Chapter 9

Metabolism: Energy Release and Conservation

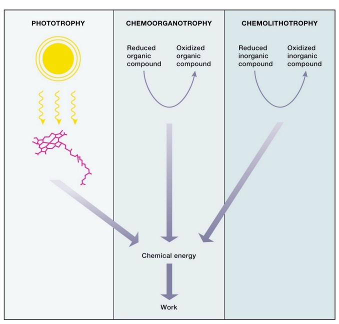
An Overview of Metabolism

- ✓ metabolism
  - ✑total of all chemical reactions occurring in cell
- ✓ catabolism
  - ✑breakdown of larger, more complex molecules into smaller, simpler ones
  - ✑energy is released and some is trapped and made available for work
- ✓ anabolism
  - ✑synthesis of complex molecules from simpler ones with the input of energy

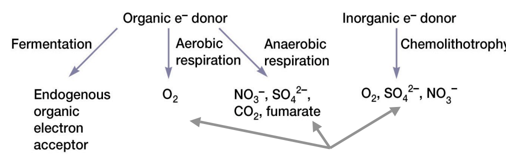
Sources of energy

electrons released during oxidation of chemical energy sources must be accepted by an electron acceptor

microorganisms vary in terms of the acceptors they use



Electron acceptors for chemotrophic processes



exogenous electron acceptors

Chemoorganotrophic metabolism

- ✓ fermentation
  - ✑energy source oxidized and degraded using endogenous electron acceptor
  - ✑often occurs under anaerobic conditions
  - ✑limited energy made available

Chemoorganotrophic metabolism

- ✓ aerobic respiration
  - ✑energy source degraded using oxygen as exogenous electron acceptor
  - ✑yields large amount of energy, primarily by electron transport activity

Chemoorganotrophic metabolism

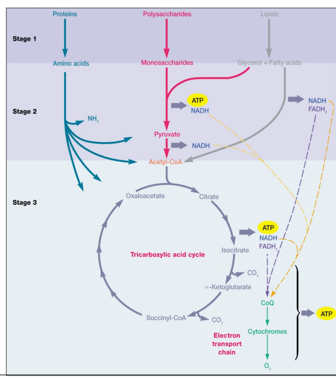
✓ anaerobic respiration
- energy source oxidized and degraded using molecules other than oxygen as exogenous electron acceptors
- can yield large amount of energy (depending on reduction potential of energy source and electron acceptor), primarily by electron transport activity

Overview of aerobic catabolism

✓ three-stage process
- large molecules (polymers)➜small molecules (monomers)
- Oxidation and degradation to pyruvate
- Oxidation and degradation of pyruvate by Krebs (TCA) cycle

Stages of catabolism

many different energy sources are funneled into common degradative pathways

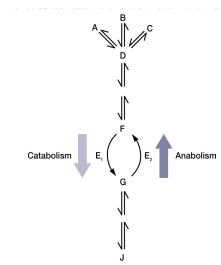


ATP made primarily by oxidative phosphorylation

Two functions of organic energy sources

✓ oxidized to release energy

✓ supply carbon and building blocks for anabolism
- amphibolic pathways
  - function both as catabolic and anabolic pathways



The Breakdown of Glucose to Pyruvate

✓ Three common routes
- glycolysis
- pentose phosphate pathway
- Entner-Doudoroff pathway

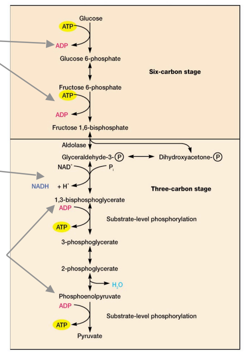
The Glycolytic Pathway

✓ also called Embden-Meyerhof pathway

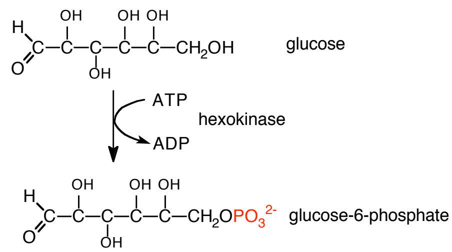
✓ occurs in cytoplasmic matrix of both procaryotes and eucaryotes addition of phosphates "primes the pump"

oxidation step – generates NADH

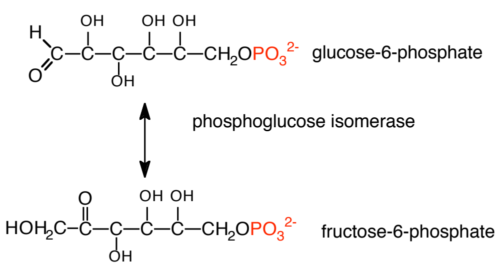
high-energy molecules – used to synthesize ATP by substrate-level phosphorylation



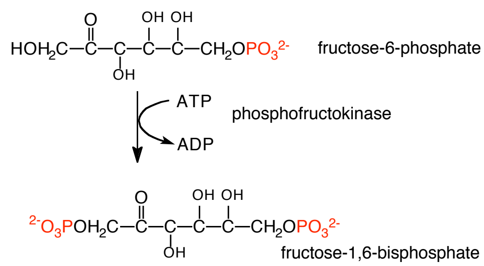
Phosphorylation prevents glucose from leaving the cell



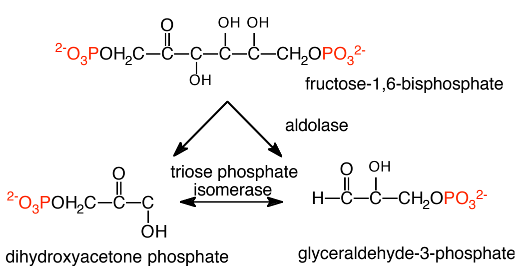
Aldose to ketose



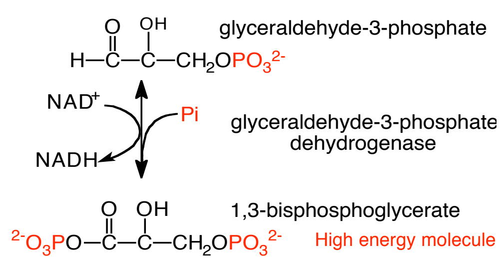
Phosphofructokinase is the key enzyme in glycolysis



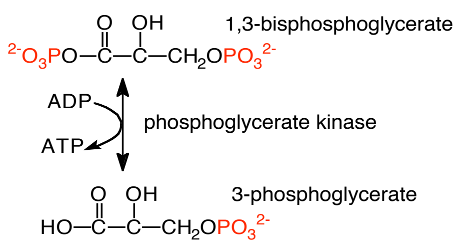
Sugar-splitting



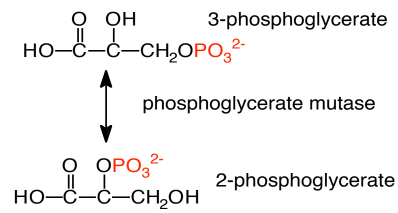
Energy transfer



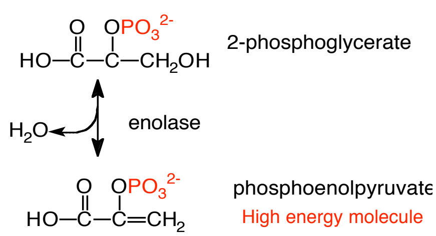
Forming ATP



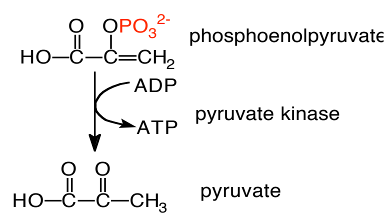
Rearrangement



Dehydration increases the potential for transfer of phosphate to ADP



Transfer of P to ADP is irreversible



Summary of glycolysis

glucose + 2ADP + $2P_i$ + $2NAD^+$

2 pyruvate + 2ATP + 2NADH + $2H^+$

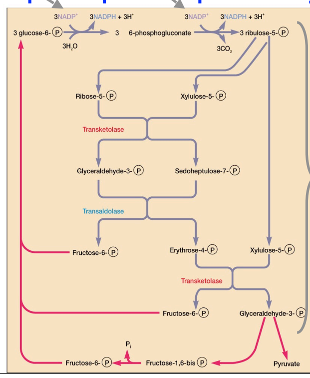
The Pentose Phosphate Pathway

- ✓ also called hexose monophosphate pathway
- ✓ can operate at same time as glycolytic or Entner-Doudoroff pathways
- ✓ can operate aerobically or anaerobically
- ✓ an amphibolic pathway

Pentose phosphate pathway

oxidation steps

produce NADPH, which is needed for biosynthesis

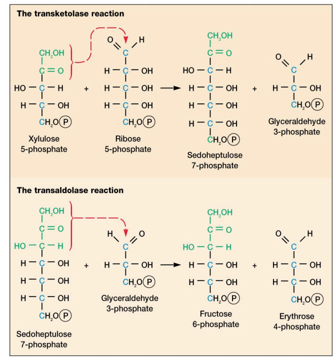


sugar trans-formation reactions

produce sugars needed for biosynthesis

sugars can also be further degraded

Transketolase & transaldolase



Summary of pentose phosphate pathway

$$\text{glucose-6-P} + 12NADP^+ + 7H_2O$$

$$6CO_2 + 12NADPH + 12H^+\ P_i$$

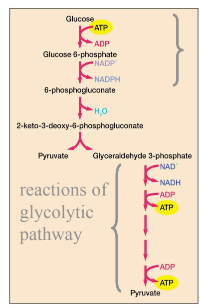
- ✓ NADPH
- ✓ 4 & 5 carbon sugars - amino acids & nucleic acids, photosynthesis, utilize pentose sugars
- ✓ Intermediates used to make ATP

The Entner-Doudoroff Pathway

✓ yield per glucose molecule:

- 1 ATP
- 1 NADPH
- 1 NADH

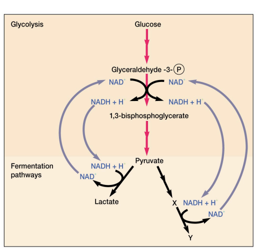
Found in *Psuedomonas*, *Rhizobium*, *Azotobacter*, *Agrobacterium*, and *Enterococcus faecalis*



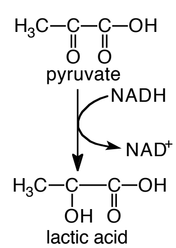
reactions of pentose phosphate pathway

Fermentations

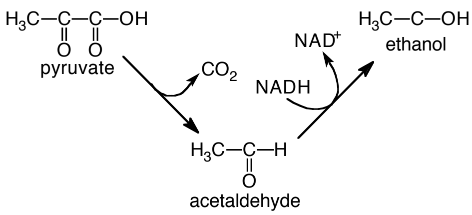
- ✓ oxidation of NADH produced by glycolysis
- ✓ pyruvate or derivative used as endogenous electron acceptor
- ✓ ATP formed by substrate-level phosphorylation



Lactic acid fermentation



Alcoholic fermentation

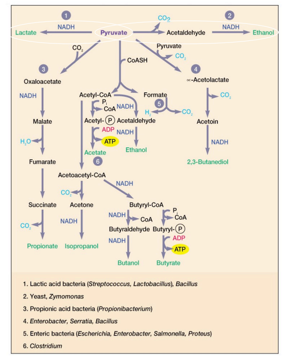


homolactic fermenters

heterolactic fermenters

food spoilage

yogurt, sauerkraut, pickles, etc.



alcoholic fermentation

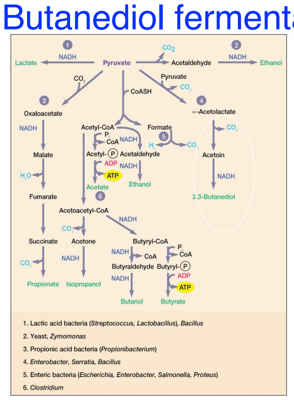
alcoholic beverages, bread, etc.

Table 9.1 Mixed Acid Fermentation Products of *Escherichia coli*

	Fermentation Balance (µM Product/100 µM Glucose)	
	Acid Growth (pH 6.0)	**Alkaline Growth (pH 8.0)**
Ethanol	50	50
Formic acid	2	86
Acetic acid	36	39
Lactic acid	80	70
Succinic acid	11	15
Carbon dioxide	88	2
Hydrogen gas	75	0.5
Butanediol	0	0

methyl red test – detects pH change in media caused by mixed acid fermentation

Butanediol fermentation



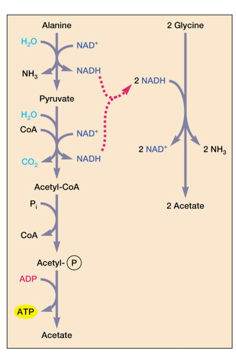
Voges-Proskauer test – detects intermediate acetoin

Methyl red test and Voges-Proskauer test important for distinguishing pathogenic members of *Enterobacteriaceae*

Enterobacter, *Serratia*, *Erwinia*, and some *Bacillus*

Fermentations of amino acids

- ✓ Strickland reaction
  - oxidation of one amino acid with use of second amino acid as electron acceptor
  - *Clostriduim sporogenes & botulinum*

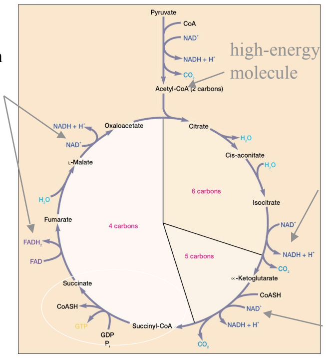


The Tricarboxylic Acid Cycle

- ✓ also called citric acid cycle and Kreb's cycle
- ✓ completes oxidation and degradation of glucose and other molecules
- ✓ common in aerobic bacteria, free-living protozoa, most algae, and fungi
- ✓ amphibolic
  - provides carbon skeletons for biosynthesis

oxidation steps – form NADH and $FADH_2$

substrate-level phosphorylation



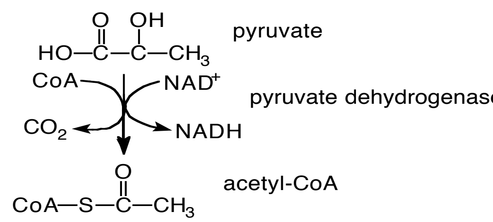
energy drives condensation of acetyl group with oxaloacetate

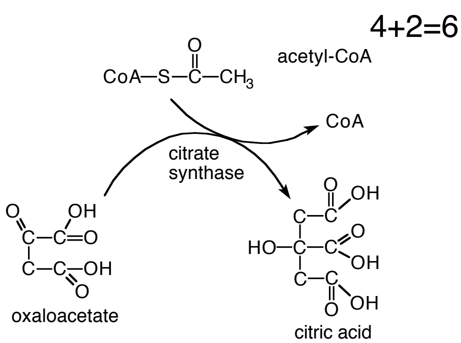
oxidation and decarboxylation steps

complete oxidation and degradation

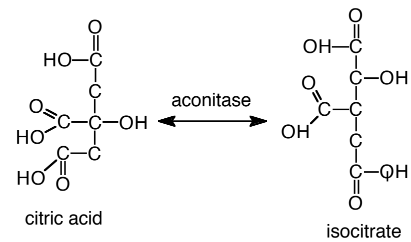
also form NADH

CoA activates the carbons from pyruvate

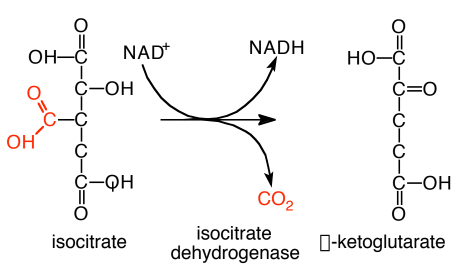




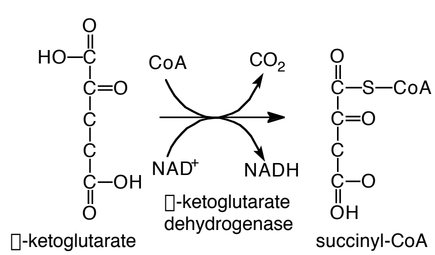
Moving an -OH



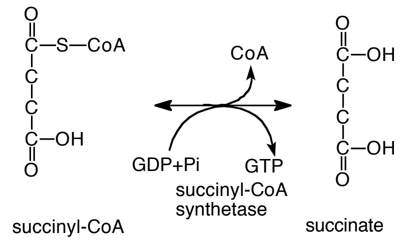
Oxidation and decarboxylation



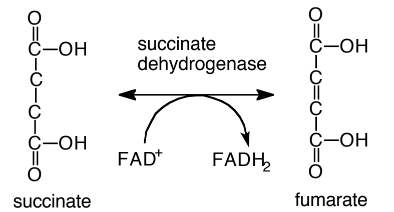
Energizing the bonds



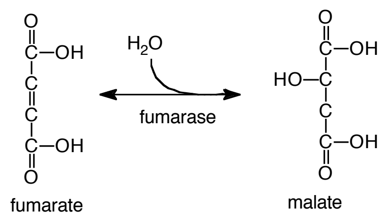
Making ATP



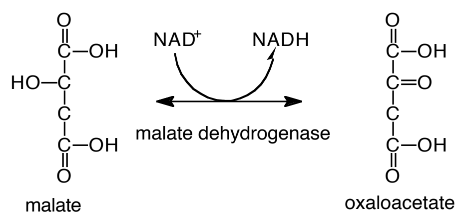
Using FAD+



Hydrating fumarate



Oxidation of malate



Krebs cycle summary

IN	OUT
2 acetyl-CoA	$2CO_2$
6 NAD^+	6NADH
2 FAD^+	$2FADH_2$
2GDP	2GTP
2Pi	$2H^+$
$2H_2O$	2 CoA

Summary

✓ for each acetyl-CoA molecule oxidized, TCA cycle generates:

- 2 molecules of CO_2
- 3 molecules of NADH
- one $FADH_2$
- one GTP

Electron Transport and Oxidative Phosphorylation

- ✓only 4 ATP molecules synthesized directly from oxidation of glucose to CO_2
- ✓most ATP made when NADH and $FADH_2$ (formed as glucose degraded) are oxidized in electron transport chain (ETC)

The Electron Transport Chain

- ✓series of electron carriers that operate together to transfer electrons from NADH and $FADH_2$ to a terminal electron acceptor
- ✓electrons flow from carriers with more negative E_0 to carriers with more positive E_0

Electron transport chain…

- ✓as electrons transferred, energy released
- ✓some released energy used to make ATP by oxidative phosphorylation
  - ✒ as many as 3 ATP molecules made per NADH using oxygen as acceptor
    - ⇨P/O ratio = 3
  - ✒P/O ratio for $FADH_2$ is 2
    - ⇨i.e., 2 ATP molecules made

large difference in E_0 of NADH and E_0 of O_2

large amount of energy released

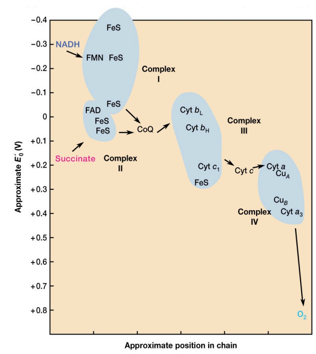


Figure 9.13

Mitochondrial ETC

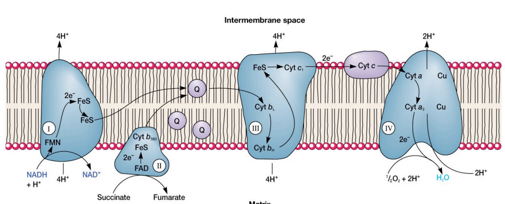


Figure 9.14

electron transfer accompanied by proton movement across inner mitochondrial membrane

Procaryotic ETCs

- ✓located in plasma membrane
- ✓some resemble mitochondrial ETC, but many are different
  - ✒different electron carriers
  - ✒may be branched
  - ✒may be shorter
  - ✒may have lower P/O ratio

ETC of *E. coli*

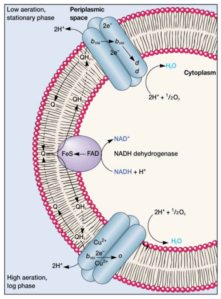


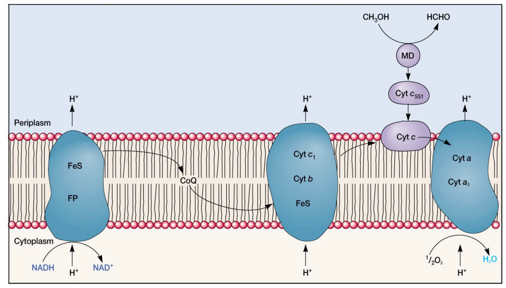
Figure 9.15

branched pathway

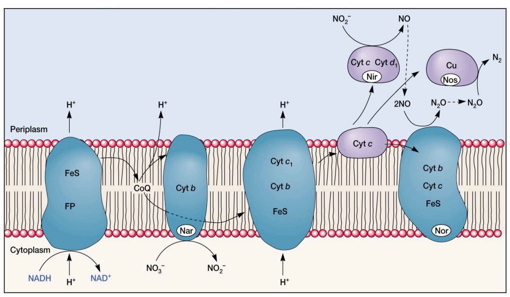
upper branch – stationary phase and low aeration

lower branch – log phase and high aeration

ETC of *Paracoccus denitrificans* - aerobic



ETC of *P. denitrificans* - anaerobic



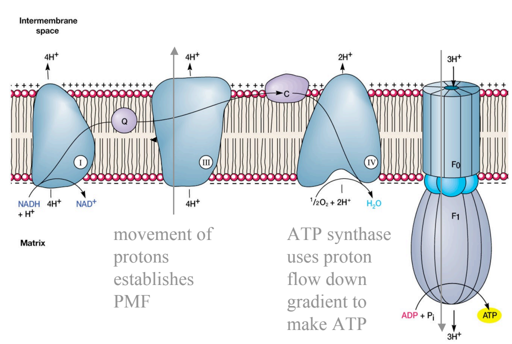
example of anaerobic respiration

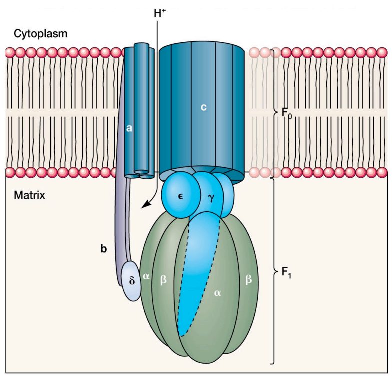
Oxidative Phosphorylation

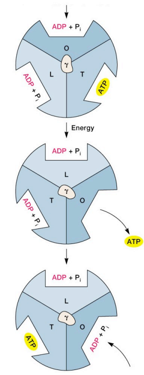
- ✓chemiosmotic hypothesis
  - ✑most widely accepted explanation of oxidative phosphorylation
  - ✑postulates that energy released during electron transport used to establish a proton gradient and charge difference across membrane
    - ✑called proton motive force (PMF)

PMF drives ATP synthesis

- ✓diffusion of protons back across membrane (down gradient) drives formation of ATP
- ✓ATP synthase
  - ✑enzyme that uses proton movement down gradient to catalyze ATP synthesis



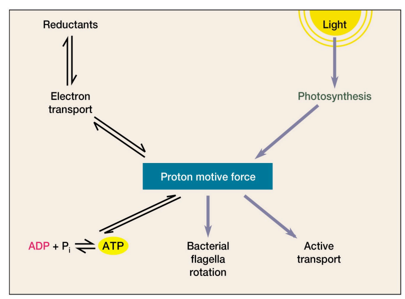




Inhibitors of ATP synthesis

- ✓ blockers
  - ✑inhibit flow of electrons through ETC
    - ⇨Piericidin, antimycin A, cyanide and azide
- ✓ Uncouplers
  - ✑allow electron flow, but disconnect it from oxidative phosphorylation
  - ✑many allow movement of ions, including protons, across membrane without activating ATP synthase
    - ⇨destroys pH and ion gradients
    - ⇨Dinitrophenol
  - ✑some may bind ATP synthase and inhibit its activity directly
    - ⇨valinomycin

Importance of PMF



The Yield of ATP in Glycolysis and Aerobic Respiration

- ✓ aerobic respiration provides much more ATP than fermentation
- ✓ Pasteur effect
  - ✑decrease in rate of sugar metabolism when microbe shifted from anaerobic to aerobic conditions
  - ✑occurs because aerobic process generates greater ATP per sugar molecule

Table 9.2 ATP Yield from the Aerobic Oxidation of Glucose by Eucaryotic Cells

Glycolytic Pathway	
Substrate-level phosphorylation (ATP)	2 ATP[a]
Oxidative phosphorylation with 2 NADH	6 ATP
2 Pyruvate to 2 Acetyl-CoA	
Oxidative phosphorylation with 2 NADH	6 ATP
Tricarboxylic Acid Cycle	
Substrate-level phosphorylation (GTP)	2 ATP
Oxidative phosphorylation with 6 NADH	18 ATP
Oxidative phosphorylation with 2 $FADH_2$	4 ATP
Total Aerobic Yield	38 ATP

[a]ATP yields are calculated with an assumed P/O ratio of 3.0 for NADH and 2.0 for $FADH_2$.

ATP yield…

- ✓ amount of ATP produced during aerobic respiration varies depending on growth conditions and nature of ETC
- ✓ under anaerobic conditions, glycolysis only yields 2 ATP molecules

Anaerobic Respiration

- ✓ uses electron carriers other than O_2
- ✓ generally yields less energy because E_0 of electron acceptor is less positive than E_0 of O_2

Table 9.3 Some Electron Acceptors Used in Respiration

	Electron Acceptor	Reduced Products	Examples of Microorganisms
Aerobic	O_2	H_2O	All aerobic bacteria, fungi, protozoa, and algae
Anaerobic	NO_3^-	NO_2^-	Enteric bacteria
	NO_3^-	NO_2^-, N_2O, N_2	*Pseudomonas, Bacillus,* and *Paracoccus*
	SO_4^{2-}	H_2S	*Desulfovibrio* and *Desulfotomaculum*
	CO_2	CH_4	All methanogens
	S^0	H_2S	*Desulfuromonas* and *Thermoproteus*
	Fe^{3+}	Fe^{2+}	*Pseudomonas, Bacillus,* and *Geobacter*
	$HAsO_4^{2-}$	$HAsO_2$	*Bacillus, Desulfotomaculum, Sulfurospirillum*
	SeO_4^{2-}	Se, $HSeO_3^-$	*Aeromonas, Bacillus, Thauera*
	Fumarate	Succinate	*Wolinella*

An example

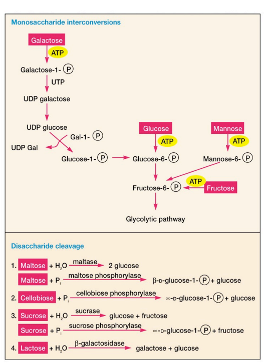
- ✓ dissimilatory nitrate reduction
  - ✑use of nitrate as terminal electron acceptor
  - ✑denitrification
    - ⇨reduction of nitrate to nitrogen gas
    - ⇨in soil, causes loss of soil fertility

Catabolism of Carbohydrates and Intracellular Reserves

- ✓ many different carbohydrates can serve as energy source
- ✓ carbohydrates can be supplied externally or internally (from internal reserves)

Carbohydrates

- ✓ monosaccharides
  - ✑converted to other sugars that enter glycolytic pathway
- ✓ disaccharides and polysaccharides
  - ✑cleaved by hydrolases or phosphorylases

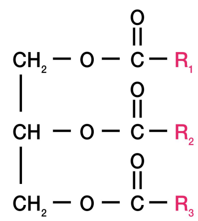


Reserve Polymers

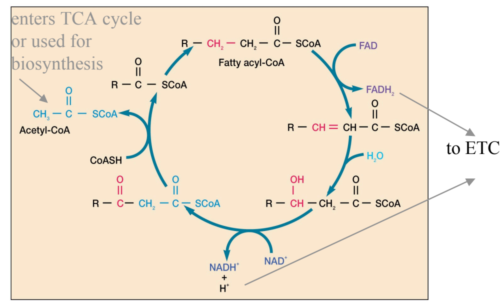
- ✓ used as energy sources in absence of external nutrients
  - ✑e.g., glycogen and starch
    - ⇨cleaved by phosphorylases
      - $(glucose)_n + P_i$ □ $(glucose)_{n-1}$ + glucose-1-P
      - • glucose-1-P enters glycolytic pathway
  - ✑e.g., PHB (poly-ß-hydroxybutyrate)
    - PHB □ □ □ acetyl-CoA
    - • acetyl-CoA enters TCA cycle

Lipid Catabolism

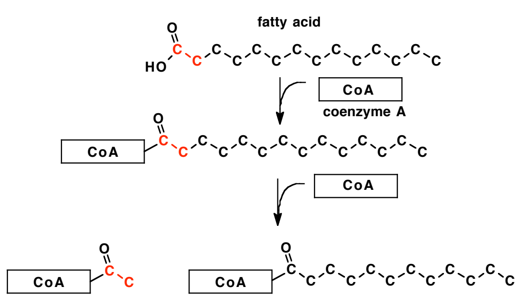
- ✓ triglycerides
  - ✑common energy sources
  - ✑hydrolyzed to glycerol and fatty acids by lipases
    - ⇨glycerol degraded via glycolytic pathway
    - ⇨fatty acids often oxidized via ß-oxidation pathway



ß-oxidation pathway



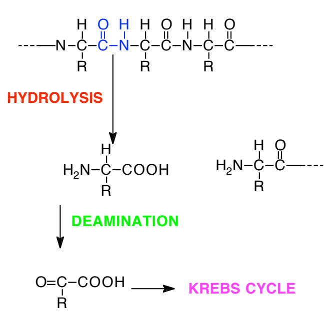
Fat metabolism



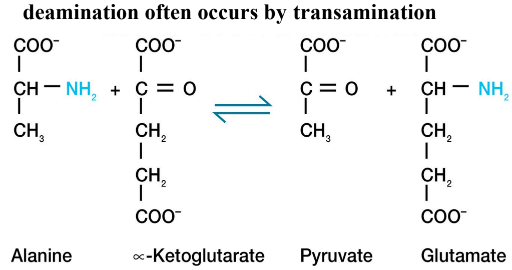
Protein and Amino Acid Catabolism

- ✓ protease
  - ✑hydrolyzes protein to amino acids
- ✓ deamination
  - ✑removal of amino group from amino acid
  - ✑resulting organic acids converted to pyruvate, acetyl-CoA, or TCA cycle intermediate
    - ⇨can be oxidized via TCA cycle
    - ⇨can be used for biosynthesis

Protein metabolism



deamination often occurs by transamination



transfer of amino group from one amino acid to □-keto acid

Oxidation of Inorganic Molecules

✓ carried out by chemolithotrophs
✓ electrons released from energy source
  - transferred to terminal electron acceptor by ETC
✓ ATP synthesized by oxidative phosphorylation

Table 9.4 Representative Chemolithotrophs and Their Energy Sources

Bacteria	Electron Donor	Electron Acceptor	Products
Alcaligenes, Hydrogenophaga, and *Pseudomonas* spp.	H_2	O_2	H_2O
Nitrobacter	NO_2^-	O_2	NO_3^-, H_2O
Nitrosomonas	NH_4^+	O_2	NO_2^-, H_2O
Thiobacillus denitrificans	S^0, H_2S	NO_3^-	SO_4^{2-}, N_2
Thiobacillus ferrooxidans	Fe^{2+}, S^0, H_2S	O_2	Fe^{3+}, H_2O, H_2SO_4

usually aerobic

Table 9.5 Energy Yields from Oxidations Used by Chemolithotrophs

Reaction	$\Delta G^{o\prime}$ (kcal/mole)[a]
$H_2 + \frac{1}{2} O_2 \longrightarrow H_2O$	−56.6
$NO_2^- + \frac{1}{2} O_2 \longrightarrow NO_3^-$	−17.4
$NH_4^+ + 1\frac{1}{2} O_2 \longrightarrow NO_2^- + H_2O + 2H^+$	−65.0
$S^0 + 1\frac{1}{2} O_2 + H_2O \longrightarrow H_2SO_4$	−118.5
$S_2O_3^{2-} + 2O_2 + H_2O \longrightarrow 2SO_4^{2-} + 2H^+$	−223.7
$2Fe^{2+} + 2H^+ + \frac{1}{2} O_2 \longrightarrow 2Fe^{3+} + H_2O$	−11.2

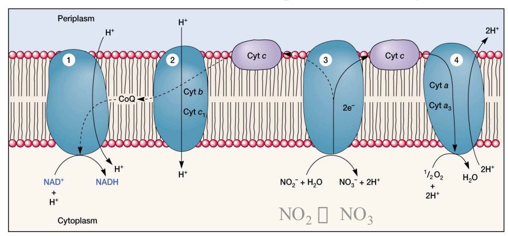
[a] The $\Delta G^{o\prime}$ for complete oxidation of glucose to CO_2 is −686 kcal/mole. A kcal is equivalent to 4.184kJ.

Nitrifying bacteria

oxidize ammonia to nitrate

NH_3 ▯ NO_2 ▯ NO_3

requires 2 different genera



Sulfur-oxidizing bacteria

ATP can be synthesized by both oxidative phosphorylation and substrate-level phosphorylation

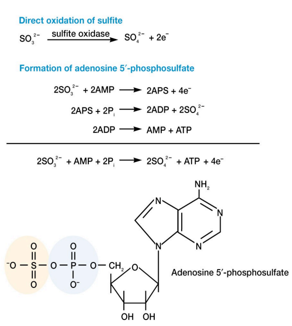


Figure 9.25

Metabolic flexibility of chemolithotrophs

✓ many switch from chemolithotrophic metabolism to chemoorganotrophic metabolism
✓ many switch from autotrophic metabolism (via Calvin cycle) to heterotrophic metabolism

Autotrophic growth by chemolithotrophs

✓Calvin cycle requires NADPH as electron source for fixing CO_2

- ✑many energy sources used by chemolithotrophs have E_0 more positive than NAD/NADH
  - ✑use reverse electron flow to generate NADH

Photosynthesis

✓light reactions

- ✑energy from light trapped and converted to chemical energy

✓dark reactions

- ✑chemical energy used to reduce CO_2 and synthesize cell constituents (discussed in Chapter 10)

Table 9.6 Diversity of Photosynthetic Organisms

Eucaryotic Organisms	Procaryotic Organisms
Higher plants	Cyanobacteria (blue-green algae)
Multicellular green, brown, and red algae	Green sulfur bacteria
Unicellular algae (e.g., euglenoids, dinoflagellates, diatoms)	Green nonsulfur bacteria
	Purple sulfur bacteria
	Purple nonsulfur bacteria
	Prochloron

oxygenic photosynthesis – eucaryotes and cyanobacteria

anoxygenic photosynthesis – all other bacteria

The Light Reaction in Eucaryotes and Cyanobacteria

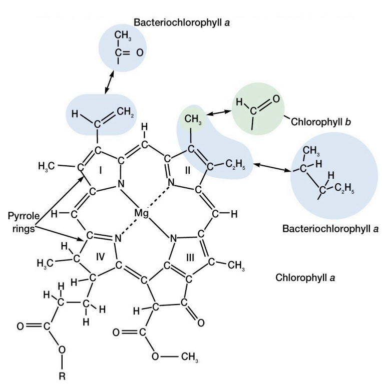
✓chlorophylls

- ✑major light-absorbing pigments

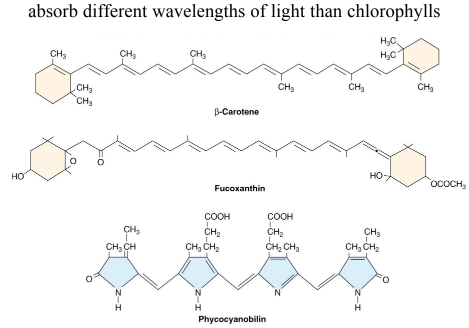
✓accessory pigments

- ✑transfer light energy to chlorophylls
  - ✑Carotenoids
  - ✑Phycobiliproteins
  - ✑Phycoerythrin
  - ✑phycocyanin

different chlorophylls have different absorption peaks



absorb different wavelengths of light than chlorophylls



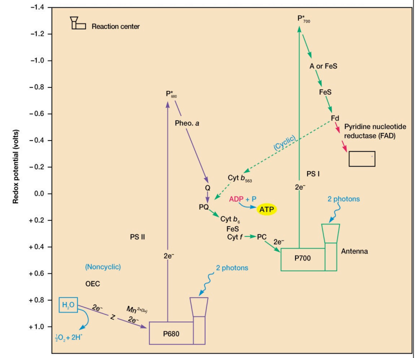
Organization of pigments

- ✓ antennas
  - ✑highly organized arrays of chlorophylls and accessory pigments
  - ✑captured light transferred to special reaction-center chlorophyll
    - ✑directly involved in photosynthetic electron transport
- ✓ photosystems
  - ✑antenna and its associated reaction-center chlorophyll

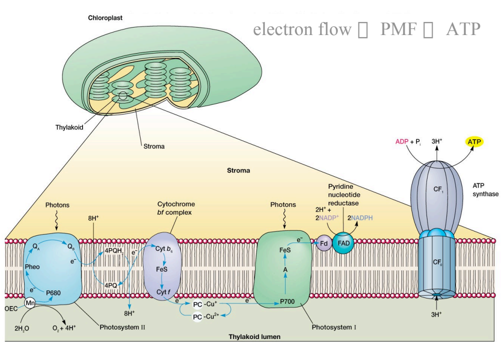
Green plant photosynthesis

noncyclic electron flow – ATP + NADPH made

cyclic electron flow – ATP made



electron flow PMF ATP

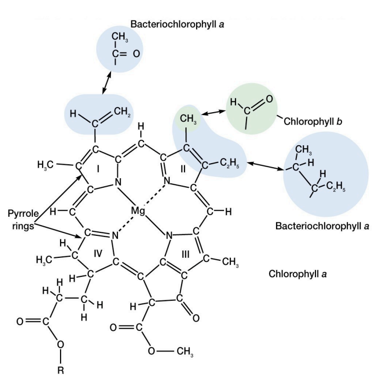


The Light Reaction in Green and Purple Bacteria

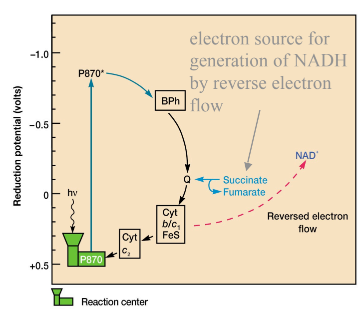
Table 9.7 Properties of Microbial Photosynthetic Systems

Property	Eucaryotes	Cyanobacteria	Green and Purple Bacteria
Photosynthetic pigment	Chlorophyll *a*	Chlorophyll *a*	Bacteriochlorophyll
Photosystem II	Present	Present	Absent
Photosynthetic electron donors	H_2O	H_2O	H_2, H_2S, S, organic matter
O_2 production pattern	Oxygenic	Oxygenic[a]	Anoxygenic
Primary products of energy conversion	ATP + NADPH	ATP + NADPH	ATP
Carbon source	CO_2	CO_2	Organic and/or CO_2

[a]Some cyanobacteria can function anoxygenically under certain conditions. For example, *Oscillatoria* can use H_2S as an electron donor instead of H_2O.



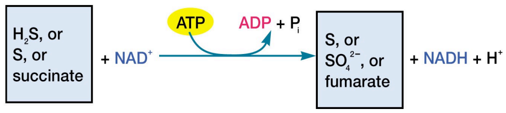
Purple nonsulfur bacteria



Reverse electron flow

more positive
E_0

more negative
E_0



ATP or PMF used to drive "upward" (reverse) flow of electrons

Green sulfur bacteria