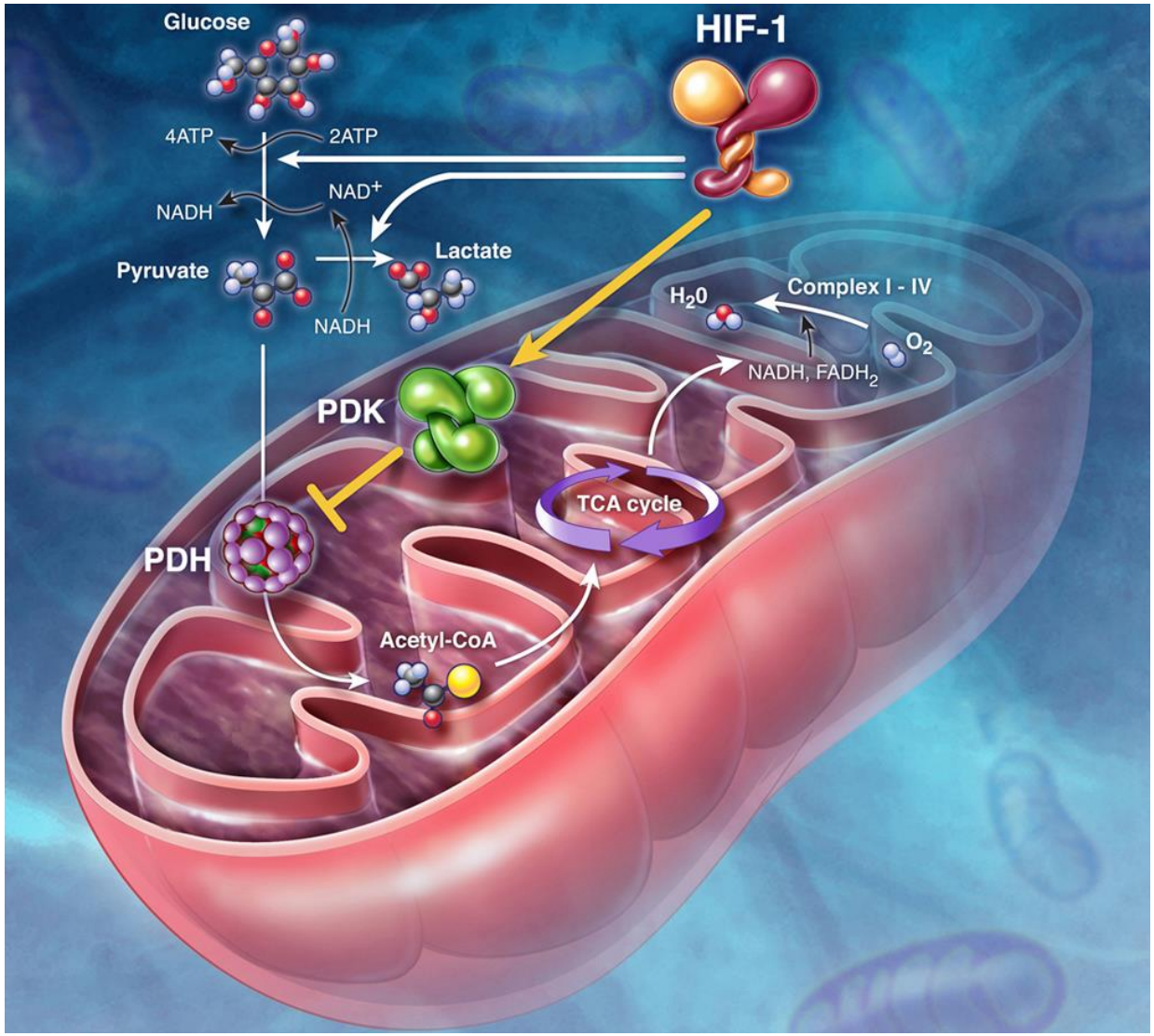
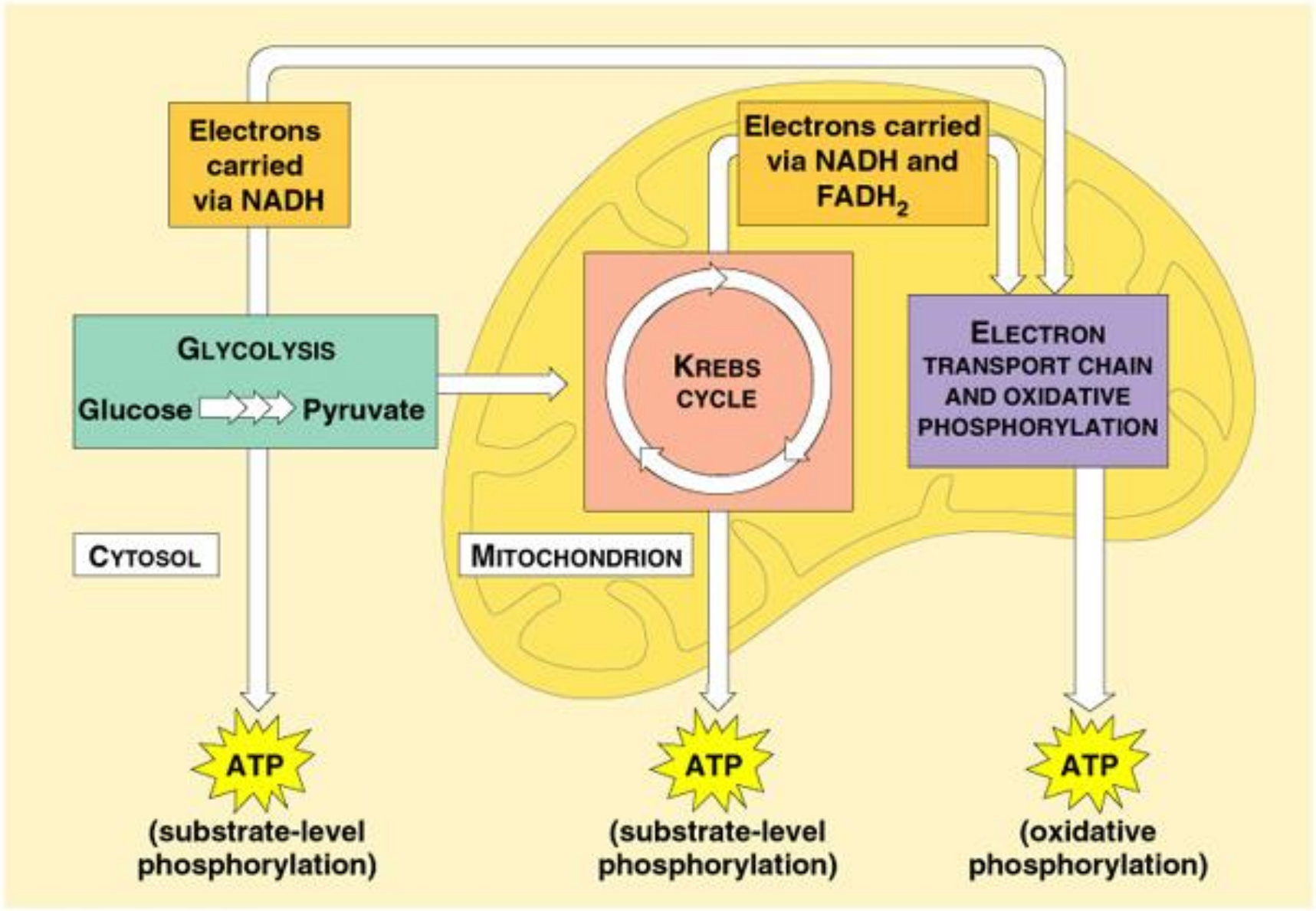
CARBOHYDRATE METABOLISM III: CITRIC ACID CYCLE





Cellular Respiration

❖Some cells obtain energy (ATP) by fermentation, breaking down glucose in the absence of oxygen.

❖For most eukaryotic cells and many bacteria, which live under aerobic conditions and oxidize their organic fuels to carbon dioxide and water, glycolysis is but the first stage in the complete oxidation of glucose.

❖Rather than being reduced to lactate, ethanol, or some other fermentation product, the pyruvate produced by glycolysis is further oxidized to H_2O and CO_2.

❖This aerobic phase of catabolism is called **respiration**.

❖Cellular respiration occurs in three major stages.

❖In the first, organic fuel molecules—glucose, fatty acids, and some amino acids—are oxidized to yield two-carbon fragments in the form of the acetyl-coenzyme A.

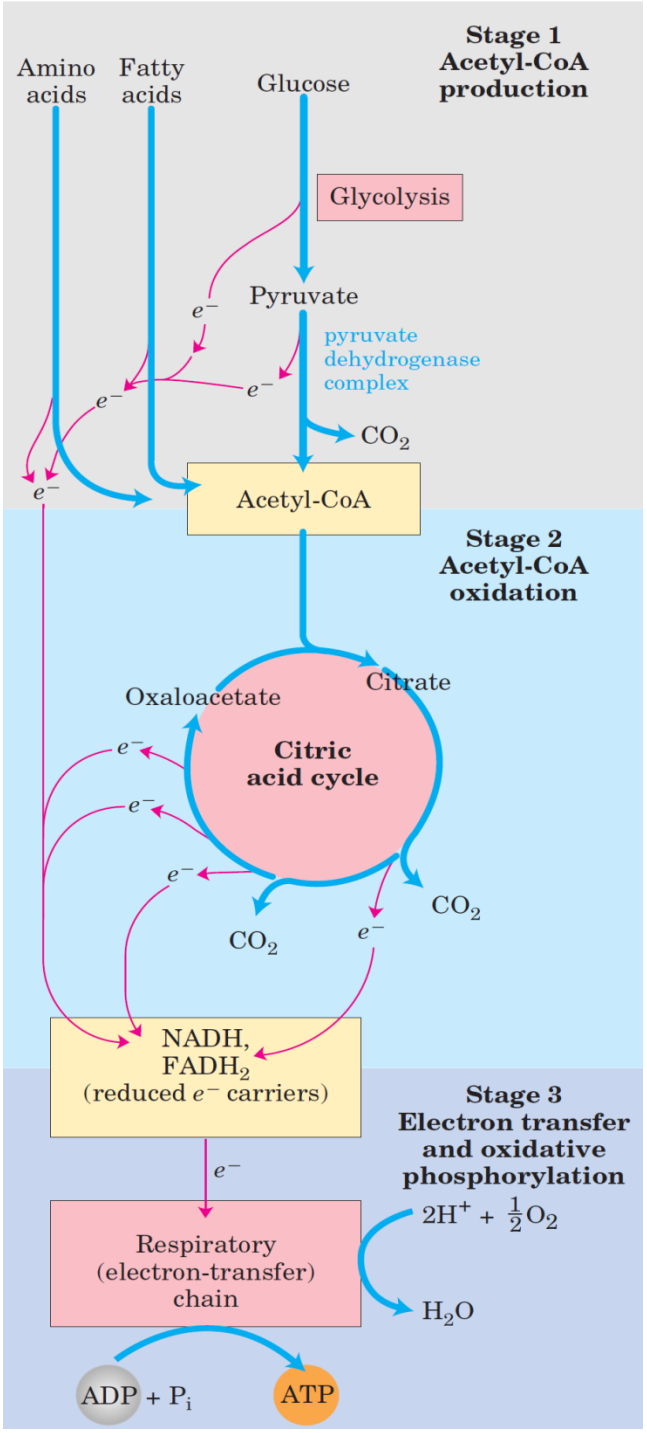
❖In the second stage, the acetyl groups are fed into the citric acid cycle, which enzymatically oxidizes them to CO_2; the energy released is conserved in the reduced electron carriers NADH and $FADH_2$.

❖In the third stage of respiration, these reduced coenzymes are themselves oxidized, giving up protons (H^+) and electrons.

❖The electrons are transferred to O_2—the final electron acceptor—via a chain of electron-carrying molecules known as the respiratory chain.

❖In the course of electron transfer, the large amount of energy released is conserved in the form of ATP, by a process called oxidative phosphorylation.

❖In aerobic organisms, glucose and other sugars, fatty acids, and most amino acids are ultimately oxidized to CO_2 and H_2O via the citric acid cycle and the respiratory chain.

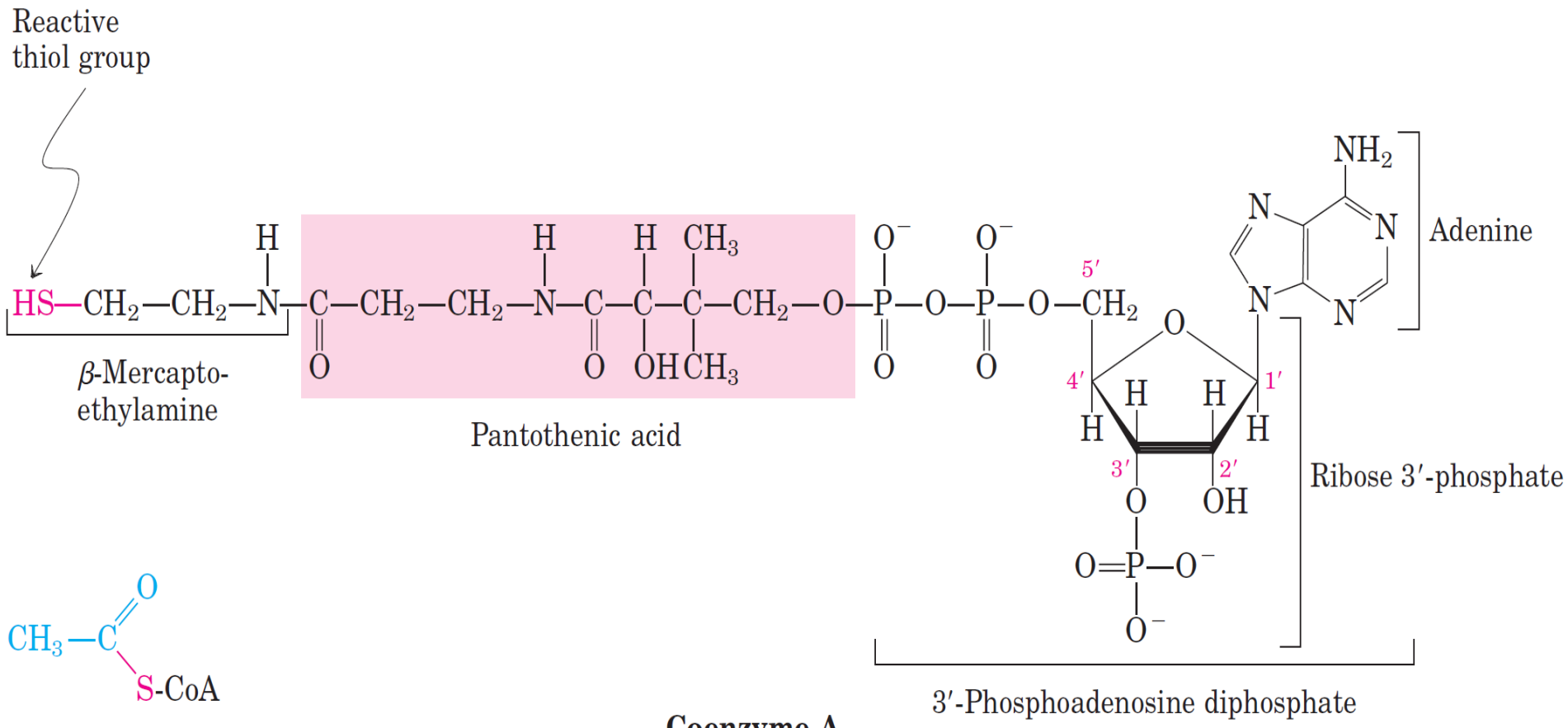


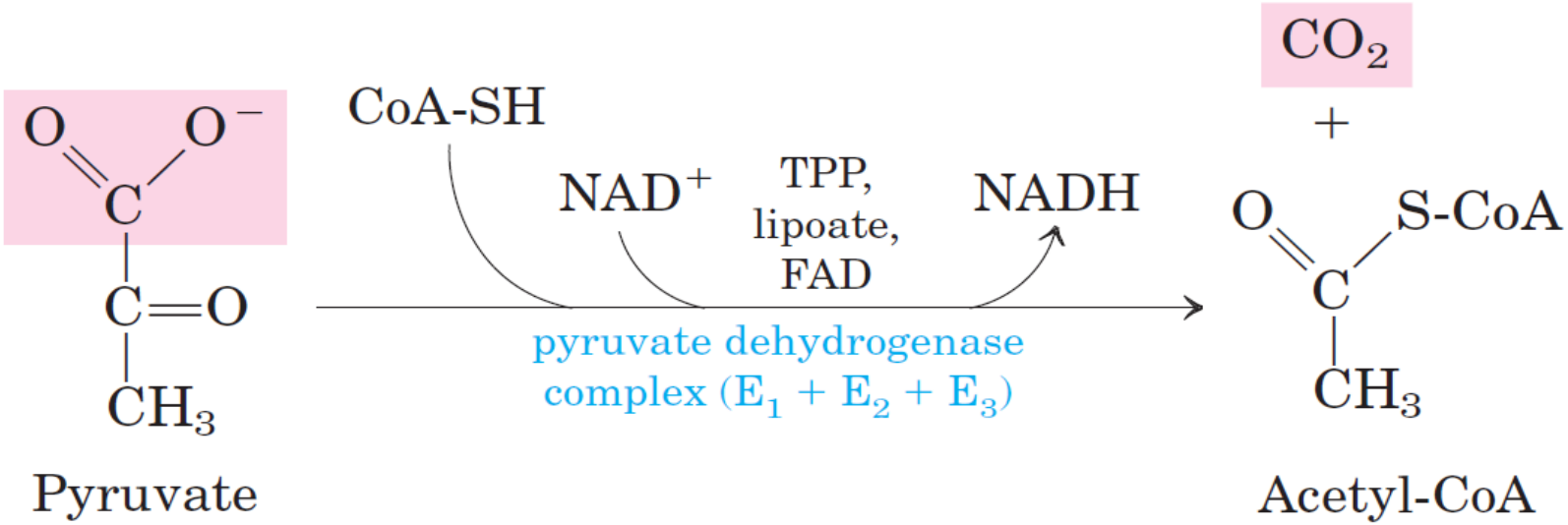
❖Before entering the citric acid cycle, the carbon skeletons of sugars, fatty acids and some amino acids are degraded to the acetyl group of acetyl-CoA, the form in which the cycle accepts most of its fuel input.

❖Pyruvate, derived from glucose and other sugars by glycolysis, is oxidized to acetyl-CoA and CO_2 by the pyruvate dehydrogenase (PDH) complex, located in the mitochondria of eukaryotic cells and in the cytosol of bacteria.

❖The overall reaction catalyzed by the pyruvate dehydrogenase complex is an oxidative decarboxylation.

❖This is an irreversible oxidation process in which the carboxyl group is removed from pyruvate as a molecule of CO_2 and the two remaining carbons become the acetyl group of acetyl-CoA


Reactive
thiol group
β-Mercapto-
ethylamine
Pantothenic acid
Adenine
Ribose 3′-phosphate
3′-Phosphoadenosine diphosphate
Acetyl-CoA
Coenzyme A



$$\Delta G'^{\circ} = -33.4 \text{ kJ/mol}$$

❖The NADH formed in this reaction gives up a hydride ion ($:H^-$) to the respiratory chain, which carries the two electrons to oxygen or, in anaerobic microorganisms, to an alternative electron acceptor such as nitrate or sulfate.

❖The transfer of electrons from NADH to oxygen ultimately generates 2.5 molecules of ATP per pair of electrons.

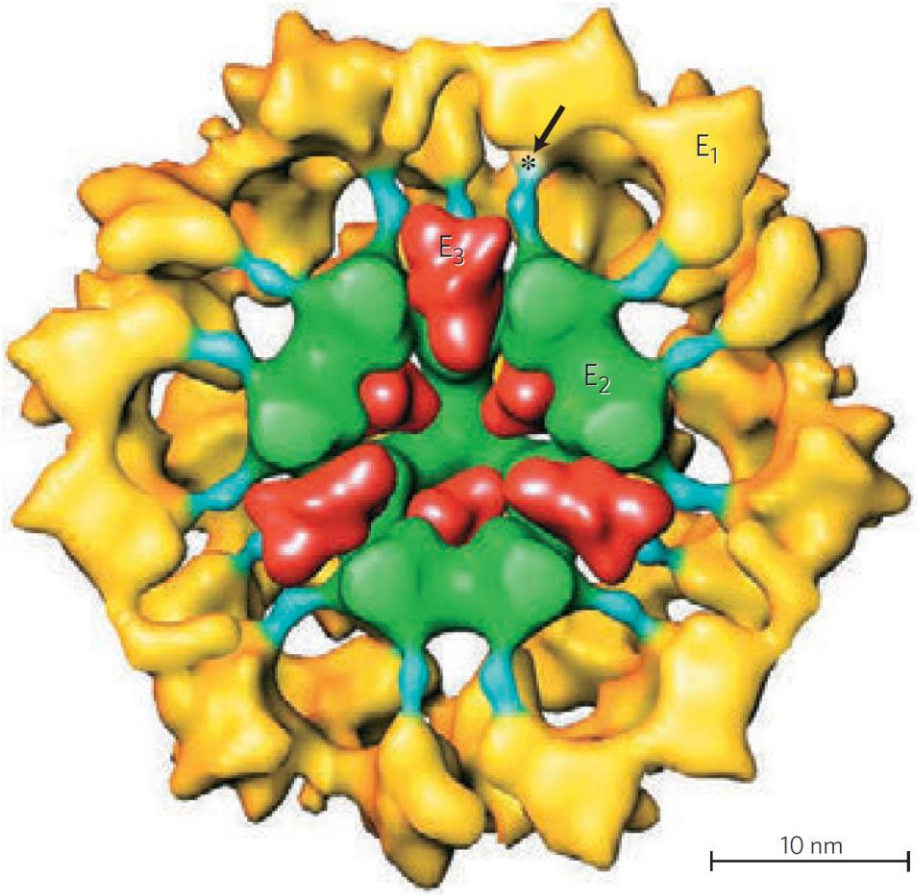
❖The combined dehydrogenation and decarboxylation of pyruvate to the acetyl group of acetyl-CoA requires the sequential action of three different enzymes and five different coenzymes or prosthetic groups—thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), coenzyme A (CoA), nicotinamide adenine dinucleotide (NAD), and lipoate.

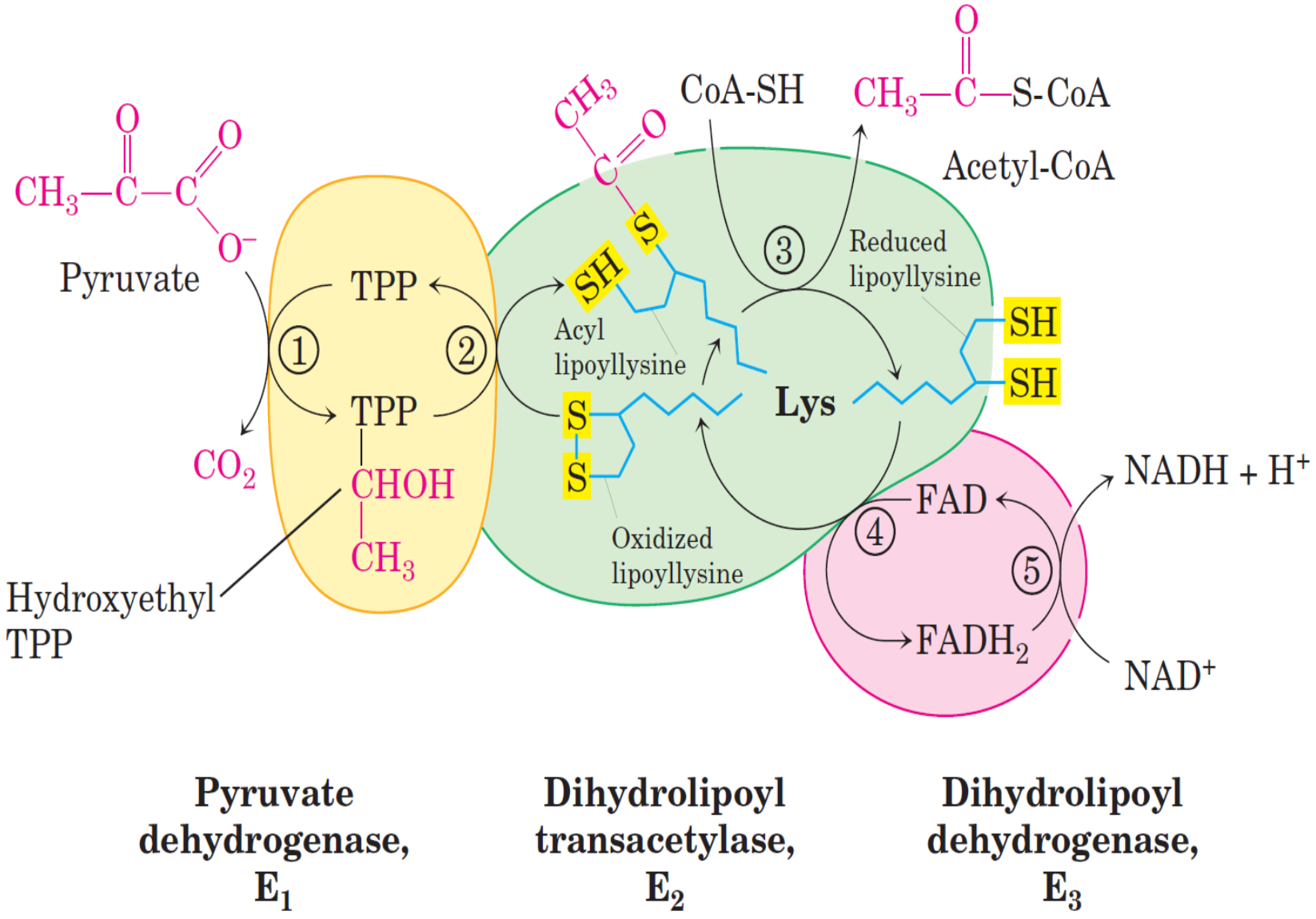
❖Four different vitamins required in human nutrition are vital components of this system: thiamine (in TPP), riboflavin (in FAD), niacin (in NAD), and pantothenate (in CoA).

❖Coenzyme A has a reactive thiol (—SH) group that is critical to the role of CoA as an acyl carrier in a number of metabolic reactions.

❖Acyl groups are covalently linked to the thiol group, forming thioesters and because of their relatively high standard free energies of hydrolysis, thioesters, have a high acyl group transfer potential and can donate their acyl groups to a variety of acceptor molecules.

❖The PDH complex contains three enzymes—pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3)—each present in multiple copies.




Pyruvate
TPP
TPP
CHOH
CH3
CO2
Hydroxyethyl TPP
1
2
CoA-SH
Acetyl-CoA
Acyl lipoyllysine
Oxidized lipoyllysine
Lys
Reduced lipoyllysine
3
SH
SH
SH
S
S
S
FAD
FADH2
4
5
NADH + H+
NAD+
Pyruvate dehydrogenase, E1
Dihydrolipoyl transacetylase, E2
Dihydrolipoyl dehydrogenase, E3

❖Pyruvate dehydrogenase complex carries out the five consecutive reactions in the decarboxylation and dehydrogenation of pyruvate.

❖Step 1 is essentially identical to the reaction catalyzed by pyruvate decarboxylase; C-1 of pyruvate is released as CO_2, and C-2, which in pyruvate has the oxidation state of an aldehyde, is attached to TPP as a hydroxyethyl group.

❖This first step is the slowest and therefore limits the rate of the overall reaction.

❖In step 2 the hydroxyethyl group is oxidized to the level of a carboxylic acid (acetate).

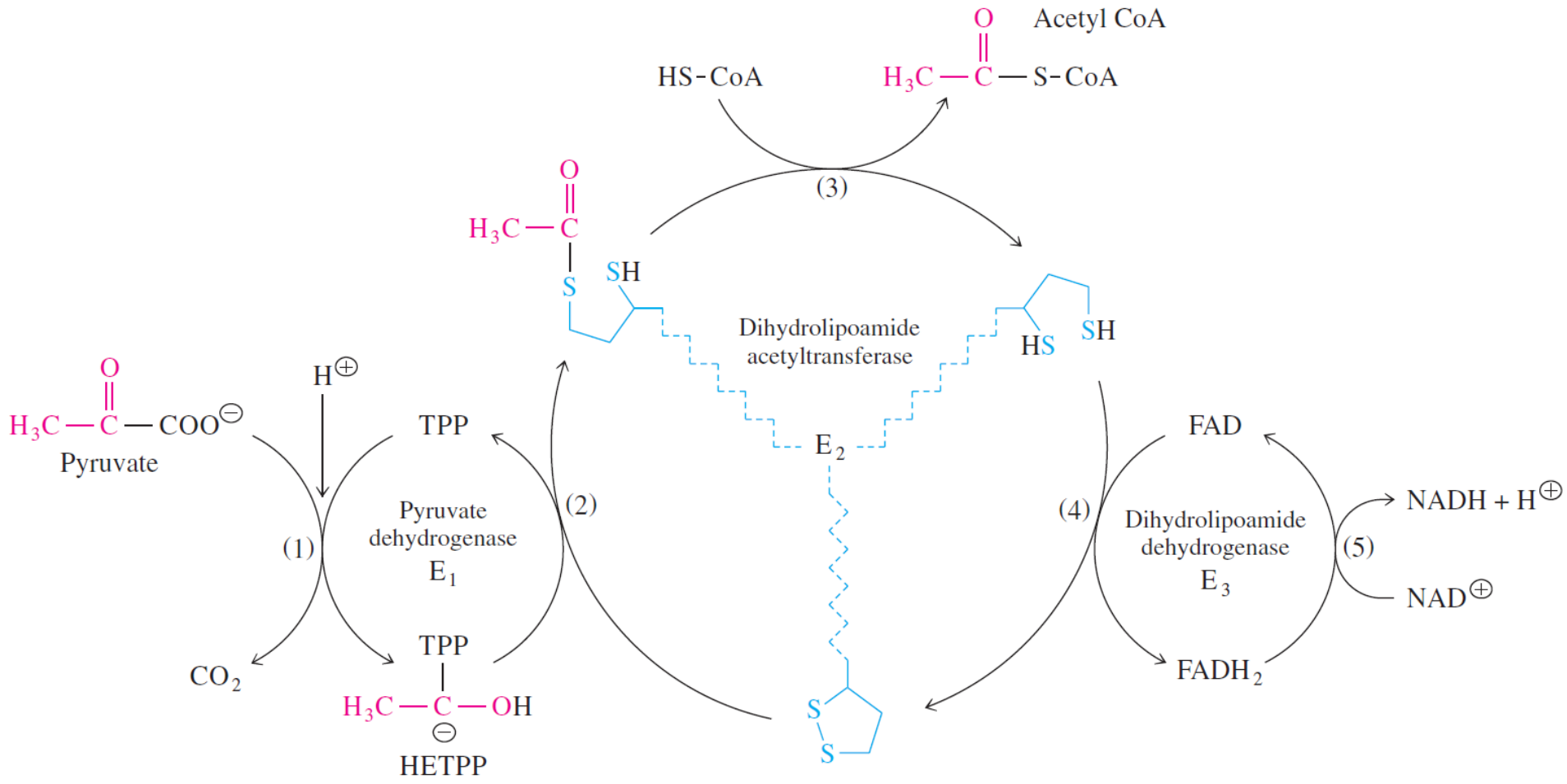
❖The two electrons removed in this reaction reduce the S-S of a lipoyl group on E2 to two thiol (—SH) groups.

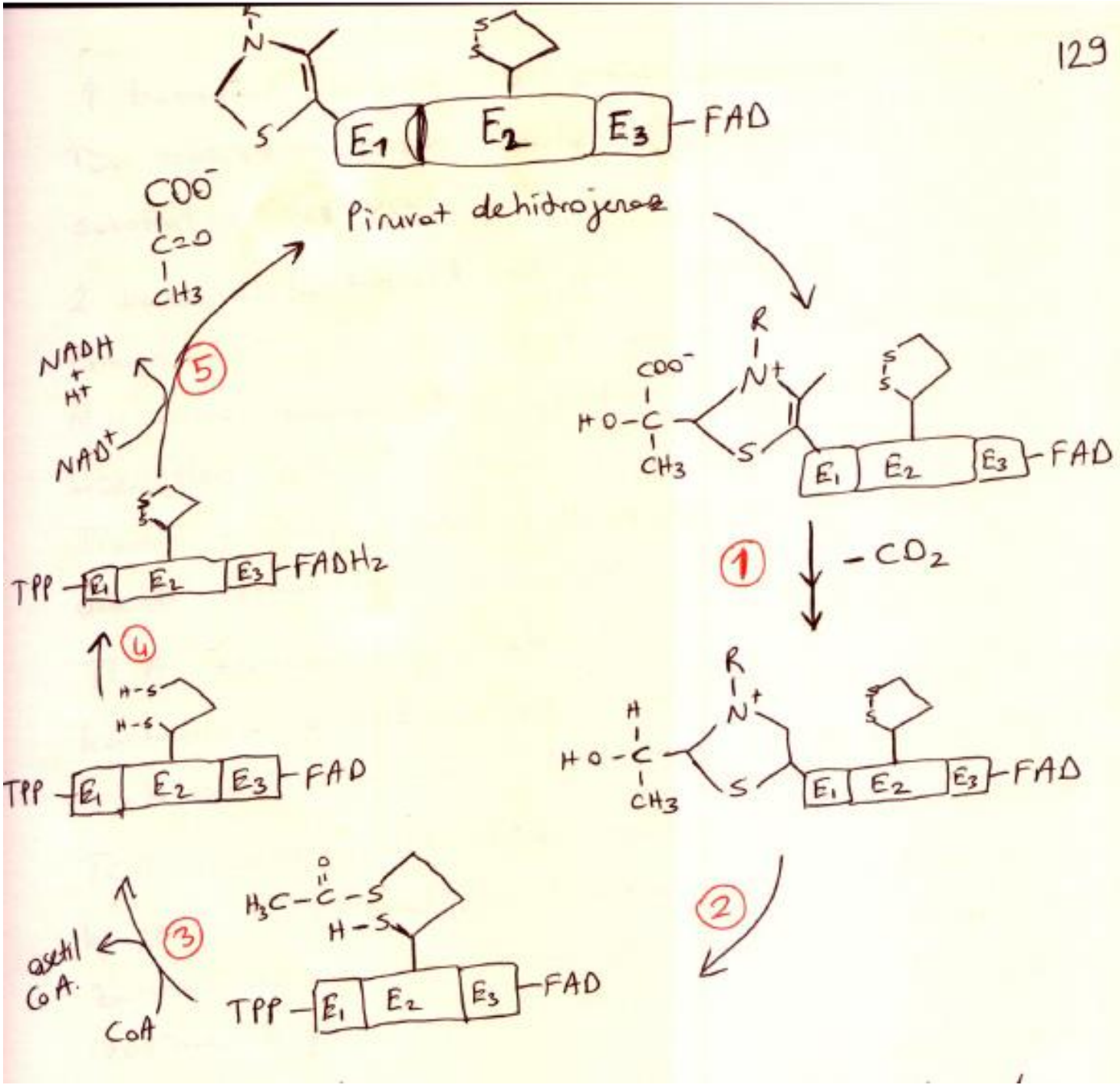
❖The acetyl moiety produced in this oxidation-reduction reaction is first esterified to one of the lipoyl —SH groups, then transesterified to CoA to form acetyl-CoA (step 3).

❖Thus the energy of oxidation drives the formation of a high-energy thioester of acetate.

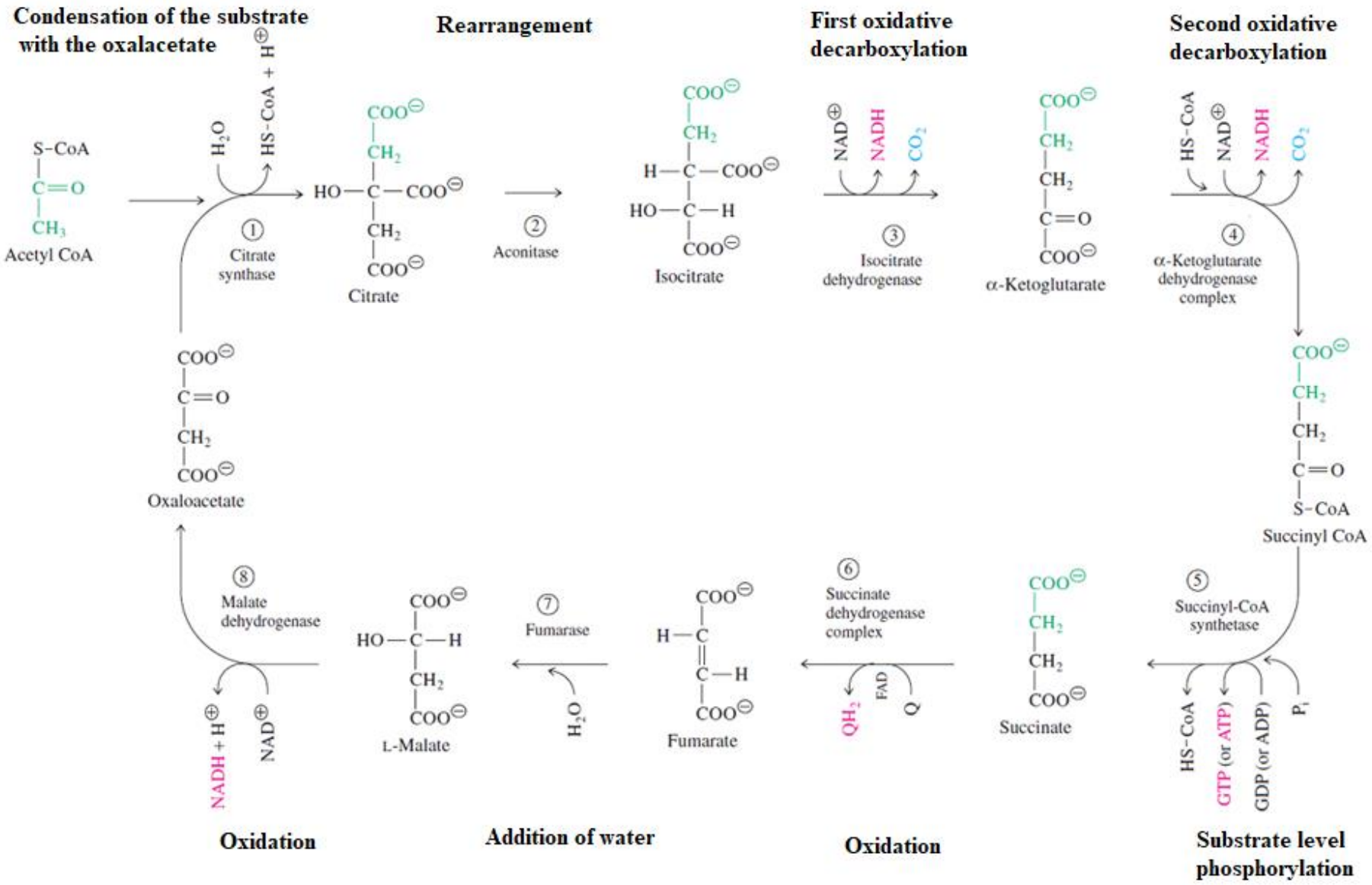
❖The remaining reactions catalyzed by the PDH complex (by E3, in steps 4 and 5) are electron transfers necessary to regenerate the oxidized (disulfide) form of the lipoyl group of E2 to prepare the enzyme complex for another round of oxidation.

❖The electrons removed from the hydroxyethyl group derived from pyruvate pass through FAD to NAD^+.


Acetyl CoA
HS-CoA
(3)
Dihydrolipoamide
acetyltransferase
E2
Pyruvate
H⊕
TPP
(1)
Pyruvate
dehydrogenase
E1
(2)
CO2
TPP
HETPP
FAD
(4)
Dihydrolipoamide
dehydrogenase
E3
(5)
NADH + H⊕
NAD⊕
FADH2


E1
E2
E3
FAD
Piruvat dehidrojenaz
COO⁻
C=O
CH3
NADH + H+
NAD+
5
TPP
FADH2
4
H-S
1
-CO2
HO-C
2
3
acetil CoA.
CoA
H3C-C
O

Reactions of the Citric Acid Cycle



❖To begin a turn of the cycle, acetyl-CoA donates its acetyl group to the four-carbon compound oxaloacetate to form the six-carbon citrate.

❖Citrate is then transformed into isocitrate, also a six-carbon molecule, which is dehydrogenated with loss of CO_2 to yield the five-carbon compound α -ketoglutarate.

❖α-Ketoglutarate undergoes loss of a second molecule of CO_2 and ultimately yields the four-carbon compound succinate.

❖Succinate is then enzymatically converted in three steps into the four-carbon oxaloacetate—which is then ready to react with another molecule of acetyl-CoA.

❖In each turn of the cycle, one acetyl group (two carbons) enters as acetyl-CoA and two molecules of CO_2 leave; one molecule of oxaloacetate is used to form citrate and one molecule of oxaloacetate is regenerated.

❖No net removal of oxaloacetate occurs; one molecule of oxaloacetate can theoretically bring about oxidation of an infinite number of acetyl groups, and, in fact, oxaloacetate is present in cells in very low concentrations.

❖Four of the eight steps in this process are oxidations, in which the energy of oxidation is very efficiently conserved in the form of the reduced coenzymes NADH and $FADH_2$.

❖Although the citric acid cycle is central to energy-yielding metabolism its role is not limited to energy conservation.

❖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.

❖The entire set of reactions of the citric acid cycle takes place in mitochondria.

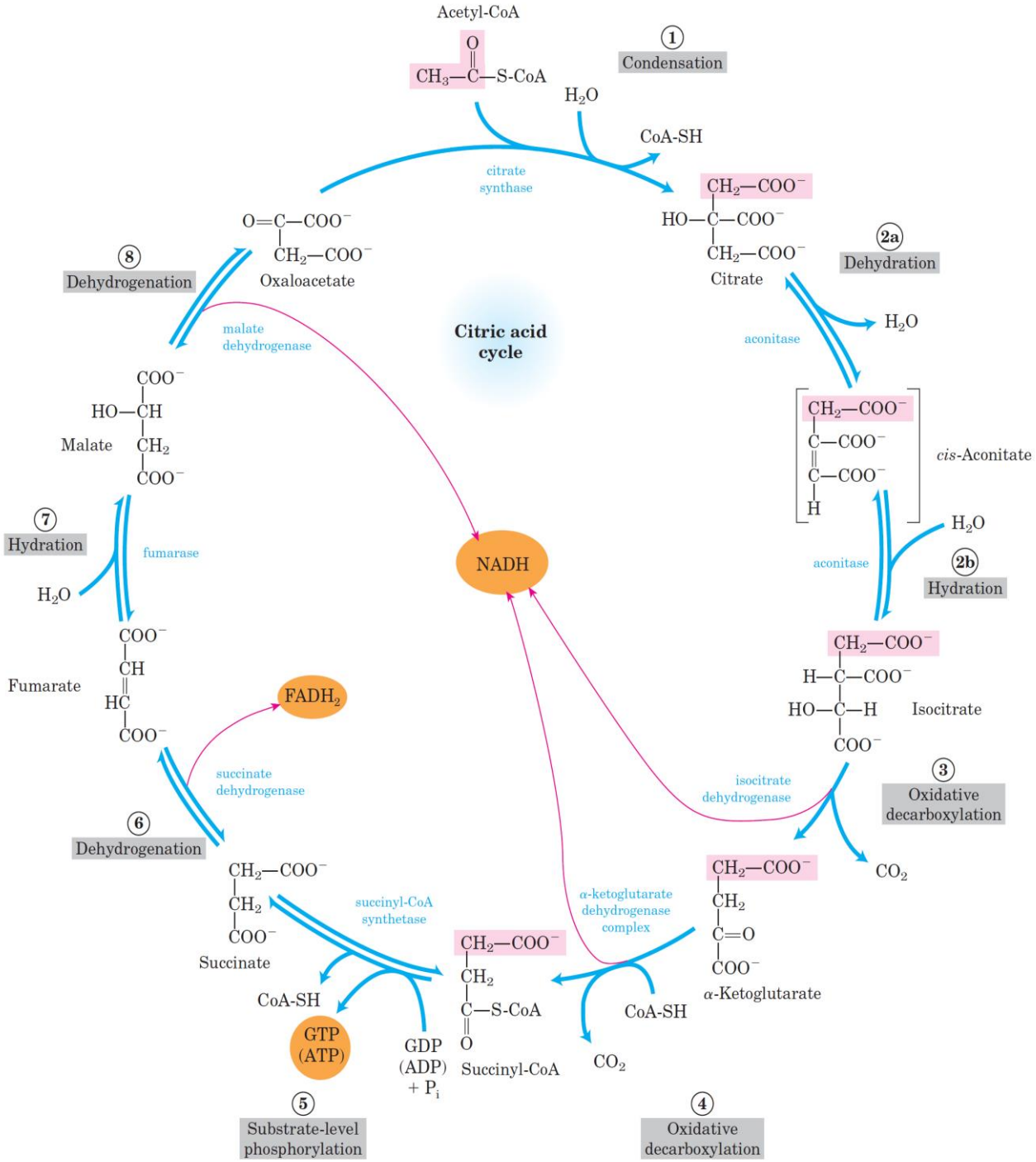
❖Isolated mitochondria were found to contain not only all the enzymes and coenzymes required for the citric acid cycle, but also all the enzymes and proteins necessary for the last stage of respiration—electron transfer and ATP synthesis by oxidative phosphorylation.

❖Mitochondria also contain the enzymes for the oxidation of fatty acids and some amino acids to acetyl-CoA, and the oxidative degradation of other amino acids to α-ketoglutarate, succinyl-CoA, or oxaloacetate.

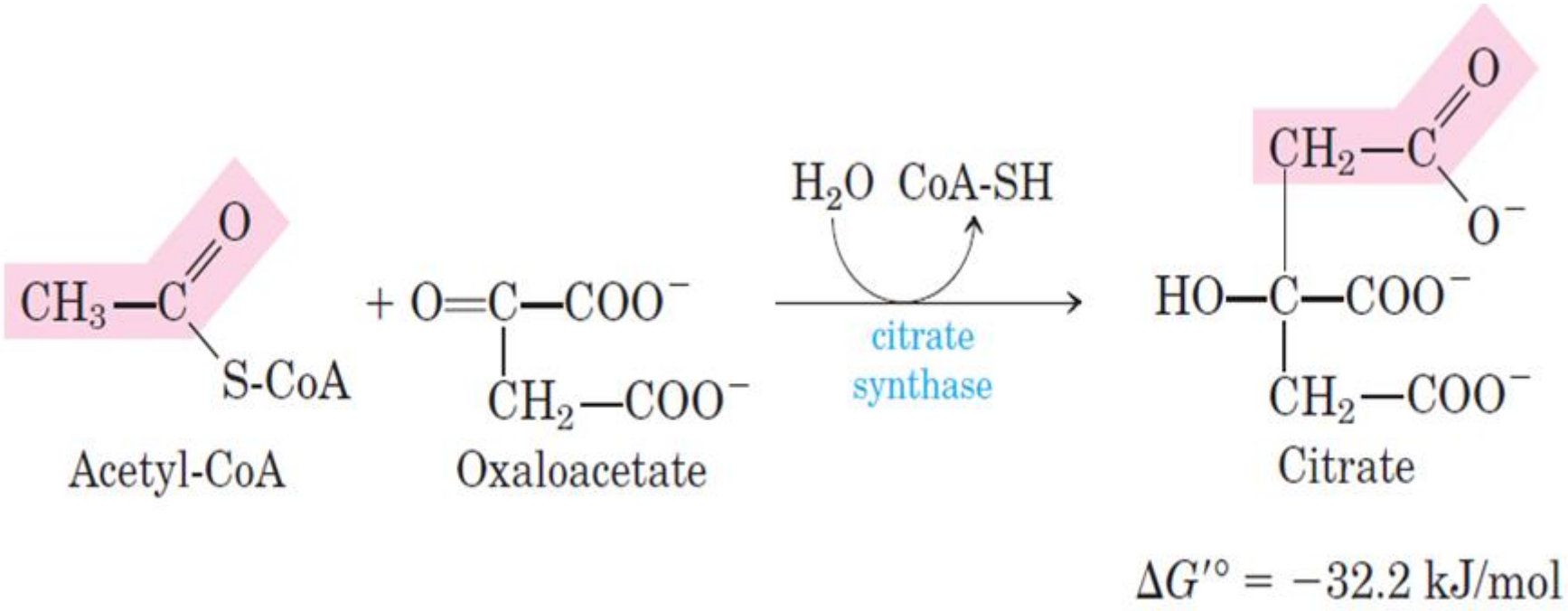
❖Thus, in nonphotosynthetic eukaryotes, the mitochondrion is the site of most energy-yielding oxidative reactions and of the coupled synthesis of ATP.

❖In photosynthetic eukaryotes, mitochondria are the major site of ATP production in the dark, but in daylight chloroplasts produce most of the organism's ATP.

❖In most bacteria, the enzymes of the citric acid cycle are in the cytosol, and the plasma membrane plays a role analogous to that of the inner mitochondrial membrane in ATP synthesis

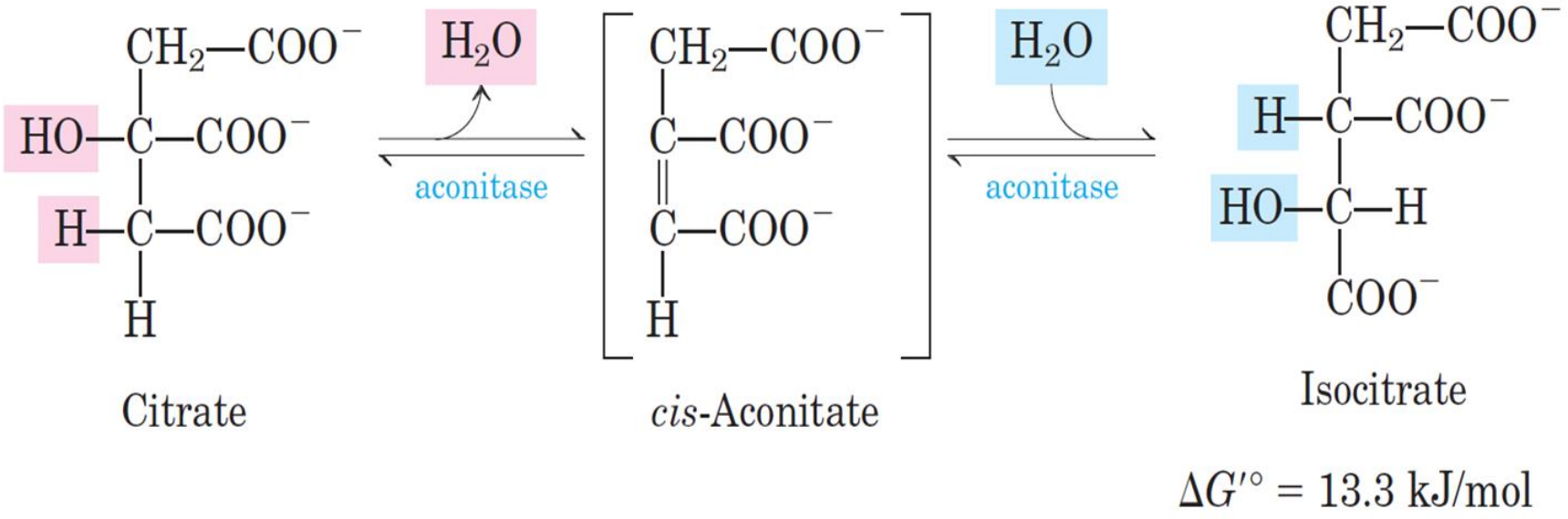

Acetyl-CoA
CH3—C—S-CoA
1
Condensation
H2O
CoA-SH
citrate synthase
CH2—COO⁻
HO—C—COO⁻
CH2—COO⁻
Citrate
2a
Dehydration
H2O
aconitase
cis-Aconitate
aconitase
H2O
2b
Hydration
CH2—COO⁻
H—C—COO⁻
HO—C—H
COO⁻
Isocitrate
3
Oxidative decarboxylation
CO2
isocitrate dehydrogenase
CH2—COO⁻
CH2
C=O
COO⁻
α-Ketoglutarate
α-ketoglutarate dehydrogenase complex
CH2—COO⁻
CH2
C—S-CoA
O
Succinyl-CoA
CO2
CoA-SH
succinyl-CoA synthetase
GTP (ATP)
GDP (ADP) + Pi
5
Substrate-level phosphorylation
CH2—COO⁻
CH2
COO⁻
Succinate
6
Dehydrogenation
succinate dehydrogenase
FADH2
COO⁻
CH
HC
COO⁻
Fumarate
7
Hydration
H2O
fumarase
COO⁻
HO—CH
CH2
COO⁻
Malate
8
Dehydrogenation
malate dehydrogenase
O=C—COO⁻
CH2—COO⁻
Oxaloacetate
NADH
Citric acid cycle
4
Oxidative decarboxylation

❖The first step of the citric acid cycle catalyzed by citrate synthase.



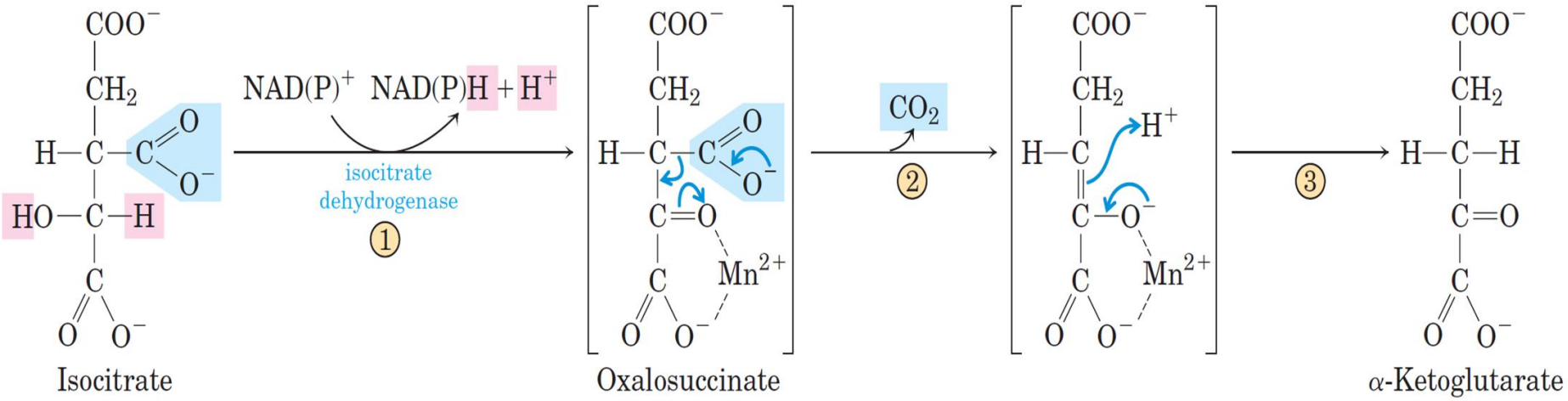
❖The carbonyl of oxaloacetate acts as an electrophilic center, which is attacked by the methyl carbon of acetyl-CoA in a Claisen condensation (reaction between a thioester (acetyl-CoA) and a ketone (oxaloacetate)) to form citrate.

❖The enzyme aconitase (more formally, aconitate hydratase) catalyzes the reversible transformation of citrate to isocitrate.



❖Aconitase contains an iron-sulfur center and promotes the reversible addition of H_2O to the double bond of enzyme-bound cis-aconitate.

❖In the next step, isocitrate dehydrogenase catalyzes oxidative decarboxylation of isocitrate to form α-ketoglutarate.

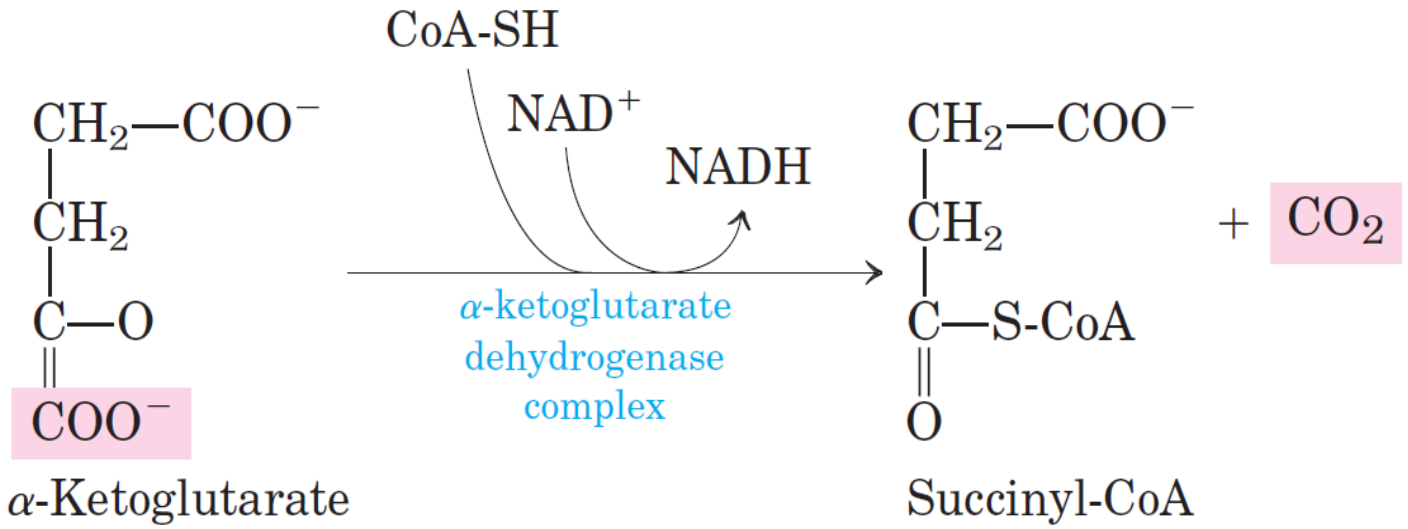


❖Mn^{2+} in the active site interacts with the carbonyl group of the intermediate oxalosuccinate, which is formed transiently but does not leave the binding site until decarboxylation converts it to α-ketoglutarate.

❖There are two different forms of isocitrate dehydrogenase in all cells, one requiring NAD^+ as electron acceptor and the other requiring $NADP^+$.

❖The next step is another oxidative decarboxylation, in which α-ketoglutarate is converted to succinyl-CoA and CO_2 by the action of the a-ketoglutarate dehydrogenase complex; NAD+ serves as electron acceptor and CoA as the carrier of the succinyl group.

❖The energy of oxidation of α-ketoglutarate is conserved in the formation of the thioester bond of succinyl-CoA.



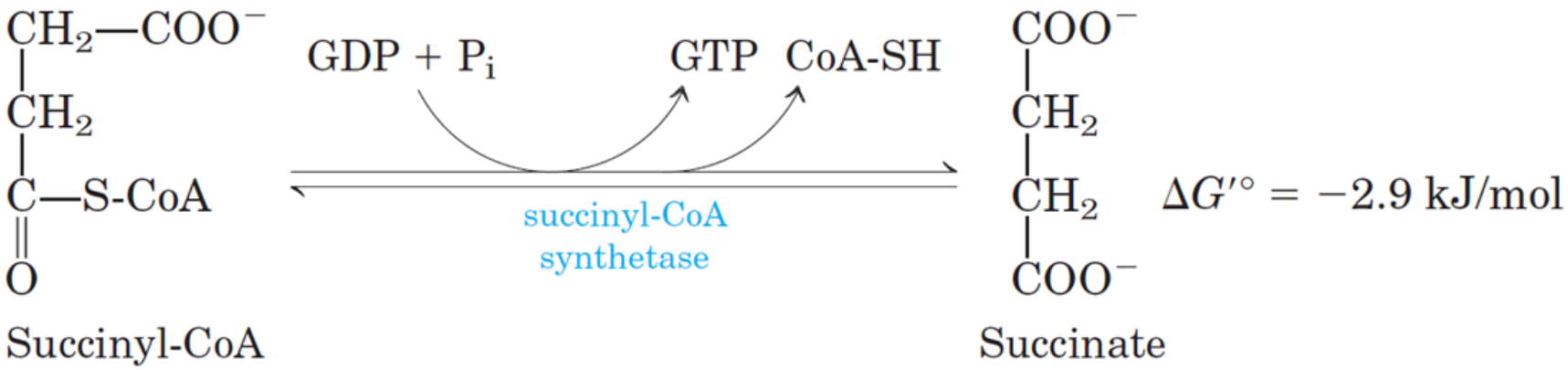
$$\Delta G'^{\circ} = -33.5 \text{ kJ/mol}$$

❖This reaction is virtually identical to the pyruvate dehydrogenase reaction.

❖Succinyl-CoA, like acetyl-CoA, has a thioester bond with a strongly negative standard free energy of hydrolysis.

❖In the next step of the citric acid cycle, energy released in the breakage of this bond is used to drive the synthesis of a phosphoanhydride bond in either GTP or ATP.

❖The enzyme that catalyzes this reversible reaction is called succinyl-CoA synthetase and this name indicates the participation of a nucleoside triphosphate in the reaction



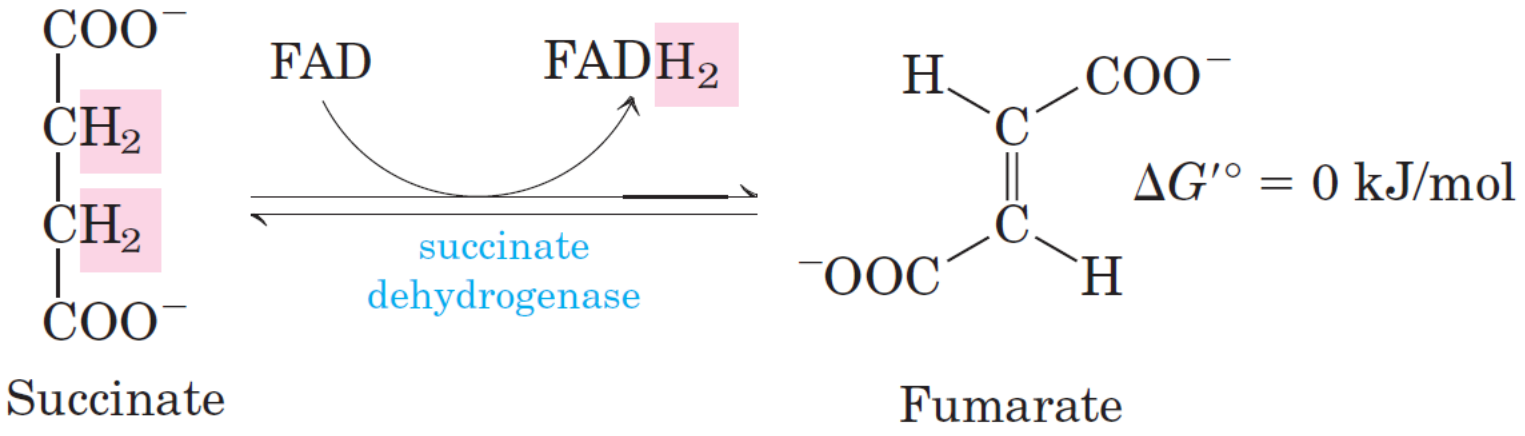
❖The formation of ATP (or GTP) at the expense of the energy released by the oxidative decarboxylation of α-ketoglutarate is a substrate-level phosphorylation, like the synthesis of ATP in the glycolytic reactions catalyzed by glyceraldehyde 3-phosphate dehydrogenase and pyruvate kinase.

❖The GTP formed by succinyl-CoA synthetase can donate its terminal phosphoryl group to ADP to form ATP, in a reversible reaction catalyzed by **nucleoside diphosphate kinase**.

$$\text{GTP} + \text{ADP} \longrightarrow \text{GDP} + \text{ATP} \qquad \Delta G'^{\circ} = 0 \text{ kJ/mol}$$

❖Thus the net result of the activity of either isozyme of succinyl-CoA synthetase is the conservation of energy as ATP.

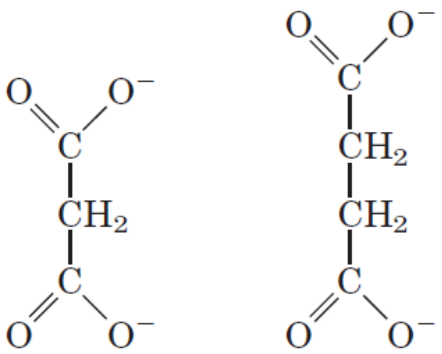
❖The succinate formed from succinyl-CoA is oxidized to fumarate by the flavoprotein succinate dehydrogenase:



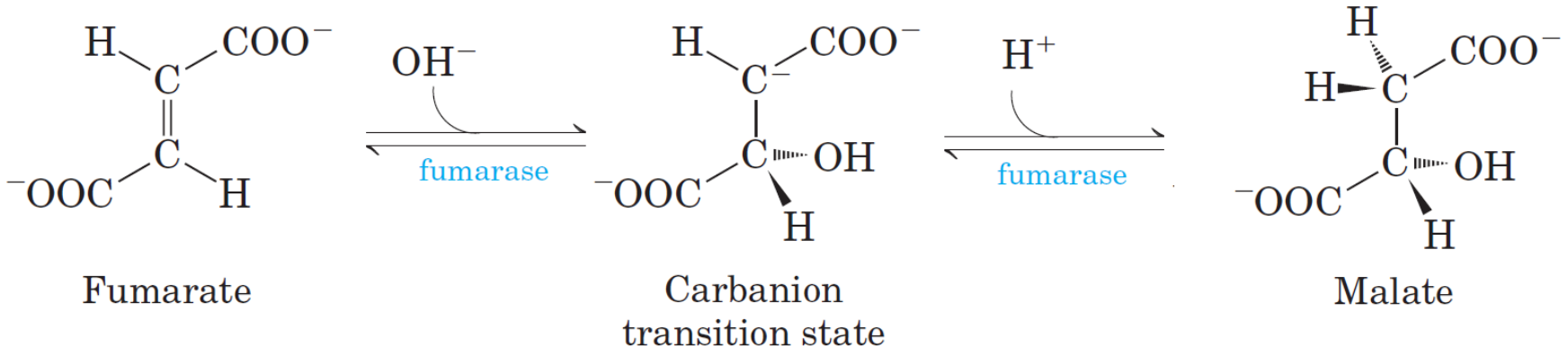
❖In eukaryotes, succinate dehydrogenase is tightly bound to the mitochondrial inner membrane and part of the electron transport system.

❖Electron flow from succinate through these carriers to the final electron acceptor, O2, is coupled to the synthesis of about 1.5 ATP molecules per pair of electrons (respiration-linked phosphorylation).

❖Malonate, an analog of succinate not normally present in cells, is a strong competitive inhibitor of succinate dehydrogenase, and its addition to mitochondria blocks the activity of the citric acid cycle.

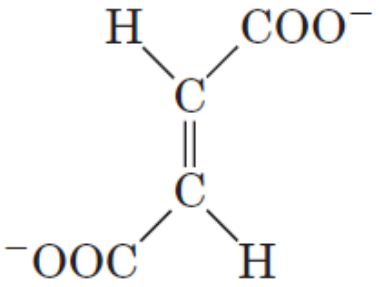


❖The reversible hydration of fumarate to L-malate is catalyzed by fumarase (formally, fumarate hydratase).

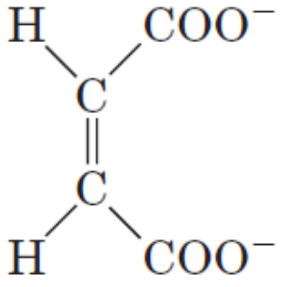


❖This enzyme is highly stereospecific; it catalyzes hydration of the trans double bond of fumarate but not the cis double bond of maleate (the cis isomer of fumarate).

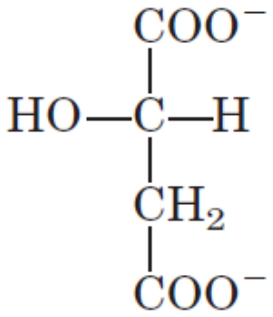
❖In the reverse direction (from L-malate to fumarate), fumarase is equally stereospecific: D-malate is not a substrate.



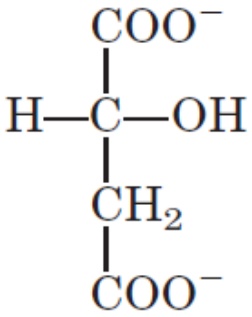
Fumarate



Maleate

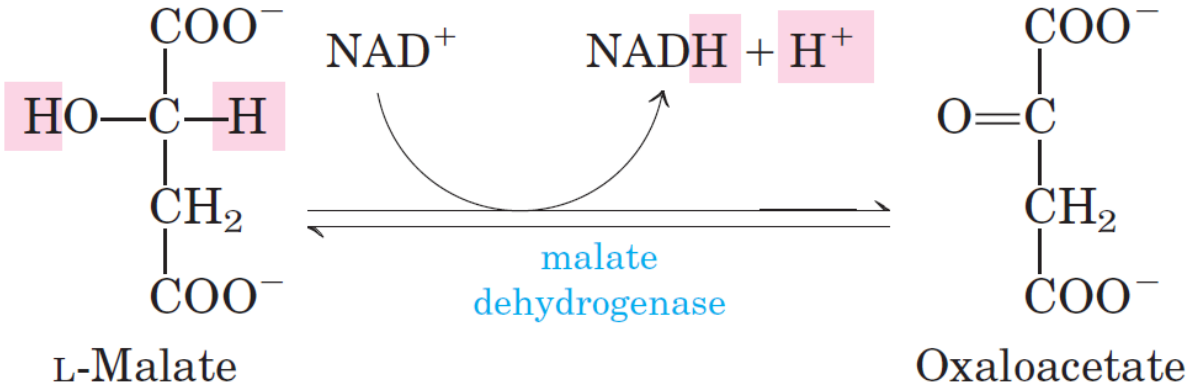


L-Malate



D-Malate

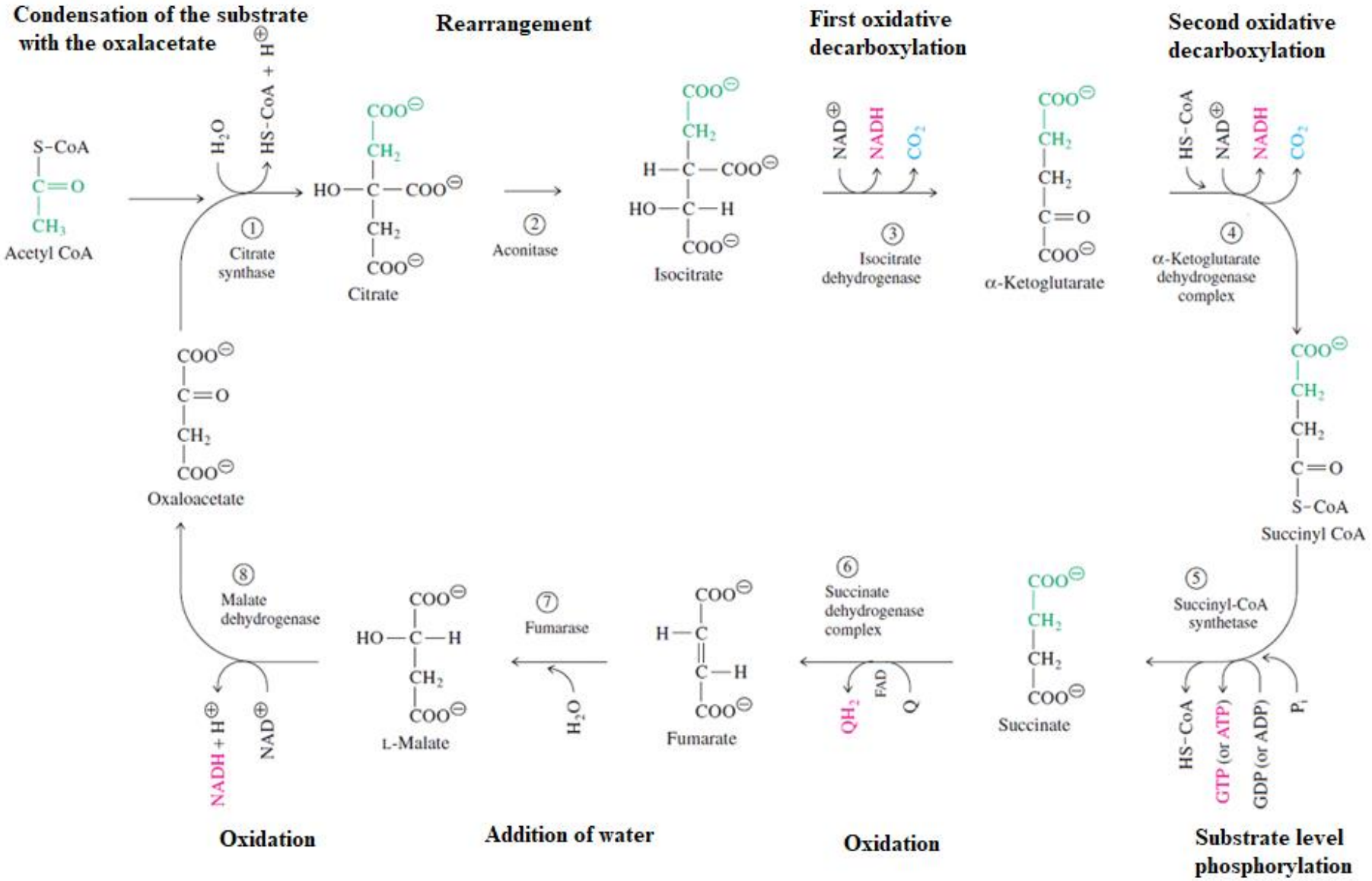
❖In the last reaction of the citric acid cycle, NAD-linked L-malate dehydrogenase catalyzes the oxidation of L-malate to oxaloacetate.

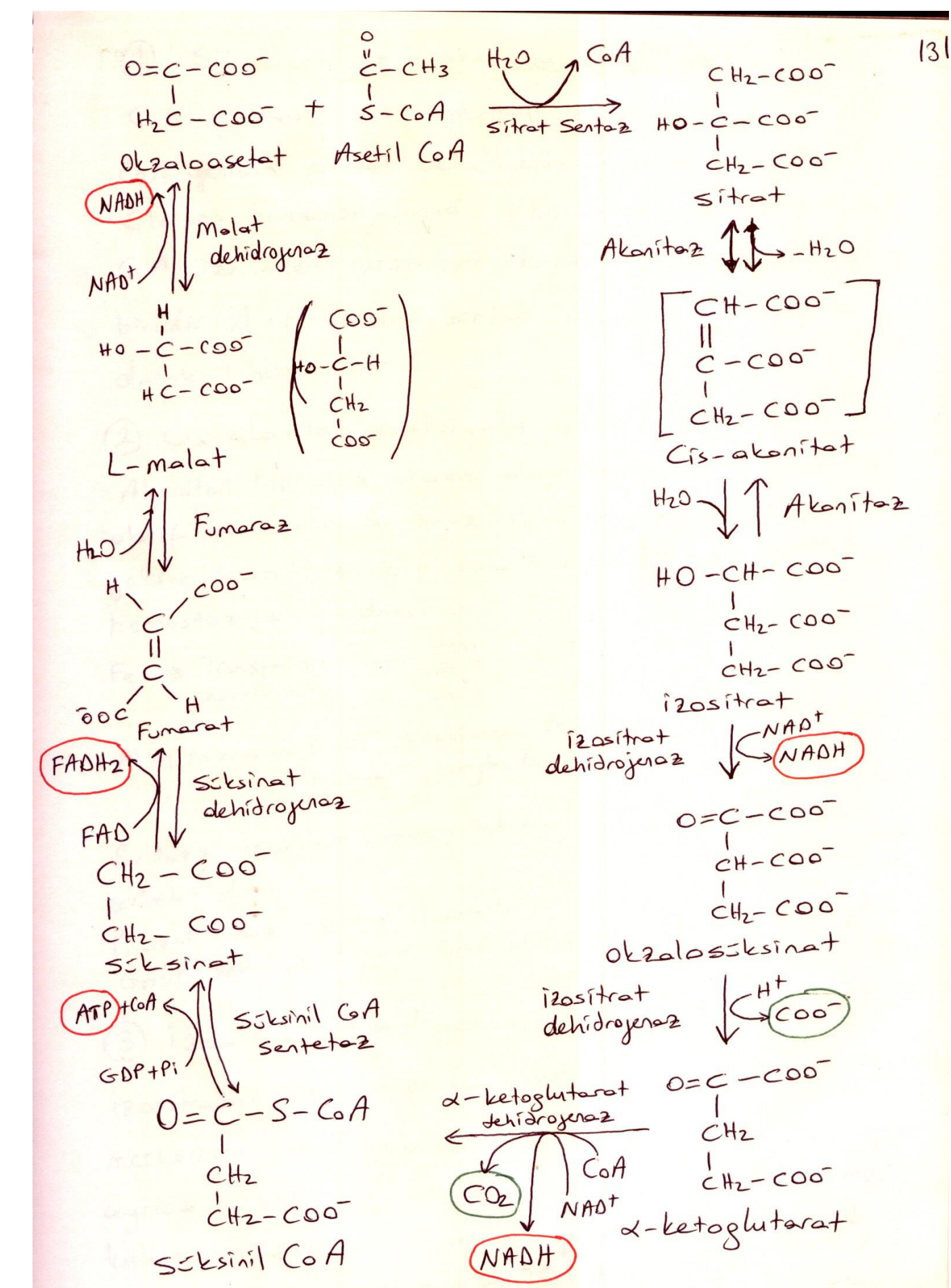


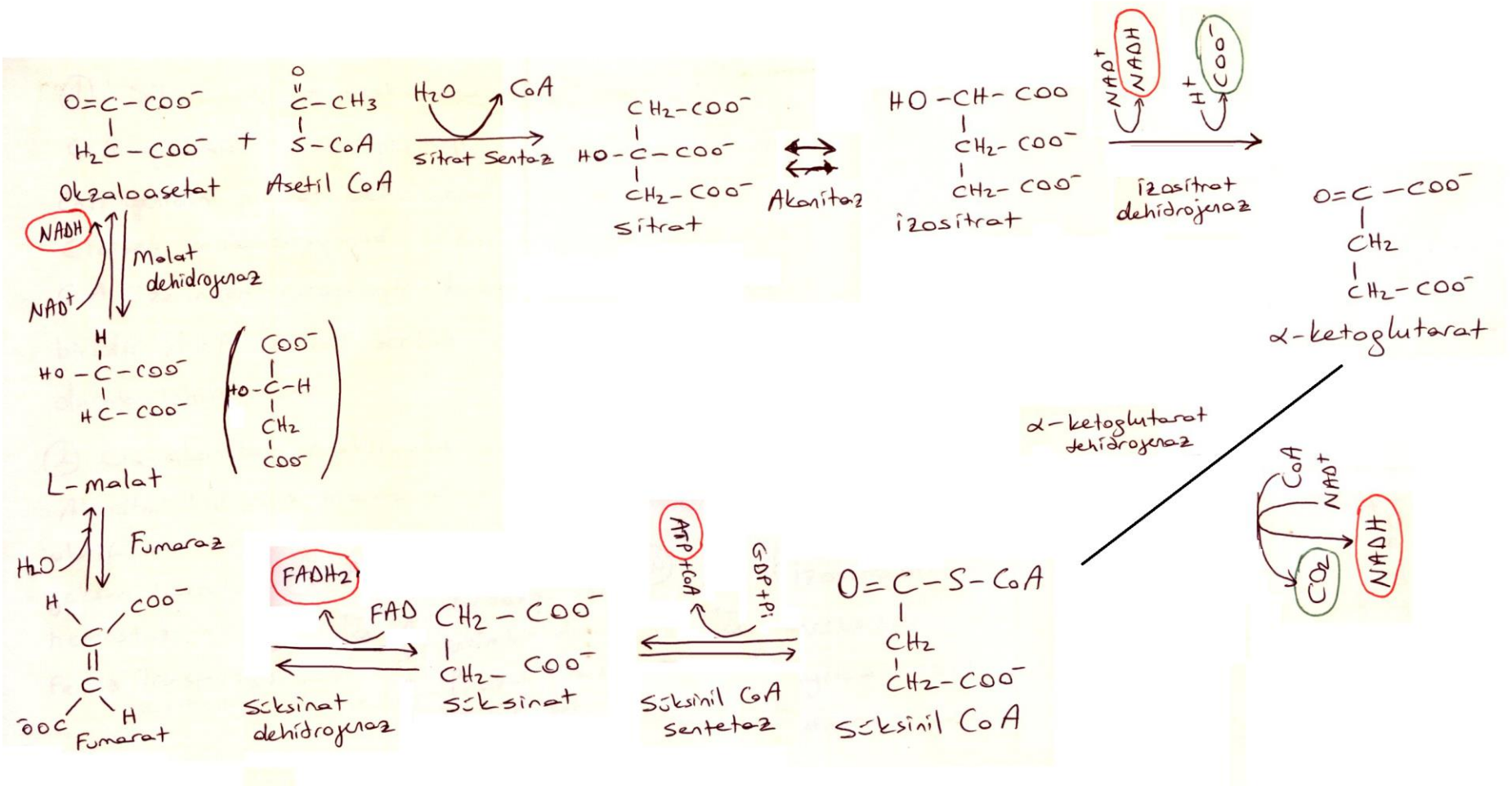
L-Malate Oxaloacetate

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

❖The equilibrium of this reaction lies far to the left under standard thermodynamic conditions, but in intact cells oxaloacetate is continually removed by the highly exergonic citrate synthase reaction and this keeps the concentration of oxaloacetate in the cell extremely low ($<10^{-6}$ M), pulling the malate dehydrogenase reaction toward the formation of oxaloacetate.


Condensation of the substrate with the oxalacetate
Rearrangement
First oxidative decarboxylation
Second oxidative decarboxylation
Acetyl CoA
Citrate synthase
Citrate
Aconitase
Isocitrate
Isocitrate dehydrogenase
α-Ketoglutarate
α-Ketoglutarate dehydrogenase complex
Succinyl CoA
Succinyl-CoA synthetase
Succinate
Succinate dehydrogenase complex
Fumarate
Fumarase
L-Malate
Malate dehydrogenase
Oxaloacetate
Oxidation
Addition of water
Oxidation
Substrate level phosphorylation


Okzaloasetat
Asetil CoA
H2O
CoA
Sitrat Sentaz
sitrat
NADH
NAD+
Malat dehidrojenaz
Akonitaz
-H2O
L-malat
Cis-akonitat
H2O
Akonitaz
Fumaraz
izositrat
izositrat dehidrojenaz
NAD+
NADH
Fumarat
FADH2
FAD
Süksinat dehidrojenaz
okzalosüksinat
süksinat
izositrat dehidrojenaz
H+
ATP+CoA
GDP+Pi
Süksinil CoA sentetaz
α-ketoglutarat dehidrojenaz
CO2
CoA
NAD+
NADH
süksinil CoA
α-ketoglutarat


Okzaloasetat
Asetil CoA
Sitrat Sentaz
Sitrat
Akonitaz
İzositrat
İzositrat dehidrojenaz
α-ketoglutarat
α-ketoglutarat dehidrojenaz
Süksinil CoA
Süksinil CoA sentetaz
Süksinat
Süksinat dehidrojenaz
Fumarat
Fumaraz
L-malat
Malat dehidrojenaz

The Energy of Oxidations in the Cycle Is Efficiently Conserved

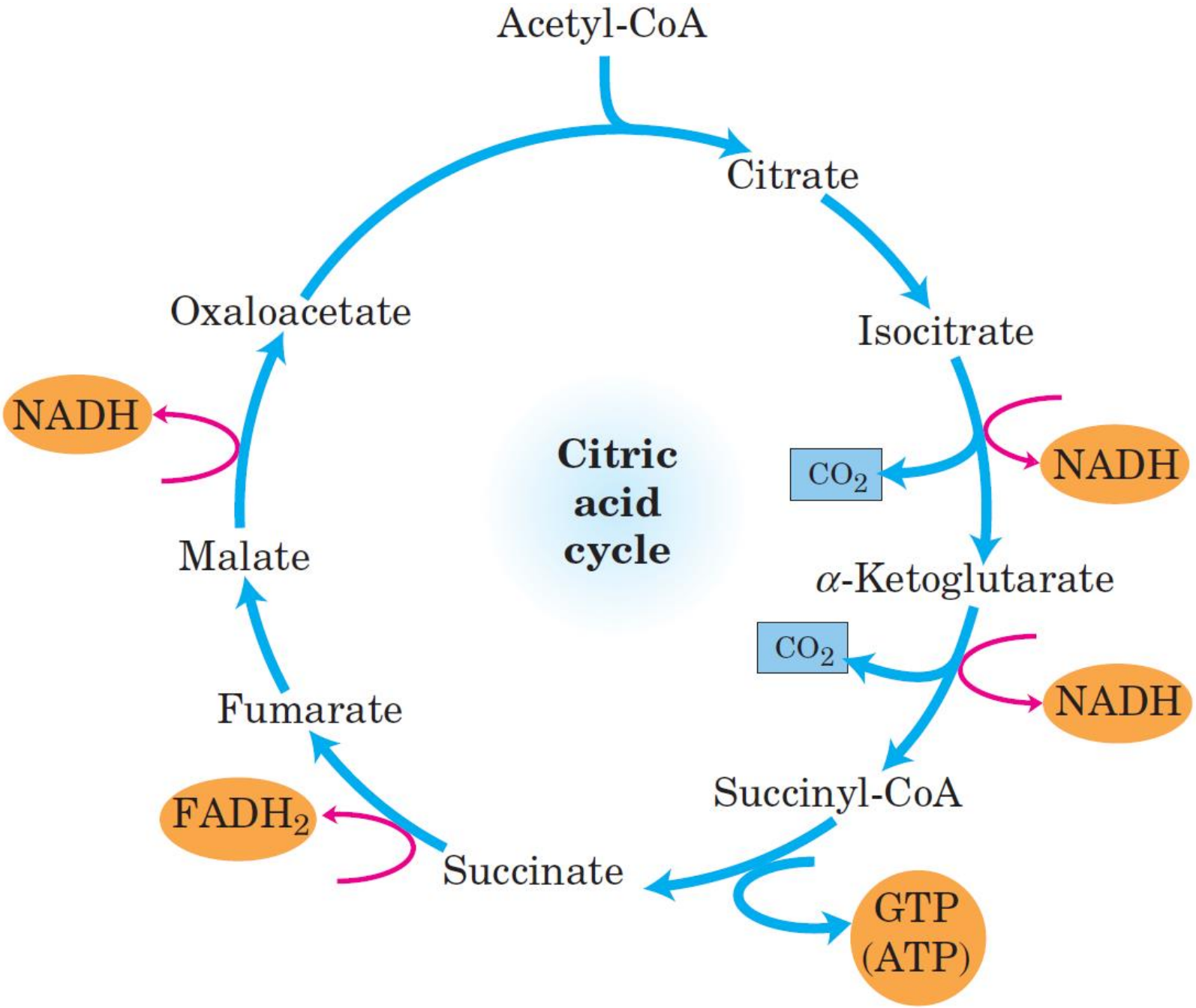
❖A two-carbon acetyl group entered the cycle by combining with oxaloacetate.

❖Two carbon atoms emerged from the cycle as CO_2 from the oxidation of isocitrate and α-ketoglutarate.

❖The energy released by these oxidations was conserved in the reduction of three NAD^+ and one FAD and the production of one ATP or GTP.

❖At the end of the cycle a molecule of oxaloacetate was regenerated.

❖Note that the two carbon atoms appearing as CO_2 are not the same two carbons that entered in the form of the acetyl group; additional turns around the cycle are required to release these carbons as CO_2


Acetyl-CoA
Citrate
Isocitrate
NADH
CO_2
α-Ketoglutarate
NADH
CO_2
Succinyl-CoA
GTP
(ATP)
Succinate
$FADH_2$
Fumarate
Malate
NADH
Oxaloacetate
Citric
acid
cycle

❖Although the citric acid cycle directly generates only one ATP per turn (in the conversion of succinyl-CoA to succinate), the four oxidation steps in the cycle provide a large flow of electrons into the respiratory chain via NADH and $FADH_2$ and thus lead to formation of a large number of ATP molecules during oxidative phosphorylation.

❖We saw in glycolysis pathway that the energy yield from the production of two molecules of pyruvate from one molecule of glucose is 2 ATP and 2 NADH.

❖In oxidative phosphorylation, passage of two electrons from NADH to O^2 drives the formation of about 2.5 ATP, and passage of two electrons from $FADH_2$ to O^2 yields about 1.5 ATP.

❖This stoichiometry allows us to calculate the overall yield of ATP from the complete oxidation of glucose.

❖When both pyruvate molecules are oxidized to 6 CO_2 via the pyruvate dehydrogenase complex and the citric acid cycle, and the electrons are transferred to O_2 via oxidative phosphorylation, as many as 32 ATP are obtained per glucose.

❖In round numbers, this represents the conservation of 32 x 30.5 kJ/mol = 976 kJ/mol or 34% of the theoretical maximum of about 2,840 kJ/mol available from the complete oxidation of glucose.

❖These calculations employ the standard free-energy changes; when corrected for the actual free energy required to form ATP within cells, the calculated efficiency of the process is closer to 65%.

TABLE 16–1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

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	3 or 5†
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

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.

†This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19–30 and 19–31.

❖The eight-step cyclic process for oxidation of simple two-carbon acetyl groups to CO_2 may seem unnecessarily cumbersome and not in keeping with the biological principle of maximum economy.

❖The role of the citric acid cycle is not confined to the oxidation of acetate, however.

❖This pathway is the hub of intermediary metabolism.

❖Four- and five-carbon end products of many catabolic processes feed into the cycle to serve as fuels.

❖Oxaloacetate and -ketoglutarate, for example, are produced from aspartate and glutamate, respectively, when proteins are degraded.

❖ Under some metabolic circumstances, intermediates are drawn out of the cycle to be used as precursors in a variety of biosynthetic pathways.

❖In aerobic organisms, the citric acid cycle is an amphibolic pathway, one that serves in both catabolic and anabolic processes.

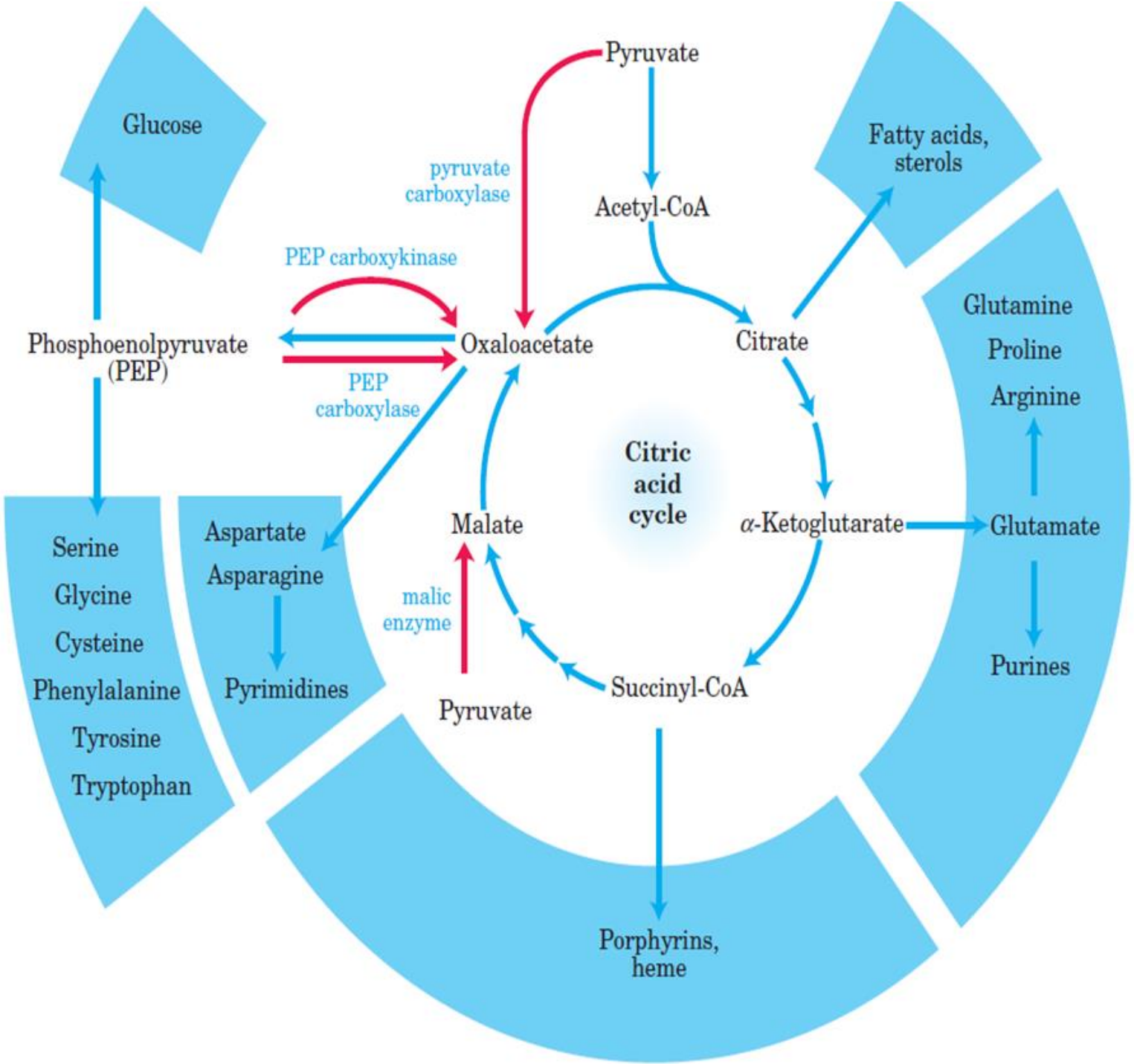
❖Besides its role in the oxidative catabolism of carbohydrates, fatty acids, and amino acids, the cycle provides precursors for many biosynthetic pathways, through reactions that served the same purpose in anaerobic ancestors.

❖α-Ketoglutarate and oxaloacetate can, for example, serve as precursors of the amino acids aspartate and glutamate by simple transamination (Chapter 22).

❖Through aspartate and glutamate, the carbons of oxaloacetate and α-ketoglutarate are then used to build other amino acids, as well as purine and pyrimidine nucleotides.

❖Oxaloacetate is converted to glucose in gluconeogenesis.

❖Succinyl-CoA is a central intermediate in the synthesis of the


Glucose
Pyruvate
pyruvate carboxylase
Acetyl-CoA
Fatty acids, sterols
PEP carboxykinase
Phosphoenolpyruvate (PEP)
Oxaloacetate
Citrate
PEP carboxylase
Glutamine
Proline
Arginine
Citric acid cycle
Serine
Glycine
Cysteine
Phenylalanine
Tyrosine
Tryptophan
Aspartate
Asparagine
Pyrimidines
Malate
α-Ketoglutarate
Glutamate
malic enzyme
Pyruvate
Succinyl-CoA
Purines
Porphyrins, heme

❖As intermediates of the citric acid cycle are removed to serve as biosynthetic precursors, they are replenished by anaplerotic reactions.

❖Under normal circumstances, the reactions by which cycle intermediates are siphoned off into other pathways and those by which they are replenished are in dynamic balance, so that the concentrations of the citric acid cycle intermediates remain almost constant.

TABLE 16–2 Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
$\text{Pyruvate} + HCO_3^- + \text{ATP} \xrightleftharpoons{\text{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + P_i$	Liver, kidney
$\text{Phosphoenolpyruvate} + CO_2 + \text{GDP} \xrightleftharpoons{\text{PEP carboxykinase}} \text{oxaloacetate} + \text{GTP}$	Heart, skeletal muscle
$\text{Phosphoenolpyruvate} + HCO_3^- \xrightleftharpoons{\text{PEP carboxylase}} \text{oxaloacetate} + P_i$	Higher plants, yeast, bacteria
$\text{Pyruvate} + HCO_3^- + \text{NAD(P)H} \xrightleftharpoons{\text{malic enzyme}} \text{malate} + \text{NAD(P)}^+$	Widely distributed in eukaryotes and bacteria

Regulation of the Citric Acid Cycle

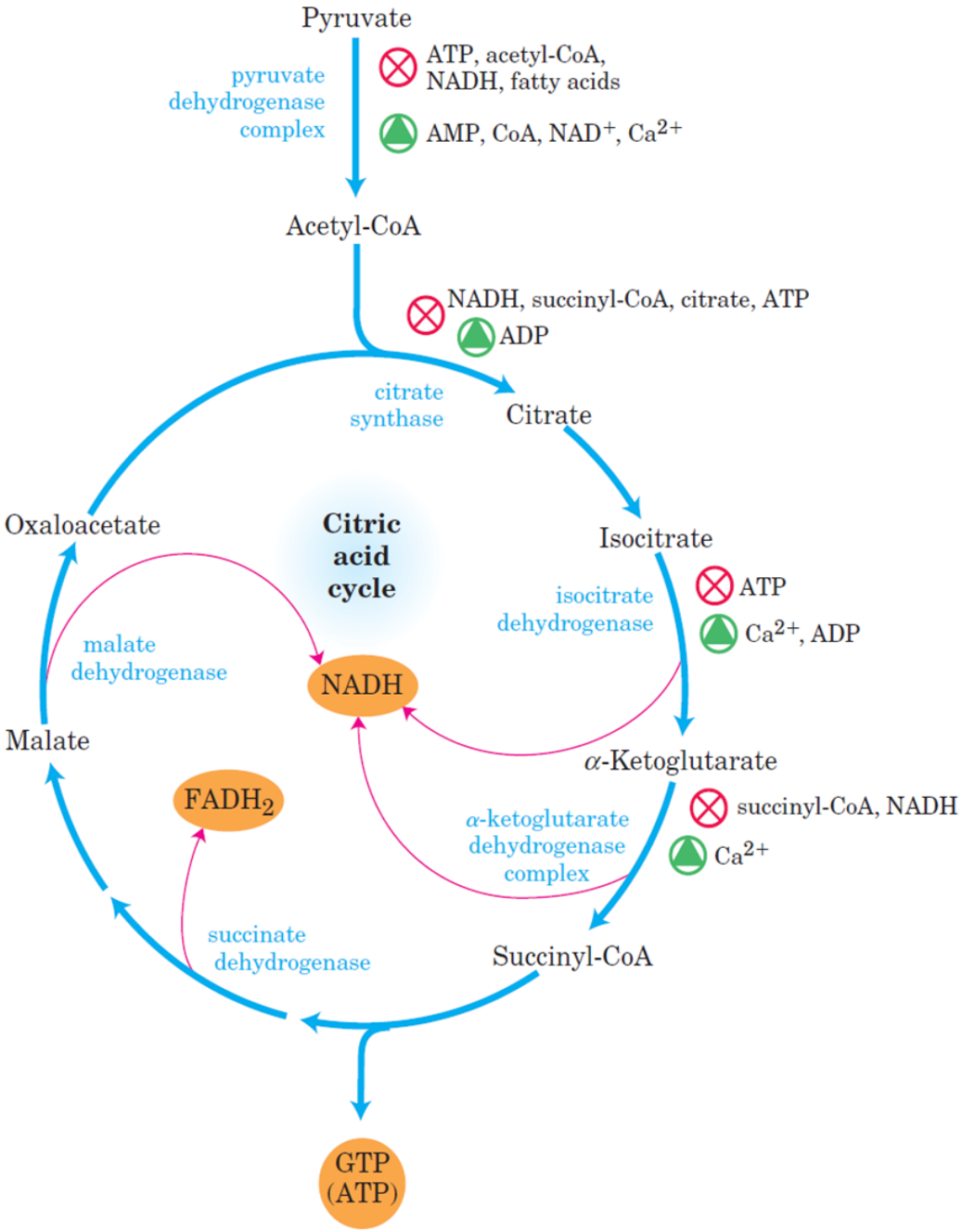
- The flow of carbon atoms from pyruvate into and through the citric acid cycle is under tight regulation at two levels:

  - Conversion of pyruvate to acetyl-CoA, (the PDH complex reaction)

  - and the entry of acetyl-CoA into the cycle (the citrate synthase reaction).

- Acetyl-CoA is also produced by pathways other than the PDH complex reaction—most cells produce acetyl-CoA from the oxidation of fatty acids and certain amino acids—and the availability of intermediates from these other pathways is important in the regulation of pyruvate oxidation and of the citric acid cycle.

- The cycle is also regulated at the isocitrate dehydrogenase and α-ketoglutarate dehydrogenase reactions.


Pyruvate
pyruvate dehydrogenase complex
ATP, acetyl-CoA, NADH, fatty acids
AMP, CoA, NAD+, Ca2+
Acetyl-CoA
NADH, succinyl-CoA, citrate, ATP
ADP
citrate synthase
Citrate
Isocitrate
ATP
isocitrate dehydrogenase
Ca2+, ADP
Citric acid cycle
Oxaloacetate
malate dehydrogenase
NADH
Malate
α-Ketoglutarate
succinyl-CoA, NADH
α-ketoglutarate dehydrogenase complex
Ca2+
FADH2
succinate dehydrogenase
Succinyl-CoA
GTP (ATP)

❖Three factors govern the rate of flux through the cycle: substrate availability, inhibition by accumulating products, and allosteric feedback inhibition of the enzymes that catalyze early steps in the cycle.

❖Each of the three strongly exergonic steps in the cycle—those catalyzed by citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase —can become the rate-limiting step under some circumstances.

❖Under normal conditions, the rates of glycolysis and of the citric acid cycle are integrated so that only as much glucose is metabolized to pyruvate as is needed to supply the citric acid cycle with its fuel, the acetyl groups of acetyl-CoA.

❖Citrate, the product of the first step of the citric acid cycle, is an important allosteric inhibitor of phosphofructokinase-1 in the glycolytic pathway.

The Glyoxylate Cycle

❖Vertebrates cannot convert fatty acids, or the acetate derived from them, to carbohydrates.

❖Conversion of phosphoenolpyruvate to pyruvate and of pyruvate to acetyl-CoA are so exergonic as to be essentially irreversible.

❖If a cell cannot convert acetate into phosphoenolpyruvate, acetate cannot serve as the starting material for the gluconeogenic pathway, which leads from phosphoenolpyruvate to glucose.

❖Without this capacity, then, a cell or organism is unable to convert fuels or metabolites that are degraded to acetate (fatty acids and certain amino acids) into carbohydrates.

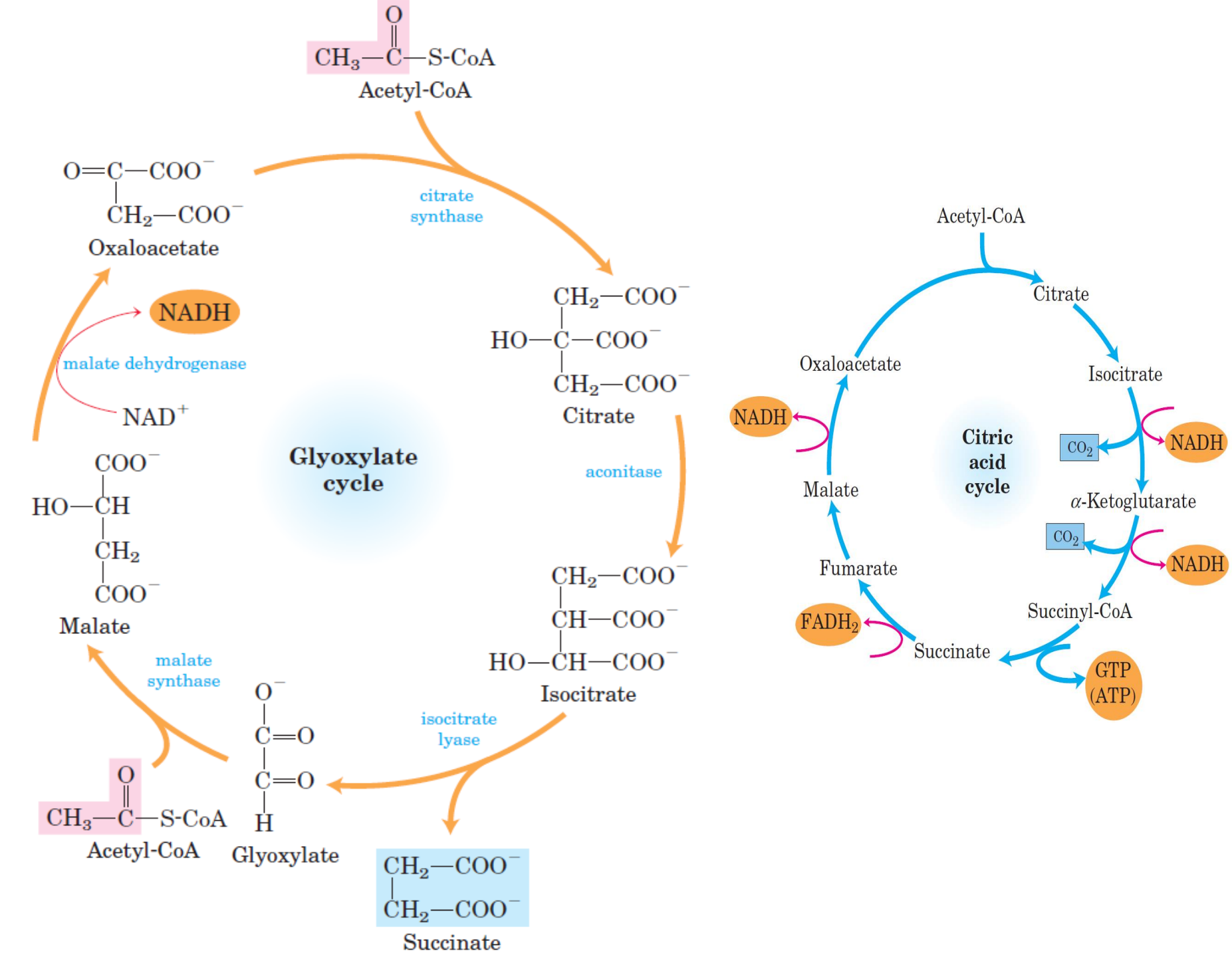
❖As noted in the discussion of anaplerotic reactions, phosphoenolpyruvate can be synthesized from oxaloacetate in the reversible reaction catalyzed by PEP carboxykinase:

$$\text{Oxaloacetate} + \text{GTP} \rightleftharpoons \text{phosphoenolpyruvate} + CO_2 + \text{GDP}$$

❖Because the carbon atoms of acetate molecules that enter the citric acid cycle appear eight steps later in oxaloacetate, it might seem that this pathway could generate oxaloacetate from acetate and thus generate phosphoenolpyruvate for gluconeogenesis.

❖However, as an examination of the stoichiometry of the citric acid cycle shows, there is no net conversion of acetate to oxaloacetate; in vertebrates, for every two carbons that enter the cycle as acetyl-CoA, two leave as CO2.

❖In many organisms other than vertebrates, the glyoxylate cycle serves as a mechanism for converting acetate to carbohydrate.


Acetyl-CoA
citrate synthase
Oxaloacetate
NADH
malate dehydrogenase
NAD+
Citrate
Glyoxylate cycle
aconitase
Malate
Isocitrate
malate synthase
isocitrate lyase
Acetyl-CoA
Glyoxylate
Succinate
Acetyl-CoA
Citrate
Isocitrate
NADH
CO2
NADH
Oxaloacetate
Citric acid cycle
α-Ketoglutarate
CO2
NADH
Malate
Fumarate
Succinyl-CoA
FADH2
Succinate
GTP (ATP)

❖In plants, certain invertebrates, and some microorganisms (including *E. coli* and yeast) acetate can serve both as an energy-rich fuel and as a source of phosphoenolpyruvate for carbohydrate synthesis.

❖Each turn of the glyoxylate cycle consumes two molecules of acetyl-CoA and produces one molecule of succinate, which is then available for biosynthetic purposes.

❖The succinate may be converted through fumarate and malate into oxaloacetate, which can then be converted to phosphoenolpyruvate by PEP carboxykinase, and thus to glucose by gluconeogenesis.

❖Vertebrates do not have the enzymes specific to the glyoxylate cycle (isocitrate lyase and malate synthase) and therefore cannot bring about the net synthesis of glucose from fatty acids.

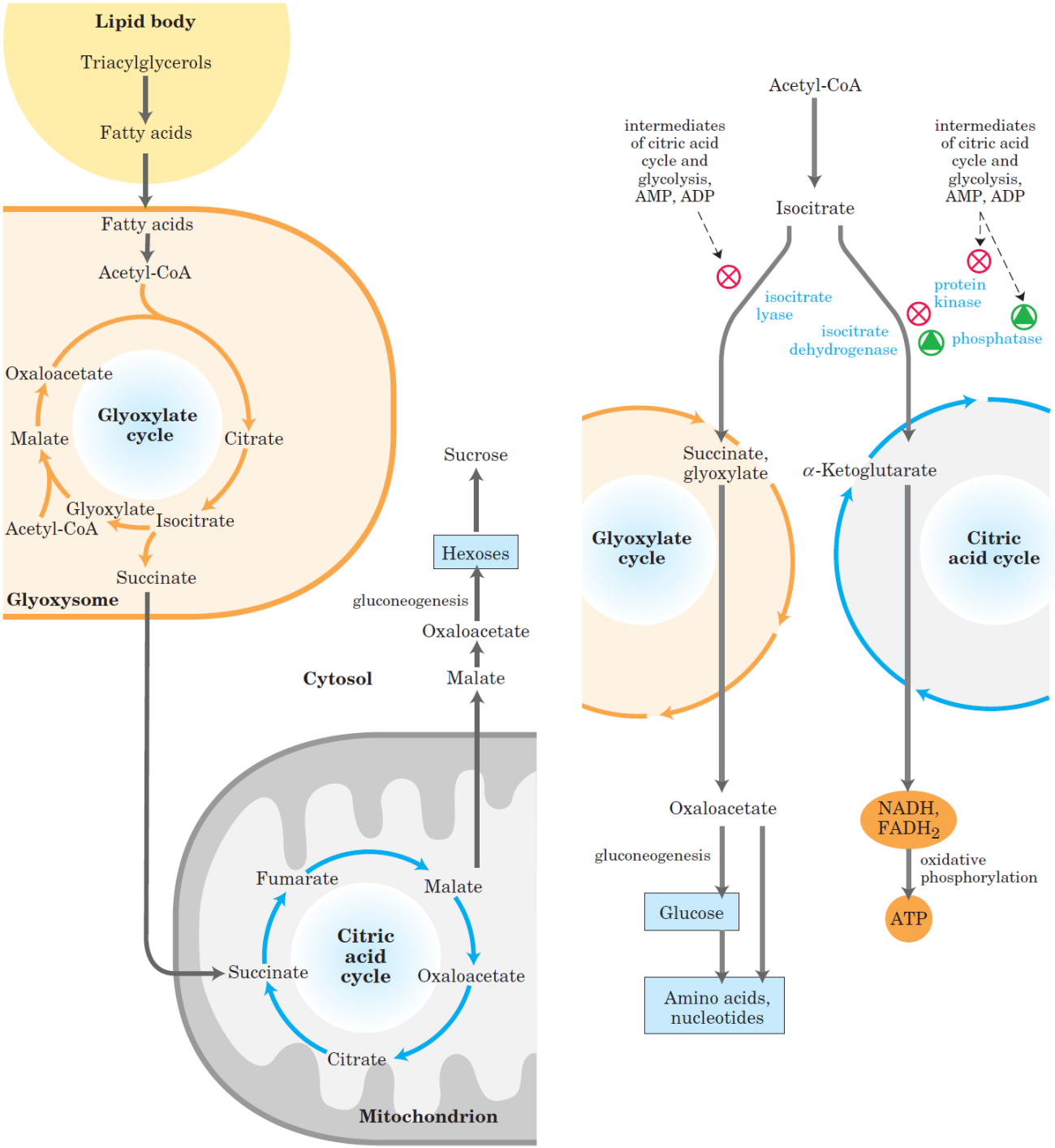
❖Isocitrate is a crucial intermediate, at the branch point between the glyoxylate and citric acid cycles.

❖Isocitrate dehydrogenase is regulated by covalent modification: a specific protein kinase phosphorylates and thereby inactivates the dehydrogenase.

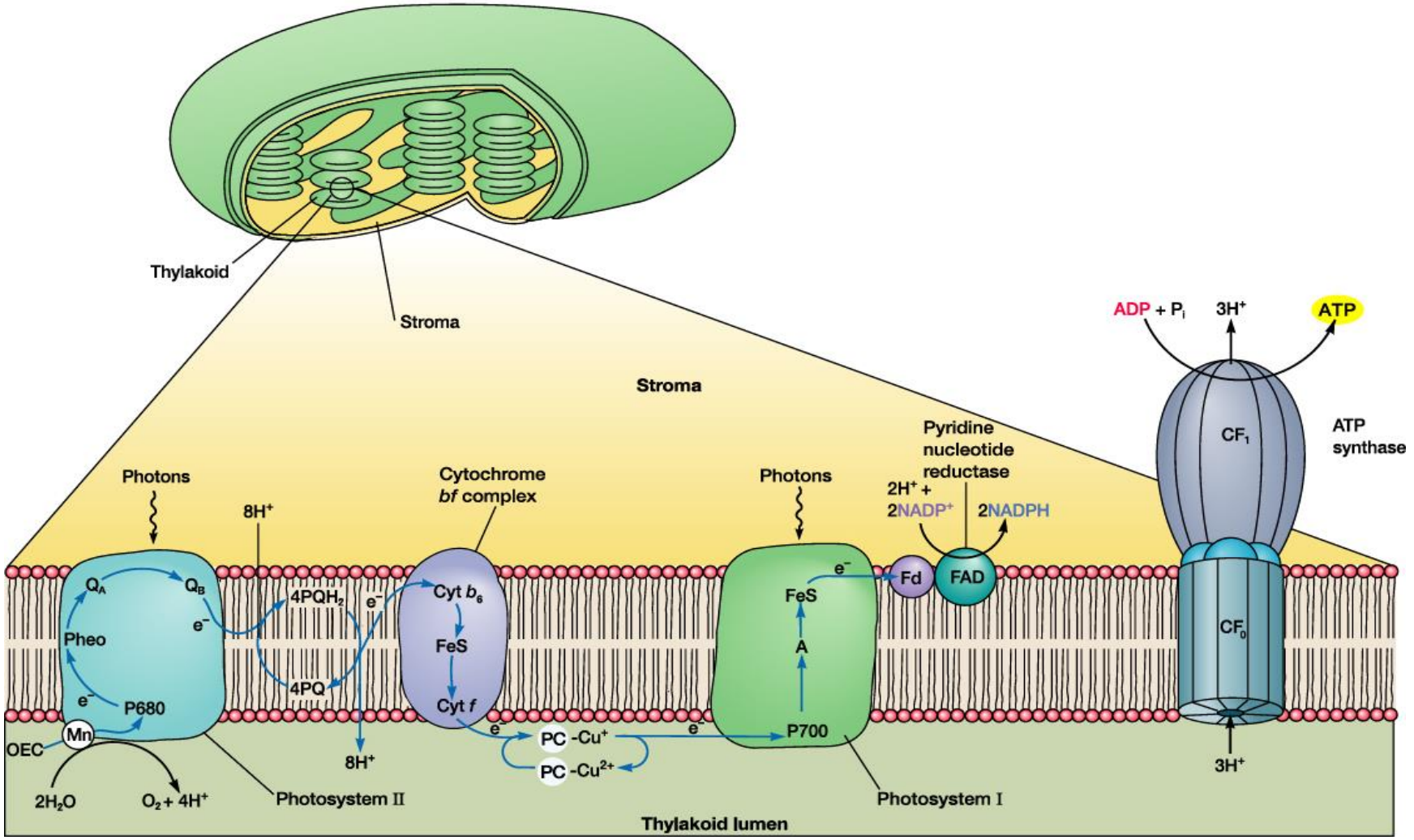
❖This inactivation shunts isocitrate to the glyoxylate cycle, where it begins the synthetic route toward glucose.

❖A phosphoprotein phosphatase removes the phosphoryl group from isocitrate dehydrogenase, reactivating the enzyme and sending more isocitrate through the energy-yielding citric acid cycle.

❖The regulatory protein kinase and phosphoprotein phosphatase are separate enzymatic activities of a single polypeptide.


Lipid body
Triacylglycerols
Fatty acids
Fatty acids
Acetyl-CoA
Oxaloacetate
Glyoxylate cycle
Malate
Citrate
Glyoxylate
Acetyl-CoA
Isocitrate
Succinate
Glyoxysome
Sucrose
Hexoses
gluconeogenesis
Oxaloacetate
Cytosol
Malate
Fumarate
Malate
Citric acid cycle
Succinate
Oxaloacetate
Citrate
Mitochondrion
Acetyl-CoA
intermediates of citric acid cycle and glycolysis, AMP, ADP
Isocitrate
intermediates of citric acid cycle and glycolysis, AMP, ADP
isocitrate lyase
protein kinase
isocitrate dehydrogenase
phosphatase
Succinate, glyoxylate
α-Ketoglutarate
Glyoxylate cycle
Citric acid cycle
Oxaloacetate
NADH, FADH2
gluconeogenesis
oxidative phosphorylation
Glucose
ATP
Amino acids, nucleotides

Carbohydrate Metabolism IV
Oxidative Phosphorylation and Photophosphorylation



Oxidative Phosphorylation and Photophosphorylation

❖Oxidative phosphorylation is the culmination of energy-yielding metabolism in aerobic organisms.

❖All oxidative steps in the degradation of carbohydrates, fats, and amino acids converge at this final stage of cellular respiration, in which the energy of oxidation drives the synthesis of ATP.

❖Photophosphorylation is the means by which photosynthetic organisms capture the energy of sunlight—the ultimate source of energy in the biosphere— and harness it to make ATP.

❖Together, oxidative phosphorylation and photophosphorylation account for most of the ATP synthesized by most organisms most of the time.

❖In eukaryotes, oxidative phosphorylation occurs in mitochondria, photophosphorylation in chloroplasts.

❖The pathways to ATP synthesis in mitochondria and chloroplasts have challenged and fascinated biochemists for more than half a century, and the fascination has grown with our deepening appreciation of these fundamental mechanisms in living organisms, their conservation in evolution, and their structural bases.

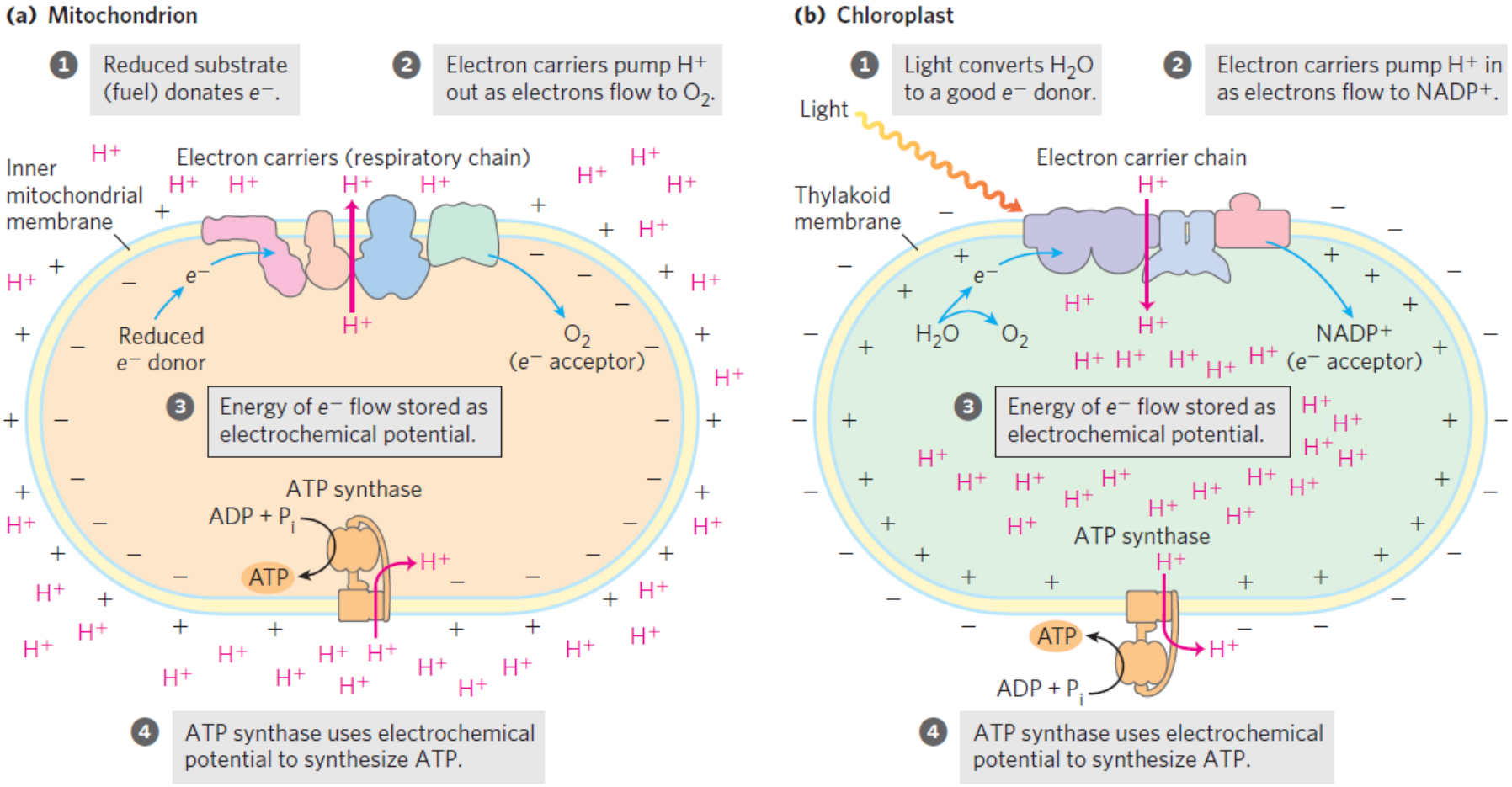
❖Our current understanding of ATP synthesis in mitochondria and chloroplasts is based on the hypothesis, introduced by Peter Mitchell in 1961, that transmembrane differences in proton concentration are the reservoir for the energy extracted from biological oxidation reactions which called as chemiosmotic theory.

❖Oxidative phosphorylation and photophosphorylation are mechanistically similar in three respects.

❖(1) Both processes involve the flow of electrons through a chain of membrane-bound carriers.

❖(2) The free energy made available by this “downhill” (exergonic) electron flow is coupled to the “uphill” transport of protons across a proton-impermeable membrane, conserving the free energy of fuel oxidation as a transmembrane electrochemical potential.

❖(3) The transmembrane flow of protons back down their concentration gradient through specific protein channels provides the free energy for synthesis of ATP, catalyzed by a membrane protein complex (ATP synthase) that couples proton flow to phosphorylation of ADP.


(a) Mitochondrion
1 Reduced substrate (fuel) donates e⁻.
2 Electron carriers pump H⁺ out as electrons flow to O2.
Inner mitochondrial membrane
Electron carriers (respiratory chain)
Reduced e⁻ donor
O2 (e⁻ acceptor)
3 Energy of e⁻ flow stored as electrochemical potential.
ATP synthase
ADP + Pi
ATP
4 ATP synthase uses electrochemical potential to synthesize ATP.
(b) Chloroplast
1 Light converts H2O to a good e⁻ donor.
2 Electron carriers pump H⁺ in as electrons flow to NADP⁺.
Light
Thylakoid membrane
Electron carrier chain
H2O
O2
NADP+ (e⁻ acceptor)
3 Energy of e⁻ flow stored as electrochemical potential.
ATP synthase
ATP
ADP + Pi
4 ATP synthase uses electrochemical potential to synthesize ATP.

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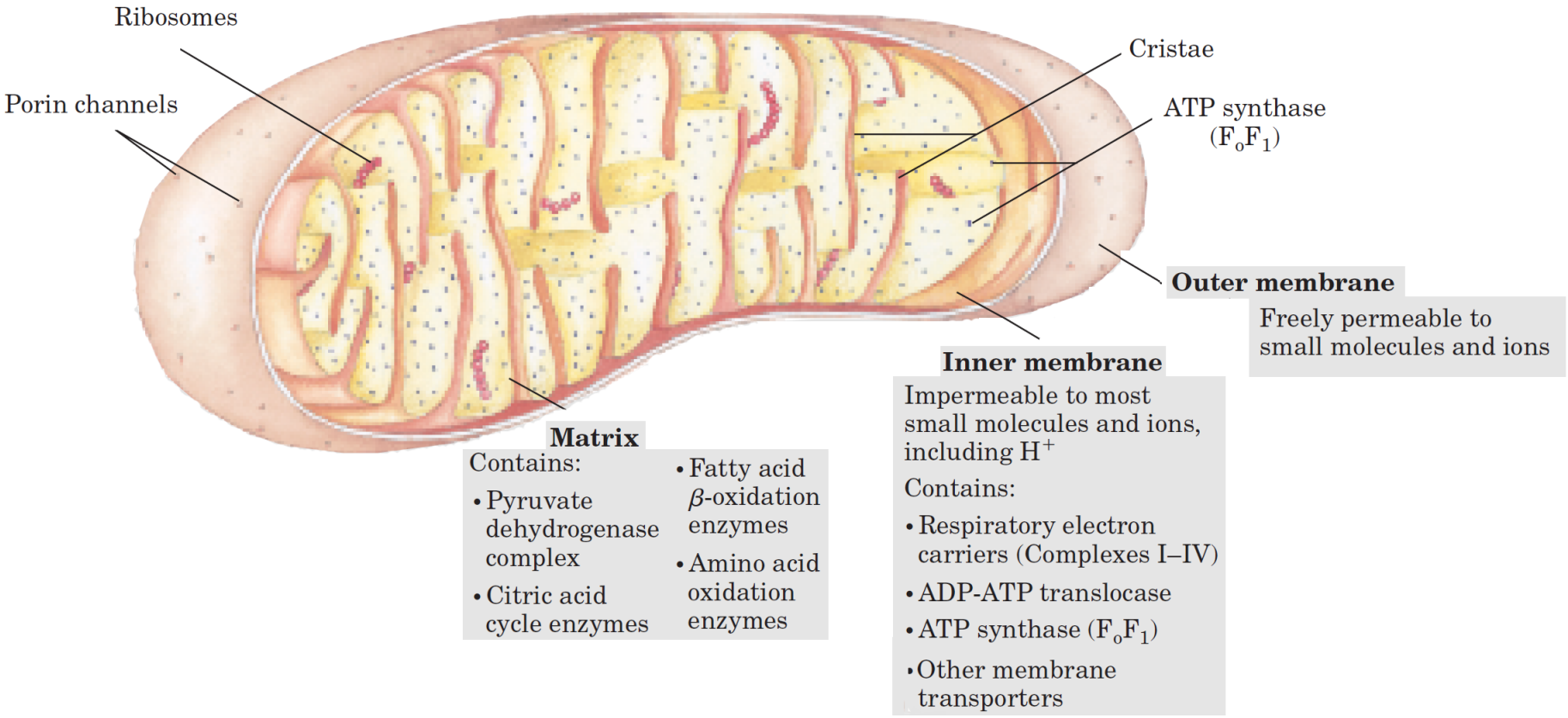
❖Mitochondria are the site of oxidative phosphorylation in eukaryotes.

❖Mitochondria, like gramnegative bacteria, have two membranes.



❖The outer mitochondrial membrane is readily permeable to small molecules (Mr, 5,000) and ions, which move freely through transmembrane channels formed by a family of integral membrane proteins called porins.

❖The inner membrane is impermeable to most small molecules and ions, including protons; the only species that cross this membrane do so through specific transporters.

❖The inner membrane bears the components of the respiratory chain and the ATP synthase.

❖The mitochondrial matrix, enclosed by the inner membrane, contains the pyruvate dehydrogenase complex and the enzymes of the citric acid cycle, the fatty acid β-oxidation pathway, and the pathways of amino acid oxidation—all the pathways of fuel oxidation except glycolysis, which takes place in the cytosol.

❖The selectively permeable inner membrane segregates the intermediates and enzymes of cytosolic metabolic pathways from those of metabolic processes occurring in the matrix.

❖However, specific transporters carry pyruvate, fatty acids, and amino acids or their -keto derivatives into the matrix for access to the machinery of the citric acid cycle.

❖ADP and Pi are specifically transported into the matrix as newly synthesized ATP is transported out.

❖Oxidative phosphorylation begins with the entry of electrons into the chain of electron carriers called the respiratory chain.

❖Most of these 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).

❖Nicotinamide nucleotide–linked dehydrogenases catalyze reversible reactions of the following general types:

$$\text{Reduced substrate} + \text{NAD(P)}^+ \rightleftharpoons \text{oxidized substrate} + \text{NAD(P)H} + \text{H}^+$$

❖NAD-linked dehydrogenases remove two hydrogen atoms from their substrates. One of these is transferred as a hydride ion ($:H^+$) to NAD^+, the other is released as H in the medium.

❖NADH and NADPH are water-soluble electron carriers that associate reversibly with dehydrogenases.

❖Flavoproteins contain a very tightly, sometimes covalently, bound flavin nucleotide, either FMN or FAD.

❖The oxidized flavin nucleotide can accept either one electron (yielding the semiquinone form) or two (yielding $FADH_2$ or $FMNH_2$).

❖The mitochondrial respiratory chain consists of a series of sequentially acting electron carriers, most of which are integral proteins with prosthetic groups capable of accepting and donating either one or two electrons.

❖Three types of electron transfers occur in oxidative phosphorylation:

❖(1) direct transfer of electrons, as in the reduction of Fe^{3+} to Fe^{2+},

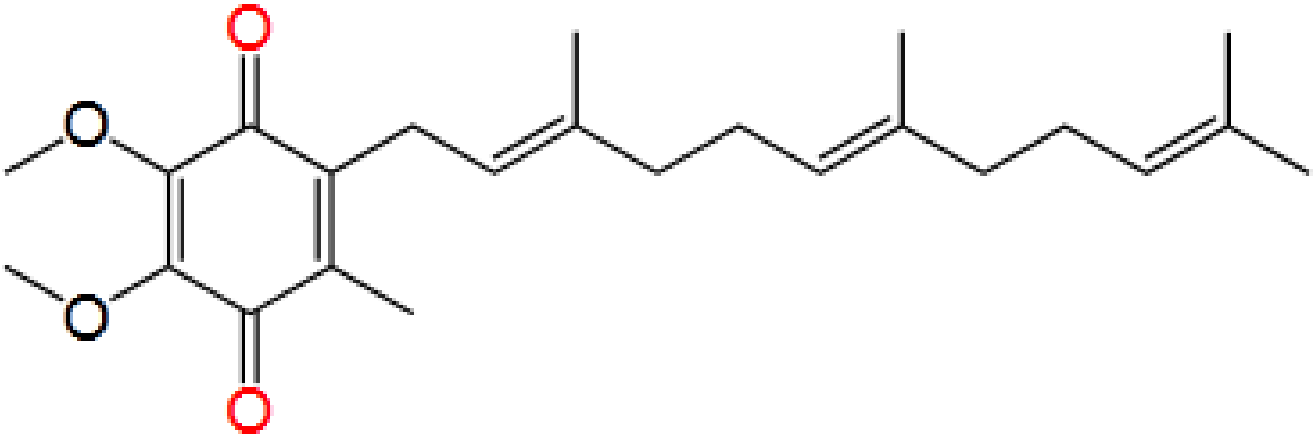
❖(2) transfer as a hydrogen atom ($H^+ + e^-$), and

❖(3) transfer as a hydride ion ($:H^-$), which bears two electrons.

❖The term reducing equivalent is used to designate a single electron equivalent transferred in an oxidation-reduction reaction.

❖In addition to NAD and flavoproteins, three other types of electron-carrying molecules function in the respiratory chain: a hydrophobic quinone (**ubiquinone**) and two different types of iron-containing proteins (**cytochromes** and **iron-sulfur proteins**).

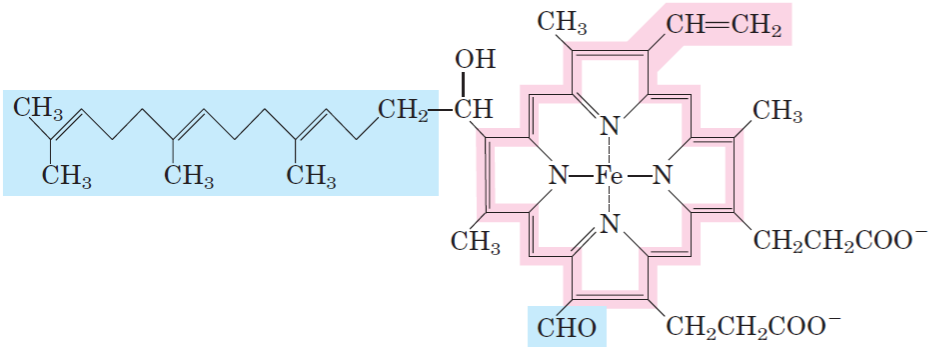
❖Ubiquinone (also called coenzyme Q, or simply Q) is a lipid-soluble benzoquinone with a long isoprenoid side chain.



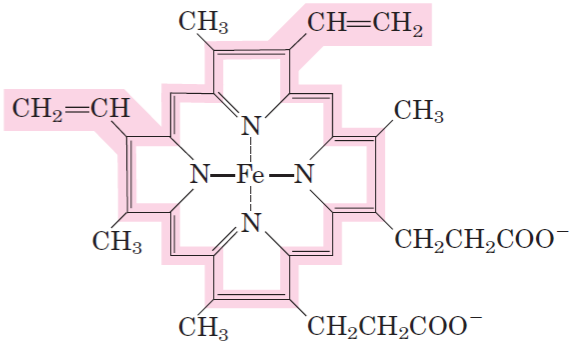
❖The closely related compounds plastoquinone (of plant chloroplasts) and menaquinone (of bacteria) play roles analogous to that of ubiquinone, carrying electrons in membrane-associated electron-transfer chains.

❖The cytochromes are proteins with characteristic strong absorption of visible light, due to their iron-containing heme prosthetic groups.

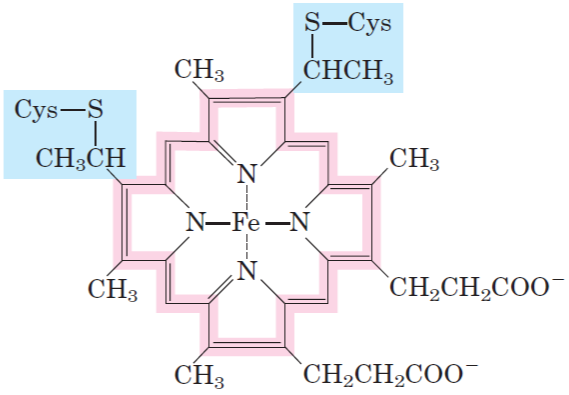
❖Mitochondria contain three classes of cytochromes, designated a, b, and c, which are distinguished by differences in their light-absorption spectra.



Heme A
(in *a*-type cytochromes)

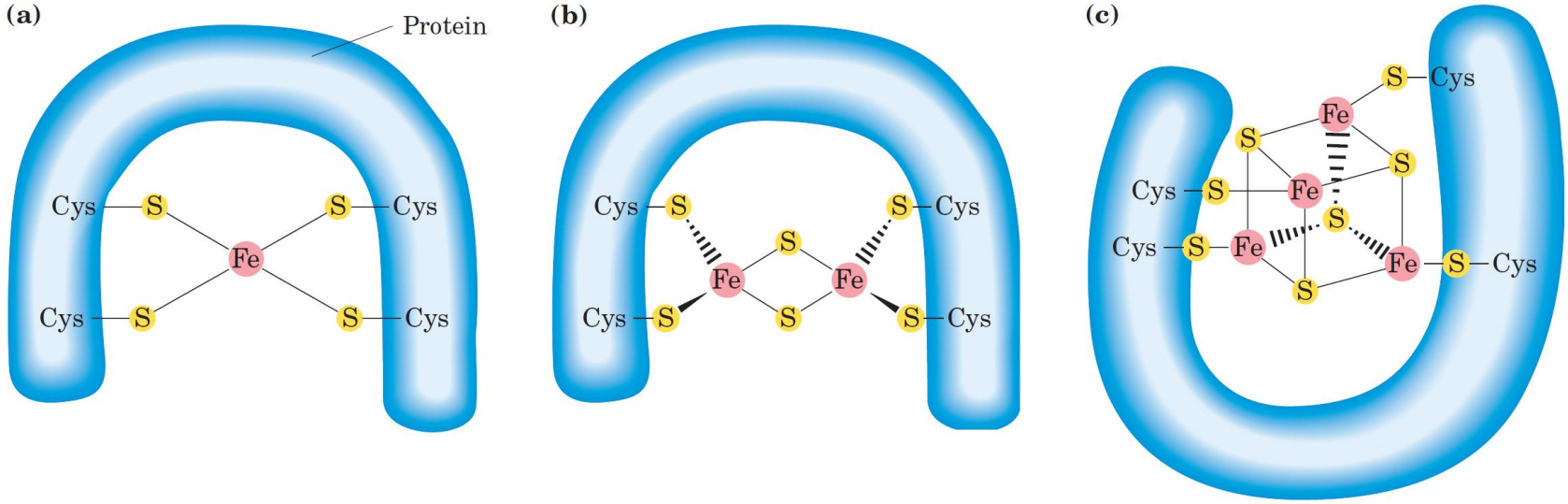


Iron protoporphyrin IX
(in *b*-type cytochromes)



Heme C
(in *c*-type cytochromes)

❖In iron-sulfur proteins, the iron is present not in heme but in association with inorganic sulfur atoms or with the sulfur atoms of Cys residues in the protein, or both.



❖Rieske iron-sulfur proteins are a variation on this theme, in which one Fe atom is coordinated to two His residues rather than two Cys residues.

❖In the overall reaction catalyzed by the mitochondrial respiratory chain, electrons move from NADH, succinate, or some other primary electron donor through flavoproteins, ubiquinone, iron-sulfur proteins, and cytochromes, and finally to O2.

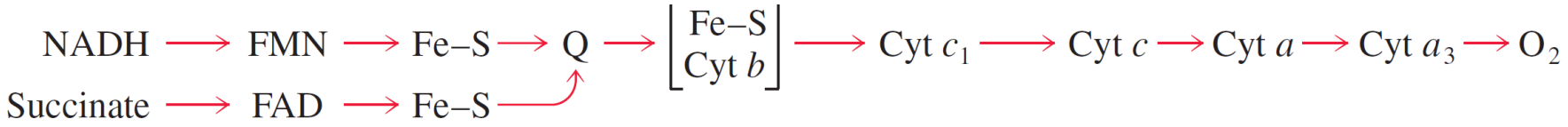
❖The electron carriers of the respiratory chain are organized into membrane-embedded supramolecular complexes that can be physically separated.

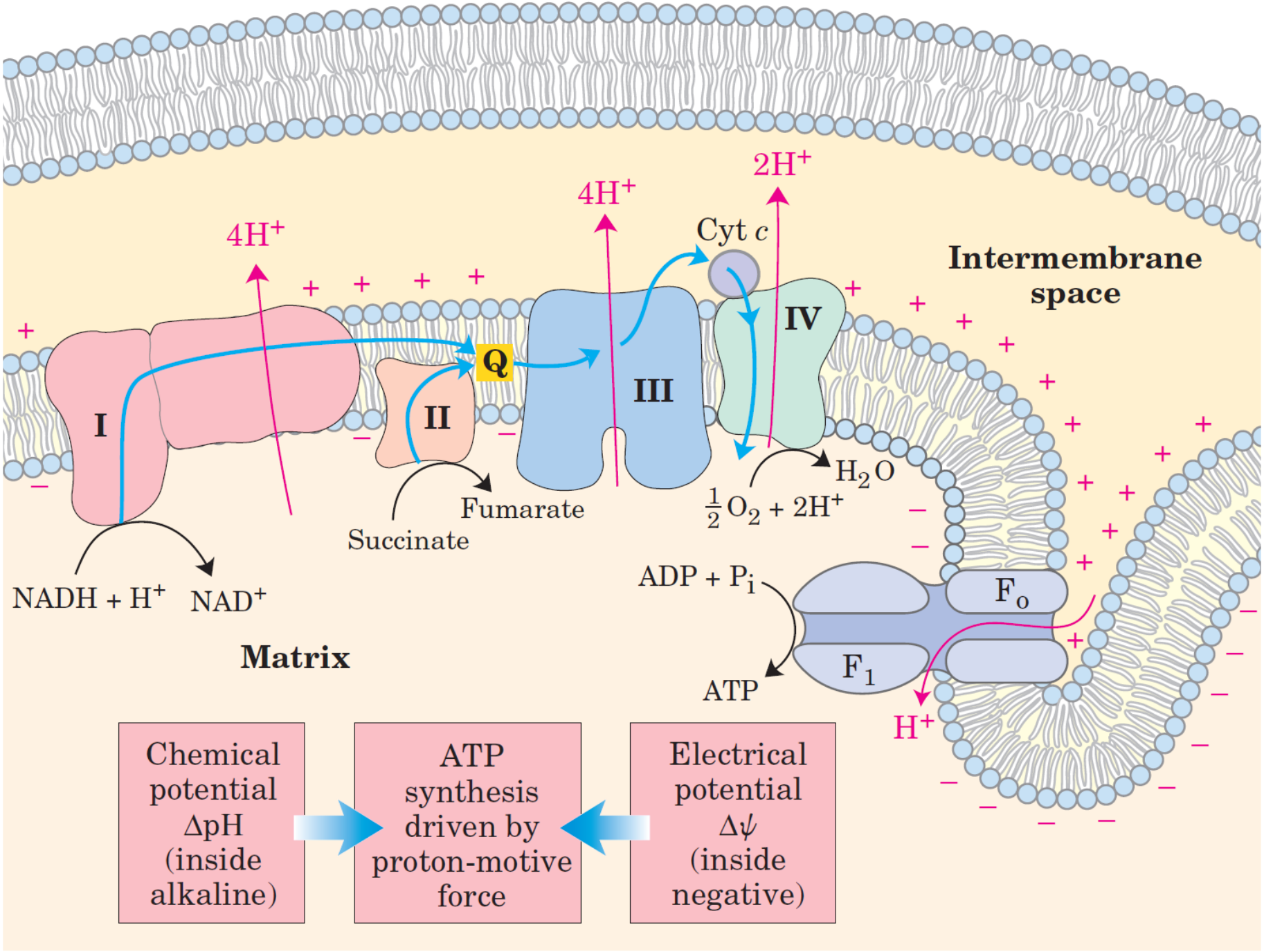
❖Gentle treatment of the inner mitochondrial membrane with detergents allows the resolution of four unique electron-carrier complexes, each capable of catalyzing electron transfer through a portion of the chain.

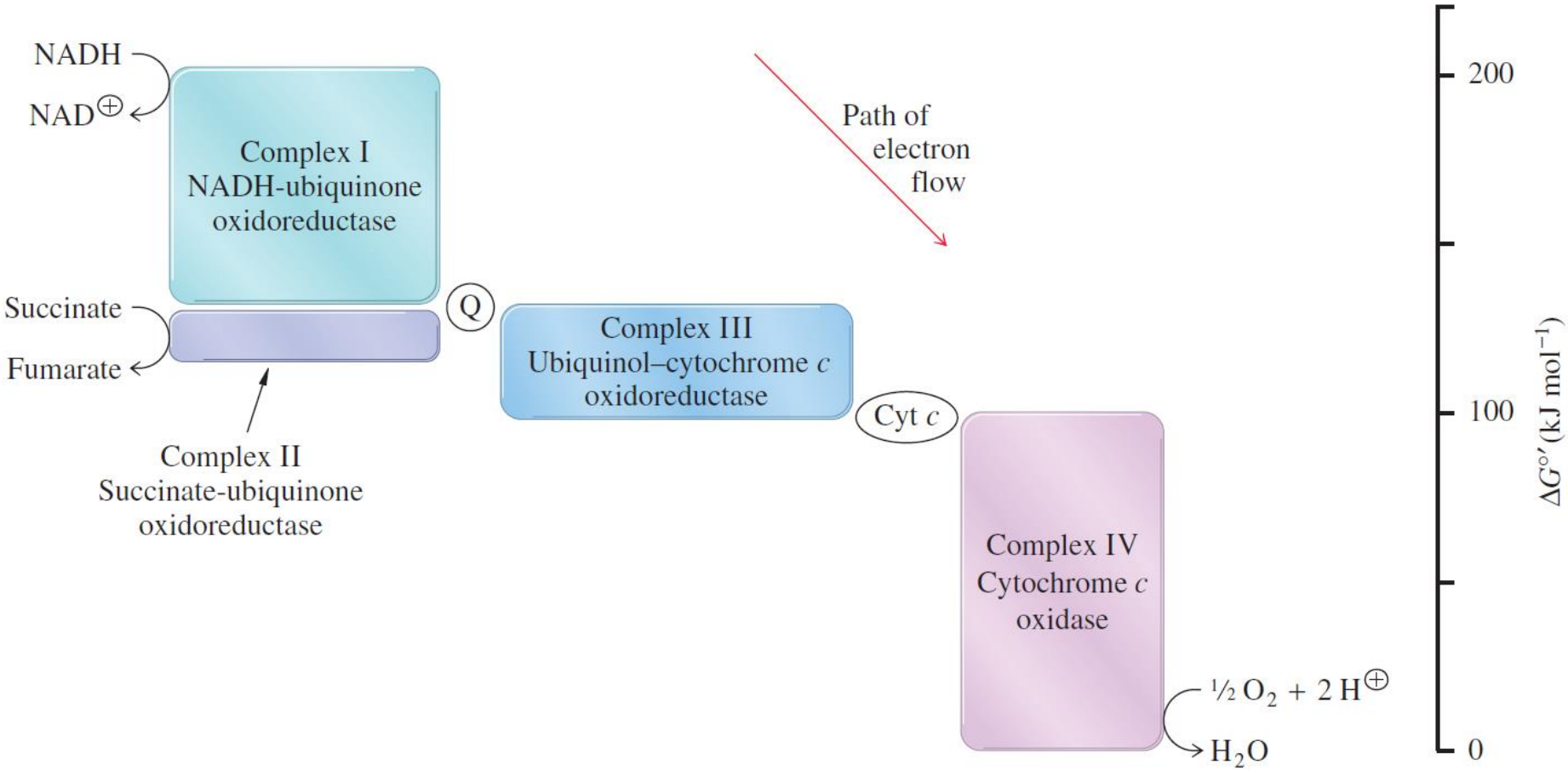
❖Complexes I and II catalyze electron transfer to ubiquinone from two different electron donors: NADH (Complex I) and succinate (Complex II).

❖Complex III carries electrons from reduced ubiquinone to cytochrome c, and Complex IV completes the sequence by transferring electrons from cytochrome c to O_2.

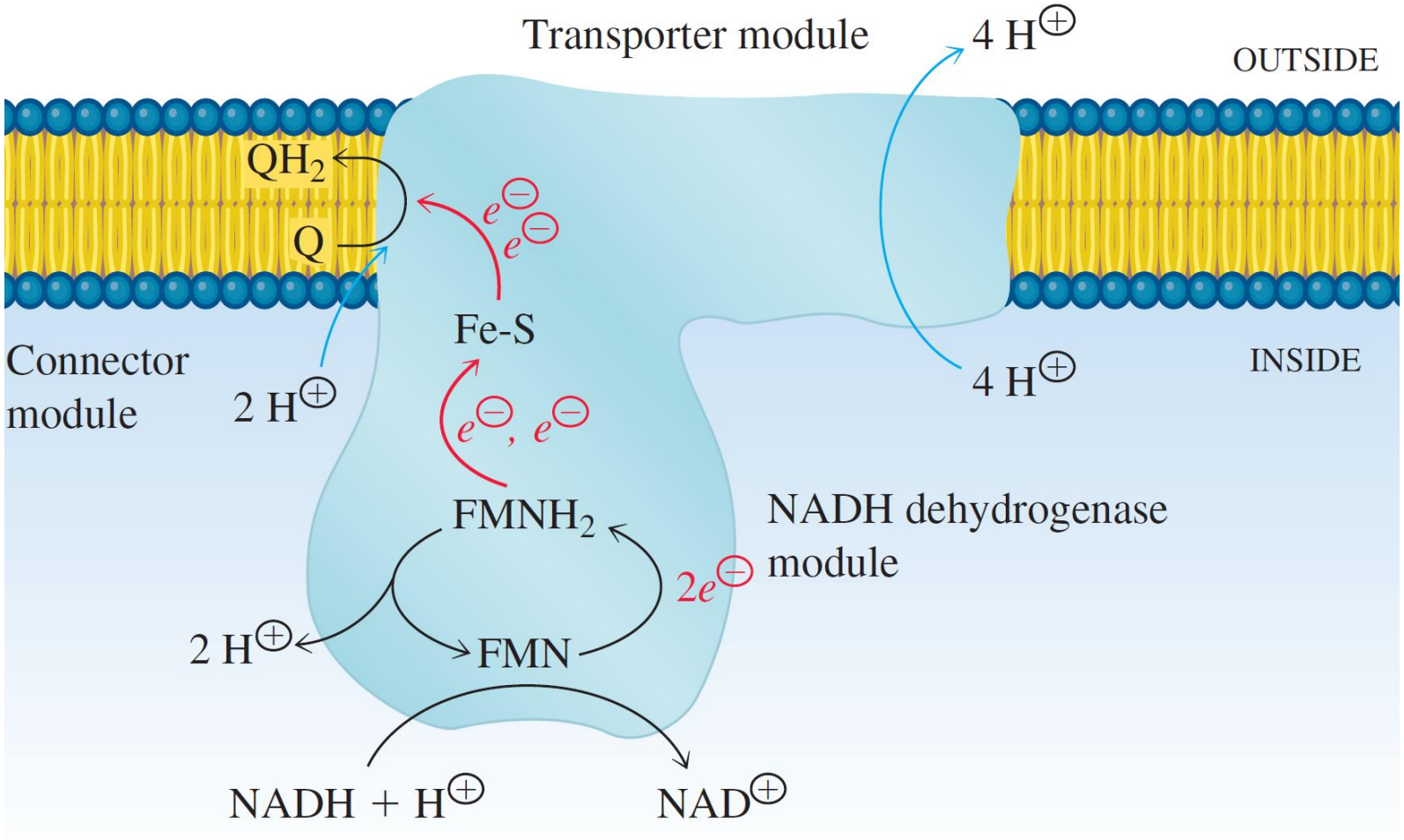
❖Complex V is an ATP synthase.



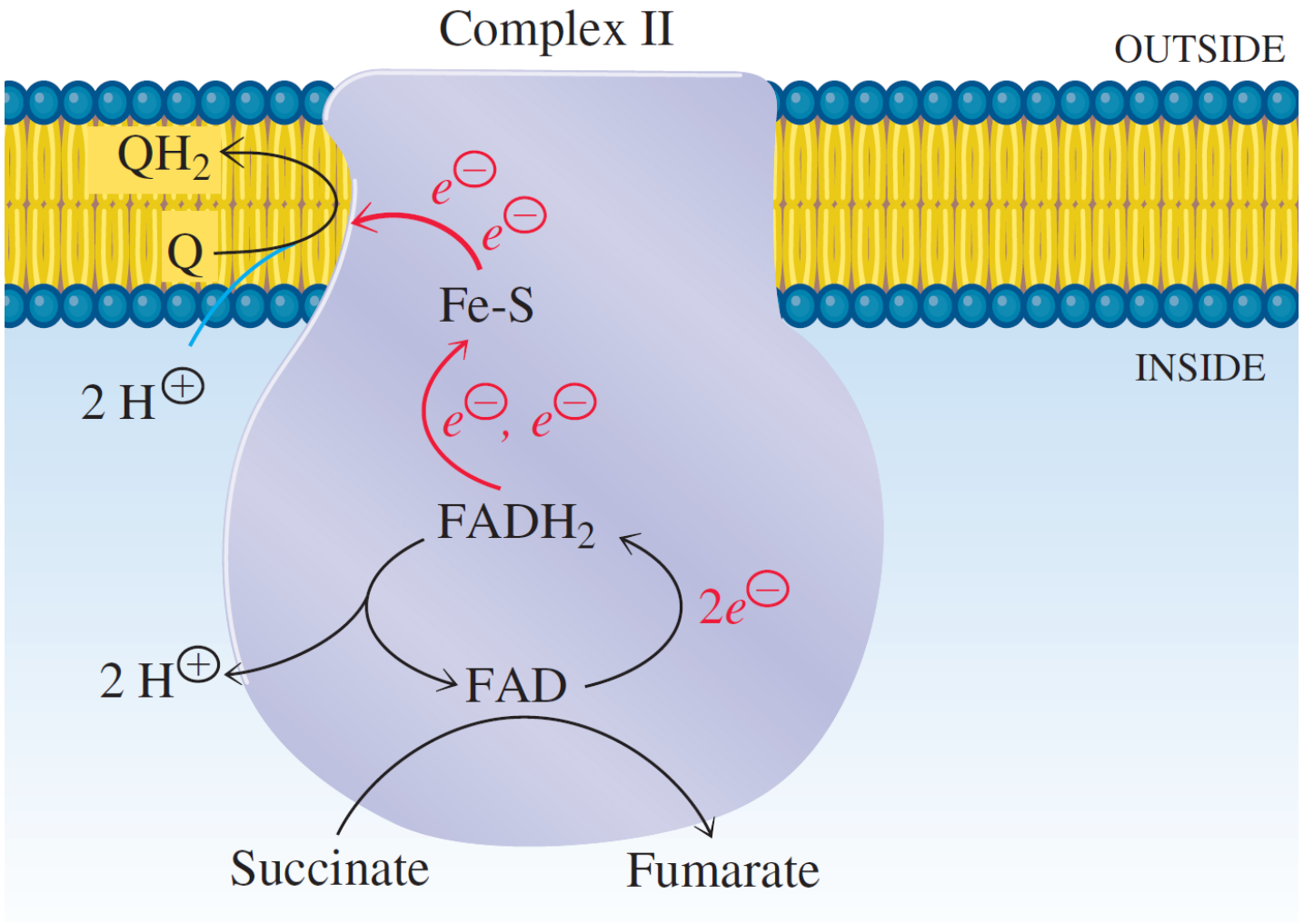

4H+
4H+
2H+
Cyt c
Intermembrane space
I
II
Q
III
IV
Succinate
Fumarate
H2O
½ O2 + 2H+
NADH + H+
NAD+
Matrix
ADP + Pi
ATP
Fo
F1
H+
Chemical potential ΔpH (inside alkaline)
ATP synthesis driven by proton-motive force
Electrical potential Δψ (inside negative)


NADH
NAD⊕
Complex I
NADH-ubiquinone
oxidoreductase
Succinate
Fumarate
Complex II
Succinate-ubiquinone
oxidoreductase
Q
Complex III
Ubiquinol–cytochrome c
oxidoreductase
Cyt c
Complex IV
Cytochrome c
oxidase
½ O2 + 2 H⊕
H2O
Path of
electron
flow
200
100
0
ΔG°′ (kJ mol−1)

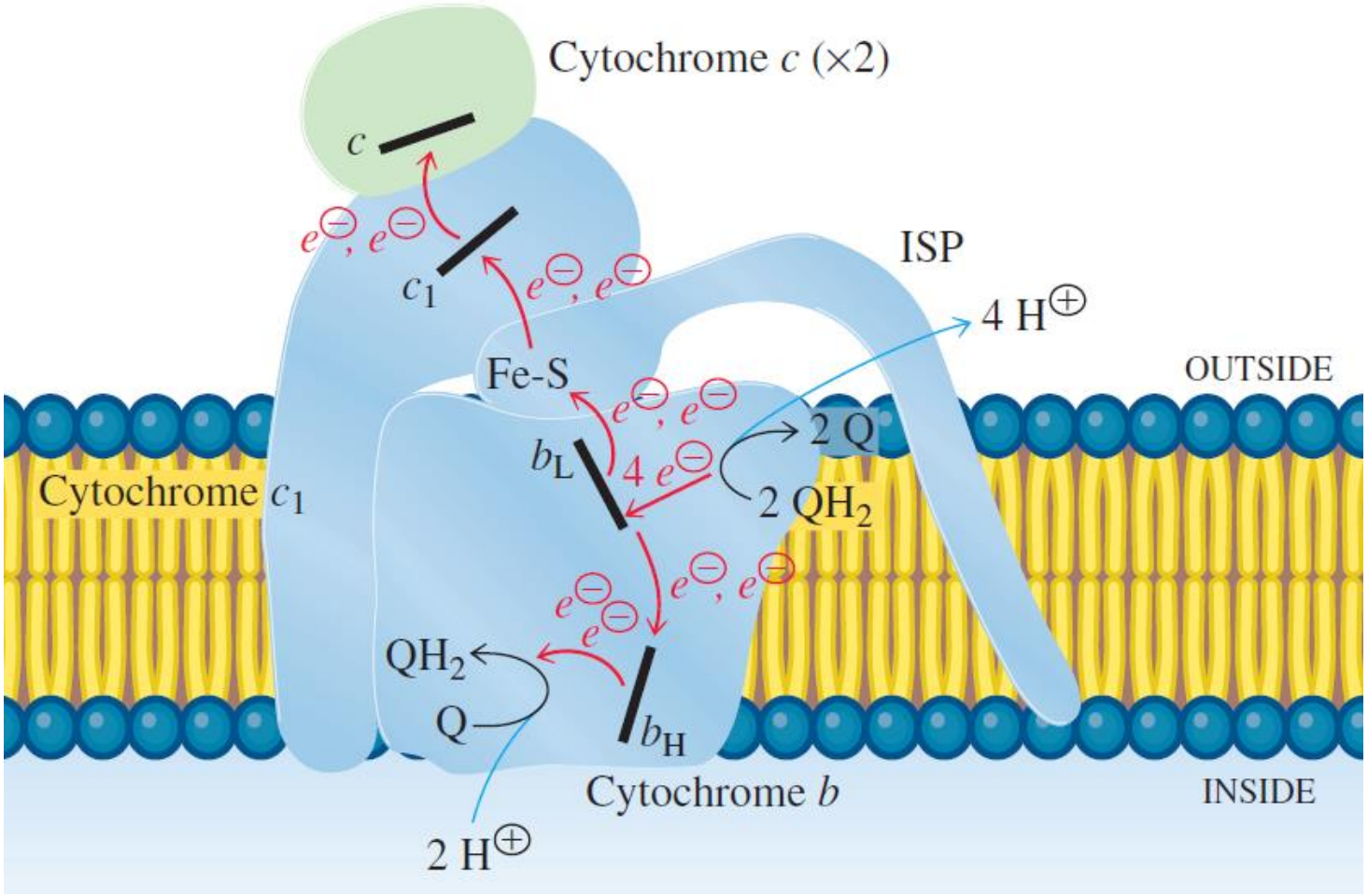
❖Complex I, also called NADH:ubiquinone oxidoreductase or NADH dehydrogenase catalyzes two simultaneous and obligately coupled processes: (1) the exergonic transfer a hydride ion from NADH and a proton from the matrix to ubiquinone, and (2) the endergonic transfer of four protons from the matrix to the intermembrane space.



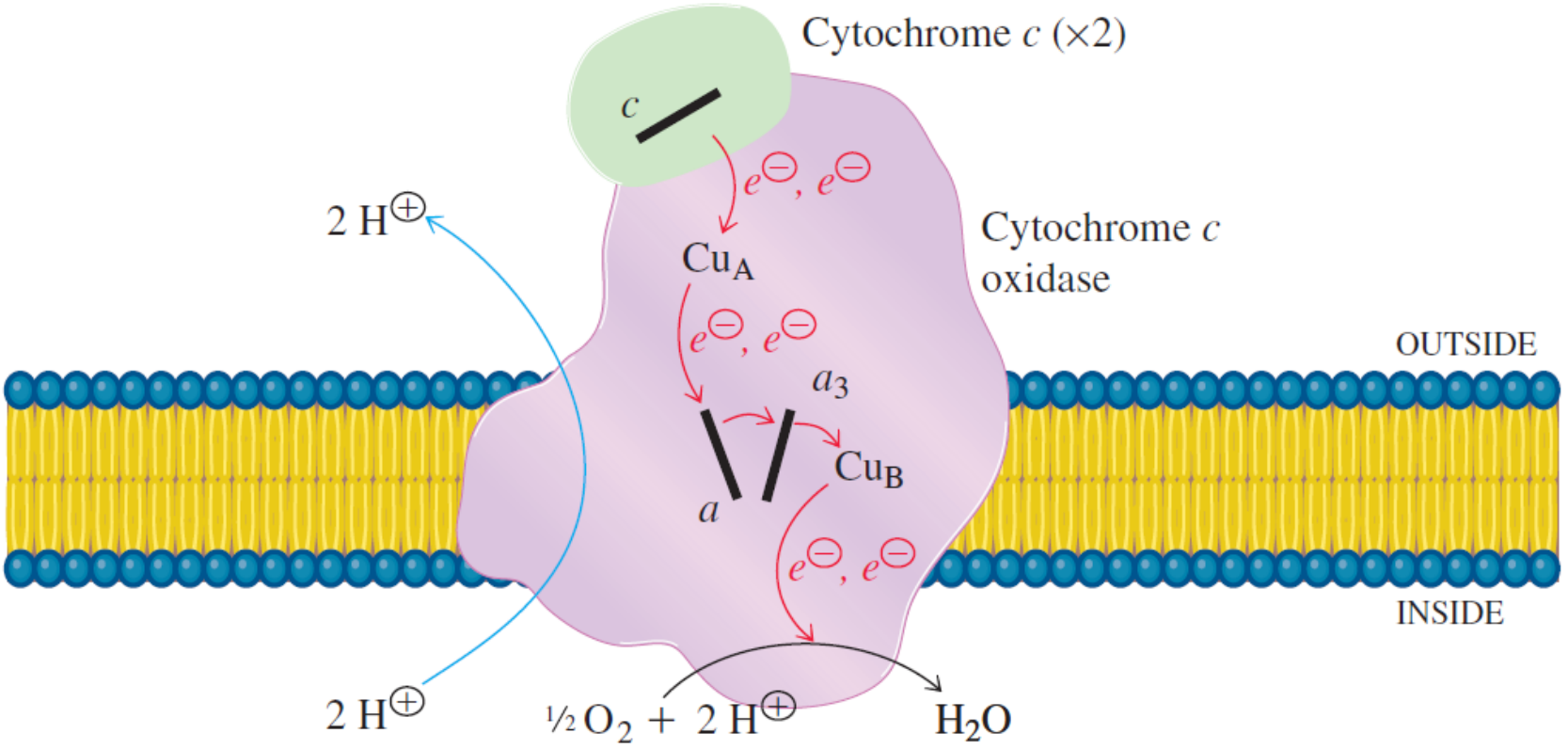
❖Complex II succinate:ubiquinon oxidoreductase or succinate dehydrogenase is an in the citric acid and transfer elestrons from succinate to ubiquinon.



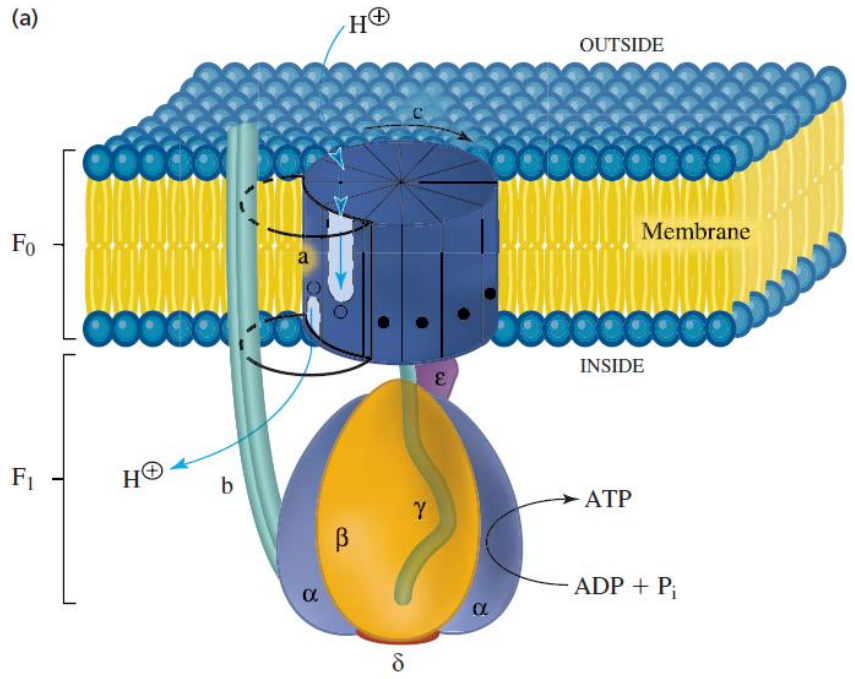
❖Complex III, also called cytochrome bc1 complex or ubiquinone : cytochrome c oxidoreductase, couples the transfer of electrons from ubiquinol (QH2) to cytochrome c with the vectorial transport of protons from the matrix to the intermembrane space.

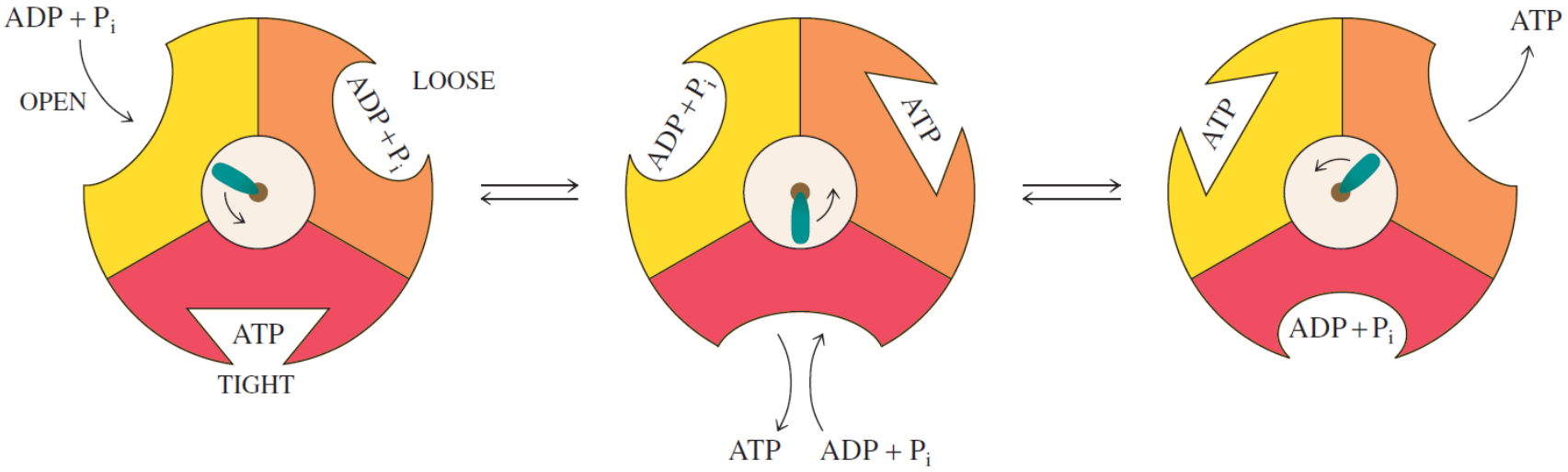


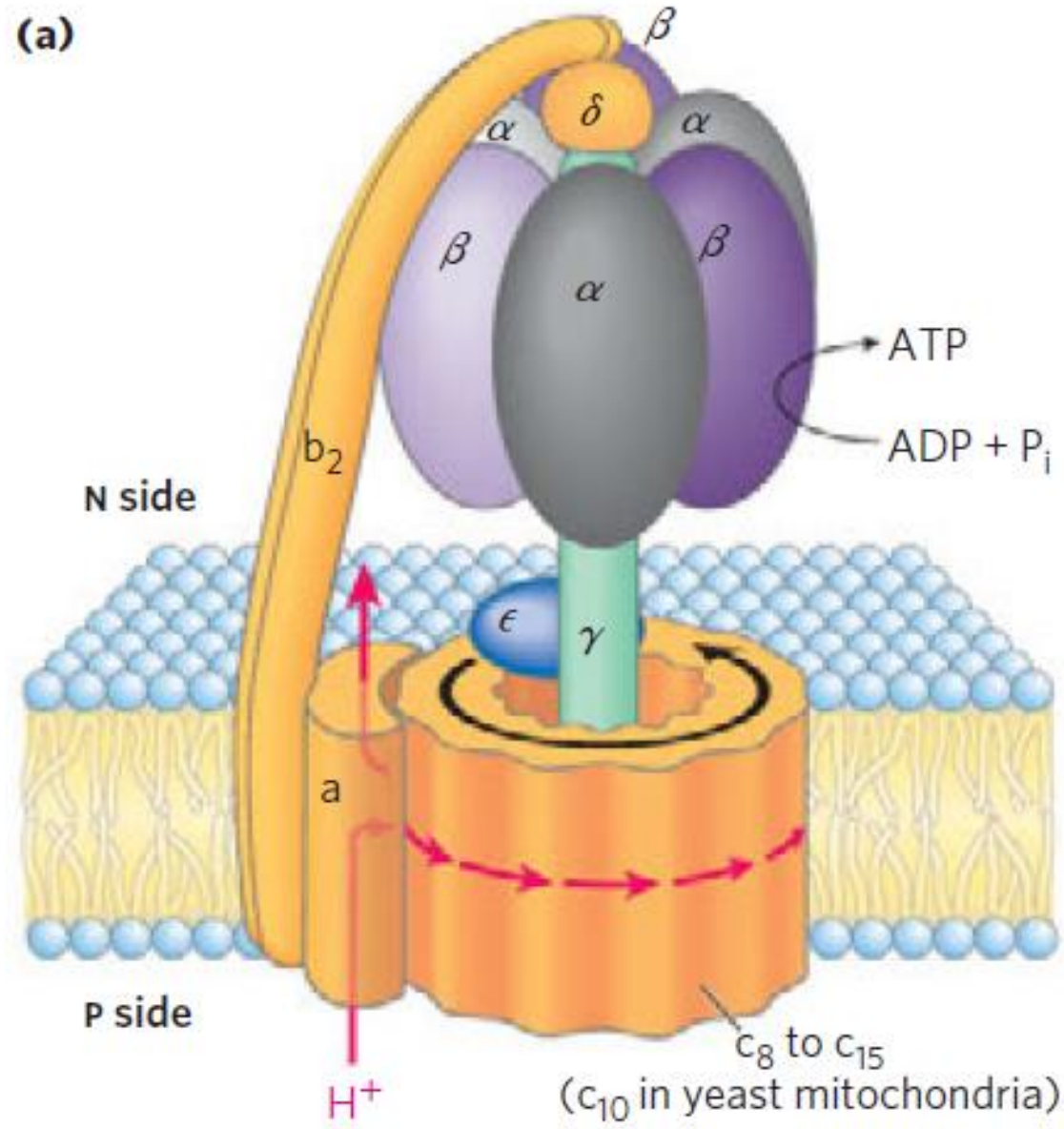
❖In the final step of the respiratory chain, Complex IV, also called cytochrome oxidase, carries electrons from cytochrome c to molecular oxygen, reducing it to H_2O.

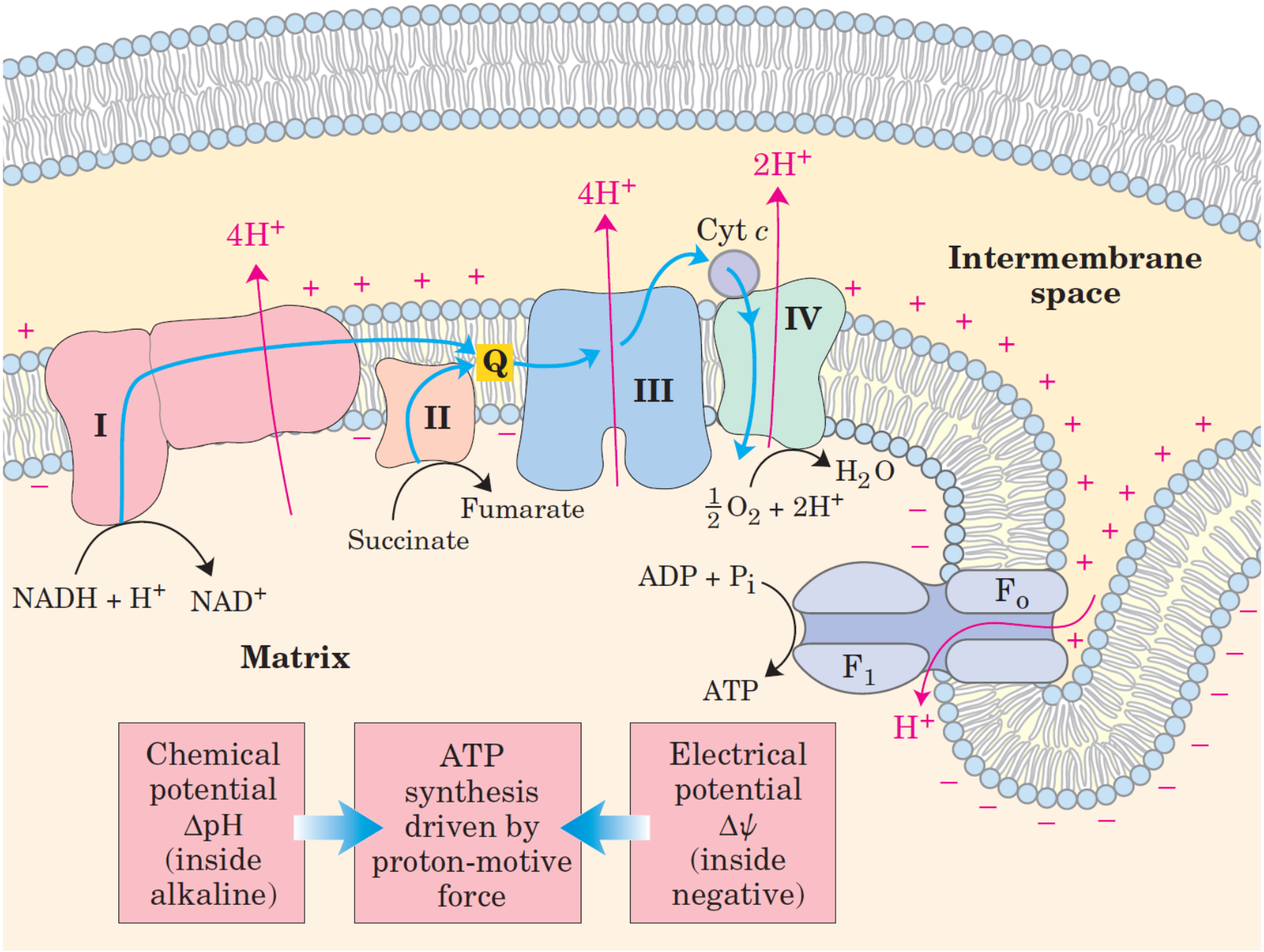


❖Complex V is an ATP synthase and using proton gradient generated through membran bounded electron transport synthesize the production of ATP from ADP and Pi.



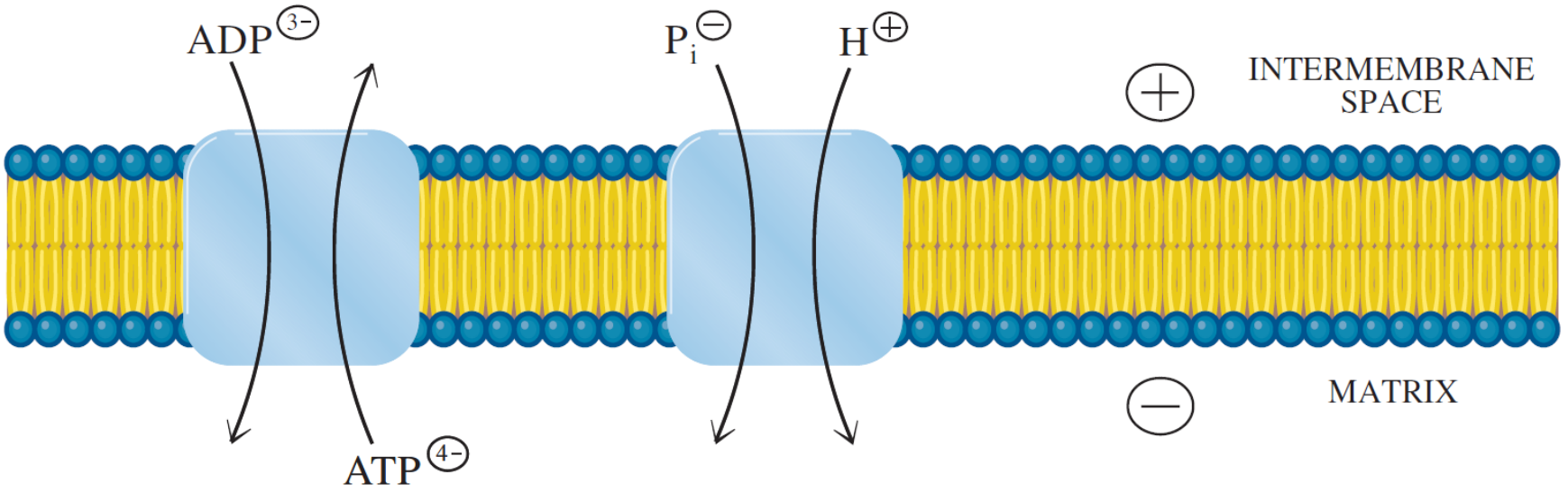



(a)
β
δ
α
α
β
α
β
ATP
ADP + Pi
b2
N side
ε
γ
a
P side
H+
c8 to c15
(c10 in yeast mitochondria)


4H+
4H+
2H+
Cyt c
Intermembrane space
I
II
Q
III
IV
Succinate
Fumarate
H2O
½ O2 + 2H+
NADH + H+
NAD+
Matrix
ADP + Pi
ATP
Fo
F1
H+
Chemical potential ΔpH (inside alkaline)
ATP synthesis driven by proton-motive force
Electrical potential Δψ (inside negative)

❖Although the primary role of the proton gradient in mitochondria is to furnish energy for the synthesis of ATP, the proton-motive force also drives several transport processes essential to oxidative phosphorylation.

❖The inner mitochondrial membrane is generally impermeable to charged species, but two specific systems transport ADP and Pi into the matrix and ATP out to the cytosol; adenine nucleotide translocase and phosphate translocase.



❖The NADH dehydrogenase of the inner mitochondrial membrane of animal cells can accept electrons only from NADH in the matrix.

❖Given that the inner membrane is not permeable to NADH, how can the NADH generated by glycolysis in the cytosol be reoxidized to NAD^+ by O2 via the respiratory chain?

❖Special shuttle systems carry reducing equivalents from cytosolic NADH into mitochondria by an indirect route.

❖The most active NADH shuttle, which functions in liver, kidney, and heart mitochondria, is the malate-aspartate shuttle.

❖The reducing equivalents of cytosolic NADH are first transferred to cytosolic oxaloacetate to yield malate, catalyzed by cytosolic malate dehydrogenase.

❖The malate thus formed passes through the inner membrane via the malate–α-ketoglutarate transporter.

❖Within the matrix the reducing equivalents are passed to NAD^+ by the action of matrix malate dehydrogenase, forming NADH; this NADH can pass electrons directly to the respiratory chain.

❖About 2.5 molecules of ATP are generated as this pair of electrons passes to O_2.

❖Cytosolic oxaloacetate must be regenerated by transamination reactions and the activity of membrane transporters to start another cycle of the shuttle.

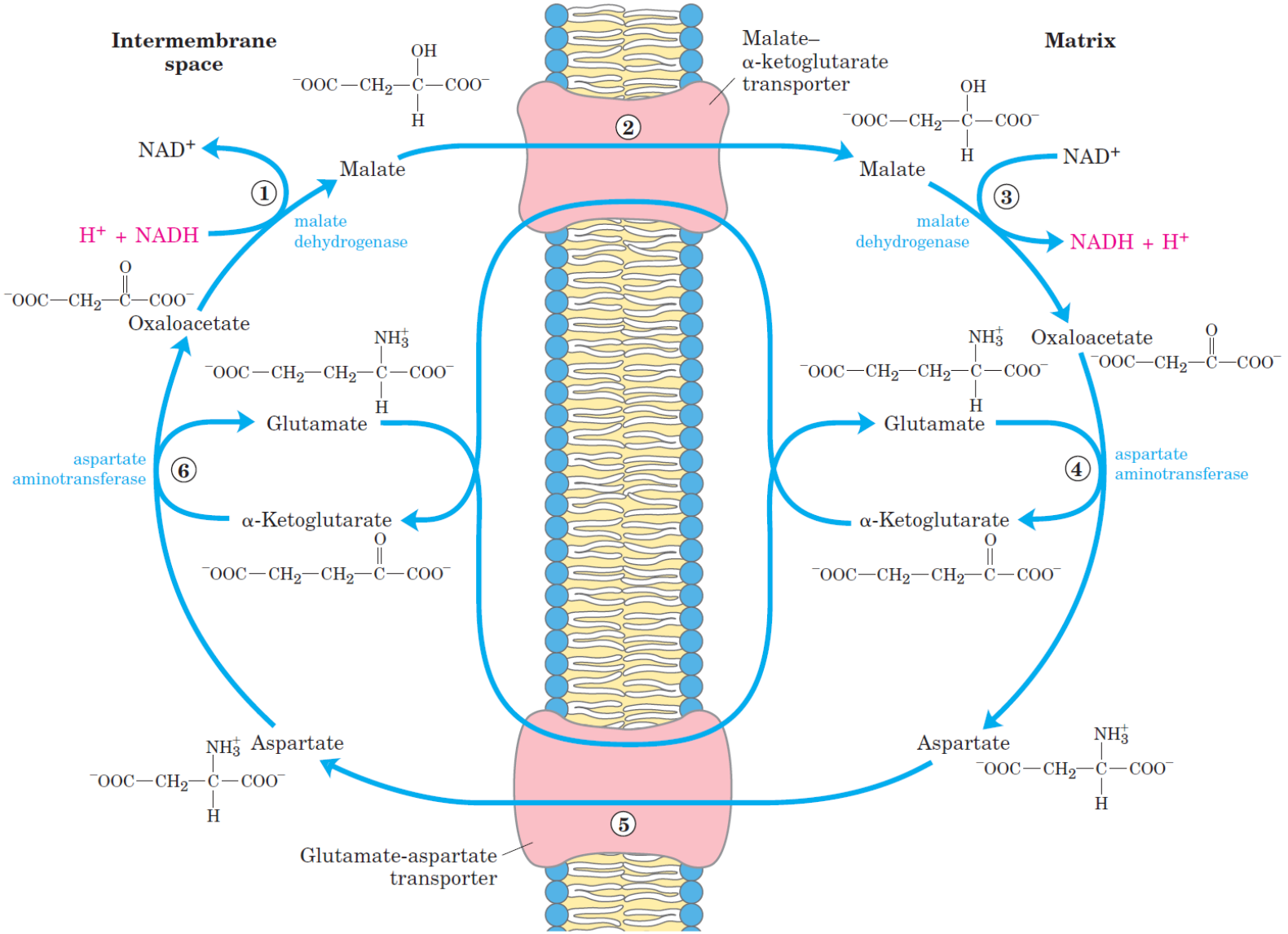
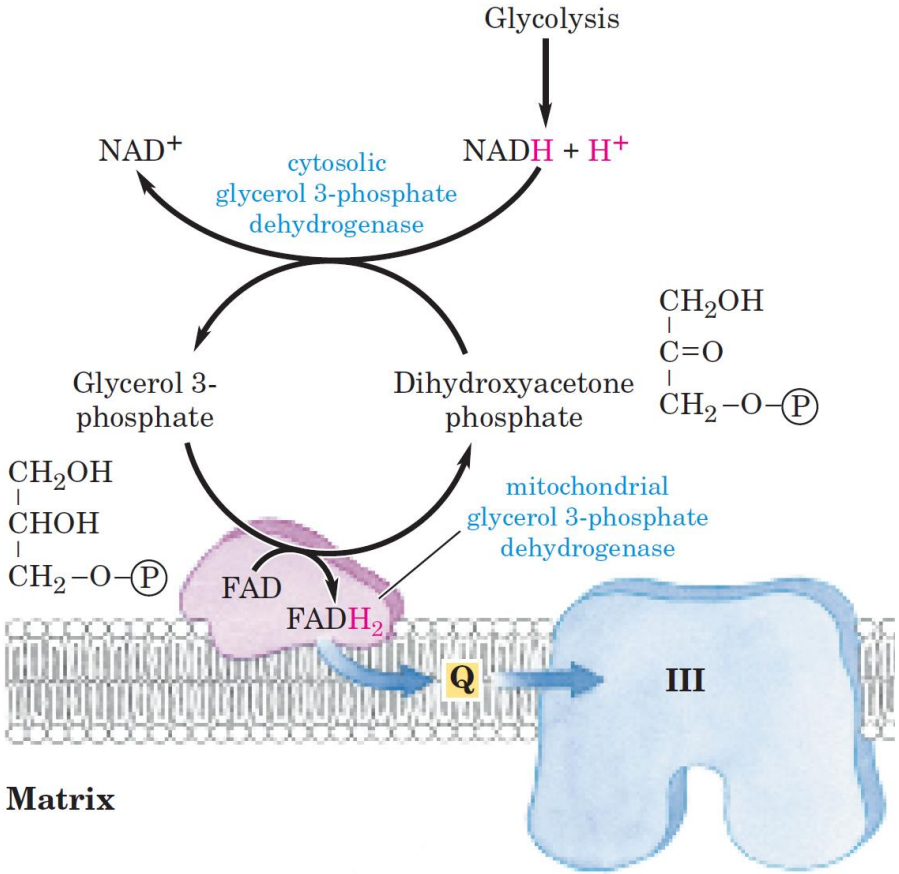
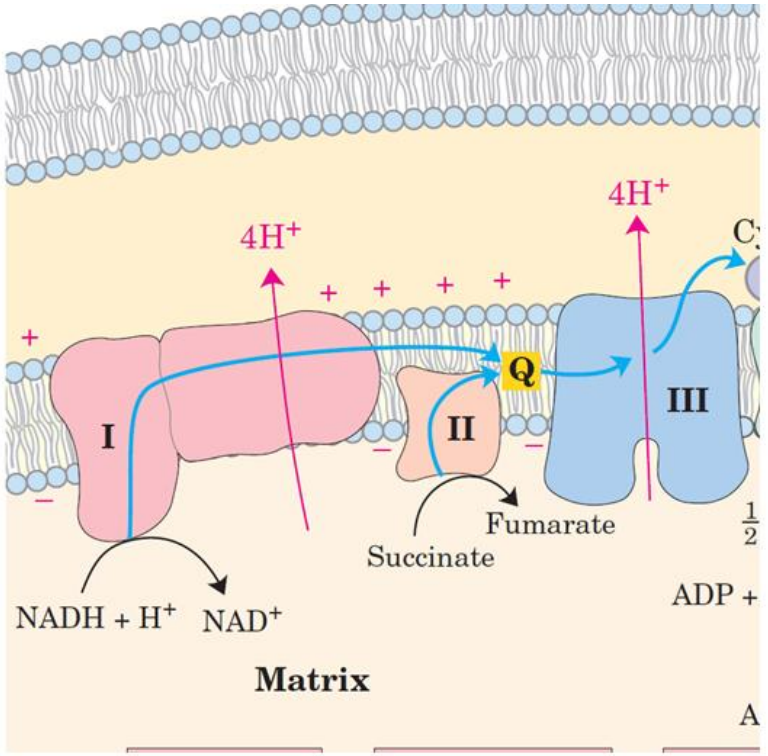


FIGURE 19–27 Malate-aspartate shuttle. This shuttle for transporting reducing equivalents from cytosolic NADH into the mitochondrial matrix is used in liver, kidney, and heart. (1) NADH in the cytosol (intermembrane space) passes two reducing equivalents to oxaloacetate, producing malate. (2) Malate crosses the inner membrane via the malate–α-ketoglutarate transporter. (3) In the matrix, malate passes two reducing equivalents to NAD^+, and the resulting NADH is oxidized by the respiratory chain. The oxaloacetate formed from malate cannot pass directly into the cytosol. (4) It is first transaminated to aspartate, which (5) can leave via the glutamate-aspartate transporter. (6) Oxaloacetate is regenerated in the cytosol, completing the cycle.

❖Skeletal muscle and brain use a different NADH shuttle, the glycerol 3-phosphate shuttle.

❖It differs from the malate-aspartate shuttle in that it delivers the reducing equivalents from NADH to ubiquinone and thus into Complex III, not Complex I, providing only enough energy to synthesize 1.5 ATP molecules per pair of electrons.





Regulation of Oxidative Phosphorylation

❖Complete oxidation of a molecule of glucose to CO2 yields 30 or 32 ATP

TABLE 19–5 ATP Yield from Complete Oxidation of Glucose

Process	*Direct product*	*Final ATP*
Glycolysis	2 NADH (cytosolic)	3 or 5*
	2 ATP	2
Pyruvate oxidation (two per glucose)	2 NADH (mitochondrial matrix)	5
Acetyl-CoA oxidation in citric acid cycle (two per glucose)	6 NADH (mitochondrial matrix)	15
	2 $FADH_2$	3
	2 ATP or 2 GTP	2
Total yield per glucose		30 or 32

*The number depends on which shuttle system transfers reducing equivalents into the mitochondrion.

❖By comparison, glycolysis under anaerobic conditions (lactate fermentation) yields only 2 ATP per glucose.

❖Aerobic oxidative pathways that result in electron transfer to O_2 accompanied by oxidative phosphorylation therefore account for the vast majority of the ATP produced in catabolism.

❖Therefore the regulation of ATP production by oxidative phosphorylation to match the cell's fluctuating needs for ATP is absolutely essential.

❖The rate of respiration (O2 consumption) in mitochondria is tightly regulated; it is generally limited by the availability of ADP as a substrate for phosphorylation.

❖The intracellular concentration of ADP is one measure of the energy status of cells.

❖Another, related measure is the mass-action ratio of the ATP-ADP system.

❖Usually this ratio is very high, so the ATP-ADP system is almost fully phosphorylated.

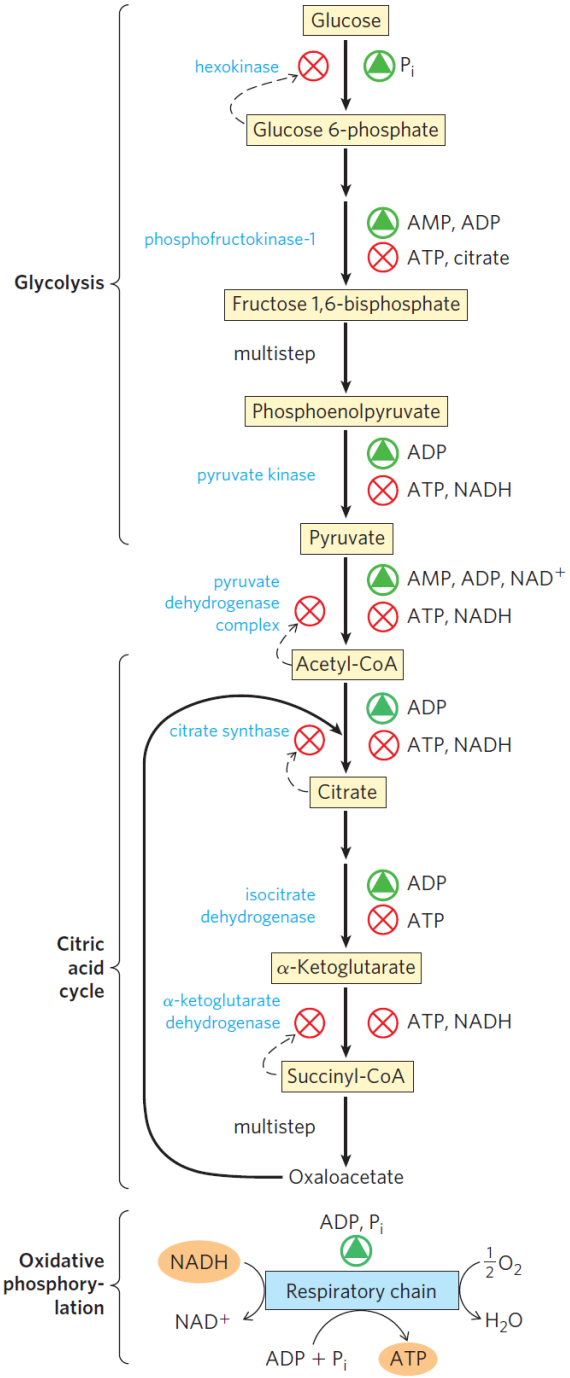
❖When the rate of some energy-requiring process (protein synthesis, for example) increases, the rate of breakdown of ATP to ADP and Pi increases, lowering the mass-action ratio.

❖With more ADP available for oxidative phosphorylation, the rate of respiration increases, causing regeneration of ATP.

❖This continues until the mass-action ratio returns to its normal high level, at which point respiration slows again.

❖The rate of oxidation of cellular fuels is regulated with such sensitivity and precision that the [ATP]/([ADP][Pi]) ratio fluctuates only slightly in most tissues, even during extreme variations in energy demand.

❖In short, ATP is formed only as fast as it is used in energy-requiring cellular activities.


Glycolysis
Glucose
hexokinase
Pi
Glucose 6-phosphate
phosphofructokinase-1
AMP, ADP
ATP, citrate
Fructose 1,6-bisphosphate
multistep
Phosphoenolpyruvate
pyruvate kinase
ADP
ATP, NADH
Pyruvate
pyruvate dehydrogenase complex
AMP, ADP, NAD+
ATP, NADH
Acetyl-CoA
Citric acid cycle
citrate synthase
ADP
ATP, NADH
Citrate
isocitrate dehydrogenase
ADP
ATP
α-Ketoglutarate
α-ketoglutarate dehydrogenase
ATP, NADH
Succinyl-CoA
multistep
Oxaloacetate
Oxidative phosphory-lation
ADP, Pi
NADH
Respiratory chain
½O2
H2O
NAD+
ADP + Pi
ATP

PHOTOSYNTHESIS

❖The capture of solar energy by photosynthetic organisms and its conversion to the chemical energy of reduced organic compounds is the ultimate source of nearly all biological energy on Earth.

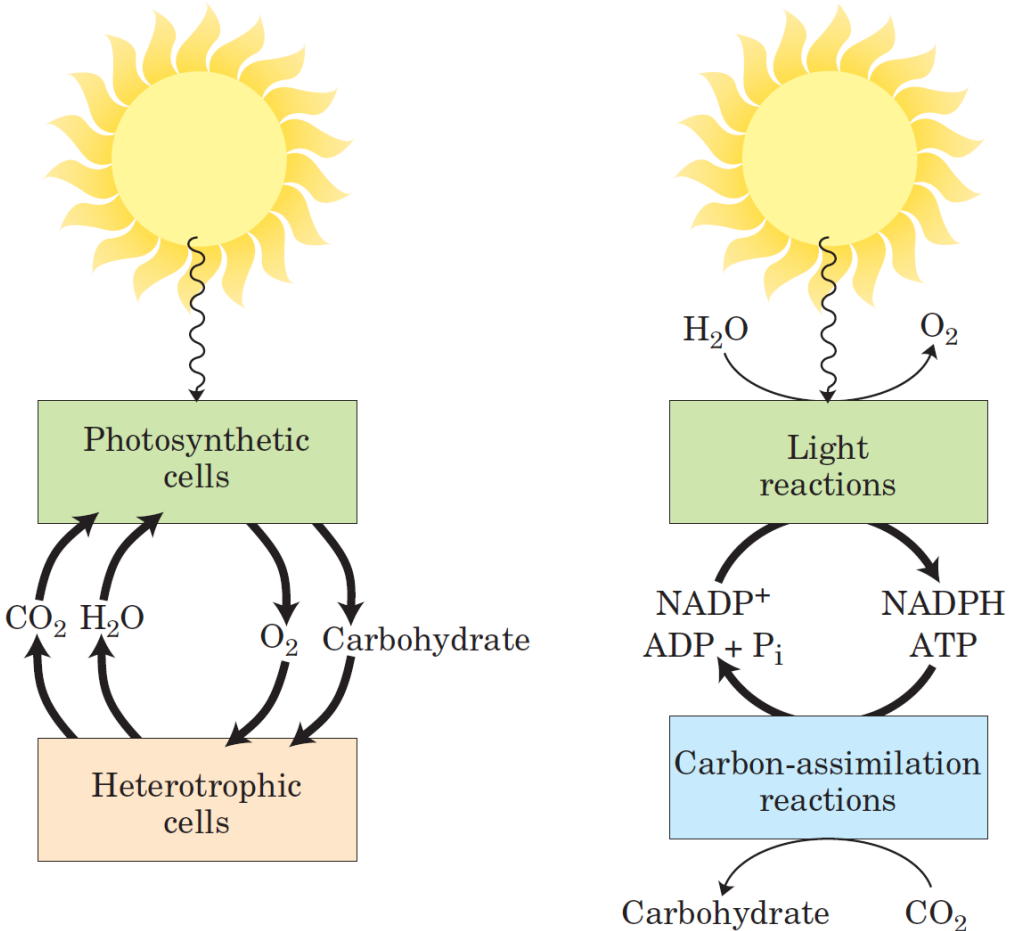
❖Photosynthetic and heterotrophic organisms live in a balanced steady state in the biosphere.

❖Photosynthetic organisms trap solar energy and form ATP and NADPH, which they use as energy sources to make carbohydrates and other organic compounds from CO_2 and H_2O; simultaneously, they release O_2 into the atmosphere.

❖Aerobic heterotrophs (humans, for example, as well as plants during dark periods) use the O_2 so formed to degrade the energy-rich organic products of photosynthesis to CO_2 and H_2O, generating ATP.

❖The CO_2 returns to the atmosphere, to be used again by photosynthetic organisms.

❖Solar energy thus provides the driving force for the continuous cycling of CO_2 and O_2 through the biosphere and provides the reduced substrates on which nonphotosynthetic organisms depend.



❖Photosynthesis occurs in a variety of bacteria and in unicellular eukaryotes (algae) as well as in plants.

❖Although the process in these organisms differs in detail, the underlying mechanisms are remarkably similar, and much of our understanding of photosynthesis in vascular plants is derived from studies of simpler organisms.

❖The overall equation for photosynthesis in plants describes an oxidation-reduction reaction in which H_2O donates electrons (as hydrogen) for the reduction of CO_2 to carbohydrate (CH_2O):

$$CO_2 + H_2O \xrightarrow{\text{light}} O_2 + (CH_2O)_n$$

❖Unlike NADH (the major electron donor in oxidative phosphorylation), H_2O is a poor donor of electrons; its standard reduction potential is 0.816 V, compared with -0.320 V for NADH.

❖Photophosphorylation differs from oxidative phosphorylation in requiring the input of energy in the form of light to create a good electron donor and a good electron acceptor.

❖In photophosphorylation, electrons flow through a series of membrane-bound carriers including cytochromes, quinones, and iron-sulfur proteins, while protons are pumped across a membrane to create an electrochemical potential.

❖Electron transfer and proton pumping are catalyzed by membrane complexes homologous in structure and function to Complex III of mitochondria.

❖The electrochemical potential they produce is the driving force for ATP synthesis from ADP and Pi, catalyzed by a membrane-bound ATP synthase complex closely similar to that of mitochondria and bacteria.

❖Photosynthesis in plants encompasses two processes:

- the light-dependent reactions, or light reactions, which occur only when plants are illuminated,

- and the carbon-assimilation reactions (or carbon fixation reactions), sometimes misleadingly called the dark reactions, which are driven by products of the light reactions.

❖In the light reactions, chlorophyll and other pigments of photosynthetic cells absorb light energy and conserve it as ATP and NADPH; simultaneously, O_2 is evolved.

❖In the carbon-assimilation reactions, ATP and NADPH are used to reduce CO_2 to form triose phosphates, starch, and sucrose, and other products derived from them.

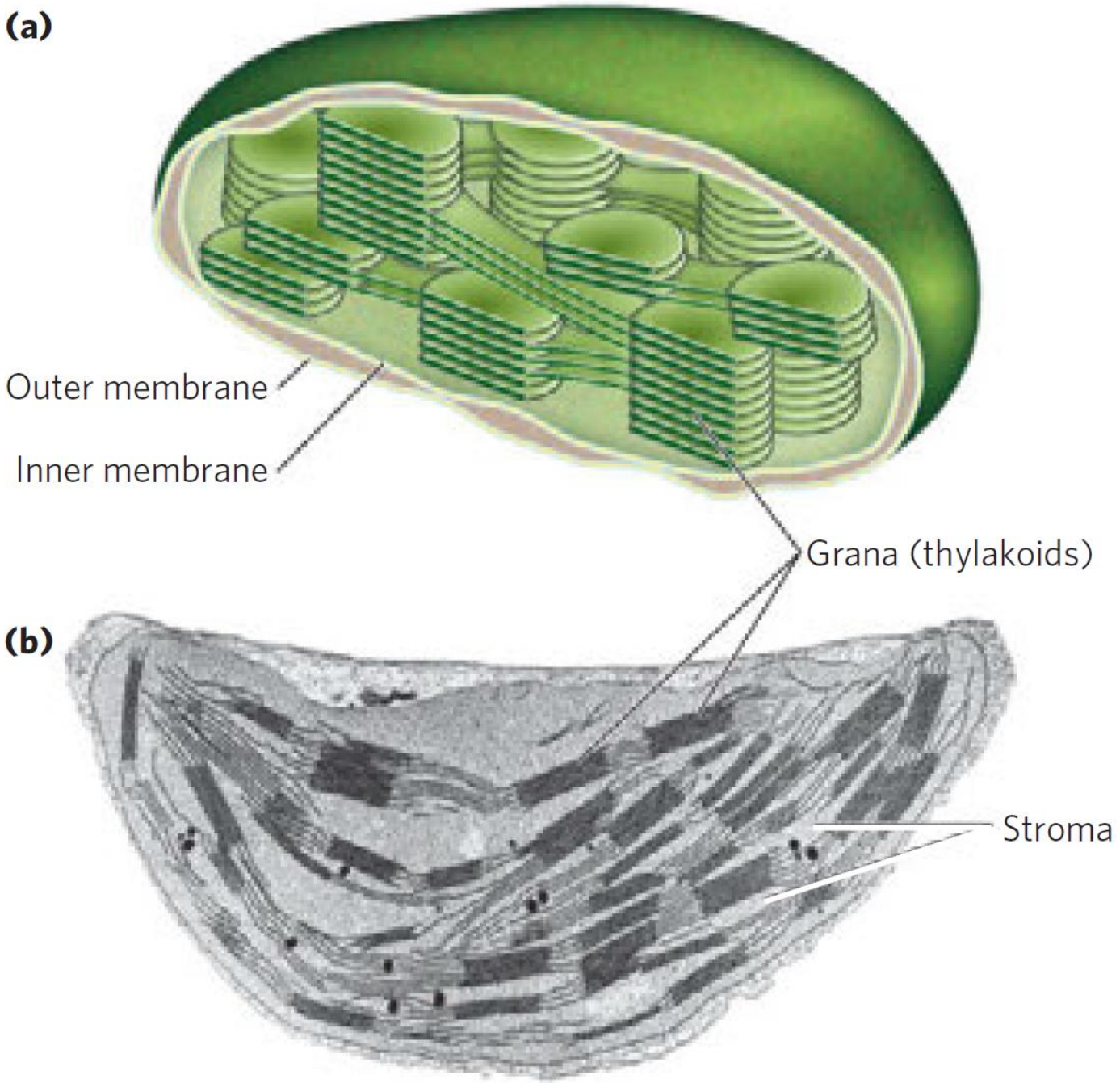
❖In photosynthetic eukaryotic cells, both the light dependent and the carbon-assimilation reactions take place in the chloroplasts.

❖Like mitochondria, they are surrounded by two membranes, an outer membrane that is permeable to small molecules and ions, and an inner membrane that encloses the internal compartment.

❖This compartment contains many flattened, membrane-surrounded vesicles or sacs, the thylakoids, usually arranged in stacks called grana.

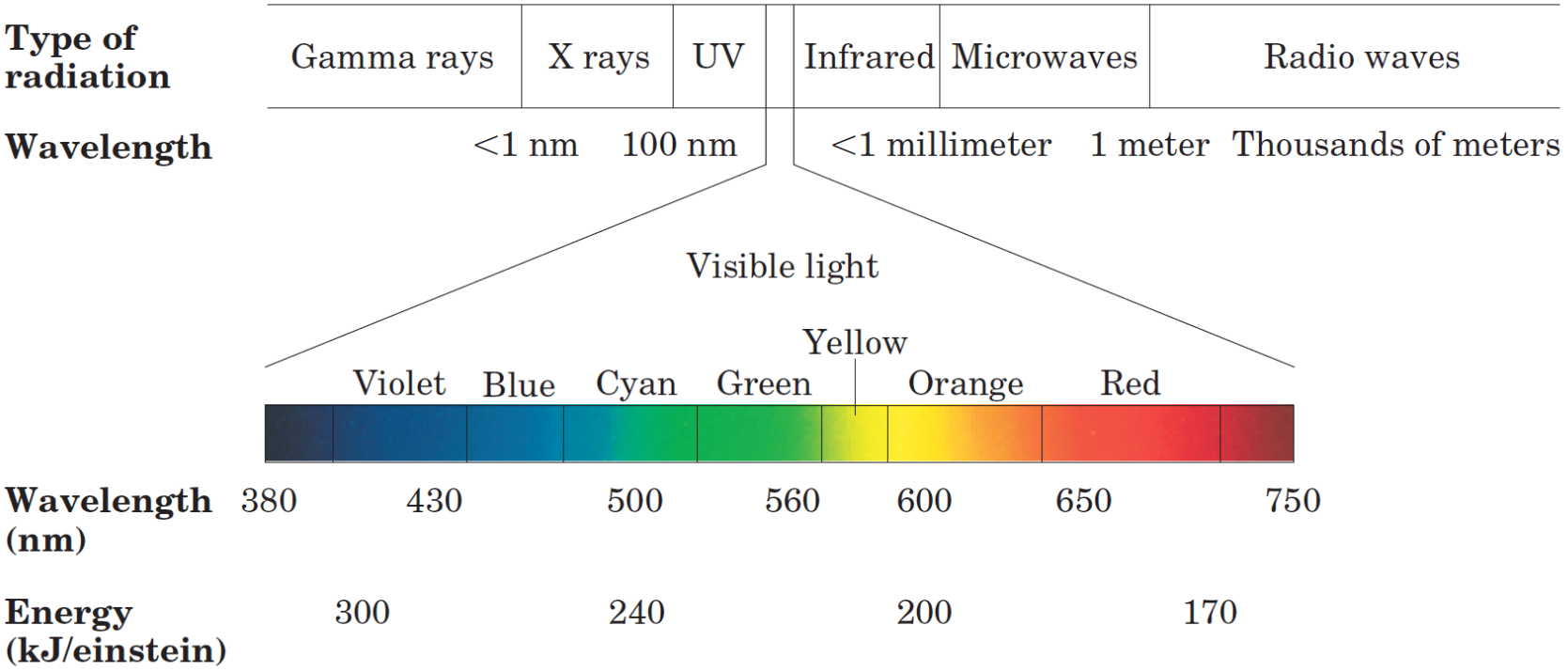
❖Embedded in the thylakoid membranes (commonly called lamellae) are the photosynthetic pigments and the enzyme complexes that carry out the light reactions and ATP synthesis.

❖The stroma (the aqueous phase enclosed by the inner membrane) contains most of the enzymes required for the carbon-assimilation reactions.


(a)
Outer membrane
Inner membrane
Grana (thylakoids)
(b)
Stroma

❖Visible light is electromagnetic radiation of wavelengths 400 to 700 nm, a small part of the electromagnetic spectrum, ranging from violet to red.

❖The energy of a single photon (a quantum of light) is greater at the violet end of the spectrum than at the red end; shorter wavelength (and higher frequency) corresponds to higher energy.



❖When a photon is absorbed, an electron in the absorbing molecule (chromophore) is lifted to a higher energy level.

❖This is an all-or-nothing event: to be absorbed, the photon must contain a quantity of energy (a quantum) that exactly matches the energy of the electronic transition.

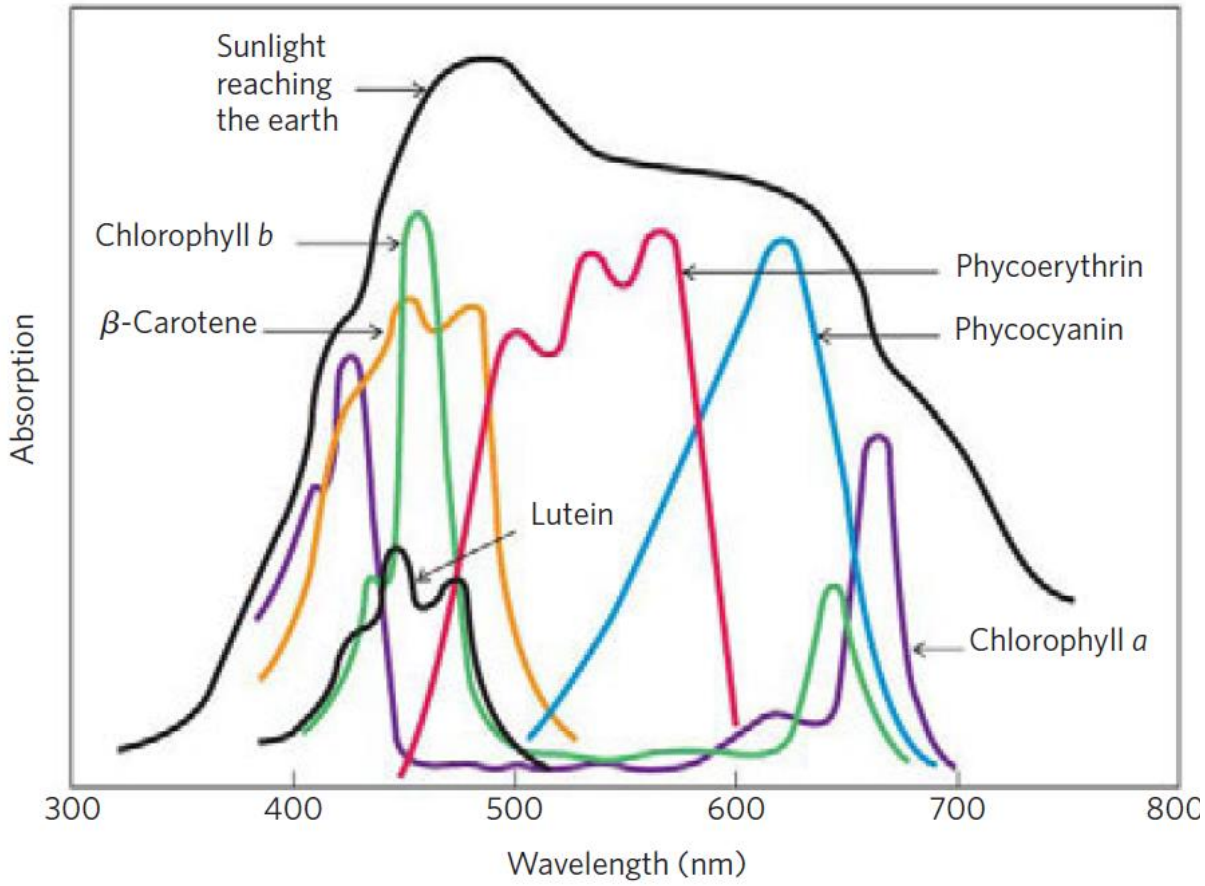
❖A molecule that has absorbed a photon is in an excited state, which is generally unstable.

❖An electron lifted into a higher-energy orbital usually returns rapidly to its lower-energy orbital; the excited molecule decays to the stable ground state, giving up the absorbed quantum as light or heat or using it to do chemical work.

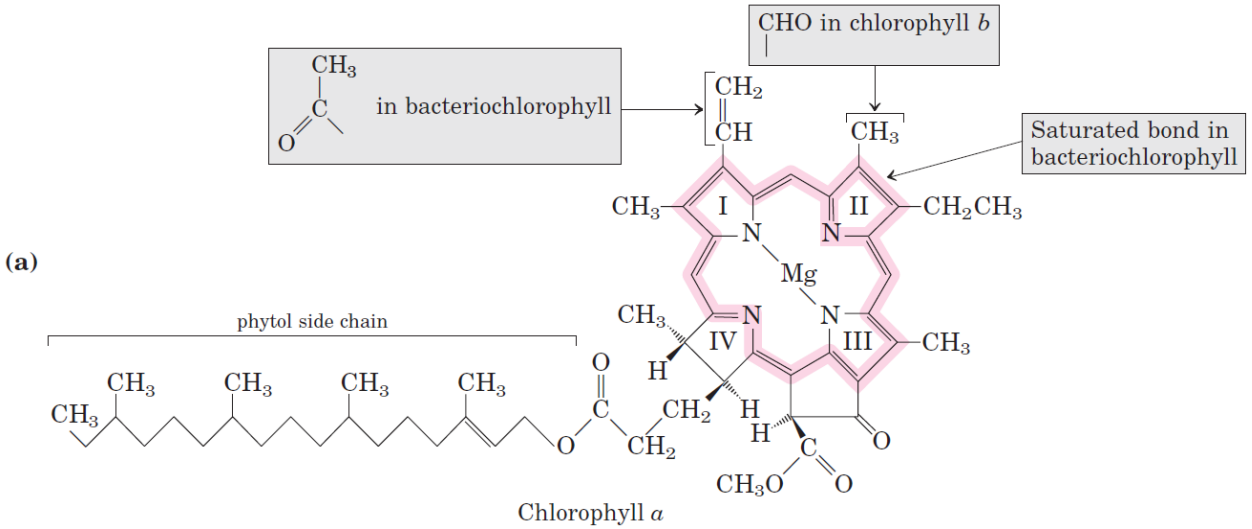
❖Light emission accompanying decay of excited molecules (called fluorescence) is always at a longer wavelength (lower energy) than that of the absorbed light.

❖An alternative mode of decay important in photosynthesis involves direct transfer of excitation energy from an excited molecule to a neighboring molecule.

❖Just as the photon is a quantum of light energy, so the exciton is a quantum of energy passed from an excited molecule to another molecule in a process called exciton transfer.

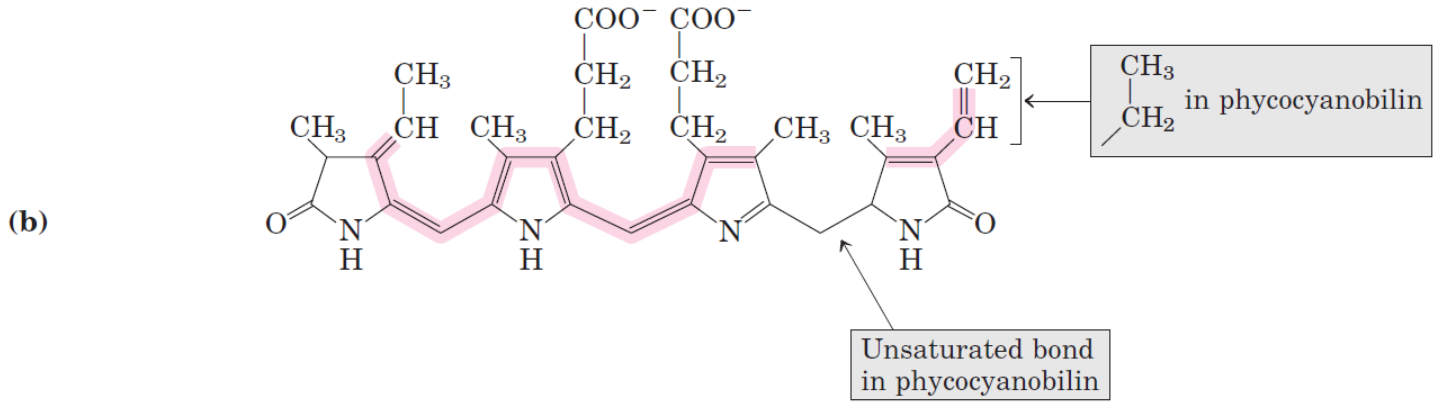


❖The most important light-absorbing pigments in the thylakoid membranes are the chlorophylls, green pigments with polycyclic, planar structures resembling the protoporphyrin of hemoglobin



Chlorophyll *a*

❖Cyanobacteria and red algae employ phycobilins such as phycoerythrobilin and phycocyanobilin as their light-harvesting pigments.

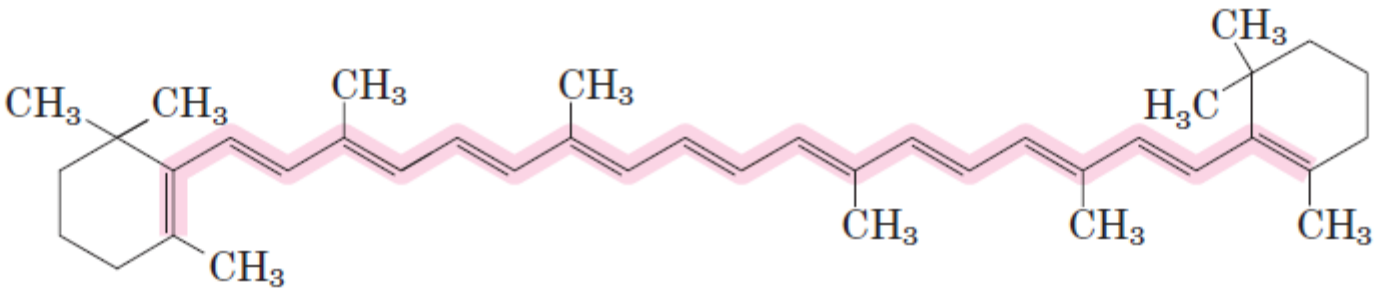


Phycoerythrobilin

❖In addition to chlorophylls, thylakoid membranes contain secondary light-absorbing pigments, or accessory pigments, called carotenoids.

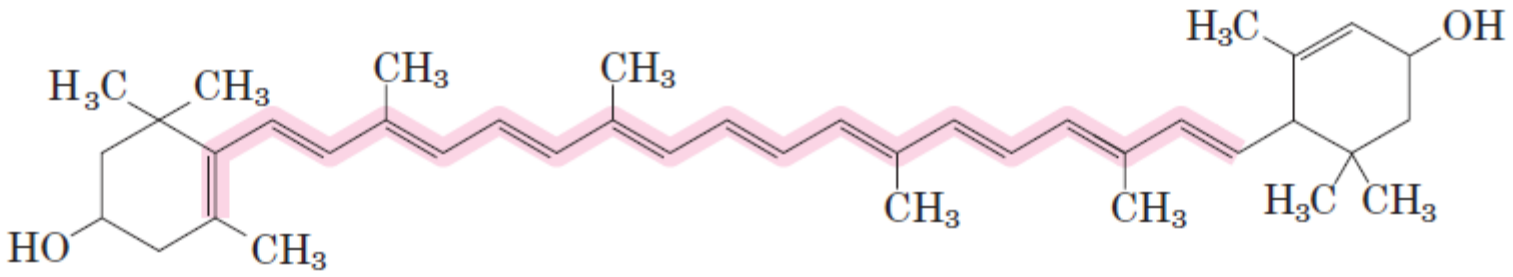
❖The carotenoid pigments absorb light at wavelengths not absorbed by the chlorophylls and thus are supplementary light receptors.

(c)



β-Carotene

(d)

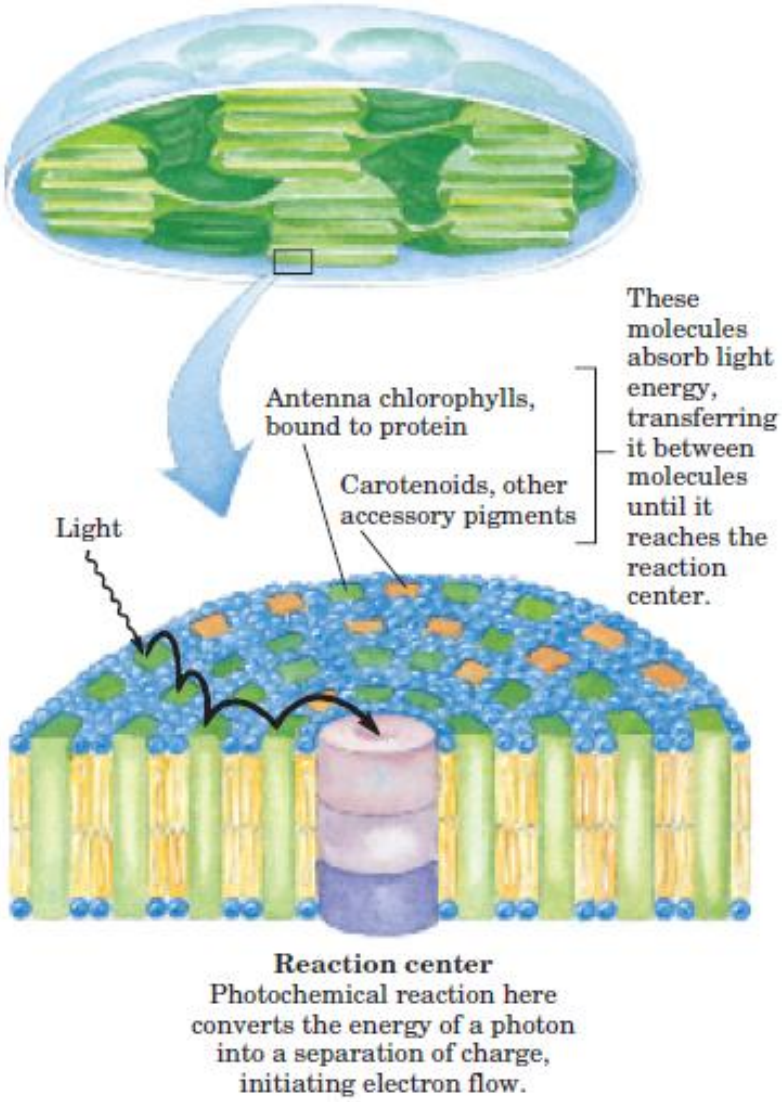


Lutein (xanthophyll)

❖The light-absorbing pigments of thylakoid or bacterial membranes are arranged in functional arrays called photosystems.

❖All the pigment molecules in a photosystem can absorb photons, but only a few chlorophyll molecules associated with the photochemical reaction center are specialized to transduce light into chemical energy.

❖The other pigment molecules in a photosystem are called light-harvesting or antenna molecules and they absorb light energy and transmit it rapidly and efficiently to the reaction center



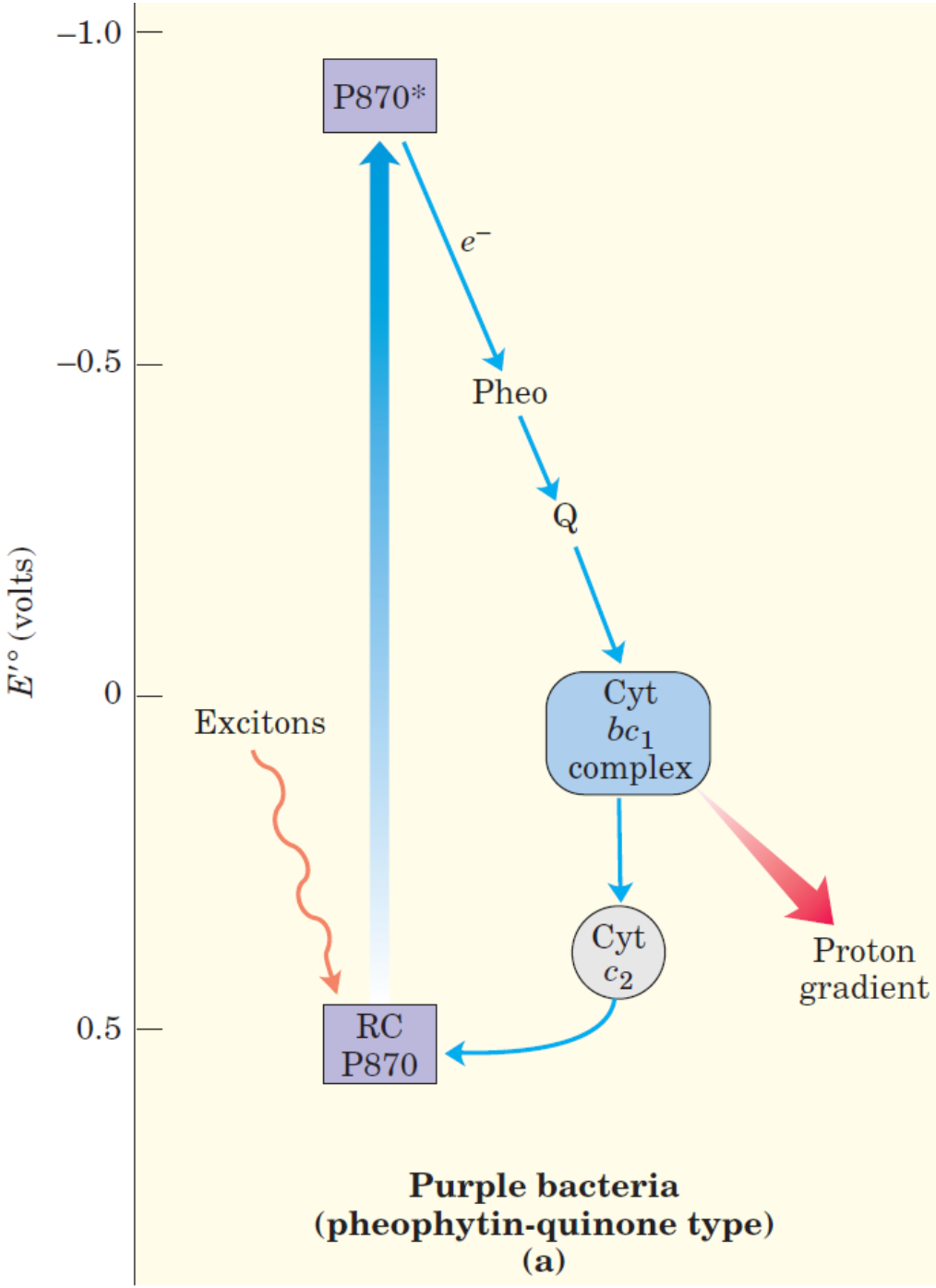
❖The photosynthetic machinery in purple bacteria consists of three basic modules:

- a single reaction center (P870),
- a cytochrome bc1 electron-transfer complex similar to Complex III of the mitochondrial electron-transfer chain,
- and an ATP synthase, also similar to that of mitochondria.

❖Illumination drives electrons through pheophytin and a quinone to the cytochrome bc1 complex; after passing through the complex, electrons flow through cytochrome c2 back to the reaction center, restoring its pre-illumination state.

❖This light-driven cyclic flow of electrons provides the energy for proton pumping by the cytochrome bc1 complex.

❖Powered by the resulting proton gradient, ATP synthase produces ATP, exactly as in mitochondria.


–1.0
–0.5
0
0.5
E'° (volts)
P870*
e⁻
Pheo
Q
Cyt
bc1
complex
Excitons
Cyt
c2
Proton
gradient
RC
P870
Purple bacteria
(pheophytin-quinone type)
(a)

❖Photosynthesis in green sulfur bacteria involves the same three modules as in purple bacteria, but the process differs in several respects and involves additional enzymatic reactions.

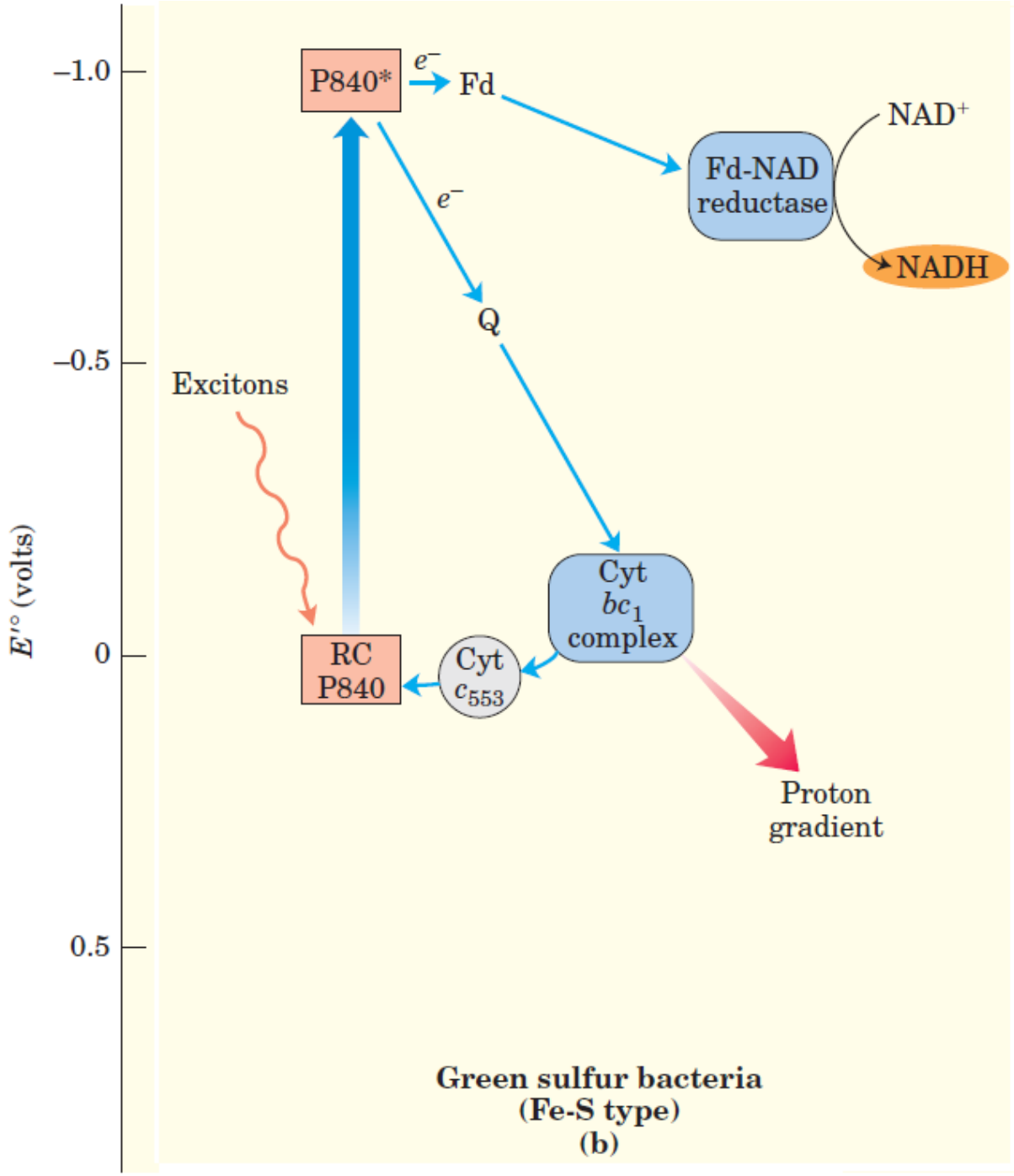
❖Excitation causes an electron to move from the reaction center to the cytochrome bc1 complex via a quinone carrier.

❖Electron transfer through this complex used for ATP synthesis.

❖However, in contrast to the cyclic flow of electrons in purple bacteria, some electrons flow from the reaction center to an iron-sulfur protein, ferredoxin, which then passes electrons via ferredoxin:NAD reductase to NAD^+, producing NADH.

❖The electrons taken from the reaction center to reduce NAD^+ are replaced by the oxidation of H_2S to elemental S, then to SO_4^{2-}, in the reaction that defines the green sulfur bacteria.

❖This oxidation of H_2S by bacteria is chemically analogous to the oxidation of H_2O by oxygenic plants.


–1.0
–0.5
0
0.5
E'° (volts)
P840*
e⁻
Fd
NAD⁺
Fd-NAD reductase
NADH
e⁻
Q
Excitons
Cyt bc1 complex
RC P840
Cyt c553
Proton gradient
Green sulfur bacteria (Fe-S type) (b)

❖The photosynthetic apparatus of modern cyanobacteria, algae, and vascular plants is more complex than the one-center bacterial systems, and it seems to have evolved through the combination of two simpler bacterial photocenters.

❖The thylakoid membranes of chloroplasts have two different kinds of photosystems, each with its own type of photochemical reaction center and set of antenna molecules.

❖The two systems have distinct and complementary functions.

❖Photosystem II (PSII) is a pheophytin-quinone type of system (like the single photosystem of purple bacteria) containing roughly equal amounts of chlorophylls a and b.

❖Excitation of its reaction-center P680 drives electrons through the cytochrome b6 f complex with concomitant movement of protons across the thylakoid membrane.

❖Photosystem I (PSI) is structurally and functionally related to the type I reaction center of green sulfur bacteria. It has a reaction center designated P700 and a high ratio of chlorophyll a to chlorophyll b.

❖Excited P700 passes electrons to the Fe-S protein ferredoxin, then to $NADP^+$, producing NADPH.

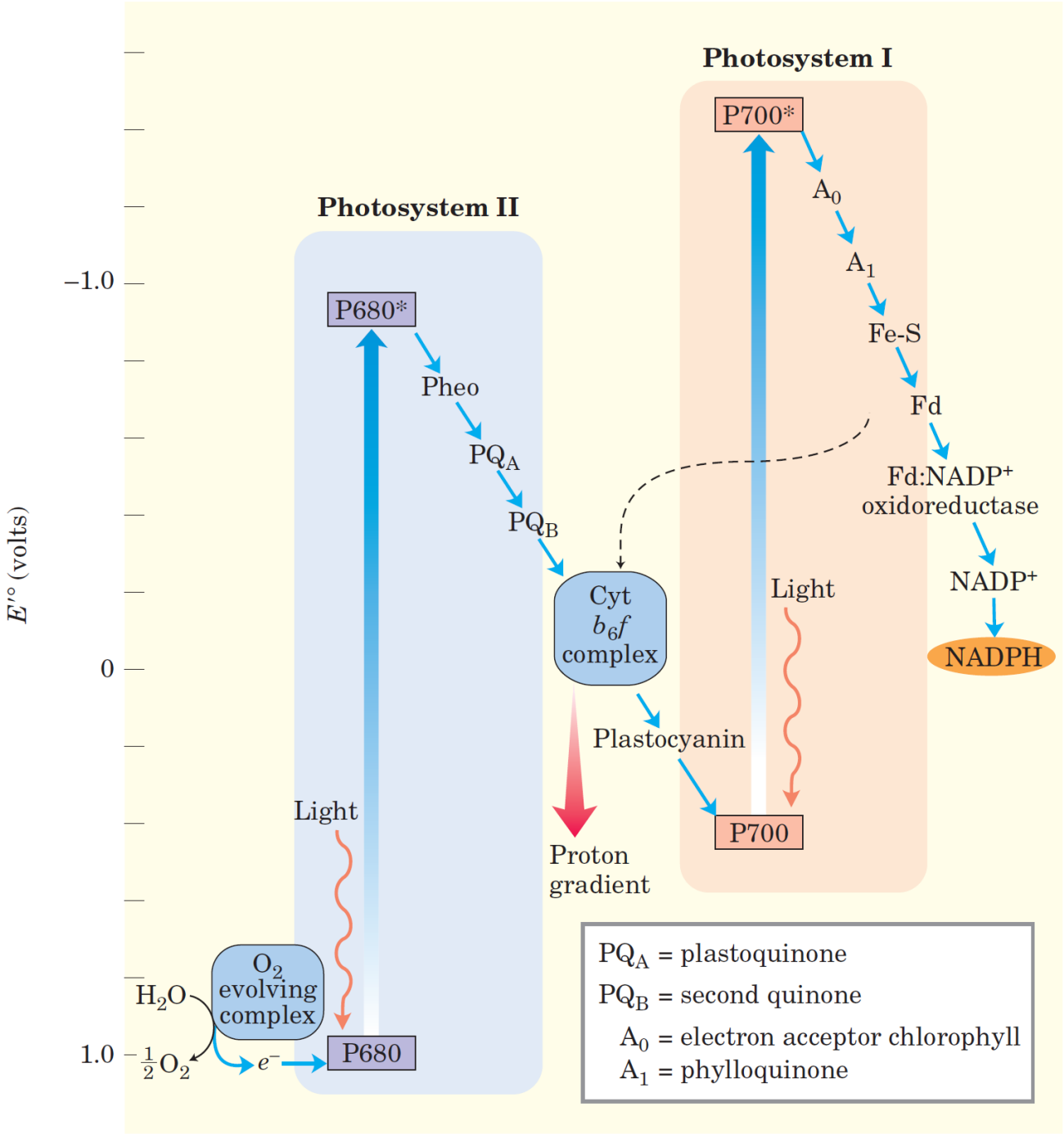
❖These two reaction centers in plants act in tandem to catalyze the light-driven movement of electrons from H_2O to $NADP^+$.

❖Electrons are carried between the two photosystems by the soluble protein plastocyanin, a one-electron carrier functionally similar to cytochrome c of mitochondria.

❖To replace the electrons that move from PSII through PSI to $NADP^+$, cyanobacteria and plants oxidize H_2O (as green sulfur bacteria oxidize H2S), producing O_2.

❖This process is called oxygenic photosynthesis to distinguish it from the anoxygenic photosynthesis of purple and green sulfur bacteria.

❖All O_2-evolving photosynthetic cells—those of plants, algae, and cyanobacteria— contain both PSI and PSII; organisms with only one photosystem do not evolve O_2.


Photosystem I
Photosystem II
P700*
A0
A1
Fe-S
Fd
Fd:NADP+ oxidoreductase
NADP+
NADPH
P680*
Pheo
PQA
PQB
Cyt b6f complex
Light
Plastocyanin
P700
Proton gradient
Light
O2 evolving complex
H2O
½ O2
e−
P680
E'° (volts)
−1.0
0
1.0
PQA = plastoquinone
PQB = second quinone
A0 = electron acceptor chlorophyll
A1 = phylloquinone

❖This diagram, often called the Z scheme because of its overall form, outlines the pathway of electron flow between the two photosystems and the energy relationships in the light reactions.

❖The Z scheme thus describes the complete route by which electrons flow from H2O to $NADP^+$, according to the equation.

$$2H_2O + 2NADP^+ + 8 \text{ photons} \longrightarrow O_2 + 2NADPH + 2H^+$$

❖For every two photons absorbed (one by each photosystem), one electron is transferred from H2O to $NADP^+$.

❖To form one molecule of O_2, which requires transfer of four electrons from two H_2O to two $NADP^+$, a total of eight photons must be absorbed, four by each photosystem.

ATP Synthesis by Photophosphorylation

❖The combined activities of the two plant photosystems move electrons from water to NADP+, conserving some of the energy of absorbed light as NADPH.

❖Simultaneously, protons are pumped across the thylakoid thylakoid membrane and energy is conserved as an electrochemical potential.

❖This proton gradient drives the synthesis of ATP, the other energy conserving product of the light-dependent reactions.

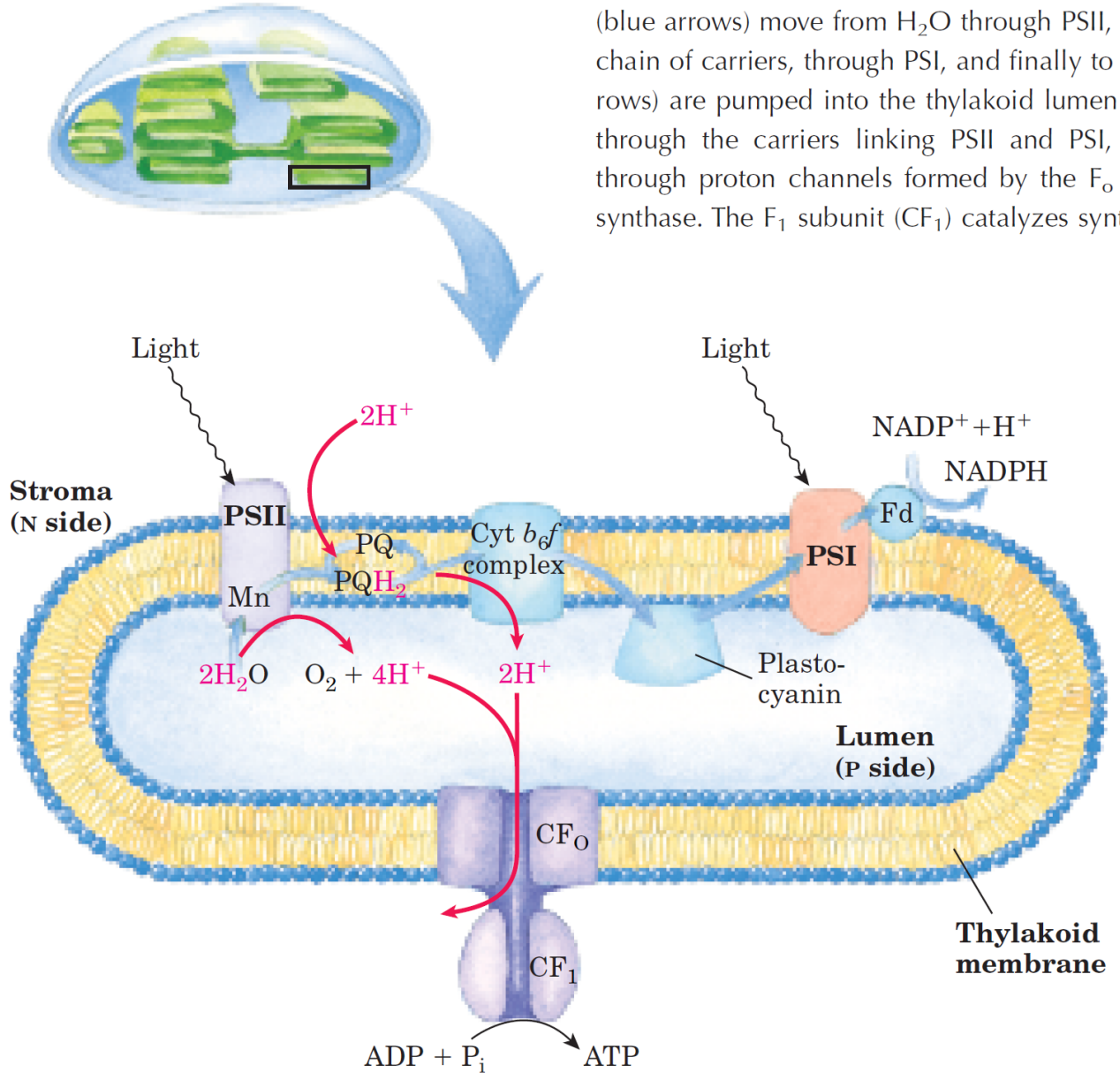


FIGURE 19–57 Proton and electron circuits in thylakoids. Electrons (blue arrows) move from H_2O through PSII, through the intermediate chain of carriers, through PSI, and finally to $NADP^+$. Protons (red arrows) are pumped into the thylakoid lumen by the flow of electrons through the carriers linking PSII and PSI, and reenter the stroma through proton channels formed by the F_o (designated CF_o) of ATP synthase. The F_1 subunit (CF_1) catalyzes synthesis of ATP.

Carbohydrate Biosynthesis in Plants and Bacteria

❖The synthesis of carbohydrates in animal cells always employs precursors having at least three carbons, all of which are less oxidized than the carbon in CO2.

❖Plants and photosynthetic microorganisms, by contrast, can synthesize carbohydrates from CO2 and water, reducing CO2 at the expense of the energy and reducing power furnished by the ATP and NADPH that are generated by the light-dependent reactions of photosynthesis.

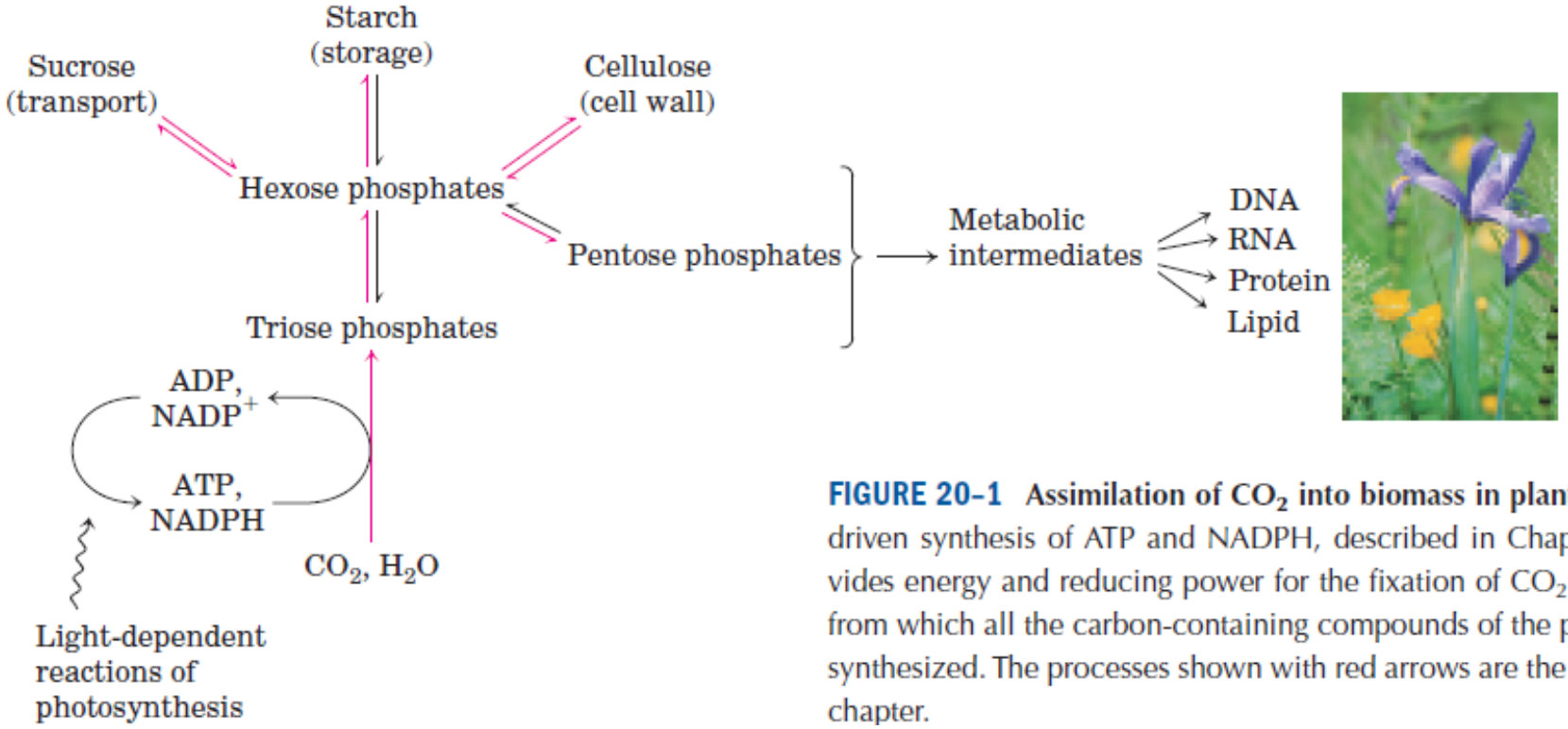


FIGURE 20–1 Assimilation of CO_2 into biomass in plants. The light-driven synthesis of ATP and NADPH, described in Chapter 19, provides energy and reducing power for the fixation of CO_2 into trioses, from which all the carbon-containing compounds of the plant cell are synthesized. The processes shown with red arrows are the focus of this chapter.

❖Most of the biosynthetic activities in plants (including CO_2 assimilation) occur in plastids, a family of self-reproducing organelles bounded by a double membrane and containing a small genome that encodes some of their proteins.

❖Chloroplasts are the sites of CO_2 assimilation.

❖The first stage in the assimilation of CO_2 into biomolecules is the carbon-fixation reaction: condensation of CO_2 with a five-carbon acceptor, ribulose 1,5-bisphosphate, to form two molecules of 3-phosphoglycerate.

❖In the second stage, the 3-phosphoglycerate is reduced to triose phosphates.

❖Overall, three molecules of CO_2 are fixed to three molecules of ribulose 1,5-bisphosphate to form six molecules of glyceraldehyde 3-phosphate (18 carbons) in equilibrium with dihydroxyacetone phosphate.

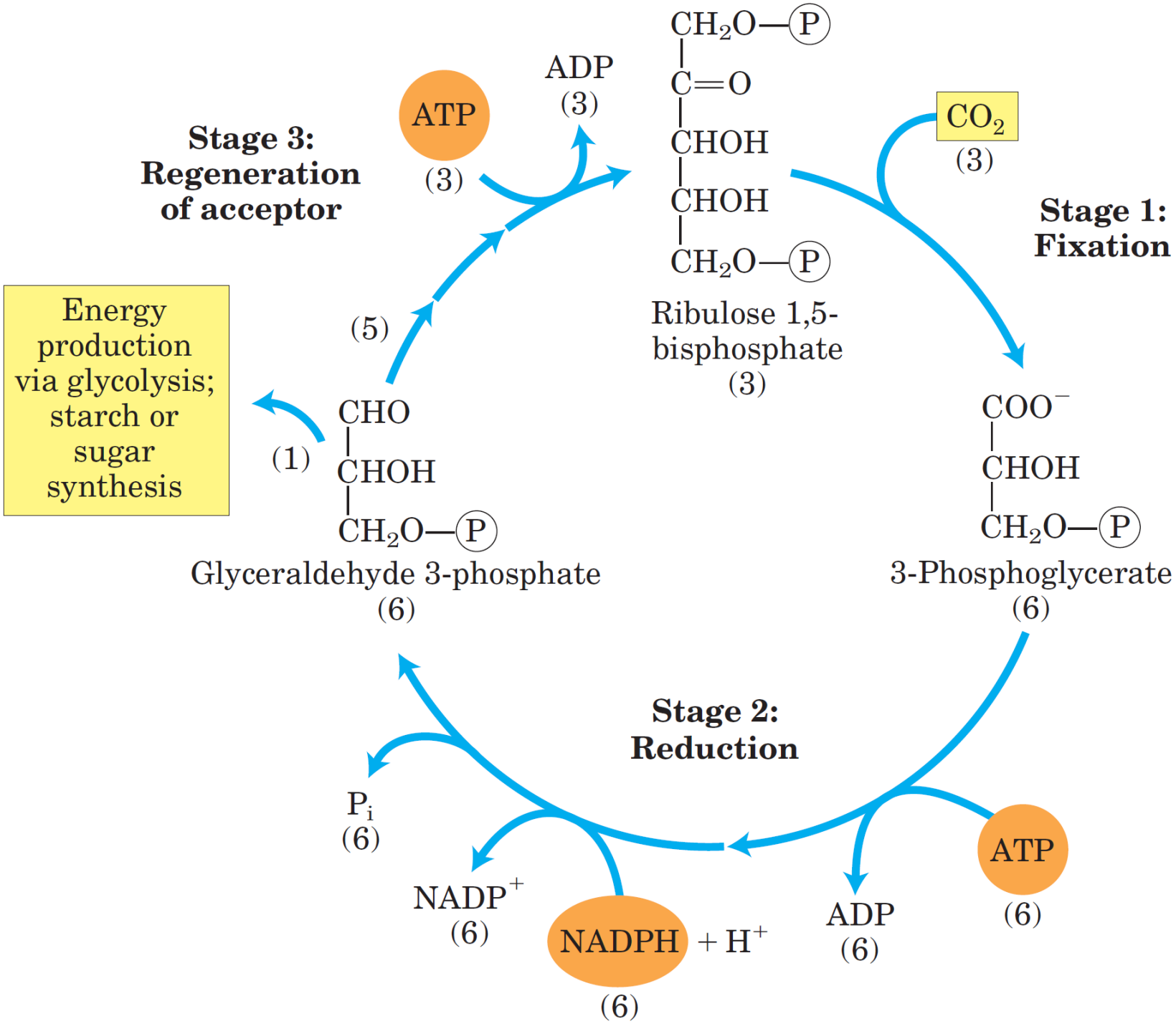
❖In the third stage, five of the six molecules of triose phosphate (15 carbons) are used to regenerate three molecules of ribulose 1,5-bisphosphate (15 carbons), the starting material.

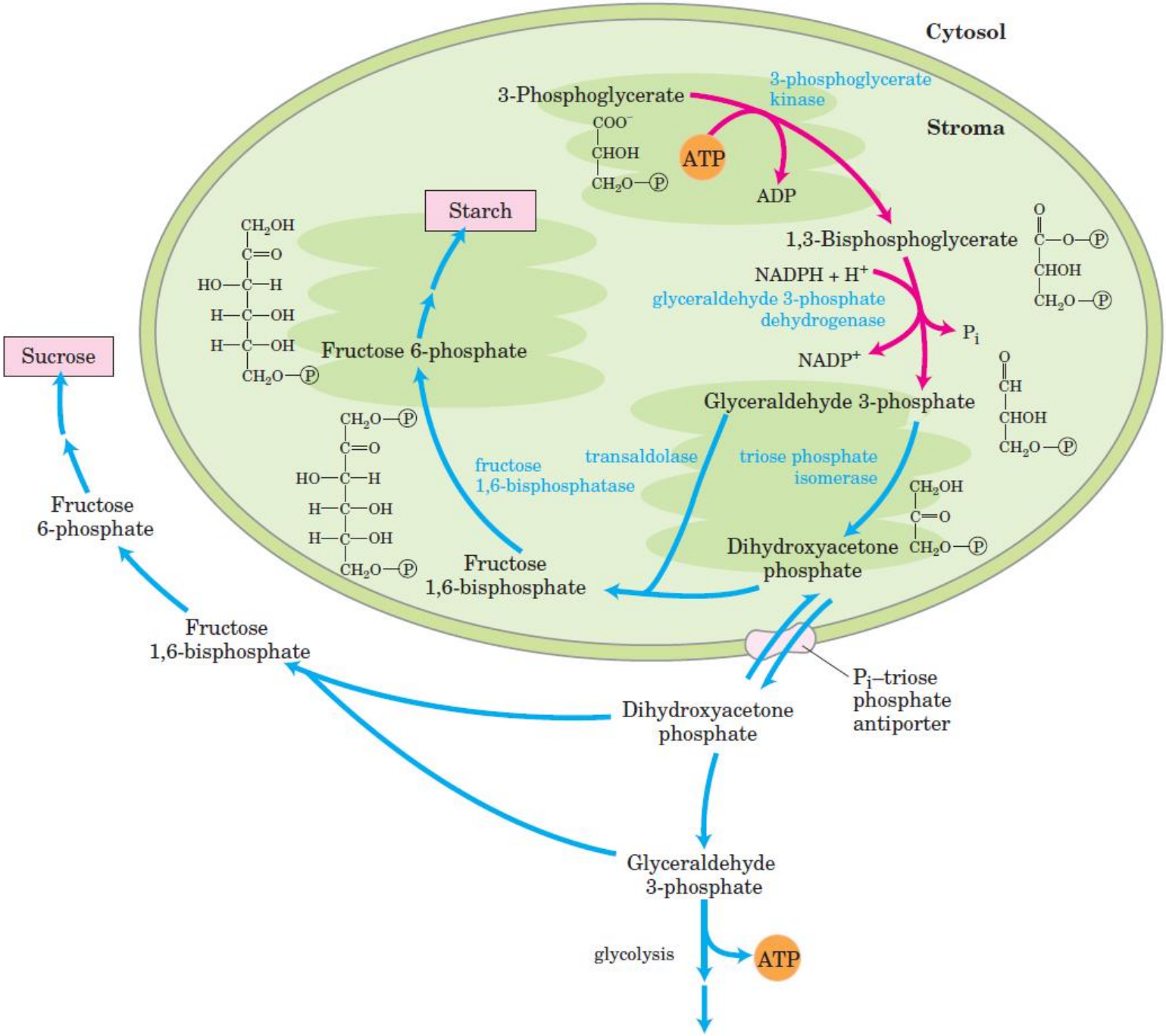
❖The sixth molecule of triose phosphate, the net product of photosynthesis, can be used to make hexoses for fuel and building materials, sucrose for transport to non-photosynthetic tissues, or starch for storage.

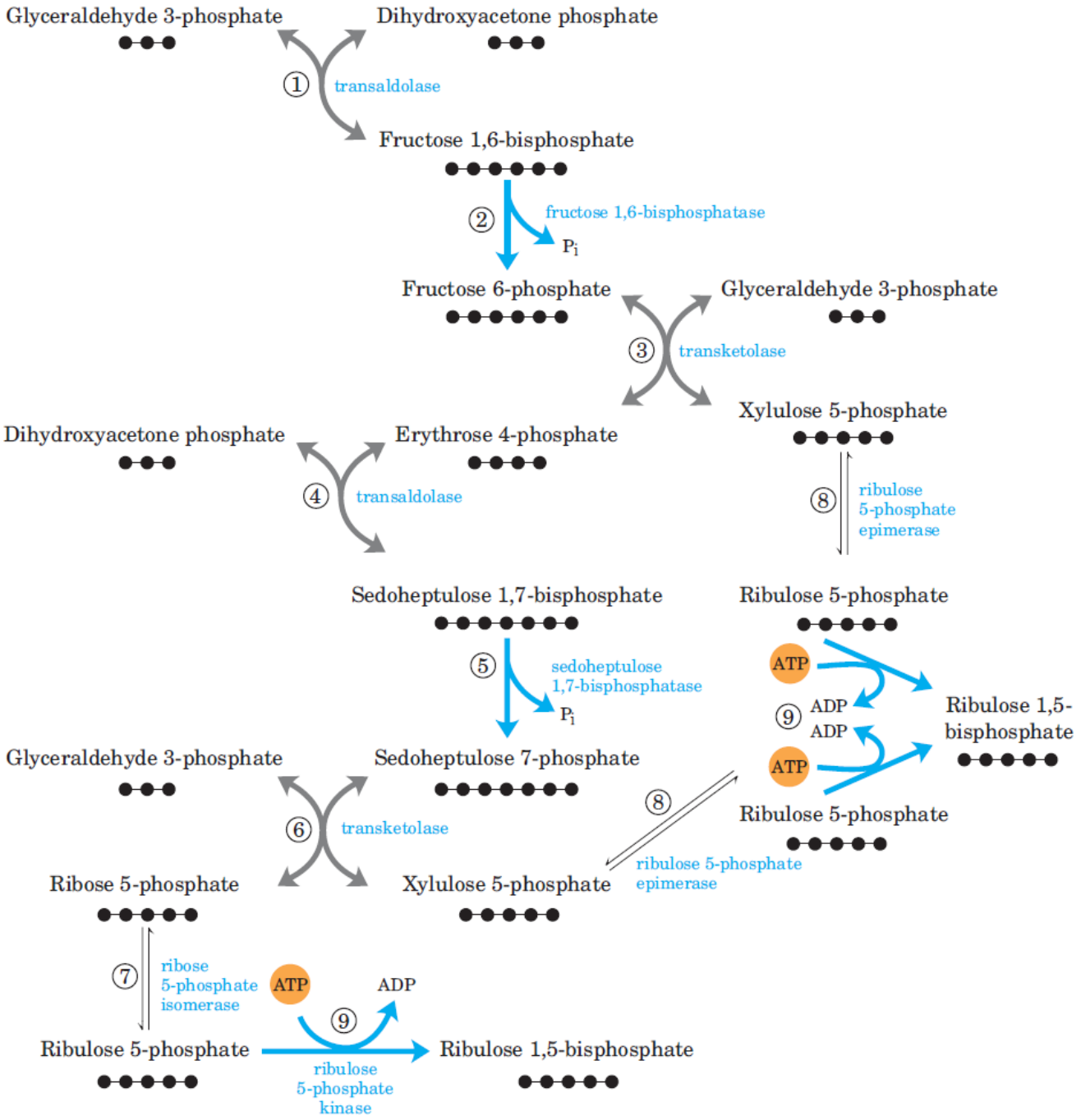
❖Thus the overall process is cyclical, with the continuous conversion of CO_2 to triose and hexose phosphates.

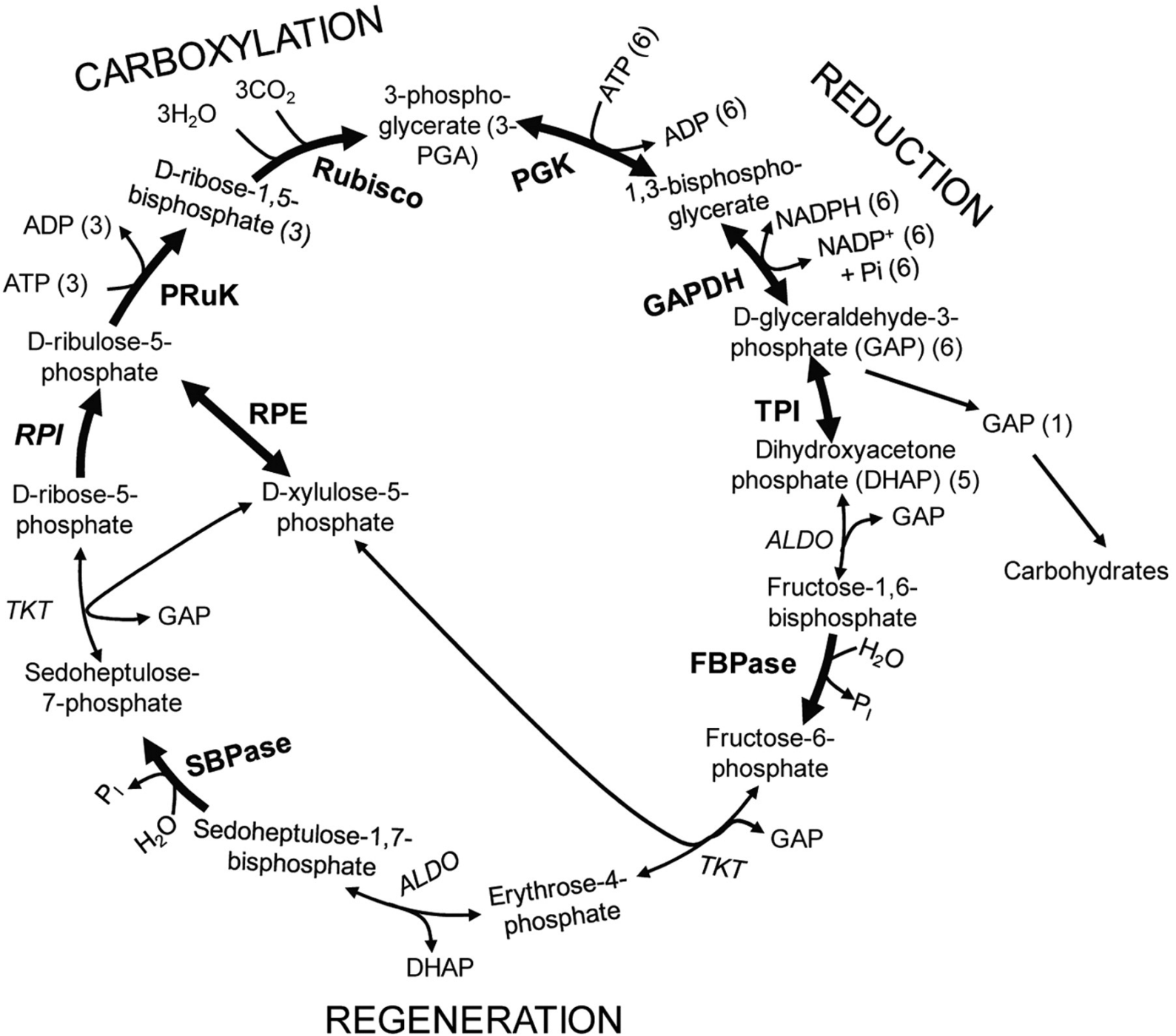
❖Fructose 6-phosphate is a key intermediate in stage 3 of CO_2 assimilation; it stands at a branch point, leading either to regeneration of ribulose 1,5-bisphosphate or to synthesis of starch.

❖The pathway from hexose phosphate to pentose bisphosphate involves many of the same reactions used in animal cells for the conversion of pentose phosphates to hexose phosphates during the nonoxidative phase of the pentose phosphate pathway


Stage 3:
Regeneration
of acceptor
ATP
(3)
ADP
(3)
CH2O—P
C=O
CHOH
CHOH
CH2O—P
Ribulose 1,5-
bisphosphate
(3)
CO2
(3)
Stage 1:
Fixation
Energy
production
via glycolysis;
starch or
sugar
synthesis
(5)
(1)
CHO
CHOH
CH2O—P
Glyceraldehyde 3-phosphate
(6)
COO−
CHOH
CH2O—P
3-Phosphoglycerate
(6)
Stage 2:
Reduction
Pi
(6)
NADP+
(6)
NADPH + H+
(6)
ADP
(6)
ATP
(6)


Cytosol
Stroma
3-Phosphoglycerate
3-phosphoglycerate kinase
ATP
ADP
1,3-Bisphosphoglycerate
NADPH + H+
glyceraldehyde 3-phosphate dehydrogenase
Pi
NADP+
Glyceraldehyde 3-phosphate
transaldolase
triose phosphate isomerase
Dihydroxyacetone phosphate
fructose 1,6-bisphosphatase
Fructose 1,6-bisphosphate
Fructose 6-phosphate
Starch
Sucrose
Fructose 6-phosphate
Fructose 1,6-bisphosphate
Dihydroxyacetone phosphate
Pi–triose phosphate antiporter
Glyceraldehyde 3-phosphate
glycolysis
ATP


Glyceraldehyde 3-phosphate
Dihydroxyacetone phosphate
① transaldolase
Fructose 1,6-bisphosphate
② fructose 1,6-bisphosphatase
Pi
Fructose 6-phosphate
Glyceraldehyde 3-phosphate
③ transketolase
Xylulose 5-phosphate
Dihydroxyacetone phosphate
Erythrose 4-phosphate
④ transaldolase
⑧ ribulose 5-phosphate epimerase
Sedoheptulose 1,7-bisphosphate
Ribulose 5-phosphate
⑤ sedoheptulose 1,7-bisphosphatase
Pi
ATP
⑨ ADP
ADP
ATP
Ribulose 1,5-bisphosphate
Glyceraldehyde 3-phosphate
Sedoheptulose 7-phosphate
⑥ transketolase
⑧
Ribulose 5-phosphate
ribulose 5-phosphate epimerase
Ribose 5-phosphate
Xylulose 5-phosphate
⑦ ribose 5-phosphate isomerase
ATP
ADP
⑨
Ribulose 5-phosphate
Ribulose 1,5-bisphosphate
ribulose 5-phosphate kinase


CARBOXYLATION
REDUCTION
REGENERATION
3CO2
3H2O
Rubisco
D-ribose-1,5-bisphosphate (3)
3-phospho-glycerate (3-PGA)
ATP (6)
ADP (6)
PGK
1,3-bisphospho-glycerate
NADPH (6)
NADP+ (6) + Pi (6)
GAPDH
D-glyceraldehyde-3-phosphate (GAP) (6)
ADP (3)
ATP (3)
PRuK
D-ribulose-5-phosphate
RPI
RPE
D-ribose-5-phosphate
D-xylulose-5-phosphate
TPI
Dihydroxyacetone phosphate (DHAP) (5)
GAP (1)
Carbohydrates
ALDO
GAP
Fructose-1,6-bisphosphate
TKT
GAP
Sedoheptulose-7-phosphate
FBPase
H2O
Pi
Fructose-6-phosphate
SBPase
Pi
H2O
Sedoheptulose-1,7-bisphosphate
ALDO
Erythrose-4-phosphate
TKT
GAP
DHAP

❖The net result of three turns of the Calvin cycle is the conversion of three molecules of CO2 and one molecule of phosphate to a molecule of triose phosphate.