

## Chapter 12 Homework Assignment

- We will not cover the entire chapter. We will cover sections 12.1, 12.3, 12.4, 12.8 and 12.10.
- The material in 12.2 (Gated Ion Channels) was covered in CHP 11 in part.
- The following problems will be due once we finish the chapter:

**2, 3, 9, 10, 11, 13, 17, 19**

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## Biosignaling

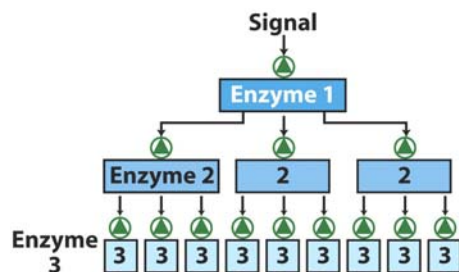
### Signal Transduction in Biological Systems

- Why is signaling necessary for living organisms?
- Are there differences between uni- and multicellular organisms?
- Are there differences between prokaryotes and eukaryotes?
- What are the mechanisms for carrying out signal transduction?

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## Chapter 12 Biosignaling



## Biosignaling Signal Transduction in Biological Systems

**TABLE 12-1**

Some Signals to Which Cells Respond

Antigens	Light
Cell surface glycoproteins/ oligosaccharides	Mechanical touch
Developmental signals	Neurotransmitters
Extracellular matrix components	Nutrients
Growth factors	Odorants
Hormones	Pheromones
	Tastants

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## Biosignaling

### Biosignaling Requirements

- (1) The cell (tissue) must have some kind of receptor for the signal
- (2) The cell (tissue) must have the metabolic machinery to amplify and respond to the signal

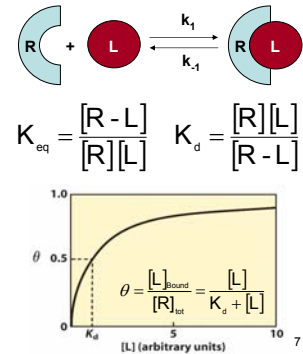
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## Biosignaling

### Ligand Binding is Similar to Ionization

- Remember, from the Myoglobin/Hemoglobin chapter, the term  $K_d$  is the **dissociation constant** and is the inverse of the  $K_{eq}$  (or the association constant,  $K_a$ )
- As the  $K_d$  value decreases in value, the affinity of the ligand for the receptor is increasing.
- For most binding equilibria in cells (including ligand-receptor interactions), the  $[L]$  is much greater than the number of binding sites
- Therefore, the binding of L to the receptor does not appreciably effect the  $[L]$  so it is considered a constant



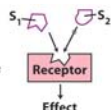
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## Biosignaling

### Molecular Mechanisms of Signal Transduction

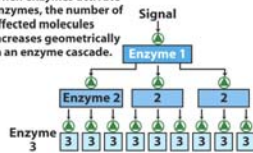
**(a) Specificity**

Signal molecule fits binding site on its complementary receptor; other signals do not fit.



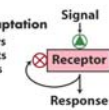
**(b) Amplification**

When enzymes activate enzymes, the number of affected molecules increases geometrically in an enzyme cascade.



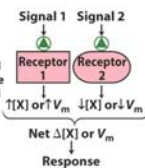
**(c) Desensitization/Adaptation**

Receptor activation triggers a feedback circuit that shuts off the receptor or removes it from the cell surface.



**(d) Integration**

When two signals have opposite effects on a metabolic characteristic such as the concentration of a second messenger  $X$ , or the membrane potential  $V_m$ , the regulatory outcome results from the integrated input from both receptors.



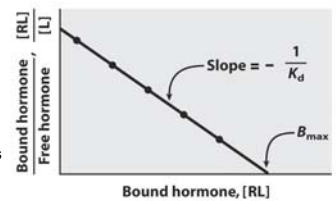
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## Biosignaling

### Ligand Binding is Similar to Ionization

- Using **Scatchard Analysis** we can estimate both the  $K_d$  and the # of receptor-binding sites.
- When binding reaches equilibrium, the total number of binding sites ( $B_{max}$ ) equals the number of unoccupied sites ( $[R]$ ) plus the number of occupied sites ( $[R-L]$ )



$$B_{max} = [R] + [R-L]$$

$$[R] = B_{max} - [R-L]$$

$$K_d = \frac{[R-L]}{[R][L]} = \frac{[R-L]}{(B_{max} - [R-L])[L]}$$

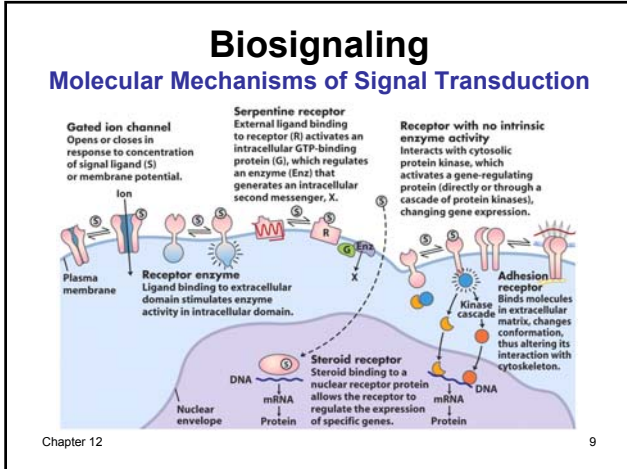
$$\frac{[R-L]}{[L]} = \frac{1}{K_d} \cdot B_{max} - \frac{1}{K_d} \cdot [R-L]$$

$$y = b + m \cdot x$$

This analysis only works for simple systems

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## Receptor Enzymes

### Overview of Hormones (“stir up, excite”)

- Mobile signals secreted by the endocrine system for distant cell-cell communication
- Occur as four major classes (and several minor ones)
  - **Peptide hormones**: tiny “proteins” from 3-200 aa’s, water-soluble, active only after cleavage of the targeting “pre-sequence” and the inactive “prohormone” (**insulin, glucagon**)
  - **Catecholamine hormones**: derived from tyrosine, water-soluble, many are neurotransmitters (**epinephrine**)
  - **Eicosanoids**: derived from arachidonate, minimally water-soluble, act locally (rather than through the bloodstream), mediate pain, inflammation, and smooth muscle contraction
  - **Steroid hormones**: derived from cholesterol, fat-soluble, are carried to targets by carrier proteins

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## Receptor Enzymes

- Receptor Enzymes are usually Type I or II integral proteins with two distinct domains:
  - A Ligand Binding Domain is located on the exterior of the membrane
  - A Catalytic Domain is located on the interior of the membrane
  - The two domains are connected by a single transmembrane segment
- Typically, the catalytic portion is a protein kinase (PK) that phosphorylates target proteins or enzymes once the signal has been received
  - A typical signal for these receptors are hormones
  - An example of this type is the Insulin Receptor
- The phosphorylation target is usually a Tyr in animals and either a Ser or Thr in plants
- Other Receptor Enzymes may synthesize a secondary messenger such as cyclic GMP (cGMP)

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## Receptor Enzymes

### Common Characteristics of Hormones

- They occur and function at very low concentrations –  $10^{-6}$  to  $10^{-12}$  M
- Deliberately unstable – levels rise rapidly upon secretion, but fall fast when it stops
- Biochemical response may be very rapid, by altering existing enzyme activities, or slower, where gene expression levels change
- Act through two receptor types: cell surface and nuclear
- Display remarkable specificity
- Operate through the “cascade” principle

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## Receptor Enzymes

### Common Characteristics of Hormones

TABLE 23-1 Classes of Hormones

Type	Example	Synthetic path	Mode of action
Peptide	Insulin, glucagon	Proteolytic processing of prohormone	Plasma membrane receptors; second messengers
Catecholamine	Epinephrine	From tyrosine	
Eicosanoid	PGE <sub>1</sub>	From arachidonate (20:4 fatty acid)	
Steroid	Testosterone	From cholesterol	Nuclear receptors; transcriptional regulation
Vitamin D	1,25-Dihydroxycholecalciferol	From cholesterol	
Retinoid	Retinoic acid	From vitamin A	
Thyroid	Triiodothyronine (T <sub>3</sub> )	From Tyr in thyroglobulin	
Nitric oxide	Nitric oxide	From arginine + O <sub>2</sub>	Cytosolic receptor (guanylate cyclase) and second messenger (cGMP)

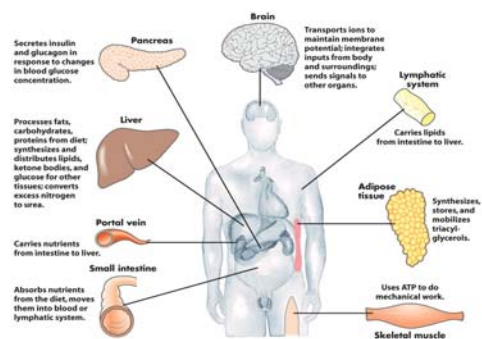
- The first and last pair are water-soluble, the middle five are fat-soluble – we need both!

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## Receptor Enzymes

### Specialized Functions of Mammalian Tissues



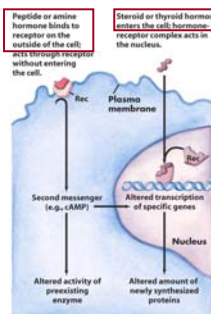
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Fig. 23-12

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## Receptor Enzymes

### Two Types of Hormone Receptors



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Fig. 23-4

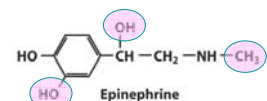
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- Hormones are present at very low concentration, thus receptor affinity must be very tight
- Cell surface receptors** bind hormone outside – a conformational change transduces the signal to inside, resulting in a “second messenger” (e.g. cAMP)
- Cytosolic/nuclear receptors** bind and retain hormone, migrating to the nucleus where, as a hormone-receptor complex, they alter gene transcription

## Receptor Enzymes

### Three Key Players in Glucose Regulation

- Insulin** (a 51 amino acid peptide hormone)
  - signals *satiety*: that blood glucose levels are too high
- Glucagon** (a 29 amino acid peptide hormone)
  - signals *starvation*: that blood glucose levels are too low
- Epinephrine** (an amine hormone derived from tyrosine)
  - signals *sudden stress*: “fight or flight” response



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## Receptor Enzymes

### Insulin is a Signal

- Where is insulin produced?
- When is insulin produced?
- What are the first order results of insulin production?
- What are the second order results of insulin production?
- How does the signaling actually occur?

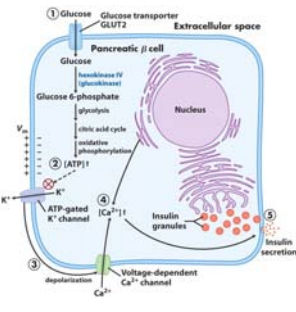


Fig. 23-25

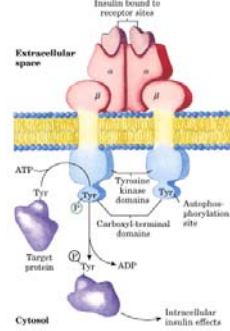
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## Receptor Enzymes

### A Typical Protein Tyrosine Kinase Receptor?

- The insulin receptor is a dimer, but differs in its dimerization from other PTKs, which dimerize only after hormone binding
- Why is the membrane association essential?
- What kind of proteins are the  $\alpha$ -subunits?
- What kind of proteins are the  $\beta$ -subunits?

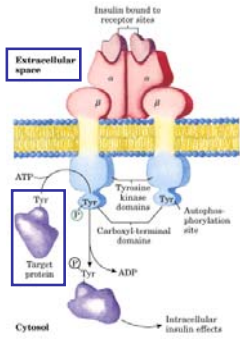


Chapter 12 3eFig. 13-6

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## Receptor Enzymes

### Receptor-Enzyme: The Insulin Receptor



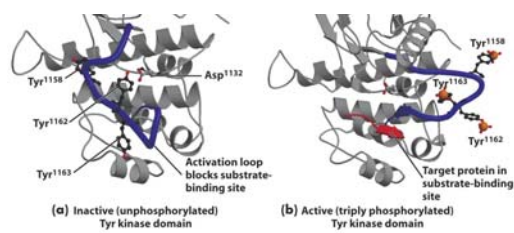
- Insulin binds to its receptor on surface of a cell, activating protein tyrosine kinase activity.
- There follows a cascade of phosphorylation events that ultimately stimulate glycogen synthesis
- In addition, the binding affects expression of certain genes at the transcriptional level, through interaction with promoters/ repressors.

Chapter 12 3eFig. 13-6

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## Receptor Enzymes

### What Happens When It Phosphorylates Itself?



- Its own loop sits in its active site, and gets phosphorylated when hormone binds
- Thus expelling the loop, and making the active site available for the appropriate segments of a target protein

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## Receptor Enzymes

### Phosphorylation of Insulin Receptor Substrate-1

- ① Insulin receptor binds insulin and undergoes autophosphorylation on its carboxyl-terminal Tyr residues.
- ② Insulin receptor phosphorylates IRS-1 on its Tyr residues.
- ③ SH2 domain of Grb2 binds to P-Tyr of IRS-1. Sos binds to Grb2, then to Ras, causing GDP release and GTP binding to Ras.

Cytosol

- We will ignore the cascade pathway by which phosphorylated IRS-1 activates nuclear transcription factors... (see the rest of Fig. 12-6 if you're interested)

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## Receptor Enzymes

### When Phosphorylated IRS-1 Stimulates Another Cascade...

With two complementary results:

- (1) Glycogen synthase is maintained in an active form
- (2) Cellular glucose uptake rises due to an increase in plasma membrane glucose transporters (GLUT4)

*What is PKB?*

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## Receptor Enzymes

### But remember this glycolipid?

Phosphatidylinositol 4,5-bisphosphate, aka. PIP<sub>2</sub>

↓ PI-3K

Phosphatidylinositol 3,4,5-triphosphate, aka. PIP<sub>3</sub>

- It serves an important signaling role in the insulin cascade, as shown on the next slide...

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## Receptor Enzymes

### How is a Pathway Turned Off?

- In this instance, there is a phosphatase specific for PIP<sub>3</sub> that reconverts it to PIP<sub>2</sub>
- Thus removing the binding site for PKB
- In some advanced cancers, this phosphatase is defective, leading to high continuous levels of PKB activity
- In turn, this seems to signal further cell proliferation

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## G Protein-Coupled Receptors

- A G Protein-Coupled Receptor requires three components:
  - A plasma membrane receptor with seven TM helical segments
  - An enzyme that generates an intracellular second messenger
  - A Guanosine nucleotide-binding protein (G protein)
- The G protein is stimulated by the active receptor and converts GDP into GTP.
- The GTP-bound G Protein can then dissociate and interact with a nearby enzyme, altering its activity

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## G Protein-Coupled Receptors Why cAMP and PKA are so important

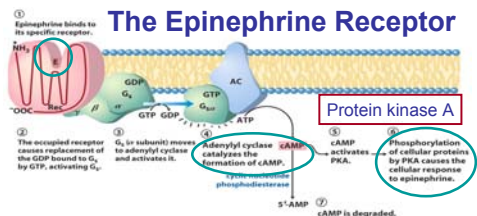


- cAMP is made in response to, but only as long as, receptor is binding to hormone
- As a soluble 2<sup>nd</sup> messenger, cAMP is short-lived, rapidly degraded by cyclic nucleotide phosphodiesterase
  - Blockers like methyl xanthines potentiate adenyl cyclase-stimulating agents, e.g. caffeine inhibits this enzyme, thereby mimicking the effects of epinephrine.
- cAMP activates protein kinase A to continue the signaling cascade, in an unusual manner...

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## G Protein-Coupled Receptors



- Activated *adenyl cyclase* makes cAMP, the “2<sup>nd</sup> messenger”
- cAMP activates “PKA” so it can phosphorylate other proteins, such as phosphorylase *b* kinase (PbK)
- PbK then phosphorylates glycogen phosphorylase *b*

**Do you remember what glycogen phosphorylase *b* does?**

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## G Protein-Coupled Receptors Why cAMP and PKA are so important

TABLE 12-3 Some Enzymes and Other Proteins Regulated by cAMP-Dependent Phosphorylation (by PKA)

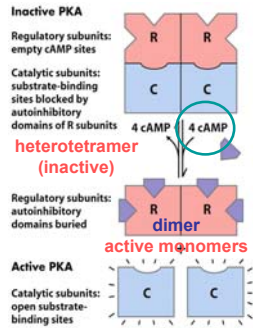
Enzyme/protein	Sequence phosphorylated*	Pathway/process regulated
Glycogen synthase	RAICSSS	Glycogen synthesis
Phosphorylase <i>b</i> kinase		
α subunit	VERRLI	Glycogen breakdown
β subunit	RMKRGSV	
Pyruvate kinase (rat liver)	GVRRKRAEL	Glycolysis
Pyruvate dehydrogenase complex (type I)	GVRRALV	Pyruvate to acetyl-CoA
Hormone-sensitive lipase	PMRRIV	Triacylglycerol mobilization and fatty acid oxidation
Phosphofructokinase-2/fructose 2,6-bisphosphatase	LQRRRISFQ	Glycolysis/gluconeogenesis
Tyrosine hydroxylase	FGRRQIL	Synthesis of L-DOPA, dopamine, norepinephrine, and epinephrine

- Protein kinase A is a heterotetramer (like Hb) which is inactive unless bound to cAMP,
- Which displaces the “autoinhibitory” dimer, releasing two active catalytic subunits
- In its active form, this kinase phosphorylates specific serine (or threonine) residues embedded in target “consensus sequences” on other proteins (see middle column above)

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## G Protein-Coupled Receptors The Role of PKA in Activation Cascades



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Fig. 12-15

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- In terms of glucose production, the activated PKA phosphorylates P<sub>gk</sub> to give active P<sub>gk</sub>
- P<sub>gk</sub> in turn, phosphorylates G<sub>p</sub> to yield the active G<sub>p</sub> form
- G<sub>p</sub> then catalyzes the production and release of glucose-1-phosphate monomers from glycogen polymers

## G Protein-Coupled Receptors Epinephrine At Work – Suddenly Startled?

- Epinephrine, released from the adrenal medulla in response to stress, has effects at several levels (see Table 23-6)
  - **Physiological**: raises heart rate, blood pressure, and dilates respiratory passages (get more oxygen to tissues!)
  - **Metabolic**: stimulates liver and muscle glycogen breakdown, and adipose TAG-lipase; stimulates glycolysis in muscle
  - **Hormonal**: stimulates pancreatic release of glucagon, inhibits release of insulin

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## The Role of Epinephrine and Glucagon in Glucose Liberation

- In the liver, Glucagon is the signal that activates the cascade for the production of glucose.
- This peptide hormone is a signal that the body is in a starvation stage and produced in pancreatic  $\alpha$  cells in response to low blood glucose levels.
- Glucagon interacts with the Glucagon "serpentine" receptor that is very similar to the  $\beta$ -Adrenergic receptor of Epinephrine
- The resulting cAMP acts as a second messenger.

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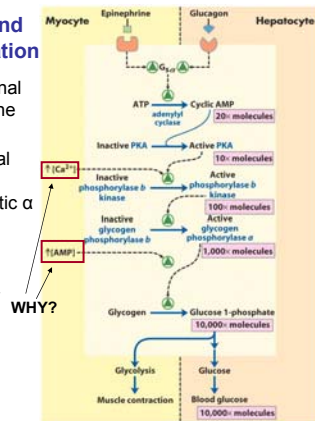


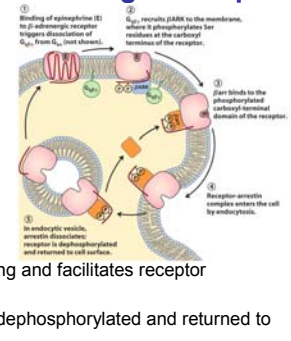
Fig. 15-25

## G Protein-Coupled Receptors Desensitization of the $\beta$ -Adrenergic Receptor

- Signal-transduction systems will undergo **desensitization** when the signal persists
- The Epinephrine receptor is no exception.
- Here, this process is mediated by a kinase ( $\beta$ ARK) that phosphorylates the receptor two times at the carboxy terminus, providing a binding site for  $\beta$ -Arrestin ( $\beta$ arr)
- The binding of  $\beta$ arr blocks G<sub>s</sub> binding and facilitates receptor sequestration via endocytosis
- In the vesicle, these receptors are dephosphorylated and returned to the surface

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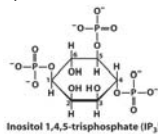
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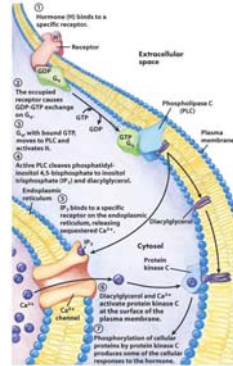
## G Protein-Coupled Receptors

### G Protein interaction with Phospholipase C

- Another class of serpentine receptors are coupled via a G protein to phospholipase C (PLC)
- PLC is responsible for the formation of diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP<sub>3</sub>) from PIP<sub>2</sub>.
- DAG and IP<sub>3</sub> are both highly potent second messengers that both effect the presence of Ca<sup>2+</sup> in the cell.



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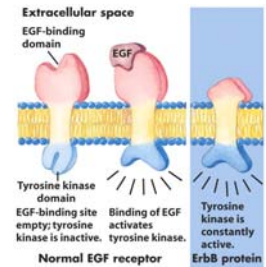


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## Oncogenes

### The Defective EFG Receptor

- Some oncogenes encode for surface receptor enzymes with defective or missing signal-binding sites
- What do you think this lack of signal binding would do for the catalytic portion of the enzyme?
- For the EFG receptor, the tyrosine kinase domain is always active, resulting in the cell thinking that the "divide" signal is always present
- What would this cause?
- What if the catalytic portion of the insulin receptor never turned off?



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## Oncogenes

### Mutant Forms of Cell Cycle Regulatory Proteins

- **Oncogenes** are cancer causing genes that have been mutated from their original form resulting in protein products that facilitate rapid, uncontrolled cell proliferation
- **Proto-oncogenes** are cellular genes that usually encode a regulatory protein and can be converted into an oncogene by mutation.
  - Chromosomal rearrangement, chemical agents and radiation are some of the common factors that produce this mutation.
- The mutations that produce the oncogenes are genetically **dominant**, meaning that you only need to be heterozygous for the oncogene to translate and a tumor result.
- An oncogene can transcribe any of a number of proteins involved in the regulation and/or mechanism of cell division
- One example is the oncogene that encodes the epidermal growth factor receptor (EGF), the *erbB* gene

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## Tumor Suppressor Genes

### Defects Result in Run-away Cell Division

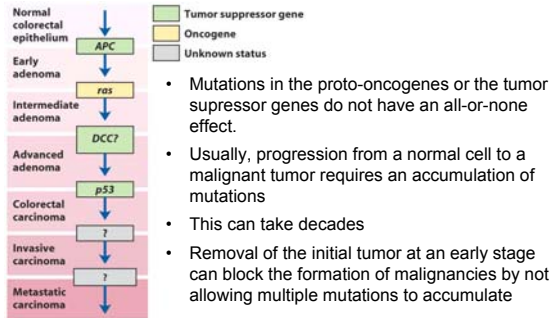
- **Tumor suppressor genes** encode proteins that normally restrain cell division
- Unregulated growth due to these defective genes is genetically **recessive**, meaning you must have two copies of the defective gene for it to present.
- In a person who inherits one correct copy and one defective copy of the gene, every cell has one defective gene.
- This would require a mutation of the correct copy in any one of the cells containing the defective copy to yield a doubly mutant cell that could produce a tumor.
- It is very unlikely that a person homozygous for the correct gene would manage to mutate both copies in a single cell, but it can occur
- Being inherently homozygous for the defective copy results in organisms that are not viable

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## Tumor Suppressor Genes & Oncogenes

### Development of Malignancies



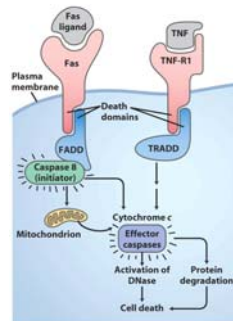
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## Apoptosis

### Programmed Cell Death

- Many cells control the time of their own demise through **programmed cell death** (aka. Apoptosis)
- The regulatory mechanisms that control apoptosis involve proteins that regulate the cell cycle
- The signal for apoptosis can come from within the cell or from the external environment.
- The monomeric products of protein and DNA degradation are released in a controlled manner that allows them to be taken up and reused by neighboring cells



**Waste Not, Want Not**

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