Outline

I. Cell Signaling
II. Forms of cell signaling
III. Quick review of cell membrane
IV. Cell Surface Receptors
   I. G-protein Coupled Receptors
   II. Tyrosine Kinase Receptors
   III. Control of cell division
   IV. Ligand-Gated Ion Channels
V. Intracellular Receptors
VI. Down regulation

Overview: Cellular Messaging

- Cell-to-cell communication is essential for both multicellular and unicellular organisms
- Biologists have discovered some universal mechanisms of cellular regulation
- Cells most often communicate with each other via chemical signals
- For example, the fight-or-flight response is triggered by a signaling molecule called epinephrine

Evolution of Signaling

- Pathway similarities suggest that ancestral signaling molecules evolved in prokaryotes and were modified later in eukaryotes
- The concentration of signaling molecules allows bacteria to sense local population density

Local and Long-Distance Signaling

- Cells in a multicellular organism communicate by chemical messengers
- Animal and plant cells have cell junctions that directly connect the cytoplasm of adjacent cells
- In local signaling, animal cells may communicate by direct contact, or cell-cell recognition
Local and Long-Distance Signaling

- In many other cases, animal cells communicate using **local regulators**, messenger molecules that travel only short distances.
- In long-distance signaling, plants and animals use chemicals called **hormones**.
- The ability of a cell to respond to a signal depends on whether or not it has a receptor specific to that signal.

**Forms of Signaling**

1. Gap Junctions
2. Autocrine - A cell secretes a molecule that binds back onto its own receptor
3. Paracrine - Local mediators
4. Synaptic – Nerve cell signal transmission  
   - Neurotransmitters, released into synaptic cleft
5. Endocrine - long acting, wide spread  
   - Hormones, secreted into the bloodstream

**The Three Stages of Cell Signaling**

- Earl W. Sutherland discovered how the hormone epinephrine acts on cells.
- Sutherland suggested that cells receiving signals went through three processes:
  - Reception
  - Transduction
  - Response
Reception: A signaling molecule binds to a receptor protein, causing it to change shape

- The binding between a signal molecule, the ligand, and receptor is highly specific
- A shape change in a receptor is often the initial transduction of the signal
- Most receptors are plasma membrane proteins

Transduction: Cascades of molecular interactions relay signals from receptors to target molecules in the cell

- Signal transduction usually involves multiple steps
- Multistep pathways can amplify a signal: A few molecules can produce a large cellular response
- Multistep pathways provide more opportunities for coordination and regulation of the cellular response

Protein Phosphorylation

- In many pathways, the signal is transmitted by a cascade of protein phosphorylations
- Protein kinases transfer phosphates from ATP to protein, a process called phosphorylation
Protein Dephosphorylation

- **Protein phosphatases** remove the phosphates from proteins, a process called dephosphorylation.

- This phosphorylation and dephosphorylation system acts as a molecular switch, turning activities on and off or up or down, as required.

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Which type of ligand would bind to a receptor in the plasma membrane

1. Water soluble
2. Lipid soluble

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The following can freely pass through a membrane:

1. Ions
2. Hydrophobic molecules
3. Hydrophillic molecules
4. Ions and hydrophillic molecules
Receptors in the Plasma Membrane

- Most water-soluble signal molecules bind to specific sites on receptor proteins that span the plasma membrane = cell surface receptors

- There are three main types of membrane receptors
  - G protein-coupled receptors
  - Receptor tyrosine kinases
  - Ion channel receptors

Receptors in the Plasma Membrane - GPCRs

- G protein-coupled receptors (GPCRs) are the largest family of cell-surface receptors

- A GPCR is a plasma membrane receptor that works with the help of a peripheral G protein

G Proteins

- Trimeric GTP-binding protein = G Proteins

- G Proteins are either in the active or inactive state

- Active state has GTP bound

- When the ligand binds the G-protein Coupled Receptor, this causes a change in shape, that activates the G Protein

- This starts a chain of events that involve intracellular mediators = secondary messengers

Trimeric G Proteins

- G Proteins are composed of three proteins: α and β and γ subunits

- When the G Protein is activated (GTP is bound) the α subunit (with GTP) goes one way and the β and γ go together another way.

- β and γ are active when they are not attached to the α subunit

- the α subunit has GTPase activity
**GTPase activity in G proteins**

- The G protein is active only when it has GTP bound to it.
- The G protein has **GTPase activity**, which means it will automatically hydrolyze a phosphate and have GDP bound.
- This way it inactivates itself automatically.
- This GTPase activity is enhanced by GTPase Activating Proteins (GAPs).

**Activation and deactivation of G proteins**

- When the G protein binds to the GPCR, the alpha subunit ejects a GDP and accepts a GTP. Alpha detaches from beta/gamma subunits, they are now active.
- After a period of time, the G protein self hydrolyses a phosphate from the GTP, forming GDP. Alpha will now reform with beta/gamma subunits, they are now inactive.

**Small Molecules and Ions as Second Messengers**

- The extracellular ligand that binds to the receptor is a pathway’s “first messenger”
- **Second messengers** are small, nonprotein, water-soluble molecules or ions that spread throughout a cell by diffusion.
- Second messengers participate in pathways initiated by GPCRs and RTKs.
- Cyclic AMP and calcium ions are common second messengers.

**Cyclic AMP**

- **Cyclic AMP (cAMP)** is one of the most widely used second messengers.
- **Adenylyl cyclase**, an enzyme in the plasma membrane, converts ATP to cAMP in response to an extracellular signal.
cAMP Pathway

1. A ligand binds to the G-protein Coupled Receptor (GPCR).
2. The binding of the ligand (hormone) activates the GPCR.
3. The active GPCR is able to bind the G-protein
4. The G-protein ejects a GDP and accepts a GTP molecule. The G-protein is now active
5. The α subunit of the G-protein with the GTP disassociates from the β and γ subunits
6. The α subunit of the G-protein with the GTP goes to adenylyl cyclase and activates it
7. The active adenylyl cyclase transforms ATP into cAMP
8. cAMP activates a protein kinase A (PKA)
9. cAMP is inactivated by phosphodiesterases
10. The PKA phosphorylates proteins
11. The phosphorylated proteins are now active and can change the cell activity
12. The g-protein with GTP bound will hydrolyze the phosphate from GTP, now has GDP bound and is inactive. It will reform with the β and γ subunits and this inactivates them
Gene transcription by cAMP

- The cAMP pathway can also activate the transcription of specific genes.
- The protein kinase A (PKA) activated by cAMP can turn on transcription of DNA using a CRE-binding protein (cAMP response element).

Examples of Hormone induced responses mediated by cAMP

<table>
<thead>
<tr>
<th>Target Tissue</th>
<th>Hormone</th>
<th>Major Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal Cortex</td>
<td>ACTH</td>
<td>Cortisol Secretion</td>
</tr>
<tr>
<td>Ovary</td>
<td>LH</td>
<td>Progesterone secretion</td>
</tr>
<tr>
<td>Muscle</td>
<td>Adrenaline</td>
<td>Glycogen breakdown</td>
</tr>
<tr>
<td>Bone</td>
<td>Parathyroid hormone (PTH)</td>
<td>Bone reabsorption</td>
</tr>
<tr>
<td>Heart</td>
<td>Adrenaline</td>
<td>Increase heart rate</td>
</tr>
<tr>
<td>Liver</td>
<td>Glucagon</td>
<td>Glycogen breakdown</td>
</tr>
<tr>
<td>Kidney</td>
<td>Vasopressin</td>
<td>Water reabsorption</td>
</tr>
<tr>
<td>Fat</td>
<td>Adrenaline, ACTH, glucagon</td>
<td>Triglyceride breakdown</td>
</tr>
</tbody>
</table>

Calcium ions as a secondary messenger

- Calcium ions (Ca^{2+}) act as a secondary messenger in many pathways.
- Calcium is an important second messenger because cells can regulate its concentration.
Receptor tyrosine kinases (RTK)

- Receptor tyrosine kinases (RTK)
  - Phosphorylates tyrosine amino acids on signaling proteins

Receptor Tyrosine Kinase

- These receptors just cross the plasma membrane once – single transmembrane proteins
  - RTKs are dimers – it takes two RTKs to function
    - Binding of the ligand causes two RTKs to come together and link to form a dimer = dimerize

Receptor tyrosine kinases (RTKs)

- Receptor tyrosine kinases (RTKs) are membrane receptors that attach phosphates to tyrosines
  - A receptor tyrosine kinase can trigger multiple signal transduction pathways at once
  - Abnormal functioning of RTKs is associated with many types of cancers

Receptor Tyrosine Kinase

- When the two receptors link together, they now have enzyme activity
  - They phosphorylate tyrosine residues on each other = autophosphorylation
  - This requires ATP
Receptor Tyrosine Kinase Ligands

- Examples of ligands for receptor tyrosine kinases:
  - Insulin
  - Growth hormone
  - Erythropoietin

MAP Cascades

- Mitogen-activated protein kinases (MAPK)
- Mitogens are important in normal cell division
- MAP kinases are a series of protein kinases that phosphorylate other protein kinases
- Ending in a cellular response including gene transcription
- These cascades amplify the response

MAP Cascades

- The process starts with a growth factor binding to a RTK
- RTK dimerizes and autophosphorylates
- The activated RTK bind activator proteins
- The activator proteins (GRB2 and SOS) activate a GTP-binding proteins (G protein) called Ras
- When Ras is activated, it releases GDP and accepts GTP
- Ras activates MKKK, which activates MKK....
**Summary of RTK/Ras/MAP Pathway**

1. Two ligands bind to two RTKs (one ligand/receptor)
2. The two RTK dimerize and phosphorylate each other, activating each other
3. The active RTKs activate GRB2 and SOS proteins activate a GTP-binding protein (G protein) called Ras causing it to eject GDP and accept GTP
4. The now active Ras activates the MAP kinase cascade until the MAP kinase is activated
5. The MAP kinase activates proteins which leads to cellular activity.
6. Ras will automatically inactivate by dephosphorylating the GTP to GDP
The eukaryotic cell cycle is regulated by a molecular control system

- The frequency of cell division varies with the type of cell
- These differences result from regulation at the molecular level
- Cancer cells manage to escape the usual controls on the cell cycle

The Cell Cycle Control System

- The sequential events of the cell cycle are directed by a distinct cell cycle control system, which is similar to a clock
- The cell cycle control system is regulated by both internal and external controls
- The clock has specific checkpoints where the cell cycle stops until a go-ahead signal is received

The Cell Cycle Clock: Cyclins and Cyclin-Dependent Kinases

- Two types of regulatory proteins are involved in cell cycle control: cyclins and cyclin-dependent kinases (Cdks)
- Cdks activity fluctuates during the cell cycle because it is controlled by cyclins, so named because their concentrations vary with the cell cycle
- MPF (maturation-promoting factor) is a cyclin-Cdk complex that triggers a cell’s passage past the G2 checkpoint into the M phase
Stop and Go Signs at the Checkpoints

- Some external signals are growth factors, proteins released by certain cells that stimulate other cells to divide.
- For example, platelet-derived growth factor (PDGF) stimulates the division of human fibroblast cells in culture.

Proto-Oncogenes

- **Proto-Oncogenes** – Part of the DNA that codes for “gas pedal” proteins.
- When proto-oncogenes mutate they become cancer-causing genes called oncogenes.
- Example of a proto-oncogene = ras gene
  - The ras protein is a G protein that relays a signal from a growth factor receptor to a cascade of protein kinases, the end result of the cascade is the synthesis of a protein that stimulates the cell cycle.

Ligand-gated ion channel

- A ligand-gated ion channel receptor acts as a gate when the receptor changes shape.
- When a signal molecule binds as a ligand to the receptor, the gate allows specific ions, such as Na⁺ or Ca²⁺, through a channel in the receptor.
- Important in neuron signal transmission.
The Nerve Impulse Is an Electrochemical Signal

- A nerve impulse, or action potential, involves sodium ions (Na⁺) and potassium ions (K⁺) that cross the cell membrane through the ion channels

- Each ion channel is designed to allow only certain ions to pass through

Membrane Potential

- The difference in charge between the inside and outside of the neuron is the membrane potential

Resting Membrane Potential

- A neuron that is not conducting a message is said to be "Resting"

- When a neuron is resting there is more sodium (Na⁺) outside the neuron cell and more potassium (K⁺) inside the cell

- The inside of the cell has a negative charge compared to the outside the cell

The Nerve Impulse

Resting Neuron
Plasma membrane is charged, with the inside negative relative to the outside.
Sodium Potassium Pump

- To maintain this resting membrane potential the neuron pumps Na\(^+\) out of the cell and K\(^+\) into the cell.
- The transport proteins take 3 Na\(^+\) ions out for every 2 K\(^+\) ions into the cell = Na\(^+\)/K\(^+\) pump
- This is Active Transport – requiring ATP

Action Potential

- An electrochemical signal conducted along an axon. It is a wave of depolarization followed by repolarization
- Depolarization is caused by sodium ions entering the axon
- Repolarization is caused by potassium ions leaving axon

Steps of an Action Potential

1. The axon is depolarized when voltage gated sodium ion channels open and Na\(^+\) comes rushing in, causing the inside of the neuron to become positively charged

Steps of an Action Potential

2. The axon is repolarized when voltage gated potassium ion channels open up and allow K\(^+\) to go out of the axon
   - This returns the membrane potential to be negative on the inside of the neuron
   - The action potential travels down the axon
Action Potential

- After the action potential, the sodium potassium pump restores the original conditions by pumping sodium (Na+) out of the cell and potassium (K+) back into the cell.

The Nerve Impulse

When a neuron is resting, sodium ions have a greater concentration:
1. inside the neuron cell
2. outside the neuron cell
3. concentration is the same

When a neuron is depolarizing, which ions come into the neuron?
1. Calcium (Ca++)
2. Sodium (Na+)
3. Potassium (K+)
4. Chlorine (Cl-)

When a neuron is depolarizing, the inside of the neuron cell becomes
1. Positively charged
2. Negatively charged
### Nerve Synapse

- How are messages passed from one nerve to the next or from the nerve to a muscle?

- The junction between two neurons or between a neuron and a muscle is called a **synapse**

### Components of the Synapse

1. Presynaptic neuron is the transmitting neuron
2. Postsynaptic neuron is the receiving neuron or the muscle
3. And the gap in between them = **synaptic cleft**

### Presynaptic neuron

- Presynaptic neuron has synaptic vesicles that contain neurotransmitters

### Synaptic Transmission

**Step 1:** The impulse reaches the axon ending of the presynaptic membrane.

**Step 2:** Synaptic vesicles release neurotransmitter into the synaptic cleft.

**Step 3:** Neurotransmitter diffuses across synaptic cleft.

**Step 4:** Neurotransmitter molecules bind to receptors on the postsynaptic neuron.

**Step 5:** Sodium ion channels open.

**Step 6:** Sodium ions enter the postsynaptic neuron, causing depolarization and possible action potential.
Transmission across synaptic cleft

1. The action potential gets to the end of the presynaptic axon
2. The action potential triggers Ca\(^{2+}\) to enter the presynaptic axon terminal
3. The Ca\(^{2+}\) triggers synaptic vesicles located at the axon terminal to merge with the neural membrane

4. The synaptic vesicles release the neurotransmitters into the synaptic cleft
5. These neurotransmitters travel across the synaptic cleft to the postsynaptic neuron (or the muscle)
6. Neurotransmitter binds to receptors on the postsynaptic neuron (or muscle)

7. These receptors are ligand gated sodium ion channels which allow Na\(^+\) to enter the postsynaptic neuron (or muscle) and triggers an action potential in the postsynaptic neuron (or muscle contraction)

8. Once the neurotransmitters are released they need to be destroyed or contained quickly or they will continue to stimulate the nerve

**Intracellular Receptors**

- Intracellular receptor proteins are found in the cytosol or nucleus of target cells
- Small or hydrophobic chemical messengers can readily cross the membrane and activate receptors
- Examples of hydrophobic messengers are the steroid and thyroid hormones of animals
- An activated hormone-receptor complex can act as a transcription factor, turning on specific genes
**Intracellular Receptors**

- Lipid soluble and small signaling molecules bind to receptors located inside the cell.
- Intracellular receptors are located in the cytosol or the nucleus.
- The intracellular receptors in the cytosol will bind with the ligand, the binding will cause the receptor/ligand complex to travel to the nucleus.

**Examples of Intracellular Receptors**

- Steroid hormones
  - Estrogen, testosterone, cortisol
  - Effects gene expression
- Nitric Oxide (gas)
  - Binds to receptor that is an enzyme = guanylyl cyclase (Viagra works through this pathway)

**Steroid Hormone Intracellular Receptors**

- These hormones cross the plasma membrane and bind to an intracellular receptor in the cytosol.
- The binding of the ligand to the receptor causes the ligand/receptor to go to the nucleus.
- The ligand/receptor regulates gene expression.

**Intracellular Receptors**

- Prior to the ligand binding, the receptor may have proteins that block the DNA binding domain.
- The binding of the ligand signals the receptor to go to the nucleus and allow the receptor to bind to the DNA.
Controlling Cell Signaling

- To control cell signaling:
  1. The G-proteins have GTPase activity
  2. Ligand levels fall
  3. Ca²⁺ is sequestered
  4. There are phosphatases that inactivate the proteins that were activated by phosphorylation
  5. Phosphodiesterases
  6. Receptors are internalized
Important Concepts

- Know the vocabulary covered in this lecture
- Know examples of signaling molecules
- Know the forms of cell signaling
- Know the main classifications of signaling receptors, what are the main differences and what type of ligands generally bind these receptors, examples of secondary messengers
- Understand how intracellular receptors work, what are the steps from the binding of a ligand to the cellular activity and what type of activity is regulated by intracellular receptors.

Important Concepts

- Know the classes of Cell Surface Receptor Proteins
- What is the function of protein kinases and phosphotases?
- Know the properties of RTKs. Be able to describe the steps of how the receptors regulate cellular activity
- What is the benefit of having kinase cascades?
- Understand signal transmission in neurons, the steps of the action potential and transmission between neurons (or neuron to muscle).

Important Concepts

- Understand the state of the neuron during an action potential and resting state.
- Know the examples of ligands for the receptors
- Be able to describe how cellular activity is regulated by the cAMP pathway, starting with the binding of a ligand to the GCPR, including how the g-protein is inactivated
- Know examples of a cellular response mediated by cAMP

Important Concepts

- Understand how the cell cycle is controlled.
- How are cell signaling pathways controlled?
- What are differences and similarities between G-protein coupled receptors, enzyme linked receptors (like RTK) and intracellular receptors