Cell Communication :

Signal Transduction

Using this book: This book is designed to be used in both introductory and advanced cell biology courses. The primary text is generally on the left side of the vertical divider, and printed in black. Details that are usually left to an advanced course are printed in blue and found on the right side of the divider. Finally, additional biomedically relevant information can be found in red print on either side of the divider.

Metazoan organisms are not just conglomerations of cells that happen to stick together. The cells each have specific functions that must be coordinated with one another in order to assure the survival of the organism and thus the shared survival of the component cells. If coordination is required, then a method of communication between cells is also required. In fact, it is even more complicated than that because the communications between the cells only scratches the surface (yes, bad pun intended) and the intracellular communication that goes on to coordinate multiple cellular activities in response to an external signal is usually far more complex than the initial transmission of that signal.

There are three primary modes of intercellular communication. These are (1) direct contact between signaling molecules bound to the membranes of two adjacent cells, (2) short-range soluble signals that diffuse over short distances, and (3) long-range soluble signals that are secreted into the circulation to be carried anywhere in the body.

An example of juxtacrine signaling is exemplified by the activity of some cell adhesion or ECM proteins, such as laminin, that do not just allow a cell to move over them, but act as signals to promote increased motility. This likely happens by activation of integrin receptors on the moving cell, which then initiate and coordinate changes through the rest of the cell to accomplish the change in activity. Another example is the Delta-Notch pathway used in embryonic patterning. Delta, a

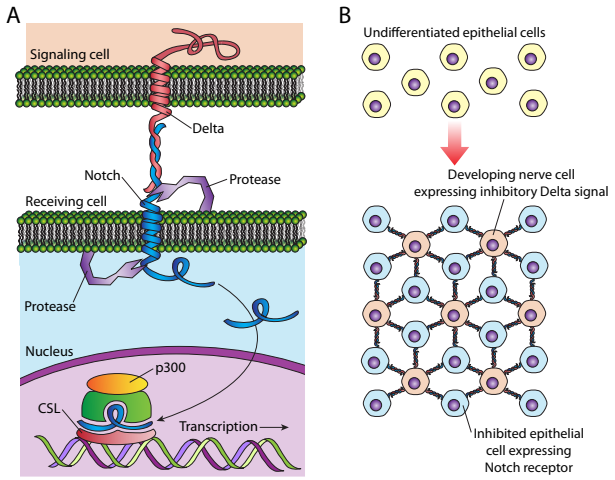

Figure 1. Delta-Notch signaling.

transmembrane protein on the signaling cell, binds to Notch, a receptor on the receiving cell. Notch alters its conformation, allowing its cytoplasmic domain to be cut off by γ-secretase. The cytoplasmic domain then translocates into the nucleus, where it acts as an activating transcription factor by binding with CSL. In the example sketched in fig. 1B, stochastic upregulation of delta in one cell activates notch in the surrounding cells, which then activates a specific differentiation pathway for them. Thus the central cell may be a sensory neuron, like a hair cell, while those immediately surrounding it are support cells like glia. This type of signaling imposes a spacing pattern on the expression of neurons (or other cell).

Diffusion limited signals from near neighbors is called *paracrine* signaling, and sometimes the signals can act on receptors right on the cell that secreted the signal, which would be *autocrine* signaling. Paracrine signals are only active if they can bind to a cell above a critical concentration to activate a signaling pathway. Therefore, as the signals diffuse away from the source, there is a cutoff, beyond which the concentration of signal is insufficient to activate a receiving cell. Growth factors are often paracrine signals. Although they do often encourage growth, they are also often survival factors. In that context, Nerve Growth Factor (NGF) is secreted by target cells that then reward the neurons that make the right connections by providing NGF for their survival. Those neurons that head off in the wrong direction, are unable to obtain NGF, and they do not survive, promoting efficiency and a better signal:noise ratio within the nervous system.

Endocrine signaling is essentially whole-body signaling. A signal produced by a hormone-producing gland is secreted into the bloodstream, where it becomes accessible to nearly any cell in the body. Of course, not every cell will respond to the hormone: like every other case of intercellular signaling, response is wholly dependent on receptors, so only the cells that have receptors to recognize the signal will react. For example, estrogen is released into the circulation, but in females, only some organs show significant impact when estrogen levels are significantly altered. Most tissues are unaffected. Endocrine signals may circulate in other extracellular fluids such as lymph.

Receptors and Ligands

A protein that happens to bind something is not necessarily a receptor. A receptor is defined as a protein that binds to an extracellular ligand, and then undergoes a conformational or biochemical shift in such a way that it initiates a chain of intracellular events by which the cell reacts to the extracellular signal. What are these ligands and their receptors?

The Delta-Notch pathway is well characterized and somewhat more complicated than portrayed in the paragraph to the left. The cleavage of Notch involves two proteases and two sites. Once the Notch cytoplasmic domain binds to CSL, it displaces a number of co-repressors bound to the CSL, and also recruits MAM (Mastermind-1) as a coactivator. MAM recruits histone acetylases to allow further increase transcription of targeted genes, but also recruits kinases that initiate the process of targeting the Notch cytoplasmic domain for ubiquitin-mediated destruction. The expression of Notch-controlled genes is thus self-regulated and shuts off soon after Delta is no longer available. Reviewed in R.A. Kovall, *Curr. Opin. Struct. Biol.* **17**: 117-27, 2007.

Intercellular signals span a very wide range of molecule types. Some are simple gases, like NO, while others are amino acids or derivatives, including glutamate, dopamine, or epinephrine. Lipids such as steroids (e.g. estrogen, cortisol) or eicosanoids (e.g. prostaglandins, leukotrienes) can be intercellular messengers. Finally many signals are peptides or even complex proteins (recall our juxtacrine signaling example). Although most are recognized by cell surface receptors, this is not always the case since, for example, steroids are lipid-soluble and can diffuse through the plasma membrane.

Receptors are a far less varied group of molecules, since they are all proteins, though it must be said that they represent many different protein structures and functions. In general, receptors are very specific for their ligands, but the specificity is not mutual: ligands can be rather promiscuous and bind with multiple receptors. This is part of the coordination aspect of signaling, though as a single ligand can initiate different effects in different cells depending on what receptor is expressed. The remainder of this chapter will delve into some of the intracellular signaling cascades that are characteristic of particular receptor types.

Because receptors, even at high density, represent only a minute fraction of the surface area of the cell, and therefore an even tinier fraction of the volume of the cell, the activation of a receptor must be amplified in order for it to initiate cellular activities (e.g. locomotion, growth, cell cycle progression). Thus one of the first things a receptor does upon activation is to initiate a signaling cascade. This aptly named sequence of events begins with the receptor activating an enzyme. The enzyme may be the cytoplasmic domain of the receptor itself, or it may be an independent protein but closely linked to the receptor. The enzyme does what enzymes do: it rapidly converts substrate molecules into product molecules. In this case, sometimes the product is an activator for another enzyme, and sometimes, the substrate is an inactive enzyme and the product is an activated enzyme. Either way, because of the high activity rates, the single activation of the receptor has increased first to tens or hundreds of enzyme activations, and each of those activates hundreds, and so on, so that the effect of the receptor can be rapidly distributed throughout the cell.

7-TM receptors (G-protein-coupled)

The 7-transmembrane receptors, or G-protein-coupled receptors are, unsurprisingly, a family of proteins that pass through the cell membrane 7 times. The amino terminal is extracellular and the carboxyl terminal is intracellular. Figure 2 shows the transmembrane regions spread out for clarity, but the transmembrane domains actually

form together in more of a cylindrical shape. 7-TM proteins are used as receptors for neurotransmitters such as epinephrine (β-adrenergic receptor), acetylcholine (muscarinic receptor), and serotonin, as well as hormones like glucagon or thyroid-stimulating hormone, and even non-molecular ligands such as light! Rhodopsins are a class of 7-TM receptors that are activated when they absorb a photon (fig. 5). Activating this family of receptors, whether by photon or by more conventional ligand binding, induces a conformational change in the cytoplasmic domain that alters the interaction between the receptor and a protein complex known as a heterotrimeric G protein.

The heterotrimeric G protein consists of an α, β, and γ subunit, of which the α subunit can bind either GTP or GDP, and can hydrolyze GTP to GDP. When the 7-TM receptor is inactive, the G protein complex is usually nearby associated with the membrane by myristoylation or palmitoylation of the α subunit and farnesylation or geranylgeranylation of the γ subunit. Once the 7-TM receptor is activated, it associates with the heterotrimeric G-protein, which causes the G_α to let go of the GDP and bind to a GTP. This then dissociates the G_α from the other two subunits. It can then associate with and activate an enzyme to expand the signaling cascade. One of the two classical pathways starts with G_α activation of adenylate (adenylyl) cyclase. Adenylate cyclase (AC) converts ATP to cAMP. Since ATP is plentiful and AC is a relatively fast enzyme, the first amplification of the signal comes with generation of the "second messenger" molecule

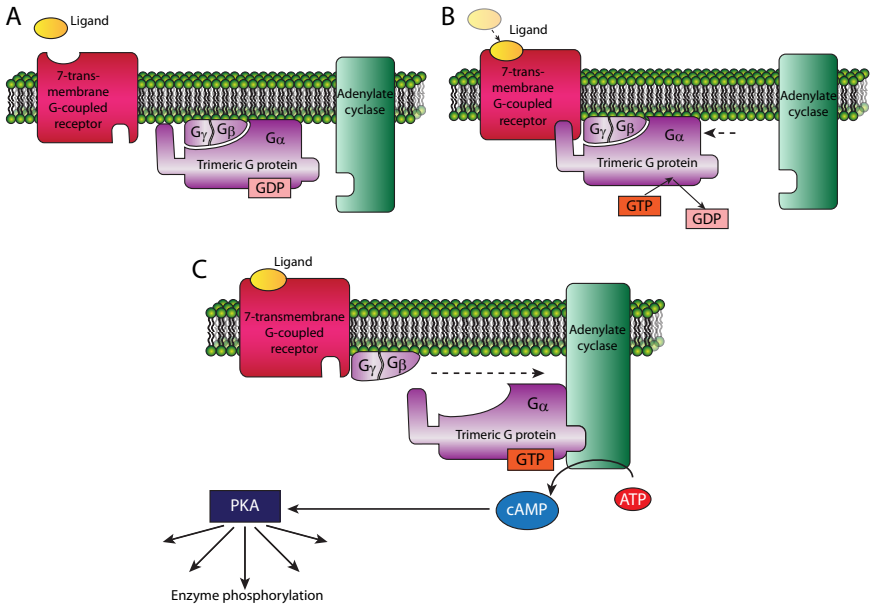

Figure 3. The heterotrimeric G-protein can act as a timed activator of adenylate cyclase.

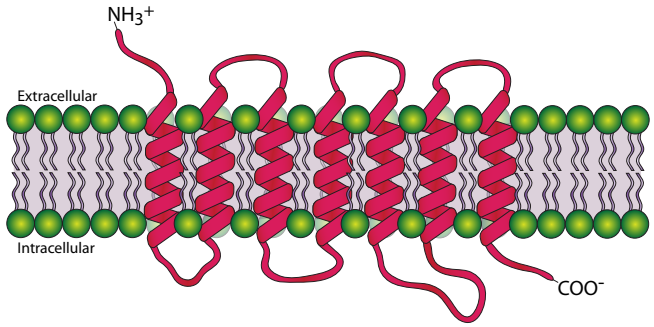

Figure 2. 7-TM receptor.

cAMP. Each cAMP molecule can then activate other enzymes, the primary one being protein kinase A. PKA can then phosphorylate a variety of substrates to alter cellular activity by gene expression, molecular motors, or metabolic changes.

The other classical pathway for 7-TM receptors is the activation of phosphlipase Cβ, also by Gα. PLCβ actually produces two second messenger molecules: it hydrolyzes phosphatidylinositol into diacylgylcerol (DAG) and inositol triphosphate (IP_3). IP_3 primarily induces the release of Ca^{++} from the endoplasmic reticulum. The DAG can activate protein kinase C. PKC is also activated by Ca^{++} and both Ca^{++} and DAG can activate PKC synergistically. Protein kinase C is an important central kinase that has been shown to phosphorylate and control the activity of numerous other enzymes from cytoskeletal elements to transcription factors.

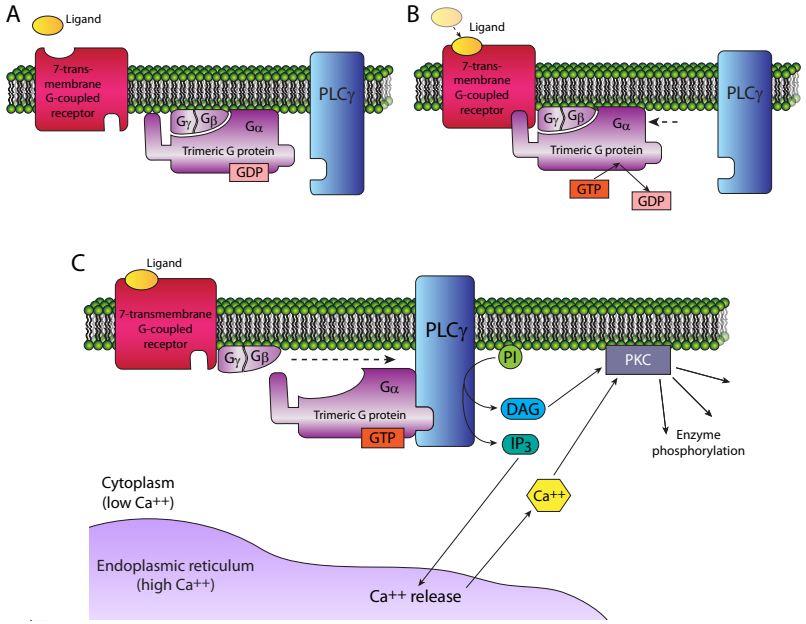

Figure 4. G-protein activated Phospholipase C. (A) The G-protein is inactive with GDP bound. (B) Upon ligand binding, G-protein binds receptor, exchanges GDP for GTP, and G_α dissociates. (C) Gα-GTP activates PLCγ, whic produces DAG and IP_3. The latter induces Ca^{++} release into the cytoplasm and together with DAG, activates PKC.

Second messengers must have two properties. They must be small enough to diffuse effectively, and the cell must be able to generate them quickly. Ca^{++} and cAMP fall into this category. Furthermore they can both be removed from circulation fairly quickly: the former by Ca^{++} pumps in the ER and Golgi, and the latter by phosphodiesterase activity. When the G-protein-pathway was discovered, the use of lipid second messengers

An interesting variation from the classic 7-TM pathways starts with the rhodopsin receptors in rod cells. These receptors bind photons for activation, and engage a heterotrimeric G protein. The Gα-GTP then binds to the g subunit of phosphodiesterase (PDE), activating it and catalyzing conversion of cGMP to GMP. As cGMP decreases, ion channels close, polarizing the membrane and changing the signal from the rod cell to the brain (via connecting neurons).

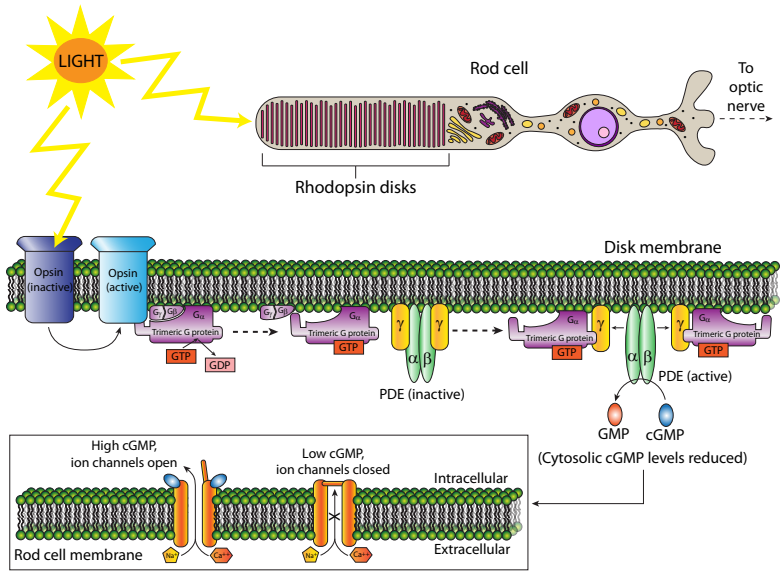

Figure 5. Activation of the 7-TM receptor rhodopsin by light.

was surprising. Membrane phospholipids were largely ignored at the time as simple static components of membranes. It is now clear that some of the phospholipids are biochemically active, with several enzymes that modify them, including phospholipases, phospholipid kinases, and phospholipid phosphatases. Some of these enzymes have a variety of functions because their substrate of product may be an important messenger molecule. For example, PI3K (phosphatidylinositol-3-kinase) is a central signaling kinase because its product, PIP3 (phosphatidylinositol (3,4,5)-triphosphate) is an activator for Akt/PKB and other enzymes that can activate several signaling pathways.

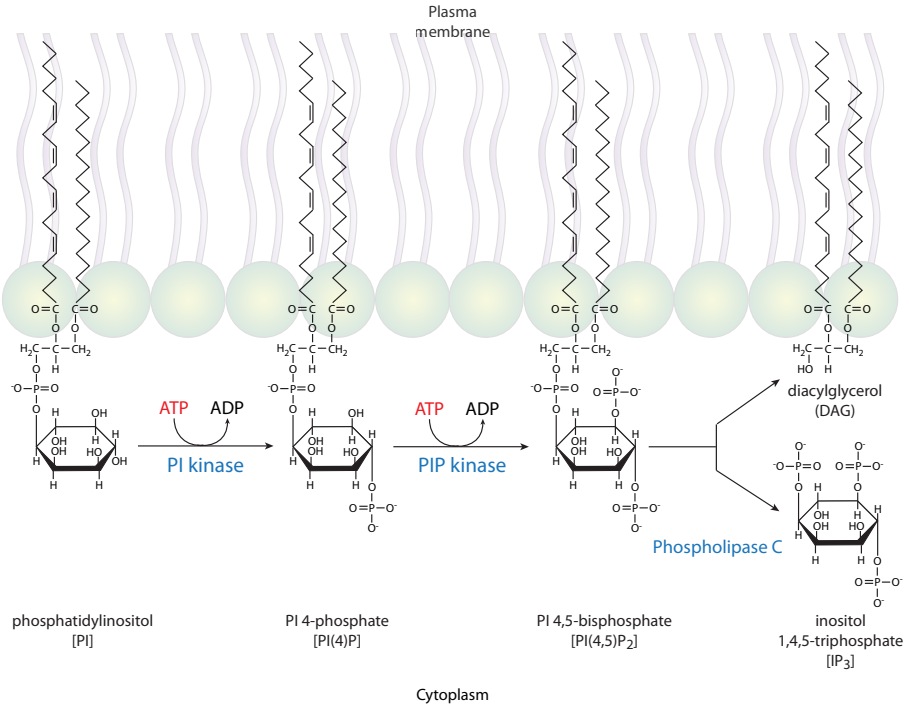

Figure 6. Modifications of Phosphatidylinositol generate various biologically active species.

The activation must eventually end, and it does so when G_α hydrolyzes the GTP bound to it. In this way, the G_α acts as a kind of timer for the signaling cascade. This is important because for signaling to be effective, it must be tightly controlled. Very early in this course, it was pointed out that Ca^{++} is kept at a very low concentration in the cytoplasm of the cell because we want Ca^{++}-sensitive mechanisms to be able to react quickly to an influx of calcium, but we equally want to be able to quickly turn off the signal as needed, and that is obviously much easier to do by sequestering a small amount of Ca^{++} than a lot of it. Similarly, if sustained activity of a recipient cell is called for, it is accomplished by continuous activation of the receptor and not by a long-lasting effect

from a single activation. This ensures that if a cellular effect must be abruptly and quickly cut off, it can be accomplished without a significant lag period between cessation of hormone secretion and cessation of intracellular signaling.

The receptor is a part of another shutoff mechanism as well: to prevent overstimulation, the receptors are desensitized for a short time after they have activated. G-protein-coupled receptor kinase (GRK) phosphorylates the 7-TM receptor. The phosphorylation creates recognition sites for arrestins. The arrestins have a variety of functions, the simplest of which is to act as a competitive inhibitor of G-protein binding by the receptor. This is a relatively short-lived desensitization.

For longer desensitization, arrestin binds to AP2 and clathrin, nucleating formation of a clathrin-coated endocytic vesicle. This removes the receptor from the cell surface, desensitizing the cell for a much longer period of time than simple competition between arrestin and G-protein.

The arrestins have another potential function. They can act as scaffolding proteins that bring a completely different signaling complex to the 7-TM receptor. Figure 8 shows an example in which the 7-TM receptor is used to activate a Jun transcription factor.

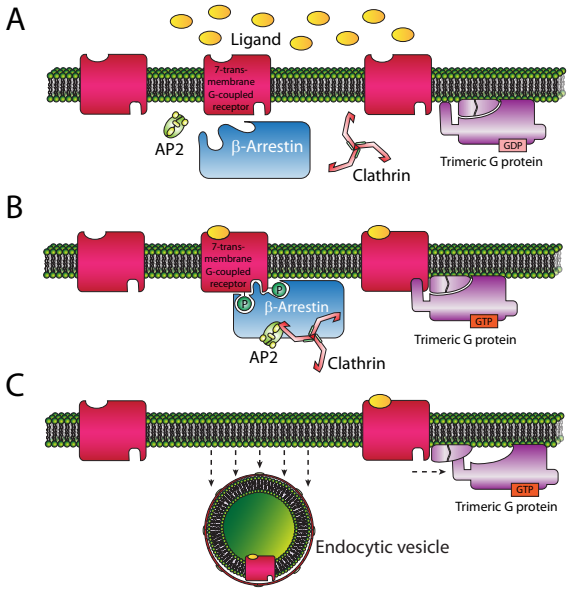

Figure 7. (A) After GRK phosphorylates a 7-TM receptor, arrestin can bind. In some cases, arresting can also bind AP1 and clathrin, (B) nucleating the formation of a clathrin-coated vesicle and endocytosis of the receptor (C).

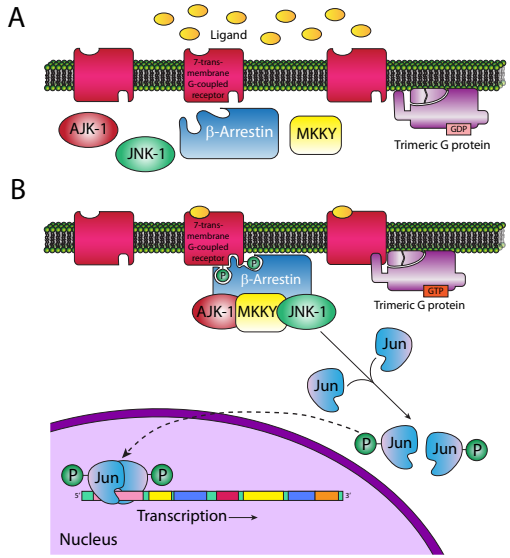

Figure 8. Interestingly, arrestins can also act to initiate a completely different type of signaling cascade from a 7-TM receptor. Here, the Jun transcription factor is activated.

The β-arrestin brings AJK-1, MKKY, and JNK-1 to activate JNK-1, which can then phosphorylate Jun. This allows translocation of phospho-Jun into the nucleus and subsequent dimerization, converting it into an active transcription factor.

What are some of the cellular actions that can be evoked by 7-TM receptor activation? Ca^{++} dynamics will be addressed in a separate section. IP_3 has been shown to evoke contraction of smooth and skeletal muscle, actin polymerization and cell shape changes, calcium release from intracellular stores, opening of potassium channels, and membrane depolarization. cAMP has been implicated in control of glycogen breakdown and gluconeogenesis, triacyglycerol metabolism, secretion of estrogens by ovarian cells, secretion of glucocorticoids, and increased permeability of kidney cells to water.

Receptor Tyrosine Kinases

In contrast to the 7-TM receptors, the receptor tyrosine kinases (RTK) pass through the membrane only once, and have a built-in enzyme domain - a protein tyrosine kinase. RTKs must dimerize to be functional receptors, although individual RTKs can bind to their ligands. The ligands also dimerize, and when a dimerized receptor is activated, the kinase domains cross-phosphorylate the cytoplasmic domain on the other receptor

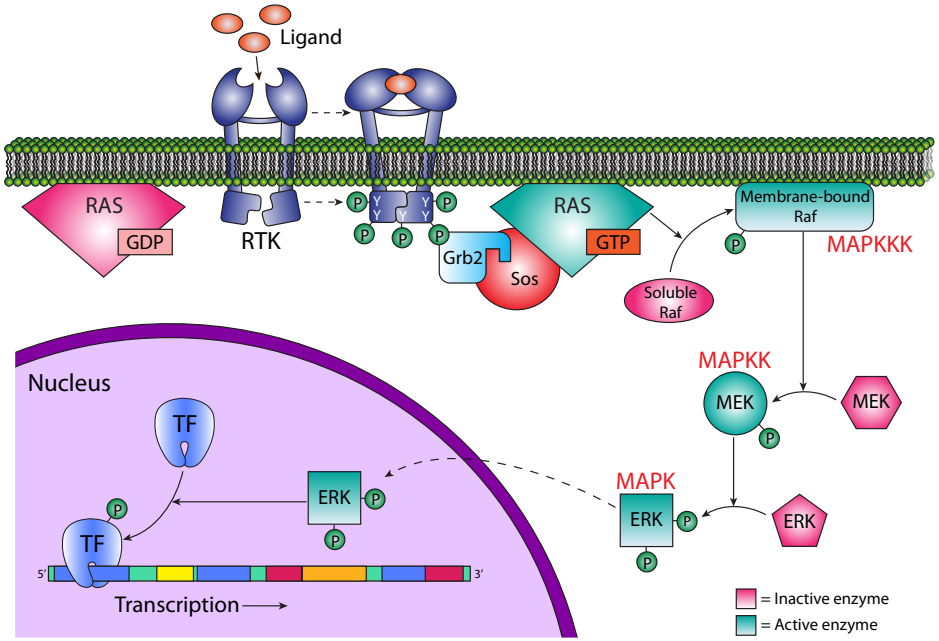

Figure 9. Receptor Tyrosine Kinases can activate the MAP pathway.

unit. This phosphorylation is necessary to form recognition sites for scaffolding or effector proteins. Figure 9 shows an example of an adapter protein, Grb2, which binds to a phosphorylated SH2/SH3 type domain on the receptor as well as to Sos (a guanine nucelotide exchange factor), which binds to and activates the GTPase Ras by exchanging a GDP for a GTP. This is the start of a very common RTK intracellular signaling pathway, the MAP kinase pathway. Following activation of Ras, it can activate Raf by phosphorylation and translocating it from the cytoplasm to the inner surface of the plasma membrane. Raf is a Ser/Thr kinase (also known by the unwieldy but fun to say, MAP kinase kinase kinase) that phosphorylates MEK (aka MAP kinase kinase). MEK is interesting because it is a dual-specificity kinase, phosphorylating both Ser/Thr sites as well as Tyr sites. The targets we are particularly interested here though, are MAP kinases (mitogen activated protein kinase), also known as ERKs (extracellular signal regulated kinases).

Each kinase along the canonical MAP kinase pathway has other potential substrates besides the next one in the MAPK sequence, so the variety of cellular responses that can be initiated by this pathway is very broad. There are at least 20 classes of RTK by structural similarity, including the fibroblast growth factor receptor (FGFR) class, epidermal growth factor receptor (EGFR) class, neurotrophin receptor (Trk) class, and insulin receptor class. Some growth factors not only induce growth, but survival, and sometimes proliferation. In fact, mutations to growth factors can be oncogenic (cancer-causing).

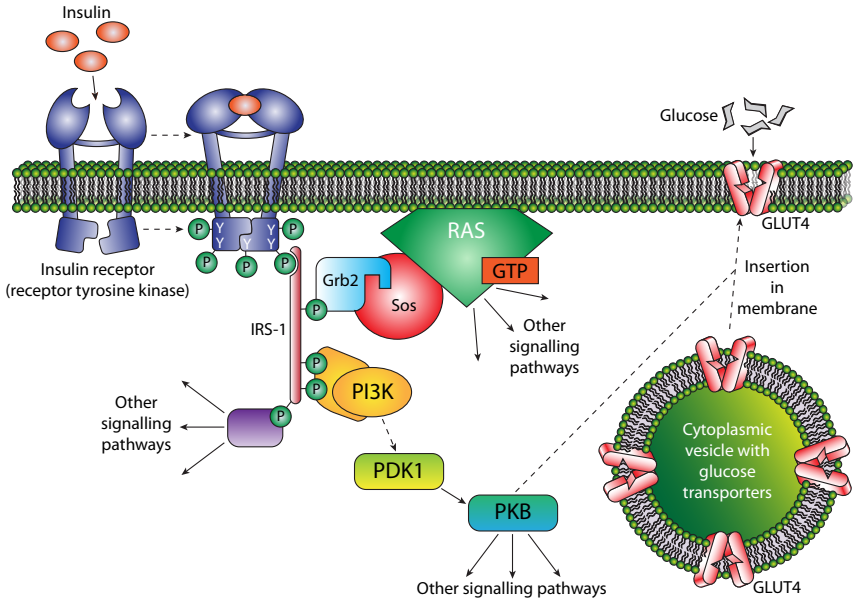

Figure 10. Insulin receptor signaling pathways.

One of the aspects of cell signalling that make studying it both fun and frustrating is the immensity of possibilities. The insulin receptor example above (fig. 10) demonstrates this. When the receptor is activated, the IRS-1 scaffolding protein binds to it, and brings with it binding sites to recruit a number of different signaling molecules such as Grb2-Sos-Ras to head down the MAPK pathway, but also PI3K, which can lead to activation of PDK1 and Protein Kinase B, important in regulation of glucose transport. PKB (also known as Akt), is also an important mediator of cell survival (by inhibiting BAD), cell proliferation, and angiogenesis.

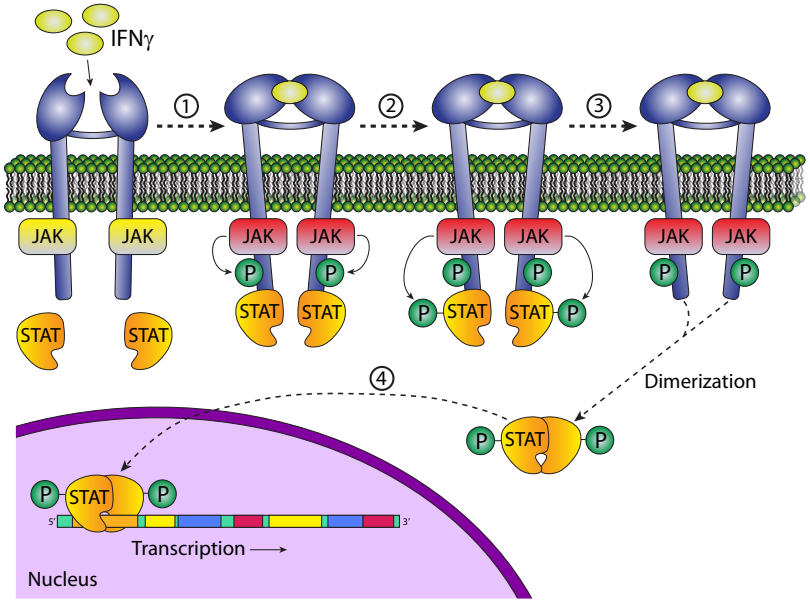

Figure 11. The JAK-STAT pathway.

Activation of cytokine receptors can initiate the JAK-STAT pathway. Cytokines are generally immunomodulatory signals, some of which act as hormones and others in a paracrine fashion. Interferon-γ is an example (fig. 11) of a cytokine, and the inactive RTK receptor binds to JAK (Janus kinase) in the inactive state. Upon ligand binding to the dimerized receptor, the JAK units are activated and the phosphorylate the receptor. This receptor phosphorylation leads to binding of STATs (the creatively named "signal transducers and activators of transcription"), which are then phosphorylated by the still-active JAK. Upon phosphorylation, the STAT-P proteins dissociate from the receptor and dimerize in the cytoplasm, where they are bound by importins and translocated into the nucleus where they act as transcription factors.

Ca^{++} Signaling

Signaling by increasing cytosolic calcium is an important and ubiquitous intracellular coordination mechanism. We already saw that release of Ca++ in muscle cells is required to allow contraction of each sarcomere, and the positioning of the sarcoplasmic reticulum makes possible rapid changes in concentration nearly simultaneous across the entire cell. Another extremely important physiological mechanism that relies on calcium is fertilization. Immediately upon penetration of the egg by the sperm, a wave of intracellular Ca++ increase spreads across the egg starting from the point of fertilization. This activates CaMKII (a kinase) and calcineurin (a phosphatase). Both are needed to overcome meiotic arrest and may also be necessary in initial embryonic development by control of chromatin decondensation, nuclear-envelope formation, and the movement and fusion of the two nuclei.

The aforementioned CaMKII is Ca++/calmodulin-dependent kinase II, and illustrates a fairly common theme, which is the use of Ca^{++}-binding proteins as intermediate Ca++ sensing activators. Calmodulin is a ubiquitous calcium-binding protein in eukaryotes and its importance is highlighted by the extraordinarily high homology across species. In normal cytosolic Ca^{++} levels, the relatively low affinity of the 4 Ca^{++} binding sites on calmodulin are unfilled. But when Ca^{++} concentrations rise, they occupy the sites, causing a conformational change in calmodulin and allowing it to interact with other proteins. In addition to calmodulin, troponin-C, and PKC, a few other important calcium-sensitive proteins are calsequestrin, a Ca^{++} buffer protein, gelsolin, the f-actin severing enzyme, the protease calpain, and calretinin, an activator of guanylyl cyclase (which makes the second messenger cGMP).

Guanylate (guanylyl) cyclase is also an important player in signal transduction by nitric oxide (NO). Nitric oxide is a gas produced by the action of nitric oxide synthase (NOS) on the substrate amino acid arginine. It is used as a super-soluble signal that passes through cells easily. However, it requires relatively high concentrations for physiological effect, so it is strictly a paracrine factor working on near neighbors. Perhaps the best studied example of NO signaling is vasodilation, in which the NOS-expressing endothelial cells of a blood vessel release NO to the smooth muscle cells surrounding them. The NO binds to and stimulates guanylate cyclase. The resulting increase in cGMP concentration leads to relaxation through multiple targets of protein kinase G.

Finally, no discussion of signal transduction would be complete without at least a fleeting mention of the extraordinary crosstalk (fig. 12) that can occur between the different pathways mentioned.

Sildenafil (Viagra) and its chemical siblings take advantage of this pathway by inhibiting cGMP-specific phosphodiesterases (PDE5) which normally break down cGMP to limit the response to NO. However, it should be noted that though PDE5 expression is limited, it is expressed not only in the genitalia but in the retina as well.

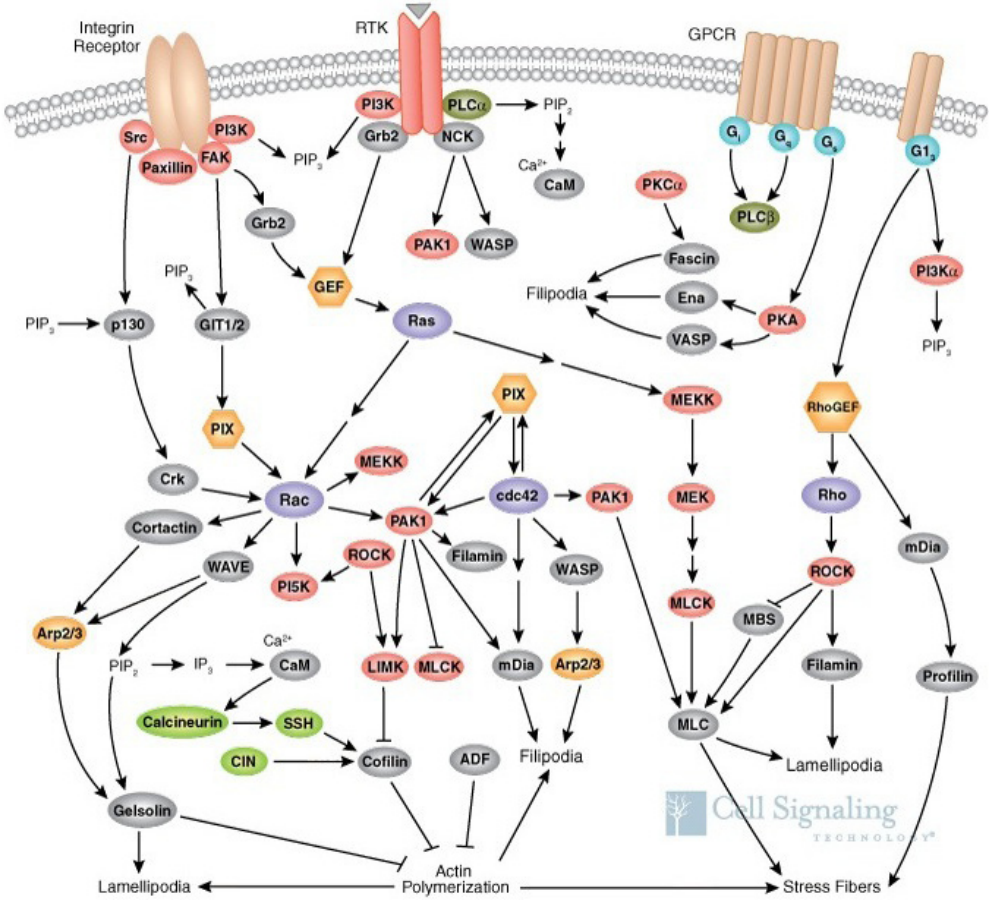

Figure 12. Signal transduction in actin dynamics. This figure comes from Cell Signaling Technology, Inc.

The figure represents only one small part of the signaling that happens inside a moving cell. Not only are some parts of the cell forming filopodia to help determine where to go, other parts or ruffling up the lamellipodia, and still others inducing motor proteins to rearrange the cytoskeleton in the proper way to facilitate bulk transport internally even as the leading edge of the cell is thrusting forward to make contacts externally. All of this must be coordinated by crosstalk between signaling systems as depicted, not to mention signaling related to metabolism, or gene expression, or even cell cycle, all of which are happening simultaneously inside the cell.