

Chapter 10

Metabolism: The Use of Energy in Biosynthesis

Anabolism

- ✓ synthesis of complex molecules and cellular structures
- ✓ turnover
 - continual degradation and resynthesis of cellular constituents
- ✓ rate of biosynthesis approximately balanced by rate of catabolism
- ✓ requires much energy

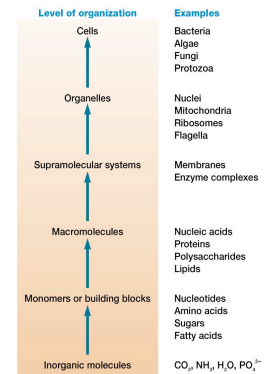


Table 10.1 Biosynthesis in *Escherichia coli*

Cell Constituent	Number of Molecules per Cell ^a	Molecules Synthesized per Second	Molecules of ATP Required per Second for Synthesis
DNA	1 ^b	0.00083	60,000
RNA	15,000	12.5	75,000
Polysaccharides	39,000	32.5	65,000
Lipids	15,000,000	12,500.0	87,000
Proteins	1,700,000	1,400.0	2,120,000

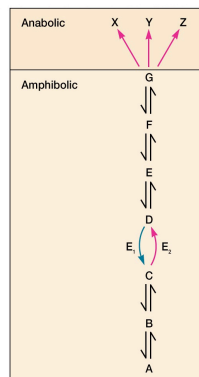
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^aEstimates for a cell with a volume of $2.25 \mu\text{m}^3$, a total weight of $1 \times 10^{-12} \text{g}$, a dry weight of $2.5 \times 10^{-13} \text{g}$, and a 20 minute cell division cycle.
^bIt should be noted that bacteria can contain multiple copies of their genomic DNA.

Principles Governing Biosynthesis

- ✓ 1. macromolecules are synthesized from limited number of simple structural units (monomers) (i.e. 20 amino acids)
 - saves genetic storage capacity, biosynthetic raw material, and energy
- ✓ 2. many enzymes used for both catabolism and anabolism
 - saves materials and energy

More principles...

- ✓ 3. catabolic and anabolic pathways are not identical, despite sharing many enzymes
 - permits independent regulation



More principles...

- ✓ 4. breakdown of ATP coupled to certain reactions in biosynthetic pathways
 - drives the biosynthetic reaction to completion
- ✓ 5. in eucaryotes, anabolic and catabolic reactions located in separate compartments
 - allows pathways to operate simultaneously but independently

More principles...

- ✓ 6. catabolic and anabolic pathways use different cofactors
 - catabolism produces NADH
 - NADPH used as reductant for anabolism
- ✓ large assemblies (e.g., ribosomes) form spontaneously from macromolecules by self-assembly

The Photosynthetic Fixation of CO₂

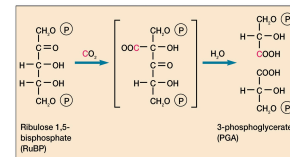
- ✓ 3 major processes used by autotrophs
 - Calvin cycle
 - Most eukaryotes and most photosynthetic prokaryotes
 - not found in archaea, some obligate anaerobes, and some microaerophilic bacteria
 - reductive TCA pathway (discussed in Chapter 20)
 - Archaea (*thermoproteus*, *Sulfolobus*)
 - *Chlorobium* and *Desulfobacter*
 - acetyl-CoA pathway (discussed in Chapter 20)
 - Methogens, sulfate reducers, acetogens

Calvin cycle

- ✓ in eucaryotes, occurs in stroma of chloroplast
- ✓ in cyanobacteria, some nitrifying bacteria, and thiobacilli, may occur in carboxysomes
 - inclusion bodies that contain ribulose-1,5-bisphosphate carboxylase (rubisco)
- ✓ consists of 3 phases

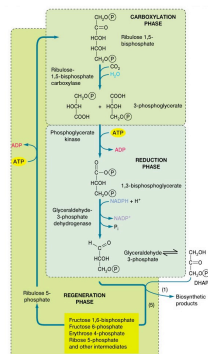
The Carboxylation Phase

- ✓ rubisco catalyzes addition of CO₂ to ribulose-1,5-bisphosphate (RuBP), forming 2 molecules of 3-phosphoglycerate



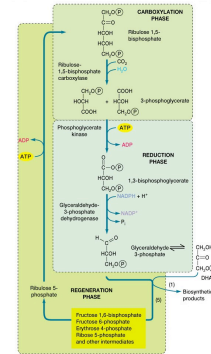
The Reduction Phase

- ✓ 3-phospho-glycerate reduced to glyceraldehyde 3-phosphate
 - Reverse of glycolysis?
 - Different enzymes

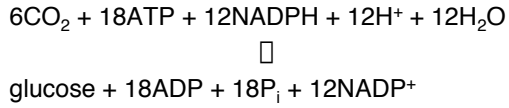


The Regeneration Phase

- ✓ RuBP regenerated
- ✓ carbohydrates (e.g., fructose and glucose) are produced



Summary



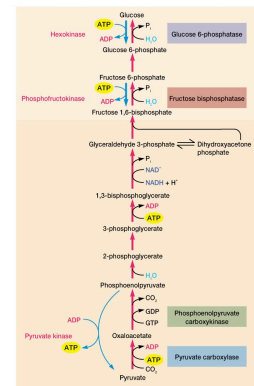
Synthesis of Sugars and Polysaccharides

- ✓ gluconeogenesis
 - used to synthesize glucose and fructose from noncarbohydrate precursors
 - No CO_2 fixation
- ✓ sugar nucleoside diphosphates
 - important in synthesis of other sugars, polysaccharides, and bacterial cell walls

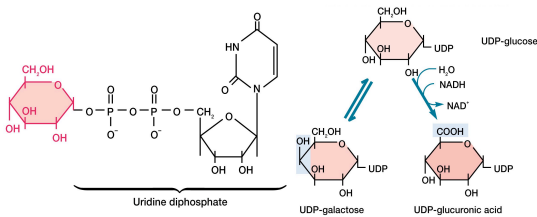
Gluconeogenesis

- ✓ generates glucose and fructose
 - most other sugars made from them
- ✓ functional reversal of glycolysis
 - 7 enzymes shared
 - 4 enzymes are unique to gluconeogenesis

Gluconeogenesis



Uridine diphosphate glucose (UDPG) and the synthesis of sugars



Synthesis of polysaccharides

- ✓ also involves nucleoside diphosphate sugars
 - e.g., starch and glycogen synthesis
- $$\text{ATP} + \text{glucose 1-P} \rightarrow \text{ADP-glucose} + \text{PP}_i$$
- $$(\text{glucose})_n + \text{ADP-glucose} \rightarrow (\text{glucose})_{n+1} + \text{ADP}$$

The Assimilation of Inorganic Phosphorus, Sulfur, and Nitrogen

- ✓ each assimilated by different routes

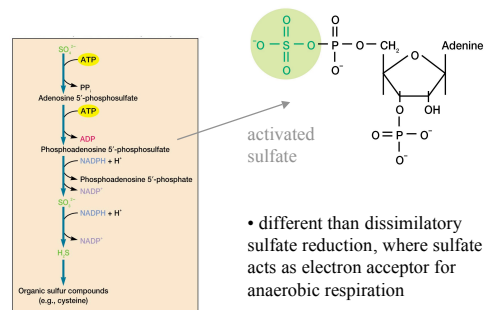
Phosphorus Assimilation

- ✓ inorganic phosphate (P_i)
 - incorporated into ATP by:
 - photophosphorylation
 - oxidative phosphorylation
 - substrate-level phosphorylation
- ✓ organic phosphate esters
 - hydrolyzed by phosphatases, releasing P_i

Sulfur Assimilation

- ✓ organic sulfur
 - obtained in form of cysteine and methionine
 - supplied by external sources or internal reserves
- ✓ inorganic sulfate
 - assimilatory sulfate reduction
 - sulfate reduced to H_2S and then used to synthesize cysteine

Assimilatory sulfate reduction



Formation of cysteine

- ✓ two processes used
 - 1) $H_2S + \text{serine} \rightarrow \text{cysteine} + H_2O$
 - 2) $\text{serine} + \text{acetyl-CoA} \rightarrow \text{O-acetylserine} + \text{Co-A}$
 $\rightarrow \text{O-acetylserine} + H_2S \rightarrow \text{acetate} + \text{cysteine}$

Nitrogen Assimilation

- ✓ 2 forms of nitrogen are commonly used as nitrogen sources
 - ammonia
 - nitrate

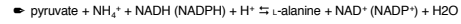
Ammonia incorporation

✓ 2 major mechanisms

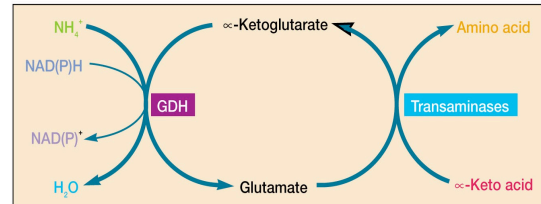
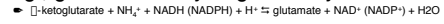
- reductive amination/transamination pathways
- glutamine synthetase/glutamate synthase system

Reductive amination/transamination pathways

✓ Alanine formation

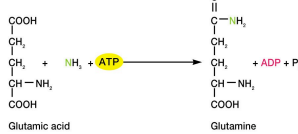


✓ e.g., glutamate dehydrogenase system

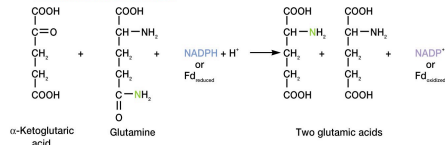


Glutamine synthetase/ glutamate synthase system

Glutamine synthetase reaction

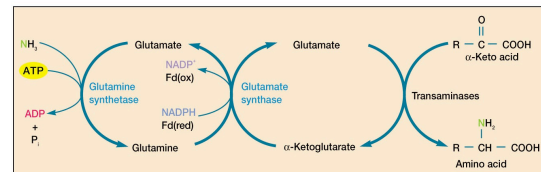


Glutamate synthase reaction



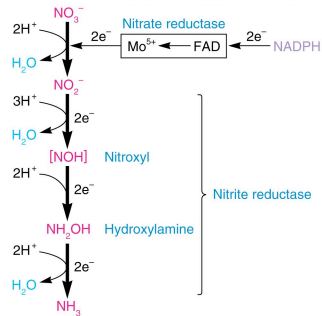
Glutamine synthetase/ glutamate synthase system

Present in *E. coli* and *Bacillus megaterium*



Nitrate

✓ incorporated by assimilatory nitrate reduction



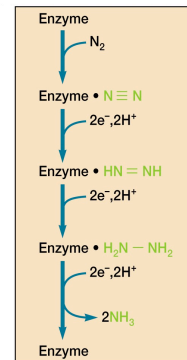
Nitrogen Fixation

✓ reduction of atmospheric nitrogen to ammonia

✓ catalyzed by nitrogenase

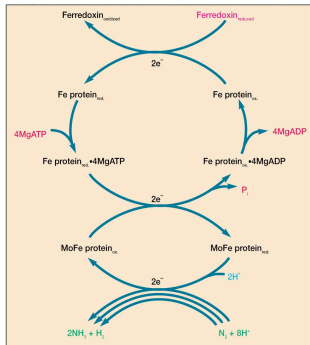
- found only in a few species of bacteria
- *Azotobacter*, *Klebsiella*, *Clostridium*, and *Methanococcus*
- *Rizobium*
- *Nostoc* and *Anabaena*

✓ requires large energy expenditure



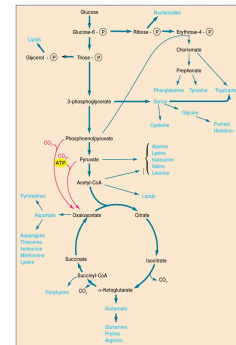
Mechanism of nitrogenase activity

- ✓ repeated 3 times to reduce N_2 to 2 molecules of Ammonia
- ✓ O_2 sensitive

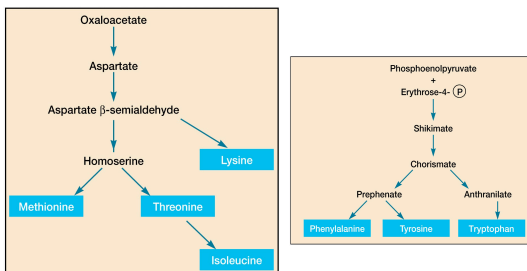


The Synthesis of Amino Acids

- ✓ intermediates of glycolytic pathway, pentose phosphate pathway, and TCA cycle used as substrates for synthesis of carbon skeletons
- ✓ ammonia added



Sample pathways

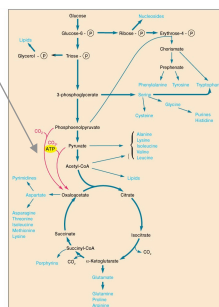


Anaplerotic Reactions (filling up)

- ✓ replenish TCA cycle intermediates
- ✓ allow TCA cycle to function during periods of active biosynthesis
- ✓ e.g., anaplerotic CO_2 fixation
- ✓ e.g., glyoxylate cycle

Anaplerotic CO_2 fixation

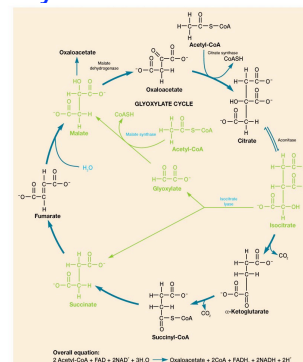
phosphoenolpyruvate (PEP) carboxylase:
 $PEP + CO_2 \rightarrow$ oxaloacetate



pyruvate carboxylase:
 $pyruvate + CO_2 \rightarrow$ oxaloacetate

Glyoxalate cycle

a modified TCA cycle

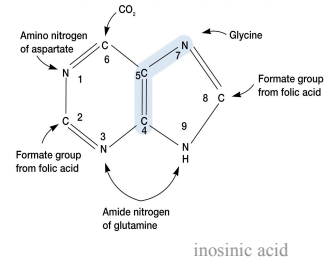


The Synthesis of Purines, Pyrimidines, and Nucleotides

- ✓ purines
 - cyclic nitrogenous bases consisting of 2 joined rings
 - adenine and guanine
- ✓ pyrimidines
 - cyclic nitrogenous bases consisting of single ring
 - uracil, cytosine, and thymine
- ✓ nucleoside = nitrogenase base-pentose sugar
- ✓ nucleotide = nucleoside-phosphate

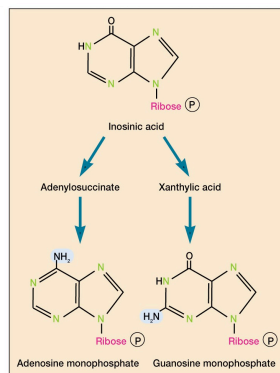
Purine Biosynthesis

- ✓ complex pathway in which several different molecules contribute parts to the final purine skeleton



initial products are ribonucleotides

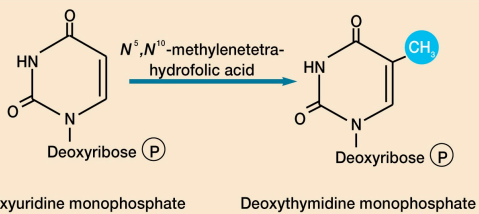
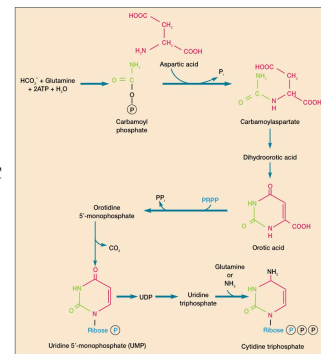
deoxyribonucleotides formed by reduction of nucleoside diphosphates or nucleoside triphosphates



Pyrimidine Biosynthesis

ribonucleotides are initial products

deoxy forms of U and C nucleotides formed by reduction of ribose to deoxyribose

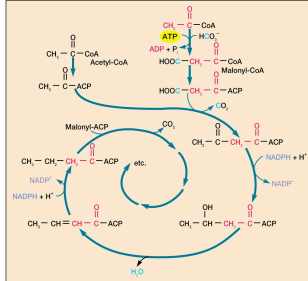


Lipid Synthesis

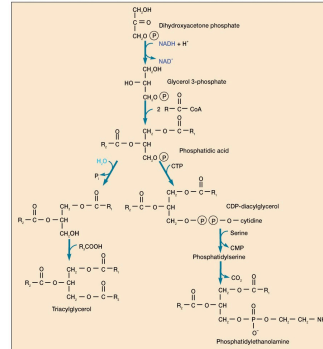
- ✓ fatty acids
 - synthesized then added to other molecules to form other lipids such as triacylglycerols and phospholipids

Fatty acid synthesis

- ✓ catalyzed by fatty acid synthetase
- ✓ involves activity of acyl carrier protein (ACP)

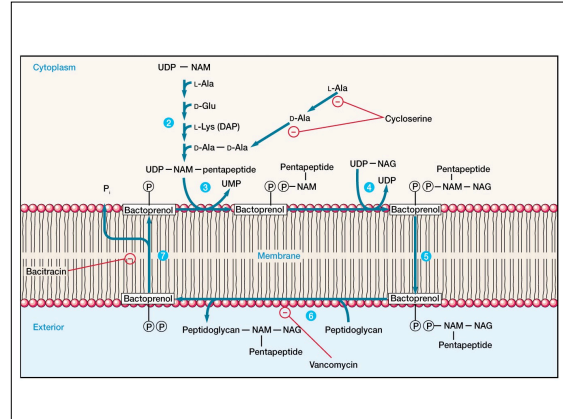


Triacylglycerols and phospholipids

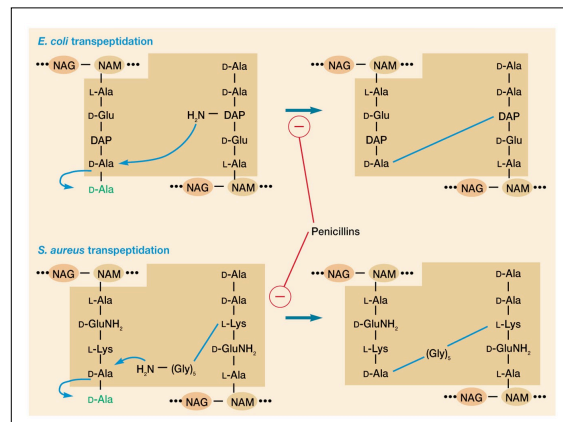
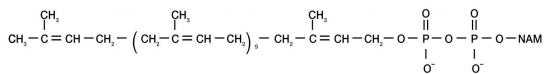


Peptidoglycan Synthesis

- ✓ complex multi-stage process
 - first forms peptidoglycan repeat unit in cytoplasm
 - involves use of uridine diphosphate and bactoprenol as carriers
 - repeat unit then transported across membrane by bactoprenol
 - repeat unit attached to growing peptidoglycan chain
 - cross-links formed by transpeptidation



bactoprenol attached to N-acetylmuramic acid



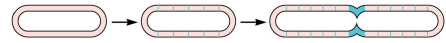
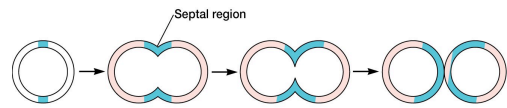
Patterns of Cell Wall Formation

✓ autolysins

- carry out limited digestion of peptidoglycan
- provide acceptor ends for addition of new peptidoglycan units

✓ 2 general patterns of cell wall formation

- growth at one or just a few sites
- usually at site of septum formation
- observed in many gram-positive bacteria



- growth sites scattered along length of cell
- growth also at site of septum formation
- observed in many rod-shaped bacteria