Second Messenger Systems

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Signaling pathways are complex systems in which a single extracellular signal can elicit multiple intracellular events, some of which may also be triggered by other signaling pathways. We will discuss how this process occurs through kinase cascades and second messengers, and then apply these to processes to the regulation of protein expression and the fight or flight response, respectively.

Receptor Tyrosine Kinase Pathways

Receptor tyrosine kinases transmit signals across the membrane

Ligand binding to its receptor protein is the first step in all biochemical-signaling pathways. For example, insulin is a protein growth factor that binds to a specific receptor whose C-terminal domain has tyrosine kinase activity. Receptor tyrosine kinases (RTKs) such as this usually have a single transmembrane segment remain as monomers in the unliganded state.

Receptor tyrosine kinases are activated by autophosphorylation, which is the cross-phosphorylation that occurs between cytoplasmic protein tyrosine kinase domains.
brought into close proximity of each other as RTK protein dimerizes. How does autophosphorylation activate the protein tyrosine kinase activity of the insulin receptor?

The human protein is a single 1382-residue precursor peptide that is proteolytically cleaved to give rise to a mature insulin receptor with α and β subunits that are linked together by disulfide bridges. Insulin binds to the receptor’s α domains on the extracellular face of the membrane, whereas the protein tyrosine kinase β domains are on the intracellular side.

![Diagram](Image created by Lecturio)

**Kinase cascades relay signals to the nucleus**

The advantage of a kinase cascade is that a small signal can be amplified much fold inside the cell. In the previous example, we discussed how the insulin receptor is directly activated by autophosphorylation, but many target proteins are activated through a highly diverse and sophisticated set of interconnected signaling pathways that involve cascades of associating proteins. In the case of the G protein Ras, which is anchored on the inner surface of the plasma membrane, is activated by growth factor binding its cognate receptor tyrosine kinase before it activates a kinase cascade to signal the transcriptional apparatus inside the nucleus.
The binding of growth factor to its receptor tyrosine kinase leads to autophosphorylation of the receptor tyrosine kinase, which then interacts with an SH2-containing protein, whose function is to activate Ras to release bound GDP and replace it with GTP. The kinase cascade downstream of Ras completes the signaling pathway, first by the phosphorylation of either MEK or MAPS kinase, which migrate from the cytosol to the nucleus to phosphorylate a variety of protein transcription factors.

Transcription factors stimulate various genes to produce the effects commissioned by the extracellular presence of the growth factor that initiated the signaling cascade. When insulin activates the Ras signaling pathway, the result is an increase in protein synthesis that supports cell growth and differentiation, a response consistent with insulin’s function as a signal of fuel abundance.

In addition, phosphorylation of more than one target protein can lead to the simultaneous activation of several intracellular processes, such as the MAP Kinase cascade. Thus, insulin signaling mediates changes in vesicle trafficking, enzyme activation, and gene expression.

G-Protein Coupled Receptors
Another major class of signal transduction pathway involves **Heterotrimeric G proteins**, which are members of the superfamily of GTPases known as the G proteins. These proteins have the ability to bind and hydrolyze guanine nucleotides GTP and GDP. The monomeric G proteins are essential in a few key processes like signal transduction, translation, protein targeting, and the growth of actin microfilaments.

The signal transduction pathways that many G proteins participate in consist of three components depicted in. **G protein-coupled receptors (GPCRs)** are transmembrane proteins that bind hormone ligands on the extracellular side, and induce a conformation inside the cell on the cytoplasmic face of the membrane. This binding of the ligand activates the G protein to in turn activate or inhibit a transmembrane enzyme called **Adenylate cyclase (AC)**.

Activated Adenylate cyclase synthesizes a compound called **adenosine-3', 5'-cyclic monophosphate (3', 5'-cyclic AMP or cAMP)** from ATP. cAMP, activates numerous cellular processes by binding and activating a variety of proteins. cAMP is also known as a **second messenger** because it intracellularly transmits a signal originated by an extracellular ligand.
G Protein-coupled receptors contain seven transmembrane helices

**G protein-coupled receptors (GPCRs),** such as the β2-adrenergic receptor depicted in, exists as integral membrane proteins with seven transmembrane α-helices. The N-and C-terminal amino acid residues vary in sequence and participate in ligand binding on the extracellular face of the cell and confer a Heterotrimeric G protein function on the cytoplasmic side. Many of these proteins are posttranslationally modified by N-glycosylation and/or by the palmitoylation of a cysteine residue, making them lipid-linked glycoproteins.

There are few other structural similarities between GPCR binding sites other than the general location. They function much like hemoglobin, which is an allosteric protein that alternates between two discrete conformations. Once conformation has bound ligand, and can transmit an extracellular signal to the cell’s interior.

**Adenylate cyclase synthesizes cAMP to activate protein kinase A**

**Protein Kinase A (PKA)** is activated when it binds four molecules of cAMP, which is a polar molecule capable of acting as a freely diffusing second messenger. Protein Kinase A is an enzyme that specifically phosphorylates serine and threonine residues of many cellular proteins containing the consensus kinase-recognition sequence Arg-Arg-X-Ser/Thr-Y, where Ser/Thr is the phosphorylation site, X is any small residue, and Y is a large hydrophobic residue.

In the absence of cAMP, PKA is an inactive heterotetramer consisting of two regulatory and two catalytic subunits, R2C2. cAMP binds to the regulatory subunits to cause the dissociation of active catalytic monomers. The intracellular concentration of cAMP, therefore, determines the fraction of PKA in its active form and thus the rate at which it phosphorylates its substrates.

The targets of PKA include enzymes involved in glycogen metabolism. For example, when epinephrine binds to the β-adrenergic receptor of a muscle cell, the sequential activation
of a heterotrimeric G protein, adenylate cyclase, and PKA leads to the activation of
glycogen phosphorylase, thereby making glucose-6-phosphate available for glycolysis in
a “fight-or-flight” response. In the end, signaling activity is limited by the destruction of
the second messenger, cAMP.

Review Questions

The answers are below the references.

1. **Some receptors for growth factors activate a protein kinase cascade, with
the participation of multiple enzymes to effect a change in gene expression.**
Which of the following statements about a protein kinase cascade are true?

A. Multiple steps allow the amplification of the signal
B. External signals can lead to changes in gene expression.
C. Multiple steps leading to kinase activations can result in cells having different
responses, depending on the presence or absence of target proteins.
D. Multiple steps in an activation mean that abnormal stimulation of a cellular
response such as growth can occur with mutations in more than one gene.
E. All of the above is true.

2. **The endogenous GTPase activity of G-proteins serves to:**

A. Stimulate the activity of enzymes by producing energy.
B. Synthesize cGMP as a second messenger.
C. Synthesize GTP as an energy source.
D. Hydrolyze GTP returning the G protein to a pre-stimulated level of activity.

3. In a fight-or-flight response, epinephrine released by the adrenal glands
binds to a membrane receptor on muscle cells. Which of the following events
result from ligand binding?

A. G-protein is activated by binding GTP, causing adenylyl cyclase to produce cAMP.
B. The second messenger, cAMP, activates protein kinase A, which inhibits glycogen
synthase and thus blocks glycogen synthesis.
C. Protein kinase A activates phosphorylase kinase, which transfers a phosphate that
activates glycogen phosphorylase. This leads to the breakdown of glycogen and
the production of glucose-1-phosphate.
D. Glucose produced by glucose-1-P stimulates glycolysis, producing energy for
muscle contraction.
E. All of the above.

References

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**Correct answers:** 1E, 2D, 3E

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