Paper: BIOMOLECULES AND BIOENERGETICS

UNIT 4: Bioenergetics

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Bioenergetics-Basic principles

- The quantitative study of **energy transductions** in living cells.
- And also study of the nature and function of the chemical processes during the reaction.
- Two fundamental laws of thermodynamics.

First law is the principle of the conservation of energy:

"For any physical or chemical change, the total amount of energy in the universe remains constant; energy may change form or it may be transported from one region to another, but it cannot be created or destroyed".

The second law of thermodynamics:

"In all natural processes, the entropy of the universe increases"

"Now, in the second law of thermodynamics . . ."

The Three thermodynamic quantities

Universe = reacting system + surrounding environment

Free energy, *G*

It is the amount of energy capable of doing work during a reaction at constant temperature and pressure.

The free energy change is denoted by ΔG

In exergonic reaction → release of free energy → ΔG *is negative.*

In endergonic reaction → gain of free energy → ΔG *is positive*

The Three thermodynamic quantities

Enthalpy (*H*):

The heat content of the reacting system.

It reflects the number and kinds of chemical bonds (covalent and non-covalent) in the reactants and products.

- Releases heat – exothermic. The change in enthalpy, ΔH - a negative value.
- Take-up the energy – endothermic. The change in enthalpy, ΔH - a positive value

The Three thermodynamic quantities

Entropy (*S*)

- Quantitative expression for the randomness or disorder in a system.
- When the products of a reacting system are less complex and more disordered than the reactants, the reaction is said to proceed with a gain in entropy.
- The units of ΔG and ΔH are joules/mole or calories/mole
- The unit of ΔS *is* joules/mole * Kelvin (J/mol * K)
- 1 cal = 4.184 J

Gas constant, $R = 8.315\ \text{J/mol} \bullet \text{K}$

$(= 1.987\ \text{cal/mol} \bullet \text{K})$

Units of absolute temperature, *T*, are Kelvin, K

$25\ ^\circ\text{C} = 298\ \text{K}$

At 25 °C, $RT = 2.478\ \text{kJ/mol}$

$(= 0.592\ \text{kcal/mol})$

Relation between ΔG, ΔH and ΔS

The changes in free energy, enthalpy, and entropy are related to each other quantitatively by the equation:

$$\Delta G = \Delta H - T\,\Delta S$$

Equilibrium constant

The concentrations of reactants and products at equilibrium define the equilibrium constant, Keq

$aA + bB \rightleftharpoons cC + dD$

where a, b, c, and d are the number of molecules of A, B, C, and D participating, the equilibrium constant is given by

where [A] eq, [B] eq, [C] eq, and [D] eq are the molar concentrations of the reaction components at the point of equilibrium.

$$K_{eq} = \frac{[C]_{eq}^{c}[D]_{eq}^{d}}{[A]_{eq}^{a}[B]_{eq}^{b}}$$

Free energy change in a reaction is expressed as…

$$\Delta G_{\mathrm{p}} = \Delta G'^{\circ} + RT \ln \frac{[\text{products}]}{[\text{Reactants}]}$$

Equilibrium constant & std concept of free energy

Transformed constants as **standard free-energy changes** and **standard equilibrium constants**.

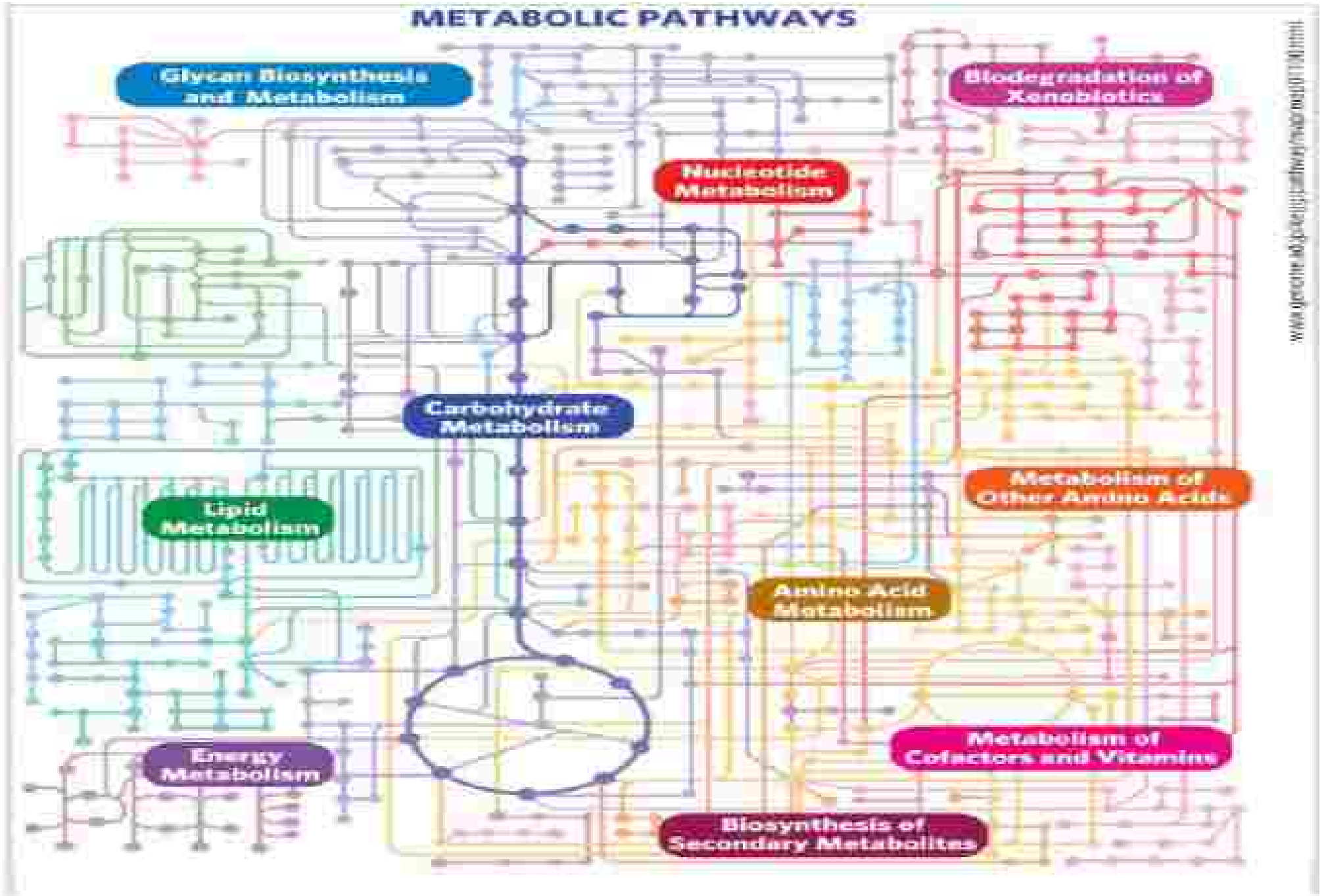
$$\Delta G'^{\circ} = -RT \ln K'_{eq}$$

TABLE 13-3 Relationships among K'_{eq}, $\Delta G'^{\circ}$, and the Direction of Chemical Reactions

When K'_{eq} is ...	$\Delta G'^{\circ}$ is ...	Starting with all components at 1 M, the reaction ...
>1.0	negative	proceeds forward
1.0	zero	is at equilibrium
<1.0	positive	proceeds in reverse

Equilibrium constant & std concept of free energy

- When ΔG is large and negative, the reaction tends to go in the forward direction;
- when ΔG is large and positive, the reaction tends to go in the reverse direction; and when $\Delta G = 0$, the system is at equilibrium.
- The free-energy change for a reaction is independent of the pathway by which the reaction occurs.


METABOLIC PATHWAYS
Glycan Biosynthesis and Metabolism
Biodegradation of Xenobiotics
Nucleotide Metabolism
Carbohydrate Metabolism
Metabolism of Other Amino Acids
Lipid Metabolism
Amino Acid Metabolism
Metabolism of Cofactors and Vitamins
Energy Metabolism
Biosynthesis of Secondary Metabolites

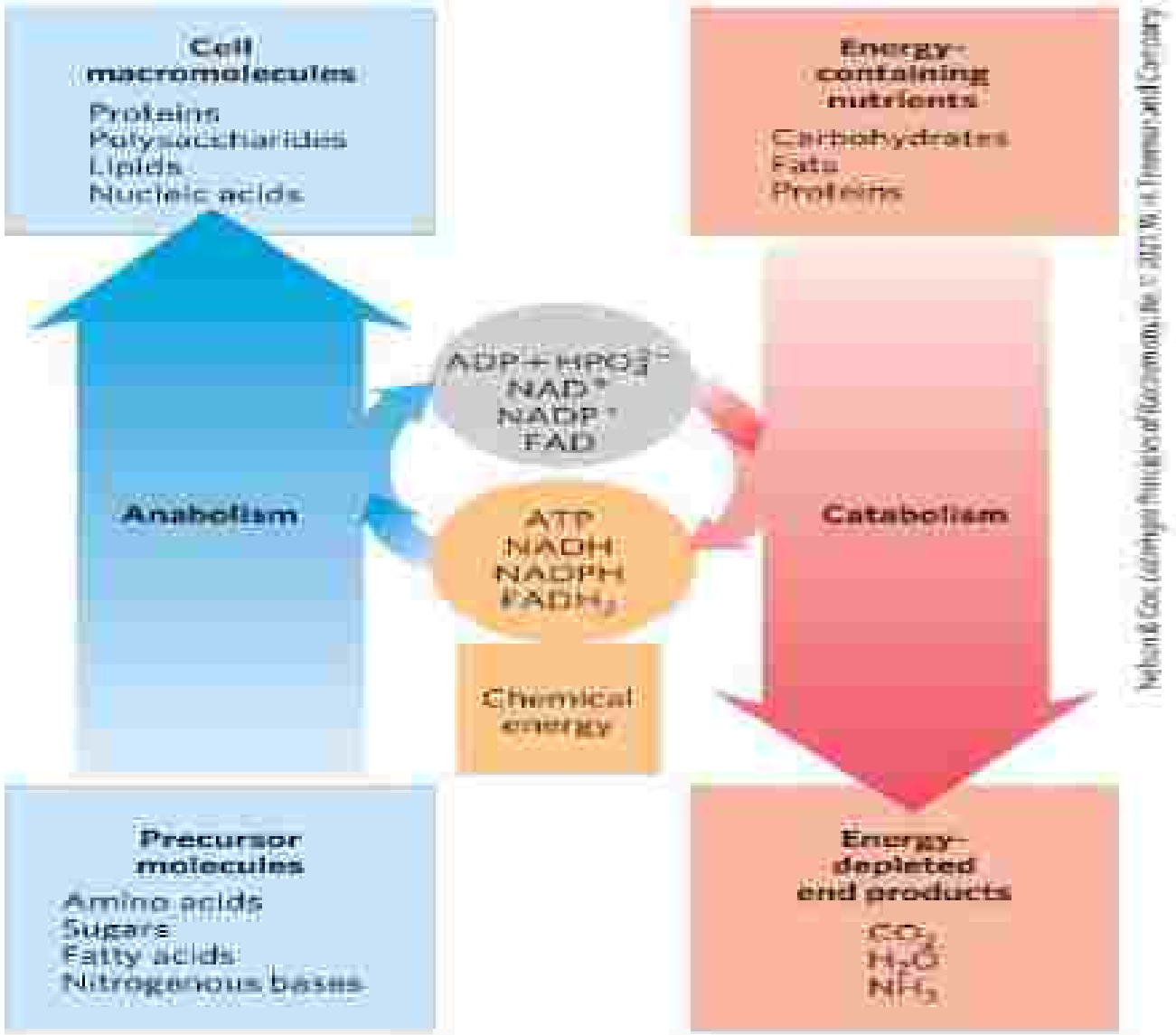
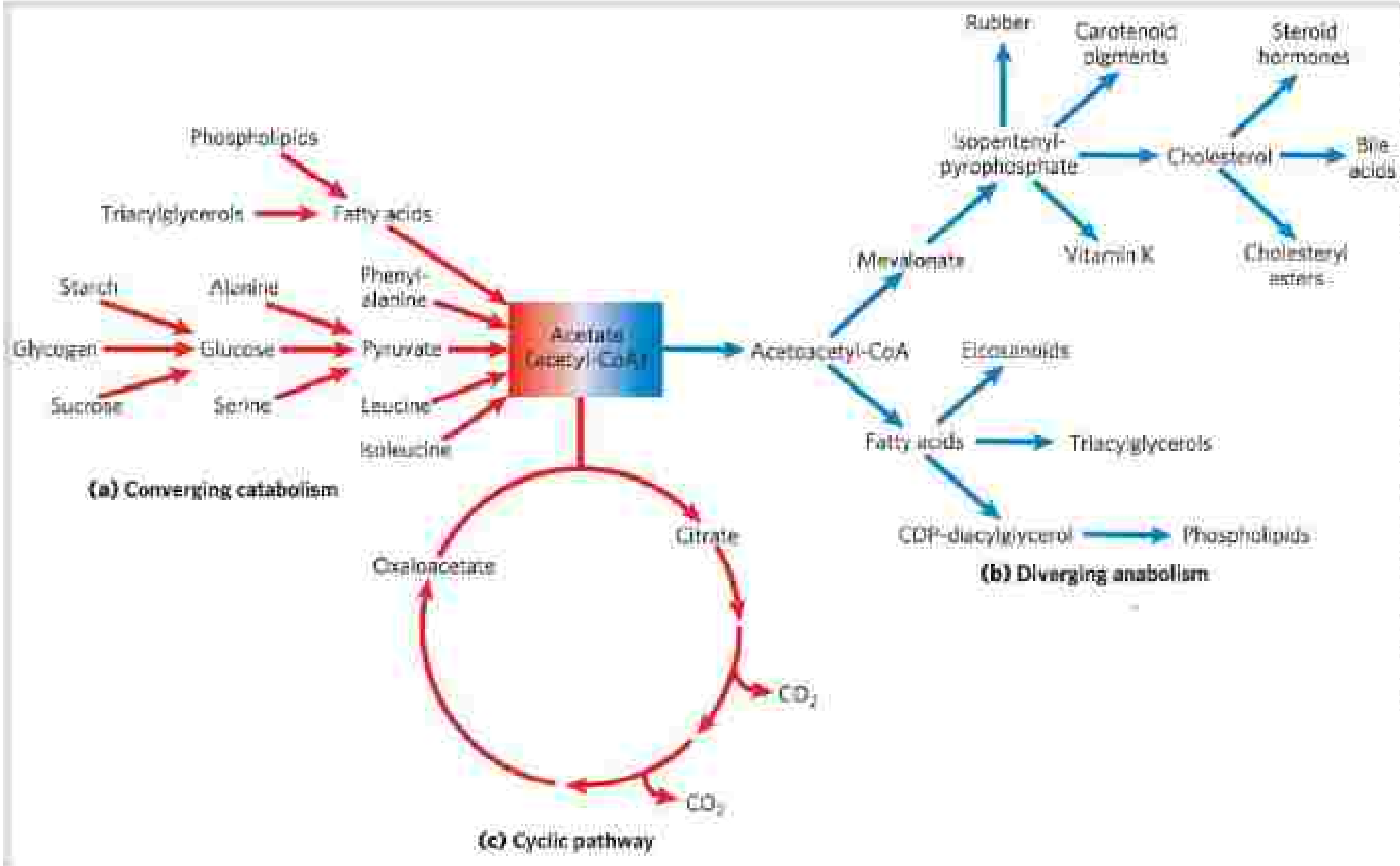


FIGURE 2 The big picture: energy relationships between catabolic and anabolic pathways. Catabolic pathways deliver chemical energy in the form of ATP, NADH, NADPH, and $FADH_2$. These energy carriers are used in anabolic pathways to convert small precursor molecules into cellular macromolecules.



Bioenergetics

- Glucose occupies a central position in the metabolism of plants, animals, and many microorganisms.
- rich in potential energy, oxidation of glucose results in standard free-energy change of −2,840 kJ/mol.
- Remarkably versatile precursor, capable of supplying a huge array of metabolic intermediates for biosynthetic reactions

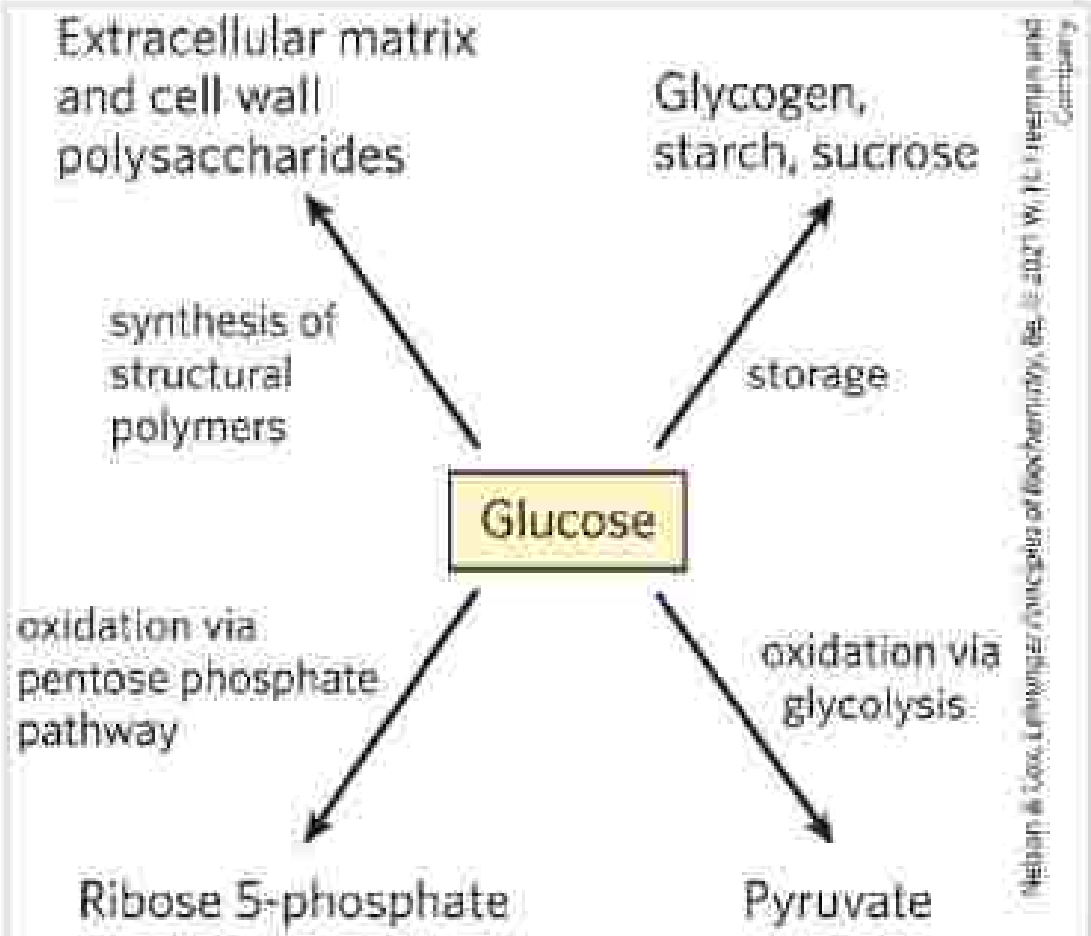


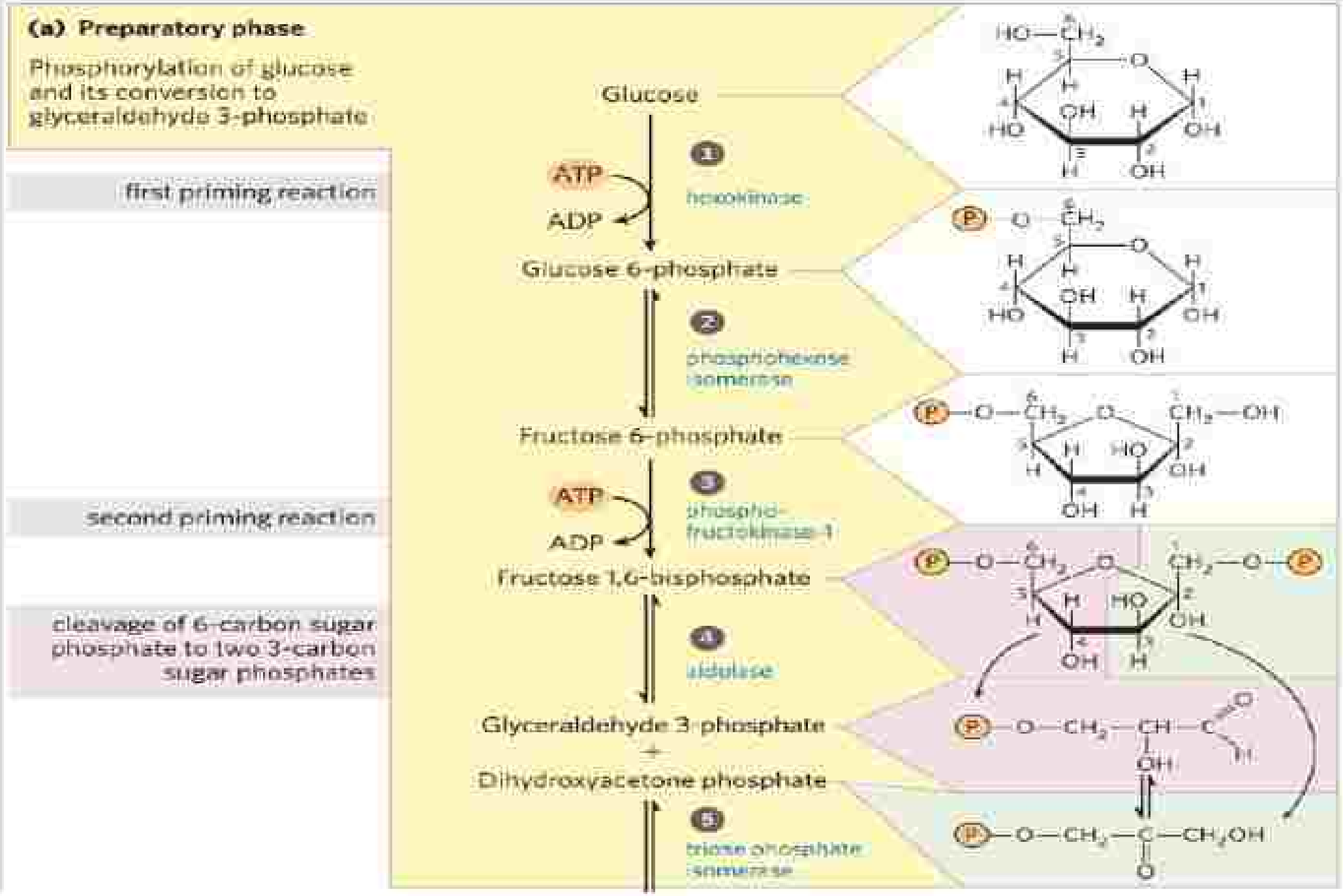
FIGURE 14-1 Major pathways of glucose utilization. Although not the only possible fates for glucose, these four pathways are the most significant in most cells.

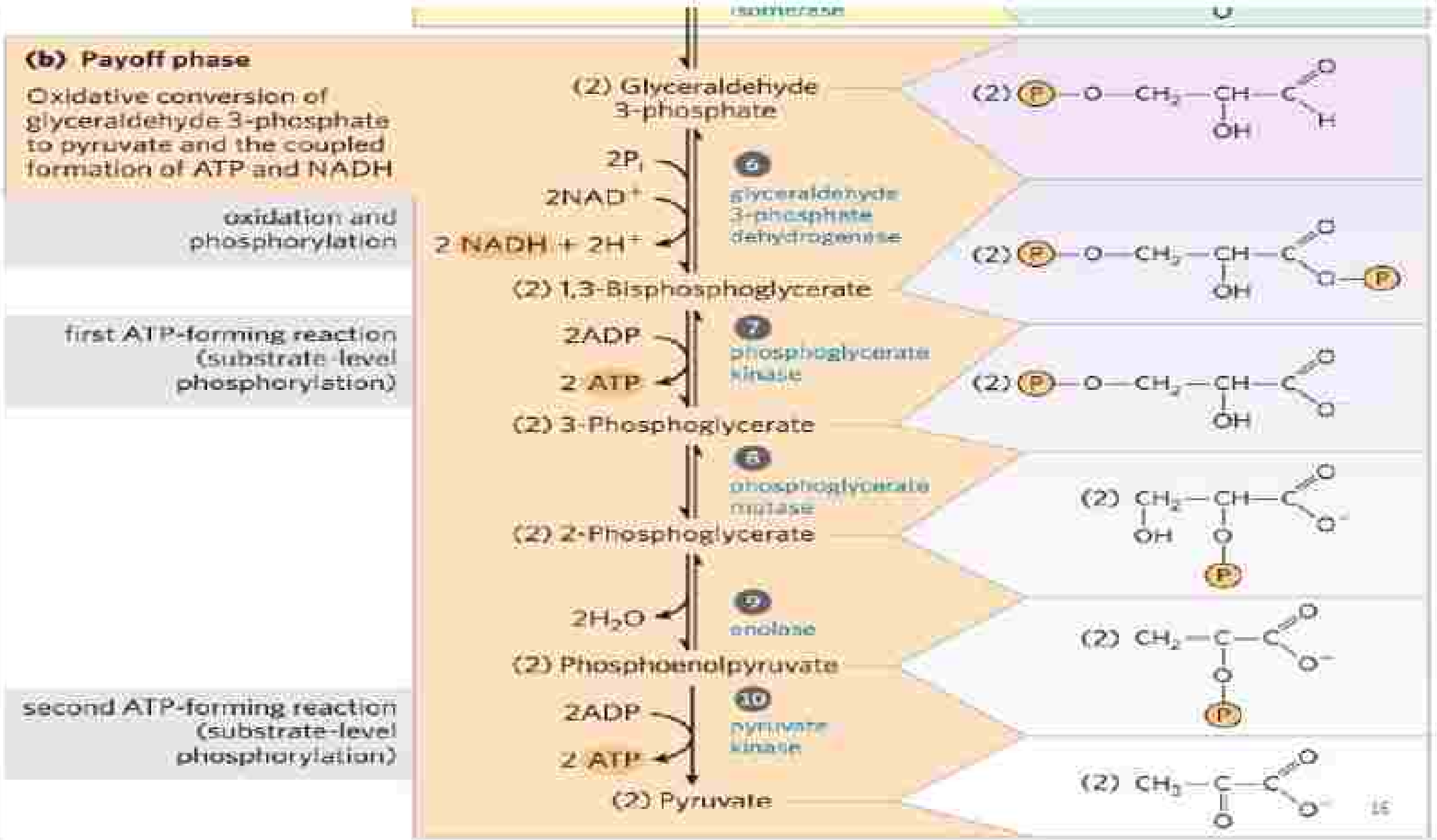
Bioenergetics > Glycolysis Pathway

- **Metabolites like glucose are ofen activated with a high energy group before their catabolism.**
- Glycolysis – 10steps
- In **glycolysis:** 2 molecules of the three-carbon (3C) compound pyruvate
- first metabolic pathway to be elucidated by Eduard Buchner's in 1897
- The glycolytic breakdown of glucose is the sole source of metabolic energy in some mammalian tissues and cell types (erythrocytes, renal medulla, brain, and sperm).
- Glycolysis – 2 phases:

I) *preparatory phase (1 to 5th step) investment of energy;*

II) payoff phase of glycolysis (6th – 10th step) gain of energy.


(a) Preparatory phase
Phosphorylation of glucose and its conversion to glyceraldehyde 3-phosphate
first priming reaction
second priming reaction
cleavage of 6-carbon sugar phosphate to two 3-carbon sugar phosphates
Glucose
ATP
ADP
1
hexokinase
Glucose 6-phosphate
2
phosphohexose isomerase
Fructose 6-phosphate
ATP
ADP
3
phospho-fructokinase-1
Fructose 1,6-bisphosphate
4
aldolase
Glyceraldehyde 3-phosphate
+
Dihydroxyacetone phosphate
5
triose phosphate isomerase
Nelson & Cox, Lehninger Principles of Biochemistry, 8e, © 2021 W. H. Freeman and Company


(b) Payoff phase
Oxidative conversion of glyceraldehyde 3-phosphate to pyruvate and the coupled formation of ATP and NADH
oxidation and phosphorylation
first ATP-forming reaction (substrate-level phosphorylation)
second ATP-forming reaction (substrate-level phosphorylation)
(2) Glyceraldehyde 3-phosphate
2Pi
2NAD+
2 NADH + 2H+
6
glyceraldehyde 3-phosphate dehydrogenase
(2) 1,3-Bisphosphoglycerate
2ADP
2 ATP
7
phosphoglycerate kinase
(2) 3-Phosphoglycerate
8
phosphoglycerate mutase
(2) 2-Phosphoglycerate
2H2O
9
enolase
(2) Phosphoenolpyruvate
2ADP
2 ATP
10
pyruvate kinase
(2) Pyruvate

Steps	Enzyme	Cofactor	End product	ΔG	EC & other info	
1	Hexokinase (I-IV) (IR)	Mg^{2+} ATP -> ADP + Pi	G-6-phosphate	-16.5 kJ/mol	Transferase	Preparatory phase / investment of energy i.e. 2ATP is hydrolysed
2	Phosphofructo isomerase (R)	Mg^{2+}	F-6-P	1.76kJ/mol	Isomerase	
3	PFK-1 (IR)	Mg^{2+} ATP -> ADP + Pi	F-1,6-bisphosphate	-14.2kJ/mol	Transferase Committed step	
4	Aldose (R) F-1,6-bisphosphate aldose (class 1 & 2: A/P & F/B; Zn^{2+})	-	Glyceraldheyde 3 P (an aldose) Dihydroxyacetone P (a ketose)	23.8kJ/mol		
5	Triose phosphate isomerase (R)	-	Glyceraldheyde 3 P (2molecules)	7.5kJ/mol	Isomerase	
6	Glyceraldheyde 3 P dehydrogenase (R)	- NAD^{+} -> NADH +H^{+}	1,3 bisphoglycerate		Oxidoreductases	Payoff steps
7	Phosphoglycerate kinase (R)	Mg^{2+}	2ATP and 3-phosphoglycerate	-12.2kJ/mol	Transferase Substrate level phosphorylation	
8	phosphoglycerate mutase (R)	Mg^{2+}	2-phosphoglycerate	4.4kJ/mol	Isomerase	
9	Enolase (R)	Mg^{2+} : stabilizer	phosphoenolpyruvate (PEP)	7.5kJ/mol	Lyases	
10	pyruvate kinase (IR)	Mg^{2+} & K^{+} ADP - > 2ATP	Pyruvate	-31.4kJ/mol	Transferase	

Balance sheet.....?

$$\text{Glucose} + 2\text{ATP} + 2\text{NAD}^+ + 4\text{ADP} + 2\text{P}_i \rightarrow$$
$$2\,\text{pyruvate} + 2\text{ADP} + 2\text{NADH} + 2\text{H}^+ + 4\text{ATP} + 2\text{H}_2\text{O}$$

Canceling out common terms on both sides of the equation gives the overall equation for glycolysis:

$$\text{Glucose} + 2\text{NAD}^+ + 2\text{ADP} + 2\text{P}_i \rightarrow$$
$$2\,\text{pyruvate} + 2\text{NADH} + 2\text{H}^+ + 2\text{ATP} + 2\text{H}_2\text{O}$$

Regulation of Glycolysis

- There are three major enzymatic control points within the glycolytic pathway.
- These include hexokinase, phosphofructokinase, and pyruvate kinase reactions.
- The three steps catalyzed by these enzymes are Irrevesible.
- Key drivers for regulating the pathway are energy demand within the cell as determined by local indicators such as ATP and AMP, as well as energy demand within the organism as a whole, which can be influenced by hormone signaling pathways

REGULATION OF GLYCOLYSIS

- Glycolysis is controlled or regulated by Allosteric inhibition mechanism of 3 enzymes as mentioned earlier.
- Hexokinase: It is allosterically inhibited by the product Glucose-6-Phosphate (G6P) in the process of ***negative feedback inhibition.***

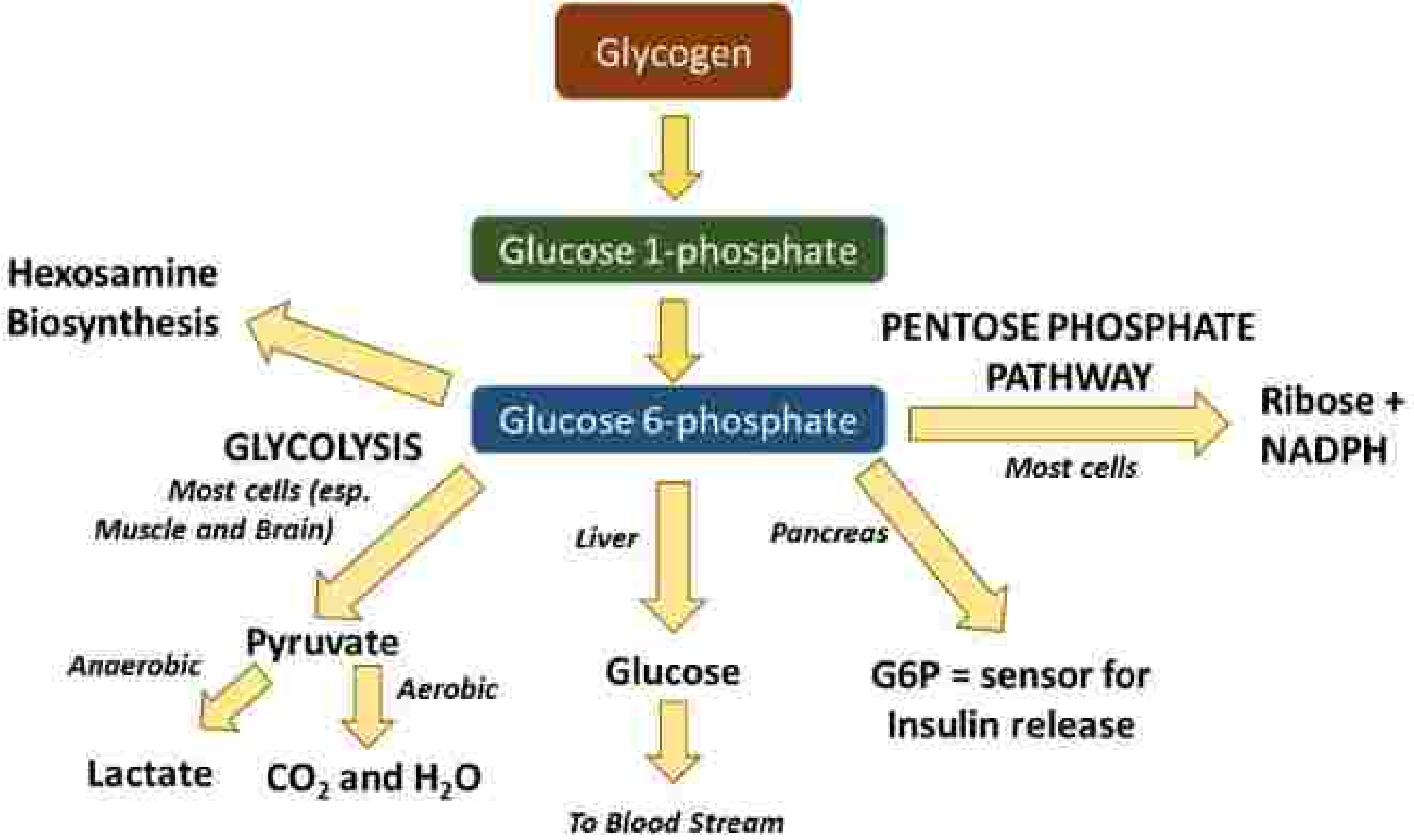


Figure 15.4.2: Cellular Fates of Glucose 6-Phosphate

Regulation of Glycolysis: PFK 1

- *PFK1 is one of the most important control points in the glycolytic pathway.*
- *PFK1 is an allosteric enzyme and has a structure similar to that of hemoglobin. PFK 1 undergoes complex* allosteric regulation.
- One half of each dimer contains the ATP binding site, whereas the other half the substrate (fructose-6-phosphate or (F6P)) binding site, as well as a separate allosteric binding site, that can bind with ADP or AMP.
- PFK 1 activity is increased whenever the cell's ATP supply is depleted.
- PFK 1 activity is decreased whenever the cell's ADP + Pi is accumulated.
- ATP and citrate (4rm CCA) acts as an inhibitor

Regulation of Glycolysis: Pyruvate Kinase

- key regulatory component within the pathway.
- There are three major isozymes of pyruvate kinase:
- the L form that is predominantly found in the liver,
- the R form that is predominantly found in erythrocytes, and
- the M1 form in muscle and brain, and the M2 form that is expressed in fetal tissue and at some level in most adult tissues.

General regulatory mechanisms common to most of the isozymes of pyruvate kinase

- FBP is an earlier product within the same metabolic cascade.
- the activation of pyruvate kinase enzymes by FBP is known as ***feed forward stimulation***.
- All of the pyruvate kinase isozymes are inhibited by the product of the reaction, ATP (or high energy load), and high levels of alanine.
- If high levels of alanine are present, this indicates that there is a high energy load within the cell (ie that the cell is full of building blocks to make new macromolecules and is not in the need of more energy).
- Thus, high levels of alanine serve as a negative regulator of the pyruvate kinase family of enzymes.
- Protein kinase A phosphorylates Pyruvate Kinase inhibiting its activity and preventing the conversion of phosphoenolpyruvate to pyruvate

REGULATION OF GLYCOLYSIS -SUMMARY

Enzyme	Activator	Inhibitor
Hexokinase	------	Glucose-6-phosphate ((increased concentration)
PFK-1	Fructose 2,6 bisphosphate Fructose 6 bisphosphate, AMP (increased concentration)	Citrate, ATP (increased concentration)
Pyruvate kinase	Fructose 1,6 bisphosphate, AMP	Acetyl – CoA, ATP (increased concentration)

Regulation of Glycolysis: Pyruvate Kinase

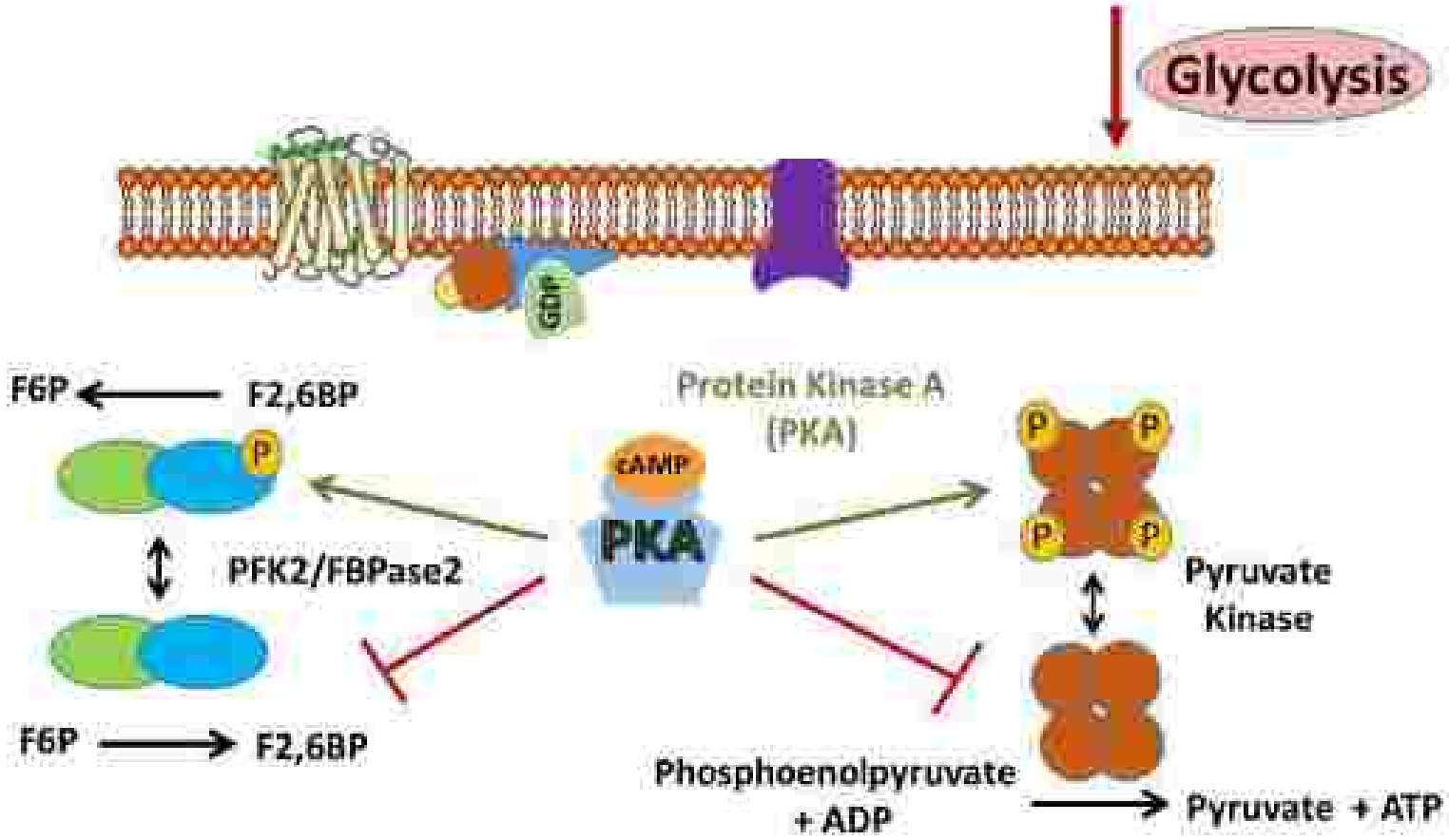


Figure 15.4.14: Glucagon Signaling in Liver Cells Down Regulates PFK2 Activity and Pyruvate Kinase Activity. Image modified from Yan, A., et al (2016)

Regulation of Glycolysis (in other steps)

- Step 6:

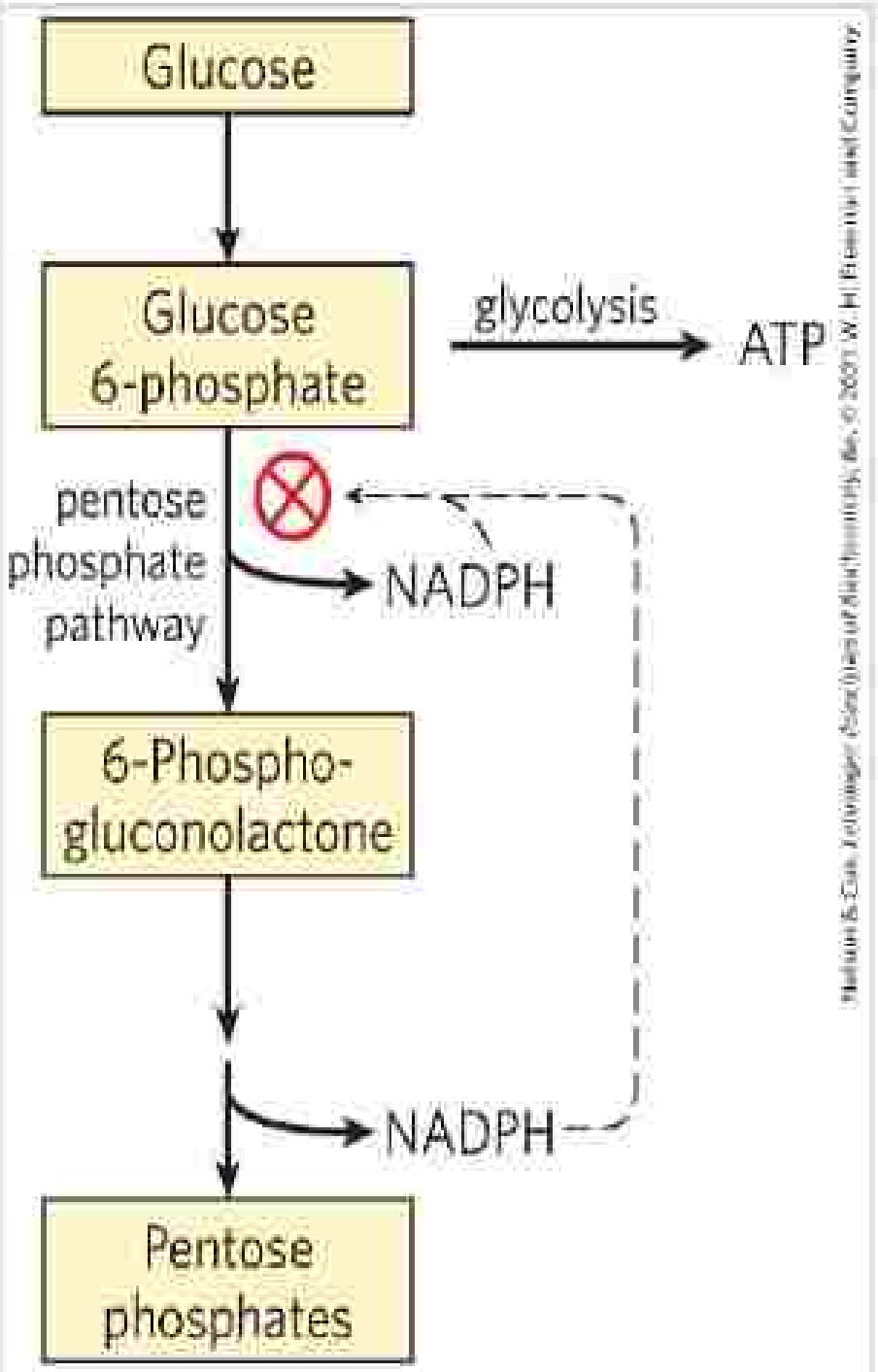
Glyceraldheyde 3 P dehydrogenase : for activity, Reaction of the essential Cys residue is necessary. *But presence of heavy metal such as Hg^{3+} irreversibly inhibits the enzyme.*

Glycolysis would soon come to a halt if the NADH concentration is increased in step 6 of glycolsis

Substrate-level phosphorylation

- The formation of ATP by phosphoryl group transfer from a substrate such as 1,3-bisphosphoglycerate is referred to as a **substrate-level phosphorylation**.
- Substrate-level phosphorylations involve soluble enzymes and chemical intermediates.

Role of NADPH in regulating the partitioning of glucose 6-phosphate between glycolysis and the pentose phosphate pathway



Role of NADPH in regulating the partitioning of glucose 6-phosphate between glycolysis and the pentose phosphate pathway. When NADPH is forming faster than it is being used for biosynthesis and glutathione reduction, [NADPH] rises and inhibits the first enzyme in the pentose phosphate pathway. As a result, more glucose 6-phosphate is available for glycolysis.

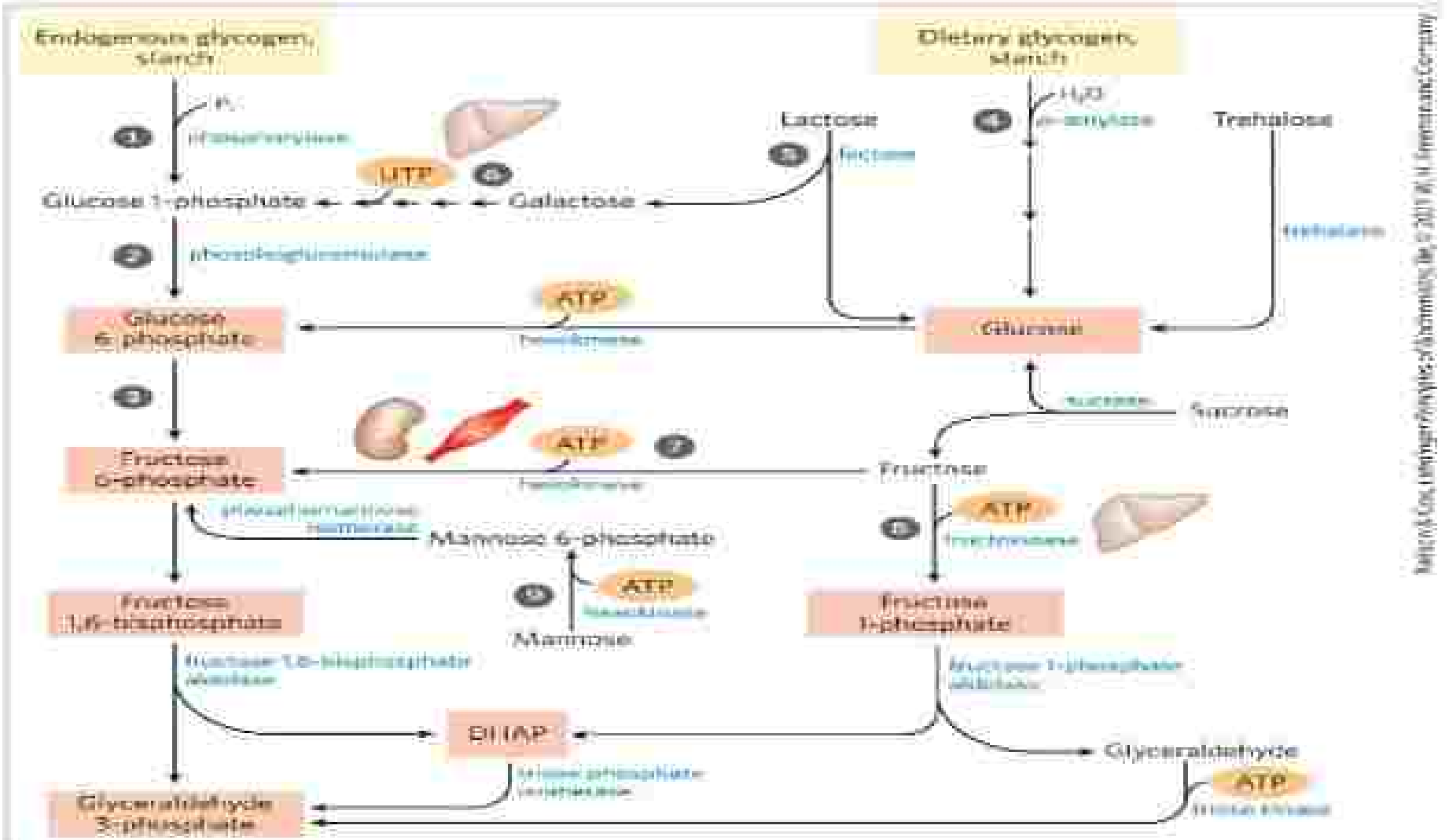


FIGURE 14-9 Entry of dietary glycogen, starch, disaccharides, and hexoses into the preparatory stage of glycolysis. The numbered steps are described

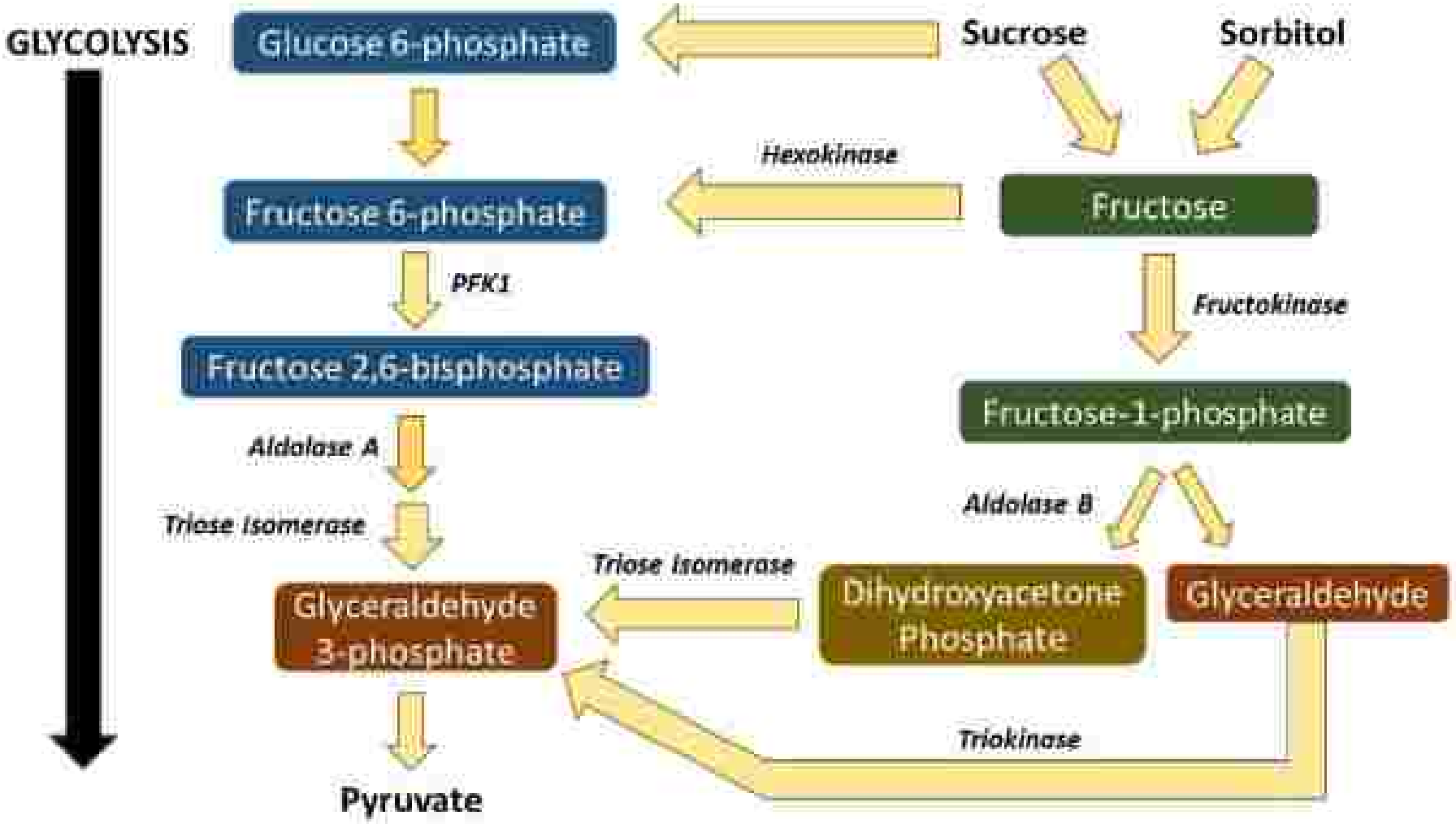


Figure 15.4.15: Alternate Sugar Sources for Glycolysis

Time to find a solution……..?

Calculate the standard free-energy change of the reaction catalyzed by the enzyme phosphoglucomutase.

Glucose 1-phosphate ⇌ glucose 6-phosphate

For the above reaction, starting with 65 mM glucose 1-phosphate and no glucose 6-phosphate, the final equilibrium mixture at 25 °C and pH 7.0 contains 2.0 mM glucose 1-phosphate and 63 mM glucose 6 phosphate. Does the reaction in the direction of glucose 6- phosphate formation proceed with a loss or a gain of free energy?

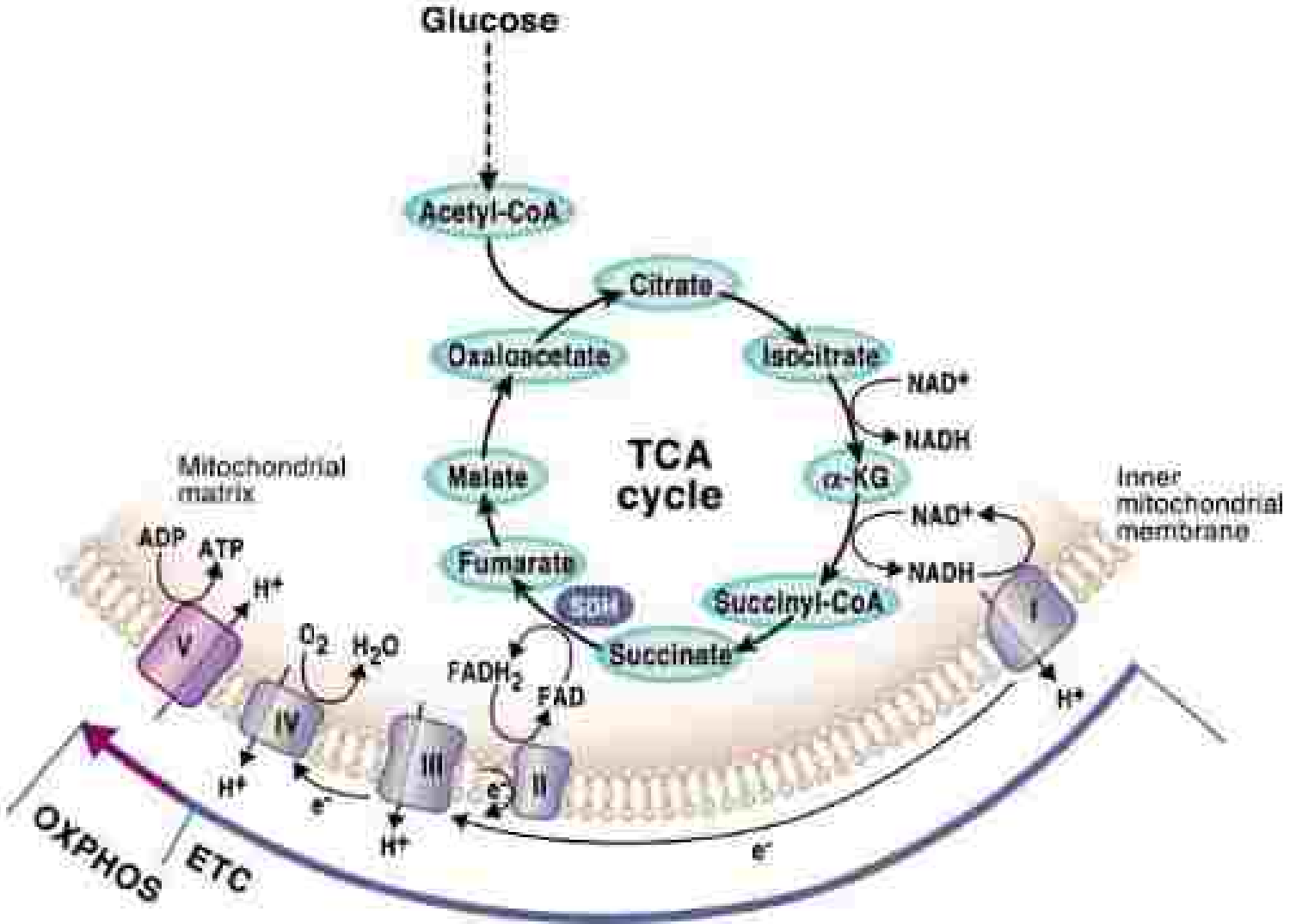
Time to find a solution……..?

Calculate the standard free-energy change of the reaction catalyzed by the enzyme phosphoglucomutase.

Glucose 1-phosphate ⇌ glucose 6-phosphate

For the above reaction, starting with 90 mM glucose 1-phosphate and no glucose 6-phosphate, the final equilibrium mixture at 25 °C and pH 7.0 contains 1.0 mM glucose 1-phosphate and 89 mM glucose 6 phosphate. Does the reaction in the direction of glucose 6- phosphate formation proceed with a loss or a gain of free energy?

Guess.....?


Glucose
Acetyl-CoA
Citrate
Isocitrate
NAD+
NADH
α-KG
NAD+
NADH
Succinyl-CoA
SDH
Succinate
FADH2
FAD
Fumarate
Malate
Oxaloacetate
TCA cycle
Mitochondrial matrix
Inner mitochondrial membrane
ADP
ATP
H+
V
O2
H2O
IV
III
II
I
H+
e-
H+
e-
H+
OXPHOS
ETC

TRICARBOXYLIC ACID CYCLE (TCA)/CITRIC ACID CYCLE -INTRODUCTION

- Pyruvate: A node in the metabolism of carbohydrates, proteins, and fats.
- Anaerobic conditions: lactate in the cytosol, regenerating NAD+ for continued ATP production by glycolysis.
- Pyruvate has a diffusion capabilities: cytoplasm to mitochondrial matrix.
- In 2 steps: a) diffusion through outer membrane pores, then via inner membrane carrier/symporter.
- By the carrier called : H^-- coupled pyruvate-specific symporter : the **mitochondrial pyruvate carrier (MPC) .**
- Pyruvate : amino acid synthesis.
- Or Citric acid cycle for ATP/NADH (energy)
- Synthesis of fatty acids and sterols (after getting converted to acetyl co A).

TRICARBOXYLIC ACID CYCLE (TCA)/CITRIC ACID CYCLE = INTRODUCTION

- Pyruvate ----- > Acetyl-CoA + CO_2
- By the enzyme **pyruvate dehydrogenase (PDH) complex**
- **PDH : 3 enzymes, 5 coenzymes; 4 cofactors derived from vitamins.**

Enzyme complex	Enzymes	Co-Enzymes/ Prosthetic groups	Co-factors
PDH complex	E1: **Pyruvate dehydrogenase**	TPP	Thiamine
	E2: **Dihydrolipoyl transacetylase**	Lipoate, coenzyme A (CoA-SH)	Pantothenate
	E3: **dihydrolipoyl dehydrogenase**	FAD, NAD	Niacin & Riboflavin

Conversion of Pyruvate to acetyl-CoA

- **oxidative decarboxylation :** an irreversible oxidation process in which the carboxyl group is removed from pyruvate as a molecule of CO2 and the two remaining carbons become the acetyl group of acetyl-CoA.
- A starting material for TCA
- NADH is produced : ETC

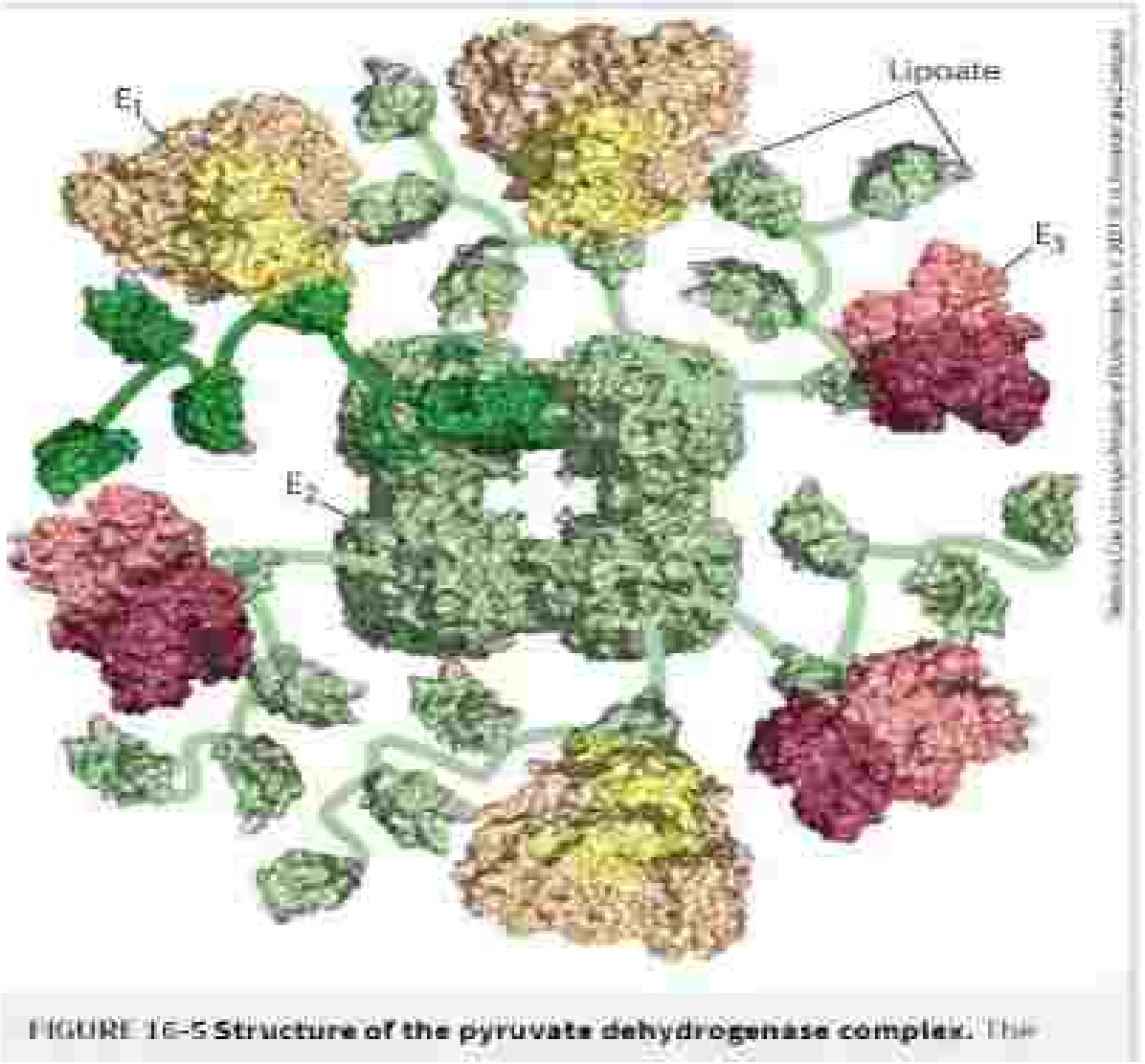


FIGURE 16-5 Structure of the pyruvate dehydrogenase complex. The

Conversion of Pyruvate to acetyl-CoA

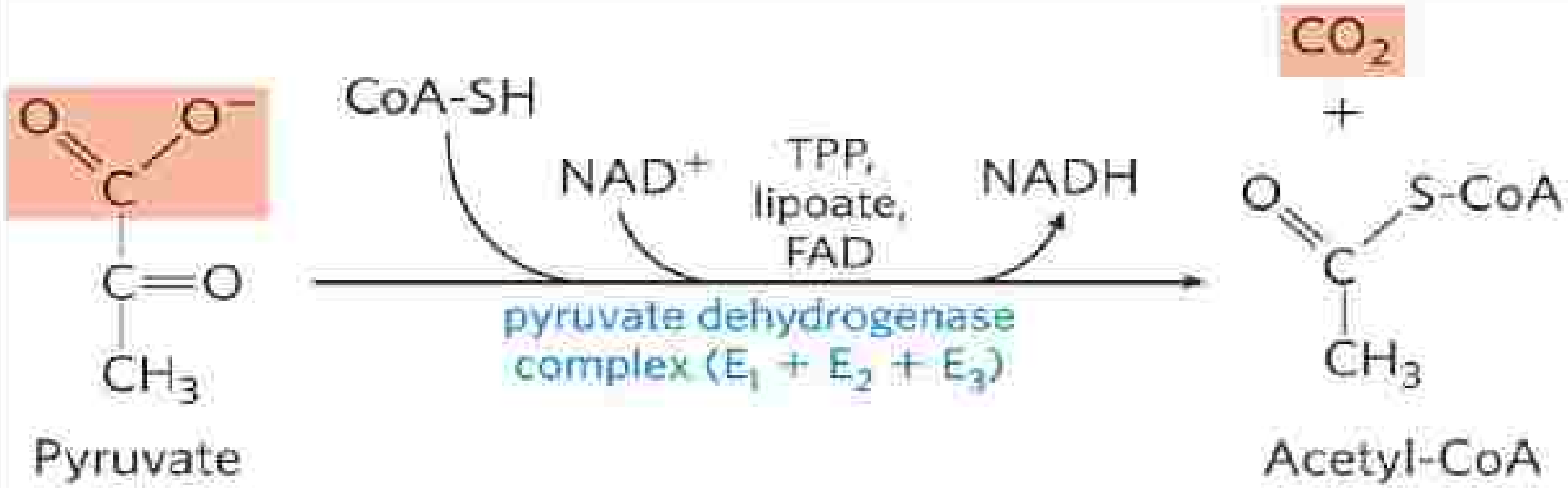


FIGURE 16-3 Overall reaction catalyzed by the pyruvate dehydrogenase complex. The five coenzymes participating in this reaction, and the three enzymes that make up the enzyme complex, are discussed in the text.

Conversion of Pyruvate to acetyl-CoA

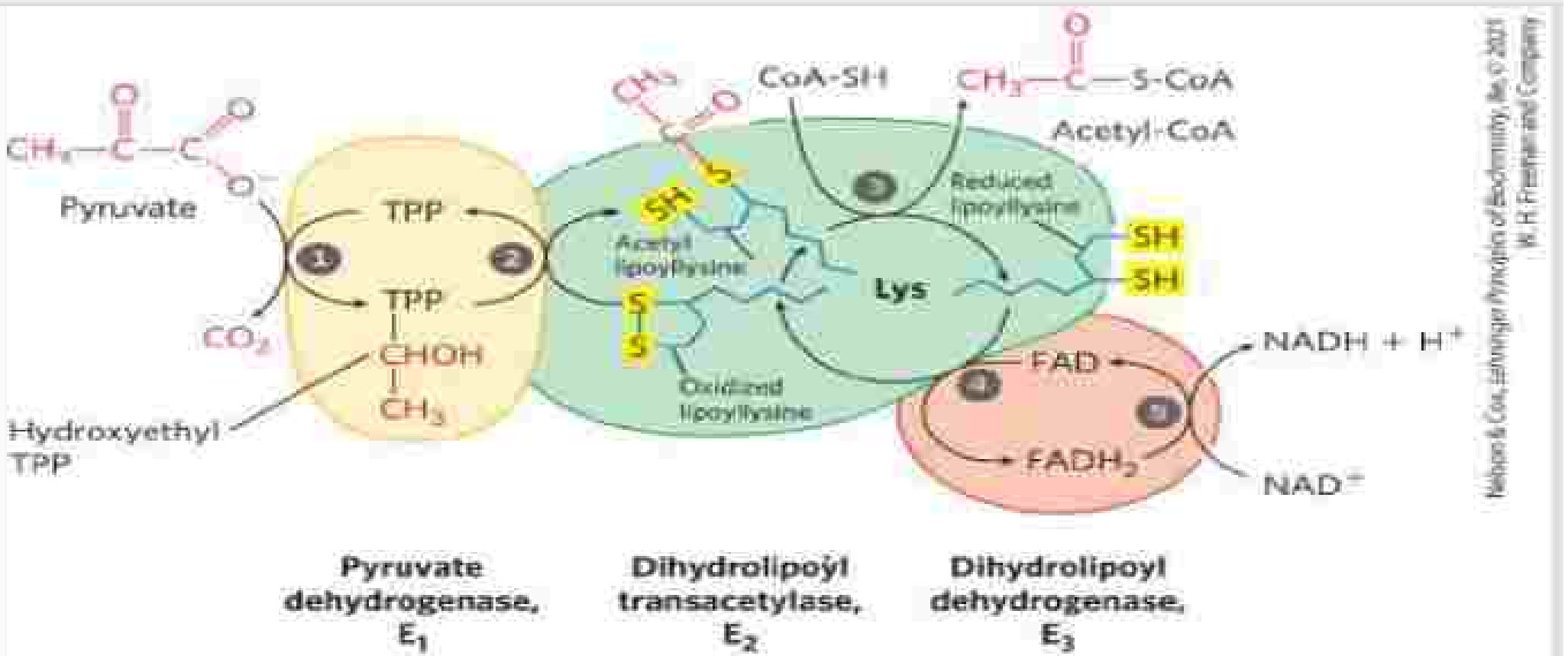


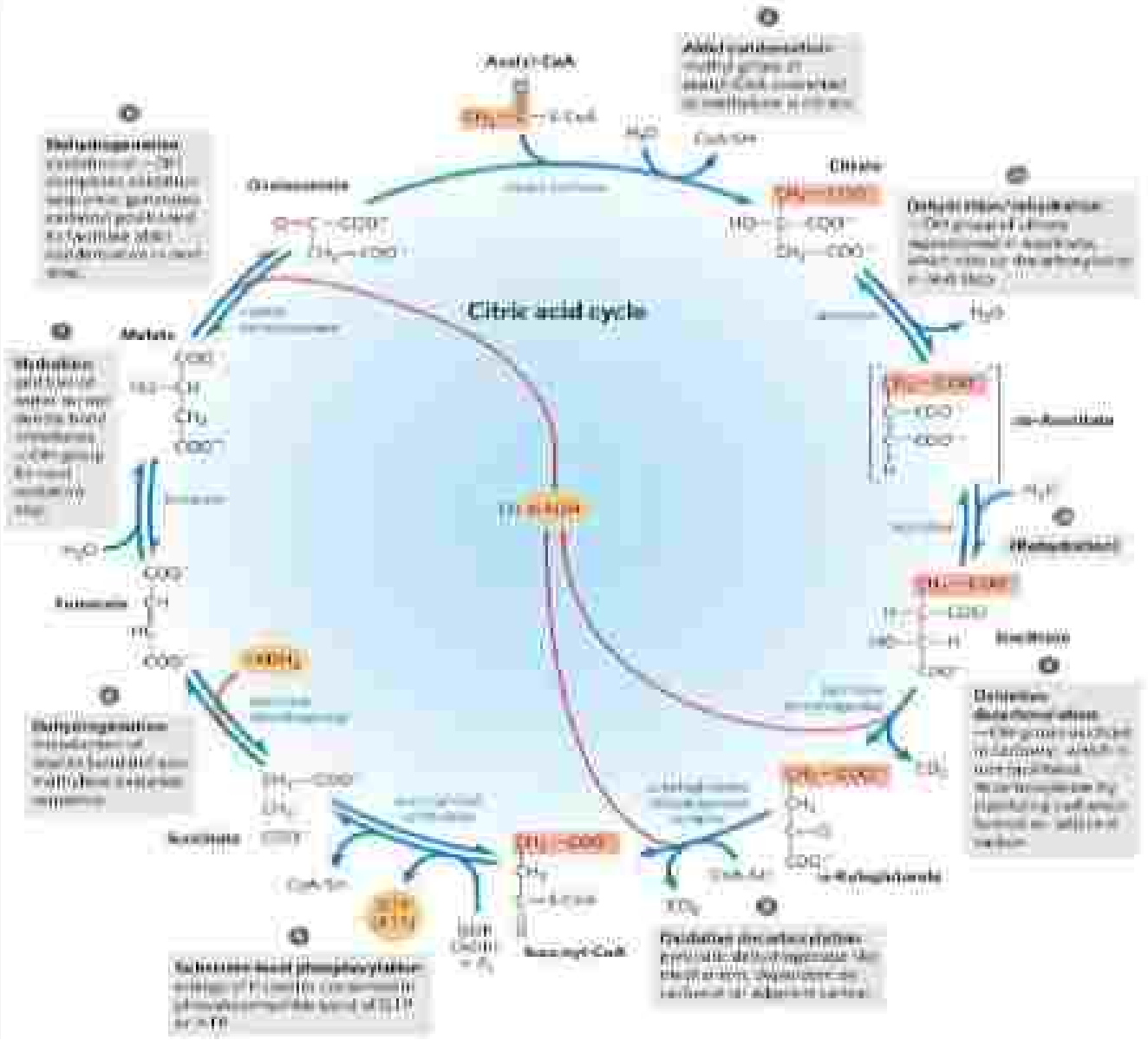
FIGURE 16-6 Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex. The fate of pyruvate is traced in red. In step 1 pyruvate

TCA cycle/Kreb's cycle/Citric acid cycle

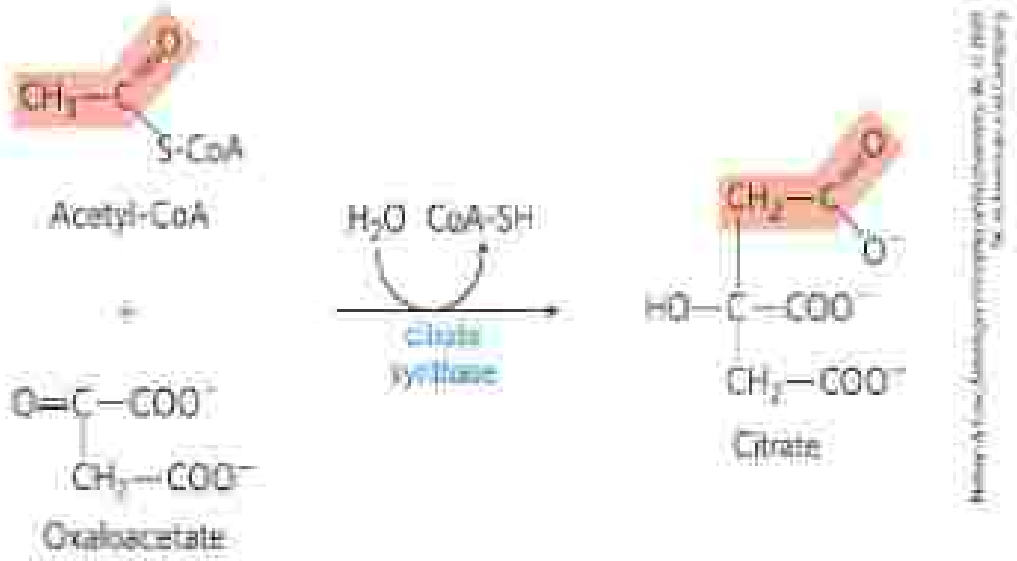
1. first *cyclic* pathway
2. Oxidation of Acetyl CoA
3. Citrate (6C) is first produced compound
4. 8 different steps
5. 3 irreversible steps

(key regulatory steps)

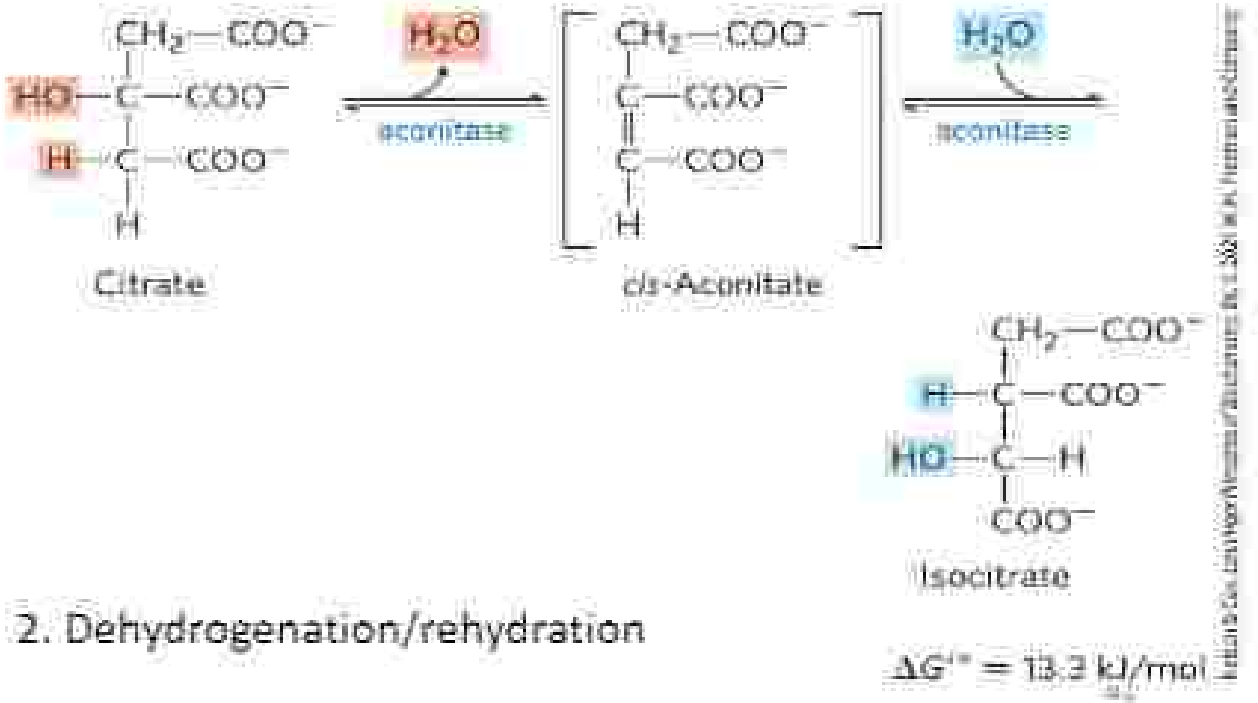
6. 3NADH, 1 FADH2 and 1GTP/ATP Is produced /pyruvate via acetyl coA entering the Cycle.
7. No net removal of oxaloacetate occurs.
8. NADH and FADH2 donate their electrons to the respiratory chain where electron flow drives ATP synthesis.
9. German chemist, Hans Adolf Krebs



TCA cycle/Kreb's cycle/Citric acid cycle

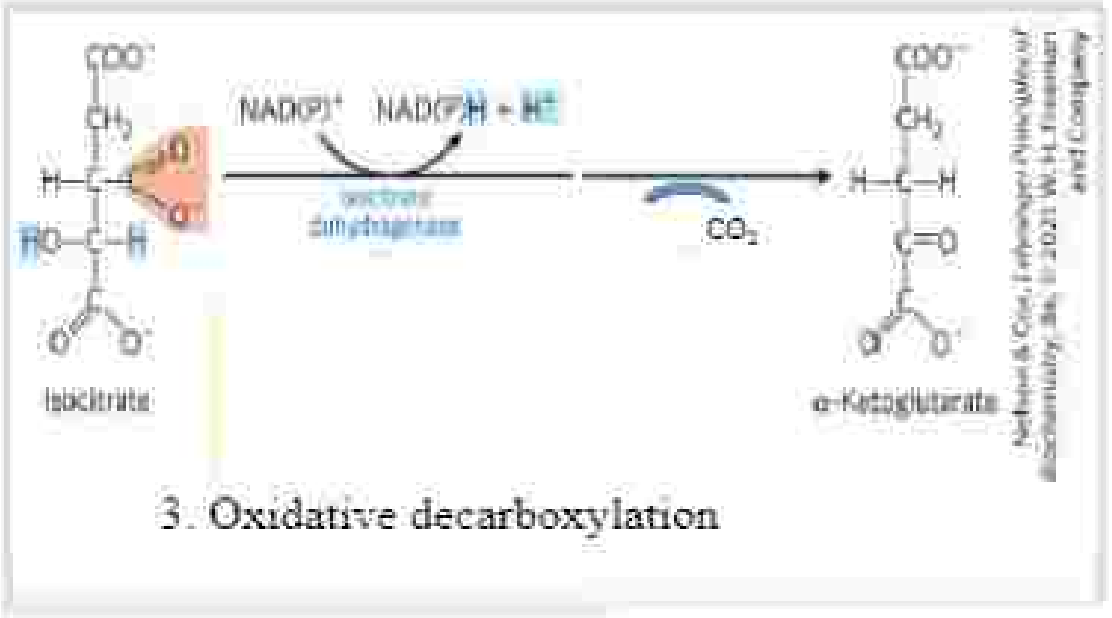


1. Condensation

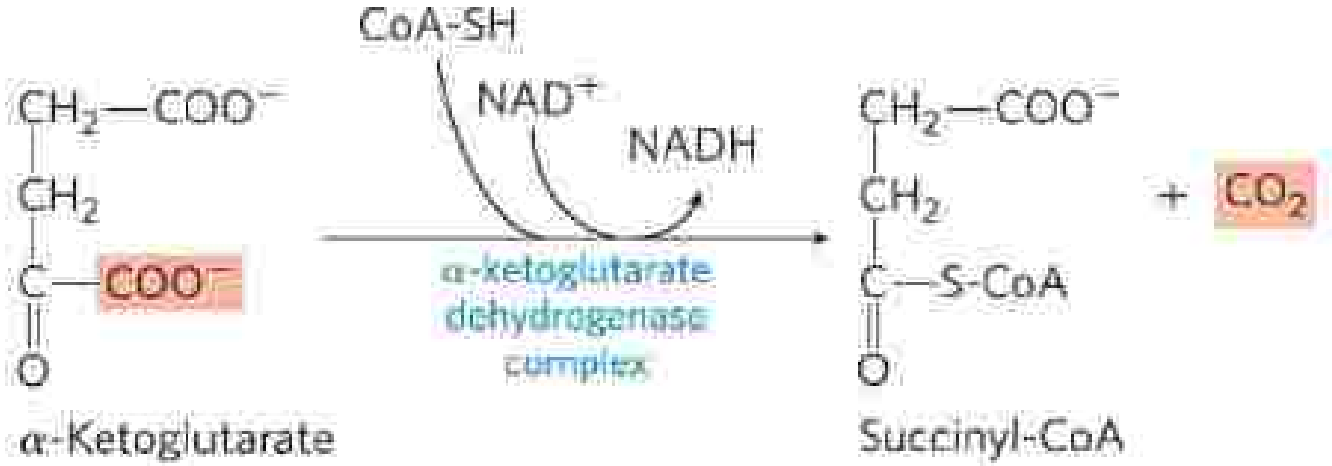


2. Dehydrogenation/rehydration

TCA cycle/Kreb's cycle/Citric acid cycle



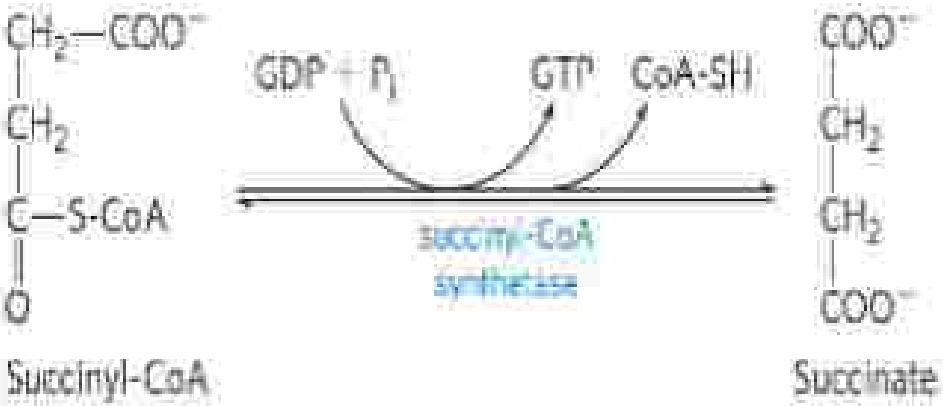
3. Oxidative decarboxylation



4. Oxidative decarboxylation

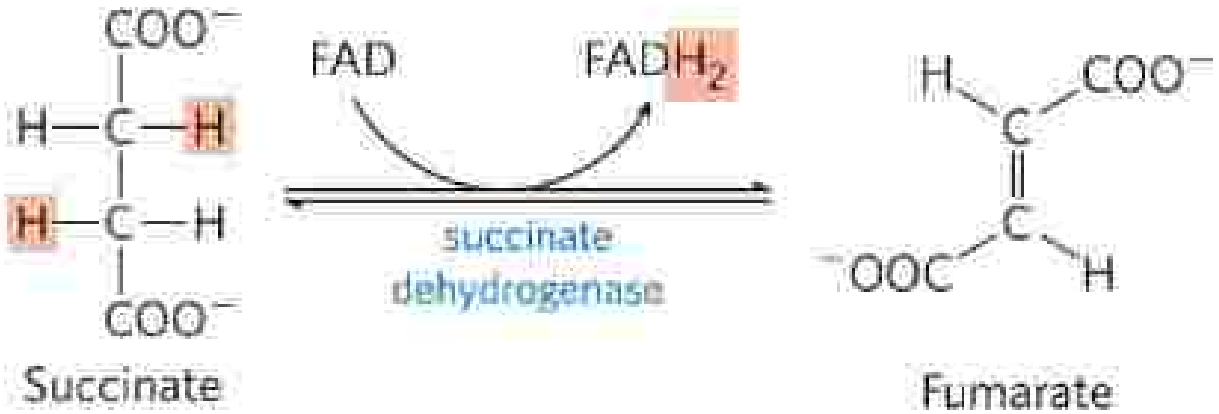
$\Delta G'^{\circ} = -33.5$ kJ/mol

TCA cycle/Kreb's cycle/Citric acid cycle



5. Substrate level phosphorylation

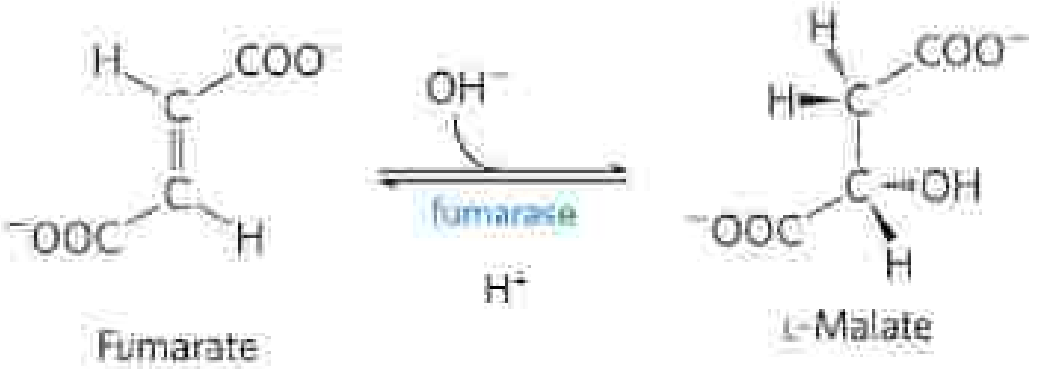
$\Delta G'^{\circ} = -2.9$ kJ/mol



6. Dehydrogenation

$\Delta G'^{\circ} = 0$ kJ/mol

TCA cycle/Kreb's cycle/Citric acid cycle



Fumarate

L-Malate

7. Hydration

$\Delta G'^{0} = -3.8$ kJ/mol

L-Malate

Oxaloacetate

8. Dehydrogenation

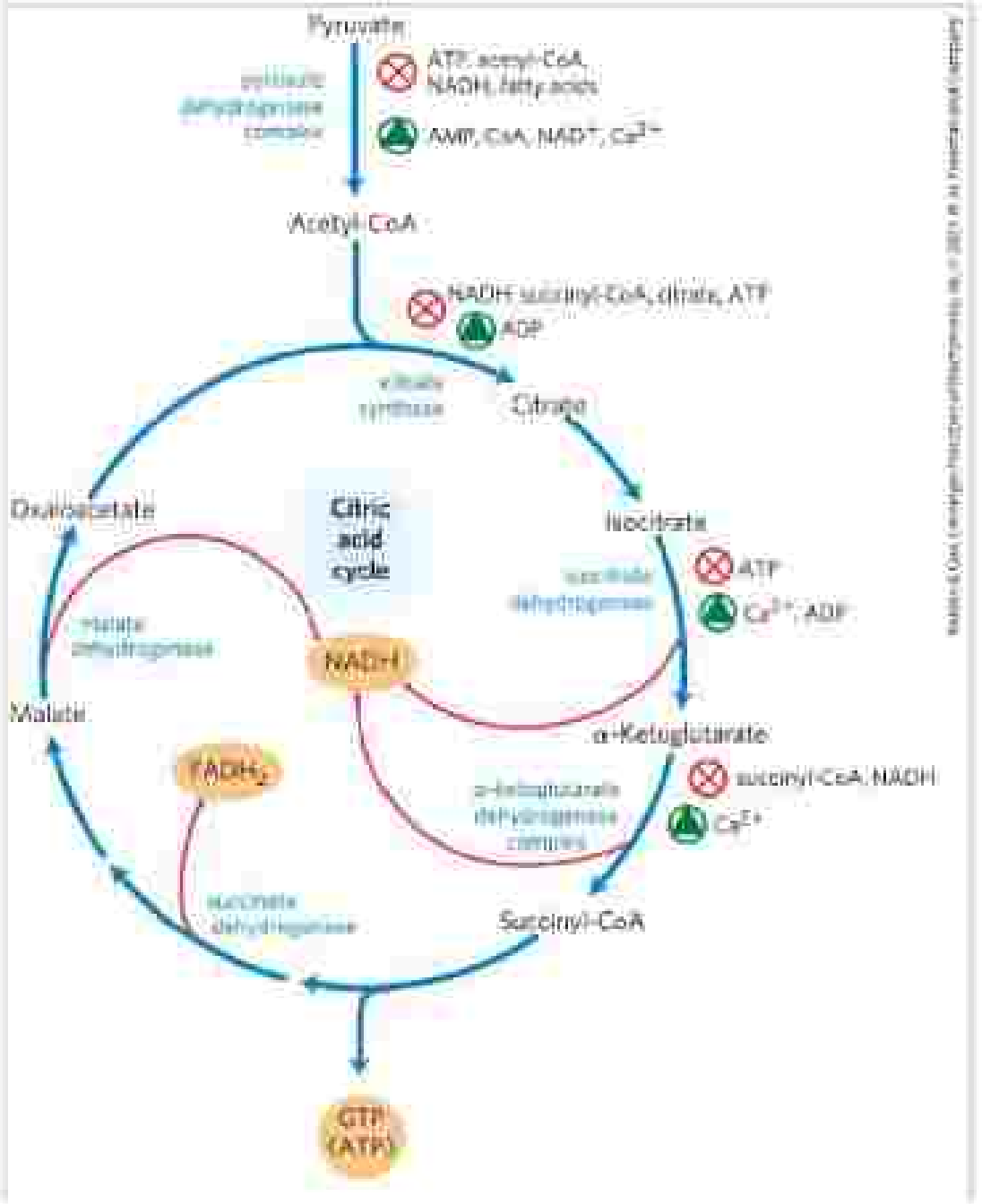
$\Delta G'^{0} = 29.7$ kJ/mol

Regulation of TCA

- stringent regulation : to balance the supply of key intermediates with the demands of energy production and biosynthetic processes.
- Regulation at several steps

1. Oxidation of pyruvate to acetyl-CoA (catalyzed by the PDH complex)

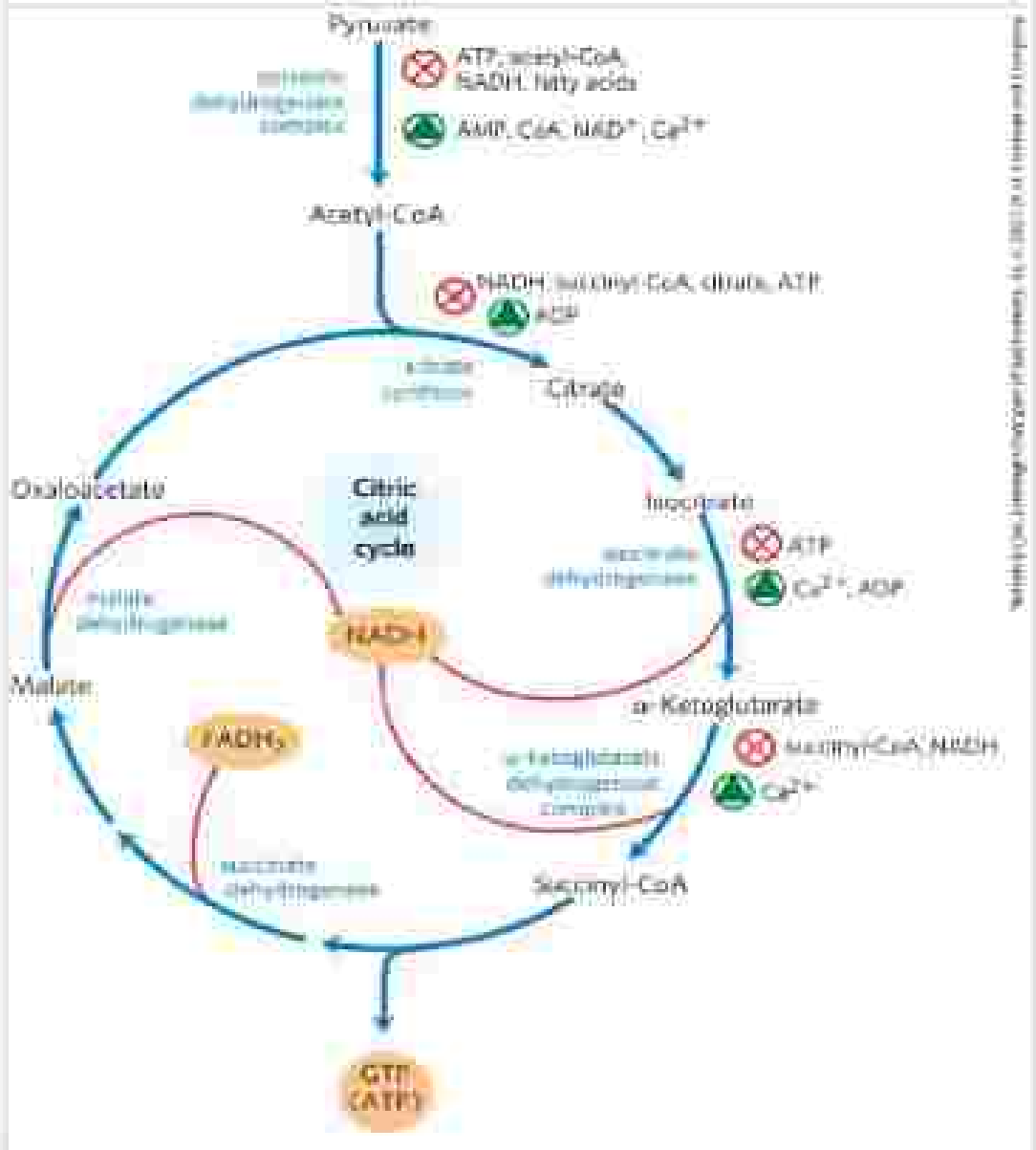
- Is inhibited allosterically by *ATP and by acetyl-CoA and NADH via phosphorylation of PDH.*
- When [ATP]/[ADP], [NADH]/[NAD+], and [acetyl-CoA]/[CoA] ratios *are high*, all of which indicate an energy sufficient metabolic state.
- When [ATP]/[ADP], [NADH]/[NAD+], and [acetyl-CoA]/[CoA] ratios *decrease, allosteric activation of pyruvate oxidation results and activation of TCA.*



Regulation of TCA

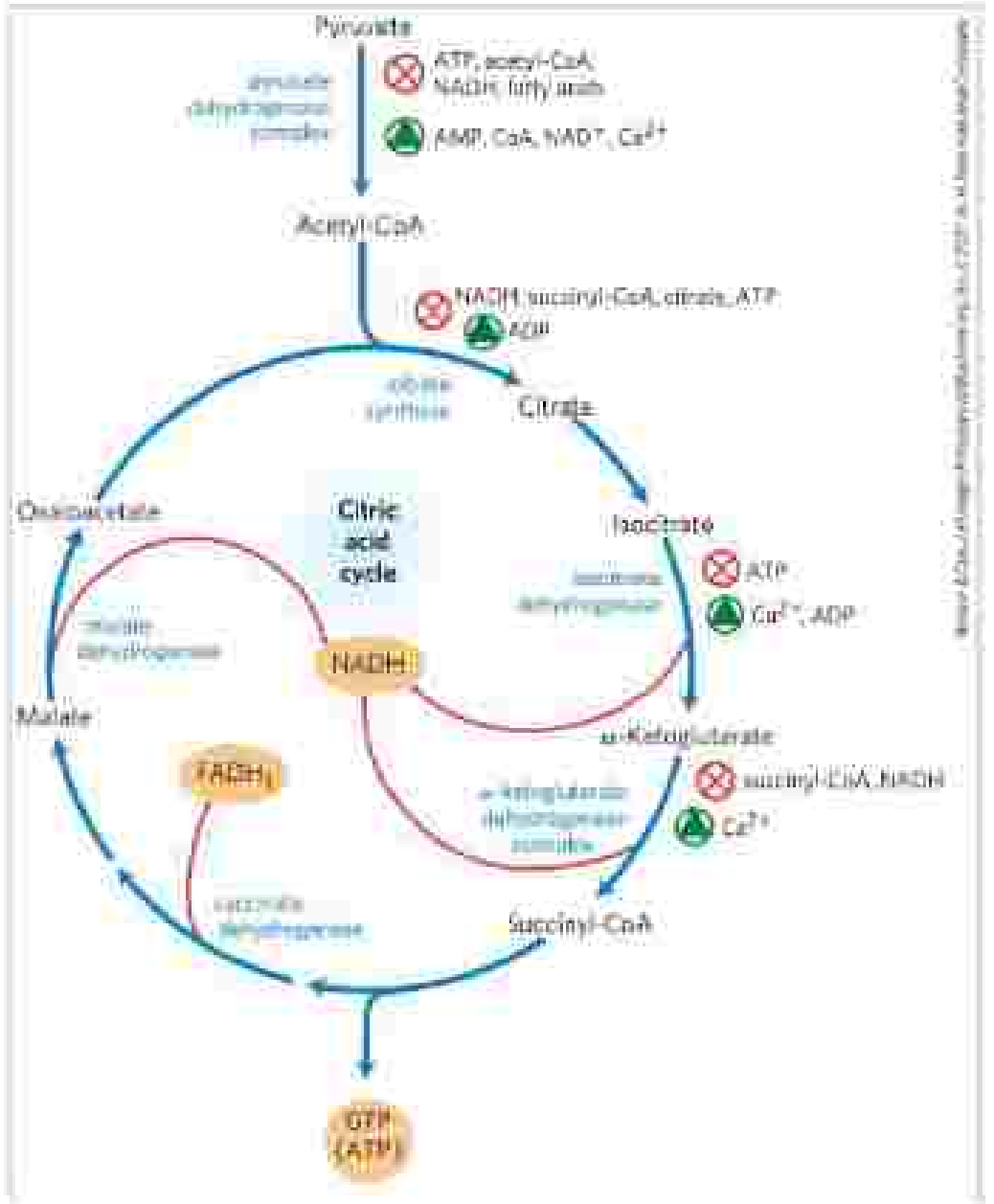
2. Citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase : exergonic steps.

- High [NADH]/[NAD+] inhibits the dehydrogenase reactions (isocitrate dehydrogenase, and α- ketoglutarate dehydrogenase) (through mass action).
- Malate dehydrogenase reaction is essentially at equilibrium in the cell when [NADH]/[NAD+] is in high the concentration, oxaloacetate is low, slowing the first step in the cycle.
- Product accumulation inhibits all 3 steps.
- Succinyl-CoA inhibits α-ketoglutarate dehydrogenase and citrate synthase.
- Citrate blocks citrate synthase
- ATP, inhibits both citrate synthase and isocitrate dehydrogenase.



Regulation of TCA

- Ca^{2+}: the signal for contraction and for a concomitant increase in demand for ATP, activates both isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, as well as the PDH complex.
- The concentration of citrate, the product of the first step of the citric acid cycle, and an important allosteric inhibitor of phosphofructokinase-1 of the glycolytic pathway
- Dichloroacetate (DCA): inhibits PDH kinase in the laboratory and so relieves the inhibition of the PDH complex ---> TCA ---> apoptosis



Bioenergetics of Glycolysis and TCA cycle

Reaction	Number of ATP or reduced coenzyme directly formed	Number of ATP ultimately formed
Glucose → glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate → fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate → 2 1,3-bisphosphoglycerate	2 NADH	5 or 6
2 1,3-Bisphosphoglycerate → 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate → 2 pyruvate	2 ATP	2
2 Pyruvate → 2 acetyl-CoA	2 NADH	5
2 Isocitrate → 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate → 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA → 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate → 2 fumarate	2 $FADH_2$	3
2 Malate → 2 oxaloacetate	2 NADH	5
Total		30–32

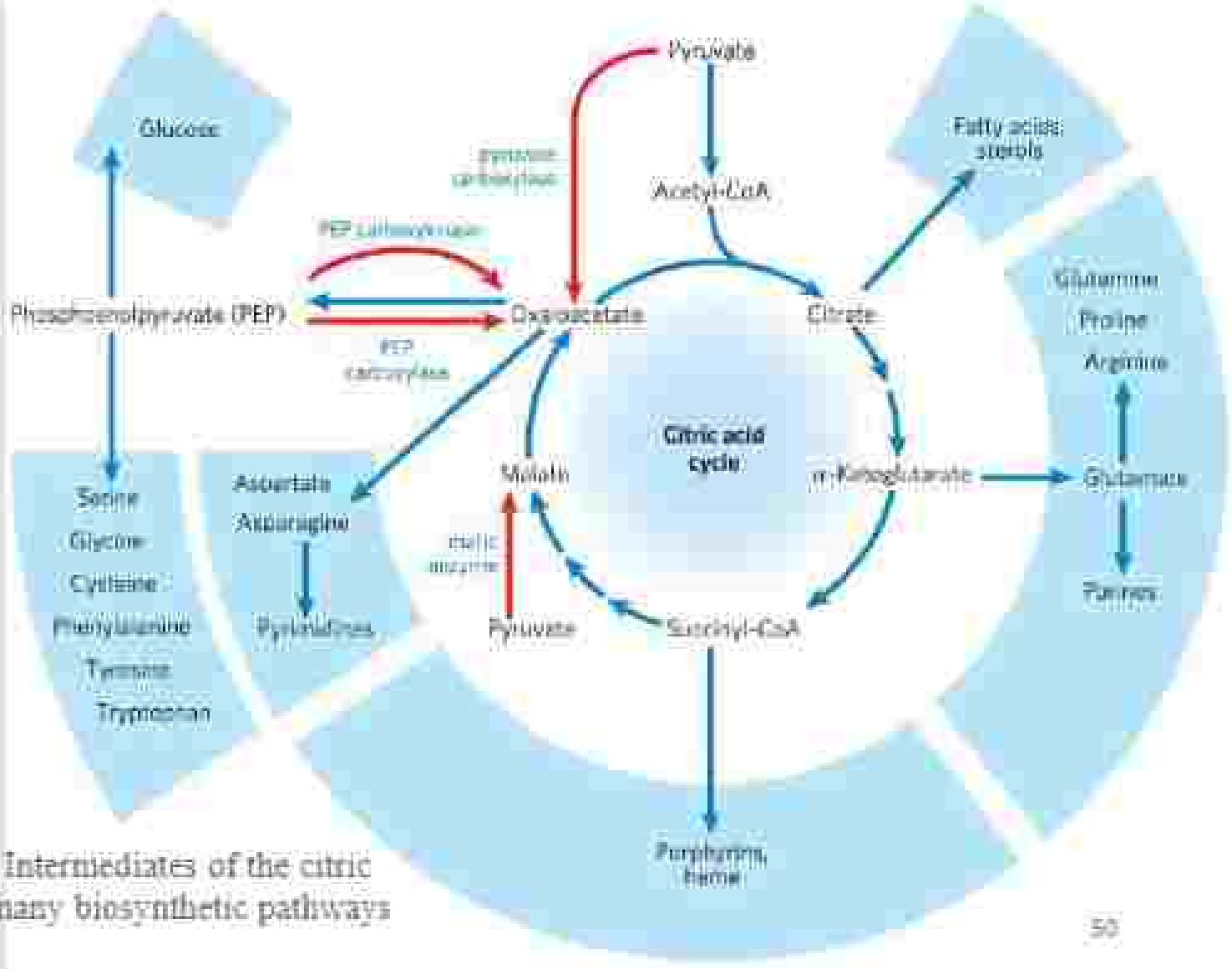
TCA cycle's Anaplerotic reaction

TABLE 16-2 Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
Pyruvate + HCO_3^- + ATP ⟶ oxaloacetate + ADP + P_i	Liver, kidney
Phosphoenolpyruvate + CO_2 + GDP ⟶ oxaloacetate + GTP	Heart, skeletal muscle
Phosphoenolpyruvate + HCO_3^- ⟶ oxaloacetate + P_i	Higher plants, yeast, bacteria
Pyruvate + HCO_3^- + NAD(P)H ⟶ malate + NAD(P)$^+$	Widely distributed in eukaryotes and bacteria

Pyruvate carboxylase is a regulatory enzyme and is virtually inactive in the absence of acetyl-CoA, its positive allosteric modulator. Whenever acetyl-CoA, the fuel for the citric acid cycle, is present in excess, it stimulates the pyruvate carboxylase reaction to produce more oxaloacetate, enabling the cycle to use more acetyl-CoA in the citrate synthase reaction.

TCA cycle's Anaplerotic reaction



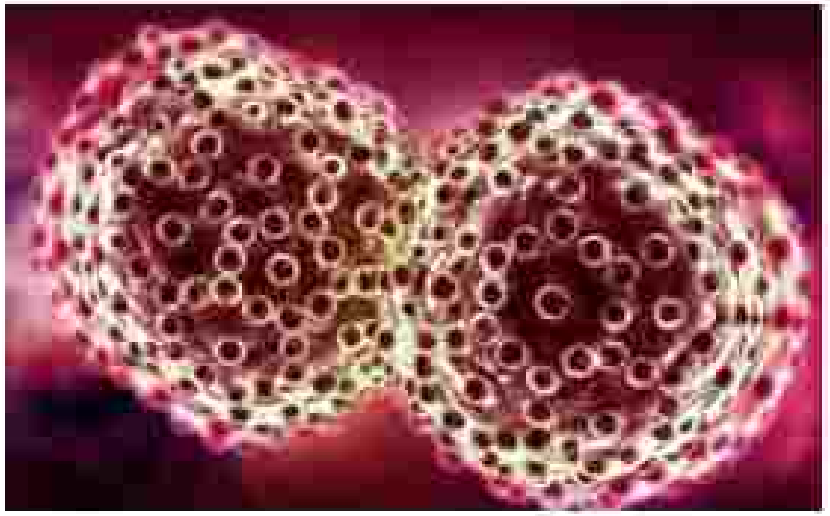
Role of the citric acid cycle in anabolism. Intermediates of the citric acid cycle are drawn off as precursors in many biosynthetic pathways

The Citric Acid Cycle Serves in Both Catabolic and Anabolic Processes & some interesting info….

- Four- and five-carbon intermediates of the cycle serve as precursors for a wide variety of products.
- To replace intermediates removed for this purpose, cells employ anaplerotic (replenishing) reactions.
- All the enzymes are in the matrix of mitochondria except succinate dehydrogenase which is in inner mitochondrial membrane.
- **Succinyl CoA synthetase** or **succinic thiokinase:** indicate the participation of a nucleoside triphosphate in the reaction
- In aerobic organisms, the citric acid cycle is an **amphibolic pathway**, one that serves in both catabolic and anabolic processes.
- oxaloacetate and α- ketoglutarate: precursors of aspartate and glutamate by simple transamination, & purine and pyrimidine nucleotides synthesis.
- Succinyl-CoA central intermediate in the synthesis of the porphyrin ring of heme groups, which serve as oxygen carriers (in hemoglobin and myoglobin) and electron carriers (in cytochromes)
- oxaloacetate can be converted to glucose via gluconeogenesis
- Conversion of acetate or acetyl-CoA to glucose occurs in bacteria, plants, fungi, and protists: the glyoxylate cycle
- Metabolon: A supramolecular assembly of sequential metabolic enzymes, example: malate dehydrogenase, citrate synthase, and aconitase – metabolon.

Citric Acid Cycle Activity Changes in Tumors…

- Down regulation of mitochondrial pyruvate carrier (MPC) & PDH
- Leads to pyruvate accumulation in cytosol.
- Accumulation of Lactate and succinate: **oncometabolites**
- **Leading to** tumor growth, acting through specific G protein–coupled receptors GPCRs.
- Mutations in citric acid cycle enzymes: succinate dehydrogenase lead to tumors of the adrenal gland;
- Mutations in the fumarase gene lead to tumors of smooth muscle (leiomyomas) and kidney



Questions to workout……

- 1. **Net Equation for Glycolysis and the Citric Acid Cycle.** Write the net biochemical equation for the metabolism of a molecule of glucose by glycolysis and the citric acid cycle, including all cofactors.
- **2. Thiamine Deficiency** Individuals with a thiamine-deficient diet have relatively high levels of pyruvate in their blood. Explain this in biochemical terms.
- **3. Formation of Oxaloacetate in a Mitochondrion** In the last reaction of the citric acid cycle, malate is dehydrogenated to regenerate the oxaloacetate necessary for the entry of acetyl-CoA into the cycle:

  L-Malate + NAD^+ → oxaloacetate + NADH + H^+; $\Delta G'^{\circ} = 30.0$ kJ/mol

  3.a. Calculate the equilibrium constant for this reaction at 25 °C.
- **Role of the Vitamin Thiamine** People with beriberi, a disease caused by thiamine deficiency, have elevated levels of blood pyruvate and α-ketoglutarate, especially after consuming a meal rich in glucose. How are these effects related to a deficiency of thiamine?

Electron Transport Chain and Oxidative phosphorylation

- Oxidative phosphorylation - is the culmination of energy-yielding metabolism (catabolism) in aerobic organisms.
- Mitochondria, with the help of protein complexes embedded in the inner mitochondrial membrane.
- **Electrons flow from electron donors (oxidizable substrates) through a chain of membrane-bound carriers to a final electron acceptor with a large reduction potential.** The final acceptor is molecular oxygen, O_2.
- The free energy made available by "downhill" (exergonic) electron flow is coupled to the "uphill" transport of protons across a proton-impermeable membrane.
- **The transmembrane flow of protons back down their electrochemical gradient through specific protein channels provides the free energy for synthesis of ATP.** This process is catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.
- **chemiosmotic theory:** Peter Mitchell in 1961.
- It is The movement of ions across a selectively permeable membrane, down their electrochemical gradient, leading to the formation of ATP.

Electron Transport Chain and Oxidative phosphorylation and

- The production of ATP using the process of chemlosmosis in mitochondria is called oxidative phosphorylation.

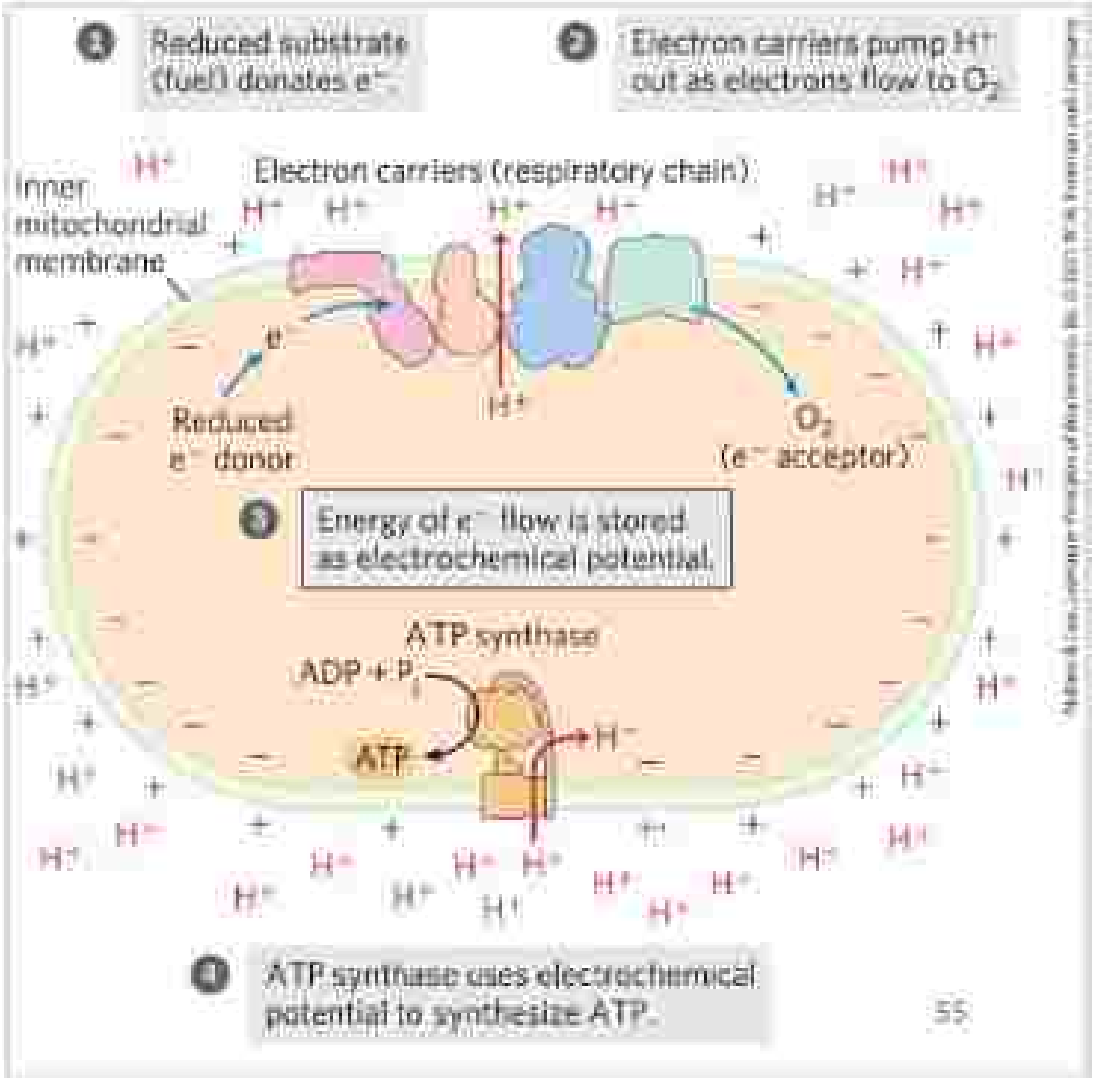


FIGURE 19-1 The chemiosmotic mechanism for ATP synthesis in mitochondria. Electrons move spontaneously through a chain of membrane-bound carriers, the respiratory chain, driven by the high reduction potential of oxygen and the relatively low reduction potentials of the various reduced substrates (fuels) that undergo oxidation in the mitochondrion. Electron flow creates an electrochemical potential by the transmembrane movement of protons and positive charge. This electrochemical potential drives ATP synthesis by a membrane-bound enzyme (ATP synthase) that is fundamentally similar in mitochondria and chloroplasts, and in bacteria and archaea as well.

Electron Transport Chain and Oxidative phosphorylation

- Eugene Kennedy and Albert Lehninger: mitochindria as a site of OP
- mitochondria have about 1,200 proteins
- The functions of up to 25% of these remain partly or entirely enigmatic.
- Under stressful conditions: trigger mitochondrial fission and sometimes **Mitophagy.**
- Oxidative phosphorylation begins with the entry of electrons into the series of electron carriers called the **Respiratory chain**.
- electrons arise from the action of dehydrogenases that collect electrons from catabolic pathways and funnel them into universal electron acceptors — Nicotinamide nucleotides (NAD+ or NADP+) or flavin nucleotides (FMN or FAD).

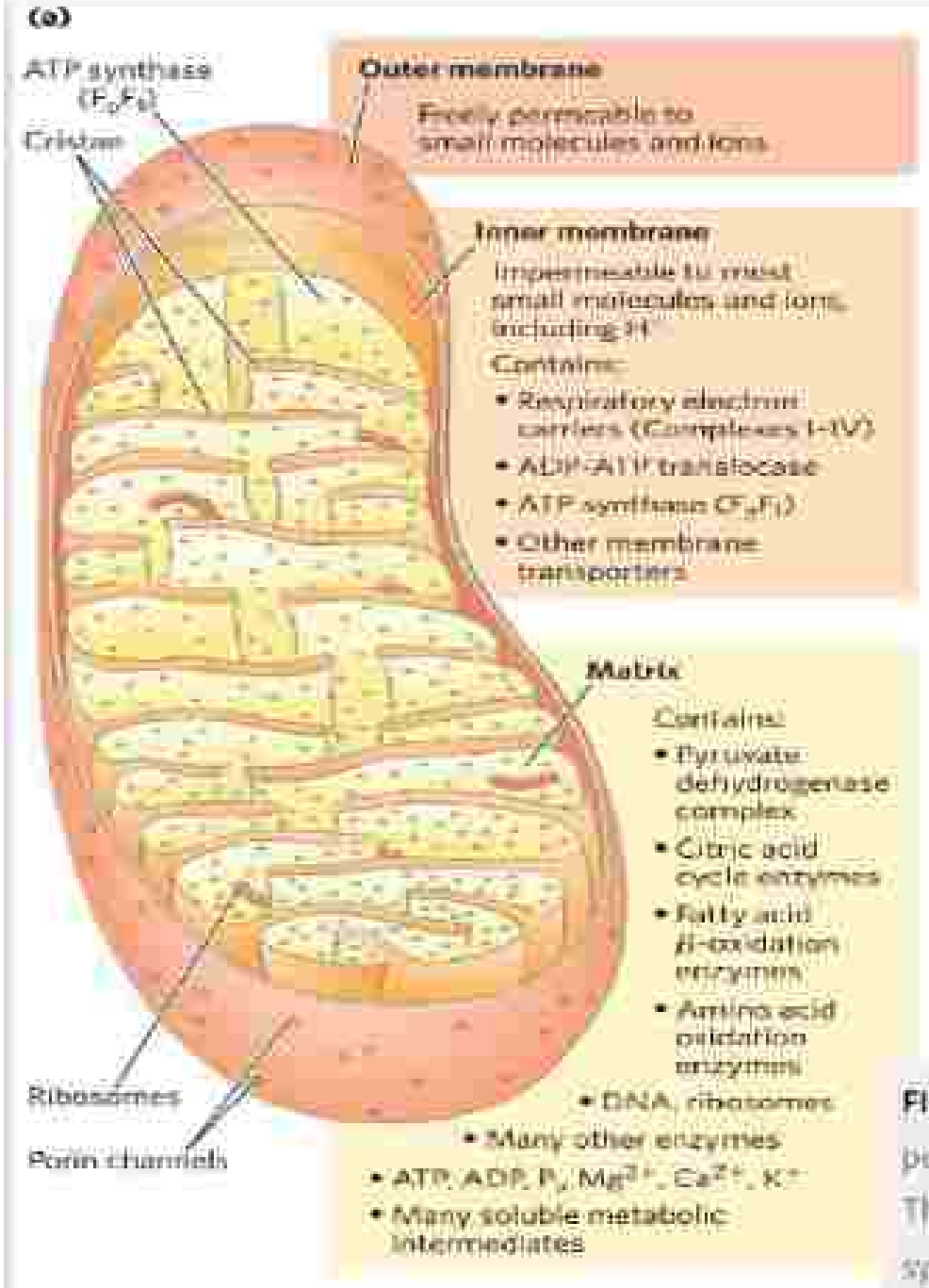
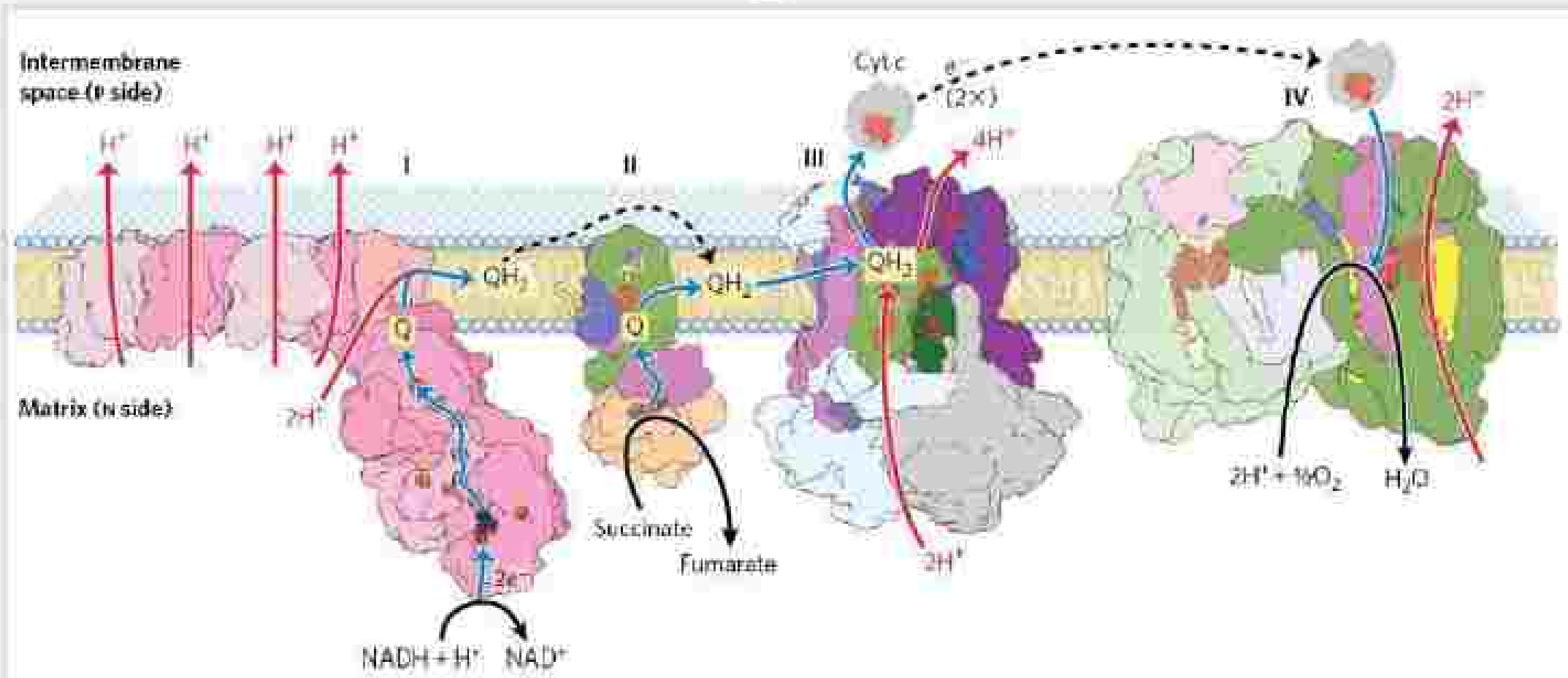


FIGURE 19-2 Biochemical anatomy of a mitochondrion. (a) The outer membrane has pores that make it permeable to small molecules and ions, but not to proteins. The cristae provide a very large surface area. The inner membrane of a single liver mitochondrion may have more than 10,000 sets of electron-transfer systems (respiratory chains) and ATP synthase molecules, distributed over the membrane surface. (b) A

Electron Transport Chain and Oxidative phosphorylation

- Three types of electron transfers occur in oxidative phosphorylation:
- (1) direct transfer of electrons, as in the reduction of Fe^{3+} to Fe^{2+}
- (2) transfer as a hydrogen atom ($H^+ + e^-$)
- (3) transfer as a hydride ion ($:H^-$), which bears two electrons.
- Three other types of electron carrying molecules function in the respiratory chain. They are
- A) ubiquinone: A type hydrophobic quinone (small),
- B & c) cytochromes and iron-sulfur proteins
- **Ubiquinone** (also called **coenzyme Q**, or simply **Q**) is a lipid-soluble benzoquinone with a long isoprenoid side chain.
- (·QH or ubisemiquinone, or ubiquinol (QH_2).
- junction between a two electron donor and a one-electron acceptor.
- freely diffusible, not bound to protein, shuttles between inner membrane and matrix.
- The **cytochromes** are proteins with characteristic strong absorption of visible light, due to their iron-containing heme prosthetic groups.
- Three types : *a*, *b*, and *c*,

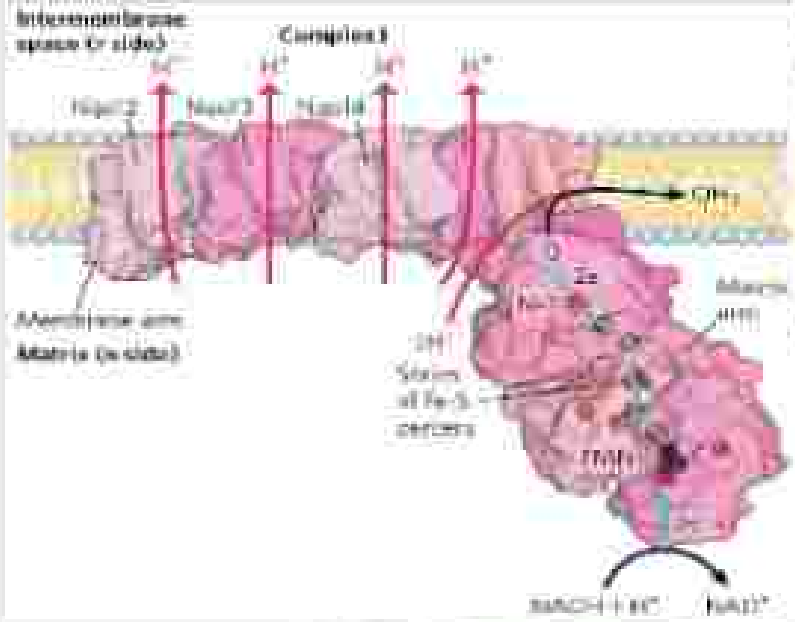
Electron Transport Chain & OP

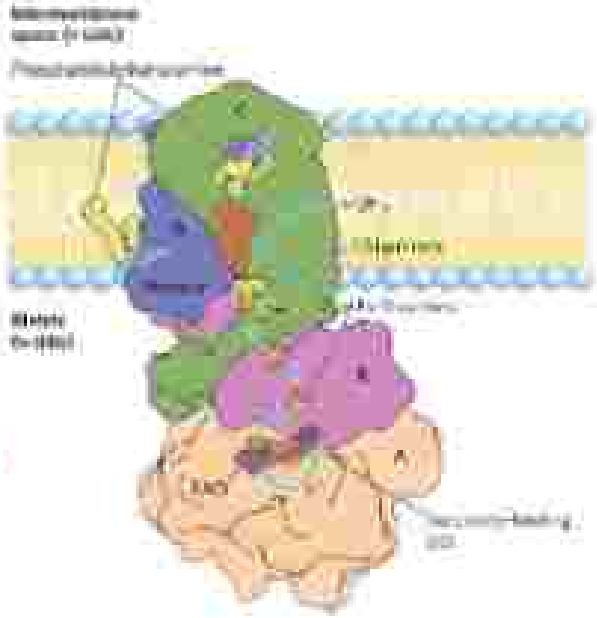


Schematic representation of ETC. Broken lines indicate the diffusion of Q in the plane of the inner membrane, and of cytochrome *c* through the inter membrane space (P).

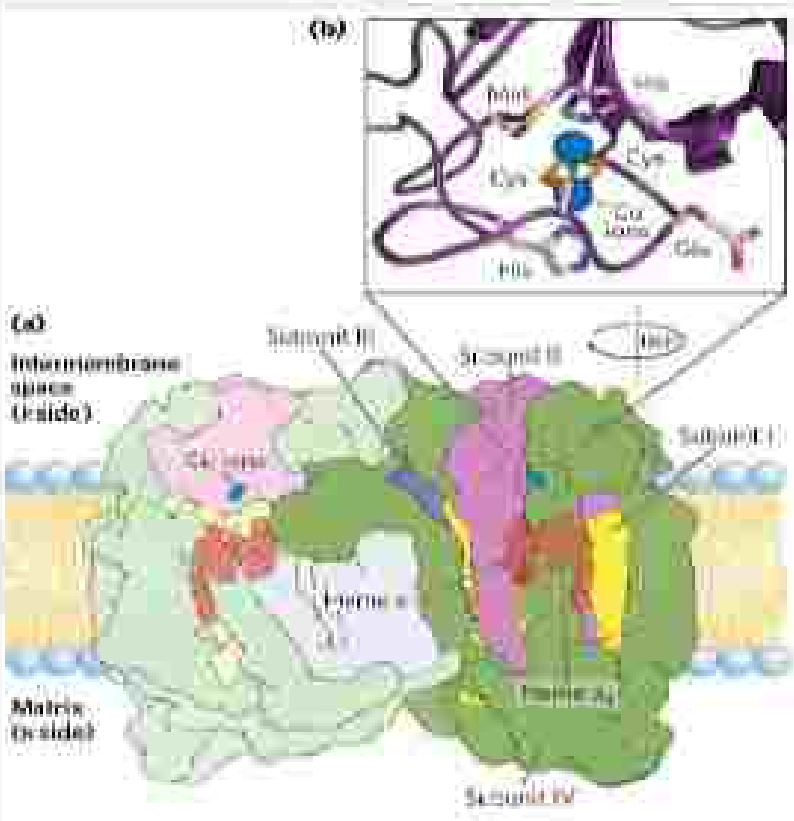
Complex name	Nature/components/subunits/PGs	work
Complex I - NADH dehydrogenase (NADH:ubiquinone oxidoreductase)	45 poly peptide(14), FMN (flavoprotein), Fe-S (min 8)	L-shaped, embedded in IM and matrix. $NADH + 5H^+_N + Q \rightarrow NAD^+ + QH_2 + 4H^+_P$
Complex II -Succinate dehydrogenase	4 subunits (C& D: IM; A & B: matrix), FAD, 2Fe-2S, heme b (BS to UQ)	oxidation of succinate and reduction of ubiquinone at another site.
Complex III- Ubiquinone: cytochrome c oxidoreductase	Dimer & contains *cytochrome b, cytochrome c1, and the Rieske iron-sulfur protein!!*	Transfers electrons from ubiquinol to cytochrome c. *This is a dimer.* $QH_2 + 2$ cyt c (oxidized) $+ 2H^+_N \rightarrow Q + 2$ cyt c (reduced) $+ 4H^+_P$
Cytochrome c	1; Heme	Soluble protein; associated at P side of Mt.M. cytochrome c moves in the intermembrane space to Complex IV to donate the electron to a binuclear copper center.
Complex IV- Cytochrome oxidase	Dimeric enzymes – 13 subunits each, Hemes, CuA, CuB	Dimeric enzyme; Electron transfer through Complex IV is from cytochrome c to the CuA center, to heme a, to the heme a3–CuB center, and finally to O_2 4 cyt c(reduced) $+ 8H^+_N + O_2 \rightarrow 4$ cyt c (oxidized) $+ 4H^+_P + 2H_2O$

*Complex IV- Cytochrome oxidase every four electrons passing through this complex, the enzyme consumes four "substrate" H- from the matrix (N side) in converting O_2 to two H_2O.

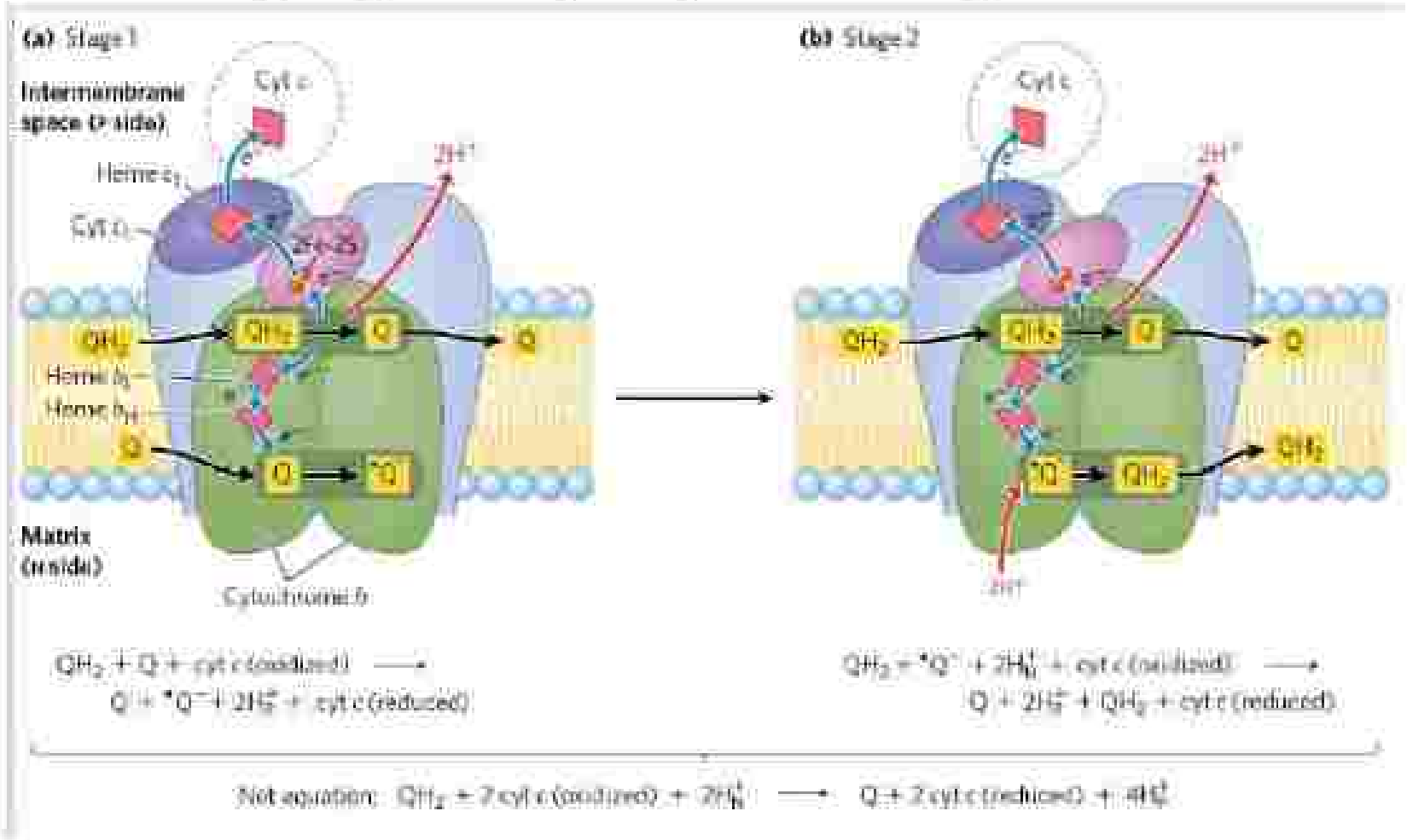

Intermembrane space (+ side)
Complex I
Membrane arm
Matrix (– side)


Intermembrane space (+ side)
Matrix (– side)


Intermembrane space (+ side)
Matrix (– side)


(b)
(a)
Intermembrane space (+ side)
Matrix (– side)

Q cycle- proposed by Mitchell



Q cycle. As electrons move from QH_2 through Complex III, QH_2 is oxidized with the release of protons on one side of the membrane (at Q_P), while at the other site (Q_N), Q is reduced and protons are taken up.

- Electrons reach Q through Complexes I and II
- Reduced Q (QH_2) serves as a mobile carrier of electrons and protons.
- It passes electrons to Complex III, which passes them to another mobile connecting link, cytochrome *c*
- Complex IV then transfers electrons from reduced cytochrome *c* to O_2.
- Electron flow through Complexes I, III, and IV is accompanied by proton efflux from the matrix into the intermembrane space.

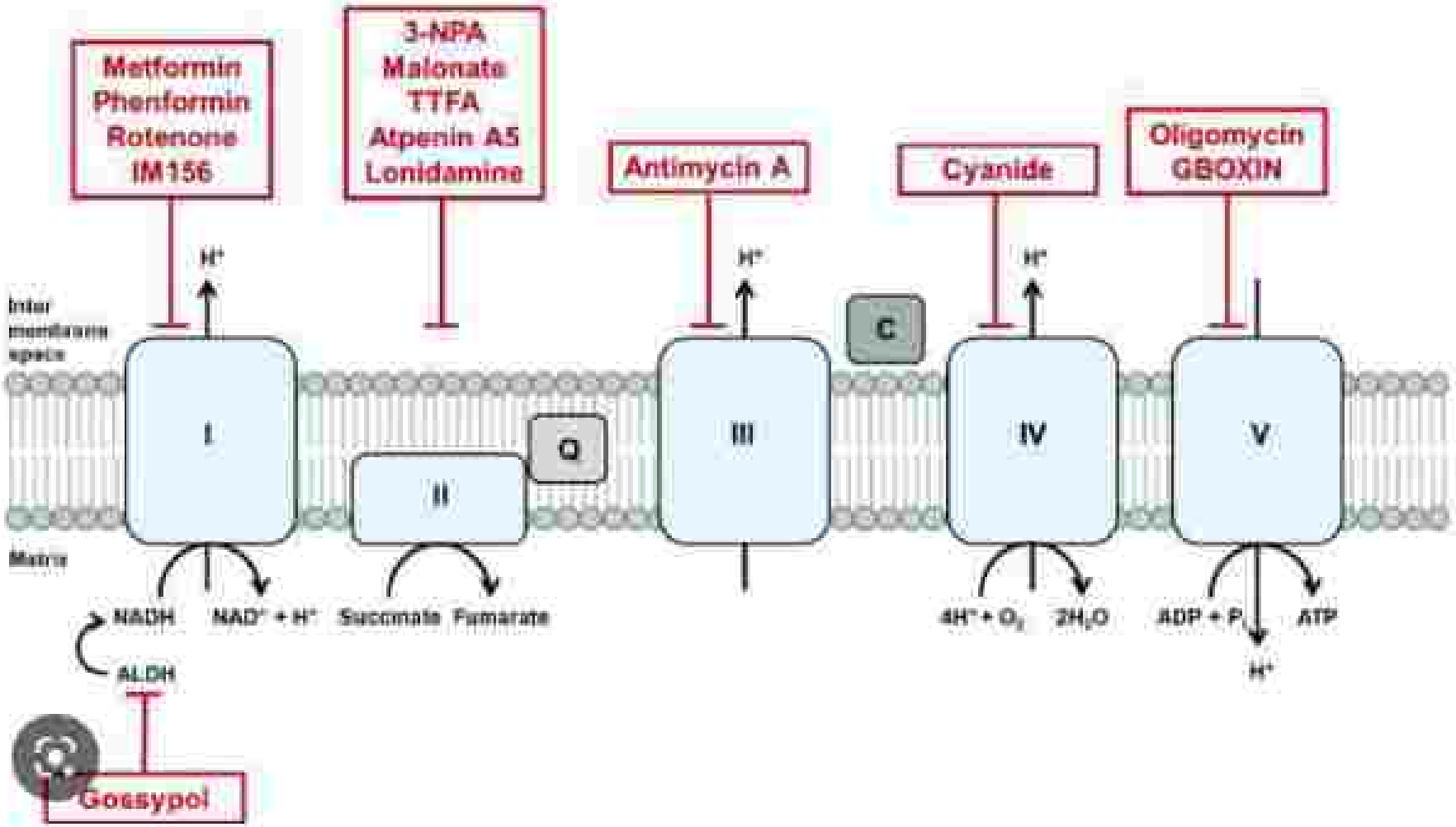
- **Chemiosmotic coupling:** Coupling of ATP synthesis to electron transfer by a transmembrane difference in charge and pH.

Inhibitors of ETC and ATP synthesis

Complex name	Inhibitors	
Complex I - NADH dehydrogenase **(NADH:ubiquinone oxidoreductase)**	Amytal, rotenone (an insecticide), and piericidin A (an antibiotic) inhibit electron flow from the Fe-S centers of Complex I to ubiquinone.	2,4. Dinitrophenol – uncoupling agent
Complex II -Succinate dehydrogenase	Carboxin	Pentachlorophenol - UPA
Complex III- Ubiquinone: cytochrome *c* oxidoreductase	Antimycin A	Oligomycin – Inhibits ATP synthase
Cytochrome *c*		
Complex IV- Cytochrome oxidase	Cyanide, CO, Azide	

Name	Function	Site of Action
Rotenone	e^- transport inhibitor	Complex I
Amytal	e^- transport inhibitor	Complex I
Antimycin A	e^- transport inhibitor	Complex III
Cyanide	e^- transport inhibitor	Complex IV
Carbon monoxide	e^- transport inhibitor	Complex IV
Azide	e^- transport inhibitor	Complex IV
2,4 Dinitrophenol	Uncoupling agent	Transmembrane H^+ carrier
Pentachloro-phenol	Uncoupling agent	Transmembrane H^+ carrier
Oligomycin	Inhibits ATP synthase	OSCP fraction of ATP synthase

Inhibitors of ETC and ATP synthesis



PROTON – MOTIVE FORCE

The free-energy change for the creation of an electrochemical gradient by an ion pump is:

$\Delta G = RT \ln(C2/C1) + ZF \Delta\psi$

C2 and C1 are the concentrations of an ion in two regions. C2 > C1; Z is the absolute value of its electrical charge (1 for a proton); and $\Delta\psi$ is the transmembrane difference in electrical potential, measured in volts.

For protons,

$\ln (C2/C1) = 2.3 (\log[H+]P - \log[H+]N)$

$= 2.3 (pHN - pHP)$

$= 2.3 \Delta pH$

Further, it reduces to $\Delta G = 2.3RT \Delta pH + F\Delta\psi$

In actively respiring mitochondria, the measured $\Delta\psi$ is 0.15 to 0.20 V, and the pH of the matrix is about 0.75 units more alkaline than that of the intermembrane space.

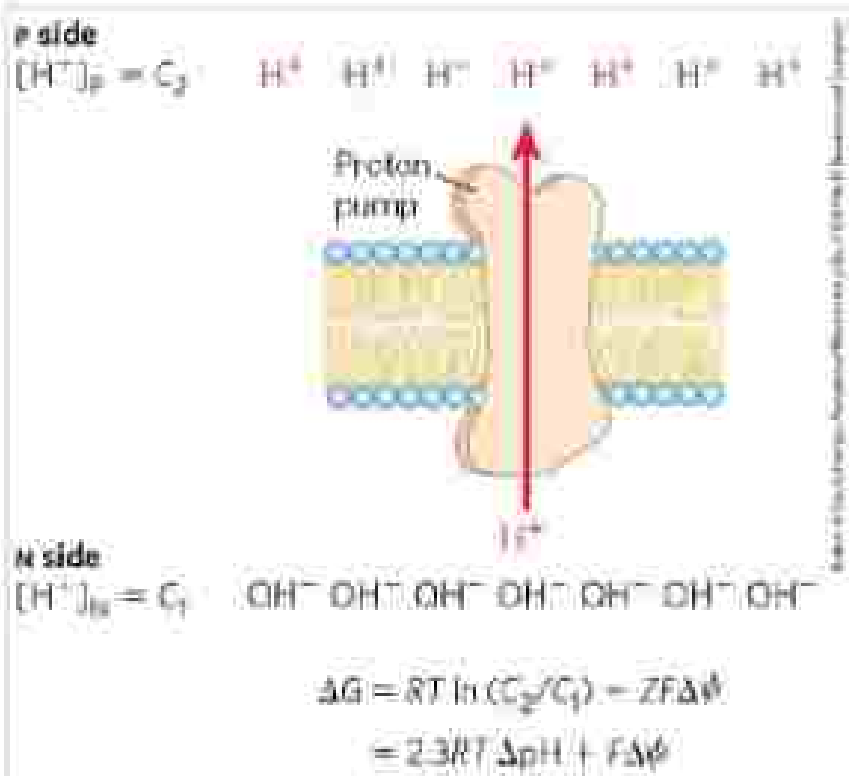


Figure: Proton-motive force. The inner mitochondrial membrane separates two compartments of different [H.], resulting in differences in chemical concentration (ΔpH) and charge distribution (Δψ) across the membrane.

ATP SYNTHESIS BY ATP SYNTHASE

- **chemiosmotic model**: Peter Mitchell
- the proton-motive force — drives the synthesis of ATP as protons flow passively back into the matrix through a proton pore in **ATP synthase**.
- It is represented as
- $ADP + P_i + nH^+_P \rightarrow ATP + H_2O + nH^+_N$
- F-type ATPase/complex V
- F1, a peripheral membrane protein, and
- Fo (*o* denoting oligomycin-sensitive), which is integral to the membrane.
- Efraim Racker : F1 component in 1960s

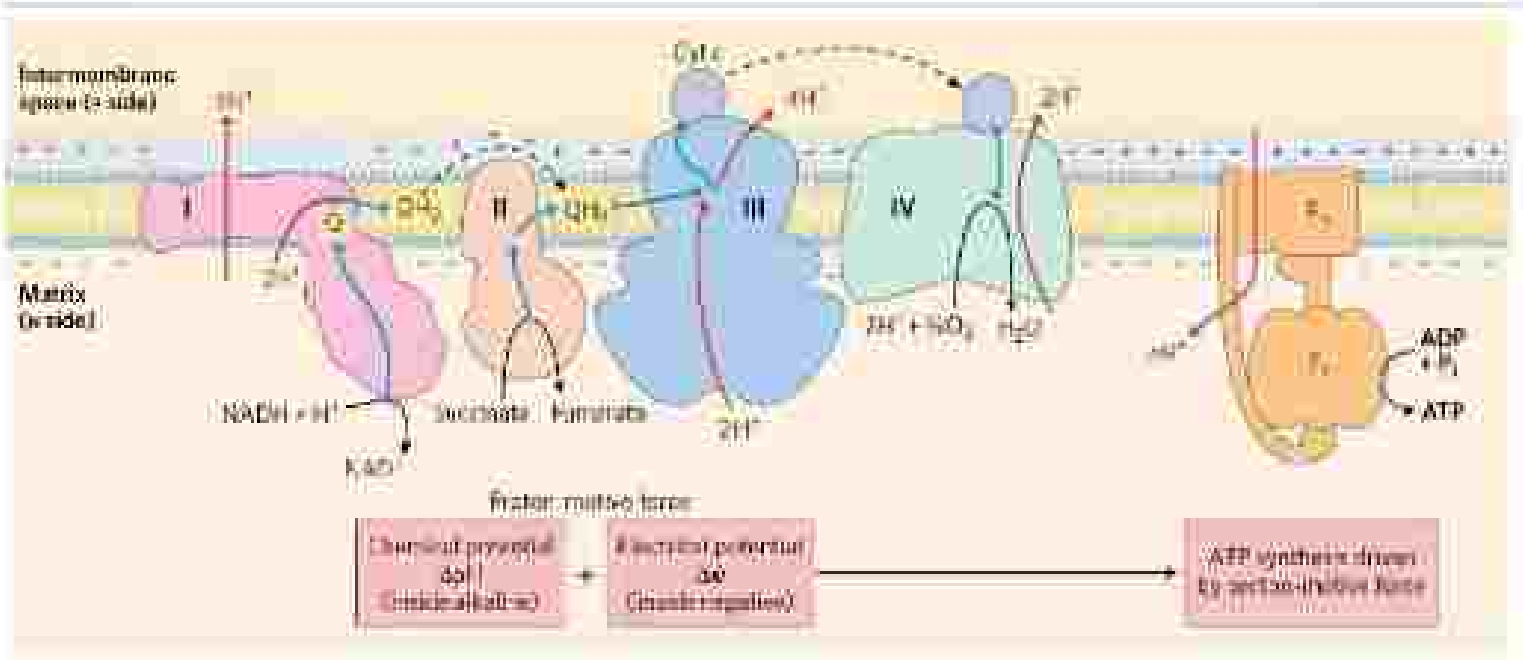
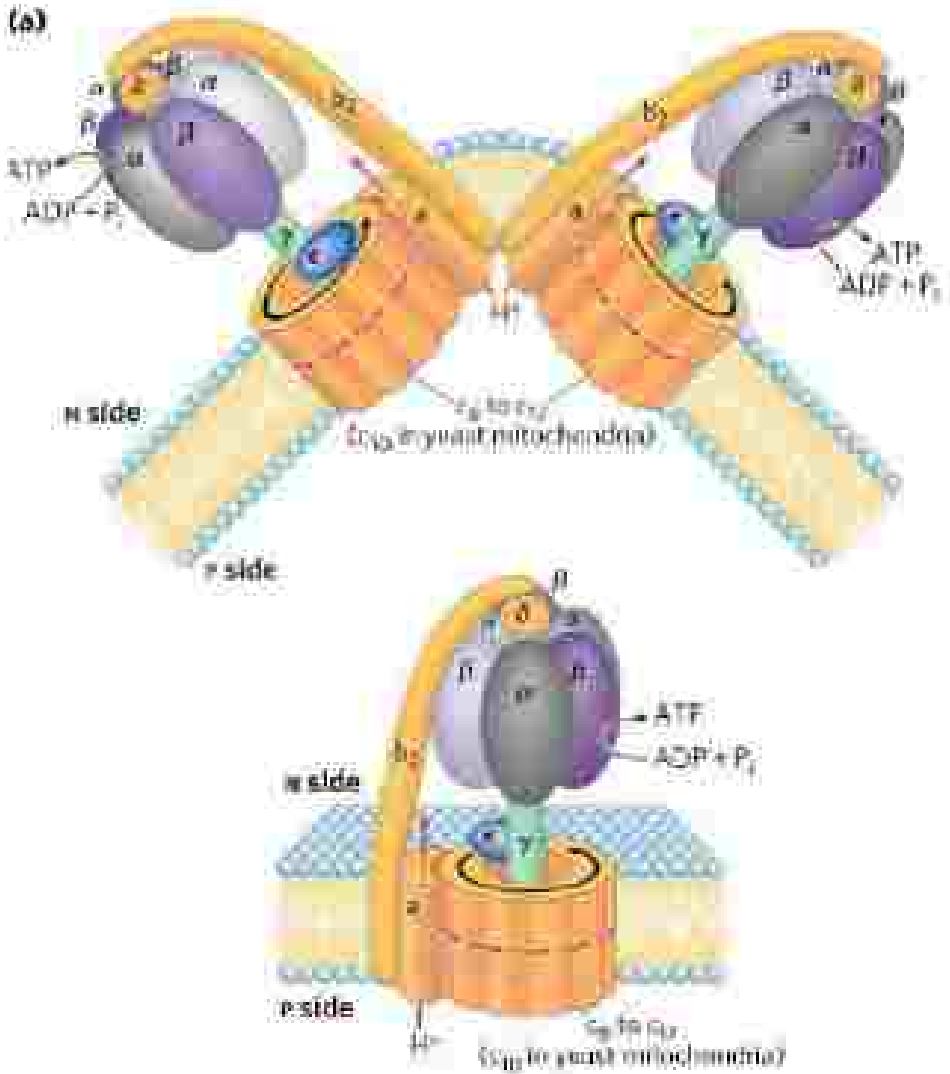


Fig: chemiosmotic model: In this simple representation of the chemiosmotic theory applied to mitochondria, electrons from NADH and other oxidizable substrates pass through a chain of carriers arranged asymmetrically in the inner membrane. Electron flow is accompanied by proton transfer across the membrane, producing both a chemical gradient (ΔpH) and an electrical gradient ($\Delta\psi$), which, combined, create the proton-motive force. The inner mitochondrial membrane is impermeable to protons; protons can reenter the matrix only through proton-specific channels (F_o). The protonmotive force that drives protons back into the matrix provides the energy for ATP synthesis, catalyzed by the F_1 complex associated with F_o.

ATP SYNTHESIS - ATP SYNTHASE



- F1: three β & α subunits. One δ units, central shaft - γ subunit.
- δ subunit - oligomycin sensitivity
- Fo – has a c ring, made up of a number C subunits, small, hydrophobic proteins; provides path for proton movement.
- Paul Boyer proposed a **rotational catalysis** mechanism ---- ATP synthesis

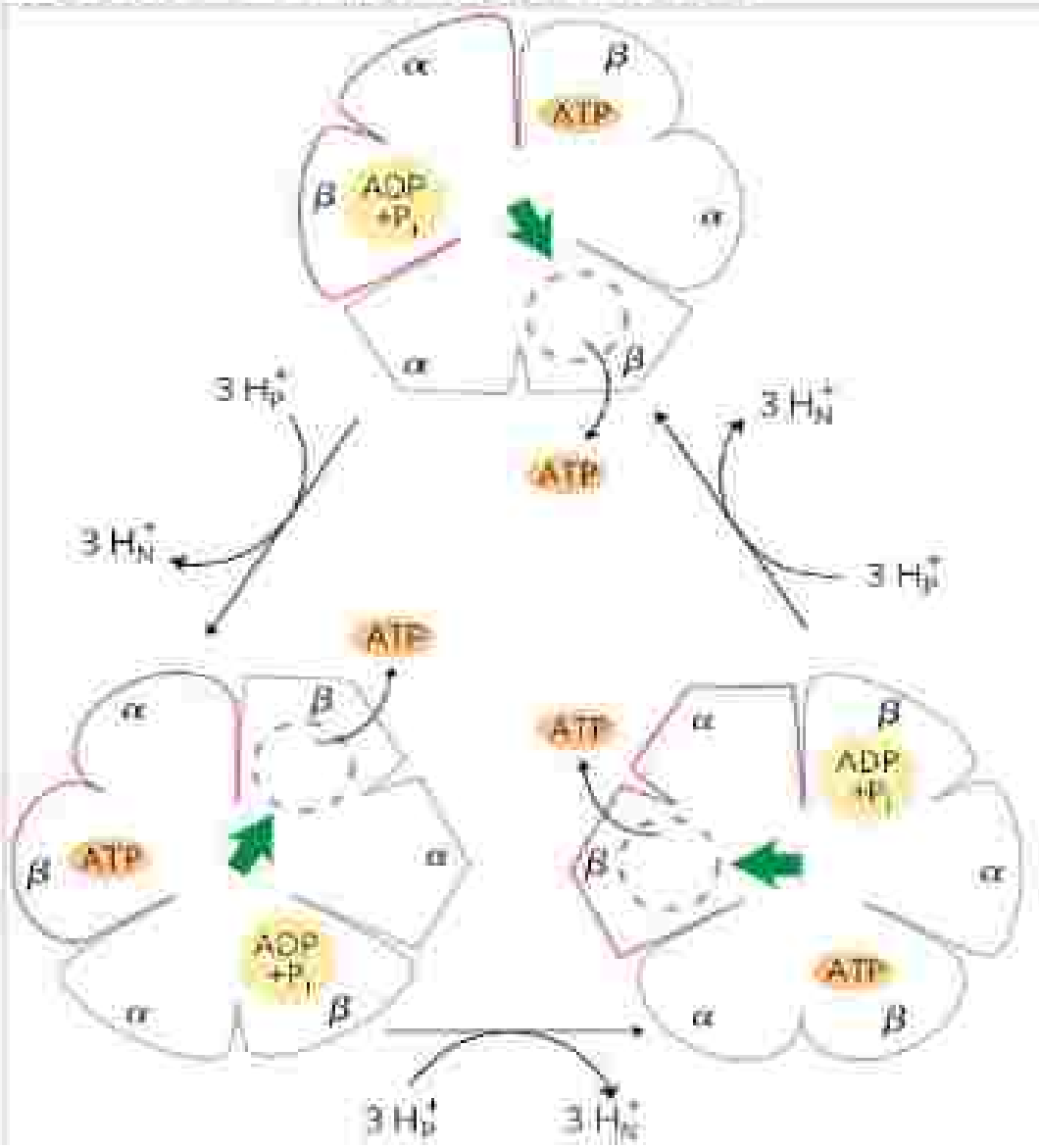
BINDING-CHANGE MODEL OF ATP SYNTHASE DURING ATP SYNTHESIS

The F1 complex has three nonequivalent adenine nucleotide–binding sites, one for each pair of α and β subunits. At any given moment, one of these sites is in the β-ATP conformation (which binds ATP tightly), a second is in the β-ADP (loose-binding) conformation, and a third is in the β-empty (very-loose-binding) conformation.

In this view from the N side, the proton-motive force causes rotation of the central shaft — the γ subunit, shown as a green arrowhead — which comes into contact with each αβ subunit pair in succession.

This produces a cooperative conformational change in which the β-ATP site is converted to the β-empty conformation, and ATP dissociates; the β-ADP site is converted to the β-ATP conformation, which promotes condensation of bound ADP + P_i to form ATP; and the β- empty site becomes a β-ADP site, which loosely binds ADP + Pi entering from the solvent.

Note that the direction of rotation reverses when the ATP synthase is acting as an ATPase



Regulation mechanism

- In hypoxic (oxygen-deprived) cells, a protein inhibitor blocks ATP hydrolysis by the reverse activity of ATP synthase, preventing a drastic drop in [ATP].
- The adaptive responses to hypoxia, mediated by HIF-1, slow electron transfer into the respiratory chain and modify Complex IV to act more efficiently under low-oxygen conditions.
- ATP and ADP concentrations set the rate of electron transfer through the respiratory chain via a series of coordinated controls on respiration, glycolysis, and the citric acid cycle.

Additional information…..

- The **cytochromes** are proteins with characteristic strong absorption of visible light, due to their iron-containing heme prosthetic groups.
- cytochromes, designated *a*, *b*, and *c*,
- type *a* cytochromes : longest wavelength 600nm;
- type *b* cytochromes: 560nm
- type *a* cytochromes: 550nm

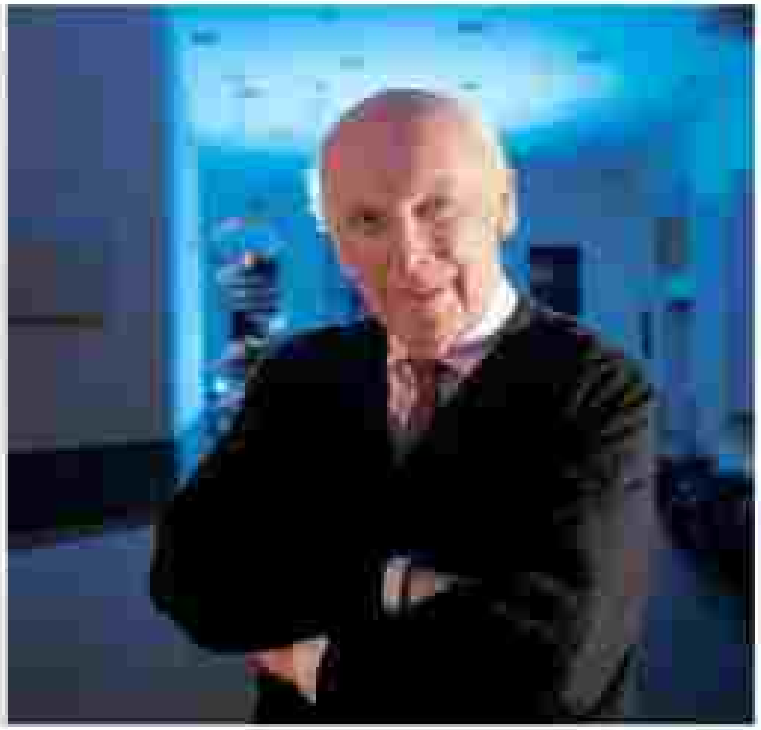
The ratio of ATP synthesized per $\frac{1}{2}O_2$ reduced to H_2O (the P/O ratio) is about 2.5 when electrons enter the respiratory chain at Complex I, and 1.5 when electrons enter at ubiquinone. This ratio varies among species, depending on the number of c subunits in the F_o complex.

Additional Information….

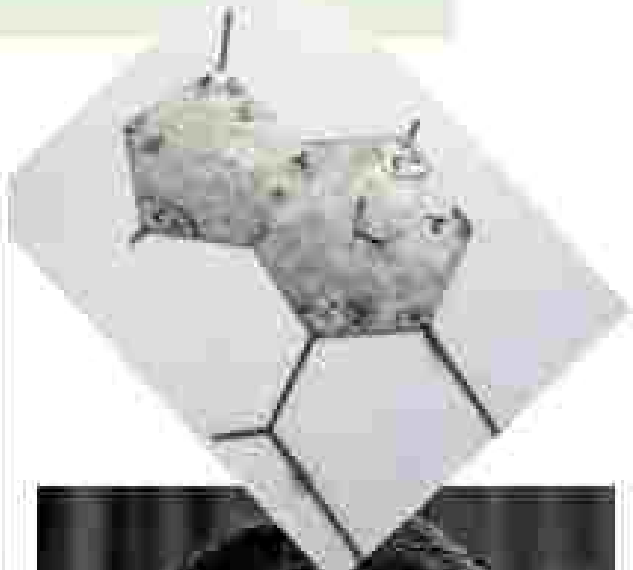
- **iron-sulfur protein:** One of a large family of electron-transfer proteins in which the electron carrier is *one or more iron ions associated with two or more sulfur atoms of Cys residues or of inorganic sulphide.*
- participate in one-electron transfers in which one iron atom of the Fe-S cluster is oxidized or reduced.
- Eight Fe-S proteins function in mitochondrial electron transfer
- The reduction potential of Fe-S proteins varies from −0.65V to +0.45V, depending on the microenvironment of the iron within the protein.
- **Rieske iron-sulfur protein:** A type of iron-sulfur protein in which two of the ligands to the central *iron ion are His side chains*; act in many electron-transfer sequences. including oxidative phosphorylation and photophosphorylation.

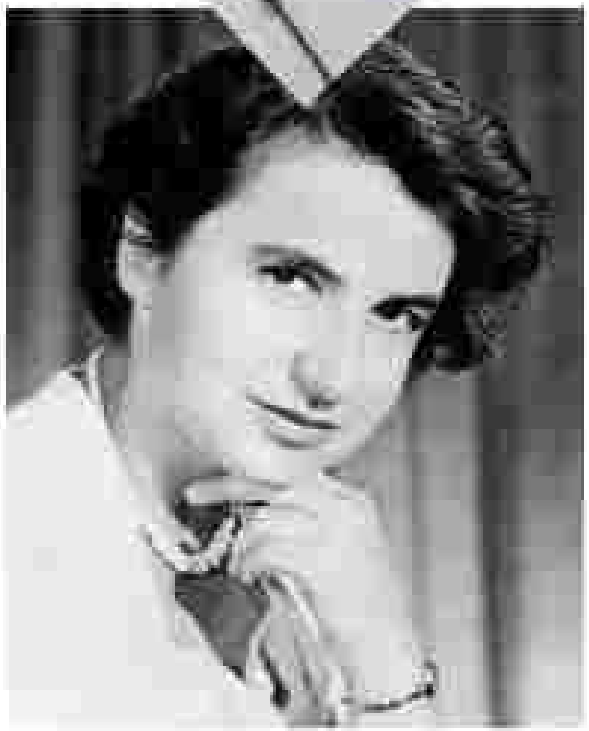
Nucleosides, nucleotides, nucleic acids - structure, diversity and function

The Swiss scientist Friedrich Miescher discovered nucleic acids (DNA) in 1869.[1] Later, he voiced the idea that they could be involved in heredity.[1]



Francis Crick, 1916–2004





A structure this pretty just had to exist.
James Watson, The Double Helix,1968

Nucleosides, nucleotides, nucleic acids - structure, diversity and function

- Nucleic acids
- Friedrich Meischer (1869)
- These are biopolymers, or small biomolecules, held by 3' & 5' Phosphate bridges and has high molecular weight.
- Made up Nucleotides: energy currency : DNA, RNA's

Importance:….

- Chemical links in the response of cells to hormones
- Involved in every facet of cellular life
- Structural components: some co-enzyme, cofactors, b-complex vitamins and metabolic intermediates
- Constituents of nucleic acids: molecular repositories & functional expressions of biological information.
- Storage, transfer and decoding of genetic materials : DNA/RNA.

Two types – nucleic acid

- DNA – deoxyribo nucleic acid
- RNA – Ribo nucleic acids

Made up of – ***Nucleotides***

Nitrogenous base linked to a 1C of ribose /deoxyribose *sugar* by glycoside bond, along with at least a *Phosphate group*

Nucleoside: Nitrogenous base linked 1C of ribose /deoxyribose sugar by glycoside bond

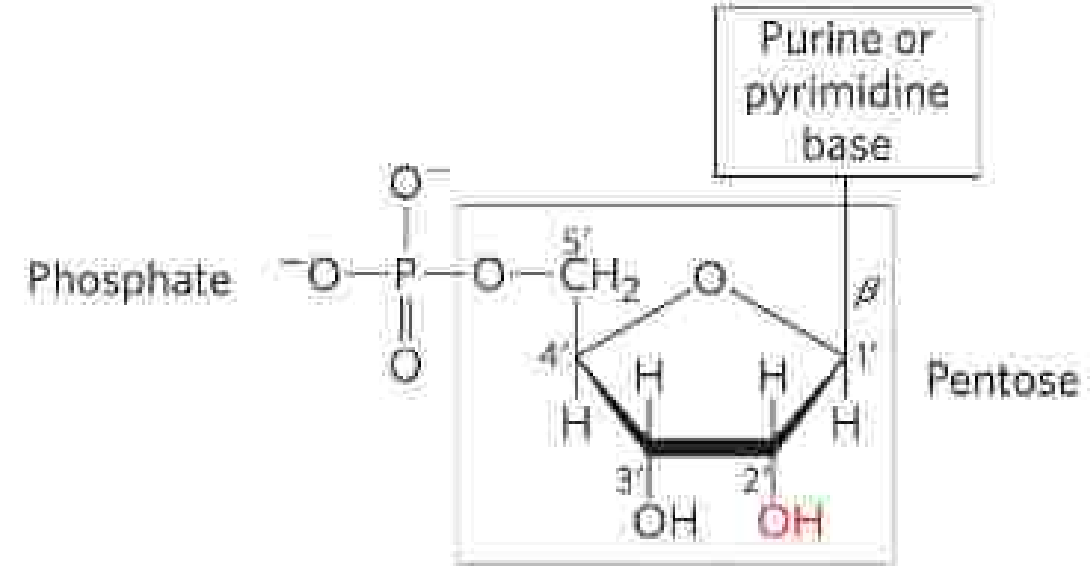
* The base of a nucleotide is joined covalently (at N-1 of pyrimidines and N-9 of purines) in an *N*-β-glycosyl bond to the 1′ carbon of the pentose, and the phosphate is esterified to the 5′ carbon.

* The *N*-β-glycosyl bond is formed by removal of the elements of water.

Nucleoside and Nucleotide

Nucleotide

- (1) A nitrogenous (nitrogen-containing) base
- (2) A pentose sugar
- (3) One or more phosphate group

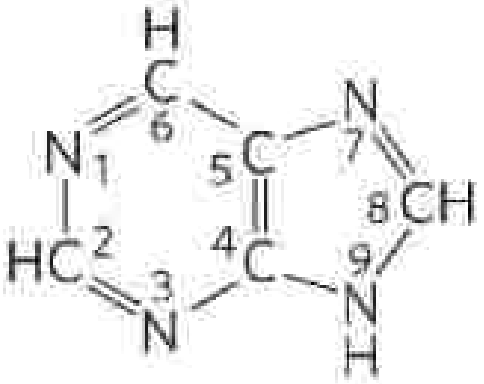


(a)

Nucleoside

- (1) A nitrogenous (nitrogen-containing) base
- (2) A pentose sugar

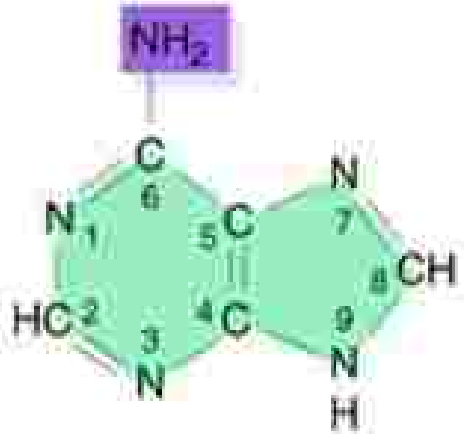
A nitrogenous (nitrogen-containing) base



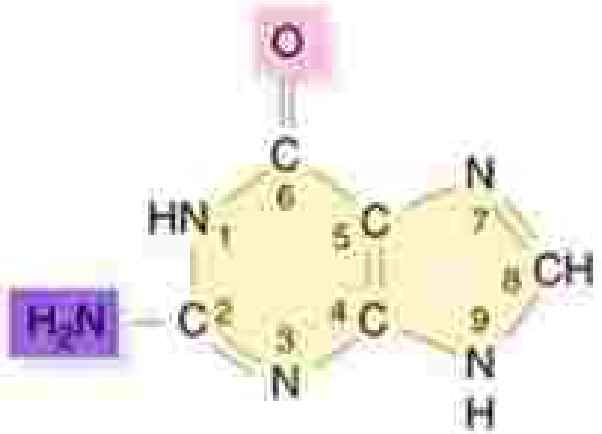
Purine

Planar, aromatic, heterocyclic molecules (N).
Two types: Purine & Pyrimidine.
Purines: is a heterocyclic aromatic organic compound that consists of a pyrimidine ring fused to an imidazole ring.

2 types:
Adenine-6-amino purine
Guanine: 6-oxy-2-amino purine



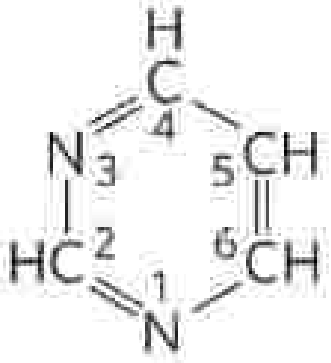
Adenine (A)



Guanine (G)

A nitrogenous (nitrogen-containing) base

Pyrimidine: heterocyclic aromatic Single ring structure



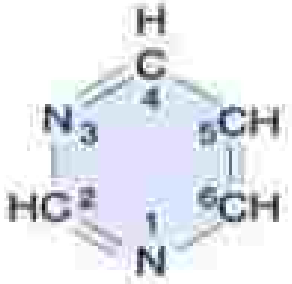
Pyrimidine

3 TYPES:

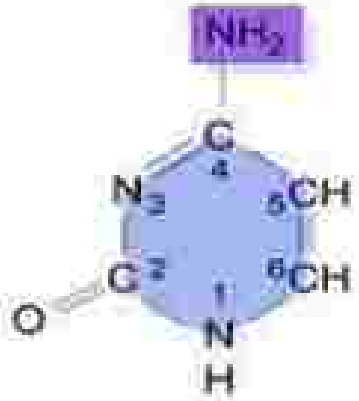
Cytosine (C): 2-oxy,4aminoPyrimidine; DNA/RNA

Uracil (U): 2,4–dioxy-Pyrimidine; RNA

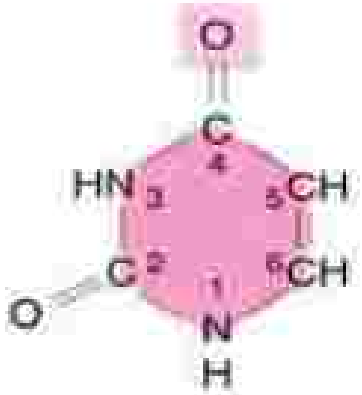
Thymine (T): 2,4–dioxy-5methylPyrimidine; DNA



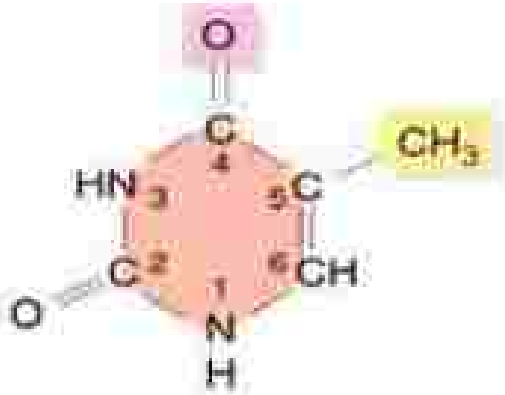
Pyrimidine



Cytosine (C)



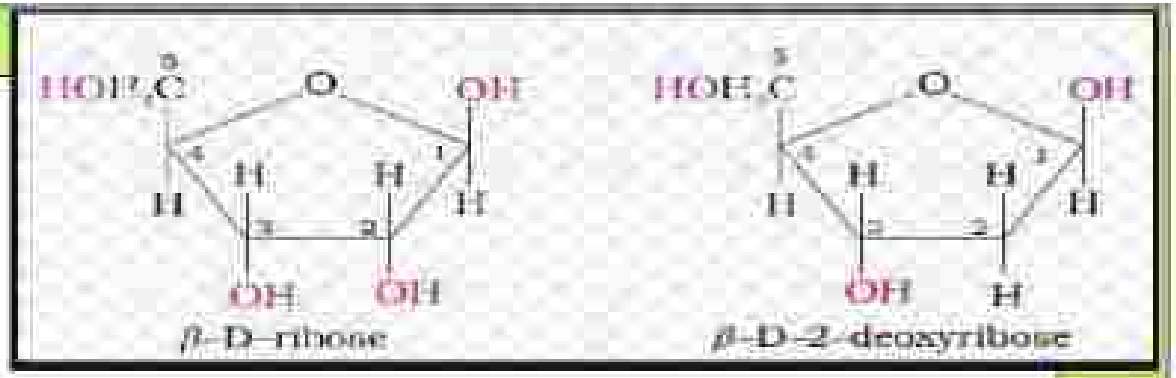
Uracil (U)
(found in RNA)



Thymine (T)
(found in DNA)

SUGAR: 2TYPES

- DeoxyRibose; DNA
- Ribose; RNA
- β-D Ribose; β-D deoxyRibose
- 5 Carbon atom
- Stable structure

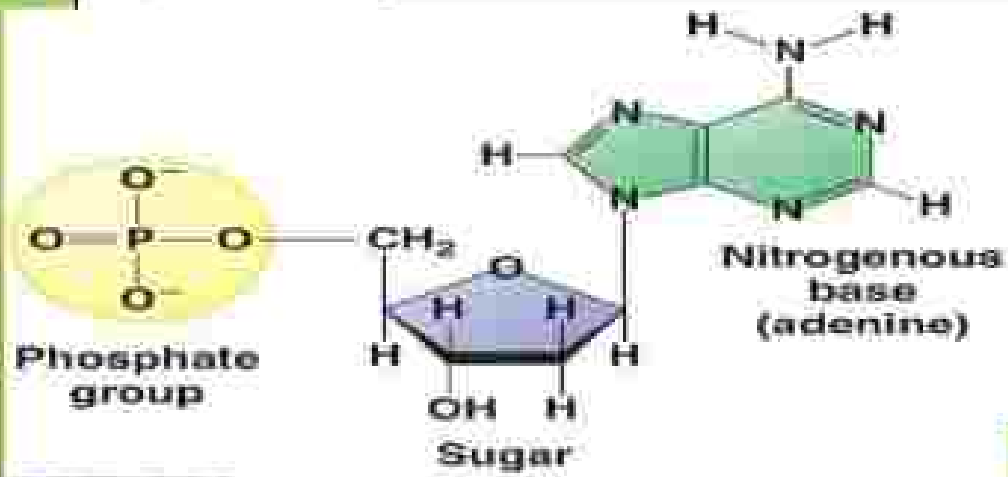


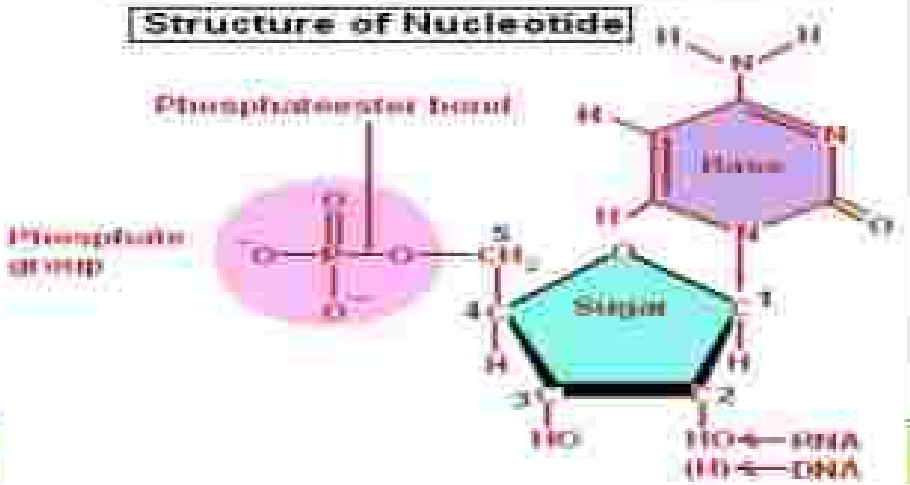
Bonding:

III. Phosphate group
Imparts negative charge.
Forms P-di-ester bond b/w two ribose @ C5 in DNA.

A & G: *N9 atom links to 1C β-D* ribose sugar by *glycosidic bond*

U,C & T: *N1 atoms links to 1C ribose* sugar by *glycosidic bond*





Nucleotide and Nucleic acid Nomenclature

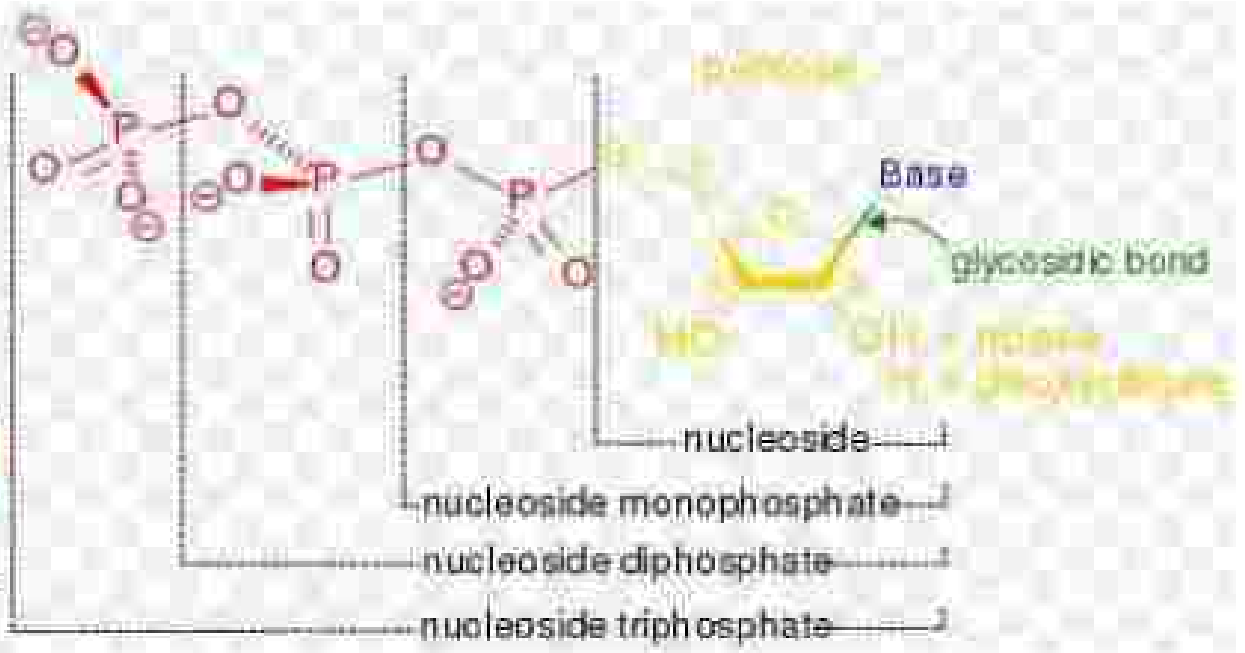


TABLE 8–1 Nucleoside and Nucleic Acid Nomenclature

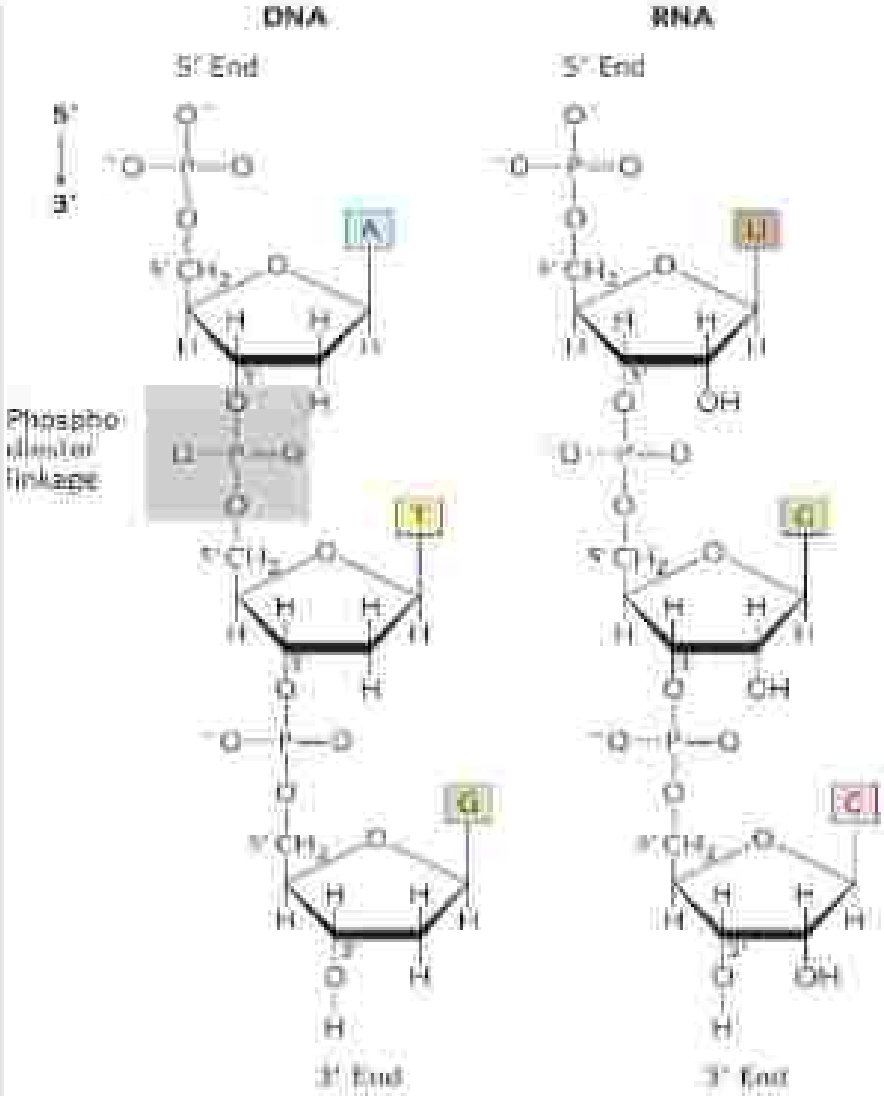
Base	Nucleoside	Nucleotide	Nucleic acid
Purines			
Adenine	Adenosine	Adenylate	RNA
	Deoxyadenosine	Deoxyadenylate	DNA
Guanine	Guanosine	Guanylate	RNA
	Deoxyguanosine	Deoxyguanylate	DNA
Pyrimidines			
Cytosine	Cytidine	Cytidylate	RNA
	Deoxycytidine	Deoxycytidylate	DNA
Thymine	Thymidine or deoxythymidine	Thymidylate or deoxythymidylate	DNA
Uracil	Uridine	Uridylate	RNA

NITROGENOUS BASES AND THEIR DERIVATIVES FOUND IN RNA AND DNA

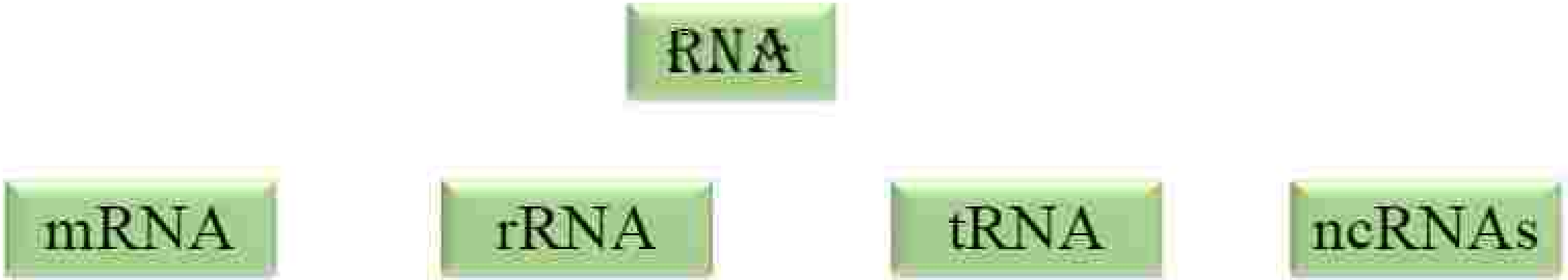
Base	Ribonucleoside	Ribonucleotide	Deoxyribonucleoside	Deoxyribonucleotide
Adenine (A)	Adenosine	Adenosine monophosphate (AMP)	Dexoyadenosine	Deoxyadenosine monophosphate (dAMP)
Guanine (E)	Guanosine	Guanosine monophosphate (GMP)	Deoxyguanosine	Deoxyguanosine monophosphate (dGMP)
Cystosine (C)	Cytidine	Cytidine monophosphate (UMP)	Deoxycytidine	Deoxycytidine monophosphate (dCMP)
Uracil (U)	Uridine	Uridine monophosphate (UMP)	Deoxyuridine	Deoxyuridine monophosphate (dUMP)
Thymine (T)	Thymidine	Thymidine monophsophate (TMP)	Deoxythymidine	Deoxythymidine monophosphate (dTMP)

Phosphodiester Bonds Link Successive Nucleotides in Nucleic Acids

- 5′- phosphate group of one nucleotide unit is joined to the 3′- hydroxyl group of the next nucleotide; covalent bonds: phosphodiester linkage
- alternating phosphate and pentose residues,
- Nitrogenous bases - side groups.
- The backbones of both DNA and RNA are hydrophilic.
- The hydroxyl groups of the sugar residues form hydrogen bonds with water.
- The phosphate groups, with a pKa near 0, are completely ionized and negatively charged at pH 7, and the negative charges are generally neutralized by ionic interactions with positive charges on proteins, metal ions, and polyamines.



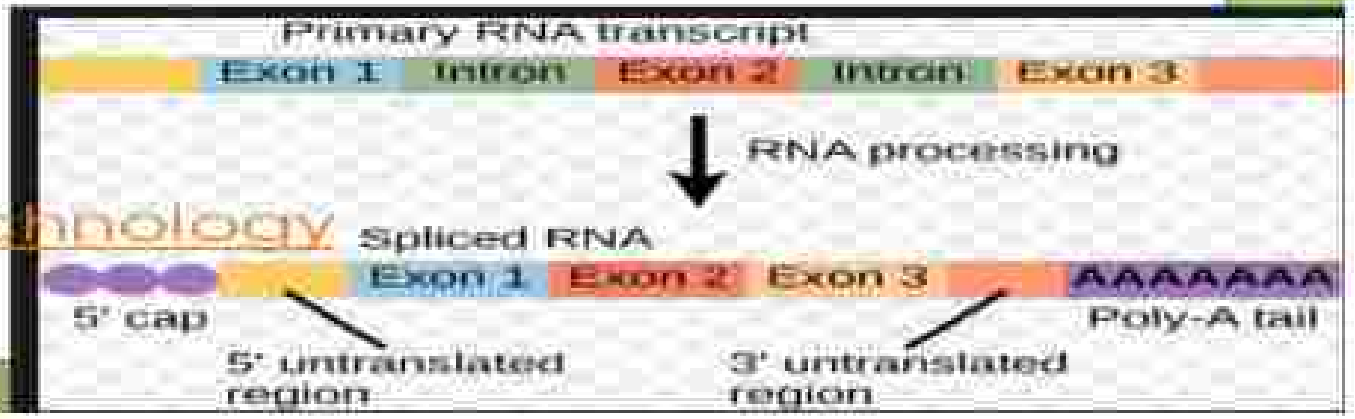
Diversity of RNA and their function…..



Diversity of RNA and their function….
mRNA

RIBONUCLEIC ACID – TYPES
a. mRNA (messenger RNA)

- large family of RNA molecules
- Convey the Genetic information for biosynthesis of proteins
- Transcribed in Nucleus in native form by the enz RNA Polymerase, processed & transported to cytoplasm.
- Exons: coding seq; introns: noncoding;
- Only exons (triplet codon)- mature mRNA;
- 5' (UTR) capping & 3' (UTR) Poly A tail
- discovered :
- Jacob, Sydney Brenner
- Matthew Meselson
- California Institute of Technology
- 1961..



Diversity of RNA and their function….
rRNA

RIBONUCLEIC ACID – TYPES
b. rRNA (ribosomal RNA)

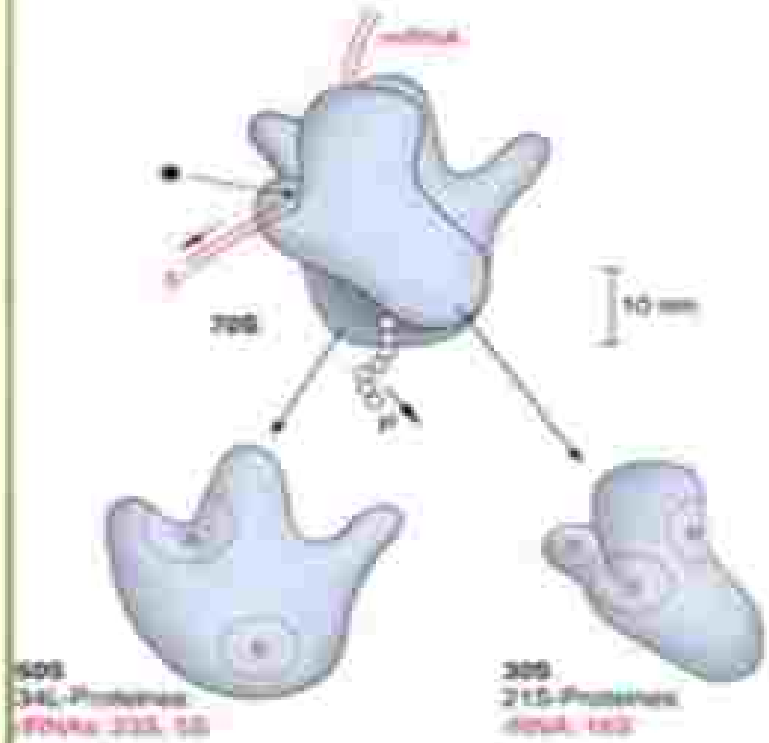
- component of the ribosome
- predominant
- 60% rRNA and 40% protein by weight,
- has two subunits, the large subunit (LSU) and small subunit (SSU).
- Helps in protein synthesis
- Ribosomal proteins – Core and split proteins
- Enzymes - Initiation factors: IF1, IF2, IF3;
- Elongation factors: Tu, Td, G & peptidyl transferase; termination factors: R1 and R2
- Metal ions like: Mg, Ca, Mn, etc.,
- RNA characteristics are important in evolution
- rRNA is the target of numerous clinically relevant antibiotics

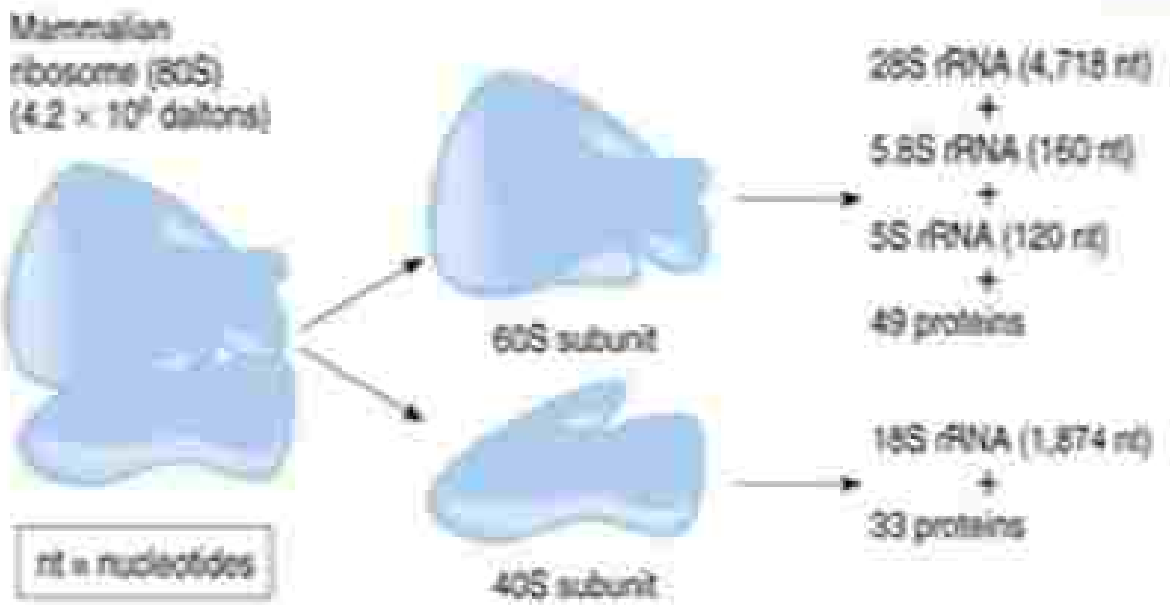
Diversity of RNA and their function….
rRNA

TYPES -BASED ON SEDIMENTATION RATE, TWO TYPES

1. 70S – Prokaryotes
2. 80S – Eukaryotes

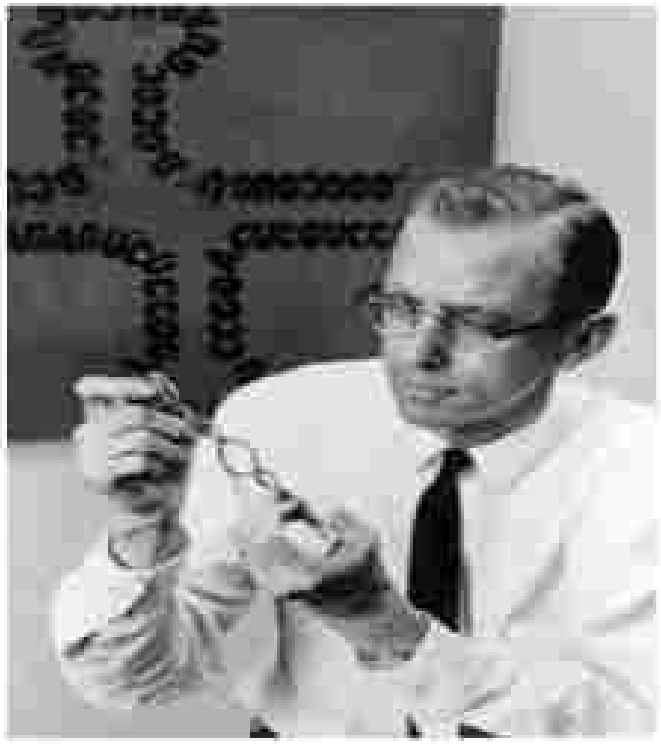
Nobel prize 2009 for studies of the structure and function of the ribosome



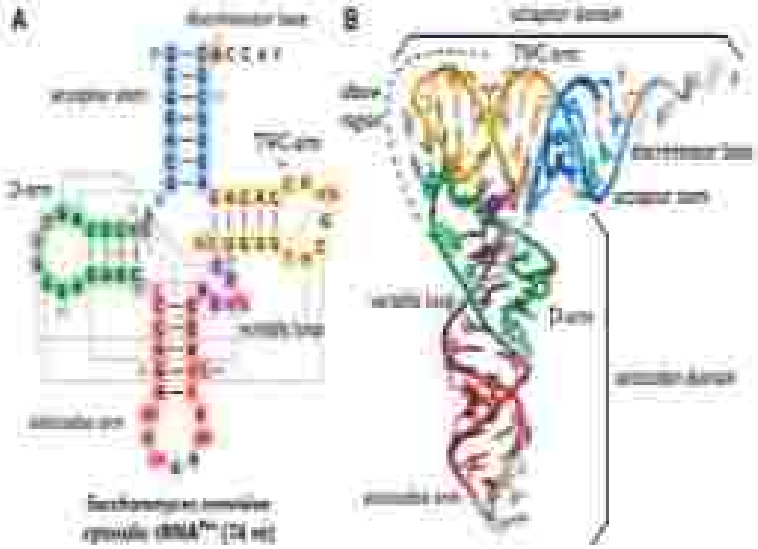


Diversity of RNA and their function….
tRNA(transfer RNA)

- Adaptor RNA
- Interface b/w NA language to Protein language
- Present in cytosol and organelles
- Robert Holley and co-workers 1st seq the tRNA in 1965

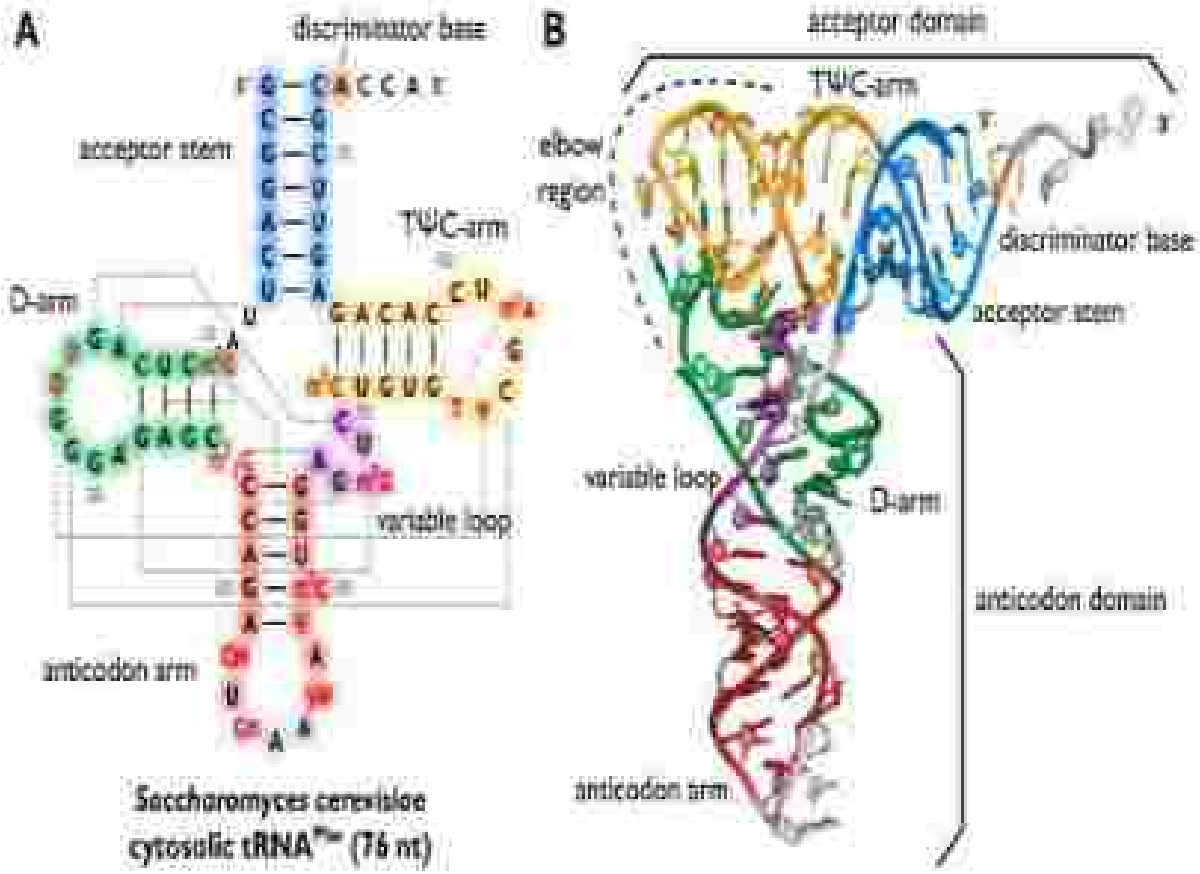


American biochemist
Ala-tRNA
Nobel Prize in Medicine and Physiology -1968

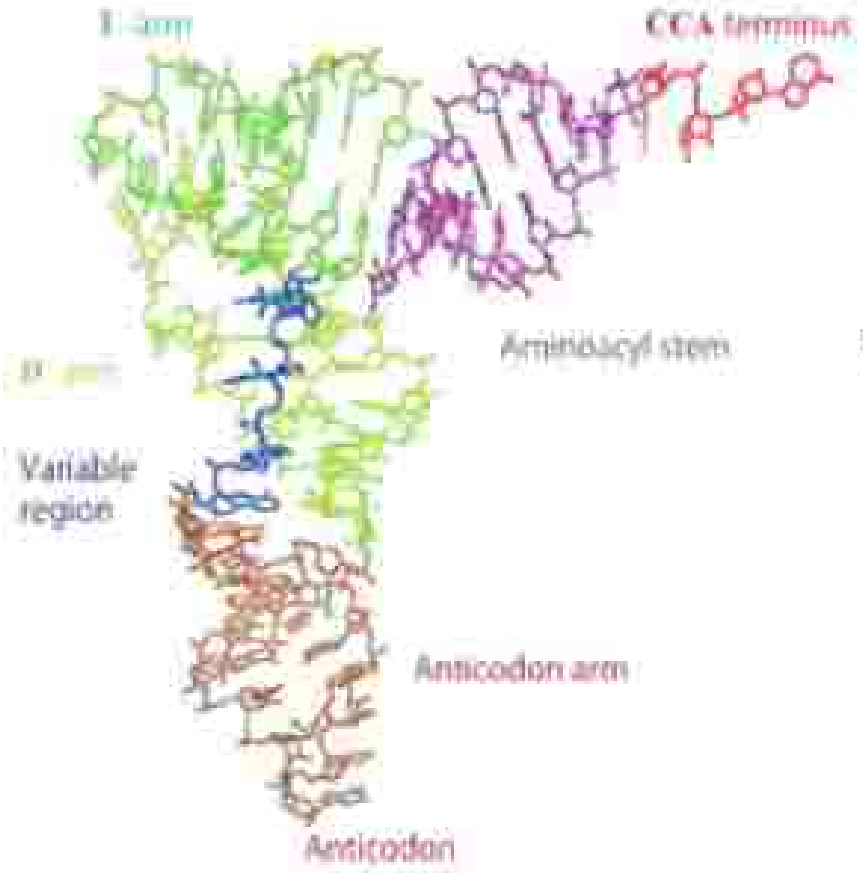


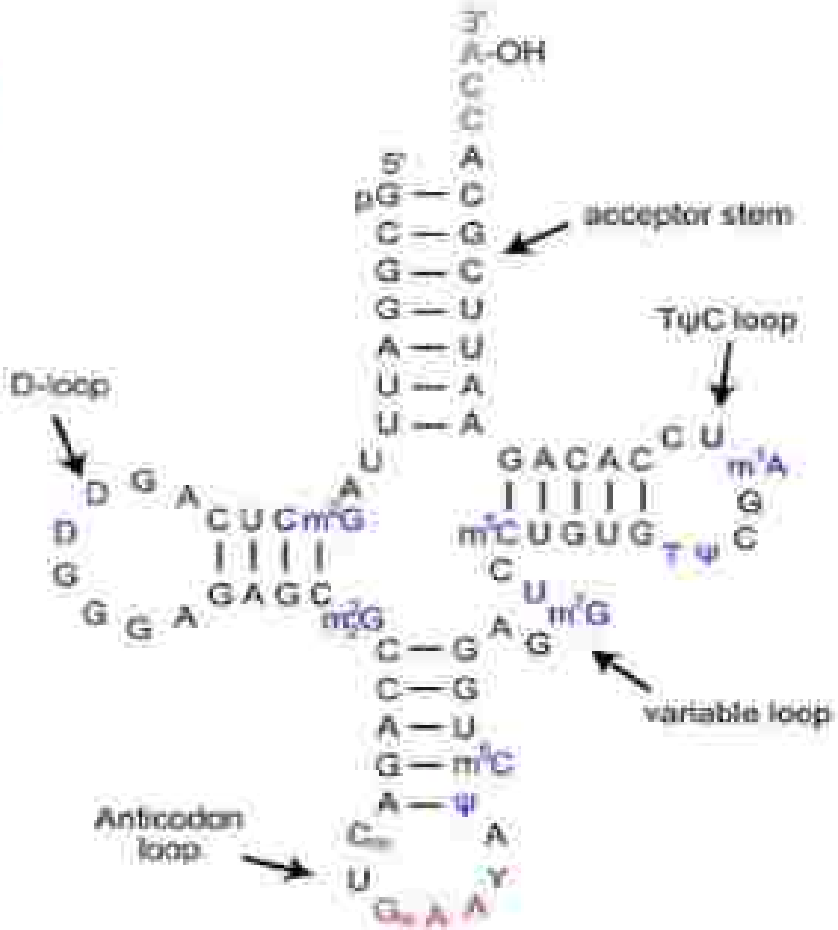
Diversity of RNA and their function….
tRNA(transfer RNA)

- Decode a messenger RNA
- Consist of 76 to 90 nucleotides in length
- Its a physical link between the mRNA and the amino acid sequence of proteins
- Structurally -primary structure, secondary structure, (*clover leaf structure*), and tertiary structure(L)
- The lengths of each arm, as well as the loop 'diameter', in a tRNA molecule vary from species to species



Diversity of RNA and their function….
tRNA(transfer RNA)



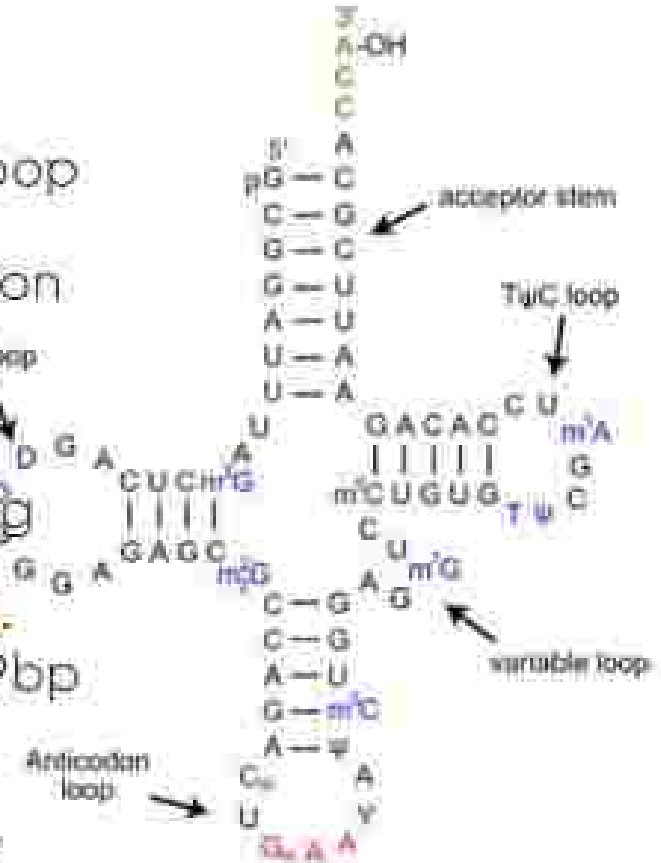


RIBONUCLEIC ACID – TYPES
c. tRNA (transfer RNA)

- Contains usual bases as a result of enzymatic modification –PTM
- Unpaired bases – loop structure, rich in usual bases.
- These loops serves as potential recognition sites for various proteins.
- The folded structure is due to the presence of complementary base in the other side.
- The usual bases are: D-dihydrouridine (D arm)
  m^2G- methylguanosine; $m^2{}_2G$-dimethylguanosine
  mC-methycytosine; Ψ –pseudouridine, T-ribothymidine
- Transfer RNAs have a sugar-phosphate backbone

The crystal structure of tRNA elucidates other important points:

- conserved structural features:
- 5'P: phospho guanine (7 base)
- D arm:4- to 6-bp stem ending in a loop that often contains dihydrouridine.
- Anticodon arm: 5-bp stem, anticodon with usual base.
- variable arm: length varies (4 – 21 nucleotides)
- TΨC arm: 4- to 5- bp stem containing the sequence TΨC where Ψ is pseudouridine, a modified uridine.
- 3' amino acid acceptor stem:7- to 9bp stem
- CCA tail is a cytosine–cytosine–adenine sequence at the 3' end to form aminoacyl-tRNA



Function

- helps decode a messenger RNA (mRNA) seque into a protein.
- tRNAs function at specific sites in the ribosome d translation.
- The codon on mRNA is specific for each a. a and recognized by anticodon region of specific tRNA.
- the D-arm and T-arm, contribute to the high level of specificity and efficiency.
- The D-arm is a highly variable region and plays an important role in stabilizing the RNA's tertiary structure and also influences the *kinetics and accuracy of translation at the ribosome*
- The T-arm is involved in the interaction of tRNA with the ribosome.

Function

- Pseudouridine - structural integrity by stiffening the nearby sugar-phosphate backbone.
- Recognition of codon is by anticodon site – wobble hypothesis
- Amino acid activation

A.A are covalently linked to 3' end by the enz amino acyl tRNA synthase (aaRS)

activates the correct a.a to be incorporated.

2steps reaction:

a. ATP+ a.a → aminoacyl-AMP+Ppi
b. aminoacyl-AMP + tRNA → aminoacyl-tRNA + AMP

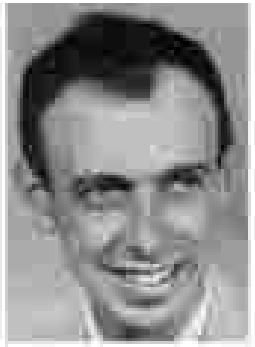
Structure of DNA - Major form of B form

Watson, Crick, & Roseland Franklin.

Based on X ray diffraction analysis of crystals of duplex oligonucleotides.

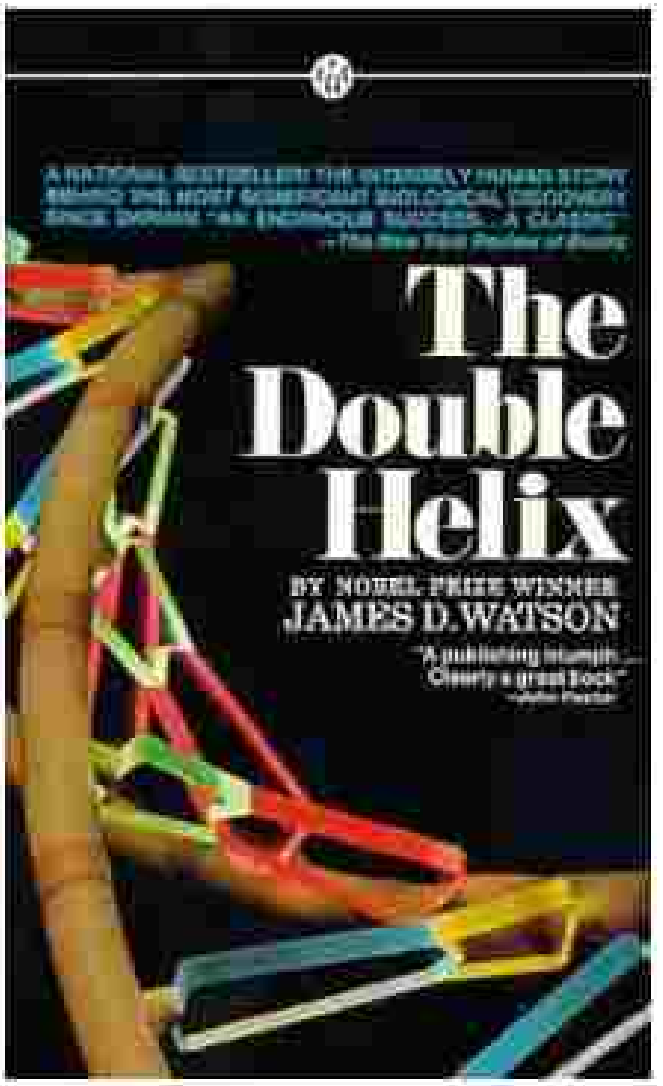
Also called as Watson and Crick model;
Nobelprize-1962

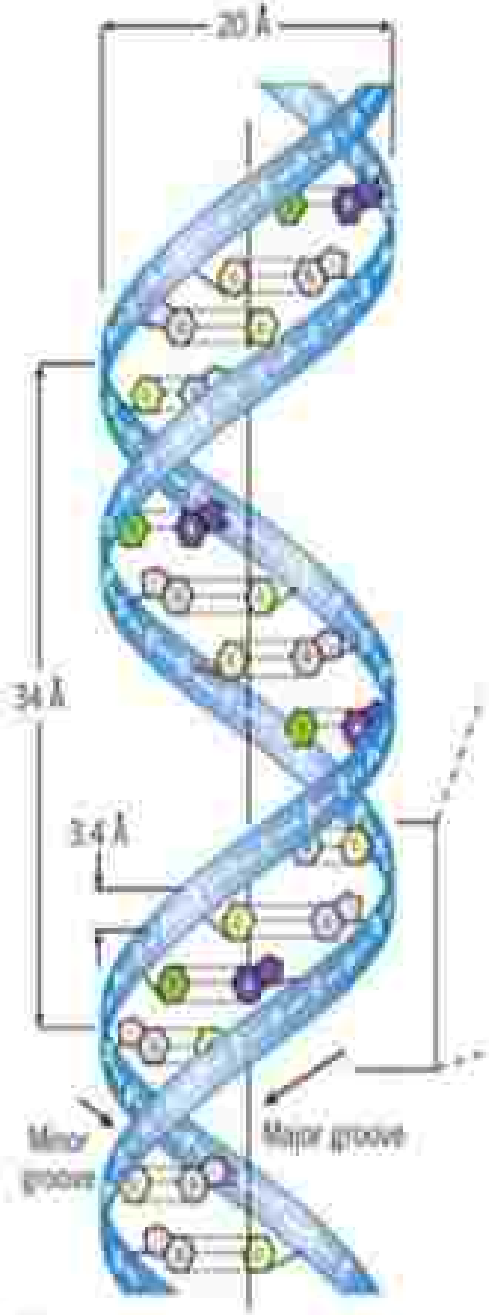

Francis Crick


James Watson


Maurice Wilkins


Rosalind Franklin





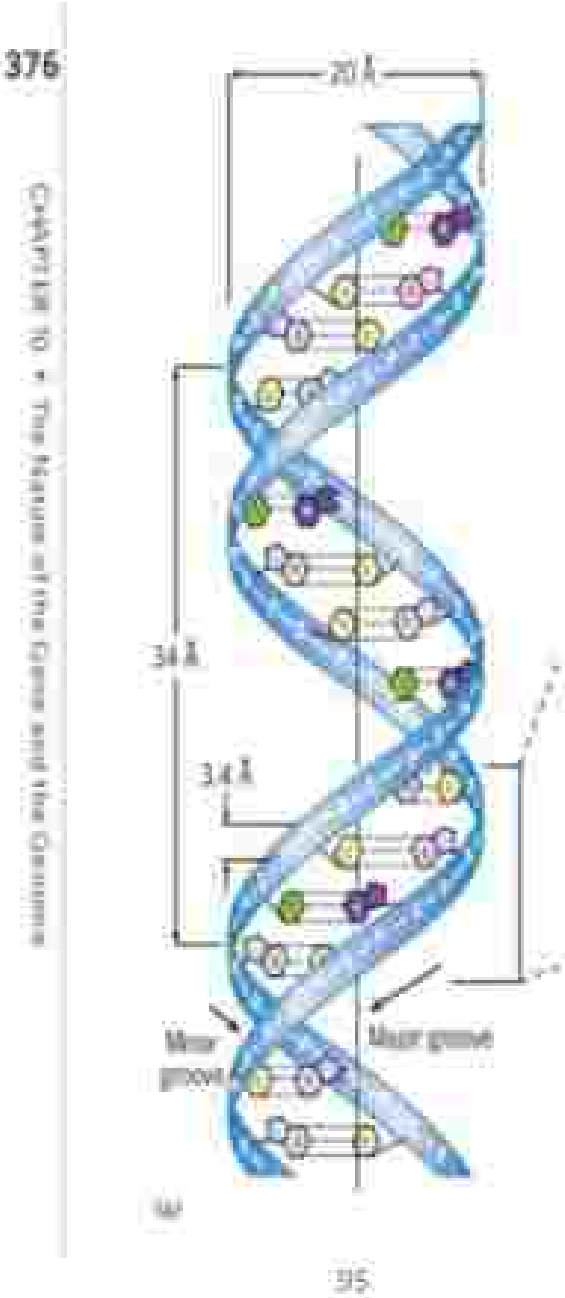
Structure of DNA - Major form of B form

- polymer of nucleotides.
- Double stranded.
- 2 chains –spiral around each other → right handed helix
- In double strand to a one strand other strand runs in antiparallel direction.
- i.e. 5'→3' & 3' → 5'
- S-P-S-P form the backbone structure of each strand
- A, T, G, C occupies the planes are ⊥ long axis.
- A::T & G:::C
- Hydrophobic & van der Waals force → stacking & stability of the structure.

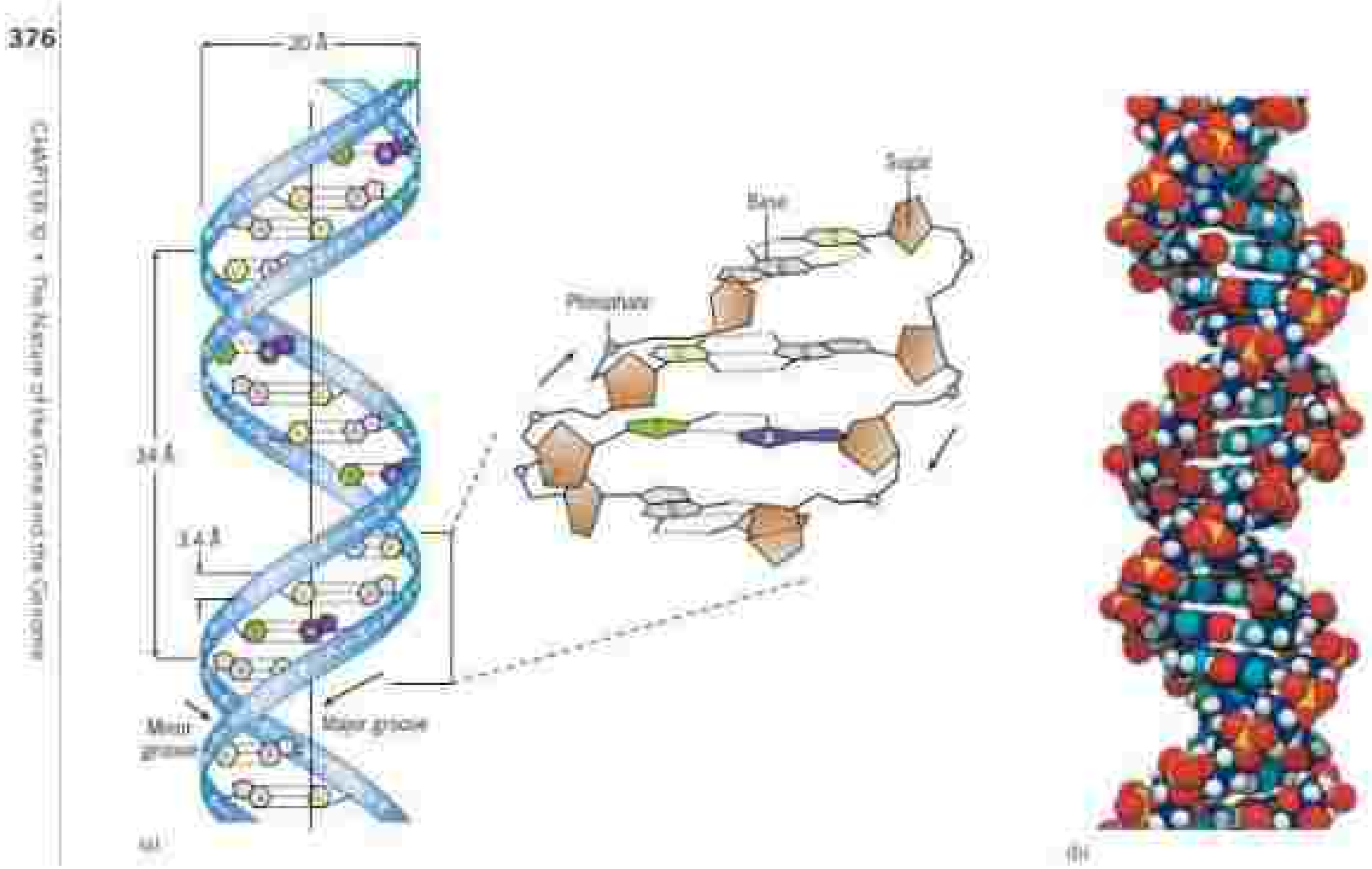
Structure of DNA - Major form of B form

2 strands are held by Hydrogen bond

- Width of the DNA is 2nm (20 A)
- Purine always paired with pyrimidine
- Space b/w adjacent turns of helix form 2 grooves: Major & Minor
- They provides the space for binding of proteins copying of codes without unwinding of
- the strand
- 10 residues -- > one complete turn → 3.4nm (34A).
- 2 strands are complementary to one another
- DNA is negatively charged and dextrorotatory



Structure of DNA - Major form of B form



Chargaff's Rules

- Purine and pyrimidine base pairs are in equal amount

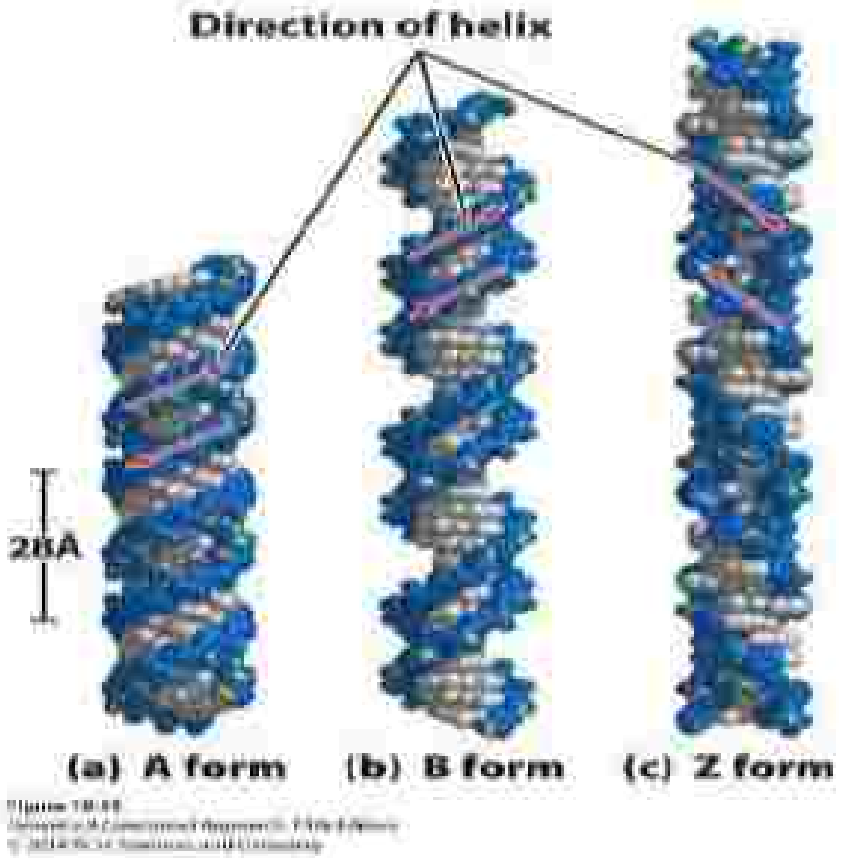
  [A + G] = [T + C], i.e., [A+G] / [T+C] = 1

- Molar amount of adenine is always equal to the molar amount of thymine

[A] = [T], i.e., [A] / [T] = 1; [G] = [C], i.e., [G] / [C] = 1

- Sugar deoxyribose and phosphate occur in equimolar proportions.
- A-T base pairs are rarely equal to C—G base pairs
- The ratio of [A+T] / [G+C] is variable but constant for a species

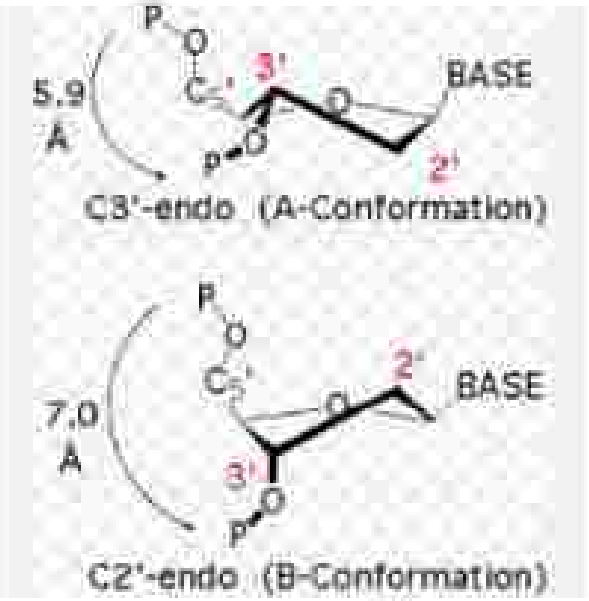
Other Types of DNA



	B	A	Z
Helix	RH	RH	LH
Bp/turn	10	11	12
Vertical bp	3.4A	2.56A	19A
Rotation /bp	$+36^0$	$+33^0$	-30^0

Understanding the Flexibility of the Sugar-phosphate Backbone

- nucleic acids are insoluble in water
- When attached to a pentose sugar and a phosphate to become a nucleotide: soluble
- But *hydrophobic face of the bases* does place strong constraints on the overall conformation of a large DNA or RNA molecule in solution
- The *sugar-phosphate backbone of the polynucleotide* also imposes strong constraints on the overall conformation of the chains
- **sugar conformation:** defines how the nucleotides are linked to each other in space
- ***sugar pucker*** refers to the conformation of the ribose or deoxyribose.
- The sugar Pucker is defined by the position of C2' and C3' atoms relative to a plane formed by the C1', O4' and C4' atoms.
- The sugar puckers in DNA/RNA structures are predominately in either C3'-endo (A-DNA or RNA) or C2'-endo (B-DNA), corresponding to the A- or B-form conformation in a duplex.



Importance……

- Importance of Watson Crick proposal
- Storage of genetic information
- Replication & inheritance
- Expression of genetic material

Comment

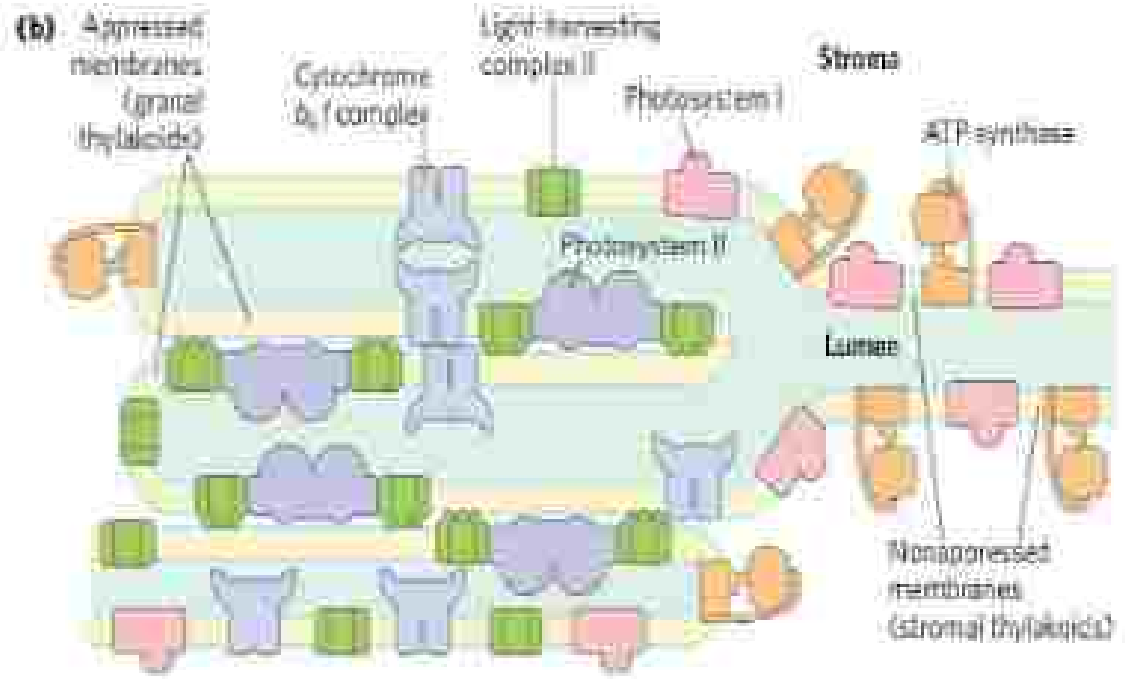
What Watson and Crick really took from Franklin

Matthew Cobb & Nathaniel Comfort

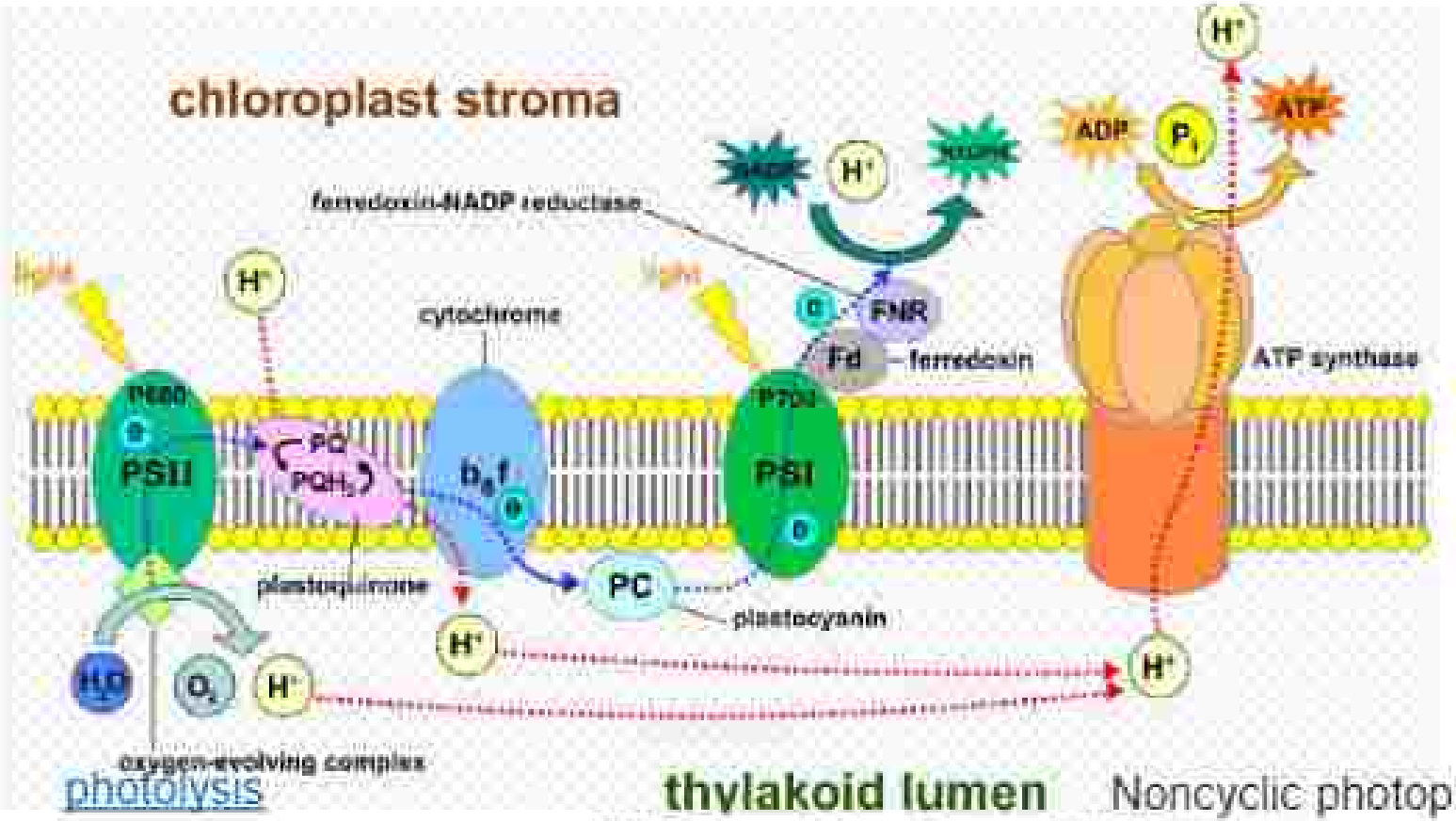
Rosalind Franklin was no victim in the discovery of DNA's structure. An overlooked letter and an unpublished news article, both from 1953, show that she was an equal contributor.

Photophosphorylation

- Hills Reaction: Robert hill
- $H_2O + 2NADP^+$ ---------- > $2NADPH + 2H^+ + O_2$
- THE EVLOVEMENT OF OXYGEN, IN PRESENCE OF SUTIABLE ELCETRON ACCEPTOR LIKE FERRICYANIDE, WHICH IS A NON-PHYSIOLOGICAL OXIDANTS.
- $NADP^+$: ELECTRON ACCEPTOR.
- Light reaction
- PS I & PS II
- PSII is present almost exclusively in the granal regions
- PSI almost exclusively in stromal regions



Photophosphorylation
Noncyclic Photophosphorylation



Involves PS I and PS II.
PS II: P680 (short-wavelength) Chla
PS I: 700 (long-wavelength).
Thylakoid membrane.

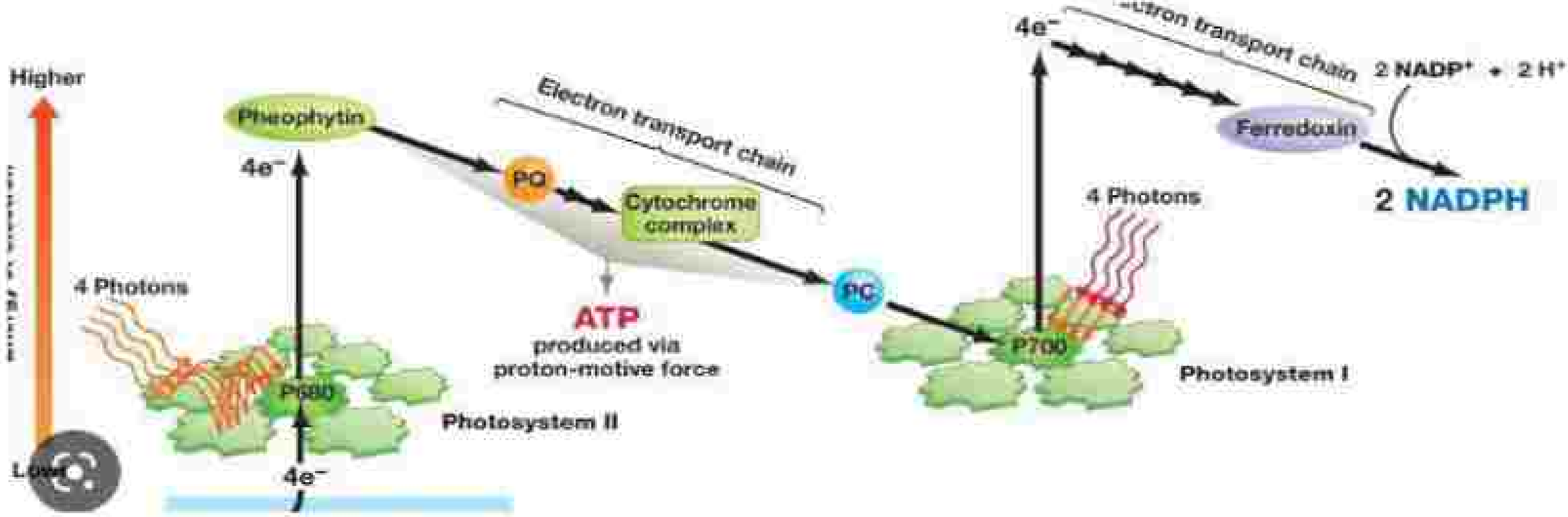
a. Absorption of light
b. ET leads to formation of O_2 from H_2O
c. Reduction of $NADP^+$ to NADPH
d. Generation of proton motive force
e. Synthesis of ATP

Plastoquinone: PS II, green plants,
Thightly bound & lossely bound electron carreirs. QA and QB.
QB ---- > PQH2 plastoquinol.

Noncyclic photophosphorylation

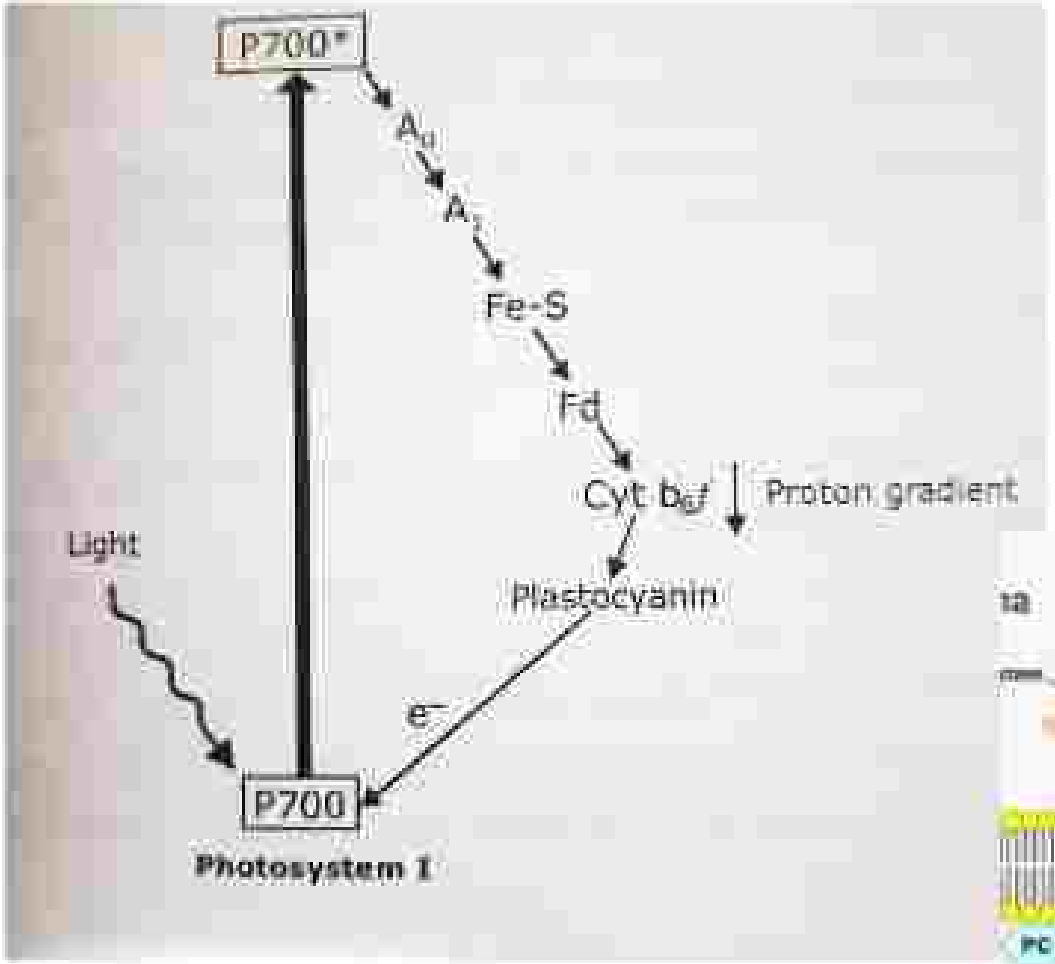
$2H^+ + 1/2\ O_2 + 2e^-$ $NADP^+ + 2H^+ + 2e^- \rightarrow NADPH + H^+$

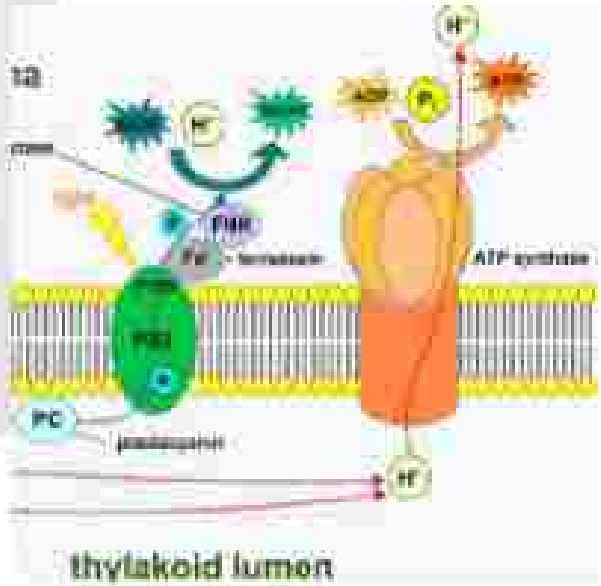
Photophosphorylation
Noncyclic Photophosphorylation



e- -→ Phenophytin (picosec) --- > Plastoquoninol --- > cytochrome bf 6 (Rieske iron-sulfur protein, 2 heme) ---- > H+ pump ---- >

Photophosphorylation
Cyclic Photophosphorylation





- Cyclic electron transfer produces only ATP and allows variability in the proportions of NADPH and ATP formed.
- When levels of NADP+ are low and levels of NADPH are high, electrons from P700 are returned to it via the cytochrome b6f complex.
- There will no reduction of NADP+ but protons are pumped across the membrane and therefore ATP is generated.

References

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