

METABOLIC BIOCHEMISTRY (6 CFU)

5 CFU Prof. Giovanna Di Nardo

1 CFU Prof. Sheila Sadeghi

48 hours lectures on:

- Monday 2-4 pm
- Tuesday 11 am -1 pm

Reference book:

- Lehninger Principles of Biochemistry (check the first slide of each lecture)
- Slides and lectures notes

EXAM:

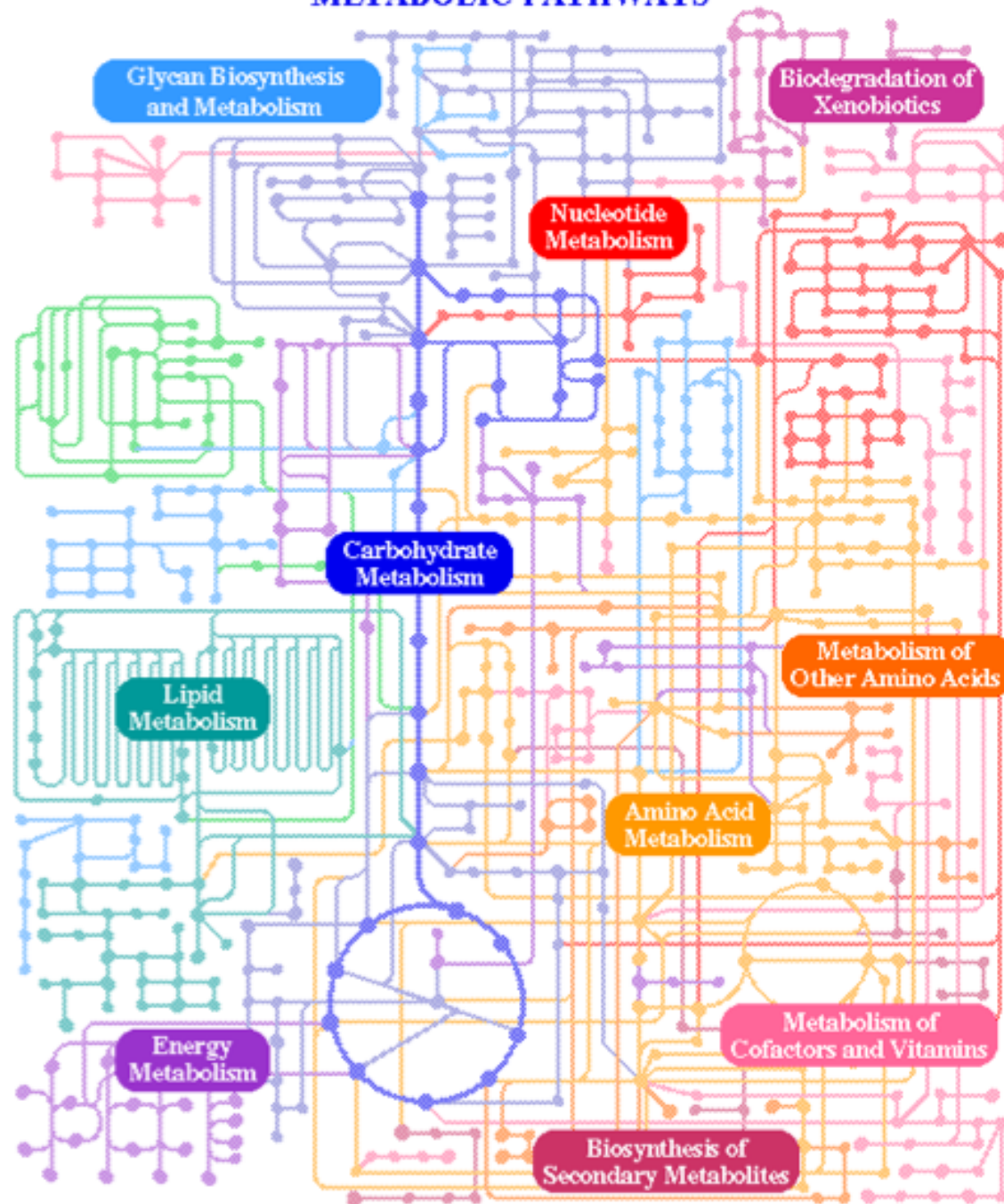
- 3 written questions (limited space) that can include all the lectures topics
- Oral exam only in specific cases.

EXTRA-ACTIVITIES (quiz on a literature paper in the last part of the course):

- Counted +1 point to the total mark, only when positive

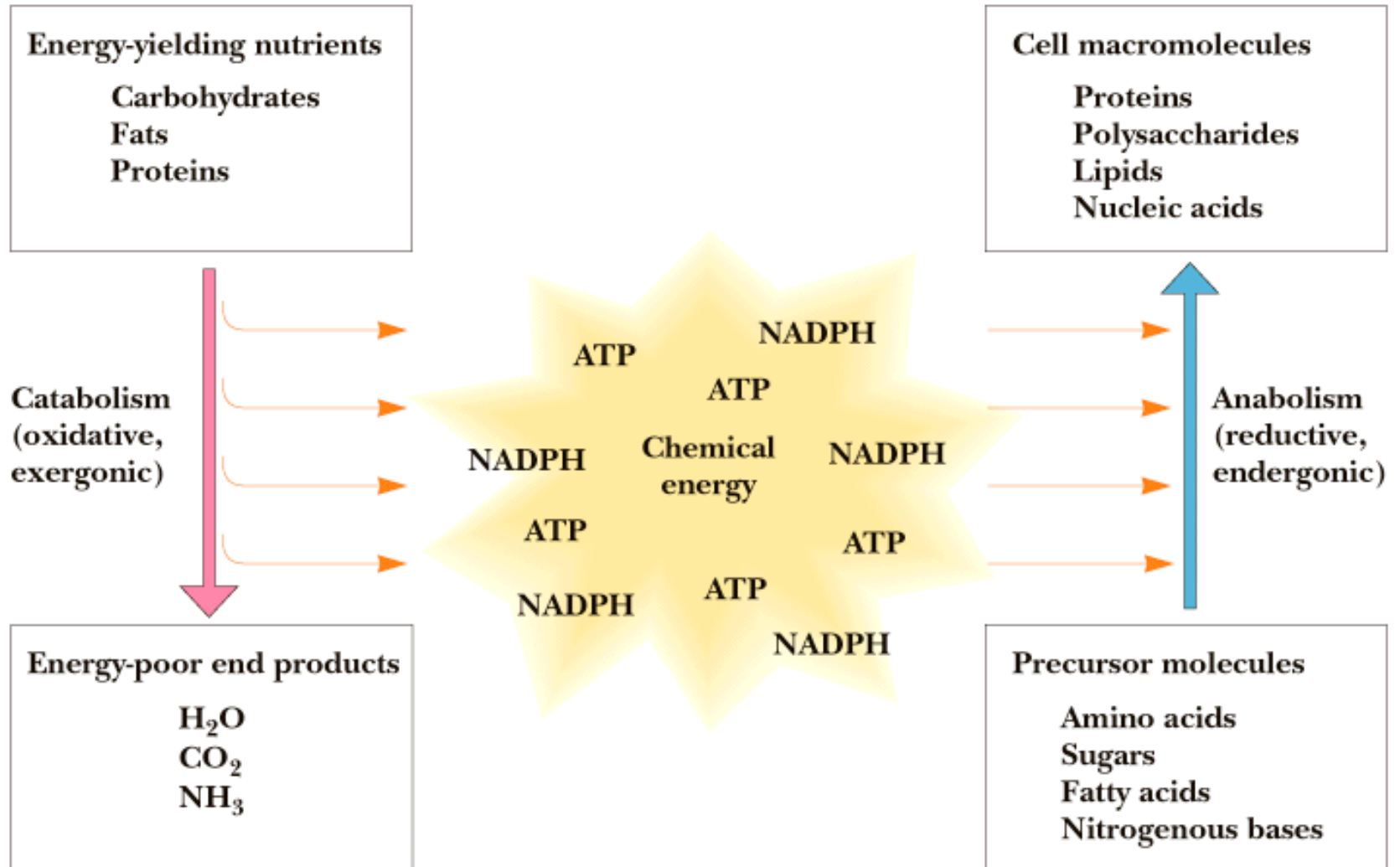
1. METABOLIC REGULATION: GENERAL CONCEPTS

METABOLIC PATHWAYS



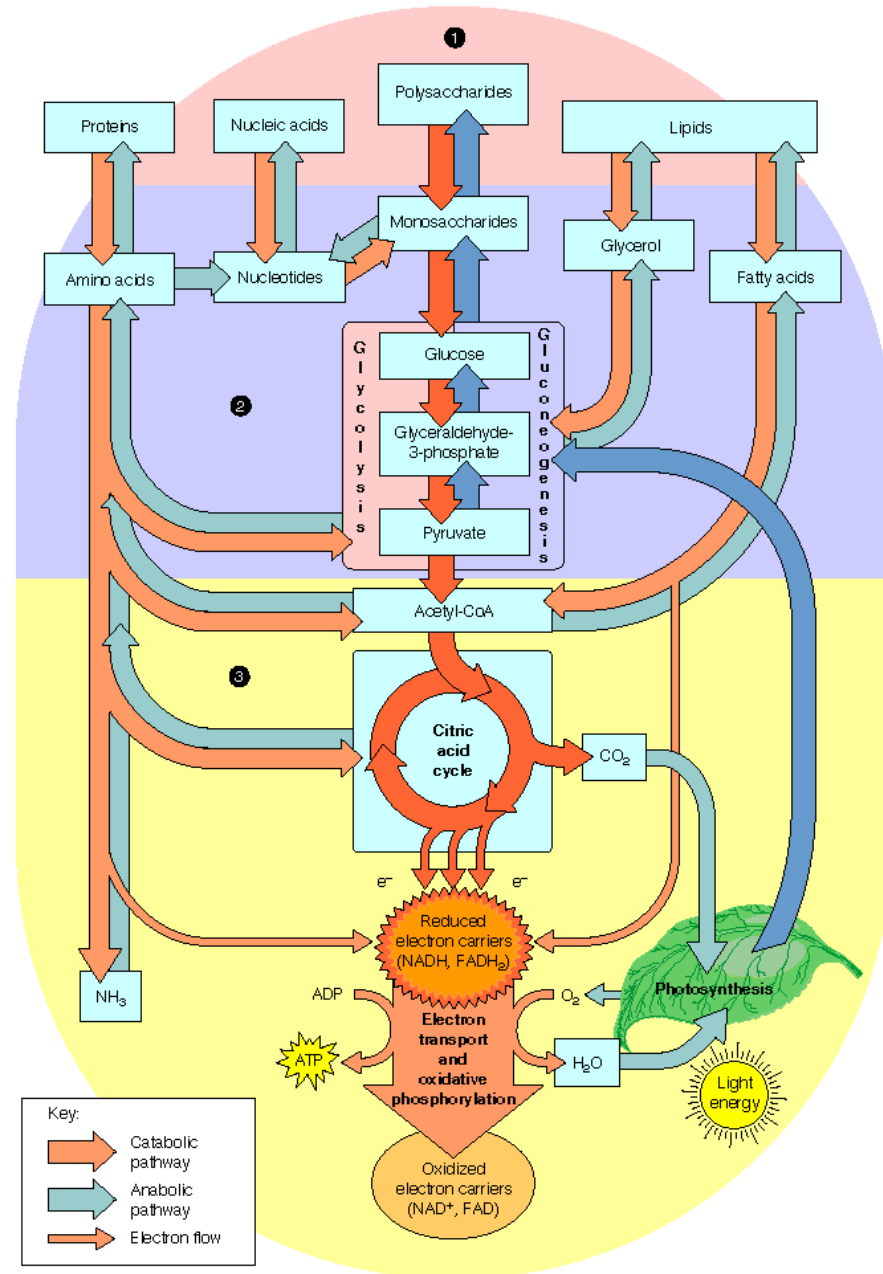
OVERVIEW

Figure 18.4

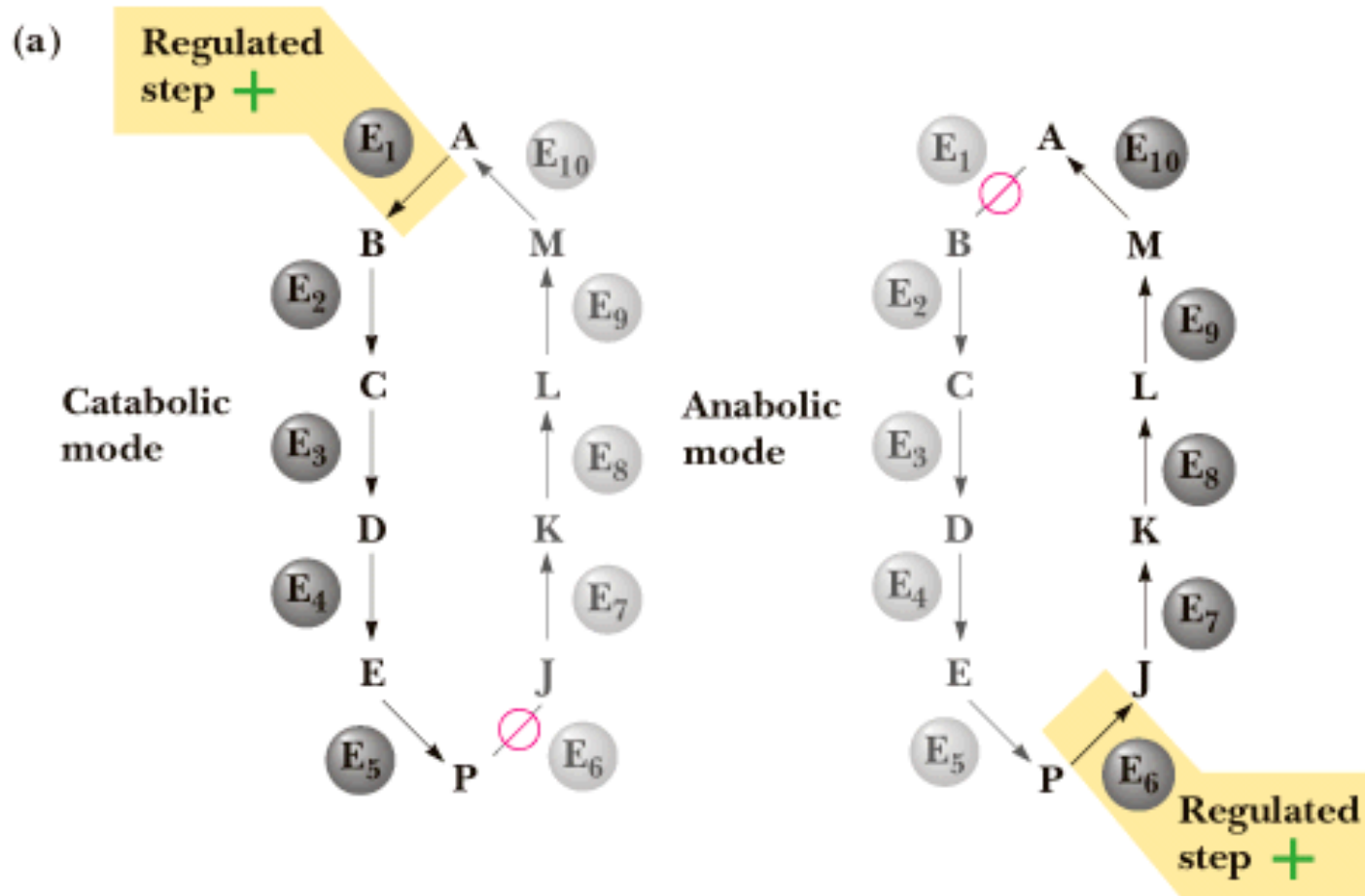


OVERVIEW

- Catabolic pathways converge to a few end products
- Anabolic pathways diverge to synthesize many biomolecules
- Some pathways serve both in catabolism and anabolism
- Such pathways are **amphibolic**
- Pathways consist of sequential steps
- The enzymes may be separate
- Or may form a multi-enzyme complex
- Or may be a membrane-bound system
- New research indicates that multi-enzyme complexes are more common than once thought



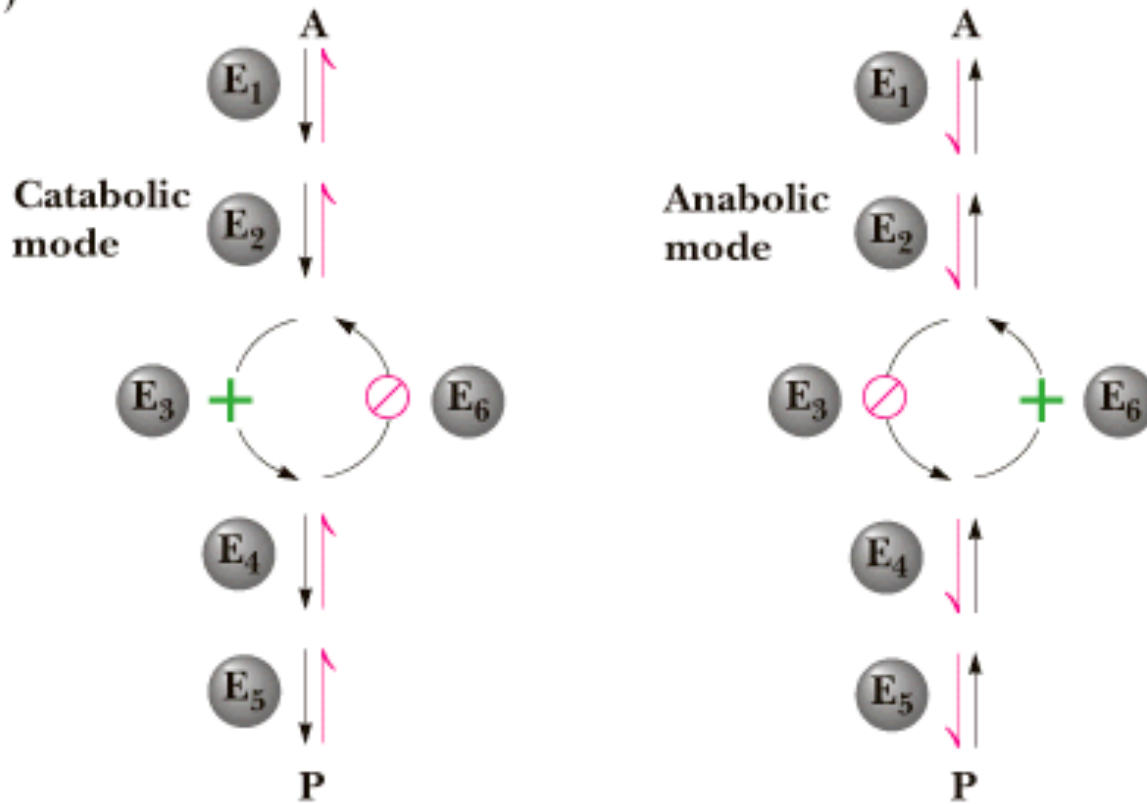
OVERVIEW



Activation of one mode is accompanied by reciprocal inhibition of the other mode.

OVERVIEW

(b)



OVERVIEW

Table 14.1

Contrasting characteristics of catabolism and anabolism

Catabolism

Leads to degradation of biomolecules

Overall process of chemical oxidation and formation of reduced cofactors of NADH, NADPH, FADH₂

Release of chemical energy (exergonic) and production of ATP from ADP

Convergence of pathways

Anabolism

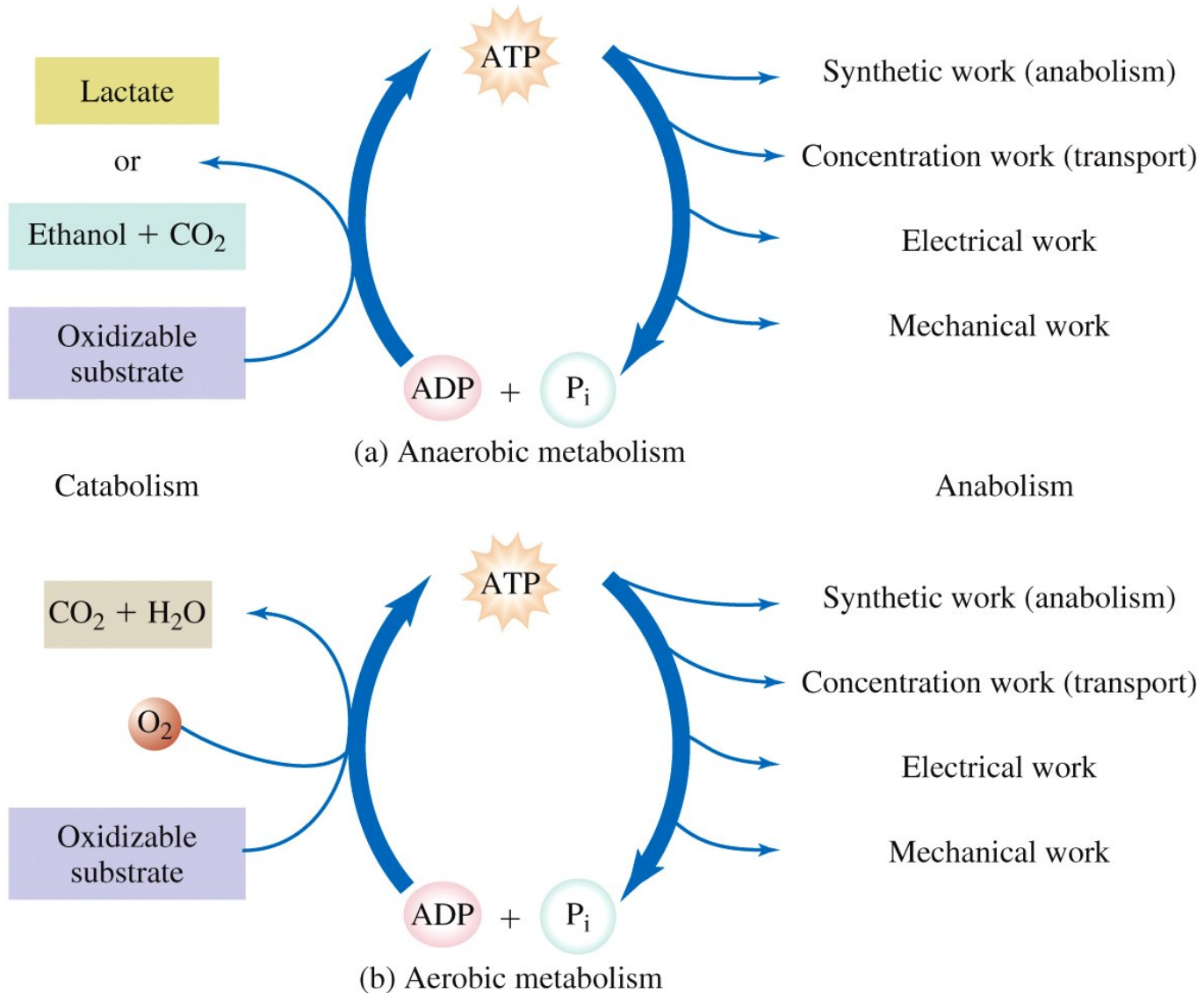
Synthesis of biomolecules

Overall process of chemical reduction and formation of oxidized cofactors NAD⁺, NADP⁺, FAD

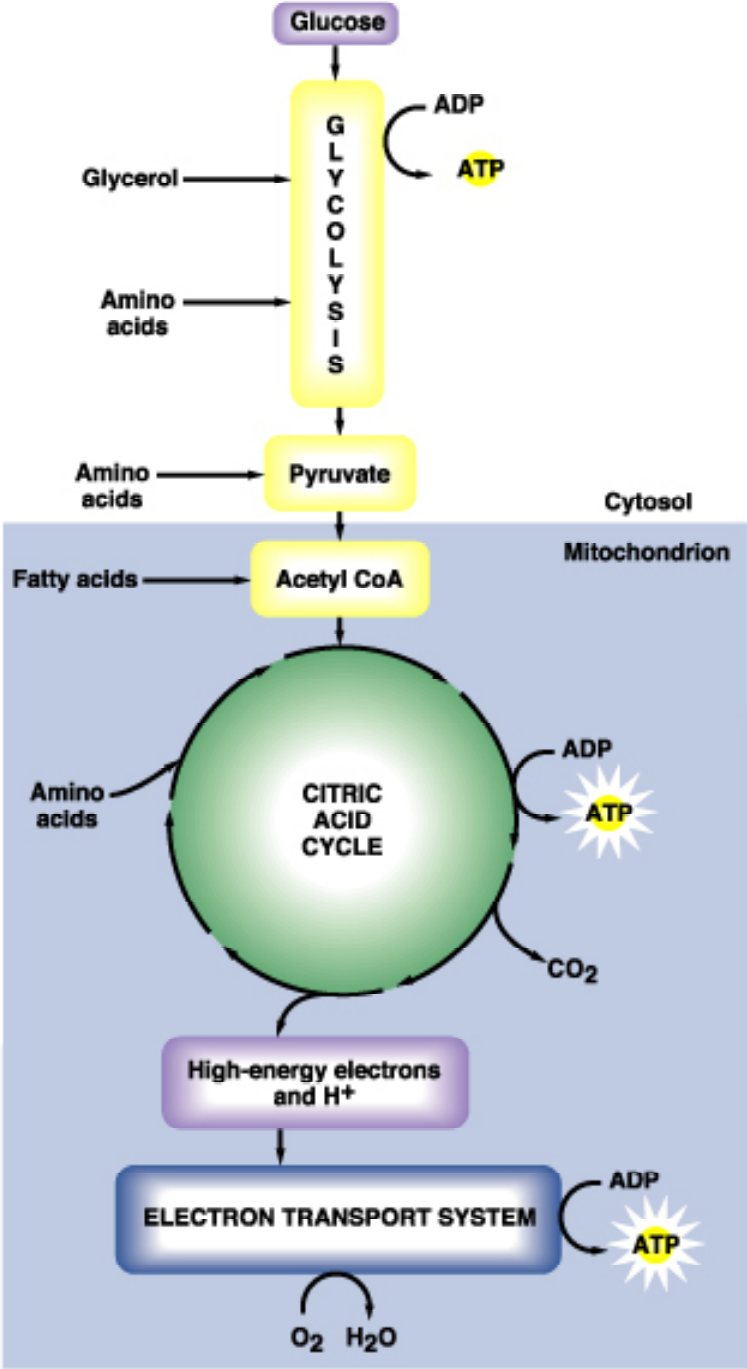
Requirement for energy input (endergonic) and use of ATP

Divergence of pathways

OVERVIEW



OVERVIEW



ENZYMES

Table 14.2

Types of chemical reactions in metabolism correlated to enzyme classes

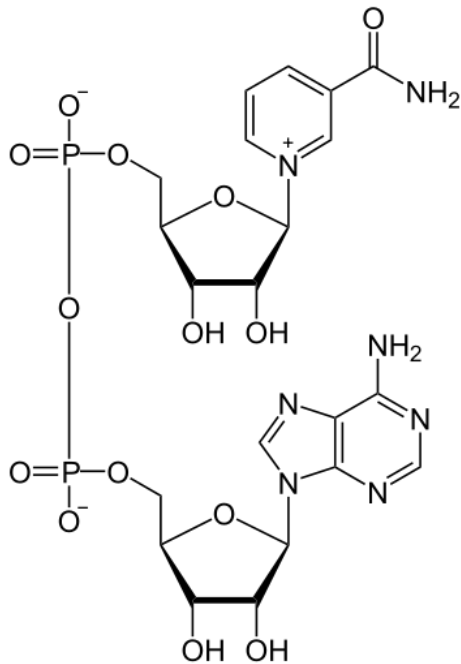
Type of Reaction	Enzyme Class	Description of Reaction
1. Oxidation–reduction	Oxidoreductases (dehydrogenases)	Transfer of electrons
2. Group transfer	Transferases	Transfer of a functional group from one molecule to another or within a single molecule
3. Hydrolytic cleavage (hydrolysis)	Hydrolases	Cleavage of bonds by water (transfer of functional groups to water)
4. Nonhydrolytic cleavage	Lyases	Splitting a molecule by nonhydrolytic processes
5. Isomerization and rearrangement	Isomerases	Rearrangement of functional groups to form isomers
6. Bond formation using energy from ATP	Ligases	Formation of carbon–carbon and other bonds with energy from ATP

Table 14-2 Concepts in Biochemistry, 3/e

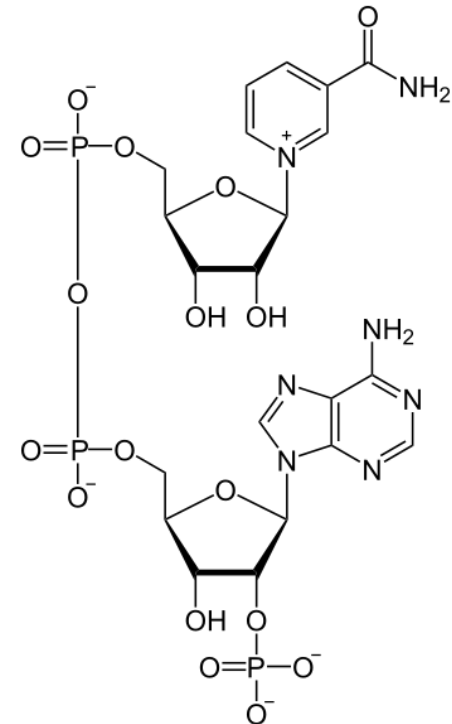
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REDOX IN METABOLISM

- NAD^+ collects electrons released in catabolism
- Catabolism is oxidative - substrates lose reducing equivalents, usually H^+ ions
- Anabolism is reductive - NADPH provides the reducing power (electrons) for anabolic processes

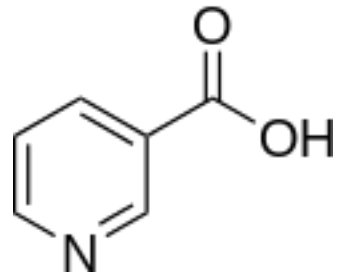
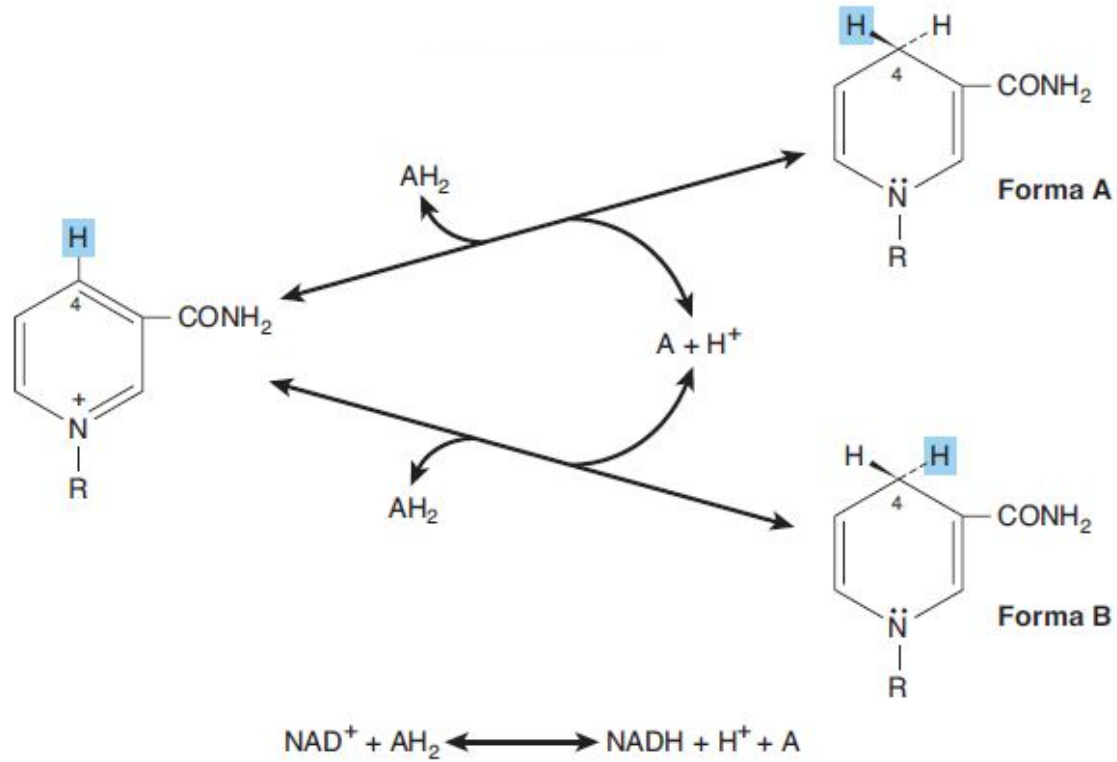


Nicotinamide adenine dinucleotide



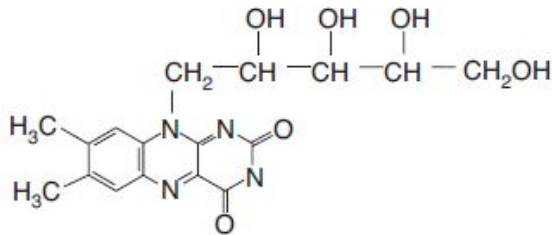
Nicotinamide adenine dinucleotide phosphate

REDOX IN METABOLISM

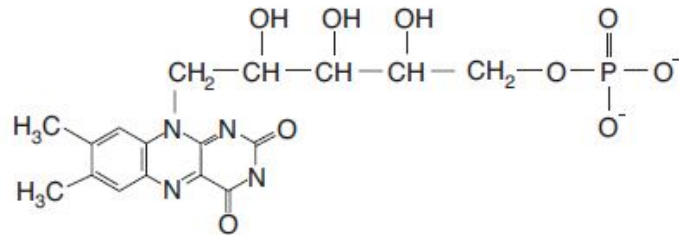


Niacin or vitamin B₃

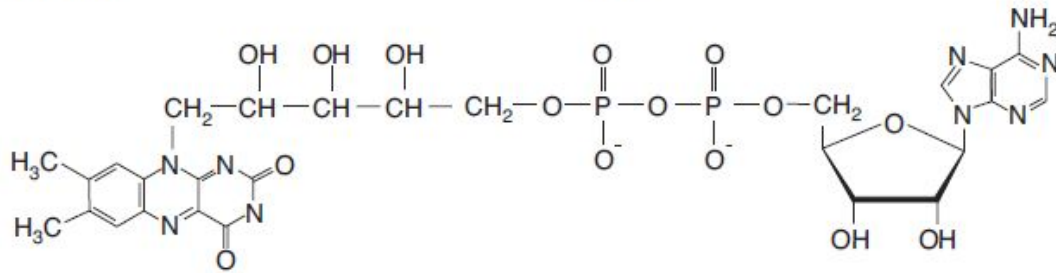
Redox in Metabolism: FMN and FAD



Riboflavina



FMN

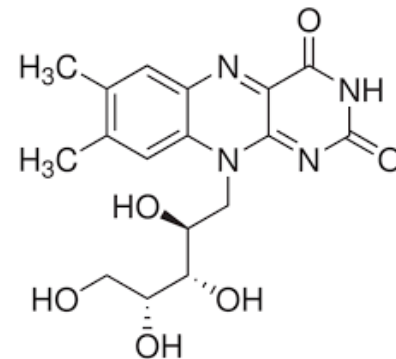


FAD

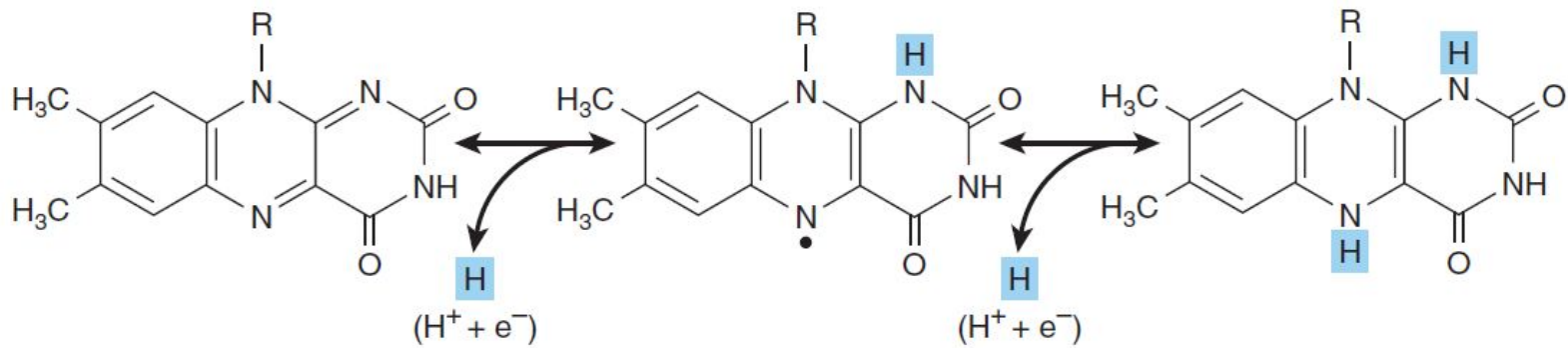
FMN: Flavin mononucleotide

FAD: Flavin adenine dinucleotide

Riboflavin or vitamin B₂



Redox in Metabolism: FMN and FAD



REDOX IN METABOLISM

Table 14.3

Relative oxidation levels of carbon in functional groups

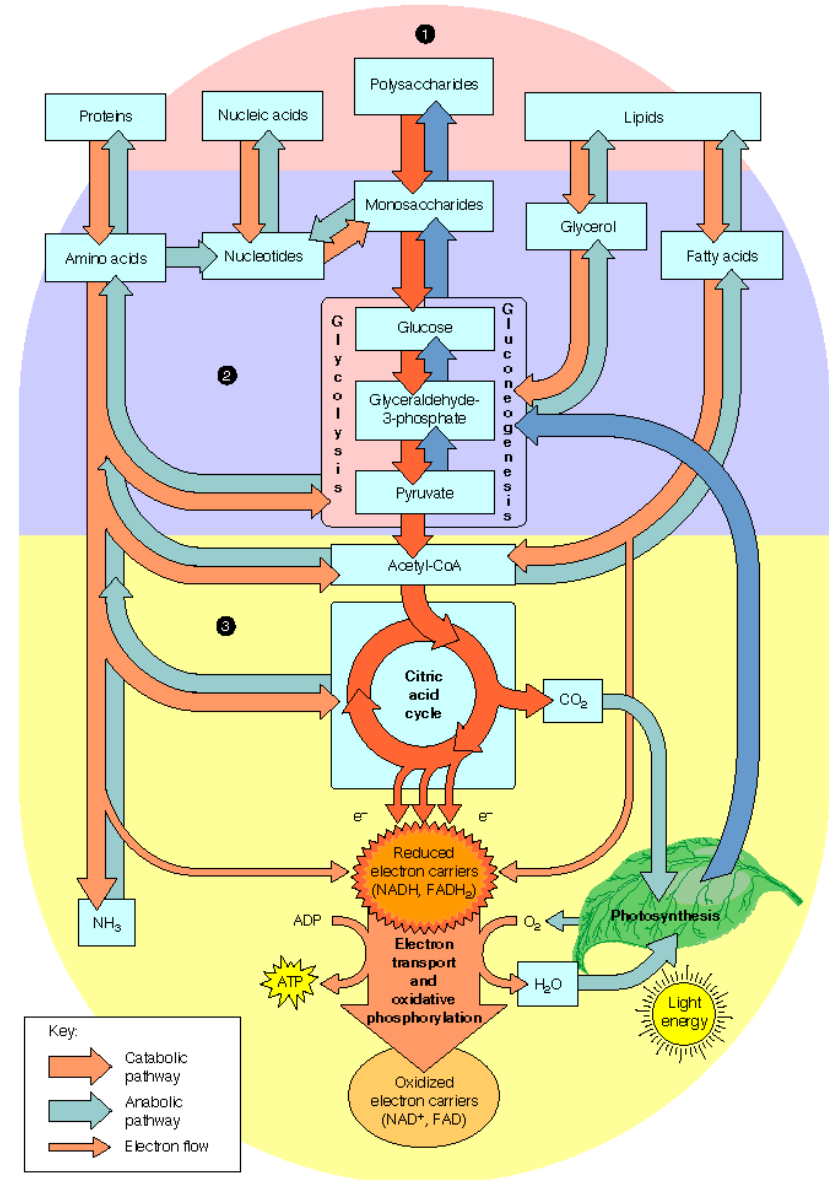
Functional Group ^{a,b}	Name	Geometry of Carbon Center	Relative Oxidation Level
CH ₄	Methane	Tetrahedral	Lowest
CH ₃ —	Methyl	Tetrahedral	
CH ₂ — 	Methylene	Tetrahedral	
RHC=CHR'	Methene	Planar	
RCHR' OH	Alcohol	Tetrahedral	
RCH O	Aldehyde	Planar	
RCR' O	Ketone	Planar	
RCX O	Carboxylic acid or derivative	Planar	
O=C=O	Carbon dioxide	Planar (linear)	

^aThe carbon center undergoing oxidation is in red.

^bR, R' = alkyl or aryl group; X = —OH (acid), —OR (ester), —NH₂ (amide).

MECHANISMS FOR CONTROLLING METABOLIC PATHWAYS

- Control of enzyme levels
- Control of enzyme activity
- Compartmentation
- Hormonal regulation
- Distributive control of metabolism
- Specialization of organs



Factors influencing enzymes levels and activity

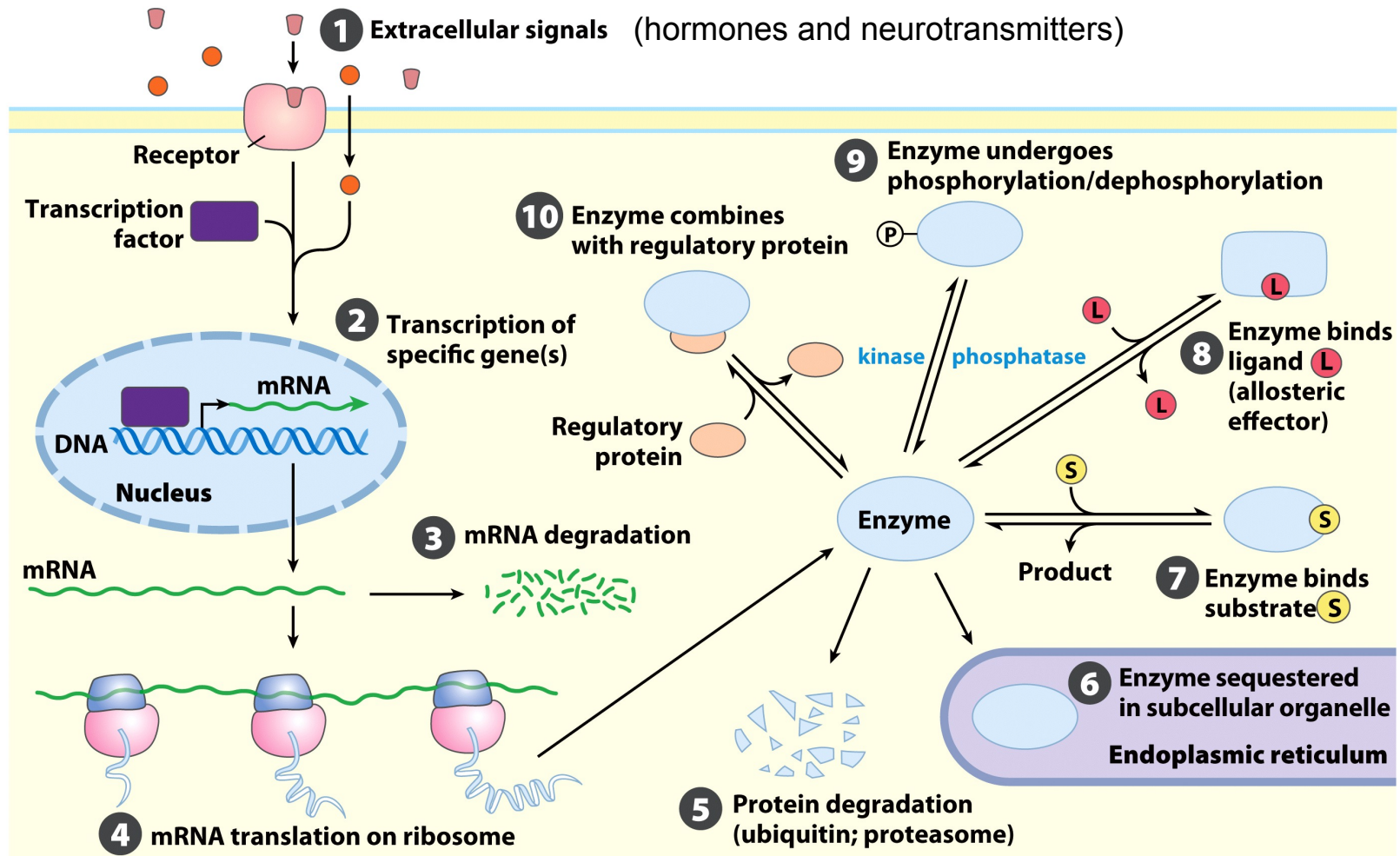


Figure 15-2
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- Control of enzyme levels

- The concentrations of different enzymes vary widely in cellular extracts.
- Enzyme levels are controlled in large part by controlling the enzyme's rate of synthesis.
- Enzyme synthesis can often be induced or repressed by the presence or absence of certain metabolites.

Mechanism of activation of the transcription factor ChREBP (carbohydrate response element binding protein). In hepatocytes it is phosphorylated and it cannot go into the nucleus. When the protein is dephosphorylated by PP2A, it can enter the nucleus, where it undergoes a second dephosphorylation and can bind to Mix. The complex ChREBP-Mix binds the carbohydrates response element (ChoRE) promoter and stimulates the transcription.

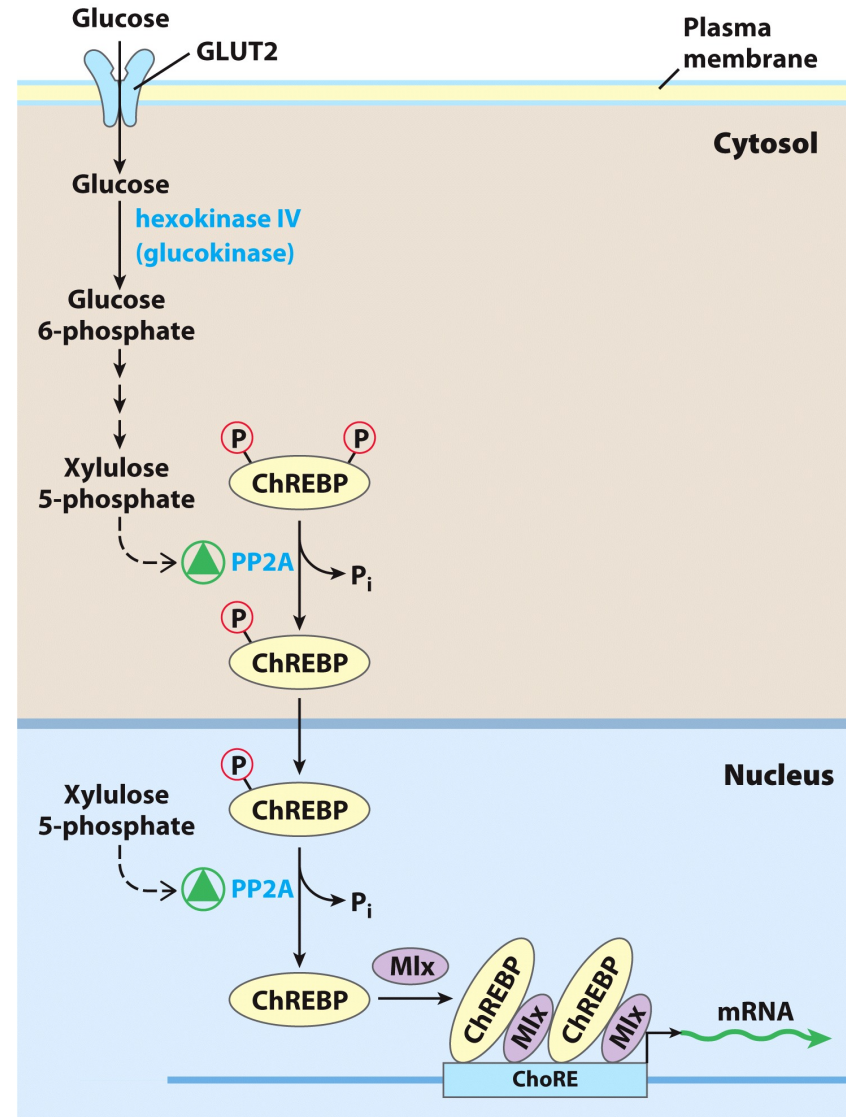


Figure 15-23
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- Control of enzyme levels

- The concentrations of different enzymes vary widely in cellular extracts.
- The rate of enzyme degradation can also be a factor in controlling enzyme levels.

TABLE 15-1**Average Half-Life of Proteins
in Mammalian Tissues**

Tissue	Average half-life (days)
Liver	0.9
Kidney	1.7
Heart	4.1
Brain	4.6
Muscle	10.7

Table 15-1
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• Control of enzyme activity

- The catalytic activity of an enzyme can be controlled in three ways:

1. by substrates concentration (Michaelis-Menten kinetics).

Intracellular substrates concentration are often similar to K_m or lower (limiting factors).

2. by reversible interaction with ligands (see next slide)

Low molecular weight ligands can interact with enzymes and exert allosteric effects. Frequently, the first or most important step in a metabolic pathway is under allosteric control in this way, enabling a cell to turn on or turn off an entire pathway easily and efficiently.

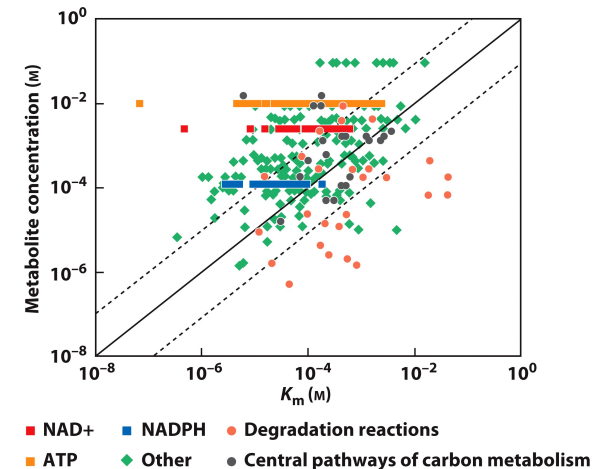
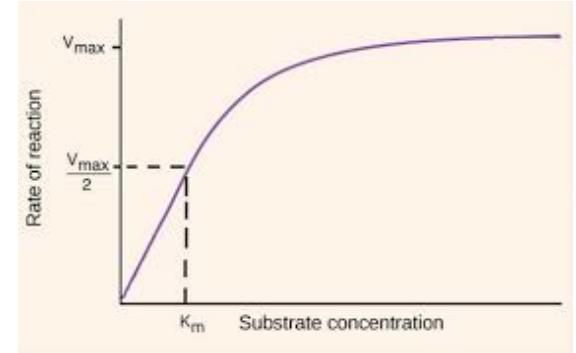
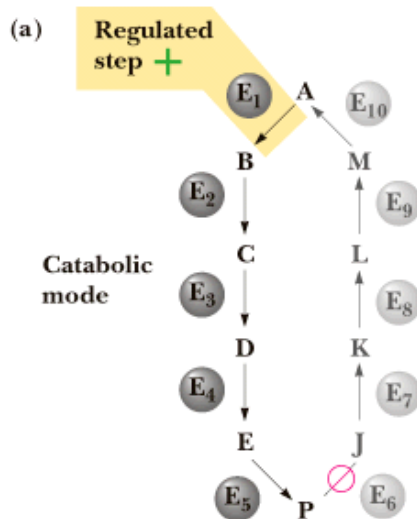


Figure 15-4
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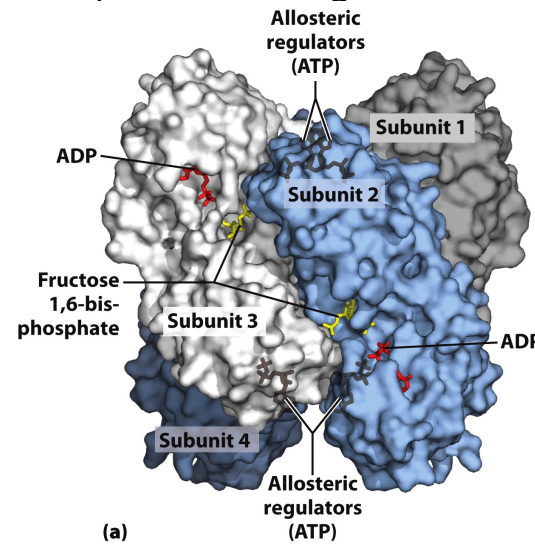


3. by covalent modification of the enzyme itself.

Covalent modifications include phosphorylations, ADP-ribosylation, and other, more complex alterations. Covalent modification often occurs as a result of action of regulatory cascades. Glycogen metabolism is regulated in this fashion. Enzymes that phosphorylate other enzymes are called protein kinases.

- Control of enzyme activity: allosteric regulation

- Typically associated with enzymes that catalyze irreversible reactions
- Allosteric regulators can cause feed back or feedforward regulation
- Allosteric regulators are often related to the energy state of the cell
- This type of regulation allows for immediate response to changes in metabolic flux (milliseconds to seconds)
- Functions at local level



Regulation of phosphofructokinase-1 (PFK-1). In *E. coli*, the protein is composed by 4 subunits, each one with a catalytic site where fructose 1,6-bisphosphate and ADP are in contact. There is a third allosteric regulatory site where ATP bind.

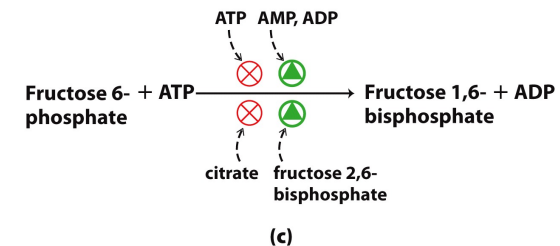
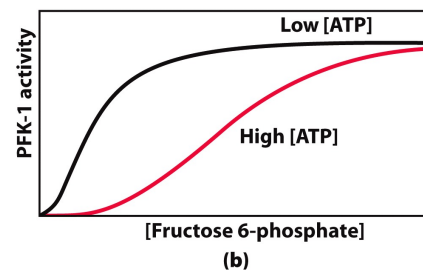
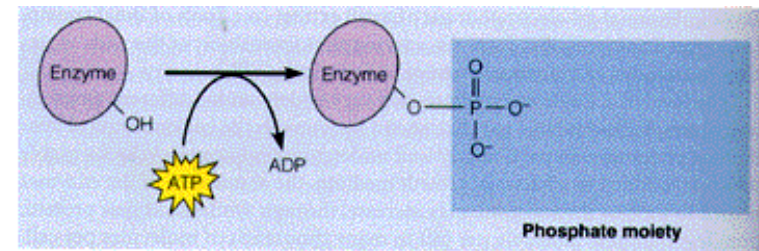


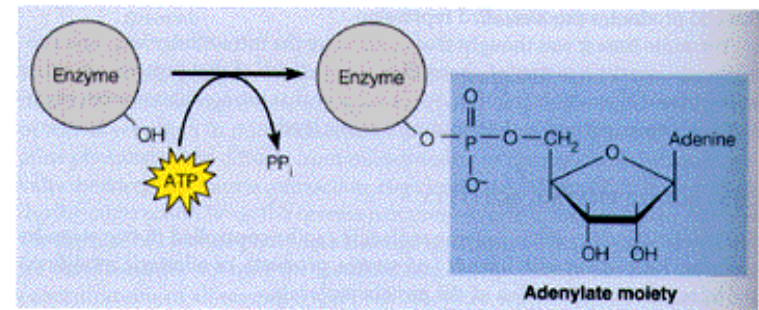
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• Control of enzyme activity: covalent modification

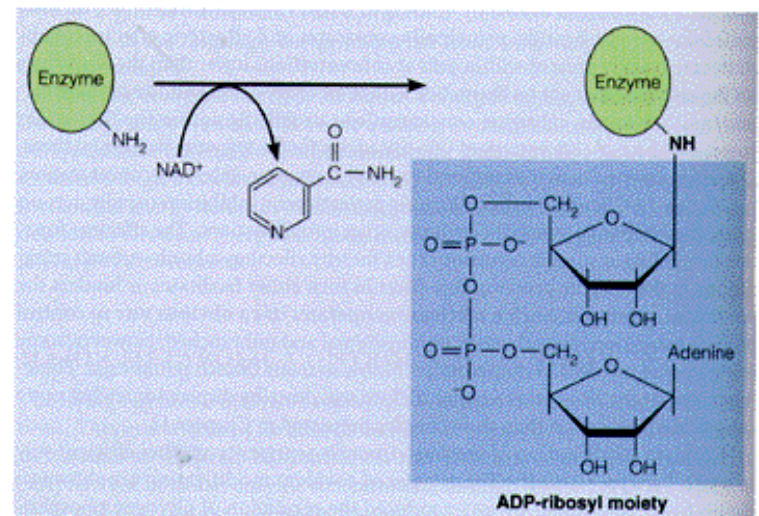
- Covalent modification is usually the last step in signal transduction pathway
- Allows pathways to be rapidly up or down regulated by small amounts of triggering signal (HORMONES)
- Last longer than do allosteric regulation (seconds to minutes)
- Functions at whole body level



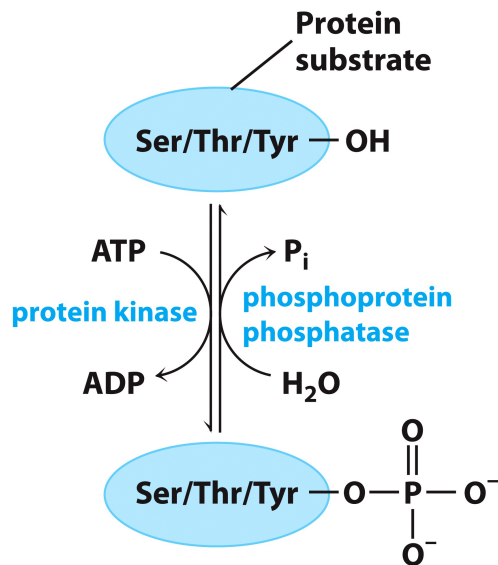
(a) Phosphorylation



(b) Adenylylation

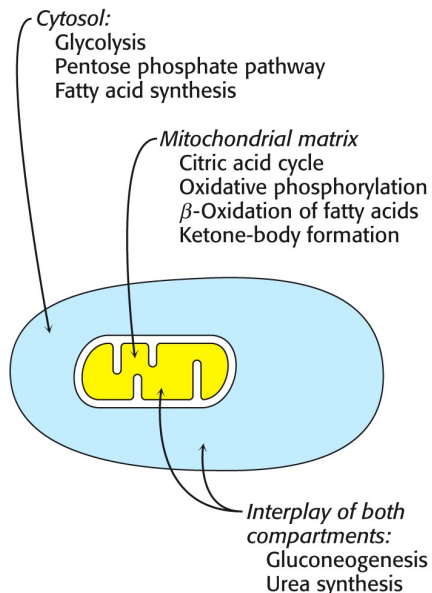
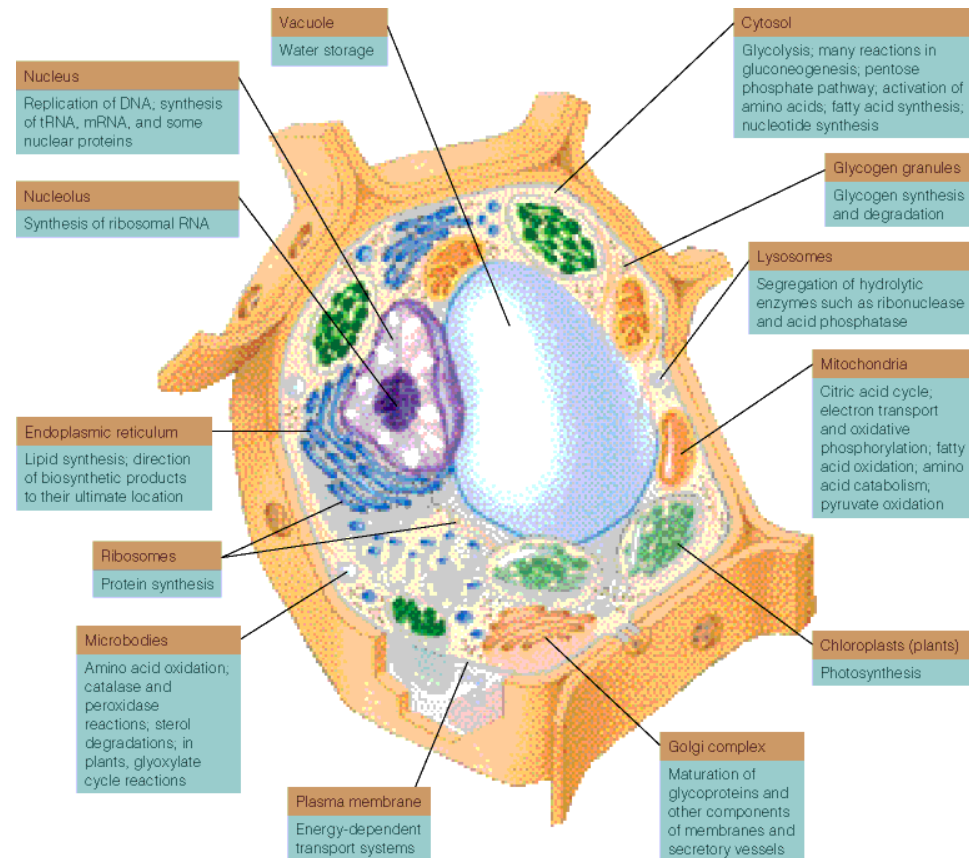


(c) ADP-ribosylation



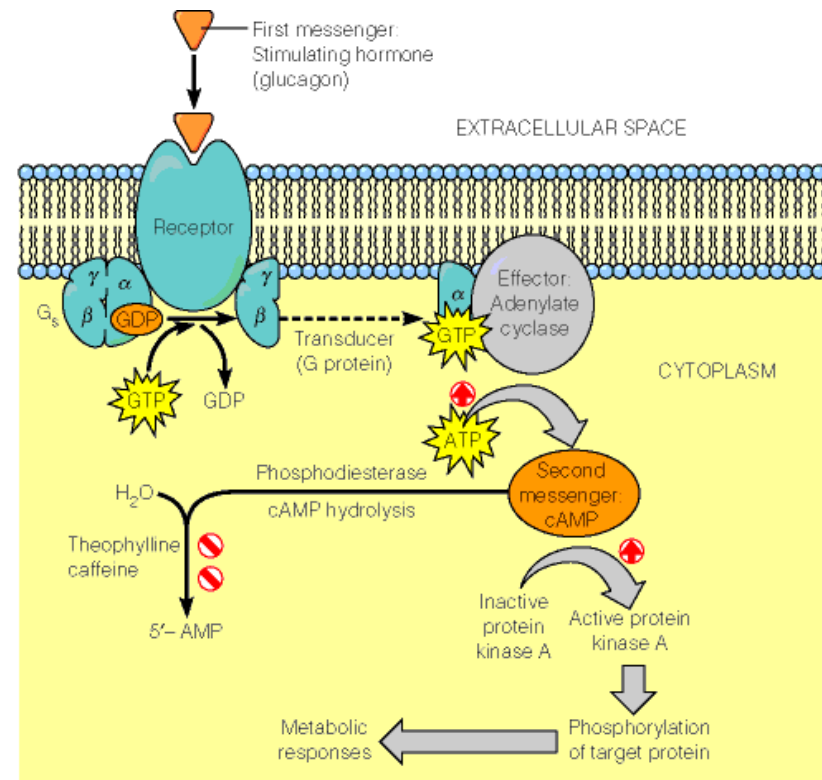
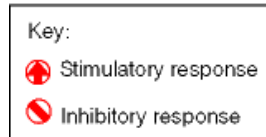
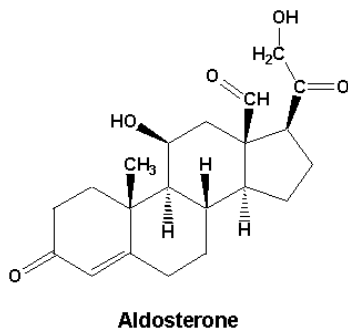
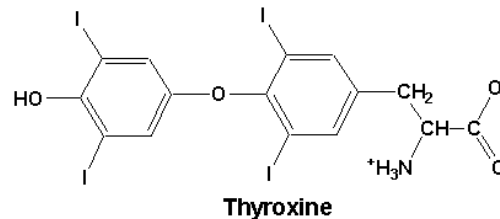
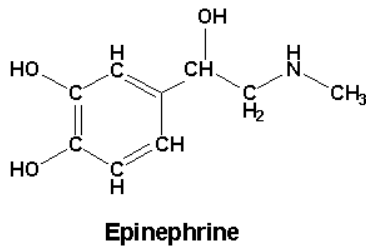
• Compartmentation

- Eukaryotic cells contain many different organelles and enzymes are distributed unevenly throughout them.
- For example, RNA polymerases are found in the nucleus and the nucleolus, where DNA transcription occurs.
- Enzymes of the citric acid cycle, on the other hand, are found in the mitochondria.
- Enzymes of fatty acid synthesis are found in the cytoplasm, but enzymes for fatty acid degradation are found in mitochondria.



• Hormonal regulation

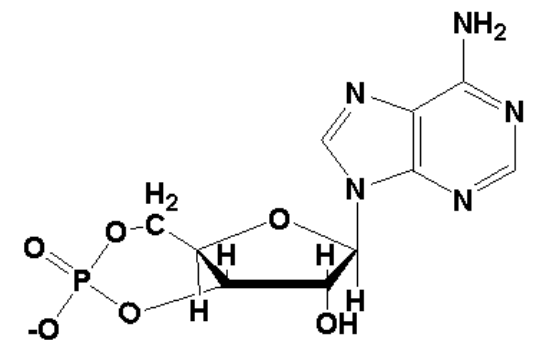
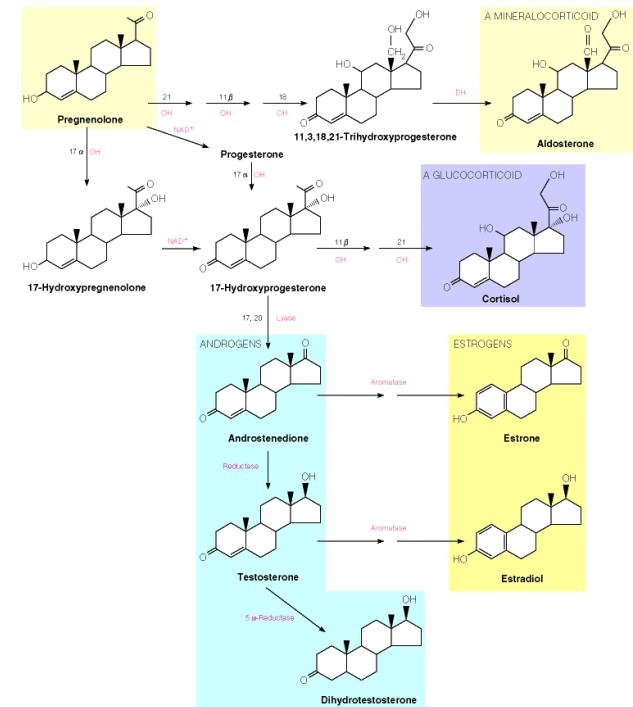
- Cells must respond to changes in the environment and/or to signals from other cells. The process of transmitting this information from outside of the cell to inside the cell is called signal transduction.
- The extracellular messengers that carry the information include hormones, growth factors, neurotransmitters, and pheromones.
- **Hormones** are substances synthesized in specialized cells and carried via the blood to remote target cells. There the hormones interact with specific receptors, resulting in specific metabolic changes in the target cell.



- Hormonal regulation

An example is the rapid generation of energy that results from secretion of adrenaline (epinephrine). The following two types of metabolic response to hormones are well understood:

1. Steroid hormones involve changes in gene expression.
2. Second messengers that control metabolic reactions are made in response to the binding of an extracellular substance (the first messenger). Common first messengers include glucagon and insulin. Common second messengers include cyclic AMP (or cAMP) and phosphoinositides.



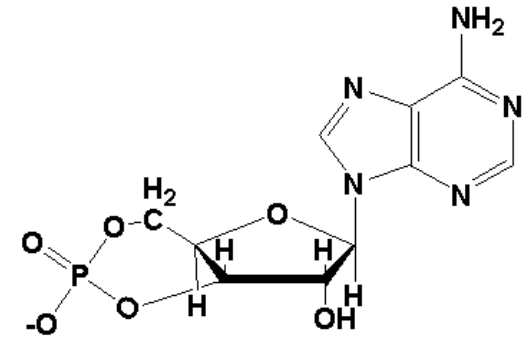
Cyclic adenosine monophosphate (cAMP)

- Second messenger system

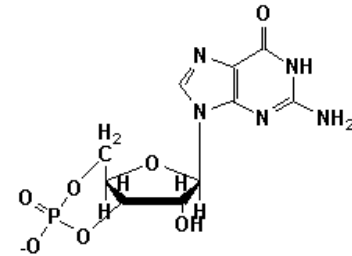
1. **Cyclic AMP (cAMP)** - Many signal transduction events involve the linked actions of a cell surface receptor, G protein, and adenylate cyclase. These events either stimulate or inhibit the synthesis of the second messenger, **cAMP**, inside the cell. Many intracellular processes are controlled, in turn, by the level of that second messenger. **cAMP** can affect transcription by binding to a protein called CREB (**cAMP** response element binding protein), and the resultant complex controls transcription of genes, including those encoding particular receptors.

2. **Cyclic GMP (cGMP)** - **Nitric oxide** stimulates the synthesis of **cGMP**. Many cells contain a **cGMP**-stimulated protein kinase that, like the cAMP-activated enzyme, contains both catalytic and regulatory subunits.

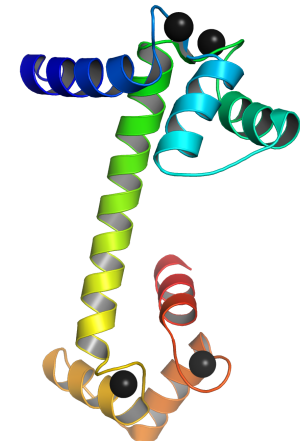
3. **Calcium** - Calcium ion can be considered a second messenger. Many cells respond to extracellular stimuli by altering their intracellular calcium concentration, which in turn effects biochemical changes either by itself or through its interaction with calmodulin. Calcium levels themselves are controlled in large part by second messengers, including cAMP. Because cAMP regulates calcium influx, calcium ion may be more of a third messenger than a second messenger.



Cyclic adenosine monophosphate (cAMP)

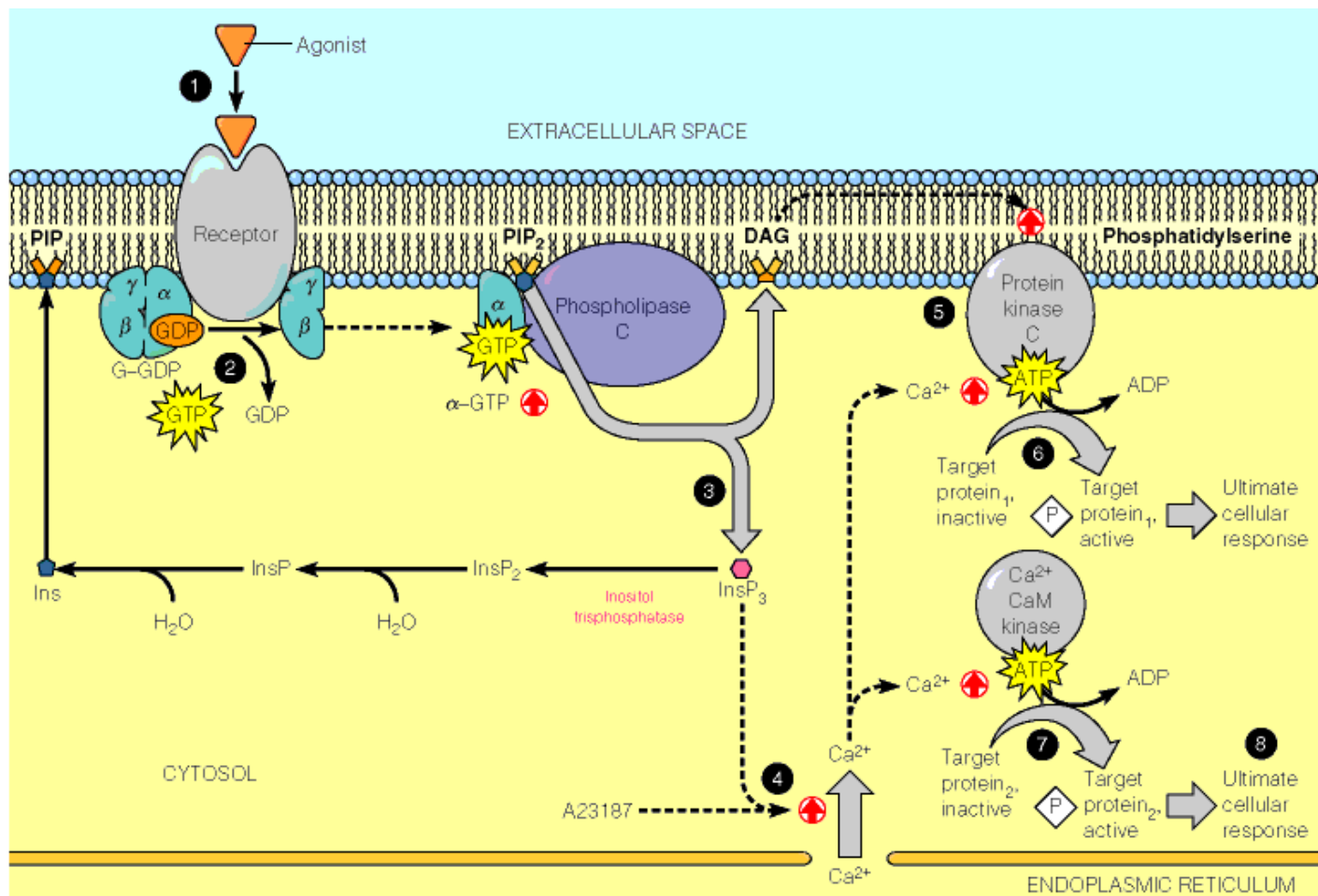


Cyclic guanosine monophosphate (cGMP)



- Second messenger system

4. **Phosphoinositide/Diacylglycerol** - Cytosolic calcium ion levels also can be increased by release from intracellular calcium stores. Access to these intracellular stores is controlled by the phosphoinositide system. In the phosphoinositide system, hormonal stimulus activates a reaction that generates two second messengers. A specific lipid in the phosphoinositide family, phosphatidylinositol 4,5-bisphosphate (PIP₂), is a membrane-associated storage form for two second messengers.



• Distributive control of metabolism

- This concept recognizes that control of metabolic pathways is not simply a function of a single allosterically regulated enzyme in the pathway, but rather a function of all of the enzymes of a pathway.
- While enzymes catalyzing committed steps in pathways often play major roles in regulating the pathway, contributions can be made by all of the enzymes of the pathway.

• Specialization of organs

- Regulation in higher eukaryotes
- Organs have different metabolic roles i.e. Liver = gluconeogenesis, Muscle = glycolysis
- Metabolic specialization is the result of differential gene expression

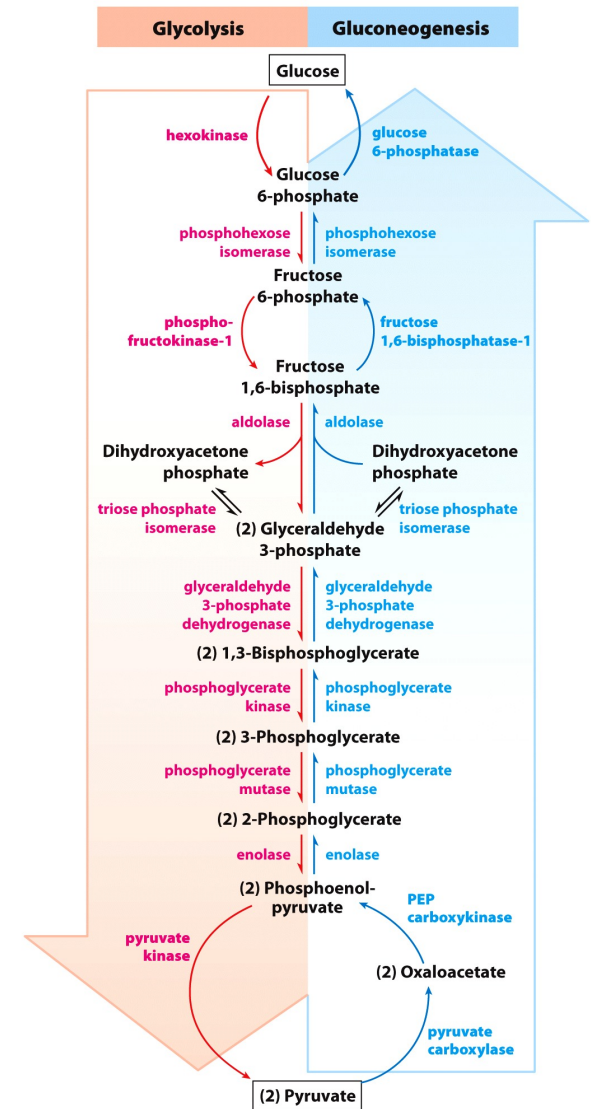


Figure 15-13
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