# Johns Hopkins Engineering

## Molecular Biology

### Protein Kinase-associated Receptors

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# Outline

- Protein kinase-associated receptors
- Receptor tyrosine kinases
- Hormone signaling and receptors

# Categories of receptors

- Receptors can be classified into several basic categories
  - Ligand-gated channels
  - Plasma membrane receptors of two types
    - Those linked to G proteins
    - **Those linked to protein kinases**

# Protein Kinase-Associated Receptors

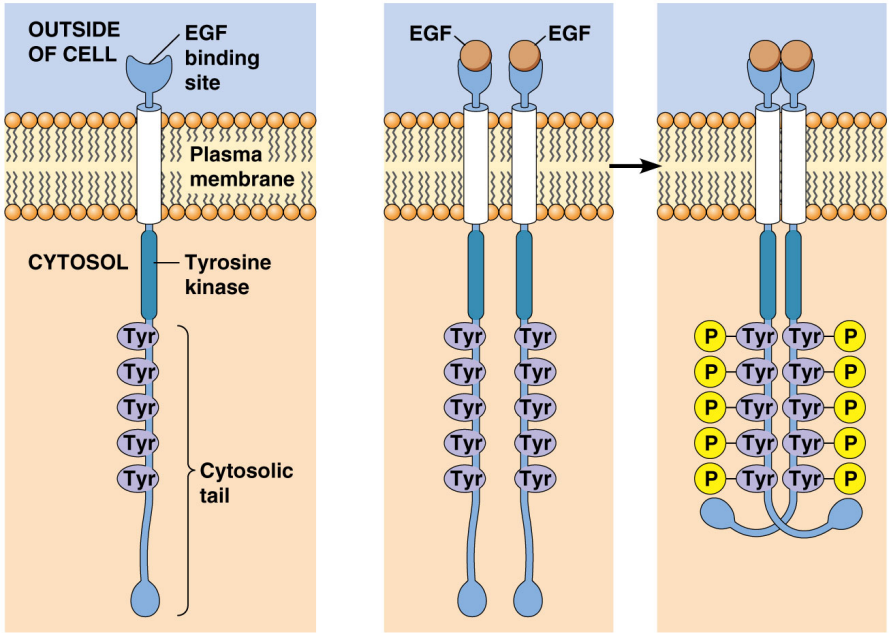
- Protein kinase-associated receptors are not only receptors, but also function as kinases (kinases *phosphorylate* proteins by transferring phosphate groups to them)
- They function in many important cellular processes
- Ligand binding stimulates their kinase activities
- Signaling of these *receptor protein kinases* is transmitted through a phosphorylation cascade

# Growth Factors Often Bind Protein Kinase-Associated Receptors

- For cells to divide they need enough nutrients for growth and signals to stimulate cell growth
- Cultured cells in vitro will not grow, even with enough nutrients, unless blood serum is provided
- Messengers in the serum that stimulate growth are called **growth factors**
- Several growth factors stimulate receptor tyrosine kinases
  - Insulin
  - Insulin-like growth factor-1
  - Fibroblast growth factor
  - Epidermal growth factor
  - Nerve growth factor

# Receptor Tyrosine Kinases Aggregate and Undergo Autophosphorylation

- Many receptor tyrosine kinases (RTKs) trigger a chain of events in the cell that culminate in cell growth, proliferation, or specialization
- Receptor tyrosine kinases often consist of a single polypeptide chain with just one transmembrane segment
- The extracellular part of the receptor contains the *ligand-binding domain*
- On the cytosolic side is the tyrosine kinase domain




**(a)** Structure of the epidermal growth factor (EGF) receptor

**(b)** Activation of the EGF receptor



https://www.youtube.com/watch?v=LT_ws4Xvj7M

# The Activation of Receptor Tyrosine Kinases

- Signal transduction is initiated upon ligand binding that causes aggregation of receptor tyrosine kinases
- In some cases, receptors dimerize upon ligand binding, and phosphorylate each other
- Because they phosphorylate the same type of receptor as themselves, this is called **autophosphorylation**
- Once autophosphorylation of the receptors occurs the receptor recruits cytosolic proteins like **Ras** and **MAP kinase**

# Receptor Tyrosine Kinases Activate a Variety of Other Signaling Pathways

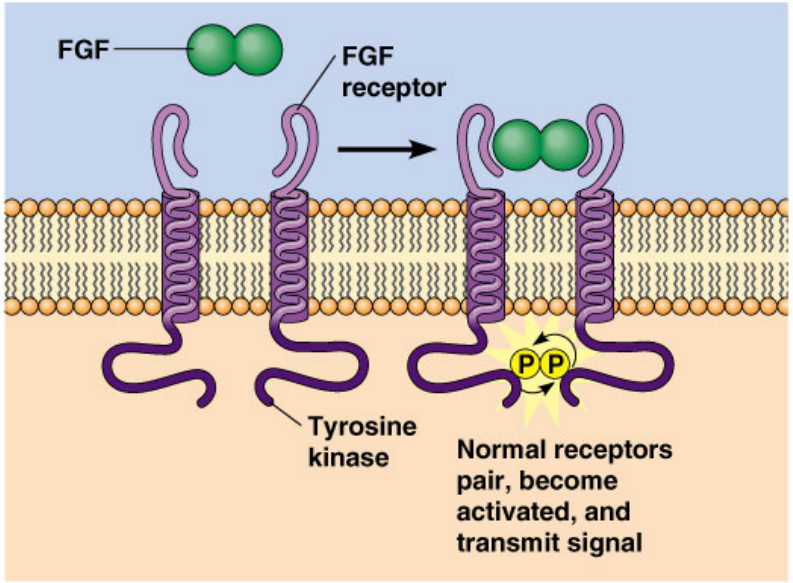
- Receptor tyrosine kinases can also activate phospholipase C, leading to production of $IP_3$ and DAG

- Signaling components such as those in the Ras pathway are sometimes assembled into large multiprotein complexes that make cascades more efficient

# Dominant Negative Mutant Receptors Are Important Tools for Studying Receptor Function

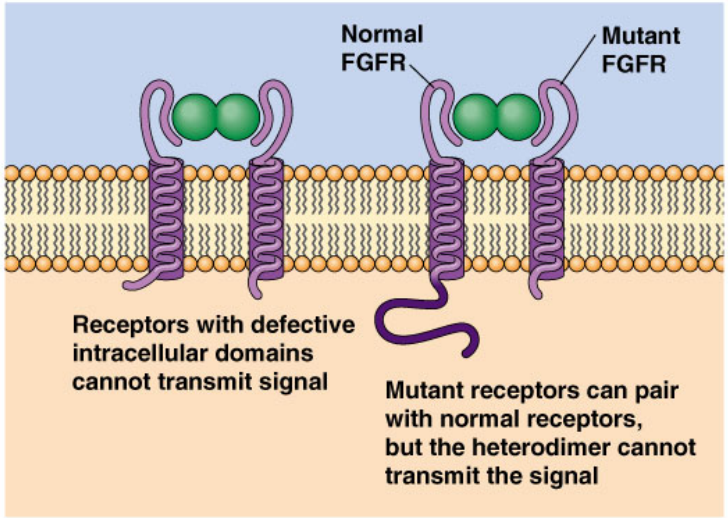
- One approach to studying receptor function involves introducing mutations into the receptor to determine the effect
- E.g., **fibroblast growth factors** (**FGFs**) and their *receptor tyrosine kinases (FGFRs)*
- Normal FGFs undergo autophosphorylation in response to ligand binding
- Some types of mutant FGFRs can bind ligands but cannot undergo autophosphorylation
- These mutant receptors interfere with normal receptor function because they can dimerize with normal receptors
- A mutant that overrides normal function in this way is called a **dominant negative mutation**

**(a) Normal FGF receptor.** Normal receptors form dimers after binding FGF, and transmit the appropriate signal via Ras and MAPK.






**(b) Dominant negative FGF receptor.** When a cell makes mutant FGF receptors, normal receptors can dimerize as in (a), or the defective receptors can bind to FGF and dimerize with normal receptors. In this case, no signal is transmitted. When sufficient quantities of the mutant receptor are present, most of the normal receptors are paired with mutant receptors, resulting in overall disruption in signaling.



# Constitutive mutations

- Some mutations make FGFRs active in signaling, even when not bound to ligand

- These mutations are called **constitutively active** mutations because they cause the receptor to stay switched “on” all the time

# Disruptions of Growth Factor Signaling Can Lead to Cancer

- Some cancers can result from the loss of regulation of growth factor signaling
- *e.g., mutations in Ras are often associated with cancer*
- Mutations in EGFR can result in breast cancer, gioblastoma (brain cancer), and fibrosarcoma (bone cancer)

# Growth Factor Receptor Pathways Share Common Themes

***Summary:***

- Ligand binding often results in activation and/or clustering of receptors, followed by a cascade of events, often phosphorylation (addition of a phosphate group)

- Phosphorylation may be catalyzed by the receptor, or by *Janus activated kinase*, when activated by a receptor

Cancer Biother Radiopharm. 2013 Jul-Aug;28(6):450-8. doi: 10.1089/cbr.2012.1399. Epub 2013 Mar 18.

**Dominant negative epidermal growth factor receptor inhibits growth of human gastric cancer cells by inducing cell cycle arrest and apoptosis.**

Liao G[1], Wang Z, Zhang N, Dong P.

- Epidermal growth factor receptor (EGFR) promotes proliferation of cancer cells.
- Dominant negative EGFR (DNEGFR) can block EGFR signal pathway by competing with endogenous EGFR for ligands.
- However, whether EGFR is overexpressed in gastric cancer and whether DNEGFR contributes to the inhibition of gastric cancer growth are not known.
- In this study, we demonstrate that EGFR is expressed in 29 of 60 of human gastric cancers. In addition, DNEGFR induces G0/G1 arrest by decreasing expression of phosphorylated retinoblastoma protein, phosphorylated GSK-3β, cyclin D1, and by increasing expression of p21 and p27 in human gastric cancer cell lines.
- Finally, DNEGFR induces apoptosis in these cells.
- These results indicate that DNFGFR may provide promising treatment strategy for a subgroup of human gastric cancers that express EGFR.

# Hormone Signaling

- Plants and animals use secreted chemical signals called **hormones** to coordinate the function of cells and tissues over long distances
- Hormones differ in many ways
- Animal hormones are better understood than plant hormones

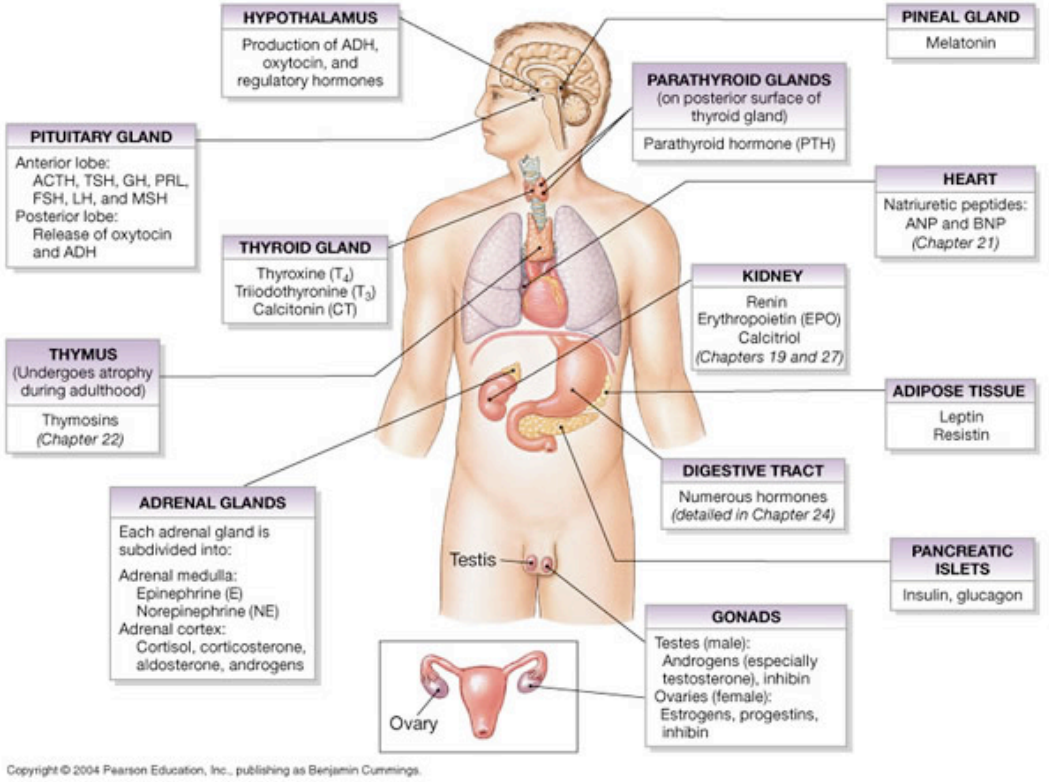
**Table 14-4** Chemical Classification and Function of Hormones

| Chemical Classification | Example | Regulated Function |
|---|---|---|
| **Endocrine Hormones** | | |
| Amino acid derivatives | Epinephrine (adrenaline) and norepinephrine (both derived from tyrosine) | Stress responses: regulation of heart rate and blood pressure; release of glucose and fatty acids from storage sites |
| | Thyroxine (derived from tyrosine) | Regulation of metabolic rate |
| Peptides | Antidiuretic hormone (vasopressin) | Regulation of body water and blood pressure |
| | Hypothalamic hormones (releasing factors) | Regulation of tropic hormone release from pituitary gland |
| Proteins | Anterior pituitary hormones | Regulation of other endocrine systems |
| Steroids | Sex hormones (androgens and estrogens) | Development and control of reproductive capacity and secondary sexual characteristics |
| | Corticosteroids | Stress responses; control of blood electrolytes |
| **Paracrine Hormones** | | |
| Amino acid derivative | Histamine | Local responses to stress and injury |
| Arachidonic acid derivatives | Prostaglandins | Local responses to stress and injury |

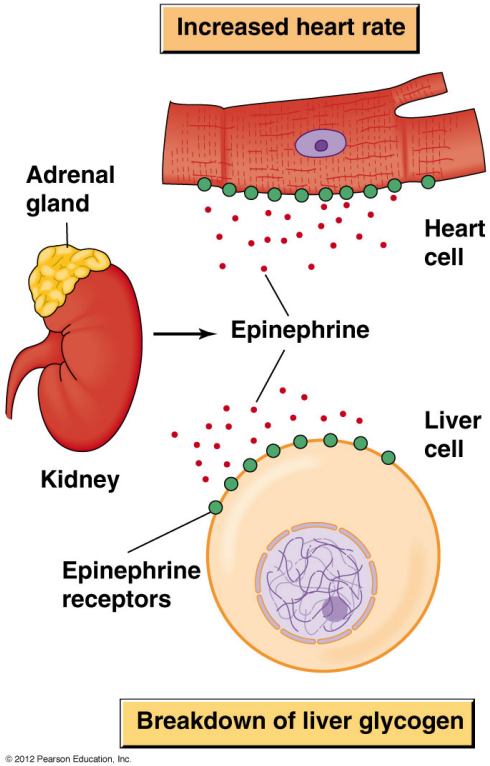
# Hormones Can Be Classified by the Distance They Travel and by Their Chemical Properties

- *Endocrine hormones* travel from sending to receiving cells via the circulatory system
- They are synthesized by *endocrine tissues* and are secreted directly into the bloodstream, with a life span ranging from a few seconds to many hours
- As they circulate, they encounter their receptors in *target tissues*

# The Endocrine System



The cells in target tissues have hormone-specific receptors embedded in their plasma membranes (or, in the case of steroid hormones, in their nucleus or cytosol).



# Chemical classification of endocrine hormones

- Endocrine hormones fall into four categories
    - Amino acid derivatives (e.g., epinephrine)
    - Peptides (e.g., *vasopressin*)
    - Proteins (e.g., insulin)
    - Lipid-like hormones such as steroids (e.g., *testosterone*)

# Control of Glucose Metabolism Is a Good Example of Endocrine Regulation

- **Adrenergic hormones**, epinephrine and norepinephrine, function to put body functions on hold and redirect resources to the heart and skeletal muscles in dangerous or stressful situations

- They bind to a family of G protein-linked receptors, **adrenergic receptors**, classified as $\alpha$- and $\beta$-*adrenergic receptors*

- *These receptors stimulate* different pathways *(and have different effects) because they are linked to* different G proteins

# Intracellular Effects of Adrenergic Hormonal Control of Glycogen Degradation

- One effect of adrenergic hormones is to stimulate the breakdown of glycogen to provide muscle cells with an adequate supply of glucose (energy)
- The breakdown of glycogen is facilitated by the enzyme *glycogen phosphorylase*, resulting in release of a glucose-1-phosphate
- Glycogen degradation begins when an epinephrine molecule binds to a $\beta$-adrenergic receptor on a liver or muscle cell

# Insulin Signaling Acts through PI 3-Kinase to Regulate Resting Glucose Levels

- Specialized cells in the pancreas called the *islets of Langerhans* secrete two peptide hormones that regulate normal glucose levels
- **Glucagon** acts to increase blood glucose through glycogen breakdown
- **Insulin** reduces blood glucose levels by stimulating uptake into muscle and adipose cells, and stimulating glycogen synthesis

# Diabetes

- *Type I diabetes* is an autoimmune disorder resulting in loss of insulin-producing cells in the islets of Langerhans (only 5% of people diagnosed with diabetes have this form)
- It can be somewhat successfully treated with insulin
- Type II diabetes appears to result from resistance to insulin and so is not as effectively treated with insulin
- Insulin is a peptide hormone that has rapid and longer-lasting effects on a variety of cells (compared to adrenergic hormones)

# Steroid Hormone Receptors Act Primarily in the Nucleus, Not the Cell Surface

- Steroid receptor proteins mediate the actions of steroid hormones such as progesterone, estrogen, testosterone, and glucocorticoids
- Steroid hormones are **lipid signaling molecules**
- The hormone enters the target cell and binds its receptor, triggering a cascade of events that activate (sometimes inhibit) transcription of a set of target genes
  - Can diffuse through plasma membrane

# Importance of Cell Signaling- for Biomedical Engineering Applications

- Implantable materials
- Drug delivery
- Biomedical Imaging

  - Surface topography
  - Drug diffusion
  - Cellular growth rates / spreading
  - Inflammation
  - Data modeling and integration

# Summary

- Protein kinase-associated receptors
  - Kinase activity
  - Growth factors
  - Mutations
  - Disease associations
  - Hormone signaling and the endocrine system
- Cell signaling considerations for biomedical engineering