MACQUARIE University

Introduction to Metabolism

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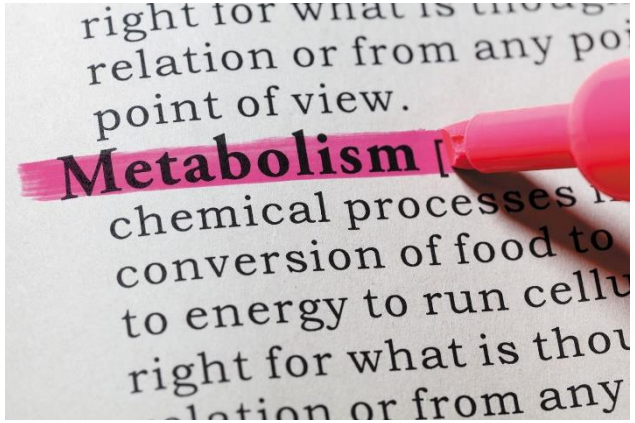
What is metabolism?

Metabolism is the **process** by which your body **converts what you eat and drink** into **energy**.

During this complex biochemical process, calories in food and beverages are combined with oxygen to release the energy your body needs to function.



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Why metabolism!

Metabolism ("change" in Greek) is the set of life-sustaining chemical reactions in organisms.

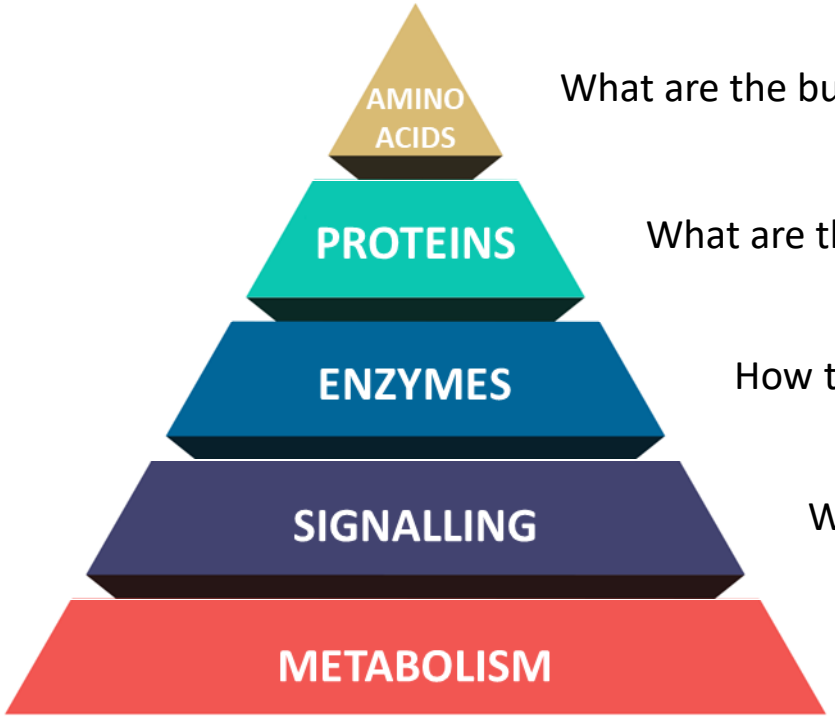
The three main purposes of metabolism are:

1. the conversion of food to energy to run cellular processes;
2. the conversion of food/fuel to building blocks for proteins, lipids, nucleic acids, and some carbohydrates;
3. the elimination of nitrogenous wastes.

These enzyme-catalyzed reactions allow organisms to grow and reproduce, maintain their structures, and respond to their environments.

Since metabolism now means all chemical reactions in an organism, the above reasons are considered part of
intermediary *or intermediate* ***metabolism***

Why did we not start with metabolism?



What are the building blocks of proteins?

What are these enzymes?

How to carry out biochemical reactions?

When do we need resources or energy?

Objectives

- Metabolism
  - Catabolism - breaking down
  - Anabolism – building up
- Enzymes assembled into teams: pathways
  - Metabolic strategies for enzyme clustering
- Thermodynamic control of metabolism
- Regulation of enzymes in a pathway
- Energy for biochemical processes

- Textbook Chapters 11 & 3

About metabolism

- The **synthesis** and **degradation** of ***small molecules*** (called intermediary metabolism) is essential for life
- All living cells require both a supply of small molecules and energy
- The demand for energy and small molecules varies over a broad range depending on the biological process
  - the rates of the reactions supplying energy and small molecules must be adjusted to account for this demand
  - the rates can be adjusted so that the concentration of key intermediates are kept remarkably constant

Metabolic strategies

- Organisms differ in their sources of energy, reducing power and starting materials

  - All organisms require ATP (adenosine triphosphate) as a **universal energy source** for driving unfavourable reactions
  - All organisms require NADPH (**reduced form** of *nicotinamide adenine dinucleotide phosphate*) which supplies reducing power for biosynthetic reactions
  - All organisms require a carbon source

- The differences lie in where organisms obtain their ATP, NADPH and carbon.

Metabolic classification of organisms

Type of organism	Source of ATP	Source of NADPH (reducing agent)	Source of carbon	Examples
Chemautotroph	Oxidation of inorganic compounds	Oxidation of inorganic compounds	CO_2	H, S, Fe and denitrifying bacteria
Photoautotroph	Sunlight	H_2O	CO_2	Higher plants, blue-green algae, photosynthetic bacteria
Photoheterotroph	Sunlight	Oxidation of organic compounds	Organic compounds	Nonsulphur purple bacteria
Heterotroph	**Oxidation of organic compounds**	**Oxidation of organic compounds**	**Organic compounds**	**All higher animals, most micro-organisms, non-photosynthetic plant cells**

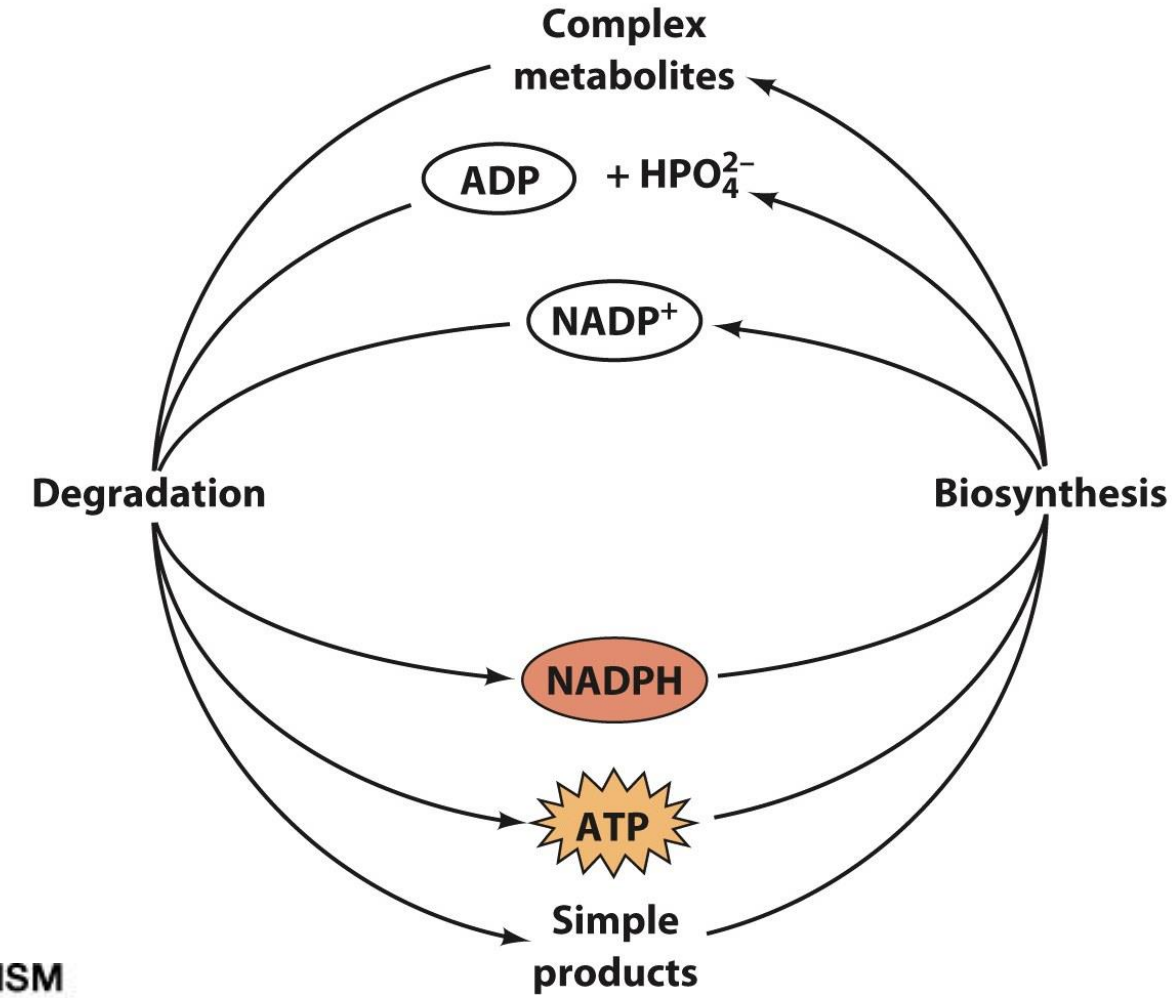
Overview of Metabolism

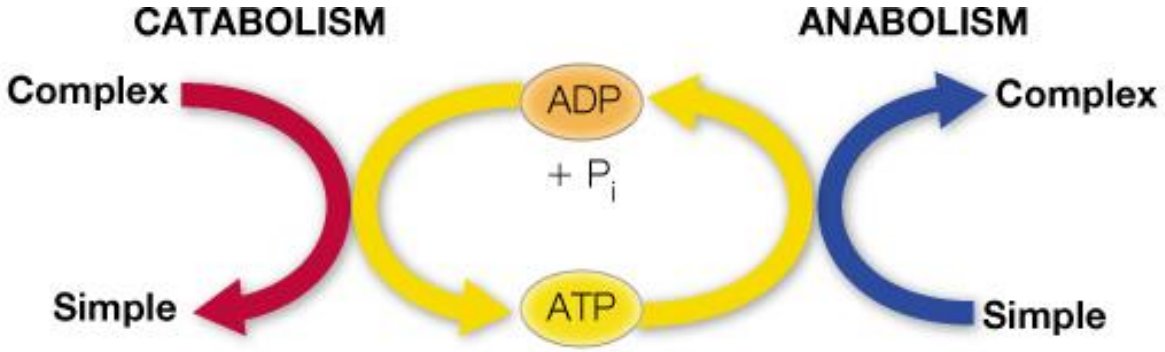
- Intermediary metabolism: synthesis (anabolic reactions) and degradation (catabolic reactions) of small molecules (metabolic intermediates)
- Energy metabolism: intermediary metabolic pathways that generate or store energy
- Central pathways: pathways accounting for large amounts of mass transfer and energy generation in a cell (heaviest traffic and highly conserved across organisms)

Catabolic (Degradative) & Anabolic (Biosynthetic) Pathways are Related

- Catabolism: nutrients and cell components are broken down to salvage their components and/or to generate energy

- Anabolism: biomolecules are synthesized from simpler components.

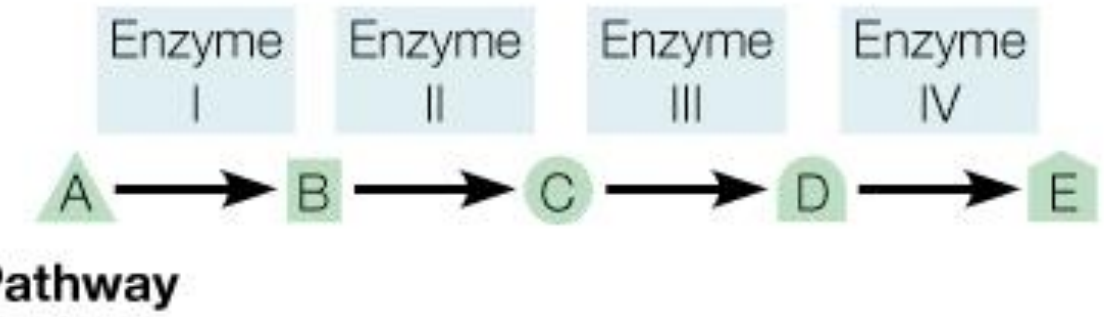




(a)

Metabolic pathways consist of a series of Enzymatic Reactions

- The energy and substances released by some reactions are utilized by others. Thus, several reactions get grouped together into “metabolic pathways”
- In most cases, it is the overall sequence of reactions that serves a function, and not the individual reactions
  - **Tryptophan** (W) synthesis from *chorismate*
    - requires **five** steps with ***five separate*** enzymatic activities
    - the intermediates in this conversion serve no function except as precursors of tryptophan



Metabolism is made up of Pathways

- Pathways of catabolism (carbohydrates, lipids, proteins) converge on a few common intermediates
- Biosynthetic pathways carry out the opposite process
  - ➢ Relatively few metabolites serve as starting materials for several diverse products!
  - ➢ "Metabolomics" is the study of intracellular concentrations of each metabolite. Metabolites (small molecules) vary in physical and chemical properties allowing thousands of low-MW metabolites to be extracted and assayed in one operation

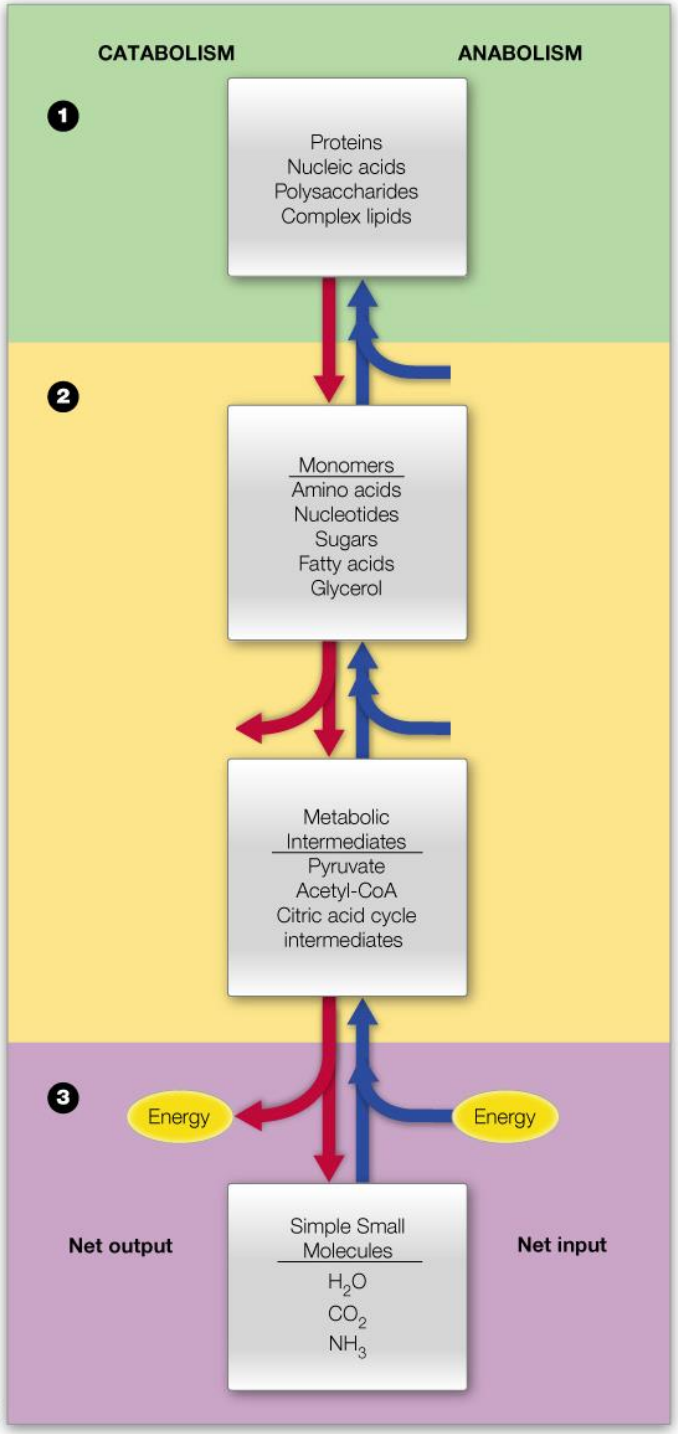


FIGURE 11.1 A brief overview of metabolism.

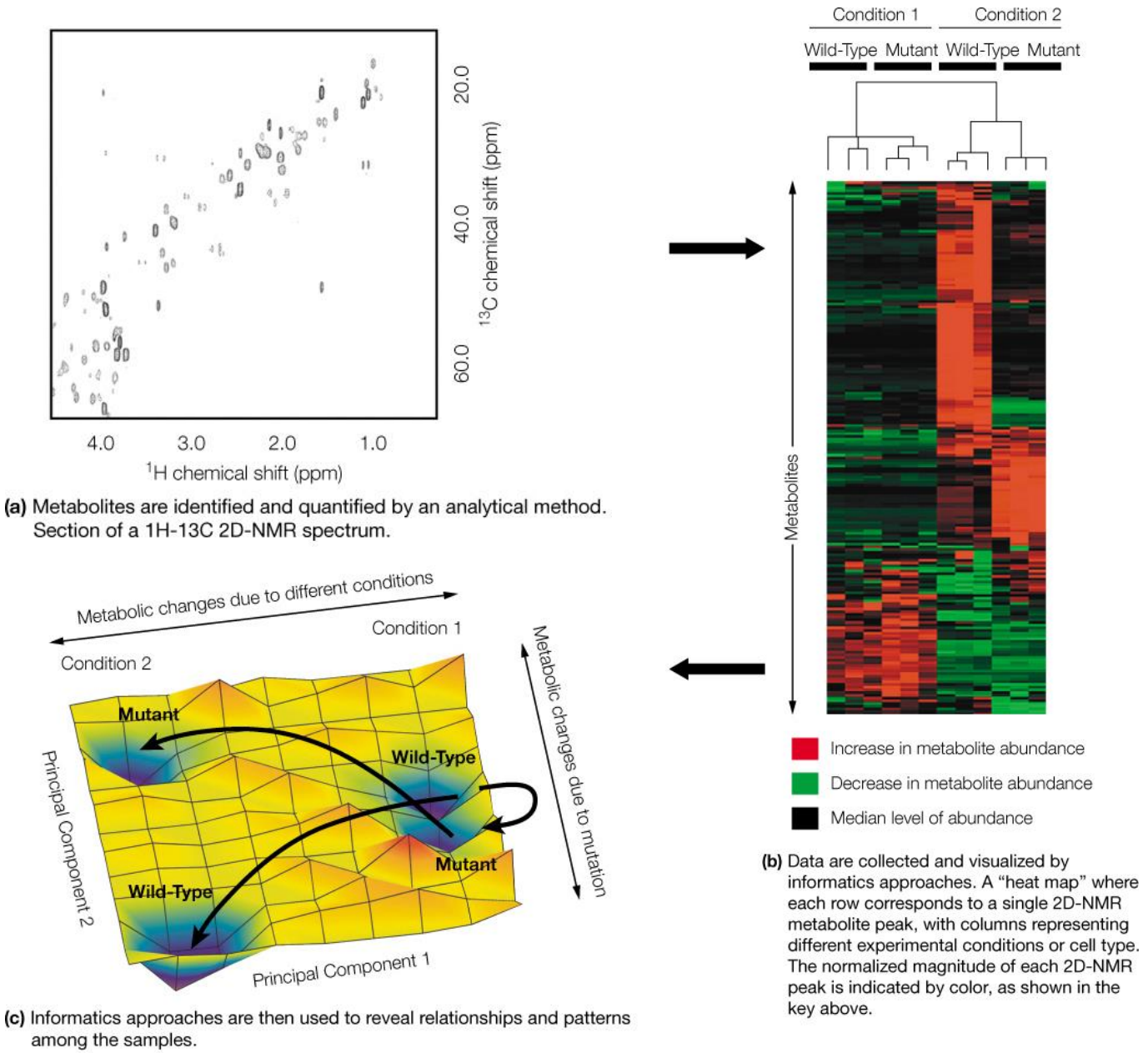
Investigating Metabolism

Metabolism is investigated using various tools and techniques:

- noninvasive metabolic monitoring by nuclear magnetic resonance (measures shift in the frequency of absorbed electromagnetic radiation)
- steps of a hypothetical metabolic pathway are also identified by analyzing mutants defective in individual steps of the pathway

Metabolic Profiling

- Metabolites can be identified by analytical methods such as 2D NMR
- Levels of metabolites under different conditions can be visualized using a "heat map"
- Informatics approaches can then be used to reveal patterns in the data.



(a) Metabolites are identified and quantified by an analytical method. Section of a 1H-13C 2D-NMR spectrum.

(c) Informatics approaches are then used to reveal relationships and patterns among the samples.

(b) Data are collected and visualized by informatics approaches. A "heat map" where each row corresponds to a single 2D-NMR metabolite peak, with columns representing different experimental conditions or cell type. The normalized magnitude of each 2D-NMR peak is indicated by color, as shown in the key above.

FIGURE 11A.1 Basic process of metabolic profiling.

Mutants identify Individual Steps of a Metabolic Pathway

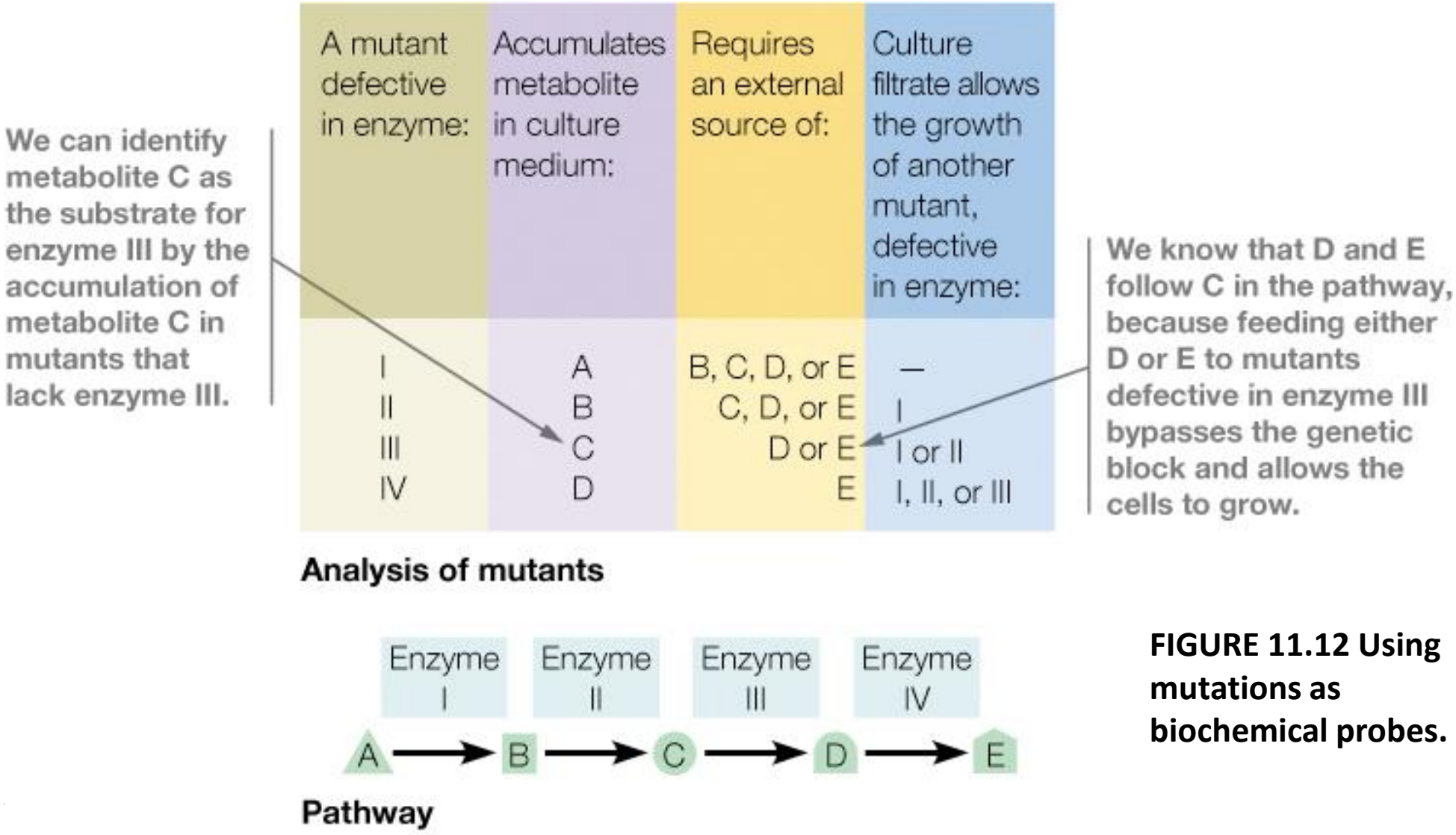


FIGURE 11.12 Using mutations as biochemical probes.

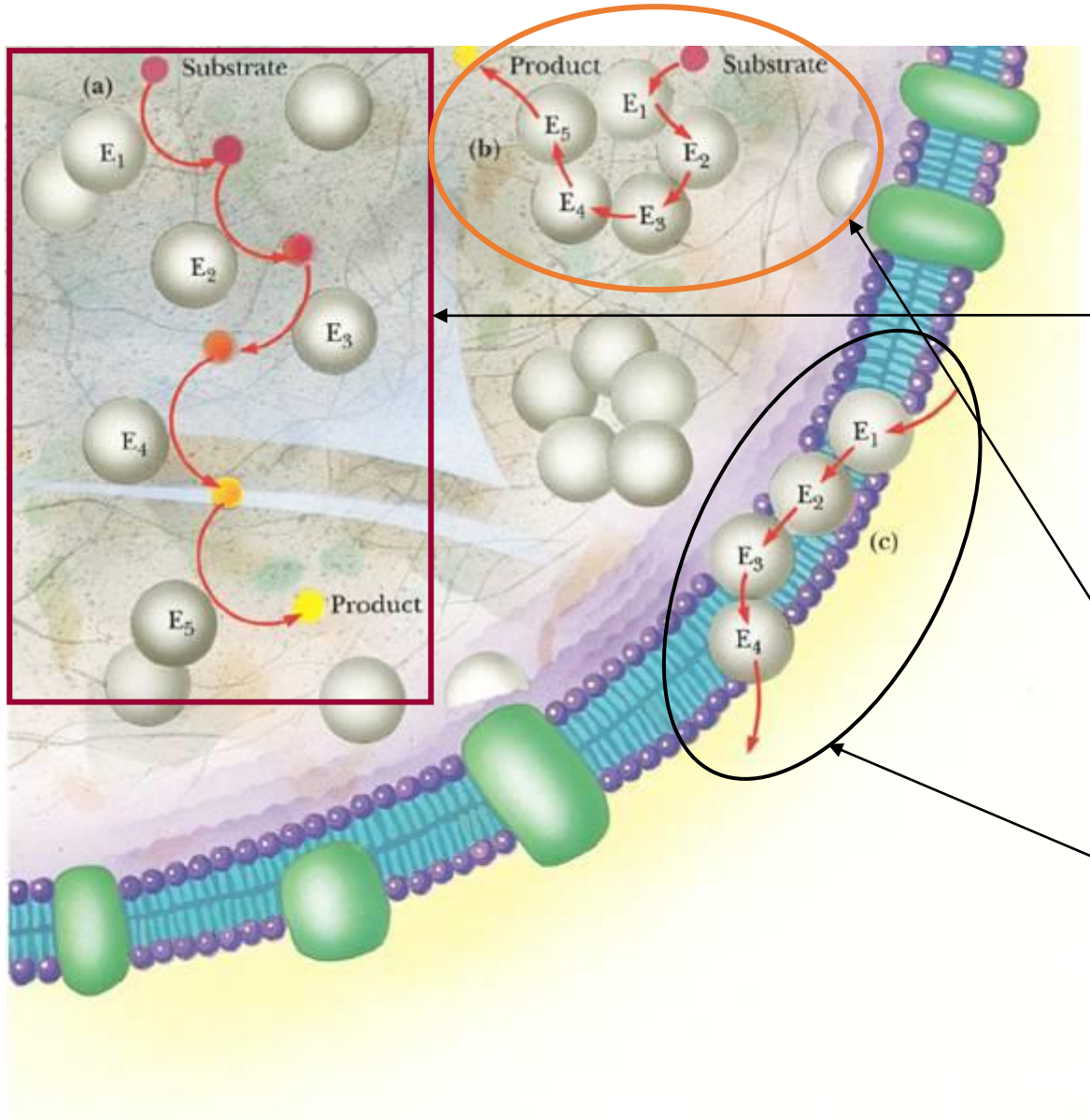
Major Metabolic Control Mechanisms

- *Control of enzyme levels (genetic regulation)*
- Control of enzyme activity: substrate-level, allosteric, covalent modification (Lectures 7 & 8)
- Signal transduction (hormones, growth factors): intercellular control often exercised through action of a second messenger (the hormone is the first messenger) (Lectures 10 & 11)
- Compartmentation: both physical (involving membranes)
  - Example: increase in membrane permeability to glucose in response to insulin secretion
  - Functional compartmentation, brought about by physical juxtaposition of sequentially acting enzymes; this allows high local concentration of intermediates, while maintaining low average concentration; this is important because the solvent capacity of the cell is limited

Enzyme clustering

- Enzymes in biochemical pathways are usually **clustered**
- Clustering allows intermediates to rapidly go from one enzyme to the next, thus allowing rapid formation of the end product without the loss of intermediates
- Three types of clustering
  1. Independent soluble enzymes in a pathway are present in the same cellular compartment
     - e.g. *fatty acid synthesis* is in the *cytosol* and fatty acid breakdown occurs in the mitochondria (later lectures)
  2. Enzyme activities are arranged in a multisubunit complex (major part of some pathways: e.g. pyruvate dehydrogenase in citric acid cycle – later lectures)
  3. The pathway is membrane-bound (electron transport chain; oxidative phosphorylation – later lectures)

Enzyme clustering



a) **Unlinked enzymes** associated in the aqueous cell compartment, with diffusing intermediates

b) Enzymes as components of a **multienzyme** complex

c) Enzymes linked as components of a **membrane-bound multienzyme complex**

Cellular Location of Major Metabolic Pathways

Compartmentation prevents biosynthetic and degradative pathways from co-occurring: “futile cycling” – reducing energy and material waste

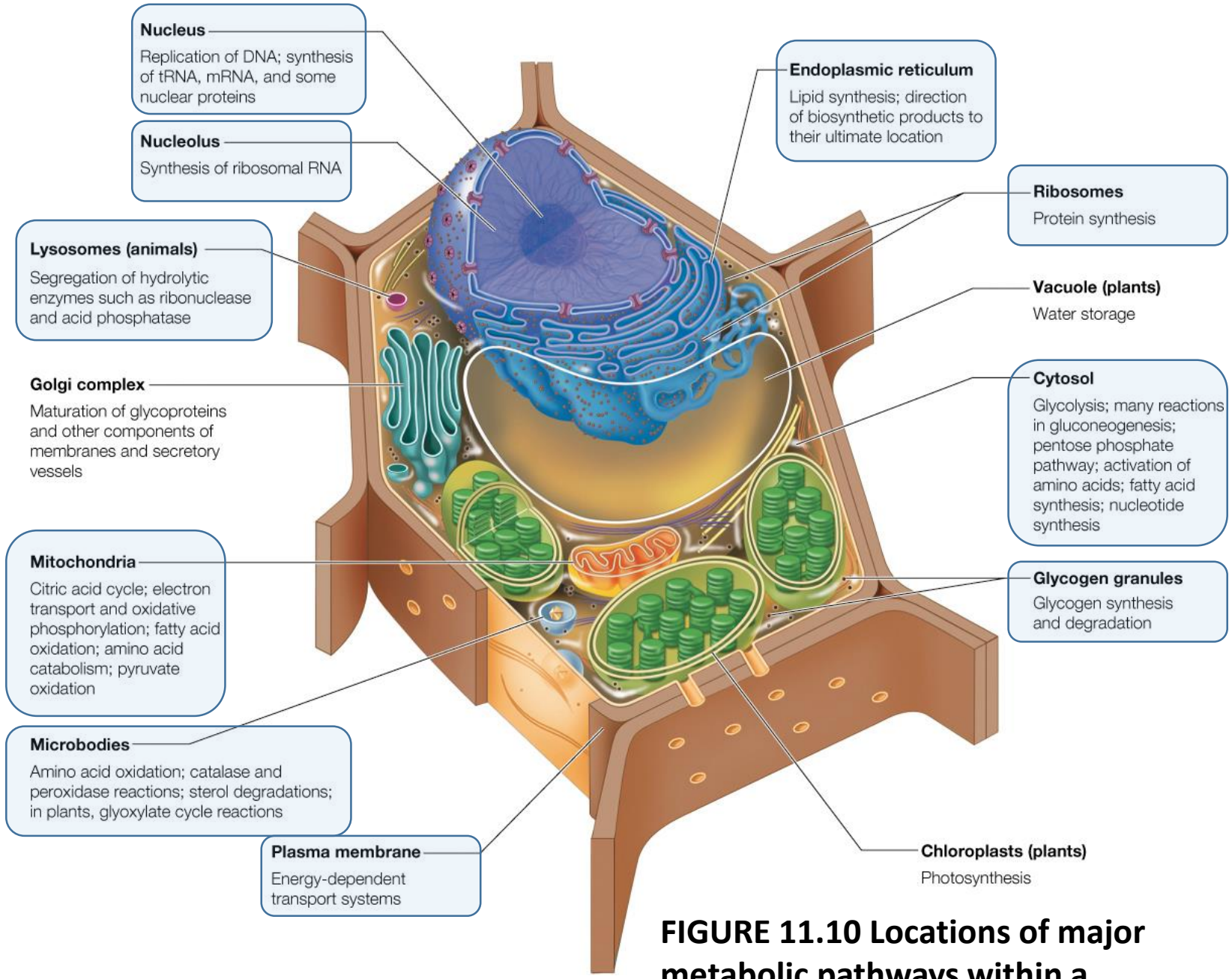
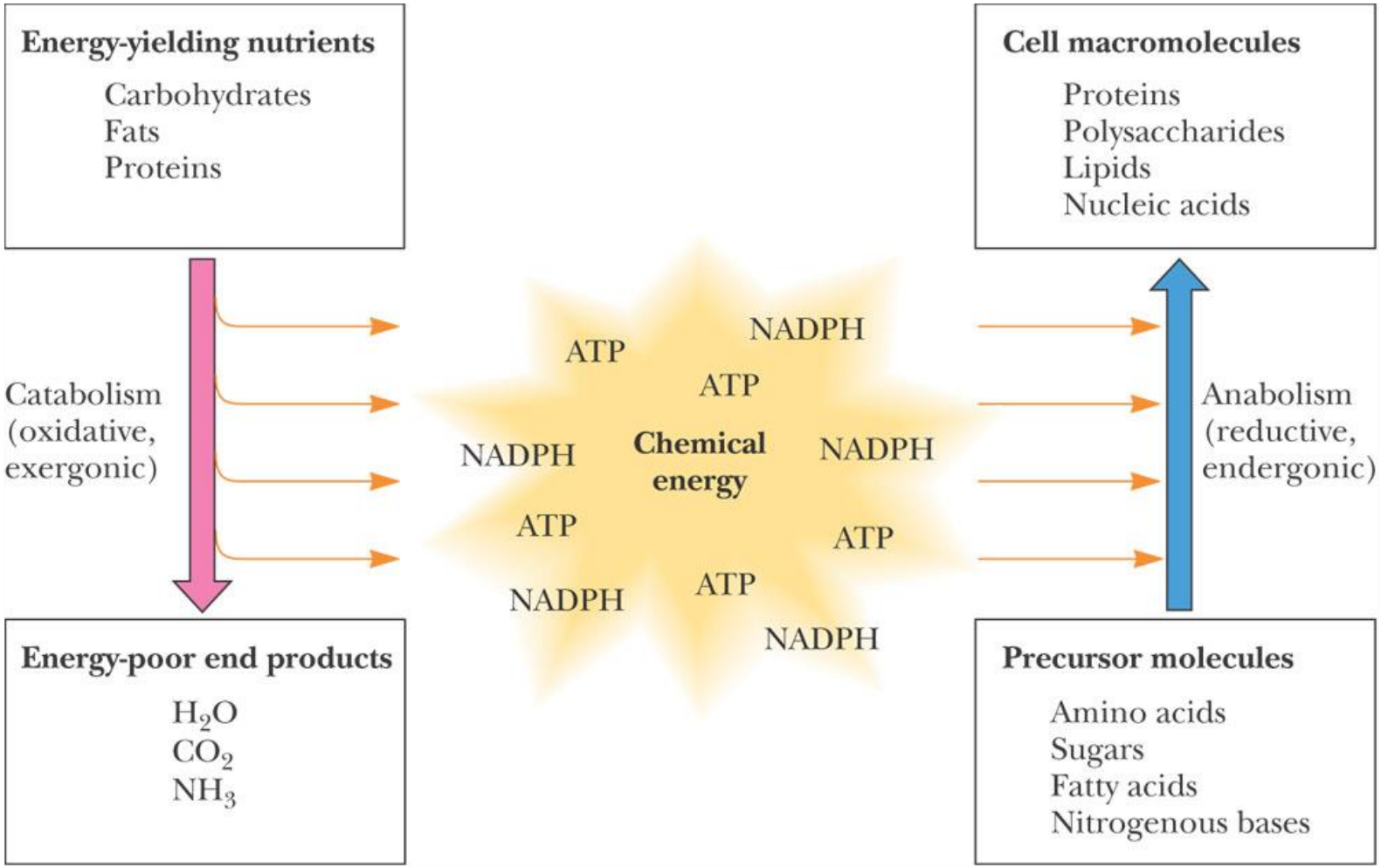


FIGURE 11.10 Locations of major metabolic pathways within a eukaryotic cell.

Metabolic pathways are functionally coupled

- Many pathways are interconnected
- Catabolic (or degradative) pathways yield energy (exergonic)
  - Foods are oxidized to produce ATP and NADPH
- Anabolic (or biosynthetic) pathways require energy (endergonic)
  - Use ATP and NADPH and various small molecules as starting materials produced by the catabolic pathways

Balancing catabolism and anabolism

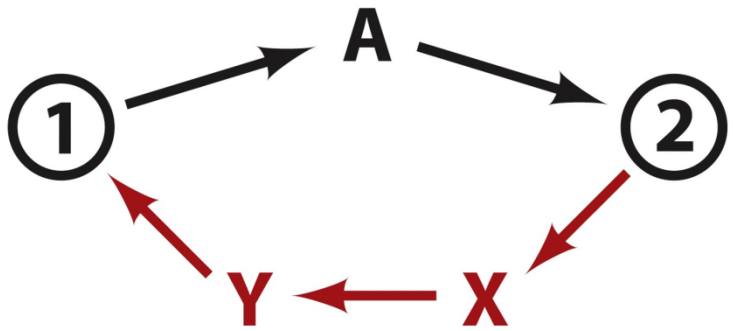


Thermodynamic control of direction and regulation of metabolic pathways

- Many metabolic enzymes catalyse near-equilibrium reactions and act quickly to maintain the relative concentrations of substrates and products
- Some key reactions function far from equilibrium and regulate the flow (i.e. flux) of reactants
- Overall, metabolic pathways are **irreversible**: one highly exergonic reaction ($\Delta G << 0$) will force the pathway to go in one direction
- Every metabolic pathway has a first committed step
- Catabolic and anabolic pathways differ and are therefore not exactly the reverse of each other

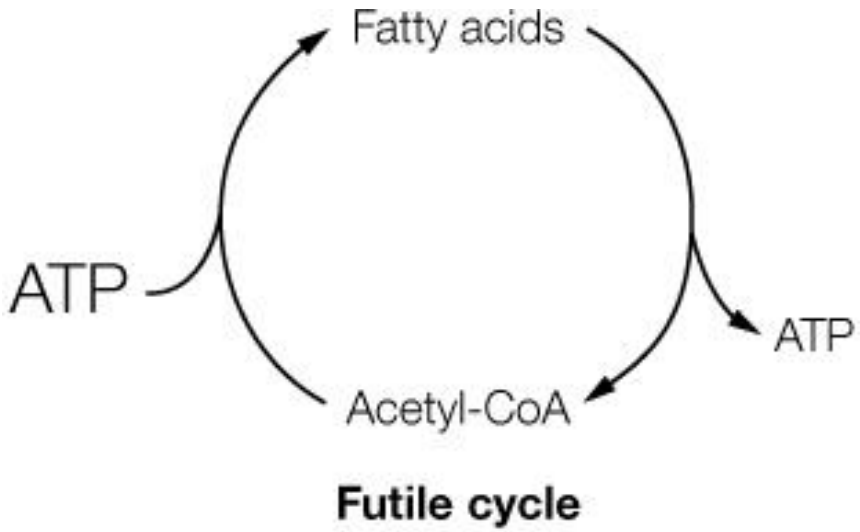
TABLE 3.2 Free energy rules

If ΔG is . . .	Free energy is . . .	The process is . . .
Negative	Available to do work	Thermodynamically favorable (and the reverse process is unfavorable)
Zero	Zero	Reversible; the system is at equilibrium
Positive	Required to do work	Thermodynamically unfavorable (and the reverse process is favorable)



Separate Pathways for Biosynthesis and Degradation

- A pathway must be exergonic in the direction in which it proceeds
- A pathway must be regulated to meet current physiological requirements
- Reciprocal regulation ensures that a futile cycle will not occur, even though both pathways are localized in the same cell compartment (e.g., glycolysis and gluconeogenesis both take place in the cytosol)
- Substrate cycle: enzymes of pathways that operate in opposite directions (glycolysis, gluconeogenesis) show control at enzymes catalyzing exergonic reactions that operate in opposite directions



Futile cycle

Metabolic flux must be controlled

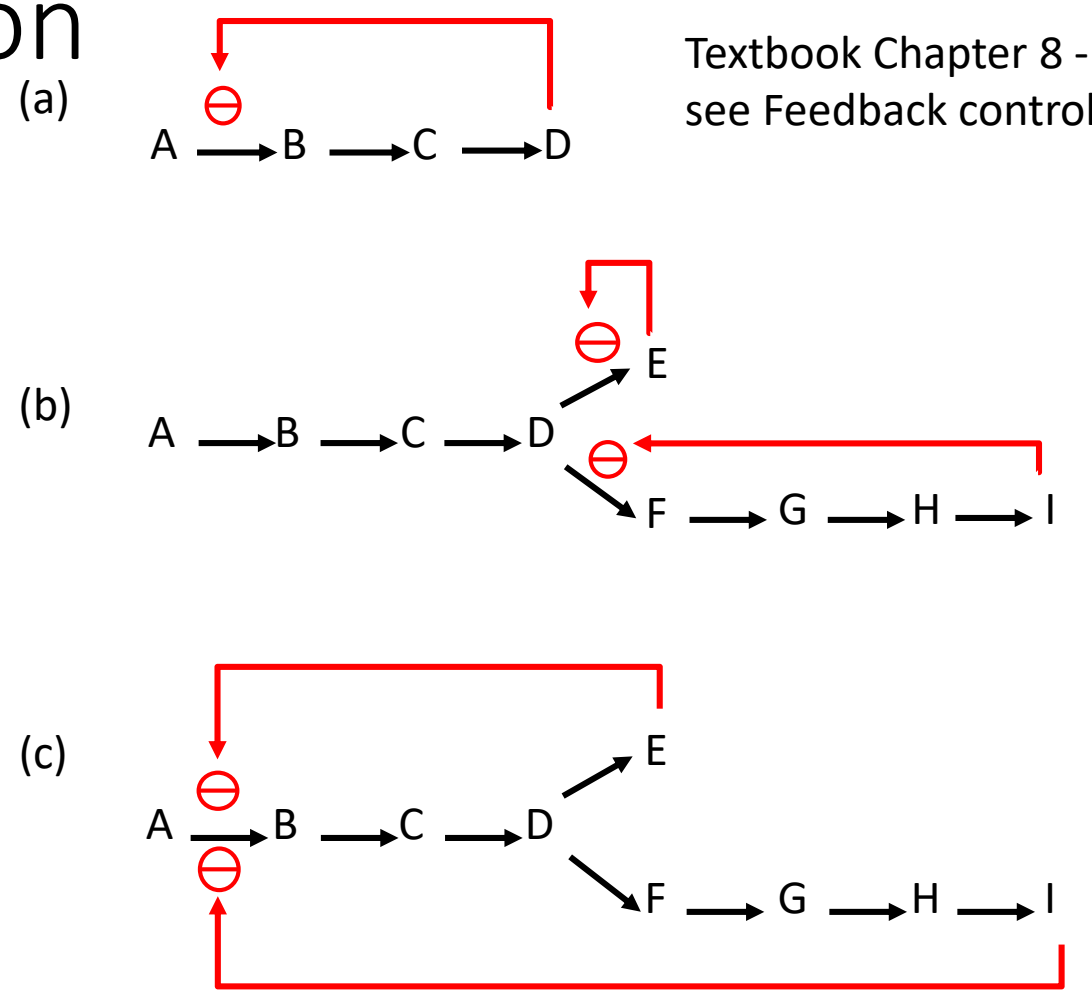
- Organisms regulate their catabolic and biosynthetic pathways
- This avoids deficiencies or excess of products
- **Regulation** is achieved by direct control of the activity of key enzymes in a pathway by
  - **Allosteric control:** non-covalent interaction of small molecule regulatory factors with the enzyme
  - **Covalent modification** of key enzymes in the pathway
  - **Substrate cycles:** where different enzymes catalyze the forward and reverse reactions
  - **Genetic control:** enzyme production is controlled at the transcription level
- Regulation can be ***either positive or negative***

Enzyme regulation

(a) End product (D) inhibits first reaction in the pathway

(b) End product inhibition of first reactions (E, I) after a branch point (D)

(c) Inhibition of first reaction by end products of both branch points (E, I)



Textbook Chapter 8 - Section 8.8: see Feedback control

Enzymes, Feedback Inhibition, and Allosteric Regulation
https://youtu.be/LKiXfqaWNHI

When to regulate an enzyme?

- A regulated reaction is only effective if it is exergonic (ΔG is negative; i.e. $\Delta G < 0$)
- The majority of enzyme reactions are under concentration regulation and are equilibrium processes ($\Delta G = 0$) – they self-regulate!
- If the reaction will not proceed rapidly, then there is probably no point in regulating it
  - ➢ Analogy of a dam with varying water levels on each side
    - ❖ The flood gates are the regulatory mechanism that allows for the flow of water
- The roles of hormones is to essentially provide immediate response; and countermand normal regulation under homeostasis.

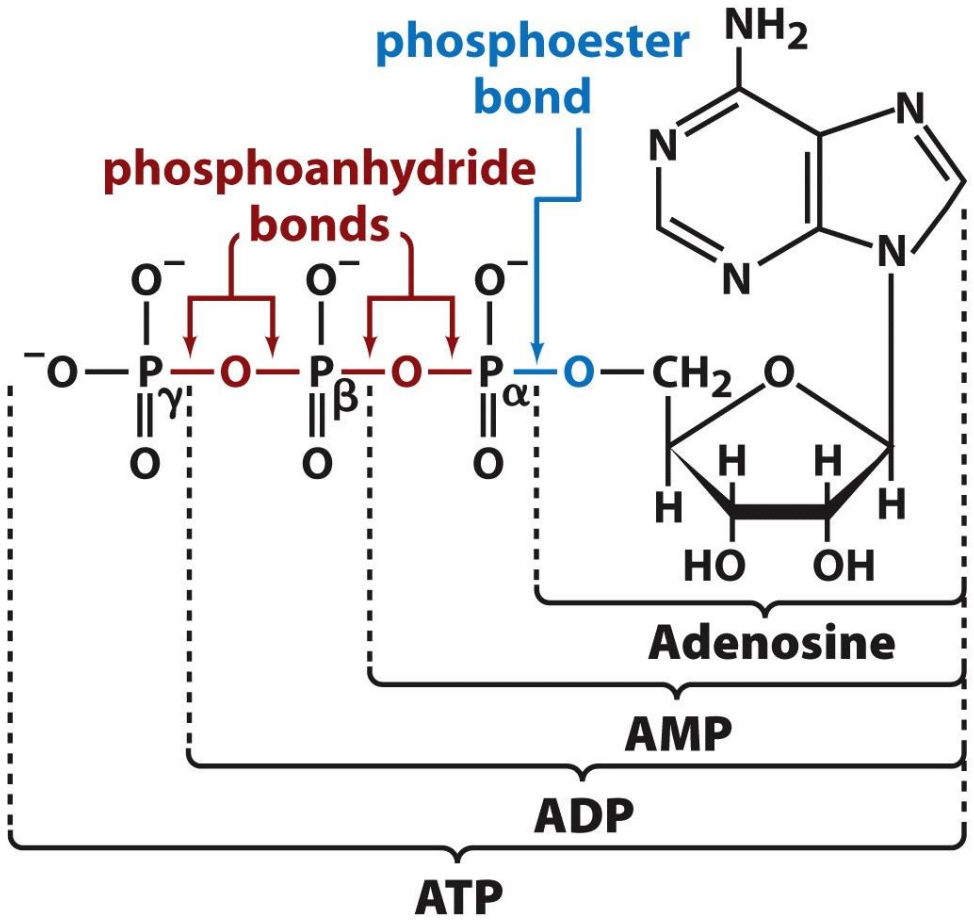
Intro to Metabolism – Summary

- Different organisms use different strategies for capturing free energy from their environment and can be classified by their requirement for oxygen.
- Mammalian nutrition involves the intake of macronutrients (proteins, carbohydrates, and lipids) and micronutrients (vitamins and minerals).
- Metabolism is the totality of all chemical reactions occurring within a living system (such as a cell)
  - ➢ catabolism is the sum of breakdown phases, while anabolism is the sum of biosynthetic phases
  - ➢ most metabolic energy comes from the oxidation of substrates
- A metabolic pathway is a series of enzyme-catalyzed reactions, often located in a specific part of the cell, by compartmentation.

Intro to Metabolism – Summary

- Metabolic pathways are defined sequences of chemical reactions in a cell that produce energy or valuable materials, and are catalyzed by enzymes
- The flux through metabolic pathways is controlled by regulating various parameters, such as enzyme concentration, enzyme activity, compartmentation, and presence of hormones
  - ➢ The flux of material through a metabolic pathway varies with the activities of the enzymes that catalyze irreversible reactions.
  - ➢ These flux-controlling enzymes are regulated by allosteric mechanisms, covalent modification, substrate cycling, and changes in gene expression.

Energy for biochemical reactions from high energy chemical bonds



Adenosine triphosphate (ATP):

- ***The energy currency in the cell***
- Usually forms ADP
- ADP can be phosphorylated back to ATP (in the mitochondria)
- In rare cases, AMP is formed.
- ADP to AMP also rarely occurs in a biochemical context.

ATP hydrolysis to ADP and AMP

- Large negative free energy change on hydrolysis is due to:
  - ➢electrostatic repulsion
  - ➢stabilization of products by ionization and resonance
  - ➢entropy factors

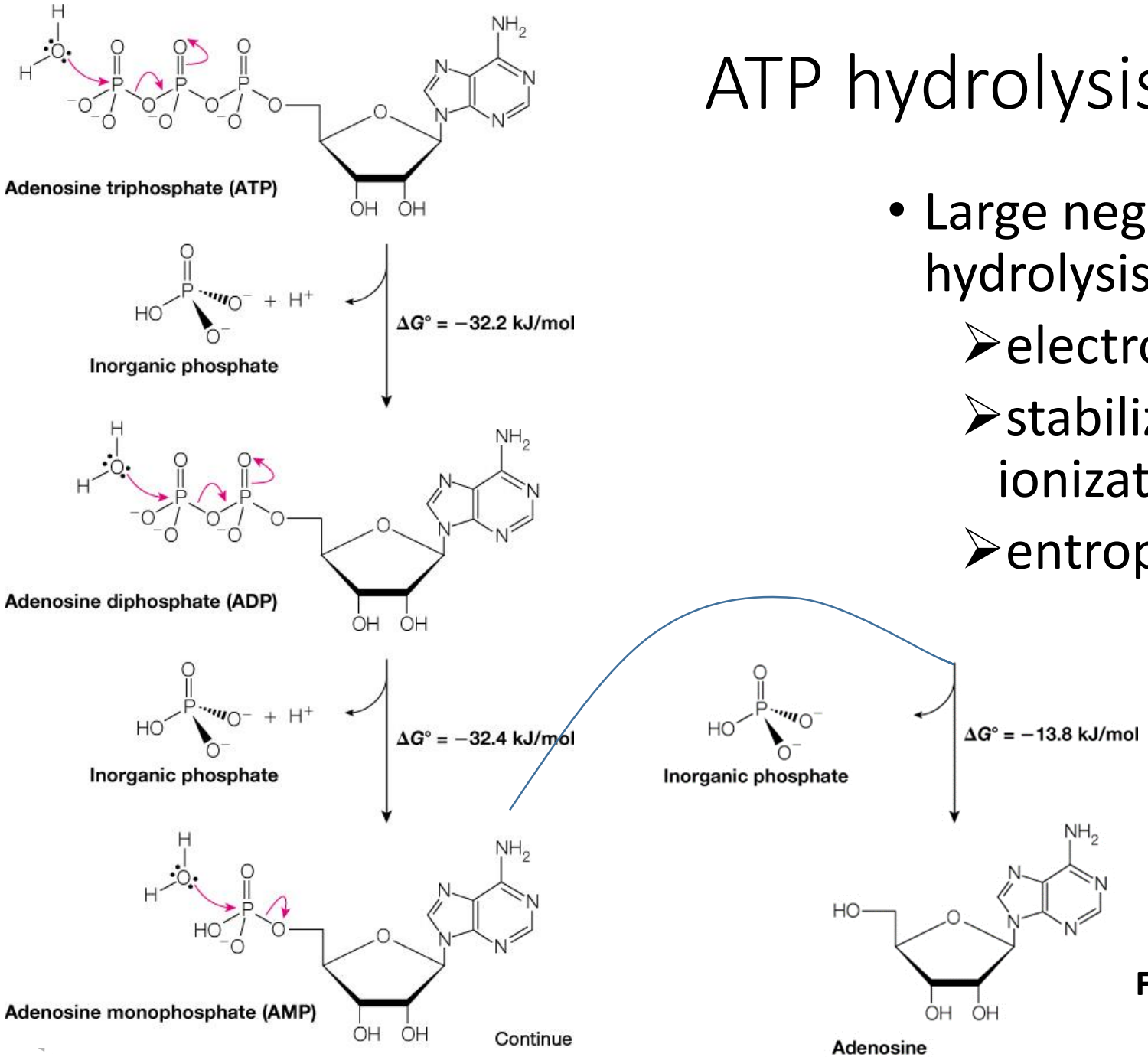


FIGURE 3.6 Hydrolysis of ATP, ADP, and AMP.

Phosphate bonds: biological energy carriers

TABLE 3.5 $\Delta G^{\circ\prime}$ for hydrolysis of some phosphate compounds

Hydrolysis Reaction	$\Delta G^{\circ\prime}$ (kJ/mol)
Phosphoenolpyruvate + H_2O ⟶ pyruvate + P_i	−61.9
1, 3-Bisphosphoglycerate + H_2O ⟶ 3-phosphoglycerate + P_i + H^+	−49.4
ATP + H_2O ⟶ AMP + PP_i + H^+	−45.6
Acetyl phosphate + H_2O ⟶ acetate + P_i + H^+	−43.1
Creatine phosphate + H_2O ⟶ creatine + P_i	−43.1
ADP + H_2O ⟶ AMP + P_i + H^+	−32.4
ATP + H_2O ⟶ ADP + P_i + H^+	−32.2
PP_i + H_2O ⟶ 2P_i	−19.2
Glucose-1-phosphate + H_2O ⟶ glucose + P_i	−20.9
Glucose-6-phosphate + H_2O ⟶ glucose + P_i	−13.8

Based on R. A. Alberty (1994) Recommendations for nomenclature and tables in biochemical thermodynamics. *Pure & Appl. Chem.* 66:1641–1666; P. Frey and A. Arabshahi (1995) Standard free energy change for the hydrolysis of the α, β-bridge in ATP. *Biochemistry* 34:11307–11310.

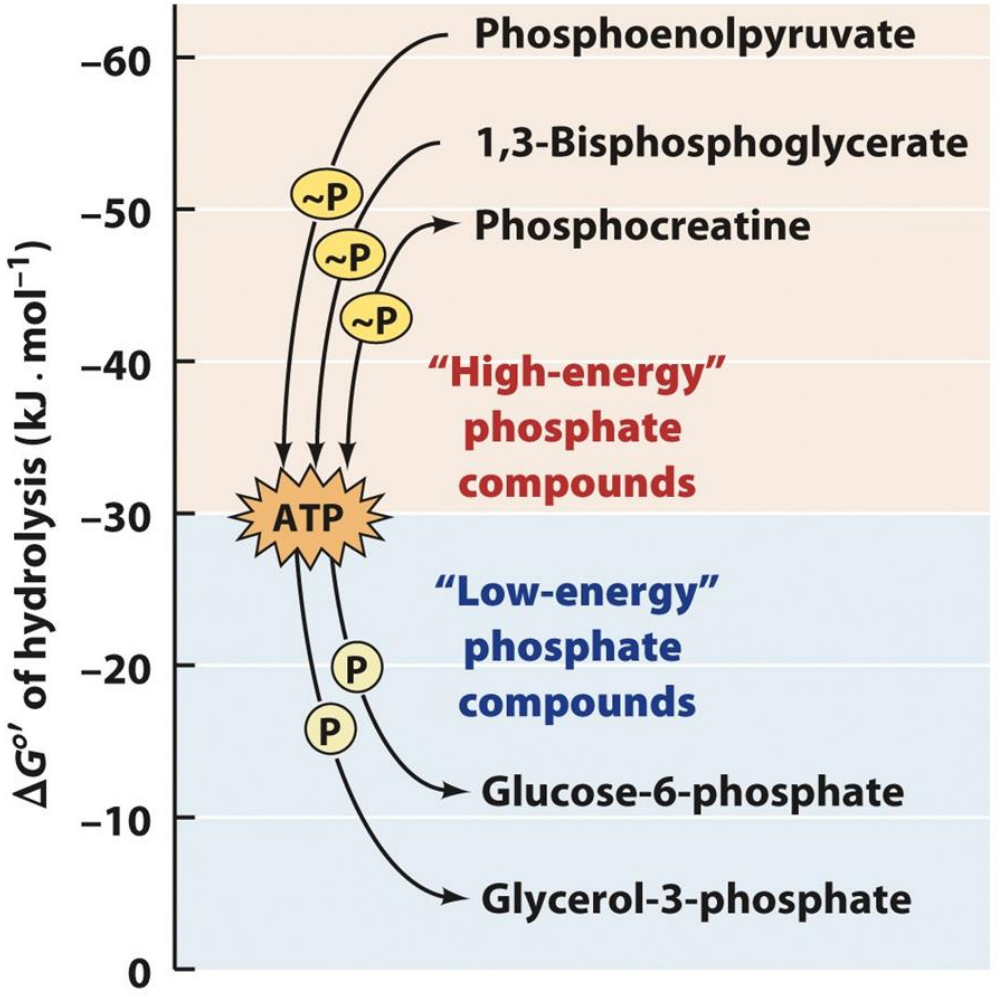


Figure 14-9

Endergonic Reactions Coupled to ATP Hydrolysis

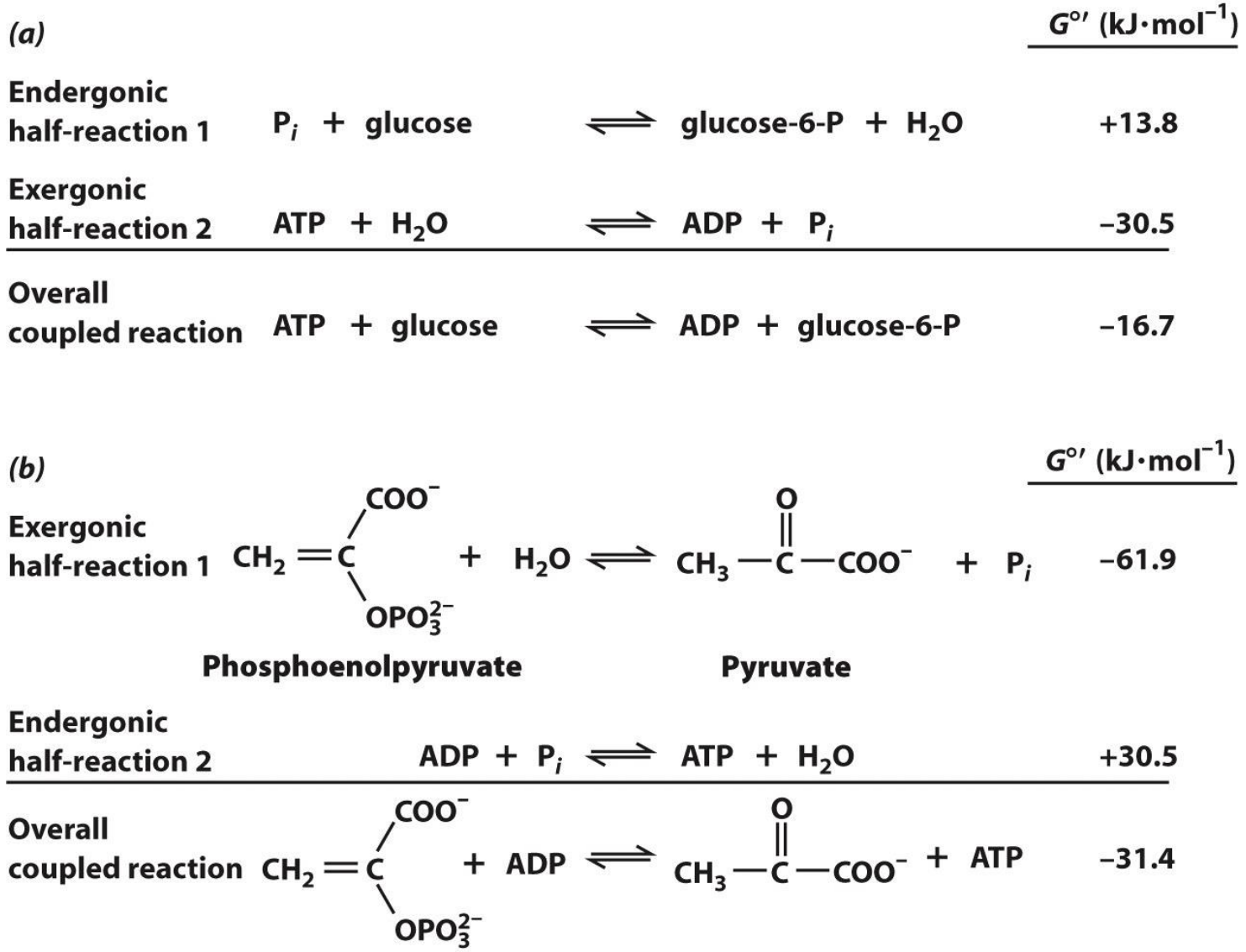


Figure 14-7

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Other High energy bonds

The thioester bond:

- ….S~COAc powers many reactions
- The hydrolysis of this bond to …SH is slightly more exergonic than ATP hydrolysis to ADP: -31.5 kJ.mol^{-1}
- E.g. Citric acid cycle

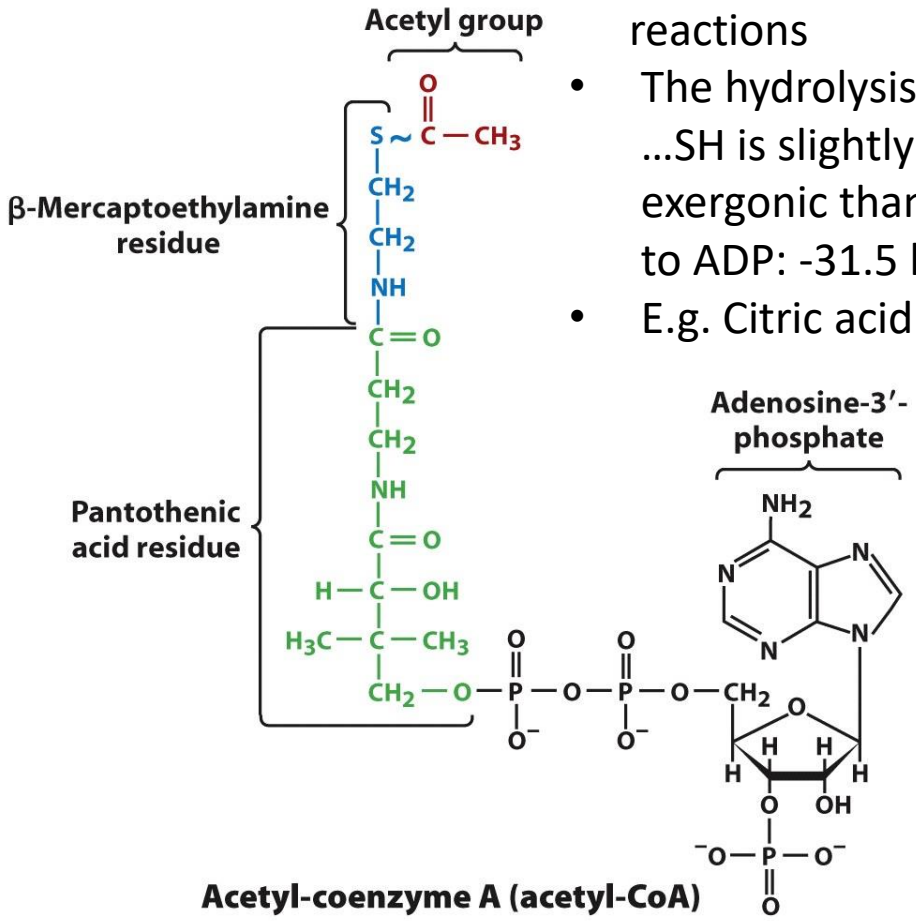
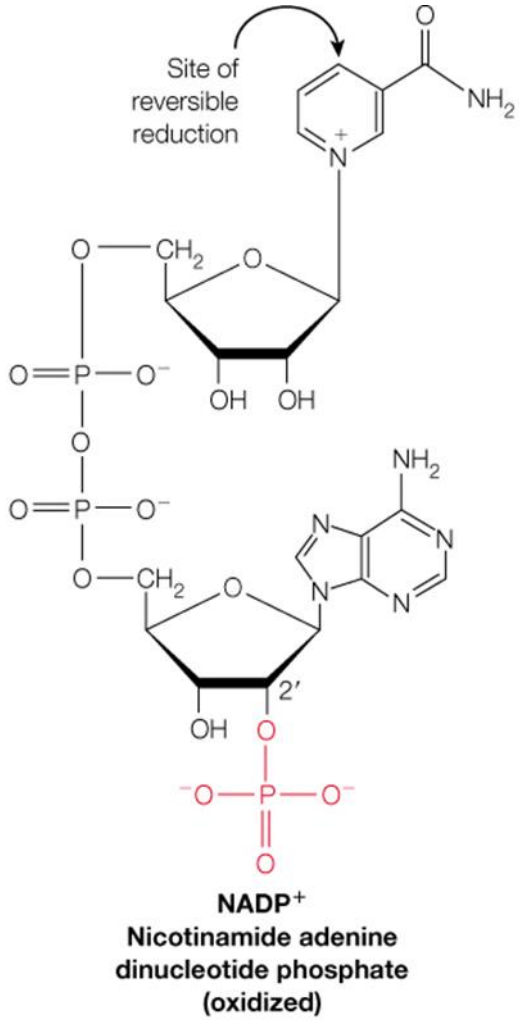


Figure 14-11



Nicotinamide Adenine Dinucleotide (Phosphate)

- NAD$^+$/NADH (Nicotinamide Adenine Dinucleotide; oxidized/reduced) and NADP$^+$/NADPH (NAD Phosphate; oxidized/reduced) are identical in standard reduction potential
- Cells maintain high [NAD$^+$]/[NADH] and high [NADPH]/[NADP$^+$] to support the roles of electron acceptor and electron donor, respectively
- NAD$^+$ is a major oxidant; NADPH is a major reductant

Powering the pathways

- A major control over all pathways is the energy status of the cell
- ATP couples energy release with unfavourable reactions. So, if its concentration decreases, this will determine which pathways are active and which ones are not.
  - Both catabolic and biosynthetic pathways can require ATP
- **ATP + ADP + AMP** = **Total [ATP]** attainable in a cell
- Energy of a cell is equal to:

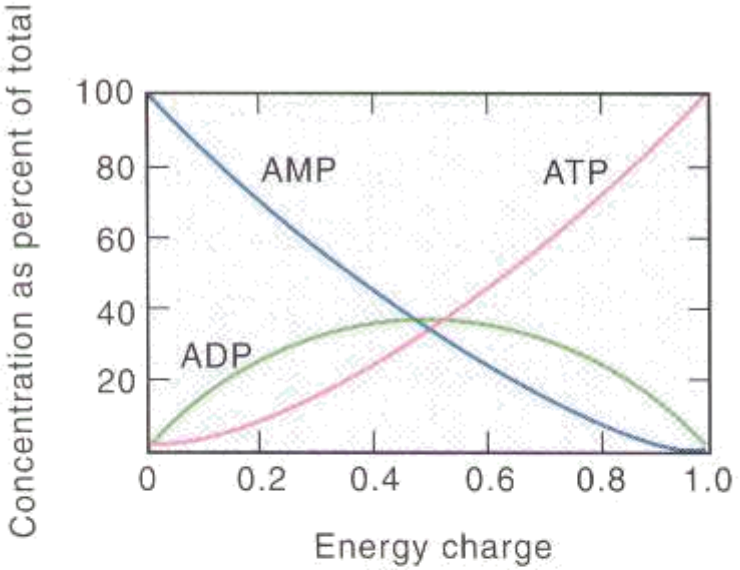
$$\frac{[ATP] + 0.5\,[ADP]}{[ATP] + [ADP] + [AMP]}$$

(energy carried by ADP is about half of ATP

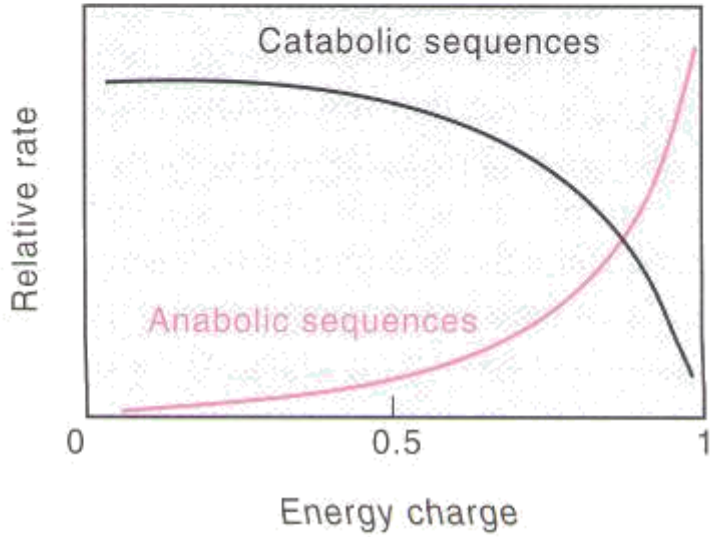
because ADP can form ATP or AMP)

Energy charge

- Concentrations of the three adenylate phosphates, i.e. ATP, ADP and AMP determine the energy charge of a cell



- Variation of reaction rates with energy charge
- Catabolic and anabolic activities are stabilized at around 0.9 energy charge



Energy for Metabolic Pathways from "High-Energy" Compounds – Summary

- Organisms capture the free energy released on degradation of nutrients as "high-energy" compounds such as ATP, whose subsequent breakdown is used to power otherwise endergonic reactions.
- The "high energy" of ATP is related to the large negative free energy change for hydrolysis of its phosphoanhydride bonds.
- ATP hydrolysis can be coupled to an endergonic reaction such that the net reaction is favorable.
- Phosphoryl groups are transferred from compounds with high phosphoryl group-transfer potentials to those with low phosphoryl group-transfer potentials.
- The thioester bond in acetyl-CoA is a "high-energy" bond.