Information Processing: Signaling

Cellular communication

Up to this point we have considered how cells carry out biochemical reactions and how they regulate the expression of the genes in response to their internal and external environments. It is intuitively obvious that even unicellular organisms must be able to sense features of their environment, such as the presence of nutrients, if they are to survive. In addition to being able to receive and respond to information from the environment, multicellular organisms must also find ways by which their cells can communicate among themselves.

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Coordination

Since different cells take on specialized functions in a multicellular organism, they must be able to coordinate activities. Cells grow, divide, or differentiate in response to specific signals. They may change shape or migrate to another location. At the physiological level, cells in a multicellular organism, must respond to everything from a meal

Signal Category	Examples
Indoleamine	Serotonin, melatonin
Catecholamine	Epinephrine, norepinephrine
Peptide hormone	Insulin, glucagon, EGF
Steroid hormone	Cortisol, estrogen, testoterone
Eicosanoids	Leukotrienes, prostaglandins, endocannabinoids

Figure 7.129 - Some examples of signal molecules

just eaten to injury, threat, or the availability of a mate. They must know when to divide, when to undergo apoptosis (programmed cell death), when to store food, and when to break it down. A variety of mechanisms have arisen to ensure that cell-cell communication is not only possible, but astonishingly swift, accurate and reliable.

How are signals sent between cells? Like pretty much everything that happens in cells, signaling is dependent on molecular recognition. The basic principle of cell-cell signaling is simple. A particular kind of molecule, sent by a signaling cell, is recognized and bound by a receptor protein in (or on the surface of) the target cell. The signal molecules are chemically varied- they may be proteins, short peptides, lipids, nucleotides or catecholamines, to name a few.

Signal properties

The chemical properties of the signal determine whether its receptors are on the cell surface or intracellular. If the signal is small and hydrophobic it can cross the cell membrane and bind a receptor inside the cell. If, on the other hand, the signal is charged, or very large, it would not be able to diffuse through the plasma membrane. Such signals need receptors on the cell surface, typically transmembrane proteins that have an extracellular portion that binds the signal and an intracellular part that passes on the message within the cell (Figure 7.130).

Receptors are specific for each type of signal, so each cell has many different kinds of receptors that can recognize and bind the many signals it receives. Because different

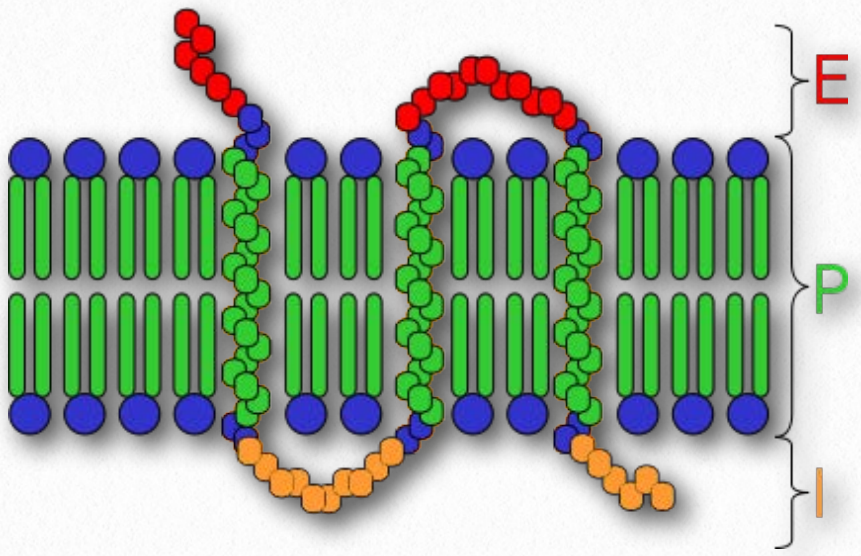

Figure 7.130 - Schematic representation of a transmembrane receptor protein. E = extracellular; P = plasma membrane; I = intracellular

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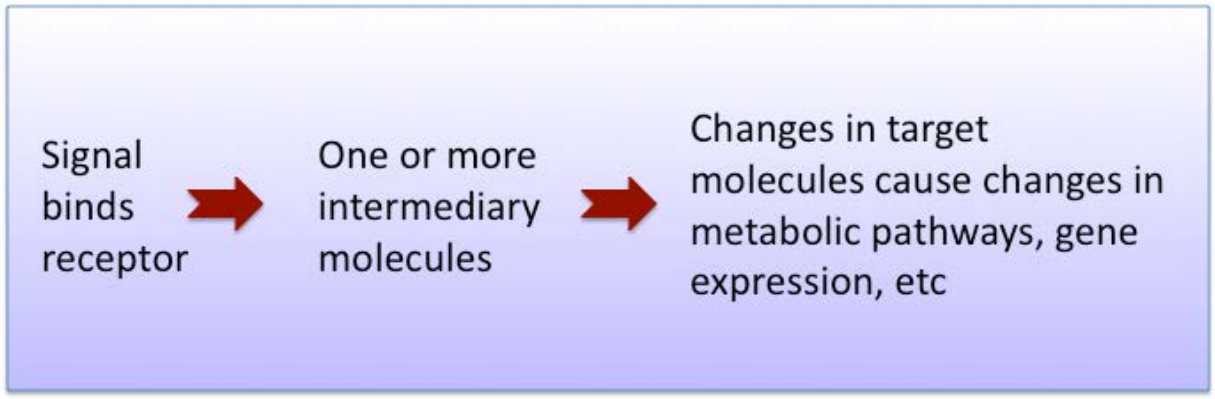

Figure 7.131 -General features of signal transduction pathways

cells have different sets of receptors, they respond to different signals or combinations of signals. The binding of a signal molecule to a receptor sets off a chain of events in the target cell. These events could cause change in various ways, including, but not limited to, alterations in metabolic pathways or gene expression in the target cell.

How the binding of a signal to a receptor brings about change in cells is the topic of this section. We will examine a few of the major receptor types and the consequences of signal binding to these receptors. Although the specific molecular components of the various signal transduction pathways differ, they all have some features in common (Figure 7.131):

• The binding of a signal to its receptor is usually followed by the generation of a new signal(s) within the cell.

The process by which the original signal is converted to a different form and passed on within the cell to bring about change is called signal transduction.

• Most signaling pathways have multiple signal transduction steps by which the signal is relayed through a series of molecular messengers that can amplify and distribute the message to various parts of the cell.

• The last of these messengers usually interacts with a target protein(s) and changes its activity, often by phosphorylation.

• When a signal sets a particular pathway in motion, it is acting like an ON switch. This means that once the desired result has been

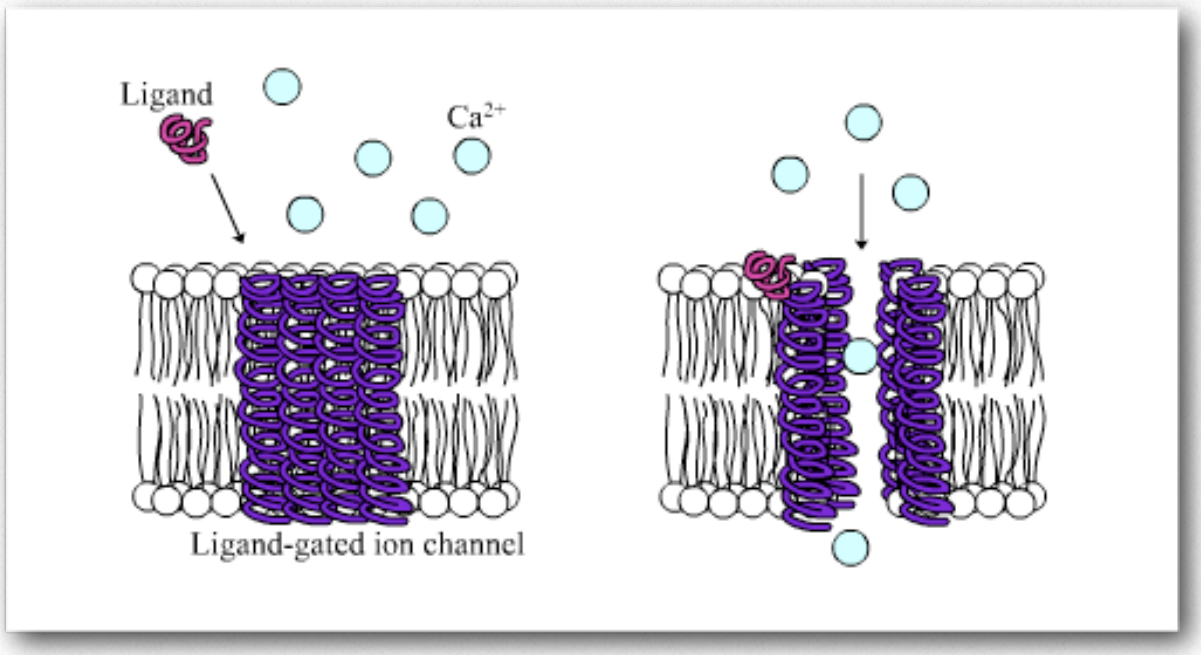

Figure 7.132 - Ligand-gated ion channel receptor opening in response to a signal (ligand)

Wikipedia

obtained, the cell must have a mechanism that acts as an OFF switch.

Understanding this underlying similarity is helpful, because learning the details of the different pathways becomes merely a matter of identifying which molecular component performs a particular function in each individual case. We will consider several different signal transduction pathways, each mediated by a different kind of receptor.

Ligand-gated ion channel receptors

The simplest and fastest of signal pathways is seen in the case of signals whose receptors are gated ion channels (Figure 7.132). Gated ion channels are made up of multiple transmembrane proteins that create a pore, or channel, in the cell membrane. Depending upon its type, each ion channel is specific to the passage of a particular ionic species. The term "gated" refers to the fact that the ion channel is controlled by a "gate" which must be opened to allow the ions through. The gates are opened by the binding of an incoming signal (ligand) to the receptor, allowing the almost instantaneous passage of millions of ions from one side of the membrane to the other. Changes in the interior environment

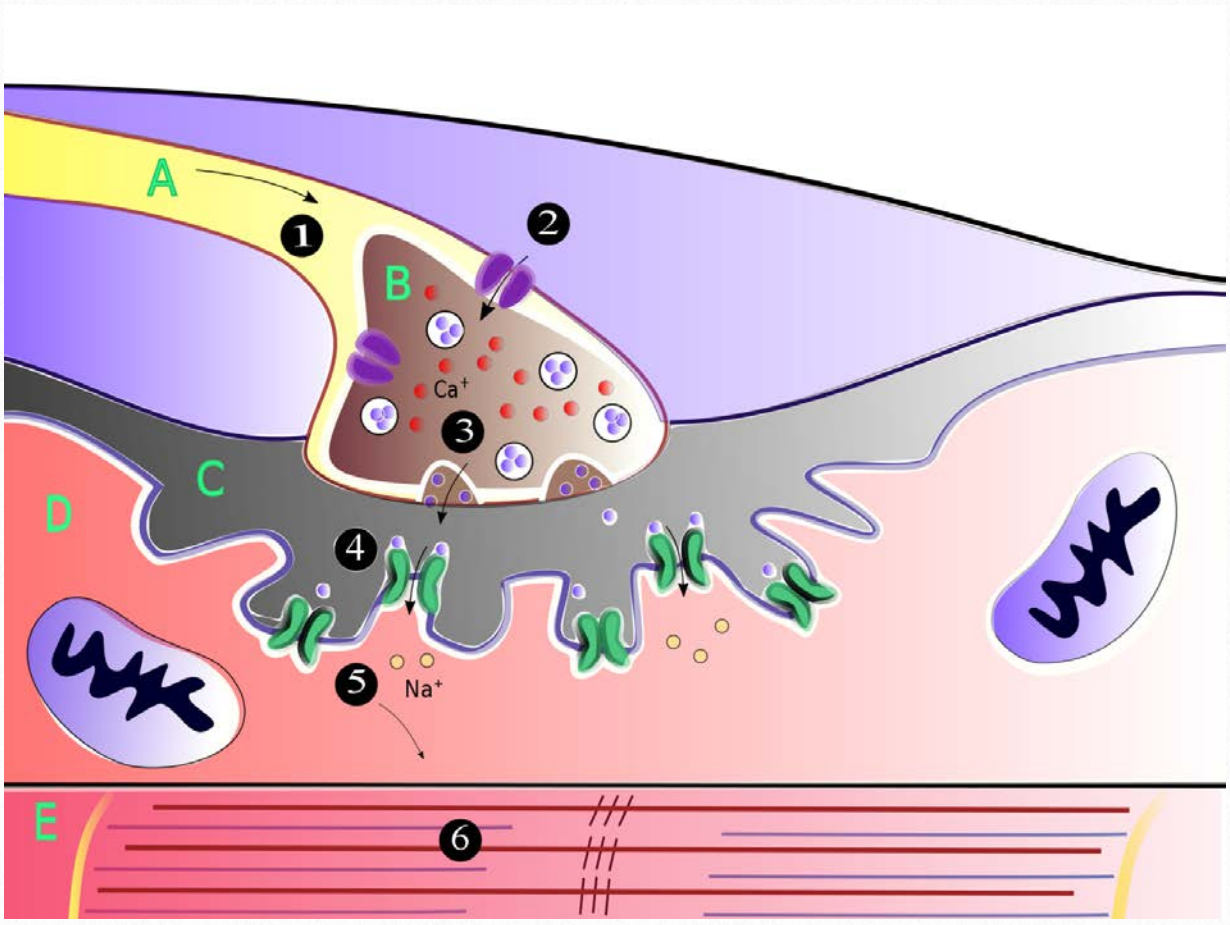

Figure 7.133 - Neuromuscular signaling - A = motor neuron axon; B = axon terminal; C = synaptic cleft; D = muscle cell; E = myofibril . Steps in the process - 1) action potential reaches the axon terminal; 2) voltage-dependent calcium gates open; (3) neurotransmitter vesicles fuse with the presynaptic membrane and acetylcholine (ACh) released into the synaptic cleft; (4) ACh binds to postsynaptic receptors on the sarcolemma; (5) ACh binding causes ion channels to open and allows sodium ions to flow across the membrane into the muscle cell; 6) flow of sodium ions across the membrane into the muscle cell generates action potential which travels to the myofibril and results in muscle contraction.

Wikipedia

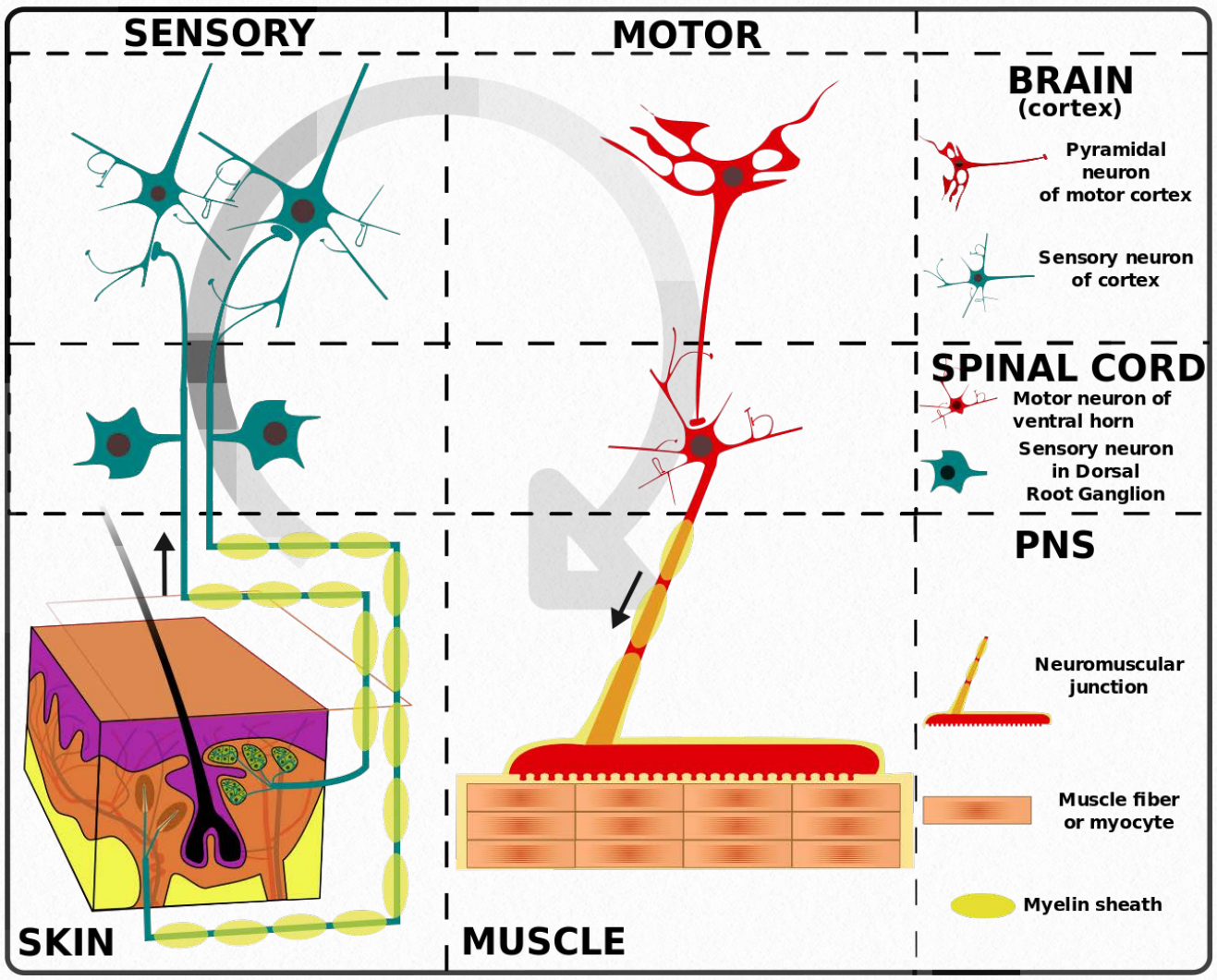

Figure 7.134 - Nerve systems

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of the cell are thus brought about in microseconds and in a single step.

Swift response

This type of swift response is seen, for example, in neuromuscular junctions, where muscle cells respond to a message from the neighboring nerve cell (Figure 7.133). The nerve cell releases a neurotransmitter signal into the synaptic cleft, which is the space between the nerve cell and the muscle cell it is "talking to". An example of such a neurotransmitter signal is acetylcholine. When the acetylcholine molecules are released into the synaptic cleft, they diffuse rapidly till they reach their receptors on the membrane of the muscle cell. The binding of the acetylcholine to its receptor, an ion channel on the membrane of the muscle cell, causes the gate in the ion channel to open. The resulting ion flow through the channel can immediately change the membrane potential of the cell. This, in turn, can trigger other changes in the cell.

The speed with which changes are brought about in neurotransmitter signaling is evident when you think about how quickly you remove your hand from a hot surface. Sensory neurons carry information to the brain from your hand on the hot surface and motor neurons signal to your muscles to move the hand, in less time than it took you to read this sentence!

Nuclear hormone receptors

The receptors for signals like steroid hormones are part of a large group of proteins known as the nuclear hormone receptor superfamily. These receptors recognize and bind not only steroid hormones, but also reti-

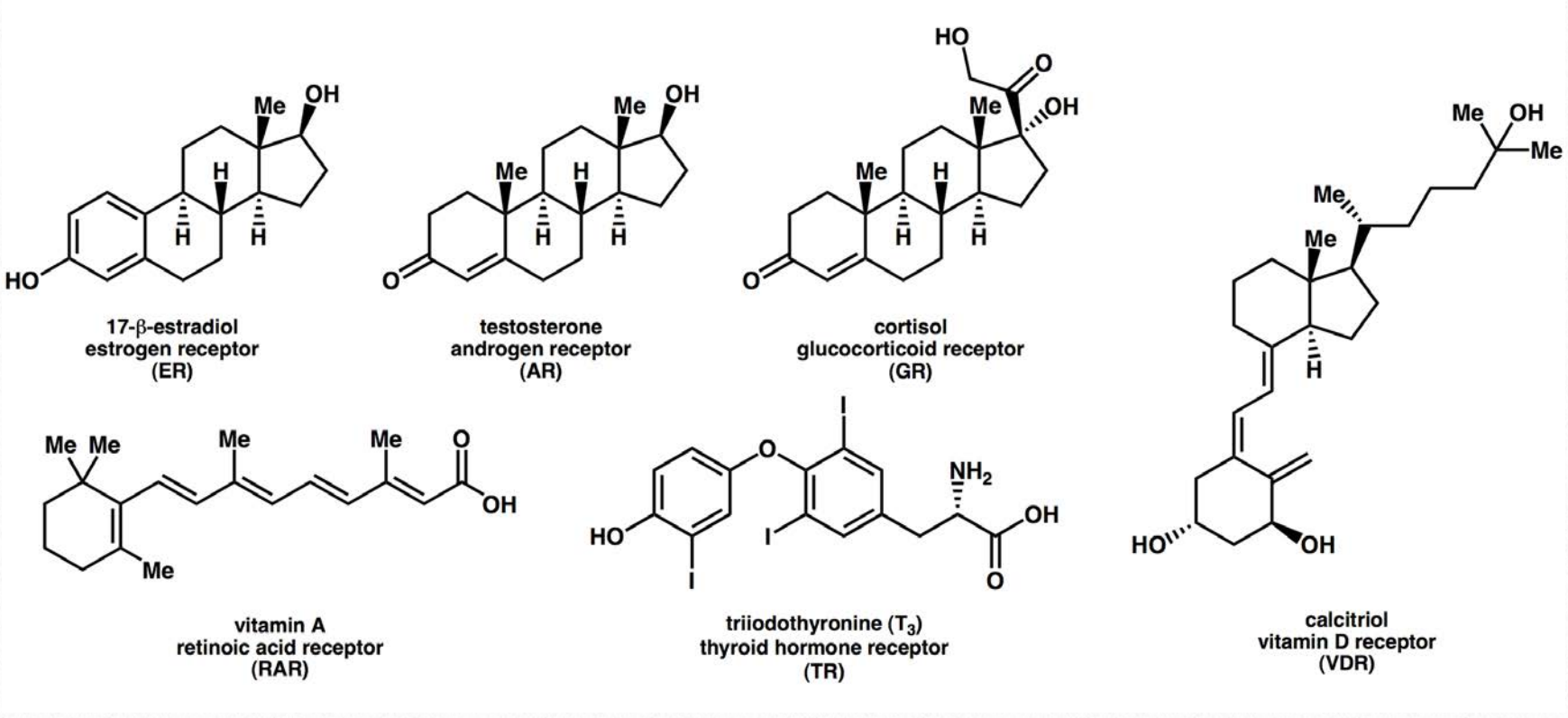

Figure 7.135 - Steroid hormones structures, with the names of their receptors

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noic acid, thyroid hormone, vitamin D and other signals. The subset of the nuclear hormone receptors that bind steroid hormones are intracellular proteins. Steroid hormones (Figure 7.135), as you are aware, are related to cholesterol, and as hydrophobic molecules, they are able to cross the cell membrane by themselves. This is unusual, as most signals coming to cells are incapable of crossing the plasma membrane, and thus, must have cell surface receptors.

Once within the cell, steroid hormones bind to their receptors, which may reside in the cytoplasm or in the nucleus. Steroid hormone receptors are proteins with a double life: they are actually dormant transcription regulators that are inactive till a steroid hormone binds and causes a conformational change in them. When this happens, the receptors, with the hormone bound, bind to regulatory sequences in the DNA and modulate gene expression. Because steroid hormone receptors act by modulating gene expression, the responses to steroid hormones are relatively slow. (There are also some effects of steroid hormones that do not involve transcriptional regulation, but the majority work through changing gene expression.) Like other transcriptional activators, steroid receptors have a DNA-binding domain (DBD) and an activation domain. They also have a ligand-binding domain (LBD) that binds the hormone.

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Glucocorticoid receptor

Examples of such signaling path-

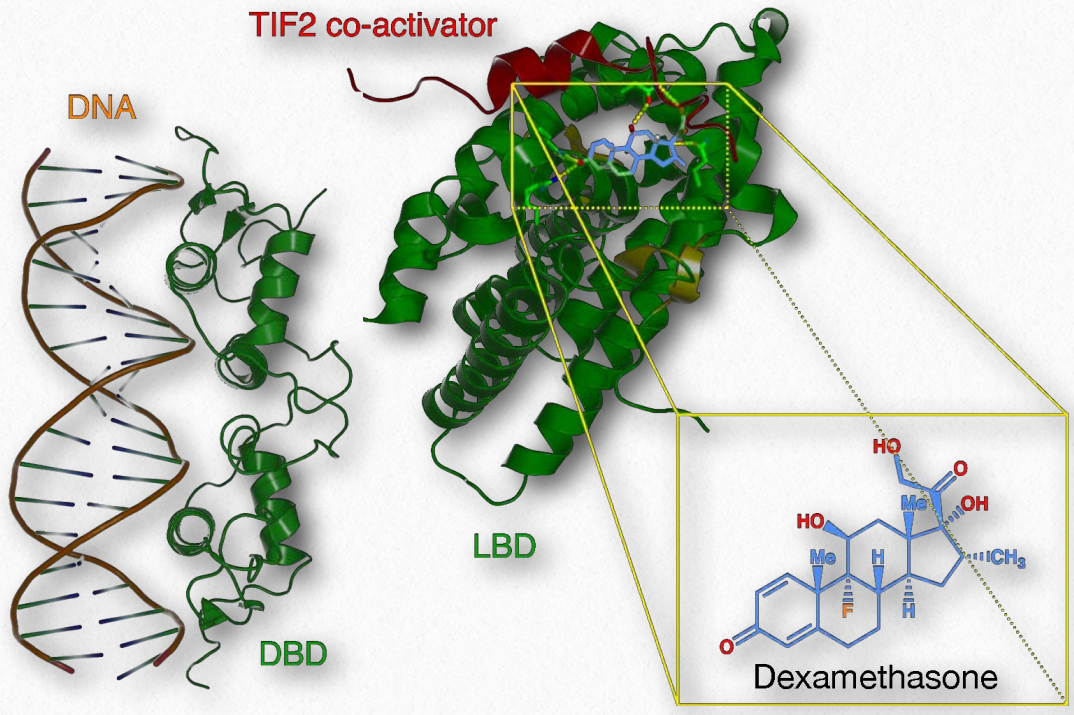

Figure 7.136 - Glucocorticoid receptor with its three domains - DNA binding (left), activator domain (top), and ligand binding domain (boxed).

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ways are those mediated by the glucocorticoid receptor (Figures 7.136 & 7.137). Glucocorticoids, sometimes described as stress hormones, are made and secreted by the adrenal cortex. Physiologically, they serve to maintain homeostasis in the face of stress and exhibit strong anti-inflammatory and immunosuppressive properties. Because of these effects, synthetic glucocorticoids are used in the treatment of a number of diseases from asthma and rheuma-

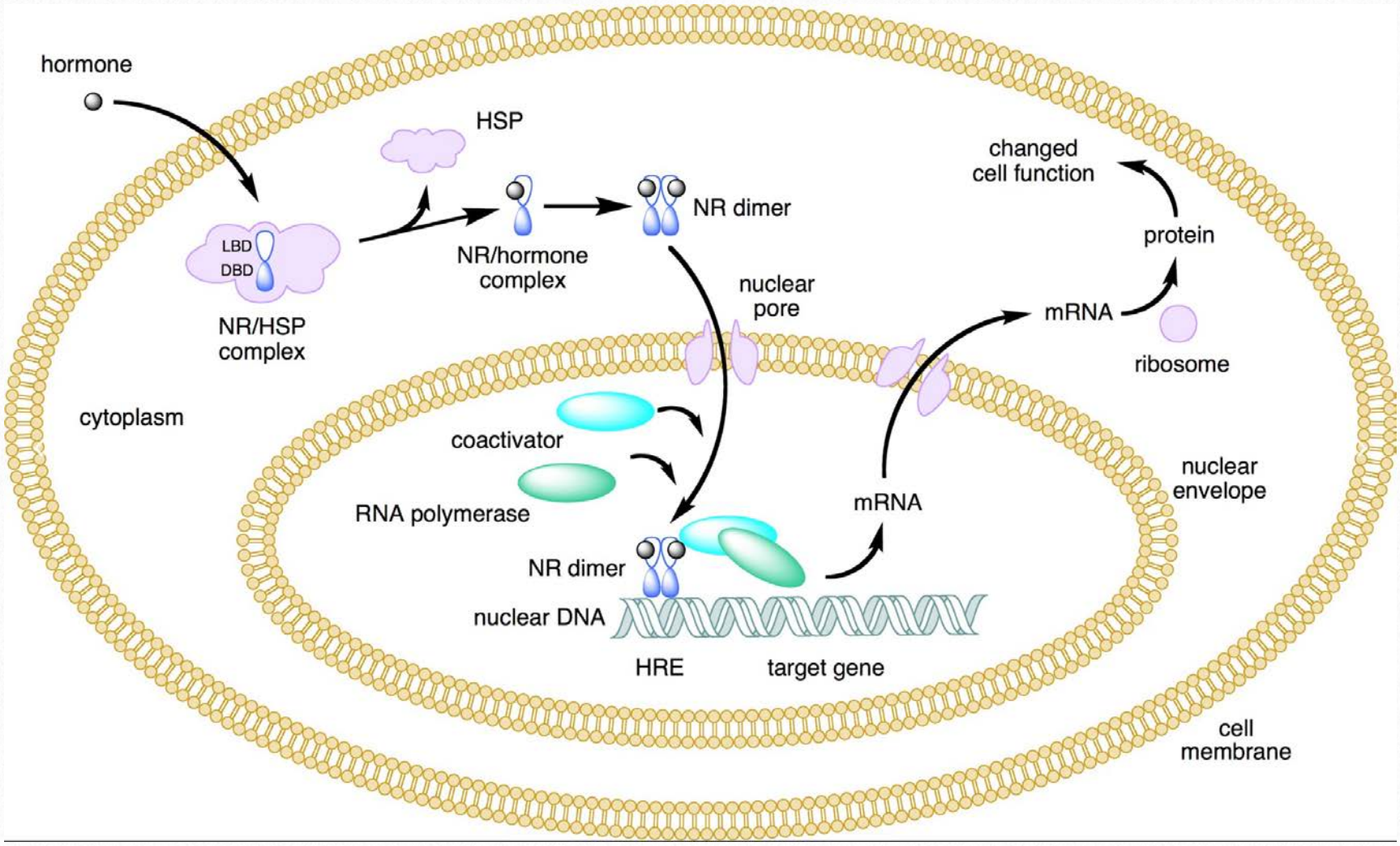

Figure 7.137 - Glucocorticoid signaling pathway

Wikipedia

toid arthritis to multiple sclerosis. All of these effects are mediated through the signaling pathway which starts with the binding of a glucocorticoid hormone to its receptor. Recall that steroids can cross the plasma membrane, so glucocorticoids can diffuse into the cell and bind their receptors which are in the cytoplasm.

In the absence of the signal, glucocorticoid receptors are found bound to a protein chaperone, Hsp90 (Figure 7.137). This keeps the receptors from being transported to the nucleus. When a glucocorticoid molecule binds the receptor, the receptor undergoes a conformational change and dissociates from the Hsp90. The receptor, then, with the hormone bound, translocates into the nucleus. In the nucleus, it can increase the transcription of target genes by binding to specific regulatory sequences (labeled HRE for hormone-response elements). The binding of the hormone-receptor complex to the regulatory elements of hormone-responsive genes modulates their expression. Many of these genes encode anti-inflammatory proteins, and their increased production accounts for the physiological effect of corticosteroid therapies.

The steroid receptor pathways are relatively simple and have only a couple of steps (Figure 7.138). Most other signaling pathways involve multiple steps in which the original signal is passed on and amplified through

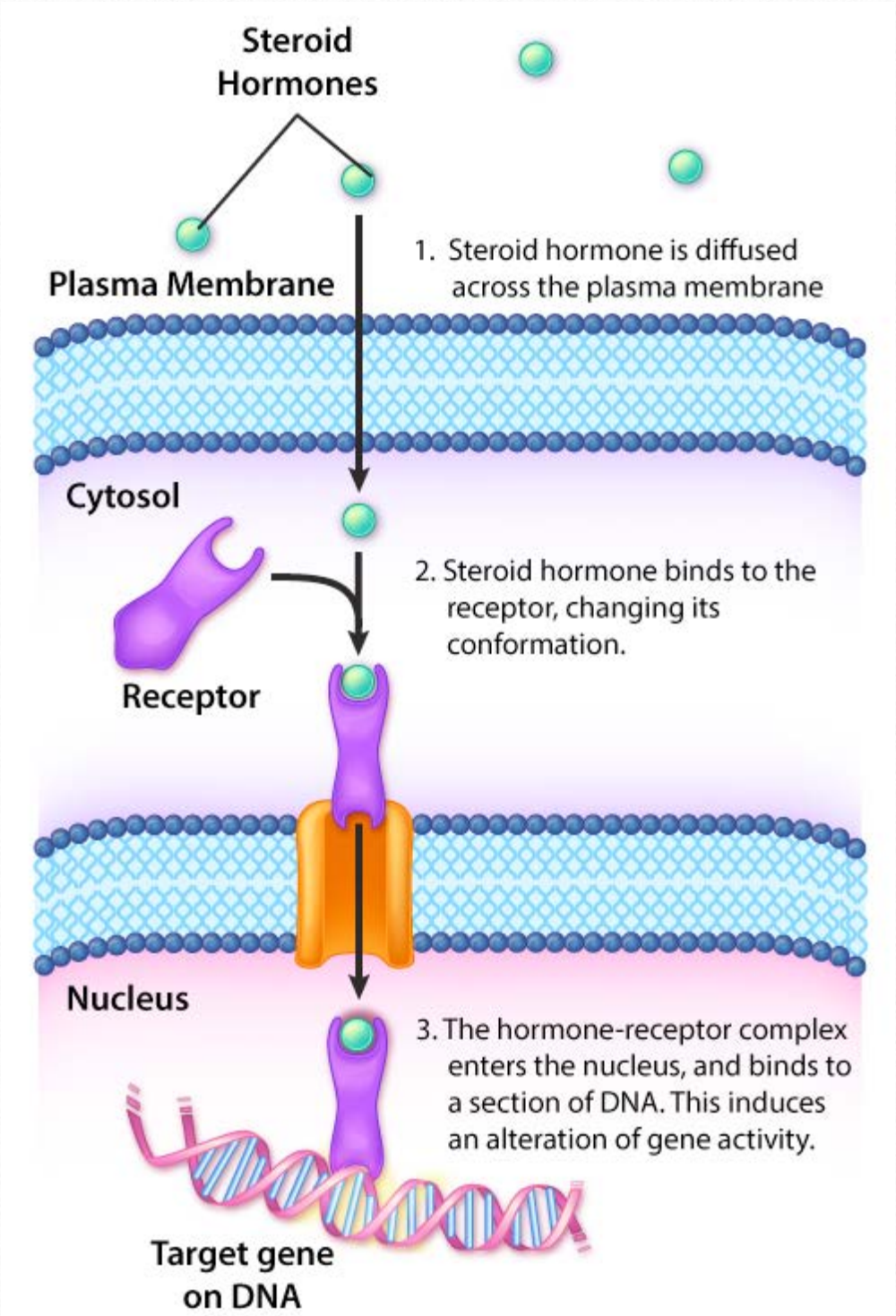

Figure 7.138 - Steroid hormone signaling

Image by Aleia Kim

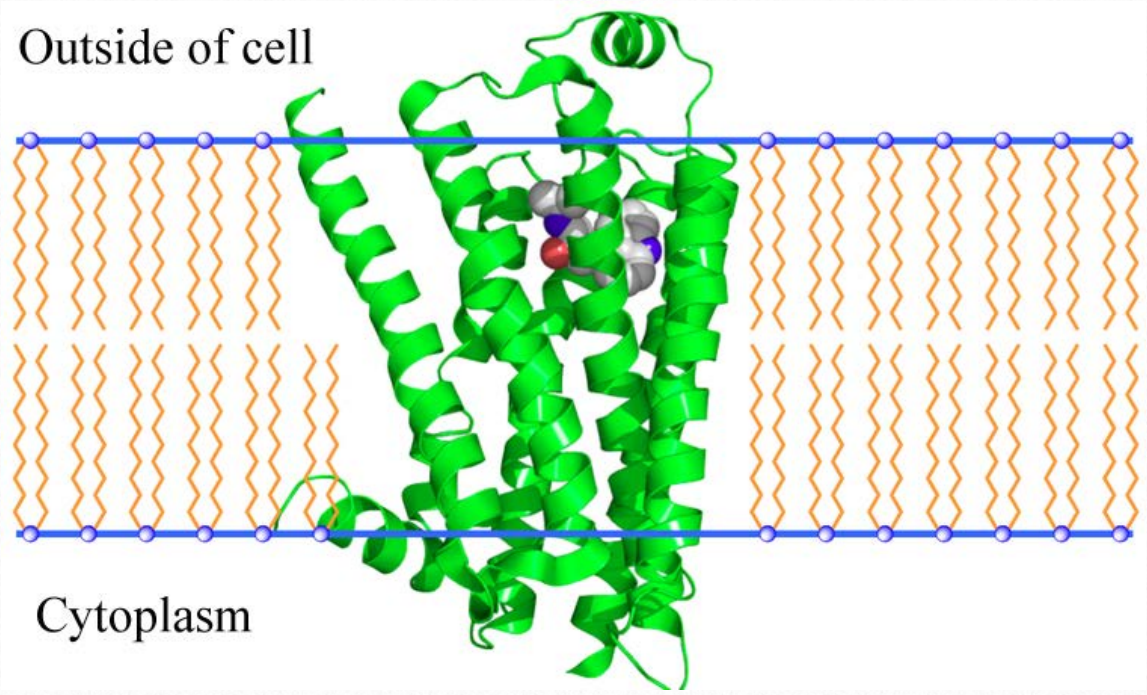

Figure 7.139 - Structure of a G-protein linked receptor
Wikipedia

a number of intermediate steps, before the cell responds to the signal.

Cell surface receptors

We will now take a look at two signaling pathways, each mediated by a major class of cell surface receptor- the G-protein coupled receptors (GPCRs) and the receptor tyrosine kinases (RTKs). While the specific details of the signaling pathways that follow the binding of signals to each of these receptor types are different, it is easier to learn them when you can see what the pathways have in common, namely, interaction of the signal with a receptor, followed by relaying and amplification of the signal through a variable number of intermediate molecules, with the last of these molecules interacting with a target or target proteins and modifying their activity in the cell.

G-protein coupled receptors

G-protein coupled receptors (GPCRs) are involved in responses of cells to many different kinds of signals, from epinephrine, to odors, to light. In fact, a variety of physiological phenomena including vision, taste, smell, and the fight-or-flight response are mediated by GPCRs. What are G-protein coupled receptors?

G-protein coupled receptors are cell surface receptors that pass on the signals that they receive with the help of guanine nucleotide binding proteins (a.k.a. G-proteins). Before thinking any further about the signaling pathways downstream of GPCRs, it is necessary to know a few important facts about these receptors and the G-proteins that assist them.

GPCR structure

Though there are hundreds of different G-protein coupled receptors, they all have the same basic structure (Figure 7.139):

They all consist of a single polypeptide chain that threads back and forth seven times through the lipid bilayer of the plasma membrane. For this reason, they are sometimes called seven-pass transmembrane

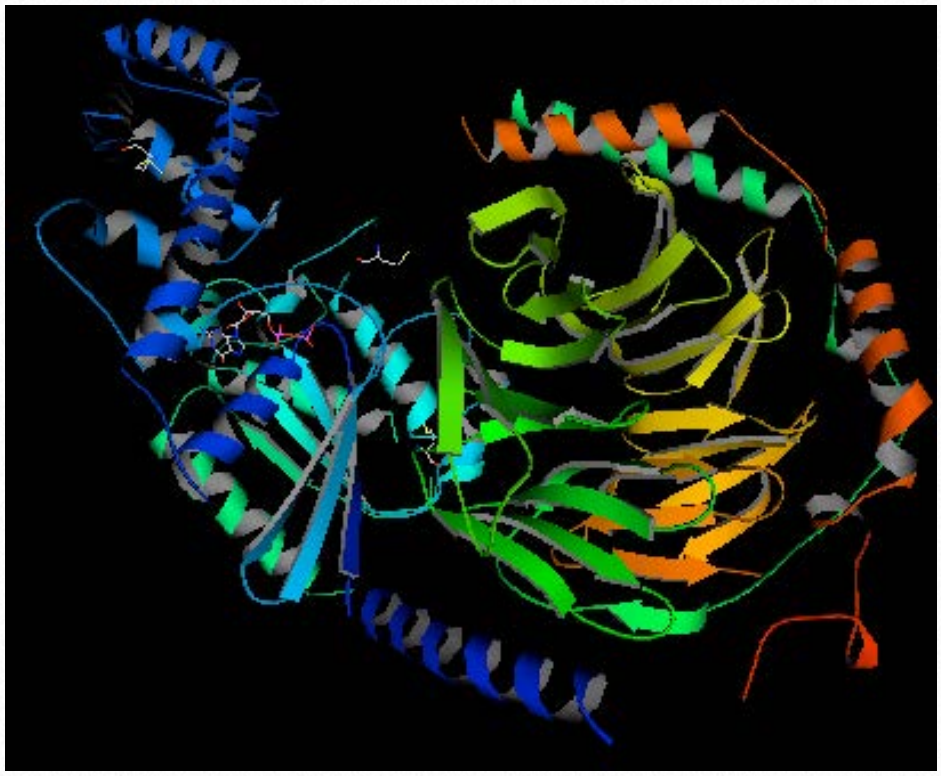

Figure 7.140- A heterotrimeric G-protein: α subunit in blue, βγ subunits red and green

(7TM) receptors. One end of the polypeptide forms the extracellular domain that binds the signal while the other end is in the cytosol of the cell.

When a ligand (signal) binds the extracellular domain of a GPCR, the receptor undergoes a conformational change, on its cytoplasmic side, that allows it to interact with a G-protein that will then pass the signal on to other intermediates in the signaling pathway.

G-proteins

What is a G-protein? As noted above, a G-protein is a guanine nucleotide-binding protein that can interact with a G-protein linked receptor. G-proteins are associated with the cytosolic side of the plasma membrane, where they are ideally situated to interact with the tail of the GPCR, when a signal binds to the GPCR. There are many different G-proteins, all of which share a characteristic structure- they are composed of three subunits called α, β and γ (Figure 7.140). Because of this, they are sometimes called heterotrimeric G proteins (hetero=different, trimeric= having three parts).

Ligand binding

The guanine nucleotide binding site is on the α subunit of the G-protein. This site can bind GDP or GTP. The α subunit also has a GTPase activity, i.e., it is capable of hydrolyzing a GTP molecule bound to it into GDP.

In the unstimulated state of the cell, that is, in the absence of a signal bound to the GPCR, the G-proteins are found in the trimeric form (α-β-γ bound together) and the α subunit has a GDP molecule bound to it. In this form, the α subunit is inactive. With this background on the structure and general properties of the GPCRs and the G-proteins, we can now look at what happens when a signal arrives at the cell surface and binds to a GPCR (Figure 7.141).

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The signaling pathway

The binding of a signal molecule by the extracellular part of the G-protein linked recep-

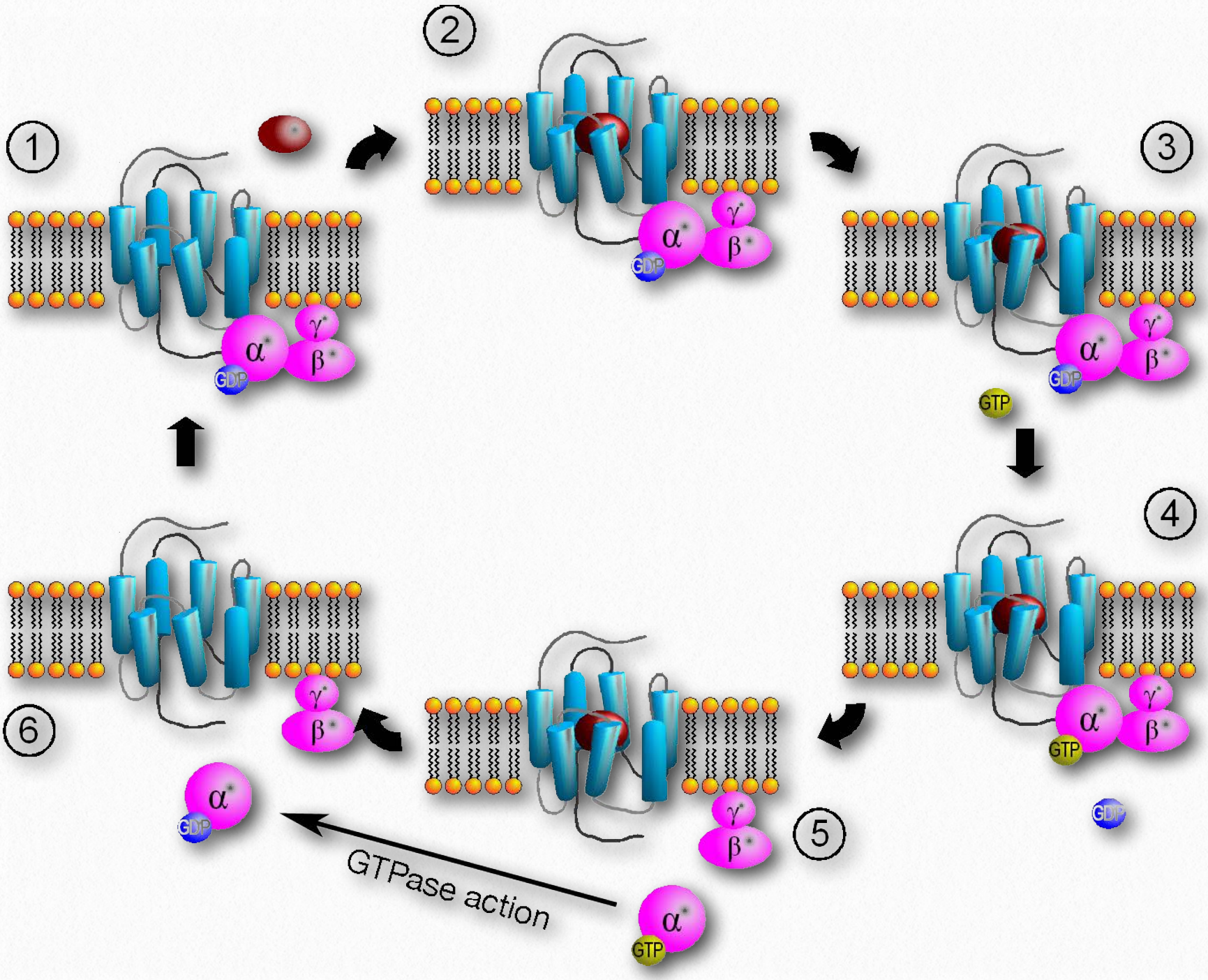

Figure 7.141 - Cycle of G-protein activation - 1) binding of ligand; 2) change of receptor structure; 3) stimulation of α-subunit; 4) binding of GTP, release of GDP; 5) separation of α-subunit from β-γ; 6) hydrolysis of GTP by α-subunit and return to inactive state.

Wikipedia

tor causes the cytosolic tail of the receptor to interact with, and alter the conformation of, a G-protein associated with the inner face of the plasma membrane.

This has two consequences. First, the α subunit of the G-protein loses its GDP and binds a GTP, instead. Second, the G-protein breaks up into the GTP-bound α part and the β-γ part.

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The binding of GTP to the α subunit and its dissociation from the β-γ subunits activate the α subunit. The activated α subunit can diffuse freely along the cytosolic face

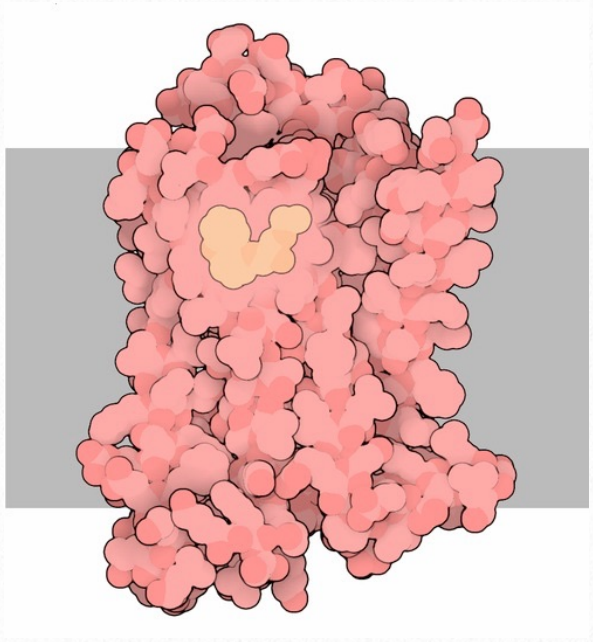

Figure 7.142 - β_2-adrenergic receptor embedded in membrane (gray)

Wikipedia

of the plasma membrane and act upon its targets. (The β-γ unit is also capable of activating its own targets.)

What happens when G-proteins interact with their target proteins? That depends on what the target is. G-proteins interact with different kinds of target proteins, of which we will examine two major categories:

Ion channels

We have earlier seen that some gated ion channels can be opened or closed by the direct binding of neurotransmitters to a receptor that is an ion-channel protein. In other cases, ion channels are regulated by the binding of G-proteins. That is, instead of the signal directly binding to the ion channel, it binds to a GPCR, which activates a G-protein that then may cause opening of the ion channel, either directly, by binding to the channel, or indirectly, through activating other proteins that can bind to the channel. The change in the distribution of ions across the plasma membrane causes a change in the membrane potential.

Enzyme activation

The interaction of G-proteins with their target enzymes can regulate the activity of the enzyme, either increasing or decreasing its activity. The change in activity of the target enzyme, in turn, results in downstream changes in other proteins in the cell, and alters the metabolic state of the cell. This is best understood by examining the well-studied response of cells to epinephrine, mediated through the β-adrenergic receptor, a type of G-protein coupled receptor.

Epinephrine (Figure 7.142), also known as adrenaline, is a catecholamine that plays an important role in the body's 'fight or flight' response. In response to stressful stimuli, epinephrine is secreted into the blood, to be carried to target organs whose cells will respond

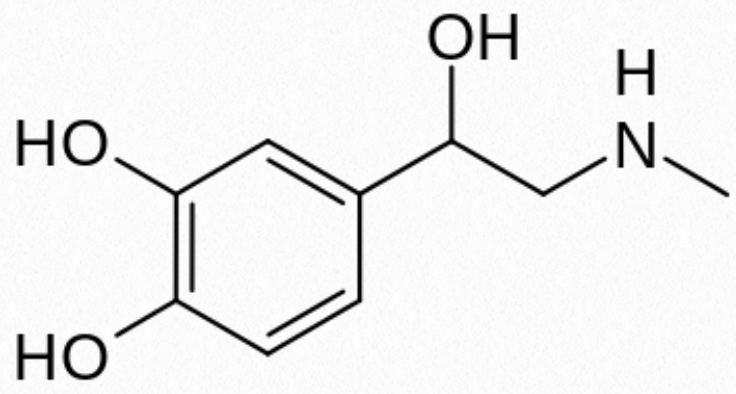

Figure 7.143 - Epinephrine

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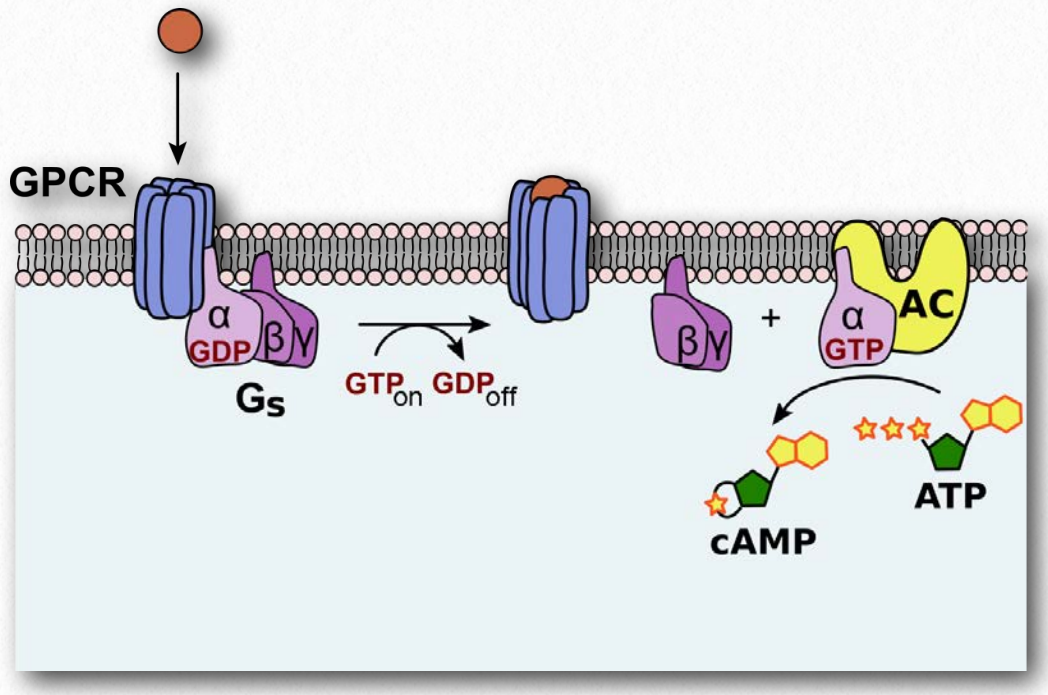

Figure 7.144 - G-protein coupled receptor. Signal starts with ligand binding (orange circle). G_s = G-protein; AC = adenylate cyclase.

Wikipedia

to this signal. If you were walking down a dark alley in an iffy neighborhood, and you heard footsteps behind you, your brain would respond to potential danger by sending signals that ultimately cause the adrenal cortex to secrete epinephrine into the blood stream. The epinephrine circulating in your system has many effects, including increasing your heart rate, but among its prime targets are your muscle cells. The reason for this is that your muscle cells store energy in the form of glycogen, a polymer of glucose. If you need to run or fight off an assailant, your cells will need energy in the form of glucose.

But how does epinephrine get your cells to break down the glycogen into glucose? Binding of epinephrine to the β-adrenergic receptor on the surface of the cells causes the receptor to activate a G-protein associated with its cytoplasmic tail. As described above, this leads to the α subunit exchanging its GDP for GTP and dissociating from the β-γ subunits. The activated α subunit then interacts with the enzyme adenylate cyclase (also known as adenylyl cyclase) stimulating it to produce cyclic AMP (cAMP) from ATP. Cyclic AMP is often described as a "second messenger", in that it serves to spread the

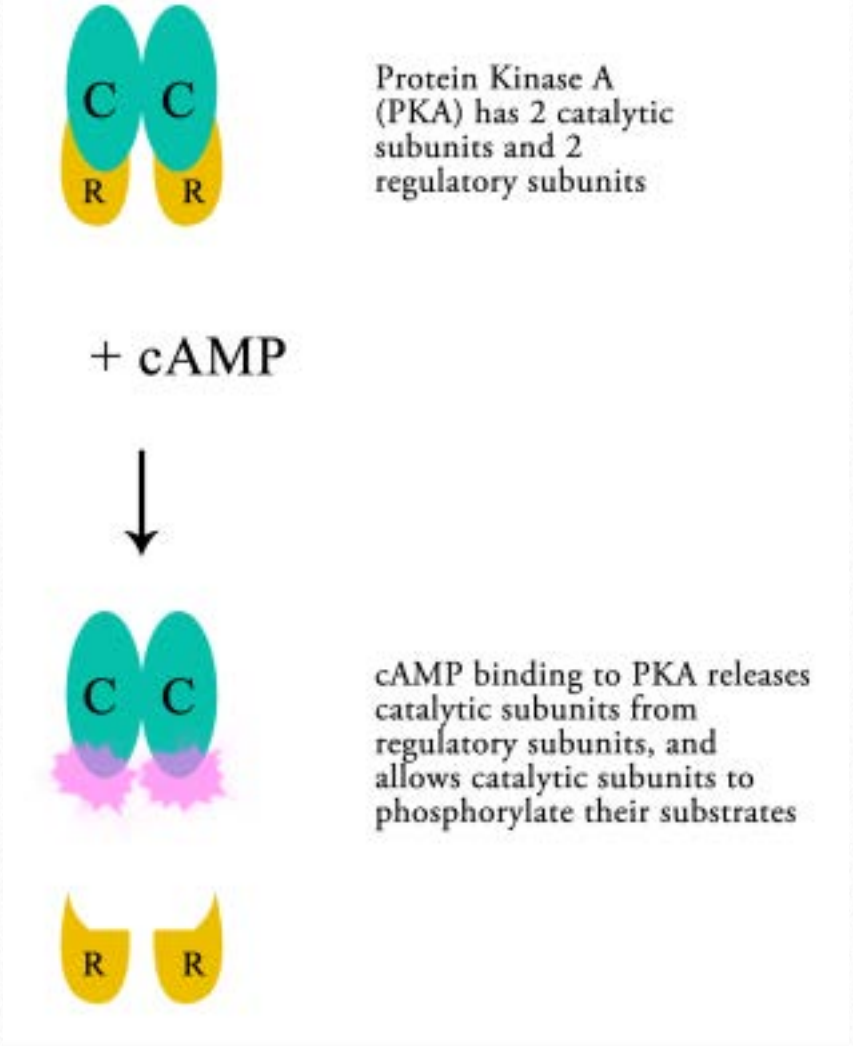

Figure 7.145 - Activation of Protein Kinase A by cAMP

Image by Martha Baker

signal received by the cell. How does cAMP accomplish this?

cAMP molecules bind to, and activate an enzyme, protein kinase A (PKA - Figure 7.145). PKA is composed of two catalytic and two regulatory subunits that are bound tightly together. Upon binding of cAMP, the catalytic subunits are released from the regulatory subunits, allowing the enzyme to carry out its function, namely phosphorylating other proteins. Thus, cAMP can regulate the activity of PKA, which in turn, by phosphorylating other proteins can change their activity. In this case, the relevant protein that is activated is an enzyme, phosphorylase kinase. This enzyme can then phosphorylate and activate glycogen phosphorylase, the enzyme ultimately responsible for breaking glycogen down into glucose-1-phosphate - readily converted to glucose. The activation of glycogen phosphorylase supplies the cells with the glucose they need, allowing you to fight or flee, as you might see fit. Simultaneously, PKA also phosphorylates another enzyme, glycogen synthase. In the case of glycogen synthase, phosphorylation inactivates it, and prevents free glucose from being used up for glycogen synthesis, ensuring that your cells are amply supplied with glucose (Figure 7.146).

Common pattern

Although the steps described above seem complicated, they follow the simple pattern outlined at the beginning of this section:

• Binding of signal to receptor

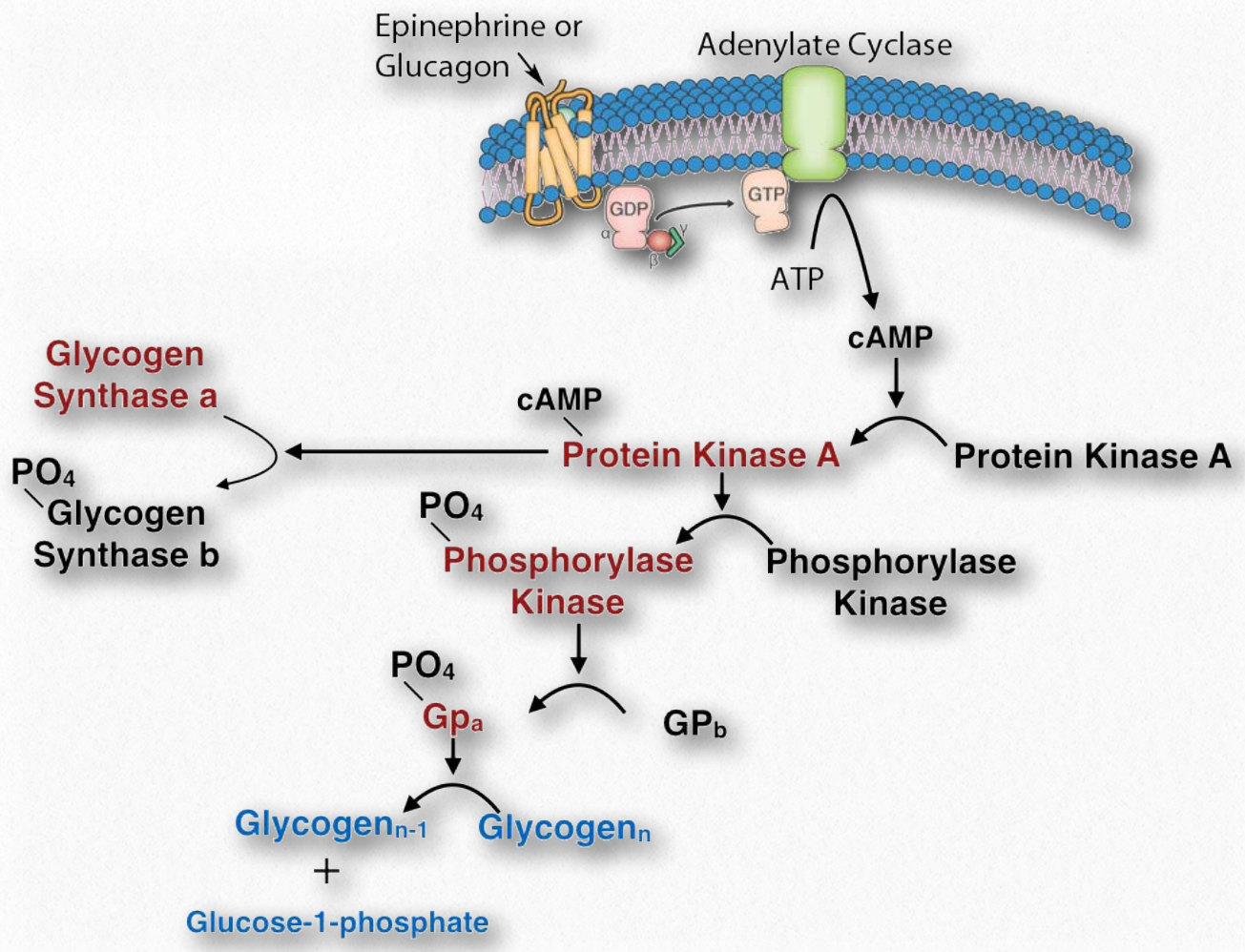

Figure 7.146 - Simultaneous activation of glycogen breakdown and inhibition of glycogen synthesis by epinephrine's binding of b-adrenergic receptor. Red enzyme names = activated forms; black enzyme names = inactivated forms; GPb = glycogen phosphorylase b; GPa = glycogen phosphorylase a.

Image by Penelope Irving

• Several steps where the signal is passed on through intermediate molecules (G-proteins, adenylate cyclase, cAMP, and finally, PKA)
• Phosphorylation of target proteins by the kinase, leading to changes in the cell. The specific changes depend on the proteins that are phosphorylated by the PKA.

Why so many steps? If you need to activate glycogen phosphorylase to break down glucose in a hurry, why not have a system in which binding of a signal to the receptor directly activated the target enzyme?

The answer to this puzzle is simple: there is amplification of the signal at every step of the pathway. A single signal molecule binding to a receptor sets in motion a cascade of reactions, with the signal getting larger at each step, so that binding of one epinephrine molecule to its receptor results in the activation of a million glycogen phosphorylase enzyme molecules!

Turning signals off

If the signal binding to the receptor serves as a switch that sets these events in motion, there must be mechanisms to turn the pathway off. The first is at the level of the receptor itself. A kinase called G-protein receptor kinase (GRK) phosphorylates the cytoplasmic tail of the receptor. The phosphorylated tail is then bound by a protein called arrestin, preventing further interaction with a G-protein.

The next point of control is at the G-protein. Recall that the α subunit of the G-protein is in its free and activated state when it has GTP bound, and that it associates with the β-γ subunits and has a GDP bound when it is inactive. We also

β-Adrenergic Signaling On Switches

1. Binding of Signal Molecule to Receptor
2. Passage of Signal Through Several Molecules (G-proteins, Adenylate Cyclase, cAMP, PKA)
3. Phosphorylation of Target Proteins

β-Adrenergic Signaling Off Switches

1. GRK Phosphorylates Receptor Tail
   Receptor Tail Bound by Arrestin
2. α Subunit G-protein Cleaves GTP to GDP
   β-γ subunits Reassociate with α Subunit
3. cAMP Hydrolyzed by Phosphodiesterase
   PKA Becomes Inactive
4. Dephosphorylation of Phosphorylated Proteins by Phosphoprotein Phosphatase

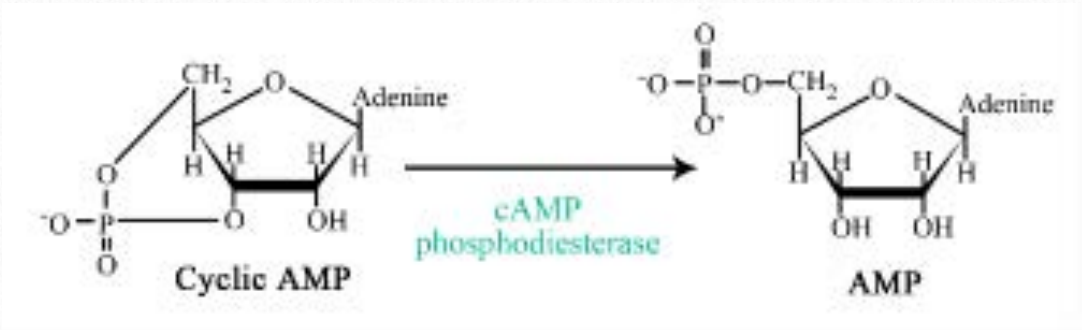

Figure 7.147 - Cyclic AMP is broken down by phosphodiesterase

know that the α subunit has an activity that enables it to hydrolyze GTP to GDP. This GTP-hydrolyzing activity makes it possible for the α subunit, once it has completed its task, to return to its GDP bound state, re-associate with the β-γ part and become inactive again.

A third "off switch" is further down the signaling pathway, and controls the level of cAMP. We just noted that cAMP levels increase when adenylate cyclase is activated. When its job is done, cAMP is broken down by an enzyme called phosphodiesterase (Figure 7.147). When cAMP levels drop, PKA returns to its inactive state, putting a halt to the changes brought about by the activation of adenylate cyclase by an activated G-protein.

Yet another way that the effects of this pathway can be turned off is at the level of the phosphorylated target proteins. These proteins, which are activated by phosphorylation, can be returned to their inactive state by the removal of the phosphates by phosphatases.

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Receptor tyrosine kinases

Another major class of cell surface receptors are the receptor tyrosine kinases or RTKs. Like the GPCRs, receptor tyrosine kinases bind a signal, then pass the message on through a series of intracellular molecules, the last of which acts on target proteins to change the state of the cell.

As the name suggests, a receptor tyrosine kinase is a cell surface receptor that also has a tyrosine kinase activity. The signal binding domain of the receptor tyrosine kinase is on the cell surface, while the tyrosine kinase enzymatic activity resides in the cytoplasmic part of the protein (Figure 7.148). A transmembrane α helix connects these two regions of the receptor.

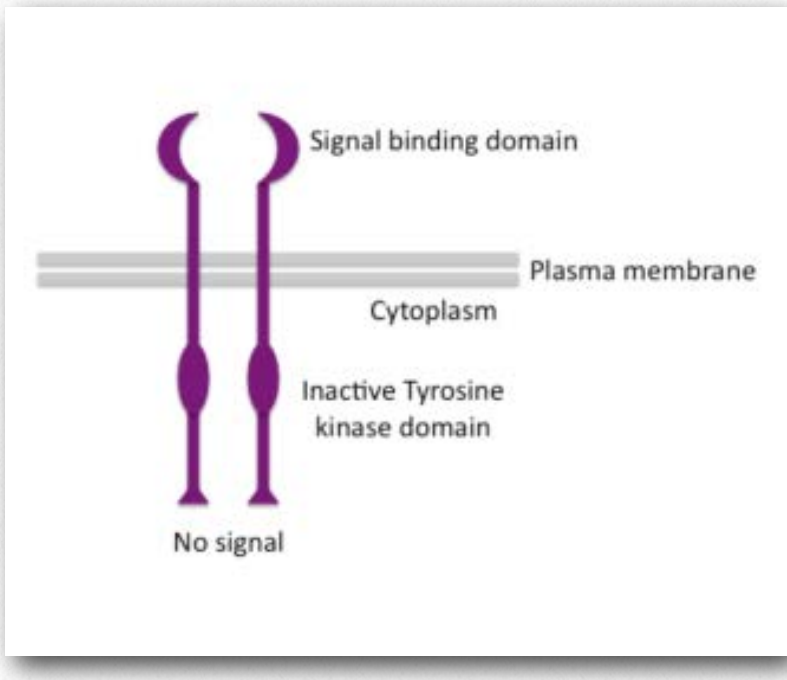

Figure 7.148 - Structure of a receptor tyrosine kinase

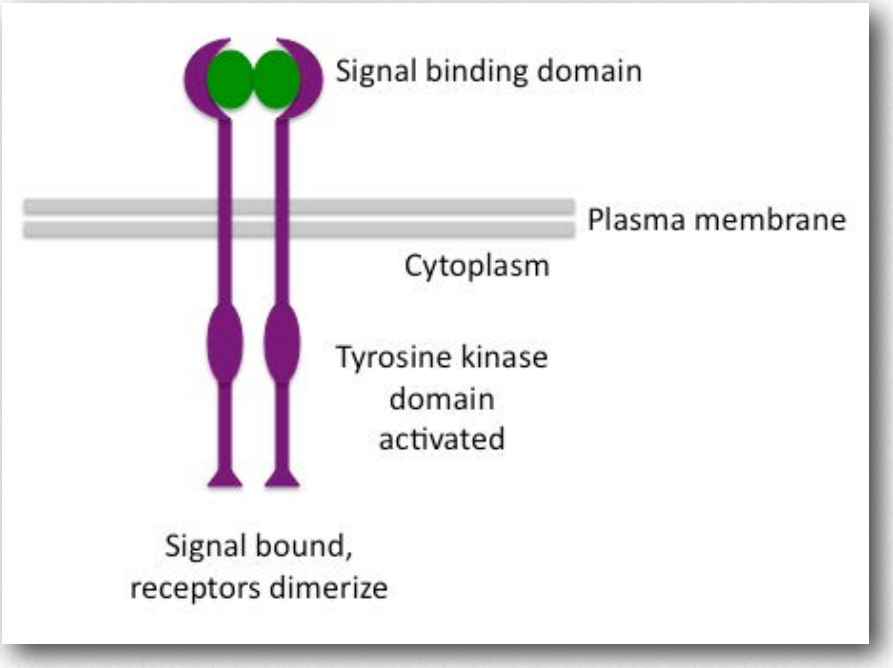

Figure 7.149 - Signal binding results in receptor dimerization and activation of tyrosine kinase activity

What happens when signal molecules bind to receptor tyrosine kinases? Binding of signal molecules to the extracellular domains of receptor tyrosine kinase proteins causes two receptor molecules to dimerize (come together and associate - Figure 7.149). This brings the cytoplasmic tails of the receptors close to each other and causes the tyrosine kinase activity of these tails to be turned on. The activated tails then phosphorylate each other on several tyrosine residues (Figure 7.150). This is called autophosphorylation.

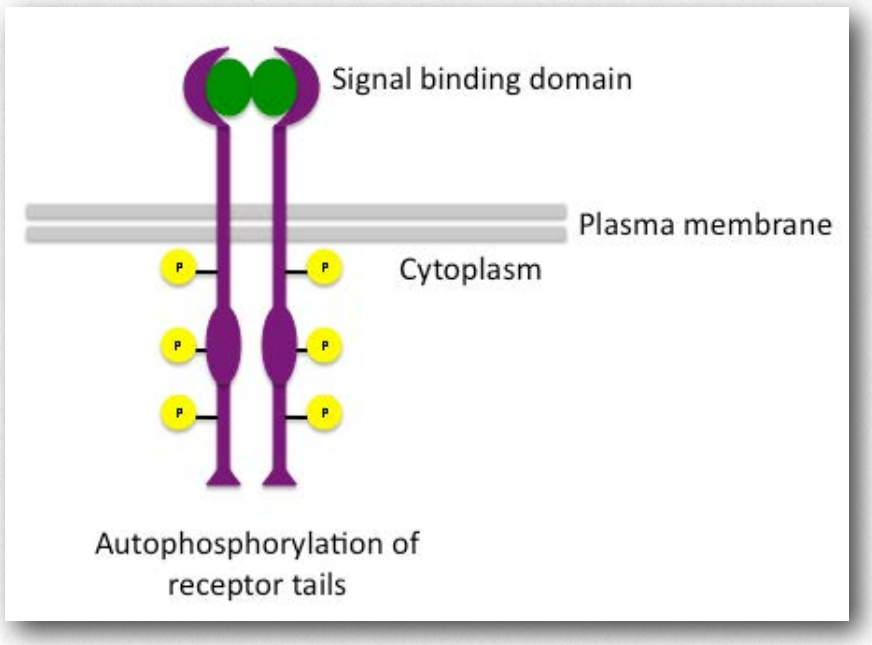

Figure 7.150 - Activated tyrosine kinases phosphorylate tyrosines on the receptor tails.

The phosphorylation of tyrosines on the receptor tails triggers the assembly of an intracellular signaling complex on the tails. The newly phosphorylated tyrosines serve as binding sites for a variety of signaling proteins that then pass the message on to yet other proteins to bring about changes in the cell. Receptor tyrosine kinases mediate responses to a large number of signals, including peptide hormones like insulin and growth factors like epidermal growth factor (EGF). We will examine how insulin

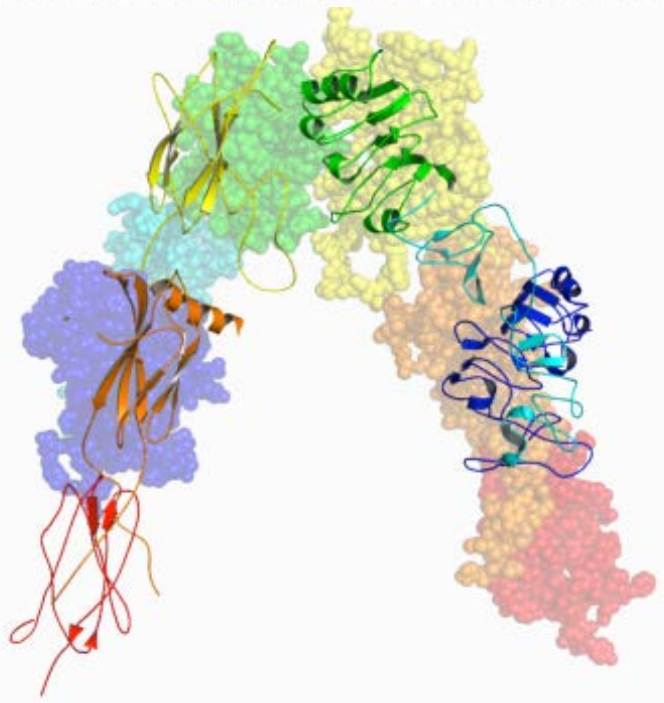

Figure 7.151 -The insulin receptor, a receptor tyrosine kinase

Wikipedia

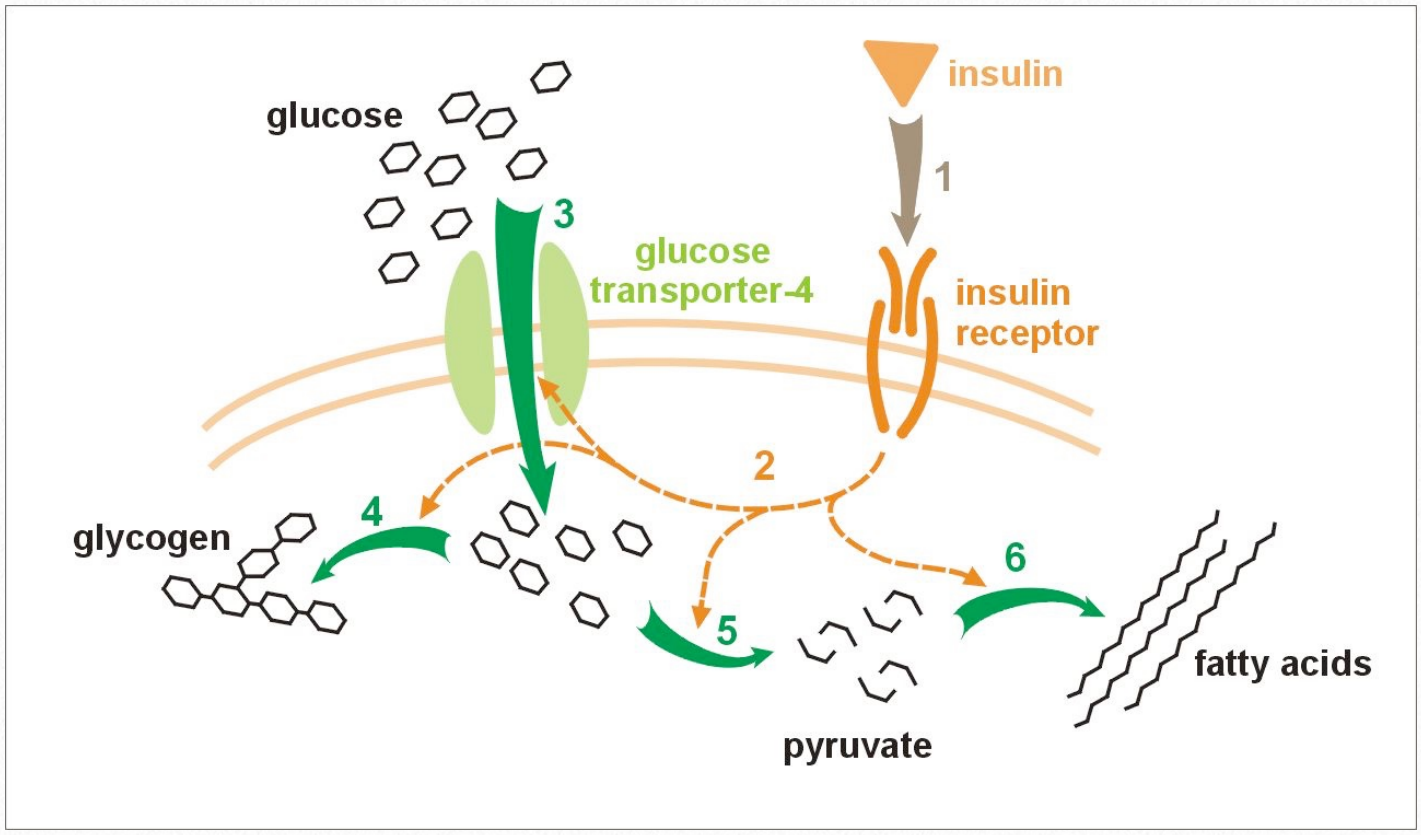

Figure 7.152 - Effects of insulin binding to its receptor tyrosine kinase: 1) insulin binding; 2) activation of protein activation cascades. These include: 3) translocation of Glut-4 transporter to plasma membrane and influx of glucose; 4) glycogen synthesis; 5) glycolysis; and 6) fatty acid synthesis.

Wikipedia

and EGF act on cells by binding to receptor tyrosine kinases.

Insulin receptor

Insulin plays a central role in the uptake of glucose from the bloodstream. It increases glucose uptake by stimulating the movement of glucose receptor GLUT4 to the plasma membrane of cells.

How does insulin increase GLUT4 concentrations in the cell membrane? The binding of insulin to the insulin receptor (IR - Figure 7.151), results in dimerization of the receptor monomers and subsequent autophosphorylation of the cytosolic kinase domains. The activated tyrosine kinase domains also phosphorylate intracellular proteins called Insulin Receptor Substrates or IRS proteins. These proteins interact with, and activate another kinase called the PI_3-kinase. PI_3-kinase then catalyzes the formation of the lipid molecule PIP_3, which serves to activate yet another kinase, PDK1, which in turn, activates the Akt group of kinases. It is this group of enzymes that appears to increase the translocation of the GLUT4 to the plasma membrane (Figure 7.152), as cells that lack functional Akts exhibit poor glucose uptake and insulin resistance.

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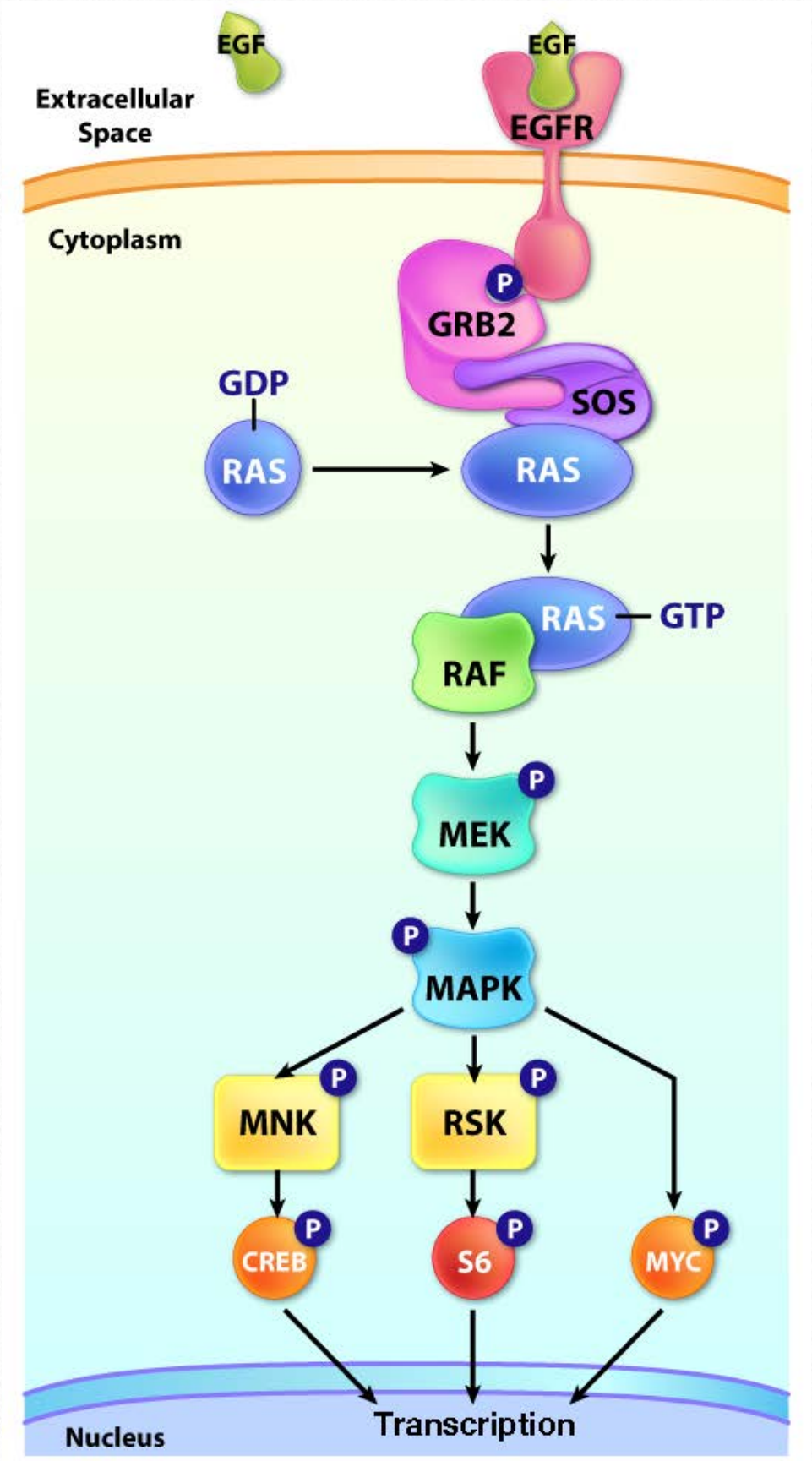

Figure 7.153 - EGFR signaling beginning at top with binding of EGF, dimerization of receptor, transmission of signal through proteins, activation of kinases, phosphorylation of transcription factors and effects on transcription

Image by Aleia Kim

EGFR pathway

Epidermal growth factor, EGF, is an important signaling molecule involved in growth, proliferation and differentiation in mammalian cells. EGF acts through the EGF receptor, EGFR, a receptor tyrosine kinase (Figure 7.153). Because of its role in stimulating cell proliferation and because overexpression of EGFR is associated with some kinds of cancers, EGFR is the target for many anti-cancer therapies. We can trace the signal transduction pathway from the binding of EGF to its receptor to the stimulation of cell division.

EGF binding to the EGFR is followed by receptor dimerization and stimulation of the tyrosine kinase activity of the cytosolic domains of the EGFR. Autophosphorylation of the receptor tails is followed by the assembly of a signaling

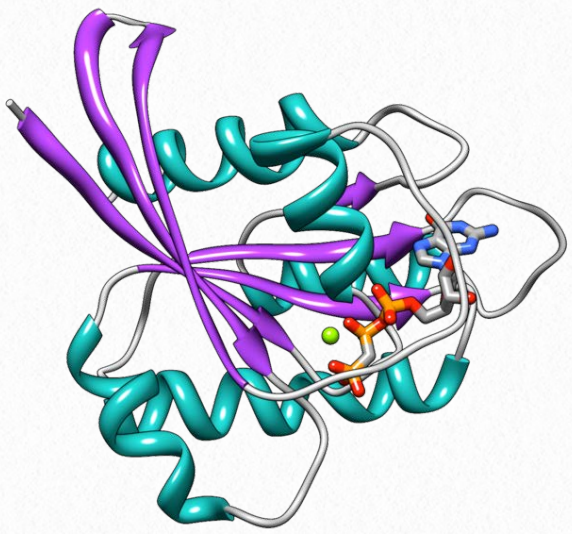

Figure 7.154 - Ras with GTP bound

Wikipedia

complex nucleated by the binding of proteins that recognize phosphotyrosine residues. An important protein that is subsequently activated by the signaling complexes on the receptor tyrosine kinases is called Ras (Figure 7.154). The Ras protein is a monomeric guanine nucleotide binding protein that is associated with the cytosolic face of the plasma membrane (in fact, it is a lot like the α subunit of trimeric G-proteins). Just like the α subunit of a G-protein, Ras is active when GTP is bound to it and inactive when GDP is bound to it. Also, like the α subunit, Ras can hydrolyze the GTP to GDP.

Ras activation

Activation of Ras accompanies the exchange of the GDP bound to the inactive Ras for a GTP. Activated Ras triggers a phosphorylation cascade of three protein kinases, which relay and distribute the signal. These protein kinases are members of a group called the MAP kinases (Mitogen Activated Protein Kinases). The final kinase in this cascade phosphorylates various target proteins, including enzymes and transcriptional activators that regulate gene expression.

YouTube Lectures by Kevin HERE & HERE

RTK Signal Transduction

1. Receptor binding of signal
2. Receptor dimerization
3. Autophosphorylation of cystosolic tails
4. Passing of message to proteins via signaling complex
5. Stimulation of kinase cascade
6. Terminal kinase acts on target proteins

The phosphorylation of various enzymes can alter their activities, and set off new chemical reactions in the cell, while the phosphorylation of transcriptional activators can change which genes are expressed. The combined effect of changes in gene expression and protein activity alter the cell's physiological state and promote cell division.

Once again, in following the path of signal transduction mediated by RTKs, it is possible to discern the same basic pattern of events: a signal is bound by the extracellular domains of receptor tyrosine kinases, resulting in receptor dimerization and autophosphorylation of the cytosolic tails, thus conveying the message to the interior of the cell.

The message is then passed on *via* a signaling complex to proteins that stimulate a series of kinases. The terminal kinase in the cascade acts on target proteins and brings about in changes in protein activities.

What is the OFF switch for RTKs? It turns out that RTKs with the signal bound can be endocytosed into the cell and broken down. That is, the region of the plasma membrane that the RTK is on can be internally pinched off into a vesicle containing the ligand-bound receptor which is then targeted for degradation.

Ras, which is activated by GTP binding, can also be deactivated by hydrolysis of the GTP to GDP. The importance of this mechanism for shutting down the pathway is evident in cells that have a mutant *ras* gene encoding a Ras protein with defective GTPase activity. Unable to shut off Ras, the cells continue to receive a signal to proliferate. The National Cancer Institute estimates that more than

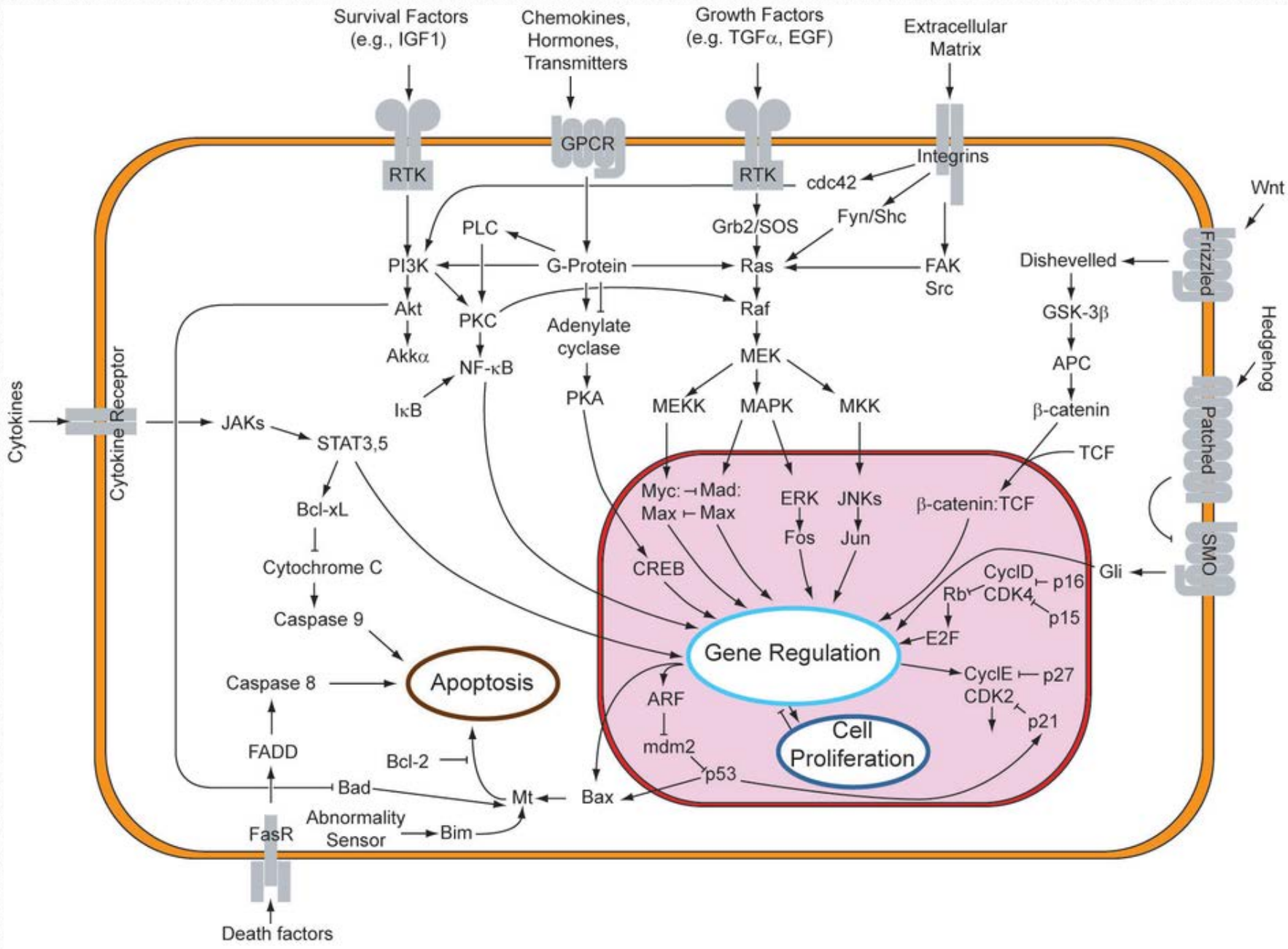

Figure 7.155 - Overview of some cellular signaling pathways

Wikipedia

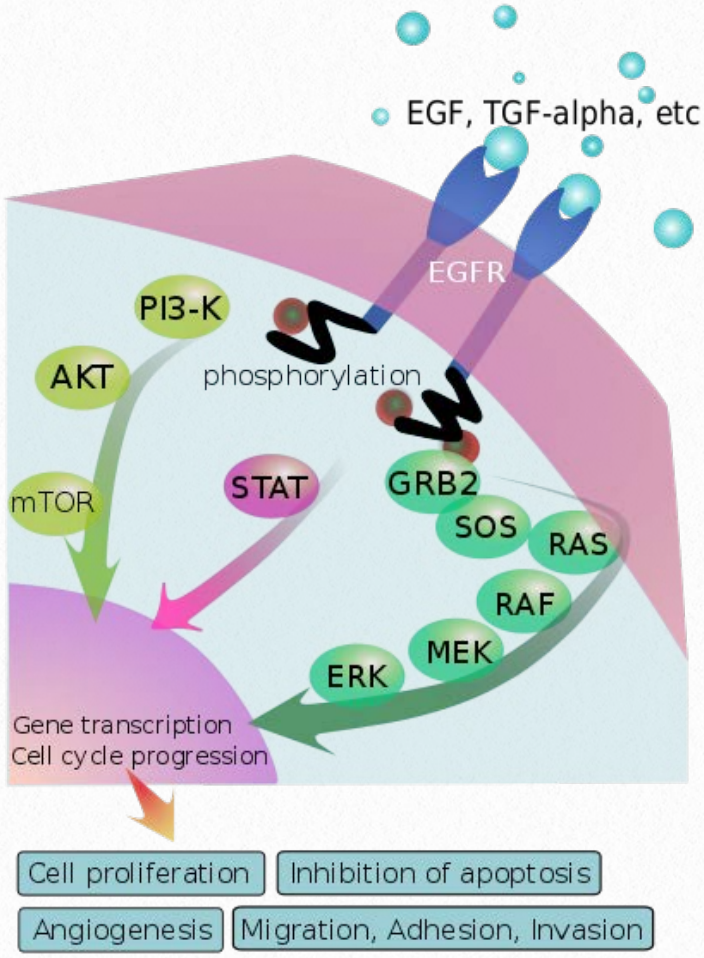

Figure 7.156 -Normal EGF Receptor signaling pathway
Wikipedia

30% of human cancers are driven by mutations in *ras* genes.

The descriptions above provide a very simple sketch of some of the major classes of receptors and deal primarily with the mechanistic details of the steps by which signals received by various types of receptors bring about changes in cells. A major take-home lesson is the essential similarity of the different pathways. Another point to keep in mind is that while we have looked at each individual pathway in isolation, a cell, at any given time receives multiple signals that set off a variety of different responses at once (Figure 7.155).

The pathways described above show a considerable degree of "cross-talk" and the response to any given signal is affected by the other signals that the cell receives simultaneously. The multitude of different receptors, signals, and the combinations thereof are the means by which cells are able to respond to an enormous variety of different circumstances.

RTKs, cancer and cancer therapies

As described above, binding of EGF to its receptor triggers a signaling pathway that results in the activation of a series of Mitogen Activated Protein Kinases (MAP kinases). These kinases are so-called because they are activated by a mitogen, a molecule, like EGF and other growth factors, that stimulates mitosis or cell division. The final kinase in the MAP kinase cascade phosphorylates a number of target proteins, many of them transcription factors, that when activated, increase the expression of genes associated with cell proliferation.

Given that the EGF-receptor pathway normally functions to stimulate cell division, it is not surprising that malfunctions in the pathway could lead to uncontrolled cell proliferation, or cancer. Next, we will take a brief look at some examples of such defects.

HER2

The human EGF receptor (HER) family has four members, HER1, HER2, HER3 and HER4. These are all receptor tyrosine ki-

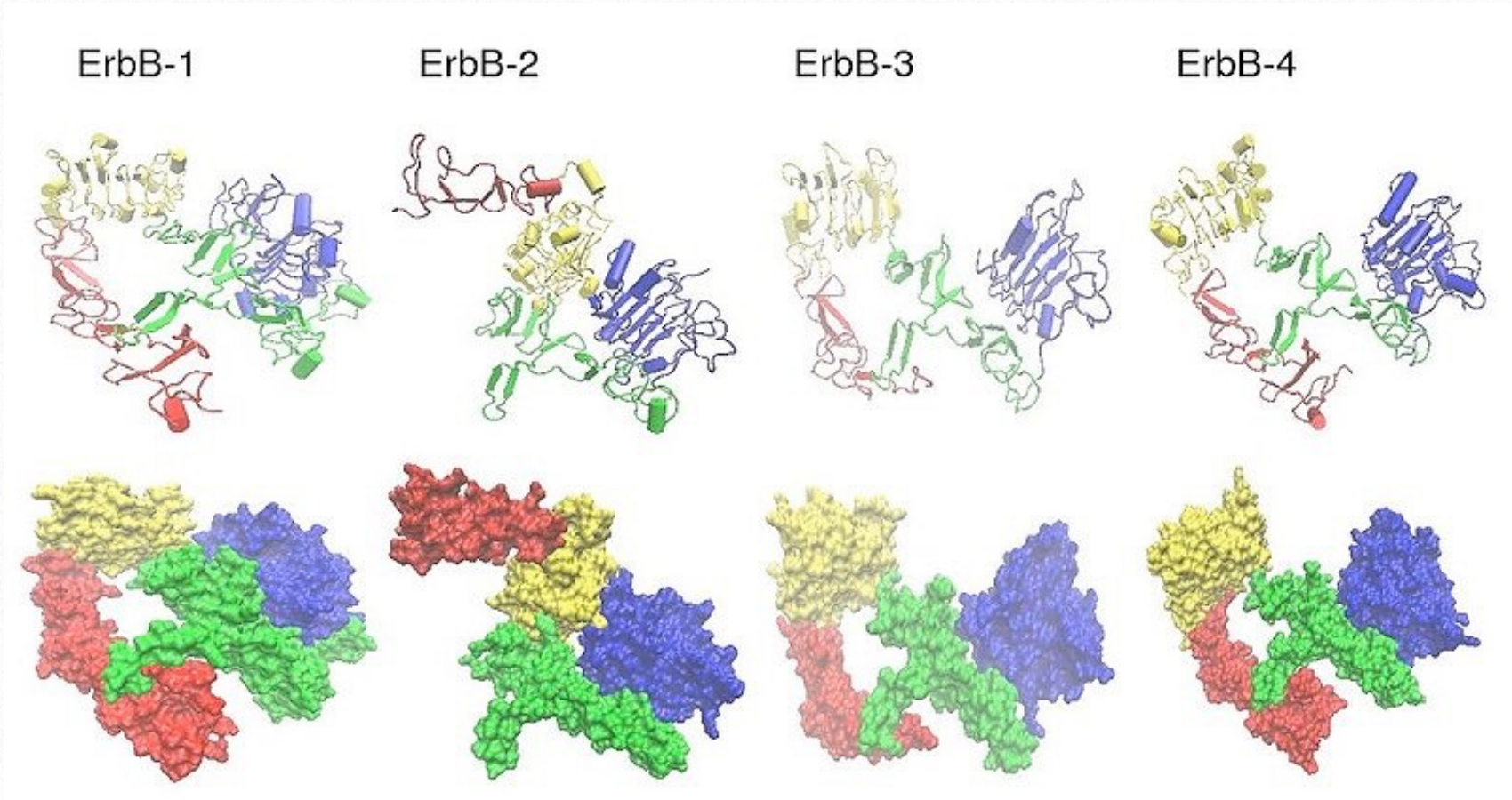

Figure 7.157 - The extracellular domains of the four members of the HER (ErbB) family

nases, cell surface receptors that bind EGF (Figure 7.157) and stimulate cell proliferation.

A crucial step in the signal transduction pathway is the dimerization of the receptors following binding of the signal, EGF, to the receptor. While HER1, HER3 and HER4 must bind the signal to dimerize, the structure of the HER2 receptor can, apparently, allow the receptor monomers to dimerize independently of EGF binding.

This means that the downstream events of the signaling pathway can be triggered even in the absence of a growth signal. In normal cells, only a few HER2 receptors are expressed at the cell surface, so this property of HER2 plays a relatively minor role in stimulating cell division. However, in about a quarter

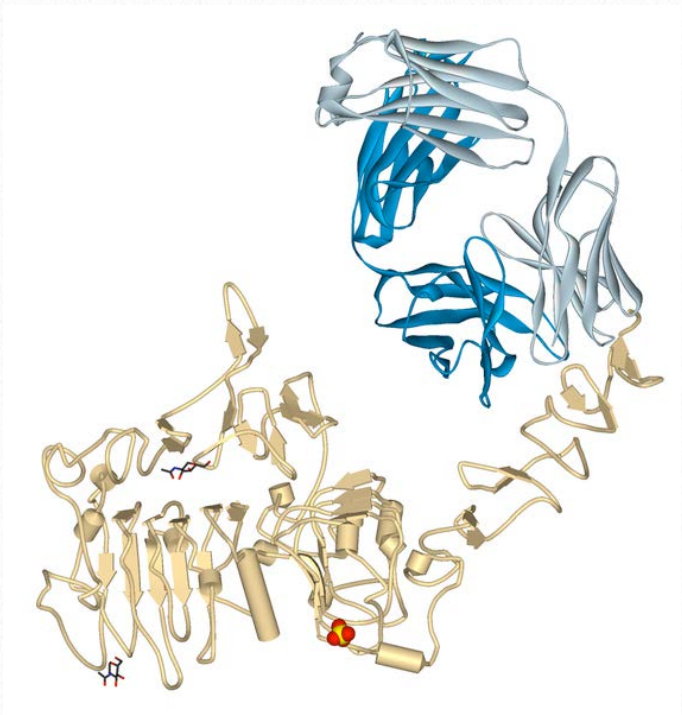

Figure 7.158 - Herceptin (blue) bound to the extracellular domain of HER2 (yellow)

Wikipedia

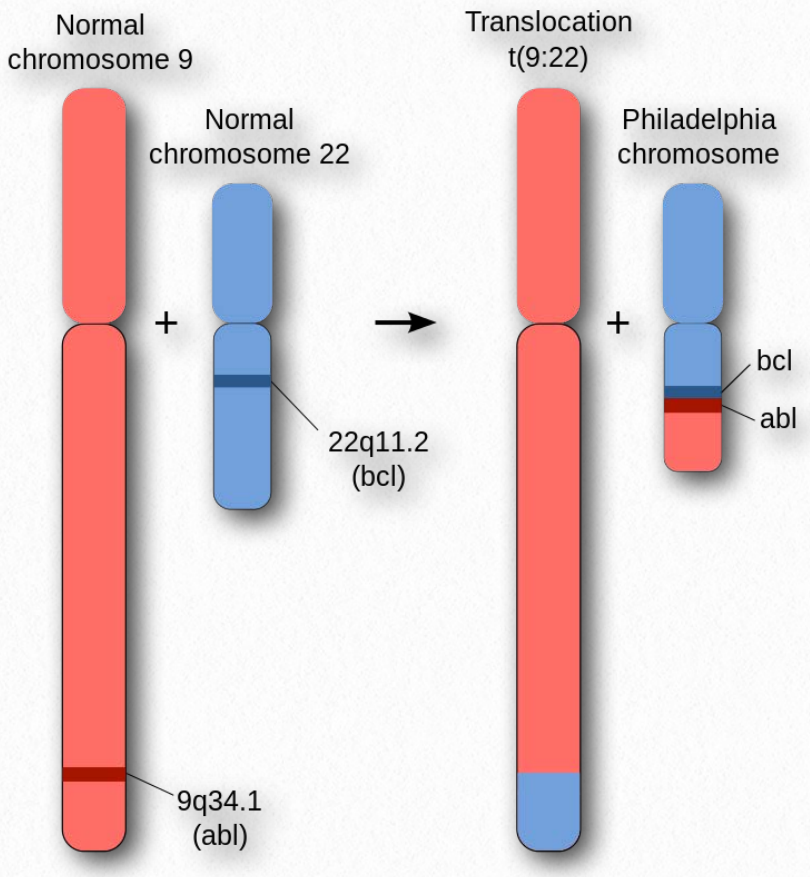

Figure 7.159 - Normal chromosomes (left) and altered chromosomes giving rise to the bcr-abl fusion (right)

Wikipedia

of breast cancer patients, HER2 receptors are overexpressed, leading to increased dimerization and subsequent uncontrolled cell proliferation.

Breast cancers that are HER2-positive can be more aggressive with a greater tendency to metastasize (spread) so therapy that blocks HER2 signaling is key in successful treatment of such cancers. Herceptin, a monoclonal antibody against the HER2 receptor, has been shown to be an effective treatment against Her2-positive breast cancers. Herceptin works by binding specifically to the extracellular domain of the HER2 receptor (Figure 7.158). This prevents dimerization of the receptor and thus blocks downstream signaling. Additionally, the binding of the Herceptin antibody to the receptor signals the immune system to destroy the HER2-positive cells.

Bcr-abl

Another example of a cancer caused by defects in an RTK signaling pathway is chronic myeloid leukemia (CML). Patients with CML have an abnormal receptor tyrosine kinase that is the product of a hybrid gene called bcr-abl, formed by the breakage and rejoining of chromosomes 9 and 22. This abnormal tyrosine kinase is constitutively dimerized, even when no signal is bound. As a result, it continuously signals cells to divide, leading to the massive proliferation of a type of blood cells called granulocytes.

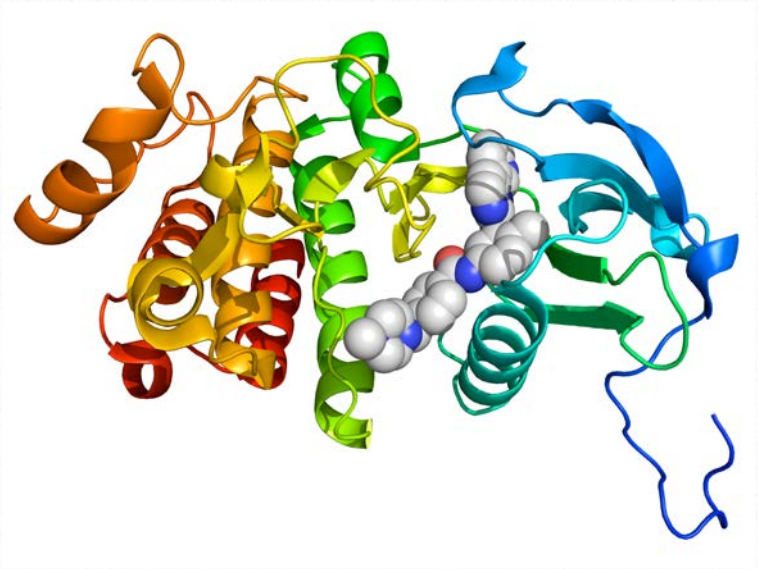

Figure 7.160 - Gleevec bound to the tyrosine kinase portion of abl

Wikipedia

As with HER2, the problem in CML is a receptor tyrosine kinase that dimerizes in the absence of a growth signal. The approach in this case was to target the next step in the signaling pathway. As you know, dimerization of RTKs activates the tyrosine kinase domain of the receptor, which results in the autophosphorylation of the cytoplasmic domains of both monomers. The phosphorylated tyrosines serve to recruit a number of other signaling proteins that pass the signal on within the cell.

In the case of the bcr-abl RTK, the drug Gleevec (imatinib) was designed to bind near the ATP-binding site of the tyrosine kinase domain. This "locks" the site in a conformation that inhibits the enzymatic activity of the tyrosine kinase and thus blocks downstream signaling. With no "grow" signal passed on, cells stop proliferating.

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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The Tao of Hormones

To the tune of "*The Sound of Silence*"

Metabolic Melodies Website HERE

Biochemistry my friend
It's time to study you again
Mechanisms that I need to know
Are the things that really stress me so
"Get these pathways planted firmly in your head,"
Ahern said
Let's start with ep-inephrine

Membrane proteins are well known
Changed on binding this hormone
Rearranging selves without protest
Stimulating a G alpha S
To go open up and displace its GDP
With GTP
Because of ep-inephrine

Active G then moves a ways
Stimulating ad cyclase
So a bunch of cyclic AMP
Binds to kinase and then sets it free
All the active sites of the kinases await
Triphosphate
Because of ep-inephrine

Muscles are affected then
Breaking down their glycogen
So they get a wad of energy
In the form of lots of G-1-P
And the synthases that could make a glucose chain
All refrain
Because of ep-inephrine

Now I've reached the pathway end
Going from adrenalin
Here's a trick I learned to get it right
Linking memory to flight or fright
So the mechanism that's the source of anxious fears
Reappears
When I make ep-inephrine

Recording by Tim Karplus
Lyrics by Kevin Ahern

Glucagon is Coming Around

To the tune of "*Santa Claus is Coming to Town*"

Metabolic Melodies Website HERE

You've gotta admire
What molecules do
Their cellular fire
Is ready on cue
Glucagon is coming around

If hormone should bind
Receptor outside
G proteins find
G nucleotides
Glucagon is coming around

They activate cyclases
That make cAMPs
Which bind to Protein Kinase A
And pull the R's from C's

The glycogen shrinks
In liver quite fast
The glucose into
Your bloodstream is passed
Thanks to this you have energy

And muscles uptake
The glucose in turn
Obtaining a substrate
All of them burn
Thanks to this you have energy

The pool of phosphatidyl
Inositides in you
Can send two separate signals
When they get split in two

The muscles contract
When calcium's free
Lowering levels
Of Creatine-P
Now you're gonna need energy

Those little calcium ions
I hope you've learned them well
Are just like Martha Stewart
All locked up in a cell

This story's complete
I know it's a load
My hope is your head
Ain't gonna explode
You will need it in finals week

Recording by Tim Karplus
Lyrics by Kevin Ahern