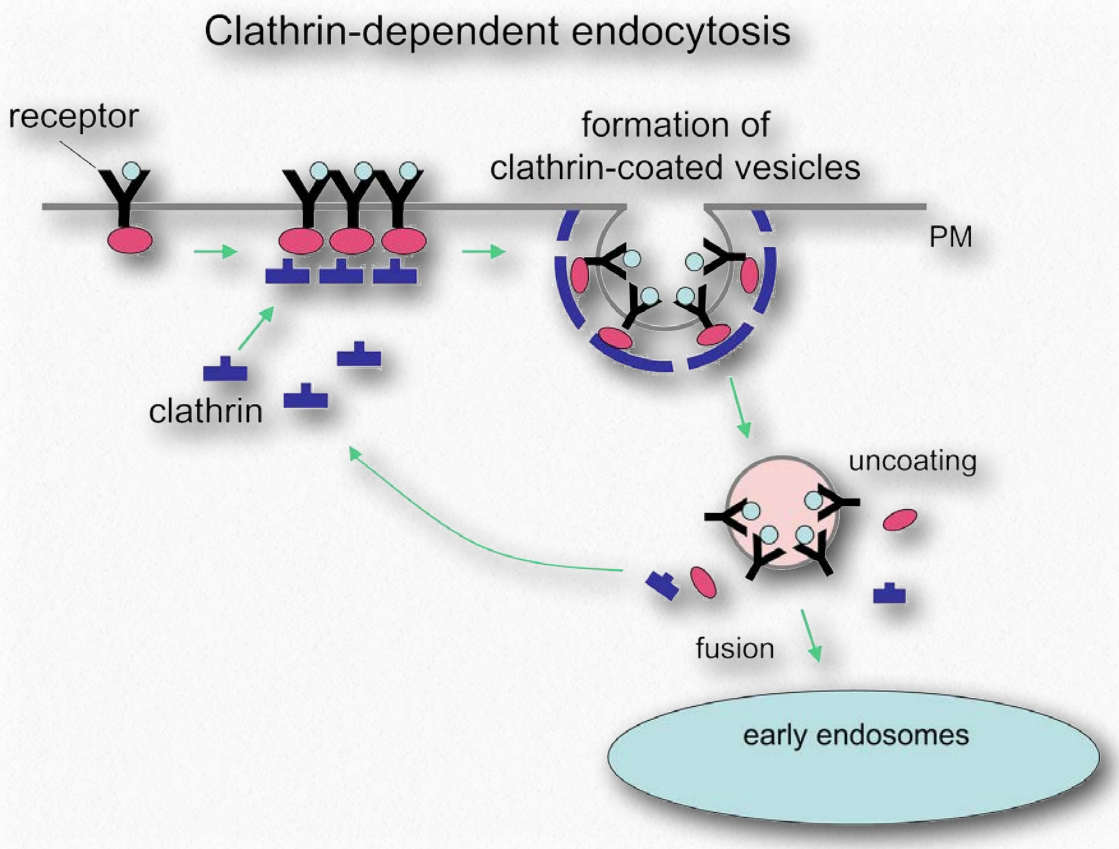

Figure 3.55 - Clathrin-mediated endocytosis of receptors

Wikipedia

shown in the figure are known as “coated pits.” The mechanism proceeds with an inward budding of the plasma membrane receptor (coated vesicles). Binding of the ligand (ApoB-100 of the LDL, for example, in Figure 3.54) to the LDL receptor leads to formation of a membrane invagination. The absorbed LDL particle fuses to form an early endosome (Figure 3.55) and contents are subsequently sorted and processed for use by the cell.

The components from the coated vesicle are recycled to the plasma membrane for additional actions. Receptor mediated endocytosis can also play a role in internalization of cellular receptors that function in the process of signaling. Here, a receptor bound to a ligand is brought into the cell and may ultimately generate a response in the nucleus.

While receptor mediated endocytosis of receptors can have the effect of communicating a signal inwards to the cell, it can also reduce the total amount of signaling occurring, since the number of receptors on the cell surface is decreased by the process.

Non-clathrin endocytosis

There are three types of endocytosis occurring in cells that do not involve clathrin. They are 1) caveolae-based endocytosis, 2) macropinocytosis, and 3) phagocytosis. Caveolae-based endocytosis is based on a receptor molecule known as caveolin. Caveolins are a class of integral membrane proteins that compartmentalize and concentrate signaling molecules in the process of endocytosis. They are named for the cave-like caveolae structures of the plasma membrane where they are found.

Caveolins

Caveolins have affinity for cholesterol and associate with it in the membrane of cells, causing the formation of membrane invaginations of about 50 nm. The caveolin proteins can oligomerize and this is important for

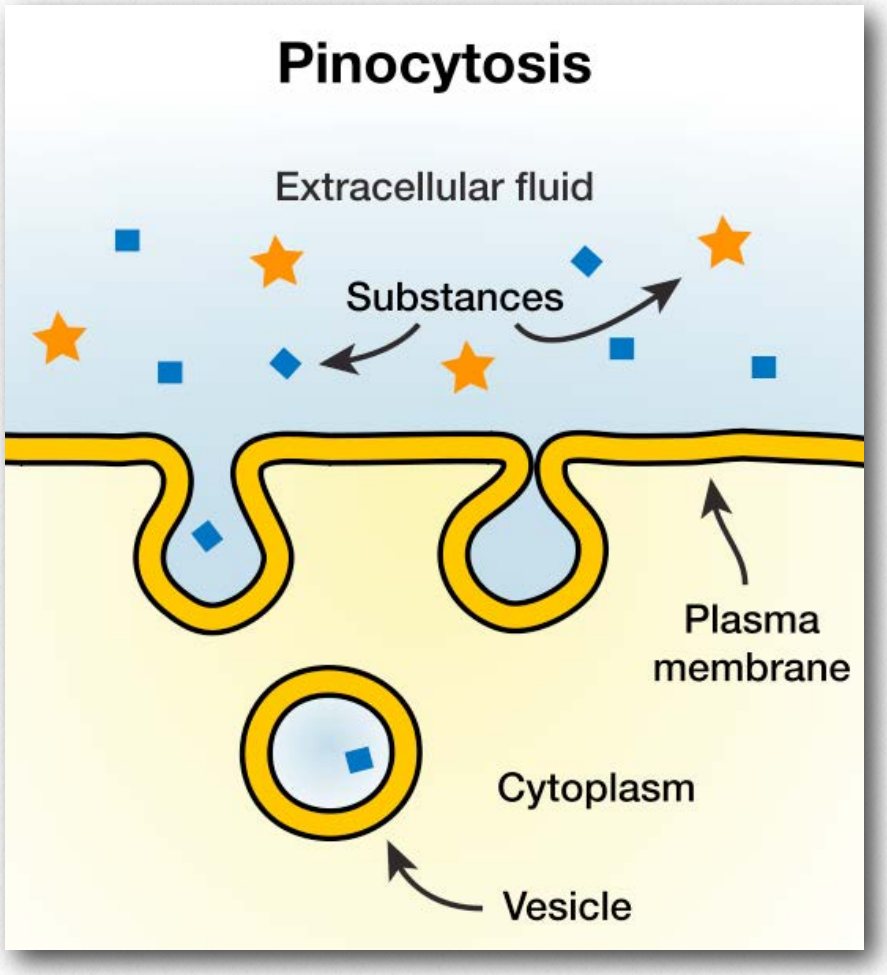

Figure 3.56 - **Macropinocytosis**

the coating and formation of the cave-like structures.

There are three caveolin genes found in vertebrate cells, CAV1, CAV2, and CAV3. Down-regulation of caveolin-1 results in less efficient cellular migration *in vitro*. Caveolins are implicated in both formation and suppression of tumors. High expression of them inhibits cancer-related growth factor signaling pathways, but some caveolin-expressing cancer cells are more aggressive and metastatic, possible due to an enhanced capacity for anchorage-independent growth.

Macropinocytosis

A phenomenon known as "cell drinking," macropinocytosis literally involves a cell "taking a gulp" of the extracellular fluid. It does this, as shown in Figure 3.56, by a simple invagination of ruffled surface features of the plasma membrane. A pocket results, which pinches off internally to create a vesicle containing extracellular fluid and dissolved molecules. Within the cytosol, this internalized vesicle will fuse with endosomes and lysosomes. The process is non-specific for materials internalized.

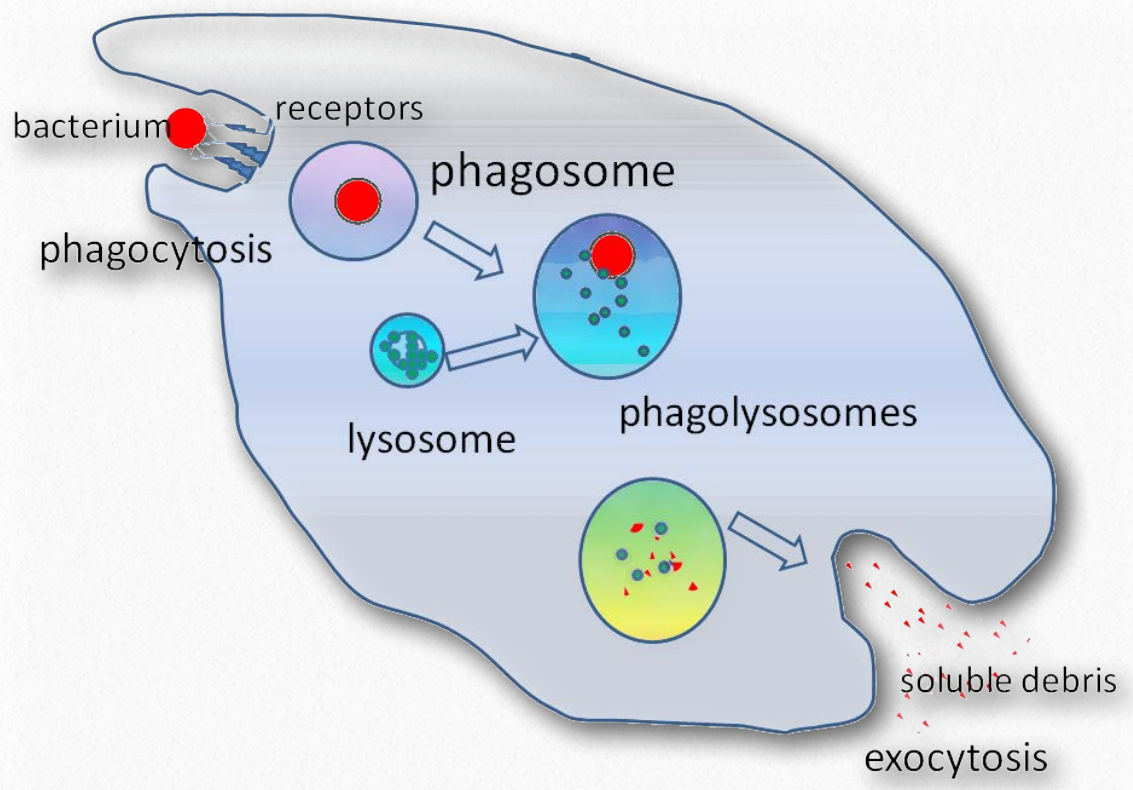

Figure 3.57 - **Generalized scheme for phagocytosis of a bacterium**

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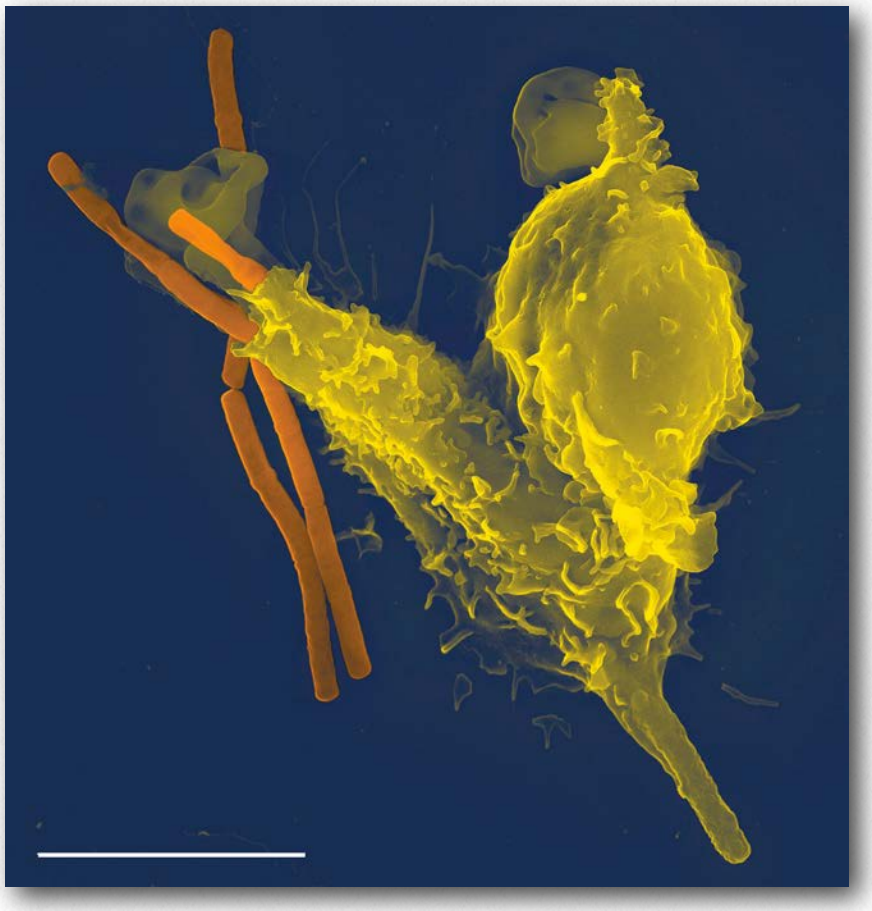

Figure 3.58 - Phagocytosis by a neutrophil (yellow) of an *Anthrax bacillus* (orange)

Wikipedia

Phagocytosis

Phagocytosis is a process whereby relatively large particles (0.75 µm in diameter) are internalized. Cells of the immune system, such as neutrophils, macrophages, and others, use phagocytosis to internalize cell debris, apoptotic cells, and microorganisms.

The process operates through specific receptors on the surface of the cell and phagocytosing cell engulfs its target by growing around it. The internalized structure is known as a phagosome, which quickly merges with a lysosome to create a phagolysosome (Figure 3.58), which subjects the engulfed particle to toxic conditions to kill it, if it is a cell, and/or to digest it into smaller pieces. In some cases, as shown in the figure, soluble debris may be released by the phagocytosing cell.

Endosomes

Internalized material from endocytosis that doesn't involve phagocytosis passes through an internalized structure called an endosome. Endosomes are membrane bounded structures inside of eukaryotic cells that play a role in endocytosis (Figure 3.59). They have a sorting function for material internalized into the cell, providing for retrieval of

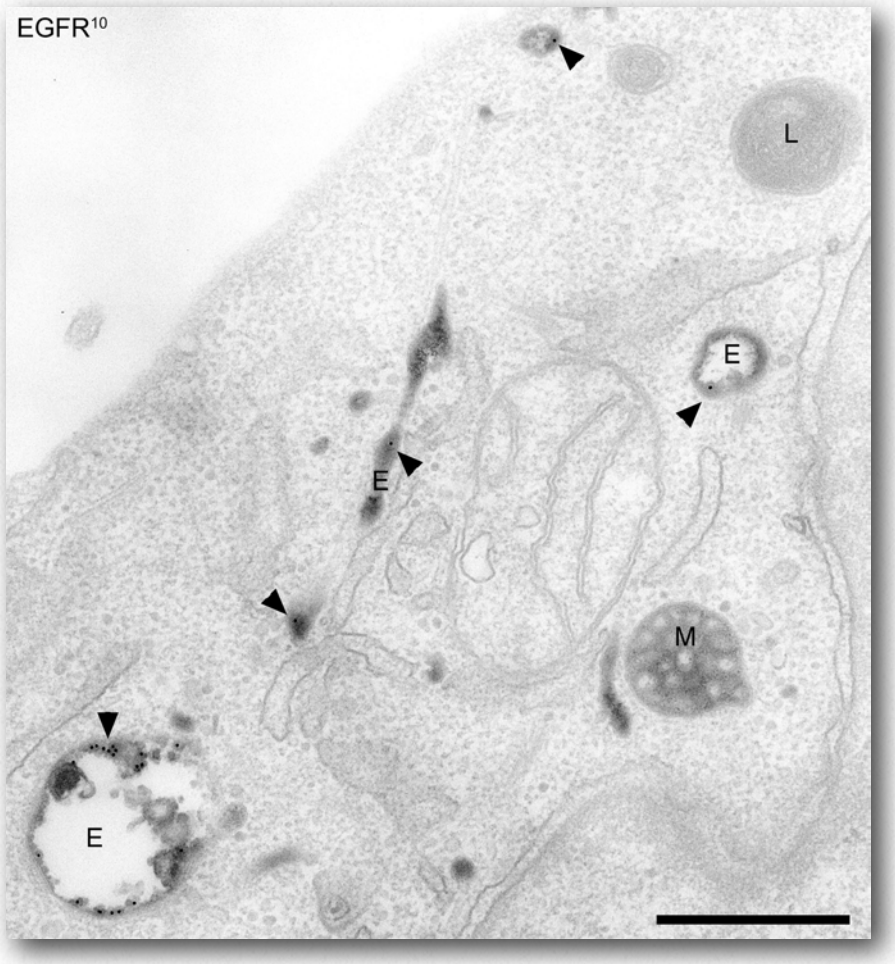

Figure 3.59 - Internalization of the epidermal growth factor receptor (EGFR) into endosomes. Early (E) and late (M) endosomes and lysosomes (L) are labeled.

Wikipedia

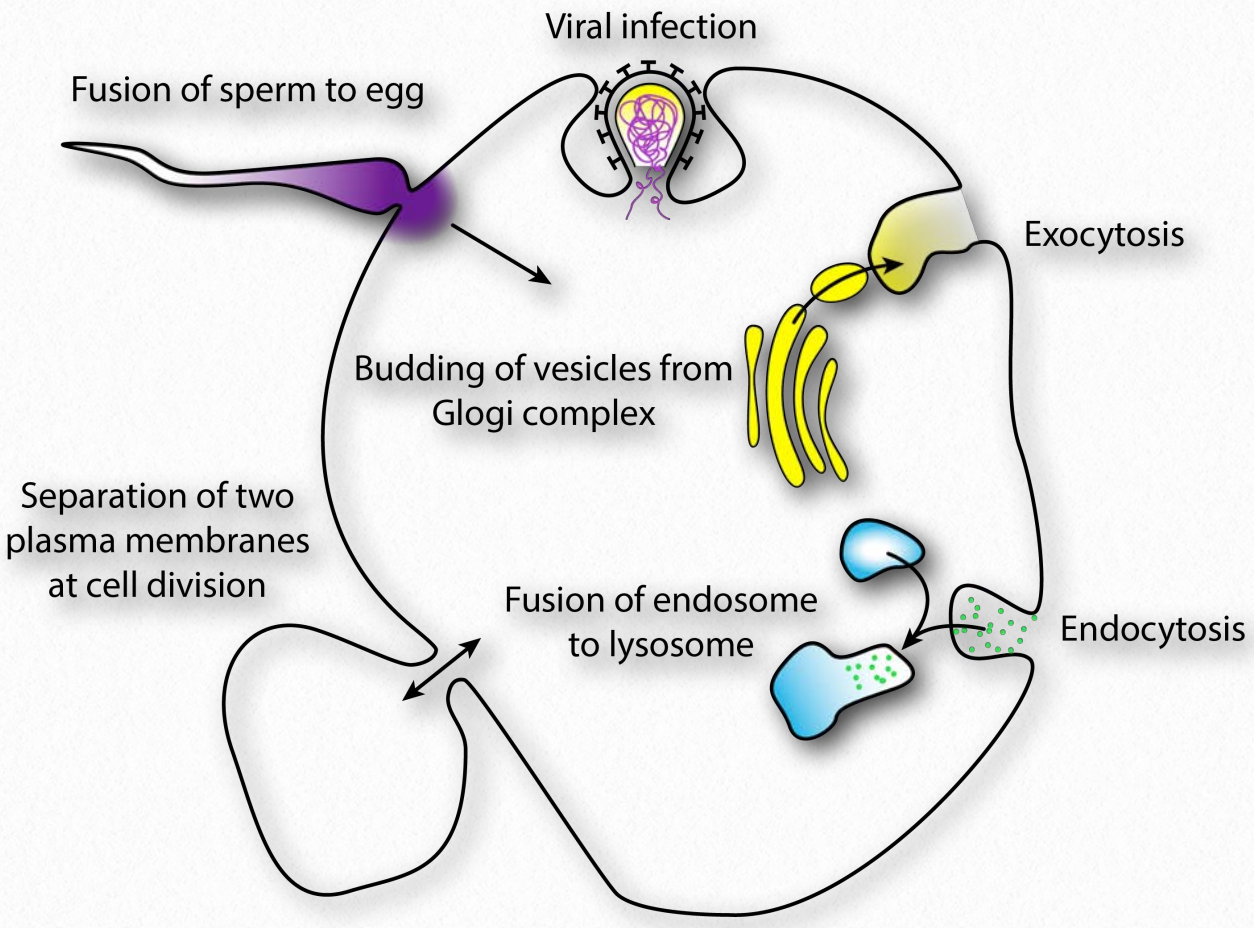

Figure 3.60 - Cell membrane fusions
Image by Pehr Jacobson

materials not destined for destruction in the lysosomes. LDLs, for example, are targeted after endocytosis to the endosomes for processing before part of them is delivered to the lysosome. The endosomes can also receive molecules from the *trans*-Golgi network. These can be delivered to the lysosomes, as well, or redirected back to the Golgi. Endosomes come in three forms - 1) early, 2) late, and 3) recycling.

Exocytosis

The process of exocytosis is used by cells to export molecules out of cells that would not otherwise pass easily through the plasma membrane. In the process, secretory vesicles fuse with the plasma membrane and release their contents extracellularly. Materials, such as proteins and lipids embedded in the membranes of the vesicles become a part of the plasma membrane when fusion between it and the vesicles occurs.

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Membrane fusion

Fusion is a membrane process where two distinct lipid bilayers merge their hydrophobic cores, producing one interconnected structure. Membrane fusion involving vesicles is the mechanism by which the processes of endocytosis and exocytosis occur.

When the fusion proceeds through both leaflets of both bilayers, an aqueous bridge results and the contents of the two structures mix.

Common processes involving membrane fusion (Figure 3.60) include fertilization of an egg by a sperm, separation of membranes in cell division, transport of waste products, and neurotransmitter release (Figure 3.61). Artificial membranes such as liposomes can also fuse with cellular mem-

branes. Fusion is also important for transporting lipids from the point of synthesis inside the cell to the membrane where they are used. Entry of pathogens can also be governed by fusion, as many bilayer-coated viruses use fusion proteins in entering host cells.

SNARE proteins

Mediation of fusion of vesicles in exocytosis is carried out by proteins known as SNAREs (Soluble NSF Attachment Protein REceptor). This large superfamily of proteins spans a wide biological range, from yeast to mammals. Common vesicle fusions occur when synaptic vesicles dock with neurons (Figure 3.61) and release neurotransmitters. These are well-studied. The SNAREs involved in this process can be proteolytically cleaved by bacterial neurotoxins that give rise to the conditions of botulism and tetanus.

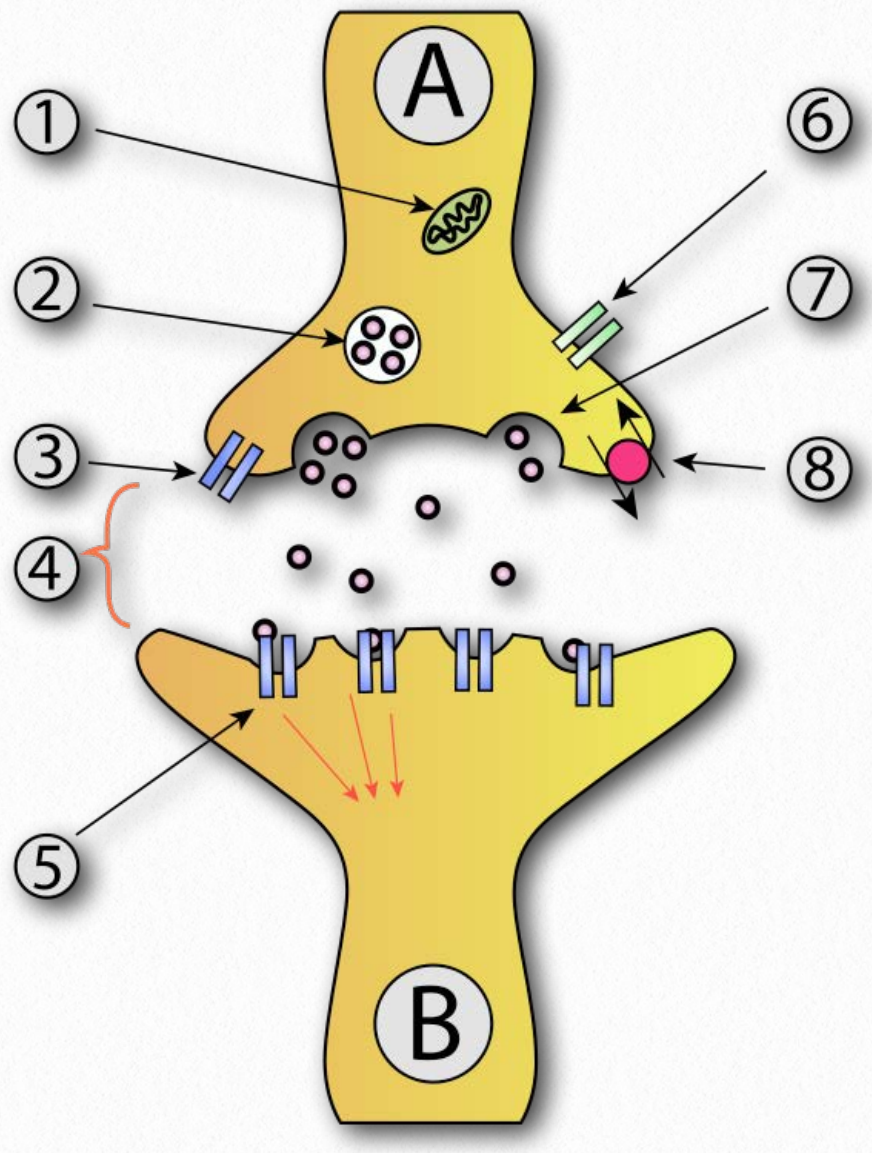

Figure 3.61 - Release of neurotransmitters (small circles) from presynaptic neuron A to postsynaptic neuron B. 1 = Mitochondrion / 2 = Synaptic vesicle with neurotransmitter / 3 = Autoreceptor / 4 = Synaptic cleft / 5 = Neurotransmitter receptor / 6 = Calcium channel / 7 = Fused vesicle releasing neurotransmitter / 8 = Neurotransmitter re-uptake pump

Wikipedia

SNAREs are found in two locations. v-SNAREs are found in the membranes of transport vesicles during the budding process, whereas t-SNARES can be found in the membranes of targeted compartments.

The act of vesicle fusion coincides with increases of intracellular calcium. Fusion of synaptic vesicles in neurotransmission results in activation of voltage-dependent calcium channels in the targeted cell. Influx of calcium helps to stimulate vesicle fusion. In the endocrine system, binding of hormones to G protein coupled receptors activate the IP_3/DAG system to increase levels of calcium.

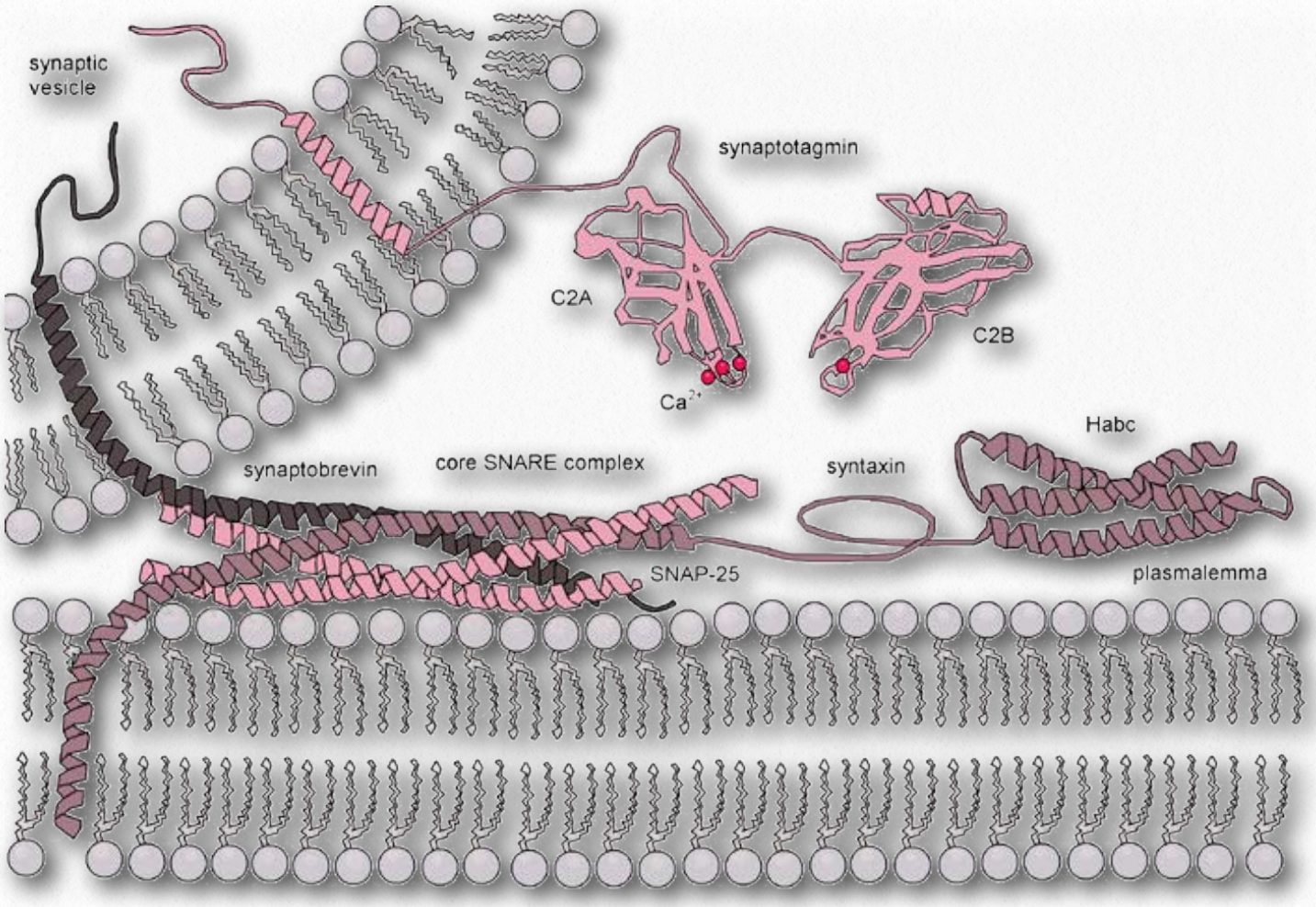

Figure 3.62 - Proteins involved in vesicle fusion in neurotransmission. A SNARE complex between α-helices of synaptobrevin, syntaxin and SNAP-25 intertwine and "zip" membranes together. Synaptotagmin is a calcium sensor regulating the process of zipping

Wikipedia

In the process of membrane fusion (Figure 3.62), the v-SNAREs of a secretory vesicle (upper left) interact with the t-SNAREs of a target membrane (bottom). The v- and t-SNAREs "zipper" themselves together to bring the membrane vesicle and the target membrane closer together.

Zippering also causes flattening and lateral tension of the curved membrane surfaces, favoring hemifusion of the outer layers of each membrane. Continued tension results in subsequent fusion of the inner membranes as well, yielding opening of the contents of the vesicle chamber to its target (usually outside the cell).

Shuttles

Another way to transport items across a membrane for which there is no specific transport system available is the use of shuttles. Shuttles are important when there is no transport mechanism for moving material across a membrane for which no transport system exists.

A great example is NADH. NADH is an important electron carrier that is produced in the cytoplasm and mitochondria of the cell. NADH produced in the mitochondrion goes directly to the electron transport system and delivers electrons to Complex I. NADH produced in the cytoplasm (such as from glycolysis) does not have this option, since the inner membrane of the mitochondrion is impermeable to the molecule

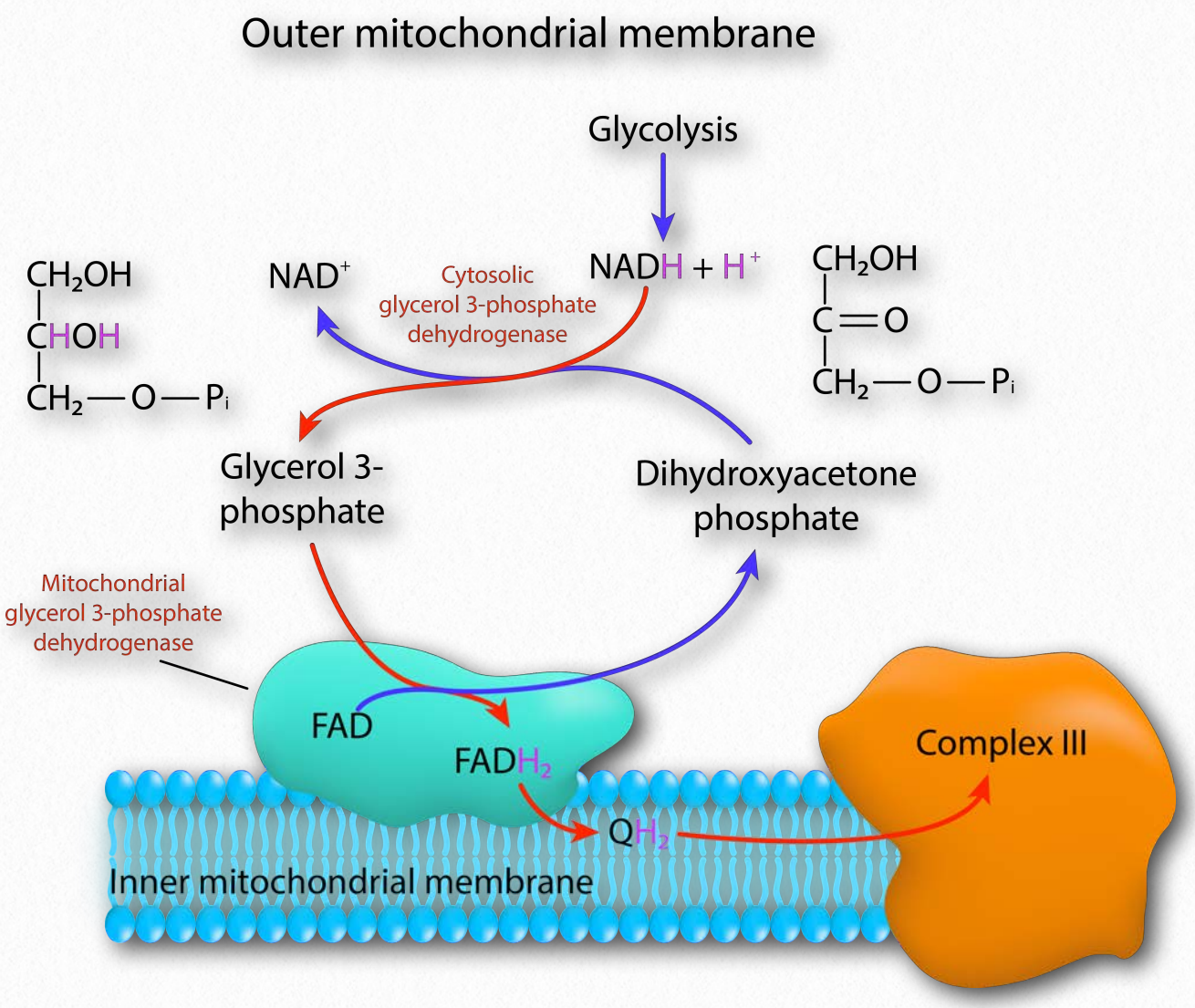

Figure 3.63 - Glycerol phosphate shuttle system in the intermembrane space of a mitochondrion

Image by Pehr Jacobson

Glycerol phosphate shuttle

The first of these methods is the least efficient, but it is rapid. It found commonly in muscles which have needs for rapid energy and brain tissue. This shuttle is referred to as the glycerol phosphate shuttle and is shown in Figure 3.63. It operates in the intermembrane space between the inner and outer mitochondrial membranes. The outer mitochondrial membrane is very porous, allowing many materials to pass freely through it. In the intermembrane space, the cytoplasmic enzyme, glyceraldehyde-3-phosphate dehydrogenase (cGPD) catalyzes transfer of electrons from NADH to dihdydroxyacetone phosphate (#2 in the figure), yielding NAD^+ and glyceraldehyde-3-phosphate (#1 in the figure). The glyceraldehyde-3-phosphate then binds to a glyceraldehyde-3-phosphate dehydrogenase (mGPD) embedded in the outer portion of the inner mitochondrial membrane. mGPD catalyzes the transfer of electrons from glyceraldehyde-3-phosphate to FAD, producing dihycroxyacetone phosphate

and no transporter exists to move it across. The important part of the NADH is its electron cargo, so cells have evolved two ways to move the electrons into the mitochondrial matrix apart from NADH.

Both methods involve shuttles. In each case, an acceptor molecule receives electrons from NADH and the reduced form of the acceptor molecule is transported. It gets transported into the matrix where it is oxidized (electrons are lost) and then donated to the electron transport system.

and $FADH_2$. $FADH_2$ then transfers its electrons to the electron transport system through CoQ (Q above), forming $CoQH_2$ (QH_2 above). As will be discussed in the section on electron transport, this is not an efficient shuttle system because it does not result in production of as much ATP as occurs when electrons are transferred to NAD^+ instead of FAD.

Malate-aspartate shuttle

A more efficient system of transferring electrons is the malate-aspartate shuttle and it is shown in Figure 3.64. As is apparent in the figure, this shuttle involves more steps than the glycerol phosphate shuttle, but the advantage of the malate-aspartate shuttle is that it is more efficient. NADH outside of

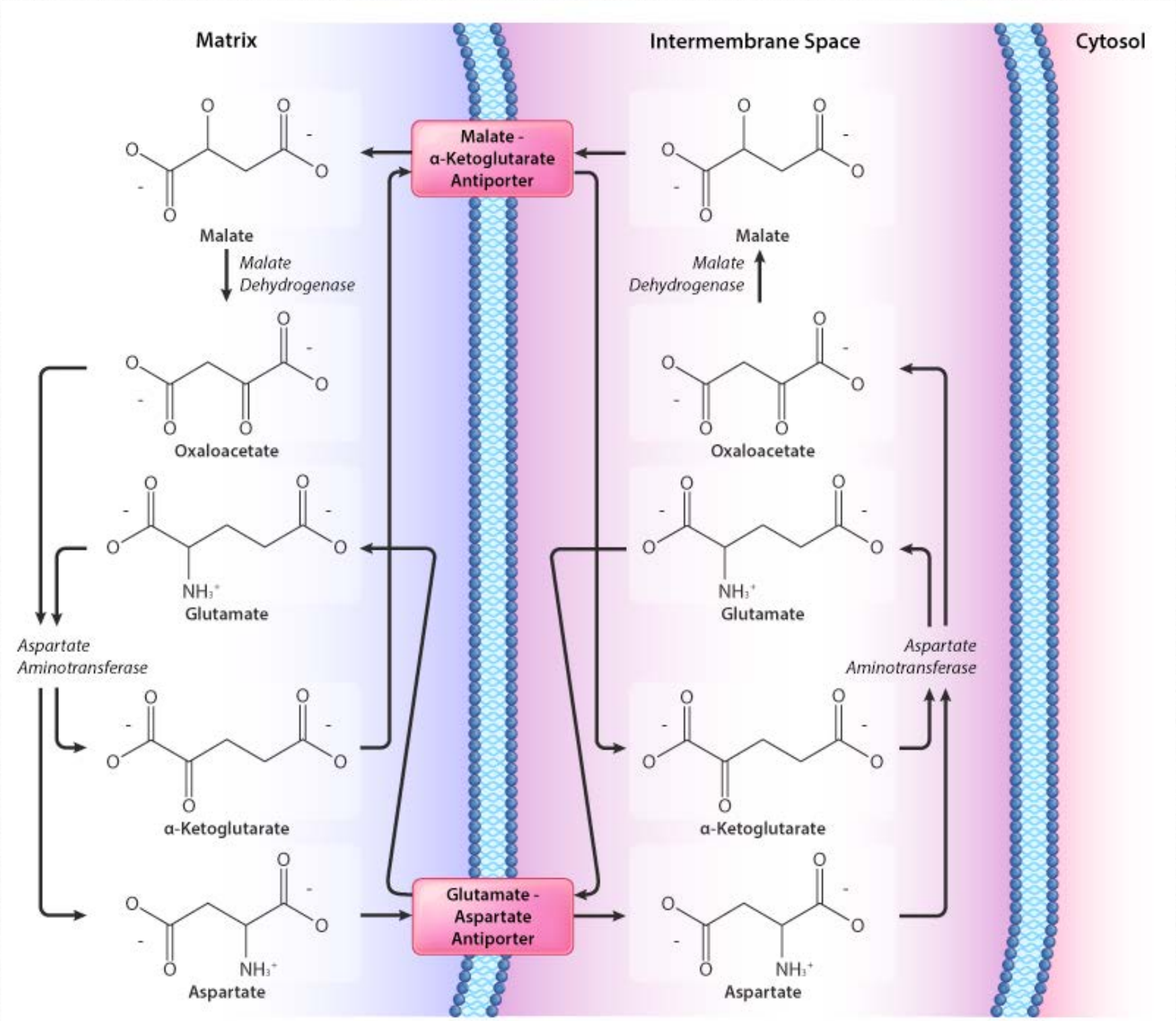

Figure 3.64 - The malate aspartate shuttle

Image by Aleia Kim

the mitochondrion transfers its electrons to the shuttle and then NADH is re-made on the inside of the shuttle. No energy is expended in the process.

When NADH accumulates in the cytoplasm, it moves to the intermembrane space where the enzyme malate dehydrogenase catalyzes the transfer of electrons to oxaloacetate to yield NAD^+ and malate. A transport system for malate moves malate into the mitochondrial matrix in exchange for α-ketoglutarate.

Inside the mitochondrion, malate is reoxidized to oxaloacetate and electrons are given to NAD^+ to recreate NADH. NADH then donates electrons to Complex I of the electron transport system. That's really all there is to the shuttle. The remaining steps are simply to balance the equation of the process. Oxaloacetate accepts an amine group from glutamic acid to yield aspartic acid and α-ketoglutarate. Aspartate then moves out of the mitochondrion through an antiport transport protein that swaps it for glutamate. A series of reactions in the intermembrane space balance the equation.

It is easy to get lost in the mess of balancing equations. The most important thing to understand here is that oxaloacetate accepts electrons on the outside to become malate which is the carrier of electrons across the membrane. Once inside the matrix, malate is converted back to oxaloacetate and its electrons are given to NAD^+, forming NADH. Everything else is simple equation balancing.

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Acetyl-CoA shuttle

Another kind of shuttle also involves the mitochondrion and in this case, the item being moved is a molecule, not a pair of electrons. The molecule of interest here is acetyl-CoA, for which no transport system operates, but which is needed in the cytoplasm for fatty acid synthesis when the cell has abundant energy.

Acetyl-CoA is mostly in the mitochondrion and so long as the citric acid cycle is operating efficiently, its concentration is relatively stable. However, when the citric acid cycle slows, acetyl-CoA and the citrate made from it in the cycle begin to accumulate.

A membrane transport system for citrate exists, so it gets moved out to the cytoplasm. In the cytoplasm, an enzyme known as citrate lyase cleaves citrate to acetyl-CoA and oxaloacetate. Oxaloacetate can be reduced to malate and moved back into the mitochondrion.

As for acetyl-CoA, the more of that cells have in the cytoplasm, the more likely they will begin making fatty acids and fat, since acetyl-CoA is the starting material for fatty acid syn-

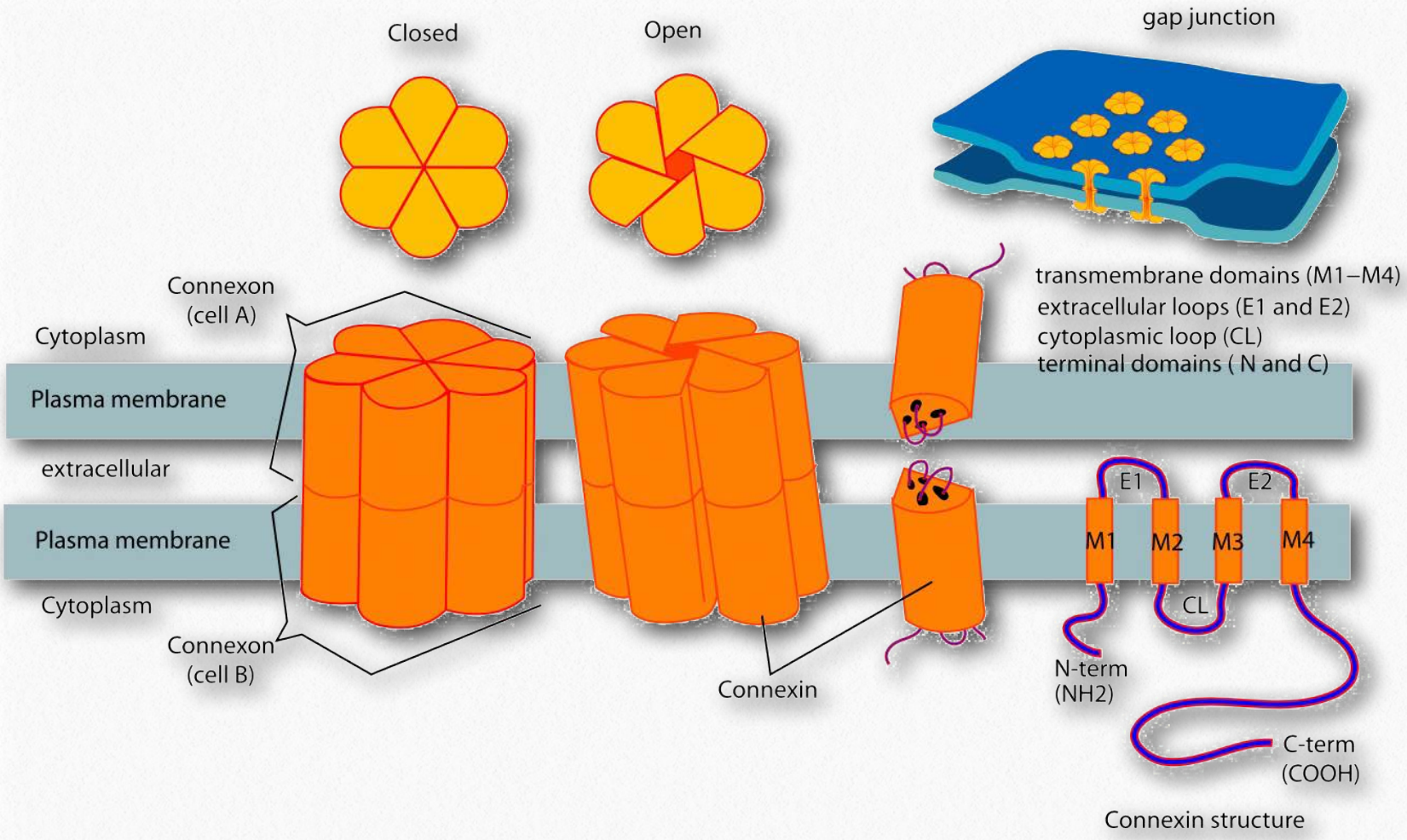

Figure 3.65 - Structure of connexons joining two cells. Bundles of six copies of connexin proteins in the plasma membrane of each cell comprise the connexon structures

thesis, which occurs in the cytoplasm. When does this process occur? As noted above, it occurs when the citric acid cycle stops and this occurs when levels of NADH and $FADH_2$ increase. These, of course, increase when one is not burning off as many calories as one is consuming as a byproduct of respiratory control. Lack of exercise leads to higher levels of reduced electron carriers.

Cell junctions

Cells in multicellular organisms are in close contact with each other and links between them are called junctions. In vertebrate organisms, there are three main types of cell junctions and one of them (gap junctions) is important for movement of materials between cells. The three types are

1. Gap junctions

2. Adherens junctions, (Anchoring Junctions, desmosomes and hemidesmosomes)

3. Tight junctions

Cell junctions in multicellular plants are structured differently from those in vertebrates and are called plasmodesmata. They too

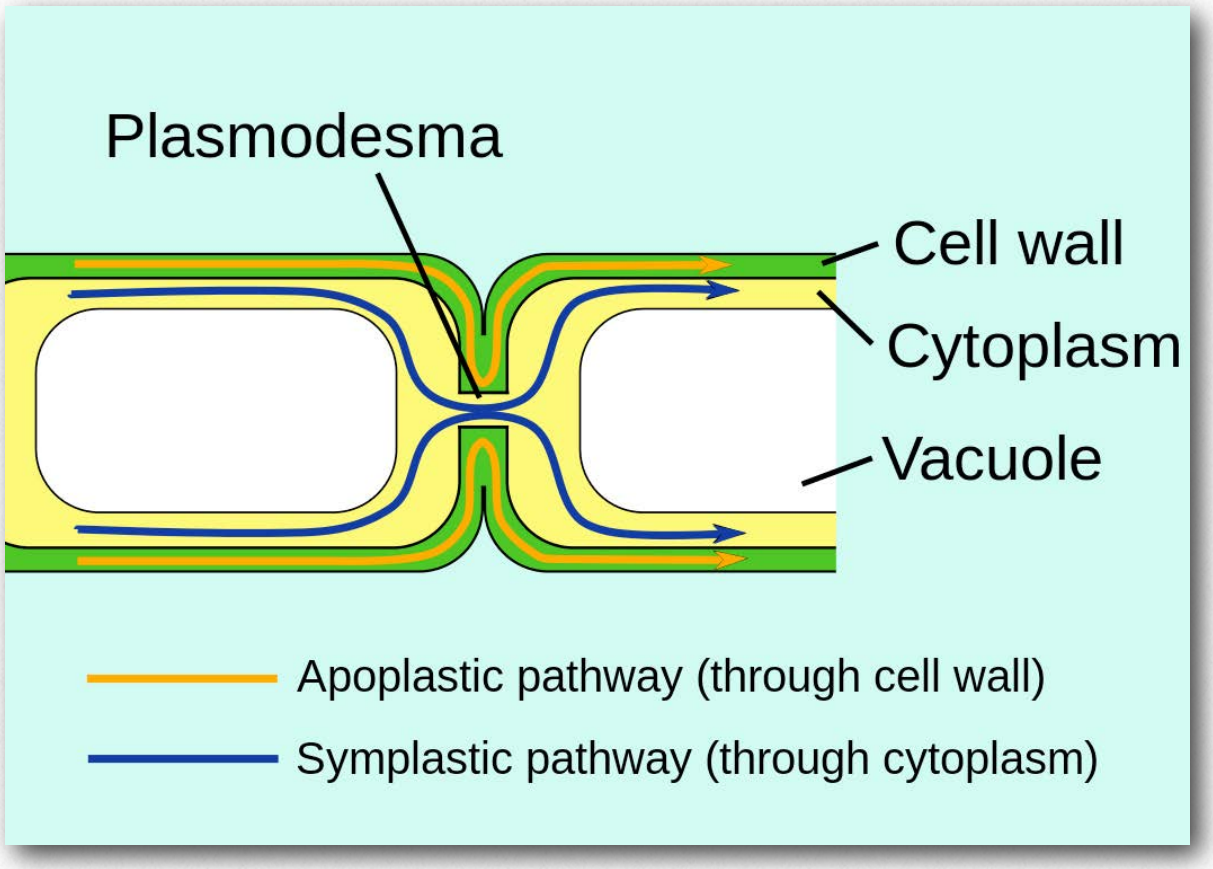

Figure 3.66 - Two means of intercellular communication in plant cells - apoplastic pathway (through cell wall) and symplastic pathway (through the plasodesma)

function in exchange of materials between individual cells.

Gap junctions

Gap junctions are specialized structures made up of two sets of structures called connexons (one from each cell - see Figure 3.65) directly link the cytoplasms of the connected cells. Gap junctions are regulated to control the flow of molecules, ions, and electrical impulses between cells. In plants, similar structures known as plasmodesmata traverse the cell wall (Figure 3.66) and perform similar functions.

Adherens junctions

Adherens junctions (Figure 3.67) are protein complexes on the cytoplasmic side of the cell membranes of epithelial and endothelial tissues that link cells to each other or to the extracellular matrix. They correspond to the fascia adherens found in non-epithelial/non-endothelial cells.

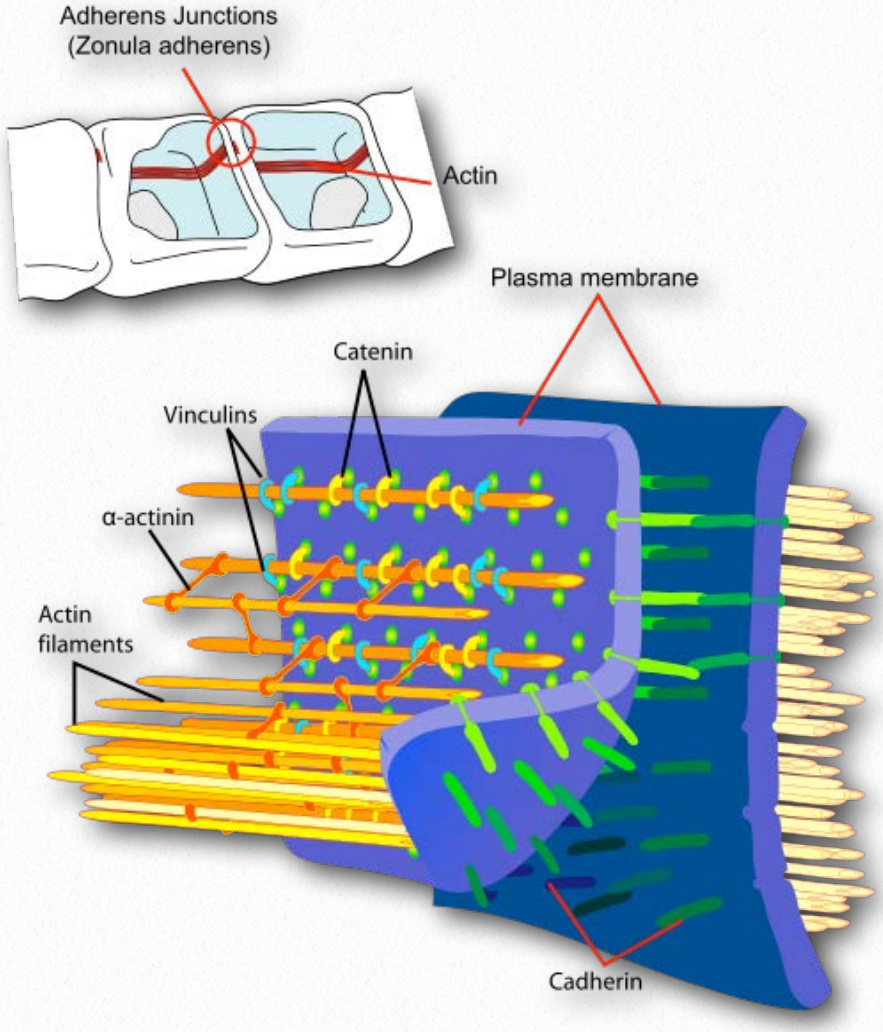

Figure 3.67 - Adherens junction

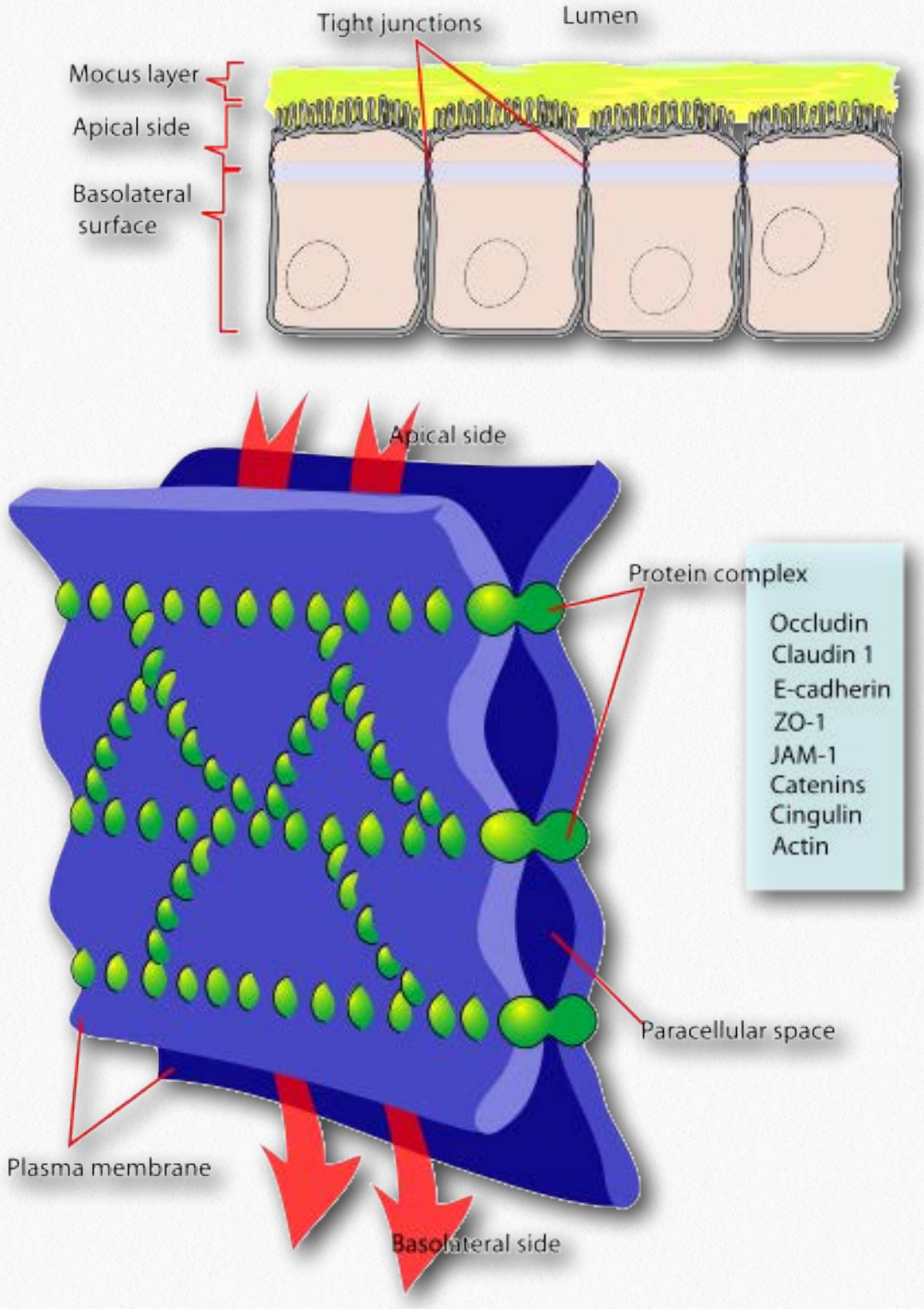

Figure 3.68 - Tight junctions

Adherens junctions contain the following proteins - 1) α-catenin (binds cadherin through β-catenin); 2) β-catenin (attachment for α-catenin to cadherin; 3) γ-catenin (binds to cadherin); 4) cadherins (group of transmembrane proteins that dimerize with cadherins on adjacent cells; 5) p120 (also called Δ-catenin - binds to cadherin); 6) plakoglobin (catenin family protein homologous to and acting like β-catenin); 7) actin; 8) actinin; and 9) vinculin. Adherens junctions may help to maintain the actin contractile ring which forms in the process of cytokinesis.

Tight junctions

Tight junctions (Figure 3.68) are a network of protein strands that seal cells together and restrict the flow of ions in the spaces between them. The effect of their structure is to restrict the movement of materials through tissues by requiring them to pass through cells instead of around them. Tight junctions join together the cytoskeletons of cells and through their structure maintain their apical and basolateral polarity.

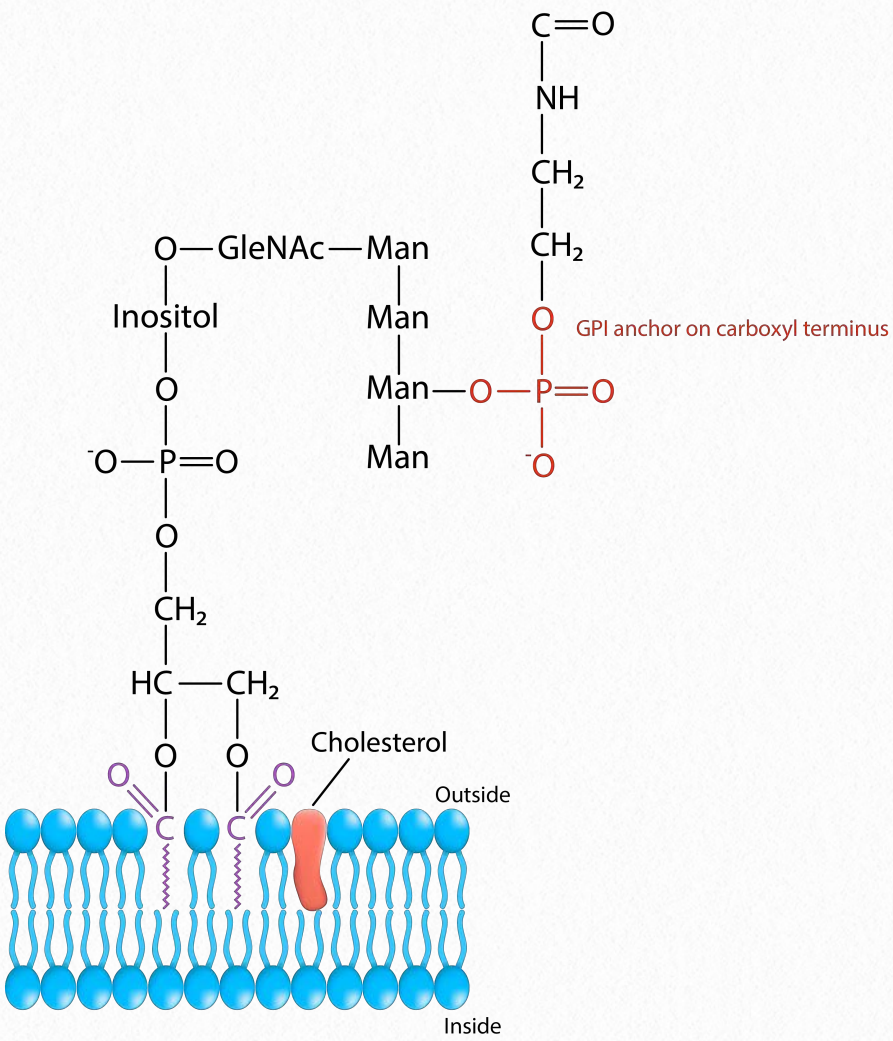

Figure 3.69 - A glypiated protein linked to a membrane-embedded inositol-based molecule. The protein portion is above the red phosphate ion

Image by Pehr Jacobson

GPI anchors

Membrane proteins attached to glycosylphosphatidylinositol (also known as a GPI anchor) are referred to as being glypiated. The proteins, which play important roles in many biochemical processes, are attached to the GPI anchor at their carboxyl terminus. Phospholipases, such as phospholipase C can cut the bond between the protein and the GPI, freeing the protein from the outer cell membrane. Proteins destined to be glypiated have two signal sequences. They are 1) An N-terminal signal sequence and 2) A C-terminal signal sequence that is recognized by a GPI transamidase (GPIT). The N-terminal signal sequences is responsible for directing co-translational transport into the endoplasmic reticulum. The C-terminal sequence is recognized by GPI transamidase, which links the carboxy terminus of a protein to the GPI anchor.

Liposomes

The spontaneous ability of phosphoglycerolipid and sphingolipid compounds to form lipid bilayers is exploited in the formation of artificial membranous structures called liposomes (Figure 3.69). Liposomes are useful for delivering their contents into cells via

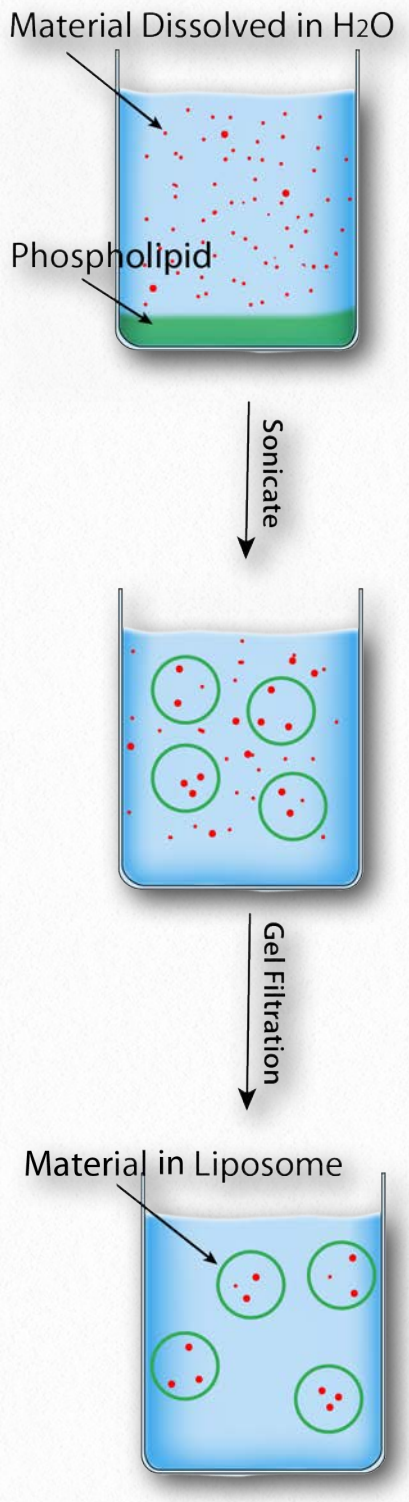

Figure 3.70 - Formation of liposomes from phospholipids in water

Image by Pehr Jacobson

membrane fusion. In the figure, items targeted for delivery to cells would be encased in the middle circular region of the liposome and when the liposome fuses with the cell membrane, it will deliver the contents directly into the cytoplasm.

Hydropathy index

The interior portion of the lipid bilayer is very hydrophobic, which makes it very restrictive to movement of ions and polar substances across it. This property also places limitations on the types of amino acids that will interact with it as well. Because of this, transmembrane protein domains found in integral membrane proteins are biased in the amino acids that interact with either the lipid bilayer or the aqueous material on either side of it.

Hydrophobic amino acids are found within the bilayer, whereas hydrophilic amino acids are found predominantly on the surfaces. An additional clue to identifying membrane crossing regions of a protein is that tryptophan or tyrosine is commonly positioned at nonpolar/polar interface(s) of the lipid bilayer for integral proteins. Such an organization of amino acids can be recognized by computer analysis of amino acid sequences using what is called a hydropathy index/score (Figure 3.71). Though the names and the scorings vary, the idea is to assign a number (usually positive) to amino acid side chains with higher hydrophobicity and negative to those that are ionic. With these scores, a computer program can easily find the average scores of short amino acid segments (say 3 amino acids long) and then plot those values on a graph of hydrophobicity index versus position in polypeptide chain. Doing that for a transmembrane protein such as glycophorin results in the plot shown in Figure 3.72. It is apparent in the analysis that this is a transmembrane protein that has seven domains crossing the lipid bilayer, as labeled.

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Amino Acid	Hydropathy index
Alanine	1.8
Arginine	–4.5
Asparagine	–3.5
Aspartic acid	–3.5
Cysteine	2.5
Glutamic acid	–3.5
Glutamine	–3.5
Glycine	–0.4
Histidine	–3.2
Isoleucine	4.5
Leucine	3.8
Lysine	–3.9
Methionine	1.9
Phenylalanine	2.8
Proline	–1.6
Serine	–0.8
Threonine	–0.7
Tryptophan	–0.9
Tyrosine	–1.3
Valine	4.2

Figure 3.71 - Hydropathy index for amino acids. More positive values indicate higher hydrophobicity.

Wikipedia

Cell walls

Cells walls are found in many cells, including plants, fungi, and bacteria, but are not found

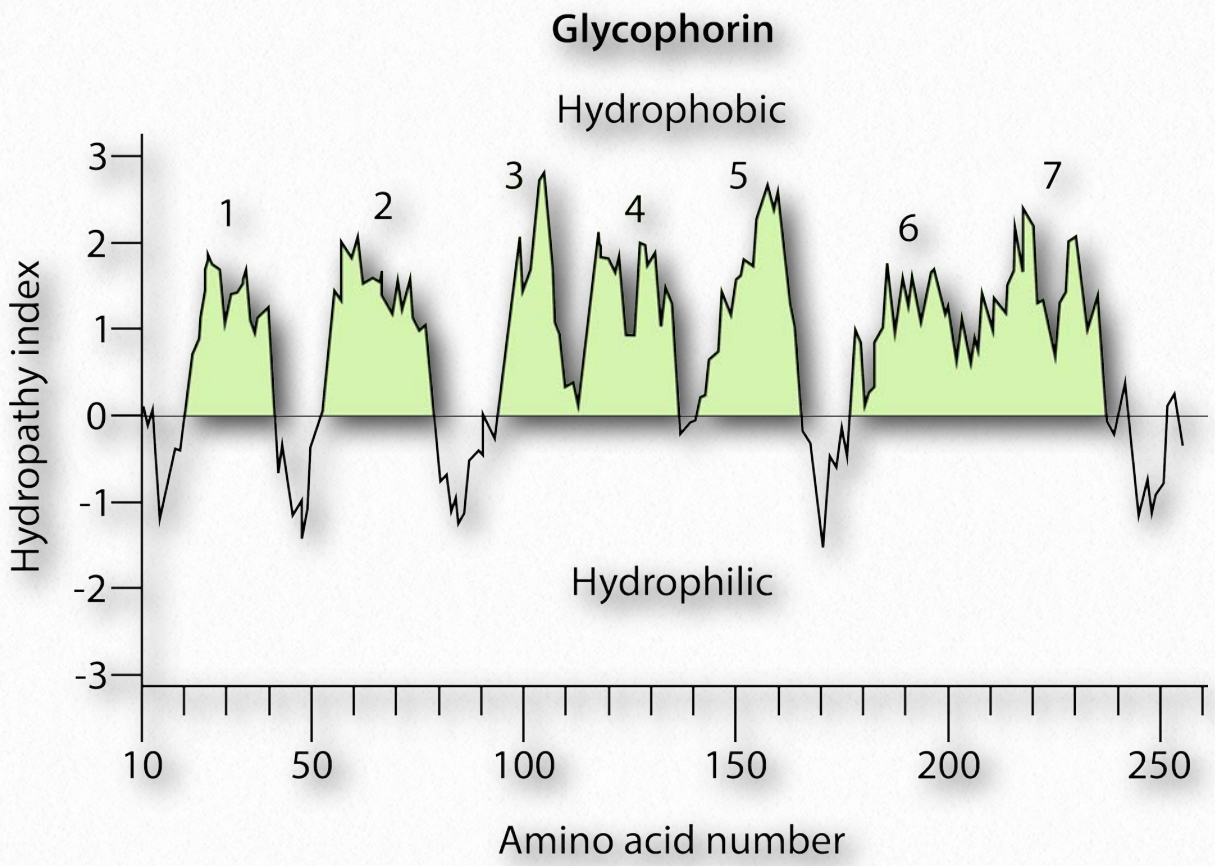

Figure 3.72 - Hydropathy index plot for glycophorin. Each lipid bilayer-crossing domain noted with a number

Image by Pehr Jacobson

in animal cells. They are designed to provide strength and integrity and at least some protection against bursting from osmotic pressure (Figures 3.73-3.75).

Gram positive bacteria (Figure 3.75) have the simplest cell wall design. Moving from outside the cell towards the cytoplasm there is an outer peptidoglycan layer for the cell wall followed by a periplasmic space, a plasma membrane, and then the cytoplasm. Gram negative bacteria add a second protective layer external to all of this, so for them,

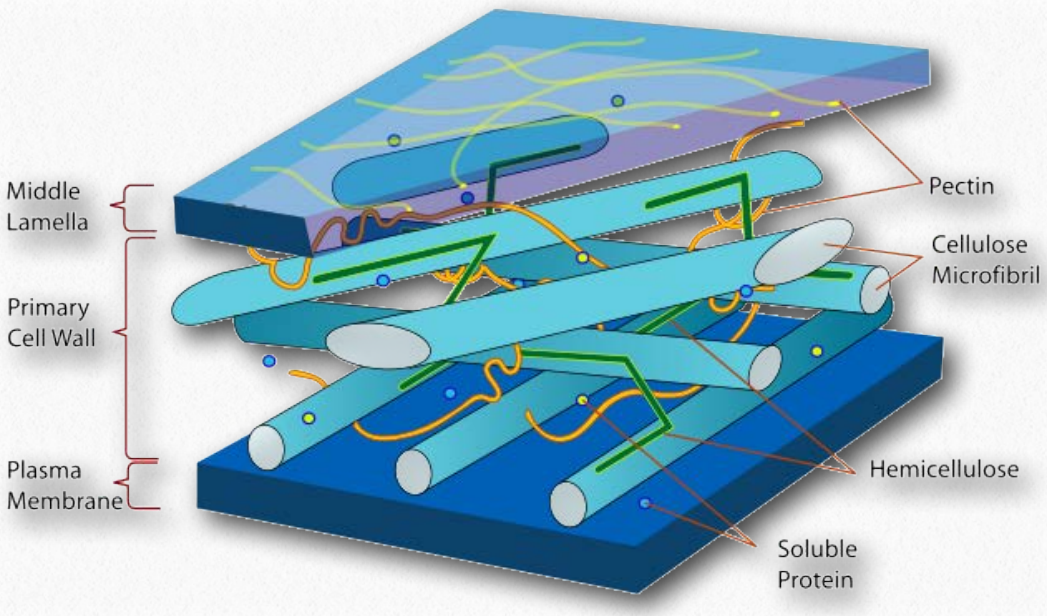

Figure 3.73 - Plant cell wall. Direction of the cytoplasm is down

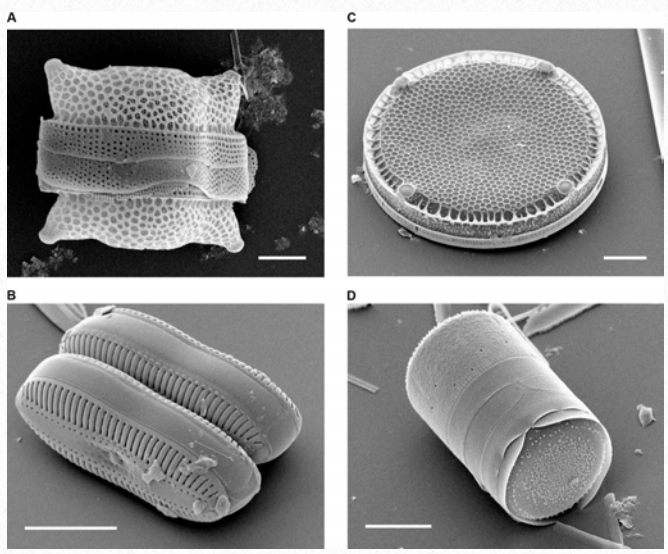

Figure 3.74 - Cell walls of diatoms

Wikipedia

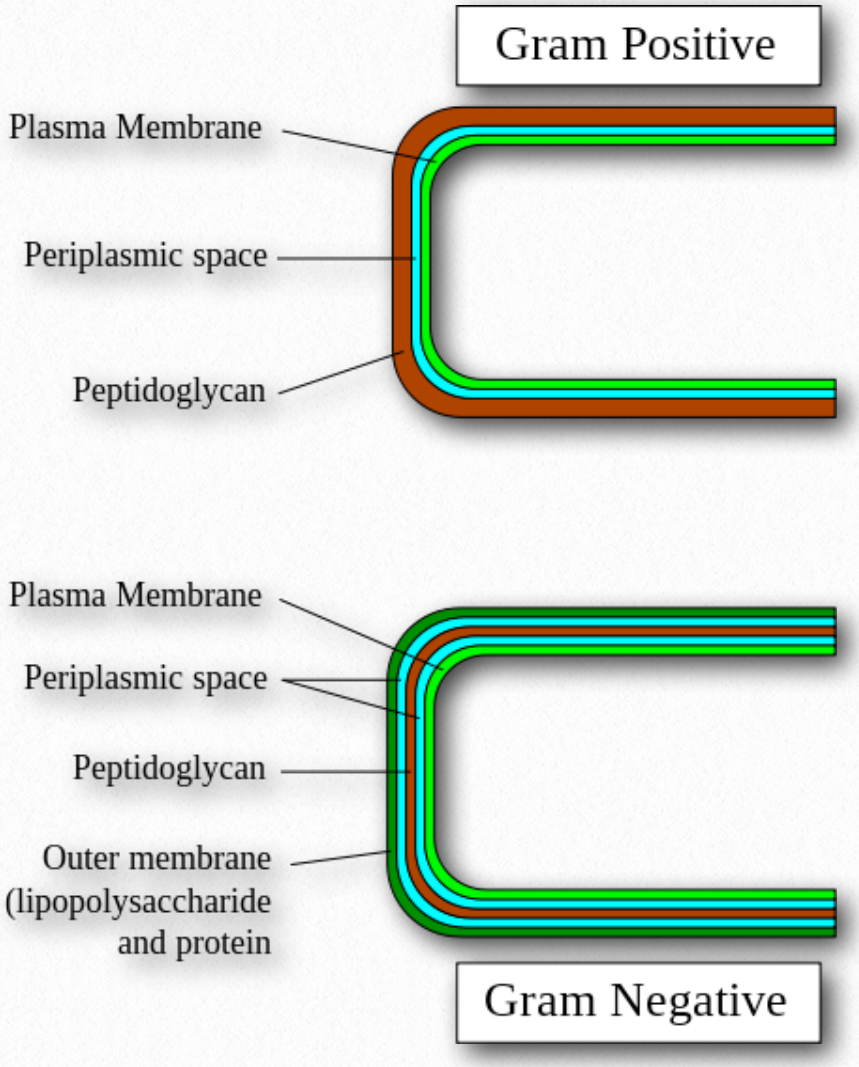

Figure 3.75 - Gram positive versus Gram negative bacteria cell coverings
Wikipedia

starting at the outside and moving inwards, one encounters an outer lipopolysaccharide layer, a periplasmic space, the peptidoglycan cell wall, a second periplasmic space, a plasma membrane and then the cytoplasm.

Herbaceous plants have a rigid outer cell wall (primarily composed of cellulose, hemicellulose, and pectin) and an inner plasma membrane. Woody plants add a second level of wall with lignin between the cellulosic wall and the plasma membrane of herbaceous plants.

Graphic images in this book were products of the work of several talented students. Links to their Web pages are below

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BB Wonderland

To the tune of "*Winter Wonderland*"

Metabolic Melodies Website HERE

Milam Hall - It's 12:30
And Ahern's gettin' wordy

He walks to and fro'
While not talkin' slow
Givin' it to B-B-4-5-0

I was happy when the term got started
Lecture notes and videos galore
MP3s got added to my iPod
But recitations sometimes were a bore

And exams bit me roughly
When the curve turned out ugly

I don't think it's so
My scores are too low
Slidin' by in B-B-4-5-0

Final-LY there's an examination
On December 9th at 6:00 pm
I'll have my card packed with information
So I don't have to memorize it then

And I'll feel like a smarty
With my jam-packed note-cardy
Just one more to go
And then ho-ho-ho
I'll be done with B-B-4-5-0

Recording by David Simmons
Lyrics by Kevin Ahern

Thank God There's a Video

To the tune of "*Thank God I'm a Country Boy*"

Metabolic Melodies Website HERE

There's a bundle of things a student oughta know
And Ahern's talk isn't really very slow
Learnin' ain't easy / the lectures kinda blow
Thank God there's a video

Well we've gone through the cycles and their enzymes too
Studying the regulation everything is new
I gotta admit that I haven't got a clue
What am I gonna do?

So I got me a note card and bought me a Stryer
Got the enzymes down and the names he requires
I hope that I can muster up a little more desire
Thank God there's a video

Just got up to speed about the N-A-D
Protons moving through Complex Vee
Electrons dance in the cytochrome C
Gotta hear the MP3

Fatty acid oxidation makes acetyl-CoA
Inside the inner matrix of the mitochondri-ay
It's very complicated, I guess I gotta say
Thank God there's a video

So I got me a note card and bought me a Stryer
Got the enzymes down and the names he requires
I hope that I can muster up a little more desire
Thank God there's a video

Replication's kind of easy in a simple kind of way
Copyin' the bases in the plasmid DNAs
Gs goes with Cs and Ts go with As
Thanks to polymerase

And the DNA's a template for the RNA
Helices unwinding at T-A-T-A
Termination happens, then the enzyme goes away
Don't forget the poly-A

So I got me a note card and bought me a Stryer
Got the enzymes down and the names he requires
I think that I can muster up a little more desire

Thank
there's a

God
video

Recording by David Simmons
Lyrics by Kevin Ahern

4

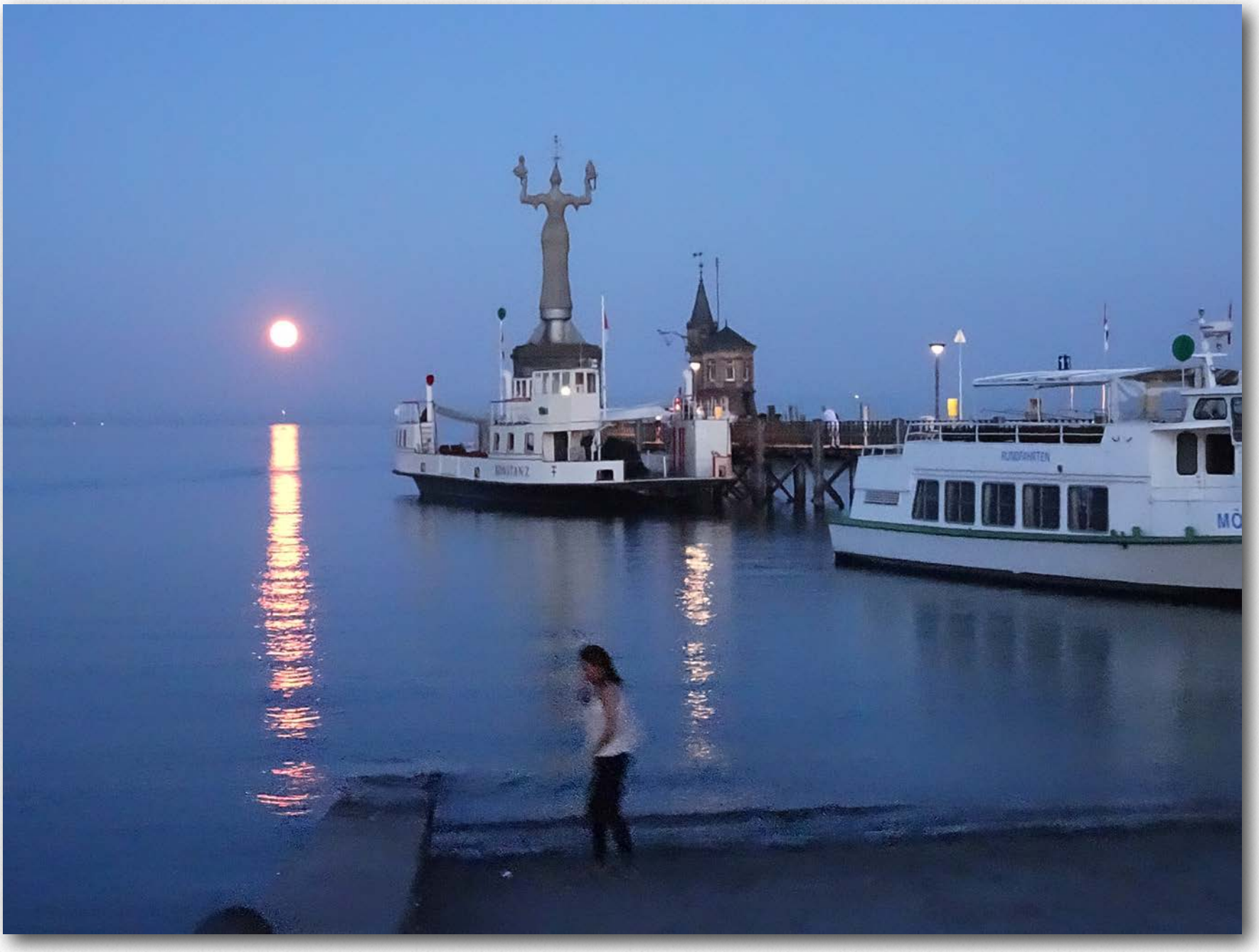
Catalysis

"A clever man commits no minor blunders."

Goethe

Where a good chemical catalyst might accelerate a reaction by a few orders of magnitude, the speed of the best enzyme catalyst boggles the mind, boosting reaction rates by 10^{24} - over 1 trillion trillion times faster than an uncatalyzed one. In this chapter we explore the ways in which the process of enzymatic catalysis occurs.

Catalysis: Basic Principles



If there is a magical component to life, an argument can surely be made for it being catalysis. Thanks to catalysis, reactions that can take hundreds of years to complete in the uncatalyzed "real world," occur in seconds in the presence of a catalyst. Chemical catalysts, such as platinum, can speed reactions, but enzymes (which are simply super-catalysts with a "twist," as we shall see) put chemical catalysts to shame (Figure 4.1). To understand enzymatic catalysis, it is necessary first to understand energy. Chemical reactions follow the universal trend of moving towards lower energy, but they often have a barrier in place that must be overcome. The secret to catalytic action is reducing the magnitude of that barrier.

YouTube Lectures by Kevin HERE & HERE

Equilibrium

Before discussing enzymes, it is appropriate to pause and discuss an important concept relating to chemical/biochemical reactions. That concept is equilibrium and it is very of-

ten misunderstood. The “equi" part of the word relates to equal, as one might expect, but it does not relate to absolute concentrations. What happens when a biochemical reaction is at equilibrium is that the concentrations of reactants and products do not change over time. This does not mean that the reactions have stopped. Remember that reactions are reversible, so there is a forward reaction and a reverse reaction: if you had 8 molecules of A, and 4 of B at the beginning, and 2 molecules of A were converted to B, while 2 molecules of B were simultaneously converted back to A, the number of molecules of A and B remain unchanged, i.e., the reaction is at equilibrium. However, you will notice that this does not mean that there are equal numbers of A and B molecules.

Concentration matters

So, contrary to the perceptions of many students, the concentrations of products and reactants are not equal at equilibrium, *unless* the **Δ**G°’ for a reaction is zero, because when this is the case,

$\Delta G = \ln\{[Products]/[Reactants]$,

since the ΔG°’ is zero. Because ΔG itself is zero at equilibrium, then [Products] = [Reactants]. This is the only circumstance where [Products] = [Reactants] at equilibrium.

Reiterating, at equilibrium, the concentrations of reactant and product do not change over time. That is, for a reaction

A <=> B

[A] at time zero when equilibrium is reached, $[A]_{T0,}$ will be the same 5 minutes later (assuming A and B are chemically stable). Thus,

$[A]_{T0} = [A]_{T+5}$.

Similarly,

$[B]_{T0} = [B]_{T+5}$

Enzyme	Nonenzymatic Half-Life	Uncatalyzed Rate (k_{un} s^{-1})	Catalyzed Rate (k_{cat} s^{-1})	Rate Enhancement (k_{cat} s^{-1}/k_{un} s^{-1})
OMP decarboxylase	78,000,000 years	28×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9 days	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

Abbreviations:
OMP - Orotine monophosphate
AMP - Adenosine monophosphate

Figure 4.1 - Rate enhancement for several enzymes

Image by Aleia Kim

For that matter, at any amount of time X after equilibrium has been reached,

$$[A]_{T0} = [A]_{T+5} = [A]_{TX}$$

and

$$[B]_{T0} = [B]_{T+5} = [B]_{TX}$$

However, unless $\Delta G^{\circ\prime} = 0$, it is *wrong* to say

$$[A]_{T0} = [B]_{T0}$$

As we study biochemical reactions and reaction rates, it is important to remember that 1) reactions do not generally start at equilibrium; 2) all reactions move in the direction of equilibrium; and 3) reactions in cells behave just like those in test tubes - they do not begin at equilibrium, but they move towards it.

Dynamic reactions

The reactions occurring in cells, though, are very dynamic and complex. In a test tube, they can be studied one at a time. In cells, the product of one reaction is often the substrate for another one. Reactions in cells are interconnected in this way, giving rise to what are called metabolic pathways.

There are, in fact, thousands of different interconnected reactions going on continuously in cells. Attempts to study a single reaction in the chaos of a cell is daunting to say the least. For this reason, biochemists isolate enzymes from cells and study reactions individually.

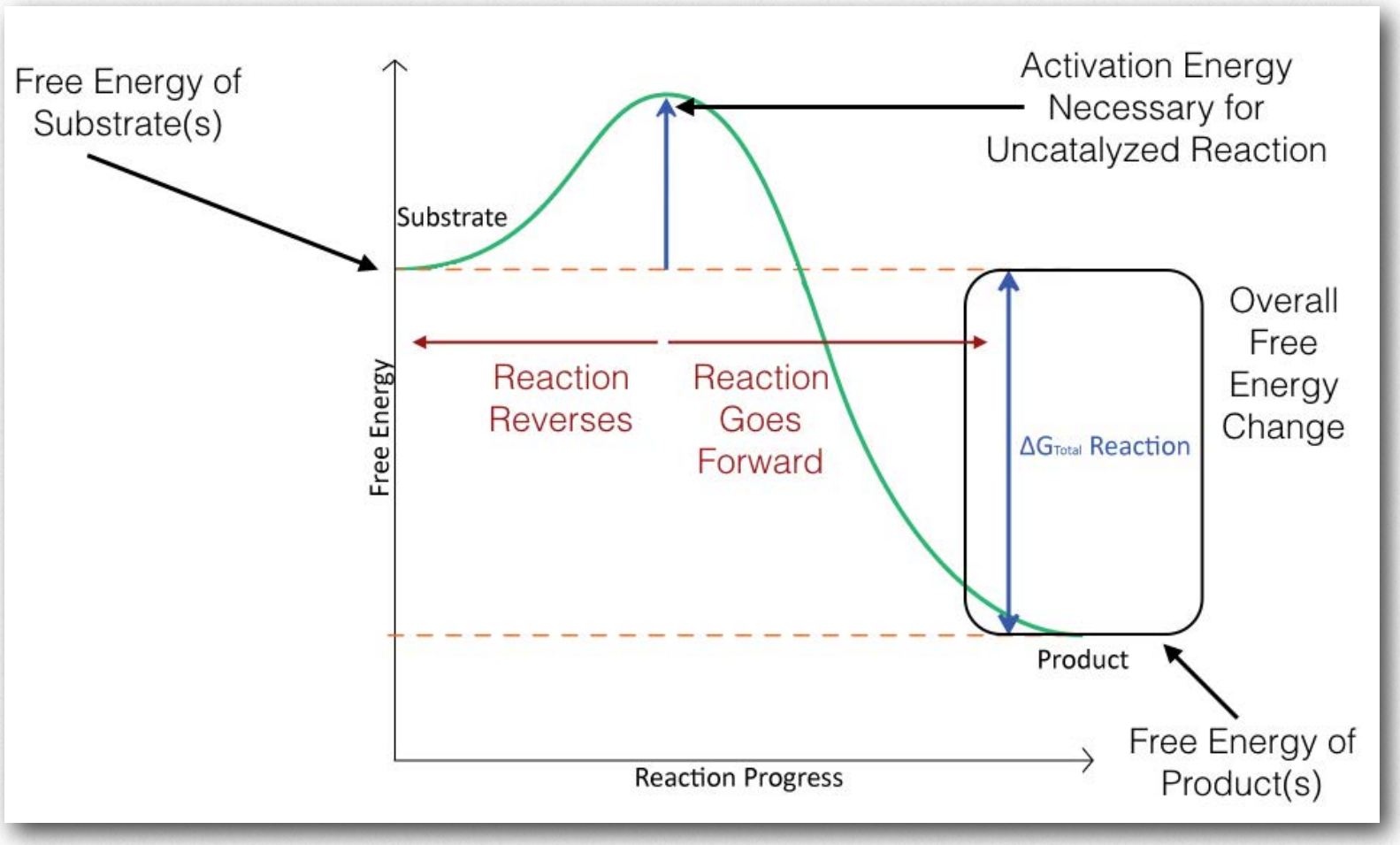


Figure 4.2 - Energy changes during the course of an uncatalyzed reaction

Image by Aleia Kim

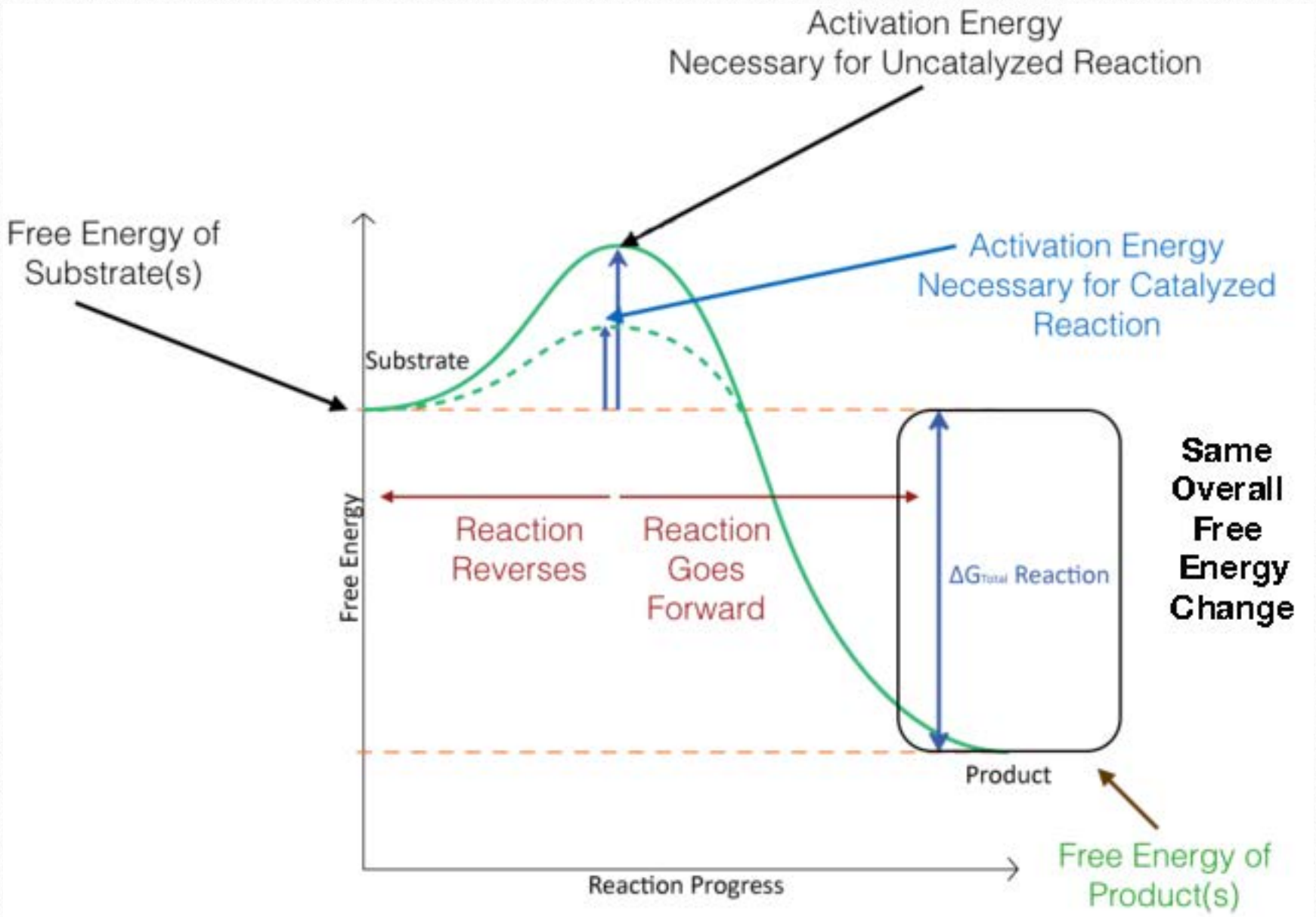


Figure 4.3 - Energy changes during the course of an uncatalyzed reaction (solid green line) and a catalyzed reaction (dotted green line).

Image by Aleia Kim

It is with this in mind that we begin our consideration of the phenomenon of catalysis by describing, first, the way in which enzymes work.

Activation energy

Figure 4.2 schematically depicts the energy changes that occur during the progression of a simple reaction. In order for the reaction to proceed, an activation energy must be overcome in order for the reaction to occur.

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In Figure 4.3, the activation energy for a catalyzed reaction is overlaid. As you can see, the reactants start at the same energy level for both catalyzed and uncatalyzed reactions and that the products end at the same energy for both as well. The catalyzed reaction, however, has a lower energy of activation (dotted line) than the un-

catalyzed reaction. This is the secret to catalysis - overall ΔG for a reaction does NOT change with catalysis, but the activation energy is lowered.

Reversibility

The extent to which reactions will proceed forward is a function of the size of the energy difference between the product and reactant states. The lower the energy of the products compared to the reactants, the larger the percentage of molecules that will be present as *products* at equilibrium. It is worth noting that since an enzyme lowers the activation energy for a reaction that it can speed the reversal of a reaction just as it speeds a reaction in the forward direction. At equilibrium, of course, no change in concentration of reactants and products occurs. Thus, enzymes speed the time required to reach equilibrium, but do not affect the balance of products and reactants at equilibrium.

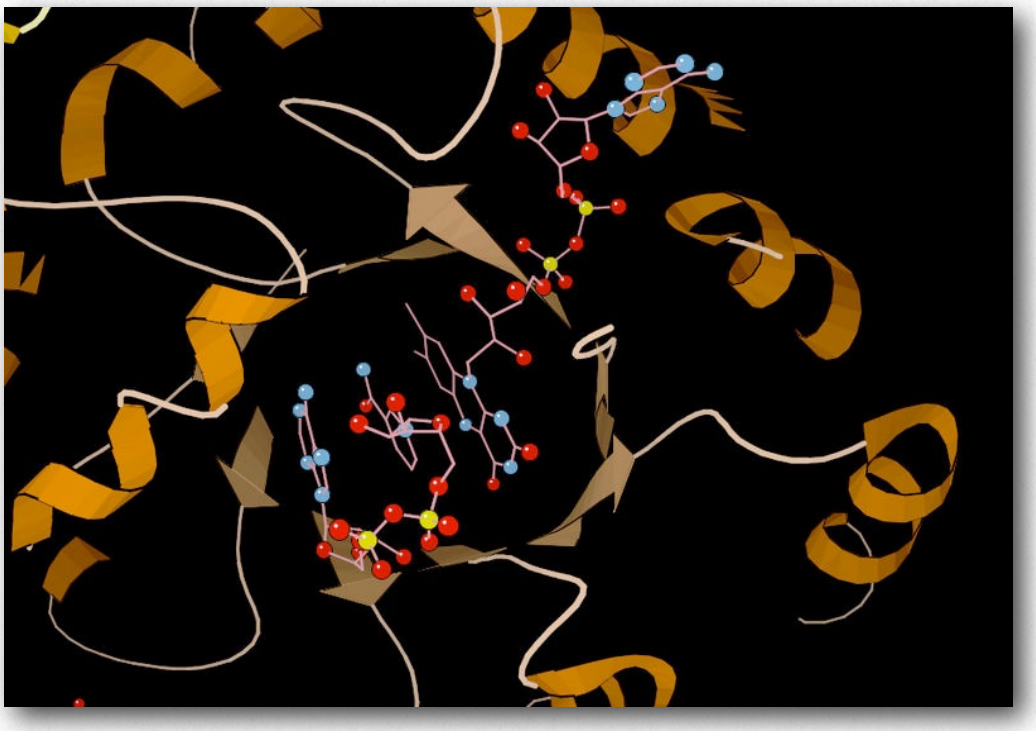

Figure 4.4 - Substrate binding by methylenetetrahydrofolate reductase

YouTube Lectures by Kevin
HERE & HERE

Exceptions

The reversibility of enzymatic reactions is an important consideration for equilibrium, the measurement of enzyme kinetics, for Gibbs free energy, for metabolic pathways, and for physiology.

There are some minor exceptions to the reversibility of reactions, though. They are related to the disappearance of a substrate or product of a reaction. Consider the first reaction below which is catalyzed by the enzyme carbonic anhydrase:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

In the forward direction, carbonic acid is produced from water and carbon dioxide. It can either remain intact in the solution or ionize to produce bicarbonate ion and a proton. In the reverse direction, water and carbon dioxide are produced. Carbon dioxide, of course, is a gas and can leave the solution and escape.

When reaction molecules are removed, as they would be if carbon dioxide escaped, the reaction is pulled in the direction of the molecule being lost and reversal cannot occur unless the missing molecule is replaced. In the second reaction occurring on the right, carbonic acid (H_2CO_3) is "removed" by ionization. This too would limit the reaction going back to carbon dioxide in water. This last type of "removal" is what occurs in metabolic pathways. In this case, the product of one reaction (carbonic acid) is the substrate for the next (formation of bicarbonate and a proton).

In the metabolic pathway of glycolysis, ten reactions are connected in this manner and reversing the process is much more complicated than if just one reaction was being considered.

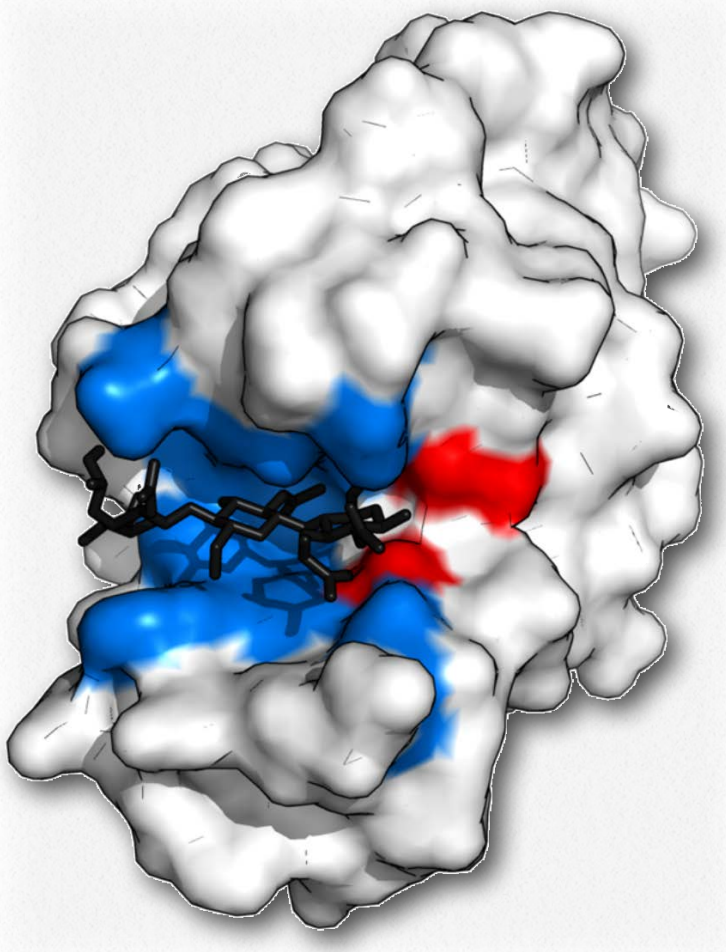

Figure 4.5 - Lysozyme with substrate binding site (blue), active site (red) and bound substrate (black).

Wikipedia

General mechanisms of action

As noted above, enzymatically catalyzed reactions are orders of magnitude faster than uncatalyzed and chemical-catalyzed reactions.

The secret of their success lies in a fundamental difference in their mechanisms of action.

Every chemistry student has been taught that a catalyst speeds a reaction without being consumed by it. In other words, the catalyst ends up after a reaction just the way it started so it can catalyze other reactions, as well. Enzymes share this property, but in the middle, during the catalytic action, an enzyme is transiently changed. In fact, it is the ability of an enzyme to change that leads to its incredible efficiency as a catalyst.

Changes

These changes may be subtle electronic ones, more significant covalent modifications, or structural changes arising from the flexibility inherent in enzymes, but not present in chemical catalysts. Flexibility allows movement and movement facilitates alteration of electronic environments necessary for catalysis. Enzymes are, thus, much more efficient than rigid chemical catalysts as a result of their abilities to facilitate the changes necessary to optimize the catalytic process.

Substrate binding

Another important difference between the mechanism of action of an enzyme and a chemical catalyst is that an enzyme has binding sites that not only 'grab' the substrate (molecule involved in the reaction being catalyzed), but also place it in a position to be electronically induced to react, either within itself or with another substrate.

The enzyme itself may play a role in the electronic induction or the induction may occur as a result of substrates being placed in very close proximity to each other. Chemical catalysts have no such ability to bind substrates and are dependent upon them colliding in the right orientation at or near their surfaces.

Active site

Reactions in an enzyme are catalyzed at a specific location within it known as the 'active site'. Substrates bind at the active site and are oriented to provide access for the relevant portion of the molecule to the electronic environment of the enzyme where catalysis occurs.

Enzyme flexibility

As mentioned earlier, a difference between an enzyme and a chemical catalyst is that an enzyme is flexible. Its slight changes in shape (often arising from the binding of the substrate itself) help to optimally position substrates for reaction after they bind.

Induced fit

These changes in shape are explained, in part, by Koshland's Induced Fit Model of Catalysis (Figure 4.6), which illustrates that not only do enzymes change substrates, but that substrates also transiently change enzyme struc-

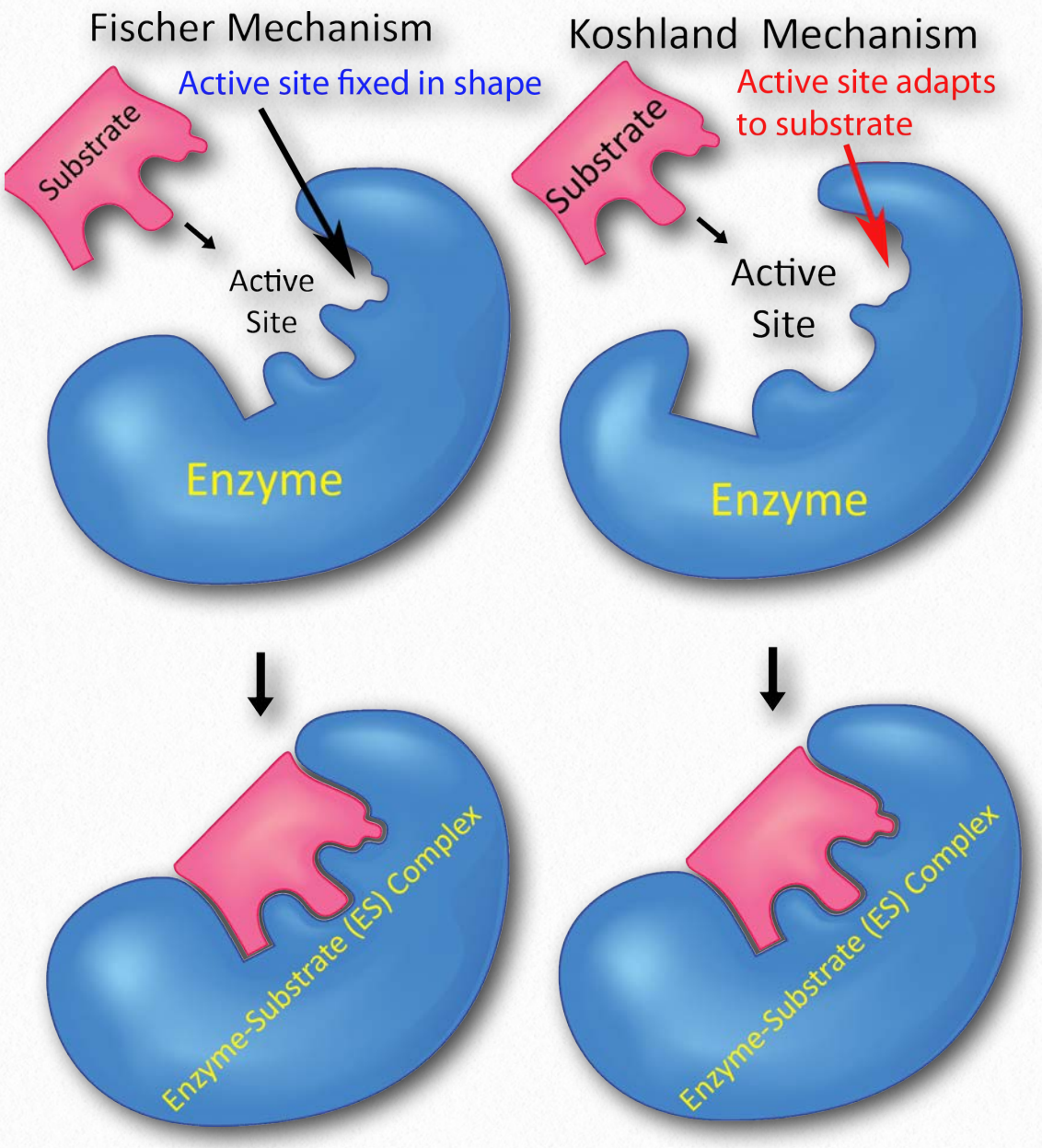

Figure 4.6 - Fischer's lock and key model (left) Vs. Koshland's induced fit model (right)

Image by Aleia Kim

ture. At the end of the catalysis, the enzyme is returned to its original state. Koshland's model is in contrast to the Fischer Lock and Key model, which says simply that an enzyme has a fixed shape that is perfectly matched for binding its substrate(s). Enzyme flexibility also is important for control of enzyme activity. Enzymes alternate between the T (tight) state, which is a lower activity state and the R (relaxed) state, which has greater activity.

Induced Fit

The Koshland Induced Fit model of catalysis postulates that enzymes are flexible and change shape on binding substrate. Changes in shape help to 1) aid binding of additional substrates in reactions involving more than one substrate and/or 2) facilitate formation of an electronic environment in the enzyme that favors catalysis. This model is in contrast to the Fischer Lock and Key Model of catalysis which considers enzymes as having pre-formed substrate binding sites.

Ordered binding

The Koshland model is consistent with multi-substrate binding enzymes that exhibit ordered binding of substrates. For these systems, binding of the first substrate induces structural changes in the enzyme necessary for binding the second substrate.

There is considerable experimental evidence supporting the Koshland model. Hexokinase, for example, is one of many enzymes known to undergo significant structural alteration after binding of substrate. In this case, the two substrates are brought into very close proximity by the induced fit and catalysis is made possible as a result.

Types of Reactions

Single Substrate - Single Product : $A \rightleftarrows B$

Single Substrate - Multiple Products : $A \rightleftarrows B + C$

Multiple Substrates - Single Products : $A + B \rightleftarrows C$

Multiple Substrates - Multiple Products : $A + B \rightleftarrows C + D$

Figure 4.7 - Categories of enzymatic reactions

Reaction types

Enzymatic reactions can be of several types, as shown in Figure 4.7.

Enzymes that catalyze reactions involving more than one substrate, such as

$$A + B \rightleftarrows C + D$$

can act in two different ways. In one mechanism, called sequential reactions, at some point in the reaction, both substrates will be bound to the enzyme. There are, in turn, two