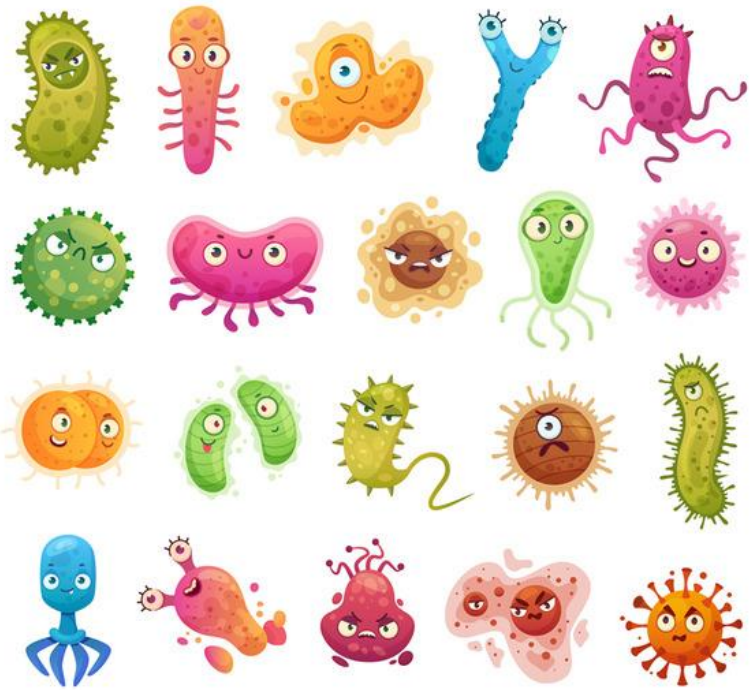
MICROBIAL METABOLISM

Energy Release & Conservation



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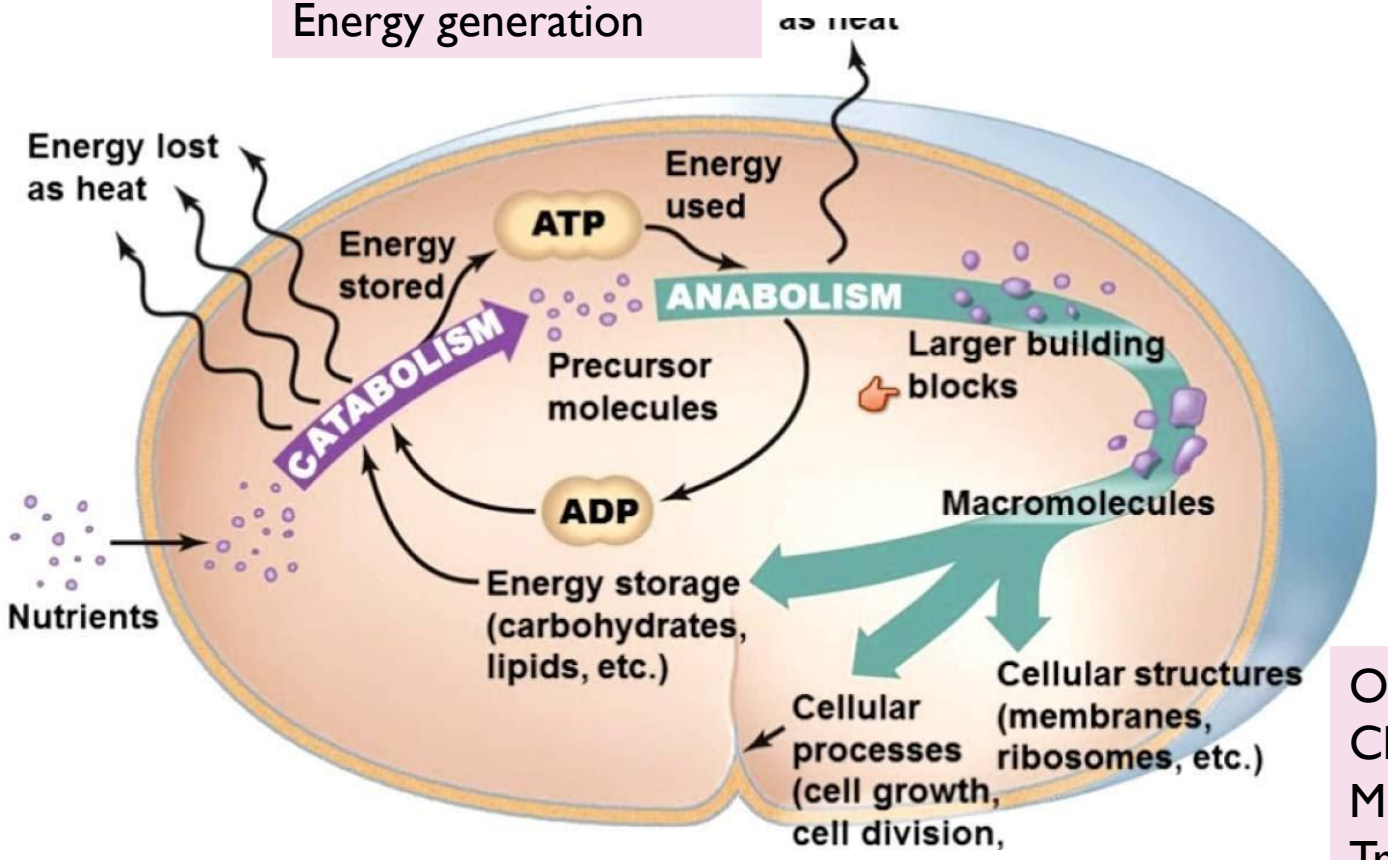
CONTENT

What is microbial METABOLISM?

The series of biochemical reaction in cells

Energy generation



Use of energy to synthesize cell material (lipids, polysaccharides, proteins, etc.)

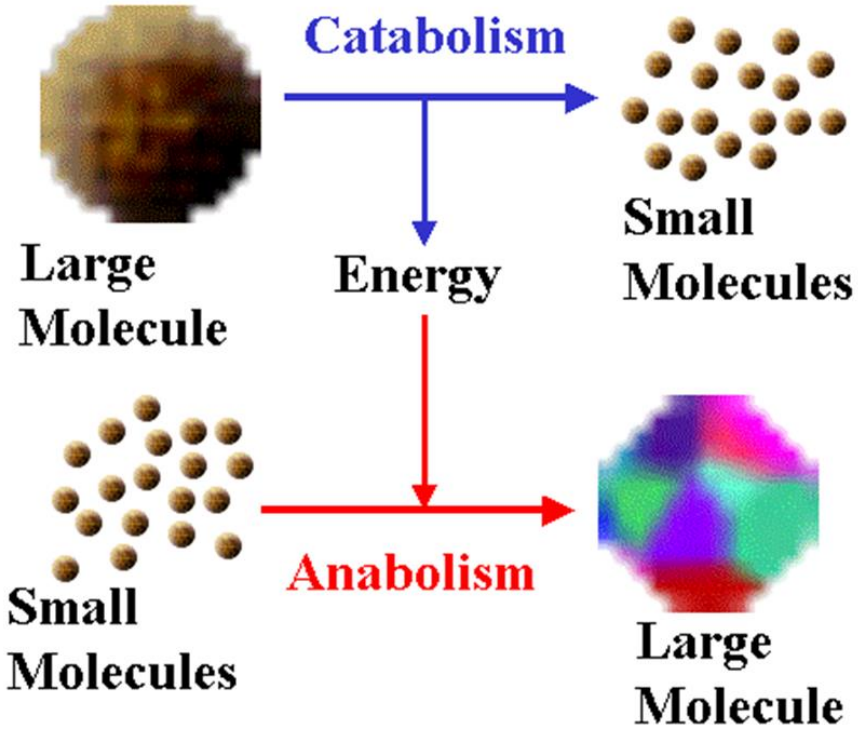
Breaks down of various biomolecules such as nutrients (carbohydrates, fat, protein)

Organelles, enzymes,
Chemical work
Mechanical work –movement
Transportation

BASIC CONCEPT OF METABOLISM

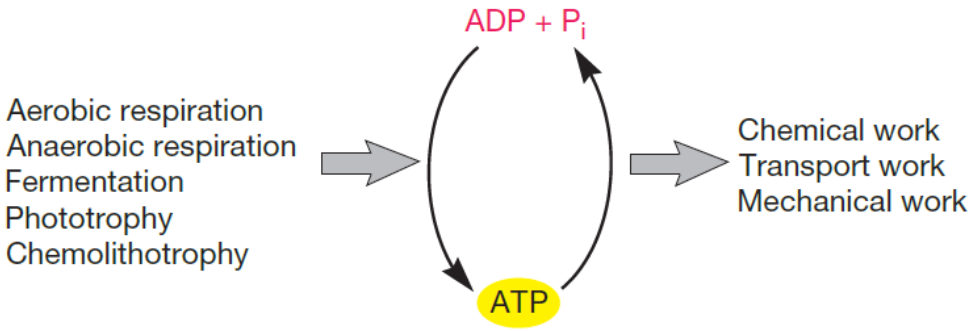
Metabolism - The series of biochemical reactions by which the cells breaks down various biomolecules such as nutrients (carbohydrates, fat, protein) for energy generation and the use of energy to synthesize cell material (lipids, polysaccharides, proteins) from small molecules.

1. **Catabolism**
   - The breakdown of larger molecules to smaller ones
   - Produce energy (ATP)
2. **Anabolism**
   - The synthesis (build up) of complex molecule from simple molecules
   - Require energy (ATP)



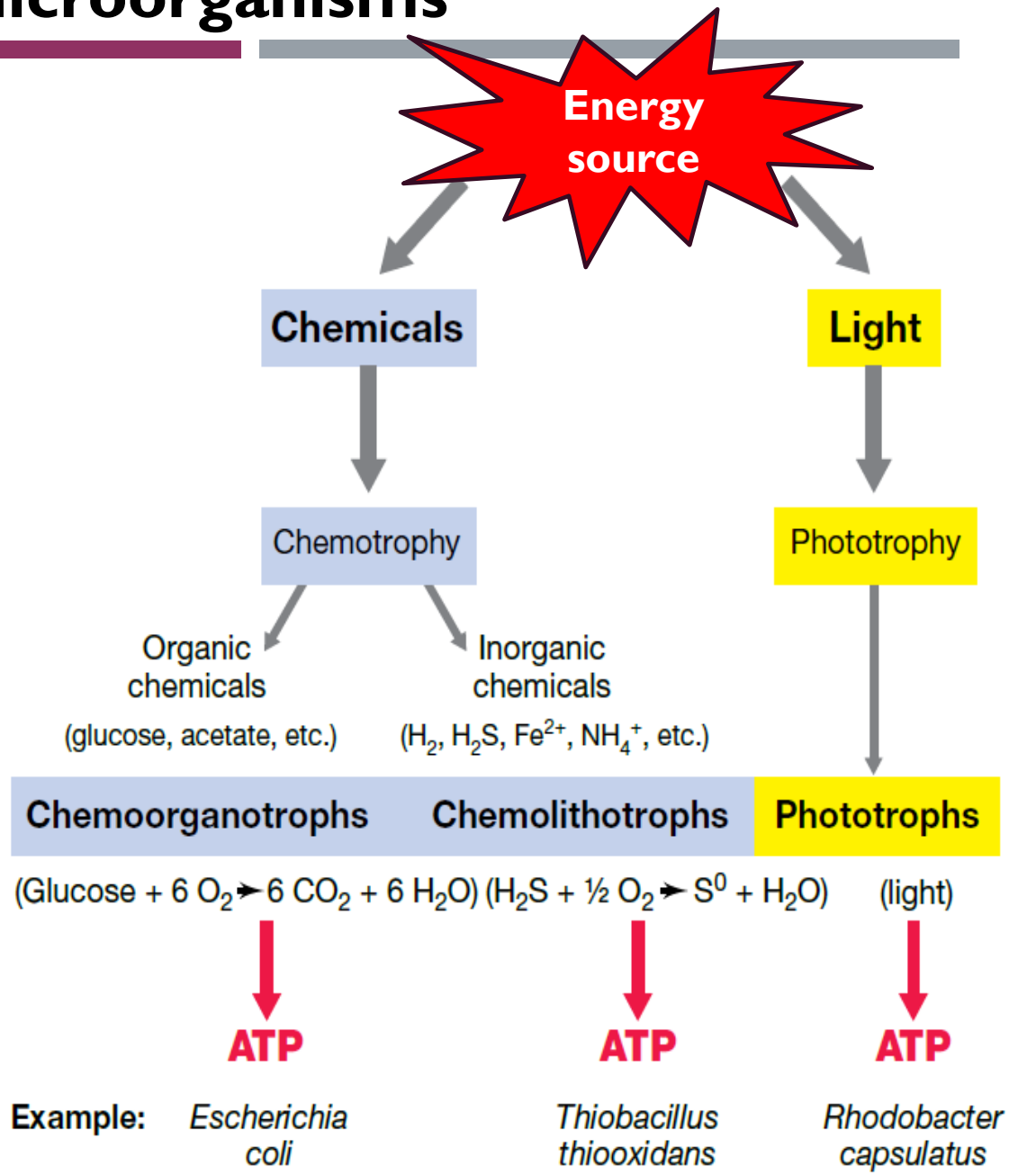
Cellular work & energy transfers

- Cells carry out 3 major types of work in order to survive and reproduce.
  1. **Chemical work** – synthesis of complex molecules (e.g. organelles, enzymes, etc.)
  2. **Transport work** – take up nutrients, eliminates wastes, and maintain ion balances (sodium potassium ion channel)
  3. **Mechanical work** – cell motility and movement of structures within cells, binary fission
- Cells need **ENERGY** to do work
- Organisms obtain the energy from an **energy source present in their environment** → convert it to a useful form (**ATP**).



Energy source for microorganisms

- All microorganisms can be defined metabolically in terms of their **energy source** **and** **electron source**.
  1. **Chemoorganotrophs** - Energy source is organic molecules, that is also the source for carbon and electrons. The organic compounds can be catabolized and the energy released is conserved in the ATP.
  2. **Chemolithotrophs** - Energy source is inorganic molecules that is also the electron source. Carbon source can be either CO_2 (autotrophs) or an organic molecule (heterotroph). E.g. "sulphur" bacteria and "nitrifying" bacteria.
  3. **Phototrophs** - Energy source is light, the carbon source can be CO_2 or organic molecules, and the electron source can be water (oxygenic phototrophs) or another reduced molecule such HS (anoxygenic phototroph). Phototrophs contain chlorophylls & other pigment that convert light into ATP. E.g. cyanobacteria (oxygenic), purple and green bacteria (anoxygenic).
- **Carbon source**:
  1. **Heterotroph** – Carbon obtained from organic compound
  2. **Autotroph** – CO_2 as carbon source



Major Nutritional Types Of Microorganisms

Nutritional type	Carbon source	Energy Source	Electron source	Representative microorganisms
Photolithoautrotroph	CO_2	Light	Inorganic e- donor	Purple & green sulfur bacteria, cyanobacteria
Photoorganoheterotroph	Organic carbon	Light	Organic e- donor	Purple & green non-sulfur bacteria
Chemolithoautotroph	CO_2	Inorganic chemicals	Inorganic e- donor	Sulfur-oxidizing bacteria, methanogens
Chemolithoheterotroph	Organic carbon	Inorganic chemicals	Inorganic e- donor	Sulfur-oxidizing bacteria
Chemoorganoheterotroph	Organic carbon	Organic chemicals *often same as C source	Organic e- donor *often same as C source	Most nonphotosynthetic microbes, including pathogens, fungi, animal, etc

PHOTOSYNTHETIC BACTERIA

Bacteria that contain light absorbing pigments capable of converting light energy into chemical energy.

They are widely distributed occupying several habitats like soil, lakes, paddy fields, oceans, and rivers.

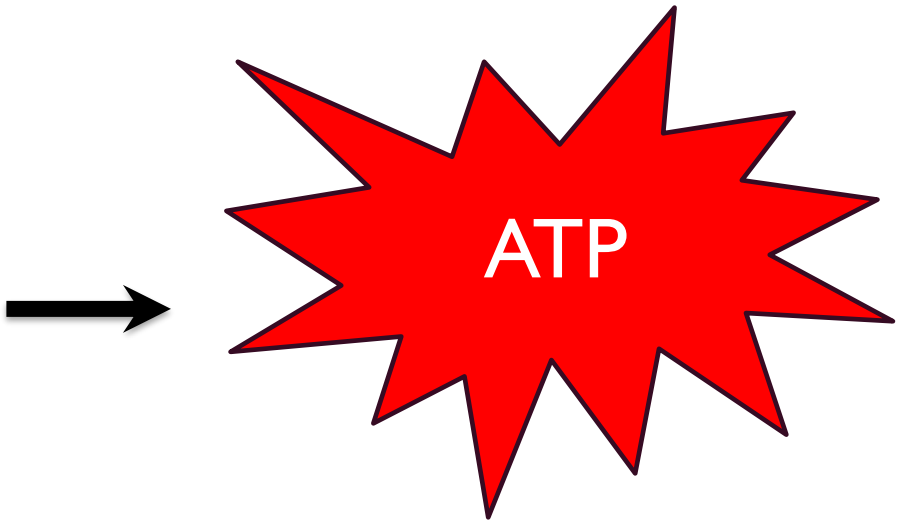
Cyanobacteria

Purple bacteria

CHEMOORGANOHETEROTROPH

e.g. Foodborne pathogens (bacteria, parasites, protozoa)

- Bacterial cell cannot use the energy source (sunlight or chemical compounds) to run cellular reactions.
- So, food needs to be turned into **ATP** because that is what actually runs the metabolic process!



What is the role of ATP in an organism?

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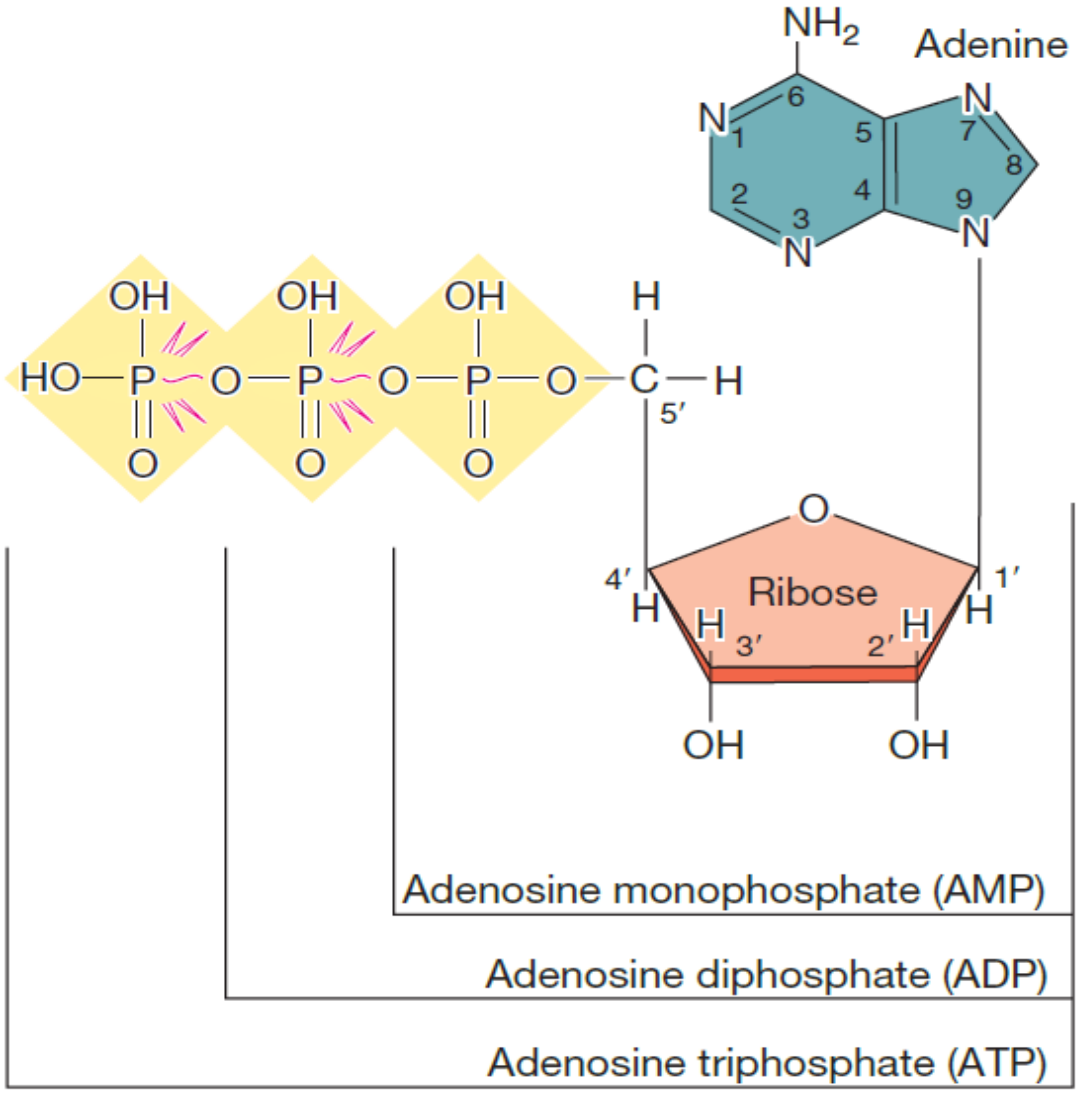
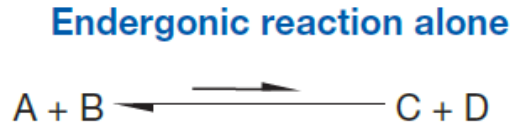
ATP: THE MAJOR ENERGY CURRENCY OF CELLS

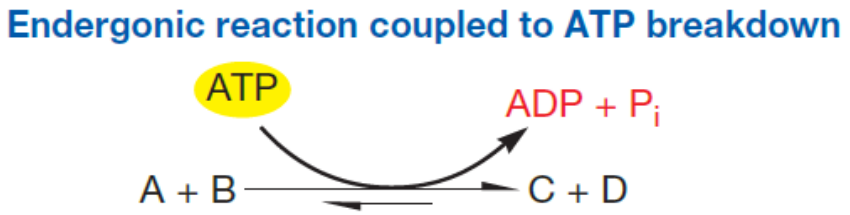


Adenosine Triphosphate

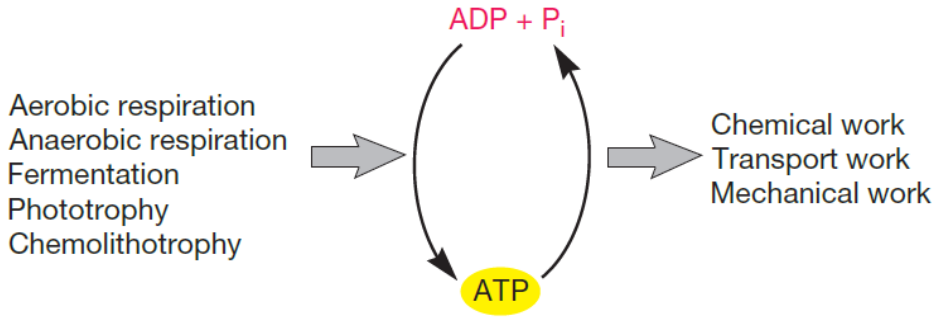
- Cell carry out certain process so that they can "earn" ATP and carry out other processes in which they "spend" their ATP – ATP as the energy currency!!

- Characteristic of ATP:
  - ATP is a high energy compound with three phosphate groups linked in a small chain.
  - It has a high phosphate transfer potential - can donate a phosphoryl group to other molecules and energy is released (ATP becomes ADP).
  - The energy released is used to power endergonic reactions





ATP is formed by exergonic reactions and then used to drive endergonic reactions.



Exergonic reactions.

Endergonic reactions.

THE ROLE OF REDOX REACTIONS IN CELL METABOLISM

- Redox reactions is also known as oxidation and reduction reactions.
- Many metabolic processes involve oxidation-reduction reactions (electron transfers).
- Redox reaction requires an enzyme to catalyse the reaction.
- Electron carriers are often used to transfer electrons from an electron donor to an electron acceptor.
- The reaction an result in energy release, which can be conserved and used to form ATP.

❑OXIDATION

- Loss of electrons
- Produce energy (catabolic reaction)
- The substance that loses electrons (donor) is oxidized, and called the reducing agent (reductant)

❑REDUCTION

- Gains of electrons
- Require energy (anabolic reaction)
- The substance that gain electrons (acceptor) is reduced, and called the oxidizing agent (oxidant)

Basic example of redox reaction

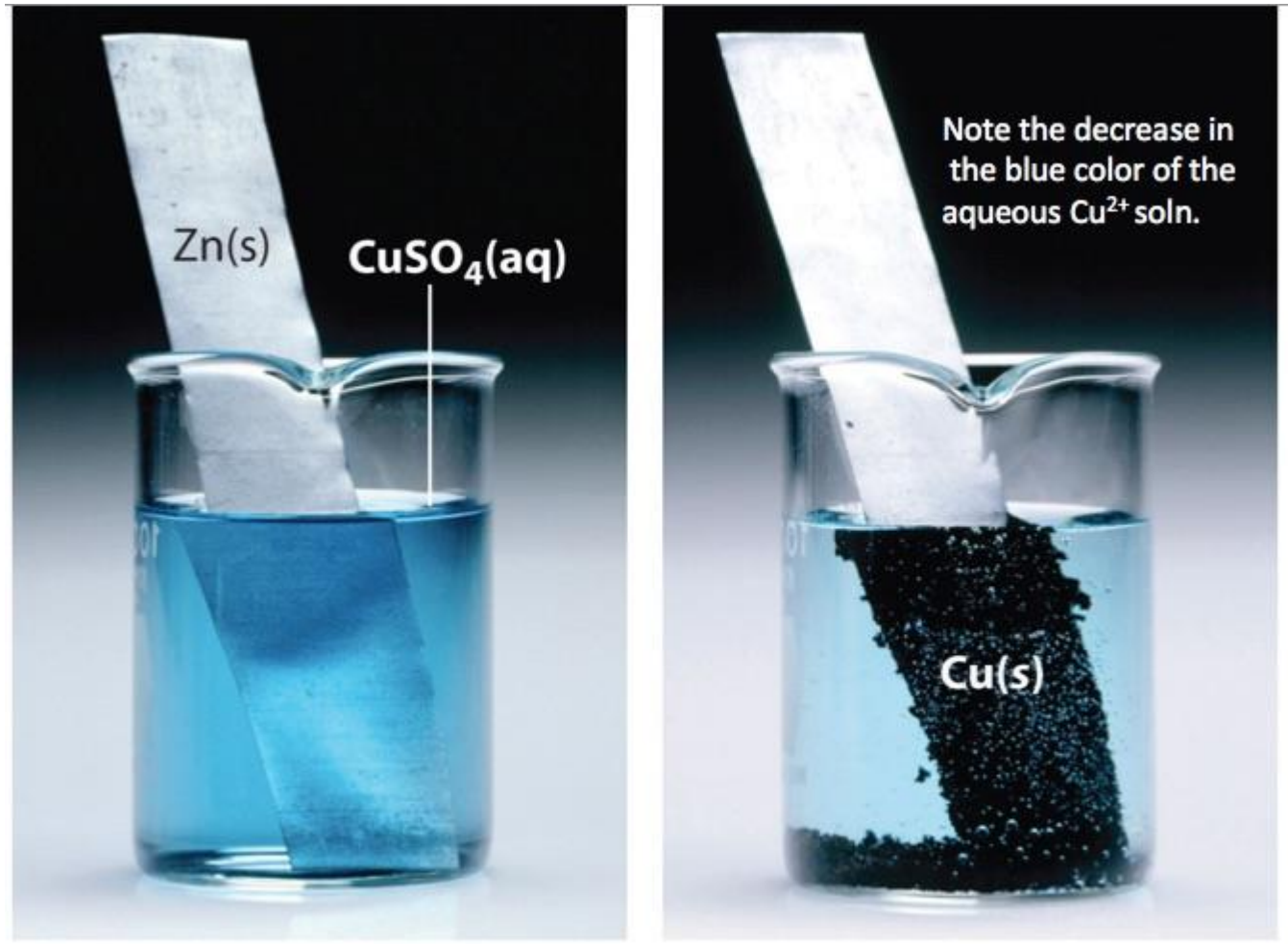
$$Zn(s) + Cu^{2+}(aq) \rightarrow Zn^{2+}(aq) + Cu(s)$$

Metallic zinc is placed in an aqueous solution containing copper ions (Cu^{2+})

Observation:
Zinc metal disappear, zinc ions (Zn^{2+}) go into solution
Copper ions (Cu^{2+}) are removed from solution, copper metal (Cu) deposited

$$\begin{array}{rl} Zn \rightarrow Zn^{2+} + 2e^- & \text{Oxidation} \\ Cu^{2+} + 2e^- \rightarrow Cu & \text{Reduction} \\ \hline Zn + Cu^{2} \rightarrow Zn^{2} + Cu & \text{Overall reaction} \end{array}$$

Zn : reducing agent (it loses electrons, donor, oxidized)
Cu^{2+} ion: oxidizing agent (it gain electrons, acceptor, reduced)



$$Zn(s) + Cu^{2+}(aq) \longrightarrow Zn^{2+}(aq) + Cu(s)$$

Redox reaction in cells (Fermentation reaction)

$$\mathrm{NADH} \rightarrow \mathrm{NAD^+} + \mathrm{H^+} + 2e^- \quad \text{Half reaction of oxidation}$$

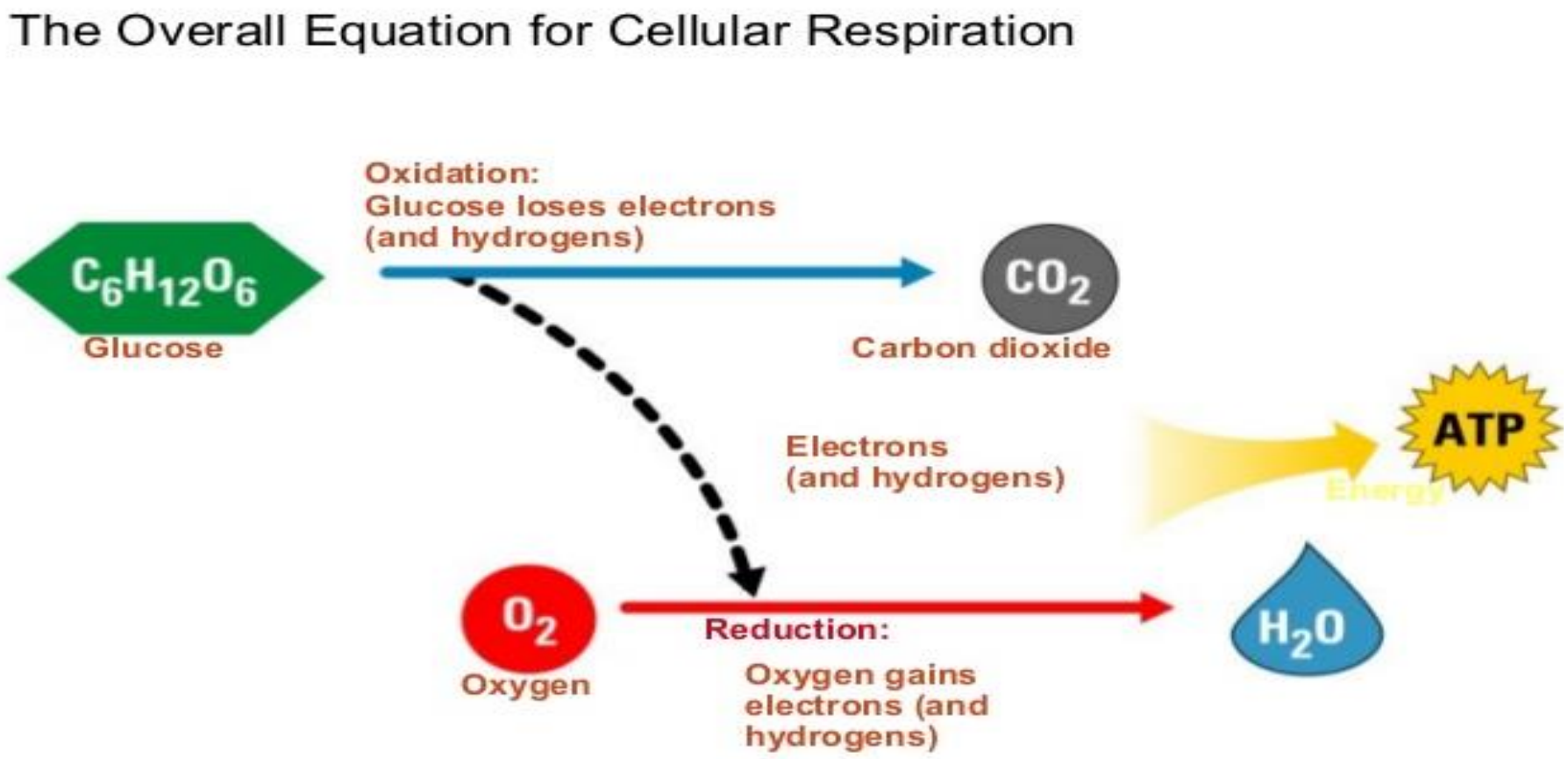
$$\mathrm{CH_3CHO} + 2\mathrm{H^+} + 2e^- \rightarrow \mathrm{CH_3CH_2OH} \quad \text{Half reaction of reduction}$$

$$\mathrm{NADH} + \mathrm{H^+} + \mathrm{CH_3CHO} \rightarrow \mathrm{NAD^+} + \mathrm{CH_3CH_2OH} \quad \text{Overall reaction}$$

Acetaldehyde — Ethanol

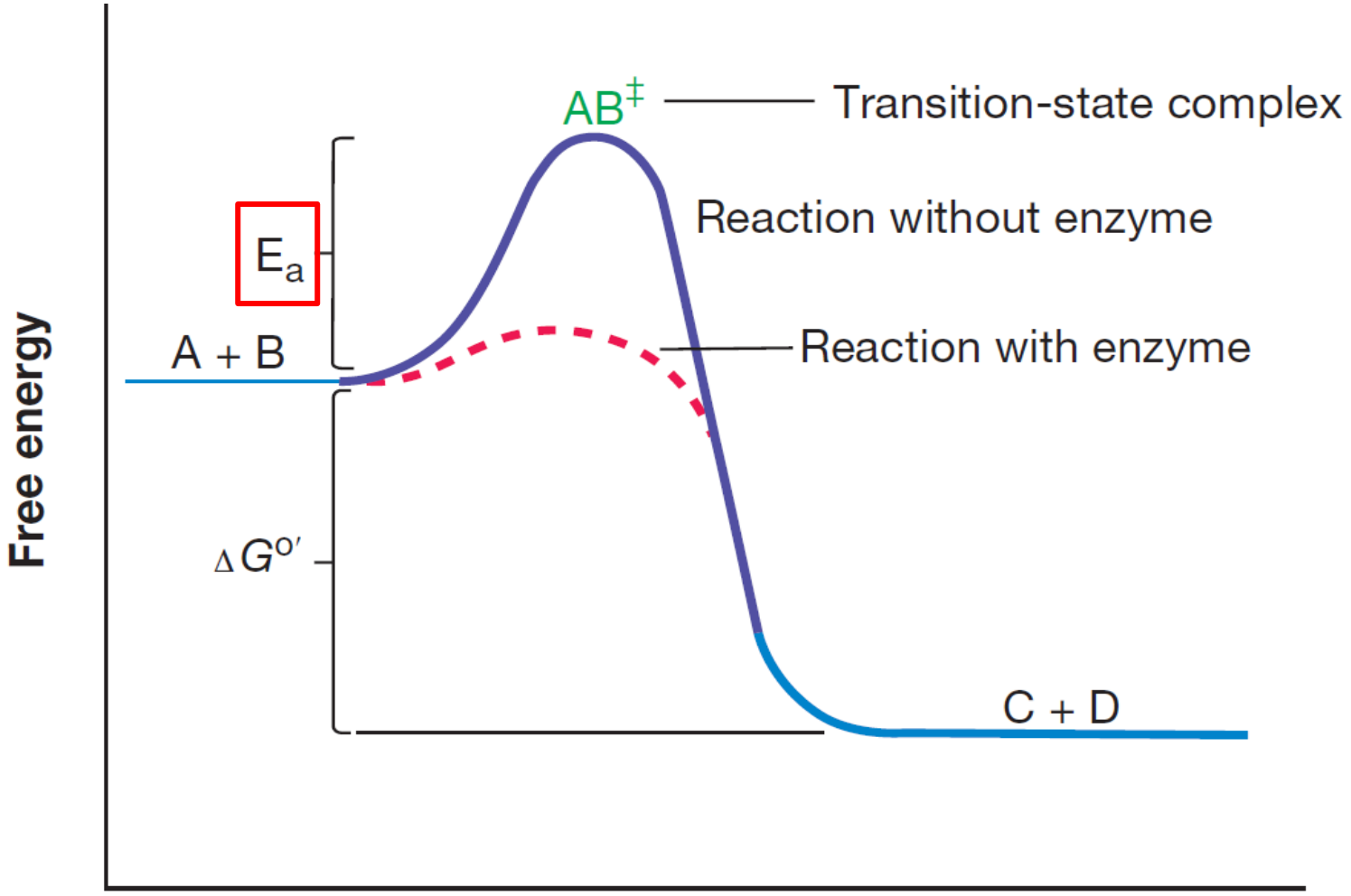
Biochemical reaction in cells need biological catalyst (enzymes) to speed up the reactions!!!

Redox reaction in cells (Aerobic respiration)



$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + H_2O + \text{Energy (ATP)}$$

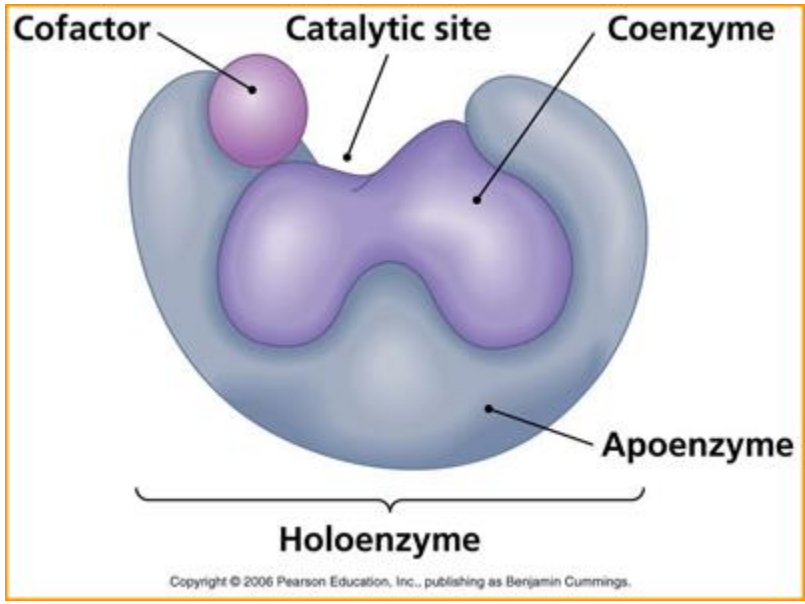
How do enzymes catalyse reactions?



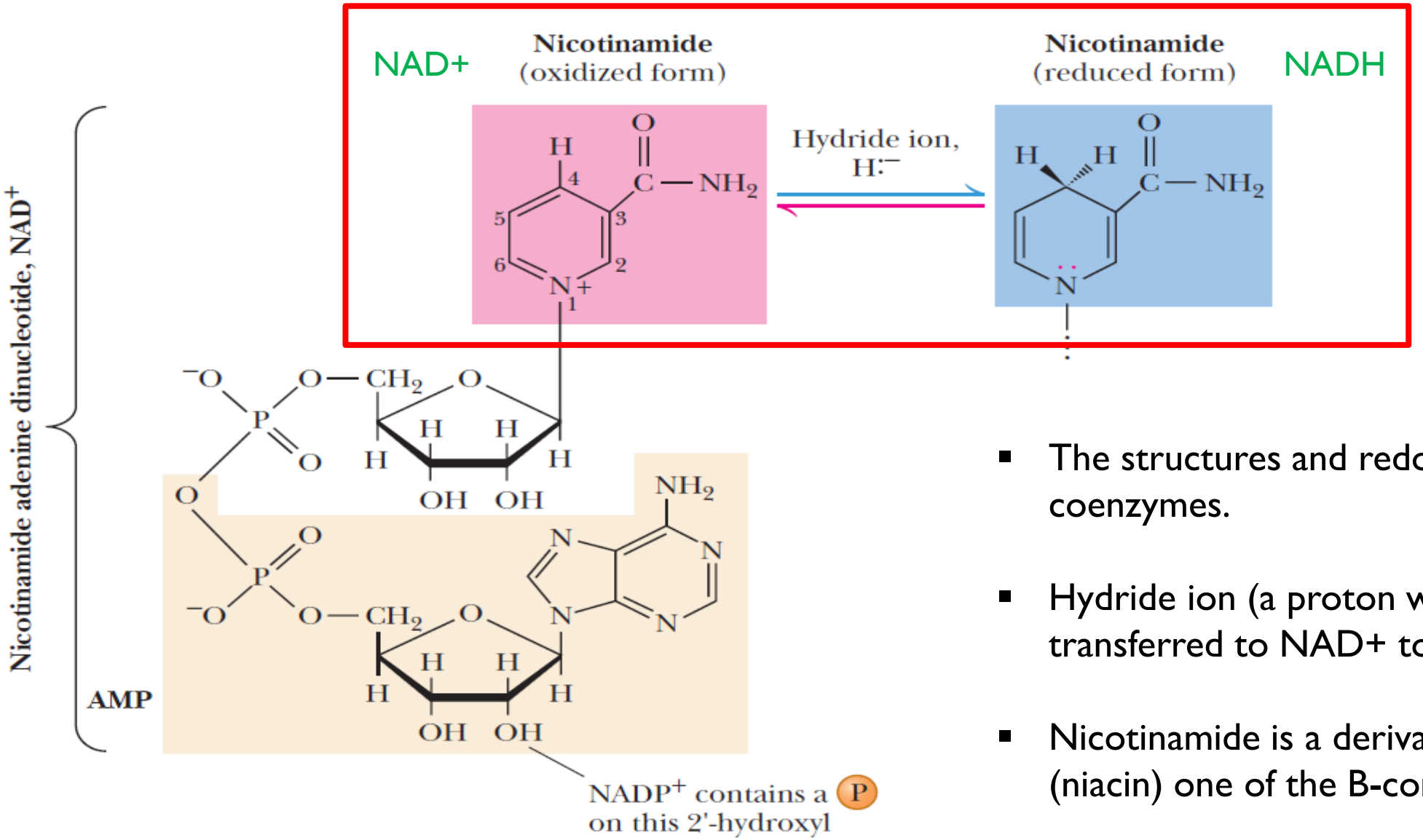
- Enzymes is a biological catalyst that speed up biochemical reaction by lowering the activation energy (E_a)
- E_a – Energy required to bring the reactants together in the correct way to reach transition state
- Transition-state complex resembles both the substrates and products

Cofactors / Coenzymes For Redox Reactions

- Enzymes that catalyze redox reactions require a cofactor to "shuttle" electrons from one part of the metabolic pathway to another part.
  - E.g. Oxidation of glucose. Electrons is passed to oxygen (final acceptor) by intermediate electron acceptors
- Enzyme component:
  - Protein – Apoenzyme
  - Nonprotein – Cofactor (metal ions, e.g. Mg^{2+}, Fe^{2+},Zn^{2+})
    – Coenzymes (organic molecules, e.g. NAD^+,FAD^+)
- Cofactor is a small inorganic molecules in which part of the structure can either be reduced (accept a pair of electrons) or oxidized (donate a pair of electrons)
- Coenzyme plays a significant role as electron carriers in metabolism
  - Most common coenzyme: Nicotamine Adenine Dinucleotide (NAD^+), Flavin Adenine Dinucleotide (FAD^+), coenzyme A, etc..



Coenzyme Nicotamine Adenine Dinucleotide (NAD+)



- The structures and redox states of the NAD coenzymes.
- Hydride ion (a proton with two electrons) is transferred to NAD+ to produce NADH.
- Nicotinamide is a derivatives of nicotinic acid (niacin) one of the B-complex vitamins.

NAD⁺/NADH CYLING IN REDOX REACTION

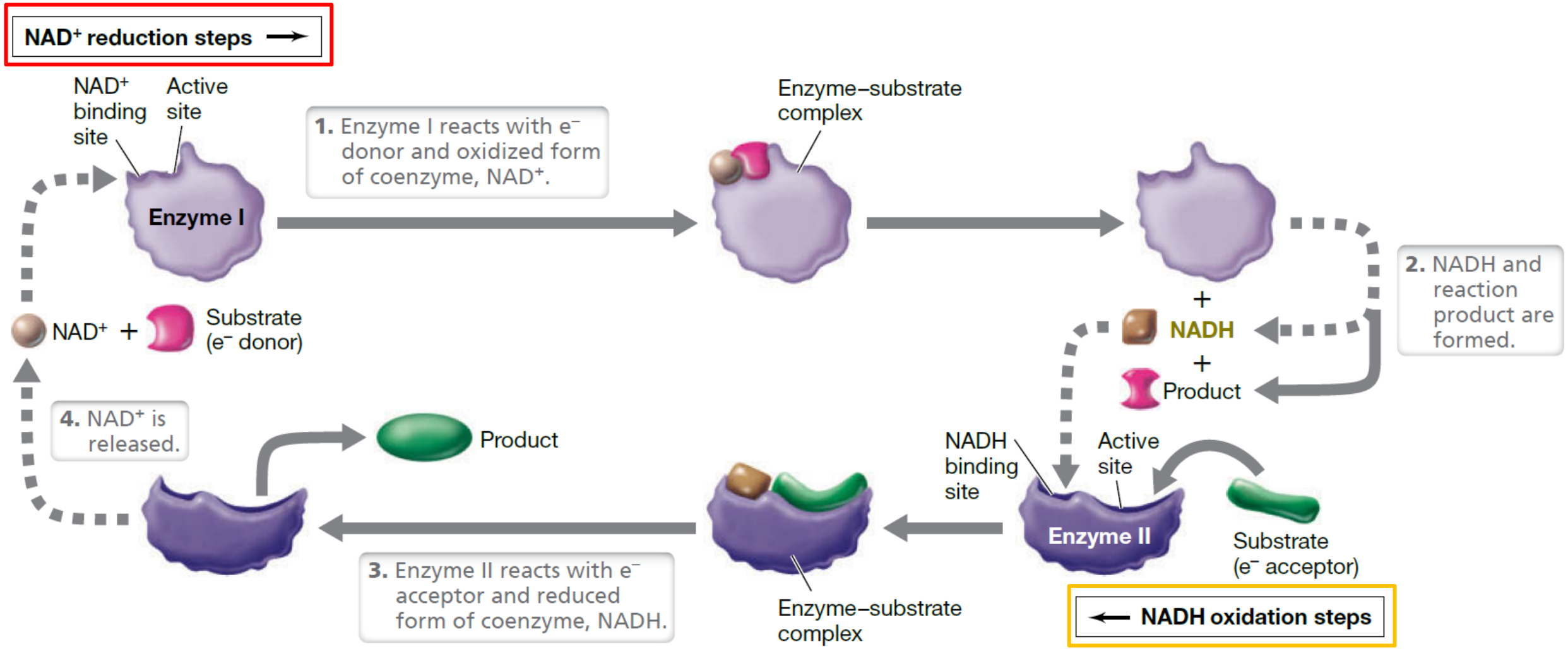


Figure 3.12 NAD⁺/NADH cycling. A schematic example of redox reactions in which two different enzymes are linked by their requirement for either NAD^+ or NADH.

Standard Reduction Potential (E_0)

- Equilibrium constant for an oxidation-reduction reaction.
- The standard reduction potential (E_0) measures the tendency of the donor to lose electrons
  - more negative E_0 ⇒ better electron donor
  - more positive E_0 ⇒ better electron acceptor
- E_0 is measured in volts.
- Each **half reaction** consist of a molecule that can accept electrons, no. of electrons, and the molecule it become after accepting electrons.
  - These molecules are called a conjugate redox pair (e.g. NAD+/NADH)

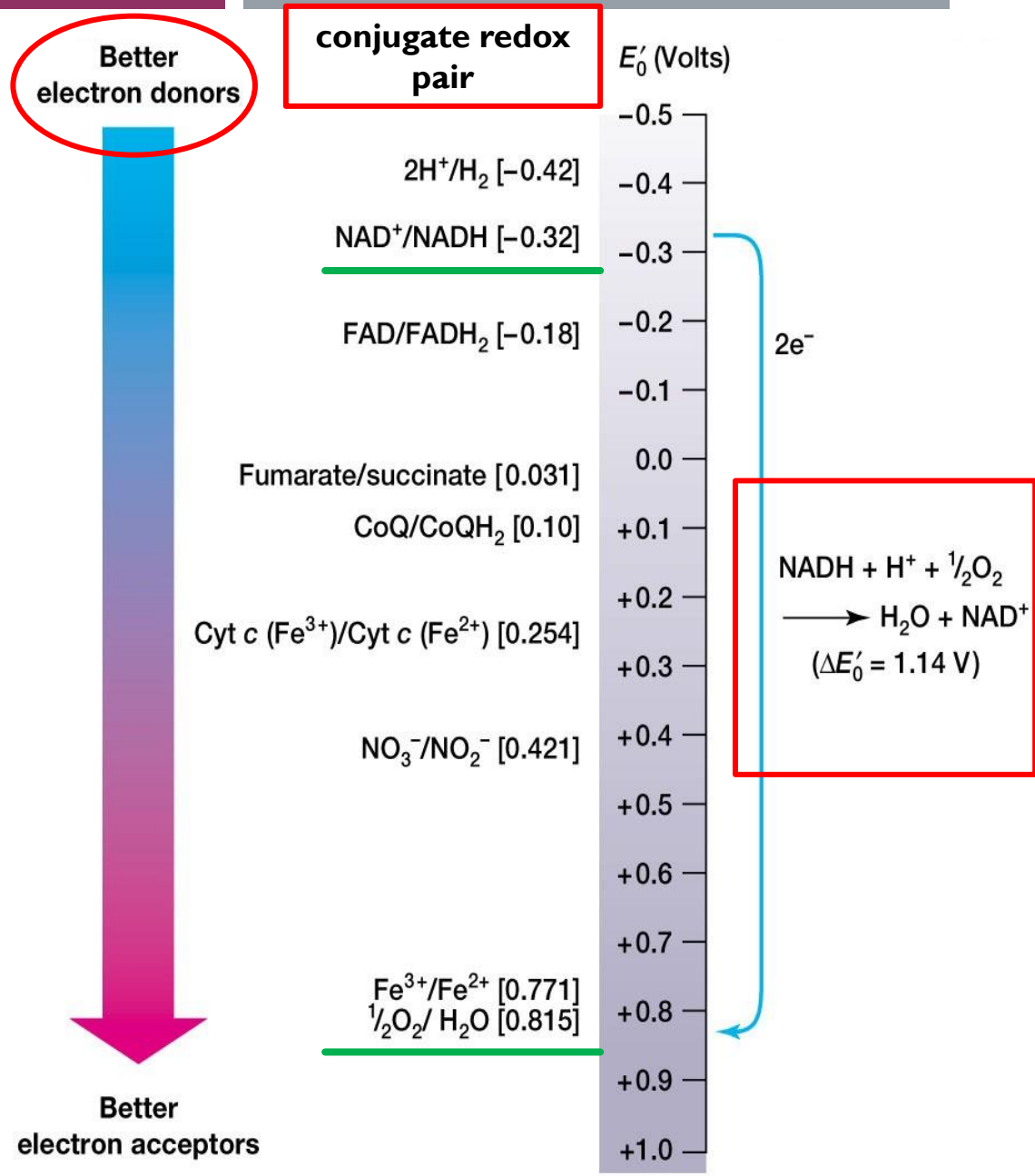
Table 10.2 Selected Biologically Important Half Reactions

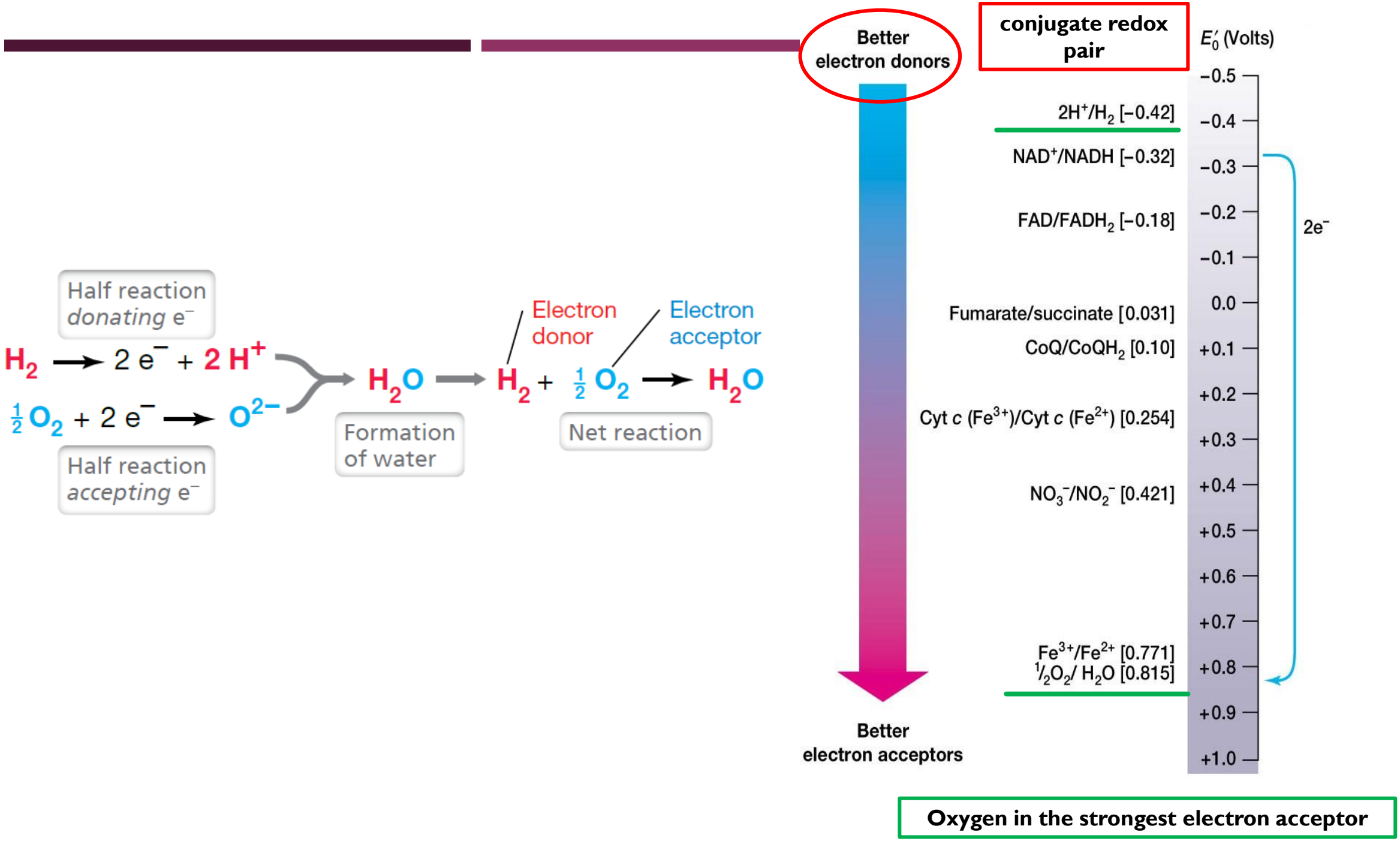
Half Reaction	E'_0 (Volts)[1]
$2H^+ + 2e^- \rightarrow H_2$	−0.42
Ferredoxin (Fe^{3+}) + e^- → ferredoxin (Fe^{2+})	−0.42
$NAD(P)^+ + H^+ + 2e^- \rightarrow NAD(P)H$	−0.32
$S + 2H^+ + 2e^- \rightarrow H_2S$	−0.27
Acetaldehyde + $2H^+ + 2e^-$ → ethanol	−0.20
$Pyruvate^- + 2H^+ + 2e^-$ → $lactate^{2-}$	−0.19
$FAD + 2H^+ + 2e^- \rightarrow FADH_2$	−0.18[2]
$Oxaloacetate^{2-} + 2H^+ + 2e^-$ → $malate^{2-}$	−0.17
$Fumarate^{2-} + 2H^+ + 2e^-$ → $succinate^{2-}$	0.03
Cytochrome *b* (Fe^{3+}) + e^- → cytochrome *b* (Fe^{2+})	0.08
Ubiquinone + $2H^+ + 2e^-$ → ubiquinone H_2	0.10
Cytochrome *c* (Fe^{3+}) + e^- → cytochrome *c* (Fe^{2+})	0.25
Cytochrome *a* (Fe^{3+}) + e^- → cytochrome *a* (Fe^{2+})	0.29
Cytochrome a_3 (Fe^{3+}) + e^- → cytochrome a_3 (Fe^{2+})	0.35
$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$	0.42
$NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O$	0.44
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.77[3]
$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$	0.82

- Electrons spontaneously move from donors, higher on the tower (more -ve potentials) to acceptors lower on the tower (more +ve potentials).

- E.g. Reduction potential (E_0) of NAD+/NADH conjugate redox pair is more negative than that of ½ O_2/H_2O, electrons flow from NADH (donor) to the O_2 (acceptor) – occur in Electron Transport Chain (ETC)

- Free energy (ΔG^o) is release and can be used to synthetize ATP.

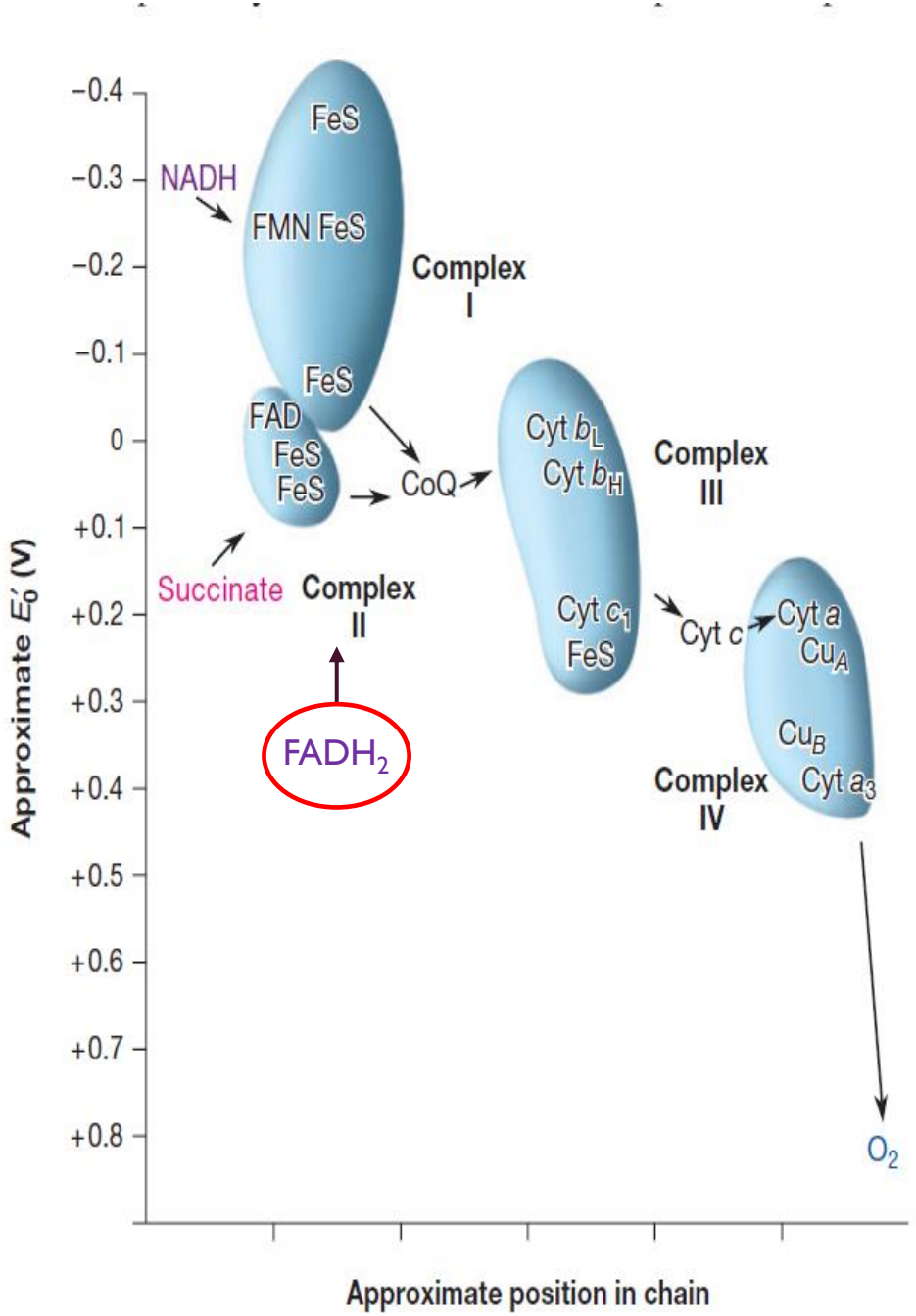




Oxygen in the strongest electron acceptor

ELECTRON TRANSPORT CHAIN

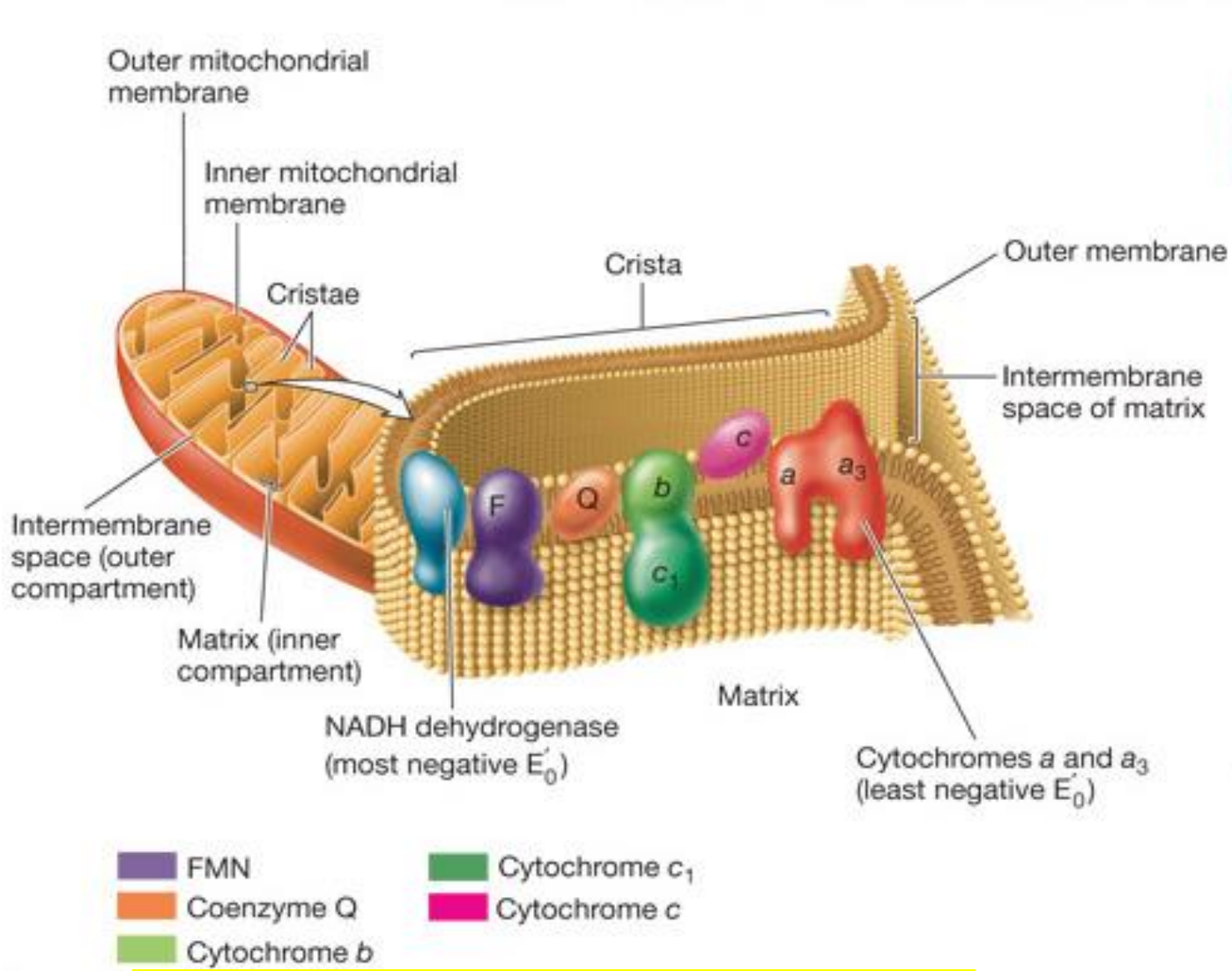
- Electron transport chain (ETC) is a series of electron carriers with the first electron carrier having the most negative E_o (standard reduction potential)
- As a result, the potential energy stored in first redox couple is released and used to form ATP.



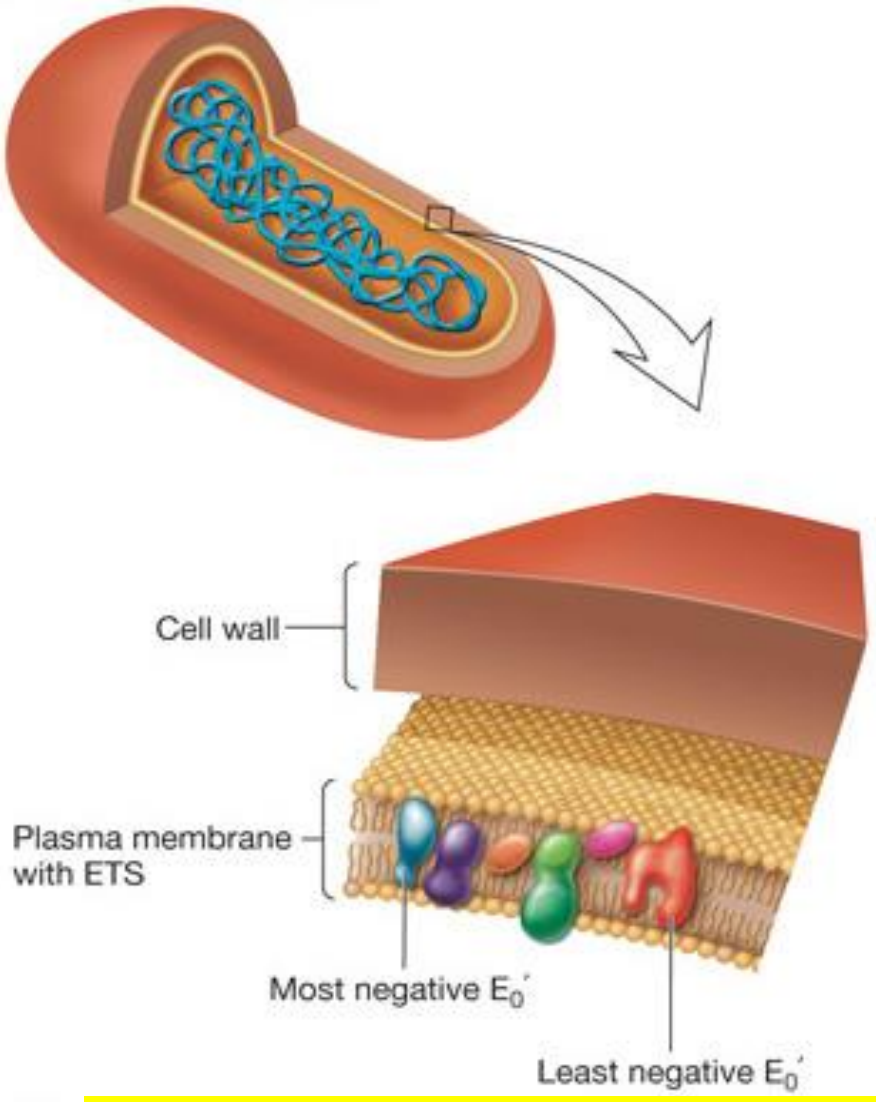
- Electron carriers:
  - NAD (nicotinamide adenine dinucleotide)
  - NADP (nicotinamide adenine dinucleotide phosphate)
  - FAD (flavin adenine dinucleotide)
  - FMN (flavin mononucleotide, riboflavin phosphate)
  - Coenzyme Q (CoQ) (a quinone, also called ubiquinone)
  - Cytochromes (iron is part of a heme group)
  - nonheme iron proteins (ferrodoxin)
    - Ferredoxin is a Fe-S protein active in photosynthetic electron transport and several other ET processes. Fe-S carries only one electron a time.
    - use iron to transport electrons
    - iron is not part of a heme group

Where is the location of Electron Transport Chain (ETC)??

Where is the location of Electron Transport Chain (ETC)??



Mitochondrial ETC – in eukaryotes



Bacterial ETC – in prokaryotes

What are the processes that generate ATP in prokaryotes??

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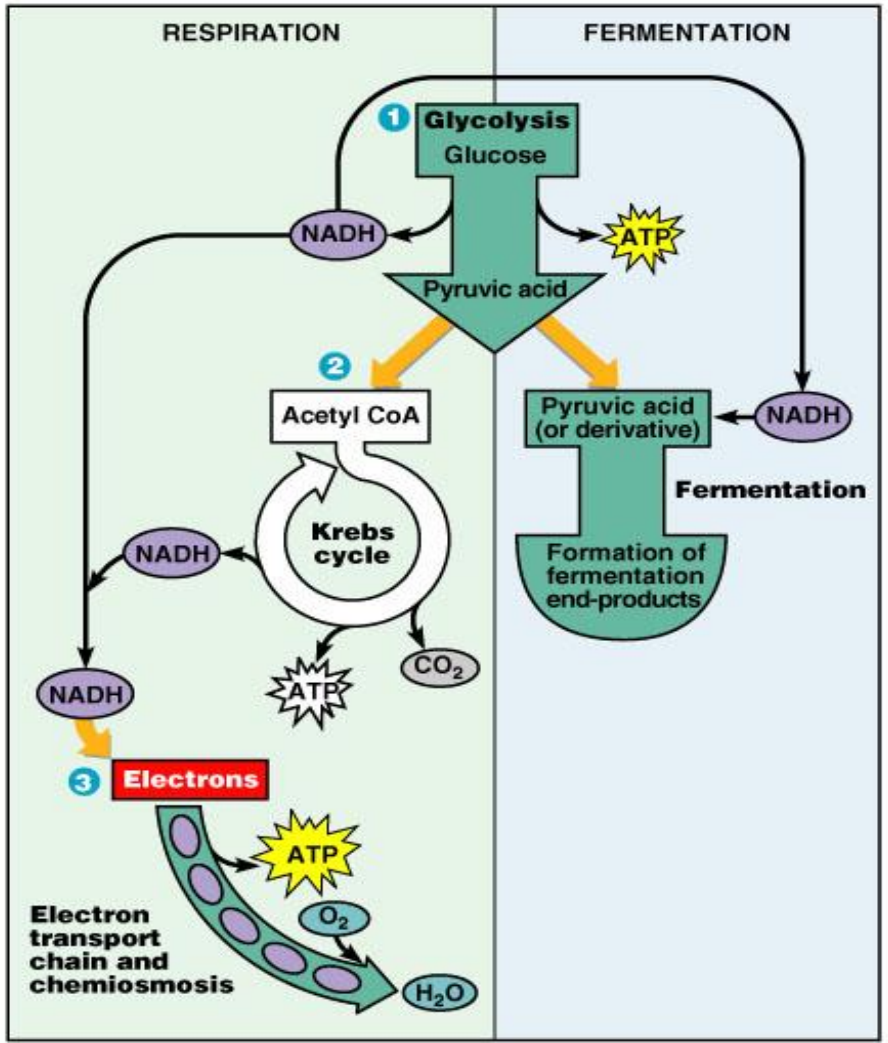
CATABOLIC PATHWAY

(CELLULAR RESPIRATION)

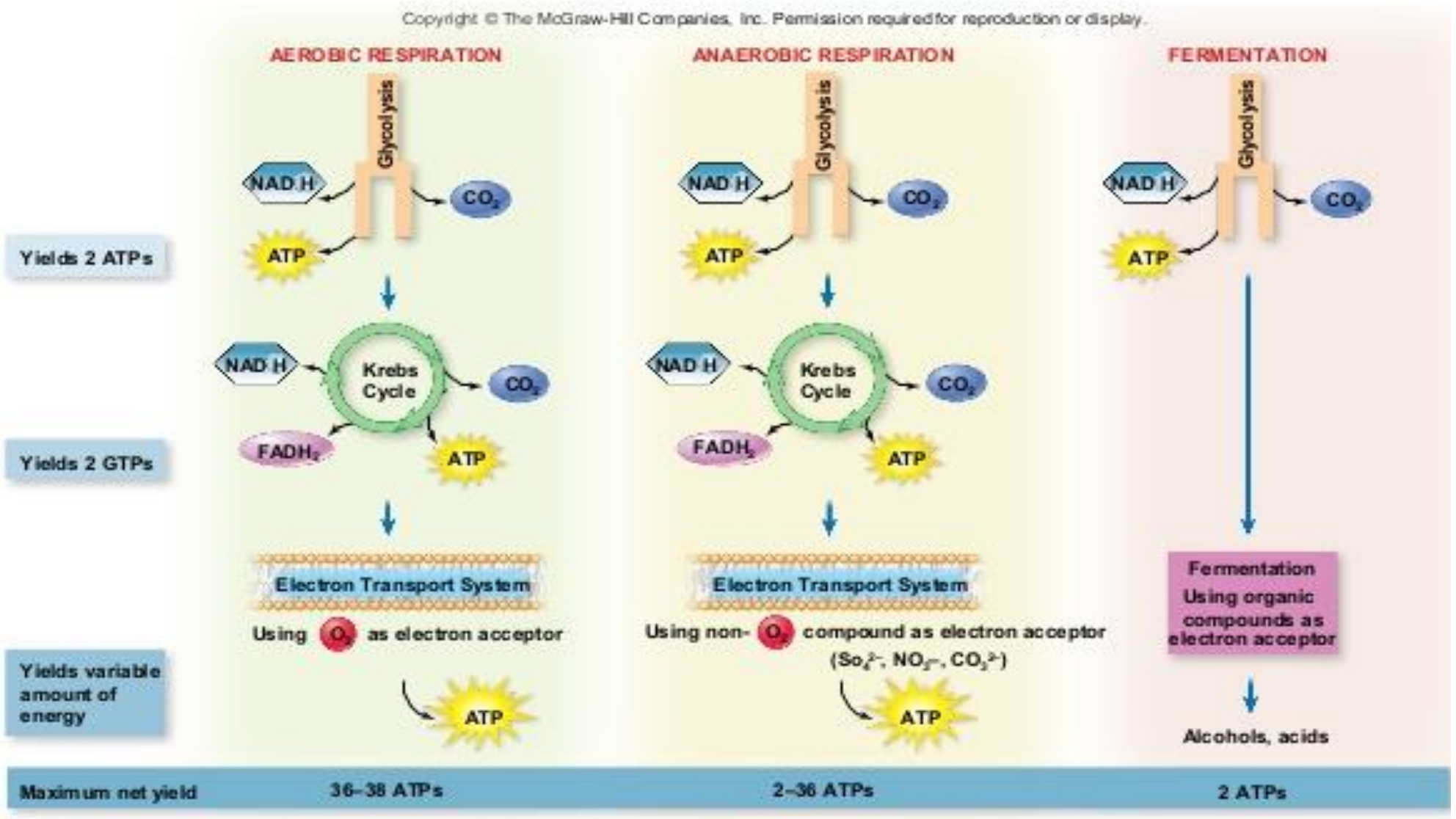
Aerobic respiration
Anaerobic respiration
Fermentation

CATABOLIC PATHWAY

- Cellular respiration is a catabolic pathway that breaks down glucose and produces ATP.
  1. Aerobic → electron donor is oxidized with O_2
  2. Anaerobic → O_2 substitute as the terminal electron acceptor
  3. Fermentation → Partial degradation of sugar without O_2, organic compound as electron acceptor
- When O_2 is available, respiration will take place instead of fermentation, therefore, more ATP is produced
- The catabolic pathway results in energy release through:
  1. Glycolysis (Embden-Meherhof-Parnas pathway)
  2. Krebs cycle (Citric acid /Tricarboxylic acid cycle (TCA))
  3. Electron transport chain (Oxidative phosphorylation)



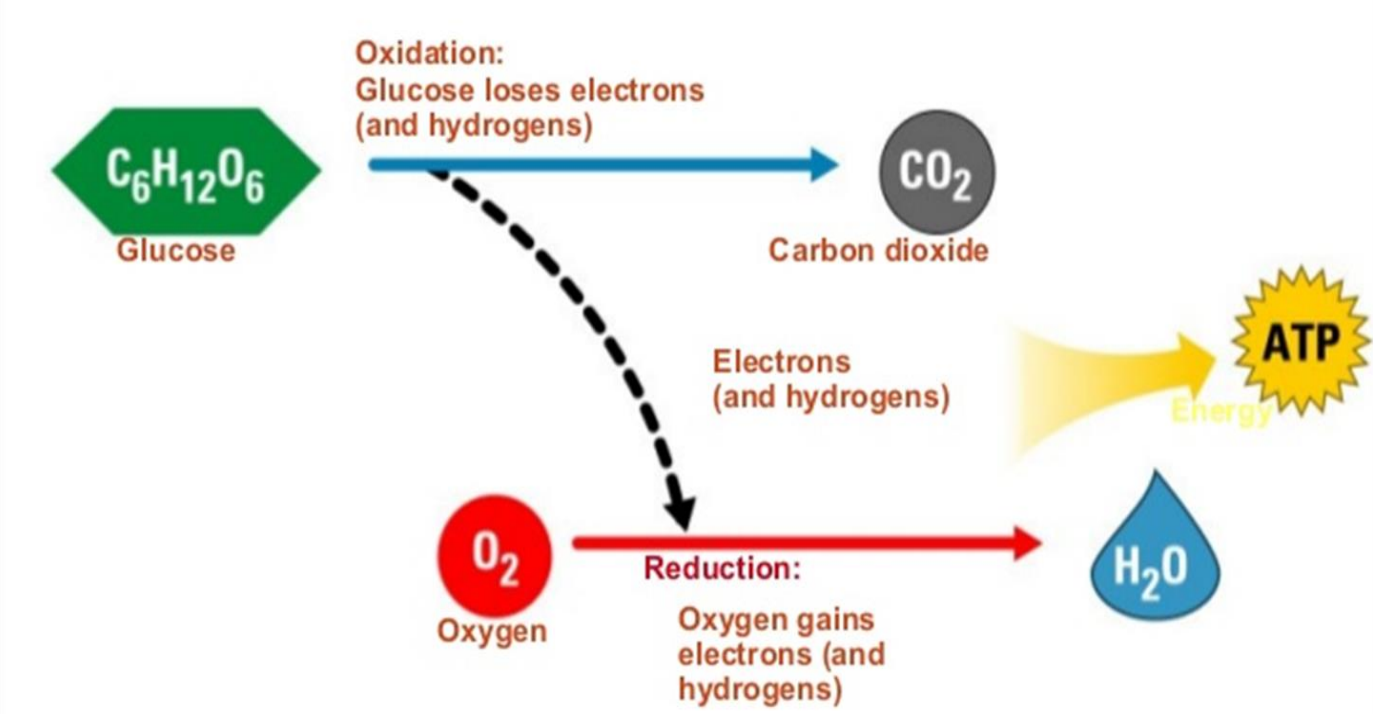
Overview of the Three Main Catabolic Pathways



38 ATPS **2-36 ATPS** **2 ATPS**

Aerobic Respiration

- Electrons released by oxidation of glucose are passed down to the Electron Transport Chain with oxygen being the final electron acceptor



$$C_6H_{12}O_6 + 6O_2 \Rightarrow 6CO_2 + H_2O + \text{Energy (ATP)}$$

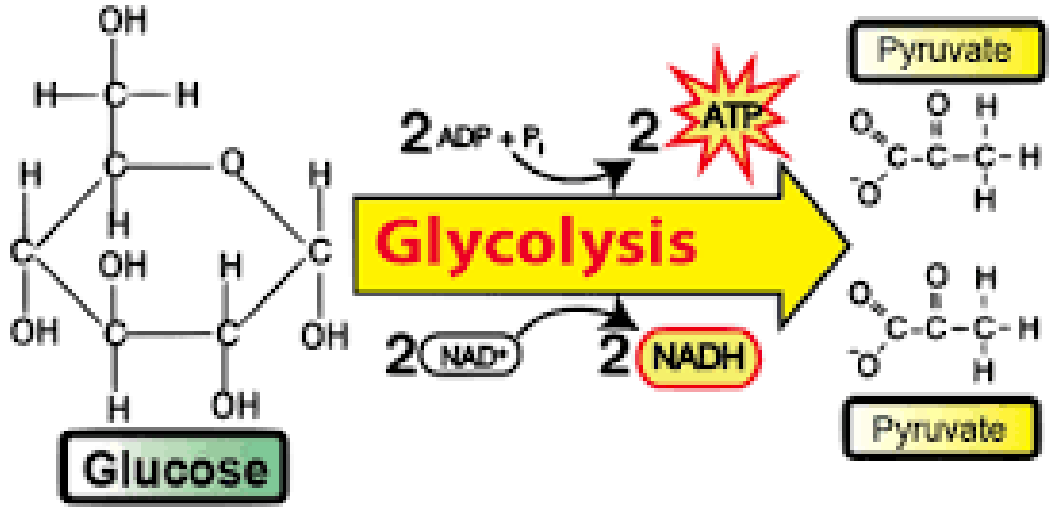
$$(38\ ADP + 38\ Pi) \Rightarrow (38\ ATP)$$

- Aerobic respiration is the most efficient way to extract energy from glucose.

- Microbes used 4 different mechanism to build ATP:
  1. Glycolysis
  2. Transition Reaction
  3. Kreb's Cycle
  4. Electron Transport System

1. Glycolysis: Splitting Of Sugar

- Oxidation of Glucose into 2 molecules of Pyruvic acid / Pyruvate
- 2 ATPs are used and 4ATPs are generated
- End Products of Glycolysis:
  - 2 Pyruvic acid
  - 2 $NADH_2$
  - 2 ATP (net gain)



Glycolysis

Glucose is phosphorylated at the expense of one ATP, generating glucose 6-phosphate, a precursor metabolite and the starting molecule for the pentose phosphate pathway.

Isomerization of glucose 6-phosphate (an aldehyde) to fructose 6-phosphate (a ketone and a precursor metabolite)

ATP is consumed to phosphorylate C1 of fructose. The cell is spending some of its energy currency in order to earn more in the next part of the pathway.

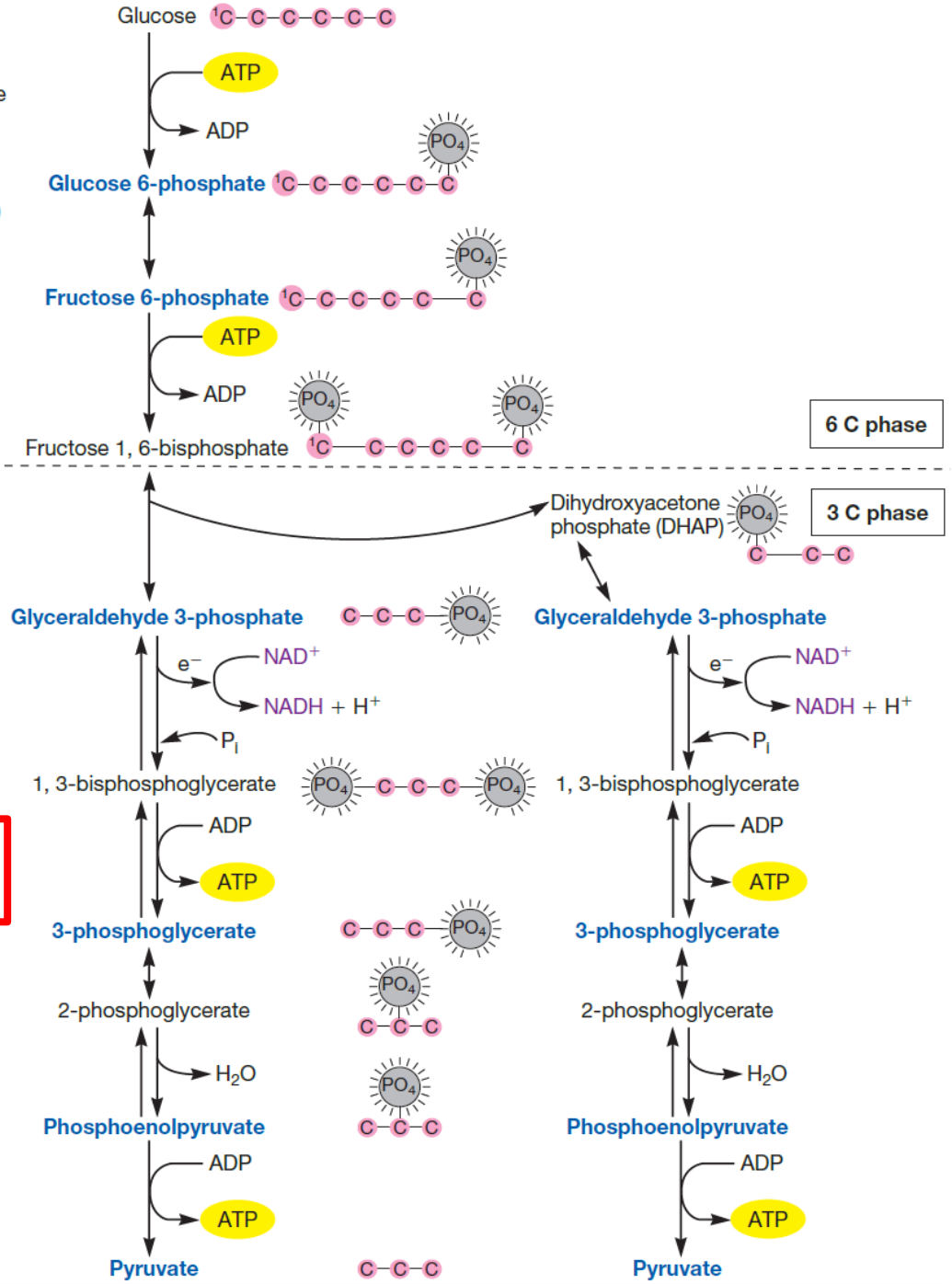
Fructose 1, 6-bisphosphate is split into two 3-carbon molecules, one of which is a precursor metabolite. DHAP is readily converted to glyceraldehyde 3-phosphate.

Glyceraldehyde 3-phosphate is oxidized and simultaneously phosphorylated, generating a high-energy molecule. The electrons released reduce NAD^+ to NADH.

ATP is made by substrate-level phosphorylation. Another precursor metabolite is made.

Another precursor metabolite is made.

The oxidative breakdown of one glucose results in the formation of two pyruvate molecules. Pyruvate is one of the most important precursor metabolites.



Substrate –level phosphorylation

- The synthesis of ATP by direct transfer of phosphate group from energy-rich substrate to a molecule of ADP
- Substrate-level phosphorylation occurs in glycolysis and TCA cycle
- The phosphate is coming from a high energy molecule and given to ADP to form ATP, rather than adding the inorganic phosphate to ADP as in oxidative phosphorylation (occur in ETC)
- Reaction is catalyzed by kinases

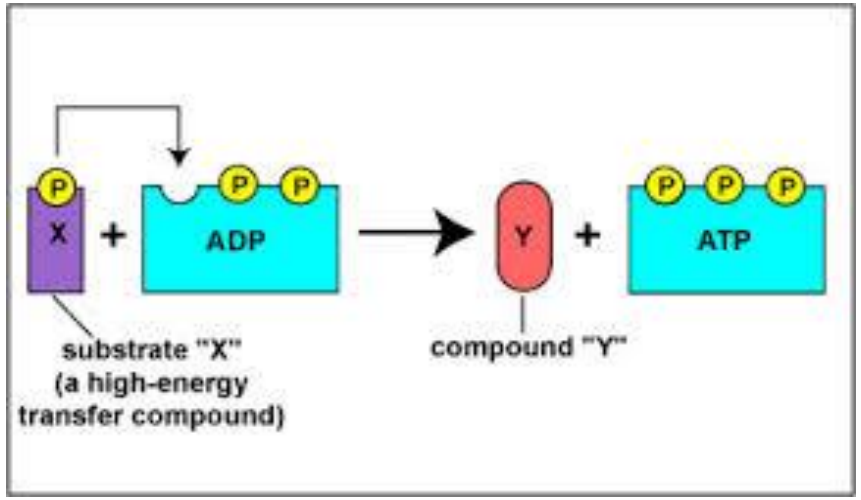


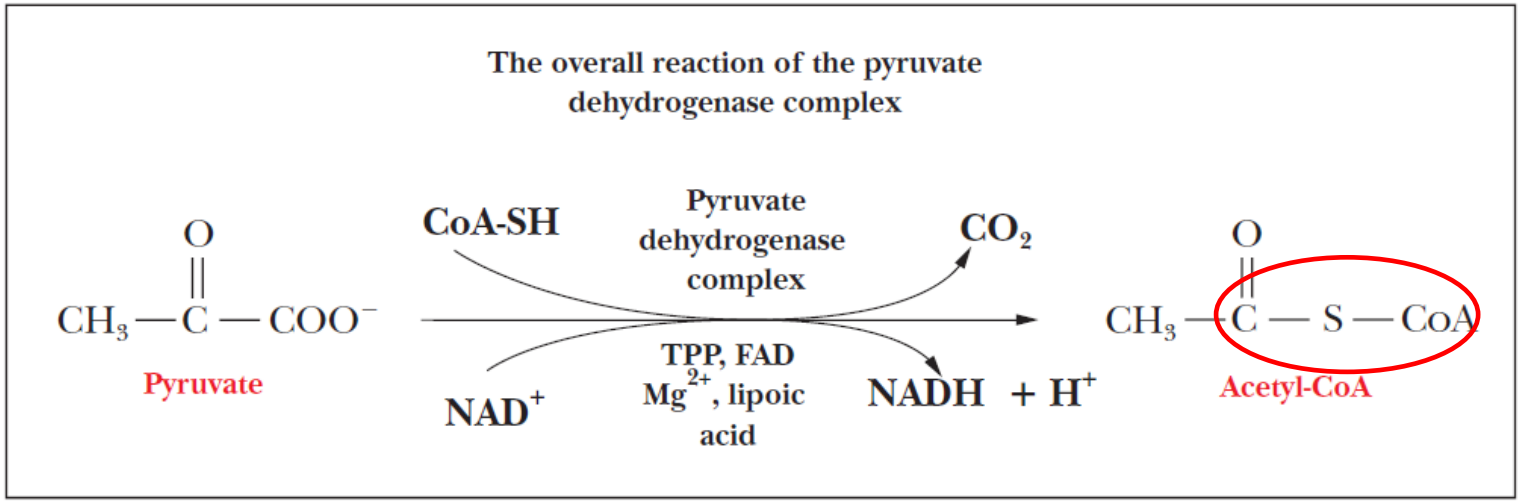
Table 10.1 Phosphate Transfer Potential of Common Phosphorylated Compounds[1]

Phosphorylated Molecule	ΔG°′ of Hydrolytic Removal of Phosphate (KJ/mol)	Phosphate Transfer Potential
High-Energy Phosphorylated Compounds		
Phosphoenolpyruvate[2]	−61.9	61.9
1, 3-bisphosphoglycerate[2]	−49.3	49.3
ATP (hydrolysis to AMP)	−45.6	45.6
ATP (hydrolysis to ADP)	−30.5	30.5
Low-Energy Phosphorylated Compounds		
Glucose 6-phosphate	−13.8	13.8
Glycerol 1-phosphate	−9.2	9.2

2. Transition reaction

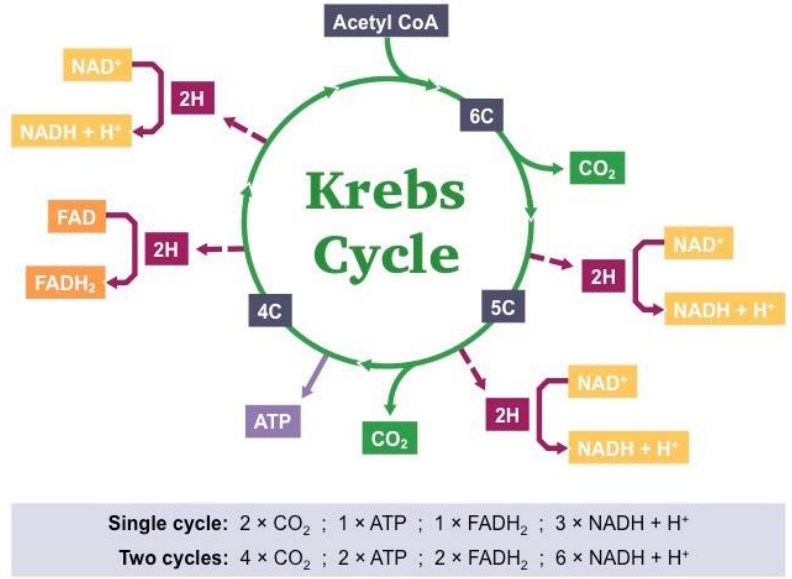
- Pre-Krebs (connects Glycolysis to Krebs Cycle)
- Pyruvates are decarboxylated, then attached to coenzyme A → acetyl-CoA
- Occur twice with the end products:
  - 2 Acetyl-CoA
  - 2 CO_2
  - 2 $NADH_2$

- Acetyl-CoA is a thioester (high energy compound)
- Sulfur atom replacing an oxygen of the usual carboxylic ester
- The hydrolysis of thioesters release enough energy to drive another reaction.



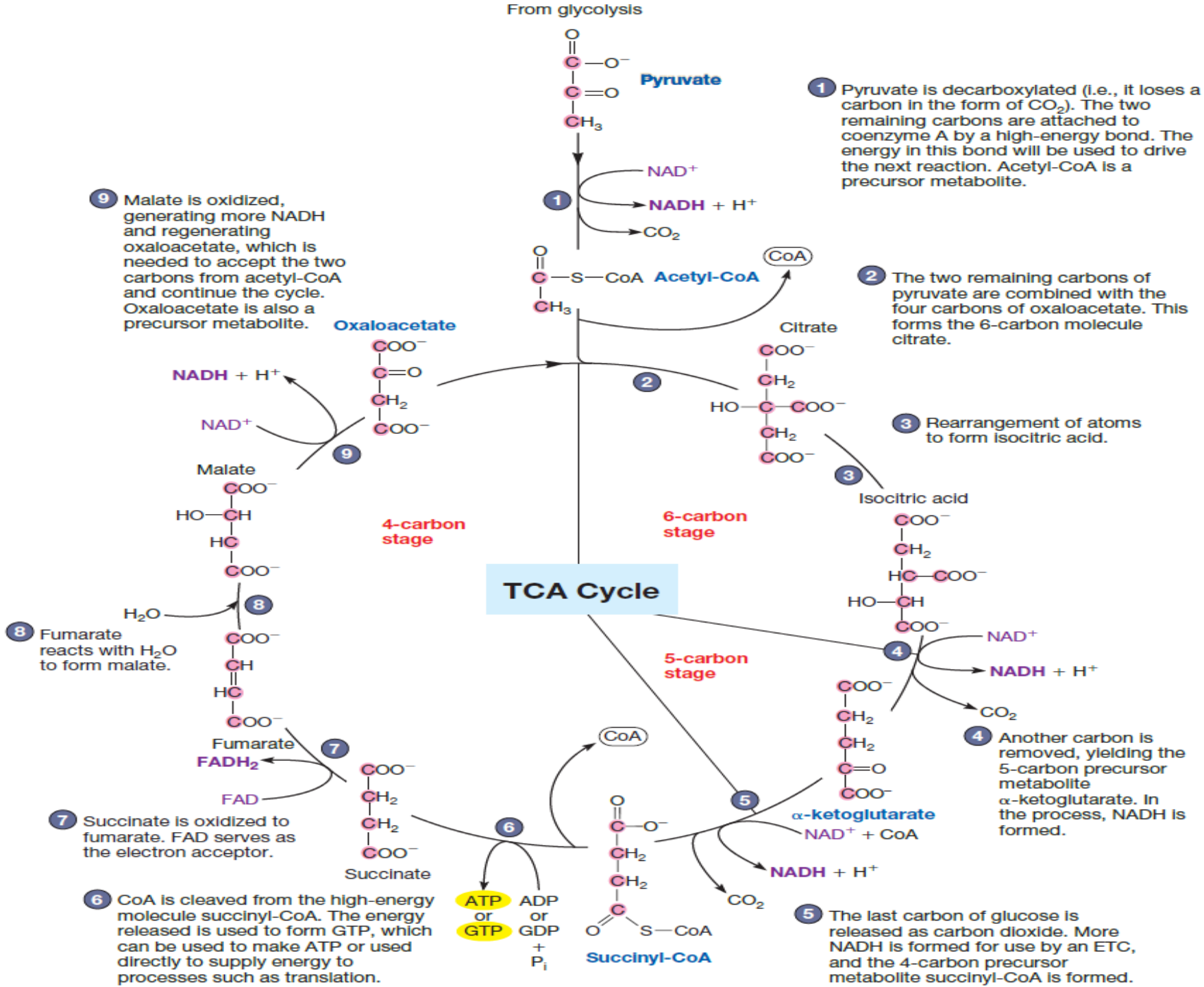
3. Krebs cycle

- Series of chemical reactions that begin and end with citric acid
- Also known as Tricarboxylic Acid cycle (TCA cycle) or Citric acid cycle.
  - Occurs twice
  - Results in the oxidation of the last 4 carbon atoms
  - Acetyl-CoA binds with oxaloacetic acid to form citric acid
  - Citric acid then progresses through a series of reactions ultimately resulting in the reformation of oxaloacetic acid
- End products:
  - 2 ATP
  - 6 $NADH_2$
  - 2 $FADH_2$
  - 4 CO_2



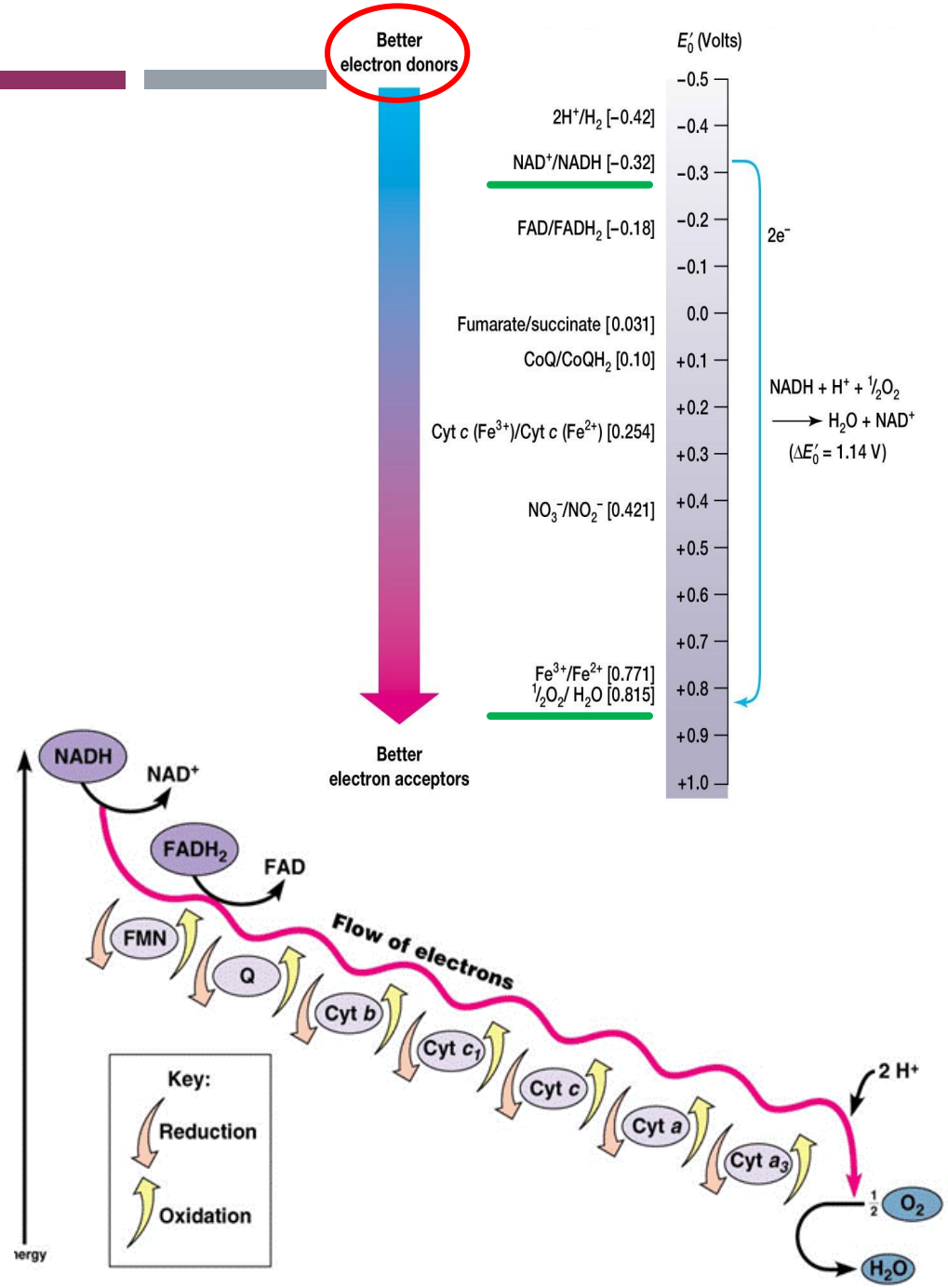
KREBS CYCLE

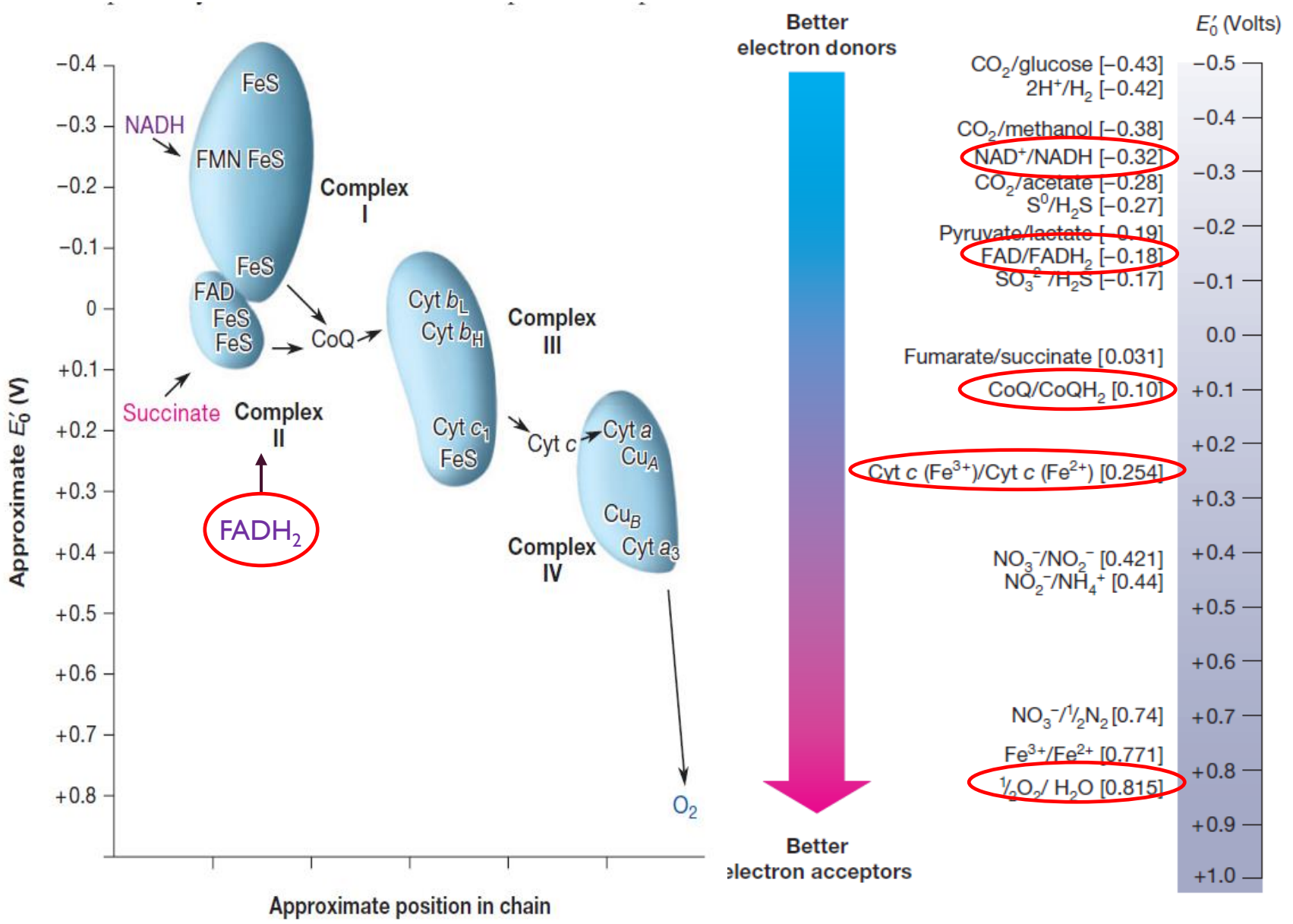
CITRIC ACID CYCLE.pdf



4. Electron Transport Chain

- ETC is located in the plasma membrane of bacteria
- It is composed of a series of electron carriers
  - Arranged in 4 complexes that are linked by coenzyme Q (CoQ) and cytochrome *c* (Cyt *c*).
  - Series of redox reactions occur – each carrier is reduced and then reoxidized.
  - The electrons flow from carriers with more negative reduction potentials to those with positive potentials
    - ✓ Transfer electrons from NADH & $FADH_2$ to O_2 resulting in H_2O
    - ✓ O_2 is the final electron acceptor
- The difference in reduction potentials between O_2 and NADH is large (E'_o = 1.14V) – release high energy for ATP production.
- ATP is synthesized by oxidative phosphorylation.



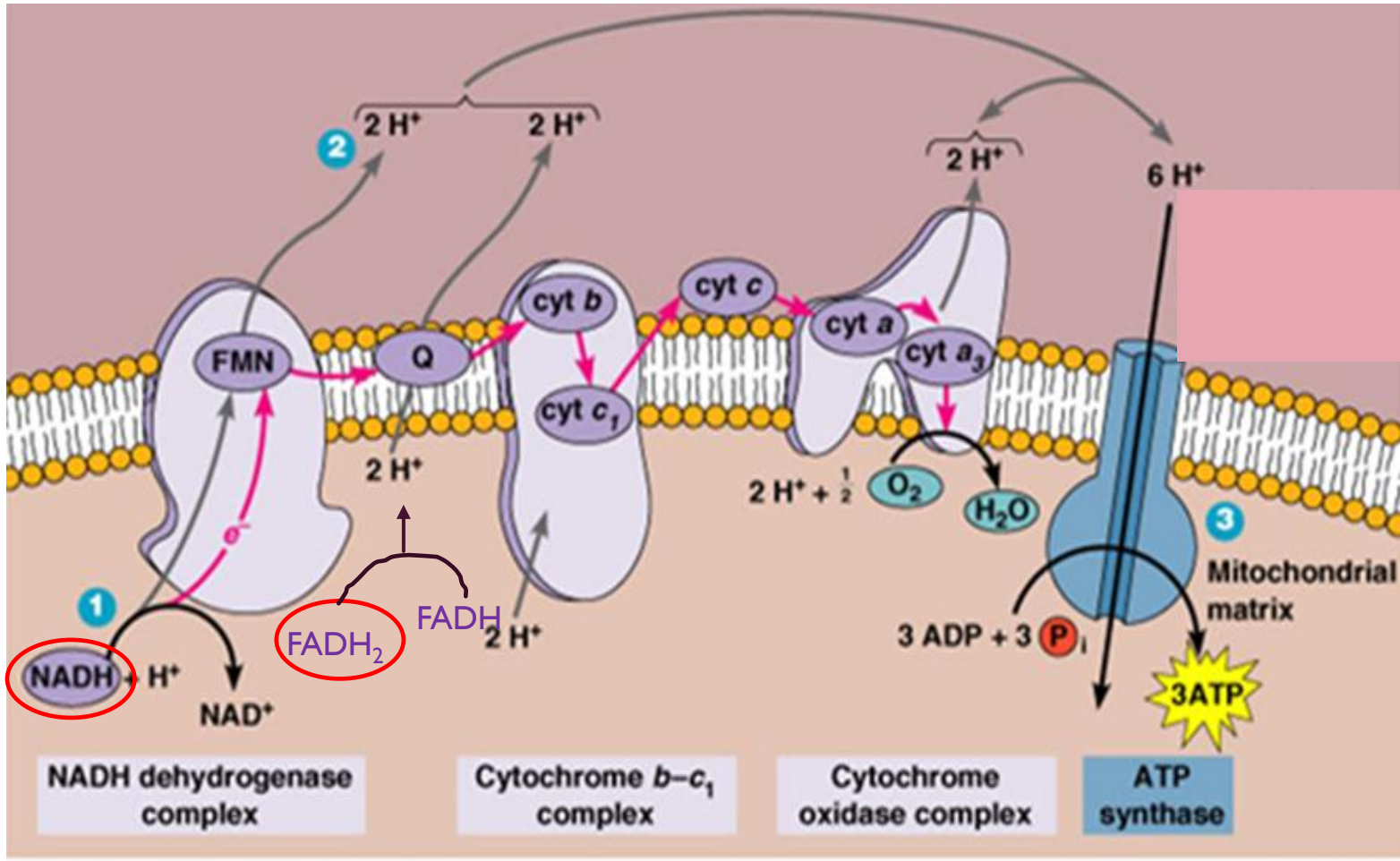


- The electron carriers are organized into four complexes that are linked by coenzyme Q (CoQ) and cytochrome c (Cyt *c*).
- Electrons flow from NADH and $FADH_2$ down the reduction potential gradient to oxygen (final electron acceptor)

Oxidative phosphorylation

Involves two processes:

1. Electron transport chain
   - ✓ NADH and $FADH_2$ are oxidized and the electrons are transferred to the electron carrier (Complex I – IV) in the ETC.
   - ✓ Electron falls to oxygen (final electron acceptor) and reduced to **water.**
2. Chemiosmosis
   - ✓ As electrons flow through the ETC, protons are moved across the membrane, generating proton motive force (PMF) / proton gradient – high potential energy.
   - ✓ PMF can drive the production of ATP when the protons flow back into the cytoplasm through the ion channel in the ATP synthase.
   - ✓ The flow of proton is exergonic and the energy is used to phosphorylate ADP to **ATP.**



Maximum theoretical ATP yield from aerobic respiration

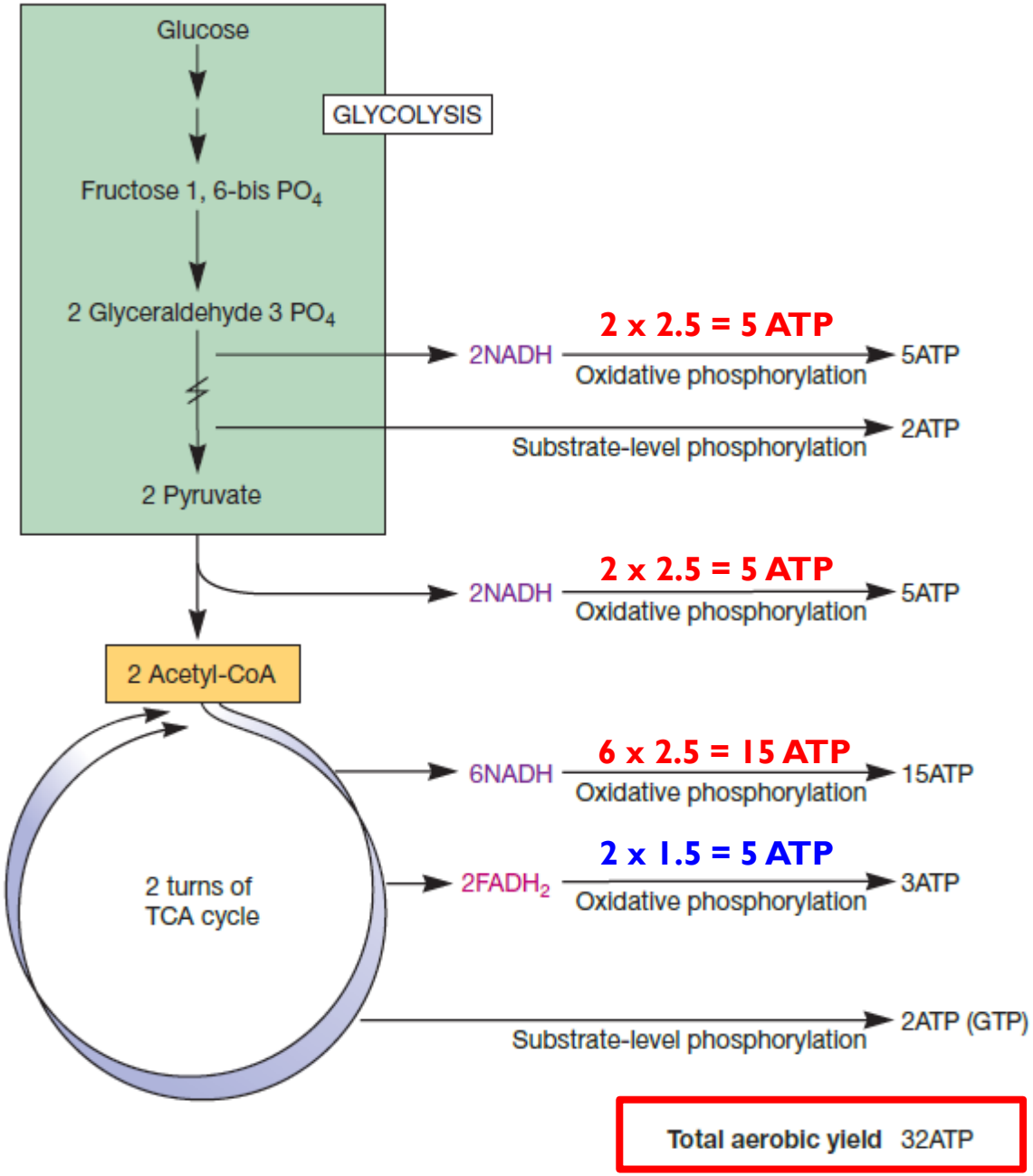


Figure 11.17 Maximum Theoretic ATP Yield from Aerobic Respiration. To calculate the theoretic maximum yield of ATP, P/O ratios of 2.5 for the oxidation of NADH and 1.5 for $FADH_2$ are assumed.

The phosphorus to oxygen ratio (P/O) is used as a measure of the number of ATP molecule generated per oxygen

P/O ratio:
NADH → 2.5 / (3)
FADH2 → 1.5 / (2)

Anaerobic Respiration

- Identical to aerobic respiration except O_2 is not the final electron acceptor in the electron transport chain.
  - Other acceptor: Nitrate, sulfate, CO_2, metals, etc
- Microbes that can carry out anaerobic respiration will perform aerobic respiration instead if oxygen is available (facultative anaerobes).
- Lower ATP yield because the final electron acceptor such as NO_3^- has less positive reduction potential than O_2.
  - The difference in reduction potential between NADH and NO_3^- is smaller compared to NADH and O_2.
  - So, less energy is available to make ATP.

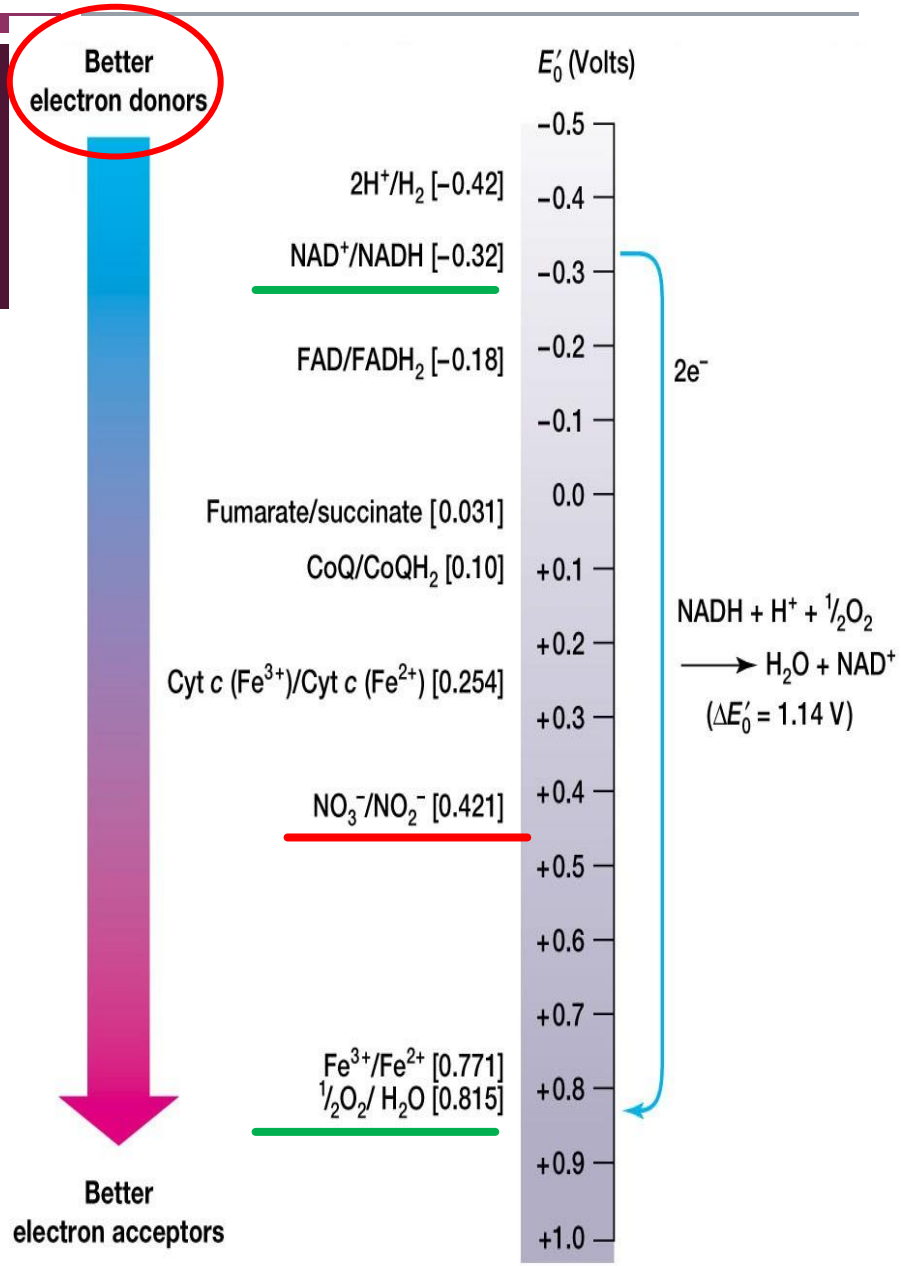


Table 11.3 Some Electron Acceptors Used in Respiration

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

The anaerobic ETC is more complex than the aerobic ETC.

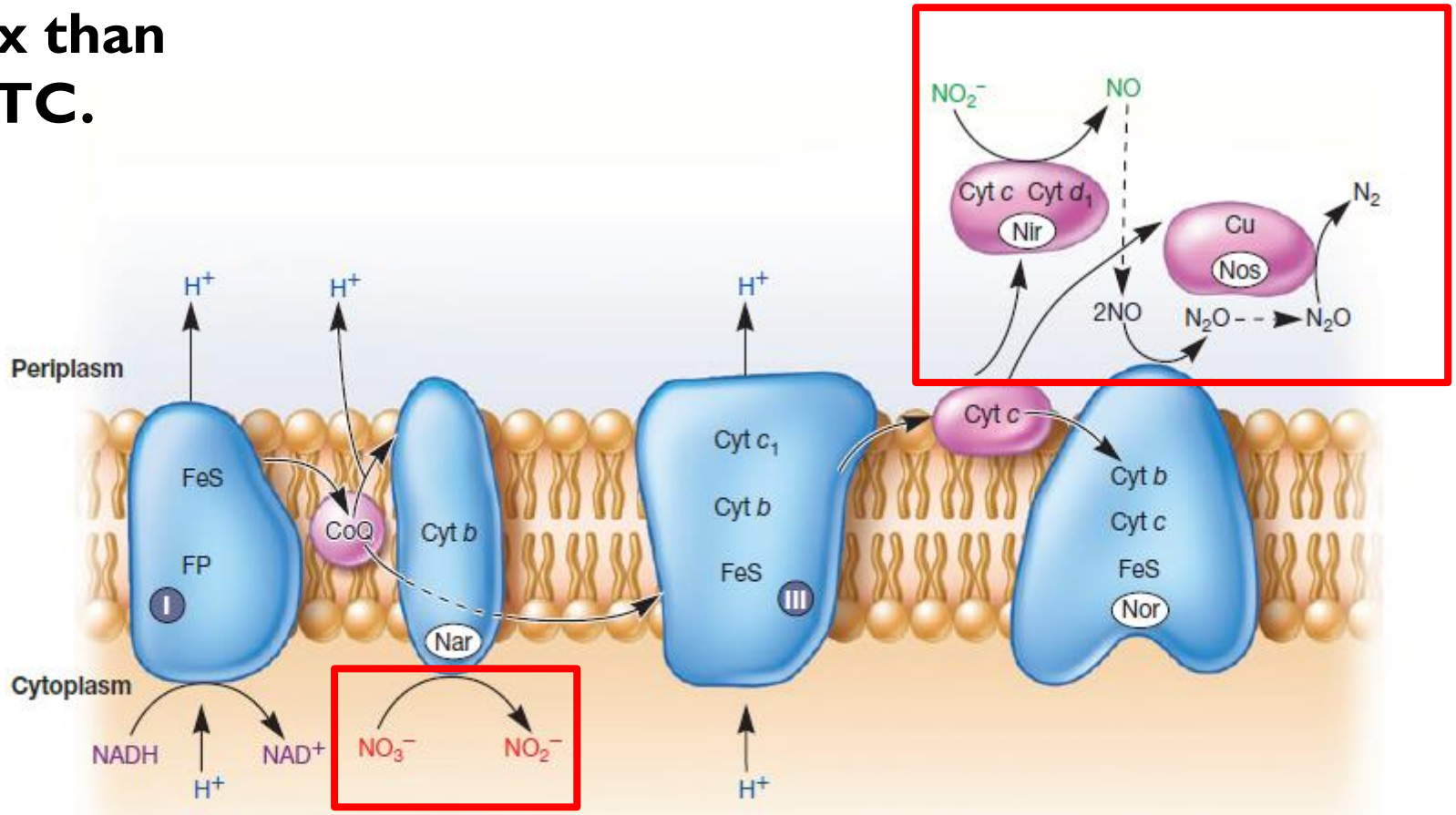
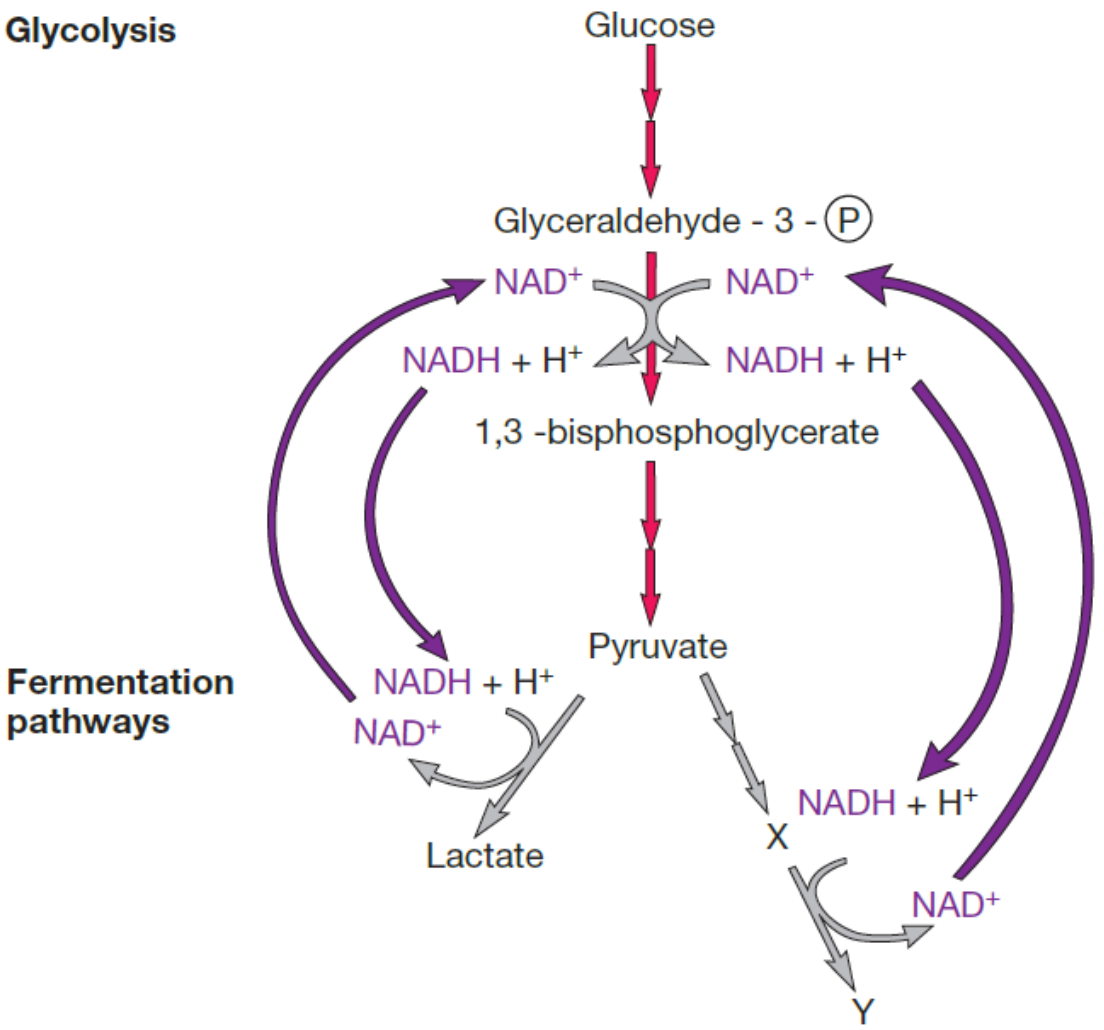


Figure 11.18 ***Paracoccus denitrificans*** **Electron Transport Chain Used During Anaerobic Respiration.** This branched ETC is made of both membrane and periplasmic proteins. Nitrate is reduced to diatomic nitrogen (N_2) by the collective action of four different reductases that receive electrons from CoQ and cytochrome *c*. Locations of proton movement are indicated. Four protons are pumped into the periplasm by complex I, two by nitrate reductase (Nar), and two by complex III. However, two protons are used by nitric oxide reductase (Nor) to reduce nitric oxide to nitrous oxide. Thus six protons, net, are used to create a PMF. Abbreviations used: flavoprotein (FP), nitrite reductase (Nir), and nitrous oxide reductase (Nos).

Fermentation

- Some microbes do not respire
  - Lack of electron transport chain or
  - They repress the synthesis of ETC components under anoxic conditions
- However, NADH must still be oxidized back to NAD^+ to continue the glycolysis.
  - Use the pyruvate or its derivatives as the electron acceptor
- **Fermentation**
  - Partial degradation of sugar without O_2 (only glycolysis)
  - NADH is oxidized to NAD^+
  - O_2 is not needed
  - The electron acceptor is either the pyruvate or the its derivatives
  - No ETC → reduce ATP yield.
  - Substrate-level phosphorylation in glycolysis is the only source of ATP



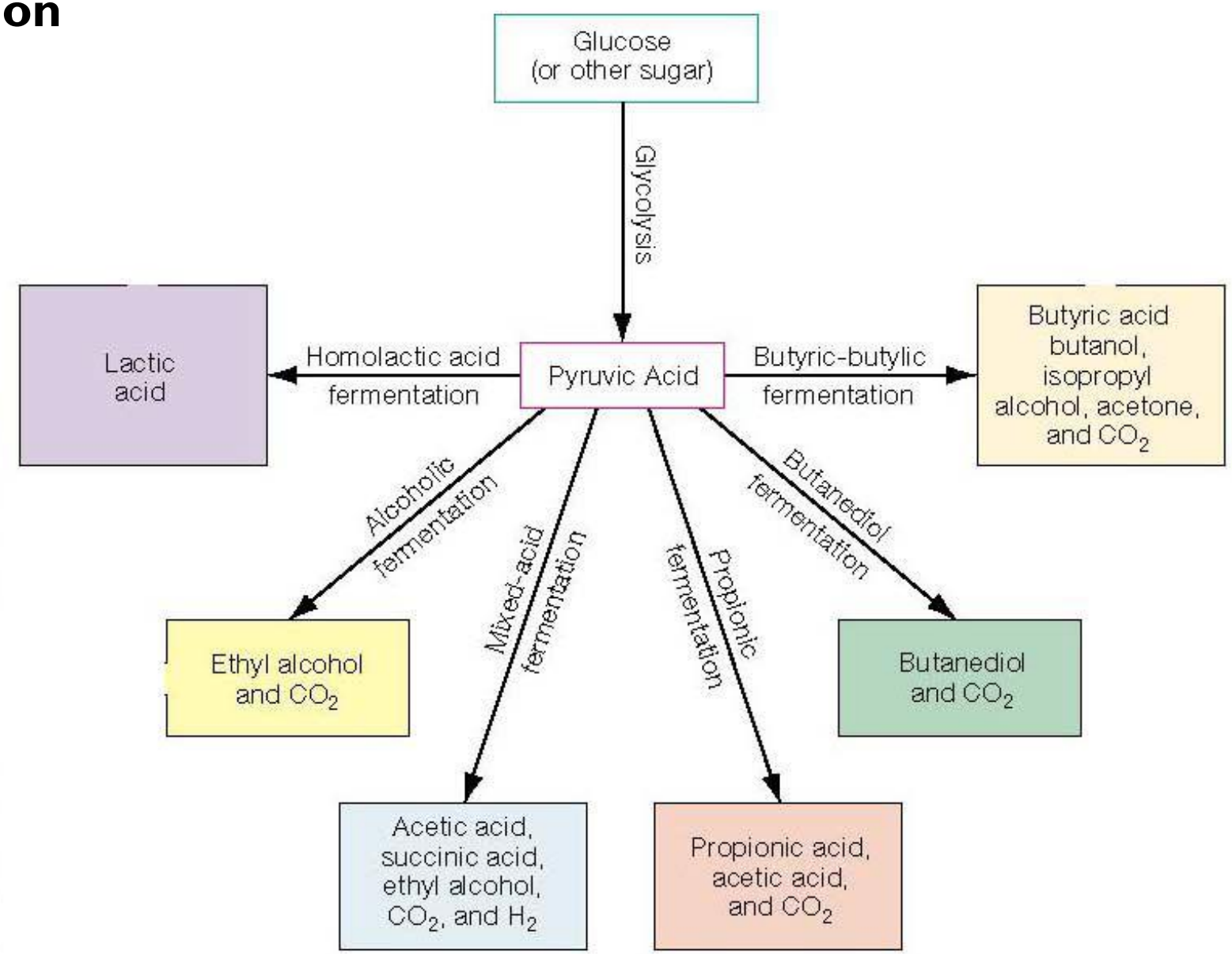
Reoxidation of NADH during fermentation

Fermentation

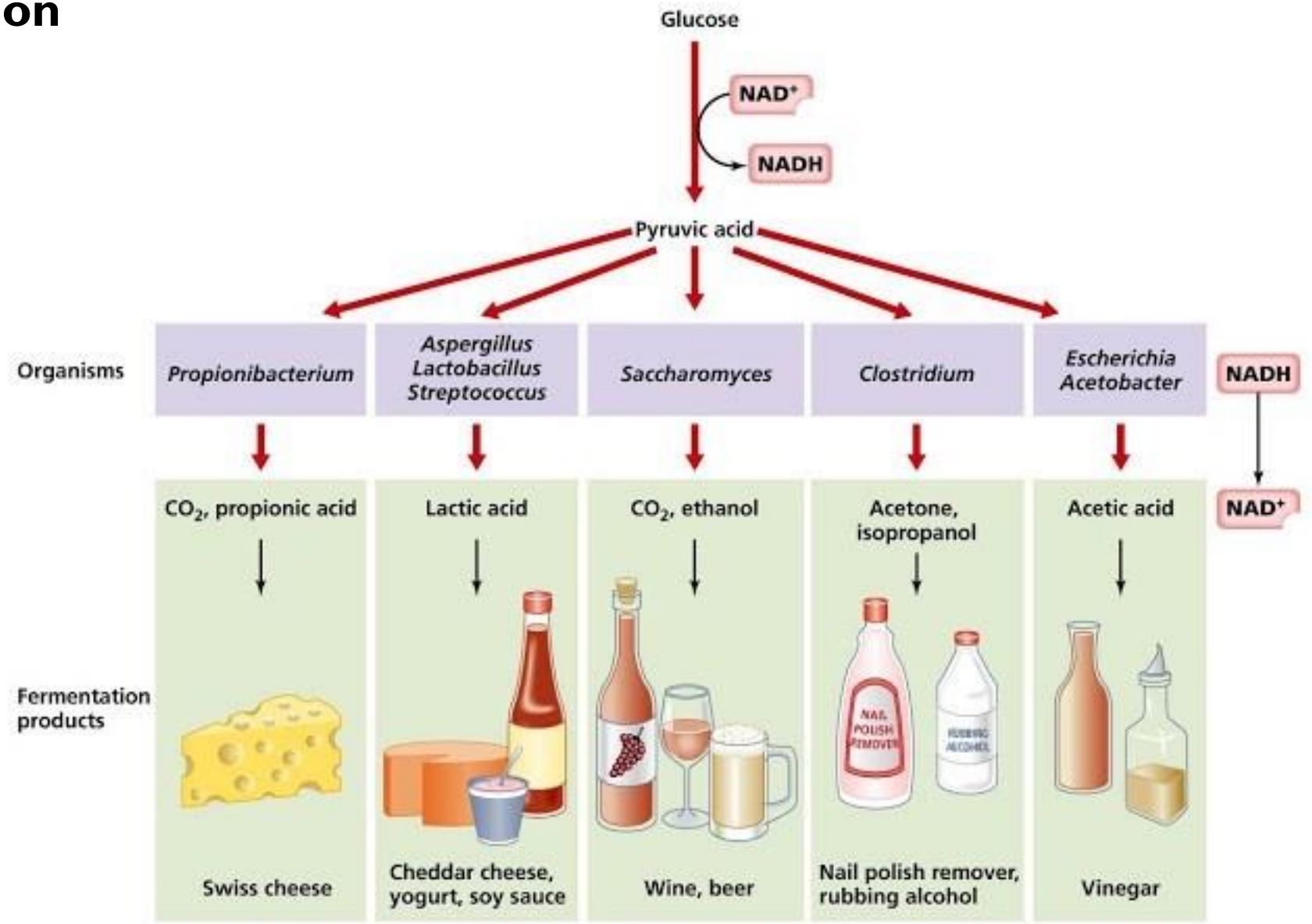
- Fermentation is a metabolic process by which organic molecules such as glucose are broken down anaerobically to release energy.
- Products: lactic acids, carbon dioxide, alcohol, etc..
- It occurs in yeast and bacteria.
- Fermentation pathways are useful as tools in biochemical identification of bacteria
- In industry, fermentation is used for food production

- Examples of fermentation pathways
  - **Alcohol fermentation** *(Saccharomyces cerevisiae)*
  - **Lactic acid fermentation** *(Lactobacillus)*
  - **Mixed acid fermentation** (*E. coli*)
  - **2,3-Butanediol fermentation** (*Enterobacter aerogenes*)
  - **Propionic acid fermentation** (*Propionibacterium*)
  - **Acetone, butaraldehyde and butanol** (*Clostridium acetobutyricum*)

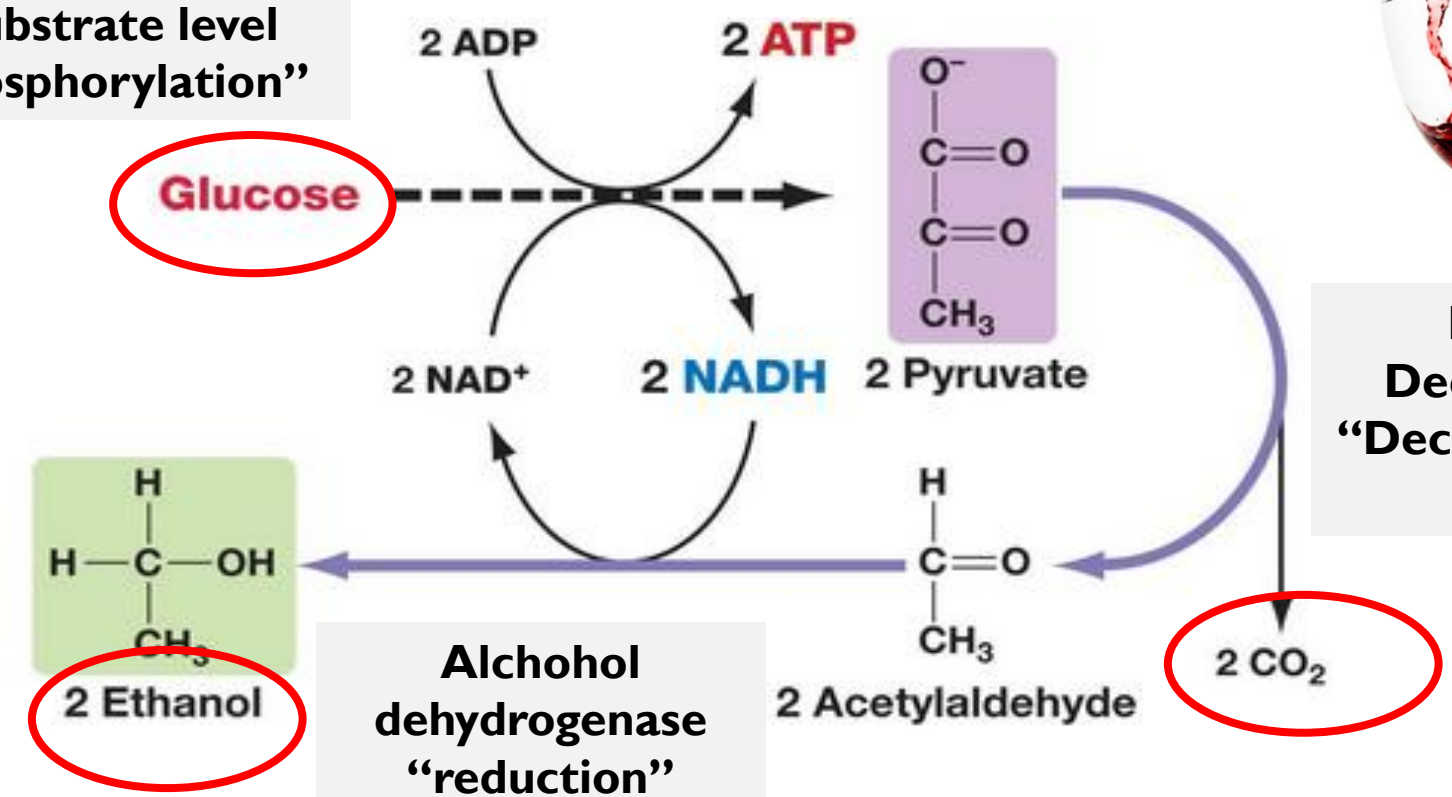
Fermentation pathway



Fermentation products



Alcohol fermentation



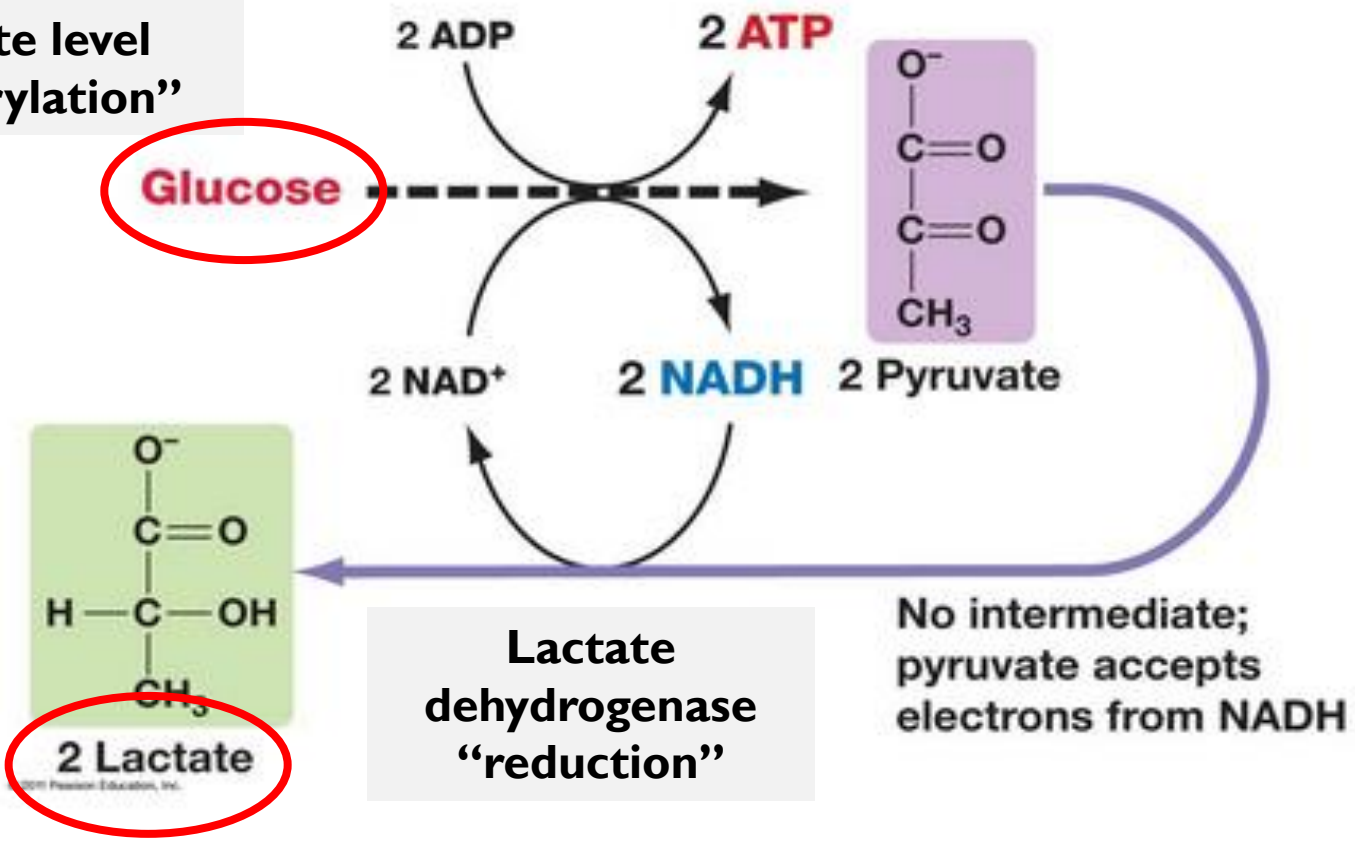
E.g. Saccharomyces cerevisiae

Glucose → 2 Ethanol + 2 Carbon Dioxide

$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$

Lactic acid fermentation

"Substrate level phosphorylation"

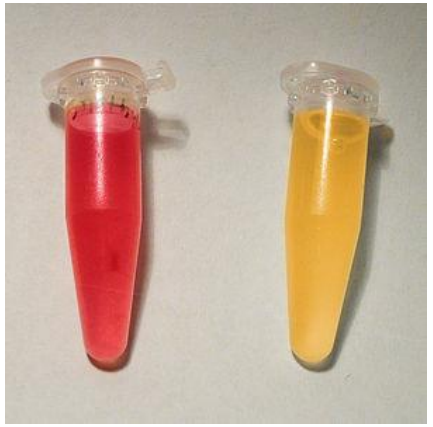


***E.g.* lactic acid bacteria (*Lactobacillus, Lactococcus, Leuconostoc, Streptococcus*

$Glucose \rightarrow 2\ Lactic\ Acid$

$C_6h_{12}o_6 \rightarrow 2\ C_3H_6O_3$

Mix acids fermentation



Fermentation Pathways: Mixed acid fermentation

Pyruvic Acid + NADH → Lactic Acid + NAD

→ Acetic Acid + Formic Acid

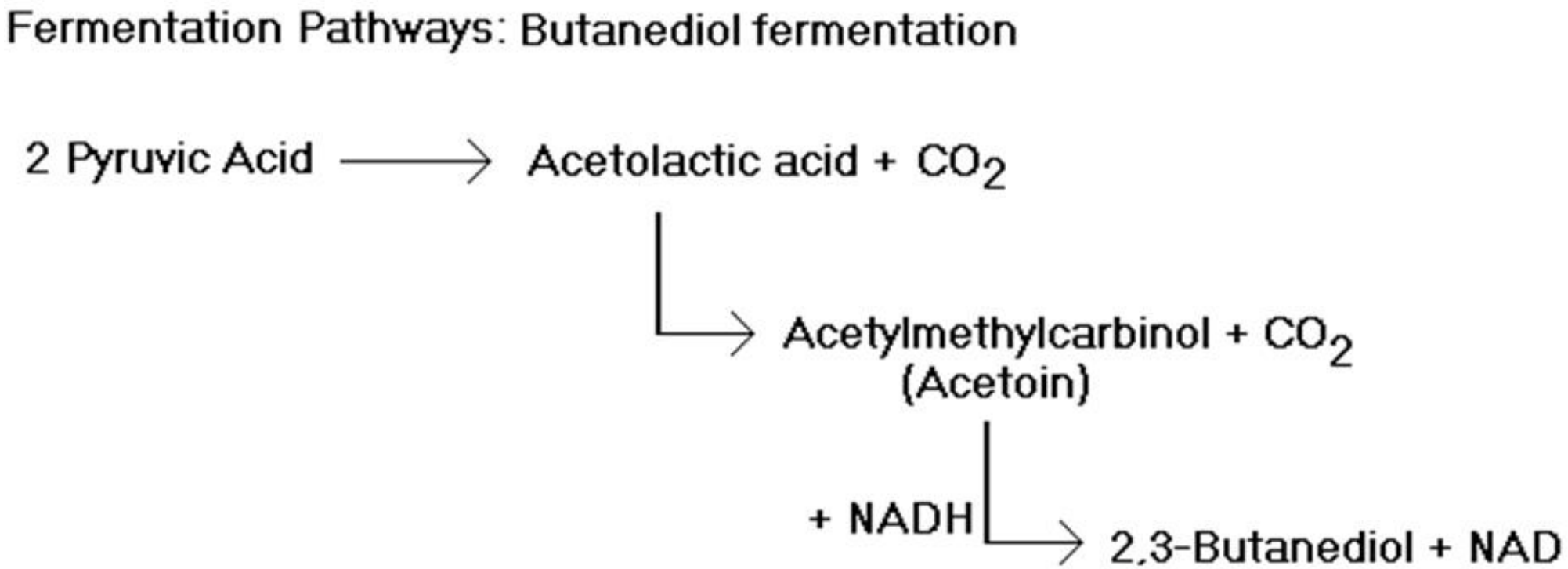
→ $H_2 + CO_2$

→ Carbonic Acid

- The methyl red (MR) test can detect whether the mixed acid fermentation pathway occurs in microbes when given glucose.
- A pH indicator is used that turns the test solution red if the pH drops below 4.4.

E.g. Escherichia coli, Salmonella, Proteus

Butanediol fermentation



E.g. Enterobacter, Serratia, Erwinia, Bacillus

Catabolism Of Organic Molecule Other Than Glucose

- Organic molecule that can be fermented:
  - Sugar (glucose, lactose, fructose, mannose, galactose,)
  - Amino acid (Trytophan, proline, arginine, isoleucine, etc.)
  - Lipid (triglyceride- hydrolyzed to glycerol and fatty acid)
  - Organic acid (acetate, lactate, propionate, citrate)

Breakdown of nutrient by enzymes:

- Carbohydrate to sugar monomer
- Lipid to fatty acid & glycerols
- Proteins to amino acids

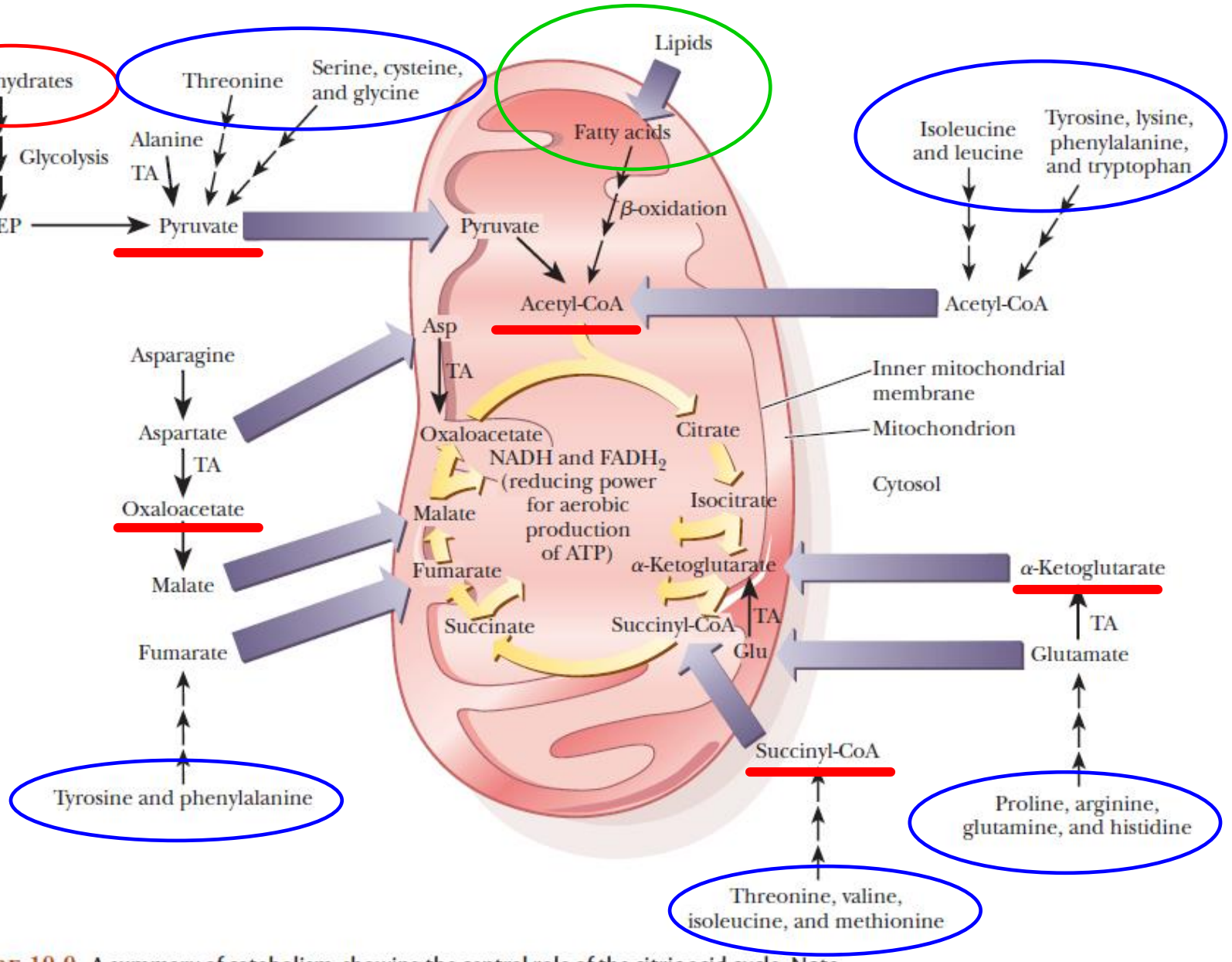
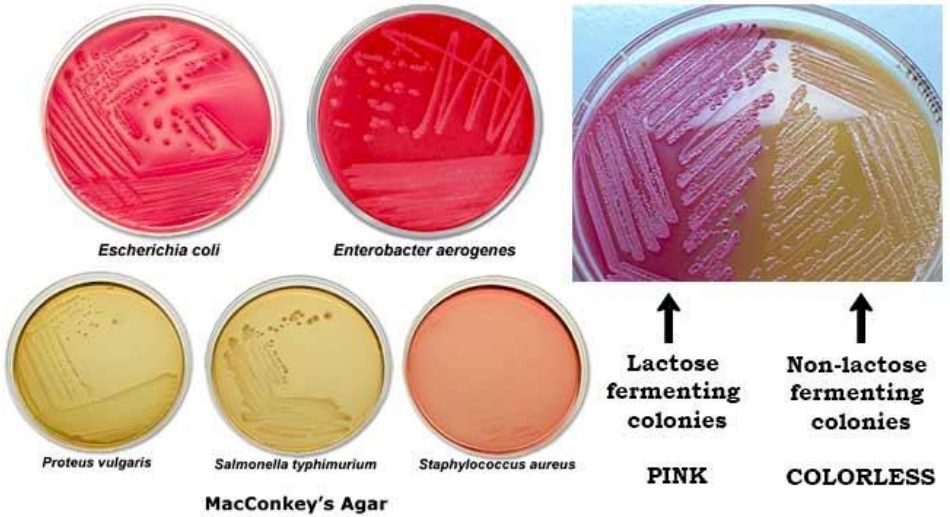


FIGURE 19.9 A summary of catabolism, showing the central role of the citric acid cycle. Note that the end products of the catabolism of carbohydrates, lipids, and amino acids all appear. (PEP is phosphoenolpyruvate; α-KG is α-ketoglutarate; TA is transamination; →→→ is a multistep pathway.)

Why Bacterial Metabolism Is Important?

- Scientists learned how to make different types of culture media by understanding bacterial metabolism.
  - MacConkey Agar
    - ✓ Selective & differential medium
    - ✓ Differentiate Gram –ve bacteria based on their ability to ferment lactose (source of fermentable carbohydrate)
    - ✓ Bile salt & crystal violet inhibit the Gram +ve bacteria



Biochemical test for identification of bacteria

Biochemical test		Aim	Result
IMViC Test	Indole test	To determine the ability of certain bacteria to decompose amino acid tryptophane to indole.	**Positive**: Formation of pink to red colour in the reagent layer on top of the medium **Negative**: No colour changes
	Methyl red (MR) test	To test for mixed acid fermentation.	**Positive test:** red colour change **Negative test:** no colour change.
	Voges-Proskauer (VP) test	To test if the bacteria has butanediol fermentation 2 pyruvate + NADH → $2CO_2$ + 2,3-butanediol.	**Positive test:** colour change to pink (e.g *Klebsiella* spp.) **Negative test:** no colour change.
	Citrate utilisation test	The test looks for the ability of a bacteria to utilise citrate as a sole source of carbon	**Positive**: Growth with colour change from green to intense blue **Negative**: No growth and no colour change

Biochemical test	Aim	Result
Triple Sugar Iron (TSI) Test	To test the ability of bacteria to utilise lactose or sucrose (or both) under aerobic/anaerobic condition.	**Ferment glucose**: Red slant, yellow butt **Ferment glucose and lactose/sucrose:** yellow slant and butt **Non-fermenter:** red slant, butt no colour changes
Oxidase test	To test whether the bacteria have cytochrome c and cytochrome c oxidase (aerobic respiration)	**Positive test result:** Dark blue-purple colour change within 10-30 sec. (E.g. *Aeromonas*, *Campylobacter*) **Negative test result:** No colour change or colour change after more than 30 sec.

Bacterial fermentation

Lactic acid bacteria

Clostridium thermocellum	• Conversion of cellulose directly to ethanol and acetic acid. • Bioethanol concentrations are generally less than 5 g/L.
Escherichia coli	• Native strains are able to ferment xylose to bioethanol, succinic, and acetic acids. • Native strains are ethanol-intolerant. • Genetically engineered strains can produce bioethanol.
Klebsiella oxytoca	• Native strains rapidly ferment xylose and cellobiose. • Genetically engineered strains can ferment cellulose and produce bioethanol predominantly.
Lactobacillus pentoaceticus	• Consumtion of xylose and arabinose. • Slow use of glucose and cellobiose.
Lactobacillus casei	• Good fermentation of lactose.
Lactobacillus xylosus	• Good use of *n*-glucose, d-xylose, and l-arabinose. • Can use cellobiose, if nutrients are supplied.
Lactobacillus pentosus	• Useful for homolactic fermentation. • Some strains are capable to produce lactic acid from sulfite waste liquors.
Lactobacillus plantarum	• Consumption of cellobiose more rapidly than glucose, xylose, or arabinose. • It is supposed to depolymerize pectins. • Production of lactic acid from agricultural residues.
Zymomonas mobilis	• Capable of fermenting glucose and fructose. • Genetically engineered strains can ferment xylose.

Why Bacterial Metabolism Is Important?

- Understanding bacterial metabolism also can lead us to use bacteria for a good cause.
  - Microbial metabolism produces product that can be useful to human. E.g. Fermented product
  - Yeast, lactic acid bacteria - fermentation

Why fermentation is important for food production?

- Preserving food
- Lowering of pH levels, restricts the growth of competing microorganisms
  - *Leuconostoc* and lactic *Streptococci* - pH 4.0
  - Some of the *Lactobacilli* and *Pediococci* - pH 3.5
- Improve shelf life of selected food products
- No heat required during preparation
- Flavor modification and nutritive value improvements
  - *Lactococcus lactis* subsp. *diacetylactis* covert milk citrate to diacetyl and give richer buttery flavour to the finished product (e.g. cultured buttermilk, sour cream)

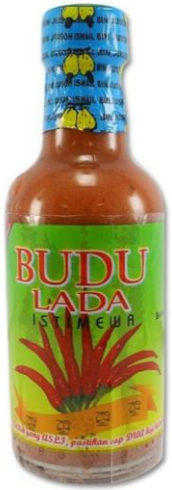
Importance of fermentative pathways to food production

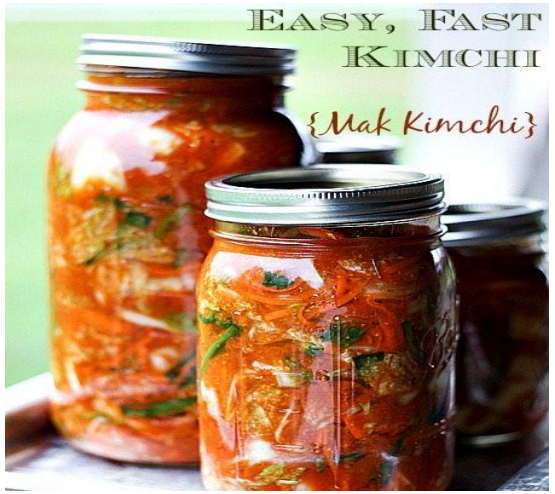
- **Yogurt**
  - Starter culture
    - ✓ *Lactobacillus bulgaricus*
    - ✓ *Streptococcus thermophilus*
  - Lactic acid increase
  - pH decrease
  - Facilitate coagulation of milk
  - Lactose fermentation produces the flavor of yogurt

Importance of fermentative pathways to food production

- **Sauerkraut (Sour cabbage)**
  - Starter culture
    - ✓ *Leuconostoc*
    - ✓ *Lactobacillus*
  - Lactic acid increase
  - pH decrease
  - Other pathogenic organisms are killed
  - Fermentation produces the unique flavor of sauerkraut

Fermented food products





Why Bacterial Metabolism Is Important?

- We know how to inhibit or stop bacterial growth by controlling their metabolism.
  - Nutrient availability plays important role in the growth control
  - Enzyme for metabolic reaction can be denatured heat, low pH
  - Oxygen is required for aerobic respiration

SUMMARY

- ✓ Basic concept of metabolism- catabolism & anabolism, endergonic & exergonic reaction
- ✓ ATP as the major energy currency
- ✓ Redox reaction – cofactors, standard reduction potential
- ✓ Catabolic pathway – Aerobic and anaerobic respiration, fermentation
- ✓ Glycolysis
- ✓ Krebs cycle
- ✓ Oxidative phosphorylation & substrate level phosphorylation
- ✓ Fermentation

THANK YOU