Chapt. 11 Cell signaling by chemical messengers

Cell signaling by chemical messengers

Student Learning Outcomes:
• Describe major chemical messengers used by cells
• Explain the function of intracellular receptors
• Explain function of major cell surface receptors:
  - G protein coupled
  - Receptor tyrosine kinases
• Describe major signal transduction pathways by small molecules
• Describe importance of termination of signal

Chemical messengers include: hormones, neurotransmitters, cytokines, retinoids, growth factors

• Some bind intracellular receptors
  - Nuclear hormone
• Some bind surface receptors
  - Ion channels,
  - Tyrosine kinase
  - G-protein-coupled

Second messengers transmit

Example of Nicotinic Acetylcholine Receptor

Nicotinic ACh receptor:
• High specificity
• Nerve signal
• Ach vesicles released
• Bind ACh receptors on muscle
• Opens ion channel
• Triggers muscle contraction

Fig. 11.2; Acetylcholine receptors at neuromuscular junction

Nicotinic Acetylcholine Receptor

Nicotinic ACh receptor:
• 5 subunits, 2 bind ACh
• Opens ion channel:
  - K+ out, Na+ in
  - Muscle contracton
• Acetylcholinesterase in synaptic cleft stops signal

Fig. 11.3
**Models of Cell-Cell signaling:**

**Endocrine:** Distant targets
- Estrogen hormone

**Paracrine:** Local targets
- Neurotransmitter

**Autocrine:** self
- T cells, cancer cells

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**Some Chemical Messengers**

- **Nervous system:**
  - Small molecule neurotransmitters
  - Neuropeptides (4-35 aa)

- **Endocrine system:**
  - Polypeptide hormones (insulin)
  - Steroid hormones
  - Thyroid hormone
  - Retinoids

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**Properties of messenger determine receptor type:**

- **Hyrophilic hormones**
  - bind surface receptors

- **Lipophilic hormones**
  - cross membrane, bind intracellular receptors:
    - Often regulate transcription

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**Some Chemical Messengers**

- Immune system:
  - Cytokines are small proteins
  - Eicosanoids – prostaglandins: respond to injuries, PGI₂ vasodilation
  - Growth factors – polypeptides:
    - PDGF (Platelet-derived)
    - EGF (epidermal)
Nuclear hormone superfamily

Steroid hormone-thyroid hormone superfamily:
- Nuclear hormone receptors
- RAR, TR, VDR, AR
- Heterodimers with RXR bind DNA, activate transcription

III. Plasma membrane receptors

Plasma membrane receptors function by signal transduction inside cell:
- Receptors have membrane-spanning a-helices
- Extracellular domain binds messenger
- Intracellular domain initiates signal cascade, amplifies signal
- Rapid response: ion channels, enzyme activity
- Slower effects on gene expression

- 1. Ion channel receptors (ACh Figs. 2, 3)
- 2. Kinase receptors or bind kinases (RTK)
- 3. Heptahelical – G-protein coupled (epinephrine)

Pathways of Intracellular Signal Transduction

Intracellular signal transduction:
- Chain of reactions that transmits signals from cell surface, amplifies, to intracellular targets.
- Different major mechanisms:
  - cAMP and protein phosphorylation (PKA)
  - cGMP
  - Phospholipids and Ca**
    - DAG and PKC, IP3 and Ca**, PIP3/AKT
  - Ras, Raf, MAP kinase
  - JAK/STAT; TGFβ/Smad

Receptor Tyrosine Kinases and related

2. Kinases or bind Kinase receptors: signal by first phosphorylating proteins, binding other proteins
- *Tyrosine kinase receptors
- JAK-STAT receptors bind Januses Kinases
- Ser-thr kinase receptors
3. Heptahelical Receptors signal by G proteins

3. Heptahelical receptors signal through heterotrimeric G proteins, second messengers

- Binding hormone initiates series of events
- GDP-GTP $\alpha$ subunit
- Second messengers are small molecules:
  - cAMP
  - DAG = diacylglycerol
  - IP3 = phosphatidylinositol

RTK – growth factor receptor and Ras

Tyrosine Kinase Receptor signals through Ras:
- Growth factor binds; self-phosphorylation of RTK
- Adaptor proteins bind to P-tyr through SH2 domain
- Convey signal to membrane-bound Ras
- GTP activates Ras (small GTP-binding protein),
- Activated Ras binds Raf, signals via MAP kinase pathway

Regulation of Ras proteins

Ras-GTP activity is terminated by GTP hydrolysis, stimulated by interaction of Ras-GTP with GTPase-activating proteins.

Ras is mutated in cancers:
Mutated Ras proteins are continuously in active GTP-bound form, driving proliferation of cancer cells in absence of growth factor

Phosphatidyl inositol signaling molecules

Phosphatidyl inositol phosphates (PIP) function in signal transduction:
- either RTK or heptahelical paths
- PI is glycolipid
- PI -> PI-4,5-bisP
- PLC (phospholipase) -> DAG + IP3
  - DAG in membrane activates PKC,
  - IP3 cytoplasm
  - PLC$\gamma$ from RTK path
  - PLC$\beta$ from G-protein coupled path

Fig. 11.10
Fig. 11.11
Fig. 11.12
PLC forms DAG + IP₃

**Two forms of phospholipase C:**
PLC-β stimulated by G proteins (G-coupled receptors). PLC-γ has SH2 domains, associates with (RTK).

Tyr phosphorylation increases PLC-γ activity, stimulating hydrolysis of PIP₂ to DAG, IP₃

DAG remains in membrane, activates protein-ser/thr kinases of PKC family (protein kinase C):
- Diverse substrates for PKC:
  - Transcription factors
  - Actin binding proteins
  - Phorbol esters activate PKC

**IP₃ mobilizes Ca²⁺**

IP₃ is a small polar molecule released to cytosol; signals release of Ca²⁺ from ER

IP₃ binds receptors that are ligand-gated Ca²⁺ channels.
Cytosol concentration of Ca²⁺ maintained at extremely low level by Ca²⁺ pumps.

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RTK Insulin receptor has divergent signaling paths

* Insulin receptor signals through several paths:
  - Binding of hormone causes autophosphorylation
  - Binds IRS (insulin receptor substrates), PO₄ those:
    - Grb2 can signal through Ras and MAPK path
    - Other proteins bind, interact with PIPs in membrane

Fig. 11.13 Insulin signaling:
PLC - phospholipase
PIP – phosphatidyl
inositol forms

JAK-STAT receptors

**JAK-STAT receptors: tyrosine kinase-associated**
- Often for cytokine signaling – more direct to nucleus
- JAK = Janus kinase (just another kinase);
- STAT = signal transducer, activator of transcription

Fig. 11.15
Receptor ser-thr kinases for proteins of TGF superfamily

- TGF-β cytokine/hormone for tissue repair
- Two different membrane-spanning subunits
- Smad proteins are receptor-specific, except Co-smad (Smad4)
- Smad complex activates or inhibits transcription

Fig. 11.16

Table 1 Subunits for Heterotrimeric G proteins

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>Gα(s)</td>
<td>Stimulates Adenylyl cyclase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(ex. glucagon, epinephrine regulate metabolic enzymes) cholera toxin modifies, keeps it active</td>
</tr>
<tr>
<td>αi/o</td>
<td>Gα(i/o)</td>
<td>Inhibits Adenylyl cyclase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(epinephrine, neurotransmitters pertussis toxin modifies and inactivates)</td>
</tr>
<tr>
<td>αq/11</td>
<td>Gα(q/11)</td>
<td>Activates PLCβ</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(epinephrine, acetylcholine, histamine)</td>
</tr>
</tbody>
</table>

Adenylyl cyclase forms cAMP, second messenger;
cAMP phosphodiesterase cleaves to stop signal

Fig. 11.18
cAMP Regulates protein kinase A

Glucagon & epinephrine signal through G-coupled receptors to increase cAMP

Effects mediated by cAMP-dependent protein kinase, or protein kinase A (PKA)
- Inactive form has 2 regulatory, 2 catalytic subunits.
- cAMP binds to regulatory subunits, which dissociate.
- Free catalytic subunits phosphorylate serine on target proteins

PKA stimulates glycogen breakdown:

Ex. PKA stimulates breakdown of glycogen:

PKA phosphorylates 2 enzymes:
- Phosphorylase kinase activated, -> activates glycogen phosphorylase.
- Glycogen synthase is inactivated

Glycogen breakdown stimulated
Glycogen synthesis blocked.

Cyclic AMP induces gene expression

Increased cAMP can activate transcription of genes
- That have regulatory sequence — cAMP response element, or CRE
- Free catalytic subunit of PKA goes to nucleus, phosphorylates transcription factor CREB (CRE-binding protein).

Pathways of Intracellular Signal Transduction

cAMP can also directly regulate ion channels:
- second messenger in sensing smells — odorant receptors are G protein-coupled, stimulate adenylyl cyclase, leading to an increase in cAMP.
- cAMP opens Na\(^+\) channels in plasma membrane, leading to initiation of a nerve impulse.
**Phosphatidyl inositol signaling molecules**

**Phosphatidyl inositol phosphates (PIP) function in signal transduction:**
- either RTK or heptahelical paths
- PI → PI-4,5-bisP
- PLC (phospholipase) → DAG + IP$_3$
- DAG in membrane activates PKC;
- IP$_3$ in cytoplasm
- PLC$\beta$ from G-protein coupled path

**IP$_3$ mobilizes Ca$^{2+}$**

IP$_3$ is small polar molecule released to cytosol; signals release of Ca$^{2+}$ from ER

IP$_3$ binds receptors that are ligand-gated Ca$^{2+}$ channels.

Cytosol concentration of Ca$^{2+}$ maintained at extremely low level by Ca$^{2+}$ pumps.

**Function of Ca$^{2+}$ and calmodulin**

Increased Ca$^{2+}$ affects activity of several proteins, including protein kinases and phosphatases:

- **Calmodulin** is activated when Ca$^{2+}$ concentration increases.
- Ca$^{2+}$/calmodulin binds to target proteins, e.g., some protein kinases
- **CaM kinase** family activated by Ca$^{2+}$/calmodulin:
  - phosphorylates metabolic enzymes, ion channels, transcription factors, regulate synthesis and release of neurotransmitters.

**Termination of signal:**

- Some turn off quickly, others slowly
- Many different steps
- Diseases from persistence of signal:
  - Cancer and Ras
### Key concepts

Cells communicate to integrate cellular functions.

Chemical messages bind receptors on cells (intracellular or plasma membrane bound)

Intracellular receptors primarily activate transcription

Plasma membrane receptors are two main types:
- Tyrosine kinase and kinase-associated
- G-protein-coupled receptors

Various mechanisms for second messenger, can converge from different hormones

### Review questions

Pseudohypoparathyroidism is heritable disorder caused by target-organ unresponsiveness to parathyroid hormone (a polypeptide hormone secreted by the parathyroid gland). One of the mutations that causes this disease occurs in the gene encoding $G_\alpha$ in certain cells.

3. The receptor for parathyroid hormone is most likely which one of the following:
   - A. An intracellular transcription factor
   - B. A cytoplasmic guanylyl cyclase
   - C. A receptor that must be endocytosed in clathrin-coated pits to transmit its signal
   - D. A heptahelical receptor
   - E. A tyrosine kinase receptor

4. This mutation (from question 3) likely has which one of the following characteristics?
   - A. It is a gain-of-function mutation
   - B. It decreases the GTPase activity of the Gas subunit
   - C. It decreases synthesis of cAMP in response to parathyroid hormone.
   - D. It decreases generation of IP$_3$ in response to parathyroid hormone.
   - E. It decreases synthesis of phosphatidylinositol 3,4,5-triphosphate in response to parathyroid hormone.

### Clinical comments

Mya Sthenia has myasthenia gravis,
- autoimmune – Antibodies directed against nicotinic ACh receptor in skeletal muscle.
- Fatigue, inability to do repeated tasks; numbers of ACh receptors greatly reduced
- Inhibitor of acetylcholinesterase briefly increases muscle strength

Ann O-Rexia - anorexia nervosa.
- Endocrine hormones mobilize fuels from adipose tissue
- Epinephrine (adrenaline) (GPCR) promotes fuel mobilization
- Different receptors on different cells (ex. Glucagon receptors on liver, not on muscle; liver does gluconeogenesis)
- Insulin (special RTK) promotes fuel storage