Principles of cell signaling
Lecture 1

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Molecular Cell Biology (1BG320), 2014
Cells communicate by generating, transmitting and receiving chemical signals.

- Growth factors
- Hormones
- Cytokines

Change in activity of existing proteins
Change in gene expression

Behavioral change

Signal transduction pathways

Receptors
Why is it worthwhile study cell signaling?

- Most pharmaceuticals (and many environmentally hazardous substances) act on signaling pathways.

- Understanding cell communication can (and have) in many cases explain the basis for diseases and thus allowed the development of new therapeutic strategies.

- Many of the breakthrough discoveries in medical sciences has come from studies of signaling molecules.
In a multicellular organism cells need to talk to each other to coordinate their activities.
Signaling may be “bad” for a given cell but “good” for the organism.
Steps involved in cell–cell communication

1. **Synthesis of signal molecule**
   - signaling proteins encoded by genes
   - organic molecules synthesized by enzymes

2. **Storage in membrane vesicles**
   - exception: lipid molecules and some continously released molecules which are stored in the extracellular matrix

3. **Exocytosis**
   - controlled & stimulated
   - \( \text{Ca}^{2+} \) – dependent

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**Insulin-like growth factor 1**

**Testosterone**

**Acetylcholine containing vesicles at a synapse**

**Insulin exocytosis from \( \beta \)-cells in pancreas**
Steps involved in cell-cell communication

4. **Processing of signaling molecule**
   - Some protein signaling molecules are produced as inactive precursor that needs to be activated.

5. **Transport from signaling cell to target cell**
   - Diffusion in bloodstream or tissue
   - Carrier proteins
Steps involved in cell – cell communication

6. Binding to receptor protein
- receptors can be found on cell surface or within the cell
- receptors can be enzymes, transcription factors
Steps involved in cell–cell communication

7. **Signal transmission from activated receptor to effectors molecules**
   - conversion of the activated state of the receptor into a chemical form that the cell can interpret

8. **Interpretation of the signal and change in cell behavior**
   - altered function of already existing proteins
   - change in gene expression

9. **Signal termination**
   - destruction of signaling molecule
   - destruction of active receptors
   - inactivation of signal transduction pathways
1. **Metabolism**

   insulin is a key signal molecule in regulation of glucose metabolism

   - **Insulin**
   - **Insulin receptor** (RTK)
   - **Glucagon**
   - **Glucagon receptor** (GPCR)
What type of processes are regulated by cell-cell signaling

2. Cell growth and division
What type of processes are regulated by cell–cell signaling

3. **Cell movement**
What type of processes are regulated by cell–cell signaling

4. Differentiation

- cell specialization
- change in gene expression pattern often irreversible
What type of processes are regulated by cell–cell signaling

5. Development

The process by which the fertilized egg turns into an organism
What type of processes are regulated by cell–cell signaling

6. Processing of sensory information

- Vision
- Smell
- Touch

![Chemical structure of 11-cis-retinal and all-trans-retinal](image)

11-cis-retinal (vitamin A)

all-trans-retinal (vitamin A)
Cell behavior is controlled signal molecules in a combinatorial manner.

- **Survive** state: Signal molecules A, B, C lead to survival.
- **Grow and Divide** state: Signal molecules A, B, C lead to growth and division with additional input from D and E.
- **Differentiate** state: Signal molecules A, B, C lead to differentiation with additional input from D, E, F, and G.
- **Die** state: Signal molecules B and C lead to apoptosis.

The default state of the cell is to die!
How can signaling molecules give different responses in different cell types?

**Intestinal blood vessels**
- **α-receptor**
- **Constriction**

**Skeletal muscle blood vessels**
- **β-receptor**
- **Dialation**

**Liver cell**
- Different intracellular proteins

**Different receptors for adrenaline**

**Different intracellular proteins**
Cell – cell communication can occur over small or large distances

**Endocrine signaling**
- Blood stream distributes hormone throughout the body
- Place of action determined by receptor expression of target cells

**Paracrine signaling**
- Signal molecule diffuse to close neighbours (mm–mm range)

**Autocrine signaling**
- Signal molecule acts back on the secreting cell
- Self-reinforcement of proliferation or differentiation
Cell – cell communication can occur over small or large distances

**Juxtacrine signaling**
- Signal molecule located on surface of signaling cells, or on extracellular matrix
- No diffusion of signal molecule

**Gap junctions**
- Open channels through the plasma membrane
- Signaling molecule passes directly between cytoplasms of two adjacent cells

**Synaptic signaling**
- Extremely precise delivery of signal molecule
- Short diffusion distance (20 nm), resulting in very high speed of signaling
Cellular response to signaling can be fast or slow.

Signal molecule (=ligand) bind to its receptor through multiple weak interactions:
- Electrostatic interactions
- Van der Waals interaction
- Hydrophobic interactions
Post-translational modifications

Covalent additions to existing proteins that regulate their activity state, cellular location and dynamic interactions with other proteins

- Are > 200 known types
- Present on at least 80% of eukaryotic proteins
- Found in all species
- Located at specific sequence motifs
- Often transient
Post-translational modifications

MSTVKHGNSTTLNITNNSSINITNATNASVL
Examples of post-translational modifications

**Phosphorylation**
Target amino acids: S, T & Y

Regulate protein activity and/or localization

**Ubiquitination**
Target amino acid: K

Many different functions including protein activation, localization, endocytosis, sorting......
Signal transduction is the biochemical mechanism of transmitting signals inside the cell.
Signal transduction proteins: enzymes

- **Kinases** (add phosphate groups)
- **Phosphatases** (remove phosphate groups)

\[
\text{ATP} \rightarrow \text{ADP} + P_i + H_2O
\]

- **Phospholipases** (hydrolyze membrane phospholipids)
Signal transduction proteins: enzymes

- **Ubiquitin-ligases** (add ubiquitin moieties to target proteins)

- **Acetyltransferases** (add acetyl-groups to K, S, T, Y)
Signal transduction proteins: enzymes

- **Proteases** (hydrolyze peptide bonds)

- **GTPases** (hydrolyze GTP to GDP)
Signal transduction proteins often contain several interaction domains

- **Modified peptide**
  - SH2
  - PTB
  - FHA
  - WW
  - WD40
  - MH2
  - Chr
  - Bromo
  - UIM
  - UBA

- **Peptide**
  - NPY/RRXK
  - PXXP
  - PXY
  - FPPPP
  - Pro
  - D/E-XXLL
  - Val-COOH

- **Nucleic Acid**
  - RNA
  - DNA
  - PUM
  - Tubby

- **Domain-domain**
  - PDZ
  - SAM
  - DD
  - DED
  - CARD
  - PyD
  - PB1
  - BRCT

- **Phospholipids**
  - PI-3,4,5-P3
  - PI-3-P
  - DAG
  - PI-4,5-P2
  - PA/PS
  - PI-4,5-P2
  - PI-3-P
  - PI-3,4,5-P3
  - PH
  - FYVE
  - C1
  - FERM
  - C2
  - Tubby
  - PX
  - ENTH
Signal transduction proteins: enzymes

Examples of domain structure of a few signal transduction enzymes
Adaptor proteins

- Lack intrinsic enzymatic activity
- Contain two or more interaction domains
- Mediate specific protein–protein interactions and thereby drive formation of protein complexes

Adaptor-mediated complex formation

Examples of adaptor protein domain structure

- SH3
- SH2
- SH3
- SH3
- SH2
- SH2
- SH3
- SH2
- PTB
- PH

- Nck1, Nck2
- Crk, Crkl
- Grb2, Grap
- Shp-76
- Shc
- Dapp1
Signaling pathways often have a cascade architecture which results in strong signal amplification.
Eukaryotic regulation of gene expression

- RNA-polymerase II (RNA-pol II) release it from the pre-initiation complex and allows it to move down the gene producing mRNA.
- Sequence specific transcription factors (TFs) bind to promoter and enhancer elements and stabilize the RNA-pol II complex on the promoter (trans-activation) through the mediator complex.
- Chromatin-modifying enzymes acetylates histones which opens up the chromatin.
- The stability and frequency with which pre-initiation complexes are formed determines the rate of initiation of transcription.
- General TF bind to promoter and recruit RNA-pol II.

Signal transduction proteins: transcription factors

 Condensed chromatin

(Gene "OFF"

Condensed chromatin

Gene "ON"

Decondensed chromatin

Mediator

RNA polymerase

Repressors

Activators

(HDACs)

General TF bind to promoter and recruit RNA-pol II

Chromatin-modifying enzymes acetylates histones which opens up the chromatin

Sequence specific TFs bind to promoter and enhancer elements and stabilize the RNA-pol II complex on the promoter (trans-activation) through the mediator complex.
Signal transduction proteins: transcription factors

How does transcription factors recognize DNA?

- $\alpha$-helix often inserts into the DNA major groove

- Usually 4–10 base-pairs are in used for recognition (“boxes”)

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**Consensus sequence**

T C C G G A T G C
Signal transduction proteins: transcription factors
Common DNA binding motifs

- Leucine zipper (e.g. AP-1)
- Helix-loop-helix (e.g. c-Myc)
- Homeodomain (Helix-turn-helix)
- Zinc finger
Transcription factors interact with DNA through hydrogen bonds and electrostatic interactions.

Glutamine (or asparagine) interacts with DNA through hydrogen bonds.

Arginine interacts with DNA through electrostatic interactions.

Thymine ≡ Adenine

Cytosine ≡ Guanine
Signal transduction pathways often affect the activity of transcription factors

How can the activity of a transcription factor be changed?
1. Changed concentration of the transcription factor in the nucleus
Signal transduction pathways can influence the concentration of transcription factors by:

1. Subcellular localization (e.g. Foxo)
2. Regulated proteolysis (e.g. p53)
3. Regulation of the transcription factor gene expression or translation
Regulation of gene expression by miRNA

1. **Drosha**
2. **Dicer**
3. **miRISC complex**
4. Target mRNA cleaved
5. Target mRNA blocked for translation
2. Post-translational modifications affecting transcriptional activity

Examples of modification:
1. Phosphorylation
2. Methylation
3. Acetylation
4. Redox modification (NO)
5. Proteolysis

Modifications can regulate:
1. Oligomerization
2. DNA binding
3. Transactivation
4. Nuclear localization
5. Protein stability
3. Changed concentration of co-operating proteins
Most signaling molecules affect expression of many genes

**PDGF**

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Regulation of gene expression

**Short term regulation:**
a way to respond and adapt to the environment (e.g. change metabolism)

**Long term regulation:**
Cell differentiation
A cell senses signals from its surroundings via receptor proteins.
Nuclear receptors are ligand-activated transcription factors

Nuclear receptors are intracellular and depending on the type can be found in cytoplasm or nucleus
All nuclear receptors share a common domain architecture.
Nuclear receptors are ligand-activated transcription factors
Nuclear receptors functions as dimers

The DNA binding domain of nuclear receptor bind to consensus sequences (dimeric) in promoter regions
There are 48 nuclear receptors in humans and out of those 50% are orphan.

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<th>Ligands:</th>
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<th>Adopted Orphan Receptors</th>
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Ligands for nuclear receptor are small lipophilic molecules

- **Steroid hormones** (estrogens, testosterone, cortisol)

- **Lipid vitamins**

- **Dietary lipids**
Nuclear receptor signal transduction

Increased gene expression

Repressor

Absence of hormone

Corepressor

HDAC

Thyroid hormone receptor

Transcription repressed

Activator

Hormone added

Transcription activated

Increased gene expression
Glucocorticoid receptor

- hormone

+ hormone
Nuclear receptors can act as sensors for xenobiotics and stimulate expression of detoxification enzymes.

Xenobiotics: pharmaceutical drugs, environmental pollutants, herbal medicines, dietary supplements.
Nuclear receptor can have cytoplasmic functions

GRP30 is a G-protein coupled receptor
A cell senses signals from its surroundings via receptor proteins
Ligand-regulated ion channels

- Hydrophilic pore through the membrane whose conductivity is regulated by ligand-binding

- Abundant on electrically active cells (nervous system, muscle)

- Many medicines and neurotoxins affect ion channels
Ion channel receptors translate a chemical signal into an electric signal.

Acetylcholine receptor/channel

Chemical signal → Electrical signal
Cell – cell communication at the synapse

1. Action potentials cause voltage-gated Ca^{2+} channels to open.
2. Ca^{2+} diffuse into the cell and cause synaptic vesicles to release acetylcholine.
3. Acetylcholine diffuses across the synaptic cleft.
4. Acetylcholine binds their receptor sites and cause Na^{+} channels to open, causing depolarization.

If depolarization reaches threshold, an action potential is produced in the postsynaptic cell.
Action potential

- Ion gradients in resting axon maintained by ATP driven pumps
- Resting membrane is (more) permeable to $K^+$ (not to $Na^+$) making the membrane potential close to equilibrium value for $K^+$

![Diagram showing ion gradients in axon](image)
Action potential

At equilibrium forces balance and creates a voltage over the membrane (-70 mV)

At equilibrium forces balance and creates a voltage over the membrane (+50 mV)
Refractory phase: Ion channels cannot be open for a certain time, making sure action potential only goes in one direction.
The axon cytoplasm is conductive and the myelin sheath inhibits charge leak through the membrane → depolarization at one node is sufficient to elevate the voltage at the next node.

If the sum of depolarization in the nerve cells rises above a threshold value at the Hillock → action potential starts in the axon.
Action potential

Refractory phase channels

Resting state

Axon

Cell body

Region of depolarization

Direction of impulse movement

Resting state re-established
Which ions can flow through the ion channel is determined by the ion filter.

For ions with opposite charge this is explained by attraction or opposing electrostatic interactions between ion and side chains in the ion filter.
How can selectivity occur for very similar ions for example Na\(^+\) and K\(^+\)?

**Na\(^+\) channels:** hydrated Na\(^+\) ions can pass but the hydrated K\(^+\) ion is too large

The ion filter does not efficiently stabilize dehydrated ions, so the cost of dehydration is not returned thus ions have to be hydrated to pass

**K\(^+\) channels:** narrow ion filter that requires dehydration. K\(^+\) big enough be stabilized by carbonyl groups in the filter, whereas Na\(^+\) is too small for efficient stabilization and the hydrated Na\(^+\) is too big

Dehydration costs energy that have be compensated for by favorable interactions with the ion filter
Ion channel receptors can be regulated by G-protein coupled receptors

Activation of G-protein coupled receptors generate cAMP, which can bind and open certain ion channels

Depolarization and generation of action potential
Ion channel receptors can be regulated by other ion channels

Increased cytoplasmic Ca$^{2+}$ concentration can trigger opening of Cl$^{-}$ ion channels

Depolarization and generation of action potential
A cell senses signals from its surroundings via receptor proteins.

- G protein-coupled receptors
- Enzyme-linked receptors
- TNF family receptors
- Ion channel receptors

**Diagram:**
- Heterotrimeric G protein
- Adaptor proteins
- Adaptor proteins
- Ion flow
- Steroids
- Nuclear receptors
- Gene regulation
Tumor necrosis factor (TNF) receptor family

- TNF receptor (TNFR) family includes: TNFR, Trail, Fas

- TNFR family members have death domains (DD) that enable protein–protein interaction

- The protein complex formed via DD-interactions is called death-inducing signaling complex (DISC)

- The DISC complex is required for proximity-based processing and activation of initiator caspases-8 and -10

- Activation of TNF receptor family members often result in apoptosis (cell suicide)
Caspases are key effectors downstream tumor necrosis factor (TNF) receptor family

Caspases can be activated through:
- aggregation
- proteolytic cleavage by other proteases (caspases, granzymeB)

![Diagram of procaspase activation by cleavage](image)
Active initiator caspases unleash a cascade of caspase activation

Initiator caspase are activated by upstream receptors

Executioner caspases cleave proteins vital for cell survival thereby killing the cell (apoptosis)

Figure 18-5b Molecular Biology of the Cell 5/e (© Garland Science 2008)
Executioner caspases proteolytically degrade proteins necessary for cell survival.
Apoptosis can be induced by activation of TNF family receptors (death receptors)

- TNFα membrane bound and soluble
- FasL membrane bound
- Trail membrane bound

![Diagram of apoptosis](image-url)
Cytotoxic T-cells express FasL on the surface and use it to kill target cells.
Macrophage and natural killer cells use soluble TNFα to kill target cells
**Death promoting signal**
TRADD binds receptor and recruits FADD that binds and promote activation of procaspase 8

**Activation of caspase cascade (death)**

**Survival promoting signal:**
- TRADD recruits TRAF2 which recruits NIK
- TRAF2 can also recruit RIP kinase

**Activation of NF-κB transcription factor (survival)**

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FADD: Fas-associated death domain protein
TRADD: TNFR-associated death domain protein
DD: death domain
DED: death effector domain
TRAF: TNFR-associated associated factor
NIK: NF-κB-inducing kinase
Inhibition of pro-caspase-8 and -10 by c-FLIP

- c-FLIP is a catalytically inactive pro-caspase-8/10 homologue

- Competes with pro-caspase-8/10 for access to active death receptor
Inhibitor of apoptosis (IAPs) block caspase activity
Inhibitor of apoptosis (IAPs) block caspase activity

The second BIR domain of XIAP (green) binds into the substrate binding groove of caspase 3, preventing access of a protein substrate and terminating apoptosis.
TNF receptor 1-induced signal transduction

Death promoting signal
TRADD binds receptor and recruits FADD that binds and promote activation of procaspase 8

Survival promoting signal
- TRADD recruits TRAF2 which recruits NIK
- TRAF2 can also recruit RIP kinase

How does the cell know whether to die or survive in response to TNF receptor 1 activation?

Activation of NF-κB transcription factor (survival)
The expression of proteins that promote apoptosis and survival determine the outcome in a given cell.
Apoptotic stimulus induced by TNF receptors also mobilize the intrinsic apoptotic pathway.