Molecular Physiology: 
Enzymes and Cell Signaling

Proteins
- Polymers of amino acids
- Have complex 3D structures
- Are the basis of most of the structure and physiological function of cells

See Boxes 2.1 and 2.2

Binding
- Much of protein function involves binding ligands
  - Ions, small molecules, macromolecules, etc.
- Form noncovalent bonds with ligands
  - Individual bonds weak
  - Stable binding requires multiple bonds to be formed

Protein Specificity
- Proteins and ligands come together through random collisions
- incorrect ligand
  - few bonds form, ligand dissociates quickly
- correct ligand
  - multiple bonds form, ligand and protein remain associated for a long time interval

Enzymes
- Protein catalysts
- Bind ligand and chemically alter the ligand
  - Transform substrates into products
- Lower activation energy of reactions
  - Increase reaction rate, allow non-spontaneous reactions to occur
- Are not permanently altered in the reaction
- very specific in the reactions they catalyze
  - hexokinase adds PO_4 to D-glucose but not L-glucose

Enzymatic Reactions
Enzyme + Substrate
→ Enzyme-Substrate Complex
→ Enzyme-Product Complex
→ Enzyme + Product
Enzyme Activity

• Ability of enzyme to convert substrate into product (i.e. reaction rate)
• Key factors influencing enzyme activity
  1. Enzyme Concentration
  2. Substrate Concentration
  3. Catalytic Effectiveness
  4. Affinity of the enzyme for the substrate
  5. Binding of additional ligands

Enzyme Concentration

• Increased concentration = more points for catalysis to take place
• Proportional (linear) increase in reaction rate with increasing enzyme concentration

Substrate Concentration

• Hyperbolic or sigmoidal relationship
• low [S], low reaction rate
• ↑ [S], ↑ rate
• As ↑[S], ↑ % of enzyme molecules occupied in E-S complexes at a given time
• Eventually, enzyme is saturated with substrate
  – No further ↑ rate w/ ↑[S]

See Fig 2.12

Substrate Concentration

• $V_{\text{max}}$
  – maximum velocity of the reaction
  – velocity at the point when enzyme is saturated with substrate
• $V_{\text{max}}$ depends on…
  – Concentration of enzyme
  – Catalytic effectiveness of the enzyme

See Fig 2.12

Catalytic Effectiveness

• The ability an enzyme molecule to rapidly convert bound substrate into product
• Expressed by an enzyme’s turnover number ($k_{\text{cat}}$)
  – # substrate molecules an enzyme molecule can convert into product per second
• Influenced by
  1. The extent activation energy is lowered by the enzyme
  2. Time course of required changes in enzyme conformation (shape)

See Figs 2.13 and 2.15

Affinity for the Substrate

• Ability of enzyme to effectively bind with colliding substrate
• Michaelis-Menton constant ($K_M$)
  – [S] at which ½ $V_{\text{max}}$ is achieved
  – a measure of an enzyme’s affinity for a substrate
  – (↑ $K_M$, ↓ affinity)
**Binding of Additional Ligands**

- Enzyme may have additional binding sites for other ligands
- Binding of ligands may influence enzyme shape
  - Influences substrate affinity and catalytic effectiveness

**Regulation of Enzyme Activity**

- Enzymes are *KEY* to controlling physiological function
  - Regulate ability of enzyme to catalyze reactions, regulate biochemical function
- Enzyme activity can be controlled by
  1. Regulating the amount of enzyme present (regulating gene expression and enzyme degradation)
  2. Regulating the affinity and catalytic effectiveness of existing enzyme molecules

**Possible Ways of Controlling Gene Expression**

1. control of transcription
   - how/when a gene is transcribed into RNA
2. control of primary transcript processing and transport to the cytosol
   - splicing, etc.
3. control of translation
   - selecting which mRNAs are translated

**Gene Regulatory Proteins**

- **Repressors**
  - proteins that bind DNA and block transcription
- **Activators**
  - proteins bind to promoters that are only marginally functional in binding RNA polymerase alone
  - Enable RNA polymerase to bind
- Function influenced by concentrations of specific ligands

**Gene Regulatory Proteins**

- *eukaryotic transcription involves…*
  - Transcription factors
  - Activators and repressors
  - Moderator complexes
  - Nucleosome proteins
- Any/all can be affected by the specific concentration of various ligands

**Ligand Function in Gene Regulation**

- Example: Glucocorticoid Receptor
  - bonds glucocorticoid
    - Steroid hormone produced by adrenal glands
    - activates multiple genes for increased glucose production in the liver
Modifying Activity of Existing Enzymes

- Metabolic pathways
  - Long chains of linked enzyme-catalyzed reactions
- Require coordinated function of the enzymes
  - Reaction rates (enzyme activity) must be regulated
  - Regulated by controlling enzyme-substrate affinity and/or catalytic effectiveness

Feedback Inhibition

- Feedback Inhibition
  - enzyme activity inhibited by binding of a product from a subsequent reaction
  - As $[P_{\text{final}}]$ ↑, enzyme affinity ↓, and $P_{\text{final}}$ production ↓

Allosteric Modification

- Allosteric enzymes
  - can assume > 1 shape
- Enzyme may have multiple binding sites (active site + site for regulating ligand)
- Binding of ligand alters shape of enzyme, altering affinity

Regulation of Enzyme Activity

- Positive Regulation
  - enzyme activity increased by binding of a product from a subsequent reaction As $[P_{\text{final}}]$ ↑, enzyme affinity ↑

Protein Phosphorylation (Covalent Modification)

- Common method of altering shape of allosteric enzymes and other proteins
  - $\text{PO}_4^2-$ has large influence on protein shape
  - Reversible
- protein phosphorylation catalyzed by protein kinases
- dephosphorylated by protein phosphatases

Cell Signaling

- Animal cells need to coordinate their activities in order to maintain homeostasis
- Must communicate chemically with one another to influence each other's physiology
Methods of Signaling Between Cells

1. **Endocrine signaling**
   - Broadcast to entire organism
2. **Paracrine signaling**
   - Signal molecules sent locally through ECF to neighboring cells
3. **Neuronal signaling**
   - High speed conduction of information between individual cells through a neuron
4. **Contact-dependent signaling**
   - Direct contact through signaling molecules in the cell membrane

Receptor Function

- Signal molecule binds to a receptor protein (reception)
- Intracellular signal generated in response (transduction)
- **Signaling cascades**
  - Signals passes from one intracellular carrier to another until an enzyme is activated
  - Behavior of cell changes

Signaling Cascade Function

1. **Transfer** signal from point of reception to cell machinery responsible for the response
2. **Transform** signal into a molecule that can elicit a response
3. **Amplify** the signal received

Hydrophobic Signals

- **Steroid and Thyroid Hormones**
  - Nonpolar
    - Pass directly through the cell membrane
    - Bind to protein receptor in cytoplasm or in nucleus
    - To regulatory sequences for specific genes on DNA
    - Modifies expression
Hydrophilic Signals
- Cannot penetrate plasma membrane
- Bind to surface receptors (transmembrane proteins)
- Three types of receptors
  1. Ion-channel-linked receptors
  2. G-protein-linked receptors
  3. Enzyme-linked receptors

Ion Channel-Linked Receptors
- Binding of chemical messenger causes ion channel to open
- Electrical response (neuronal and muscular function)

G-protein-linked receptors
- Binding of signal induces release of a subunit of a membrane-bound G-protein
  - Initiates a cascade of effects
  - 2nd messenger system

Enzyme-linked receptors
- Enzyme activity is switched on at the cytoplasmic end of the receptor
- Enzyme generates further intracellular signals