Cell to cell communication are important for Multi-cell organisms

- Survival depends on an elaborate intercellular communication network that coordinates growth, differentiation and metabolism.
- Cells adjacent to one another frequently communicate through cell-cell contact
- Other forms of communication cover long distance, the extracellular signaling molecules

Steps of cell-cell communication

- Synthesis of signal.
- Release of the signaling molecule (ligand) by the signaling cell: exocytosis, diffusion, cell-cell contact.
- Transport of the signal to the target cell.
- Detection of the signal by a specific receptor protein (receptor)
- A change in cellular metabolism, function or development triggered by the receptor-ligand interaction
- Removal of the signal
Definition of ligand and receptor

**Ligand:**
A molecule that is able to bind to and form a complex with a protein to serve a biological purpose, such as a neurotransmitter, hormone, or other substance.

**Receptor:**
A protein on the cell membrane or within the cytoplasm or nucleus that binds to a specific molecule (a ligand), and initiates the cellular response to the ligand.

Agonists and Antagonists

If a molecule binds a(743,934),(995,970)(52,936),(154,972), i.e., cannot generate a signal, it acts as an **antagonist**. It competes with and blocks the activity of other endogenous ligands.

If a molecule binds to a receptor and activates the downstream signal transduction pathways, it acts as an **agonist**.

Action of hydrophobic Hormones

- Hormone diffuses through phospholipid bilayer & into cell
- Binds to receptor turning on/off specific genes
- New mRNA is formed & directs synthesis of new proteins
- New protein alters cell’s activity

Action of hydrophilic Hormones

- Can not diffuse through plasma membrane
- Receptors are cell-surface proteins — act as first messenger
- Receptor protein activates G-protein on membrane
- G-protein activates adenylate cyclase to convert ATP to cAMP in the cytosol
- Cyclic AMP is the 2nd messenger
- Activates kinases in the cytosol to speed up/slow down physiological responses
- Phosphodiesterase inactivates cAMP quickly
- Cell response is turned off unless new hormone molecules arrive
There are two broad classes of receptors: intracellular and cell-surface.

Hydrophilic ligands bind to cell-surface (plasma membrane) receptors.

Hydrophobic ligands diffuse across the plasma membrane and bind to intracellular receptors in the cytoplasm or nucleus.

How can ligand concentrations be measured?

- Using antibodies specific for the ligand in an immunochemical assay, e.g., ELISA assay
- Using analytical or physical methods, such as HPLC

Such assays show that many ligands are present at very low concentrations: $10^{-9}$ to $10^{-10}$ M. Receptors that recognize the ligands bind them with high affinity. Thus, ligand-receptor binding shows great sensitivity.

How to measure receptor concentrations?

Ligand binding assay:
Add labeled ligand to a constant amount of tissue, membranes or cells and determine the amount of labeled ligand associated with the sample.

From such studies, $N$, the number of receptors per unit tissue, membrane or cell and the affinity constant $K_d$ for the receptor-ligand interaction can be determined.

The affinity constant $K_d$ for receptor-ligand interaction is equivalent to Michaelis constant $K_m$ for the affinity of enzyme-substrate binding.
Receptor-ligand interaction

Thermodynamics

\[
K_d = \frac{k_{\text{off}}}{k_{\text{on}}} = \frac{1}{K_a} = \frac{k_{\text{on}}}{k_{\text{off}}}
\]

- \( K_a \) is second-order rate constant for the bimolecular association reaction.
- \( K_d \) is first-order rate constant for the unimolecular dissociation reaction.
- \( K_a = K_d = \text{equilibrium constant for dissociation} \)

- \( K_d \) values in the mM range are considered as rather weak, values in the nM range or below as strong.
- The strength of a biological response to a ligand is usually proportional to the number of occupied receptors.
- Cells with more receptors will, for a given concentration of ligand, have more occupied receptors. Thus, cells with more receptors are more sensitive to lower concentrations of a ligand.

Cells often have "spare" receptors

Concentration of ligand needed to induce maximum response is less than the concentration of ligand needed to saturate all receptors.

Residues on growth hormone and its receptors for interaction

Only 8 residues on growth hormone contribute 85% of binding energy. Two tryptophan residues (blue) on receptors contribute most of the binding energy. Binding of the second set receptor to the opposing side of hormone involves different residues. Hormone-induced dimerization of receptors is common for receptor activation.

Outline

- Definition of receptor and ligand
- Interaction between ligands and receptors
- Signal transduction cascades
  - Hormone receptors in mammalian systems
  - G-protein coupled receptors
  - Receptors that are ion channels
  - Receptors with enzyme activities
    - Pathways involving proteolysis
    - Intracellular pathways
  - Hormone receptors in plants
    - Auxin
    - Ethylene
    - Cytokinin
    - ABA
    - GR
- Receptors without ligand: Photoreceptors
  - Rhodopsin
  - Phytochrome
The basics of signal transduction

- Signal Reception
- Signal Amplification
- Signal Transduction
- Response is usually a change in protein levels or associations
- Specificity possible at all levels
- Feedback possible
- Conservation between many organisms and pathways

How does ligand-receptor binding transduce signals to the cell?

Responses of cells to ligands can be divided into:

- **Rapid responses**: Does not require gene expression. These involve alterations in existing proteins, and everything occurs in the cytoplasm.
- **Persistent responses**: Require changes in gene expression. To alter the transcription of genes, signals must travel from the plasma membrane to the nucleus.

Signal amplification

- Small signal produces large cell response
- Amplification enzyme
- Cascade

Signal transduction

- Transforms signal energy
- Protein kinase
- Second messenger
- Activate proteins
  - GTPase
  - Phosphorylation
  - Calcium binding
- Cell response
One common consequence of ligands binding to cell surface receptors is the activation of intracellular kinases, resulting in the phosphorylation of intracellular protein (on serine, threonine or tyrosine residues).

Another common consequence of ligands binding to cell surface receptors is the activation of small GTPase, resulting in subsequent activation of downstream factors.

Switching mechanism for monomeric and trimeric G proteins

- Switch I and II are bound to γ phosphate of GTP through interactions with backbone amide groups of a conserved threonine and glycine residue.
- Release of γ phosphate by GTPase hydrolysis causes Switch I and II to relax into different conformation.

Four common intracellular second messengers

- 1,2-Diacylglycerol (1,2-DAG): Activates protein kinase C (PKC)
- 1,4,5-Inositol triphosphate (1,4,5-IP3): Activates Ca²⁺ channels in the endoplasmic reticulum
- Cyclic AMP (cAMP): Activates protein kinase A (PKA)
- Cyclic GMP (cGMP): Activates cGMP-dependent protein kinase
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Major classes of cell-surface receptors

- G protein-coupled receptors
- Cysine-receptors
- Receptor tyrosine kinases
- TGFβ receptors
- Hexaglycer (Hh) receptors
- Wnt receptors
- Notch receptor

G-Protein-coupled Receptors

<table>
<thead>
<tr>
<th>TABLE 14: Overview of Major Receptor Classes and Signaling Pathways</th>
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<tbody>
<tr>
<td>Receptor Class/Pathway</td>
</tr>
<tr>
<td>C-protein-coupled receptor</td>
</tr>
</tbody>
</table>
Operational model for ligand-induced activation of effector proteins associated with G protein-coupled receptors

1. Binding of ligand to receptor.
2. Activation of G protein (Gα and Gβγ). (GTP bound)
4. Activation of effector protein.
5. Binding of second messenger (e.g., cAMP) to effector.
6. Effector activation and downstream signaling.

**G protein-coupled signal transduction**

- Epinephrine binds to receptor, which associates with a heterotrimeric G protein.
- The G protein associates with adenylyl cyclase that converts ATP to cAMP.
- The second messenger relays extracellular signals to intracellular effectors.

**Receptor-mediated activation of coupled G proteins occurs in a few seconds**

- Fluorescence emission at 400 nm (cyan).
- Excitation light at 440 nm.
- Gα binds GTP.
- GTP hydrolysis.
- Effector activation.
- Gα, Gβγ, and GTP dissociate.

**Table 1.1 Major Classes of Mammalian Y-Linked G Proteins and Their Effectors**

<table>
<thead>
<tr>
<th>Class</th>
<th>Associated Effector</th>
<th>2nd Messenger</th>
<th>Signal Example</th>
</tr>
</thead>
</table>
| Gα    | Adenylyl cyclase     | cAMP (increased) | β2 Adrenergic receptor
|       | Nc      | cAMP (decreased) | s2 Adrenergic receptor |
| Gαγ   | Phospholipase C     | IP3, DAG (increased) | Activates PLC, increases intracellular calcium |
|       | s2      | cAMP (decreased) | s2 Adrenergic receptor |
| Gβγ   | s2      | cAMP (decreased) | s2 Adrenergic receptor |

*1. The Gα subunit may be associated with more than one effector protein.
2. To date, only one class C protein has been identified, but multiple C proteins are postulated. (Redrawn from Magnasco, M. O., 1993, *Science* 260(5113), 1045-1047)
Receptor-mediated activation of coupled G proteins occurs in a few seconds.

Schematic diagram of mammalian adenylyl cyclases

Adenylyl cyclase contains two similar catalytic domains on the cytosolic side and two transmembrane domains.

Structure of Gsα complexed with catalytic domains of Adenylyl cyclase

α3-β5 loop and Switch II regions of Gsα contact with a specific region of adenylyl cyclase. Forskolin locks adenylyl cyclase in its active conformation.

Hormone-induced activation and inhibition of adenylyl cyclase

Ligand binding to Gs-coupled receptors causes activation of adenylyl cyclase, whereas ligand binding to Gi-coupled receptors causes inhibition.
Acetylcholine receptor in the heart muscle plasma membrane

Binding of acetylcholine to receptor triggers activation of G\textalpha\textsubscript{i} subunit. In this case, the G\textbeta\gamma subunit binds to and open the associated K\textsuperscript{+} channel.

Synthesis of DAG and IP\textsubscript{3} from membrane-bound Phosphatidylinositol (PI)

DAG/IP\textsubscript{3} pathway and the elevation of cytosolic Ca\textsuperscript{2+}

Trigger by ligand binding to certain G protein-coupled receptors and other receptor types, lead to activate phospholipase C.
Nitric oxide is synthesized in endothelial cells in response to acetylcholine and the elevated cytosolic Ca²⁺, then diffuses locally through tissues and activates the NO receptor with guanylyl cyclase activity in the nearby smooth muscle cells, resulting in activation of protein kinase G.

In resting cells, Tubby is bound to PIP₂ in the plasma membrane. Receptor stimulation leads to activation of phospholipase C, hydrolysis of PIP₂, and release of Tubby into the cytosol.
Transforming Growth Factor β (TGFβ)

- A superfamily in mammalian system
- Induce cell transformation, antiproliferative effects; promote expression of cell adhesion molecules; promote synthesis and secretion of growth factors
- Synthesized as precursor form, cleaved after secretion
- 110-140 residues with compact and four anti-parallel β-strand. Three conserved intracellular disulfide linkages form the cystine knot (red). A disulfide chain between two monomers link two monomers into the functional homodimer and heterodimer.

TGFβ-Smad signaling pathway

1. (1a&1b). TGFβ bind to RI receptor (constitutively active kinase) either directly or presented by RIII receptor.
2. (2). Recruit RI receptor and release the de-activated kinase activity from RI.
3. (3). Activated RI phosphorylates Smad3 and unmask the NLS.
4. Two phosphorylated Smad3 complex with Smad4 and importin β (Imp-β)
5. (5&6). Translocation and Ran-GTP dissociate the complex.
6. (7). TFE3 associates with Smad3-Smad4 complex to form a activating complex for gene expression.

Dimerization of the receptor for Epidermal Growth Factor (EGF)

- Binding of EGF to a monomeric receptor causes an alteration in the structure of a loop located in-between the EGF-binding domains. Dimerization of two ligand-bound receptors occurs through interactions between the two activated loop segments.

General structure and activation of receptor tyrosine kinase (RTKs) and cytokine receptors
Recruitment of signaling proteins to cell membrane by binding to phosphotyrosine residues

- Cytosolic proteins with SH2 and PTB domains bind to specific phosphotyrosine residues in activated RTKs or cytokine receptors.
- Certain receptors use multi-docking proteins (e.g., IRS-1) to increase the number of signaling proteins that are recruited and activated.

JAK-STAT pathway

- Following ligand binding to a cytokine receptor and activation of an associated JAK kinase, JAK phosphorylates several tyrosine residues on the cytosolic domain.
- STAT transcription factor binds to the phosphotyrosine, then get phosphorylated by JAK, release from receptor, dimerize, and move into the nucleus.

Surface model of SH2 domain bound to a phosphotyrosine-containing peptide

- The bound peptide is shown in spacefill. The phosphotyrosine (Tyr0 and OPO3) and Ile3 fit into two sockets on the surface.

Two mechanisms for terminating signal transduction from Erythropoietin receptor (EpoR)

- SH2 domain on SHP1 bind to phosphotyrosine on EpoR, which unmask the phosphatase active site for binding to the phosphotyrosine on JAK.
- SOCS block the phosphotyrosines on receptor. SOCS box targets proteins for degradation.
Structure of Ras bind to GDP and GTP

(a). In Ras GDP form, the switch I and switch II segments do not directly interact with GDP.
(b). Sos binds to both switch regions, leading to a conformation change of Ras, allowing GDP to release.
(c). In Ras-GTP form, conformational change of the protein allows Switch I & II bind to the GTP γ phosphate, promoting the interaction with its effectors.

Kinase cascade that transmits signals downstream from activated Ras protein to MAP kinase

In the cytosol, MAP kinase phosphorylates and activates p90RSK, then both move into the nucleus to phosphorylate downstream transcription factors.

Structure of MAP kinase in inactive and active forms

Phosphorylation of MAP kinase at Y185 and T183 leads to a conformational change in the activation lip. The change promotes dimerization of MAP kinase and binding to its substrate, certain proteins and ATP.
**NF-κB signaling pathway**

- Ubiquitin-dependent degradation of I-κBα, which is promoted by kinase phosphorylation, will re-activate p65/p50 transcription factor (NF-κB).
- I-κB kinase is activated by TNF-α, IL-1 signaling and ionizing radiation.

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**Notch/Delta signaling pathway**

- Binding of Notch to Delta on adjacent signaling cell triggers cleavage of Notch by TACE metallocprotease.
- Presenilin 1 catalyze the cleavage on the intracellular side to release cytoplasmic domain of Notch for nuclear gene activation.
Nitric oxide is synthesized in endothelial cells in response to acetylcholine and the elevated cytosolic Ca²⁺, then diffuses locally through tissues and activates the NO receptor with guanylyl cyclase activity in the nearby smooth muscle cells, resulting in activation of protein kinase G.
Auxin binding to TIR1/ABF auxin receptors

Model of ethylene signaling in Arabidopsis

Gibberellins (GA):
Regulators of plant height and seed germination

With-type repressor

Mutant repressor

No growth

Growth response to GA

No growth even in presence of GA

Growth even in absence of GA
Model of cytokinin signaling: Regulators of cell division

Abscisic acid (ABA): Seed maturation and anti-stress signal

Brassinosteroid signaling
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Human rod cell
Human retina contains two types of photoreceptors, rods and cones. Cones are involved in color vision. Rods are stimulated by weak light. Rhodopsin is located in the flattened membrane disks of the outer segment on rod cells.

Light-triggered step in vision
The light-absorbing pigment 11-cis-retinal is covalently linked to a amino group of lysine in opsin, the protein part of rhodopsin. Absorption of light induces rapid photoisomerization of 11-cis-retinal to all-trans-retinal, forming meta-rhodopsin II, the activated opsin for activating G protein.

Model for rhodopsin-induced closing of cation channels
Light-absorption by rhodopsin activates cGMP phosphodiesterase to remove cytosolic cGMP, which promotes the dissociation of cGMP from cGMP-gated ion channel and closing of the channel.
Structural models of rhodopsin and its associated Gα protein

Light regulates growth and development throughout the life cycle of plants

Corn (A and B) and kidney bean (C and D) seedlings grown either in light or dark
Phytochromes: Sensors of the light environment

Phytochromes are present in different organisms

Avena sativa
Arabidopsis thaliana
Mesotaenium caldarium
Synechocystis sp 6803

Phytochrome structure

Phytochromes are photochromic, nonfluorescent biliprotein light sensors
Phytochrome activity is modulated by light reception and phosphorylation

Phytochrome is an autophosphorylating protein kinase

Phytochrome is an autophosphorylating protein kinase
Questions?