微生物学
Microbiology

Lecture 7

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Part Three

Microbial Metabolism

CHAPTER 10

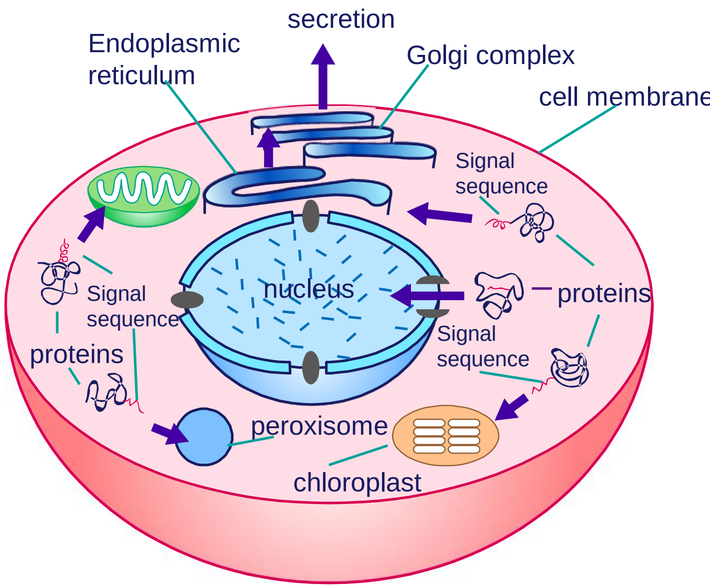
Introduction to Metabolism

OUTLINE

- **What is metabolism: Important principles and concepts**
- **Work and Energy**
- **What is the role of ATP in metabolism?**
- **Oxidation-reduction Reactions**
- **Electron Transport Chains: Sets of Sequential Redox Reactions**
- **Biochemical Pathways**
- **Enzymes and Regulation of Metabolism**

Unifying principle in biology

- **Construction to its function**
- **The trapping and use of energy**

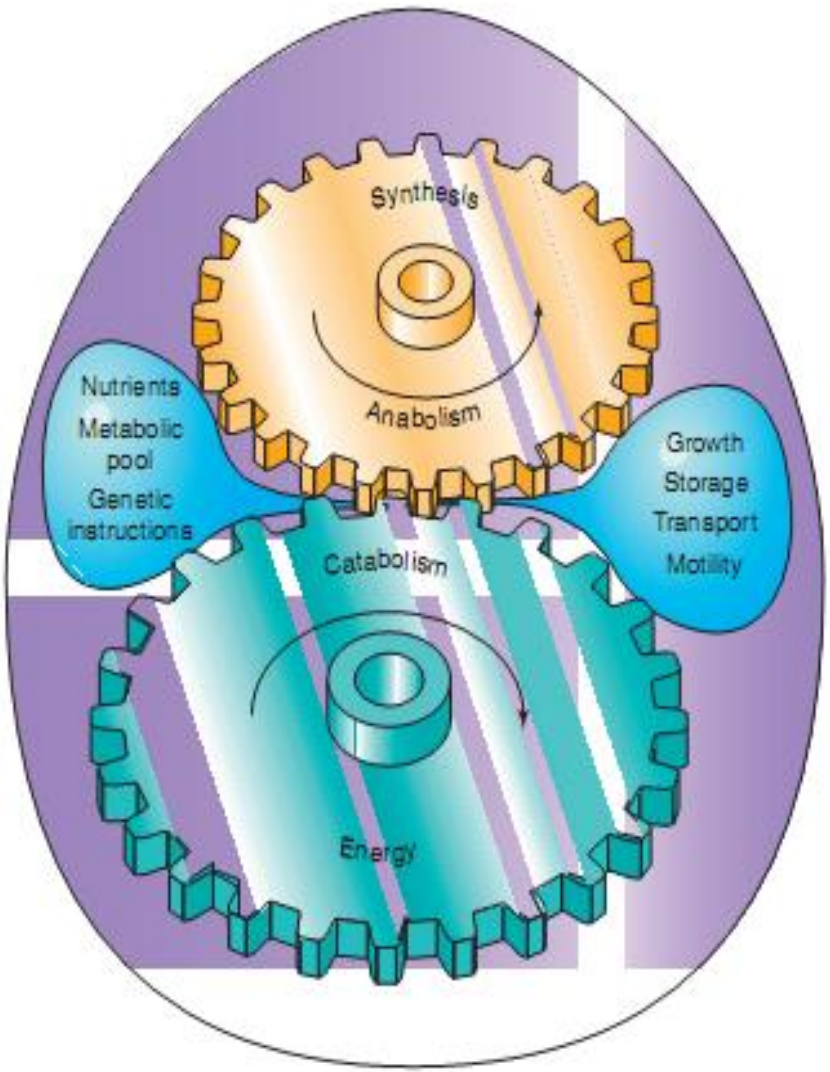


Metabolism is central to all life. 代谢是所有生命的核心

Living cells are self-regulating chemical engines, tuned to operate on the principle of maximum economy.

—*Lehninger*

Metabolism is central to all life 代谢是所有生命的核心



A marvelous metabolic machine

This simplified model summarizes cell metabolism.

The chemical reactions in cells are interactive and highly balanced.

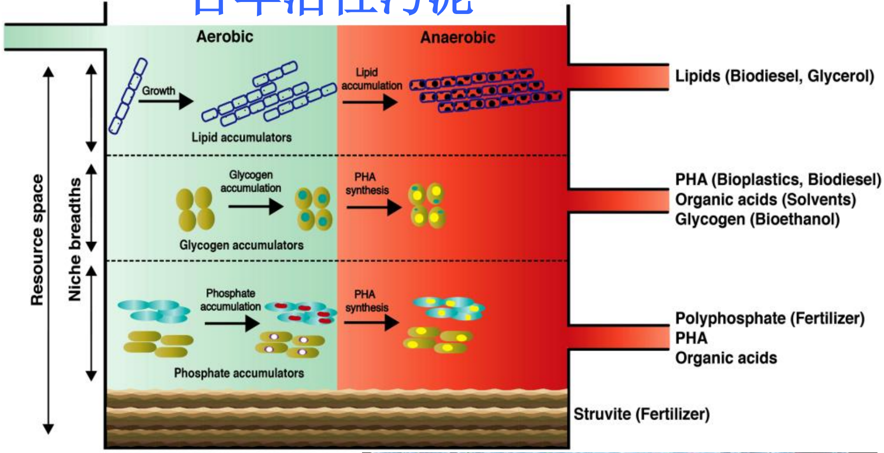
Synthetic actions of **Anabolism** work with the energy- producing actions of **Catabolism**.

They mutually drive each other and keep the basic actions of the cell constantly in play.

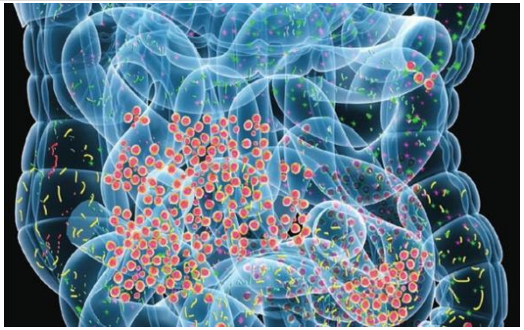
Microbial metabolism and its importance

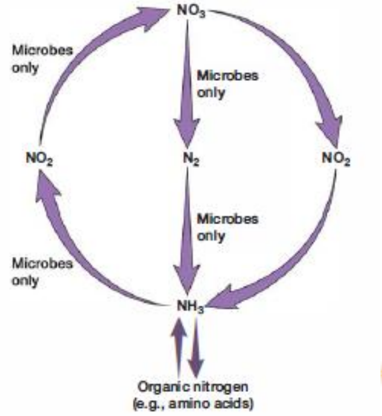
- the <u>metabolic prowess</u> of microbes in industry and food production

百年活性污泥

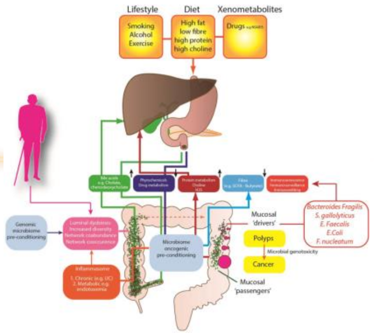


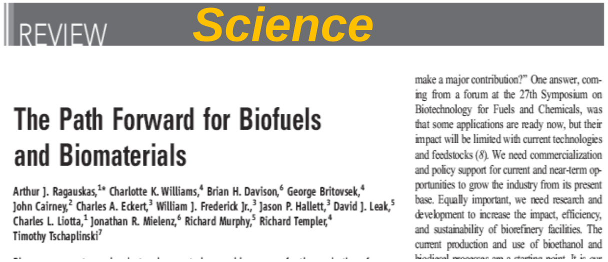
- <u>contribute</u> to cycling of elements in ecosystems
  - some cycling reactions performed <u>only</u> by microbes





人体肠道微生物





REVIEW Science

The Path Forward for Biofuels and Biomaterials

Arthur J. Ragauskas,[1*] Charlotte K. Williams,[4] Brian H. Davison,[6] George Britovsek,[4] John Cairney,[2] Charles A. Eckert,[3] William J. Frederick Jr.,[3] Jason P. Hallett,[3] David J. Leak,[5] Charles L. Liotta,[1] Jonathan R. Mielenz,[6] Richard Murphy,[5] Richard Templer,[4] Timothy Tschaplinski[7]

make a major contribution?" One answer, coming from a forum at the 27th Symposium on Biotechnology for Fuels and Chemicals, was that some applications are ready now, but their impact will be limited with current technologies and feedstocks (8). We need commercialization and policy support for current and near-term opportunities to grow the industry from its present base. Equally important, we need research and development to increase the impact, efficiency, and sustainability of biorefinery facilities. The current production and use of bioethanol and

Biorefinery
生物冶炼-微生物细胞工厂

Metabolism common features

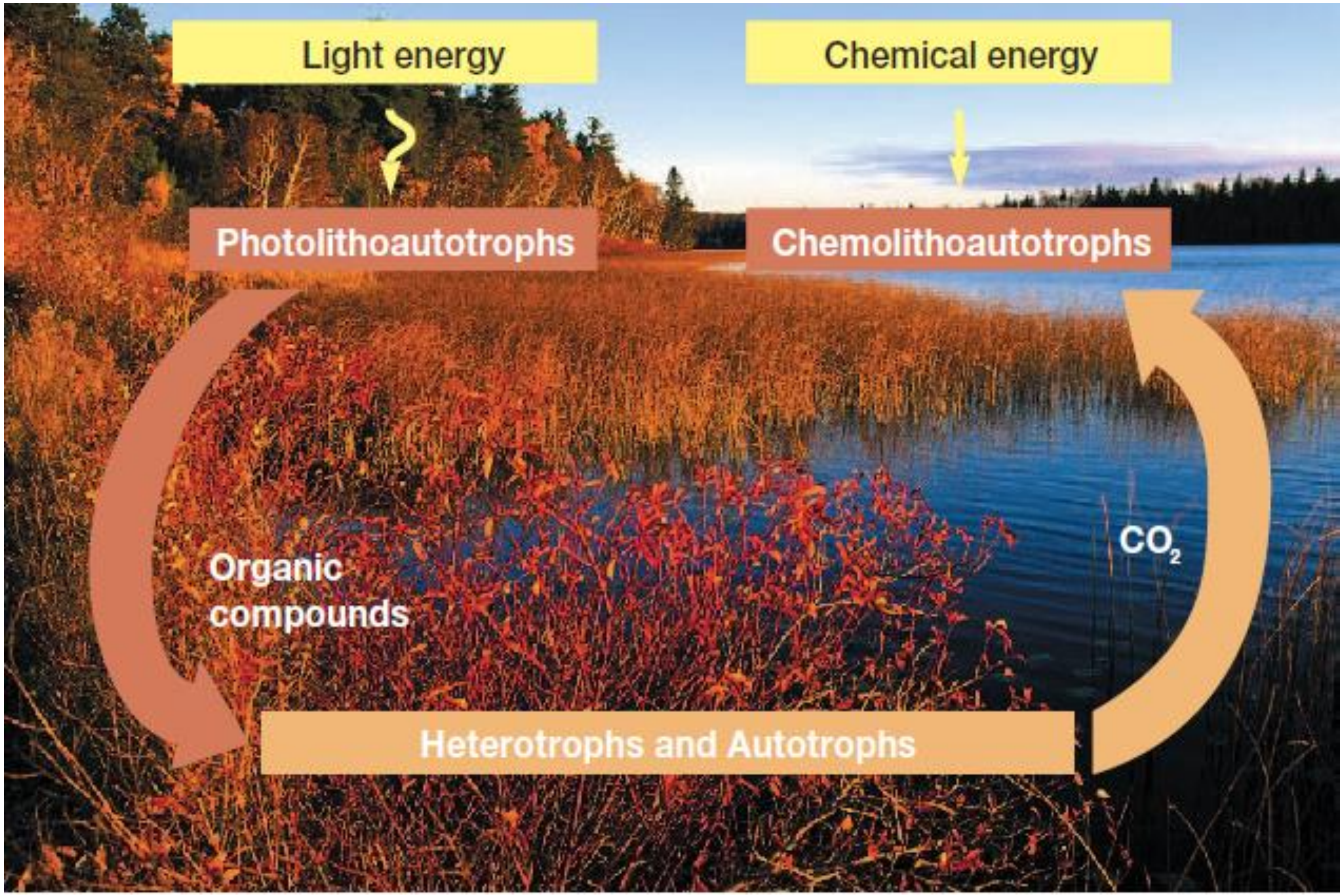
- Life obeys the laws of thermodynamics.
- The energy cells obtain from their environment is most often conserved as a molecule called ATP.
- Oxidation-reduction (redox) reactions play a critical role in energy conservation.
- The chemical reactions that occur in cells are organized into pathways.
- Each reaction of a pathway is catalyzed by an enzyme or a ribozyme.
- The functioning of biochemical pathways is regulated.

Microbial cells must do work

- chemical work 化学功
  - synthesis of complex molecules
- transport work 运输功
  - take up of nutrients, elimination of wastes, and maintenance of ion balances
- mechanical work 机械功
  - cell motility and movement of structures within cells

All these works need energy. Where is from?

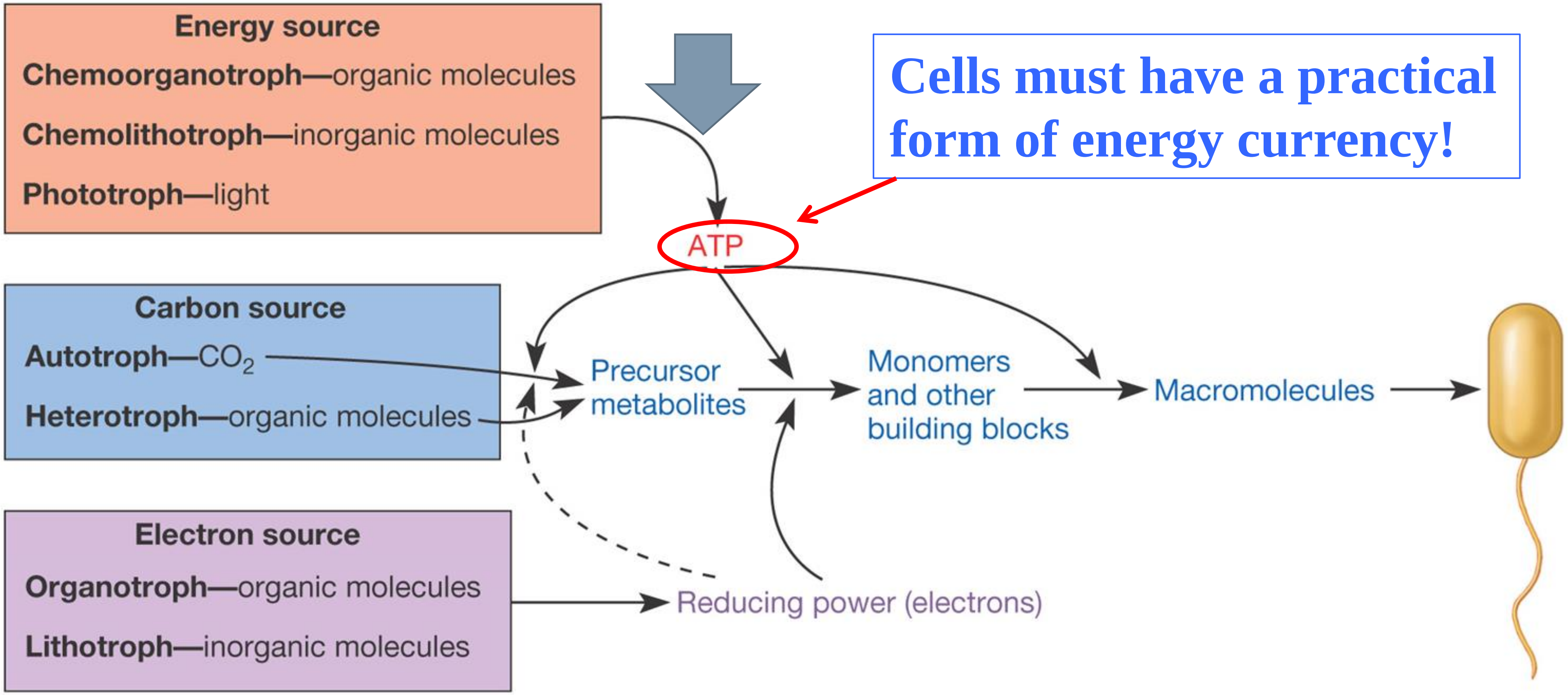
√**Energy** the capacity to do work or to cause particular changes.



The Flow of Carbon and Energy in an Ecosystem.
This diagram depicts the flow of energy and carbon in general terms.

在生态系统中碳的流动和能量密切相关

Microbial metabolism



Cells must efficiently transfer energy from their energy generating or trapping apparatus to the systems actually carrying out work.

Free Energy and Reactions

- The **free energy change** is the amount of energy in a system available to do useful work at constant temperature and pressure.

放能反应

Exergonic reactions

$$A + B \rightleftharpoons C + D$$

$$K_{eq} = \frac{[C]\,[D]}{[A]\,[B]} > 1.0$$

$\Delta G^{o\prime}$ is negative.

吸能反应

Endergonic reactions

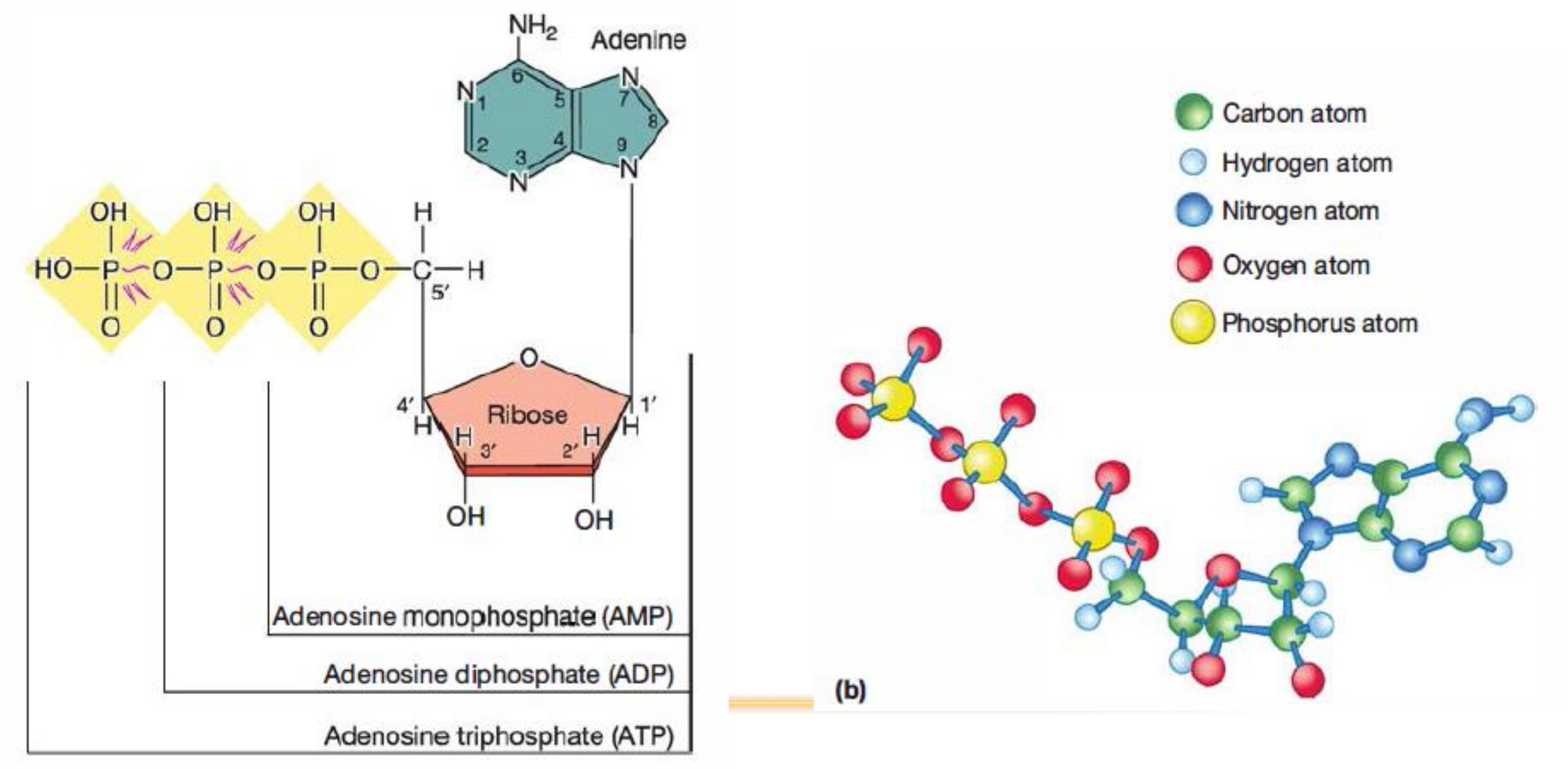
$$A + B \rightleftharpoons C + D$$

$$K_{eq} = \frac{[C]\,[D]}{[A]\,[B]} < 1.0$$

$\Delta G^{o\prime}$ is positive.

$\Delta G^{o\prime}$ the **standard free energy change at pH 7**

ATP: The Major Energy Currency of Cells

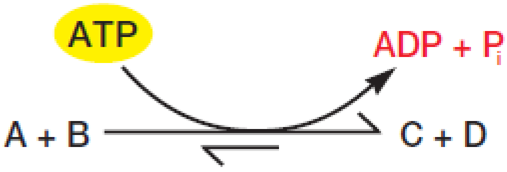


(a) ∽ Bond that releases energy when broken

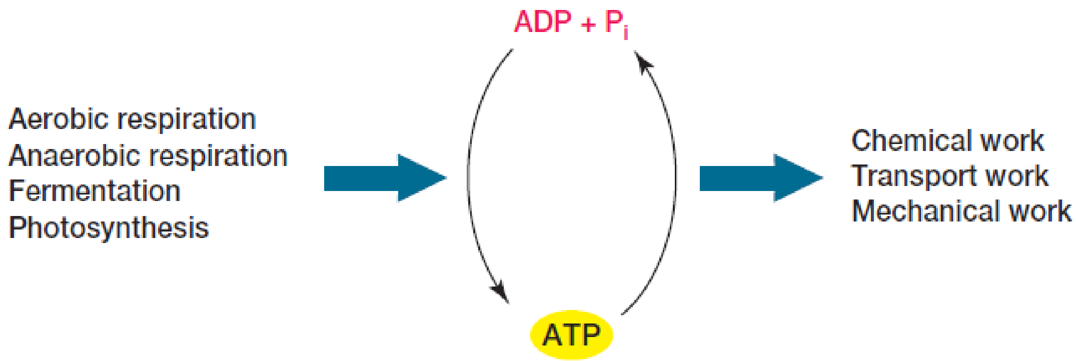
What makes ATP suited for its role as energy currency?

Endergonic reaction alone

Endergonic reaction coupled to ATP breakdown



ATP as a Coupling Agent



The Cell's Energy Cycle.
ATP is formed from energy made available during aerobic respiration, anaerobic respiration, fermentation, and photosynthesis. Its breakdown to ADP and phosphate (Pi) makes chemical, transport, and mechanical work possible.

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

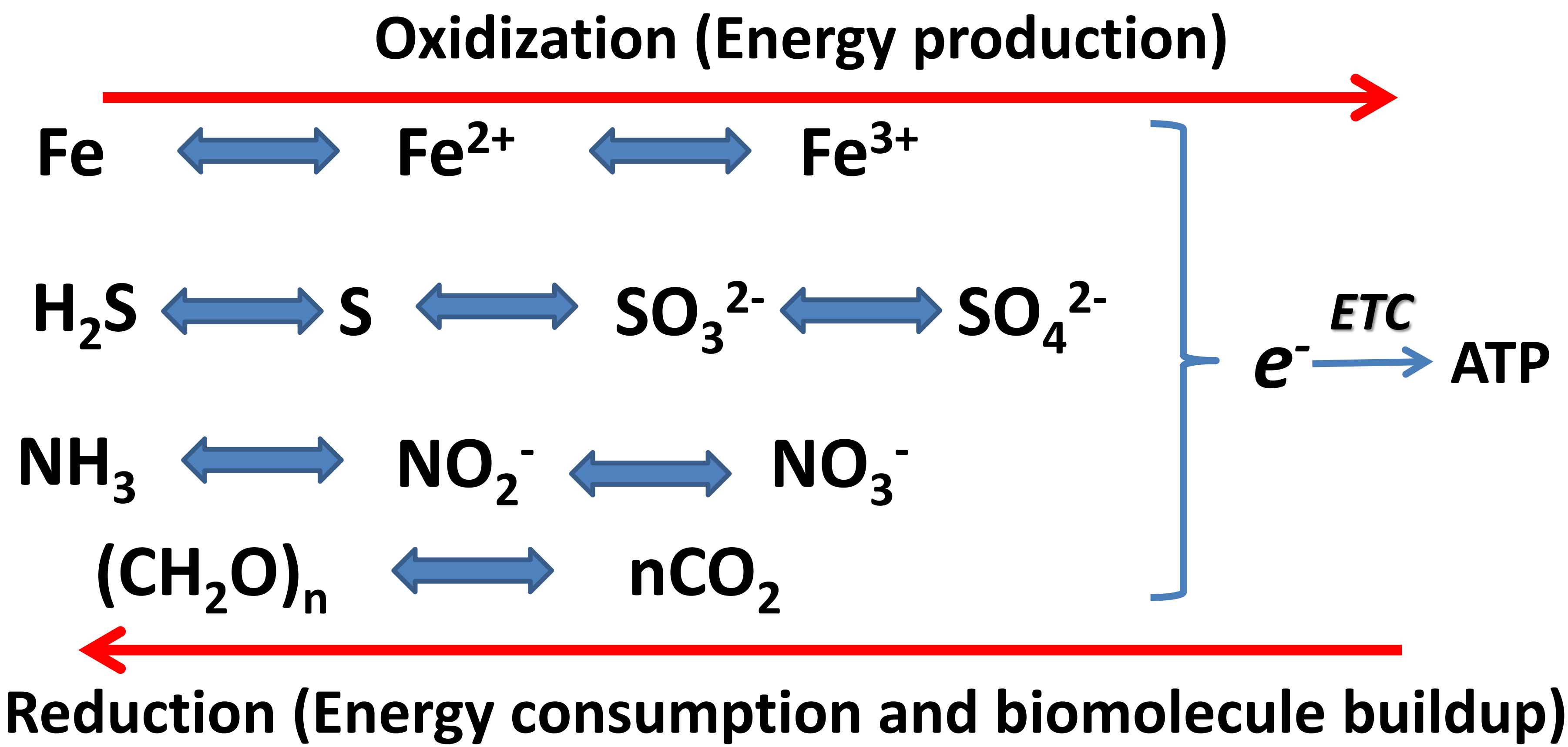
Phosphorylated Molecule	ΔG°′ of Hydrolytic Removal of Phosphate (KJ/mol)	Phosphate Transfer Potential
High-Energy Phosphorylated Compounds		
Phosphoenolpyruvate[2]	−61.9	61.9
1, 3-bisphosphoglycerate[2]	−49.3	49.3
ATP (hydrolysis to AMP)	−45.6	45.6
ATP (hydrolysis to ADP)	−30.5	30.5
Low-Energy Phosphorylated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

ATP is the major energy currency for cells, but it is not the only energy currency.

Do you know Other nucleoside triphosphates (NTPs)?

How to get ATP?

Oxidation-reduction reactions



Oxidation-reduction reactions

- **Metabolism** is the total of all chemical reactions in the cell.
- **Oxidation-reduction** (redox) reactions, electrons move from an **electron donor**(电子供体） to an **electron acceptor**（电子受体）.
- **conjugate redox pair**

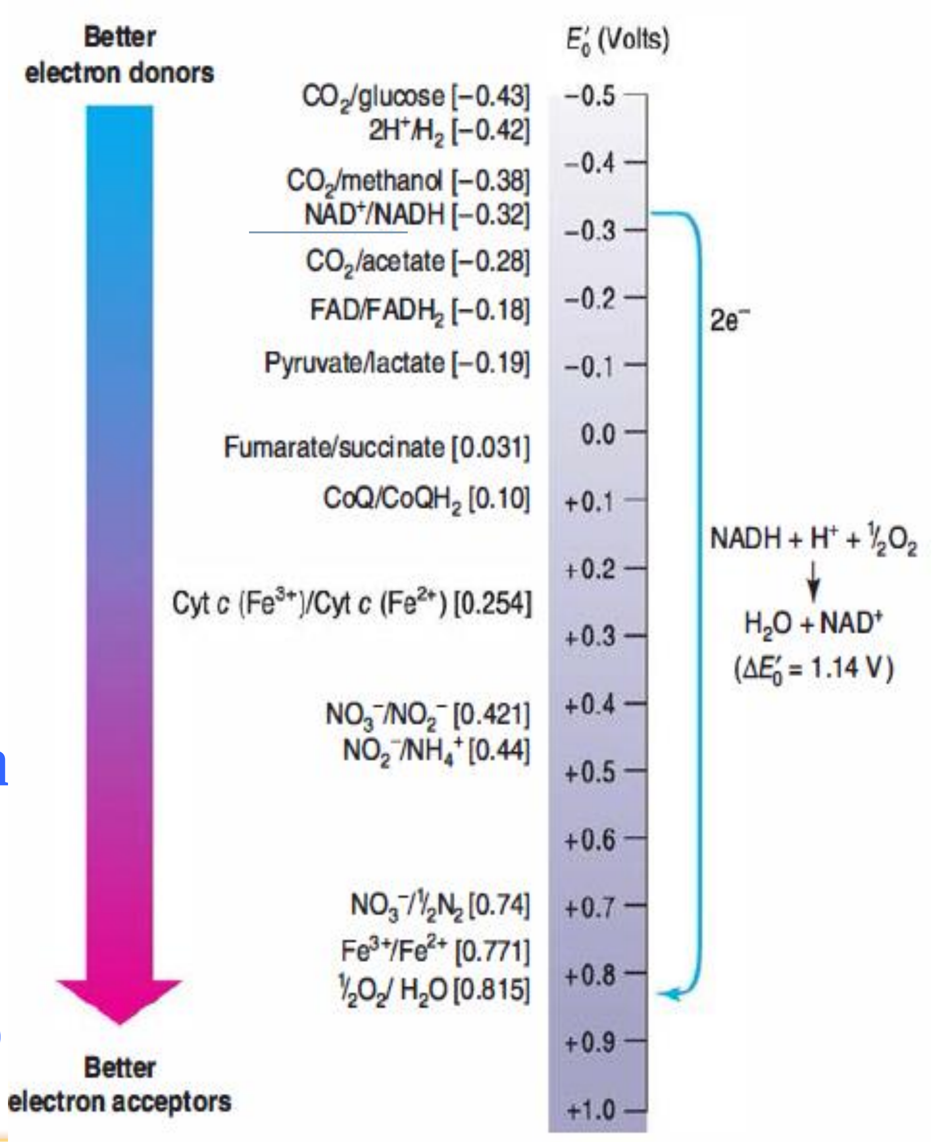
$$\text{Acceptor} + ne^- \rightleftharpoons \text{donor}$$

- E_0' **Standard reduction potentials** (标准还原电势）**pH 7**

$$\Delta G^{\circ\prime} = -nF \cdot \Delta E_0'$$

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

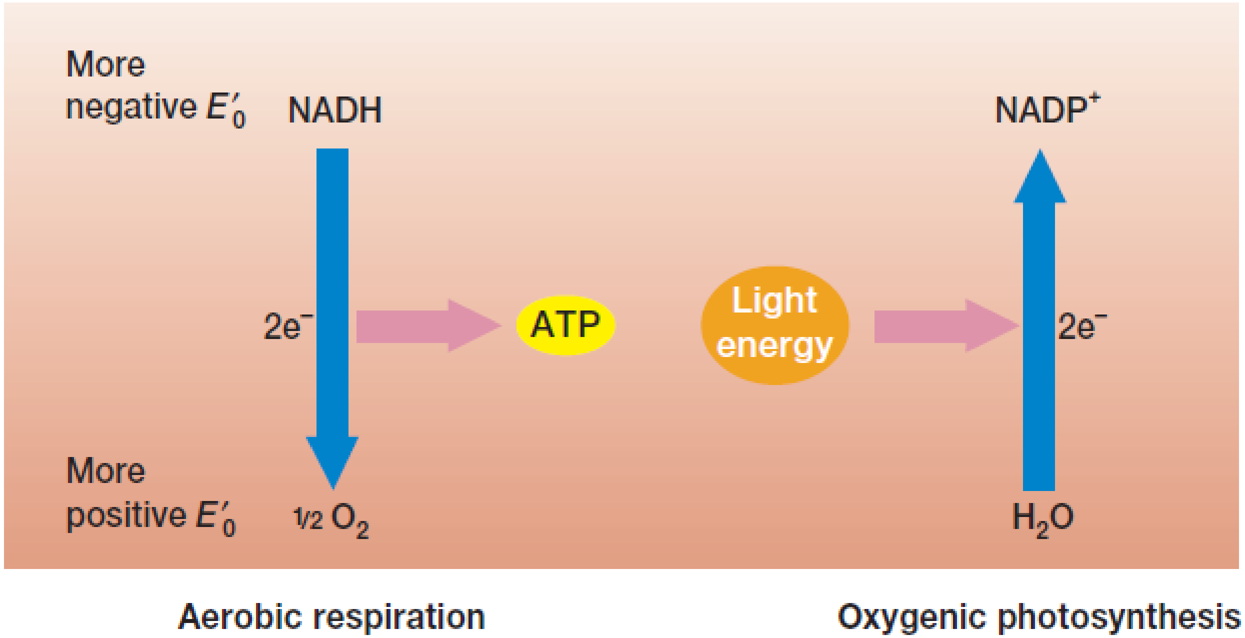
- The relatively negative E_0' of conjugate redox pair stores more potential energy than redox pairs with or more positive E_0' values.
- Electrons move from a donor to an acceptor with a more positive redox potential, free energy is released and can be used to synthesize ATP and do other work.



Electron tower

DISCUSSION

- **How to predict which molecule will act as an electron donor, which molecule will act as an electron acceptor, and the relative amount of energy released by a redox reaction, using the standard reduction potentials of the reaction's conjugate redox pairs?**



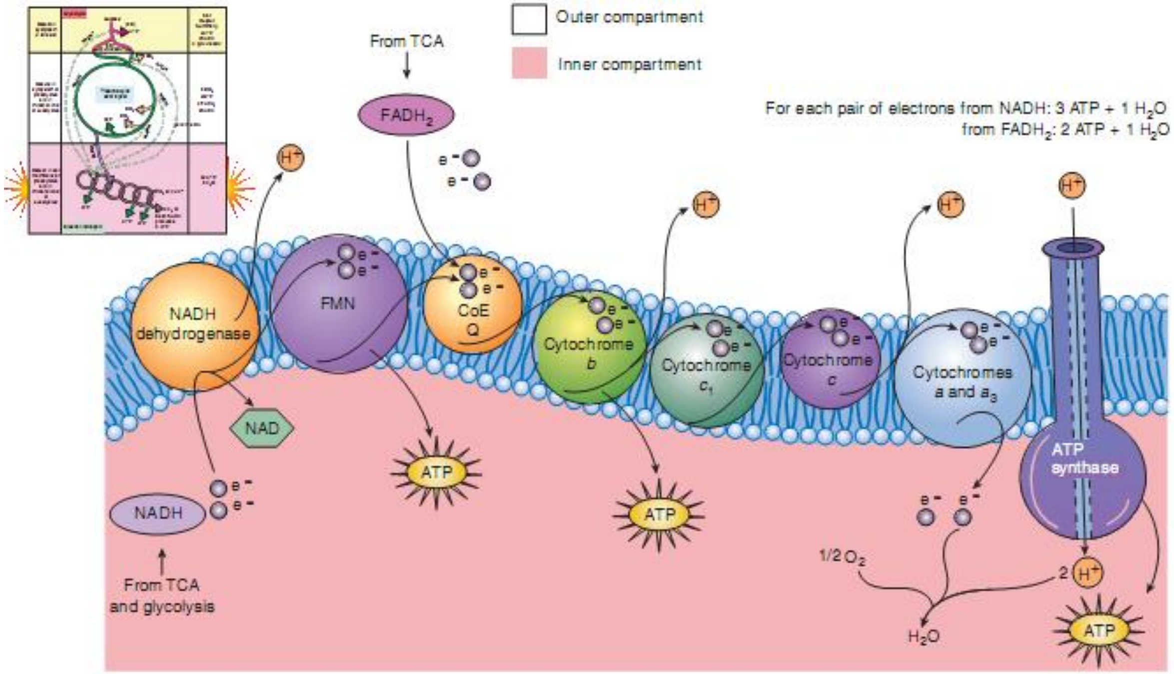
Energy Flow in Metabolism.

Examples of the relationship between electron flow and energy in metabolism. Oxygen and NADP serve as electron acceptors for NADH and water, respectively.

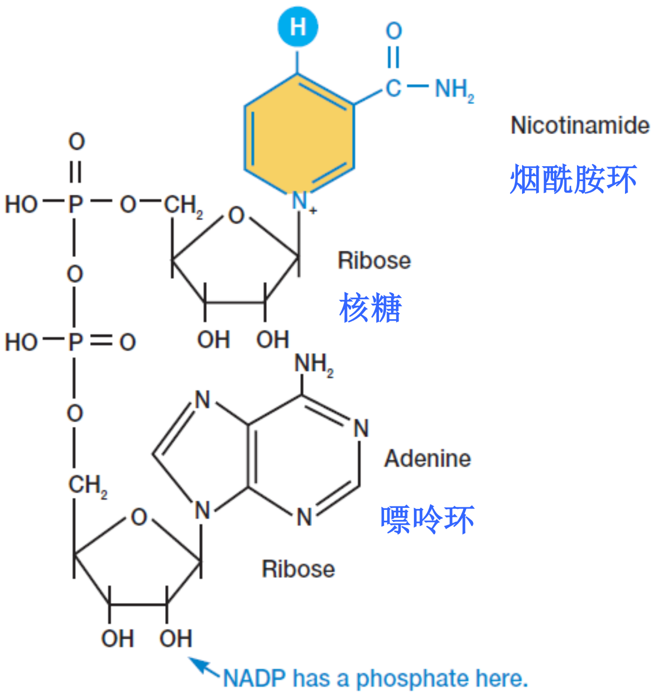
Nicotinamide adenine dinucleotide (NAD^+)烟酰胺鸟嘌呤二核苷酸

Electron carriers

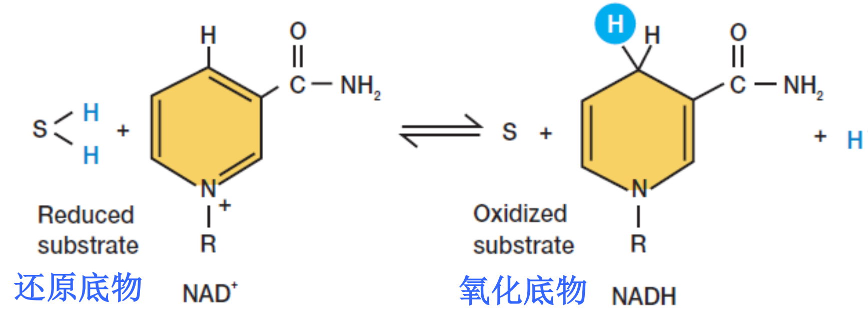
- The electrons are transferred to O_2 via a series of electron carriers that are organized into a system called an **Electron Transport Chain (ETC)**



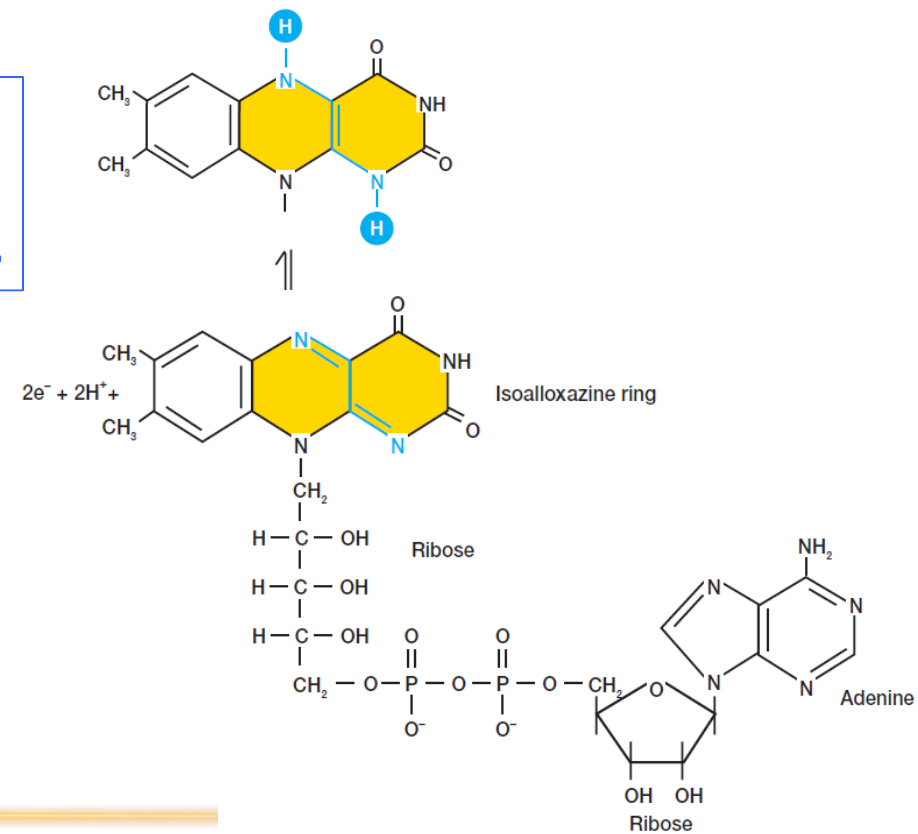
How many members in ETC?

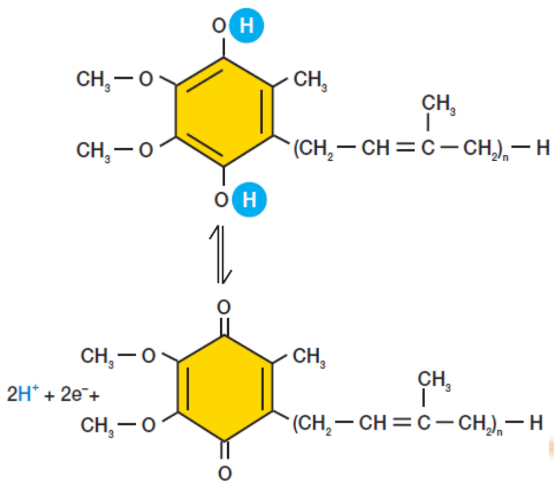


The Structure and Function of **NAD**



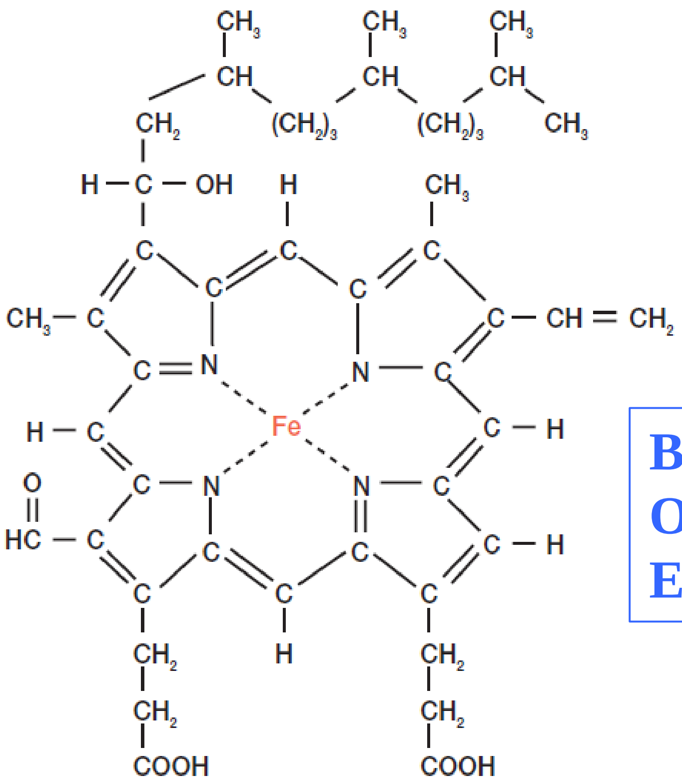
Bear Two Electrons





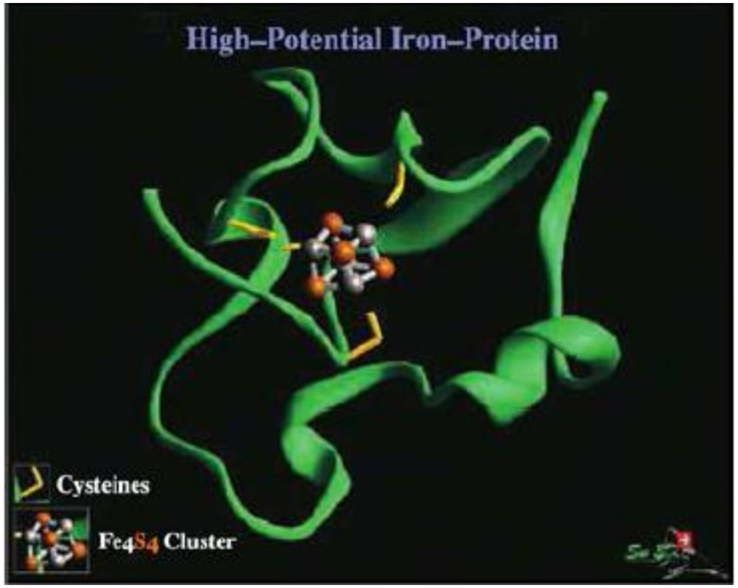
Coenzyme Q or Ubiquinone 辅酶Q 或泛醌

The Structure and Function of **FAD**
黄素腺嘌呤二核苷酸



Fe^{3+} (ferric iron) + e^- $\rightleftharpoons$ Fe^{2+} (ferrous iron)

Bear One Electrons



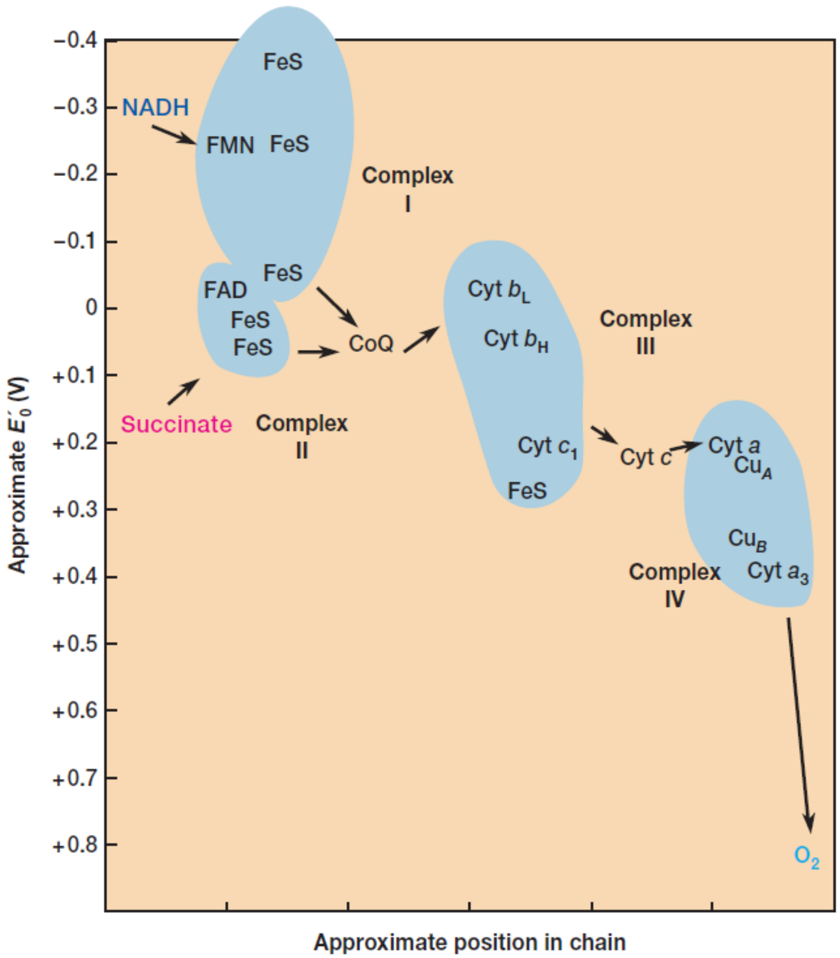
The Structure of Heme
血红素

An Iron-Sulfur Protein
非血红素铁蛋白

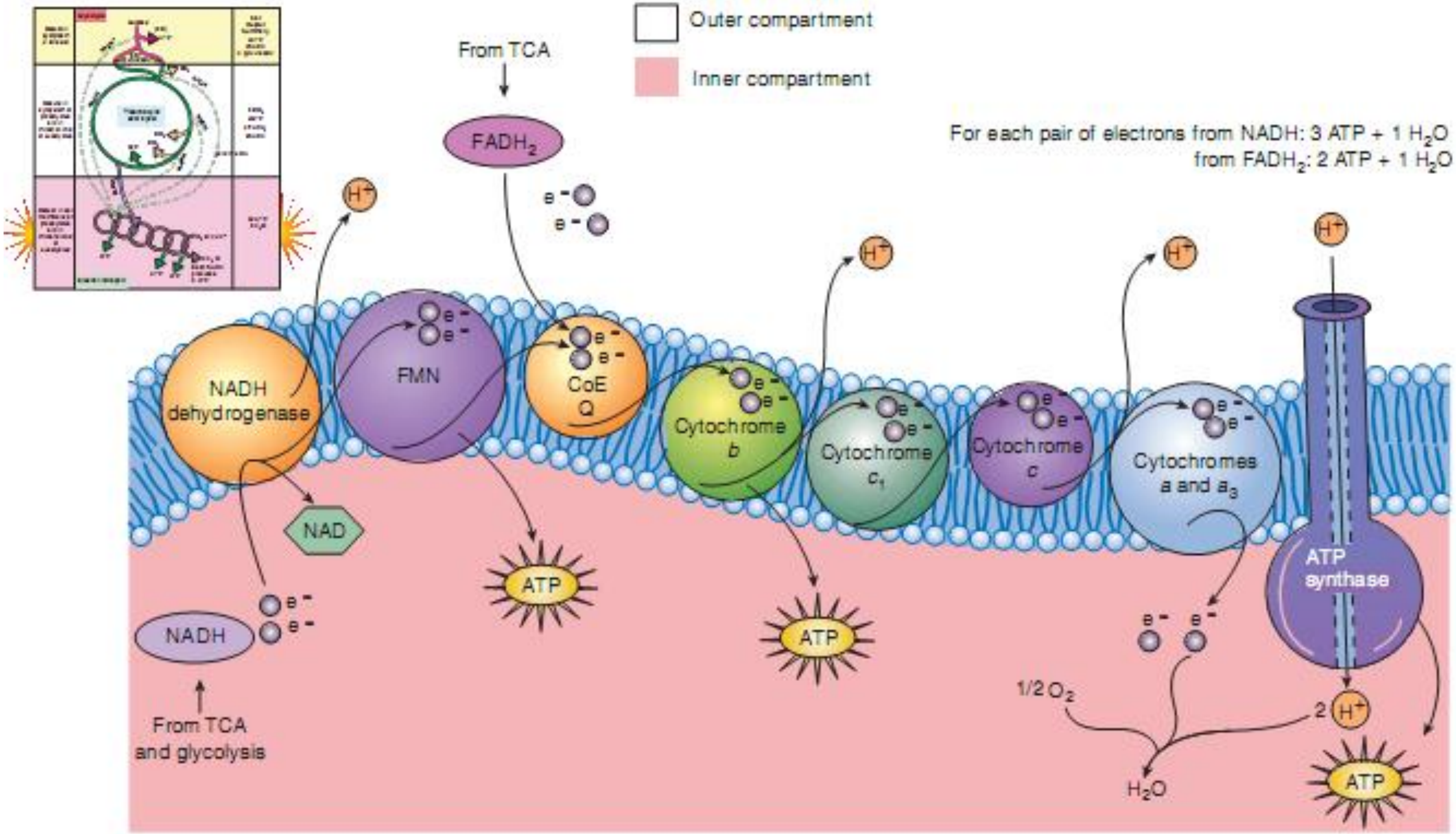
Use iron atoms to transport electrons

Arrangement of electron carriers in an ETC

- Carriers are arranged at approximately the correct **reduction potential** and sequence.
- Organized into **four complexes** linked by coenzyme Q (CoQ) and cytochrome c (Cyt c)
- Electrons flow from NADH and succinate down the reduction potential gradient to oxygen.



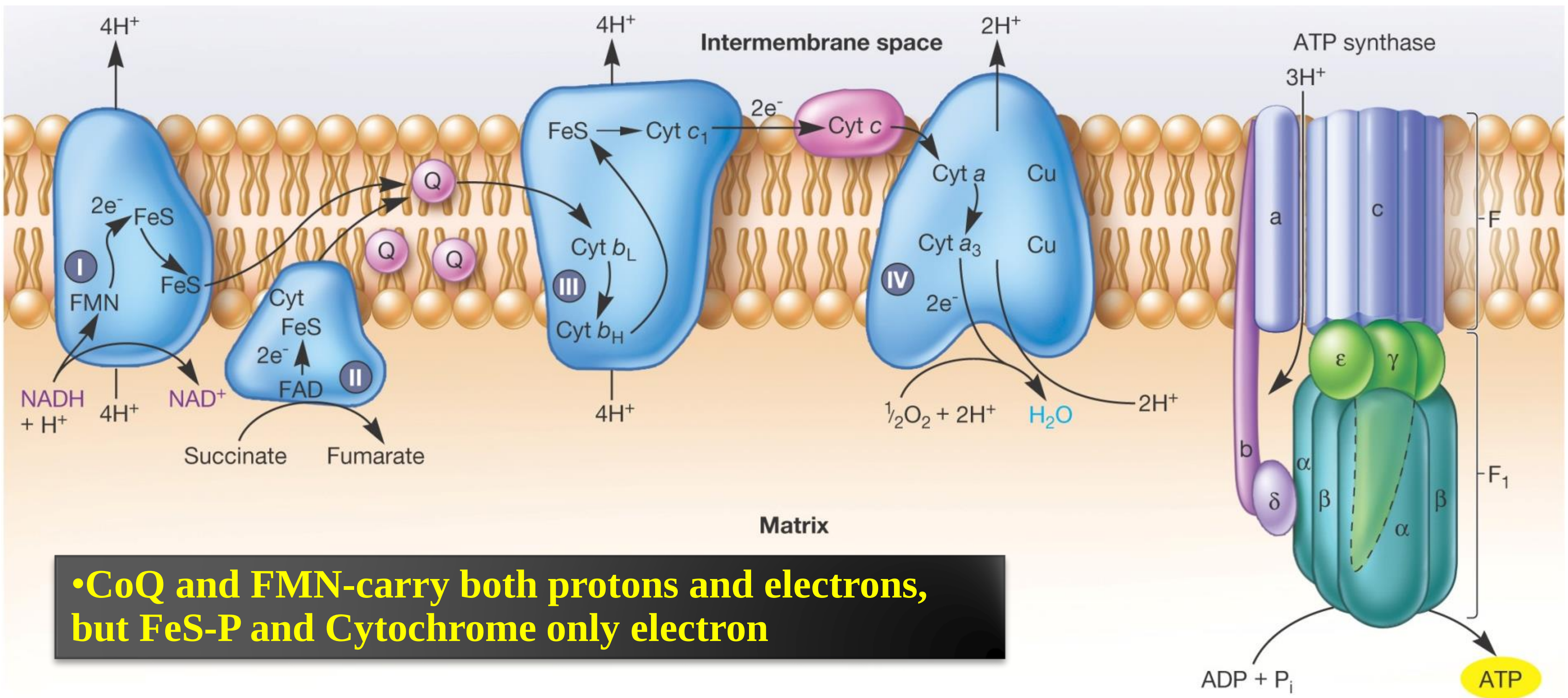
An ETC



Where is ETC ?

Eucaryotic ETCs

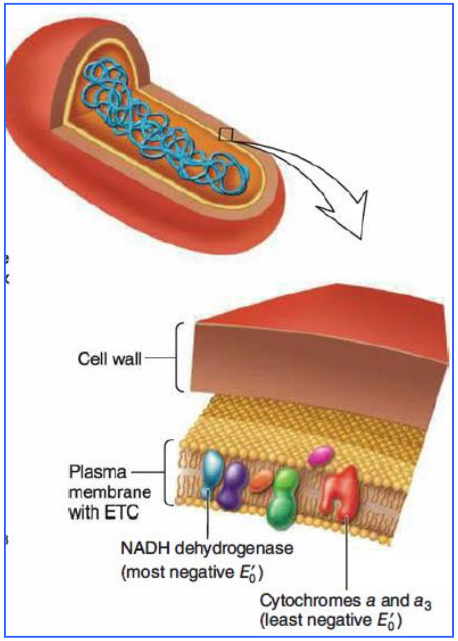
- within the inner <u>mitochondrial(线粒体） membrane</u>
- electron transfer accompanied by proton movement across inner mitochondrial membrane



•CoQ and FMN-carry both protons and electrons, but FeS-P and Cytochrome only electron

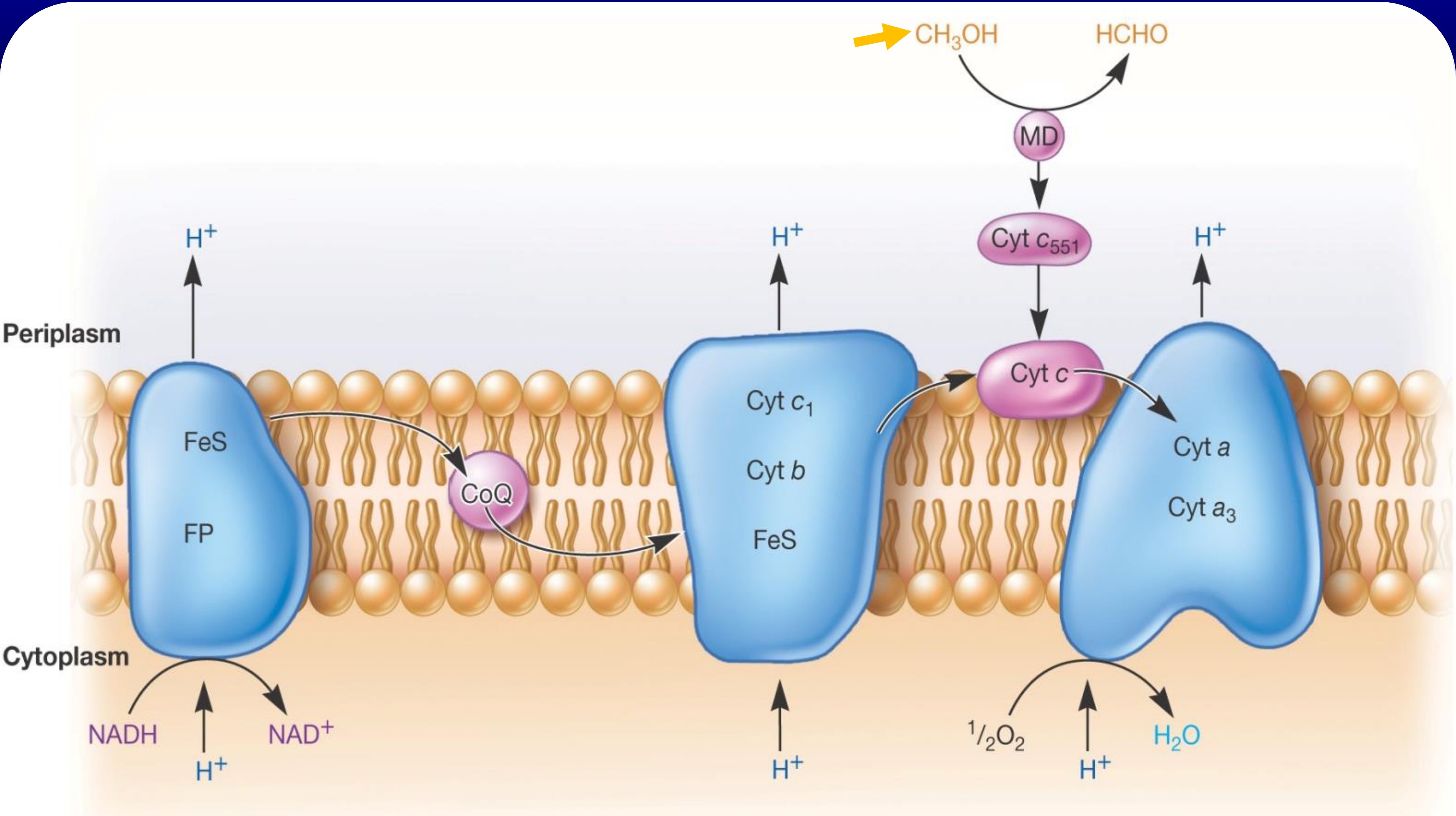
Bacterial and archaeal ETCs

- located in plasma membrane
- some resemble mitochondrial ETC, but many are different
  - different electron carriers
  - may be branched(Bo/Bd)
  - may be shorter
  - may have lower P/O ratio(1.3/0.67)



*Paracoccus denitrificans*脱氮副球菌

- facultative, soil bacterium兼性厌氧土壤细菌
- extremely versatile metabolically
- under oxic conditions, uses aerobic respiration
  - similar electron carriers and transport mechanism as mitochondria
  - protons transported to periplasmic space rather than inner mitochondrial membrane
  - can use one carbon molecules instead of glucose

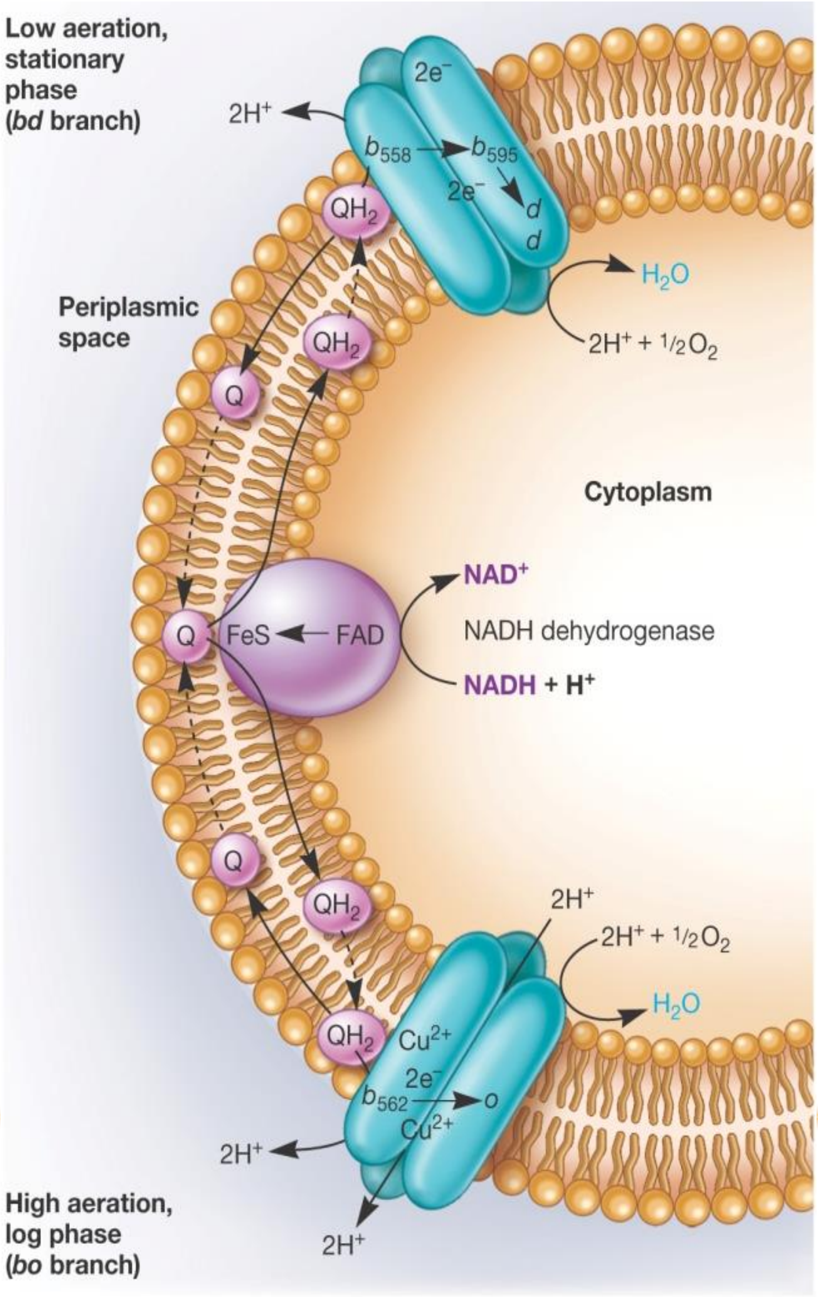


glucose

Paracoccus denitrificans (G⁻)

Electron transport chain of *E. Coli*

Different array of cytochromes used than in mitochondrial



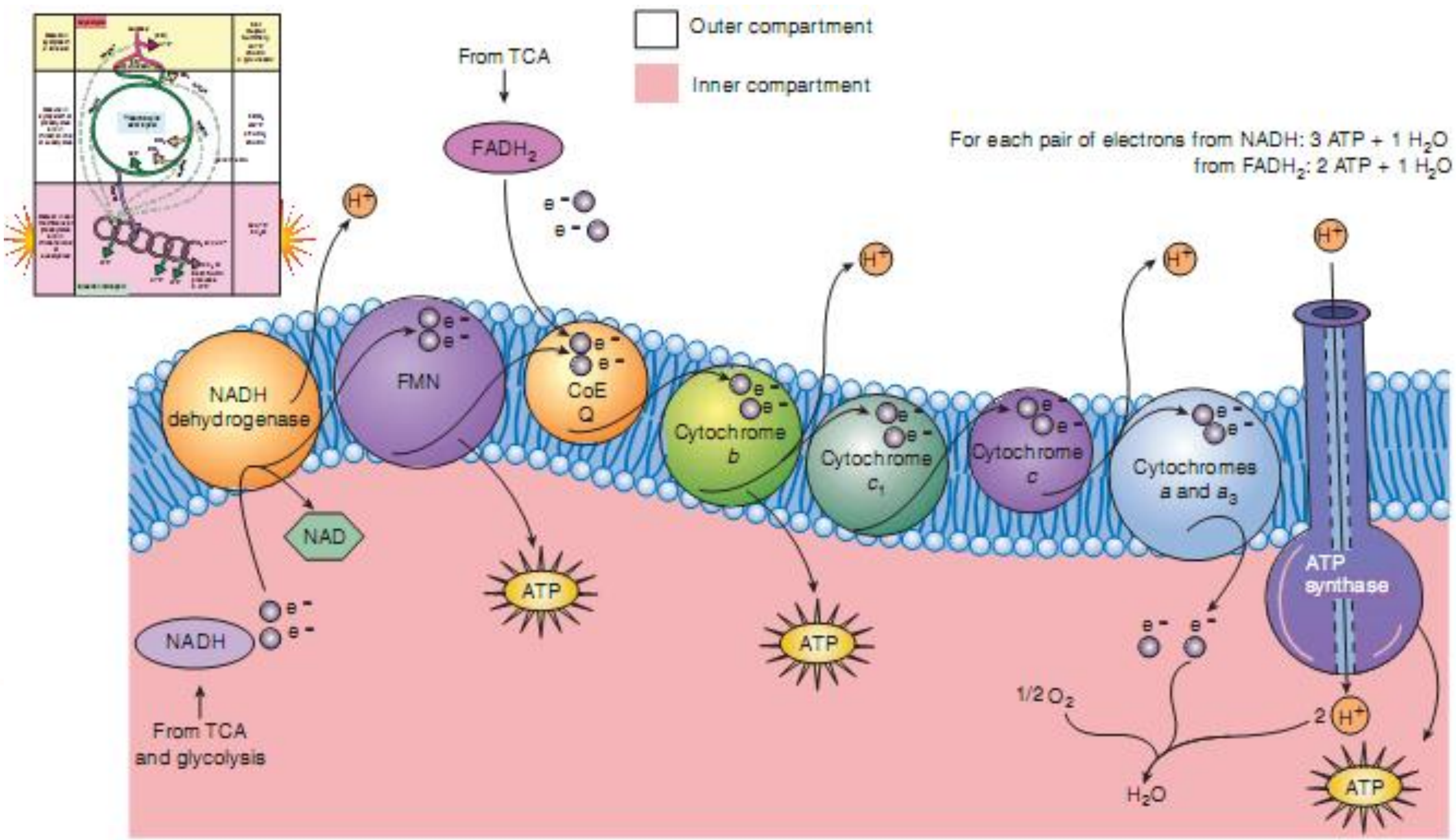
Two branches- oxygen level

upper branch – stationary phase and low aeration(Bd)

lower branch – log phase and high aeration(Bo)

Oxidative phosphorylation

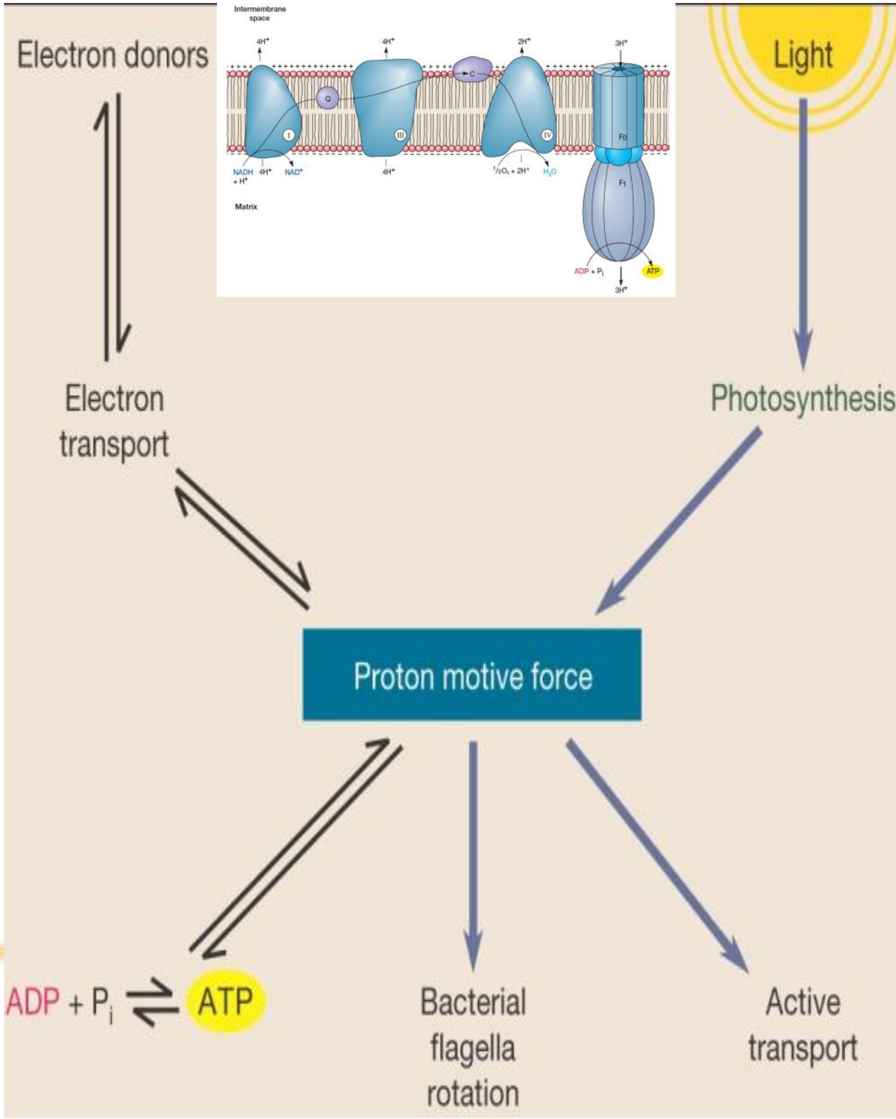
ATP is synthesized as the result of electron transport driven by the oxidation of a chemical energy source.

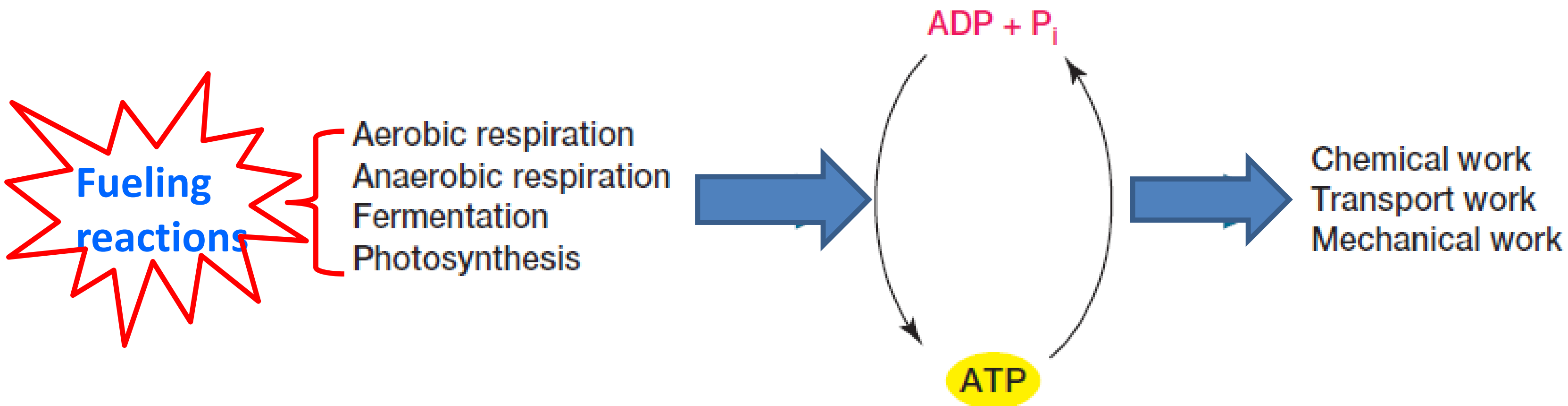


Chemiosmotic hypothesis 化学渗透假说

PMF drives ATP synthesis

- **diffusion of protons back across membrane (down gradient) drives formation of ATP**
- **ATP synthase**
  - **enzyme that uses PMF down gradient to catalyze ATP synthesis**
  - **functions like rotary engine with conformational changes**





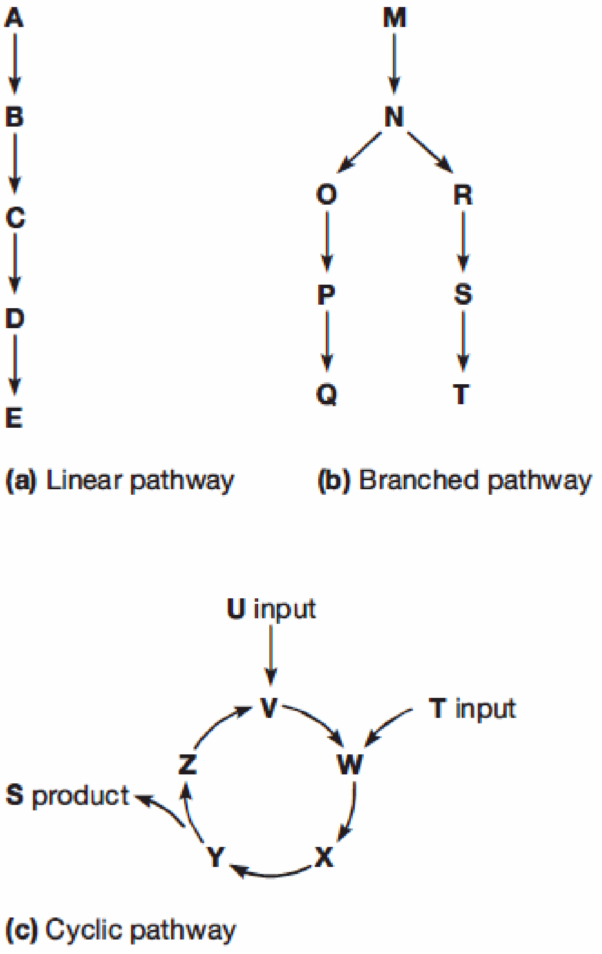
The Cell's Energy Cycle.

ATP is formed from energy made available during aerobic respiration, anaerobic respiration, fermentation, and photosynthesis. Its breakdown to ADP and phosphate (Pi) makes chemical, transport, and mechanical work possible.

How to organize the chemical reactions?

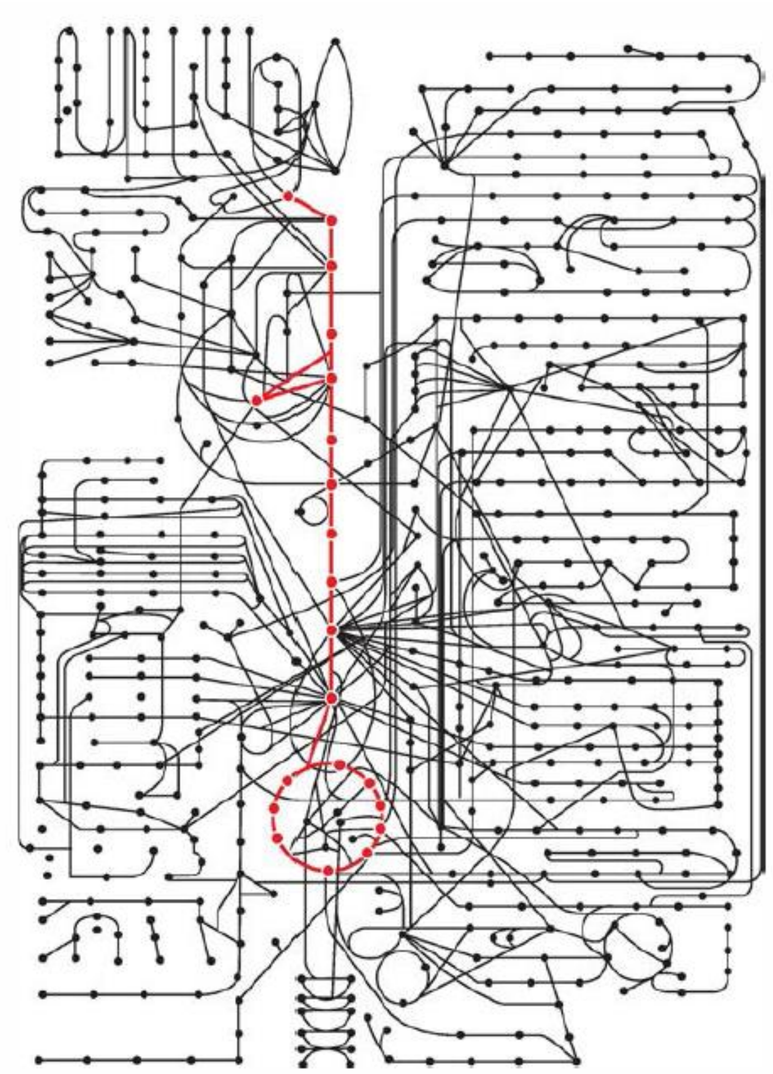
Biochemical Pathways

- Chemical reactions carried out by cells are organized into biochemical pathways.
- **Metabolic pathways** are treated as a sequence of enzymes functioning as a unit, with each enzyme using as its substrate a product of the preceding enzyme-catalyzed reaction.

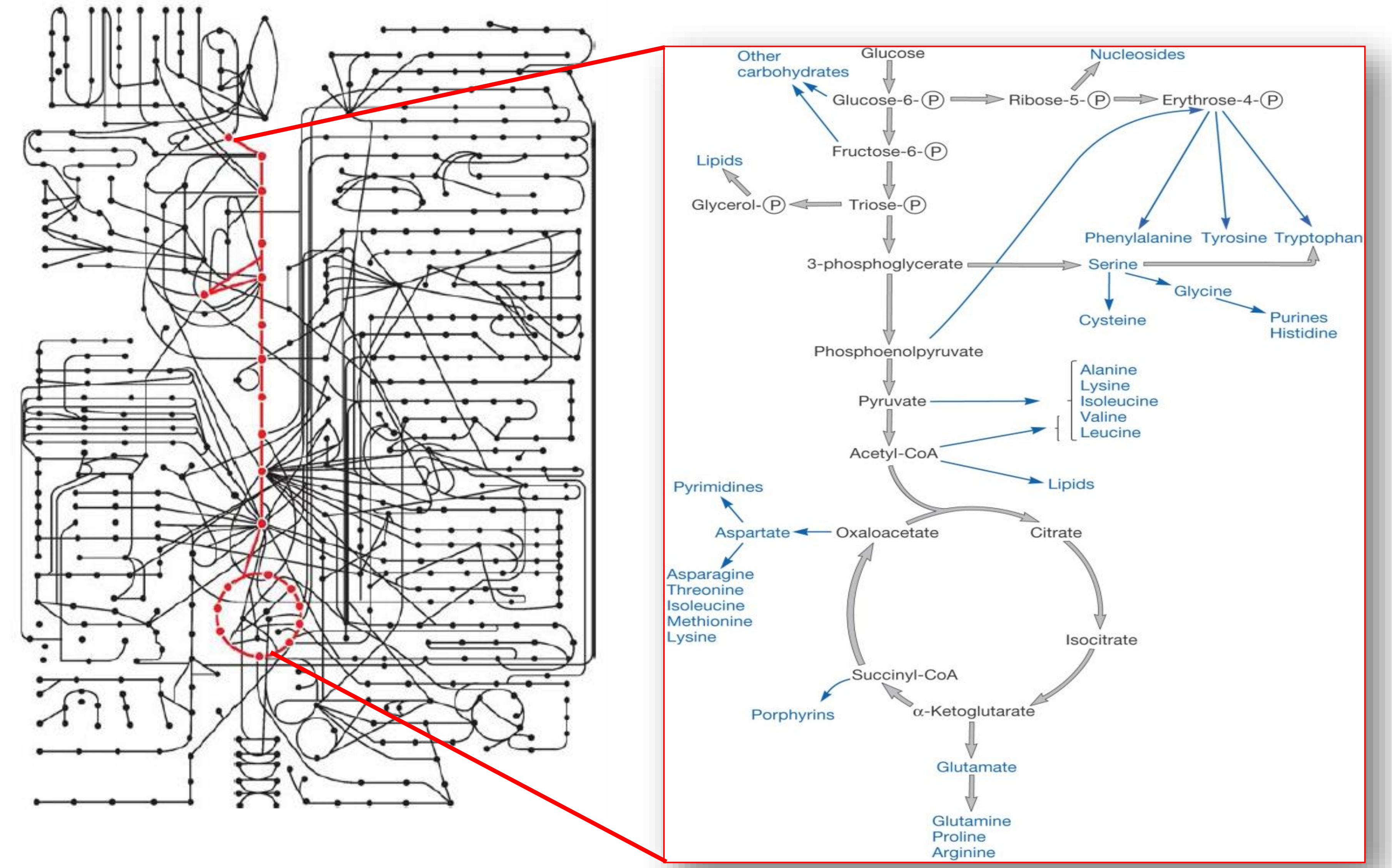


Biochemical Pathways

- Biochemical pathways are connected and form a complex **network**.
- Biochemical pathways are **dynamic**.
- **Metabolite flux** is the rate of turnover of a metabolite, used as a measure of pathway activity.



This picture of metabolic pathways is incomplete WITHOUT the **Regulation of pathway operation.**



This picture of metabolic pathways is incomplete WITHOUT the **Regulation of pathway operation.**

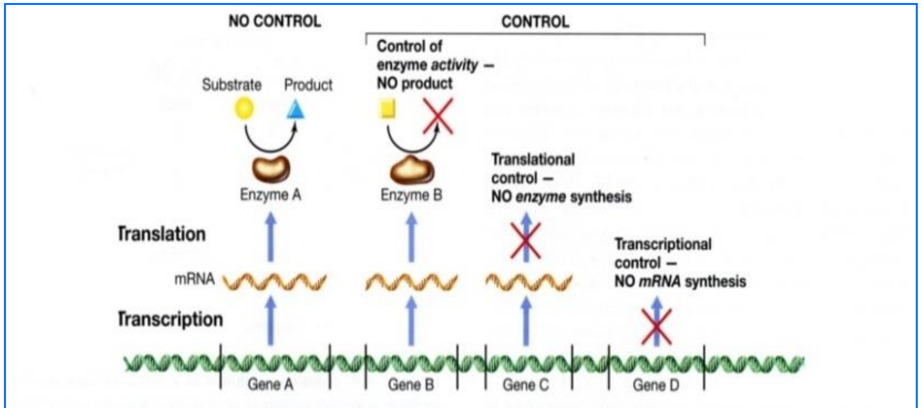
Regulation of metabolism

- **important for conservation of energy and materials**
- **maintenance of metabolic balance despite changes in environment**

Metabolic pathways can be regulated in

Three major ways:

- metabolic channeling代谢通道
- regulation of the synthesis of a particular enzyme
- direct stimulation or inhibition of the activity of a critical enzyme-
    - post-translational modification



Metabolic channeling

- differential localization of enzymes and metabolites
- Compartmentation (distribution)区室作用
  - differential distribution of enzymes and metabolites among separate cell structures or organelles(eucaryotic) makes possible the simultaneous, but separate, operation and regulation of similar pathways.
  - pathway activities can be coordinated through regulation of the transport of metabolites and coenzymes between cell compartments.

Regulation of Gene Expression

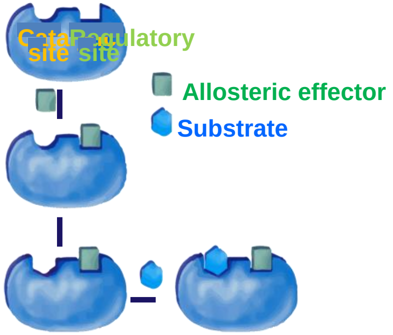
- transcriptional and translational
- control the amount of an enzyme present in the cell.
- relatively slow

Post-translational regulation of enzyme activity

- Rapidly alters pathway activity
- Some are irreversible
- Two important reversible control measures
  - allosteric regulation变构调节(non-covalent)
  - covalent modification共价修饰

Allosteric regulation

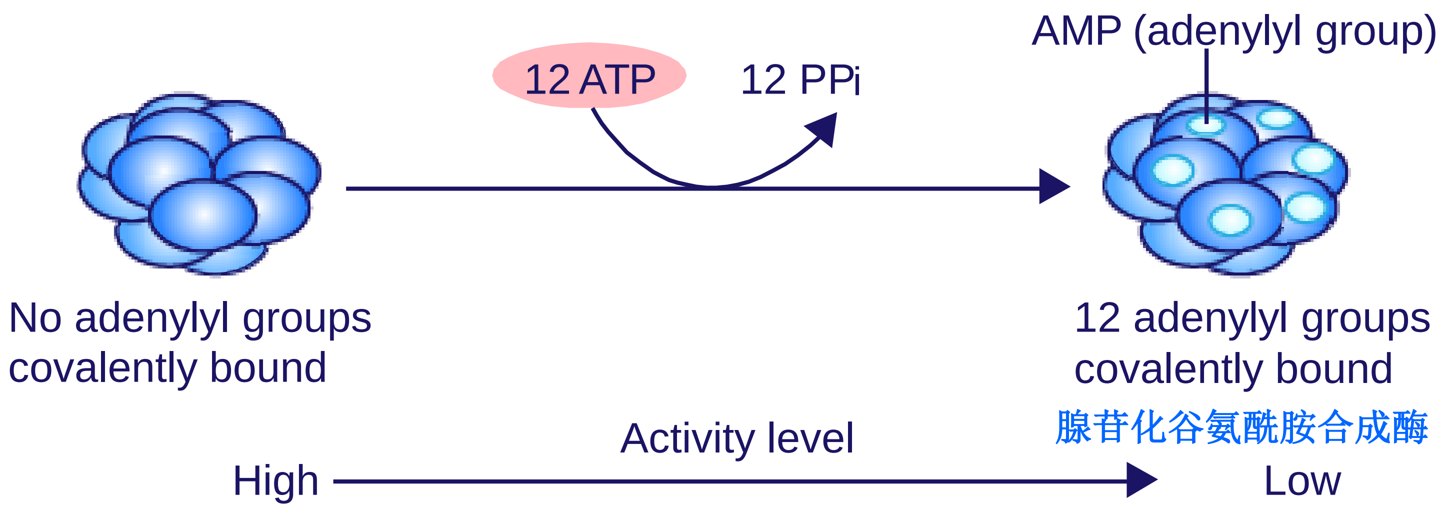
- most regulatory enzymes-allosteric enzymes别构酶
- activity altered by small molecule
- allosteric effector效应子
  - binds non-covalently at regulatory site调控部位
  - changes shape of enzyme and alters activity of catalytic site催化部位



  - positive effector increases enzyme activity
  - negative effector inhibits the enzyme

Covalent modification of enzymes

- **reversible on and off switch**
- **addition or removal of a chemical group (phosphate, methyl, adenyl, acetyl)**

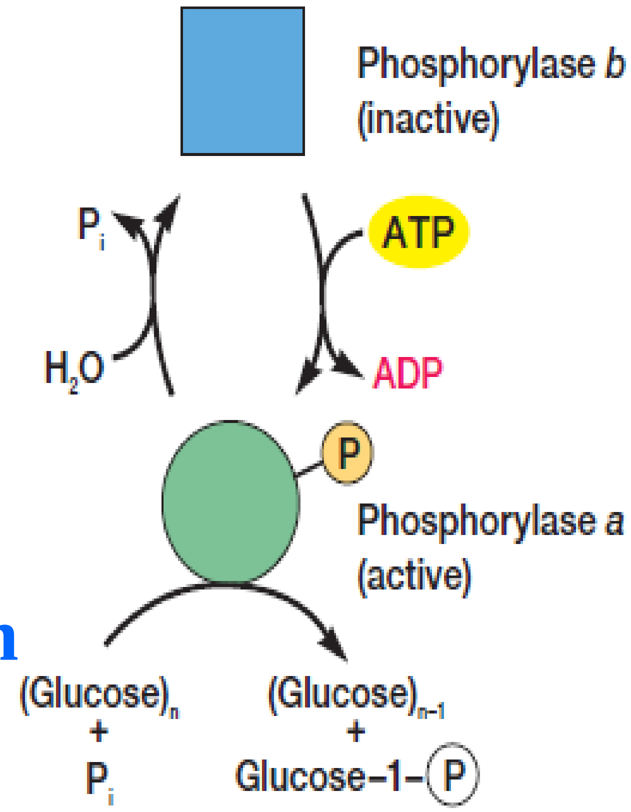


- ***E. coli*** **-nitrogen assimilation**
- **glutamine synthetase-glutamate synthase 12 subunits**
- **Glutamic acid +ammonia –glutamine- 2 glutamic acid**

Advantages

*respond to more stimuli in varied and sophisticated *ways*.

*regulation on enzymes that catalyze covalent modification in second level.



Reversible Covalent Modification of glycogen phosphorylase糖原磷酸化酶

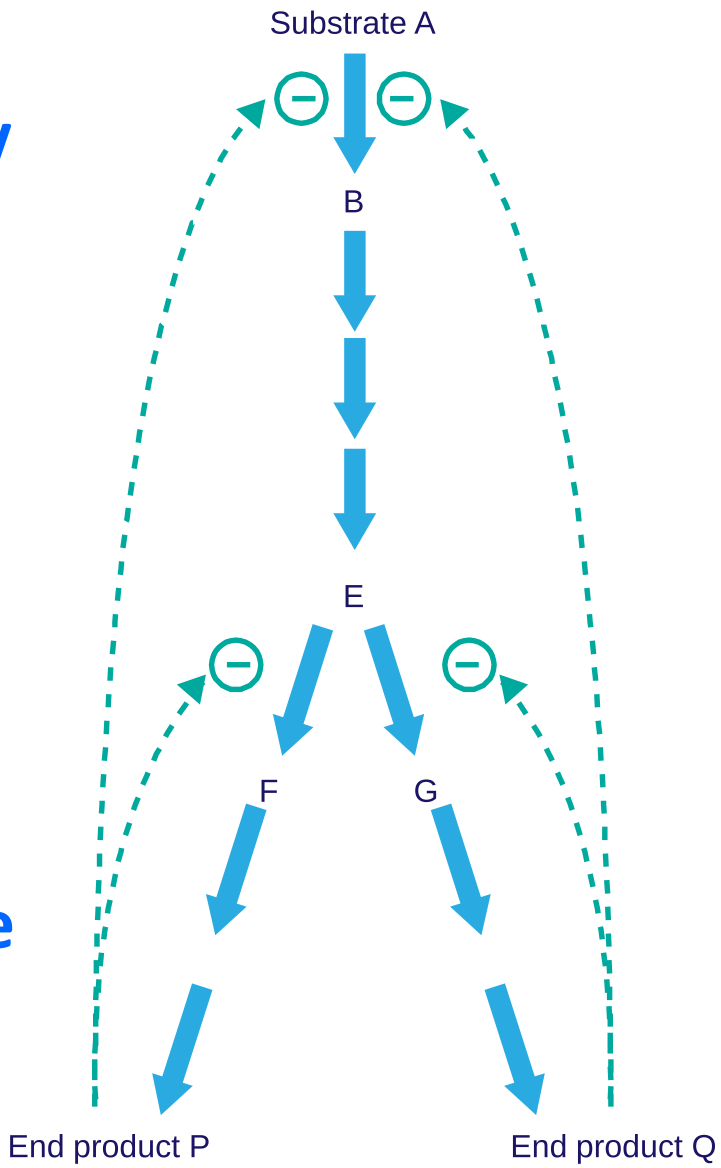
Phosphorylation and dephosphorylation are catalyzed by separate enzymes, which also are regulated.

Feedback inhibition反馈抑制作用

- also called end-product inhibition末端产物抑制作用
- inhibition of one or more critical enzymes in a pathway regulates entire pathway
  - pacemaker enzyme定步酶-regulatory E
    - catalyzes the slowest or rate-limiting reaction in the pathway

Feedback inhibition

- Usually the first step in a pathway is a pacemaker reaction catalyzed by a regulatory enzyme
- each end product regulates the initial <u>pacemaker enzyme</u>
- each end product regulates its own branch of the pathway
- Isoenzymes同工酶
  - different enzymes that catalyze same reaction



Summary

- **Energy is the capacity to do work.**
- **ATP is the major energy currency and connects energy-generating processes with energy-using processes.**
- **Redox couples with more negative reduction potentials donate electrons to those with more positive potentials, and energy is made available during the transfer.**
- **Some most important electron carriers in cells are NAD^+, $NADP^+$, FAD, FMN, coenzyme Q, cytochromes, and the nonheme iron proteins.**
- **Chemical reactions carried out by cells are organized into biochemical pathways.**

Discussion

1. Describe in general terms how energy from sunlight is spread throughout the biosphere.

2. What makes ATP suited for its role as energy currency?

3. How to predict which molecule will act as an electron donor, which molecule will act as an electron acceptor, and the relative amount of energy released by a redox reaction, using the standard reduction potentials of the reaction's conjugate redox pairs?

4. Could electron transport be driven in the opposite direction? If yes, why would it be desirable to do this?

5. How can regulatory enzymes be influenced by reversible covalent modification? What groups are used for this purpose with glycogen phosphorylase and glutamine synthetase, and which forms of these enzymes are active?