Signal transduction, IV
Calmodulin, Insulin receptor; IRS-1, 2; MAP kinases
(readings MBC 759-770, V&V 1234-1244)

I. Signaling by cytosolic calcium
As we’ve seen, the α1-adrenergic receptor activates the G protein Gq, which in turn activates PLCβ, which catalyzes the breakdown of PIP2 to form DAG and IP3. IP3 then binds to calcium channels in the ER membrane and induces the release of calcium into the cytoplasm. How does this rise in calcium concentration lead to the activation of glycogen phosphorylase?

Calmodulin, a 150 amino acid protein abundant in all eukaryotic cells, is one of the main sensors for the level of calcium in the cytoplasm. Calmodulin has a structure similar to that of troponin C, with two globular domains connected by a helix. Each globular domain has two calcium-binding sites. (See Alberts p. 750 Fig. 15-34 and Stryer p. 349 Fig. 13-45.)

The sites on the C-terminal domain have a high affinity for Ca++ and are always saturated with Ca++, while the domain at the N-terminus acts as a Ca++ sensor. This domain has a low affinity for Ca++ and so only binds to Ca++ when its cellular concentration increases. When both domains are loaded with calcium, calmodulin undergoes a conformational change which is sensed by other molecules that have binding sites for calmodulin.

One of these calmodulin-sensitive molecules is phosphorylase kinase. Phosphorylase kinase has the subunit composition (αβγδ)4. (See Alberts p. 752 Fig. 15-36.)

The α and β subunits are the substrates for PKA, and when phosphorylated they activate the γ subunit, which is the kinase. However, PKA is only one of the routes to phosphorylase kinase activation; calcium alone can also activate the γ subunit via
calmodulin. It turns out that δ subunit of phosphorylase kinase is calmodulin itself; thus, when calcium concentration rises and induces a conformational change in calmodulin, calmodulin activates the adjacent γ subunit kinase. The kinase then activates phosphorylase by phosphorylating its Ser14 residue.

The activation of phosphorylase by an increase in cytoplasmic calcium fits in with the function and needs of skeletal muscle cells. An incoming action potential leads to depolarization of the cell, triggering the release of calcium from the sarcoplasmic reticulum. The rise in cytoplasmic calcium concentration leads to contraction, which requires ATP. Where does the cell get its fuel? In the short term creatine phosphate provides energy, but in the long term the cell relies on glycogen. Thus it makes sense that the signal for contraction also triggers the breakdown of glycogen.

**CaM kinase II** (calmodulin-dependent kinase) is another calmodulin-associated protein and is of particular interest to neurobiologists due to its abundance in the nervous system, especially in the hippocampus. The hippocampus is an area of the brain associated with memory formation, which may occur via a process called long term potentiation in which cells appear to remember that they have been stimulated. To see whether CaM kinase II is involved in memory, researchers made a mouse that lacked the CaM kinase II gene. On first inspection the mouse appeared to be normal, but when dropped into water it had a harder time remembering how to swim through a maze than did its wild type counterparts. The result was interpreted as evidence that CaM kinase II is involved in memory. (See also Alberts p. 751 Fig. 15-35.)

So far we’ve seen that the formation of IP₃ leads to a rise in calcium, which activates phosphorylase kinase. DAG, the other half of the hydrolyzed PIP₂, stays at the membrane and activates another kinase, protein kinase C (PKC). Thus, both branches of the PLCβ-mediated signaling pathway ultimately lead to the activation of kinases.

**II. Insulin receptor**  
Epinephrine triggers the breakdown of glycogen, but what gives the signal for muscle cells to start making glycogen? **Insulin**, a polypeptide hormone that binds to and activates the **insulin receptor (IR)**, acts as a glucose sensor that tells the cell to activate glycogen synthetase and start making glycogen when the extracellular glucose concentration is high, such as after eating.

**A. Receptor structure**  
Unlike the adrenergic receptors, the IR is not a seven transmembrane receptor; rather, it belongs to another class of receptors known as **receptor-associated kinases**. These receptors have a ligand-binding domain outside the cell and a kinase domain in the cytoplasm, and the two domains are linked by a single transmembrane helix. (See Alberts p. 760 Fig. 15-47 and Stryer p. 351 Fig. 13-49.) The IR is made as a single polypeptide, but a proteolytic processing event snips out a piece to give the mature IR consisting of an α chain, which is the external insulin binding site and a β chain which is the internal kinase domain.
The kinase domain phosphorylates tyrosine residues; the insulin receptor belongs to a family of receptors called receptor tyrosine kinases (RTK's). RTK's have become well-known for the fact that many of them are protooncogenes that can acquire mutations that lead to cancer-causing, constitutively activated kinases. A related family of receptor kinases are specific for Ser/Thr residues.

An intriguing feature of these receptors is the fact that they manage to transmit a signal from the extracellular ligand-binding domain to the cytoplasmic kinase domain through just the single small transmembrane helix. A common model for signal transduction through this type of receptor is that the ligand induces dimerization of the ligand-binding domains, and as a result the kinase domains also associate and activate themselves. However, it turns out that in the case of the IR, information can travel through the single strand. In this sense, it is not that different from other allosteric proteins, but how does the information get across the membrane? This question has not been answered.

The 1350 amino acid IR occurs naturally as a dimer linked by disulfide bonds. (The α chain and the β chain are also linked together by disulfide bonds.) The structure deduced by electron microscopy shows that the insulin-binding N-terminal domain is rich in cysteine and is linked to the binding domain on the adjacent α chain by disulfide bonds. Next are structural domains that contain fibronectin-like repeats that lift the binding domain off the membrane, the same way the carbohydrate bristles on the LDL receptor keep its extracellular region rigid. The β chain contains the transmembrane helix, followed by the tyrosine kinase domain in the cytoplasm.

B. Insulin receptor substrate (IRS) While the main phosphorylation substrates of many RTK's are their own tyrosine residues, the IR is different. The IR phosphorylates itself on 5 tyrosine residues, but its main target is a separate substrate called the insulin receptor substrate (IRS). The two forms of IRS, IRS-1 and IRS-2, are large polypeptide chains with 20 tyrosines and 15 serines and threonines that can all be phosphorylated. Thus the signal from the IR is read out by the phosphorylation state of IRS-1 and -2.

C. Signaling downstream of IRS What does the phosphorylated IRS (or the autophosphorylated RTK's) do? Each of the phosphorylated tyrosines is part of a four or five amino acid sequence that recognizes a specific SH2 domain on another protein. The SH2 domain is a sequence on one protein that specifically recognizes Tyr-containing sequences on another protein when those tyrosine residues are
phosphorylated, and the specific pairing between certain phosphotyrosine-containing sequences and certain SH2 domains leads to the phosphorylation-dependent interaction of specific proteins. As a result of several such interactions, the signal leads to the localized collection of downstream signaling molecules. (See Alberts p. 762 Fig. 15-49.)

In the case of insulin signaling, once the IR has phosphorylated both itself and IRS, the phosphorylated IRS binds to the SH2 domain of GRB2, a protein that serves as a linker molecule between the IRS and a third protein, SOS. SOS, a GNRP (guanine nucleotide releasing factor similar to the one we discussed for ARF), then binds and is hence localized to the membrane where the small GTP-binding protein ras is located by means of its hydrophobic tail. SOS then catalyzes the exchange of GDP for GTP on ras. Once ras binds GTP, it activates a kinase called Raf. Raf phosphorylates and thus activates MAP kinase kinase (MAPKK), which in turn phosphorylates and activates MAP kinase (MAPK). MAPK then signals to a wide assortment of targets, including transcription factors, that then activate transcription and lead to cell growth. The ability of insulin to activate cell growth and division through MAPK activation makes insulin a growth factor, and, in fact, the receptor phosphorylation----> SH2 binding----> ras complex formation----> MAPK activation pathway through which insulin activates growth is common to many growth factors. (See also Alberts pp. 763-6 Figs. 15-50, 53 and 54.)

In addition to triggering long term growth, insulin-dependent activation of MAPK also triggers rapid changes in the cell. Most relevant to the discussion of glycogen
metabolism is the fact that MAPK phosphorylates pp90rsk, or IRSK, a kinase first isolated in muscle as an insulin-dependent kinase.

D. pp90rsk-dependent activation of glycogen synthesis When insulin activates pp90rsk through this signaling cascade, pp90rsk puts a single phosphate on the muscle phosphatase PP1G. We’ve seen before that PP1G with no phosphate is basally active and becomes inactive when doubly phosphorylated on its regulatory subunit G. However, when PP1G is only singly phosphorylated on a serine in its regulatory subunit, it becomes very active and starts removing phosphates from glycogen synthetase. Remember that glycogen synthetase is more active when it is less phosphorylated; thus the insulin-dependent activation of pp90rsk triggers the synthesis of glycogen through the activation of PP1G, which leads to the activation of glycogen synthetase. At the same time, activated PP1G inactivates glycogen phosphorylase by dephosphorylating its Ser14. Thus, pp90rsk both triggers glycogen synthesis and prevents glycogen breakdown.

E. Insulin-triggered glucose uptake At the same time insulin signals the synthesis of glycogen from glucose, it also increases the influx of glucose into the cell through specific glucose transporters (GLUT4) in the membrane. Most of these glucose transporters reside in vesicles, and upon insulin-dependent phosphorylation IRS interacts with a kinase called PI3 kinase (PI3K) that then triggers the vesicles to fuse with the plasma membrane. The increased number of glucose transporters at the membrane allows more glucose to enter the cell. Since the process involves the secretion of GLUT from vesicles to the plasma membrane, it is a form of regulated secretion. [See problem 11-7 from The Problem's Book.]

III. Conclusion In the lectures on signal transduction we’ve seen three types of situations. In the first, a seven transmembrane receptor (such as the β-adrenergic receptors) triggers the formation of cAMP, which activates PKA, a kinase. In the second, a seven transmembrane receptor (such as the α1-adrenergic receptor) leads both to an increase in cytoplasmic calcium, which activates CaM kinase, and to the activation of PKC, a kinase. Finally, in the third case, a single transmembrane receptor-associated kinase such as the insulin receptor triggers the phosphorylation of itself and of a cascade of factors leading to the activation of MAP kinase. Thus, whatever receptor on the cell surface is activated, the signal is interpreted downstream as the activation of one or more kinases.