Cell Signaling 1- Cell Biology

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Objectives

- Understand main types of signaling mechanisms
- Understand concept of receptor and ligand
- Understand membrane, cytoplasmic and nuclear receptors
- Brief understanding of steroid hormone pathway
- Brief understanding protein growth factor pathway
- Understand differences between select membrane receptors (G protein structure, tyrosine kinases)
Cell signaling basics

1. Receptor-ligand binding
2. Signal transduction (via second messengers)
3. Cellular responses
4. Changes in gene expression

Signal molecule

Ligand (primary messenger)

Specific receptor

Target cell

Initiates a response in the target cell
Many different kinds of molecules transmit information between the cells of multicellular organisms.

These molecules act as ligands that bind to receptors expressed by their target cells.

There is considerable variation in the structure and function of the different types of molecules that serve as signal transmitters.

Some molecules carry signals over long distances, whereas others act locally to convey information between neighbouring cells.

Some signaling molecules are able to cross the plasma membrane and bind to intracellular receptors in the cytoplasm or nucleus, whereas others bind to receptors expressed on the target cell surface.
Cell Communication

- Cells in higher animals communicate by means of hundreds of kinds of signal molecules.
- These include proteins, small peptides, amino acids, nucleotides, steroids, retinoids, fatty acid derivatives, and even dissolved gases such as nitric oxide and carbon monoxide.

Contact Mediated Communication
- display molecules on cell surface, recognized by receptor on another cell

Non-Contact Mediated Communication
- chemical signal, nearby or at a distance
Modes of Signaling

Direct Cell-Cell Signaling
Direct cell contact

Example - integrins and cadherins are cell adhesion molecules (CAMs). CAMs are located on the cell surface and are involved in binding with other cells or with the extracellular matrix in the process called cell adhesion.

But integrins and cadherins function not only as CAMs but also as signaling molecules that regulate cell proliferation and survival in response to cell-cell and cell-matrix contacts.
Modes of Signaling

**Signaling via the action of secreted signaling molecules**

**Endocrine signaling**
Hormones (at a distance) are carried through the circulatory system to act on distant target cells.
Example: Estrogen produced by ovary, transported to the brain where it acts to regulate female reproductive system.

**Paracrine signaling**
A molecule released from one cell acts locally to affect nearby target cells.
Example: The action of neurotransmitters in carrying signals between nerve cells at a synapse.

**Autocrine signaling**
Cell produces a signaling molecule to which it also responds.
Example: The response of cells of the vertebrate immune system to foreign antigens. T lymphocytes can drive their own proliferation.
Chemical Signal Types

- Receptors can be located on the target cell surface or inside the target cell.
- If inside cell, signal molecule has to enter the cell to activate it.

**Water soluble signal molecules**

Water-soluble signaling molecules cannot diffuse across the plasma membrane so all bind to cell-surface receptors.

**Lipid soluble signal molecules**

Many lipid-soluble hormones diffuse across the plasma membrane and interact with receptors in the cytosol or nucleus.
**Extracellular Signal Steps**

**Signaling Molecule**
- Synthesis
- Release by signaling cell
- Transport to target cell
- Detection by a specific receptor protein
- Change by receptor-signal complex (trigger)
  - The binding of a ligand to its receptor causes a conformational change in the receptor that initiates a sequence of reactions leading to a specific cellular response.
- There can also be a feedback mechanism following signaling.
- Different cells may have different sets of receptors for the same ligand, each of which induces a different response.
- Or the same receptor may occur on various cells, and binding of the same ligand may trigger a different response in each type of cell.
Cell Surface Receptors

Four main classes
• **G protein** - coupled receptors - Ligand binding activates a G protein which activates or inhibits an enzyme that generates a response.

• **Ion-channel receptors** - Ligand binding changes the conformation of the receptor so that specific ions flow through it; this alters the electric potential across the cell membrane.

• **Tyrosine kinases** - linked receptors - These receptors lack intrinsic catalytic activity, but ligand binding stimulates formation of a dimeric receptor, which then interacts with and activates one or more cytosolic protein-tyrosine kinases.

• **Receptors with intrinsic catalytic activity** - Several types of receptors have intrinsic catalytic activity, which is activated by binding of ligand.
Cell Surface Receptors

Ligand binding to cell-surface receptor
  • trigger intracellular pathways
  • modulate cellular metabolism, function, or development

Removal of the signal
  • Often terminates cellular response
Second Messengers

The binding of ligands to many cell-surface receptors leads to a short-lived increase (or decrease) in the concentration of the intracellular signaling molecules termed second messengers.

- Cyclic nucleotides - cAMP, cGMP (see G protein signalling lecture)
- Calcium Ions
- Inositol 1,4,5-trisphosphate (IP3) - IP3 receptor plays in the conversion of numerous external stimuli to intracellular Ca2+ signal
- Diacylglycerol (DAG) - modified lipid activates PKC
- Protein Kinase A - PKA, B, C (phosphorylate other proteins)

- Act to regulate the activity of cellular proteins
- The elevated intracellular concentration of one or more second messengers following ligand binding triggers a rapid alteration in the activity of one or more enzymes or non-enzymatic proteins.
The two cell-two-gonadotrophin system for estradiol synthesis in the ovarian follicle.

LH and FSH are shown to stimulate adenylate cyclase via G-protein-couples receptors.

The cyclic AMP (cAMP) generated from ATP activates protein kinase A to stimulate expression of the respective steroidogenic enzymes in theca and granulosa cells.

GDP, guanosine diphosphate, GTP, guanosine triphosphate
Intracellular Signaling/Steroid Responses

- Intracellular Receptors respond to signaling molecules that are able to diffuse across the plasma membrane.
- Steroid Hormones (androgens, glucocorticoids, mineralocorticoids)
  - thyroxine (thyroid, not a steroid)
  - vitamin D3
  - retinoic acid
  - cortisol (adrenal)
  - estrogens and testosterone (gonad)
  - (longest acting of hormone classes, hours-days)
- Steroid hormones are less soluble in aqueous solution, most are transported in the blood by carrier proteins such as specific plasma globulins or albumin (synthesised in the liver)

Example- DHT, T bind to sex hormone-binding globulin (SHBG)
Main function: transport steroid within blood stream to target tissues
Steroid Synthesis

- All steroid hormones are synthesized from cholesterol.
Steroid actions

Female HPG Axis

- Hypothalamus
  - GnRH
  - Anterior pituitary
    - LH
    - FSH
    - Ovaries
    - Estrogen
    - Progesterone

- Theca cells
- Granulosa cells

- Cholesterol
- ANDROSTENEDIONE
- Aromatase
- Estrone
- ANDROSTENEDIONE
- 17β-hydroxysteroid dehydrogenase
- Estradiol

FSH
LH

Anterior Pituitary Hormones

FSH
LH

Ovarian Hormones

- Estradiol
- Progesterone

Ovary
- Follicle
- Corpus Luteum
- Corpus Albicans

Recruitment
- Selection
- Dominance
- Recruitment

Uterine Endometrium

Recruitment

Steroid Hormone Receptors (SHRs)

When steroid hormones bind to receptors, they activate the receptors, which bind to DNA to regulate the transcription of specific genes.

Cytosol location
- receptor bound to inhibitor
- ligand binding activates receptor
- translocates to nucleus on ligand binding

Nuclear location
- binds ligand and DNA
- becomes transcription factor

Diagram:
- Steroid hormone binds to receptor in cytoplasm.
- Receptor binds to inhibitor complex, making the receptor inactive.
- Ligand binds and alters the conformation of the receptor protein.
- Inhibitory complex dissociates.
- Receptor binds to coactivator proteins that induce gene transcription.

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Steroid Receptor Structure

Nuclear receptors can directly bind to DNA and regulate the expression of adjacent genes, hence they are classified as transcription factors.

Most members have a common domain structure consisting of:

- amino-terminal activation domain (AF-1)
- DNA-binding domain- controls which gene will be activated
- hinge region - controls movement of receptor to the nucleus
- a carboxy-terminal ligand-binding domain (LBD).

LBD specifies the ligand-binding properties and ligand-regulated interactions with co-activators and co-repressors (region known as AF-2).
Nuclear receptor (NR), in the absence of ligand, is located in the cytosol. Hormone binding to the NR triggers dissociation of heat shock proteins (HSP), dimerization, and translocation to the nucleus, where the NR binds to a specific sequence of DNA known as a hormone response element (HRE). The nuclear receptor DNA complex in turn recruits other proteins that are responsible for transcription of downstream DNA into mRNA, which is eventually translated into protein, which results in a change in cell function.
Receptor Types

48 human nuclear receptor types
Steroid hormone receptor family

**Type I Receptors** - located in the cytosol
- Sex hormone receptors (sex hormones) - Androgen receptor, Estrogen receptor, Progesterone receptor
- Glucocorticoid receptor (glucocorticoids)
- Mineralocorticoid receptor (mineralocorticoids)

**Type II Receptors** - located in the nucleus
- Vitamin A receptor
- Vitamin D receptor
- Retinoid receptor
- Thyroid hormone receptor

**Orphan receptors** - receptor that has a similar structure to other identified receptors but whose ligand has not yet been identified.
Several types of lipids serve as signaling molecules that, in contrast to the steroid hormones, act by binding to cell surface receptors.

The most important of these molecules are members of a class of lipids called the eicosanoids, which includes prostaglandins, prostacyclins, thromboxanes and leukotrienes.

- lipids acting as signaling molecules
  - made from essential fatty acids (EFAs) oxidation (arachidonic acid)

act by binding to cell surface receptors

- rapidly broken down

- act locally in autocrine or parocrine signaling pathways

Stimulate variety of responses in target cell including blood platelet aggregation and inflammation

(anti-inflammatory drugs act by downregulating eicosanoid synthesis)
Nitric Oxide

- The gas nitric oxide (NO) is a simple gas, very quick short-lived signal
- Synthesised from arginine by the enzyme nitric oxide synthase
- NO diffuses out of the cell and can act locally to affect nearby cells.
- Major paracrine signaling molecule in the nervous, immune, and circulatory systems.
- Like steroid hormones NO is able to diffuse directly across the plasma membrane of its target cells.
- Rather than binding to a receptor that regulates transcription, NO alters the activity of intracellular target enzymes

Example of NO action is signaling the dilation of blood vessels. (The medical use of nitroglycerin in treatment of heart disease is based on its conversion to NO, which dilates coronary blood vessels and increases blood flow to the heart)
Summary of action of proteins and steroids

Protein hormone (P) binds to cell surface receptor:
- Activates effector enzymes
- Generates second messengers
- Changes enzyme activity
- Activates transcription factors
  - Regulates gene transcription
  - mRNA synthesis
  - Protein synthesis

Steroid hormone (S) diffuses across cell membrane:
- Diffuses across cell membrane
- Enters cytoplasmic or nuclear receptor
- Hormone-activated receptor
- Receptor binds to DNA elements in target genes as a dimer
- Regulates gene transcription
  - mRNA synthesis
  - Protein synthesis

Biological output

Cytoplasm
- Cytoplasm
- Nucleus
- Receptor binds to DNA elements in target genes as a dimer
- Regulation of gene transcription
  - mRNA synthesis
  - Protein synthesis

Plasma membrane
- Plasma membrane
- Cytoplasmic or nuclear receptor
- Hormone-activated receptor
- Receptor binds to DNA elements in target genes as a dimer
- Regulation of gene transcription
  - mRNA synthesis
  - Protein synthesis